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MICROSCOPIC AND AUTORADIOGRAPHIC  
STUDIES OF DISTRIBUTION OF URANIUM  
IN THE RAT KIDNEY  
Edith Seymour Jones



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MICROSCOPIC AND AUTORADIOGRAPHIC STUDIES OF DISTRIBUTION  
OF URANIUM IN THE RAT KIDNEY

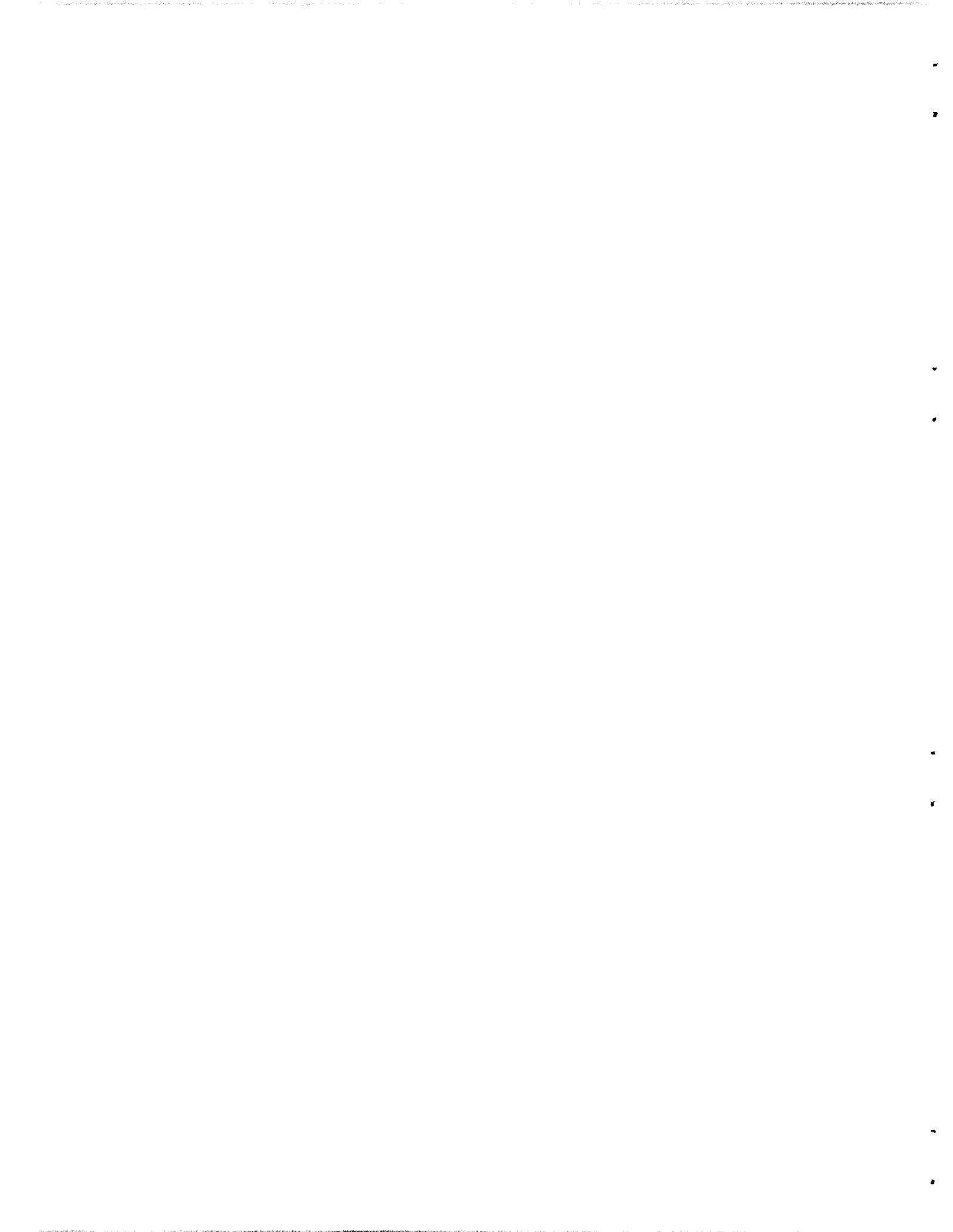
Edith Seymour Jones, Consultant

FEBRUARY 1965

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MICROSCOPIC AND AUTORADIOGRAPHIC STUDIES  
OF DISTRIBUTION OF URANIUM IN THE RAT KIDNEY

By

Edith Seymour Jones

Abstract

The distribution of uranium in rat kidney has been studied by autoradiography and the data compared with that found by radiochemical analysis in order to obtain more information relating to the phenomenon of prolonged retention of uranium by kidneys of rats given an intravenous injection of a large mass of uranium. Dose levels of 10  $\mu\text{g U/kg}$ , 100  $\mu\text{g U/kg}$ , and 1,000  $\mu\text{g U/kg}$  were used. These experiments indicate that retention is significantly higher at the two higher dosage levels than at the lowest level. A concentration ratio, the ratio of the average concentration in the cortex to the average concentration in the entire kidney, was approximately 1.3. At early times postinjection the uranium tracks were observed mainly in proximal tubules, but an increasing proportion of total tracks were in distal tubules as postinjection time increased. Leaching and redistribution of uranium, in the processes of preparation, appeared to be negligible. There were indications of a gradient in the cortex when aggregates were counted, but with aggregates excluded the concentrations were similar throughout the cortex. Proximal tubules of damaged kidneys did not show a change in diameter from those of normal kidneys, but glomeruli in injected rats tended to be smaller than in uninjected rats. Histo-pathological changes up to 28 days postinjection were striking at high dose levels.

### Introduction

This study was prompted by considerations of the data reported in the literature<sup>(1)</sup> from terminal-brain-tumor patients into whom rather large doses of natural uranium enriched with  $U^{233}$  or  $U^{235}$  were injected to determine the uptake of uranium by the tumor and the feasibility of certain courses of therapy. At autopsy, tissues, including kidney, were obtained from these patients. The findings revealed that there was a prolonged retention of uranium in the kidney, and this is of concern for occupational exposure to uranium. Hence, some additional studies were needed to test whether the prolonged retention in kidney was correlated with the gram-mass of uranium injected<sup>(2)</sup> and whether the effect of the toxicity was to prolong the retention in the kidney and slow the clearance from the body.

In some small-animal experiments,<sup>(3)</sup> it had been observed that the skeleton was an important long-term-storage organ for uranium retention. Extensive studies on rats, mice, and dogs by Tannenbaum et al.<sup>(4)</sup> led those authors to consider the kidney and bone to be so significant that "limiting of analyses to the kidney and bone, in studies on accumulation or elimination of uranium, is justified." Kisielecki et al.<sup>(5)</sup> performed an experiment with mice, injecting two dose levels of  $U^{233}$  and observed that "at the higher dose level (0.05  $\mu\text{c}/\text{gm}$ ) renal function was affected to such a degree that the kidneys could not clear the  $U^{233}$  as fast as those of the animal receiving the lower dose of 0.005  $\mu\text{c}/\text{gm}$ ." They question whether this condition is purely a uranium concentration effect or reflects some anatomical change.

Some experiments were performed by Fish and Muir<sup>(6,7)</sup> to elucidate this effect of the mass of injected amount of uranium on the retention in the kidney. Working with rats, these investigators found that "the percentage deposition in the kidneys varies directly with gravimetric dose" of uranium. The plan of these experiments and the conclusions reached were presented by Fish and Bernard at the Sixth Conference on Industrial Hygiene and Air Pollution and published in Industrial Hygiene News Report.<sup>(8)</sup> They reported that a prolonged retention of uranium in kidneys was observed in the rats but not in dogs and indicated that a species difference might be operative.

The autoradiographic observations of the microscopic distribution of uranium in the rat kidney, to be discussed below, augment the studies of this effect of the mass of uranium administered. Autoradiograms of kidneys of rats given various levels of uranium and sacrificed at different times after injection were prepared and studied for information pertinent to correlation of prolonged kidney retention and the mass of uranium injected. Although these studies are suggestive on both of these objectives, they are only of an exploratory nature and in no way can be considered to be complete. More data are needed to establish the results suggested here.

### Methods

#### Animal Injections

Solutions of uranium consisting of four different levels were injected into the saphenous vein of female white rats (strain CD and weight about 250 g). These injection solutions of hexavalent uranyl nitrate ( $\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ), buffered with an 0.4 M sodium-acetate solution,

were prepared as described by Bernard and Struxness.<sup>(1)</sup> The injection levels and isotopic composition used (Fish<sup>(6)</sup> and Muir<sup>(7)</sup>) were the following:

1,000  $\mu\text{g}/\text{kg}$  contained 10  $\mu\text{g}$   $\text{U}^{233}$  plus 990  $\mu\text{g}$   $\text{U}^{238}$   
 100  $\mu\text{g}/\text{kg}$  contained 10  $\mu\text{g}$   $\text{U}^{233}$  plus 90  $\mu\text{g}$   $\text{U}^{238}$   
 10  $\mu\text{g}/\text{kg}$  contained 10  $\mu\text{g}$   $\text{U}^{233}$  plus 0  $\mu\text{g}$   $\text{U}^{238}$   
 1  $\mu\text{g}/\text{kg}$  contained 1  $\mu\text{g}$   $\text{U}^{233}$  plus 0  $\mu\text{g}$   $\text{U}^{238}$ .

Since the  $\text{U}^{233}$  has a high specific activity (20,000 dis/min per  $\mu\text{g}$ ) and the  $\text{U}^{238}$  a low activity (1 dis/min per  $\mu\text{g}$ ), then the amount of activity injected into the animal, except for the lowest dose, is essentially constant.

Detailed results of studies on 24 rats injected with the four levels are presented in this paper. In addition, there were two uninjected control rats. The eight rats with the high-level dose of uranium (1,000  $\mu\text{g}/\text{kg}$  of body weight) were sacrificed two at 1 day, two at 2 days, and one each at 4, 7, 10, and 28 days postinjection. The nine rats with the intermediate dose of 100  $\mu\text{g}/\text{kg}$  were sacrificed two at 1 day and one each at 2, 4, 7, 10, 28, 56, and 84 days. The five rats with the low dose of 10  $\mu\text{g}/\text{kg}$  were sacrificed two at 1 day and one each at 2, 4, and 7 days. The two rats injected with the lowest dose level of 1  $\mu\text{g}/\text{kg}$  were sacrificed at 1 and 2 days.

At sacrifice each kidney was bisected and the halves weighed. The 24 rats were divided into three groups (as specified in Table 3). One half of each kidney of a rat in Group I was used for radiochemical analysis and the other half for autoradiography. For animals in Group II, cortex and medulla of a half-kidney were separated from each other with sharp needles,<sup>(9)</sup> weighed separately, and ashed for radiochemical analysis, and the corresponding half-kidney was used in autoradiographic studies. In the case of animals in Group III, autoradiograms were made

on both half-kidneys, one fixed with 10% formalin and the other prepared by the freeze-dry method.

#### Preparation of Tissues

The solutions used for fixing and dehydrating the tissues are those commonly used in pathological work<sup>(10)</sup> and are specified in the first column of Table 1.

The freeze-dry method used is that of Pearse;<sup>(11)</sup> the apparatus used is the "Canalco Freeze-Dry Unit." In order to have sections flat on the microscope slide so that the cortex and medulla may be seen clearly, the first cutting and quenching is crucial. Immediately after autopsy the excised half-kidneys were cut with a single-edged razor blade into thin sections (1 to 2 mm thick) onto paraffin paper. Each piece was kept flat, wrapped separately in aluminum foil, and dropped into a wide-mouthed test tube containing isopentane. The test tube was held by a wire frame in a Dewar flask containing liquid nitrogen which cooled the isopentane to a thick consistency, not quite frozen. As the kidneys were being excised, this consistency was maintained by dipping the isopentane in and out of the liquid nitrogen. The test tubes were numbered to correspond with the positions on the specimen screen of the drying chamber of the freeze-dry unit. Sections from only one kidney were placed in each test tube. Immediately the tissues froze as the tube was replaced quickly in the liquid nitrogen. All tissues were then kept frozen by repeated additions of liquid nitrogen into the surrounding flask until the freeze-dry unit was ready for use.

The freeze-dry equipment was prepared by the following process. The small amount of paraffin at the bottom of the drying chamber envelope

Table 1. Leaching of Uranium from Rat Kidneys in Fixing and Dehydrating solutions

Composition of Solutions	Time in Solution (hr)	Uranium Activity of Solution (dis/min/sample)		
		For High Dose Level (1000 $\mu\text{g}/\text{kg}$ )		For Low Dose Level (10 $\mu\text{g}/\text{kg}$ )
		1 Day Postinjection	7 Days Postinjection	7 Days Postinjection
10% Formalin neutralized with excess of $\text{CaCO}_3$	24	14.8	2.7	1.8
35% Ethyl alcohol	1	7.4	2.1	0.9
	1	2.5	0.6	0.7
50% Ethyl alcohol	1	2.0	2.4	1.1
	1	0.7	0.7	0.5
70% Ethyl alcohol	1	1.1	0.5	0.3
	~ 17	9.0	0.4	4.5
80% Ethyl alcohol	0.5	0.8	0.4	0.3
	0.5	0.5	0.5	0.7
95% Ethyl alcohol	1	0.8	0.2	0.7
	1	0.4	0.1	0.2
N-Butyl alcohol	2	1.1	0.1	0.8
	2	3.1	0.1	0.4
Total:				
In solutions		44.2	10.7	12.8
In quartered kidney		8317.0	3591.0	254.0
Leached from kidney		0.5(%)	0.3(%)	4.7(%)

was melted with the warm mantle and degassed by the vacuum pump. Then the pump was turned off and the entire drying chamber cooled to liquid-nitrogen temperature by covering it momentarily with a Dewar flask containing liquid nitrogen. Transfer of the specimens from the aluminum foil in the isopentane to the precooled specimen screen was then made as quickly as possible with precooled, long forceps. This is crucial to prevent any melting of the crystals originally formed in the tissues. The drying chamber envelope was quickly replaced and surrounded with the Dewar flask of liquid nitrogen and the pump turned on. Frequent additions of liquid nitrogen during the period of the experiment made possible the operation of the whole equipment at a temperature of about  $-60^{\circ}\text{C}$  under a vacuum of  $10^{-3}$  mm Hg for three days. Then the Dewar flask containing the liquid nitrogen was removed from the drying chamber and placed over the cold trap. The paraffin was melted by warming with the heating mantle and the specimen screen dropped into the paraffin so slowly that the tissues were not disturbed from position. After about 15 minutes the specimens were removed one by one into clean paraffin in a  $60^{\circ}\text{C}$  oven. This must be done rapidly so that the paraffin does not solidify. After sufficient time, depending on the size of the tissue, the tissue was embedded in the usual way.

After cutting the block on the microtome at 8 microns, the tissue was affixed directly onto the slides without spreading liquid. To assure affixation, a small amount of Mayer's egg albumen was smeared previously onto subbed slides.<sup>(12)</sup> The combination of egg albumen over the subbing emulsion proved to be more satisfactory than either fixative alone for affixing freeze-dried tissue. Transfer from the microtome was achieved by taking

each section directly from the knife with a fine brush and placing it over a square hole in a metal slide, the edges of the paraffin being pressed onto the slide with the fingers. This metal slide with the section was then placed over the albumenized subbed slide, and all were placed on a warming pan at 45°C. As the paraffin began to expand, the section was blown slightly with the breath until it expanded and dropped through the hole onto the slide, and the edges of the paraffin were cut from the metal with sharp needles. The slides with the sections thus adhered were then left in an oven to dry at 60°C.

#### Autoradiographic and Histologic Procedures

Half-kidneys from rats of Groups I and II were cut into serial sections 8 microns thick. The tissues of rats in Group III, both those fixed in 10% formalin and those freeze-dried, were cut individually at the same thickness and made into autoradiograms. From the blocks cut serially, every 40th and 41st section were mounted for autoradiograms. In all cases, adjacent sections were mounted for pathological study and stained either with hematoxylin and eosin or other specific stain. Except for the freeze-dried tissue, subbing of slides was found not to be necessary and was a disadvantage in mounting serial sections. Mayer's egg albumen was adequate. After drying and removing the paraffin, the slides for autoradiograms were hydrated and placed in distilled water at 45°C. In the dark room they were dipped in liquid NTA emulsion,\* left in a horizontal position until the emulsion solidified, dipped a second time, and left horizontally again until solidified. After placing

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\*The author is indebted to Eastman Kodak for liquid NTA emulsion for much of the early work.

in light-tight slide boxes sealed with black tape and containing a small amount of Drierite<sup>(12)</sup> for humidity control, the autoradiograms were exposed at about 4°C for periods varying with the dose level. Those from animals receiving the two higher dose levels were exposed for 14 or 28 days, and those from the low and lowest levels for longer periods, conversion factors being used to make comparable calculations.

In developing the autoradiograms, the developer (D-19), the rinse water, and the Acid Fixer were all kept at 17°C. After development in the D-19 for 6 minutes, slides were rinsed, fixed for 30 minutes, washed 45 minutes in running tap water, and immediately stained in a Giemsa<sup>(13)</sup> mixture.\*

#### Determination of Leaching

In order to determine how much uranium is removed from the kidneys during fixing and dehydrating processes, all solutions used in the processing of tissues from Group III were analyzed radiochemically for their uranium content.

Also, a thorough study was made of possible leaching during the process of embedding in paraffin. Autoradiograms were made on samples of the used paraffin and compared with autoradiograms made of unused paraffin.

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\* Some effort was directed to a study of the various kidney stains for pathological examination and for autoradiograms. Hematoxylin and eosin also were used for the autoradiograms, but observations for counting tracks were clearer with the Giemsa stain. The stain showing histological units most distinctly without emulsion was Lillie's Allochome.<sup>(10)</sup> In this the fringe borders of the proximal tubules were clearly visible with a light purple stain. The epithelial basement membranes were red purple. The nuclei were black, the cytoplasm a soft green, hyaline droplets orange, and casts a deep purple.

## Calculations

Alpha tracks were counted in the autoradiograms by the visual method, first under the compound microscope and later under a microviewscope.\* Usually tracks in 100 fields in the cortex and 50 or 100 fields in the medulla were counted.

When the work first started, the method of randomizing consisted in letting the microscope down onto a field, determining that field to be within the general area desired (cortex or medulla), and then counting eight nonoverlapping fields from that point. The next section was counted likewise. In going from one section to another, care was used to approach each section from a different angle so far as possible. In this way the majority of the tissues were counted.

To check this method, four different half-kidneys were counted by a statistically random method. The comparative results are given in Table 2. A diagram of a typical section showing four chosen fields is shown in Plate 1. The method of finding the random fields is as follows: Instead of using a random table, pennies were numbered, shaken together, and drawn to determine random digits. In this way

- Step 1. Slides were randomized
- Step 2. Sections on each slide were randomized
- Step 3. The half-section was chosen
- Step 4. The microviewscope is equipped with click stops, making possible the randomizing of both vertical and horizontal sides. At the first four points of intersection, the fields were read. Sometimes groups of three or four fields would be read at each point.
- Step 5. For the second section to be counted, return was made to the second slide of step 1 and the process repeated.

---

\* Reichert Microviewscope (HALCO Co.)

Table 2. Comparison of the Two Counting Methods

Injection Level	Sacrifice Time Postinjection (days)	Regular Method			Randomized Method		
		Mean No. of Counts per Field		No. of Aggregates	Mean No. of Counts per Field		No. of Aggregates
		Cortex	Medulla		Cortex	Medulla	
1000	1	526	56.5	14	407	35.4	10
1000	10	51.5	42		43	30.5	
100	1	306	66.5		272	80	
10	4	8	1.4		7.8	1.6	

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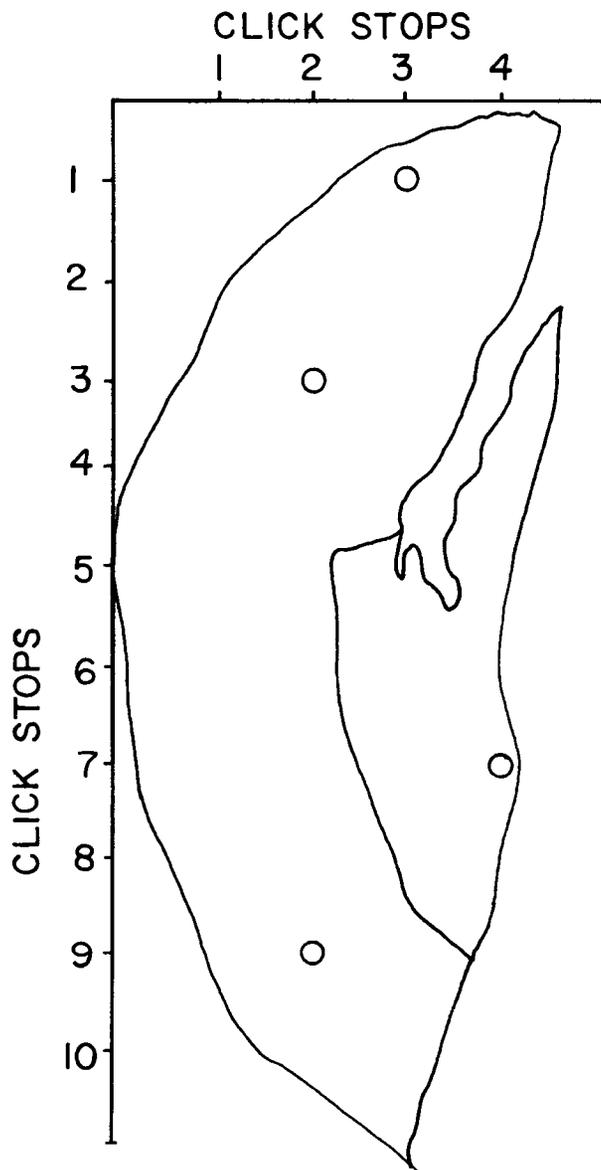


Plate 1. Location of Microscopic Fields in Rat Kidney Section

It is evident from Table 2 that the results of this random counting agreed sufficiently with the counts made by the previous method. At the high level, the difference between the results of the two methods may be due to the difference in the number of aggregates which happened to be included in the fields counted. When aggregates occurred in the field, the tracks were counted as far as visibly separate, and those in the dense areas were estimated. These counts were classified by conventional statistical methods. The number of tracks per field was converted to uranium concentration in the following manner:

Let  $r$  denote the radius of the microscopic field in microns,  
 $t$  the thickness of the tissue section in microns,  
 $c$  the number of tracks counted in the field, and  
 $D$  the number of days of exposure.

The volume of the field is considered to be  $\pi r^2 t$ .

Assuming the specific gravity of the tissue to be  $1.05^{(14)}$  and that each disintegration within the volume produces a track which is counted, then the disintegrations per minute per gram are given by

$$\text{dis min}^{-1} \text{g}^{-1} = \frac{10^{11} c}{151.2 D \pi r^2 t} .$$

Letting  $r = 185 \mu$ ,  $t = 8 \mu$ , and using  $D = 28$  days to correspond to the exposure time,

$$\text{dis min}^{-1} \text{g}^{-1} = 27.46 c .$$

A geometry factor of 2 might be assumed if stripping film were used, but the liquid photographic emulsion seems to penetrate the cellular structure so that all tracks are countable. This assumption that the emulsion penetrated the cellular structure so that essentially all alpha tracks

are visible from either side was tested by turning the slide with the autoradiogram over and recounting an identifiable field. Thus, tracks were counted in the same fields from both sides at a magnification of 200, the fields being accurately identified by cellular structure. The fields seen from the two sides were nearly always identical. Although it is possible that some disintegrations did not result in detectable tracks in the emulsion, the agreement between autoradiographic and radiochemical estimations indicates that such undetected disintegrations were few in number.

#### Estimated Uranium Concentration in Uninjected Rats and in the Emulsion of the Autoradiograms

To ascertain whether there was background activity, two uninjected rats were studied, both autoradiographically and radiochemically. The tracks visible in the tissues of the autoradiograms were negligible. The average dis  $\text{min}^{-1}\text{g}^{-1}$  found by radiochemical analysis in one rat was 3.05 and 29 in the other.

Since any background tracks might be especially significant with low-level concentrations, count was made on the emulsion of the autoradiogram of a kidney of one of the rats injected with 10  $\mu\text{g}/\text{kg}$  and sacrificed at 4 days. One hundred fields were counted and classified statistically. The graph obtained is shown in Fig. 1. The arithmetic mean of the background counts in these fields is 9.6 dis  $\text{min}^{-1}\text{g}^{-1}$ .

Before each slide was read, 10 fields in the emulsion itself on that slide were studied to ascertain if there were tracks which might affect the calculations. In no case did the emulsion itself contain activity high enough to affect significantly the mean counts per field.

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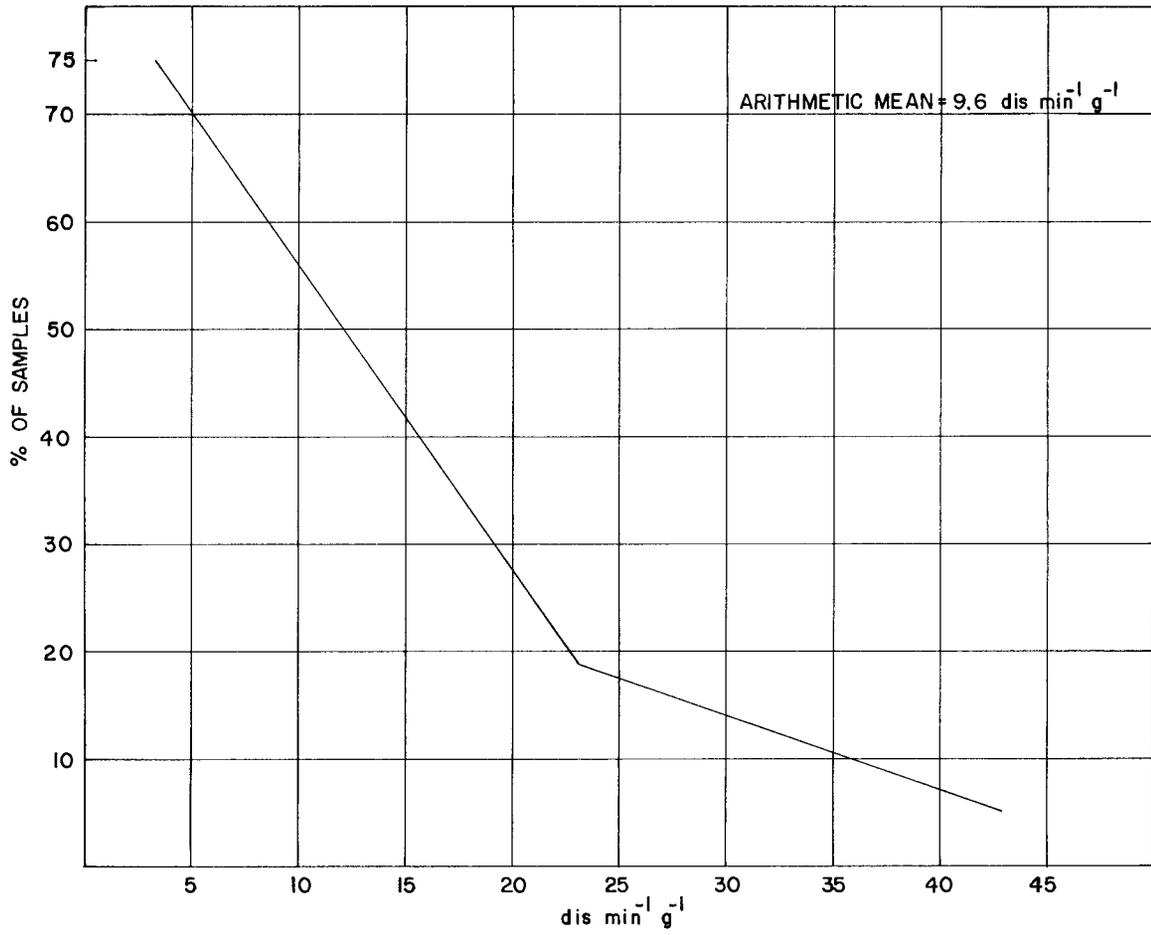


Fig. 1. Distribution of Uranium in Emulsion 4 Days Following Intravenous Injection of 10 µg U per kg of Body Weight

## Concentration Ratio

The concentration ratio, defined as the ratio of the average concentration in the cortex to the average concentration in the entire kidney, may be estimated in two ways. First, in autoradiograms, the ratio of the average number of tracks in the fields in the cortex to the average number of tracks per field, assuming the entire activity in the kidney spread uniformly over the whole kidney, is calculated and the estimate denoted by  $R_k$ . Second, in the material analyzed radiochemically, the ratio of the average of the  $\text{dis min}^{-1}\text{g}^{-1}$  in the cortex to the average  $\text{dis min}^{-1}\text{g}^{-1}$  for the entire kidney is calculated and the estimate denoted by  $R_{k1}$ . In previous reports<sup>(15,16)</sup> the concentration factor has been called a "non-uniform distribution factor." The contrast between activities in cortex and medulla is illustrated by the photographs in Plates 2 and 3.

It should be noted that the concentration ratio could be estimated from the ashed kidneys only when the cortex and medulla were weighed separately. This entailed separating cortex and medulla immediately on excising the kidneys. Miller and Carlton,<sup>(17)</sup> working with the cat, did careful separation of the tissues but fixed them first in Müller's fluid. Fischermann,<sup>(9)</sup> however, working with the rabbit, dissected before fixation and found the line of demarcation so distinct that the dissection was made without the use of a dissecting microscope.

Before proceeding, the author tested the method. With the use of a dissecting microscope, cortex and medulla of an extra half-kidney were separated, prepared for sectioning in the usual way, cut into complete serial sections at 5 microns thick, and stained with hematoxylin and eosin. In this way it was possible to make an examination of all the

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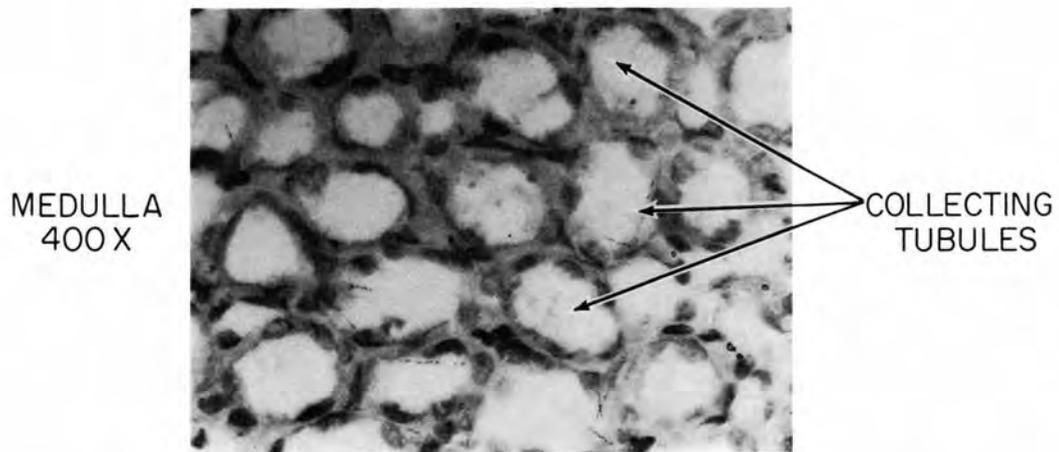
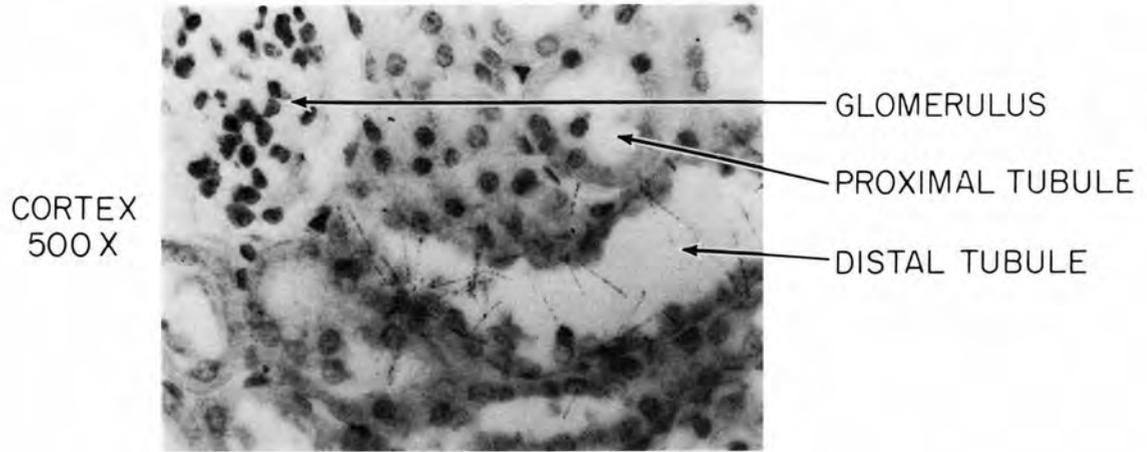


Plate 2. Autoradiograms of Kidney Cortex and Medulla from a Rat Injected with 100  $\mu\text{g}/\text{kg}$  of Uranium and Sacrificed 28 days Postinjection

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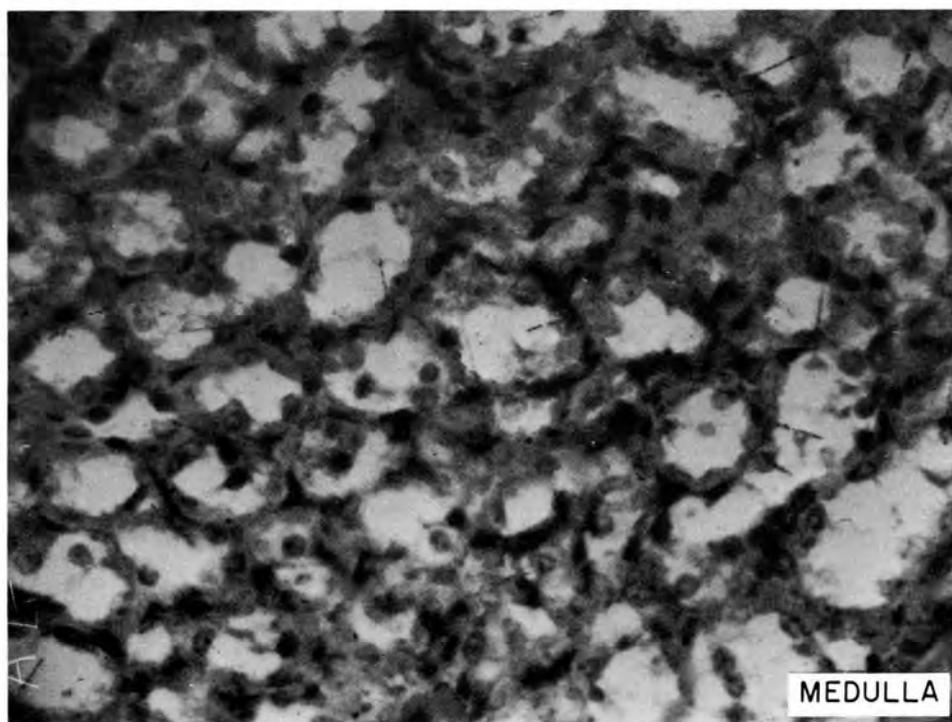
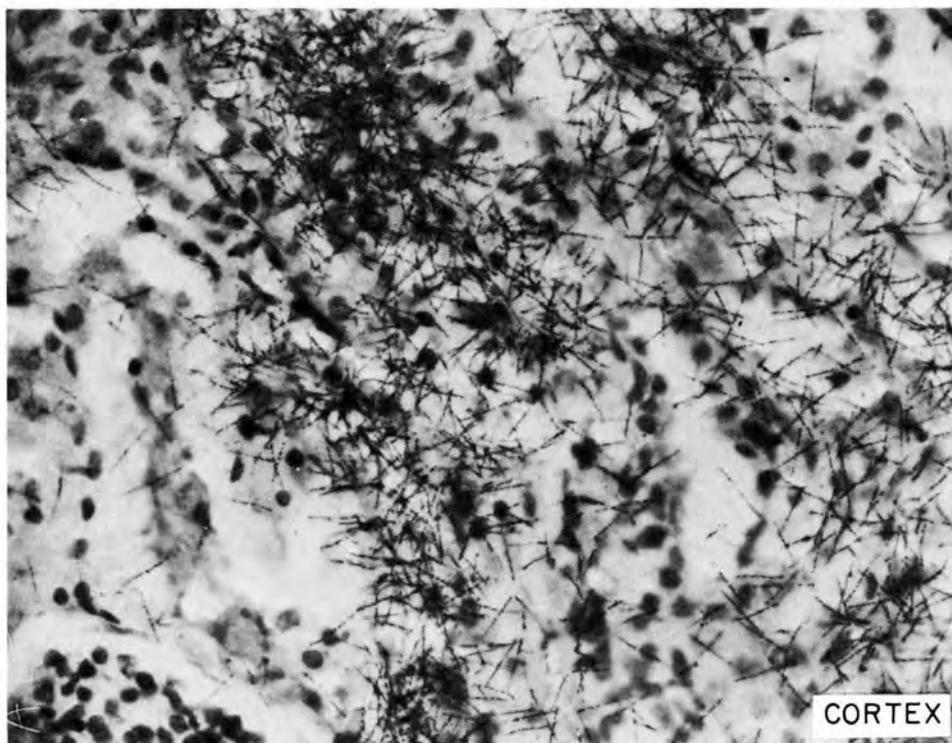


Plate 3. Autoradiograms of Kidney Cortex and Medulla from a Rat Injected with 1000  $\mu\text{g}/\text{kg}$  of Uranium and Sacrificed 1 Day Postinjection

tissue microscopically. Only four microscopic bits of cortex were found in the entire set of medulla ribbons on 12 slides. This led to the conclusion that the method was reasonably accurate. However, it also showed that bits of cortex might be ashed with medulla, and so error might be introduced.

The concentration ratio (Table 3) for 40 half-kidneys based on track counting on autoradiograms of rats injected with the four levels of uranium and sacrificed at the various times postinjection has been calculated and compared with the concentration ratio based on alpha activity of 30 corresponding half-kidneys measured radiochemically. Considering the cortex to be 70% of the entire mass of the kidney,<sup>(14)</sup> the formula for calculating the ratio  $R_k$  is as follows:

$$\begin{aligned} \text{Let } C &= \text{average counts per field after } D \text{ days in the cortex,} \\ M &= \text{average counts per field after } D \text{ days in the medulla,} \\ K &= \text{estimated average counts per field over entire half-} \\ &\quad \text{kidney after } D \text{ days,} \\ K &= 0.7 C + 0.3 M, \\ R_k &= C/K. \end{aligned}$$

In the kidneys ashed, let

$$\begin{aligned} C' &= \text{dis min}^{-1} \text{g}^{-1} \text{ in cortex,} \\ M' &= \text{dis min}^{-1} \text{g}^{-1} \text{ in medulla,} \\ K' &= \text{estimated dis min}^{-1} \text{g}^{-1} \text{ over entire ashed half-kidney,} \\ K' &= 0.7 C' + 0.3 M', \\ Rk' &= C'/K'. \end{aligned}$$

### Results

#### Concentration after Injection of 1 $\mu\text{g}/\text{kg}$

The tissues of the three rats injected with 1  $\mu\text{g}/\text{kg}$  and sacrificed at 1, 2, and 10 days were exposed for 147 days. After a careful count of background activity, the highest cortex concentration level found at

1 day postinjection was  $55 \text{ dis min}^{-1} \text{ g}^{-1}$ . At 2 days postinjection the activity was still lower, and at the tenth day there was no indication of activity. Because the values at 10 days were of the order of those obtained in the study of background, the results are not considered as significant and are not discussed below.

#### Concentration Ratio for Uranium in Rat Kidney

The concentration ratio did not appear to vary markedly with time or dose level; therefore, estimates for all groups have been averaged. The individual estimates are shown in Table 3, and the average values at each time and dose level are shown in Table 4. The average of all values of  $R_k$  ( $R_{k,}$ ) in Table 3 is 1.3 (1.2) with a range of 0.56 (0.8) to 1.41 (1.37) and a probable error of 0.11 (0.11). The average of all values in Table 4 for  $R_k$  is 0.01 lower than in Table 3, but for  $R_{k,}$  the average is the same. Exceptionally low ratios were observed at 4-days-postinjection time with the intermediate dose of  $100 \mu\text{g}/\text{kg}$  and with the radiochemically analyzed kidneys sacrificed at 10 days in the two highest levels. The kidneys of animals given  $100 \mu\text{g}/\text{kg}$  and sacrificed at 84 days showed greater activity in the medulla, reversing the general trend; and when activity was observed in the cortex, it was more frequently in the distal than in the proximal tubules.

#### Comparison between Activity in Autoradiograms and Activity in Ashed Material

Activities determined by track counting are compared with the activities obtained by chemical analysis and appear in Table 5. Activities in the whole half-kidneys observed in autoradiograms are calculated with the formula given above, i.e.,  $0.7 C + 0.3 M = K$ . When the radiochemical

Table 3. Rat Kidney Concentration Ratios Based on Autoradiographic Data ( $R_k$ ) and Radiochemical Analysis ( $R_{k'}$ ) of Individual Half-Kidneys

Postinjection Time (days)	Group*	D O S A G E							
		1000 $\mu\text{g}/\text{kg}$		100 $\mu\text{g}/\text{kg}$		10 $\mu\text{g}/\text{kg}$		1 $\mu\text{g}/\text{kg}$	
		$R_k$	$R_{k'}$	$R_k$	$R_{k'}$	$R_k$	$R_{k'}$	$R_k$	$R_{k'}$
1	II	1.37	1.34	1.28	1.31	1.35	1.37	1.32	1.17
		1.36	1.33	1.31	1.37	1.39	1.10	1.33	1.35
	III	1.36		1.31		1.33			
2	II	1.39	1.34	1.22	1.13	1.41	1.28	1.26	1.13
		1.38	1.16	1.08	1.30	1.40	1.34	1.30	
		1.35	1.37						
4	II	1.26	1.31	1.03	1.02	1.32	1.27		
		1.24	1.26	1.07	1.11	1.31			
7	III	1.30		1.28		1.30			
10	II	1.06	0.8	1.20	1.20				
		1.19	1.37	1.24	0.97				
28	II	1.36	1.19	1.16	1.24				
		1.36	1.06						
56	I			1.33					
				1.11					
84	I			0.79					
				0.56					

\* See page 4 for treatment groups.

Table 4. Average Concentration Ratio of Uranium in Rat Kidney  
Based on Autoradiographic Data ( $R_k$ ) and Radiochemical Analysis ( $R_{k'}$ )

Post-injection Time (days)	1,000 $\mu\text{g}/\text{kg}$				100 $\mu\text{g}/\text{kg}$				10 $\mu\text{g}/\text{kg}$				1 $\mu\text{g}/\text{kg}$			
	No. of 1/2 Kidneys	$R_k$	No. of 1/2 Kidneys	$R_{k'}$	No. of 1/2 Kidneys	$R_k$	No. of 1/2 Kidneys	$R_{k'}$	No. of 1/2 Kidneys	$R_k$	No. of 1/2 Kidneys	$R_{k'}$	No. of 1/2 Kidneys	$R_k$	No. of 1/2 Kidneys	$R_{k'}$
1	3	1.36	2	1.34	3	1.30	2	1.34	3	1.36	2	1.24	2	1.33	2	1.21
2	3	1.37	3	1.29	2	1.15	2	1.22	2	1.41	2	1.31	2	1.28	1	1.13
4	2	1.25	2	1.29	2	1.05	2	1.06	2	1.32	1	1.27				
7	1	1.30			1	1.28			1	1.30						
10	2	1.13	2	1.09	2	1.22	2	1.09								
28	2	1.36	2	1.13	1	1.16	1	1.24								
56					2	1.22										
84					2	0.63										

Table 5. Part I. Arithmetic Means of Uranium Concentrations in Half Kidneys Autoradiographic Method Compared with Radiochemical in  $\text{dis min}^{-1} \text{g}^{-1}$

Level ( $\mu\text{g}/\text{kg}$ )	Postinjection Time (days)	Autoradiographic Mean ( $\text{dis min}^{-1} \text{g}^{-1}$ )	Radiochemical Mean ( $\text{dis min}^{-1} \text{g}^{-1}$ )	Ratio Between the two Methods
1000	1	8,923	11,642	0.76
1000	1	10,579	6,209	1.7
1000	2	5,793	6,807	0.85
1000	2	7,807	4,841	1.6
1000	2	8,129	6,227	1.3
1000	4	2,032	2,983	0.7
1000	4	2,101	4,347	0.48
1000	10	1,337	614	2.17
1000	10	805	1,209	0.66
1000	28	1,065	1,115	0.96
1000	28	917	1,393	0.66
100	1	6,923	7,286	0.95
100	1	6,437	4,681	1.38
100	2	6,418	5,570	1.15
100	4	2,208	2,590	0.85
100	4	2,057	3,073	0.67
100	10	836	605	1.38
100	10	1,022	840	1.22
100	28	2,192	4,471	0.49
10	1	2,882	1,987	1.45
10	1	4,896	3,626	1.35
10	2	3,753	2,556	1.47
10	2	3,122	2,380	1.31
10	4	169	184	0.92
				Av 1.1

Probable error of the ratio = 0.28.

**Table 5. Part II. Arithmetic Means of Uranium Concentration in Cortex Autoradiographic Method Compared with Radiochemical in  $\text{dis min}^{-1} \text{g}^{-1}$**

Level ( $\mu\text{g}/\text{kg}$ )	Postinjection Time (days)	Autoradiographic Mean ( $\text{dis min}^{-1} \text{g}^{-1}$ )	Radiochemical Mean ( $\text{dis min}^{-1} \text{g}^{-1}$ )	Ratio Between the two Methods
1000	1	12,181	13,124	0.93
1000	1	14,447	6,740	2.14
1000	2	8,031	7,790	1.03
1000	2	10,736	5,482	1.96
1000	2	11,012	7,251	1.52
1000	4	2,546	3,218	0.79
1000	4	2,568	4,538	0.57
1000	10	956	1,509	0.63
1000	10	1,415	512	2.76
1000	28	1,458	1,275	1.14
1000	28	1,245	1,504	0.83
100	1	8,827	7,693	1.15
100	1	8,413	4,935	1.7
100	2	7,832	5,815	1.35
100	4	2,282	2,622	0.87
100	4	2,189	3,059	0.72
100	10	994	645	1.54
100	10	1,252	821	1.52
100	28	2,537	4,731	0.54
10	1	3,902	2,274	1.72
10	1	6,819	3,694	1.85
10	2	5,306	2,953	1.80
10	2	4,404	2,766	1.60
10	4	222	200	1.11
			Av	1.32

Probable error of the ratio 0.35.

Table 5. Part III. Arithmetic Means of Uranium Concentrations in Medulla, Autoradiographic Method Compared with Radiochemical in  $\text{dis min}^{-1} \text{g}^{-1}$

Level ( $\mu\text{g}/\text{kg}$ )	Postinjection Time (days)	Autoradiographic Mean ( $\text{dis min}^{-1} \text{g}^{-1}$ )	Radiochemical Mean ( $\text{dis min}^{-1} \text{g}^{-1}$ )	Ratio Between the two Methods
1000	1	1321	2015	0.65
1000	1	1552	1124	1.38
1000	2	569	1155	0.49
1000	2	973	2913	0.33
1000	2	1401	782	1.79
1000	4	799	709	1.13
1000	4	969	1436	0.67
1000	10*	1154	877	1.32
1000	28	147	591	0.25
1000	28	151	1203	0.13
100	1	2480	1701	1.46
100	1	1826	524	1.23
100	2	312	3527	0.88
100	4	2038	2452	0.83
100	4	1752	1743	1.00
100	10	467	290	1.61
100	10	483	904	0.53
100	28	1386	1705	0.81
10	1	503	236	2.13
10	1	410	2545	0.16
10	2	129	829	0.16
10	2	132	439	0.3
10	4	44	58	0.76
				Av 0.87

Probable error of the ratio = 0.36.

\*Values for right kidney or rejected because of error in analysis.

method of analysis was used, the activity concentration (disintegrations per minute) in the ashed material of the cortex, medulla, or half-kidney was calculated using the actual weight.

These values, so compared, gave a gross check. Due to the difficulties of separating rapidly the fresh medulla from the cortex and weighing the proportionately very small amounts of medulla, the method could not be sufficiently accurate for detailed comparison. Also, the presence of aggregates may give a slight bias. This will be discussed later when considering a possible gradient.

The ratio of the estimate of activity by autoradiographic counting to the estimate by radiochemical analysis was averaged. A mean ratio of 1.3 with a probable error of 0.35 was obtained for the cortex, 0.87 with a probable error of 0.36 for the medulla, and 1.1 with a probable error of 0.28 for the half-kidney.

#### Location of Uranium Tracks in Histological Units

A study was made of the histological cells from which the uranium tracks seemed to originate. In the process of counting tracks, the location of these tracks in the histologic units was noted. When the deposition of uranium was heavy, it was difficult to recognize all the cells beneath the tracks. An attempt was made to distinguish, as accurately as possible, the individual cells in the tissues of four animals, two injected with 1,000  $\mu\text{g}/\text{kg}$  of uranium and two with 100  $\mu\text{g}/\text{kg}$  with postinjection times of 1, 28, 56, and 84 days. Table 6 gives the total number of tracks counted and the number of tracks whose point of deposition could be recognized. The number of tracks present in proximal tubules, distal tubules, and glomeruli were recorded, and all other tracks in identified

**Table 6. Histologic Units in Cortex in Which Tracks Were Observed**

Dose ( $\mu\text{g}/\text{kg}$ )	Postinjection Time of Sacrifice (days)	Total No. of Tracks Counted	No. of Tracks in Identified Cells	% of Tracks in Identified Cells	% of Tracks in Proximal Convoluted Tubules	% of Tracks in Distal Tubules	% of Tracks in Glomeruli	% of Tracks in Other Units
1000	1	43,969	40,458	92	96	1	1	2
1000	1	43,231	43,055	99.6	97	0.6	0.5	1.9
1000	28	2,403	1,279	53	72	7	3	18
1000	28	2,267	884	39	47	4.8	1.8	53.6
100	56	82	79	96	80	10	3.6	6.4
100	56	107	107	100	72	10	2.8	15.2
100	84	2,774	2,549	92	30	61	0.5	8.5
100	84	3,288	2,199	67	9.6	77	0.2	13.2

cells were grouped together as a fourth category. The table gives each of these track groups as a percentage of the total number of tracks in identified cells. A high percentage of the tracks was found in the proximal tubules, their epithelial cells, nuclei, and cellular debris in the autoradiograms from animals sacrificed at 1, 28, and 56 days, and a low percentage was found in the distal tubules. However, in those sacrificed at 84 days, the high percentage of tracks was found in the distal tubules. There was a trace of uranium found in the glomeruli at both dose levels and at all four injection times. Plate 4 shows kidney cortex with an aggregate and scattered tracks of uranium. The tracks probably originate from proximal tubules. This material is from a rat injected with 1,000  $\mu\text{g}/\text{kg}$  and sacrificed at 1 day. The percentage of tracks in distal tubules increased with time. In Plate 2 the tracks in the kidney cortex of a rat injected with 100  $\mu\text{g}/\text{kg}$  and sacrificed at 28 days are seen to arise mainly from the epithelial cells of a distal tubule.

#### Leaching of Uranium from Tissues

Table 1 presents data obtained in the studies of leaching of uranium from fixing and dehydrating processes. In column 1 appears the composition of the solutions, in column 2 the length of time the kidneys remained in the solutions, while in columns 3, 4, and 5 the amount of uranium (disintegrations per minute) found in the solution after removal of the kidney material. As can be noted at the bottom of the table, very little (0.3 to 4.7% of the total uranium) was leached by the solutions, thus corroborating the work of Neuman.<sup>(3)</sup> Two control solutions of 45 cc of the formalin each showed 0.618 disintegrations per minute per sample. In general, most leaching occurred when samples remained in the solutions for extended periods (12-24 hours) of time.

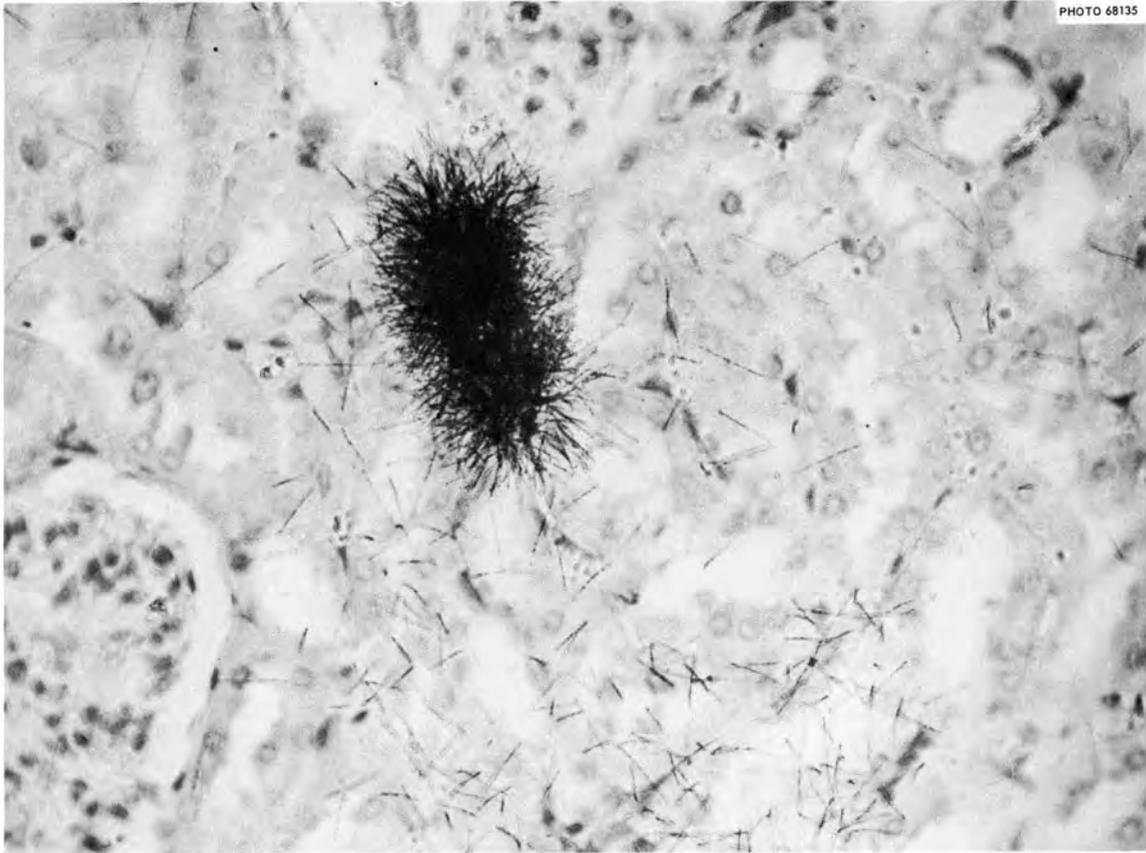


Plate 4. Uranium Tracks in Rat Kidney Cortex. Injection dose 1000  $\mu\text{g}/\text{kg}$ . Sacrificed at 1 day. Tracks are mainly from proximal tubules. At least one track is evident in the glomerulus. Mag. 250. Stain Giemsa. Reduced 41%.

From the thorough study made of possible leaching during the process of embedding in paraffin, microscopic examination revealed no appreciable concentration of uranium, leading to the conclusion that leaching during embedding is negligible.

#### Distribution Studies

##### Distribution of Uranium in Rat Kidney Tissues Prepared by the Freeze-Dry Method Compared with Formalin-Fixed Tissues

The freeze-dry method of fixation was carried through to observe the distribution of uranium and to contrast this with the distribution found in formalin-fixed material. This gave a check against possible alteration of distribution by formalin fixation and the ensuing dehydrating solutions. Kidneys from two rats administered 1,000  $\mu\text{g}/\text{kg}$  and sacrificed at 7 and 1 days, respectively, were made into autoradiograms, the left half having been fixed with formalin and the right half freeze-dried as described above.

Data obtained from both the formalin-fixed and the freeze-dried tissues were classified statistically and plotted. The distribution curves are shown in Figs. 2 and 3. Fig. 2 shows the curves compiled from the data assembled from the tissues of the animal sacrificed at 7 days postinjection. There is almost identical agreement between the one curve representing data from the freeze-dried tissue and the other from formalin-fixed tissue. Fig. 3 shows the contrasting curves constructed from the data of the two differently prepared tissues from the animal sacrificed at 1 day. These latter show a slight lack of agreement, perhaps due to the fewer number of samples obtainable from freeze-dried material or to an actual difference in distribution in the left and right kidneys of the same animal.

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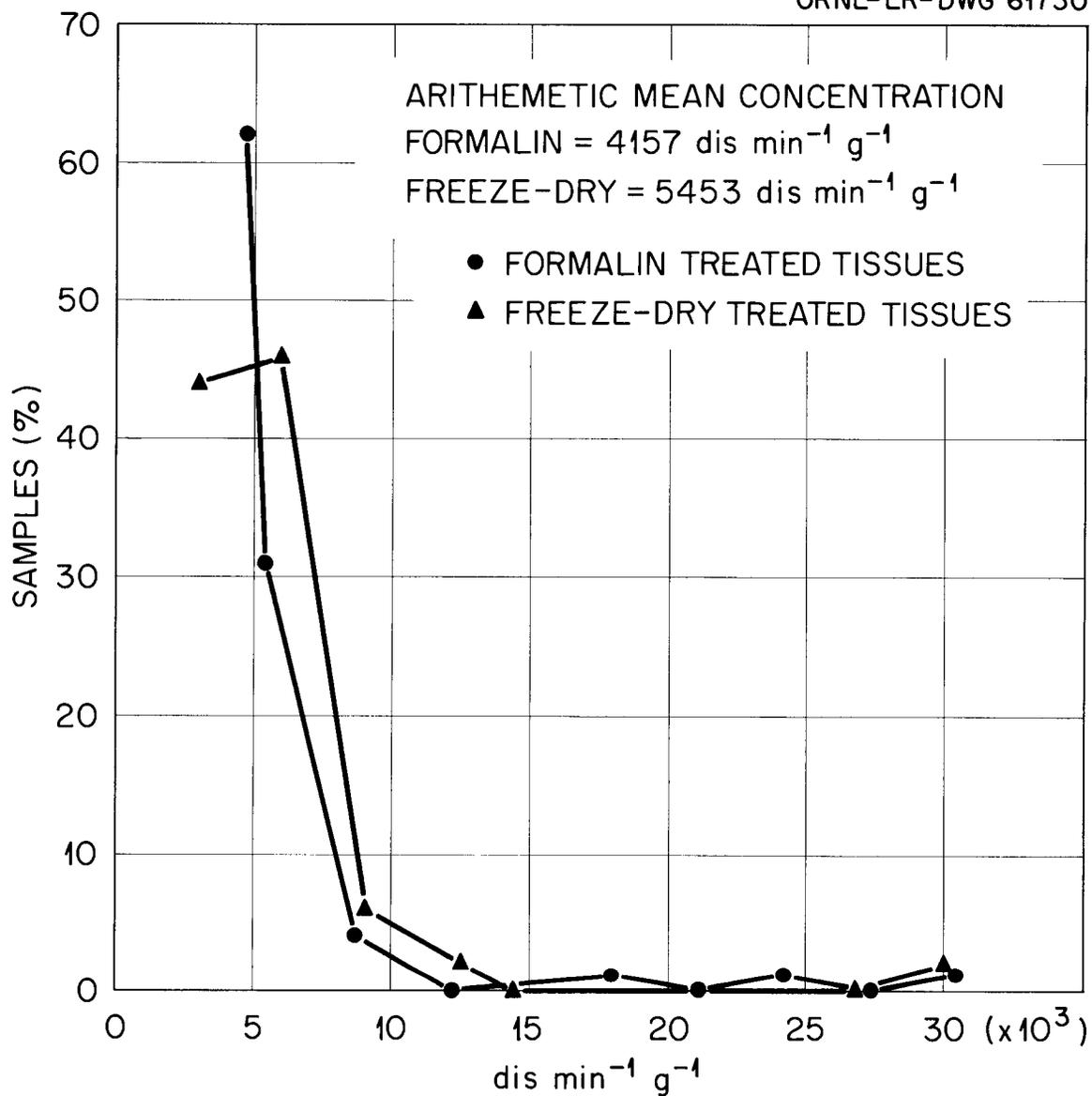


Fig. 2. Distribution of Uranium in Rat Kidney Cortex at Seven Days Following an Injection of  $1000 \mu\text{g U per kg}$  as Estimated by the Freeze-Dry Method and the Formalin-Fixation Method

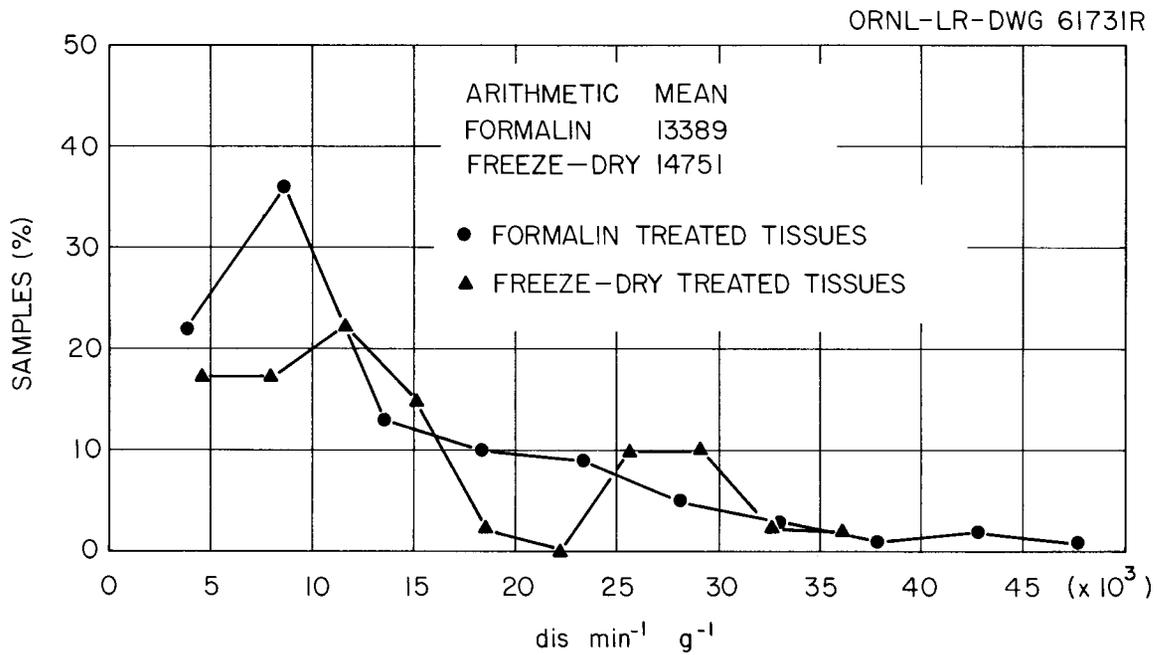


Fig. 3. Distribution of Uranium in Rat Kidney Cortex at One Day Following an Injection of 1000  $\mu\text{g}$  U per kg as Estimated by the Freeze-Dry Method and the Formalin-Fixation Method

The arithmetic means in  $\text{dis min}^{-1} \text{g}^{-1}$  appearing on the graphs (Figs. 2 and 3) for the two animals estimated from counts made on formalin and on freeze-dried tissue agree quite closely, although the mean  $\text{dis min}^{-1} \text{g}^{-1}$  based on freeze-dried data is slightly, but probably not significantly, higher.

#### Retention at Different Injected Levels and at Different Postinjection Times

The results of the work clearly suggest greater retention of uranium at the high levels, and this is expressed in several different ways. The three highest injection levels contained the same amounts of radioactivity, so the disintegration rate per field is a measure of the fraction of injected mass present in the field. Although there is some overlapping of the 95% confidence limits, the mean concentration in the tissue at 1, 2, and 4 days increases with the mass of uranium injected, as is shown in Figs 4 and 5. This increase of concentration at higher levels is observed also in considering the highest counts for each level. For example, in rats administered 1,000  $\mu\text{g}/\text{kg}$  in this study, levels of over 40,000  $\text{dis min}^{-1} \text{g}^{-1}$  have been found in the kidneys. In rats administered 100  $\mu\text{g}/\text{kg}$ , levels of around 19,000 to 22,000  $\text{dis min}^{-1} \text{g}^{-1}$  were observed, whereas the highest levels in kidneys from rats administered 10  $\mu\text{g}/\text{kg}$  are 10,000 to 17,000  $\text{dis min}^{-1} \text{g}^{-1}$ . To express this another way, a kidney cortex concentration level of 10,000  $\text{dis min}^{-1} \text{g}^{-1}$  was found in  $\sim 26\%$  of the samples from rats administered 1,000  $\mu\text{g}/\text{kg}$ , in 16% of the samples from rats administered 100  $\mu\text{g}/\text{kg}$ , and in only about 4% of the samples from rats administered 10  $\mu\text{g}/\text{kg}$ ; yet, if the fractions of dose retained in the kidney were the same for all three levels, these percentages would be the same.

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ARITHMETIC MEANS  $\text{dis min}^{-1} \text{g}^{-1}$   
OBSERVED AT DIFFERENT LEVELS AND POSTINJECTION  
TIMES WITH 95% CONFIDENCE LIMITS AUTORADIOGRAPHIC DATA

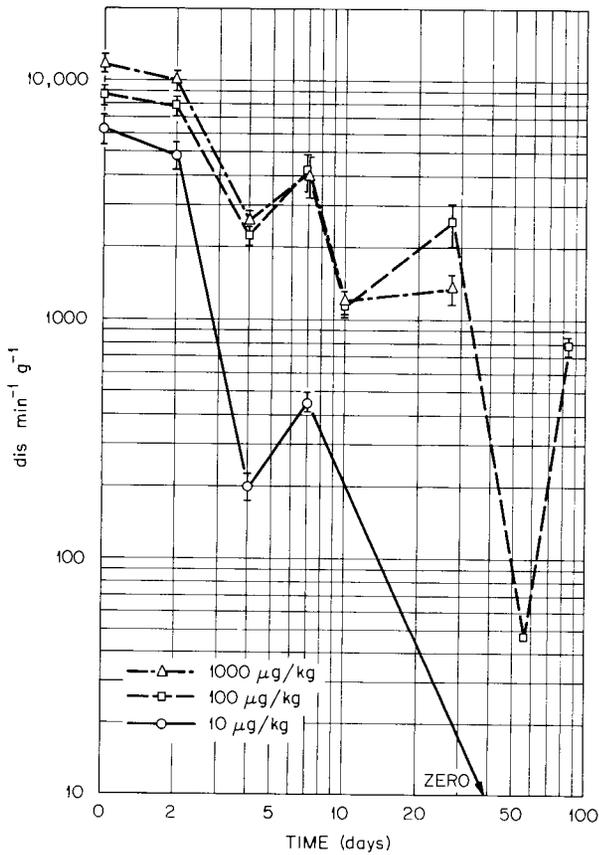


Fig. 4. Activity in the Cortex –  
Concentration in Arithmetic Means –  
Autoradiographic Data

ARITHMETIC MEANS  $\text{dis min}^{-1} \text{g}^{-1}$   
OBSERVED AT DIFFERENT LEVELS AND POSTINJECTION  
TIMES WITH 95% CONFIDENCE LIMITS

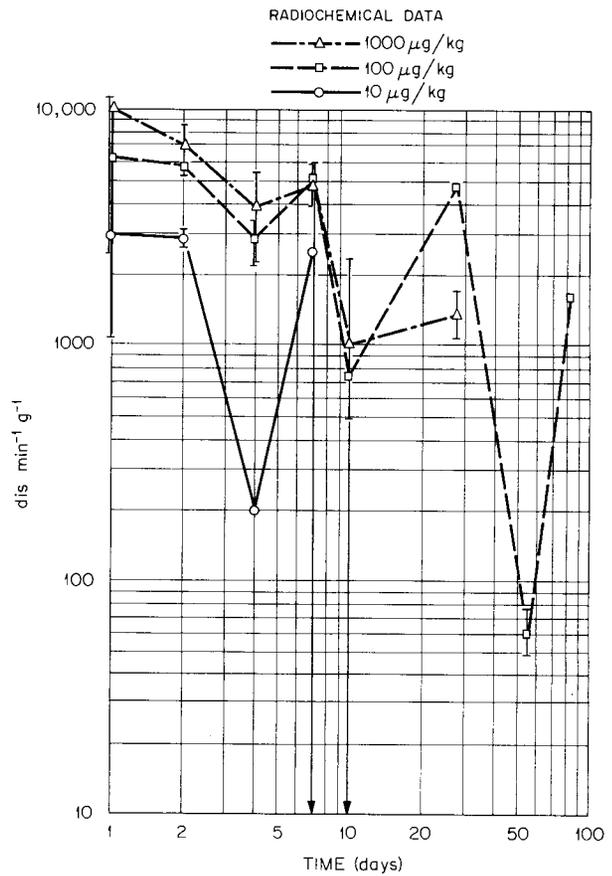


Fig. 5. Activity in the Cortex –  
Concentration in Arithmetic Means –  
Radiochemical Data

In detail, the fractions of injected dose in the kidney, as recorded by counting tracks in autoradiograms, are listed in Table 7. In animals sacrificed at 1, 2, and 10 days postinjection, the fractions are consistently higher with increasing dose levels. On the other hand, there is some nonlinearity, for at 4 and 7 days, the fraction found in the kidneys of animals injected with 100  $\mu\text{g}/\text{kg}$  are slightly higher than in animals injected with 1,000  $\mu\text{g}/\text{kg}$ . With the kidneys analyzed radiochemically (Table 8), the slightly higher fraction in rats injected with 100  $\mu\text{g}/\text{kg}$  is observable at 1, 4, and 28 days.

The dependence of the distribution on time after the injection can be noted in Fig. 6. As time after injection increases, as would be expected, the data crowd around the origin, showing a smaller number of fields of higher concentrations. The line representing the disintegrations found at 28 days lies close to the origin in contrast with the great number of fields with high concentrations found in the tissues sacrificed at 1 day.

The contrast of the retention at the high injection levels is also shown in the photographs of the cortices of rat kidneys injected with 1,000  $\mu\text{g}/\text{kg}$  and 10  $\mu\text{g}/\text{kg}$  (Plate 5).

#### Gradient Within the Cortex and Aggregates

Some exploratory studies were carried out to determine whether or not there is a gradient in concentration of uranium in the cortex.

From a section of the kidneys which had been bisected into left and right halves and these bisected again into anterior and posterior quarters, measurements were made of the tracks along rays extending inward from the peripheral edge of the cortex. The schematic diagram, Plate 6, illustrates

**Table 7. Fraction of Injected Dose in the Kidney  
Based on Autoradiographic Counting**

Dosage Level ( $\mu\text{g}/\text{kg}$ )	Postinjection Time (days)	Fraction of Injected Dose to Kidney	Standard Deviation	Number of Animals	Number of Half Kidneys
1000	1	0.209	0.015	2	3
100	1	0.146	0.001	1	2
10	1	0.087	0.008	2	3
1000	2	0.205	0.001	2	3
100	2	0.120	0.019	1	1
10	2	0.073	0.006	1	2
1000	4	0.047	0.001	1	2
100	4	0.053	0.001	1	2
10	4	0.004	0.001	1	2
1000	7	0.073		1	1
100	7	0.074		1	1
10	7	0.007		1	1
1000	10	0.034	0.004	1	2
100	10	0.023	0.006	1	2
1000	28	0.020	0.007	1	2
100	28	0.052		1	1
100	56	0.001	0.0002	1	2
100	84	0.037	0.012	1	2

Table 8. Fraction of Injected Dose in the Kidney Based on Radiochemical Analysis

Dosage Level ( $\mu\text{g}/\text{kg}$ )	Postinjection Time (days)	Fraction of Injected Dose to Kidney	Standard Deviation	Number of Animals	Number of Half Kidneys
1000	1	0.152 <sup>a</sup>	0.024	4	8
100	1	0.159 <sup>a</sup>	0.027	5	10
10	1	0.11 <sup>a</sup>	0.016	5	10
1000	2	0.228	0.035	3	6
100	2	0.193	0.011	1	2
10	2	0.074	0.005	1	2
1000	4	0.115	0.034	1	2
100	4	0.121	0.005	1	2
10	4	0.006		1	1/2
1000	7	0.134 <sup>a</sup>	0.056	3	6
100	7	0.095 <sup>a</sup>	0.004	4	8
10	7	0.04 <sup>a</sup>	0.045	4	8
1000	10	0.052	0.018	1	2
100	10	0.031	0.013	1	2
1000	28	0.049	0.005	1	2
100	28	0.147		1	1
100	56	0.002	0.0002	1	2
100	84	0.035	0.017	1	2

<sup>a</sup>Contains unpublished data of S. R. Bernard.

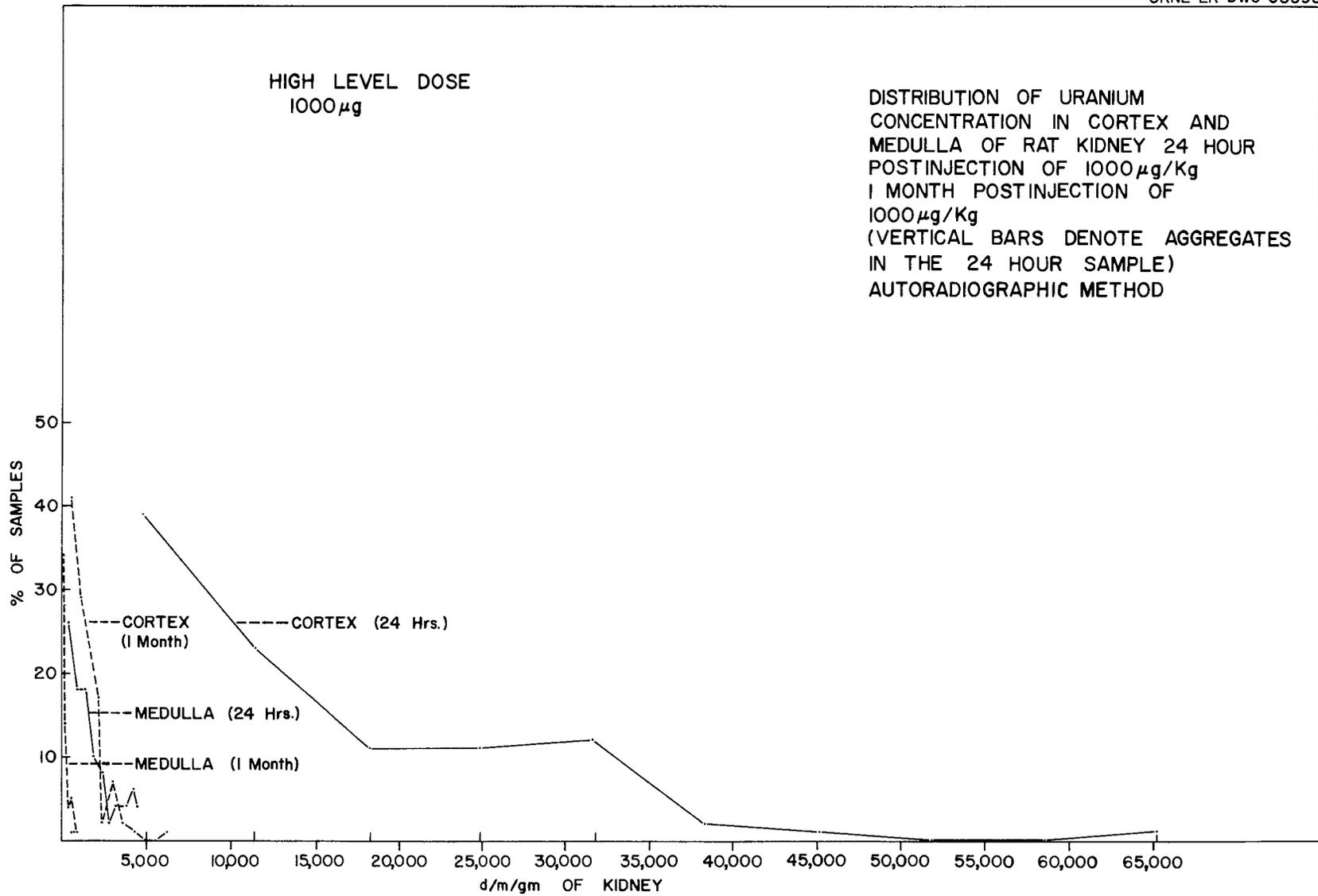


Fig. 6. Distribution of Uranium Concentration in Cortex and Medulla of Rat Kidney 24 Hours Postinjection of 1000  $\mu\text{g}/\text{kg}$  and 1 Month Postinjection of 1000  $\mu\text{g}/\text{kg}$  - Autoradiographic Method

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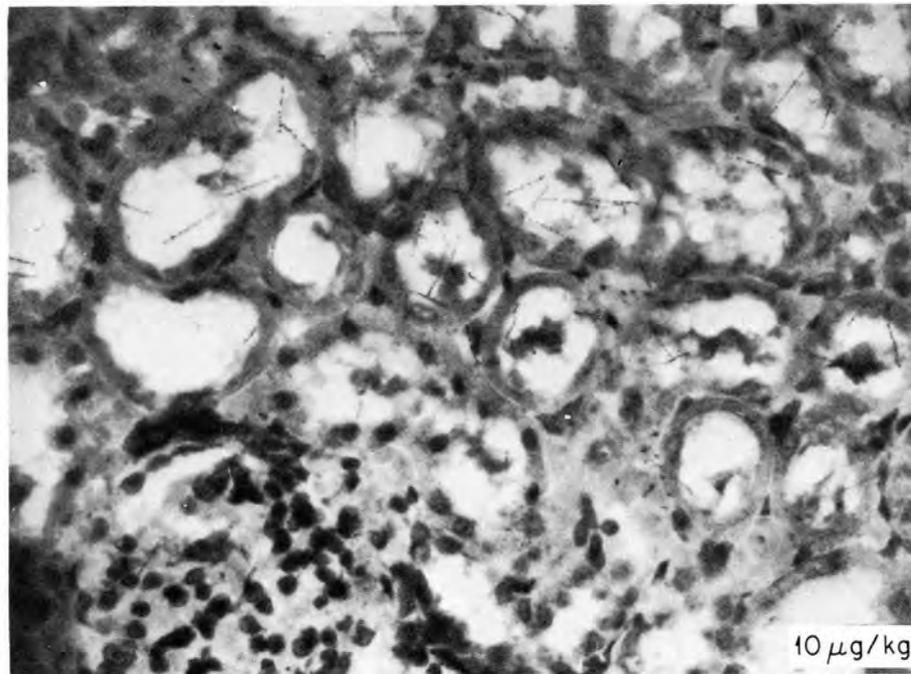
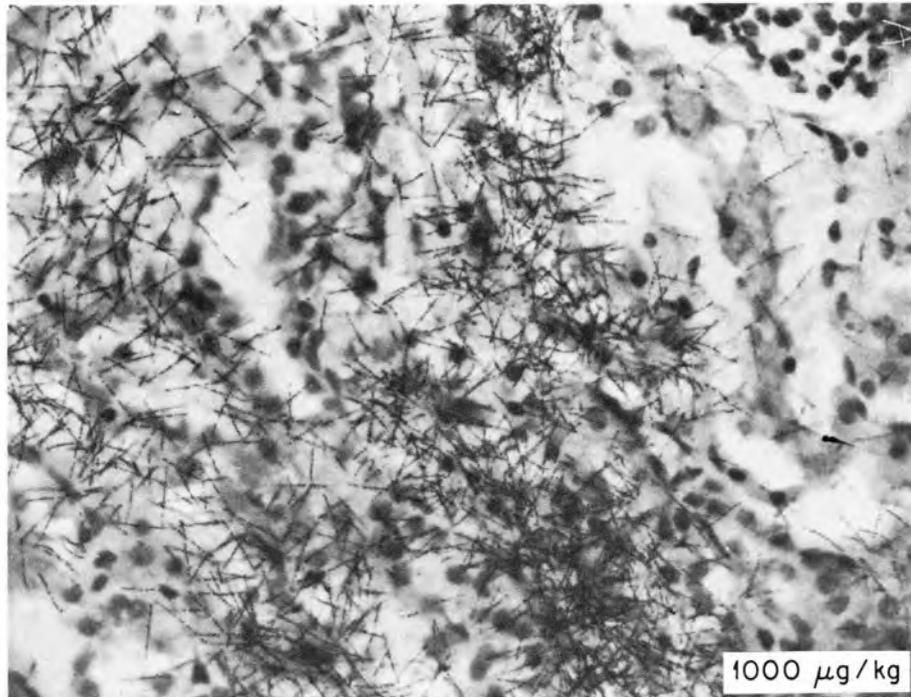


Plate 5. Kidney Cortices from Rats Injected with 1000 µg/kg and with 10 µg/kg and Sacrificed at 1 Day

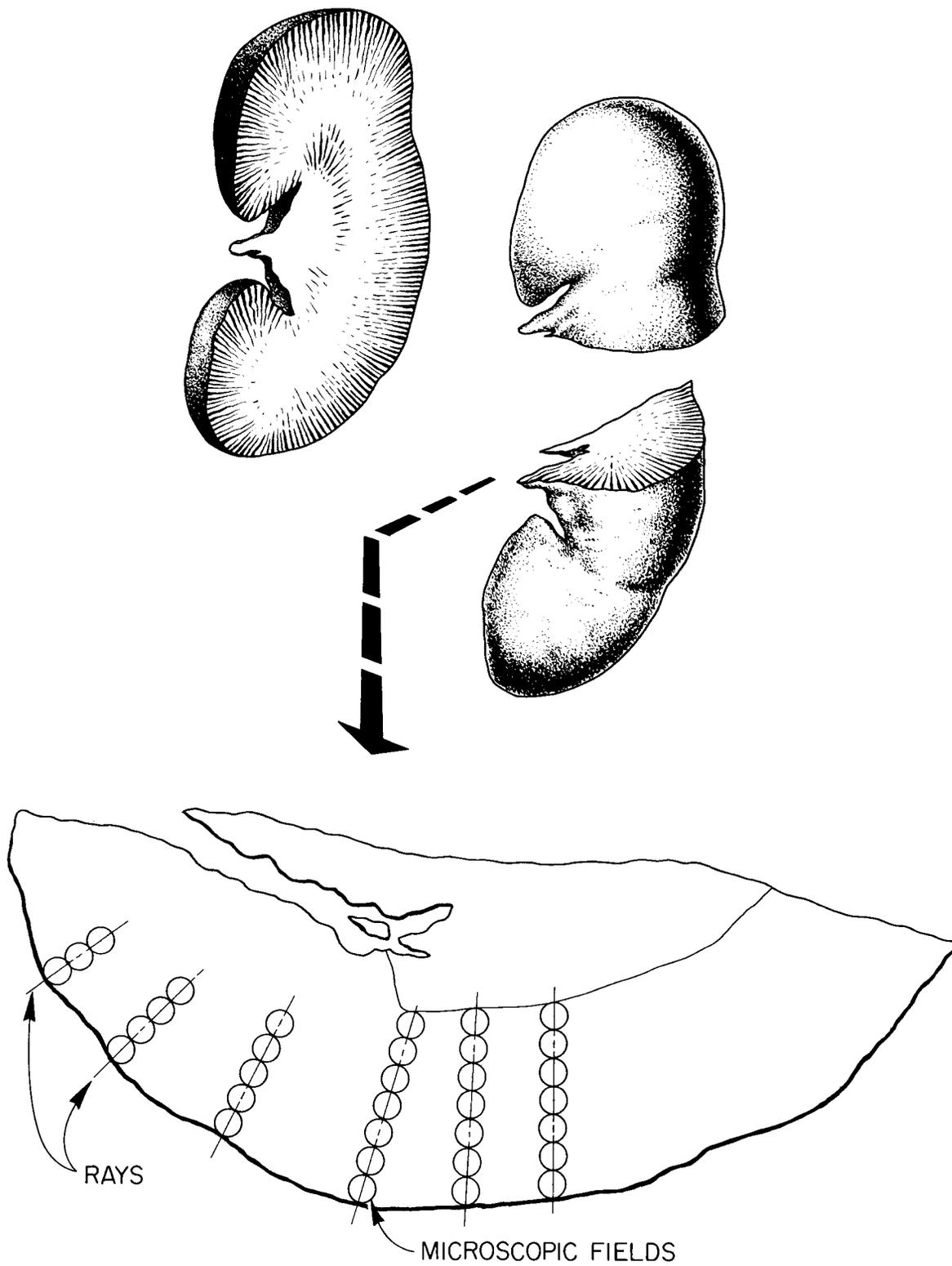


Plate 6. Schematic Diagram

the position of the section in the kidney and the location of the rays along which counts were made. As can be seen, the rays roughly converge at a point on the outer edge of the medulla. The first microscopic field was located on the periphery of the kidney. Tracks contained in this field were counted and recorded. The second microscopic field was located one diameter (370  $\mu$ ) interior, bordering on the first field, and counted. In the same way fields were counted inward along the rays. The counts per field for the six rays are shown in Table 9. At the top of each column appears the distance from the periphery. In some cases, measurements could not be made at depths of 2,035  $\mu$  due to irregularities in the sections. The average counts per field appear in the eighth row. These average counts per field decrease with increasing distance from the periphery of the cortex. Note also in the table that aggregates of activity occur (denoted by "a") at 185  $\mu$  and 555  $\mu$ . When the aggregates of activity are excluded and averages recomputed, the average counts per field appear to be relatively constant. By multiplying by 27.46, as explained above, the counts are thereby converted to  $\text{dis min}^{-1} \text{g}^{-1}$  as shown in row 9. These values are plotted as a function of distance from the periphery of the kidney, and the graph appears in Fig. 7.

The preceding preliminary work on a study of gradient has led to a continued study of aggregates. In this paper an aggregate may be defined as a set of tracks which emerge in such proximity to each other that light may not be observed through its central core when studied under a light microscope and which seem to have more than 200 tracks. With the equipment used in this study, the number of tracks can only be estimated. The number of aggregates found on the sections were counted in kidney tissues

**Table 9. Distribution of Uranium Concentrations in the Rat Kidney Cortex as a Function of Distance Along a Ray Extending from the Periphery Inward – High-level Dose (1,000  $\mu\text{g}/\text{kg}$ ) at 1 Day Postinjection**

Ray	Uranium Concentration (counts/field) at a Depth ( $\mu$ ) of					
	185	555	925	1295	1665	2035
1	132	87	234	244	235	199
2	653 <sup>a</sup>	1,393 <sup>a</sup>	332	217		
3	562	72	101			
4	822 <sup>a</sup>	150	311	342	252	
5	78	394	384	351		
6	1,086	536	316			
<b>Average</b>						
counts/field	556 (257) <sup>b</sup>	438 (248) <sup>b</sup>	279	289	244	199
dis/min/g	15,268 (7057) <sup>c</sup>	12,028 (6810) <sup>c</sup>	7661	7936	7002	5465

<sup>a</sup>Denotes an aggregate was noted in the field.

<sup>c</sup>Denotes average dis/min/g with aggregates excluded.

<sup>b</sup>Denotes average of fields with aggregates excluded.

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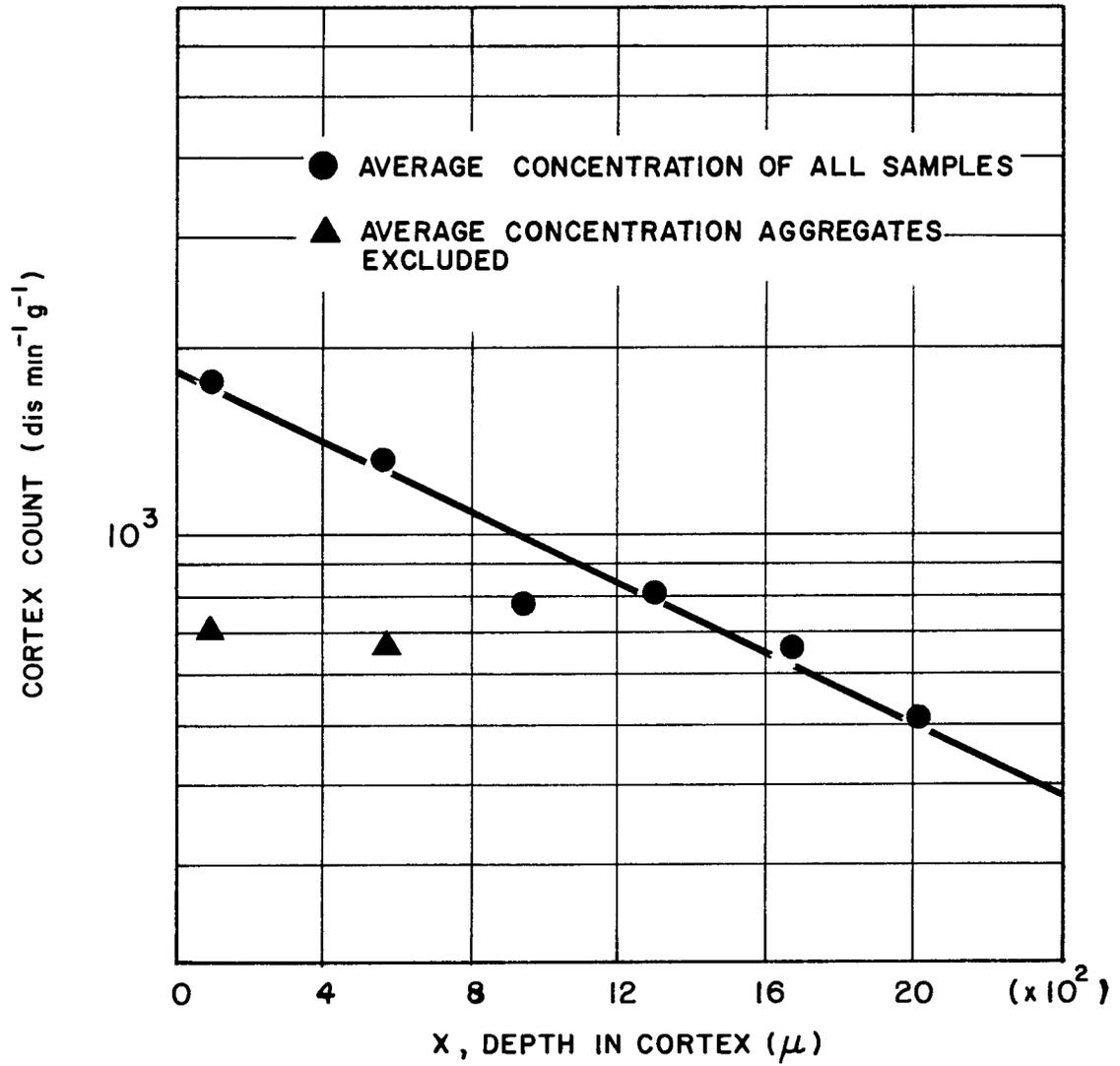


Fig. 7. Concentration in Rat Kidney as a Function of Distance from Outer Periphery of Cortex.

which had been injected with 1,000  $\mu\text{g}/\text{kg}$  and sacrificed at postinjection times of 1, 2, 4, and 10 days and are tabulated in Table 10. No aggregates have been observed in tissues sacrificed at longer postinjection times.

In rats injected with 100  $\mu\text{g}/\text{kg}$ , the only aggregate observed was found in tissues sacrificed at 2 days. None were found in rats injected with 10  $\mu\text{g}/\text{kg}$ .

### Anatomical Changes

#### Proximal Tubules

In studying the distribution curves, there were indications of some possible structural change or changes. In an attempt to throw light on this, some measurements of anatomical changes at the microscopic level were made on stained and autoradiographic sections of kidneys of rats injected with 1,000  $\mu\text{g}/\text{kg}$ . The diameters of proximal convoluted tubules, sectioned perpendicularly to their long axes, were measured. The tubules were not always uniformly circular, and thus a somewhat arbitrary procedure of measuring the narrowest diameter was adopted. Table 11 presents the measured diameters of 10 tubules observed in kidneys of control animals (Col. 1), animals injected with 10  $\mu\text{g}$  of uranium/kg (Col. 2), and animals injected with 1,000  $\mu\text{g}$  of uranium/kg (Col. 3). All animals were sacrificed at 4 days postinjection except the one stained by the Periodic Acid-Schiff method whose age relative to sacrifice is not known. As can be noted, the mean diameters of 10 tubules from experimental animals (42  $\mu$ , 40.5  $\mu$ ) do not differ significantly from the mean of the controls (41.3  $\mu$ ). Measurements were made also on tubules of kidneys of rats sacrificed at 7 days postinjection (Table 12). Here, a slightly expanded tubule is evident in the experimental animals relative to controls.

Table 10. Number of Aggregates Observed on Every 40th and 41st Section  
in Rats Injected with 1000  $\mu\text{g}/\text{kg}$

Postinjection Time (days)	Aggregates Containing 200 – 600 Tracks	Aggregates Containing 600 – 1000 Tracks	Autoradiographic Exposure Time (days)
1	390	55	28
1	592	164	28
2	159	20	28
2	219	16	28
4	77	0	28
4	155	3	28
10	3	0	35

Table 11. Diameters of Proximal Rat-Kidney Tubules  
4 Days After Injection of Uranium

	Tubule Diameter ( $\mu$ )		
	Control*	Uranium Injected**	
		10 $\mu\text{g}/\text{kg}$	1000 $\mu\text{g}/\text{kg}$
	37.50	45.00	41.25
	37.50	45.00	37.50
	37.50	41.25	39.38
	33.75	43.13	43.13
	41.25	41.25	31.88
	39.38	37.50	50.63
	48.75	41.25	43.13
	48.75	33.75	39.38
	50.63	43.13	37.50
	37.50	48.75	41.25
Mean	41.25	42.00	40.50
Std. Dev.	1.85	1.3	1.5

Table 12. Diameters of Proximal Rat-Kidney Tubules  
7 Days After Injection of Uranium

	Tubule Diameter ( $\mu$ )		
	Control	Uranium Injected	
		10 $\mu\text{g}/\text{kg}$	1000 $\mu\text{g}/\text{kg}$
	41.25	43.13	46.88
	30.00	35.63	45.00
	41.25	50.63	37.50
	39.38	43.13	48.75
	43.13	45.00	48.75
	31.88	39.38	41.25
	33.75	33.75	41.25
	30.00	37.50	48.75
	33.75	33.75	33.75
	41.25	45.00	45.00
Mean	36.56	40.69	43.68
Std. Dev.	1.65	1.77	1.66

\*Periodic acid-Schiff stain.

\*\*Hematoxylin and eosin stain.

Glomeruli

Measurements of diameters of glomeruli, following the method employed by Arataki,<sup>(14)</sup> also were made on rats sacrificed at four and 84 days postinjection. Arataki used the diameter of the Bowman's capsule as the maximum and the diameter of the glomerulus, in the strict sense, as the minimum. The average was obtained by taking the geometric mean of these quantities.

Table 13 presents the measurements of the diameters of glomeruli for control animals and for uranium-injected animals sacrificed at four days and at 84 days postinjection. The mean diameters appear at the bottom of the table. The means for the control animals are 96  $\mu$ , determined on sections stained by the Periodic Acid-Schiff method, and 104  $\mu$ , determined on sections stained with hematoxylin and eosin. Appearing at the bottom of columns 3, 4, and 5 are the mean diameters of animals injected with different levels of uranium. As can be seen, the data suggest that the mean diameter of glomeruli may become larger as the amount injected is increased.

A time trend of the diameters of glomeruli of animals injected with 100  $\mu\text{g}/\text{kg}$  is suggested. At four days the diameter is 81.7  $\mu$  (Col. 4), while at 84 days, the diameter increases to 88.3  $\mu$  (Col. 6). Unfortunately, no control animals were sacrificed at 84 days. However, Arataki studied the change in diameter with age, and his data show that the mean diameters increase with age, going from 94  $\mu$  for 90-day-old rats to 107  $\mu$  for 200-day-old rats. Since the uranium-injected rats were  $\sim$  90 days old when injected, then at 84 days postinjection they are  $\sim$  174 days old. Then, the measured diameter of 88.3  $\mu$  (Col. 6) for  $\sim$  174-day-old rats, although greater than 81.7  $\mu$  (Col. 4), still remains below the diameter of  $\sim$  100  $\mu$  (Cols. 1 and 2) for  $\sim$  90-day-old control rats.

Table 13. Diameters (in  $\mu$ ) of Glomeruli of Kidneys of Uranium-Injected Rats

Control				4 Days Postinjection						84 Days Postinjection	
Max	Min	Max	Min	10 $\mu$ g/kg		100 $\mu$ g/kg		1000 $\mu$ g/kg		100 $\mu$ g/kg	
				Max	Min	Max	Min	Max	Min	Max	Min
I <sup>a</sup>		II <sup>b</sup>		III		IV		V		VI	
86.25	73.13	93.75	82.50	103.13	80.63	106.88	86.25	84.38	65.63	80.63	73.13
127.50	93.75	120.00	103.13	108.75	90.00	80.63	71.25	91.88	71.25	103.13	90.00
108.75	93.75	103.13	84.38	90.00	75.00	71.25	60.00	103.13	84.38	75.00	50.63
112.50	78.75	84.38	56.25	91.88	82.50	82.50	71.25	84.38	61.88	90.00	63.75
86.25	67.50	112.50	93.75	90.00	69.38	103.13	82.50	110.63	69.38	90.00	80.63
106.88	84.38	131.25	108.75	90.00	56.25	90.00	75.00	78.75	61.88	105.00	86.25
78.75	63.75	103.13	90.00	90.00	65.63	88.13	82.50	93.75	78.75	105.00	86.25
127.50	97.50	127.50	108.75	108.75	71.25	90.00	69.38	93.75	71.25	103.13	71.25
101.25	75.00	120.00	108.75	108.75	75.00	71.25	56.25	78.75	78.75	93.75	67.50
108.75	88.13	112.50	112.50	93.75	58.13	82.50	67.50	103.13	75.00	75.00	75.00
120.00	120.00	125.63	108.75	108.75	88.13	112.50	93.75	106.88	84.38	103.13	80.63
90.00	71.25	135.00	123.75	97.50	76.88	93.75	80.63	93.75	73.13	80.63	60.00
125.65	93.75	108.75	93.75	101.25	75.00	90.00	78.75	84.38	76.88	98.38	80.63
121.88	103.13	108.75	97.50	112.50	93.75	90.00	63.75	112.50	88.13	78.75	67.50
112.50	90.00	112.50	93.75	82.50	69.38	93.75	71.25	112.50	88.13	65.63	56.25
112.50	101.25	131.25	123.75	112.50	97.50	80.63	75.00	103.13	84.38	116.25	112.50
103.13	84.38	98.38	90.00	112.50	93.75	63.75	56.25	90.00	60.00	90.00	82.50
93.75	75.00	71.25	63.75	97.50	84.38	86.25	76.88	88.13	67.50	135.00	118.13
112.50	84.38	138.75	123.75	105.00	75.00	98.38	88.13	101.25	71.25	121.88	110.63
108.75	90.00	108.75	86.25	103.13	82.50	106.88	93.72	78.75	63.75	121.88	103.13
Mean <sup>c</sup>	96		104		88.5		81.7		83.5		88.3

<sup>a</sup>Periodic acid-Schiff stain.<sup>b</sup>Hematoxylin and Eosin stain.<sup>c</sup>See text for definition.

### Histopathological Changes\*

Pathological conditions, recorded in Table 14 and Plate 7, become apparent in tissues 2 days after injection. These are seen in the animals injected with 100  $\mu\text{g}/\text{kg}$  and are striking in animals injected with 1,000  $\mu\text{g}/\text{kg}$ . Scattered proximal, distal, and collecting tubules show mucoid or waxy material in the lumen. Other tubules of the cortex, primarily at the junction of cortex and medulla, show hyaline droplet degeneration of epithelium. Four and 7 days after injection, these conditions are more severe in the tissues from rats given 1,000  $\mu\text{g}$  than in the tissues from rats given 100  $\mu\text{g}$ , where mildly nephrotic conditions are found. In the former there is extensive cellular degeneration of the hyaline droplet type in proximal convoluted tubules. Mucoid or waxy casts are found at all levels of the nephrons. At 10 days the diffuse hyaline droplet degeneration in the proximal convoluted tubules is less severe than at 7 days and regeneration is beginning. Focal calcification is seen occasionally at both the 1,000  $\mu\text{g}$  and the 100  $\mu\text{g}$  levels. Waxy change is evident in some epithelial cells at the cortico-medullary junction. At 28 days the tissues from animals injected with 100  $\mu\text{g}/\text{kg}$  show no abnormalities, whereas those from animals injected with the higher amount of uranium show tubular atrophy and interstitial lymphoid infiltrations. Many tubules also show dilatation and contain precipitate in the lumen. Some proximal convoluted tubules show regeneration, but others show hydropic change of the epithelium.

Kidneys of rats injected with 10 and 1  $\mu\text{g}/\text{kg}$  show no significant pathological changes.

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\* I am most grateful to Dr. A. C. Upton of the Biology Division, Oak Ridge National Laboratory, for the histopathological examinations reported.

Table 14. Pathological Anatomy of Rat Kidney After Injection of Uranium-238 + Uranium-233

Level	Postinjection Time and Animals Injected																			
	Number of Animals	1 Day	Number of Animals	2 Days	Number of Animals	4 Days	Number of Animals	7 Days	Number of Animals	10 Days	Number of Animals	14 Days	Number of Animals	15 Days	Number of Animals	28 Days	Number of Animals	56 Days	Number of Animals	84 Days
990 $\mu\text{g}$ U <sup>238</sup> + 10 $\mu\text{g}$ U <sup>233</sup> per kg body weight	2	Occasional tubules show waxy casts; small foci of calcification are also noted sporadically	3	Scattered tubules with mucoid or waxy material in lumen as well as in lining cells including proximal, distal and collecting tubules; other tubules show hyaline droplet degeneration of epithelium; these are in the cortex, not in medulla, but primarily in junction of cortex and medulla	1	Shows extensive cellular degeneration of hyaline droplet type in proximal convoluted tubules; many tubules at all levels of nephrons show waxy casts. This is a sick kidney	1	Extensive degeneration; epithelial cells sloughed off; mucoid or waxy material in lumen; hyaline droplet degeneration	1	Diffuse hyaline droplet degeneration in proximal convoluted tubules; less severe than at 7 days with beginning regeneration; occasional focal calcification	0		0		2	Atrophy and interstitial lymphoid infiltrations; many tubules show dilatation and contain precipitate in lumen; proximal convoluted tubules show regeneration but there is associated hydropic change in the epithelium	0		0	
90 $\mu\text{g}$ U <sup>238</sup> + 10 $\mu\text{g}$ U <sup>233</sup> per kg	2	Occasional waxy casts at all levels of nephron; focal calcification	1	Hyaline droplet change diffuse throughout proximal convoluted tubules	1	Hyaline droplet change diffuse throughout proximal convoluted tubules; mild; also waxy casts in many tubules	1	Slight degeneration; some waxy casts; similar but not so severe as high level	1	Focal calcification. Seems to show waxy change at cortico-medullary junction	0		0		1	Negative	1	Focal interstitial lymphoid infiltrations; otherwise negative	1	Negative
0 $\mu\text{g}$ U <sup>238</sup> + 10 $\mu\text{g}$ U <sup>233</sup> per kg	2	Negative	1	Negative	1	Negative	1	Negative	0		1	Focal calcification; otherwise negative	1	Negative	0		1	Negative		
0 $\mu\text{g}$ U <sup>238</sup> + 1 $\mu\text{g}$ U <sup>233</sup>	2	Negative; a few desquamated epithelial cells in lumen of proximal and distal convoluted tubules; these were also found in controls	1	Negative	0		1	Negative	1	Negative	0		0		0		0			0

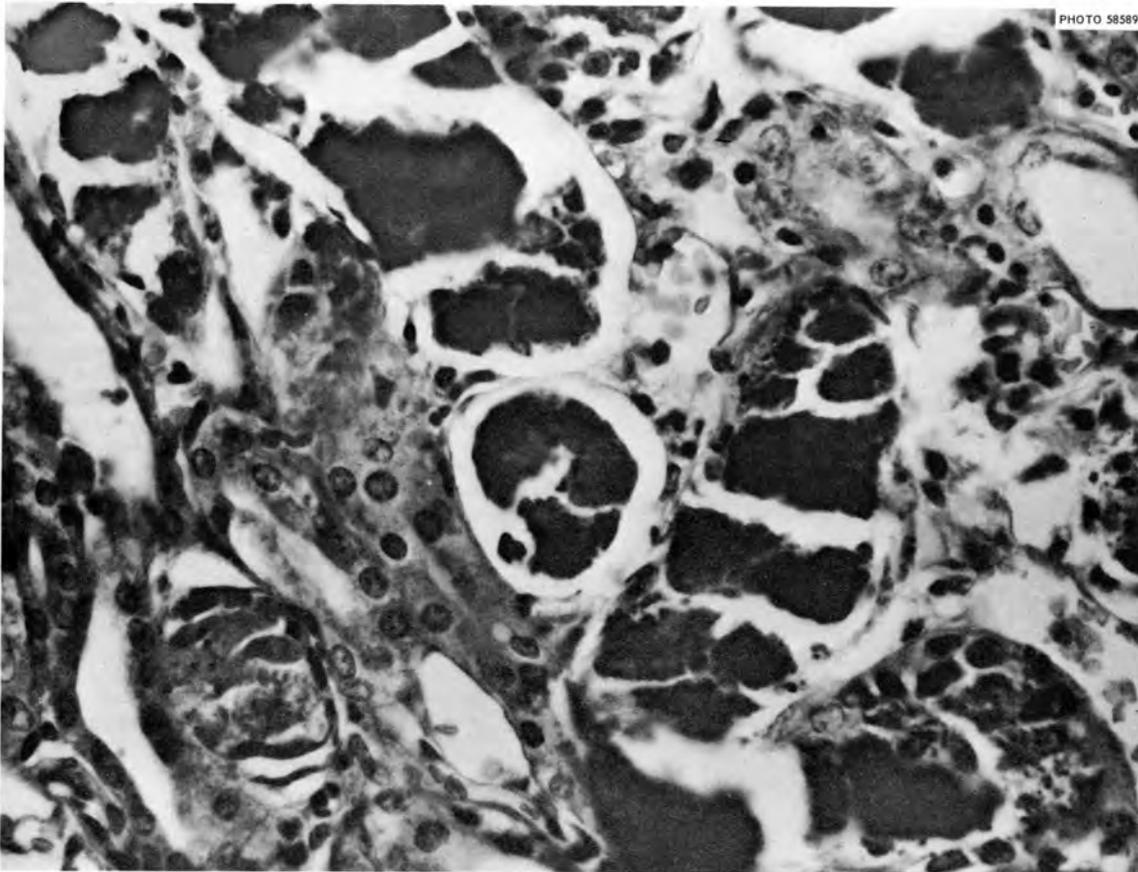


Plate 7. Pathological Conditions in Tissues 2 Days after Injection

Discussion

## Concentration Ratio

The ratios,  $R_k$ , of uranium concentration in cortex to the uranium concentration in the whole kidney, as estimated from individual half-kidneys, were averaged over all dose levels and at all times postinjection and gave an average of 1.3. In general, the averages for the various groups approximated this value. However, the average of  $R_k$  for samples taken at 10 days postinjection for the 1,000  $\mu\text{g}/\text{kg}$  level and also those taken at 4 days postinjection for the 100  $\mu\text{g}/\text{kg}$  level were close to unity. Likewise, the value of  $R_k$  at 84 days postinjection indicated a higher concentration in medulla than in cortex, but, since the data represent only one animal, this reversal cannot be considered as established. Whether these differences from the other data are significant and whether they can be correlated with uranium removal from the kidney are questions worthy of further study.

Analyzing the figure 1.3, if the uranium were evenly distributed over the kidney,  $R_k$  would be 1; if 100% of the uranium were in the cortex and none in the medulla,  $R_k$  would be 1.43. One finds that  $R_k = 1.3$  indicates that 90% of the uranium is localized in the cortex which comprises about 70% of the volume. This would lead to the conclusion that, averaged over many animals, for radioactive isotopes of uranium the absorbed radiation dose to the cortex is approximately 1.3 times greater than the average dose in the entire kidney.

### Comparison between Activity in Autoradiograms and in Ashed Material

In comparing activities found by the two methods--autoradiographic and radiochemical--the rapid separation of fresh medulla from the cortex and the weighing of proportionately very small amounts of medulla present difficulties, as mentioned earlier.

Also, the presence of aggregates may produce some bias. In the process of counting, aggregates of uranium tracks were observed in tissues of rats which had been injected with 1,000  $\mu\text{g}/\text{kg}$ . At lower injection levels only once was an aggregate observed, and this was in the cortex of a kidney from a rat injected with 100  $\mu\text{g}/\text{kg}$  and sacrificed at 2 days. With our equipment the number of tracks in these aggregates could only be estimated, roughly. Their position in the tissues and the percentage of tracks in the aggregates in relation to the total number of tracks counted are shown in Table 10. In general, they were found in the cortex, but an occasional aggregate was found in the medulla.

### Location of Uranium Tracks in Histological Units

In examining Table 6 concerned with histological units, it appears that our findings corroborate earlier work<sup>(3)</sup> indicating that the uranium is found mainly in proximal tubules. However, the percentage of uranium in recognizable distal tubules increased with postinjection time until at 84 days a higher percentage was found in distal than in proximal tubules. Studies on autoradiograms could well be continued for more information on location of tracks.

### Leaching of Uranium from Tissues

In all work with radioactive isotopes, the question of leaching enters. Although the implication has been that alpha-particle emitters are not easily leached, workers have avoided possible leaching solutions. For example, Neuman<sup>(3)</sup> followed a rapid method of fixing and dehydrating which was shown to remove only insignificant quantities of uranium. It was considered desirable to check radiochemically the formalin and dehydrating solutions. The small amount of uranium which appeared in the radiochemical analysis of these solutions was also found to be insignificant and was found only in solutions in which tissues had been immersed for 12 to 24 hours.

### Redistribution

The limited data obtained by the meticulous and time-consuming technique of mounting freeze-dried tissue can be said to indicate qualitatively that the distribution in cortex is not appreciably different from that observed in the case of formalin-fixed tissues (Figs. 2 and 3). However, these data are not adequate to establish whether or not a statistically significant difference exists. More data are needed here.

### Kidney Retention

The mean concentration values estimated autoradiographically and determined by the radiochemical method corroborate the earlier work<sup>(7)</sup> in that, in general, the greater the mass, the greater the retention. In these experiments the correlation between retention and mass is evident at 1, 2, and 4 days. At the early times of 1 or 2 days, there is a steady increase of retention with injected mass. Beyond 4 days postinjection,

the data on the two higher levels do not indicate significant differences, but both seem to differ markedly in retention from data on the lowest level. This is evident in Figs. 4 and 5.

#### Gradient within the Cortex and Aggregates

The question has been raised whether or not there is a gradient or definite pattern of deposition of uranium in the rat kidney. This matter of concentration in the cortex is discussed by Casarett<sup>(18)</sup> in connection with studies on polonium in rabbits. He finds that the major part of the radioactivity beyond 9 days concentrates in the cortico-medullary border. Since our estimate of the gradient (Table 9) is based on counts from only 25 fields, more studies on the concentration of uranium in the cortex as a function of distance from the cortical borders are needed. Also, the effect of mass of uranium injected and the effect of the length of time after injection during which biological repair of renal tubule damage is going on, as they relate to the gradient, need to be studied. However, the indications seem to be that the presence of aggregates may be statistically significant.

#### Anatomical Changes

The work on the anatomical changes is exploratory, but some very significant effects of uranium in rat kidney are thereby indicated. Evidently the uranium has produced a decrease in the diameter of the glomerulus. Diameters of glomeruli of injected rats were all smaller than those of control rats. A similar condition is recorded in rats after two years' exposure to uranium by Barnett and Metcalf<sup>(19)</sup> and suggested in the discussion by Aschoff and Susuki.<sup>(20)</sup> Nuzum and Rothschild<sup>(21)</sup> refer to "changes

in the glomeruli," and in 1958 Magee and Foreman<sup>(22)</sup> referred to the appearance of serum-like urinary proteins suggesting a defect in the glomerulus filtration apparatus. This appearance "provides further evidence that the glomerulus, as well as the tubule, is altered by uranium administration." MacNider,<sup>(23)</sup> in an extensive compilation of "acute uranium nephritis" articles, refers to "later degeneration changes in the glomeruli."

#### Histopathological Changes

The pathological conditions observed in these tissues taken adjacent to the sections used for autoradiograms and studied with the light microscope harmonize well with the excellent observations of Stone, Bencosme, Latta, and Madden<sup>(24)</sup> with the electron microscope. Naturally the identity of materials observed by the two methods differs somewhat, but the hyaline droplet degeneration, the necrotic debris in the tubules, and the nephrotic conditions are recognized in both methods.

#### Summary

Studies of the distribution of uranium in rat kidney at the microscopic level were carried out on autoradiograms to obtain more information relating to the phenomenon of prolonged retention of uranium by kidneys of animals given intravenous administrations of a large mass of uranium. The distribution of uranium in the cortex and medulla of kidneys of 24 rats has been studied at different times after injection and for different injected amounts of uranium. Although these autoradiographic studies are only exploratory, they may be considered to define an accurate method of studying deposition of uranium in rat kidney.

The concentration ratio (the ratio of the average concentration in the cortex to the average concentration in the entire kidney) was estimated as 1.3, indicating a somewhat higher average concentration in the cortex than in the whole kidney. The data used for this purpose were obtained from autoradiograms of 40 half-kidneys and also from ashed material from 29 half-kidneys.

In the study of the histological units from which the tracks seem to originate, the greater number were found in the proximal tubules except at 84-days-postinjection time in the 100  $\mu\text{g}/\text{kg}$  level when the greater number were found in the distal tubules. The proportion of tracks observed in distal tubules increased with postinjection time. Traces of uranium were found in the glomeruli. Location may have great significance in relation to the physiological behavior of kidney response to uranium.

No significant leaching of uranium into 10% formalin, the dehydrating alcohols, nor in the embedding medium was found. Tissues prepared by freeze-drying showed a similar pattern of track distribution as that prepared by fixation in 10% formalin.

The retention curves corroborate the earlier work of Fish and Muir<sup>(6,7,8)</sup> at this Laboratory, indicating a prolonged retention of uranium in kidneys, perhaps due to the effect of the toxicity which injures the tubules and so slows the clearance from the body.

Some exploratory studies were carried out to ascertain if there is a gradient in concentration from cortical border inward to the medulla. From 25 fields counted, the indications are that when aggregates are included there is a decrease inward, but, not counting the aggregates, the concentrations seem to be very similar.

Measurements of the diameters of proximal tubules from kidneys of uranium-injected rats did not differ significantly from those of control rats or correlate with the amount of uranium injected. The diameters of the glomeruli, however, were smaller in the injected rats than in the controls.

Histopathological changes were apparent in tissues 2 days after injection from animals injected with 1,000 and 100  $\mu\text{g}/\text{kg}$  of uranium. Abnormalities of tissues were found at the high level up to 28 days after injection. At the intermediate level, histopathological changes were observed only through 10 days. These observations correlate with the observed retention and indicate a toxic reaction which would prolong retention.

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