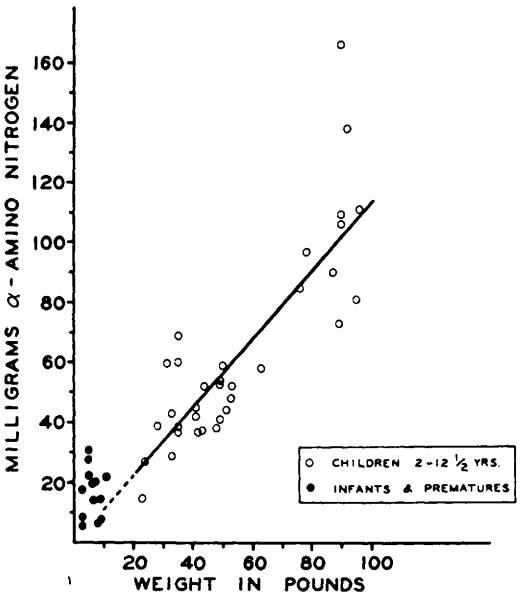

FIG. 1. Urinary excretion of free alpha-amino nitrogen by infants and children of various ages, expressed on a body weight basis.

Simon, and Goebel(6), who show that the proportion of total nitrogen excreted, attributable to alpha-amino acid nitrogen, was higher in the newborn and premature than in adults. Fig. 1 illustrates the results graphically and indicates that except for an occasional value, approximately 1 mg of alpha-amino acid nitrogen is excreted per pound of body weight, excluding the values for infants. The solid line represents the calculated mean for the determinations done on children. The broken line extension shows that among the values of free alpha-amino acid nitrogen for infants, all but 2 are above the calculated mean for children.

Summary. A series of 48 determinations of free alpha-amino acid nitrogen was done on the urine of 39 infants, premature infants, and pre-adolescent children. The children excreted approximately 1 mg per lb per day; the infants and prematures approximately 3.9 mg per lb per day. This difference is statistically significant. The values for children were lower than those reported for adults, and no sex difference was found.

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Distribution of C^{14}-Labeled Alloxan in the Tissues of the Rat and its Mode of Elimination.* (19829)

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A recent paper by Lee and Stetten(1) deals with the metabolism of alloxan-2-C^{14} and alloxan-1,3-N^{15} in rats. Following intraperitoneal administration of diabetogenic doses of these compounds, the isotopes were rapidly eliminated, chiefly by way of the urine. While the exact nature of the N^{15}-containing urinary excretory products was not determined, it was shown that they were neither ammonia nor urea, and probably not alloxan or alloxanic acid. The pancreas took up relatively small amounts of the labeled compounds.

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TABLE I. Elimination of C^{14} in Urine and Expired Air Following Administration of Alloxan Labeled in Two Different Positions. Dosage was 11 mg/kilo in each case.

	Total radioactivity in % of administered dose					
	Expired CO_2		Urine			
Hr after inj.	Alloxan-5-C^{14} Rat 1	Alloxan-2-C^{14} Rat 2	Alloxan-5-C^{14} Rat 3	Rat 4	Alloxan-2-C^{14} Rat 5	Rat 6
2	.19	.12			61†	
4½	.29	.25				
7½			68*		83	69
11			90	91		
22			93	95	91	92

* .24% of the C^{14} in this sample was due to urea.
† .27% " C^{14} " .

The present study is principally concerned with the rates of elimination of alloxan-5-C^{14} in the urine and expired air of rats, and with the distribution of this compound in the tissues of the body at various times following administration of the drug. In addition, some experiments were performed with alloxan-2-C^{14}, in order to compare its metabolism with that of the 5-labeled substance.

Methods. Male rats (250-275 g) of the Long-Evans strain were employed. Alloxan-5-C^{14} (0.86 μc/mg) and alloxan-2-C^{14} (1.85 μc/mg) were purchased from Tracerlab, Inc., Boston, on allocation from the U. S. Atomic Energy Commission. The labeled compounds were administered subcutaneously either at a "tracer" level of 11 mg/kilo body weight, or at the level of 110 mg/kilo, found to be diabetogenic in our laboratory. When the higher dosage was employed, the isotopic alloxan was diluted with 9 parts of ordinary alloxan monohydrate. At specified time intervals after injection, the animals were anesthetized with ether, and blood was withdrawn from the aorta. The tissues were quickly removed and frozen in dry ice. A sample of each tissue was homogenized with absolute ethanol. Aliquots of the resulting suspensions were pipetted upon aluminum discs. The blood was centrifuged, and 0.1 ml samples of plasma were spread uniformly on discs. After drying, the radioactivity of each sample was measured with a thin mica-window Geiger counter. The values were corrected for self-absorption. The kidney, plasma, and urine, all had relatively high C^{14} concentrations, and the samples were generally counted to within a probable error of 2 to 3%. The probable error was 5 to 8% with the other tissues studied. The values recorded in the tables and graphs are averages of duplicate determinations. These data were converted from counts per minute to % of total administered C^{14} per g fresh tissue, by reference to the specific radioactivity of the original alloxan-C^{14} preparations. In certain experiments, the rats were maintained in metabolic cages for the quantitative collection of urine. They were given food and water *ad libitum*. Suitable aliquots of the urine, collected at intervals, were plated for counting. In determining expired $C^{14}O_2$, an all-glass chamber was used, and the outgoing air was bubbled through a solution of CO_2-free NaOH. This solution was replaced by fresh NaOH at suitable intervals. Aliquots of each alkali solution were treated with excess $BaCl_2$, and the resulting $BaCO_3$ was washed with water and ethanol and plated for counting. Urea was precipitated from diluted urine samples with xanthydrol reagent, and the resulting dixanthyl urea was washed 3 times with methanol prior to plating for radioactivity measurements.

Results. Following the administration of either alloxan-5-C^{14} or alloxan-2-C^{14}, 90 to 95% of the C^{14} was accounted for in 22 hours in the urine (Table I). Lee and Stetten(1), employing alloxan at a level of approximately 200 mg/kilo, found the 2-C^{14} compound to be eliminated to the extent of only 67% in 2 days, while 57% of the alloxan-1,3-N^{15} was found in the urine in 2 days. These rates are somewhat lower than those observed in the present study.

Very low $C^{14}O_2$ concentrations were observed in analyzing the expired air. These accounted for less than 0.3% of the total

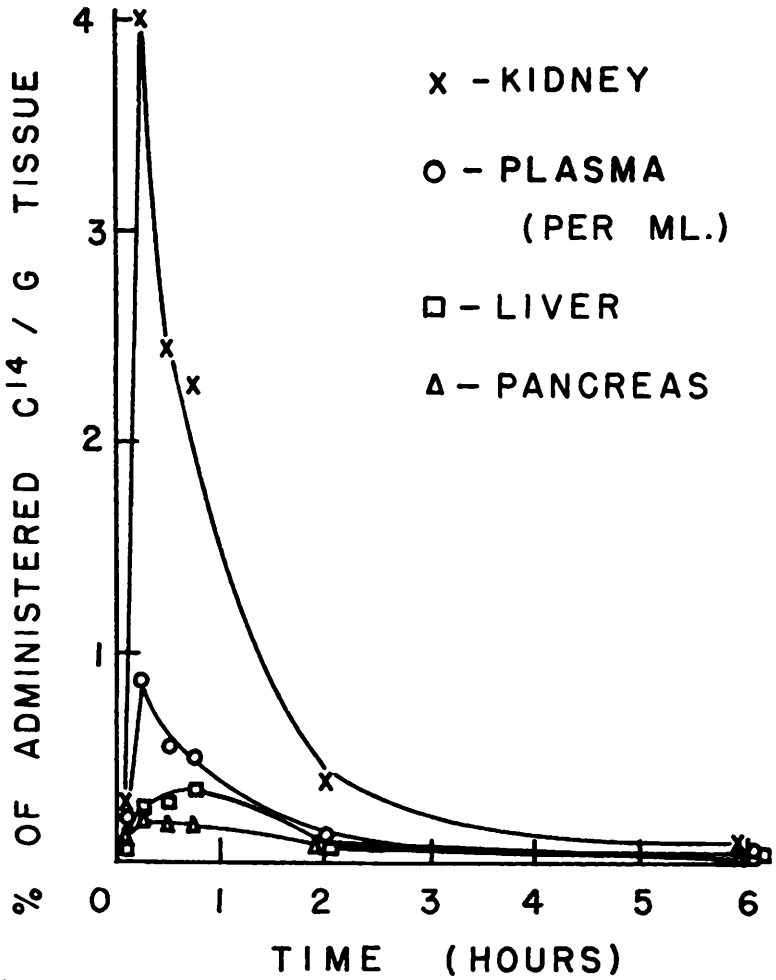

FIG. 1. Concentrations of C^{14} in tissues, following administration of 11 mg/kilo of alloxan-5-C^{14} One animal was sacrificed at 5, 15, 30, and 45 min., and at 2 and 6 hr.

dose, after 4 1/2 hours, with either alloxan-2-C^{14} or alloxan-5-C^{14}.

Only a very minor proportion of the radioactivity in the urine was due to urea, with either type of C^{14}-alloxan (Table I). Lee and Stetten similarly found 0.8% of the N^{15} of alloxan-1,3-N^{15} to be due to urea. These virtually negligible activities in CO_2 and urea suggest that the alloxan molecule is not significantly catabolized in the animal body.

In exploratory starch column chromatograms by the Moore and Stein method(2) on urine from rats given alloxan-5-C^{14}, approximately 80% of the total C^{14} emerged in a sharp peak in the region of 20 ml of effluent. The nature of this component has not as yet been determined. It may be mentioned that both alloxan and alloxanic acid, likewise have peaks near 20 ml with the starch column. However, no positive conclusion can be drawn from these chromatograms, since the resolving power of starch for this class of substances has not been studied.

The relative concentrations of alloxan-5-C^{14} in 4 tissues after different periods of time are shown in Fig. 1. While the concentration of C^{14} in kidney appears to be several times greater than that in plasma, if the total quantity of kidney tissue (about 2 g) relative to that of blood plasma (approximately 10 ml) is taken into account, the quantities of C^{14} in the 2 cases are approximately equal at the

TABLE II. Radioactivity of Various Tissues Following Administration of Radioactive Alloxan.

Tissue	% of administered C^{14}/g fresh tissue 11 mg alloxan-5-C^{14}/kg 15 min.	45 min.	110 mg alloxan-5-C^{14}/kg† 15 min.	45 min.
Kidney	4.03	2.27	3.80	2.87
Plasma	.85*	.50*	.90*	.58*
Pancreas	.20	.15	.17	.17
Liver	.18	.40	.26	.50
Spleen	.21	.15	.16	.19
Adrenals	.14	.17	.13	.14
Salivary gland	.17	.17	.13	.16
Thyroid	.24	.21	.20	.21
Pituitary	.16	.19	.10	.14
Brain	.08	.04	.08	.07
Upper intestine	.12	.09	.11	.11
Skeletal muscle	.18	.08	.05	.05
11 mg alloxan-2-C^{14}/kilo				
Kidney	3.75	2.65		
Plasma	.91*	.43*		
Pancreas	.18	.13		
Liver	.21	.24		

* Per ml plasma. † Animal was fasted 24 hr.

different time intervals. With both kidney and plasma the radioactivity was maximal at approximately 15 minutes. However, the C^{14} concentration in the liver reached a maximum 45 minutes after injection of the alloxan. It was of interest that only low concentrations of C^{14} were found in the pancreas. A similar observation has been made with alloxan-1,3-N^{15}(1).

A comparison of a variety of tissues with respect to alloxan-5-C^{14} uptake for two short intervals of time was made, with both high and low levels of the drug (Table II). The percentages of the C^{14} taken up were approximately equal with the 11 mg and 110 mg doses. The C^{14} concentration in the pancreas was approximately that in the other glands and tissues, except for kidney and plasma, which were higher, and brain, intestine, and skeletal muscle, which were somewhat lower. This finding relative to the pancreas seems of particular interest in view of the marked sensitivity of the beta-cells to injury by alloxan. The data for alloxan-2-C^{14} in Table II correlate reasonably well with those for alloxan-5-C^{14}, and this parallelism strengthens the view that the ureide and malonic acid portions of the alloxan molecule are not dissociated in the body. As with the 5-labeled compound, only relatively low C^{14} concentrations appeared in the pancreatic tissue.

In general the uptake of the two C^{14}-labeled alloxan preparations by the tissues is rather similar to the results with alloxan-1,3-N^{15}(1), particularly when the one-hour values with the latter compound are compared with the present 45 minute values. It should be pointed out that the values in Table II are for total C^{14}, and do not distinguish between protein and non-protein fractions. Actually, the experiments with alloxan-1,3-N^{15}(1) demonstrate that most of the isotope is in the protein-free filtrates of tissues.

The high radioactivity of the kidney tissue most probably reflects simply the passage of urine. Seligson and Seligson(3) have shown that alloxan is rapidly converted to alloxanic acid upon addition to blood plasma. The very high rate of elimination of the drug from the body tissues, and the negligible conversion to urea and carbon dioxide, make it seem improbable that alloxan is metabolized much beyond the alloxanic acid stage.

Summary. 1. Two alloxan preparations, labeled in the 2- and in the 5-position with C^{14}, were administered subcutaneously to rats. Both labeled compounds were rapidly excreted in the urine, to the extent of 90-95% in 22 hours. Negligible proportions of the C^{14} were found in urea. 2. With either tracer or diabetogenic doses, the radioactivity was highest in the kidneys and plasma, and reached a peak at approximately 15 minutes. The other tissues studied, including pancreas, had much lower C^{14} concentrations. 3. With either alloxan-2 or 5-C^{14}, very little radioactivity appeared as $C^{14}O_2$ in expired air. 4. It is concluded that the alloxan molecule does not undergo extensive catabolism in the rat.

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