cated that the increase in alpha amino nitrogen was due to a generalized failure of the reabsorptive mechanisms rather than inhibition of a specific transport system for groups of amino acids as described by Beyer(10).

The finding of variable potassium excretion appears to differ from the report by Robin, *et al.*(11) that hypokalemia occurs during salicylate poisoning. However, this hypokalemia was thought to be a manifestation of the respiratory alkalosis caused by salicylate rather than a specific function of salicylate.

Summary. 1. Both small and large amounts of acetylsalicylic acid have a marked inhibitory effect on renal excretion of sodium and chloride. 2. Uricosuric amounts of acetylsalicylic acid block the renal reabsorptive mechanisms for amino acids. 3. The findings of Yu and Gutman concerning the paradoxical effects of salicylate on uric acid excretion are confirmed.

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Fate of Radioactive Glucosamine Administered Parenterally to the Rat.* (27194)

M. R. SHETLAR, REAGAN BRADFORD, DANNY HERN, BOYD ENDECOTT, AND JOHN A. SCHILLING

Departments of Biochemistry and Surgery, University of Oklahoma School of Medicine, and Oklahoma Medical Research Institute, Oklahoma City

Glucosamine is found in many animal tissues, in glycoproteins and mucopolysaccharides. It has been shown that radioactively labeled glucose is converted to protein bound glucosamine(1,2,3). Furthermore, liver extracts are capable of transforming glucosamine to acetyl glucosamine-6-phosphate(4). However, few studies have been made of utilization of glucosamine by the intact animal. Boas and Foley(5) administered glucosamine intravenously to rats and found that the added glucosamine was rapidly cleared from the blood and that the hexosamine content of a number of tissues was increased.

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Recently, glucosamine-1-C^{14} has become available in amounts which make possible studies in laboratory animals. Preliminary studies indicate that glucosamine-1-C^{14} administered parenterally to rats is rapidly incorporated into the serum glycoproteins without degradation(6). This report concerns the distribution of radioactivity in various tissues of the rat following administration of radioactive glucosamine.

Methods. Three male Holtzman rats ranging in weight between 350 and 450 g were fed a stock diet of laboratory chow. During the experiment, the animals were kept in individual all glass metabolism cages(7) which permit collection of expired air, urine and feces. Feed and water were supplied *ad libitum*. One hundred microcuries of radio-

active glucosamine were administered by intraperitoneal injection in divided dosages, 3 times daily over a period of 5 days. At 3, 4, and 15 hours after the last injection, animals were anesthetized with ether, the peritoneal cavities were opened, and the animals killed by exsanguination from the abdominal aorta. Tissues were removed, washed with tap water, blotted dry on filter paper, frozen in a dry ice bath or in the deep freeze, and dried from the frozen state. The dry material was ground to pass through a 60 mesh screen. Samples of the ground tissue were weighed and suspended in 0.1 N NaOH with a motor driven tissue homogenizer with a teflon pestle (Arthur H. Thomas Co.). The suspension was made to a volume of 10 ml and an aliquot was plated out on glass planchets and counted in a flow gas counter.

The respired air was passed through a gas washing tower containing 140 ml of 3 N NaOH. These towers were changed twice daily. At the end of the experiment, the alkaline solutions were combined, mixed and made to a definite volume. An aliquot was precipitated with barium chloride, filtered, counted at infinite thickness, the proper corrections were made, and total radioactivity converted to CO_2 was calculated. Urine was collected under toluene for each day and combined at the end of experiment. An aliquot was taken for counting, and total radioactivity excreted in the urine was calculated. A 0.10 ml aliquot of serum from each sample was plated out and counted in the gas flow counter.

Results. The data concerning level of radioactivity in various tissues are summarized in Table I. High counts were found in kidney, liver, spleen, lung, brain, adrenals, pancreas, testes, and the digestive system. Skin, brain and bladder tissue have moderately high counts, while bone, cartilage, and tendons exhibit low levels of radioactivity.

Total radioactivity of the serum was estimated by assuming that serum represents 4% of body weight. Total radioactivity in other tissues and organs was estimated by using the data of Donaldson(8). From these estimations, the recovery of administered radioactivity in each tissue or organ, in serum, urine and respiratory CO_2 was calculated. Results are summarized in Table II.

TABLE I. Radioactivity in Tissues of the Rat after Administration of Glucosamine-1-C^{14}.

Tissue or organ	Counts/min./mg of tissue Avg*	Range
Kidney	1230	961–1330
Liver	710	554 – 852
Spleen	661	568 – 810
Lung	527	398 – 624
Brain	443	258 – 550
Heart	217	192 – 242
Adrenal	458	——
Pancreas	404	——
Testes	498	370 – 694
Muscle (skeletal)	61	37 – 82
Skin	183	174 – 194
Bone, femur (less marrow)	27	17 – 38
Bone marrow	328	289 – 366
Teeth	52	37 – 104
Cartilage: Xiphoid	137	193 – 257
Costal	95	79 – 126
Intervertebral disc	64	——
Tail tendon	78	49 – 99
Eye	178	119 – 248
Stomach	398	245 – 538
Small intestine	376	341 – 412
Colon	408	245 – 538
Bladder	328	175 – 454

* Avg of results from 3 animals except for adrenals, pancreas and intervertebral disc which are results on only one sample.

When so expressed, over 30% of the administered glucosamine is metabolized and converted to CO_2, and another 15% is excreted in the urine. Thus during the period of study, 50-55% of the administered radioactivity is retained. The retained radioactivity appears largely in the serum, and in skin, liver, muscle and lungs. Although the specific radioactivity is high in kidney tissue, total radioactivity is calculated to be only 2% of administered radioactivity.

Discussion. The work recorded here was designed to provide data on incorporation of glucosamine into tissue constituents when tissues are presented with glucosamine over an extended period. Distribution of radioactivity in tissues at various times after administration of one dose of glucosamine-1-C^{14} is being studied. Although the radioactivity found in tissue may not be entirely due to glucosamine, the recovery of highly radioactive glucosamine from serum proteins

TABLE II. Recovery of Administered Radioactivity from Rats Given Glucosamine-1-C^{14}.

	% of administered dose	
	Avg*	Range
Respiratory CO_2	32.2	30.0 –36.0
Urine	14.7	13.9 –15.6
Serum	10.2	8.5 –12.0
Liver	8.0	6.2 – 9.6
Kidneys	2.1	1.7 – 2.4
Lungs	5.1	3.9 – 6.0
Brain	.5	.3 – .6
Heart	.2	.15– .22
Muscle	6.2	3.8 – 8.4
Skin	14.7	12.7 –17.7
Skeleton	1.4	.9 – 2.0
Total	95.3	87.0–108.7

* Avg of results from 3 animals. Calculated by assuming that serum represents 4% of body wt. Total wt of organs used in the calculations taken from Donaldson(8).

and the occurrence of only small amounts of radioactivity in bound hexose of serum(6) would strongly indicate that most of the tissue radioactivity is due to the intact glucosamine molecule. On this basis comparison may be made with the data of Boas and Foley (5) derived with unlabeled glucosamine. They record definite increases of hexosamine in blood, liver, stomach, intestines, and kidney. All of these tissues have high radioactivity (Table I) after administration of radioactive glucosamine. In addition, however, high counts in brain, testes, lung, and spleen were found in this study, whereas Boas and Foley(5) record only slight increases for these tissues. These differences may be attributed to the difference in mode of administration and to dosage levels in the two studies. The relatively low radioactivity in cartilage is of interest. As the predominant hexosamine containing compound of cartilage is chondroitin sulfate which contains galactosamine, these data may tend to indicate little conversion of glucosamine to galactosamine. However, the relatively low turnover rate of cartilage components must be considered. It is of interest that cartilage from different sources differs in radioactivity.

Data in Table II indicate that under the conditions of this experiment about 30% of the radioactive C-1 of administered glucosamine is metabolized to carbon dioxide. In addition, labeled carbonate is found in the urine. Urine was found to have about 15% of the administered radioactivity, as compared to 5% found by Boas for a much shorter period. Figures reported here are probably too low because of difficulty of recovery of all urine. Only part of the radioactivity is free hexosamine, approximately one-third is not dialyzable, and some as mentioned above is present as carbonate.

Summary and conclusions. Distribution of radioactivity in various tissues of the rat after intraperitoneal administration of glucosamine-1-C^{14} has been studied. Highest levels of radioactivity were found in kidney, liver, spleen, lung, brain, digestive system, and blood serum. Radioactivity, however, was generally distributed throughout the animal body. The utility of glucosamine-1-C^{14} as a means of studying glycoprotein and mucopolysaccharide metabolism is indicated.

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