pressure of 190/110 to 160/100, soft aortic systolic murmur, and electrocardiographic changes suggesting diffuse non-specific myocardial disturbance.

Summary. The urinary excretion of 5-hydroxyindole acetic acid in patients with malignant hypertension and chronic glomerular nephritis is reported. Low levels were recorded for 3 patients exhibiting the malignant phase of hypertensive cardiovascular disease and in each patient with chronic glomerular nephritis. All patients exhibited extensive renal involvement and 5 of the 6 patients with malignant hypertension had severe arterial lesions. The 2 patients on whom determinations were made after removal of early carcinoid tumors did not show abnormal urinary 5-hydroxyindole acetic acid levels. Recovery studies show that proteinuria does not interfere with the determination of 5HIAA in the urine.

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Distribution of C^{14}-Labeled Aminoacetonitrile in Tissues of Rat, Metabolism and Mode of Elimination.* (22803)

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Several recent studies(1,2,3) have dealt with the effects of aminonitriles on rats. The remarkable ability of these substances to produce the characteristic skeletal lesions of lathyrism in young animals suggests the investigation of the metabolism of the aminonitriles, with the aid of the isotopic labeling technic. Very recently isotopically-labeled β-amino-proprionitrile has been reported synthesized and a study of its metabolism begun (4). For the present work, aminoacetonitrile (AAN) labeled with C^{14} in its cyanide group was employed. It has been previously established(1) that this compound resembles closely in its action, but is more potent than the higher homolog, β-aminoproprionitrile, or β-(γ-L-glutamylamino)-proprionitrile, the active principle of Lathyrus peas. Besides determining the distribution of the radioactivity in the animal organism and the modes of elimination, experiments were designed to explore the possible incorporation of the cyanide carbon atom into major body constituents, including protein, lipid, and carbohydrate. One possible route would be via hydrolysis of the cyanide radical to yield a carboxyl group, *i.e.* the transformation of AAN into glycine.

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Methods. Synthesis of $(H_2N\text{-}CH_2C^{14}N)_2\,H_2SO_4$. The following modification was employed of the method given in Organic Syntheses(5): A solution of NaCN (1 g, 20 mM, containing 1 millicurie of C^{14}) in 1.7 ml water was added slowly to a mixture of 1.1 g of NH_4Cl (20 mM) and 3 ml of 37% formaldehyde (40 mM). When half the NaCN had been added, 0.76 ml of acetic acid was added at such a rate that both the addition of the remaining cyanide and of the acid ended at the same time. The stirring was continued for 30 minutes, and the methyleneiminoacetonitrile was filtered off; yield, 0.70 g (51.5%). The methyleneiminoacetonitrile was treated with a solution of 1 g of 96% H_2SO_4 in 5 ml of ethanol, and allowed to stand over night. The yield of crystalline aminoacetonitrile hydrogen sulfate was 0.91 g or 43% based on NaCN. The recrystallized product melted at 155-7° with decomposition. Its nitrogen content, 25.9%, was lower than theory (26.65), suggesting that a small proportion of the molecules were in the form: $(H_2NCH_2C^{14}N)$ H_2SO_4. Female weanling Sprague-Dawley rats (45-50 g) were given AAN-C^{14} by either: (1) A single IP injection of a neutralized solution of the sulfate salt, or (2) Feeding ground Purina Chow containing 0.01% AAN-C^{14} plus 0.09% non-isotopic AAN for a number of days.† After stated intervals of time, animals were anesthetized, and blood samples taken by heart puncture. The tissues were rapidly removed, weighed, and homogenized with 20 parts of water. Aliquots of each homogenate were dried on steel planchets, and radioactivity measured with a flow-gas Geiger counter. All values were corrected for self-absorption. Subsequently protein was isolated(5) from the tissue homogenates and its C^{14} content determined. In one instance liver protein was hydrolyzed and the radioactivity of its glycine and serine determined(6). Also liver glycogen and lipid were isolated(7) and their radioactivity measured. In other experiments, rats were maintained in metabolic cages for the collection of respiratory CO_2 and urine(8). In studying the forms in which the C^{14} was present in the urine and in the tissues, extensive use was made of the "carrier" method, with 3 to 4 recrystallizations of the isolated substance. In employing this technic, AAN was precipitated by adding alcohol to urine and tissue extracts; urinary urea was isolated by the xanthydrol method(9), creatinine as the picrate(10), and allantoin via the silver and mercury salts(11). In one experiment sodium benzoate was injected together with AAN-C^{14}, and hippuric acid subsequently isolated from the urine(12). Lastly, the procedure of Meyer and Smyth(13) for chondroitin sulfate was employed for the isolation of a polysaccharide fraction‡ from the enlarged epiphyseal cartilage plates of rats maintained on diets containing AAN-C^{14}.

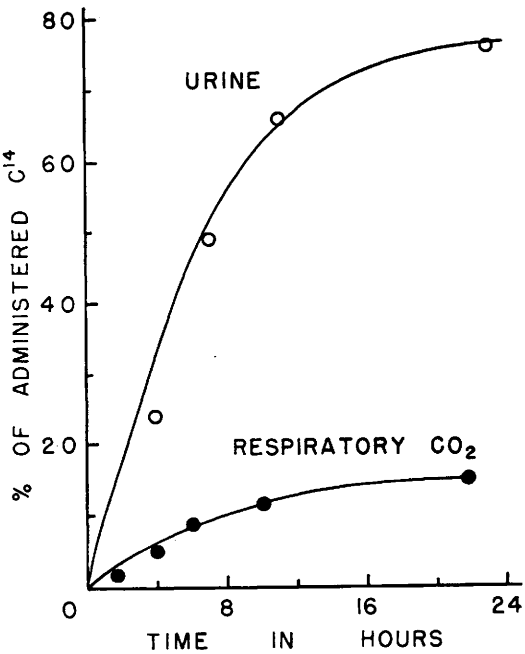

FIG. 1. Rates of elimination of C^{14} in urine and in expired air, following IP inj. of 2 mg AAN-C^{14}. The values are for a group of 6 rats maintained in a common metabolic cage.

Results. Fig. 1 shows that AAN was catabolized to a significant extent, since 15% of the administered C^{14} was found in respiratory CO_2 in 22 hours. Almost 80% of the radioactivity appeared in the urine in this time interval. Further analysis of this same urine, and of urine from rats on a diet containing AAN-C^{14}, revealed that free AAN it-

† Amounts of nitrile are expressed in terms of free AAN, as calculated from quantity of sulfate employed.

‡ This preparation differed from chondroitin sulfuric acid in its lower mobility and more diffuse pattern in paper electrophoresis at pH 8.9. It was sulfated, as indicated by metachromatic staining with toluidine blue. (Kindly performed by Drs. R. D. Montgomery and T. R. Sawyer).

TABLE I. Distribution of Isotope among Urinary Constituents.

Source of urine*	AAN	Urea	Creatinine	Allantoin
	% of C^{14} retained by carrier			
Rats inj. with 2 mg AAN-C^{14}, urine collected first 4 hr	15	8	3.0	2.1
Same, 4-7 hr urine	18	9	3.5	1.8
Rats on stock diet containing 0.1% AAN-C^{14}, urine collected 6th day	16	12	2.2	1.5

* Pooled urine of 6 rats.

self accounted for only a minor part of the C^{14} eliminated by this route (Table I). The moderate radioactivity in the isolated urea agrees with the finding for respiratory CO_2 The low, but significant isotopic values for creatinine and allantoin suggest that a certain degree of conversion of AAN-C^{14} to glycine-C^{14} occurred, since glycine is a precursor of both these compounds. Further confirmation of this pathway was obtained by injecting a mixture of 1.3 mg AAN-C^{14} and 25 mg sodium benzoate into each of 2 rats. The hippuric acid isolated from the pooled urine (collected over an 18 hour period) accounted for 1.2% of the total administered C^{14}.

Some 60-70% of the C^{14} of urine was not characterized in the present study.§ Schilling and coworkers(4) find that the C^{14} of $NH_2CH_2CH_2C^{14}N$ is likewise chiefly eliminated in the urine of rats, in an altered form. However, they state that the main excretory product "appears to be a volatile, ether-soluble, basic or neutral substance." No volatile products were found in the urine of C^{14}-AAN animals, and only a minor portion of the radioactivity (2-3%) was extractable by ether from either neutral or basic urine. Apparently the 2 nitriles are metabolized differently.

Fig. 2 gives the relative C^{14} levels in body

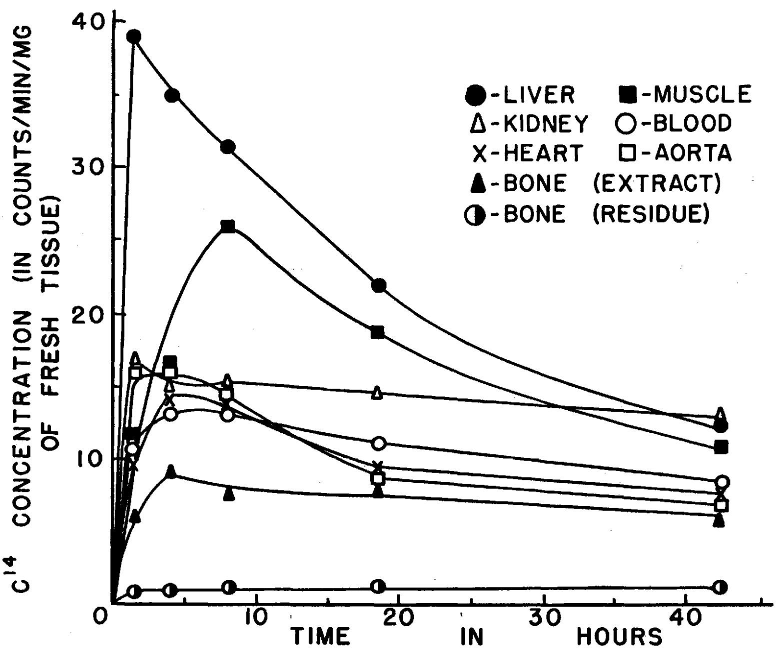

FIG. 2. Distribution of isotope in various tissues, following inj. of 10 mg of AAN-C^{14} per rat. Each point represents pooled material from 3 animals.

§ Attempts to detect C^{14}-labeled thiocyanate by paper chromatography were unsuccessful.

TABLE II. C^{14} Distribution in Rats Fed a Stock Diet Containing 0.1% AAN-C^{14}.

Tissue	C^{14} in cts/min./g fresh tissue, 6 days	C^{14} in cts/min./g fresh tissue, 10 days	μmoles of labeled substances/g tissue, 6 days	μmoles of labeled substances/g tissue, 10 days
Liver	7300	7600	2.25	2.34
Kidney	6500	9900	2.00	3.04
Skeletal muscle	2480	3000	.76	.93
Blood	2100	2250	.65	.69
Bone*	2280	2250	.69	.70
Epiphyseal plates of long bones	1770	1990	.55	.61

* Non-mineral extractives.

All values are for pooled tissues of 3 rats. The animals developed moderate lesions at 6 days, and typically severe lesions by 10 days.

tissues at varying times after the administration of a larger quantity of AAN-C^{14} to rats. It may be seen that liver was by far the most active tissue. After 4 hours the specific activity of muscle exceeded that of all other tissues excepting liver, and after about 30 hours, liver, muscle, and kidney were comparable in C^{14} concentration. Blood, heart, aorta, and bone (aqueous extractives) tended toward somewhat lower values, while bone residue was least active. It is evident that the radioactivity was retained by the animals to a greater degree than in Fig. 1, in which the dosage of AAN-C^{14} was smaller.

If the results in Fig. 2 are considered in terms of the total mass of each tissue, it is apparent that skeletal muscle actually accounts for a greater percentage of the isotope than does liver. Likewise, the skeleton, because of its relatively great weight and also its retention of the isotope, contained more C^{14} than any tissue except muscle, and possibly liver.

TABLE III. Isotopic AAN in Rat Tissue.

Tissue	% of C^{14} in each tissue recovered by carrier method: Sacrificed 1½ hr after inj. 10 mg AAN-C^{14}	Sacrificed 18 hr after inj. 10 mg AAN-C^{14}	Fed for 10 days diet containing .1% AAN-C^{14}
Blood	73	53	27
Liver	24	16	25
Kidney	33		20
Muscle	61	43	26
Bone	90	58	31
Epiphyseal plates of long bones			39

The animals were those described in Fig. 2 and Table II.

When rats were maintained for a number of days on diets containing AAN-C^{14}, relatively high concentrations of labeled material accumulated in the body tissues (Table II). This is most evident when the radioactivities are converted into μMoles. The values for bone and epiphyseal plates, while not as high as with liver or kidney, do represent substantial accumulations of labeled carbon. Furthermore, it is noteworthy (Table III) that bone was the tissue with the highest percentage of its radioactivity in the form of free AAN; 39% of the C^{14} in the epiphyseal cartilage plates of rats on the AAN-C^{14} diet was present in the form of AAN. These plates were extremely enlarged.

TABLE IV. C^{14} in Tissue Proteins of Rats.

Tissue	Sacrificed 18 hr after inj. 10 mg AAN-C^{14}	Fed 10 days diet containing .1% isotopic AAN
Blood	10	8
Liver	13	12
Kidney		6
Muscle	4	5
Bone	6	7
Epiphyseal plates of long bones		14

The values represent % of total C^{14} in each tissue.

It is of interest that a significant part of the C^{14} in the tissues was in protein combination (Table IV). The high value for epiphyseal plates again reflects the intense synthetic activity occurring in this region. Examination of the liver protein of rats given a single injection of AAN-C^{14} disclosed that 40% of the radioactivity of the protein was due to glycine, while serine accounted for 18%. The distribution of the remaining C^{14} was not determined.

It was found that the sulfated polysaccharide isolated from epiphyseal plates was moderately radioactive (Table V). The position of the labeling was not determined. The C^{14} content of the epiphyseal cartilage protein isolated in this same experiment is given for comparison.

Indication that the C^{14} of AAN partici-

TABLE V. Labeling in Epiphyseal Plate. Rats maintained for 10 days on stock diet containing 0.1% isotopic AAN.

Substance isolated	C^{14} conc./g fresh tissue Cts/min.	μM
Polysaccharide fraction*	95	.029
Protein	157	.048

* Approximately 10 mg were obtained per g of plates.

pated in a variety of reactions was the finding of small but definite concentrations of isotope in glycogen and lipids of liver, 4 hours after injecting AAN-C^{14} into rats. The glycogen accounted for 0.5%, and the lipids 0.6%, of the total C^{14} in this organ.

Summary. Aminoacetonitrile, with C^{14} in its cyanide group, was synthesized. When this compound was administered to weanling rats, the major portion of the radioactivity was eliminated in the urine, while a minor part appeared in respiratory CO_2. High initial C^{14} concentrations were observed in various body tissues. The proportion of this C^{14} due to unchanged nitrile decreased fairly rapidly with time. Labeling was found in tissue proteins, and to a lesser extent in liver lipids and glycogen. Also, in rats fed a diet containing isotopic aminoacetonitrile, radioactivity was detected in the sulfated polysaccharides of the enlarged epiphyseal plates of the bones. The presence of C^{14} in glycine and serine of liver protein and in urinary creatinine, allantoin, and hippuric acid, indicated an appreciable conversion of the cyanide radical into a carboxyl group. However, the major portion of the C^{14}-containing metabolites was not identified.

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Chronic Effect of Dietary Protein on Hypercholesteremia in the Rat.* (22804)

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A diet consisting of casein, lard and dextrin was found to be atherogenic in the rat(1). Variations in fat and cholesterol content of the diet seemed to have little influence on the consequent coronary arterial lesions(2). A reduction in quality or quantity of dietary protein has raised the serum cholesterol level and promoted atherogenesis in other experimental situations(3,4).

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The serum cholesterol level of adult cholesterol-fed rats rises gradually over a period of months to reach a plateau, where it remains for at least another 3 to 6 months. The serum cholesterol after 1 or 2 months may not reflect the ultimate level to be reached when the animal has finally adapted to the diet. On the other hand, the great frequency of a chronic form of pneumonitis(5), which is virtually inescapable in the rat after the age of