had the typical crystalline form of PAB.

The PAH extracted above melted at 198-200°C, contained 14.1-14.4% nitrogen (theoretical is 14.4), and had an acid equivalent weight of 189-195 (theoretical is 194.2).

Purification of an aqueous iso-electric solution can be effected by extracting with ether and evaporating the ether to dryness. The residue *dissolved in hot water* yields crystalline PAB on cooling. The ether-extracted aqueous solution can be crystallized directly to give PAH which may be recrystallized from absolute alcohol.

Discussion. We conclude from the above data that lot No. 236518 is essentially pure PAH, whereas lot No. 12322 contains approximately 23% of PAB. Incidentally, it is demonstrated that PAB is rapidly absorbed from the intestinal tract from dog and man, whereas PAH is slowly and poorly absorbed.

PAH is made commercially by the conjugation of p-nitrobenzoyl chloride with glycine, the nitro group being subsequently reduced. Failure to effect complete conjugation, or hydrolysis during reduction, will yield PAB. Since the clearance of PAB is less than the creatinine clearance in dogs,[1] the presence of a significant quantity of this substance in PAH would introduce a large error into the determinations of the renal plasma flow. The Medical Division of Sharp and Dohme, who prepared ampouled PAH for clinical investigation, report that lot No. 12322, which we find to be contaminated with PAB, has never been marketed, and they are now examining all samples for significant contamination by PAB.

Summary. P-aminobenzoic acid is rapidly absorbed from the gastrointestinal tract in dog and man while p-aminohippuric acid is poorly absorbed.

One commercial lot (National Aniline No. 12322) of p-aminohippuric acid was found to be contaminated with 23% of p-aminobenzoic acid.

None of the contaminated material, to our knowledge, has been marketed in ampouled form for clinical investigation and an adequate control is now being maintained on the purity of clinical material.

We are indebted to Joanne Baker Schreiner for technical assistance and to Dr. Conrad Dobriner of the Sloan-Kettering Institute, New York City, for determining the infra-red absorption spectrum of the several compounds studied.

We are also indebted to Dr. R. Keith Cannan for his assistance in chemical purification, and to Sharp and Dohme for parallel chemical studies and for rechecking their file samples of their ampouled material.

17048 P

Recovery of Isotopic Succinate from Urine of Rats Administered Isotopic Acetate.

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To demonstrate that a given pathway is involved in the metabolism of an administered isotopic compound, it would appear essential to determine the isotopic composition of the assumed intermediates in the pathway. Evidence is accumulating that the Krebs or tricarboxylic acid cycle, developed mainly on the basis of *in vitro* evidence, actually operates in the intact animal, and that this scheme participates in the metabolism of acetate.[1-4] In the present investigation it was found that after the administration of isotopic acetate

[1] Krebs, H. A., and Johnson, W. A., *Biochem. J.*, 1938, **32**, 113.

[2] Lorber, V., Lifson, N., and Wood, H. G., *J. Biol. Chem.*, 1945, **161**, 411.

[3] Lifson, N., Lorber, V., Sakami, W., and Wood, H. G., *J. Biol. Chem.*, 1948, **176**, 1263.

[4] Rittenberg, D., and Bloch, K., *J. Biol. Chem.*, 1945, **157**, 749.

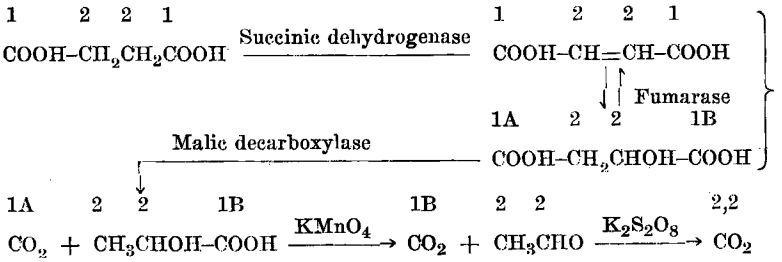
to rats, labeled carbon is found in succinate recovered from the urine, and that the location of isotope in this intermediate of the tricarboxylic acid cycle corresponds to the distribution predicted from the reactions of the cycle.

Methods. Two ml of 0.5 M sodium malonate per 100 g of body weight was administered subcutaneously and 1.10 mM of $CH_3C^{13}OONa$ (6.10 atom % excess C^{13} in the carboxyl group) per 100 g of body weight was fed by stomach tube to fasted rats weighing 120 - 200 g. The malonate was injected to augment urinary succinate excretion.[1] The acetate dose was repeated at the end of approximately 8 and 16 hours. The urine was collected in 20% H_2SO_4 for 24 hours, except during periods when respiratory carbon dioxide was being sampled.

The urine was extracted with ether in the presence of sodium bisulfite in order to bind keto acids. The ether extract was distilled with steam and the residue oxidized with acid permanganate. Succinic acid was extracted with ether from the oxidation mixture and purified by repeated silver precipitations.[5] The melting point of the white crystalline product was 180° - 185° and that of the Eastman Kodak product 185° - 187°.

The succinic acid recovered from the urine was converted into a mixture of fumaric acid and malic acid with succinic dehydrogenase prepared from pigeon breast muscle.[6] Fumaric and malic acid were extracted with ether and subjected to the action of an acetone powder of *Lactobacillus arabinosus* prepared by Ochoa's procedures[7] with the exception that fumaric acid instead of malic acid was used in the culture medium. The former proved to be more effective in developing fumarase as well as malic decarboxylase activity. This enzyme preparation converts quantitatively the mixture of fumarate and malate into lactic acid and CO_2.

The lactic acid thus formed was oxidized by permanganate into aldehyde and CO_2. The aldehyde was oxidized by potassium persulfate to CO_2.[8] The overall degradation scheme may be summarized as follows:



At each step, the yields of degradation products from urinary succinate compared satisfactorily with theoretical expectations and with those from authentic succinate.

Results and discussion. From the data in Table I, it will be noted that after the administration of $CH_3C^{13}OOH$, $C^{13}OOHCH_2$-$CH_2C^{13}OOH$ is recovered from the urine. This is the result predicted from metabolism of acetate via the tricarboxylic acid cycle, by condensation with oxalacetate.[9] Since appreciable amounts of C^{13} also appeared in the respiratory CO_2 (.02 - .55 atom% excess, depending on the time interval between sampling and acetate feeding), and since CO_2 fixation would also be anticipated to yield carboxyl labeled succinate,[9] it is not possible on the basis of these results alone to evaluate the extent to which the latter mechanism accounts for the observations. The small but significant difference in C^{13} concentration between the 2 carboxyl carbons (fractions 1A and 1B), which theoretically should be equal because

5 Wood, H. G., Werkman, C. H., Hemingway, A., and Nier, A. O., *J. Biol. Chem.*, 1942, **142**, 31.

6 Umbreit, W. W., Burris, R. H., and Stauffer, J. F., Manometric Techniques, 1945, p. 144, Burgess Publishing Co.

7 Ochoa, S., personal communications; Korkes, S., and Ochoa, S., *J. Biol. Chem.*, 1948, **176**, 463.

8 Osborn, O. L., and Werkman, C. H., *Ind. and Eng. Chem. Anal. Ed.*, 1932, **4**, 421.

9 Wood, H. G., *Physiol. Rev.*, 1946, **26**, 198.

TABLE I.
Distribution of C^{13} in Urinary Succinic Acid After Administration of Carboxyl Labeled Acetate to Rats. C^{13} Values Are in Atom % Excess.

Exp. No.	Rat No.	Labeled acetate fed mM/100 g	C^{13} in whole succinic acid molecule	C^{13} in degradation fractions: Carboxyl carbons 1A	Carboxyl carbons 1B	Methylene carbons 2,2
I	1	1.1	0.15			
II	2	3.4	0.14			
III	3-8	3.4	0.10	0.21	0.17	0,00

succinate and fumarate are both symmetrical molecules, is interpreted as due to the oxidation of a certain amount of extraneous carbon by permanganate, but other explanations are possible.[10]

These experiments are being extended.

Summary. After the administration of malonate plus carboxyl-labeled acetate to rats, there has been recovered from the urine carboxyl-labeled succinate, an intermediate of the tricarboxylic acid cycle. This result is consistent with and additional evidence for the metabolism of acetate via the tricarboxylic acid cycle in the intact mammal.

We wish to thank Mr. Richard E. Halsted and Mrs. Ruth Boe for performing the isotopic analyses under the supervision of Dr. A. O. C. Nier, Mrs. Shirley L. Michel for valuable technical assistance, and Dr. S. Ochoa for kindly supplying us with cultures of *L. arabinosus.*

[10] Calvin, M., and others. Isotopic Carbon, 1949, p. 192, John Wiley & Sons, Inc.

17049

Simultaneous Fluorocardiography and Recording of Intracardiac Pressure.

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Various studies of clinical fluorocardiography have been published in the last three years.[1-5] This method is based on the photoelectric recording of the motion of selected points of the cardiovascular contour as revealed by the fluoroscope.

Although simultaneous recordings of other graphic tracings (electrocardiogram, carotid tracing, phonocardiogram, etc.) have supported the reliability of the fluorocardiogram, an incontrovertible demonstration of the accuracy of the latter has been lacking. For this reason, fluorocardiograms of the right auricle or the right ventricle, simultaneous with tracings of pressure in the corresponding chamber, have been taken in the anesthetized dog.

Materials and methods. A Sanborn electrokymograph and a Sanborn electromanometer were used. The former is a standard apparatus for fluorocardiography; the latter is a new apparatus, based on the strain gauge principle, which permits the recording of variations of pressure with controlled amplification. A Sanborn tri-beam cardiette was employed for the transcription of the tracings so that both tracings were recorded by two separate channels while a third recorded the heart sounds through a stethoscopic micro-

[1] Henny, G. C., and Boone, B. R., *Am. J. Roentgen.*, 1945, **54**, 217.

[2] Luisada, A. A., Fleischner, F. G., and Rappaport, M. E., *Am. Heart J.*, 1948, **35**, 336 and 348.

[3] Luisada, A. A., and Fleischner, F. G., PROC. SOC. EXP. BIOL. AND MED., 1947, **66**, 436; 1948, **67**, 535; 1948, **69**, 23.

[4] Luisada, G. C., and Fleischner, F. G., *Am. J. Med.*, 1948, **4**, 791.

[5] Luisada, G. C., and Fleischner, F. G., *Acta Card.*, 1948, **3**, 308.