survived.

Effect on acid secretion by the frog gastric mucosa. The effect of maleate on acid secretion by the frog gastric mucosa was studied by the technic of Patterson and Stetten (3). Because of the buffering capacity of maleate in the physiological range of pH, it was not possible to measure the secretion of H^+ ions by the stomach into a solution containing appreciable amounts of maleate and the maleate was, therefore, added only to the chamber in contact with the external surface of the mucosa. No inhibition was evident with maleate concentrations of 100 mM/l. There may be some question of the penetration of the maleate to the region of acid formation under the circumstances of this experiment. NaF, 10 mM/l, was completely inhibitory when added under similar conditions.

Discussion. Maleate appears to exert an effect on acidification of the urine which is unique among substances whose action has been reported. Sulfanilamide impairs the excretion of titratable acid(4), but the effect is relatively slight and can be demonstrated only when acidosis is relatively mild. Cyanide (5) and tartrate(6) have been found to abolish urinary acidification but in these instances the effect is observed along with almost complete loss of renal tubular activity and a loss of the ability to excrete a urine with a pH different from that of the plasma. The latter is not the case with maleate since the capacity to excrete an alkaline urine is retained; urines with pH over 7.8 have been observed repeatedly. Although maleate appears to depress most of the renal functions studied to some extent, the effect on acidification is far greater than its other actions.

Maleate has been observed to exert an inhibitory action on a number of enzyme systems studied *in vitro*(7-11). It is not clear through which, if any, of these systems the effects described here are exerted.

Summary. Maleate administered to severely acidotic dogs at a dosage of 0.35 mM/kg abolishes the capacity to excrete an acid urine. Lesser effects on other renal functions are produced.

3. Patterson, W. B., and Stetton, DeW., Jr., *Science*, 1949, v109, 256.

4. Pitts, R. F., and Alexander, R. S., *Am. J. Physiol.*, 1945, v144, 239.

5. Nicholson, T. F., *Biochem. J.*, 1949, v45, 112.

6. Nicholson, T. F., Urquhart, R. W. I., and Selby, D. L., *J. Exp. Med.*, 1938, v68, 439.

7. Morgan, E. J., and Friedmann, E., *Biochem. J.*, 1938, v32, 733.

8. Morgan, E. J., and Friedmann, E., *Biochem. J.*, 1938, v32, 862.

9. Hopkins, F. G., Morgan, E. J., and Lutwak-Mann, C., *Biochem. J.*, 1938, v32, 1829.

10. Weil-Malherbe, H., *Biochem. J.*, 1938, v32, 2257.

11. Friedmann, E., Marrian, D. H., and Simon-Reuss, I., *Brit. J. Pharmacol. and Exp.-Chemotherapy*, 1948, v3, 263.

Received November 10, 1950. P.S.E.B.M., 1950, v75.

Biosynthesis of Radioactive Allantoin.* (18345)

J. D. VALENTINE, S. GURIN, AND D. W. WILSON.

From the Departments of Physiological Chemistry and Medicine, School of Medicine, University of Pennsylvania, Philadelphia.

Heinrich and Wilson(1) reported experiments dealing with the carbon precursors of the tissue purines of the rat. Since allantoin is one of the main end products of purine

* Aided by a grant from the American Cancer Society administered by the Committee on Growth of the National Research Council. The C^{14} used in this investigation was obtained on allocation by the United States Atomic Energy Commission.

1. Heinrich, M. R., and Wilson, D. W., *J. Biol. Chem.*, 1950, v186, 447.

metabolism in mammals, it was felt that the isolation of allantoin from urine following administration of radioactive precursors would provide a relatively simple method of studying purine metabolism. For this purpose, a simple procedure for the isolation of allantoin from rat urine was developed. $NaHC^{14}O_3$, $CH_3C^{14}OOH$ and $NH_2C^{14}H_2C^{14}OOH$ were administered to rats, allantoin was isolated from the urines, and the resulting products degraded chemically to determine the position of the isotope.†

Experimental. Organic syntheses. Sodium bicarbonate was made from C^{14} barium carbonate. Carboxyl C^{14} acetate was made by the Grignard reaction, and carboxyl C^{14} glycine from carboxyl labeled ethyl bromoacetate and potassium phthalimide(3). Doubly labeled glycine was prepared from doubly labeled acetic acid as previously described(3).

Isolation of allantoin. After rinsing each cage and funnel with distilled water, the diluted urines were combined and neutralized to approximately pH 7 with dilute sodium hydroxide. A 10% solution of $AgNO_3$ was added in excess, and the suspension chilled to 0°C. Cold 5% NH_4OH was added dropwise with stirring to pH 8-9 and the suspension was allowed to stand overnight in the cold room. After centrifugation, the precipitate was washed once with cold water, and suspended in 200 ml of water. The silver was precipitated with H_2S, the suspension centrifuged, and the supernatant aerated. A solution of 10% mercuric acetate in 20% sodium acetate was then added until the supernatant failed to become cloudy on further addition. After chilling for 2 hours, the suspension was centrifuged and the precipitate washed once by stirring with cold water. The precipitate was suspended in 200 ml of water and decomposed with H_2S. The mercury sulfide precipitate was removed by centrifugation, and, after aeration, the supernatant was concentrated *in vacuo* to dryness. The residue was then extracted with a few ml of hot water, filtered hot, and the solution chilled overnight. The precipitated crude allantoin was removed by centrifugation, dissolved in minimal hot water and decolorized with charcoal. Upon chilling, analytically pure allantoin was usually obtained. For radioactivity studies, the allantoin was invariably recrystallized several more times. The average yield from the pooled 24-hour urines of 5 rats was 40-60 mg. M.P. 216-217°. Total nitrogen (Kjeldahl) 35.2-35.6%; theory 35.4%.

Chemical degradation of allantoin. The method employed was that described by Buchanan, Sonne and Delluva(4). Under these conditions it was found that 80 mg of allantoin yielded 20-30 mg of glyoxylic acid and sufficient urea for radioactivity measurements.

Results. Labeled bicarbonate. One rat (wt. 220 g) received 440 mg of labeled sodium bicarbonate in divided doses hourly for 7 hours. A total of 680,000 counts/minute was administered. The allantoin recovered from the urine of this animal was free of radioactivity. This is to be expected if purines are converted to allantoin by the loss of carbon No. 6. It has been demonstrated that bicarbonate is incorporated preferentially into position 6 of uric acid of the pigeon(4), and guanine and adenine of the rat(1).

Labeled acetate. The 5 animals on this experiment received a total of 632,000 counts/minute over a period of 5 days (subcutaneous injection twice daily). On degradation of the allantoin from these animals no radioactivity could be demonstrated in the urea fraction representing carbons 2 and 8. A trace of radioactivity observed in the glyoxylic acid fraction representing carbons 4 and 5 is believed to be of no significance. Heinrich and Wilson have similarly demonstrated that the carboxyl carbon of acetate is not incorporated into positions 2 and 8 of the adenine and guanine isolated from the tissues of the rats used in this experiment(1). It, therefore, appears that acetate is not a precursor of positions 2 and 8 in the purines

† Preliminary report of a portion of this work has appeared elsewhere(2).

2. Valentine, J. D., Gurin, S. and Wilson, D. W., *Fed. Proc.*, 1949, v8, 262.

3. Sakami, W., Evans, W. E. and Gurin, S., *J. Am. Chem. Soc.*, 1947, v69, 1110.

4. Buchanan, J. M., Sonne, J. C., and Delluva, A. M., *J. Biol. Chem.*, 1948, v173, 81.

TABLE I. Radioactivity of Allantoin After Feeding Carboxyl Labeled Glycine.*

Days	Counts/min./mM of carbon
1	40
2	120
5	250
6	260
7	290
8	310
10	350

* 50 mg of glycine containing 10,700 counts/min./mM carbon fed daily.

TABLE II. Radioactivity of Biosynthetic Allantoin.

Individual carbon atoms of allantoinΔ	$NH_2CH_2C^{14}OOH$* C/min./mM C	$NH_2C^{14}H_2C^{14}OOH$† C/min./mM C
#2	0	15
#8	0	15
#4	410	85
#5	70	80

* See footnote to Table I.

† 18 mg of glycine containing 7900 c/m/mM of carbon fed daily for 5 days.

Δ The conventional numbering system of allantoin has been used. The individual samples were oxidized to CO_2 and analyzed in the form of $BaCO_3$. Measurements were made with a thin mica-window counter and the counts calculated for infinitely thick samples.

of the rat. Elwyn and Sprinson(5) have also obtained evidence that carboxyl-labeled acetate is not utilized by the pigeon for the synthesis of uric acid.

Carboxyl-labeled glycine. Table I shows the results obtained on allantoin isolated daily from the urine of 9 rats fed carboxyl-labeled glycine. Fifty mg of glycine (10,700 counts/min./mM of carbon) was fed daily to each animal over a 10-day period. A rapid incorporation of isotope into allantoin is apparent from the results obtained after the first day. Although there is a tendency to level off, the concentration of C^{14} in the allantoin was still increasing at the end of the experiment. In order to obtain sufficient compound for degradation, 40 mg of allantoin isolated from the second day's urine was combined with 42.5 mg of allantoin from the 5th day. This pooled specimen had a count of 170 counts/min./mM carbon. The degradation of the allantoin demonstrated that the carboxyl carbon of glycine was incorporated primarily into position 4 of allantoin (Table II). The radioactivity of carbon 5 is probably due to inherent small errors usually observed in the oxidation of glyoxylic acid semicarbazone(1).

Doubly-labeled glycine. The degradation of the allantoin from the urine of the rats fed 18 mg of doubly-labeled glycine daily for 5 days (total = 95,000 counts/min.) demonstrated that essentially equal amounts of isotope enter positions 4 and 5 of the allantoin molecule; carbon 4 originating from the carboxyl carbon and carbon 5 from the methylene carbon of glycine (Table II). The presence of isotope in small quantity in positions 2 and 8 is in agreement with the findings of Karlsson and Barker(6) who demonstrated the incorporation by pigeons of the methylene carbon of glycine into positions 2, 4, 5, and 8 of uric acid. This result is consistent with the evidence obtained by Sakami(7) that the methylene carbon of glycine is converted to "formate."

Summary. 1. A convenient method for the isolation of allantoin from the urine of rats has been developed. 2. The carbon of sodium bicarbonate is not incorporated in the urinary allantoin of rats. This result is in accord with the fact that bicarbonate is incorporated only into position 6 of purines. 3. The carboxyl carbon of acetate is not involved in the biosynthesis of allantoin in the rat. 4. The carboxyl carbon of glycine is incorporated into position 4 of allantoin and the methylene carbon into position 5.

5. Elwyn, D., and Sprinson, D. B., *J. Biol. Chem.*, 1949, v184, 465.

6. Karlsson, J. L., and Barker, H. A., *J. Biol. Chem.*, 1949, v177, 597.

7. Sakami, W., *J. Biol. Chem.*, 1949, v178, 579.

Received November 13, 1950. P.S.E.B.M., 1950, v75.