Previous work[7] demonstrated that following hepatectomy, no DNA is synthesized or labeled by orotic acid for the first 18 hours following the operation. An experiment was therefore designed in which homogenates were prepared from lobes removed at partial hepatectomy, as well as from liver allowed to regenerate 15 hours, and 24 hours and incubated in the presence of radioactive thymidine. The results presented in Table I show the incorporation observed in these preparations.

TABLE I

INCORPORATION OF THYMIDINE INTO DNA OF RAT LIVER HOMOGENATES

Each 50-ml. flask contained: 2 ml. of 25% rat liver homogenate; approximately 3×10^6 c.p.m. (3 micrograms) of thymidine[b]: 20 μM. each of K fumarate, K glutamate and K pyruvate; 5 μM. disodium ATP; 100 μM. nicotinamide; 12 μM. $MgSO_4$; 12 μM. K phosphate, *p*H 7.6; 10 μM. KCl; 20 μM. Tris:HCl buffer, *p*H 8; and 500 μM. of sucrose in a final volume of 2.55 ml.; incubation, for one hour with shaking, 38°, O_2 in the gas phase. Reaction was stopped by placing the flasks in an ice-bath. Sodium nucleates extracted and DNA purified as described previously.[7] A trace of radioactivity associated with RNA is attributed to slight degradation of radioactive DNA during the mild alkaline hydrolysis required to remove RNA.

Rat no.	Homogenate from	C.p.m./ plate (actual[a])	DNA[a] mg./ plate	Sp. act.[a] c.p.m./mg. DNA
1	Lobes removed at partial hepatectomy	350	0.35	1,000
2	Lobes removed at partial hepatectomy	270	.32	840
1	Liver 15 hours after operation	360	.30	1,200
2	Liver 24 hours after operation	12,300	.28	44,000
3	Liver 24 hours after operation	4,400	.26	16,900[c]

[a] Tritium was assayed in windowless flow counters. Since approximately the same amount of DNA was placed on each plate no correction has been made for self-absorption. Crude self absorption curves for tritium, using DNA as absorber, indicate a correction of about 2-fold could be applied to the DNA samples. [b] Colorimetric analysis using the Dische diphenylamine reagent. [c] This homogenate aged for 2 hours at 0° before incubation. All others incubated immediately after preparation.

It is apparent that a low level of incorporation is present in the zero hour and 15 hour regenerated preparations, and that the 24 hour regenerating liver shows about a 50-fold increase (Rat no. 2) in ability to incorporate thymidine into DNA. Thus the results with the homogenates are in agreement with previous studies with orotic acid *in vivo*[7] and in rat liver slices.[8]

It is our opionion that the marked incorporation seen in the 24-hour livers and the low incorporation in other samples argues against simple exchange reactions, since in all cases the same amount of tissue was incubated with the labeled precursor under identical conditions. The distribution of tritium in the isolated DNA was examined by hydrolysis with 98% formic acid for 2 hours at 165° and chromatography of the bases in 2-propanol: HCl on S. and S. no. 589 filter paper. The chromatogram showed only one radioactive spot, coincident with an ultraviolet quenching spot, with R_f and spectrum of thymine.[9]

(7) L. J. Hecht and V. R. Potter, *Cancer Res.*, **16**, 988 (1956).

(8) L. J. Hecht and V. R. Potter, *Fed. Proc.*, **15**, 271 (1956).

(9) *Cf.* G. R. Wyatt in E. Chargaff and J. N. Davidson, "The Nucleic Acids," Vol. I, Academic Press, New York, N. Y., 1955, pp. 243–265.

(10) Postdoctoral Fellow of the National Cancer Institute. This investigation was also supported by a grant (No. C-646) from the National Cancer Institute, National Institutes of Health, Public Health Service.

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FORMATION OF L-XYLULOSE FROM L-GULONOLACTONE IN RAT KIDNEY

L-Xylulose, a sugar excreted by patients with essential pentosuria, is present normally in urine of man,[1] guinea pig[1] and rat.[2] Conversion of D-glucuronolactone to L-xylulose occurs in man[1,3] and guinea pig.[1] L-Gulonic acid, which is formed from D-glucuronolactone in the rat and guinea pig,[4] has been postulated to be an intermediate in this reaction.[1] This communication reports the presence of an enzyme system in rat kidney which catalyzes the formation of L-xylulose from L-gulonolactone. It has been shown previously that L-gulonolactone is converted to L-ascorbic acid in rats.[5–7]

Carboxyl labeled and uniformly labeled L-gulonolactone[8] were incubated with the soluble fraction of rat kidney (Table I).[9] Carboxyl labeled L-gulonolactone yielded 30 to 60% of the added C^{14} in CO_2.[10] Uniformly labeled L-gulonolactone yielded about one-sixth the amount of C^{14} in CO_2.

TABLE I

DECARBOXYLATION OF L-GULONOLACTONE IN RAT KIDNEY

Expt.	% of added C^{14} found in CO_2[a] Carboxyl labeled L-gulonolactone	Uniformly labeled L-gulonolactone
1	30	4.9
2	51	7.8
3	..	4.6
4	..	7.6
5	60	..
6	35	..

[a] 2.0 mg. of either carboxyl labeled L-gulonolactone (0.05 μc./mg.) or uniformly labeled L-gulonolactone (0.20 μc./mg.) was incubated under air at 37° for 90 minutes in 6.0 ml. of a high speed supernate fraction (100,000 × g) equivalent to 600 mg. of tissue, *p*H 7.0, 0.1 *M* phosphate buffer. Cofactor additions were the same as used by Rabinowitz and Sall.[9] The methods used in preparation and assay of samples for C^{14} have been described.[5]

(1) O. Touster, R. M. Hutcheson and L. Rice, *J. Biol. Chem.*, **215**, 677 (1955).

(2) S. Futterman and J. H. Roe, *ibid.*, **215**, 257 (1955).

(3) M. Enklewitz and M. Lasker, *ibid.*, **110**, 443 (1935).

(4) J. J. Burns, THIS JOURNAL, **79**, 1257 (1957).

(5) J. J. Burns and C. Evans, *J. Biol. Chem.*, **223**, 897 (1956).

(6) M. ul Hassan and A. L. Lehninger, *ibid.*, **223**, 123 (1956).

(7) J. J. Burns, P. Peyser and A. Moltz, *Science*, **124**, 1148 (1956).

(8) The authors are grateful to Dr. Peter Dayton for carrying out the syntheses of these labeled compounds.

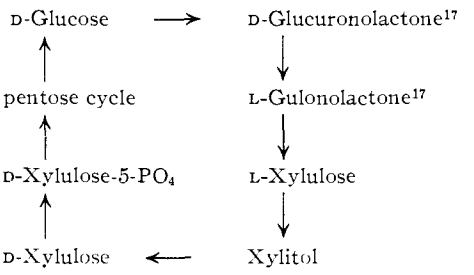
(9) This system is similar to that described for decarboxylation of D-glucuronolactone, J. L. Rabinowitz and T. Sall, *Biochim. Biophys. Acta*, **23**, 289 (1957).

(10) Parallel experiments with carboxyl labeled L-gulonic acid show that this compound is converted similarly to CO_2.

These results indicate that L-gulonolactone is decarboxylated in this system.

Evidence was found for the conversion of at least 15% of the uniformly labeled L-gulonolactone to labeled L-xylulose as follows:[11] 400 mg. of carrier L-xylulose[12] was added to the trichloroacetic acid extract of the incubation mixture and the solution was passed through an Amberlite IR-4B (acetate) column. L-Xylulose, in the effluent, was converted to L-ascorbic acid by the procedure described previously.[13] In brief, this method involved oxidizing L-xylulose to L-xylosone which was converted to imino-L-ascorbic acid by cyanide addition. After hydrolysis, the resulting L-ascorbic acid was purified by ion-exchange chromatography and converted to its 2,4-dinitrophenylosazone derivative which was recrystallized to constant specific activity.[5,14] This procedure does not distinguish between L-xylulose and L-xylose since both isomers are oxidized to L-xylosone. However, identification of the labeled pentose as L-xylulose was established and it was quantitatively determined by the cysteine-carbazole method[15] and a specific enzyme assay.[16]

Finding that L-gulonolactone is converted to L-xylulose suggests the following cyclic pathway for glucose metabolism in animals



The presence of enzyme systems in mammalian tissues for all these steps has been reported by others,[6,18–24] except the conversion of L-gulonolactone to L-xylulose. Evidence for occurrence *in vivo* of such an alternate pathway of glucose metabolism in animals will be presented elsewhere.

(11) A carrier dilution procedure[5] showed less than 0.2% of the added C^{14} present as L-ascorbic acid at the end of incubation.

(12) Carrier L-xylulose was kindly furnished by Dr. Gilbert Ashwell. In some experiments L-xylose was used as carrier instead of L-xylulose.

(13) L. L. Salomon, J. J. Burns and C. G. King, THIS JOURNAL, **74**, 5161 (1952).

(14) Repeated recrystallization of the 2,4-dinitrophenylosazone removes any labeled D-ascorbic acid osazone originating from D-xylulose. This was shown in control experiments with samples of osazone prepared from non-radioactive L-ascorbic acid which contained a trace amount of D-ascorbic acid-1-C^{14}; P. G. Dayton and J. J. Burns, to be published.

(15) Z. Dische and E. Borenfreund, *J. Biol. Chem.*, **192**, 583 (1951).

(16) L-Xylulose was determined by measuring the decrease in absorption of reduced triphosphopyridine nucleotide at 340 mμ in the presence of a purified xylitol dehydrogenase prepared from guinea pig liver. The authors are grateful to Dr. Gilbert Ashwell for carrying out the enzymatic and colorimetric assays for L-xylulose.

(17) It is not known at present whether the lactone or acid form of each compound is the intermediate in this scheme.

(18) J. L. Strominger, H. M. Kalckar, J. Axelrod and E. S. Maxwell, THIS JOURNAL, **76**, 6411 (1954).

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(23) B. L. Horecker, J. Hurwitz and P. Z. Smyrniotis, *ibid.*, **78**, 692 (1956).

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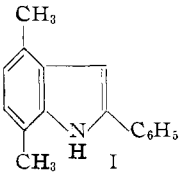
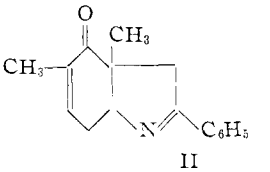
EVIDENCE FOR A HIGH ENERGY INTERMEDIATE IN THE FISCHER INDOLE SYNTHESIS. A NEW CLASS OF HYDROINDOLES

Sir:

When acetophenone 2,6-dimethylphenylhydrazone was heated at 130° in nitrobenzene solution with anhydrous zinc chloride for one hour, five products were isolated, among which 2-phenyl-4,7-dimethylindole (I), 4% yield, and 3a,4,7,7a-tetrahydro-2-phenyl-3a,5-dimethyl(3H)pseudoindolone-4 (II), 33% yield, were the most significant.



I



II

The structure of I, m.p. 65.5–66.5°, (calcd. for $C_{16}H_{15}N$: C, 86.84; H, 6.38; N, 6.33. Found: C, 86.69; H, 7.17; N, 6.24) was established by its independent synthesis from acetophenone 2,5-dimethylphenylhydrazone. The structure of II was proven by degrading it to 2-(β-phenylethyl)-2,6-dimethylcyclohexanone (VI), b.p. 143° (1–2 mm.), $n^{20}D$ 1.5205, (Calcd. for $C_{16}H_{22}O$: C, 83.43; H, 9.63. Found: C, 83.20; H, 9.66. Oxime, m.p. 115–116°, calcd. for $C_{16}H_{23}NO$: C, 78.32; H, 9.45; N, 5.71. Found: C, 78.51; H, 9.28; N, 5.64) which was synthesized by treating 2,6-dimethylcyclohexanone with sodamide and then with β-phenylethyl bromide. The infrared spectra of the two samples of VI were identical, and their oximes had the same m.p. and gave no mixed m.p. depression.

Crystalline II, m.p. 92–101°, was obtained as a hemihydrate (Calcd. for $C_{16}H_{17}NO \cdot {}^1/_2H_2O$: C, 77.39; H, 7.31; N, 5.64. Found: C, 77.46; H, 7.31; N, 5.68.), but the oxime, m.p. 230° (dec.), (Calcd. for $C_{16}H_{18}N_2O$: C, 75.56; H, 7.13; N, 11.02. Found: C, 75.42; H, 7.25; N, 11.20), picrate, m.p. 224° (dec.), (Calcd. for $C_{22}H_{20}N_4O_8$: C, 56.41; H, 4.30: N, 11.96. Found: C, 56.19; H, 4.02; N, 12.20.), and hydrochloride, m.p. 214° (dec.) (Calcd. for $C_{16}H_{17}NO \cdot HCl$: C, 69.68; H, 6.58; N, 5.08. Found: C, 69.74; H, 6.49; N, 5.16) all crystallized without bound water. The infrared spectrum ($CHCl_3$) of II featured bands at 2.72 μ (water), 6.02 μ (conj. C=O); 6.22 μ (conj. C=N, conj. phenyl) which split into two bands at