COMMUNICATIONS TO THE EDITOR

THE CLEAVAGE OF 3-TROPANYL CHLORIDE WITH POTASSIUM CYANIDE

Sir:

When 3-tropanyl chloride[1] (I) was treated with potassium cyanide in alcohol solution a sharp-boiling (b.p. 68–70°, 0.2 mm.) mixture of isomers was obtained whose analysis agreed with the formula $C_9H_{14}N_2$ (I); (*Anal.* Calcd. for $C_9H_{14}N_2$: N, 18.65, N_{AP},[2] 9.33. Found: N, 18.35; N_{AP},[2] 9.38).

The infrared spectrum[3] was characterized by the presence of two cyanide bands at 4.50 μ and an intense band at 6.08 μ. When this oil was treated with phenylmagnesium bromide a liquid (b.p. 69–70°, 0.1 mm.), presumably a mixture of isomers also, was obtained, the analysis of which was compatible with the formula $C_{14}H_{19}N$ (III); (*Anal.* Calcd. for $C_{14}H_{19}N$: C, 83.53; H, 9.51; N, 6.96. Found: C, 83.70; H, 9.81; N, 6.84). The infrared spectrum showed that bands due to the aromatic ring system and the 6.08 μ band were present but the absorption at 4.50 μ had disappeared. This displacement of a nitrile function by the radical of a Grignard reagent is manifested by α-dialkylaminoacetonitriles.[4] The band at 6.08 μ is indicative of a terminal vinyl group (confirmatory bands at 11.00 μ + 3.27 μ). Catalytic hydrogenation resulted in the uptake of one mole of hydrogen and the bases IV (b.p. 111–113°, 0.4 mm.). (*Anal.* Calcd. for $C_{14}H_{21}N$: N, 6.86. Found: N, 6.90) were isolated. The band at 6.08 μ had disappeared. Structures I–IV illustrate the course of the reactions. The mixtures which persisted throughout were due to the formation of *cis* and *trans* pyrrolidine nitriles in the first step.

The bases (IV) were converted to a mixture of methiodides (V), m.p. 126–130°, the most readily purified of which melted at 173–174° after crystallization from acetone (*Anal.* Calcd. for $C_{15}H_{20}IN$: I, 36.1. Found: I, 36.0). Hofmann degradation of the bases corresponding to V furnished an oil VI (b.p. 125–128°, 0.4 mm.). (*Anal.* Calcd. for $C_{15}H_{23}N$: N, 6.46. Found: N, 6.44) which showed typical styrene absorption, $\lambda_{max}^{95\%\ EtOH}$ 253 (log ϵ 4.07).

Reduction in the presence of Adams catalyst furnished 4-dimethylamino-1-phenylheptane, which was independently synthesized from 1-phenyl-4-heptanone via oximation, reduction and methylation. The picrates from both samples melted at 92.5–94° (*Anal.* Calcd. for $C_{21}H_{28}N_4O_7$: N, 12.49. Found: N, 12.23) and were otherwise identical in all respects. The 1-phenyl-4-heptanone (b.p. 96–98°; 0.1 mm.; *Anal.* Calcd. for $C_{13}H_{18}O$: C, 82.06; H, 9.53. Found: C, 82.07; H, 9.43) required for this synthesis was prepared from γ-phenylbutyronitrile and propylmagnesium iodide.

STERLING-WINTHROP RESEARCH INSTITUTE
RENSSELAER, NEW YORK
S. ARCHER
T. R. LEWIS
BERNARD ZENITZ

RECEIVED MAY 29, 1957

(1) M. Polonovski and M. Polonovski, *Bull. soc. chim.*, [IV] **45**, 305 (1929).

(2) Acetic–perchloric acid titration of basic nitrogen. Analyses were carried out under the supervision of Mr. K. D. Fleischer.

(3) We are indebted to Miss C. M. Martini for determination of the spectra reported here.

(4) P. Bruylants, *Bull. soc. chim. Belg.*, **33**, 467 (1924); *Chem. Abs.*, **19**, 288 (1925).

THYMIDINE INCORPORATION INTO DEOXYRIBONUCLEIC ACID OF RAT LIVER HOMOGENATES

Sir:

The incorporation of radioactive thymidine into DNA[1] of cell-free extracts of *E. coli* was reported in 1956.[2] Attempts to demonstrate thymidine incorporation in homogenates of chick embryo were unsuccessful,[3] although rabbit thymus nuclei have been reported to be active in this respect.[4] In normal or regenerating rat liver homogenates which actively incorporate radioactive orotic acid into RNA, no radioactivity was found in DNA.[5] The availability[6] of very high specific activity tritium labeled thymidine encouraged us to re-examine DNA synthesis in rat liver homogenates. Our experiments have met with some success, and will be described briefly herein.

(1) These abbreviations are used: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; ATP, adenosine triphosphate; and Tris, tris-(hydroxymethyl)-aminomethane.

(2) A. Kornberg, I. R. Lehman and E. S. Simms, *Federation Proc.*, **15**, 291 (1956).

(3) M. Friedkin, D. Tilson and D. Roberts, *J. Biol. Chem.*, **220**, 627 (1956).

(4) M. Friedkin and H. Wood, *ibid.*, **220**, 639 (1956).

(5) E. Herbert, V. R. Potter and L. I. Hecht, *ibid.*, **225**, 659 (1957).

(6) From Schwarz Laboratories, Inc., Mount Vernon, N. Y. Specific activity of the thymidine is reported to be 390 μc./μM., labeled in position 4; radiochemical purity 100%.

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.

McARDLE MEMORIAL LABORATORY
UNIVERSITY OF WISCONSIN
MADISON 6, WISCONSIN
F. J. BOLLUM[10]
VAN R. POTTER

RECEIVED MAY 24, 1957

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.