TABLE II

PHOSPHORYLATION COUPLED TO SPAN: BOH → CYTOCHROME *c*

Test system (1.0 ml) contained 0.01 *M* *dl*-BOH, 0.0024 *M* ADP, 0.005 *M* P_i (pH 6.5; $1 \cdot 10^6$ c.p.m. ^{32}P), 0.005 *M* KCN and enzyme (50 γ N). Ferricytochrome *c* titrated into medium; 5 minutes at 25°, data in mμmoles.

Components	$+ 1/2\ O_2$ / Fe^{++}	$-$ / P_i	*P : O*
1. Complete	48.2	46.5	0.96
Complete minus BOH	0.0	0.0	
Complete plus DNP	106	1.2	0.01
Complete plus 0.1 γ antimycin A	0.0	0.0	
2. Complete	36.2	32.6	0.90
Complete minus ADP	18.0	0.0	0.0
Complete minus Pi	17.5	0.0	0.0

TABLE III

PHOSPHORYLATION COUPLED TO OXIDATION OF FERROCYTOCHROME *c*

Test system (3.0 ml) contained 0.01 *M* P_i (pH 5.5; $1.3 \cdot 10^6$ c.p.m. ^{32}P), 0.01 *M* ascorbate, $4 \cdot 10^{-5}$ *M* cytochrome *c*, 0.0024 *M* ADP, and enzyme (250 γ N); 15 minutes at 23°. Data in μatoms.

Components	O_2 *uptake*	$-$ / P_i	*P : O*
1. Complete system	1.28	0.84	0.66
Complete minus ascorbate	0.00	0.00	
Complete plus DNP	1.42	0.00	0.00

The enzyme preparations have an average particle weight of about 50,000,000 as judged by sedimentation rate (equivalent to less than 1/2,000 of the "particle weight" of the rat liver mitochondrion). Considerable functional and steric organization is still retained in these particles; for instance, it has been found that added DPN and cytochrome *c* do not "mix" freely with the DPN and cytochrome *c* present in the particles.

These preparations have already revealed significant new information on oxidative phosphorylation not heretofore approachable with intact mitochondria; complete experimental details will be presented for publication shortly. This investigation was supported by grants from the U.S. Public Health Service and the Nutrition Foundation, Inc.

CECIL COOPER*
THOMAS M. DEVLIN**
ALBERT L. LEHNINGER

Department of Physiological Chemistry,
The Johns Hopkins University School of Medicine,
Baltimore 5, Maryland (U.S.A.)

1 A. L. LEHNINGER, *The Harvey Lectures*, 1953–1954, Academic Press, Inc., New York, 1955.
2 S. O. NIELSEN AND A. L. LEHNINGER, *J. Am. Chem. Soc.*, 76 (1954) 3860; *J. Biol. Chem.*, (in press).
3 B. BORGSTROM, H. C. SUDDUTH AND A. L. LEHNINGER, *J. Biol. Chem.*, (in press).
4 A. L. LEHNINGER, M. UL HASSAN AND H. C. SUDDUTH, *J. Biol. Chem.*, 210 (1954) 911.
5 I. RAW, *J. Am. Chem. Soc.*, 77 (1955) 503.
6 P. D. BOYER, A. B. FALCONE AND W. H. HARRISON, *Nature*, 174 (1954) 401.

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* Fellow of the U.S. Public Health Service.
** National Science Foundation Fellow.

Oxidative degradation of uric acid by cell extracts of a *Pseudomonas*

The aerobic degradation of uric acid by bacteria has been reported by LIEBERT[1], KREBS AND EGGLESTON[2], and DI FONZO[3]. While this work was in progress, FRANKE AND HAHN[4] reported that growing cells and washed cell suspensions of *Pseudomonas aeruginosa* oxidize uric acid to oxalic acid, ammonia and carbon dioxide. Allantoin, allantoic acid, glyoxylic acid and urea were identified as intermediates in the breakdown.

The present paper reports on the degradation of uric acid by an unidentified species of *Pseudomonas*, strain 2RCC-1, isolated from allantoin enrichment cultures[5]. Cell extracts of this organism convert uric acid to urea, carbon dioxide and water via allantoin, allantoic, glyoxylic and formic acids.

Cells of *Pseudomonas*, 2RCC-1, were grown in a medium containing uric acid, 0.3%; yeast extract, 0.1%; and K_2HPO_4, 0.1% in distilled water, pH 7.2. After 18 hours incubation at 30° C cells were removed by centrifugation, washed three times, ground with alumina[5] and extracted with 0.01 *M* phosphate buffer, pH 7.4. The supernatant obtained by high speed centrifugation of the extract contained the enzyme systems under study.

Manometric experiments revealed that 2 μM of oxygen were taken up and 3 μM of CO_2 produced per μM of uric acid utilized. Table I gives typical manometric data with uric acid and the various intermediates in the degradation.

TABLE I

MANOMETRIC DATA OF *Pseudomonas*, 2RCC-1, GROWN ON URIC ACID

Substrate	O_2 *uptake*	CO_2 *evolution*
	μM	μM
Uric acid	19.65	29.63
Allantoin	9.48	20.01
Allantoic acid	10.03	19.54
Glyoxylic acid	9.86	20.05
Formic acid	5.03	10.00

Reaction vessels contained 10 μM substrate, 50 mg of alumina extract in a total volume of 2.0 ml of 0.01 *M* phosphate buffer, pH 7.4. In O_2 uptake experiments, 0.2 ml of 10% KOH was in the center well. In CO_2 evolution experiments, 0.2 ml of 0.5 *N* HCl was tipped from a side arm at the end of the incubation period to release bound CO_2. The values have been corrected for endogenous activity. Reaction time 2 hours at 30° C with air as the gas phase.

The intermediates in uric acid degradation were identified as follows: Allantoin was identified by ascending and descending paper chromatography in phenol (saturated with an aqueous solution containing 6.3% sodium citrate and 3.7% KH_2PO_4), butanol-acetic-H_2O (80:20:20), butanol-ethanol-H_2O (80:20:20), and *iso*butyric acid-H_2O (80:20) according to BERRY *et al.*[6]. Allantoin was visualized as a green spot on spraying with phenol-hypochlorite (PHC)[6]. Allantoin from the reaction mixture, authentic allantoin and a mixture of the two gave R_F values of 0.44, 0.27, 0.23 and 0.36 in the respective solvents. Allantoin is distinguished from uric acid since the latter has an R_F value of 0.25 in phenol, 0.06 in butanol-ethanol and gives an orange color on spraying with PHC. Urea, allantoic, glyoxylic and formic acids were isolated and identified as described earlier[5].

The results of this and related studies[5] indicate that the degradation of uric acid by *Pseudomonas*, 2RCC-1, occurs according to the following reaction sequence:

$$\text{uric acid} \xrightarrow{O_2} \text{allantoin} + CO_2$$
$$\text{allantoin} \xrightarrow{+H_2O} \text{allantoic acid}$$
$$\text{allantoic acid} \xrightarrow{+H_2O} \text{glyoxylic acid} + 2\ \text{urea}$$
$$\text{glyoxylic acid} \xrightarrow{\frac{1}{2}O_2} \text{formic acid} + CO_2$$
$$\text{formic acid} \xrightarrow{\frac{1}{2}O_2} CO_2 + H_2O$$

It appears that the pathway of degradation by this organism and *Ps. aeruginosa* are identical up to glyoxylic acid and urea. *Ps. aeruginosa* oxidizes glyoxylic acid to oxalic acid and hydrolyzes urea to ammonia and carbon dioxide. *Pseudomonas*, 2RCC-1, on the other hand, oxidizes glyoxylic acid to carbon dioxide and water via formic acid and does not hydrolyze urea.

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L. LEON CAMPBELL, Jr.

Laboratory of Food Microbiology, Department of Horticulture, State College of Washington, Pullman, Washington (U.S.A.)

1 F. LIEBERT, *Proc. Koninkl. Akad. Wetenschap. Amsterdam*, 12 (1909) 54.
2 H. A. KREBS AND L. V. EGGLESTON, *Enzymologia*, 7 (1939) 310.
3 M. DI FONZO, *Am. Rev. Tuberc.*, 66 (1952) 240.
4 W. FRANKE AND G. E. HAHN, *Z. Physiol. Chem.*, 299 (1955) 15.
5 L. L. CAMPBELL, Jr., *J. Bacteriol.*, 68 (1954) 598.
6 H. K. BERRY, H. E. SUTTON, L. CAIN AND J. S. BERRY, *Univ. Texas Publication*, 5109 (1951) 22.

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