TABLE I

PROPERTIES OF HYDROLYSATES OF DNP-PEPTIDES

RF value of spot	*Products of hydrolysis of DPN peptide*	
	Ether extract	*Aqueous residue*
0.40	DNP-glycine	Glycine (?) valine (xx)
0.45	DNP-valine	Glycine (x) valine (?)

REFERENCES

1 F. SANGER, *Advances in Prot. Chem.*, 7 (1952) 1.
2 P. A. LEVENE, P. E. STEIGER, AND A. ROTHEN, *J. Biol. Chem.*, 97 (1932) 717.
3 R. L. M. SYNGE, *Biochem. J.*, 39 (1945) 351.
4 E. ABDERHALDEN AND E. KOMM, *Z. Physiol. Chem.*, 139 (1924) 147.
5 F. SANGER AND H. TUPPY, *Biochem. J.*, 49 (1951) 463.
6 S. BLACKBURN AND A. G. LOWTHER, *Biochem. J.*, 48 (1951) 126.
7 F. SANGER, *Biochem. J.*, 45 (1949) 563.

Received June 19th, 1952

6-HYDROXY NICOTINIC ACID AS AN INTERMEDIATE IN THE OXIDATION OF NICOTINIC ACID BY *PSEUDOMONAS FLUORESCENS*

by

D. E. HUGHES

Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, The University, Sheffield (England)

The ability of many strains of *Pseudomonas fluorescens* to oxidise nicotinic acid was first reported by ALLINSON[1]. Later studies[2,3,4] indicated that the oxidative system is adaptive and that CO_2 and ammonia are among the end products. There were however no indications of the intermediate steps of the oxidation. The following experiments show that 6-hydroxy nicotinic acid is an intermediate.

Five strains of *Pseudomonas fluorescens* isolated from soil were grown on a medium containing salts, 2% yeast extract and 0.1% nicotinic acid. The cells were washed and the oxidation of the nicotinic acid was followed manometrically in the Warburg apparatus. All strains oxidised both nicotinic acid and 5-fluoro nicotinic acid (cf. HUGHES[5]) at approximately the same rate (0.5–1.0 μmol nicotinic acid/mg dry wt./hr), and to the same extent (3.5–3.9 μmol O_2/μmol nicotinic acid), but none of the strains oxidised 6-fluoro nicotinic acid under the conditions tested. Variable results were obtained with 2-fluoro nicotinic acid but some batches of all the strains were able to oxidise the 2-fluoro-analogue with an uptake of 0.5 to 1.5 μmol O_2/μmol 2-fluoro nicotinic acid. These results suggested that the carbon atom six was important in the primary oxidative attack. Search for intermediates in the oxidation of the nicotinic acids in adapted cells was unsuccessful. Experiments were therefore carried out with cells not previously grown on nicotinic acid and the products of the oxidation examined as the cells became adapted. The non-adapted cells were grown on a medium containing 0.1% asparagine in the place of the nicotinic acid previously used. The cells were washed and placed in the Warburg apparatus suspended in 0.2 *M*-phosphate buffer, pH 6.5; 0.01 *M*-NH_4Cl and 0.025 *M*-nicotinic acid. No extra oxygen uptake over the endogenous respiration was found until 90–120 min had elapsed, after which the rate of oxygen uptake increased slowly until 0.5–0.8 μmol of extra O_2/μmol nicotinic acid had been taken up; then the rate of additional O_2 uptake increased until all the nicotinic acid was used. The final oxygen uptake varied between 3.8 and 4.0 μmol O_2/μmol nicotinic acid. During the initial period of increased oxygen uptake the presence of an intermediate was detected by paper chromatographic methods. This intermediate did not react with CNBr and *p*-aminobenzoic acid[6] but had an absorption curve between 215 and 300 mμ, typical of pyridine derivatives. This suggested that during the first slow extra oxygen uptake the pyridine ring was modified by substitution. From the results with the fluoro analogues it seemed likely that the substitution was in the six position and arguing by analogy to the oxidation of the benzene ring[7] it seemed possible that a hydroxylation occurred. Synthetic 6-hydroxy nicotinic acid was

therefore compared with the material from the bacterial oxidation and the two were found to give identical chromatograms and to have similar absorption curves. In addition nicotinic acid-adapted cells were found to oxidise 6-hydroxy nicotinic acid without any lag period but did not oxidise 2-hydroxy nicotinic acid. This "simultaneous adaptation"[8] supports the view that the 6-hydroxy nicotinic acid is an intermediate in the oxidation of nicotinic acid. In a large scale adaptation experiment (200 ml washed cell suspension) the reaction was stopped when the mother liquor was found to contain the maximum amount of the intermediate product. The intermediate was extracted from the mother liquor into n-butanol, recrystallised from water and found to be identical with authentic 6-hydroxy nicotinic acid (m.p. = 304°, decomposing with evolution of gas at 314°; $\varepsilon^{max}_{260\,m\mu}$ in N HCl $= 1.2 \cdot 10^4 \pm 3\%$). It may thus be concluded that 6-hydroxy nicotinic acid is formed from nicotinic acid as a step prior to the opening of the pyridine ring.

REFERENCES

[1] M. J. C. ALLINSON, *J. biol. Chem.*, 147 (1943) 785.
[2] S. A. KOSER AND G. R. BAIRD, *J. infect Dis.*, 75 (1944) 250.
[3] C. H. NICHOL AND M. MICHAELIS, *Proc. Soc. Exp. Biol. Med.*, 66 (1947) 70.
[4] W. B. JAKOBY, A. SCHATZ, S. H. HUTNER, AND M. M. WEBER, *J. Gen. Microbiol.*, 6 (1952) 278.
[5] D. E. HUGHES, *J. Biochem.*, 51 (1952) XXII.
[6] E. KODICEK AND K. K. REDDI, *Nature*, 168 (1951) 475.
[7] F. C. HAPPOLD, *Biochemical Society Symposia*, No. 5 (1950) 85.
[8] R. Y. STANIER, *Bact. Rev.*, 14 (1950) 179.

Received July 3rd, 1952

REVUE DES LIVRES

Mikroskopische und chemische Organisation der Zelle. 2. Colloquium der Deutschen Gesellschaft für Physiologische Chemie. Springer-Verlag, Berlin, Göttingen, Heidelberg, 1952, 102 pp.

Ce petit livre résume les travaux d'un Colloquium de la Société allemande de Chimie physiologique, qui s'est tenu à Mosbach en avril 1951; on y trouvera un excellent résumé des connaissances actuelles sur la structure et la biochimie des constituants cellulaires.

L'ouvrage commence par une intéressante étude de la morphologie cellulaire à l'échelle microscopique et submicroscopique, due à F. E. LEHMANN; l'auteur y tient largement compte des données récentes fournies par les méthodes cytochimiques et par la microscopie électronique.

L'article suivant, rédigé par K. LANG, porte sur la composition chimique et l'activité enzymatique des constituants cellulaires isolés (noyaux, mitochondries et microsomes). Les résultats, en ce qui concerne l'activité enzymatique des noyaux, proviennent souvent de recherches personnelles de K. LANG; ils ont été obtenus sur des noyaux isolés dans une solution de saccharose et ils diffèrent à maints égards de ceux publiés depuis par MIRSKY *et al.* (*J. gen. Physiol.*, 35 (1952) 559) qui ont opéré en milieu non aqueux. Il nous semble que certaines des conclusions tirées par LANG, notamment sur la présence dans les noyaux d'enzymes qui seraient inactifs en raison de l'absence de co-enzymes, devraient être revues à la lumière des résultats de MIRSKY; l'auteur nous semble aussi se montrer trop catégorique au sujet du rôle, primordial à son avis, du noyau dans la synthèse des protéines.

On doit à K. FELIX une intéressante mise au point sur les nucléoprotéides et les nucléoprotamines; il en résulte que les noyaux des spermatozoïdes de Poissons seraient dépourvus de protéines autres que les protamines; les enzymes y feraient sans doute défaut. Les protamines des spermatozoïdes de diverses espèces présenteraient des différences notables dans leur composition, d'autant plus marquées que ces espèces sont zoologiquement plus éloignées.

Après une bonne revue d'ensemble sur la structure macromoléculaire des acides nucléiques par G. SCHRAMM, G. PIEKARSKI discute la question des équivalents du noyau (nucléoïdes, caryoïdes) chez les Bactéries et les Cyanophycées, en se basant sur la localisation intracellulaire des deux types d'acides nucléiques chez les micro-organismes.

Chaque exposé est suivi d'une discussion animée, souvent intéressante. Tout au plus peut-on reprocher à cet ouvrage, par ailleurs hautement recommendable, que les noms des auteurs cités soient souvent orthographiés de façon incorrecte (par exemple, SCHICKELMANN pour SPIEGELMAN, MONET pour MONNÉ, D. DUNE pour DE DUVE, BERTHLET ou BERTHLETT pour BERTHET, etc.).

J. BRACHET (Bruxelles)