A Bacteriocinogenic Type 1 Strain of Klebsiella pneumoniae

Production of bacteriocins is a property found in many groups of bacteria (Hamon and Péron¹). Hamon² found that colicinogeny could be transferred from Escherichia coli to Klebsiella pneumoniae, and more recently (Hamon and Péron¹) reported that many strains of Klebsiella and Aerobacter aerogenes produce bacteriocins active on other such strains, although not upon the Escherichia coli strains used as colicin indicators.

We have investigated the nature of the inhibitory agent released by *K. pneumoniae* strain 1.2. This strain is of capsular serotype 1(A) and was kindly provided by Professor J. P. Duguid. It is derived directly via serial subcultures from N.C.T.C. strain 5054. Ciucă et al.³ reported that their strain 52144, also derived originally from N.C.T.C. 5054 was lysogenic for UV-inducible bacteriophage active on *K. pneumoniae* strain B7380.

Following the method of FREDERICQ⁴ strain 1.2 was grown for two days as stab inocula in nutrient agar plates. The surface of the plates was then sterilized by brief exposure to chloroform vapour, and a soft nutrient agar overlay seeded with potential bacterial indicator strains was added. After overnight incubation the plates were examined for zones of inhibition centered on the underlying stabs of strain 1.2. Strain 1.2 was found by this, and by other methods also, to produce inhibitory activity against 12 of 47 other Klebsiella strains, including some non-capsulate strains. No inhibition of colicin indicator strains *E. coli* K-12, *E. coli* B-1 and *Shigella dysenteriae* was observed.

Attempts were made to propagate the inhibitory agent(s) released by strain 1.2. Cores of agar were cut from zones of inhibition on test plates and shaken in small volumes of broth. These broth eluates and serial dilutions were sterilized by filtration, by chloroform

treatment, or by streptomycin and spot tested onto suitable indicator strains. In all cases there was a gradual diminution of inhibitory activity and in no case were there observed discrete plaques indicative of the presence of bacteriophage. Similar tests were performed with aged broth cultures and with UV-irradiated cells grown in broth. In the latter case a considerable increase in the titer of inhibitory activity was observed but again no phage plaques were visible. Attempts to concentrate the inhibitory activity by high-speed centrifugation gave negative results. Treatment with heat (70°C, 10 min), with α -chymotrypsin, or with trypsin resulted in destruction of activity.

We conclude that *K. pneumoniae*, strain 1.2, releases an UV-inducible bacteriocin, or bacteriocins, active on other Klebsiella strains.

Résumé. La souche 1.2 (N.C.T.C. 5054) de Klebsiella pneumoniae est bactériocinogène. Sur 47 souches de Klebsiella étudiées 25% se sont révélées sensibles à cette bactériocine.

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- ¹ Y. Hamon and Y. Péron, C. R. Acad. Sci. (Paris) 257, 309 (1963).
- ² Y. Hamon, C.R. Acad. Sci. (Paris) 242, 2064 (1956).
- ³ M. CIUCĂ, S. STAMATESCO-EUSTATZIOU, C. BARBER, M. VOINEA, and G. TULPAN, Arch. Roum. Pathol. exp. Microbiol. 18, 347 (1959).
- ⁴ P. Frederico, Ann. Rev. Microbiol. 11, 7 (1957).
- We are grateful to Professor J. P. Duguid, Drs. B. H. Park, I. Ørskov, and Y. Hamon for strains and information.

Biosynthesis of Alkaloids. On the Transformation of Tyrosine to 3,4-Dihydroxyphenylalanine in *Papaver somniferum* L. Plants

The aromatic amino acids, tyrosine and 3,4-dihydroxyphenylalanine, or their biochemical equivalents, are in general considered as alkaloid precursors in *Papaver somniferum* plants¹⁻⁴. Phenylalanine was found to be incorporated into the morphine structure to a much lesser degree⁵. The mechanism of participation of this amino acid in biosynthetic reactions leading to opium alkaloids is still ambiguous. Using tissue homogenates and infiltration technique, we did not succeed in demonstrating the hydroxylation of phenylalanine to tyrosine⁶.

Phenylalanine and tyrosine occur in some organs of the plant only in traces, 3,4-dihydroxyphenylalanine failed to be detected at all. The presence of biochemical equivalents of these substances, phenylpyruvic and phydroxypyruvic acid at certain stages of plant development was proved.

In previous experiments, we succeeded in demonstrating polyphenolase activity in *P. somniterum* plants, which might be in relation to oxidative and oxidative-coupling reactions involved in biosynthetical mecha-

nisms. We were therefore interested in investigating the individual reaction steps, first of all the transformation of tyrosine to 3,4-dihydroxyphenylalanine, which failed to be detected in poppy plants, although dopamine was demonstrated as a significant precursor in radioisotopic feeding experiments ¹⁰.

We have carried out experiments in vitro with acetone powders of roots collected in the vegetation phase of

- ¹ К. Мотнеs and H. R. Schütte, Angew. Chem. 75, 265 and 357 (1963).
- ² A. R. Battersby, Proc. chem. Soc. (London) 1963, 189.
- ³ D. H. R. Barton, Proc. chem. Soc. (London) 1963, 293.
- ⁴ A. Jindra, Chem. Listy 57, 230 (1963).
- ⁵ A. R. BATTERSBY, R. BINKS, and B. J. T. HARPER, J. chem. Soc. 1962, 3534. E. LEETE, J. Am. chem. Soc. 81, 3848 (1959); Chem. and Ind. 1958, 977.
- ⁶ P. Kovács and A. Jindra, unpublished observations.
- ⁷ G. Kleinschmidt, Planta med. 8, 114 (1960).
- 8 A. JINDRA, Z. ŠÍPAL, and V. HUDECOVÁ, Exper. 20, 371 (1964).
- P. Kovács, M. Pšenák, and A. Jindra, Herba hung. 2, 147 (1963); Čsl. Farmácia 13, 179 (1964).
- 10 E. LEETE and S. J. B. MURRILL, Tetrahedron Letters 1964, 147.