A Bacteriocinogenic Type 1 Strain of *Klebsiella pneumoniae*

Production of bacteriocins is a property found in many groups of bacteria (HAMON and PÉRON[1]). HAMON[2] found that colicinogeny could be transferred from *Escherichia coli* to *Klebsiella pneumoniae*, and more recently (HAMON and PÉRON[1]) 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 FREDERICQ[4] 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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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* plants[1–4]. Phenylalanine was found to be incorporated into the morphine structure to a much lesser degree[5]. 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 tyrosine[6].

Phenylalanine and tyrosine occur in some organs of the plant only in traces, 3,4-dihydroxyphenylalanine failed to be detected at all[7]. The presence of biochemical equivalents of these substances, phenylpyruvic and *p*-hydroxypyruvic acid at certain stages of plant development was proved[8].

In previous experiments[9], we succeeded in demonstrating polyphenolase activity in *P. somniferum* plants, which might be in relation to oxidative and oxidative-coupling reactions involved in biosynthetical mechanisms. 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

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P. somniferum L. var. 'Váhovecký' plants specified[11] as II_3 (the phase of developed leaves). The acetone powder prepared in the usual way[12] was dissolved in a buffer solution (TRIS-HCl, pH 7.05); a 2.4% preparation resulted, which was set free from endogenous amino acids by dialysis at 0°C against the same buffer solution. The transformation of tyrosine in $7.8 \cdot 10^{-3} M$ concentration was followed in the reaction mixture (incubation at 30°C, 20, 40, and 60 min respectively) by photometric determination of the disappearance of this amino acid (9.0, 20.0, and 31.0% respectively) at given conditions (after paper chromatographic separation using the method described[13]). At the same time the formation of 3,4-dihydroxyphenylalanine was analysed by ascending and descending paper chromatography in different solvent systems (*n*-butanol-CH_3COOH-H_2O [4:1:5 or 78:5:17] and *n*-propanol-H_2O [3:1]); detection with ninhydrin solution[13] or a mixture composed of equal parts of a 15% (w/v) solution of $FeCl_3$ and 1% (w/v) solution of $K_3Fe(CN)_6$[14]. As the presence of L-ascorbic acid stimulates the oxidative metabolism of tyrosine[15], we also studied the formation of 3,4-dihydroxyphenylalanine after addition of this substance in $1.8 \cdot 10^{-2}$ and $3.6 \cdot 10^{-2} M$ concentration. The identification of 3,4-dihydroxyphenylalanine was confirmed by rechromatographing the eluted substance with a standard preparation of 3,4-dihydroxyphenylalanine (Calbiochem Inc.)[16]. Other ninhydrin-positive substances were detected, especially in experiments with increased amounts of L-ascorbic acid. These substances could not be identified; nevertheless, some of them probably correspond to previous data[15].

We succeeded in demonstrating the enzymic transformation of tyrosine to 3,4-dihydroxyphenylalanine, both in presence and in absence of L-ascorbic acid. The process is of enzymic character, since heat-denaturated preparations were absolutely ineffective: the amounts of exogenous tyrosine did not decrease and the presence of 3,4-dihydroxyphenylalanine was not detected.

Zusammenfassung. In Wurzelpräparaten von *Papaver somniferum* L. wurde die enzymatische Umwandlung von Tyrosin in Dioxyphenylalanin bei Anwesenheit oder Abwesenheit von L-Ascorbinsäure nachgewiesen.

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Kinetik des Malondialdehydes im Organismus

Malondialdehyd (MDA), ein Produkt der oxydativen Degradation der Polyenfettsäuren, entsteht aus Dimethinmethanradikalen, als Enolform von β-Hydroxyakrolein. Dieser Bestandteil aus der Lipoxydationsspaltung wurde bisher praktisch nur analytisch ausgenützt, da MDA mit Hilfe kolorimetrischer Reaktion mit 2-Thiobarbitursäure (Methinfarbstoff) günstig festgestellt werden kann[1]. Die Reaktion kam bei technologischen Fettanalysen wiederholt zur Anwendung[2], ebenfalls bei Lipoperoxydationsuntersuchungen an biologischem Material[3]. Aldehyde sind zur Oxydation MDA auch in künstlichen Systemen im Organismus leicht oxydierbar, z.B. MDA + Fe^{2+} + Cystein[4].

Die Oxydationsprodukte des MDA – nicht aber Malonsemialdehyd und Malonsäure – geben keine Thiobarbitursäurereaktion, was bei Untersuchungen der Lipoperoxydationsintensität zu negativen Ergebnissen führen kann. Ausserdem kommt man zur Annahme, dass MDA als leicht reagierende Substanz eine gewisse Rolle im Stoffwechsel spielt: Bildung des Malonylcoenzyms A, welches in der Synthese der Fettsäuren einen wichtigen Platz einnimmt[5].

Darum verfolgten wir, nach Verabreichung von MDA in bestimmten Zeitabschnitten, die Konzentration des MDA in verschiedenen Organen, im Blutserum und ebenso seine Ausscheidung im Harn. Folgende Fragen sollten geklärt werden: (1) Wird MDA metabolisiert? Wenn ja, mit welcher Intensität in vivo? (2) Beeinflusst die Oxydation des MDA die Ergebnisse der Lipoperoxydation im Organismus?

MDA wurde mit vorsichtiger Hydrolyse aus 1,1,3,3-Tetraäthoxypropan gewonnen. Die Versuche wurden an 48 erwachsenen männlichen Albinoratten von 200–220 g Gewicht vorgenommen. Es wurden 6 mg MDA in 2 ml physiologischer Lösung i.p. verabreicht. MDA wurde im Homogenat der Organe oder direkt im Serum und Harn nach Enteiweissung (0,5 ml Serum, Harn oder Homogenat (25%) + 4,5 ml Phosphat-Puffer pH 5,9 + 2,0 ml 7% Perchlorsäure, vom Filtrat 4,0 ml + 2,0 ml Thiobarbitsäure 0,8%; 100°, 10 min photometrisch bei 530 mμ) gemessen. Die Kontrolltiere (6 Ratten) erhielten 6 mg in 2 ml i.p. Antipyrin/1-Phenyl-2,3-dimethylpyrazolon (Bestimmung siehe[6]); eine Substanz, die im Körper praktisch nicht metabolisiert wird.

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