tures have a helical configuration. Since the side chains are fully extended after spreading on a cytochrome c monolayer, which at pH 8.6 must be positively charged, it is likely that the side chains represent polyanionic molecules[10,11].

Electron microscopical observations on polysaccharide or protein-polysaccharide complexes are very scanty. For instance, it has been shown that hyaluronic acid prepared from human synovial fluid represents a linear molecule without branching[10]. However, uranyl-stained protein-polysaccharide complexes prepared from bovine nasal cartilage have been found to consist of a protein core filament with regularly arranged side chains representing the chondroitin sulfate molecules[11]. Although the structures present in M-DNA preparations show the same type of configuration, they differ from the molecules obtained from cartilage by the greater length of the side chains (3×), and the size-distribution of the central filaments. In our material there is a continous distribution in length, whereas the macromolecules of nasal cartilage fall within two distinct size classes, presumably representing monomers and dimers respectively.

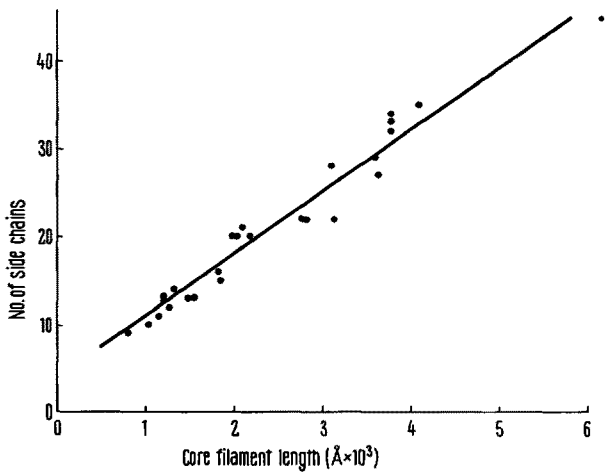

Fig. 2. Correlation between the length of central filaments and the number of side chains. The data are based on measurements of 26 well defined structures. The electron micrographs were taken at a magnification of 30,000×. The structures were enlarged to a final magnification of 635,000×, traced on paper and the length determined by tracing with a map ruler.

Owing to the scarcity of this material in our preparations, chemical identification of these molecules is still pending. Nevertheless, the regular occurrence of such molecules in DNA extracts of highly purified mitochondrial preparations suggests that protein-polysaccharide complexes (proteoglycans) may represent a genuine component of mitochondria. This view is also supported by the finding that isolated mitochondria of rat liver are capable of glycoprotein synthesis[15,16] and the recent identification of the glycoproteins as components of mitochondrial structural proteins[17]. Experiments designed to localize such molecules within structural components of mitochondria are now in progress.

Zusammenfassung. In hochgereinigten Präparaten von M-DNS aus Mausleber, die mittels Phenol extrahiert und auf einem Proteinfilm gespreitet wurde, konnten proteoglykanartige Makromoleküle mit einer Häufigkeit von 0,5% nachgewiesen werden. Diese bestehen aus einem Zentralfilament variabler Länge (790–6220 Å) und Seitenketten konstanter Länge (1574 ± 135 Å), deren Zahl mit der Länge der Zentralfilamente korreliert ist.

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The Metabolism of Biphenyl by *Pseudomonas putida*[1]

In a previous report on the microbial decontamination of waste waters, containing aromatic hydrocarbons used as nuclear reactor coolants, we have isolated, by enrichment cultures, a strain of *Pseudomonas putida* able to grow on biphenyl as sole carbon and energy source[2]. In the present work research on the degradation of biphenyl by *Ps. putida* is reported.

Materials and methods. The cells were grown and harvested and the cell-free extract was obtained as previously described for *Ps. desmolyticum*[3]. Proteins were determined by the method of Warburg and Christian[4]. The spectrophotometric determinations were carried out in a Zeiss P.M.Q. II. A model 137 Infracord spectrophotometer (Perkin-Elmer Ltd.) was used to obtain IR-absorption spectra. Mass spectra were run by a LKB 9000 spectrograph. TLC and GLC were performed as described in the experimental results. Biphenyl was supplied by Merck; 2-, 3- and 4-hydroxybiphenyls by BDH, K and K Laboratories and Eastman, respectively; 3,4-dihydroxybiphenyl by Eastman; benzoic acid by BDH. Each product was further purified by crystallization.

Results. 14–16 h cultures of *Ps. putida* incubated with biphenyl showed positive test for diols[5]. When the diol concentration in 20 l glass jars cultures was at a maximum, the culture fluid was collected and made alkaline (pH 8) with aqueous potassium carbonate. Inorganic phosphate precipitate, residual biphenyl and cells were removed by filtration and 3 extractions were performed with ethyl ether; the solvent was dried over anhydrous magnesium sulphate and evaporated in the cold under reduced pres-

sure. The crude residue (20 mg) was dissolved in a few ml of 0.1 *N* NaOH solution and re-extracted with ether. The ethereal extract was evaporated to dryness and the crude diol was refluxed with HCl in aqueous methanol (methanol: water: conc. HCl = 5:5:1) for 2 h. The solution was extracted several times with petroleum ether (b.p. 30°–50°C), washed with 5% sodium bicarbonate, dried over anhydrous magnesium sulphate and evaporated. A portion of this oily residue was redissolved in water, the solution adjusted to pH 10 and treated with 2,6-dichloroquinonechloroimide and *n*-butanol. The organic layer showed λ_{max} 655 nm. In the same conditions authentic 2-, 3- and 4-hydroxybiphenyls showed λ_{max} (in *n*-butanol) 655, 673, 662 nm, respectively. 2-, 3- and 4-hydroxybiphenyls were run on a thin silica-gel layer, activated at 100°C for $^1/_2$ h. Petroleum ether (b.p. 30°–50°C) and ethyl ether 1:1 were used as developing solvent. The spots, detected by spraying with 0.5% potassium permanganate solution, showed Rf 0.52, 0.33, 0.40, respectively. TLC of the phenolic mixture obtained by dehydration of the crude diol gave two spots: the larger one showing Rf 0.52, the other very small with Rf 0.33. Gas-chromatography of 2-, 3- and 4-hydroxybiphenyls was performed by a Carlo Erba GT mod. 200 Gas-chromatograph, with a flame ionization detector. Column: stainless steel, 2 m × 2mm I.D., packed with 10% SE on Chromosorb W (60-80 mesh). Temperatures: column 210°C, detector 230°C, injector 250°C. Carrier gas nitrogen at 1 atm (40 ml/min). Hydrogen at 0.8 atm and air at 1.4 atm. In these conditions the peaks of 2-, 3- and 4-hydroxybiphenyls showed retention times of 3.75, 4.40, 4.45 min, respectively. Gas-chromatography of the residue from the acidic treatment of biphenyl diol gave two peaks at retention times 3.75 and 4.40 min, relative amounts 95:5. The mass spectrum of the first compound (m/e = 170) was wholly superimposable upon that of an authentic sample of 2-hydroxybiphenyl. The spectrum of the second component of the mixture (m/e = 170) could not be compared with the spectra of 3- and 4-hydroxybiphenyls owing to the poor amount relative to the bleeding of the column.

In other experiments the mother liquors from the extraction were acidified to pH 3 and re-extracted with ethyl ether. The crude oily residue (70 mg) was heated with a few ml of petroleum ether (b.p. 30°–50°C), the liquid phase decanted and the solvent evaporated. White crystals were obtained after repeated crystallization from *n*-pentane (m.p. 120°C, undepressed by admixture with authentic benzoic acid). Elemental analysis was as follows: for $C_7H_6O_2$ calc. 68.80% C; 4.92% H; found 69.14% C; 4.95% H. IR, UV and mass spectra were identical with those of benzoic acid.

In Warburg experiments, cells of *Ps. putida* grown on biphenyl were simultaneously induced to oxidize biphenyl and benzoic acid, not 3,4-dihydroxybiphenyl and phenylpiruvic acid. Only the cells grown on phenylpiruvic acid were simultaneously induced to oxidize this compound. Catechol is oxidized by *meta*-cleavage with an enzymatic system that is constitutively present.

Conclusions. Like other aromatic hydrocarbons, biphenyl undergoes oxygenation to a diol. The diol isolated from biphenyl cultures of *Ps. putida* is shown to be 2,3-dihydro-2,3-dihydroxybiphenyl. In effect the dehydration product of this diol was identified as 2-hydroxybiphenyl. A small amount was also found of a compound with the same TLC and GLC features as 3-hydroxybiphenyl. The further degradation of the diol leads to the production of benzoic acid through a series of unidentified compounds.

Recently LUNT and EVANS[6] found that a gram-negative bacterium oxidized biphenyl to phenylpiruvic acid with the intermediary formation of 2,3-dihydroxybiphenyl and α-hydroxy-β-phenylmuconic semialdehyde. The metabolism of biphenyl by *Ps. putida* is different after the intermediary production of 2,3-diol as we isolated benzoic acid from the cultures and showed that the cells grown on biphenyl are simultaneously induced to oxidize benzoic acid in Warburg experiments, not phenylpiruvic acid. Only the cells grown on phenylpiruvic acid are simultaneously induced to oxidize this compound.

Research is in progress in order to clarify the metabolic pathway from the diol to benzoic acid.

Riassunto. La degradazione del bifenile da parte di *Ps. putida* inizia con la formazione di un diolo cui si attribuisce la struttura di 2,3-diidro-2,3-diossibifenile, in quanto, per disidratazione a caldo con acido cloridrico, si trasforma in 2-ossibifenile e tracce di 3-ossibifenile. Il diolo viene ulteriormente degradato ad acido benzoico. Le cellule di *Ps. putida* cresciute su bifenile sono simultaneamente indotte ad ossidare al Warburg il bifenile e l'acido benzoico, non il 3,4-diossibifenile e l'acido fenilpiruvico. Le stesse cellule contengono una metapirocatecasi costitutiva.

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Zum phototaktischen Laufverhalten junger Honigbienen (*Apis mellifica* L.)

Bevor Stockbienen zum Sammeldienst übergehen, machen sie mehrere Orientierungsflüge. Sie beginnen damit etwa am 5. Lebenstag (RÖSCH[1], SAKAGAMI[2]). Eigene Beobachtungen und Experimente zeigen, dass junge Stockbienen vor ihrem 1. Orientierungsflug ebenso wie ausfliegende Sammelbienen (JACOBS-JESSEN[3]) positiv phototaktisch gestimmt sind.

Phototaktische Laufversuche sollen zeigen, ob und inwieweit sich in den ersten Lebenstagen adulter Bienen die Reaktion gegenüber geringen Lichtintensitäten ändert. Bienen des 1.–6. Lebenstages wurden am Tage in einem verdunkelten Raum (bei 26°C) mit einer Versuchsanordnung getestet, wie sie in Figur 1 dargestellt ist (nach SCHRICKER[4]). Eine positive Reaktion war ein ununterbrochener Lauf durch das beleuchtete Rohr. Als Versuchstiere dienten um 0 Uhr ± 2 h im Brutschrank geschlüpfte Bienen, die zu dieser Zeit bei sehr schwachem Rotlicht gekäfigt und mit einem Pollen-Futterteigge-