Table I. Activities (μmoles P_i × mg protein^{-1} × min^{-1}) of the ATPases of the sarcolemma from normal and 20,25-diazacholesterol treated rats

	n	$(Na^+ + K^+)$ ATPase + (Mg^{++}) ATPase	(Mg^{++}) ATPase
Control rats	14	0.241 (0.206–0.260)	0.164 (0.135–0.200)
Myotonic rats	14	0.158 (0.152–0.165)	0.123 (0.088–0.146)

Conditions of assay: 3 m*M* [*Tris*-ATP], 20 m*M* [TES] pH 7.4, 1 m*M* [$MgCl_2$], 65 m*M* [choline chloride] and 0.1 mg sarcolemmal protein/ml in a total volume of 2 ml. T = 37 °C. For the determination of the $(Na^+ + K^+)$ ATPase + the (Mg^{++}) ATPase 60 m*M* [NaCl] and 5 m*M* [KCl] were added to the solution instead of the choline chloride[6]. After 5 and 10 min, aliquots were pipetted into an equal volume of 10% trichloroacetic acid and the P_i was determined according to FISKE and SUBBAROW[10]. n = number of experiments; the extreme values are shown in parentheses.

Table II. Inhibition of the ATPases of the sarcolemma from normal and 20,25-diazacholesterol treated rats by 2.5 m*M* 2,4-dichlorophenoxyacetate

	n	$(Na^+ + K^+)$ ATPase + (Mg^{++}) ATPase	(Mg^{++}) ATPase
Control rats	10	54% (34–79%)	64% (49–73%)
Myotonic rats	10	61% (44–73%)	58% (36–73%)

n = number of experiments; the extreme values are shown within parentheses.

Table I gives the results of ATPase determinations for control and myotonic rats. The values for the control rats are in the same region as the data given by PETER[6]. For myotonic animals, however, a decrease is found in the activities of both the (Mg^{++}) ATPase and the $(Na^+ + K^+)$ + (Mg^{++}) ATPase. The activity of the former is 75% that of the control, while the activity of the $(Na^+ + K^+)$ + (Mg^{++}) ATPase is only 66% that of the control. From these results it can be concluded that the $(Na^+ + K^+)$ ATPase in myotonic rats is more inhibited than the (Mg^{++}) ATPase. In further experiments we measured the inhibition of the sarcolemmal ATPases by 2,5 m*M* 2,4-dichlorophenoxyacetate. As can be seen from Table II, this concentration, which is able to induce myotonia in rats, causes a 60% inhibition of the ATPases from the sarcolemma of normal and of myotonic rats.

This results give further evidence that the induction of myotonia by 20,25-diazacholesterol and by 2,4-dichlorophenoxyacetate is connected with alterations in the membrane system of muscle fibers. It remains to be proved whether there are comparable changes in human hereditary myotonia.

Zusammenfassung. Die Aktivität der (Mg^{++})- und der $(Na^+ + K^+)$-stimulierten ATP-ase des Sarkolemm von Ratten mit Myotonie durch 20.25-Diazacholesterin ist gegenüber den Kontrollratten eindeutig erniedrigt. 2,5 m*M* 2,4-Dichlorphenoxyacetat im Bestimmungsansatz hemmt beide ATP-asen um ungefähr 60%.

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A Structure Resembling Proteinpolysaccharide Complexes in Preparations of Mitochondrial DNA from Mouse Liver

The presence of polysaccharides as a common contaminant in phenol-extracted DNA has been reported by various authors[1–3]. This also applies to mitochondrial DNA (M-DNA), which after phenol-extraction and centrifugation in CsCl gradients has been found to contain a contaminant of high UV-absorbancy[4–6]. On the other hand, it is also known that phenol-extracted polysaccarides are always contaminated by DNA[7]. After isopycnic centrifugation in CsCl density gradients, this material accumulates together with M-DNA at a buoyant density of 1.68–1.71 g/cm^3. However, separation of polysaccharides from M-DNA has been achieved either by the addition of ethidium bromide to CsCl density gradients[8], or by purification of M-DNA through MAK-columns (MAK = methylated albumin on Kieselguhr)[9].

During our work on the electronmicroscopical characterization of DNA extracted from mouse liver mitochondria, we found at a frequency of about 0.5% 'lampbrush-like' structures showing a characteristic configuration. They consist of a central filament, 50 Å thick, and unbranched side chains, 80–110 Å thick, which are fully extended and insert at the central filament at inervals of 200–300 Å. The side chains which possess a terminal thickening of 170–210 Å have a rather constant length 1574 ± 135 Å (S.D.). On the other hand, the central filaments vary in length (Figure 1), but there is a close correlation between the length of the central filament and the number of attached side chains (Figure 2). Since the method of spreading leads to a two-dimensional configuration of the molecules, their normal configuration is unknown. Likewise, it is impossible to deduce from the electron micrographs whether or not the filamentous struc-

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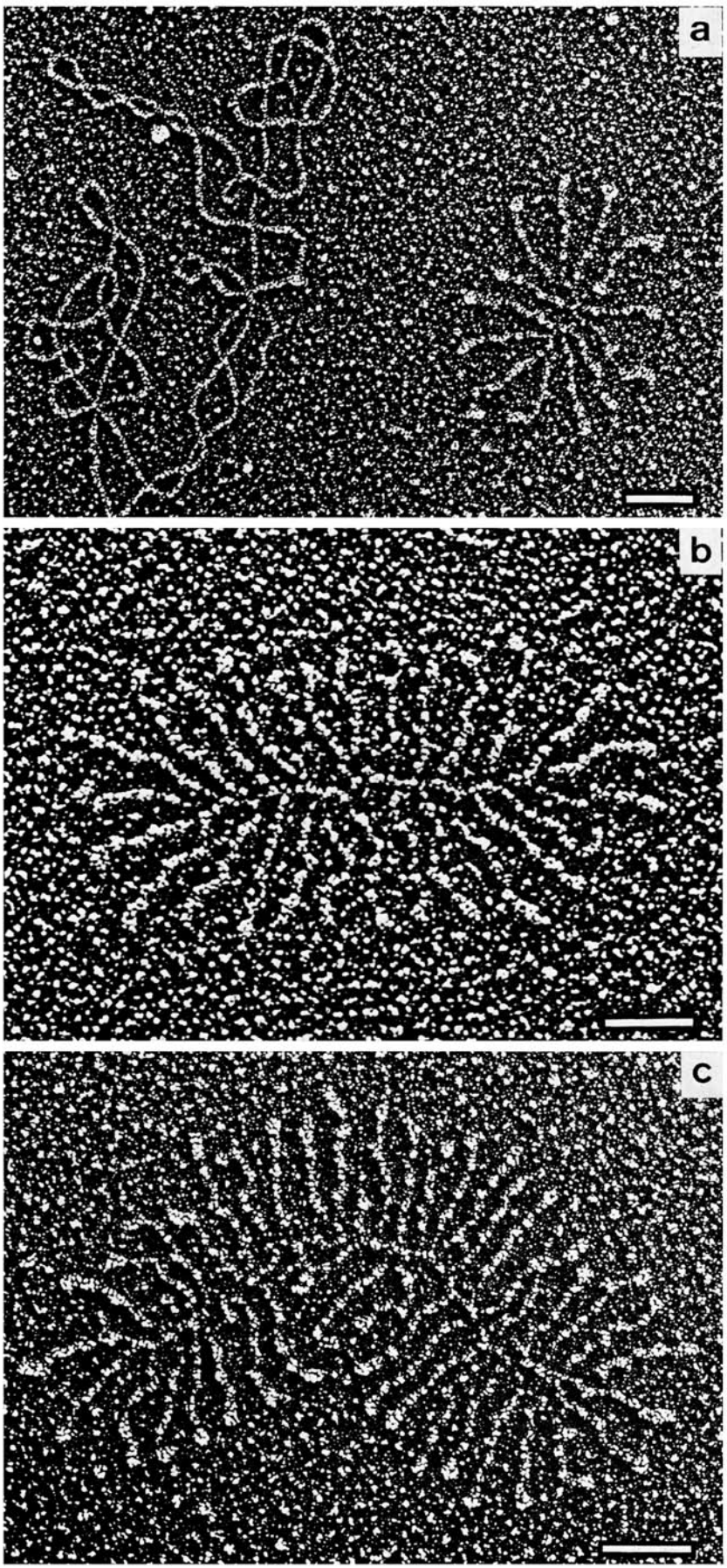

Fig. 1. Electron micrographs of 'lampbrush-like' structures of different length in preparations of M-DNA from mouse liver. a) A small unit, presumably due to fragmentation, with radially arranged side chains. A circular M-DNA molecule is shown on the left. × 99,000. b), c) Longer forms with distinct central filament and perpendiculary oriented side chains showing a club at their end. × 132,000. Bar = 100 nm. Purified mitochondrial preparations[12] were lysed in the presence of sodium lauryl sulfate (SLS), and DNA was extracted with 80% phenol; RNA was removed by treatment with RNase (50 µg/ml). DNA was isolated by centrifugation for 70 h at 33,000 *g* in a CsCl density gradient (3 ml, initial density 1.710 g/cm^3) using the SW 50.1 rotor of the SPINCO L2-50 B centrifuge. For electron microscopical analysis, the M-DNA-containing fraction was spread by a modified[13] protein monolayer technique[14]. The solution containing 50% formamide in 0.004 *M* $NaHCO_3$ (pH 8.6), 0.1 *M* NaCl, 0.001 *M* *tris* (pH 8.4), 0.001 *M* EDTA, 100 µg/ml cytochrome c and 5 µg/ml M-DNA was spread on a surface of redistilled water. The monolayer was picked up by carbon-coated grids previously coated with pyroxiline. The specimen were dehydrated in ethanol and rotary shadowed at a low angle with a Pt-Pd-Au-Ag alloy.

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.

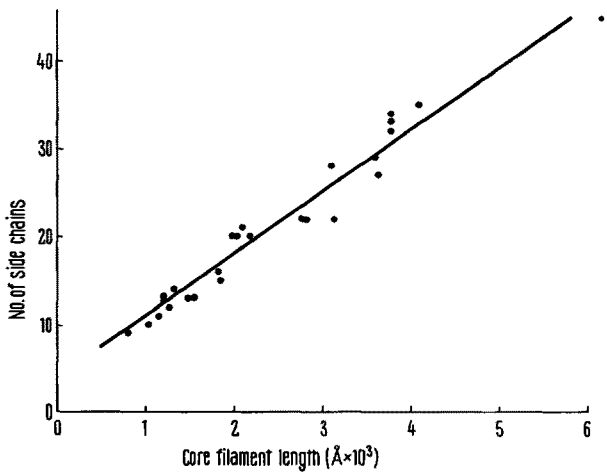

Fig. 2. Correlation between the length of central filaments and the number of side chains. The data are based on measurements of 26 well defined structures. The electron micrographs were taken at a magnification of 30,000×. The structures were enlarged to a final magnification of 635,000×, traced on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