significantly high titers of neutralizing antibodies to Coxsackie B viruses[4,5]. It may be assumed, therefore, that Coxsackie virus B_1 can cause diabetes mellitus in man and other animals. The findings in the experiments described above provide strong evidence for a viral etiology of diabetes mellitus, since the direct viral invasion of β-cells of islets of Langerhans, followed by cell degeneration and necrosis and later atrophic changes, can finally reduce the mass of functional β-cells of the islets of Langerhans.

Zusammenfassung. In den β-Zellen der Langerhans'schen Inseln des Pankreas wurden Viruskristalle von mit Encephalomyocarditis-Virus oder Coxsackie-B_1-Virus infizierten Mäusen gefunden. Die Inselzellen zeigten sowohl leichte als auch schwere Schädigung.

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Effects of Benzo(a)pyrene on Isolated Rat Liver Mitochondria

In a previous study, the authors observed that hepatocytes of rats treated with benzo(a)pyrene underwent alterations of the endoplasmic reticulum and the mitochondria; these were, in particular, swollen, showed a pale matrix, rare cristae and an increase of the mannitol-impermeable spaces[1]. It was not clear, however, whether these mitochondrial modifications were due to the benzo(a)pyrene action directly exerted on the mitochondrial membranes or to a mediate or indirect action of the same toxic substance through the sites of other sub-structures of the hepatocytes. To clarify this histogenetic question the present communication reports the treatment of isolated rat liver mitochondria in vitro with benzo(a)pyrene.

The preparation of the mitochondria was carried out at 0 °C. The rats were killed and bled. The pools of livers previously minced and washed in sucrose (0.25 *M* and 0.44 *M*) were homogenized for 3 min in a Potter-Elvehjem homogenizer with a teflon pestle in the same sucrose solution. The homogenate was then centrifuged for 10 min at 5,500 *g*. The precipitate, consisting of the mitochondria, was resuspended in sucrose with the passages of the pestle carried out manually and then centrifuged for 10 min at 10,000 *g*. The mitochondrial pellet obtained was washed twice with sucrose 0.25 *M*.

The mitochondria thus isolated were resuspended and mixed in an electromagnetic agitator in an aqueous solution of 2% 3–4 benzo(a)pyrene for 30 min[2]. Immediately thereafter the mitochondria were washed with a sucrose solution 0.25 *M* and fixed in 3% glutaraldehyde diluted in 0.13 *M* phosphate buffer at pH 7.4 for 1 h, washed with buffer, post-fixed in phosphate buffered (pH 7.4) 1% osmium tetroxide with added sucrose for 1 h, dehydrated in ethanol and embedded in araldite.

From the material treated in this way, ultra-thin sections were prepared with a Porter-Blum MT2 ultramicrotome; these were then stained with uranil acetate and lead citrate and observed under an Elmiskop 1A electron microscope.

The mitochondria thus treated increased in volume and had a roundish shape, a pale and homogenous matrix,

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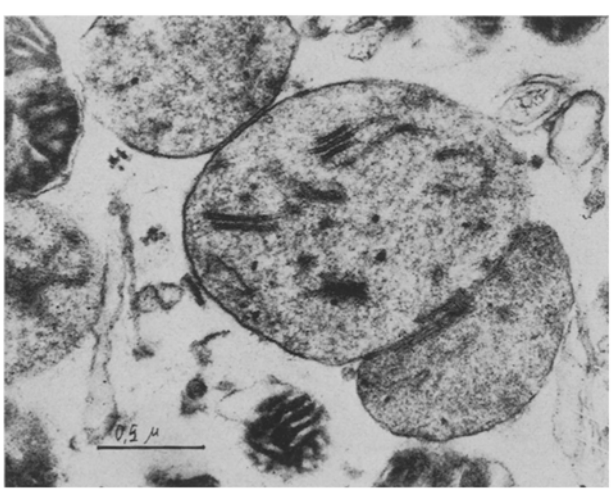

Fig. 1. Mitochondria treated increased, presented a pole, homogenous matrix and rare and broken cristae.

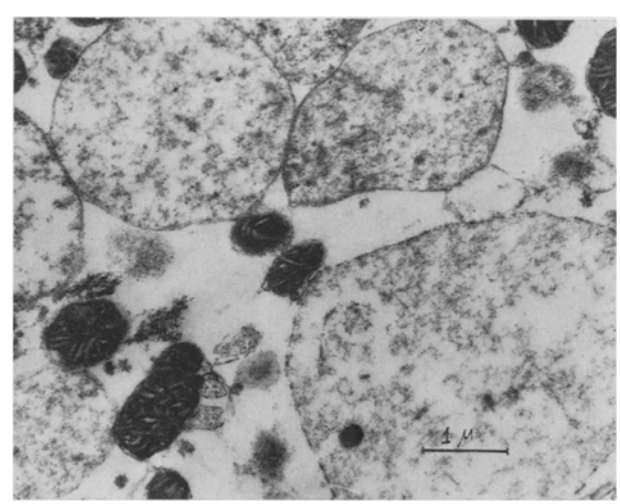

Fig. 2. The cytoplasmic matrix is swollen and in the mitochondria which reached striking volume it appeared rarefied.

and rare and broken cristae; the intracrystal spaces were reduced in size until they became almost virtual.

The cytoplasmic matrix was instead swollen, and in the mitochondria which reached striking volume, it appeared rarefied. The progressive mitochondrial swelling caused rupture of the surface membranes and external emptying of the contents.

The mitochondria constitute a double-compartment system; of these one is accessible to sucrose and various small-sized molecules; the other is permeable only to water and corresponds to the cytoplasmic matrix[3]. Swelling of the mitochondria thus treated is brought about at the expense of the second compartment (mannitol-impermeable) and is probably preceded by alterations of the internal membrane caused by benzo(a)pyrene.

The action mechanism of benzo(a)pyrene on the mitochondrial membranes is similar to that carried out by some detergents, by gramicidin and diethylstilbestrol[4,5]; these substances weaken and break up the protein-phospholipides link, and in this way provoke the leakage of phospholipidic molecules from the mitochondrial membranes. Through the gaps left open by the removal of the phospholipidic molecules, according to the diagram of GREEN[6], the passage of solutes, normally impenetrable, takes place.

Riassunto. Viene trattato mitocondri isolati di fegato di ratto con una soluzione acquosa di benzo(a)pyrene. I mitocondri cosi trattati appaiono rigonfi, hanno matrici chiare e notevole aumento degli spazi mannitolo-impermeabili. Queste modificazioni sono verosimilmente dovute all'azione del benzo(a)pyrene sulle molecole fosfolipidiche delle membrane motocondriali.

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A Cumulating Metabolite Derived from a Piperazine-Substituted Phenothiazine Drug

Accumulation of a minor metabolite during repeated administration of tricyclic psychoactive drugs has been proposed as a possible mechanism accounting for the delayed onset of antipsychotic action. We observed that, upon repeated application of the neuroleptic drug perazine (Taxilan®) to rats, a hitherto unknown metabolite accumulates in tissues.

Materials and methods. Perazine, desmethyl perazine (DMP) and γ-[phenothiazinyl-(10)]-propylamine (PPA) were kindly supplied by Chemische Fabrik Promonta, Hamburg. N-[γ-Phenothiazinyl-(10)-propyl)-ethylenediamine (PPED) was synthesized by reacting PPA with chloroacetonitrile and triethylamine in tetrahydrofuran for 4 days at room temperature. The resulting nitrile (m.p. 85–86° from methanol) was reduced with $LiAlH_4$ in ether. PPED was obtained as an oil, over-all yield 40%.

Rf values of perazine metabolites in thin layer chromatography on Kieselgel GF_{254}

Compound	Solvent[a]		
	A	B	C
Perazine	0.79	0.76	0.36
DMP	0.40	0.33	0.38
PPA	0.63	0.89	0.63
PPED natural	0.30	0.55	0.34
synthetic	0.30	0.56	0.33
PPED sulfoxide			
natural[b]	0.09	0.21	0.12
synthetic	0.09	0.21	0.12

[a] Solvent A) isopropanol/chloroform/water/25% ammonia = 40:20:2.5:2. B) acetone/isopropanol/1 *N* ammonia = 36:28:16. C) dichloroethane/ethyl acetate/ethanol/acetic acid/water = 15:26:12:8:7.5.
[b] Prepared in vitro from natural PPED.

Adult male Wistar rats were given perazine dimalonate (2 × 50 mg/kg free base) by oesophageal tube for 7 days and killed 12 h after the last dosage. Liver tissue (5 g) was homogenized with 20 ml 10% NaCl solution, mixed with 0.6 ml 10% sodium deoxycholate solution and 3.5 ml 25% NH_3 and extracted with 3 × 20 ml dichloroethane. The organic phases were evaporated and the residue chromatographed on Kieselgel GF_{254}. The plates were first washed with chloroform/isopropanol = 10:1 and then developed in solvent A (Table). UV-absorbing bands were removed and the substances isolated[1]. They were purified by rechromatography in solvent B.

Results. The liver extracts were found to contain small quantities of perazine, DMP and PPA chromatographically identical with authentic compounds in all solvent systems tested. The band with Rf 0.30 in solvent A could be resolved in solvent B into a minor yet unidentified component and a major ninhydrin-positive product whose concentration in liver exceeded that of all other metabolites. Neither this metabolite itself, nor its sulfoxide obtained by H_2O_2 oxidation, were chromatographically identical with any of the perazine metabolites isolated from human urine[1,2].

The mass spectrum (Varian MAT SM 1 A) of the unknown compound showed an intense molecular ion peak at *m/e* 299 (according to exact mass measurement $C_{17}H_{21}N_3S$), a base peak at *m/e* 226 ($C_{14}H_{12}NS$, [phenothiazinyl-(10)]-ethyl cation[2,3]) and further fragments at *m/e* 212 (10-methylene-phenothiazonium ion) and 199

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