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.

L. CUCCURULLO and G. MANOCCHIO

Department of Pathology, University of Naples, Via L. Armanni 5 (Napoli, Italy),
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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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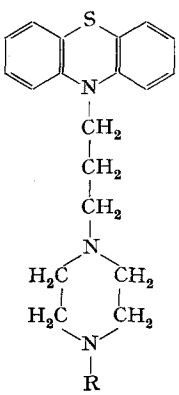
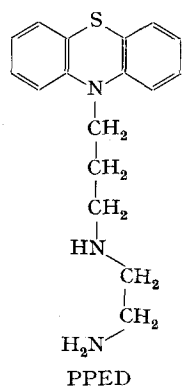
($C_{12}H_9NS$, phenothiazonium ion). Therefore, N-[γ-phenothiazinyl-(10)-propyl]-ethylenediamine (PPED) was proposed as structure of the metabolite. In accordance with this, the 100 MHz-NMR-spectrum (TMS as internal reference) showed absorptions at 6.8–7.4 (8 aromatic H), 3.92 (N^{10}-CH_2-, t, 2 H, J $\approx$ 7 Hz), 1.81 (C-CH_2-C, s, 2 H) and 2.61 ppm (N-CH_2-, m, 6 H). The UV-spectrum which closely resembled that of perazine[1] and the IR-spectrum also agreed with the proposed structure. UV-, IR- and mass-spectra of the metabolite and of synthetically prepared PPED were identical, as were the Rf values of the two compounds and of their sulfoxides in TLC (Table).

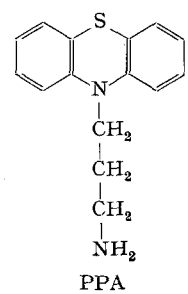
In acute experiments with 50 mg/kg perazine per os, PPED proves to be a minor metabolite in rat liver. Repeated dosage leads to a progressive increase in its tissue concentration. After termination of perazine administration, tissue levels of PPED decline much slower than those of other metabolites, and PPED is still detectable in various organs 2 weeks after the last dosage.



R = CH_3 Perazine
R = H DMP



PPED



PPA

Discussion. Though the piperazine ring forms part of a large number of pharmaceutical compounds, apparently nothing was known on its metabolic degradation in mammals. An oxidative attack on the carbon skeleton could be demonstrated by us[2] through the identification of a diketopiperazine derivative which is excreted in humans ingesting perazine. The occurrence of PPA in rat liver shows that the piperazine ring can be completely degraded, leaving only 1 amino group. PPA constitutes a common metabolite of perazine and promazine[4].

Zusammenfassung. Wiederholte orale Gabe des Neuroleptikums Perazin (Taxilan®) führt bei der Ratte zur Kumulation eines Metaboliten mit teilweise abgebautem Piperazinring, N-[γ-Phenothiazinyl-(10)-propyl]-äthylendiamin. Seine Konstitution wurde durch NMR- und Massenspektroskopie sowie durch Synthese geklärt. Als weiteres Abbauprodukt wurde γ-[Phenothiazinyl-(10)]-propylamin identifiziert.

U. Breyer, D. Krauss and J.C. Jochims

Institut für Toxikologie der Universität, Wilhelmstrasse 56, D–74 Tübingen, Organisch-chemisches Institut der Universität, D–69 Heidelberg; and Max-Planck-Institut für medizinische Forschung, D–69 Heidelberg (Deutschland), 23 September 1971.

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Neuron Populations in the Cerebellum of the Cat

Recent comparisons of the cerebellum to a neuronal machine[1] would gain in validity if consideration were given to the numerical parameters. In cerebellum research, findings made on non-primates are frequently considered applicable to man, to the extent of influencing clinical neurology[2]. The enumeration of the cerebellar nuclear cells, so clearly isolated from other neuronal groupings, renders an exact assessment of the outflow oriented cerebellar capacity[3] in a given species. Cell frequencies in the deep cerebellar grey of man were found to be but a fraction of the formerly reported range of figures[4]. Thus, estimation methods are applicable, giving a high level of confidence. Estimates of the vast number of Purkinje cells would tend to be less precise. The widest range of uncertainty is necessarily associated with attempts to enumerate the granular cells. The available estimate[5] for man ranges from 10^{10} to 10^{11}. Basic cerebellar structure on the microscopic level is remarkably constant in all vertebrates. It is of interest whether the proportionate distribution of the constituent neuronal elements is simply a function of bodyweight or size, or relates to other factors.

Neuron nucleolar counts were carried out on 25 µm Nissl stained sections of 5 cat cerebella. Brains were fixed by perfusion and subsequent immersion in neutral buffered isotonic formalin. Counts were made by periodic samples of 10 section intervals. A 5 section period was used in 1 brain. Compared to an all section count, the expected loss of accuracy[4] is 2% only.

There is little variation among cell frequencies in the deep cerebellar nuclei of 4 animals (Table I). The 5th, a young male of low bodyweight, has significantly lower cell numbers (P 0.005) in all of the nuclei. Perinatal nalnutrition is a possible but not provable correlate for this difference. Compared to cell frequencies in the same nuclei of man[4], shifts in proportions are considerable. Homologies here, comparing primates to non-primates, are accepted now with few reservations[6,7]. Whereas the medial nucleus of the cat accounts for 35% of all deep cerebellar neurons, the homologe fastigial nucleus of man contributes only 1.65% of the total. Against an assumed

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