Zusammenfassung. Elektronenoptisch wird gezeigt, dass bei der Katze die Bahnen von der unteren Olive zum Kleinhirn Kollateralen besitzen, welche an die Kleinhirnkerne ziehen und sowohl ipsilateral als auch kontralateral von einer Olive versorgt werden. Damit können elektrophysiologische Befunde sicher interpretiert werden.

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Short Term Effects of Dimethylnitrosamine and Methylmethanesulphonate on Hydrolases of the Rat

The metabolic and biochemical effects, particularly those concerned with alkylation of nucleic acid bases, caused by the administration of dimethylnitrosamine (DMN) and methylmethanesulphonate (MMS) in experimental animals have been extensively reported in the literature[1-3]. The enzyme systems that have been mostly investigated include those associated with tissue oxidation and glycolysis[4-6]. The behaviour of acid hydrolases is less well documented and the available data mainly deal with changes that result from administration of either a single lethal dose or of repeated sublethal doses over a prolonged period[7,8]. Report of acute effects induced by these alkylating agents following a single sublethal dose are scant.

In view of the above, the short term effects of administration of single sublethal doses of DMN and MMS on 2 hydrolytic enzymes, namely acid phosphatase (EC 3.1.3.2) and nonspecific esterases (EC 3.1.1.1) were investigated in the rat. These 2 enzymes were selected also because of their known dynamic nature and responsiveness to changes in cellular environment[9].

Materials and methods. 3 groups, each consisting of 10 male adult Wistar rats, were administered single i.p. injections of DMN (10 mg/kg), MMS (90 mg/kg) and normal saline (control) respectively. Liver, kidney and testis obtained from these animals at 72 h were subjected to histochemical, electrophoretic and biochemical techniques for the study of acid phosphatase and nonspecific esterases as described previously[9]. Routine histological staining was also carried out.

Results. Scattered areas of centrilobular necrosis were evident in the liver of 3 DMN-treated rats. Vascular congestion was present, although no frank haemorrhages were observed. In histochemical preparations these areas appeared as enzyme deficient islands. The kidney and testis of the DMN- and MMS-treated rats showed no histological or enzyme histochemical changes. Esterase zymograms of the testis in 3 DMN-treated rats showed a characteristic suppression of the fastest cathodally migrating isozyme with α-naphthyl butyrate, while in the control and MMS preparations this band was prominent. No other changes in acid phosphatase of esterase zymograms were noted. The findings are summarized in Table I.

Biochemical assay of both the hydrolases exhibited wider variations in enyzme levels between the individual

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Table I. Morphological and enzymological (acid phosphatase and nonspecific esterases) changes at 72 hours following administration of single sublethal doses of DMN and MMS in the liver, kidney and testis of the rat

Organs	Morphology (H & E), (paraffin sections)	Enzyme histochemistry (cryostat sections, post-fixed)	Zymogram pattern (*Tris*/boric buffer system; pH 7.3)
Liver	Scattered areas of centrilobular necrosis and vascular congestion in three DMN-treated animals	Enzyme deficient islands, corresponding to necrotic areas, in both acid phosphatase and esterase preparations	Normal
Kidney	Normal	Normal enzyme distribution	Normal
Testis	Normal	Normal enzyme distribution	Suppression of the fastest cathodally migrating isozyme with α-naphthyl butyrate in three DMN-treated animals

Substrates employed for acid phosphatase were α-naphthyl phosphate and naphthol AS-MX phosphate with Red Violet LB as the diazonium salt. For esterases, α-naphthyl acetate, α-naphthyl propionate, α-naphthyl butyrate and naphthol AS acetate were used with Fast Blue BB as the diazonium salt. Simultaneous capture technique was employed for the histochemical and electrophoretic studies.

Table II. Results of biochemical assay of acid phosphatase and nonspecific esterases with their standard deviations

Organs	Enzyme type	Enzyme activity in mU/mg of protein		
		Control	DMN	MMS
Liver	Acid. phosphatase	1.10 ± 0.14	0.95 ± 0.24	1.00 ± 0.18
	Esterases	4.70 ± 0.90	4.90 ± 1.50	4.20 ± 1.20
Kidney	Acid. phosphatase	0.78 ± 0.18	0.79 ± 0.15	0.72 ± 0.10
	Esterases	4.90 ± 0.30	4.85 ± 0.80	4.40 ± 0.85
Testis	Acid. phosphatase	0.18 ± 0.09	0.19 ± 0.12	0.18 ± 0.12
	Esterases	5.40 ± 0.40	5.50 ± 1.50	5.00 ± 0.60

mU = international milliunit. Post-coupling technique was employed.

experimental animals (DMN and MMS groups), than was observed amongst the controls. As the large standard deviations in the experimental groups overlapped those of the latter, no statistically significant differences were noted between them. The bioassay data are presented in Table II.

Discussion. In view of the available reports that larger doses of alkylating agents than used in the present investigation are required for experimental pathogenesis in rats, the findings noted may be of significance. HULTIN et al.[10] reported that the smallest dose of DMN necessary to produce definite necrosis of the liver is about 20 mg/kg. The present findings suggest that hepatic involvement, as evinced by scattered areas of centrilobular necrosis and enzyme deficient islands, can result from administration of the chemical at half the stated dosage. Such occurance of morphological and enzymological changes even in a small proportion of experimental animals underlines the role of individual susceptibility in the causation of lesions by the alkylating agents. The bioassay data exhibiting wide variations in enzyme levels amongst the individual experimental animals are corroborative in this regard. However, the absence of any statistically significant difference in enzyme activities between the experimental and control groups, as noted earlier, is relevant to the biochemical findings reported by SLATER et al.[7]. Working on hepatic lysosomal hydrolases following necrotizing doses of hepatotoxins, including DMN, these authors observed little alteration in lysosomal activity in the prenecrotic and in the early necrotic stages of hepatic involvement and concluded that the lysosomes probably play no role in the early development of hepatic lesions. Changes in activity that were observed after 10 h were attributed to the aftermath of the necrotic process, rather than with its initiation.

The selective loss of the terminal cathodal esterase of the testis in 3 DMN-treated animals is also significant, as this isozyme seems characteristic of the organ. In all zymograms with both unsubstituted and substituted naphthyl esters this particular variant features prominently. Besides, its capacity to produce hybrid esterase species is also distinctive[11]. The present finding points to the fact that cytochemical changes may occur from administration of alkylating agents before any morphological changes become evident and that such subtle changes may be detectable by appropriate analysis of the constituent isozyme systems.

Zusammenfassung. Ein kleiner Teil der mit Dimethylnitrosamin (10 mg/kg) behandelten Ratten zeigte Lebernekrose und Suppression eines Esterisozyms in den Hoden.

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Intramitochondrial Bodies in the B-Cells of Rat's Pancreas Treated by Glybenclamide

Intramitochondrial bodies, different in shape, size and structure, and bound to the matrix or the cristae, have been described in the cells of various animals. They are detached in different organs in various conditions – normal, pathological or experimental, and in different evolutionary phases – embryonal and postnatal[1–11].

Peculiar intramitochondrial structures are seen in the B-cells of the islets of Langerhans of the pancreas of the rat, treated with glybenclamide (HB 419). A single dose of glybenclamide (7 mg/kg) was given orally to 9 white rats. The animals were sacrificed by decapitation on the 2nd, 8th and 24th h. 2 rats were used as controls. The pancreas was removed immediately and fixed in 1% osmium tetroxide in Millonig's buffer at pH 7.4 for 1 h at 4 °C. After a fast rinse in the Millonig's buffer, the specimens were dehydrated and embedded in Durcopan. Reichert ultramicrotome was used to obtain specimens. The stains used were uranyl acetate after WATSON[12] and lead citrate after REYNOLDS[13]. Sections were examined by Hitachi HU-11 A electron microscope.

Dense intramitochondrial bodies, single or multiple (Figure 1), were detected in the mitochondria of some of the B-cells, more frequently in the pancreas of animals sacrificed after 24 h. These bodies were mainly observed in bigger mitochondria. The mitochondria themselves (matrix, cristae and membrane) did not show any