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Diethylene glycol (DEG)-associated myocardial changes: a pilot investigation of chronic intoxication in guinea-pigs

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Summary. The myocardium of guinea pigs fed on sublethal doses of diethylene glycol (DEG) over a period of 2–11 days was examined for microscopical and ultrastructural changes. Coagulative myocytolysis and loss of myofibrils, not observed in the controls, was patchily distributed throughout the myocardium. The accompanying ultrastructural features included swelling, pleomorphism and hyperplasia of mitochondria with an associated distension of the interfibrillary spaces and a displacement, distortion and rupture of adjacent myofibrils.

Key words: Diethylene glycol – Intoxication – Myocardium – Myocytolysis – Ultrastructure – Mitochondria – Guinea pig

Zusammenfassung. Das Myokard von Meerschweinchen, welche mit subletalen Mengen von Diethylenglycol über eine Zeitdauer von 2–11 Tagen gefüttert worden waren, wurde mikroskopisch und elektronenmikroskopisch untersucht. Eine Koagulationsmyozytolyse und Verlust von Myofibrillen war fleckförmig verstreut über das Myokard. Diese Veränderungen waren in den Kontrollen nicht zu beobachten. Die begleitenden ultrastrukturellen Veränderungen bestanden in Schwellung, Pleomorphie, Hyperplasie von Mitochondrien, begleitet von einer Erweiterung der interfibrillären Räume und Verlagerung, Verzerrung und Ruptur angrenzender Fibrillen.

Schlüsselwörter: Diethylenglycol – Intoxikation – Myokard – Myozytolyse – Ultrastruktur – Mitochondrien – Meerschweinchen

Introduction

Following several reports of cases of acute accidental lethal intoxication in man [8, 9, 16, 17, 19], the nephrotoxicity of diethylene glycol (DEG) was clinically and morphologically well documented [8, 11, 26, 31]. In contrast, little is known about the pathology of sublethal chronic exposure to DEG as would occur, for instance, through the regular consumption of wines contaminated with this chemical agent. The clinical observation that patients treated for acute DEG intoxication on occasions die suddenly and unexpectedly from cardio-respiratory arrest (after a period of apparent improvement [8]) raises the possibility of a delayed cardiotoxic effect of DEG.

This pilot study was therefore conducted to demonstrate morphological changes in the myocardium following the oral administration of sublethal quantities of DEG recurrently.

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Materials and methods

DEG (1.2 g per kg body weight) was administered orally on a once daily basis to 9 male guinea pigs weighing 300–360 g, over a period of 2 to 11 days. Since the DEG (Merck) contained traces of ethylene glycol (0.4%), 5 more guineapigs received 4.5 mg/kg body weight ethylene glycol over the same period as a control. After decapitation tissue samples were taken from the heart, the liver and the kidneys. The samples from the heart were taken from the left ventricle (anterior and posterior wall), the ventricular septum (apex and base), the right ventricle (subjacent to the tricuspid valve), and from the conus pulmonalis. Standard 5-micron thick histological sections were prepared and stained with haemalaun und eosin. The samples were fixed in 4% formaldehyde solution. Semi-thin sections for light microscopy and ultra-thin sections for electron microscopy were prepared from epon-embedded tissue samples fixed with glutaraldehyde and osmium tetraoxide. The semi-thin sections were stained with toluidine blue.

Results

1. Light microscopical examination revealed foci of vacuolar cytolytic intracytoplasmic aggregates interposed with normal myocardial fibres. The vacuoles appeared to be located between the myofibrils (Fig. 1a). Areas of recent colliquative cytolysis were present in which there was a focal disappearance of myofibrils with remnants of

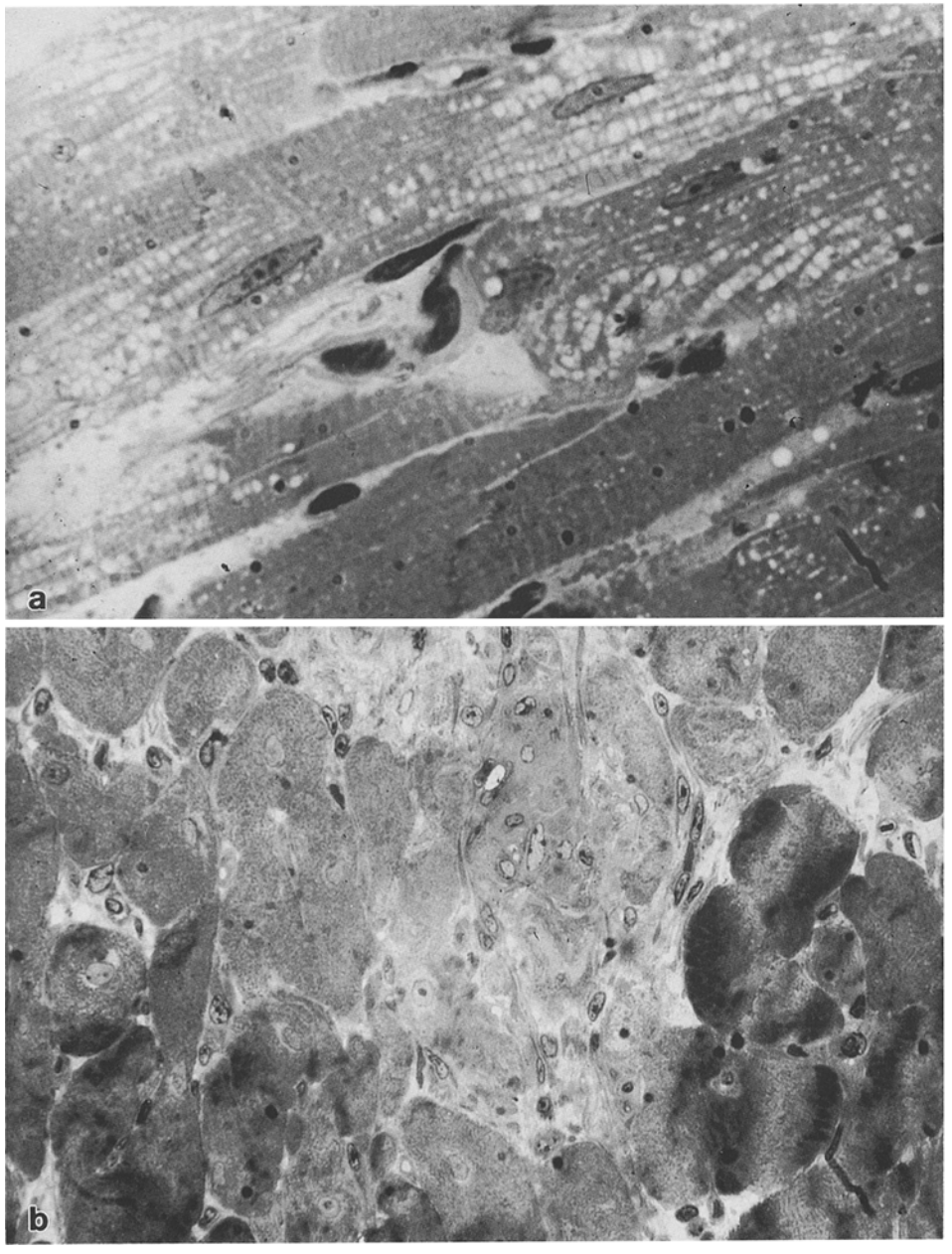

Fig. 1. a Irregular groups of vacuoles in a patchy distribution within the myocardium; (toluidine blue; semi-thin section, × 725). **b** Recent myocytolysis with focal disappearance of myofibrils. Note remnants of sarcolemma and paucity of cellular reaction; (toluidine blue; semi-thin section, × 725)

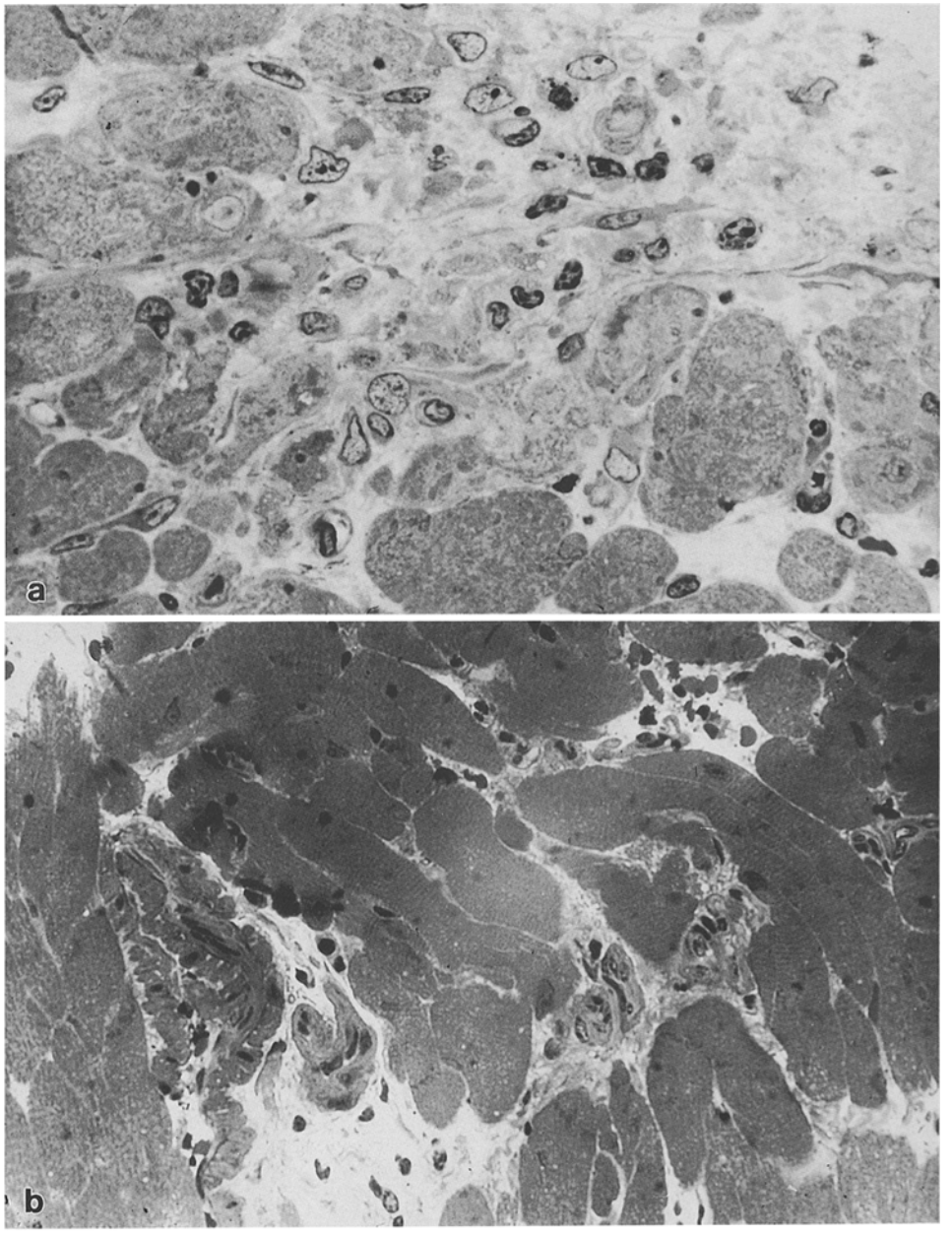

Fig. 2. a Older myocytolytic lesions with marked cellular reaction and **b** formation of multinucleated giant cells; (toluidine blue semi-thin section, × 725)

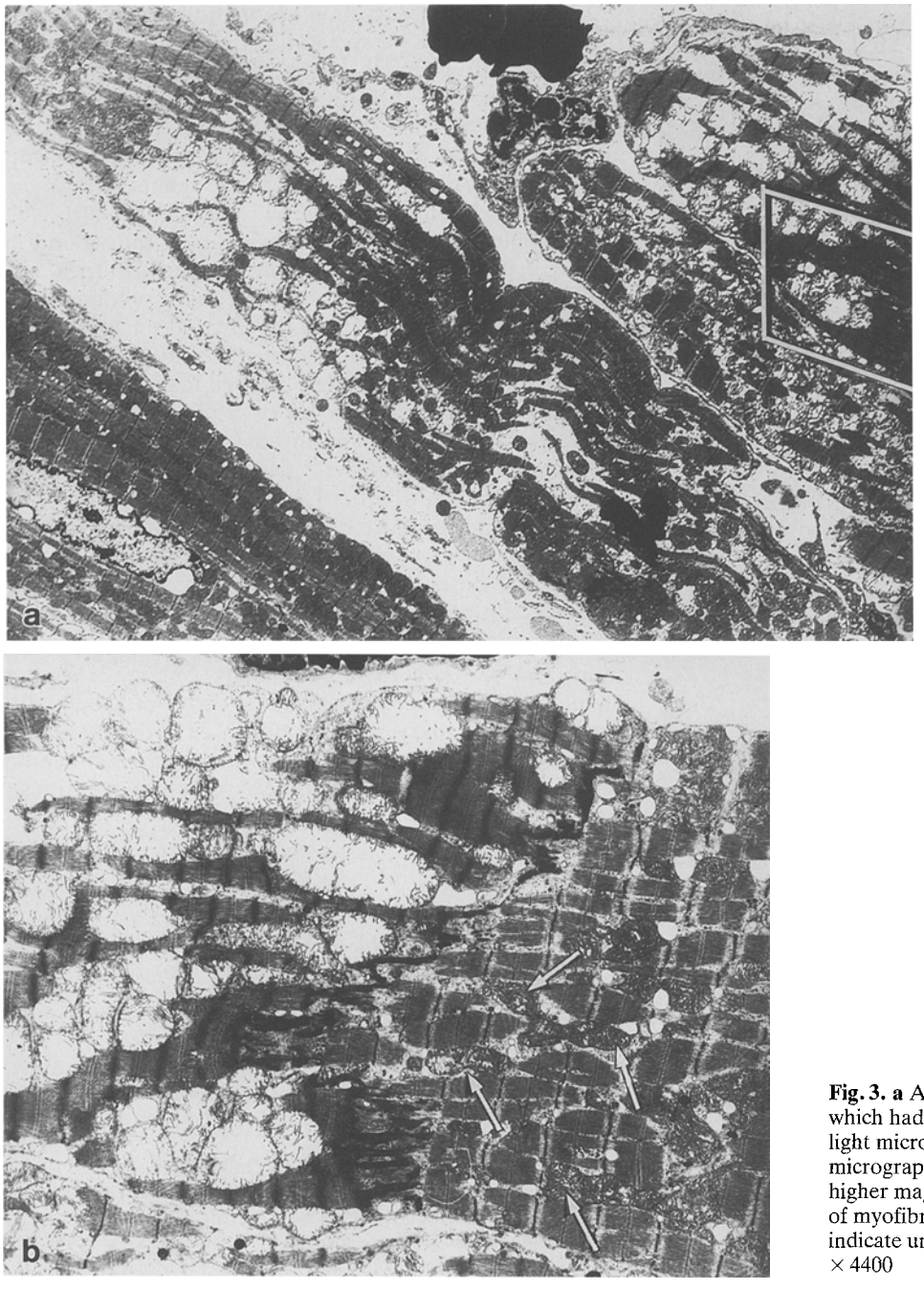

Fig. 3. a Aggregates of swollen mitochondria which had the appearance of vacuoles under the light microscope as shown in Fig. 1a; (electron micrograph, × 1100). **b** Marked part of **a** at a higher magnification: distortion and compression of myofibrils by swollen mitochondria. (*Arrows* indicate unaffected mitochondria for comparison). × 4400

sarcolemma surviving, but without cellular inflammatory reaction being recognisable (Fig. 1b). Similar but older cytolytic lesions were present and these were associated with a cellular reaction (Fig. 2a) inclusive of multinucleated giant cells (Fig. 2b).

2. The vacuoles observed under the light microscope were ultrastructurally identified as irregular aggregates of swollen mitochondria (Fig. 3a) which has caused adjacent myofibrils to be deformed, compressed and pushed apart (Fig. 3b).

3. At higher magnifications, such areas showed pleomorphism and hyperplasia of groups of mitochondria with resultant distension of the interfibrillary spaces leading to displacement, distortion, overstretching and rupture of myofibrils (Fig. 4).

In none of the control animals was vacuolisation or cytolysis present in the myocardium. Mitochondrial pleomorphism and hyperplasia were not apparent on electron microscopy.

Discussion

The quantity of DEG administered (1.12 g/kg body weight) corresponds to the average lethal dose in man [2, 16, 26] and is less than one tenth of the LD_{50} in ani-

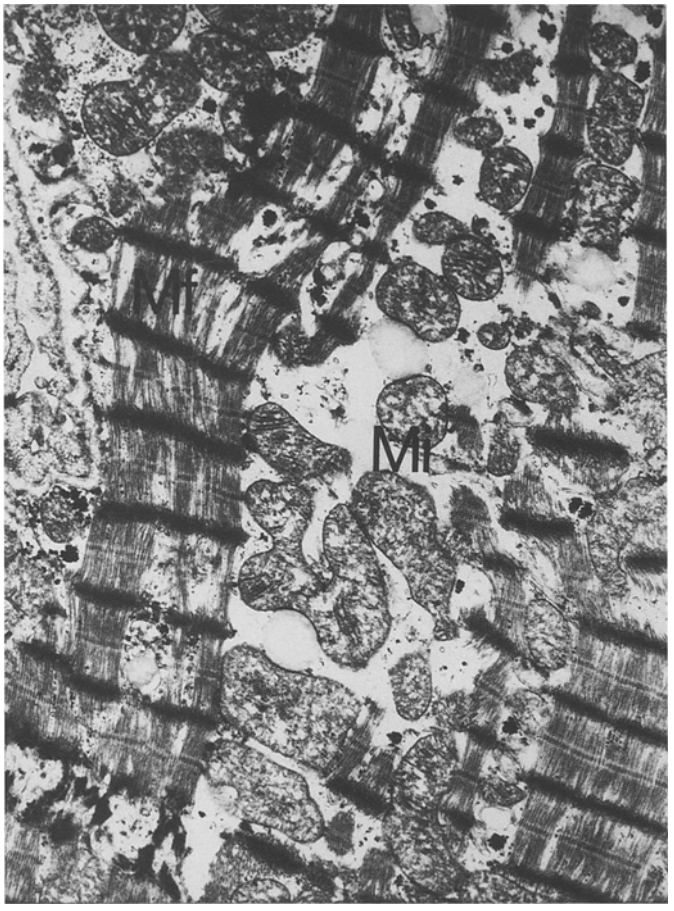

Fig. 4. Pleomorphism and hyperplasia of mitochondria. Note distension of interfibrillary spaces and rupture of myofibrils. × 3000. (enlarged portion of original electronmicrograph)

mals [2, 12, 26]; this dosage proved suitable for the induction of demonstrable morphological changes in the myocardium of the guinea-pig. Histological renal changes which were found in this study were similar to those reported in previous investigations [8, 11, 31].

In a pilot study of this nature in which a very limited number of animals (9 test animals and 5 controls) were subjected to DEG exposure, it can only be infered that the structural myocardial changes observed are directly related to this chemical.

As in many similar morphological investigations of large organs, the strategy for tissue sampling poses a problem especially when the relevant changes are discontinuous, patchy and randomly distributed such as the lesions identified in this study. An acceptable representative coverage of the organ is achieved if topographical boundaries and demarcations are respected. A more effective approach would be to apply a statistically verified sampling method for arbitrary distribution as applied in stereology [10, 18, 25].

Although the myocyte has only limited patterns of reaction to injury, it has been possible to distinguish between ischaemic coagulative necrosis, coagulative myocytolysis – equivalent to the Zenker necrosis of skeletal muscle [5, 20], and colliquative myocytolysis frequently observed in the toxic cardiomyopathies [4, 6, 20, 23, 28].

The cytolysis observed in association with DEG is of the toxic type and is associated with a focal reduction of the contractile elements. Inspite of some overlaps, the observed constellation of ultrastructural changes (swelling, hyperplasia and pleomorphism of mitochondria) appears to differ from that observed in association with other known or suspected cardiotoxic agents such as the anthracyclines and ethanol [5, 13, 21, 22, 29, 30]. The anthracyclines show a striking shrinkage of mitochondria [5, 13]. Ethanol-associated changes include mitochondrial swelling [1, 27], but unlike the changes found with DEG exposure, distensions of the sarcoplasmic reticulum [27] occur. This also differs from the mitochondrial hyperplasia and pleomorphism observed in conditions such as cardiac hypertrophy [5, 15] or Whipple's disease [3, 14, 24], in which random groups of swollen mitochondria do not constitute a feature.

It is not known whether DEG causes such myocardial changes similar to those observed in the guinea pig with similar exposures in humans. However the sudden and unexpected onset of cardio-respiratory arrest which is observed during treatment of cases of acute DEG intoxication indicate a possible involvement of the myocardium and of the cardiac conducting system. If similar changes are found in human hearts, they would be of differential diagnostic importance in cases of otherwise unexplained sudden cardiac death when there has been a history of regular intake of DEG in sublethal quantities.

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