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A cytochemical method for the identification of ischemic and necrotic myocardial fibres

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Summary. The author studied the nickel-dimethylglioxim reaction in ischemic and necrotic myocardium fibres in dogs, following ligature of the left anterior descending coronary artery. The nickel-dimethylglioxim reaction was positive in ischemic fibers, but not in necrotic ones. The cytochemical reaction, observed in ischemic fibers, was localized in the intracellular compartment and in the mitochondria.

Key words: Experimental myocardial infarction – Nickel cytochemistry – Ischemia – Necrosis

Zusammenfassung. Untersucht wurde die Nickel-Dimethylglioxim-Reaktion in ischämischen und nekrotischen Herzmuskelzellen von Hunden. Vorausgegangen war eine Ligatur des linken absteigenden Koronararterienastes. Die Nickel-Dimethylglioxim-Reaktion war positiv in ischämischen Zellen, jedoch nicht in nekrotischen. Die cytochemische Reaktion, wie sie in ischämischen Fasern beobachtet wird, war im intrazellulärem Compartment und in den Mitochondrien lokalisiert.

Schlüsselwörter: Experimenteller Myokardinfarkt – Nickel-Cytochemie – Ischämie – Nekrose

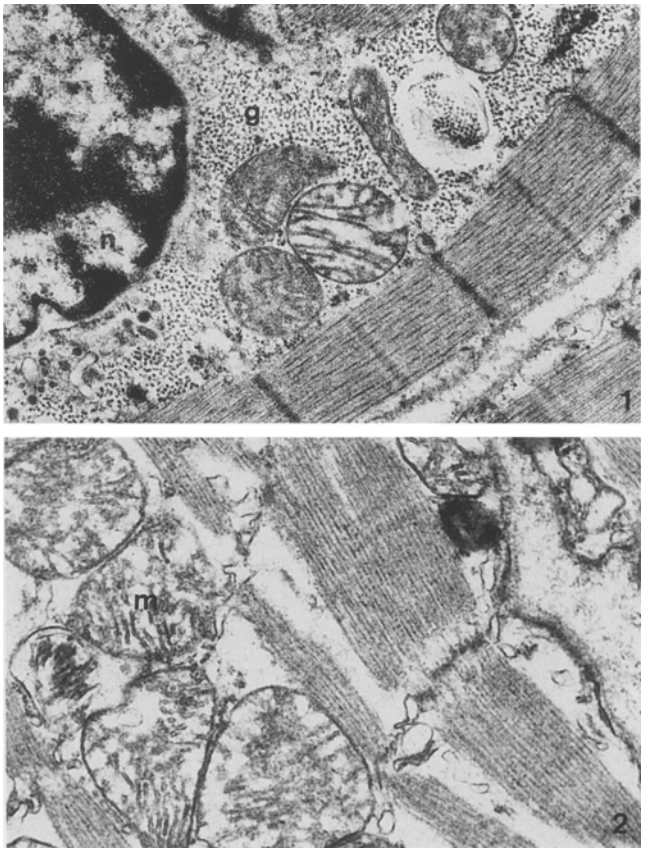
Introduction

The identification of ischemic and necrotic fibers in the myocardium and the detection of acute myocardial infarction has a great significance in forensic pathology. In cases of survival, the occurrence of myocardial infarction can be confirmed at a relatively early stage, however the findings are difficult to interpret [9, 10, 15, 18]. The dimethylglioxim cytochemical technique would seem to be suitable under the condition of autolysis, which is of special importance in forensic medicine [3].

In this study results from experimental acute myocardial infarction are presented.

Materials and methods

Mongrel dogs, both male and female, (16–33 kg bodyweight) were narcotized with glucochloralose (100 mg/kg) and the anaesthetic was maintained as necessary. The animals were immobilized using Flaxedyl (20 mg/kg), and pulmonary ventilation was accomplished by a positive pressure respirator (Harvard) with room air enriched by 100% oxygen. The left coronary ramus descendens was ligated in 12 animals during thoracotomy. In the 4 animals serving as controls thoracotomy was performed, but the descending branch of



Group I. (Figs. 1–4)

Fig. 1. Ligation of the coronary artery for 1.5 h. Mitochondrial swelling *(m)* and intracellular glycogen particles *(g)* in remote (2 cm) areas, with the sign of developing nuclear ischemia *(n)*. Transmission electron microscopy, *magnification:* × 18500

Fig. 2. Ligation of the coronary artery for 1.5 h. Severe mitochondrial damage *(m)* and intracellular edema in the close vicinity of the ligation. Glyocogen particles are absent. Transmission electron microscopy, *magnification:* × 18500

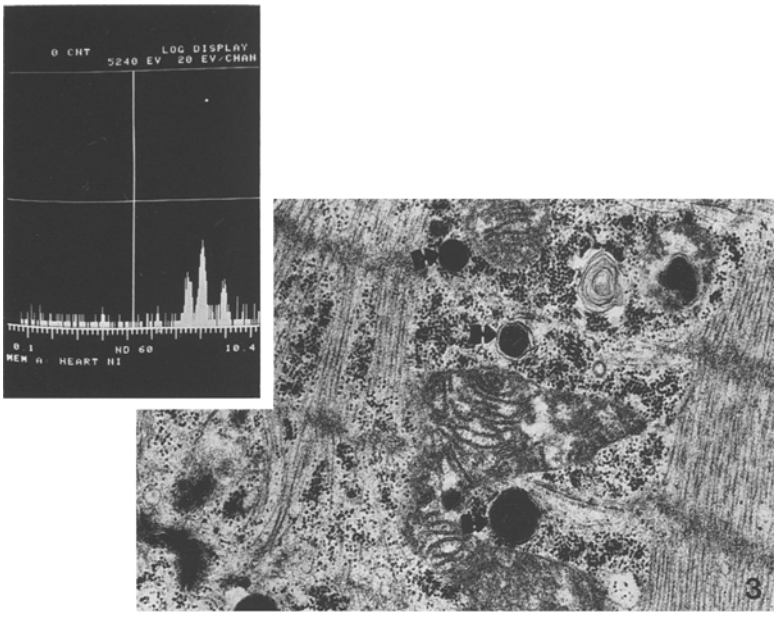


Fig. 3. Ligation of the coronary artery for 1.5 h. Mitochondrial swelling and intracellular nickel dimethylglioxim complexes *(arrows)* in remote (2 cm) areas, with intracellular glycogen particles present in abundant quantity. *Insert*: Energy dispersion microanalysis of precipitates: the third spike from the right denotes nickel, the other two spikes are caused by the copper content of the carrier layer. Cytochemical reaction for the detection of nickel. *Magnification:* × 18500

Fig. 4. Ligation of the coronary artery for 1.5 h. Severe mitochondrial damage and areas of hypercontractility *(hb)* in the close vicinity of the ligation, with the presence of intramitochondrial nickel dimethylglioxim complexes *(arrow)*. Cytochemical reaction for the detection of nickel. *Magnification*: × 18500

the left coronary artery was not ligated. Maintaining adequate respiration, a biopsy of the myocardium was performed after 1.5 h in 6 animals and after 3 h in the remaining 6 animals. Specimens were taken from the close vicinity of the ligated coronary branch and from another location, slightly further apically in the first group and from the outer zone of the visible intramural haemorrhage in the second group.

Assessment was performed on slides prepared for light microscopy and semi-thin sections prepared for electron microscopy. The specimens for the latter purpose was fixed in 2.5% glutaraldehyde and 1% osmium tetroxide. The presence of nickel was detected by the dimethylglioxim method [2, 3, 12]. Investigations were carried out in JEOL 100B and 100CX electron microscopes using an accelerating voltage of 60 kV. Microanalysis was done on an EDAX 707B device.

Results

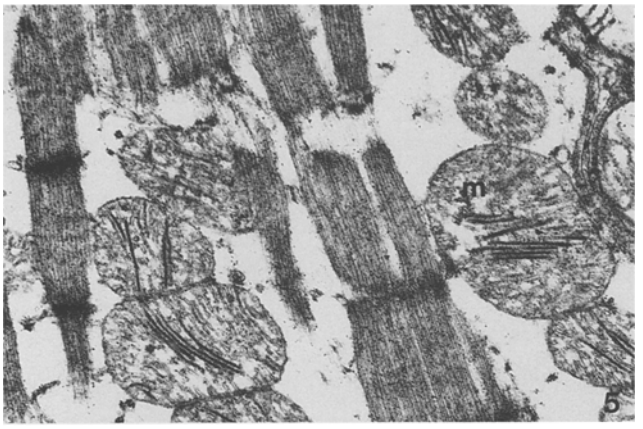
The control sections showed normal structures and reaction patterns; the dimethylglioxim test was also negative.

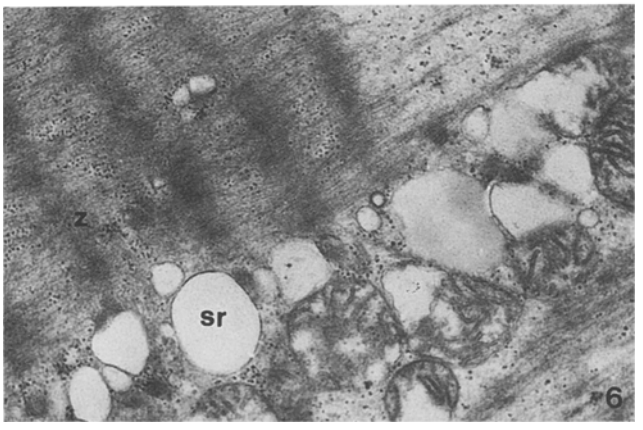
Group I

The electron microscopical investigation of specimens from the apical region taken 1.5 hours after the ligature showed ischemic damage of the nucleus (Fig. 1), swelling of the mitochondria and an abundance of intracellular glycogen particles (Fig. 1). In specimens taken from the close vicinity of the ligated coronary branch, severe mitochondrial damage, intramitochondrial nickel complex, intracellular edema were noted, and glycogen particles were absent (Fig. 2). Cytochemical investigations revealed an abundance of intracellular glycogen particles, mitochondrial swelling, and intracellular nickel-dimethylglioxim particles in specimens taken from the apical region (Fig. 3). In specimens taken from the close vicinity of the ligated coronary artery (Fig. 4), the areas of hypercontraction were characterized by intracellular edema, and the presence of nickel dimethylglioxim particles in the mitochondria.

Group II

The electron microscopical investigation of specimens taken 3 hours after the ligature from the edge of the visible intramural haemorrhage (Fig. 7) showed severe mitochondrial damage, intracellular edema and nickel dimethylglioxim particles were found in the intracellular compartment and in the mitochondria. In specimens





Group II. (Figs. 5–7)

Fig. 5. Ligation of the coronary artery for 3 h. Severe mitochondrial damage *(m)* intracellular edema in the close vicinity of the ligation, with the presence of intercristal deposits in the mitochondria. Transmission electron microscopy, *magnification*: × 18500

Fig. 6. Ligation of the coronary artery for 3 h. Significant dilatation of sarcotubules *(sr)*, severe mitochondrial damage in the close vicinity of the ligation with a widening of Z-bands *(z)*. Nickel dimethylglioxim complexes are absent, the reaction is negative. Cytochemical reaction for the detection of nickel. *Magnification:* × 18500

taken from the close vicinity of the ligation, necrosis of fibers was detected, with a few hypercontracted areas still visible and intercristal deposits were observed in the mitochondria (Fig. 5).

Cytochemical reactions were negative in this area (Fig. 6).

Discussion

The nickel ion can be substituted for calcium at the binding sites through competitive antagonism, especially in the myocardium where it is connected to the system of excitation – contraction and decreases contraction [5, 6, 11]. In experimental material nickel also causes contraction of the coronary vessels, and the nickel dimethylglioxim particles can be detected morphologically in the smooth muscle cells of coronary vessels [7]. Nickel significantly increases the resistance of coronary vessels,

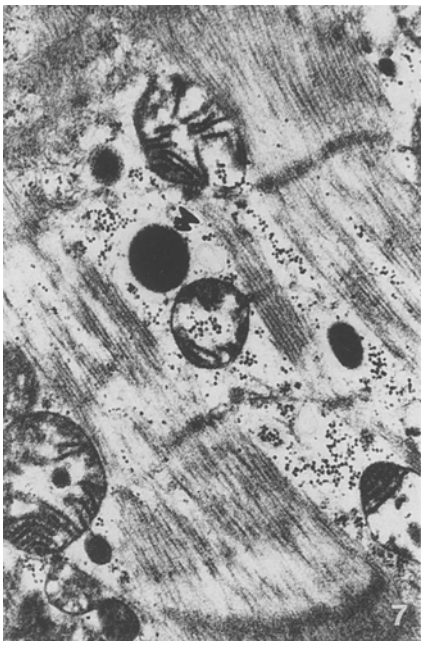


Fig. 7. Ligation of the coronary artery for 3 h. Severe mitochondrial damage in remote areas at the edge of the hemorrhage with the presence of intramitochondrial and intracellular nickel dimethylglioxim complexes. Cytochemical reaction for the detection of nickel. *Magnification*: × 18500.

even at a perfusion medium concentration of 6 µg/l. High serum nickel levels were found in the majority of patients suffering from acute myocardial infarction; the hypernickelemia was followed by coronary vasospasm. Leach et al. [8] gave 3 alternative explanations for the hypernickelemia observed in their patients suffering from acute myocardial infarction or instable angina: 1. the myocardium or coronary vessels of atherosclerotic cardiac patients may contain high pathological concentrations of nickel which leaches into the circulation; 2. the hypernickelemia is a secondary phenomenon mediated by nickel effluxed from other organs under conditions of stress, hypertension, congestive heart damage or pulmonary edema. The lungs have an especially high nickel content; 3. nickel reaches the serum during the coagulation cascade as a bound complement factor. This latter hypothesis is supported by the fact that human F VIII and C-3 may contain nickel ions in converted metal bonds [14, 16].

It is generally accepted that hypernickelemia observed after myocardial infarction cannot be regarded as specific. Several other cardiopathological states are known, where nickel accumulation and/or nickel release are observed, for example, in carbon monoxide poisoning and in burning [2, 3, 4, 13, 17].

Our experiments were intended to observe changes in the myocardium following the ligation of the coronary ramus descendens at various timeintervals. Specimens were taken from the close vicinity from the coronary branch and also from zone further away. The changes developed 1.5 hours and 3 hours after the ligation were compared by electron microscopical and cytochemical methods. The results are shown in Table 1.

Table 1

Group I	1.5 h after the ligation	Close vicinity of the coronary branch	Apical area
		Severe mitochondrial damage, intracellular edema (Fig. 2)	Smaller mitochondrial edema, several glycogen particles, damage of the nucleus (Fig. 1)
		Cytochemical reaction: positive (Fig. 4)	Cytochemical reaction: positive (Fig. 3)
Group II	3 h after the ligation	Close vicinity of the coronary branch	Apical area
		Severe mitochondrial changes, intracellular edema, intercristal deposits in the mitochondria (Fig. 5)	Severe mitochondrial changes, intracellular edema
		Cytochemical reaction: negative (Fig. 6)	Cytochemical reaction: positive (Fig. 7)

Our experiments showed that specimens taken from the close vicinity of the ligated branch after 1.5 h may be qualified as ischemic, whereas insignificant changes can be seen in specimens taken from the apical area. The cytochemical reaction was positive in the close vicinity of the coronary branch 1.5 h after the ligature, however the reaction was also positive in the apical area (1.5 hours after the ligature) indicating the sensitivity of the reaction. No changes were observed in the coronary arteries and no coronary sclerosis was detected. It is presumed that deficiency of nickel dimethylglioxim complexes in the necrotic area might be the result of a significant decrease in or termination of local blood circulation, however, the possibility of the origin of nickelemia observed in acute myocardial infarction cannot be excluded.

It is possible that the absence of nickel dimethylglioxim complexes in the necrotic areas can be attributed to a significant reduction or complete cessation of local circulation, however, it can also be the consequence of the hypernickelemia due to a release of nickel from the necrotic areas developing after myocardial infarction. The method discussed seems appropriate for the distinction between ischemic and necrotic areas in the myocardium. It must be emphasized, that the reaction is positive even in borderline lesions with minimal swelling of the mitochondria and compensatory appearance of glycogen particles. Referring to the results of other studies, the nickel dimethylglioxim complex is considered to be relatively stable, it is produced even under autolytic conditions, the reaction is interpretable and therefore its application is recommended in the field of forensic pathology [1, 2, 3].

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