Z Rechtsmed (1983) 90 : 7–14

Zeitschrift für
Rechtsmedizin

Electron-cytochemical Detection of Endogenous Nickel in the Myocardium in Acute Carbon Monoxide Poisoning

Applicability of a New Cytochemical Technique in Forensic Medicine

I. Balogh[1], E. Somogyi[1], P. Sótonyi[1], G. Pogátsa[3], G. Rubanyi[2], and E. Bellus[1]

[1] Dept. of Forensic Medicine, Semmelweis Medical School, Üllöi ut. 93, H-1450 Budapest, Hungary
[2] Dept. of Physiology, Experimental Research Laboratory, Budapest, Hungary
[3] National Institute of Cardiology, Experimental Research and Diagnostic Board, Budapest, Hungary

Summary. After acute carbon monoxide poisoning (inhalation, dogs; perfusion, rats; postmortem, humans) endogenous nickel was cytochemically detected in heart muscle by dimethylglioxime reaction. Dimethylglioxime cytochemical reaction, in each case at the COHb level, was always positive in experiments and in postmortem samples. A nickel ion accumulation in the heart muscle above 30 rel.% of COHb was suggested. A possible role of nickel ion in the pathomechanism of the acute carbon monoxide poisoning was supposed. This dimethylglioxime cytochemical technique is applicable in forensic medical practice, primarily because it is not disturbed by autolysis.

Key words: Nickel cytochemistry – Acute carbon monoxide – Poisoning, heart muscle

Zusammenfassung. Bei unterschiedlicher Einwirkung von Kohlenmonoxid (Inhalation, Perfusion, post mortem) wurde endogenes Nickel im Herzmuskel histochemisch durch die Dimethyl-Glyoxim-Reaktion nachgewiesen. Die histochemische Reaktion ist für den morphologischen Nachweis ab 30% COHb geeignet. Die Methode ist für die Routinearbeit zu empfehlen, zumal die Reaktion nicht durch Autolyse gestört wird.

Schlüsselwörter: CO-Vergiftung, endogener Nickel im Myocard – Nickel, bei CO-Vergiftung im Myocard

Introduction

A new cytochemical reaction for subcellular localization of nickel was published previously by Balogh and colleagues [3, 4, 9, 10], which proved to be a valuable

Offprint requests to: I. Balogh, MD (address see above)

tool in the visualization of exogenous $NiCl_2$ penetration into intracellular space and its distribution. It is well-known that much Ni is released under hypoxic-ischemic conditions [11]. On the other hand, up until recently electron microscopy had been of strictly limited importance in routine postmortem forensic medical practice because of the necessity of procedures to fix well. For a critical evaluation, however, the restricted utilization of electron microscopy depends to a great extent upon the characteristics of forensic pathology.

The reasons of the investigation were (1) Investigation of endogenous nickel accumulation and/or release in the heart muscles of rats and dogs poisoned experimentally by carbon monoxide, (2) detectability of nickel using dimethylglioxime cytochemical reaction in postmortem human heart muscle, and (3) evaluation of an autolyzing effect upon the nickel-dimethylglyoxime complex-forming process in tissuly circumstances.

Materials and Methods

Rat hearts ($n=20$) perfused according to Langendorff with a solution of 45.3% CO + 4.7% CO_2 (Krebs-Henseleit bicarbonic buffer) and dog hearts taken from animals ($n=20$) poisoned by inhalation of carbon monoxide have been investigated.

Human postmortem heart samples ($n=40$) removed from cadavers 30 min–4 days after death have been tested. Electroncytochemically nickel localization was performed by the following technique: After fixation of the tissues in 2.5% glutaraldehyde they were incubated for 10 min in 0.1% dimethylglioxime (Renal) dissolved in 70% ethanol. Then followed fixation in 1% osmium tetroxide. After dehydration the tissues were embedded in Durcupan (Fluka ACM), and ultrathin sections were cut by a Reichert U OM2 ultratome. The examination was performed on an JEOL 100B electron microscope at 60 kV acceleration voltage. Analysis of the composition of the reaction product was carried out by electron probe-energy dispersive microanalysis (ORTEC) and X-ray diffraction. Controls were extracted by neutral chloroform. Blood COHb content was recorded simultaneously according to Wolff and Maehly.

Results

A striking endogenous nickel ion accumulation was noted in the heart muscles of rats and dogs poisoned by carbon monoxide. Nickel-dimethylglioxime particles with a concentrically located electron-dense structure could be seen in the myocardium (Figs. 1–4), capillary walls, and in the lumen of the capillary (Figs. 9–10). Nickel-dimethylglioxime particles were observed intramitochondrially too (Figs. 5–8). In each case at the COHb level above 30 rel.%, the dimethylglioxime reaction was always positive in experiments and in the postmortem samples. In autopsy materials a highly autolyzed myocardium was noted while a striking density of nickel-dimethylglioxime complexes was detected (Figs. 13–16) while using only dimethylglioxime in the control perfusion solution nickel particles could be observed (Fig. 12). The nickel particles attached to dimethylglioxime rods could be removed with chloroform extraction (Fig. 11). A specific Ni-peak could be demonstrated in the energy spectrum obtained by electron microanalysis indicating the specificity of the cytochemical reaction. Similar results were observed by the X-ray diffraction analysis where comparison of distances

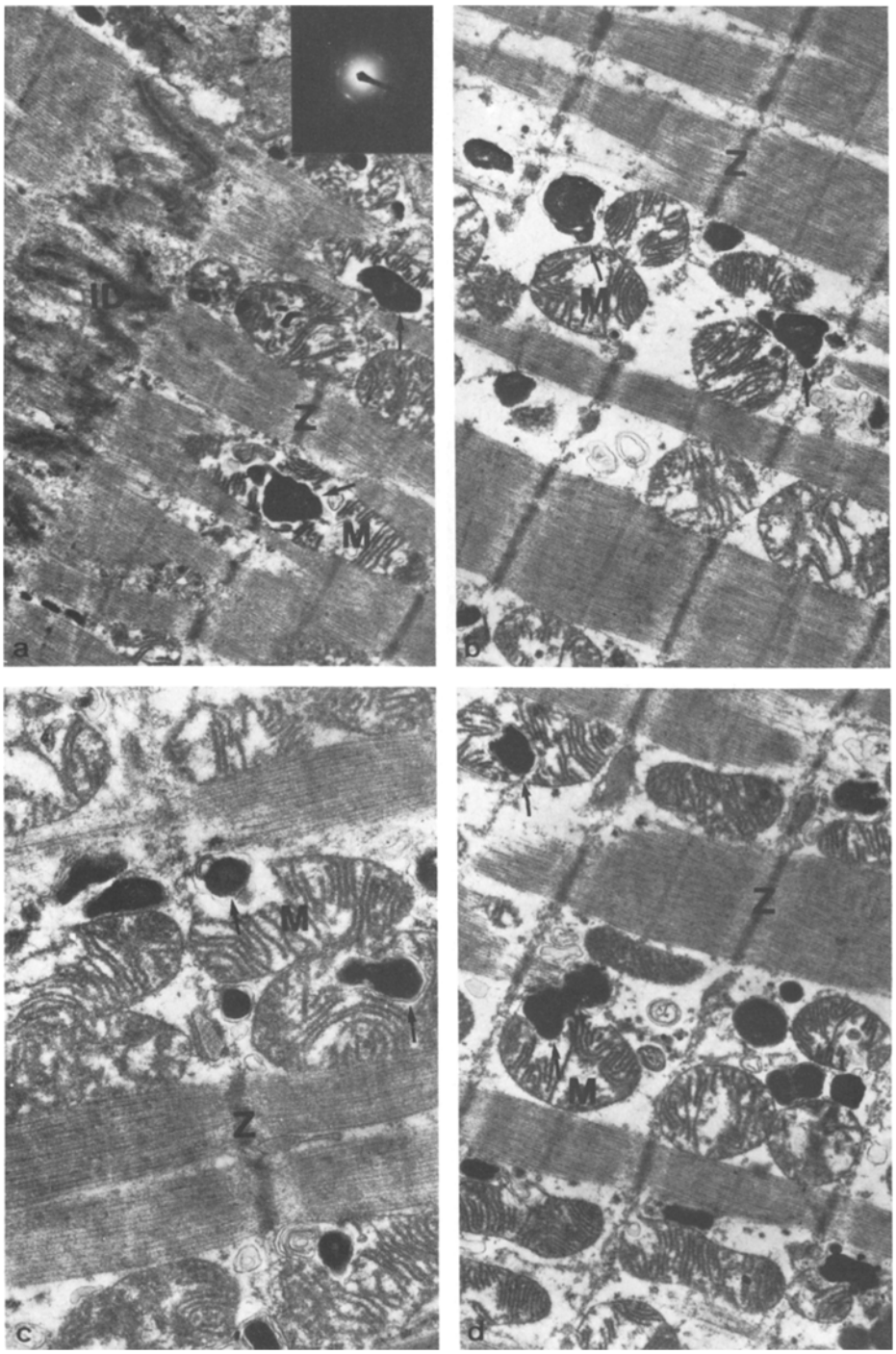

Fig. 1a–d. Dog heart muscle. CO-poisoning. **a** COHb: 35 rel.%, ×18,000. Inset: X-ray diffractogram of Ni-dimethylglioxime complexes. **b** COHb: 42 rel.%, ×18,000. **c** COHb: 50 rel.%, ×24,000. **d** COHb: 63 rel.%, ×18,000

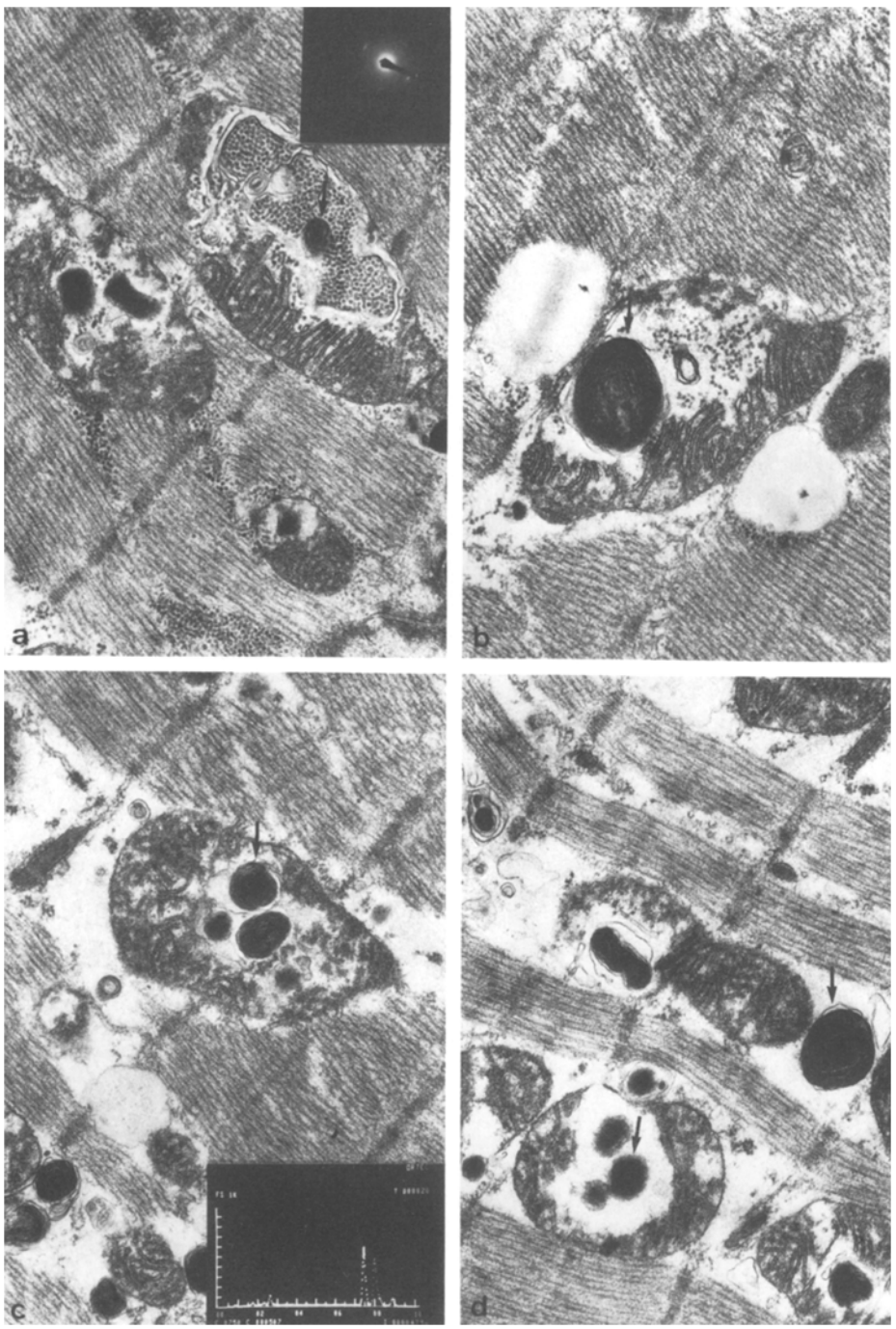

Fig. 2a–d. Dog heart muscle. CO-poisoning. **a** COHb: 42 rel.%, × 24,000. Inset: X-ray diffractogram of Ni-dimethylglioxime complexes. **b** COHb: 50 rel.%, × 24,000. **c** COHb: 63 rel.%, × 24,000. Inset: Energy-dispensive analysis. **d** COHb: 83 rel.%, × 24,000

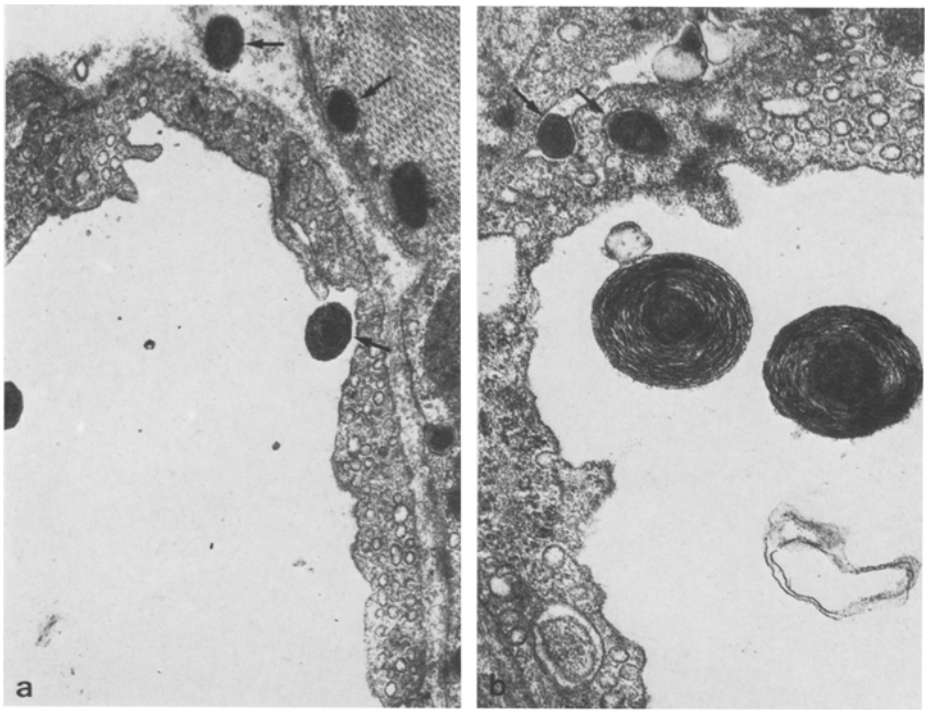

Fig. 3a, b. Dog heart muscle. CO-poisoning. **a** COHb: 42 rel.%, ×24,000. **b** COHb: 63 rel.%, ×36,000

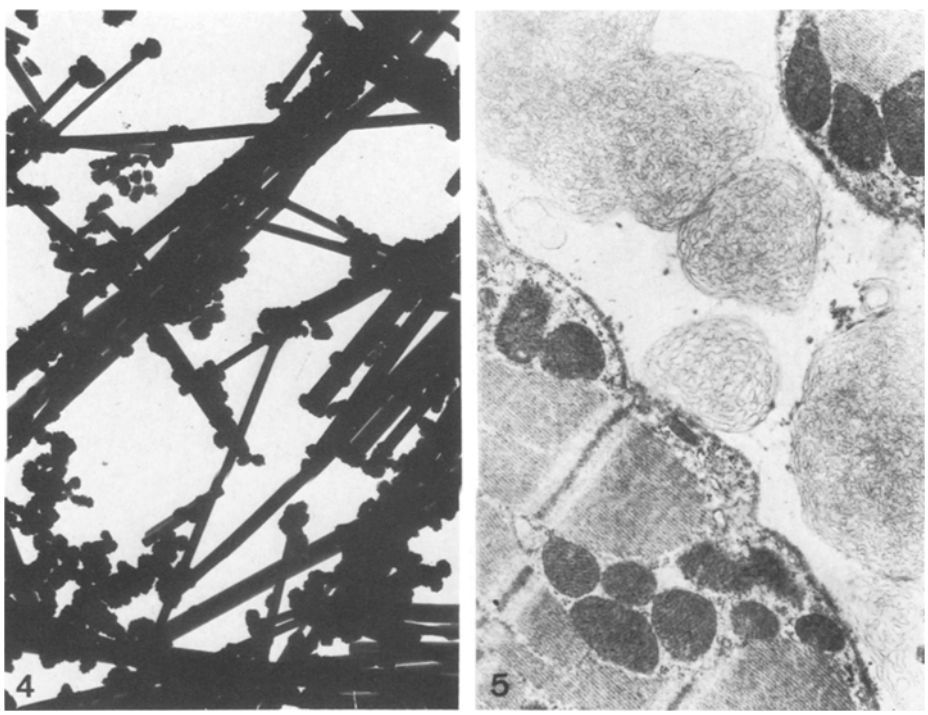

Fig. 4. Nickel-dimethylglioxime rods. ×12,000

Fig. 5. Only dimethylglioxime-perfused rat heart. Dimethylglioxime network could be seen without any nickel particles. ×18,000

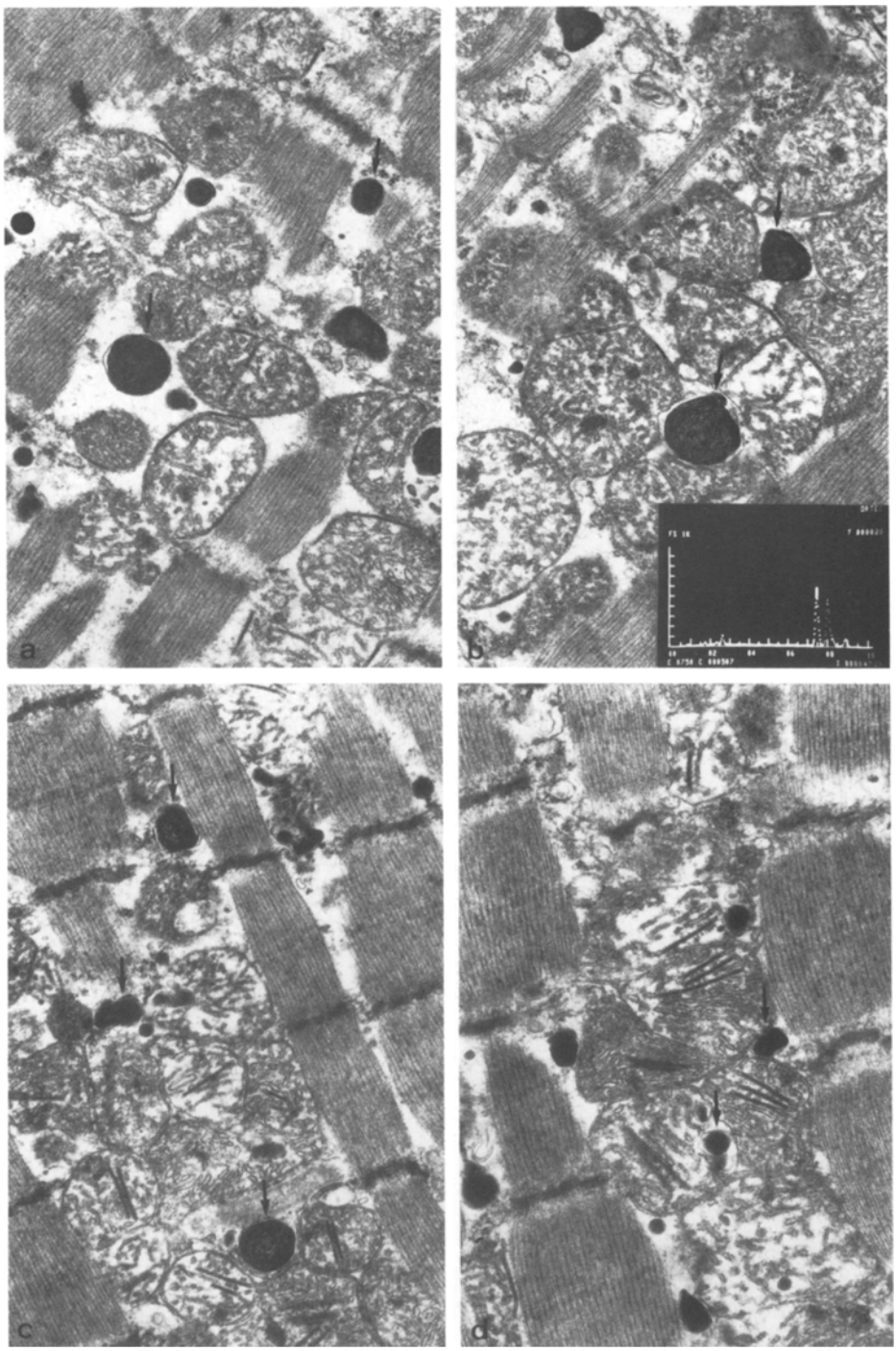

Fig. 6a–d. Postmortem human cardiac biopsy. CO-poisoning. **a** COHb: 57 rel.%, ×14,000. **b** COHb: 78 rel.%, ×24,000. Inset: Energy-dispersive analysis. **c** COHb: 38 rel.%, ×24,000. **d** COHb: 40 rel.%, ×30,000

between concentric circles and the ASTM values demonstrated the specificity of the reaction product for Ni (Figs. 1a, 5a, 7a, 14a).

Discussion

Myocardial alteration of Z-band, intercalate discs, and muscle fibers; an inhibition of cytochrome oxidase activity; loss of adenine nucleotides, particularly adenosine triphosphate and creatin phosphate; increase of intracellular calcium and that of adenylate cyclase activity have been observed within the first few minutes of acute carbon monoxide poisoning [1, 2, 6, 12]. Besides CO-hemoglobin association the binding of CO to cytochrome is one of the most significant factors in the pathogenesis of acute carbon monoxide poisoning [12]. Using dimethylglioxime after exogenous nickel load [3, 4, 9, 10] and in pathological processes endogenous nickel accumulation was cytochemically demonstrated [5, 7, 8]. Following myocardial ischemia, burn, hepatic disease, and hypertension serum nickel concentration increased significantly in humans. The newly developed dimethylglioxime cytochemical technique proved to be a valuable tool in the visualization of endogenously accumulated and/or released nickel content in the myocardia of experimentally poisoned rats and dogs as well as the heart muscles of humans poisoned by carbon monoxide. The specificity of the reaction product was verified by energy-dispersive and X-ray diffraction. It is well-known that protein enzymes—causing cytochemical reaction—have to be autolyzed very rapidly after death. However, using dimethylglioxime, a purley chemical relationship between Ni and dimethylglioxime was revealed; autolysis has thus no effect on the nickel-dimethylglioxime complex formation. This cytochemical reaction is usable even in forensic medical practice, primarily because autolysis has no effect on the method.

References

1. Balogh I, Sótonyi P, Somogyi E (1975) Effect of experimental carbon monoxide poisoning on the heart muscle. Acta Morphol Acad Sci Hung 23 : 165–172
2. Balogh I (1976) Electron-microscopic investigation of myocardial lesions induced by coal-gas poisoning. Morphol Igazsagugyi Orv Sz 16 : 85–92
3. Balogh I, Rubányi G, Somogyi E, Kovách AGB, Sótonyi P, Héjj G (1979) Effect of nickel ions on the perfused rat heart ultrastructure. Morphol Igazsagugyi Orv Sz 19 : 104–110
4. Balogh I, Rubányi G (1980) Electron-microscopic localization of nickel in the myocardium, coronary and uterine smooth muscle. In: Spurenelemente, Ni-As. Jena, pp 129–135
5. Balogh I, Rubányi G, Kovách AGB, Sótonyi P, Somogyi E (1981) Electron-cytochemical detection of nickel ions under pathological conditions in the rat heart. Acta Morphol Acad Sci Hung 29 : 87–90
6. Balogh I, Rubányi G, Schulze W, Kovách AGB, Sótonyi P, Somogyi E (1981) Electron-cytochemical studies on changes of the adenylcyclase activity following experimental hypoxia and ischemia in perfused rat hearts. Exp Pathol 20 : 121–127
7. Balogh I, Somogyi E, Rubányi G (1982) Nickel cytochemistry: applicability of a new cytochemical technique. Proc XIIth Congr Int Acad Forens Soc Med, Vienna, pp 459–464

8. Balogh I, Rubányi G, Sótonyi P, Kovách A, Somogyi E (1981) Possibilities for electron-cytochemical detection of nickel ions under certain pathological conditions. In: Elektronenmikroskopie. Leipzig, pp 160-161
9. Kovách AGB, Rubányi G, Balogh I (1979) Effect of nickel ions on contractility, metabolism, coronary resistance and ultrastructure of isolated rat heart. Bol Soc Port Cardiol [Suppl 2] 17 : 269-285
10. Rubányi G, Balogh I, Somogyi E, Kovách AGB, Sótonyi P (1980) Effect of nickel ions on ultrastructure of isolated perfused rat heart. J Mol Cell Cardiol 12 : 609-618
11. Rubányi G (1980) Effect of calcium and nickel ions on oxidative and glycolytic metabolism in the perfused rat heart. Adv Physiol Sci 8 : 139-147
12. Somogyi E, Balogh I, Rubányi G, Sótonyi P, Szegedy L (1981) New findings concerning the pathogenesis of acute carbon monoxide (CO) poisoning. Am J Forensic Med Pathol 2 : 31-39

Received September 6, 1982