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Differentiation of histochemically visualized mercury and silver

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Summary

Accumulations of silver and mercury can be visualized in tissue sections by a technique called autometallography or physical development. In order to make a histological differentiation between mercury and silver in tissue exposed to both metals, it is necessary to remove one of the metals while leaving the other untouched. The present paper describes a technique by which silver accumulations in histological sections can be removed by potassium cyanide, yet leaving mercury accumulations intact to be developed autometallographically.

Introduction

A new tool for studying the light and electron microscopical localization of silver and mercury has recently been introduced (Danscher, 1984). The technique, the basic principle of which has been known for many years by the inappropriate name of physical development, allows tiny amounts of silver and mercury in mammalian tissue to be silver amplified (Liesegang, 1928; James, 1939; Timm, 1962; Danscher, 1981b; Danscher & Møller-Madsen, 1985).

It is a prerequisite that silver is present in the tissue as either metallic silver, silver sulphide, or silver selenide, and mercury as either mercury sulphide or mercury selenide (Timm, 1962; Danscher, 1981b, 1982; Danscher & Møller-Madsen, 1985). Of these forms selenides and sulphides of the two metals are supposed to be the main chemical compounds present in living tissues (Dempsey, 1973; Shirabe, 1979; Aaseth *et al.*, 1981; Danscher & Møller-Madsen, 1985).

Accumulation of mercury or silver in the forms mentioned above catalyses the reduction of silver ions to metallic silver if reducing molecules (e.g. hydroquinone) are present simultaneously. When tissue sections are exposed to physical development/autometallography a solution containing silver ions and reducing molecules penetrates them. Silver ions and reducing molecules adhere to the surfaces of the catalytic specks (e.g. mercury sulphide accumulations) and electrons travel from the reducing molecules through the speck to the adhering silver ions and transform them to metallic silver atoms. The metal accumulation thus acts as an electrode. During the developing process, layer after layer of silver surrounds the catalyst until it becomes electron dense enough to be seen in the electron microscope or big enough to be observed in the light microscope.

Under experimental conditions it is easy to exclude one of the metals, but if an organism is at the same time exposed to both silver and mercury, differentiation of the autometallographic patterns has been impossible. This problem was aggravated by the fact that the two metals, to some extent, accumulate in identical cell types and organelles.

In the present study a chemical removal of silver from the tissue sections before autometallography permitted a differentiation of silver and mercury present in the sections.

Materials and Methods

Twelve male Wistar rats (weighing 100–110 g at the initiation of heavy metal treatment) were kept in batches of four under standard laboratory conditions. One batch received daily injections of 200 μg $HgCl_2$ (200 μg/ml distilled water) for ten days. Silver treatment was given to another batch as daily injections of 2 mg silver lactate (2 mg/ml distilled water) for ten days. The third batch was treated with both silver lactate and mercury chloride in the above mentioned doses, and for the same period of time. Two additional animals having received mercuric chloride and silver nitrate, respectively, in the drinking water were analysed.

The animals were sacrificed under nembutal anaesthesia by transcardial perfusion. The perfusion was performed with 2% glutaraldehyde and 1% formaldehyde in a 0.75 M phosphate buffer for 10 min. The liver, both kidneys and the spinal cord were removed and immersed in the same fixative.

Fixed tissue blocks were embedded in Epon 812 or paraffin according to conventional procedures. Epon sections were cut at 1–2 μm and paraffin sections at 5 μm. Immediately after cutting,

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parallel sections were immersed in either distilled water or a 1% aqueous potassium cyanide solution for 2 h. Sections were then mounted on glass slides, rinsed in distilled water and exposed to autometallographic development for 60 min according to the procedures described by Danscher (1981b) and Danscher & Møller-Madsen (1985). Finally, sections were counterstained with Toluidine Blue or Haematoxylin and Eosin. Electron microscopic sections were obtained either by reembedding the developed Epon sections into new blocks of Epon, after which trimming and cutting of ultrathin sections could be performed, or by direct development of the ultrathin sections (Danscher, 1984). The ultrathin sections were counterstained with lead acetate for 2 min and uranyl acetate for 30 min.

Epon sections 5 μm thick were used for the particle induced X-ray emission silver analyses. Six such sections were placed on a nucleopore filter material membrane. The frame was placed in a proton beam, and emission spectra were computer-analysed as described by Danscher *et al.* (1985).

Results

Sections of kidney (Figs. 1, 7), spinal cord (Fig. 3) and liver from rats intravitally exposed to silver contained silver accumulations that could be autometallographically visualized. The presence of silver in the tissues was verified by multielement analysis of parallel sections not exposed to autometallography (Fig. 11).

When tissue sections from a silver exposed animal were treated with potassium cyanide prior to being subjected to autometallography the total lack of silver grains showed that all traces of silver had been removed (Figs. 2, 4, 8). This result was confirmed by particle induced X-ray emission analyses of potassium cyanide treated, but not autometallographically developed sections (Fig. 11).

In mercury treated rats, sections from kidney, spinal cord, and liver contained accumulations of mercury sulphide or mercury selenide that could be autometallographically developed (Fig. 5). If such sections were exposed to potassium cyanide prior to being autometallographically developed they showed no qualitative changes in localization nor could any decrease in number and size of the amplified mercury be seen (Fig. 6). The catalytic mercury accumulations in the sections thus appeared unaltered by the potassium cyanide treatment.

Sections from animals treated with both silver and mercury demonstrated autometallographic patterns corresponding to a simultaneous presence of both metals (Fig. 9). Treatment with cyanide ions resulted in an altered stainability corresponding to the removal of silver catalysts from the sections (Fig. 10). By subtraction of the two autometallographic patterns the localizations of silver and mercury, respectively, can be deduced.

Discussion

The silver amplification method of autometallography is an extremely sensitive photographic technique that makes it possible to visualise silver accumulations at the size of 3–6 atoms and zinc accumulations as small as two molecules of zinc sulphide (James, 1977). The minimum number of mercury sulphide molecules that can be detected by this method is not, to our knowledge, known, but might be less than 10. The possibility of analysing the precise localization of the two metals in quantities 100–1000 times less than can be detected by other current methods (neutron activation analysis, proton-induced X-ray emission, atomic absorption spectrophotometry, electron emission X-ray spectrophotometry) makes the technique unique. Autometallography, with the present

Fig. 1. Photomicrograph of a 5 μm thick paraffin section of kidney cortex from a rat treated with intraperitoneal silver lactate injections. The section has been autometallographically developed and counterstained with Toluidine Blue. Basement membranes in the glomeruli and the luminal surfaces of the tubuli contain grains of amplified silver accumulations. × 450.

Fig. 2. Paraffin section, 5 μm thick, from the same tissue block as Fig. 1. This section was exposed to potassium cyanide prior to autometallography. The section is void of silver grains. Toluidine Blue counterstain. × 450.

Fig. 3. Motor neurones of the spinal cord from a silver treated rat. After autometallography silver is seen in the perikarya and in vessel walls. 5 μm paraffin section, Toluidine Blue counterstain. × 330.

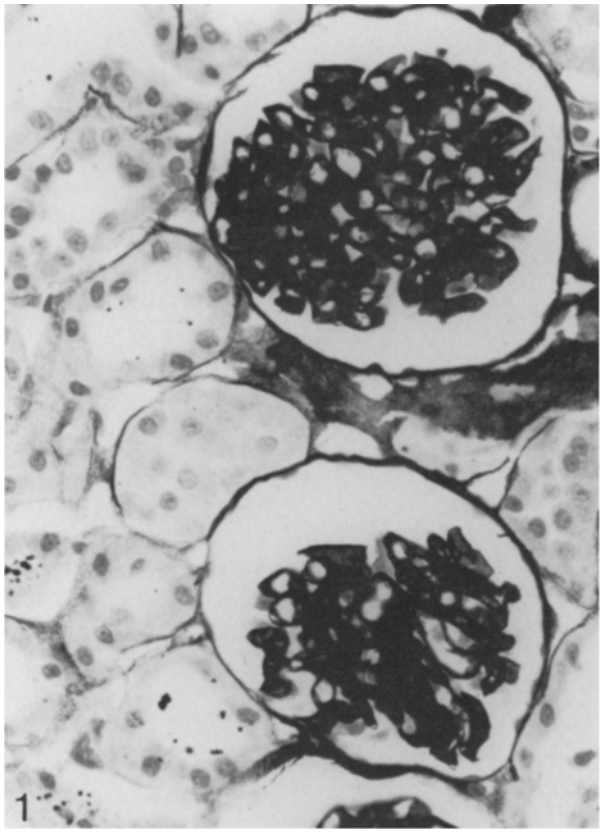
Fig. 4. Section from the same tissue block as Fig. 3 but subjected to potassium cyanide prior to silver amplification. The cyanide treatment has removed all traces of silver. 5 μm paraffin section, Toluidine Blue counterstain. × 330.

Fig. 5. Autometallographically developed section of kidney from a rat treated with mercury chloride. Grains of developed mercury traces are located primarily intracellularly, especially in the proximal tubules. The glomerular basement membrane is void. Paraffin embedded tissue cut at 5 μm and counterstained with Toluidine Blue. × 400.

Fig. 6. Section from the same tissue block as shown in Fig. 5. This section was treated with potassium cyanide prior to development. However, the localization and numbers of amplified mercury grains are comparable to those seen in Fig. 5. 5 μm section, Toluidine Blue counterstain. × 400.

Fig. 7. Electron micrography of part of a renal glomerulus from a silver treated rat. The developed silver grains are located throughout the glomerular basal laminae. Counterstained with lead and uranyl acetate. × 9000.

Fig. 8. Electron micrography showing part of a glomerulus from the same kidney as shown in Fig. 7. The developed survey section was a 2 μm thick Epon section cut in parallel. The present section was treated with potassium cyanide before being exposed to autometallography. The section is void of silver grains. Lead and uranyl acetate counterstain. × 11 000.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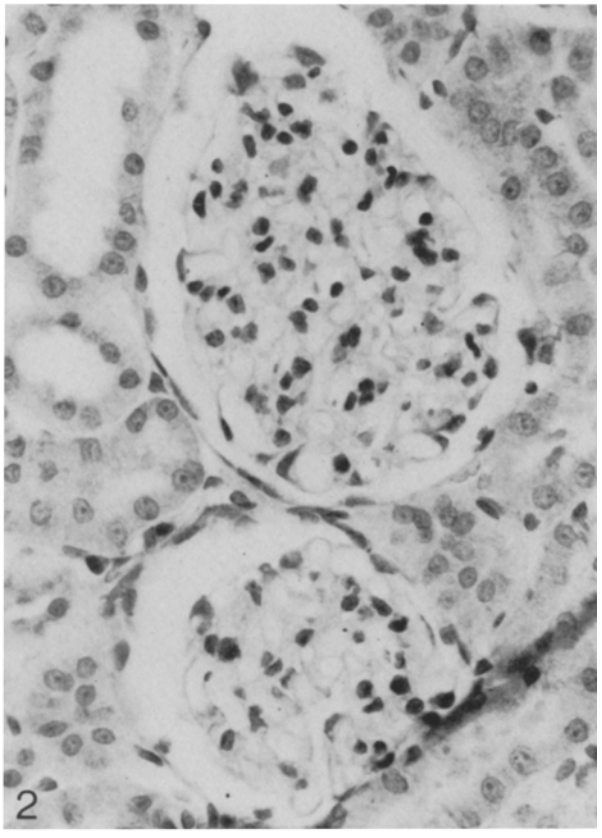
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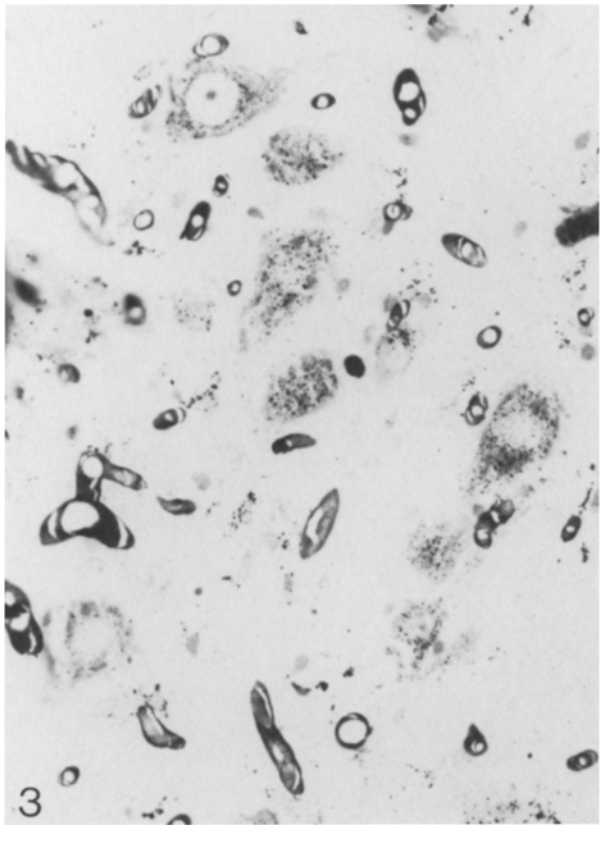
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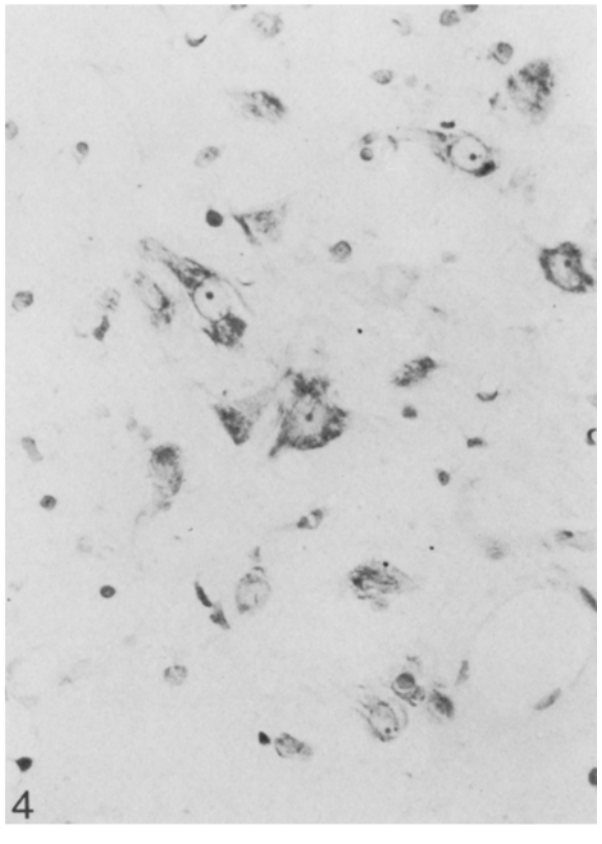
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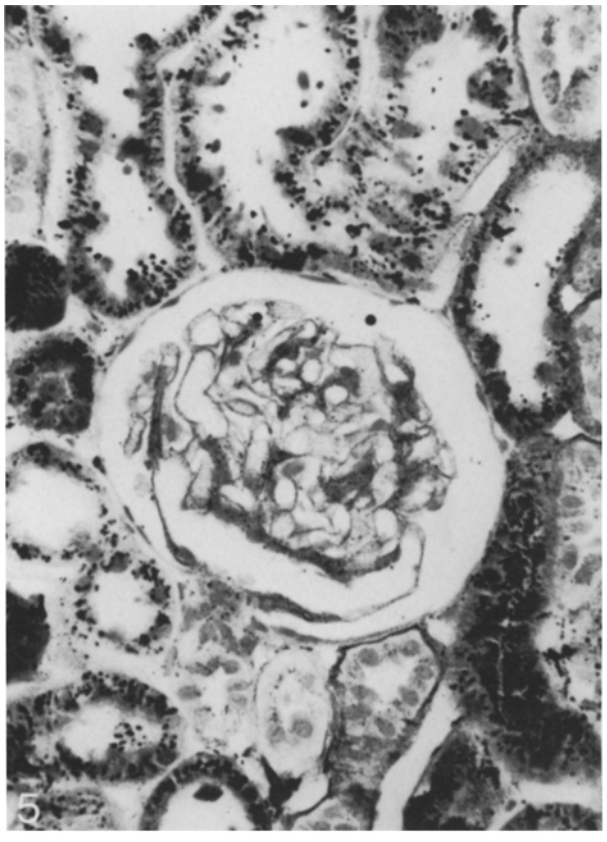

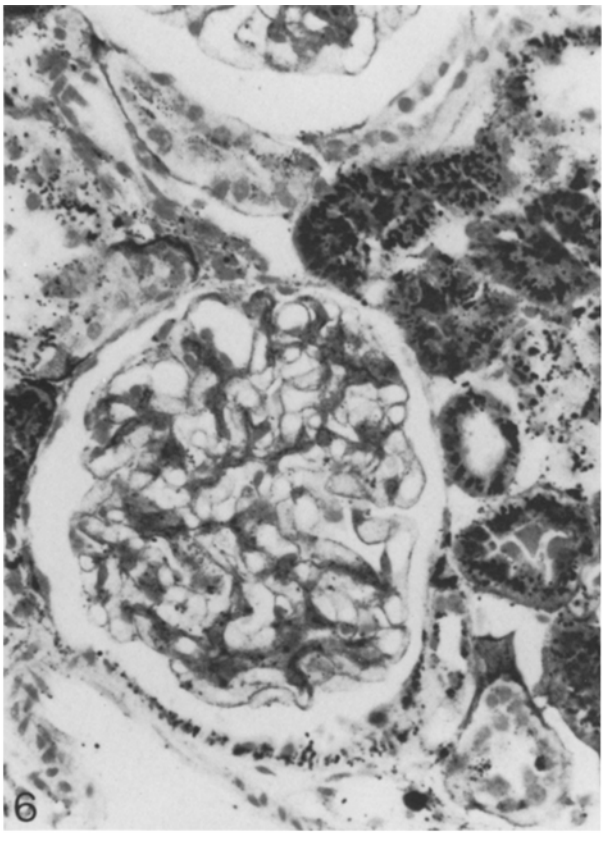

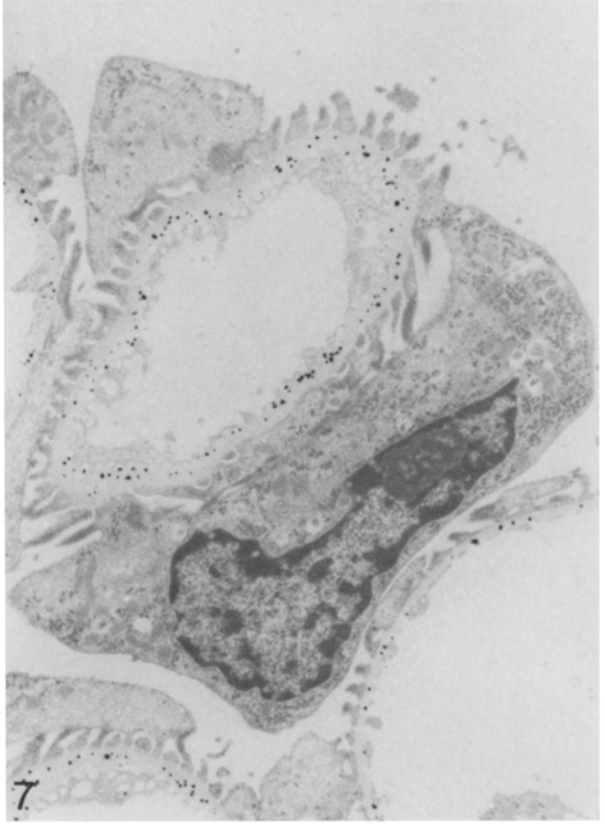

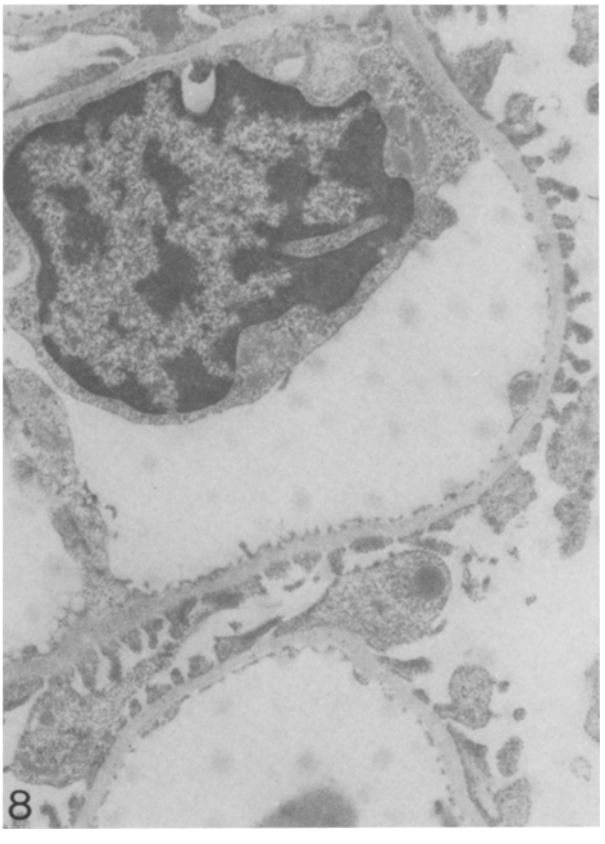

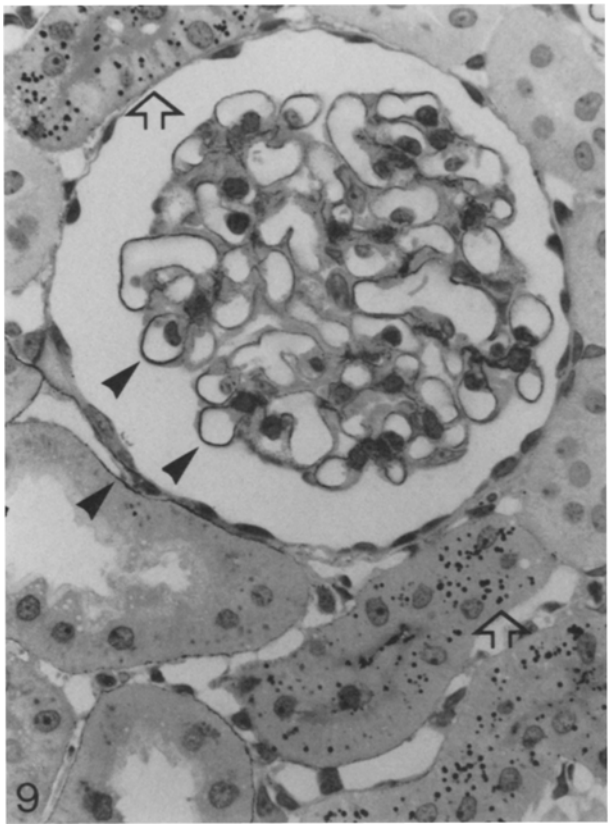

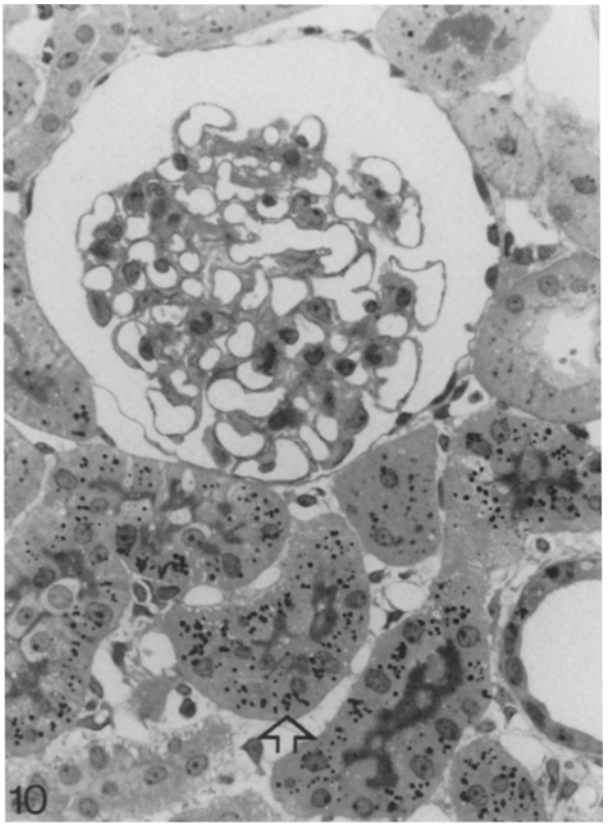

Fig. 9. Photomicrograph of a 2 μm thick Epon kidney section from a rat treated with both silver and mercury. After development grains are seen in glomerular and tubular basement membranes (arrowheads) corresponding to silver accumulations. In cells of the proximal tubuli (open arrows) grains are located corresponding to mercury accumulations. Toluidine Blue counterstain. × 500.

Fig. 10. Photomicrograph of a 2 μm Epon section obtained from the same tissue block as shown in Fig. 9. This section was cyanide treated before autometallography resulting in the disappearance of the staining in the basement membranes (silver) whereas the intracellular (mercury) traces (open arrow) were left untouched. Toluidine Blue counterstain. × 500.

modification, represents a useful tool for both localizing metal accumulations and as a guide for further analysis of the cell organelles in which the metals accumulate.

The necessity for a method that can distinguish between the two metals is underlined by the fact that they have previously been demonstrated to coexist in e.g. human brain tissue, resulting in difficulties in interpreting autometallographic sections (Rungby *et al.*, 1983).

Metals and metal molecules other than silver and mercury sulphide can be amplified. Treatment of tissue with sodium selenide either intravitally or by perfusion (Danscher, 1982) or exposure to sodium sulphide (Timm, 1958; Danscher, 1981a) will cause metal ions in the tissue (in the brain primarily zinc; Danscher *et al.*, 1985) to precipitate as crystals of metal selenides or sulphides. These crystals can be visualized by the autometallographic procedure.

If potassium cyanide is used to remove silver, all metals but mercury that could possibly be precipitated by sulphide or selenide ions will be removed. Catalysts left over in the tissue can then be either mercury sulphide or metallic gold, since they are equally insoluble. It is impossible to differentiate between these two compounds without the aid of a specific physical analytical method, and a prerequisite then is that the amounts accumulated are large enough to be recognized. Removing mercury accumulations by exposing sections to sodium sulphide solutions or by evaporating mercury by placing sections in vacuum (10^{-5} torr) has been tried with some success (Danscher & Møller-Madsen, 1985). Gold is, however, present in extremely small amounts in the human organism unless the tissue is taken from patients treated with gold salts for rheumatic diseases.

Accumulations of zinc sulphide or copper sulphide can be separated from accumulations of silver and/or mercury sulphide by treating the sections with weak acids (Timm, 1958; Danscher, 1984). Such compounds may exist if the tissue undergoes cadaverous changes before being fixed (Danscher & Zimmer, 1978).

By introducing a two-step procedure, i.e. treatment of sections first with weak acids and thereafter with potassium cyanide, these confounding factors can be eliminated.

In conclusion, silver and mercury are often co-existent in tissues because of environmental, industrial or medical (Amalgam fillings) exposure. The present method provides

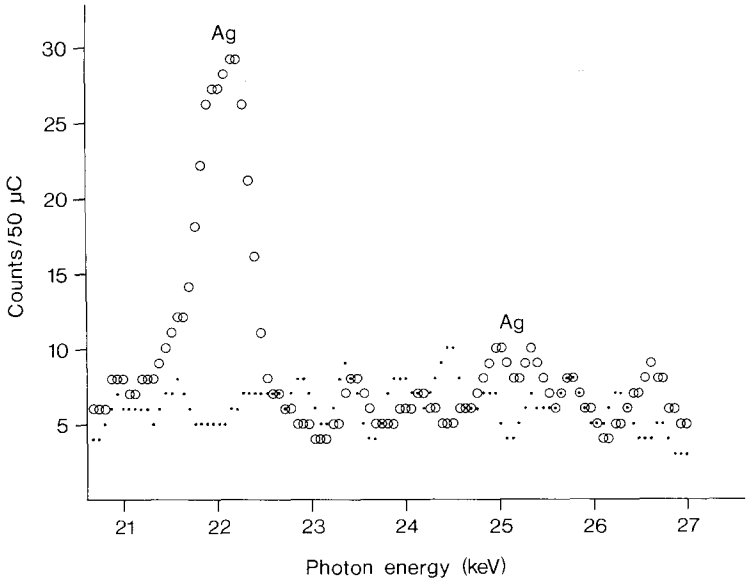

Fig. 11. Particle induced X-ray emission analyses of 5 µm thick Epon sections from the kidney of a rat treated with silver lactate. None of the analysed sections was exposed to autometallography. Circles indicate readings from sections *not* exposed to potassium cyanide. Dots are readings from parallel sections treated with potassium cyanide. In the cyanide treated sections no silver is present, indicating a removal of the metal by potassium cyanide.

a means for clearly differentiating the two metals in histological sections at both light and electron microscopical levels.

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