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Energy dispersive X-ray analysis of tissue gold after silver amplification by physical development

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Summary. Rats were treated intraperitoneally with the gold-containing compounds sodium aurothiomalate (Myocrisin), sodium aurothiosulfate (Sanocrysin), and aurothioglucose. Using stem energy dispersive X-ray analysis, gold and silver were shown to be located at the same point in lysosomes of proximal tubular cells of the kidney, in hepatocytes and in macrophages of lymph glands, spleen and liver. This result indicates that, after exposure to ultraviolet radiation, chemically bound tissue gold is transformed to metallic gold that subsequently can catalyze the reduction of silver ions to silver when subjected to physical development, i.e. exposed to a photographic developer containing silver ions in addition to the reducing molecules.

Introduction

The possibility of encapsulating metallic gold in silver was already realized in the 19th century (James 1977). Since Roberts (1935) first published his procedure for the light microscope visualization of gold in tissue by physical development, a series of modifications and improvements to his method have been made (Querido 1947; Gilg 1952; Doré and Vernon Roberts 1976; Danscher 1981b). It is now possible to use this technique at the electron microscope level (Danscher 1981b). The rate of physical development catalyzed by Ag, Au, Ag_2S, Au_2S_3 has been thoroughly analyzed (Shuman and James 1971; Faelens and Tavernier 1962). Arens and Eggert (1929) found that, after the same period of development, silver, gold and silver sulfide nuclei caused the deposition of equal amounts of silver. This observation is not invalidated by the fact that the catalytic activity is not uniquely determined by the electronic properties of the catalytic sites since other factors like absorption of reducing molecules and/or silver ions to the surface of the catalytic site might also influence the rate of activity (Shuman and James 1971). As 3–6 atoms of silver are sufficient to initiate a silver reduction (James 1977) and since gold and silver have the same catalytic capacity (Shuman and James 1971) the photochemical method for gold detection is extremely sensitive (Danscher 1981b).

In the present study Epon embedded tissue sections from gold treated animals exposed to UV light and silver amplified were analyzed with the aid of scanning transmission electron microscopy (STEM) combined with energy dispersive X-ray analysis (EDX). It was found that gold and silver were located at exactly the same point in the physically developed tissue. This demonstrates that, if animals are exposed to gold compounds, the photochemical method can be used to identify the location of the metal.

Materials and methods

Twelve adult Wistar rats of both sexes were intraperitoneally injected with aurothioglucose, aurothiomalate (Myocrisin), or aurothiosulfate (Sanocrysin). Doses from 0.2–5 mg calculated as pure gold were used. The period of survival was 30 min to 7 months. Controls were treated with 0.9% NaCl instead of a gold compound. The animals were anaesthetized with sodium barbital and killed by transcardial perfusion with 3% glutaraldehyde in 0.1 M Sørensen phosphate buffer (pH 7.4) at a pressure of 120 mm/Hg. Blocks of tissue were embedded in Epon and survey sections were exposed to UV light for a period of 30–60 min.

Physical development. The slides were immersed in a solution of gum arabic, hydroquinone and silver lactate adjusted to pH 3.5 by citrate buffer (Danscher 1981a). After development the sections were counterstained with toluidine blue. Following light microscope analysis, the sections were embedded on a blank Epon block. Ultrathin sections to be analyzed by STEM-EDX were not counterstained with lead- and uranylacetate.

EDX procedure. Using a modified Cambridge 180 scanning electron microscope and a Kevex energy dispersive detector with a 45° inclined Si(Li) crystal in a horizontal detector finger, analysis was performed at 30 KV acceleration potential. The geometry allowed analysis at a distance of 5 mm from specimen to detector. The ultrathin sections (400–700 Å) were mounted on copper grids covered by formvar films. In a specimen carrousel 15 grids could be mounted allowing interchange without breaking vacuum.

The sections to be analysed were not counterstained. In order to facilitate comparison with transmission electron microscope, every second grid in the series was counterstained.

The specimens were mounted on carbon pipes. These were made from carbon rods that had been heated to white glow in order to eliminate the background. The electrons passing through the sections travelled through a carbon pipe below the mounting pipe preventing stray electrons in the specimen chamber.

Analyses were either performed in the spot mode or made with a reduced raster at 50,000 times enlargement. The latter mode proved less damaging to the sections than the former.

Results

X-ray microanalysis of silver amplified gold in lysosomes of cells from kidney proximal tubules (Fig. 1) revealed that gold and silver were present in all middle sized and larger grains. The smallest grains contained only silver.

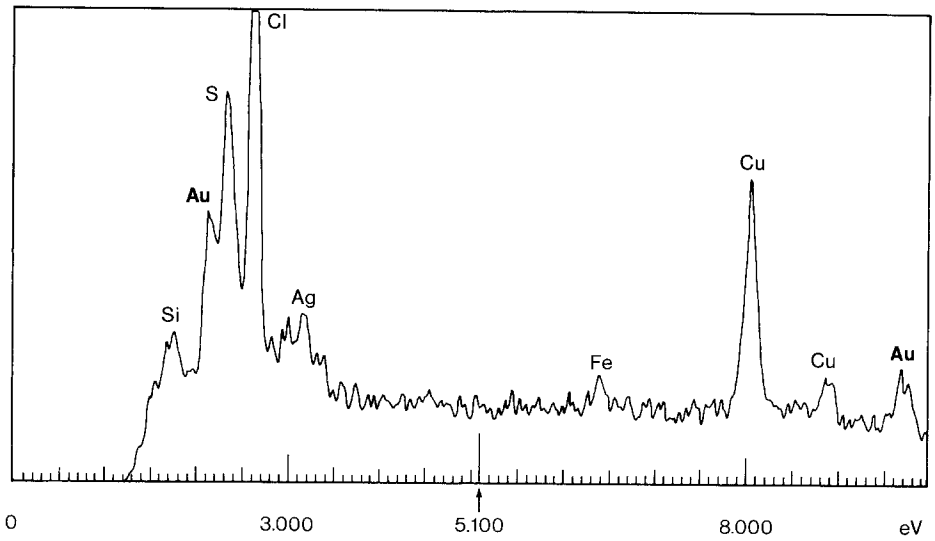

Fig. 1. X-ray spectrum from gold containing lysosome of proximal tubule cells subjected to energy dispersive X-ray microanalysis (EDX). As the gold accumulation has been physically developed prior to analysis silver, amplifying the gold spikes to visible dimensions, is recorded at the same point as gold. *Ag*, silver; *Au*, gold; *Cl*, chloride; *Cu*, copper; *S*, sulphur; *Si*, silicium; *Fe*, iron

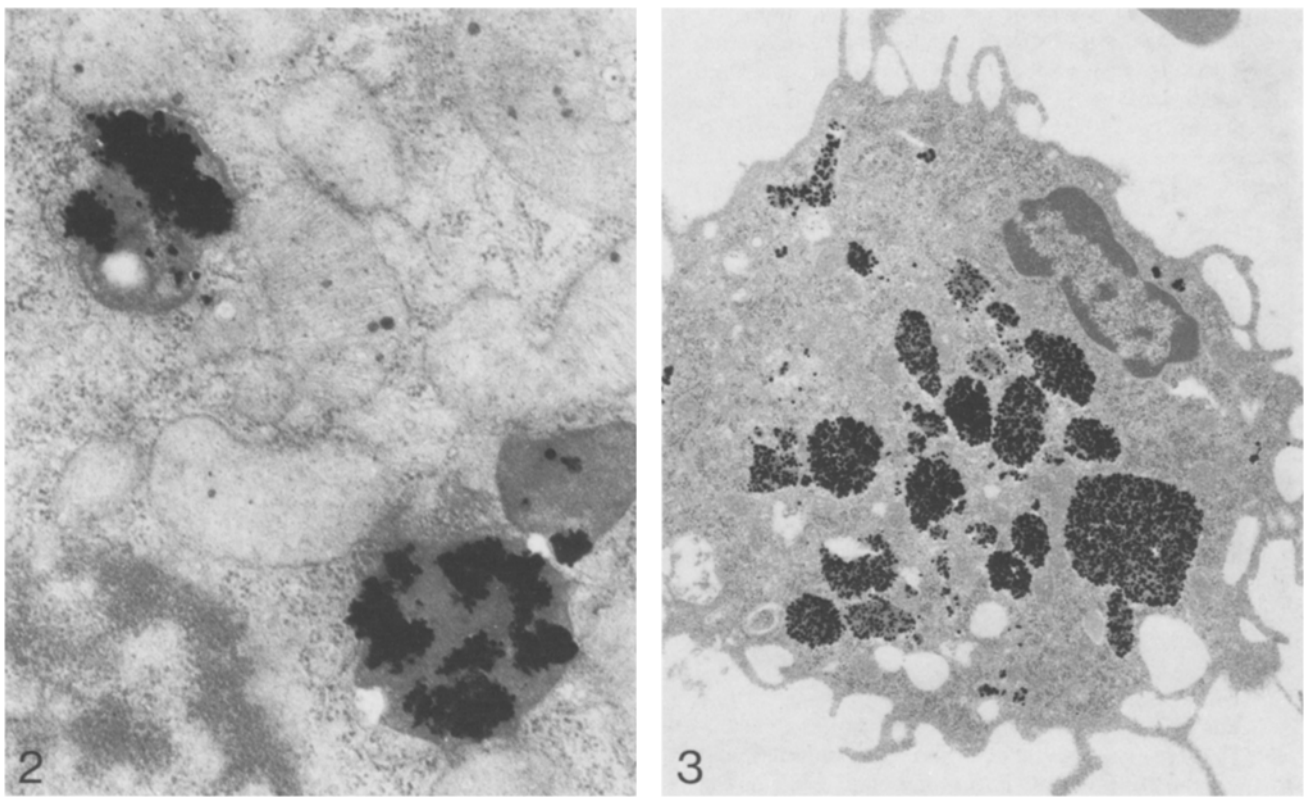

Fig. 2. Electron micrograph of a proximal tubule cell taken from a rat treated with 10 mg aurothiomalate (Myocrisin) and killed after 3 weeks. × 33,600

Fig. 3. Electron micrograph of a macrophage taken from the spleen of a rat treated with 8 mg aurothioglucose. × 12,600

In hepatocytes and in macrophages from spleen (Fig. 2), liver and lymph glands the two noble metals were always located together in the lysosomes.

Gold was recorded at 2140 eV and 9700 eV by its Mα and Lα lines, respectively. Silver was characterized by its Lα and Lβ lines (Fig. 3). In sections from control animals neither gold or silver could be detected.

Discussion

Physical development is the process by which silver ions present in the developing solution are tightly absorbed to the catalyst and subsequently reduced to metallic silver by electron transfer from the reducing agent, which is also present in the developer. The photochemical method for the detection of gold in tissue gives the precise localization of gold deposits (Danscher 1981b). The technique is very sensitive. About 3–6 gold atoms are believed to be capable of initiating the catalytic reduction of silver ions to metallic silver atoms (James 1977; Shuman and James 1971).

The exceptional sensitivity of the procedure explains why the EDX analysis does not detect gold in the small grains. Supposing a 100% relative detection efficiency and

a sensitivity at 10^{-18} g for calcium, the number of Au atoms detectable with EDX can be estimated to be around 3000.

Chemically bound gold ions in the tissue were reduced to metallic gold, by exposing the sections to UV-light, and then silver amplified by the photographic technique. As the size of the precipitates after a given time of development may be directly related to the dimensions of the original catalytic site, the small silver spheres represent gold accumulations probably below the detection limit of the STEM-EDX procedure.

Since STEM-EDX "sees" atoms and not their chemical compound the present technique is unable to differentiate between metallic gold, gold salts, and gold complexes. Only Au^0, and gold ions bound to either S^- or Se^- can be silver amplified (Timm 1958; James 1977; Danscher 1981a, 1982), and in sections not exposed to UV light only a few cells have been found to contain precipitates (Danscher 1981b). Therefore, the majority of detected gold must be present as metallic gold.

Before UV radiation most of the tissue gold must be bound to chemical groups other than sulphide or selenide. The few scattered cells containing lysosomal silver grains seen without prior exposure to UV light represent either gold bound sulphide or selenide ions or free gold (Au^0) reduced in vivo or in the post mortem treatment of the tissues.

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