## Elemental analyses of autoradiographic grains by X-ray microanalysis

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*Summary.* Electron probe X-ray microanalysis has revealed that the autoradiographic grains obtained by high resolution electron microscopic autoradiography contain deposits of lead, silicon and chlorine in addition to silver grains. These results suggest the necessity for the confirmation of the autoradiographic grains by ancillary techniques.

In the previous study[2], distribution of $H^3$-dimetacrine in rat cerebral cortex was examined using electron microscopic autoradiography. A considerable portion of the developed grains was located over the synaptic areas. However, the ultrastructural distribution study of this drug within synaptic areas was not apparent because of the limitation of the tissue-to-grain resolution.

Salpeter and Bachmann[3] indicate that the physical developing system gives better resolution than the chemical developing system. In a preliminary series[4], when autoradiograms of $H^3$-dimetacrine were developed in a Phenidon developer, the resultant grains were smaller dot-shaped particles as compared with Konidol-X, and thus a more highly resolution autoradiography was expected. However, it was very difficult to confirm whether these developed grains were the autoradiographic grains or artifacts produced by the autoradiographic processes. The objective of the present study is to analyze the developed grains of the high resolution autoradiography by electron probe X-ray microanalysis.

*Materials and methods.* Male Wistar rats (200–250 g) were given 500 μg of $H^3$-dimetacrine (1.92 mCi/mg) by the direct lateral intraventricular injection method of Noble et al.[5]. At 1 h after administration, cerebral cortices were fixed in glutaraldehyde (4%, 2 h). The samples were postfixed in osmium tetroxide (1.5%, 1.5 h), dehydrated in graded ethanols and embedded in Epoxy resin of Luft[6]. Autoradiographic procedures used here were described previously[2]; they were based on the method of Salpeter and Bachmann[7]. Thin sections (gray) were coated with Sakura NR-H2 emulsion. After 10 to 20 weeks exposure at 5 °C, they were developed in Phenidon[8] developer or Konidol-X. The energy dispersive microanalyses were done with a Hitachi 12A electron microscope operated at 75 kV with a total beam current of 10–20 μA and equipped with Kevex energy dispersive

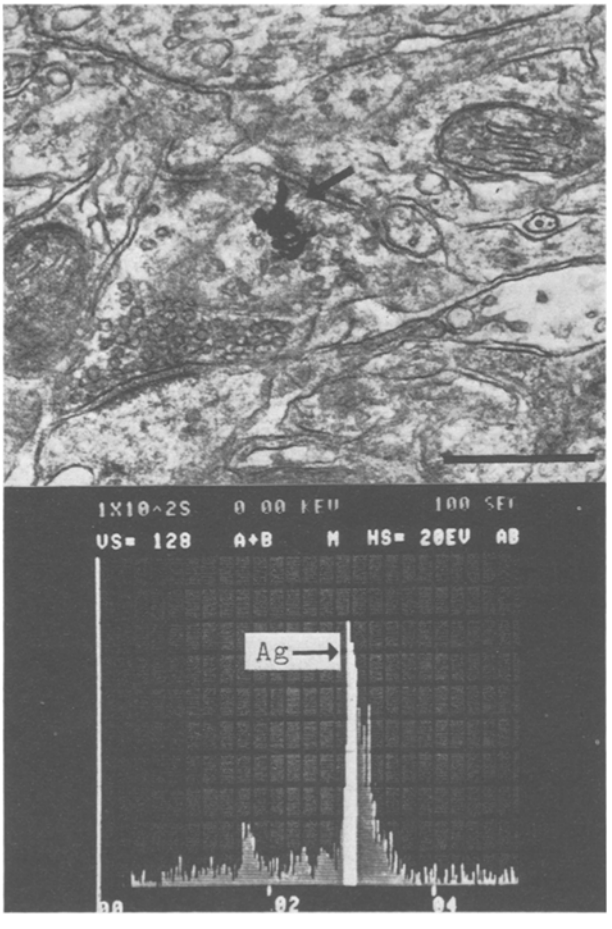


Fig. 1. Elemental spectrum from a autoradiogram. There are prominent peaks of silver ($L_\alpha$ and $L_\beta$; 2.984 and 3.150 keV, respectively). Arrow indicates the probe grain. The abscissa shows the energy of the X-ray lines. Developed in Konidol-X for 4 min at 15 °C. Probe diameter 1700 Å. Bar equals 0.5 μm.

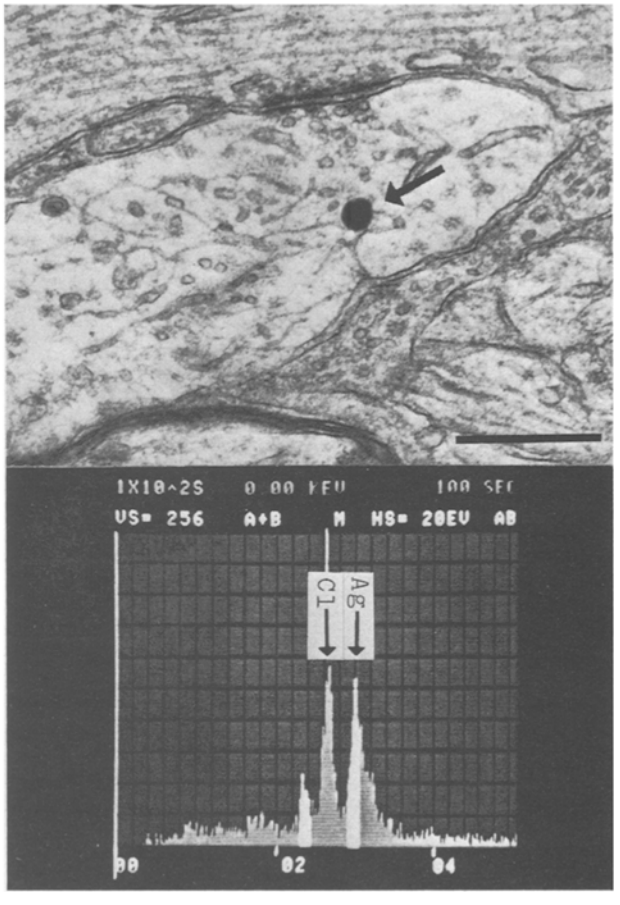


Fig. 3. Elemental spectrum from a autoradiogram. Silver and chlorine (2.621 keV) are detected from one probe grain. Arrow indicates the probe grain. Developed in Phenidon for 1 min at 11 °C. Probe diameter 1200 Å. Bar equals 0.5 μm.

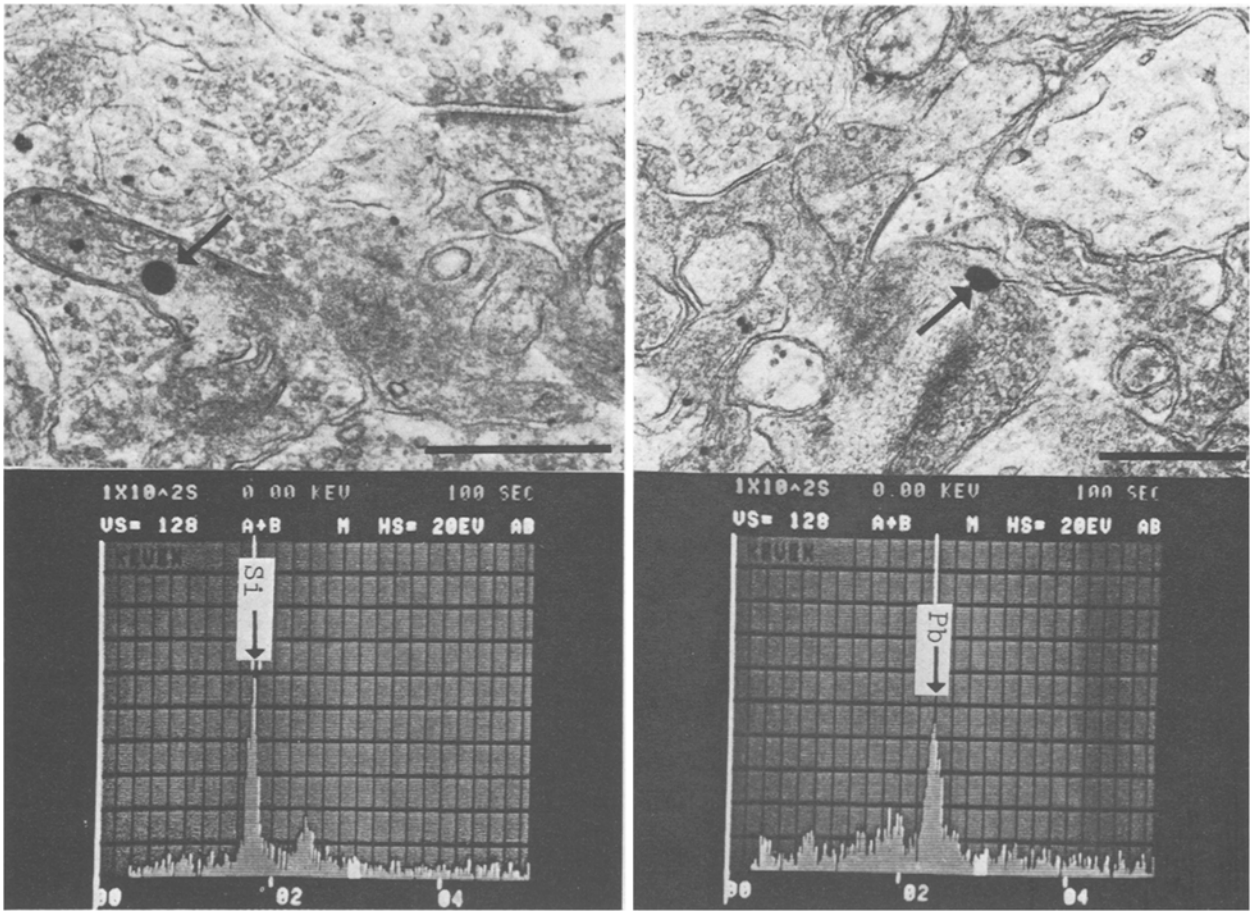


Fig. 2. Elemental spectra from autoradiograms. Silicon (1.739 keV) and lead (2.380 keV) are detected. Arrows indicate the probe grains. Developed in Phenidon for 1 min at 11 °C. Probe diameter 900 Å. Bars equal 0.5 µm.

microanalyzing system (operated at 20 eV/channel). The elemental spectra of various developed grains were photographed after 100 sec of probing. The resolution power of the energy dispersive microanalyzer was 0.16 keV.

*Results and discussion.* When autoradiograms were developed in Konidol-X, the resultant grains were dense and irregular coil particles (see figure 1). These shaped grains could easily be confirmed as the developed silver grains without use of any analyzing techniques. However, in the case of Phenidon developer, the resultant grains were rather small and dot-shaped particles as compared to Konidol-X (see figures 2 and 3). It was impossible to determine whether or not these resultant grains were the autoradiographic silver grains. Therefore, we used an electron probe X-ray microanalyzing system. The elemental spectra from the various developed grains of the high resolution autoradiography are shown in figures 2 and 3. When the probe was placed on a deposit of grain, the elemental spectrum became very distinctive. In addition to the silver emission, high peaks of lead (2.380 keV), silicon (1.739 keV) and chlorine (2.621 keV) appeared. Furthermore, sometimes two kinds of elements were detected from one deposit of grain, e.g., silver and chlorine (see figure 3). The lead emission from the deposits of grains is undoubtedly due to the artificial product with lead-electron staining. The high peaks of chlorine and silicon may be ascribed to the developing media and the nuclear track emulsion, respectively. The type and number of the probed elements varied from specimen to specimen and thus only qualitative analysis was performed. However, the lead emission showed the highest frequency as the resultant artificial product through the autoradiographic processes. These results strongly suggest that the identification of the resultant autoradiographic grains must be performed when a high resolution autoradiographic study is done. By the calculation method of Bachmann and Salpeter[9], we found that the combination of Sakura NR-H2 emulsion with a Phenidon developer gave about 1050 Å as a theoretical resolution power. This finding indicates that Phenidon is a more suitable developer than a Konidol-X for the high resolution autoradiography with Sakura NR-H2 emulsion. Finally, the high resolution autoradiography combined with electron probe X-ray microanalysis may provide a tool for studying the ultrastructural distribution of $H^3$-dimetacrine within synaptic areas.

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