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A new conductivity staining method for tissue analysis under scanning electron microscope

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Abstract: A conductivity staining method for tissue samples were developed using a solution of 10 ml of 5% carbolic acid solution, 2 g of tannic acid, and 10 ml of saturated aluminium sulfate 12-hydrated after fixation with osmium. Specimens of kidney (mouse) with the above treatment can be analized under a scanning electron microscopy without the charging phenomenon.

Key words: Conductive staing, intracellular structure, ultrastructure, scanning electron microscopy.

The three-dimensional images of intracellular structures observed under scanning electron microscope (SEM) lead to a more realistic view than that of two-dimensional images obtained from thin sections analyzed under a transmission electron microscope (TEM). Nevertheless, the majority of descriptions of normal or pathologic tissues has been made with thin sections. One reason for this situation is the current development of both methodologies. TEM is more than 50 years old, whereas, SEM first became commercially available in 1965 (Black 1974).

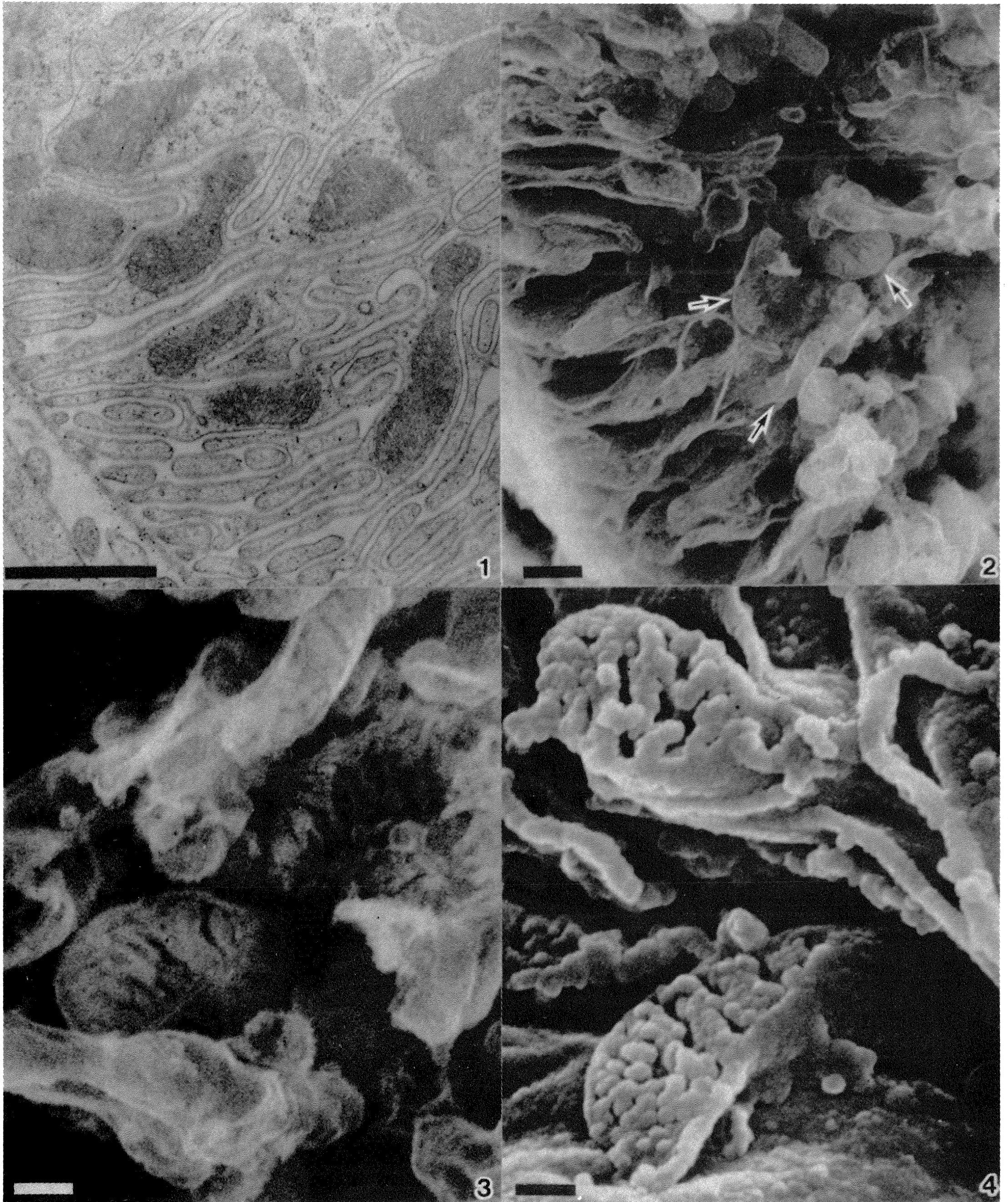
Initially SEM was a tool exclusively used for observing surfaces from biological or nobiological samples; although there were numerous attempts to design techniques to resolve the intracellular ultrastructure. Most of these used criofracture to expose organelles (Haggis 1970). Tanaka and his colleagues are pioneers in developing successful techniques to visualize the intracellular environment (Tanaka and Mitsushima 1984).

The successful application for the methodology developed by Tanaka is based on a maceration process on the fractured surface of specimens fixed in 1% Osmium (Os), without or with light aldehyde fixation; so, that the lipids of the membranes are fixed enough, while the proteins are lightly fixed. In this condition a treatment with 0.1% Os dissolves the protein of the cytoplasmic matrix where organelles are immersed; this treatment is called "maceration" by Tanaka and Mitsushima (1984). Afterwards, an increased osmication of the sample is done using sequencially Os, tannic acid, and Os again (Murakami and Jones 1980); then the sample follows a standard procedure for SEM, including dehydration, critical point dring, and a 3 nm coat of platinum (Inoué 1992).

The 3 nm coat of platinum is required to avoid the charging phenomenon; but it represents an obstacle for resolving fine structures. However, a conductivity stain, which does not need an additional metal coat, is ideal for observing intracellular structures; in this report we describe such a conductivity-stain method.

MATERIAL AND METHODS

Mouse kidneys were removed, sliced (*ca.* 2 mm thickness), fixed in 1% Os for an hour at



Figs. 1 and 2. Transmission and scanning electron micrograph of the basal area of renal epithelia, respectively. The basal plasma membrane has a labyrinthine undulating pattern of folds, with some mitochondria (arrows). Bar = 1 µm. Figs. 3 and 4. Mitochondria in samples with conductive-staining and with metal coat, respectively. Compare the thickness of membranes in each micrograph, they are thinner in the former (See the text for details). There are two structures like a coated vesicles (arrows in figure 3). Bar = 0.25 µm.

4°C, and processed according to Tanaka and Mitsushima (1984). Briefly, the fixed tissues were rinsed in distilled water, immersed in 25 and 50% DMSO for 30 minutes each, frozen, and fractured (EIKO TF-1). The specimens were macered in 0.1 % Os for three days at room temperature, rinsed with distilled water and immersed for one hour in the mordant of the bacterial flagella stain described by Kodaka *et al.* 1982. Then the tissues were rinsed again, inmersed in 1% Os for one hour, rinsed, dehydrated, critical point drying, mounted on aluminium stubs, and analyzed under SEM at 15 KW and at a work distance of 0-3 mm. In additions, some samples were rotary shadowed with platinum in a metal evaporator (Hitachi HU-S5), and observed at SEM.

The mordant of the Kodaka *et al.* (1982) stain method is: 10 ml of 5% carbolic acid solution, 2 g of tannic acid, and 10 ml of saturated aluminium sulfate 12-hydrated.

For TEM comparison, thin sections from samples fixed in Karnowski solution, dehydrated, and embedded in Spurr were analyzed.

RESULTS AND DISCUSSION

The columnar epithelium of the proximal and distal tubules are attached on a basal membrane by numerous interdigitations of the basal citoplasmic membrane; in the middle of the cell the nucleus is located and the proximal side presents numerous microvilli (Figs. 1 and 2). Identification of mitochondria is easy at TEM; the majority of them are localized in the interdigitations, and appear extremely pleomorphic, predominating the elongated shapes (Fig. 1). At SEM the mitochondria is identified by the pattern of cristae and their pleomorphism is enhanced. Also, the apical ends of these cells have elongated microvilli, as is shown in figure 3.

Figs. 2 and 3 correspond to samples processed with the conductivity stain without platinum coating. In both the appearance of the intracellular membranes are tenuous, given the appeareance of the delicate pattern of folds. On the other hand, the platinum shadowed samples present a more rigid appeareance, as is show in Figure 4. For example, the mitochondrial cristae appear more defined, but as solid structures, because the platinum coat on each membrane of a cristae gives it the aspect of a unique structure.

The enhancement osmication on the samples treated with the mordant of Kodaka *et al.* (1982) stain is based on the action of tanic acid, assisted by aluminium sulfate and phenol; the final result is an intense osmication, which imparts a deep black color to the sample, and an electric conductivity. This procedure made unnecessary the metal shadow, which was usually indispensable in the standard methodology to avoid the charging phenomenon. Moreover, the aspect of intracellular structures is more real, because the thickness of membranes is not altered by the deposit of metal grains. Furthermore, this method represents an economy, because special equipment is not required.

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RESUMEN

Se describe un nuevo método de tinción conductiva para muestras biológicas, empleando una solución de 10 ml de ácido carbólico al 5%, 2 g de ácido tánico y 10 ml de sulfato de aluminio duodecahidratado saturado, adicionado a las muestras luego de la fijación con osmio. Con este método se procesaron muestras de riñón de ratón y se analizaron al microscopio electrónico de rastreo sin que manifestaran fenómenos de carga.

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