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Ultrastructural immunogold labelling of vimentin filaments on postembedding ultrathin sections of arachnoid villi and meningiomas

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Summary. An immunoelectron microscopic technique for the labelling of vimentin intermediate filaments on postembedding ultrathin sections is reported. Arachnoid villi obtained at autopsy and meningiomas at surgery were fixed in 1% paraformaldehyde for 30 minutes, embedded without postfixation in Epon-Araldite mixture and polymerized at 37°C for 3 weeks. Ultrathin sections were etched in 2% KOH for 3 minutes and incubated with anti-vimentin monoclonal antibodies which were subsequently labelled with goat anti-mouse IgG coupled to colloidal golds. All of these labelling procedures were consistently performed within 4 hours. In both arachnoidal and meningioma cells, immunogolds preferentially decorated the intermediate filaments in proportion to the concentration. Very few gold particles were seen over the nucleus, Golgi zone, mitochondria and the extracellular connective tissue fibres. The present technique may be applied to the immunogold labelling of intermediate filaments on postembedding ultrathin sections.

Key words: Arachnoid villus, Meningioma, Vimentin, Intermediate filaments, Immunogold, Electron microscopy

Introduction

Intermediate filaments are an essential component of the cytoskeletal architecture of most eukaryotic cells and are characterized by five immunologically different classes (Osborn, 1983). For ultrastructural labelling of intermediate filaments, indirect immunocytochemistry may be performed by pre- or post-embedding method. By the preembedding method, cellular fine structure is usually not well preserved and soluble cell components are extracted during processing as a result of detergent treatment. The masking effect of dense reaction products on the underlying cellular compartment may occur with use of enzymatic techniques utilizing peroxidase and chromogens such as DAB. Although the postembedding method provides better preservation of cellular fine structure, all preparatory steps for embedding specimens affect antigenicity by causing a conformational change in proteins (Slepecky and Chamberlain, 1986). Denaturation occurs with the conventional fixation by glutaraldehyde and osmium tetroxide and with high temperature used during polymerization. Accordingly, until now the postembedding immunoelectron microscopy has been limited to the demonstration of aggregated antigenic sites such as secretory hormones (Roth et al., 1978, 1981). The author recently tried a revised postembedding technique for the immunoelectron microscopic labelling of vimentin filaments in both autopsy and biopsy materials. This paper is to report the technical procedures by which intermediate filaments of arachnoidal and meningioma cells were successfully decorated by immunogolds on ultrathin sections.

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Materials and methods

Tissues

Specimens of arachnoid villi were obtained from three subjects at autopsy within 2 hours postmortem, while tumor specimens were obtained by biopsy from three patients with angiomatous or syncytial meningiomas. The specimens were fixed in 1% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, at 4°C for 30 minutes. They were rinsed in 0.01 M phosphate buffered saline (PBS), dehydrated in graded alcohols and embedded without postfixation in Epon-Araldite mixture. The latter was polymerized in the incubator at 37°C for 3 weeks. Suitable areas to be investigated were selected in thin sections stained with toluidine blue. Ultrathin sections

(pale gold in colour) were cut with a diamond knife using an LKB ultramicrotome and mounted on nickel grids of 300 mesh which were previously coated with the meshcement of 0.5% chloropurine rubber in toluene. The remainder of each specimen was fixed in 3.7% buffered formalin, cut into thin sections, and stained with hematoxylin and eosin for histopathological analysis.

Antibodies

The following commercially available antibodies were used for the indirect immunocytochemistry. Primary antibodies were mouse monoclonal antibodies to vimentin against porcine eye lens (Dakopatts, Glostrup, Denmark). No reaction with other closely related intermediate filament proteins including desmin and glial fibrillary acidic protein was obtained by immunoblot analyses (Osborn et al., 1984). To label the primary antibodies, goat anti-mouse IgG coated with colloidal golds measuring 10 nm in diameter (Janssen, Olen, Belgium) were used as secondary antibodies.

Immunolabelling

All reactions were carried out on a sheet of Parafilm in a humid chamber at room temperature. For incubations with immune reagents, the grids were immersed at the bottom of drops of the reagents with the sections facing up. For rinsing, the grids were floated on top of drops of the washing fluid with the sections facing down. The grids were transferred from one droplet to another with a fine forceps. The excess fluid was removed from the grids by filter paper, but the sections were never allowed to dry. All solutions except for immunogold reagents were Millipore filtered prior to use.

0.1% BSA-TRIS is a buffer composed of 20 mM Tris buffered saline supplemented with 1 mg/ml of BSA (bovine serum albumin) and 20 mM NaN_3. 0.05% Tween 20 and 0.5 M Nacl were added to avoid background stains.

The following steps were carried out and ultrathin sections were examined with a Hitachi H-600 electron microscope.

(1) The grids were rinsed in distilled water for 10 minutes.

(2) They were etched on 2% saturated aqueous solution of KOH in 100% ethylalcohol for 3 minutes.

(3) After the etching the grids were washed in distilled water for 5 minutes.

(4) The washed grids were transferred on 0.1% BSA-TRIS supplemented with 5% normal goat serum for 15 minutes.

(5) The grids were incubated in monoclonal antibodies to vimentin diluted 1/10 (12.8 µg/ml) in 0.1% BSA-TRIS supplemented with 1% normal goat serum for 1 hour.

(6) For washing, the grids were transferred into 0.1% BSA-TRIS for 2 x 5 minutes.

(7) The grids were incubated in goat anti-mouse IgG coated with 10 nm colloidal golds diluted 1/20 in 0.1% BSA-TRIS for 1 hour.

(8) The grids were washed in 0.1% BSA-TRIS for 2 x 5 minutes.

(9) The grids were washed with distilled water for 2 x 5 minutes.

(10) The grids were contrasted with 2% uranyl acetate for 4 minutes and lead citrate for 2 minutes.

Controls

Labelling specificity was evaluated in three ways: (1) primary antibodies to vimentin were replaced with purified IgG fraction of non-immune serum, (2) primary antibodies were omitted, and (3) secondary antibodies were replaced with goat anti-rabbit IgG coated with colloidal golds.

Results

Conventional electron microscopic techniques were revised for processing specimens in the present method. The antigenicity of vimentin intermediate filaments was well preserved after the single fixation with low concentrations of paraformaldehyde and after the low temperature polymerization in Epon-Araldite mixture. Special attention was paid to the time of the etching procedure; as the longer the etching the more damage the ultrathin sections sustained and the shorter the etching the worse the labelling became. Although the cellular fine structure was relatively preserved following the whole procedures, the cell membranes were sometimes disrupted and the mitochondria were not preserved. The specificity of immunocytochemical labelling was confirmed by the negative results in all of the three controls. The reaction between the immunoreagents and the antigenic sites exposed on the surface of ultrathin sections were stable in both autopsy and biopsy materials. There was almost no difference in the intensity of labelling when autopsy material was compared to biopsy material.

Figures 1-4 illustrate the labelling of intermediate filaments after incubation of the ultrathin sections with both anti-vimentin antibodies and the immunogold solution. The decoration of 10 nm gold particles was closely confined to the cytoplasm of arachnoid cells (Fig. 1). Non-specific labelling over the nucleus, Golgi zone, mitochondria and the extracellular connective tissue fibres was extremely rare (Fig. 2). There was no background staining. The number of gold particles was proportional to the concentration of intermediate filaments (Fig. 3). Most of the gold particles were superimposed on the individual intermediate filaments (Fig. 4).

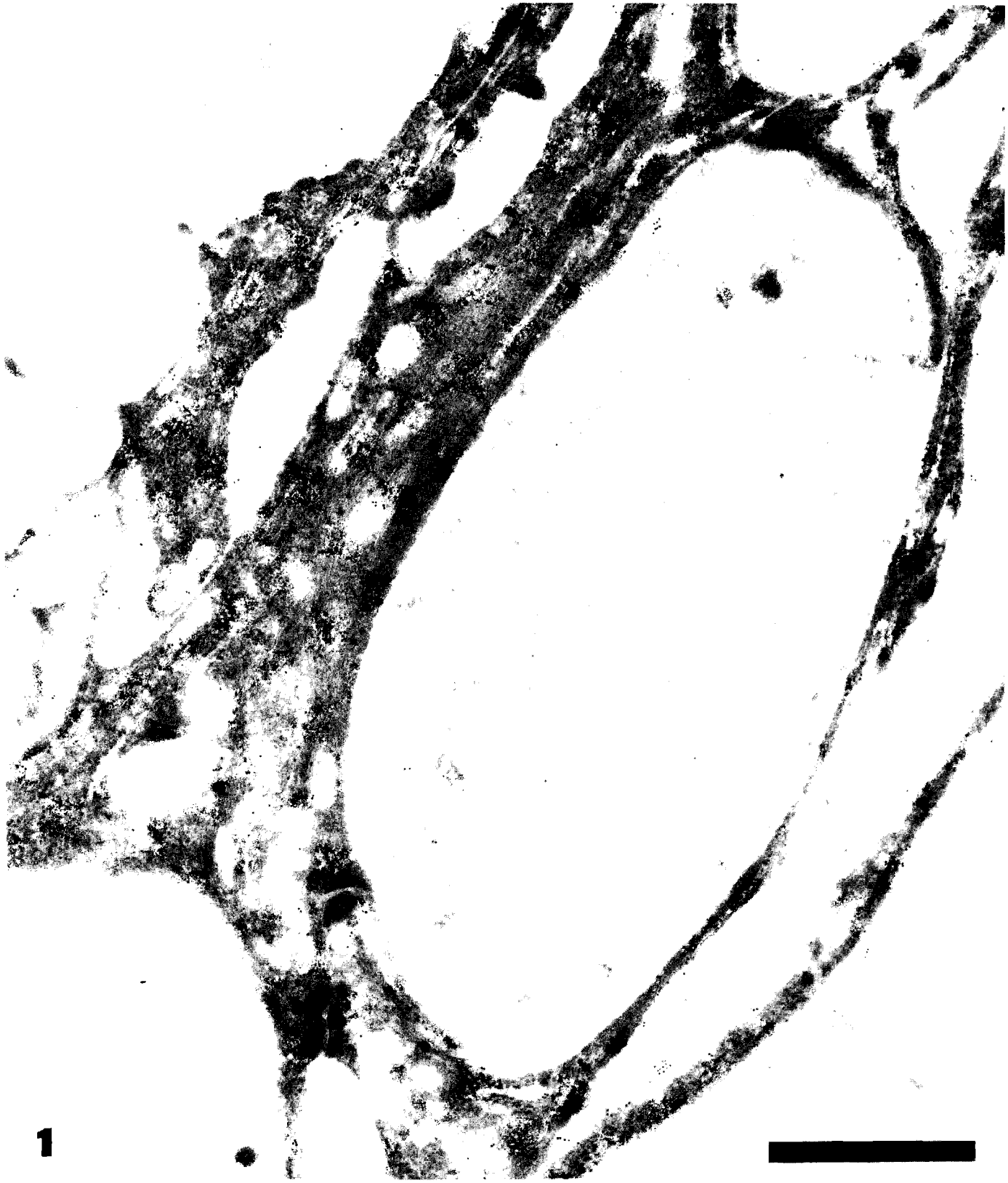

Fig. 1. Immunoelectron micrograph of a human arachnoid villus showing decoration of gold particles closely confined to the cytoplasm of arachnoid cells. × 37,500. Bar=1 μm.

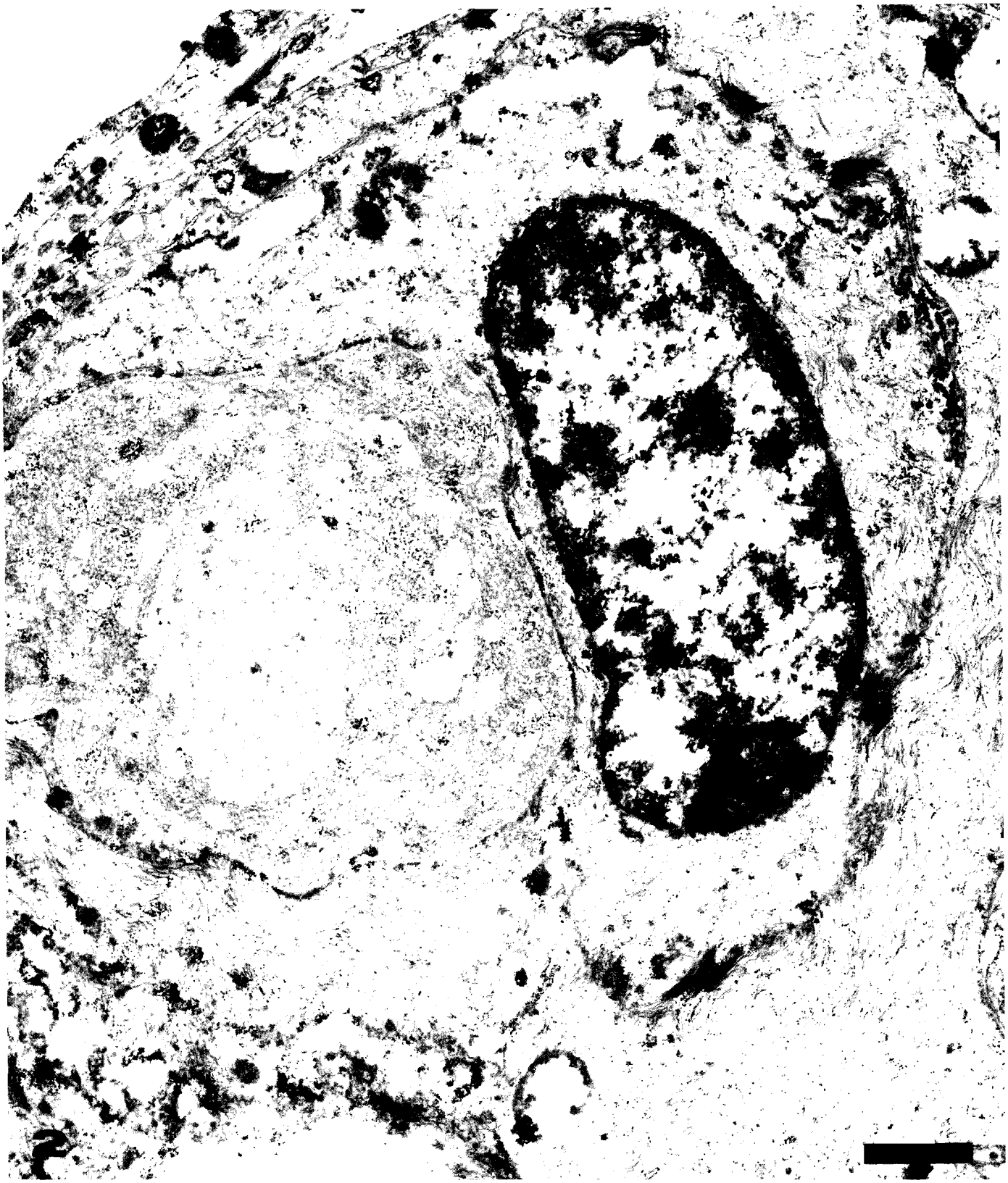

Fig. 2. Immunoelectron micrograph of a human arachnoid villus showing label of vimentin filaments without decoration on other cytoplasmic structures and extracellular connective tissue fibres. × 20,000. Bar = 1 μm.

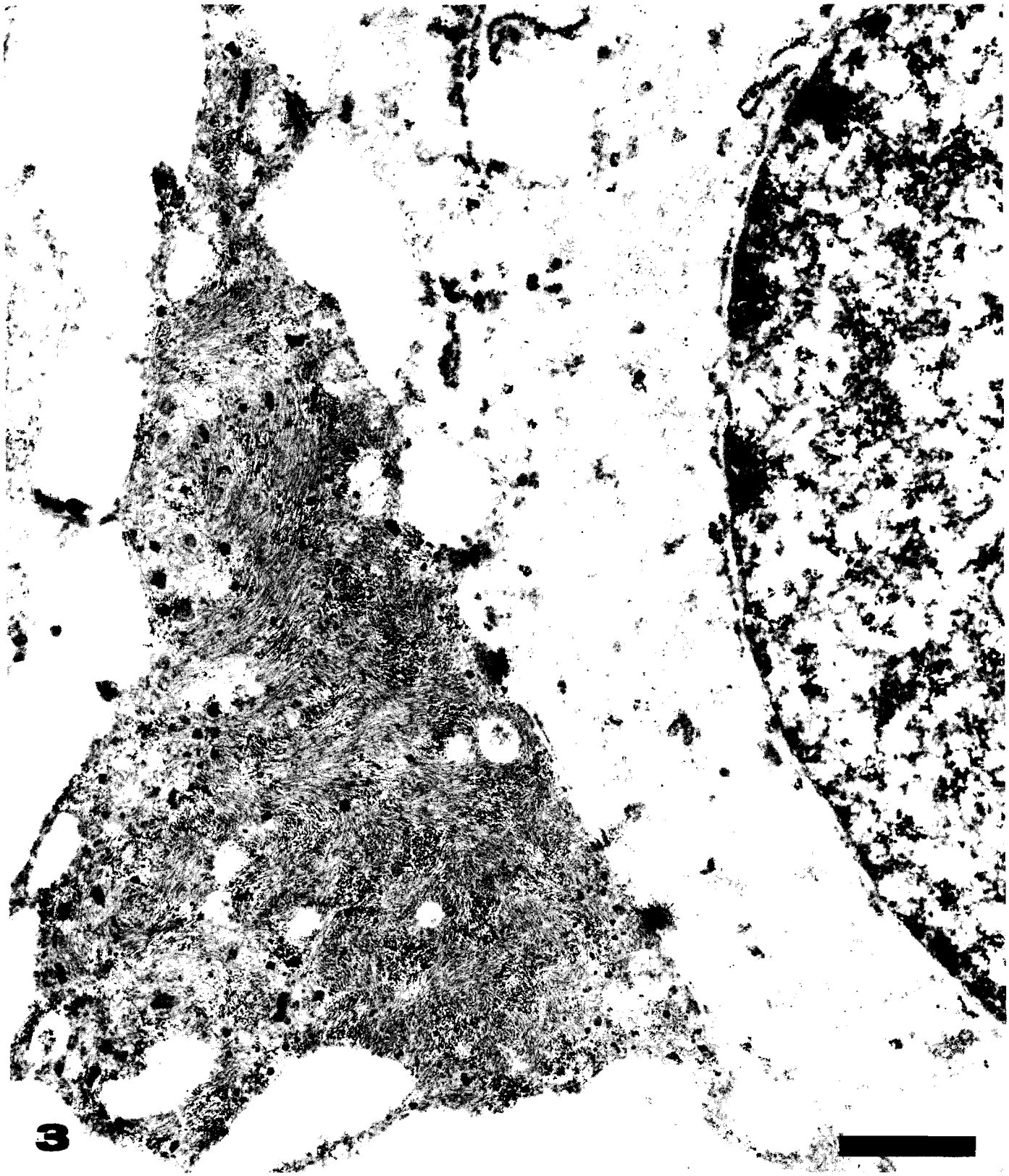

Fig. 3. Immunoelectron micrograph of an angiomatous meningioma showing distribution of gold particles in proportion to the concentration of vimentin filaments in tumor cells. × 25,000. Bar=1 μm.

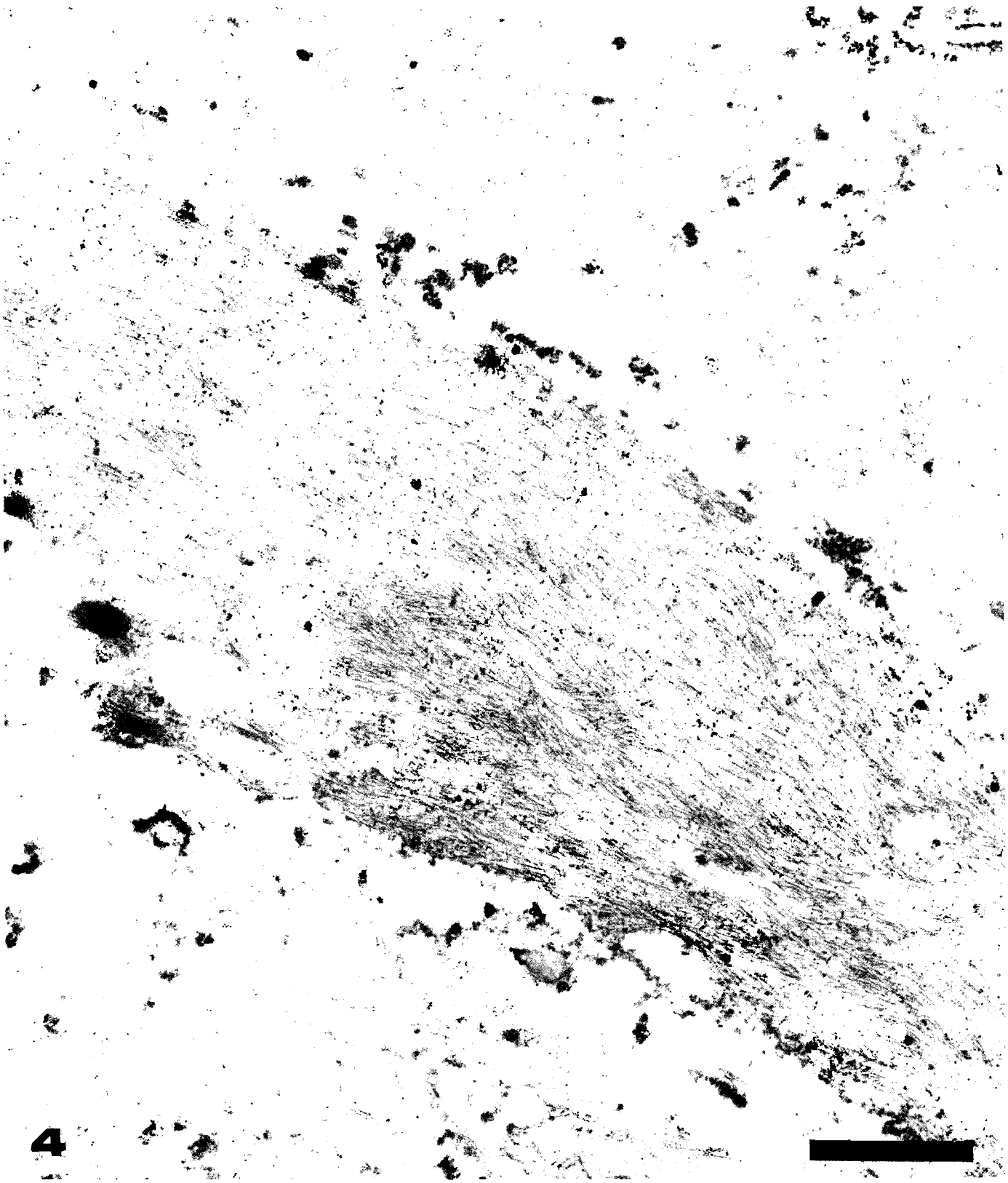

Fig. 4. Immunoelectron micrograph of a syncytial meningioma showing gold particles superimposed on the individual vimentin filaments. × 30,000. Bar = 1 μm.

Discussion

It is generally accepted that the expression of intermediate filaments is highly tissue specific. Although intermediate filaments appear morphologically identical in different cell types, biochemical and immunological characterization has established five classes including cytokeratins, neurofilaments, glial fibrillary acidic protein, desmin and vimentin (Osborn, 1983). Vimentin has been found in a variety of cells of mesenchymal origin and certain nonepithelial cell types (Osborn, 1983; Osborn et al., 1984). Schwechheimer et al. (1984) and Halliday et al. (1985) confirmed the exclusive expression of vimentin in meningioma cells by both two-dimensional gel electrophoresis and immunoblot analysis of cytoskeletal proteins. Vimentin is known to be the major intermediate filament not only of meningioma cells but also of normal arachnoidal cells (Kartenbeck et al., 1984; Theaker et al., 1986; Holden et al., 1987). As the relationship between vimentin and intermediate filaments of meningioma and arachnoidal cells is already established, the primary objective of the present study was directed toward the precise, reliable immunolabelling procedure by the postembedding method. The latter has been commonly used in immunoelectron microscopic labelling of aggregated antigenic sites as polypeptide hormone (Roth et al., 1978, 1981). An immunoelectron microscopic demonstration of cytoplasmic filaments using the postembedding method appears to be extremely difficult (Theaker et al., 1986).

The present postembedding method for labelling vimentin filaments at the ultrastructural level was successful because of modifications that could preserve antigenicity. Despite the lack of postfixation by osmium tetroxide, the single fixation by paraformaldehyde considerably preserved the fine structure. The present method had a number of theoretical and practical advantages.

(1) Conventional electron microscopic techniques could be utilized for processing specimens.

(2) The tissue could be embedded in the Epon-Araldite mixture in the slightly modified manner.

(3) The whole labelling procedure could be done within 4 hours.

(4) The labelling on ultrathin sections required only small amounts of purified IgG fragment.

(5) The fine structure of cellular organella was relatively well preserved.

(6) Ultrathin sections could be moderately contrasted with uranyl acetate and lead citrate.

(7) There was no masking effect of dense reaction products on the underlying cellular compartment.

(8) Autopsy material could be similarly labelled as biopsy material.

When the present data are compared with those of the preembedding method using immunogolds (Kartenbeck et al., 1984; Schwechheimer et al., 1984; Wang et al., 1984; Czernobilsky et al., 1987), both show a quite similar quality of labelling. However, the remarkable differences are the simplicity of the present immunoreaction method and the availability of the low magnification survey view. Furthermore, the present postembedding method can be applied to the labelling of serial ultrathin sections with various antisera or to the double labelling using immunogolds with a different diameter. The present techniques appear to be of general applicability to a variety of interesting problems of intermediate filaments in cell biology.

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