Silver Impregnation for Electron Microscopy.*

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Heavy metal impregnation of specimens for electron microscopy offers a means for differential "staining" of various tissue components and cellular constituents. Of many silver impregnation methods, one of the most useful is Gomori's silver methenamine (1) after periodic acid oxidation (PA-SM) (2, 3). Several authors have applied silver impregnation to electron microscopy (4–7), mainly for the demonstration of connective tissue fibers. Dettmer and Schwarz (3) applied Gomori's silver methenamine to the study of teased preparations. We have adapted this procedure to the impregnation of small blocks of tissue which are then embedded in plastic and sectioned in the usual manner.

Procedure

Fixation.—Small blocks of tissue (approximately 1 mm. in diameter) were fixed by one or more of the following methods: (*a*) 4 per cent formaldehyde USP (pH approximately 5.6), 12 to 24 hours at room temperature; (*b*) 4 per cent formaldehyde made isotonic by addition of 2 per cent sodium acetate (pH approximately 6.8), 12 to 24 hours at room temperature; (*c*) 4 per cent formaldehyde in M/15 phosphate buffer, pH 7.4, 24 to 48 hours in the refrigerator; and (*d*) formaldehyde-alcohol-acetic acid (5 ml. formaldehyde USP 37 to 40 per cent, 5 ml. glacial acetic acid, 90 ml. 70 per cent ethyl alcohol), 2 to 6 hours at room temperature. After fixation tissue was washed in water and stored in 70 per cent alcohol.

Fixative (*c*) affords the best preservation of tissue components. Fixative (*d*) preserves selectively collagen fibrils and basement membranes at the expense of cell structures. Fixatives (*a*) and (*b*) fall in between.

Oxidation.—One hour in 0.5 per cent solution of periodic acid in 70 per cent ethyl alcohol at 37°C. followed by several changes of distilled water.

Impregnation.—Twenty-four to 48 hours or longer, at 55°C. in a solution of silver methenamine as modified by Jones (8). The solution is changed every 24 hours. At each change one or two blocks of tissue are removed from the solution, processed through paraffin, sectioned and examined with the light microscope in order to determine the adequacy of impregnation. After impregnation is completed, the tissue is washed in several changes of distilled water. It is then postfixed for 30 minutes in a 2 per cent aqueous solution of osmium tetroxide, washed again, and stored in 70 per cent alcohol.

Embedding and Sectioning.—The blocks of tissue are dehydrated in alcohols and embedded in butyl methacrylate in the usual manner (9). One-half micron sections are cut and examined with the light microscope to determine the adequacy of impregnation of each block. Well impregnated blocks are then cut for electron microscopy.

RESULTS

Silver precipitates out in the form of fine granules which are approximately 100 angstroms in diameter, or small conglomerates up to 500 A°. Therefore the granular structure does not become conspicuous until a magnification of approximately 10,000 is reached. Basement membranes, collagen fibrils, nuclear chromatin, the granules of certain leukocytes, and the hyalin droplets in renal tubular cells are densely impregnated with silver precipitate ("specific staining"), while other tissue or cell constituents show only scattered silver granules. This is the same pattern of distribution as seen in the light microscope (Figs. 1–6).

There is some variation in the distribution of the silver following different fixatives. Neutral (pH 7.4) formalin is followed by intense nuclear "staining," slightly acid formalin by markedly weaker nuclear "staining," and the more acid fixatives by the absence of nuclear "staining." It should be noted that osmium tetroxide is not satisfactory as a first fixative because it results in diffuse precipitation of silver throughout the tissues.

DISCUSSION

We have used silver impregnation primarily in the investigation of pathologic material, especially for demonstration of connective tissue elements. The high contrast afforded by this technique makes small variations from the normal pattern readily detectable on the fluorescent screen of the electron microscope. Consequently, material can be surveyed in the microscope without

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resorting to photographic procedures. When photography is desirable, the high contrast and small size of the granules makes focusing very easy.

The granularity of the silver makes it readily distinguishable from deposits of osmium compounds (*e.g.* lipide droplets), while the small size of the granules permits a useful magnification at least 10 times as high as in the light microscope.

The mechanism of silver methenamine impregnation is not understood. We have been unable to show any simple chemical specificity; it does not appear to be an aldehyde reagent as has been claimed (1, 3). Nonetheless, we find the technique useful for distinguishing between tissue components which have similar density and structure in conventional electron microscopy. Even some tissue components of postmortem material, poorly preserved by the standards of the electron microscopist, may be studied in this way to great advantage.

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Explanation of Plates

Plate 425

All photographs taken from tissues impregnated with periodic acid-silver methenamine after formalin fixation.

Fig. 1. Portion of normal glomerulus, human kidney, routine surgical specimen. Basement membrane of the capillary loops and the nuclear chromatin are densely impregnated. × 4,000.

Fig. 2. Normal human glomerulus as seen under the light microscope. × 1,500.

Fig. 3. Same glomerulus as in Fig. 1, under higher magnification. × 12,000.

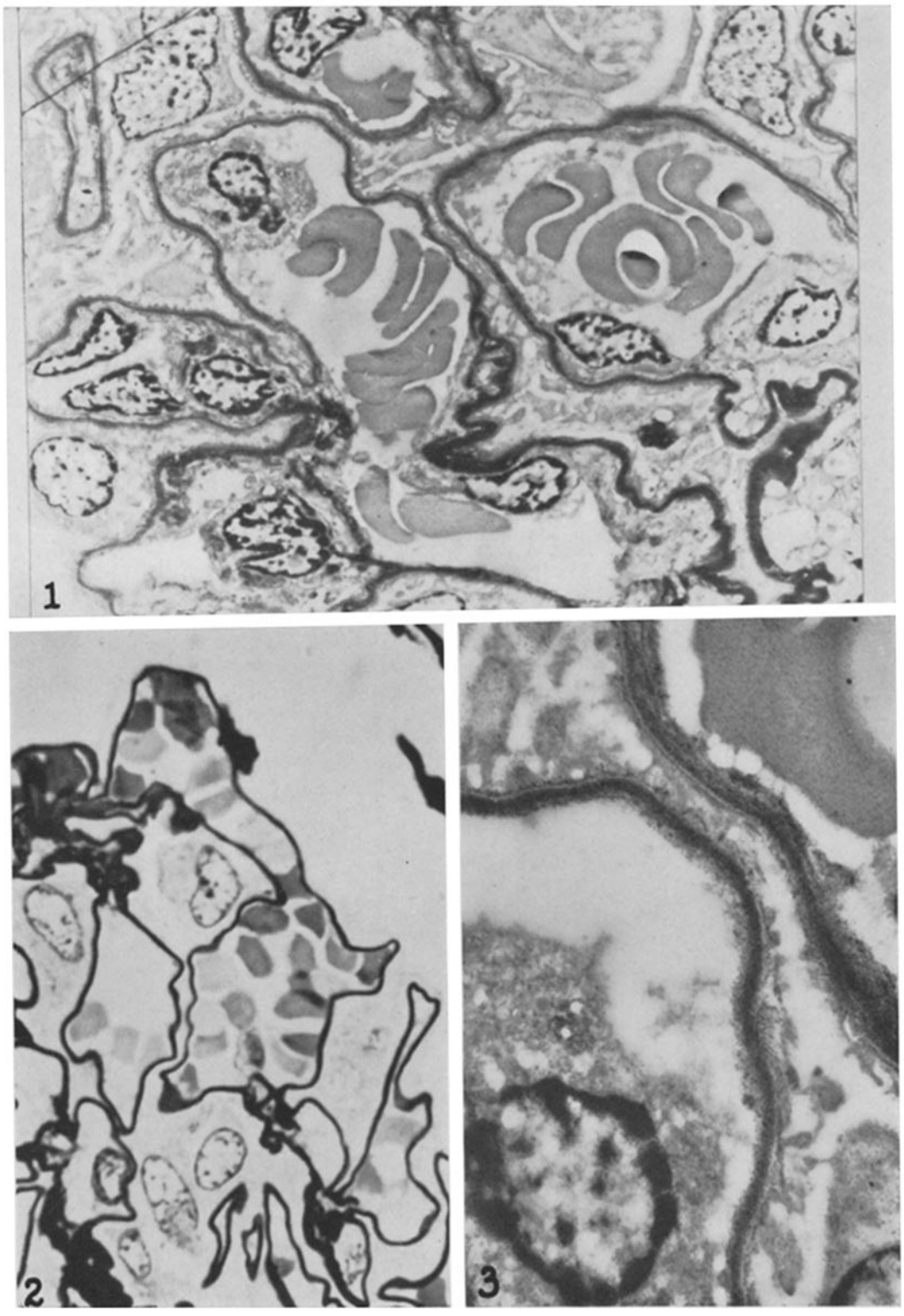

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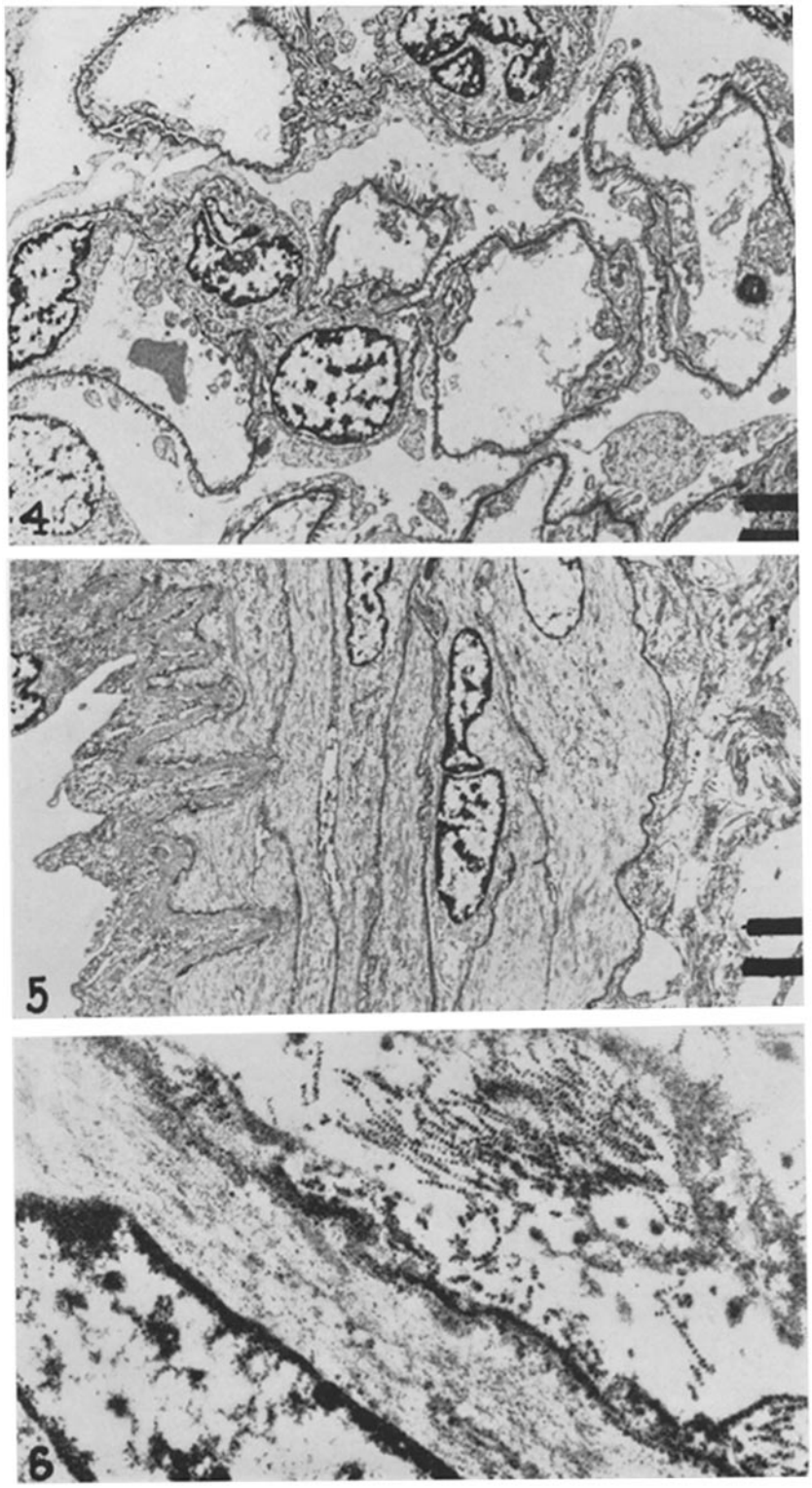
PLATE 426

FIG. 4 Portion of glomerulus, rabbit kidney, Masugi-type nephritis. There is partial splitting of capillary basement membranes in the two loops on the right. × 5,000.

FIG. 5. Part of a wall of arteriole, rabbit kidney. Nuclear chromatin, perimysial membranes, and collagen fibrils in the adventitia (right) are impregnated. × 5,000.

FIG. 6. Same arteriole as in Fig. 5, under higher magnification. Periodic structure of collagen fibrils is well demonstrated. Note that each period is represented by more than one granule of silver. × 13,500.



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