Demonstration of basement membrane in renal biopsies by silver-methenamine on thin epoxy-resin sections

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In the recent paper on an improved technique for renal biopsies, Meadows and Schoemaker (1970) describe the use of a special wax to give fine sections at 1-2 μ . They illustrate their excellent results with Jones's silver technique. In this laboratory, however, difficulty was experienced with their use of ammoniacal ethanol and as we, in agreement with Eastham and Essex (1969), believe that the thinner sections possible with polymer-embedding are of greater diagnostic value, the present note describes the successful application of a methenamine-silver method on sections from Araldite.

Fixation

Buffered formol saline is perfectly satisfactory and no differences in results have been observed with material fixed from six hours to two weeks.

Attempts to impregnate the reticulin with Foot's method have not been satisfactory with epoxy-resin sections. The silver-methenamine method of Gomori and Grocott, already successfully used on acrylic-resin sections, is now suggested as a simple way of demonstrating reticulin on epoxy-resin sections. A real advantage of epoxy resin (Araldite) is that thin sections (0.5 μ) adhere to glass and this allows 'staining' on the slide and a flat, clean, mounted section.

Methods

After fixation in formol saline, a portion of the renal biopsy, not longer than 5 mm, is selected for embedding in Araldite. The remainder of the needle

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biopsy may be processed for paraffin. The selected material is dehydrated through:

30% acetone	..	..	..	..	15 minutes
70% acetone	..	..	..	..	30 minutes (overnight)
100% acetone	..	..	..	..	3 hours
30% Araldite in dry acetone	..	..	..	..	15 minutes
70% Araldite in dry acetone	..	..	..	..	15 minutes
70% Araldite in dry acetone at 60°C	..	..	..	..	30 minutes
100% Araldite at 60°C	..	..	..	..	30 minutes

Embed in a previously warmed, size O, gelatine capsule mould and allow to polymerize at 60°C for two days.

Araldite, as used in the method, refers to the following formula (modified from Luft, 1961): 20.0 ml Ciba Araldite epoxy (Araldite M) CY212, 20.0 ml Ciba Epoxy hardener HY964, 1.5 ml Ciba accelerator, and 1.0 ml di-butyl phthalate.

Sections are cut at 0.5-1.0 μ on a Reichert OMU2 ultramicrotome using a glass knife. The sections are floated onto a clean glass slide and affixed without the use of adhesives (Eastwood *et al*, 1969), dried flat on a hotplate at 60°C for half an hour, allowed to stand at room temperature for a further 30 minutes, immersed in saturated alcoholic NaOH (Lane and Europa, 1965) for a minimum of one hour, and washed in running tap water for two to three minutes.

Impregnation Method

- 1 Rinse thoroughly in distilled water.
- 2 Treat with 1.0% aqueous periodic acid for 30 minutes. (This solution lasts for several months.)
- 3 Rinse briefly in four changes of distilled water (each four to eight seconds).
- 4 Place slides in Grocott's silver methenamine solution at room temperature then transfer the slides and silver solution, in the coplin jar, to a 60°C water bath.
- 5 After approximately 30 minutes the section, macroscopically, appears a light autumn brown colour. Check microscopically at intervals of two to three minutes after this time until the glomerular capsule is a deep black and the membrane of the capillary loops is dark brown.
- 6 Rinse thoroughly in distilled water.
- 7 Tone in 0.1% gold chloride until the capillary loops of the glomerulus have changed from dark brown to black and the background is almost colourless.
- 8 Wash well in distilled water.
- 9 Treat with 2.0% aqueous sodium thiosulphate (three to five minutes).
- 10 Wash thoroughly in running tap water, dehydrate through ethanol, clear in xylol, and mount in D.P.X.

Fig. 1

Fig. 1 Normal glomerulus. Araldite embedded, sectioned at 0.8μ . P.A.S.M. $\times 1250$.

Fig. 2

Fig. 2 Glomerulus in membranous glomerulonephritis showing spike-like projections (confirmed by electron microscopy) on the external surface of the basement membrane (arrowed). Araldite embedded, sectioned at 0.8μ . P.A.S.M. $\times 1250$.

Notes

- 1 The method is very sensitive to any free chloride and all glassware used in making or storing the distilled water and silver solution should be pre-cleaned with nitric acid.
- 2 With volumes over 50 ml of silver solution the time required for this solution to reach temperature in the water bath becomes prolonged and the silver solution has a tendency to 'break down' before the desired impregnation intensity has been reached.
- 3 After approximately 45 minutes in the water bath the silver solution produces nuclear staining as well as impregnation of the basement membrane. This is not a disadvantage as it helps to distinguish the epithelial and endothelial nuclei.
- 4 After approximately one hour at 60°C there is a tendency for 'silver plating' to occur on both slide and section. Excessive 'plating' is invariable if impregnation is carried too far but with moderate plating the contrast is still adequate and can provide good quality black and white transparencies.

The method described has been shown to give uniform and consistent silver impregnation of glomerular basement membranes on 30 biopsies. It is a simple method and is suitable for a routine diagnostic laboratory.

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