

The Effect of Short-term High-dose Treatment with Methenamine Hippurate of Urinary Infection in Geriatric Patients with Indwelling Catheters

I. The preparation and morphology of a quantified urine sediment

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ABSTRACT

A quantified sediment of the urine from patients with indwelling catheters was prepared by fixation of 0.1 ml urine in 0.9 ml 2% glutaraldehyde immediately after sampling. Slide preparations were then made from 0.2 ml of the glutaraldehyde suspension by means of a cytocentrifuge. Bacteria and epithelial cells were properly contrasted by the May-Grünwald-Giemsa stain but haematoxylin-eosin and the Papanicolaou stain were superior as regards leukocyte morphology. It is suggested that glutaraldehyde-cytocentrifuge preparations of the urine cytology may be useful in the evaluation of urinary infection and in the evaluation of the therapy of urinary infection.

INTRODUCTION

The microscopic examination of urine sediments is a rapid and simple procedure which provides essential information in many cases of kidney disease or infections in the urinary tract. The conventional urinary sediment has, however, serious pitfalls, e.g. low reproducibility, low precision and high vulnerability to delay in transport and preparation (1-10).

It nevertheless seemed desirable to make a quantified urine sediment from patients with indwelling catheters in order to evaluate urinary infection and the effects of therapy. Since delay in transport and preparation could not be avoided under the conditions prevailing, we chose to "freeze" the cell picture at the moment of sampling by fixation.

The aim of the present study was to identify a simple and rapid method of sampling, fixing and quantifying the cytology of the urine from patients with indwelling catheters. Different staining procedures were also compared as regards the contrast and detail revealed in the morphology of bacteria and leukocytes.

MATERIAL AND METHODS

The urine was sampled from 14 inpatients at the somatogeriatric wards at Saint Lars Hospital, Lund, twice a week during the pre-treatment control period, days 10-17, during treatment with methenamine hippurate (MH, Hiprex^R, Riker Laboratories, Loughborough, Leicestershire, England), 2 g x 3, days 18-52 and during the post-treatment control period, days 66-73. During days 1-9 different cytocentrifuge preparations of leukocytes and bacteria were evaluated.

All patients had an indwelling catheter with continuous flow of the urine into a bag with a valve preventing back flow (closed system) for months or years prior to the present study. The catheter was plugged 20-30 minutes before sampling. The urine, approximately 15 ml, was collected in a sterile plastic tube and thoroughly shaken in order to disperse the solid matter. The specimen (0.1 ml) was then immediately transferred to 0.9 ml 2% glutaraldehyde in phosphate buffer, 0.135 M, pH 7.4. Cells and bacteria were spun down on slides 2-4 hrs later by means of a cytocentrifuge (Shandon-Elliot Cytospin), 1,000 r.p.m., 10 minutes, 0.2 ml of the cell suspension.

During the present study 197 double preparations of urine sediments were obtained. One preparation was always stained according to May-Grünwald-Giemsa (MGG), which was chosen as reference stain to confirm with the haematological traditions of the laboratory. Other routine stains available in the laboratory were used for the remaining preparation and compared with MGG: haematoxylin-eosin (Mayer's Haematoxylin-Erytrosin), the Papanicolaou stain, periodic acid Schiff (PAS), methyl green-eosin or haematoxylin-eosin-methylene blue. In addition, staining with acridine orange at low pH (4) was tried. The evaluation of the staining procedures was performed by means of a Zeiss Photomicroscope equipped with a planachromatic x 100 objective (cf. Figs 1-3).

RESULTS

The cytological picture of urine sediments occasionally shed light on the associated disease; the renal lesions in a patient with hyperparathyroidism and urinary tract infection were confirmed by the finding of numerous hyaline casts with embedded leukocytes, suggestive of protein loss in the urine and renal inflammation (Fig. 1).

The glutaraldehyde fixation was so rapid and effective that leukocytes were caught in different stages of the locomotory cycle (Fig. 2B), in different stages of phagocytosis (Fig. 2B) and in different stages of disintegration (Figs. 1B, 3).

The staining of glutaraldehyde-cytocentrifuge preparations entailed several problems. The basic haematological stain, May-Grünwald-Giemsa (MGG), produced

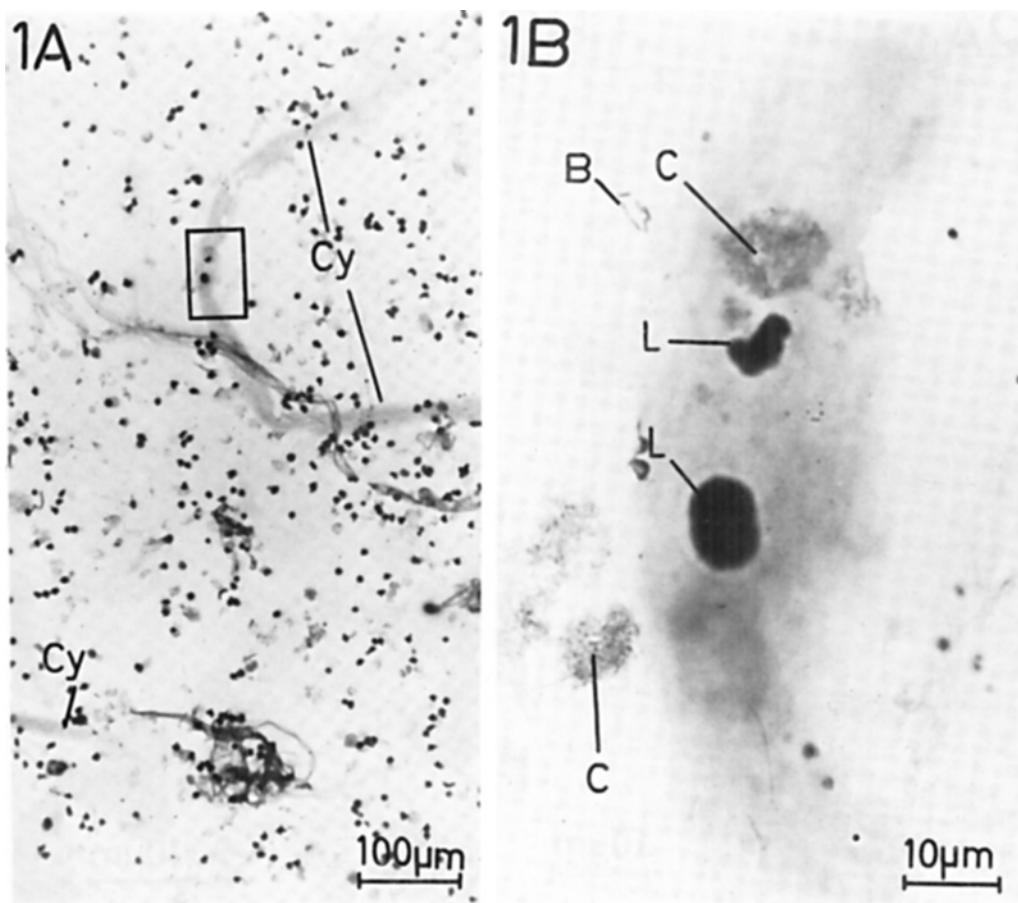


Fig. 1. From patient no. 8, an 80-year-old man with cerebral arteriosclerosis, a moderate degree of renal failure (s-creat. 130-150-140 mmol/l), and hyperparathyroidism (s-Ca 3.7-2.5-2.8 mmol/l, s-P 0.8-0.8 mmol/l, s-parathormone 690-510 pmol/l). The urine sediment contained numerous hyaline casts with embedded leukocytes. The leukocyte casts suggested renal lesions induced by hypercalcemia or urinary tract infection, or both. Staining according to May-Grünwald-Giemsa.

Fig. 1A. Low-power field, magnification x134. Two hyaline casts (Cy) with embedded leukocytes. Except for the casts, the picture is typical of the urine sediment from a patient with indwelling catheter and heavily infected urine, i.e. numerous bacteria and leukocytes, some in lysis. This specimen was obtained after treatment with methenamine hippurate, 2 g x 3, for 22 days.

Fig. 1B. Detail from Fig. 1A, magnification x1,336 of a part of a hyaline cast (enclosed area). Two leukocytes (L), surrounded by bright halos, are embedded in the cast. Two cells in lysis (C) are also seen, one embedded in the cast. B: bacteria, probably proteus mirabilis, which were repeatedly cultured from the urine of this patient.

a brilliant contrast in epithelial cells and bacteria (Fig. 2A). The leukocytes were, however, overloaded with stain which veiled intracellular details (Figs.

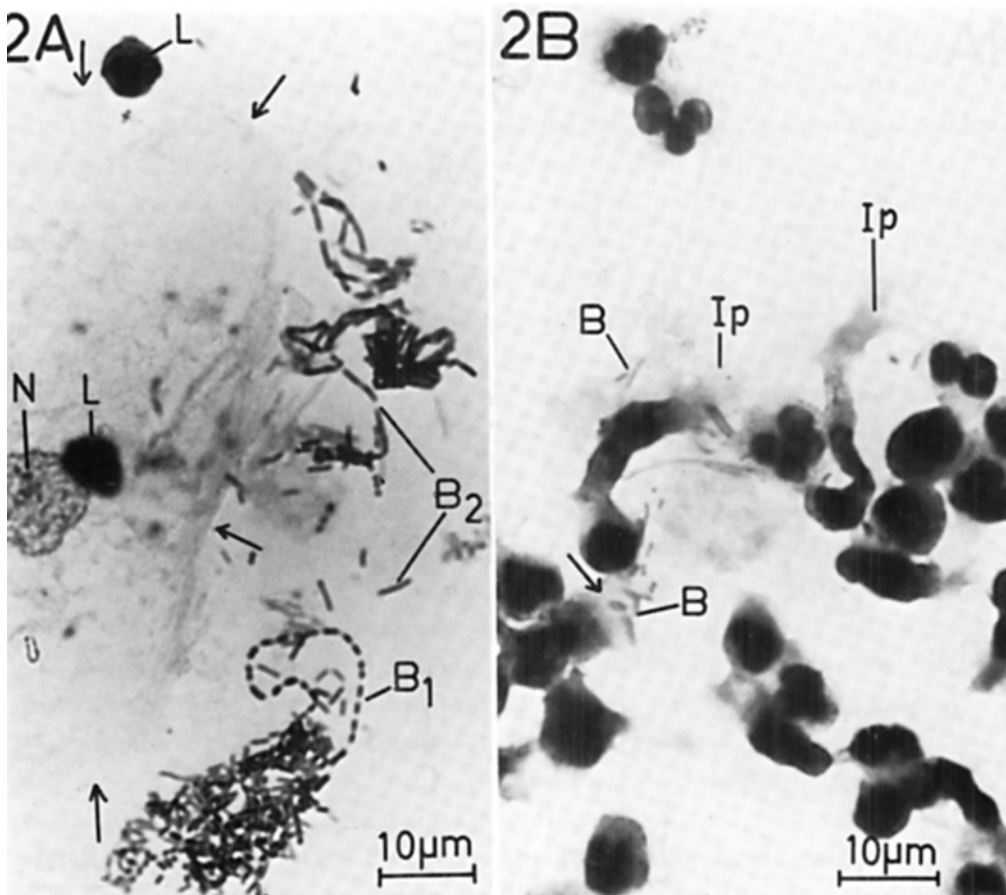


Fig. 2A. The urine sediment from an 82-year-old female, patient no. 5, prior to treatment with methenamine hippurate. The cytological picture was dominated by bacteria of which two strains are shown, B₁ apparently streptococcus faecalis, B₂ apparently E coli, both of which were found in her urine cultures. The upper part of the microphotograph is dominated by an epithelial cell, the borders of which are indicated by arrows. N: nucleus of the epithelial cell. L: leukocytes, one probably located within the cytoplasm of the epithelial cell. Staining according to May-Grünwald-Giemsa. Magnification xl,336.

Fig. 2B. Urine sediment from an 82-year-old female, pat. no. 7, prior to treatment with methenamine hippurate. Leukocytes caught in the hunt and phagocytosis of bacteria. Many of the leukocytes have the elongated shape typical of cells in locomotion, amoeboid movement configuration. Phagocytosis is performed by extension of the thin granule-free cytoplasmic veil in the front part of the leukocytes, the lamellipodium (Ip) towards and around the bacteria (B). Several bacteria are found within leukocytes and surrounded by phagocytic vacuoles (see e.g. arrow). Staining according to May-Grünwald-Giemsa. Magnification xl,336.

l, 2A) with rare exceptions (Fig. 2B). The PAS stain was nearly as bad as the MGG stain as regards leukocyte morphology and clearly inferior to MGG in the staining of bacteria.

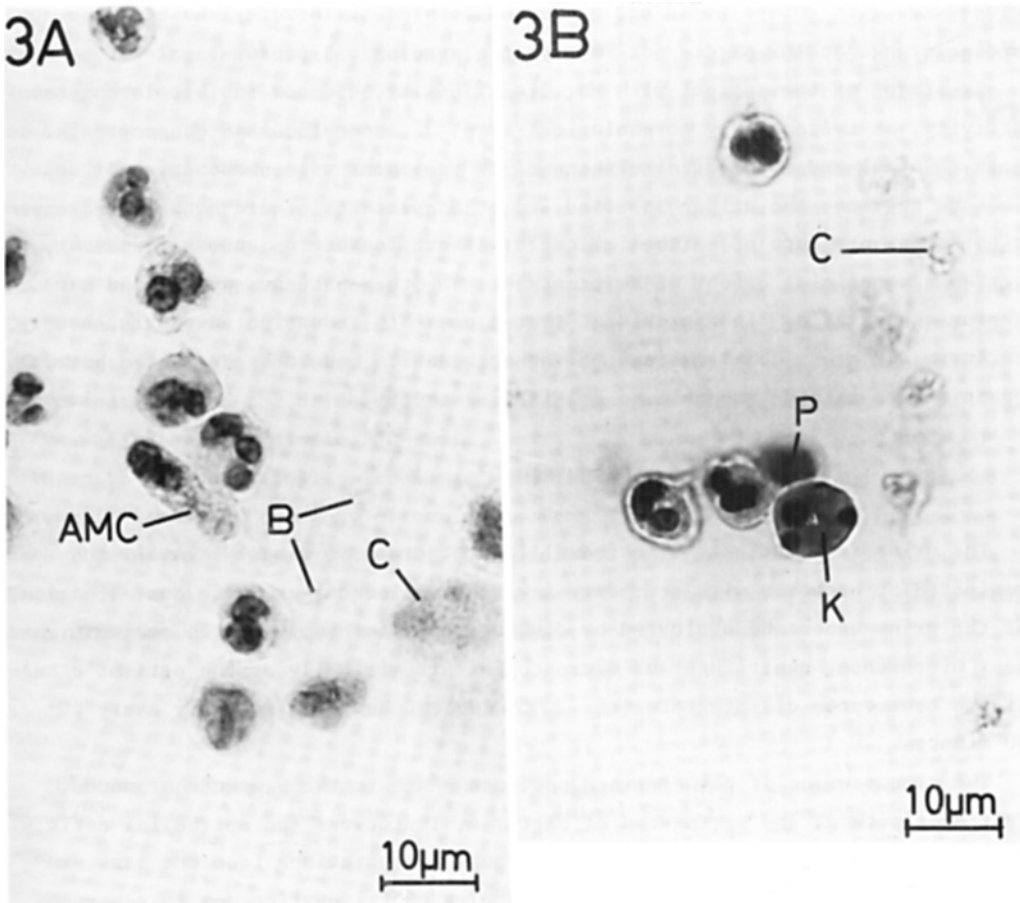


Fig. 3. The hematoxylin-eosin stain provided better transparency of cytoplasm and nucleus than the May-Grünwald-Giemsa stain but bacteria was hardly visible (B). It is evident that the vast majority of leukocytes in the urine of patients with indwelling catheters are polymorphonuclears. AMC: amoeboid movement configuration, the elongated cell shape suggestive of locomotion at the moment of fixation. Some leukocytes were fixed in the state of pycnosis (P), karyorrhexis (K) or cytolysis (C).

Urine sediment from an 81-year-old male, patient no. 2, with cerebral arteriosclerosis and a moderate degree of cardiac failure, after treatment with methenamine hippurate for 17 days. Magnification 1,336.

Haematoxylin-eosin and the Papanicolaou stain provided interesting alternatives to the MGG stain. The nuclear and cytoplasmic details of leukocytes were better visualized than in MGG-stained preparations (Fig. 3) without little difference between the HE stain and the Papanicolaou stain. At least 90-99% of the leukocytes in the urine of these catheterized patients were granulocytes. Epithelial cells were stained properly by both HE and the Papanicolaou stain. Bacteria were often hardly visible in preparations stained by HE and only slightly easier to see in preparations stained by the Papanicolaou stain. Other staining

procedures with methyl green and eosin, HE combined with methylene blue and acridine orange at low pH did not improve the cytological picture.

Treatment of the patient with MH, 2 g x 3 daily, did not inhibit leukocyte activity, as reflected by morphological signs of locomotion and phagocytosis. Both clinical and cytological effects of MH treatment were, however, ambiguous. In some patients the urine clarified, in other patients it did not. In some patients, the presence of epithelial cells without leukocytes and bacteria suggested a beneficial effect of MH treatment, in other patients pyuria and bacteriuria persisted. It was evident that a possible reduction in pyuria, bacteriuria and other complications of catheterization had to be evaluated by more quantitative methods amenable to statistical tests.

DISCUSSION

The urine from patients with indwelling catheters is usually heavily contaminated by bacteria with secondary invasion of leukocytes. The contamination of the urine cannot be evaluated by conventional microscopy of the sediment unless microscope, centrifuge and microscopist are virtually at the patient's bedside. Leukocytes disintegrate (cf. 2, 5, 10) and bacteria multiply every 20-60 minutes.

The cloudy urine of catheterized patients - due to the presence of mucus, salt precipitates and aggregates of bacteria, leukocytes and epithelial cells - was a major source of variation between double preparations from the same sample. Other pitfalls were loss of cells during centrifugation due to adherence to the walls of the plastic chamber of the centrifuge, loss of cells into the filter paper during centrifugation, and loss of cells from the slides during the drying and staining.

The leukocytes present in the glutaraldehyde-cyto-centrifuge sediments of catheter urine consisted to 90-99% of granulocytes. (Figs. 1-3.) This finding is in agreement with observations of previous authors on patients with bacteriuria (5). The morphology of the bacteria was sometimes sufficiently distinct to permit a tentative identification of bacterial species (Fig. 2A).

In conventional coverslip preparations of urine sediment, vital leukocytes are sometimes seen hunting and phagocytising bacteria. This phenomenon is not seen in air-dried preparations of leukocyte suspensions, apparently due to the slowness of the method; the leukocytes stop hunting and take up a stationary spherical form during preparation.

The glutaraldehyde fixation was rapid and effective, as evidenced by the finding of leukocytes with the elongated shape of moving cells and leukocytes in different stages of disintegration.

The leukocytes were fixed in the rounded shape of suspended cells, not in the

flattened shape of smeared and air-dried cells. The leukocytes and the bacteria thus became located in different optical planes on the slide due to the restricted depth of the visual field (0.2-0.4 μm). This fact rendered the assessment of bacteria more difficult.

The glutaraldehyde-cytocentrifuge method had some obvious advantages over the conventional coverslip preparation of urine sediment and the contrasting procedures described by previous authors (1-10). The cytological and bacteriological picture of the urine was frozen in the state existing at the moment of sampling. Cells and bacteria on the slide were linearly correlated to the cellularity of the original sample and were properly contrasted by routine cytological stains. The preparation of the urine during its transfer from the catheter to microscope was relatively simple and rapid.

In spite of the pitfalls associated with sampling, preparation and assessment of the inflammatory cytology of the urine from patients with indwelling catheters, the glutaraldehyde-cytocentrifuge sediment provided a rough quantitative measure of urine contamination. Bacteria were best visualized by the MGG stain, leukocytes by HE or by the Papanicolaou stain.

ACKNOWLEDGEMENTS

This work was supported by the Swedish Medical Research Council project no. 2294 and no. 5362 and grants from the Medical Faculty of Lund.

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Received July 1978. Accepted October 1978.

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