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Differential Count of Urinary Leukocytes and Renal Epithelial Cells

A Comparison between Phase Contrast Microscopy of Unstained Sediments and Light Microscopy of Fixed and Stained Specimens

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ABSTRACT

Urine samples from 143 patients were treated in two ways. One part of every sample was centrifuged in a conventional centrifuge and the other one in the Cytospin® centrifuge, a so-called cytocentrifuge. Cytocentrifugation seemed to be better suited for cell concentration than common centrifugation, yielding a greater number of specimens with a sufficient number of cells for a differential count. The sediments obtained by common centrifugation were examined by phase contrast microscopy, while the cytocentrifuged specimens were examined by conventional light microscopy after fixation and staining with Papanicolaou technique. By both methods were determined the percentages of renal epithelial cells, granulocytes and mononuclear leukocytes. Similar results were obtained by both methods. The percentages of renal epithelial cells and granulocytes varied from case to case. The percentage of mononuclear leukocytes was small in all cases. Use of fixation and staining seemed to enhance the possibilities of identification of histiocytes and lymphocytes.

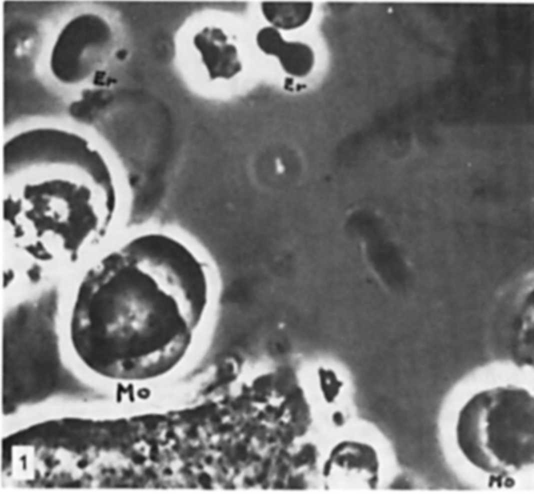
INTRODUCTION

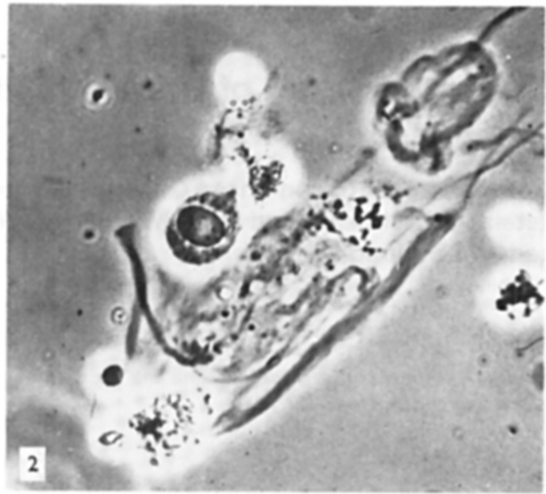
Microscopic examination of the urinary sediment is routinely performed for detection of pyuria, which is usually interpreted as a sign of urinary tract infection. Presence of renal epithelial cells (1, 3, 4, 11, 15) and of lymphocytes, histiocytes and plasma cells (15) is also recorded in some laboratories, but usually no attempt is made to count the different leukocytes and renal epithelial cells. It should be noted, that in the opinion of some authors, it is impossible to distinguish renal epithelial cells, i.e. cells derived from renal tubules or collecting ducts, from urothelial cells, except when they are part of casts (6). However, a counting of renal epithelial cells and leukocytes has been performed by some investigators (5, 12). Phase contrast microscopy, used for differential counting of granulocytes, mononuclear leukocytes and renal epithelial cells, has revealed considerable differences in the urinary cell pattern between different diseases (9, 10). The results or a similar differential count have also been published by Kozlovskaya et al. (7). Use of phase contrast microscopy (2, 3, 14), however, has some disadvantages. For example, the sediment examination has to be performed very soon after collecting the specimen, and it often fails due to too small cell number in the sediment.

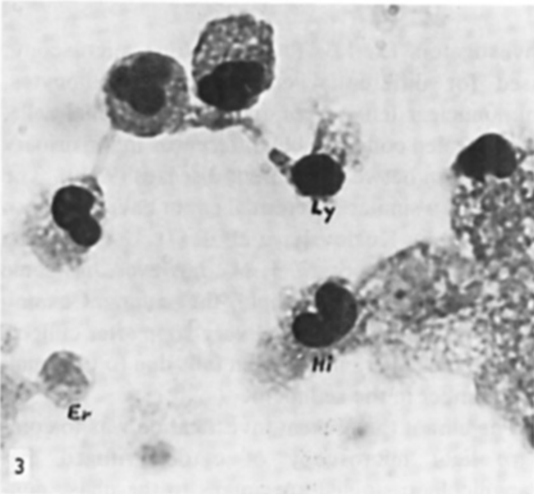
The aim of the present investigation was to compare light microscopy of cytocentrifuged and Papanicolaou stained specimens to the phase contrast technique. Cytocentrifugation is known to be a good means for concentrating cells in cellpoor specimens. Papanicolaou technique was chosen because this staining method is routine in most cytology laboratories for examination of urine specimens. Several other staining methods have been advocated for staining of urinary sediment (15). Peroxidase staining has been used for differentiation between epithelial cells and leukocytes (1, 13). Since it is known, that lymphocytes and monocytes like renal epithelial cells are usually peroxidase negative, this method does not seem to yield any advantages compared to other staining methods (16).

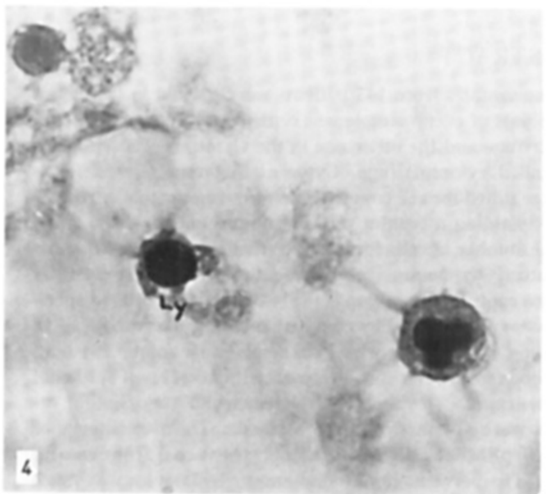
METHODS

Fresh non-morning voided midstream urine samples were obtained from 143 patients, admitted to the clinic of internal medicine. The majority of the patients had a diagnosis









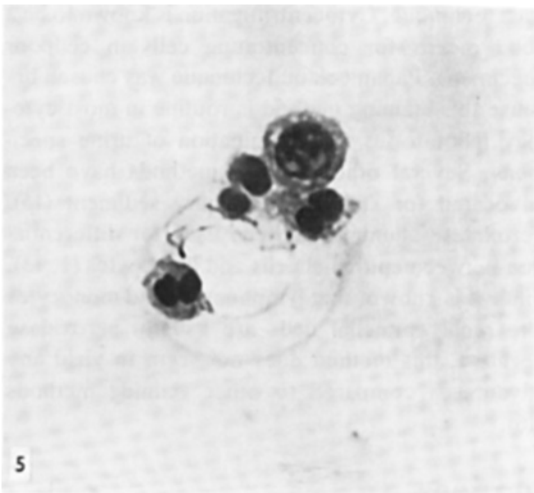


Fig. 1. Mononuclear leukocytes (*Mo*) and erythrocytes (*Er*). (Phase contrast. ×3 200.)

Fig. 2. Renal epithelial cell, adjacent to cast fragment. (Phase contrast. ×1 300.)

Fig. 3. Histiocyte (*Hi*), lymphocyte (*Ly*) and granulocytes. One erythrocyte is also indicated. (Light microscopy. ×1 600.)

Fig. 4. Lymphocyte (*Ly*), granulocyte and erythrocyte. (Light microscopy. ×1 600.)

Fig. 5. Renal epithelial cell and granulocytes. (Light microscopy. ×900.)

of pyelonephritis, interstitial nephritis, glomerulonephritis, hydronephrosis, lupus nephritis, polycystic kidney disease or had a transplanted kidney, but other kidney diseases were also represented. All of the patients were included in a previous investigation (9, 10). Every urine sample was investigated by means of the two methods described below. All microscopic examinations were performed by the author.

Phase contrast microscopy of unstained sediments

10 ml of the urine sample was centrifuged in a conventional centrifuge for 10 min at 2 500 r.p.m. and the supernatant was carefully poured off. The sediment was vigorously shaken, a small drop was placed on a slide by means of a thin plastic tube and a coverglass was applied. A differential count was performed within one hour after collecting the urine, using a phase contrast microscope with a magnification of 1 000× and oil immersion. 50–100 randomly selected cells, fulfilling the criteria described below, were identified. *Granulocytes* were generally easy to identify. Their nuclear segments were dark and the cytoplasm light gray with rapidly moving granules. Sometimes these cells had a degenerate appearance with shrunken nuclei and disappearance of granule movements. As *mononuclear leukocytes* (Fig. 1) were classified cells which did not seem to be either granulocytes or epithelial cells. The cell size varied from about half to twice the granulocyte size. The cytoplasm contained a varying amount of granules, which were slightly larger than the granules of the granulocytes. The granules exhibited Brownian movements. The smaller of these cells had a rounded nucleus and a small amount of cytoplasm with a few granules. The larger cells had a nucleus which was sometimes separated into two or more parts or elongated and bean shaped. As *renal epithelial cells* (Fig. 2) were classified oval or round cells with round nuclei and visible nucleoli. There were no movements in the slightly granular cytoplasm. The cell size was about one to two times the granulocyte size. Rounded cells, larger than the limits set up for renal epithelial cells, were regarded to be of urothelial origin and consequently not counted. The nucleus of these cells occupied a central position.

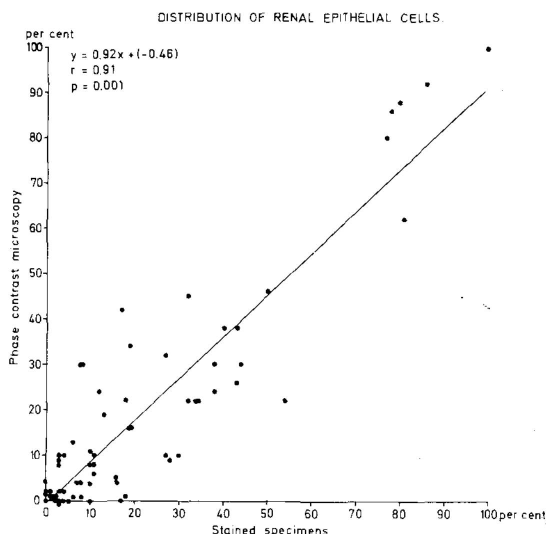


Fig. 7. Distribution of renal epithelial cells.

Light microscopy of fixed and stained specimens

1.5 ml of the urine sample was immediately fixed with the same volume of methanol-acetic acid[1] or Lillie's buffered formol solution (8) and centrifuged in a cytocentrifuge (Cytospin®) for 20 min at 1 600 r.p.m. The slides were permitted to air-dry before staining with a Papanicolaou technique. (In about 60 cases two slides were prepared from each urine sample, the second slide being used for a control stain with a hematoxylin-eosin stain or periodic acid Schiff stain. None of the methods seemed superior to the others.) The slides were mounted with coverglasses and codified before examination. Conventional microscopy was used with a magnification of 1 000× and oil immersion. One hundred cells per specimen, belonging to the cathegories described below, were counted.

Granulocytes (Fig. 3) were generally easy to recognize. No effort was made to separate them into neutrophils, basophils or eosinophils. The *mononuclear leukocytes* could be separated by this method into lymphocytes and histiocytes. For identification of lymphocytes (Fig. 4) common hematologic criteria were used. Sometimes lymphocytes were difficult to differentiate from small renal epithelial cells. As histiocytes (Fig. 3) were recorded cells, which were generally slightly larger than granulo-

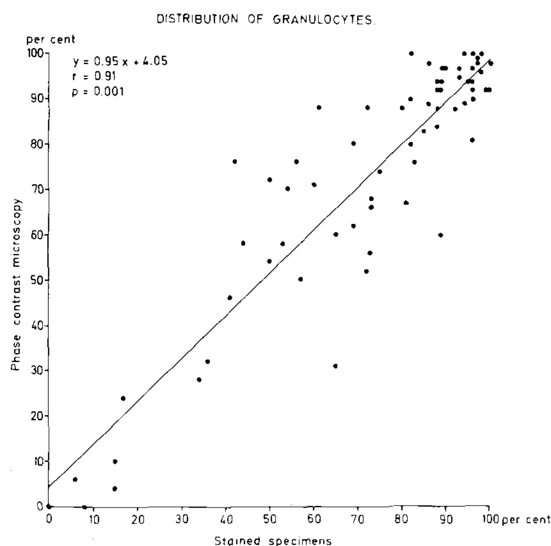


Fig. 6. Distribution of granulocytes.

[1] Methanol-acetic acid was prepared by mixing methanol 450 g, concentrated acetic acid 100 g, and distilled water 450 g.

[2] Cytospin® was supplied by Histo-Lab, Bethlehem Trading Ltd, Gothenburg, Sweden.

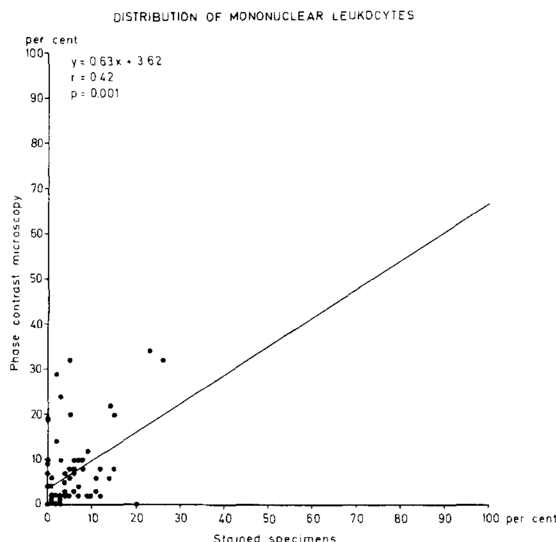


Fig. 8. Distribution of mononuclear leucocytes.

cytes. The nucleus was usually rounded or bean shaped, more seldom cleft or separated into two or more parts. The cytoplasm was slightly foamy and often contained ingested material. As *renal epithelial cells* (Fig. 5) were regarded cells with an eccentrically located nucleus, usually with clearly visible nucleoli. The cell shape was round or oval and the diameter ranged from one to two times the granulocyte diameter. The cytoplasm was homogeneous but sometimes showed inclusions. These cells were often seen adherent to or inside casts or occurring in clusters. Urothelial cells were defined in the same way as in the phase contrast investigation.

Statistical methods

For comparison of the two methods correlation coefficients were calculated according to the method of Pearson. Wilcoxon's rank sum test for paired observations was also used. $P<0.05$ was chosen as level of significance for both tests.

RESULTS

143 urine specimens were examined. By phase contrast microscopy differential counting was possible to perform on 75 sediments. Failure was due to too small cell number in 61 cases and advanced degeneration in 7 cases. By cytocentrifugation and staining with Papanicolaou technique differential counting could be performed in 104 cases. Failure was noted in 38 cases due to too small cell number and in one case due to degeneration. In 66 cases a differential count was obtained by both methods. These 66 pairs of observations were statistically compared to each other.

The individual percentages of *granulocytes and renal epithelial cells* are shown in Fig. 6 and 7. A significant linear relationship between the values obtained by the two methods was found ($r=0.91$, $p<0.001$ for both cell types). Nor did Wilcoxon's rank sum test for paired observations yield any significant difference, when applied to the percentages of granulocytes and renal epithelial cells obtained by the two methods.

The percentage of *mononuclear leukocytes* (lymphocytes and histiocytes) was small in all these cases, never exceeding 34%. (Median value 4% for both methods.) A positive correlation between the methods was found also for this cell cathegory (Fig. 8), but the correlation coefficient was small ($r=0.42$, $p<0.001$). Wilcoxon's rank sum test did not yield any significant difference between the methods.

CONCLUSIONS

For the purpose of this investigation cytocentrifugation proved to be a better method for concentration of urinary cells than common centrifugation.

Phase contrast microscopy of unstained sediments and conventional light microscopy of fixed and stained specimens gave similar results when used for cell identification. Phase contrast microscopy of unstained sediment is a simple and rapid method, suited for routine investigation of urine sediment. Fixation and staining on the other hand probably allows a more accurate identification of lymphocytes and histiocytes. Another advantage is that fixed specimens and slides can be stored, while the phase contrast examination must be carried out as soon as possible. Also, the number of specimens impossible to evaluate due to cell degeneration was smaller with this method.

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