FOREWORD

The last twenty years have seen a decline in the interest of clinicians for the examination of the urinary sediment as a tool for the diagnosis of renal diseases. Most of our attention has been focused on the study of renal tissue obtained by biopsy and on difficult and sophisticated serological tests. This trend represents quite a change from the past when the microscopic examination of the urinary sediment was considered one of the most valuable clinical laboratory determinations. Beale (L.S. Beale, "Kidney Diseases, Urinary Deposits and Calculus Disorders: Their Nature and Treatment", Lindsay and Blakiston, Philadelphia, 1870), a real pioneer in laboratory medicine, had admonished that "the different morbid states of the kidney are (not) different stages of one and the same disease .... a few months' careful study in the wards of a hospital ...., will serve to convince any unprejudiced person that the nature of renal disease may be diagnosed in many cases by the microscopical characters of the urinary deposit, and that there are several essentially distinct forms of renal disease". He correctly stated that "in many cases of congestion and in inflammation of the kidney, a spontaneous coagulable material is effused into the tubules and coagulates there forming a cast or mold of the tube .... (and) entangles in its meshes any structures which may be there at that time". These concepts were widely advertised by Addis (T. Addis, "Glomerular Nephritis. Diagnosis and Treatment", McMillan, New York, 1948) who wrote "when the patient dies the kidneys may go to the pathologist, but while he lives the urine is ours. It can provide us day by day, month by month, and year by year with a serial story of the major events going on within the kidney. The examination of the urine is the most essential part of the physical examination of any patient with Bright's disease".

The present poor attitude towards the microscopic examination of the urinary sediment is, in part, due to absence of dialogue between physicians and laboratorians. In general, an informed microscopist is a much better screener than one who is required to examine the urinary sediment without knowledge of the clinical problem. The book by G. Piccoli, D. Varese and M. Rotunno obviates the problems that, in the last two decades, have contributed to lower the stature of this important test, and correlates pertinent urinary findings with kidney pathology seen in living patients with the aid of renal biopsy and with the most rigorous and modern clinical, morphological and immunocytochemical techniques.

The book fulfills the objective to provide a practical, comprehensive guide to the examination of the urinary sediment for general microscopists, clinicians, pathologists, technologists and medical students. The modern classification of various types of renal disorders, the concise interpretation of the microscopic findings in the light of the most recent acquisitions in collateral fields of pathology and immunology, and the beautiful illustrations, all reflect the long-term interest of Dr. Piccoli and his Collaborators in the field of diagnosis of renal disease and the high standard of clinical activity attained by this group of Italian nephrologists. Their commendable effort will undoubtedly contribute to increase our understanding of renal pathophysiology and to improve the clinical management of renal patients.

GIUSEPPE A. ANDRES

PREFACE

In the course of the research which has led to the development of modern nephrology, only limited attention has been given so far to the study of urinary sediment. Ignoring this inexpensive and non invasive analysis one runs the risk of losing a patiently built up wealth of knowledge which is not exploited as fully as it might be, either for initial diagnosis or subsequent examinations.

In our opinion both the quantitative and the morphological assessment of urinary sediment are of great value. Therefore, in conjunction with other more recent techniques, we have continued to pay particular attention to urinary sediment analysis and to consider our findings systematically.

Our results have confirmed that this type of investigation is still worth consideration in modern nephrology and that its re-evaluation is justified.

In this atlas G. Piccoli, D. Varese and M. Rotunno have collected and documented considerable information on the elements of the urinary sediment and discussed their significance in the more common nephropathies.

Whilst not ignoring traditional ideas, the work gives a careful account of current thinking and, in particular, makes a strong case for the inclusion of the examination of sediment in nephrological diagnosis.

This volume is the outcome of many years of experience that I have shared with the authors, and of much eager discussion; I do hope it will contribute to the growing interest which has been apparent for some time in this field.

ANTONIO VERCELLONE

1.0
INTRODUCTION

To suggest a text on the study of urinary sediment in renal diseases, at a time when more and more sophisticated techniques are available to the nephrologist, might well seem anachronistic or even unnecessary. In fact, a critical re-assessment of what this type of examination has to offer is justified by the fact that most clinicians today still utilize, directly or indirectly, the results of tests on the urinary sediment in their diagnostic approach to nephropathies.

However, the value of these analyses may, at times, be obscured by a hurried examination of the urine and/or the use of concepts with inappropriate terminology to present day knowledge of nephrology.

Indeed, a morphological analysis of the individual cells and casts together with the general picture of the urinary sediment can provide valuable information that is as important as a careful clinical examination.

Bearing this in mind, we have made a systematic collection of a series of urinary findings and examined their morphological interpretation in the light of current diagnostic practice.

A series of photographs illustrates the individual cells and casts and also gives a general picture of sediment samples in various kidney diseases; most are unstained samples examined by ordinary light microscopy, as is common in clinical practice. In general, the critical analyses that accompany these photographs are based on recent or time-honoured classical concepts, which are considered to be clear and widely accepted. There is still much to learn, however, and some aspects are as yet obscure.

As the questions that may arise from the analysis of these samples are often as important as the answers given, we have tried to draw attention to various controversial aspects. We hope this text will not only encourage discussions that may help to clarify these aspects but will also promote a re-assessment of the importance of urinary sediment interpretation in nephrological practice.

2.0

URINARY SEDIMENT UNDER NORMAL AND PATHOLOGICAL CONDITIONS

Under normal conditions, the urine of healthy people contains a number of erythrocytes, leukocytes and hyaline casts [3], yet there is no precise information as to the upper limit of the normal cell range counts in urine excreted daily [70]. This lack of data is due in part to technical problems and to the wide variation from one individual to another, and in part to the considerable variability in the percentage of cells and casts that are destroyed and consequently not counted; the extent of this loss depends on the physicochemical conditions of the urine and the length of time these elements remain in it [62].

Quantitative assessment is therefore only empirical and approximate; nonetheless, for conventional volumes of 1250 ml of urine voided per day, it is generally agreed that the approximate, acceptable maximum limits for normal urine are 500 erythrocytes/ml, 2000 leukocytes/ml and less than 15 hyaline casts/ml [31]. Since the various techniques for obtaining total counts are too time-consuming for routine examination, they have not come into common use and an estimate of the average number of elements in highly magnified microscopic fields (generally 400x) is preferred.

This is a more approximate and even less reliable assessment, since it is based on a technique which introduces an additional number of variables, partly determined by chance (e.g. dilution of the urine) and partly connected with the execution of the test. The latter is often by no means standardized: there may be variations in the volume of urine centrifuged, the time and speed of centrifugation, the volume of the supernatant, the drop of urine examined, etc. (p. 203).

If, however, one does not intend to fix precise limits for normal and abnormal values, or to make accurate comparisons of the different examinations, this type of semi-quantitative test does provide quite a good practical basis for assessment.

Erythrocytes and leukocytes are not found in the sediment of some healthy people; yet, although opinions differ, one red cell, one to two leukocytes and only an occasional hyaline cast are generally taken, in a rather arbitrary way, to indicate the upper normal limit for each highly magnified microscopic field observed (400x).

These are, of course, only approximate values. Before drawing any definite conclusion, all borderline results require careful critical assessment, bearing in mind that the urinary sediment in certain nephropathies may, at least in some stages, be basically normal.

In pathological conditions it is generally superfluous to discuss the limits between normal and abnormal values, as in the course of most nephropathies the elimination of cells and casts is well over the limits given here.

Even in these circumstances, quantitative or semi-quantitative assessment must be considered as only approximate, especially if successive observations are being compared.

3.0
MORPHOLOGICAL STUDY OF URINARY SEDIMENT

An accurate morphological definition of the elements in the urinary sediment is essential: in the first place the identification of both typical cells of normal appearance and atypical cells showing signs of degeneration is required for any quantitative assessment.
The definite identification of some specific elements may also, in itself, make diagnosis easier. This is so, for example, in the case of large casts, apparently normal or clearly abnormal erythrocytes, renal epithelial cells and lipuria.

3.1 ERYTHROCYTES

The morphology of the red blood cells found in urinary sediment is extremely variable. To recognize some of the most typical aspects accurately can be of considerable diagnostic value since there are quite close correlations between certain pathological conditions of the kidney and the urinary tract and the morphology of the red cells in the sediment [40, 6, 18, 19, 20].

Using a morphological classification, a first category may include the following elements, typical of hematuria, generally caused by «urologic» diseases:

a) biconcave red cells similar to those in the blood;

b) red cells that have lost their biconcave shape but still appear «intact». They may be disc- or balloon-shaped, of spherocyte type; those of smaller dimensions are typical of this morphological class. The pigmentation of these red cells is often well preserved; in hypotonic urine however they may become what are known as «ghost cells»;

c) crenated red cells.

All these forms can be easily reproduced suspending normal red blood cells in solutions with varying physico-chemical properties (hypo-, iso-, or hypertonic) [40].

1   Red cells morphologically similar to those in the blood («urologic» hematuria) (400x).

2   Ring-shaped red cells. Note the similar shape of these cells (urolithiasis) (400x).

3   Balloon-shaped red cells, all of similar appearance (calculi in the renal pelvis) (400x).

4   Crenated red cells (calculi in the ureter). With red cells of this type a definite diagnosis of non-glomerular bleeding can be made (400x).

5   Leukocytes and crenated red cells (calculi in the renal pelvis; urinary infection). These red cells come from the urinary tract (400x).

6   Modifications in the morphology of normal red blood cells, suspended in the laboratory in urine from healthy people; the osmolality of the urine varies: («a» 500 mOsm/kg, «b» 300 mOsm/kg, «c» 120 mOsm/kg). Note that the «ghost» cells in «c» have lost their pigmentation, by no means a rare clinical finding (400x).

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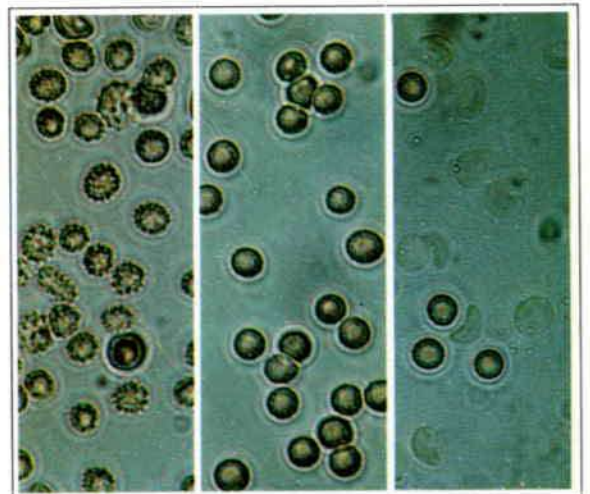
6   a   b   c

Urinary red cell morphology can be assessed best by phase-contrast microscopy.
It was Birch and Fairley [6,18,19], and more recently Fasset [21] (who also adopted the percentage count of the various types of erythrocyte), who showed by this procedure that relatively normal cells with very regular or crenated outlines are most often found in «non-glomerular» bleeding, a criterion now commonly accepted [39,46,47].

7 Relatively normal red cells in a case of «non-glomerular» hematuria. The outlines of the cells are brought out very clearly in phase-contrast examination (400x).

8 «Non-glomerular» bleeding (hematuria caused by a urethral catheter; phase-contrast examination) (400x).

9-12 «Wrinkled» red cells: red blood cells suspended in urine differing in osmolality (from 400 mOsm/kg to 1000 mOsm/kg). When the urine has a very high osmolality the red cells may be quite seriously damaged; under these conditions it may be difficult to ascertain that the cellular membrane is definitely intact (phase-contrast) (400x), (400x), (400x), (400x).
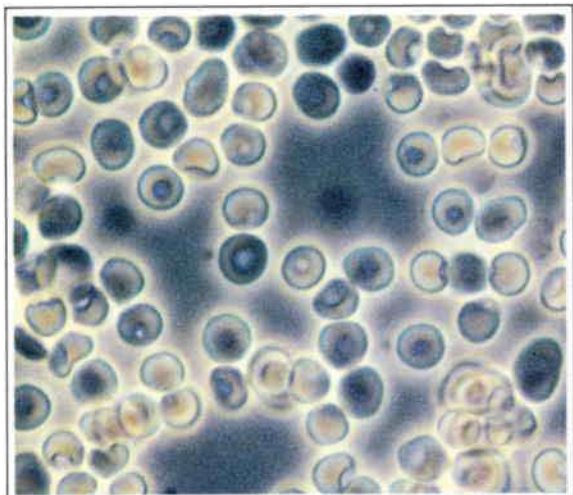
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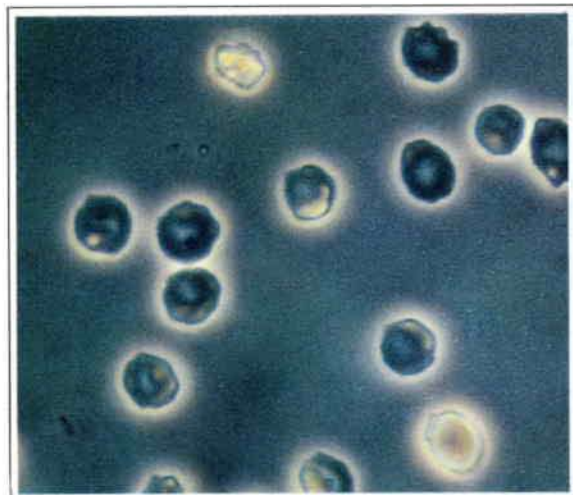
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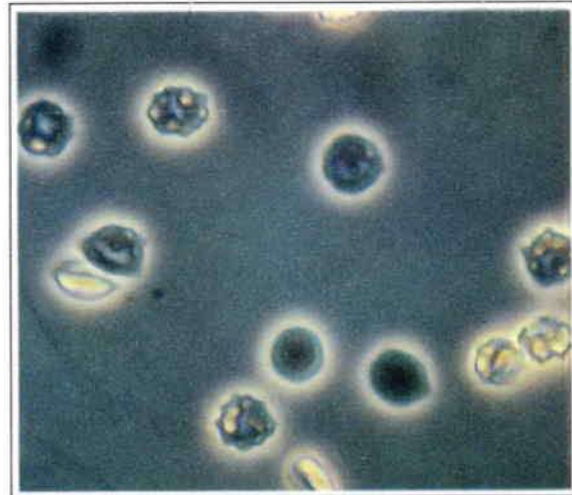
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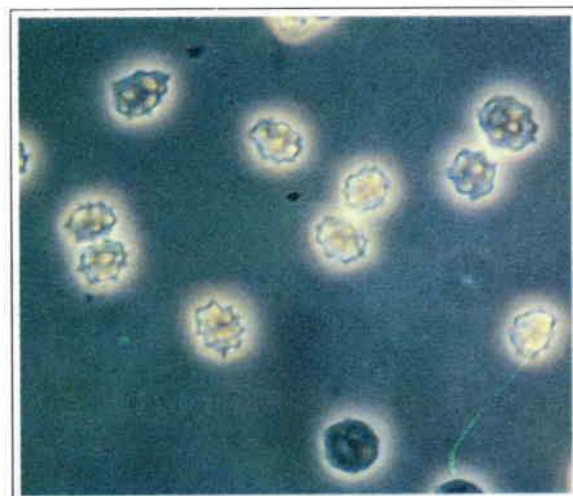
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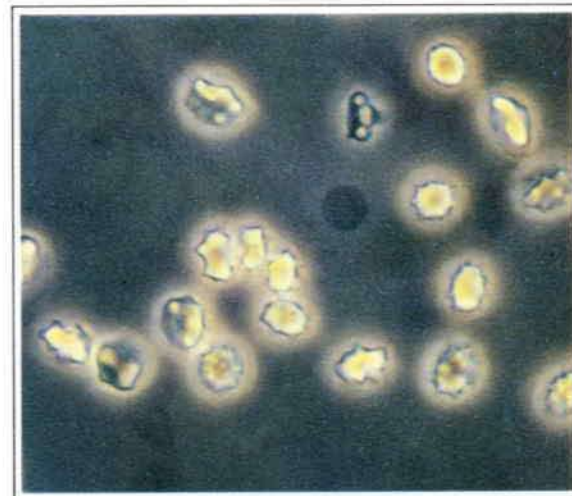
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A second morphological category of red cells in the urinary sediment includes damaged, irregularly shaped cells [6,20], showing fractured membranes, extrusion of cytoplasm, or fragmentation. If most red cells are fragmented, granular clusters are found in the sediment [40].

These cells often lose some of their hemoglobin and consequently appear paler. This behavior is not easily demonstrable in the laboratory merely by varying the solution in which normal erythrocytes are suspended. It can be hypothesized, therefore, that, apart from the effects of being kept in an extravascular, non-physiological environment, there may be traumatic damage, caused while passing through the glomerular capillaries and renal tubules [20], and/or a modification of the electric charges on the cells surface.

Substantial morphological abnormalities, with red cells of different shapes and showing various degrees of depigmentation, are typical of hematuria in glomerulonephritis and in vascular nephropathies, and are sometimes also found in interstitial nephritis.

Extensive fragmentation of the red cells is generally a sign of severe proliferative glomerulonephritis or cortical necrosis [40]. Birch and Fasset [6,20] demonstrated very clearly, by phase-contrast microscopy, the morphological changes undergone by the membranes of these damaged red cells, which they have called «glomerular» red cells.

This definition is now becoming more widely used in clinical practice [39,46,47].

The few red cells found in the urinary sediment of healthy people are very rarely normal [6]. The red cells from the so-called «exercise-induced hematuria» [48] are generally abnormal in appearance, which indicates that they have come from the glomerulus [21] rather than from the bladder [22,23].

13 Abnormal red cells (glomerulonephritis) (400x).

14 Hematuria: the cells are clearly abnormal (glomerulonephritis). Findings of this kind indicate the red cells have almost certainly come from the glomerulus (400x).

15 Extensive fragmentation of the red cells (acute glomerulonephritis in the initial phase); this finding is pathognomonic (400x).

16-18 The abnormal appearance of these red cells is demonstrated particularly well by phase-contrast microscopy (sediments in glomerulonephritis) (400x), (400x), (250x).
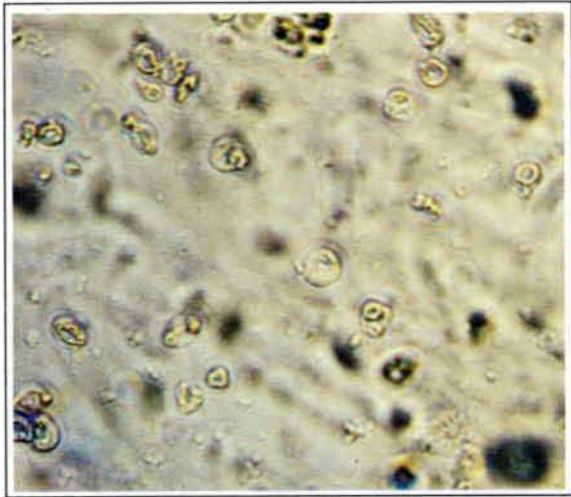
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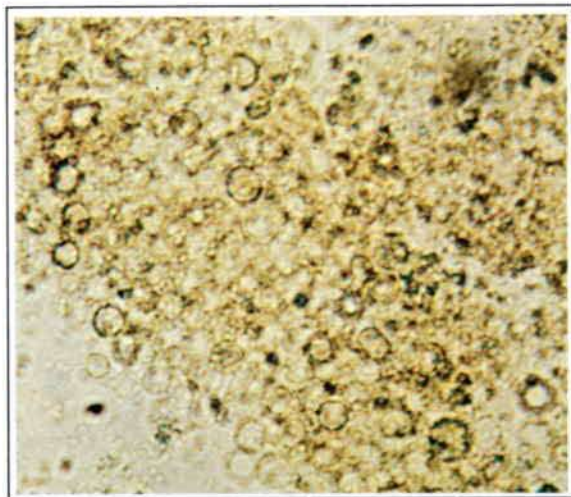
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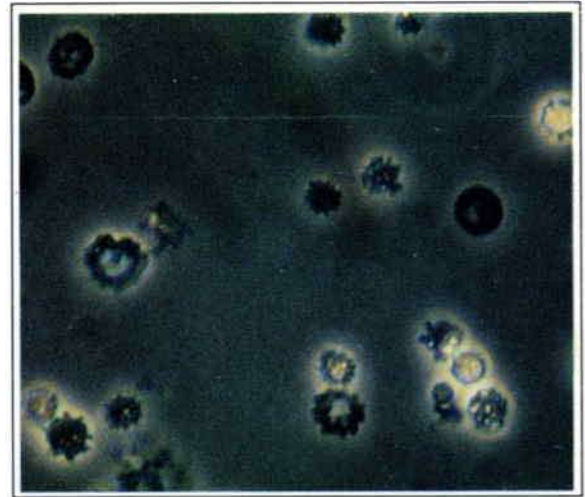
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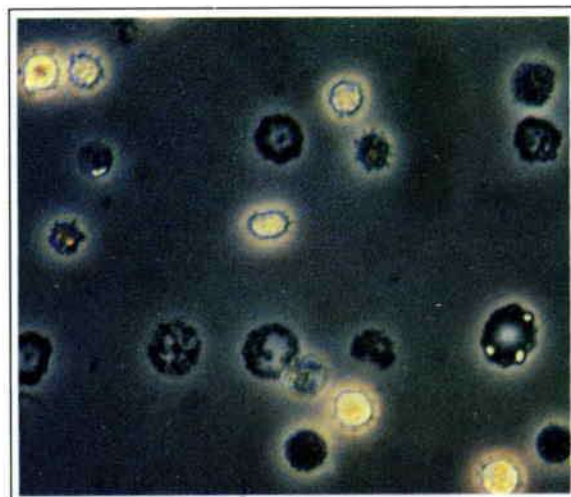
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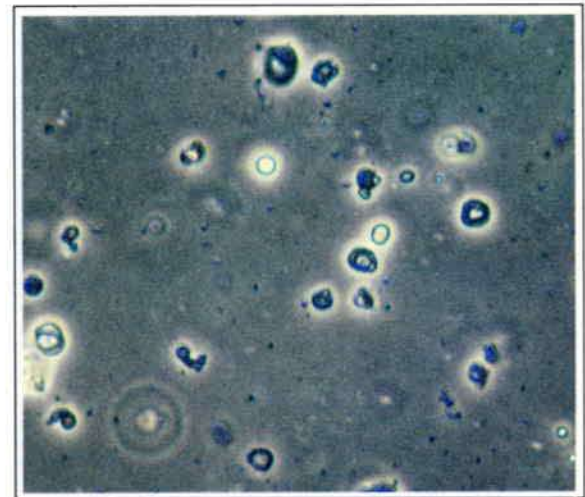

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19 Many red cells showing obvious morphological changes. Numerous examples of cytoplasmic extrusion can be seen here (phase-contrast) (400x).

20 A field similar to the above (400x).

21-22 «Glomerular» red cells in two cases of glomerulonephritis (phase-contrast and ordinary light microscopy, respectively) (250x), (250x).

23 Though phase-contrast microscopy generally gives very good definition, it has not entirely replaced conventional light microscopy, which can be used to assess depigmentation of the erythrocytes (glomerulonephritis) (400x).

24 Red cells, mostly abnormal in shape, in an advanced stage of depigmentation (glomerulonephritis) (400x).
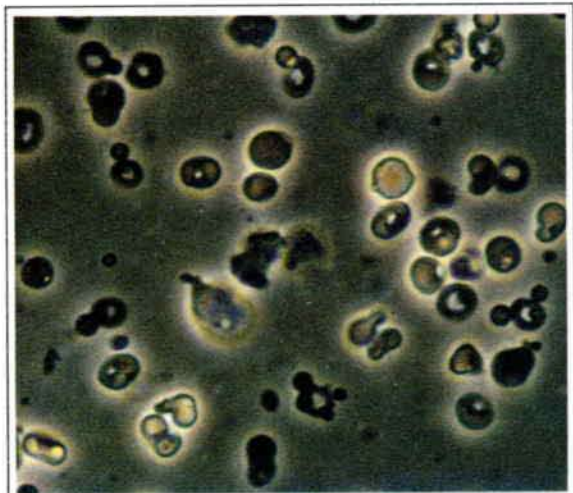
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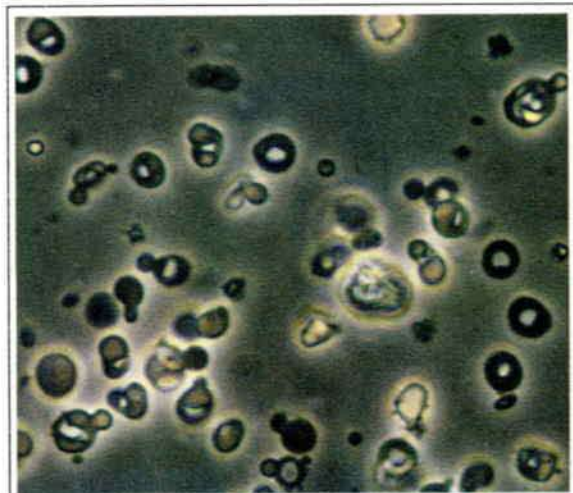
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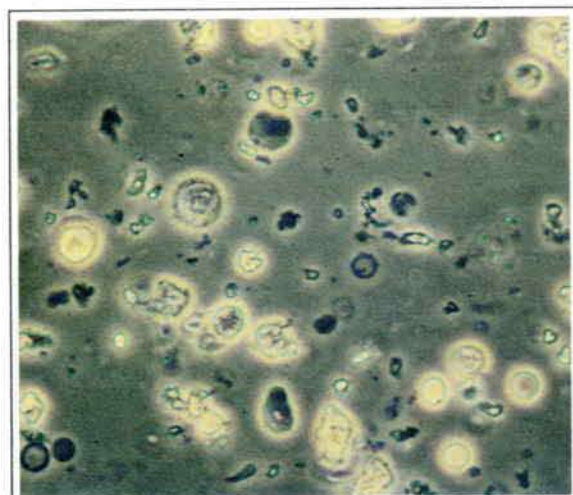
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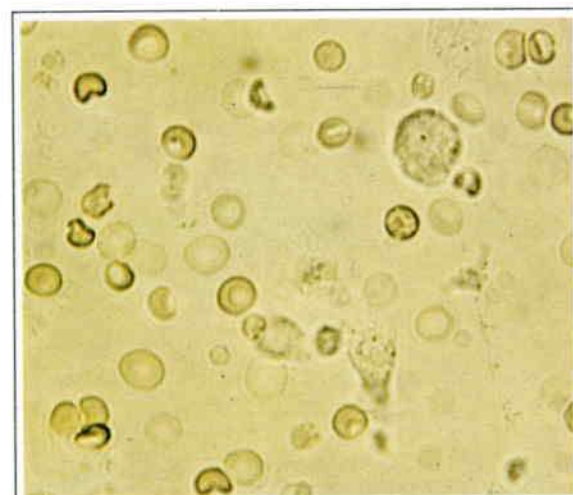
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25-28 Evidence of the glomerular origin of morphologically abnormal red cells is provided by the presence of cells of this kind in casts [19] (160x), (400x), (400x), (250x).

29-30 Relatively undamaged and well pigmented red cells, in a mucous thread and a clot, respectively (calculi of the ureter) (400x), (400x).

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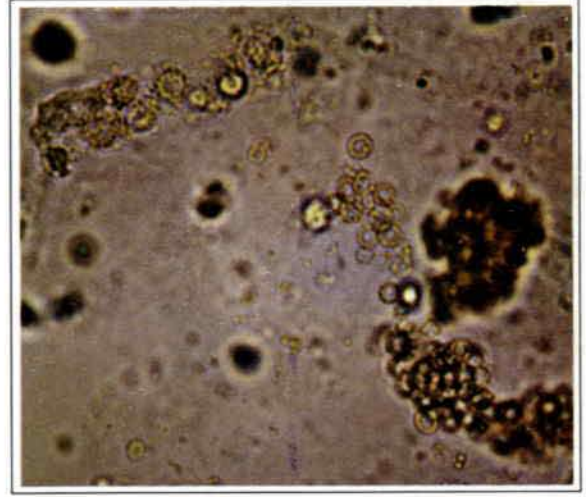
The characteristics of the red cells (relatively normal, «non glomerular», or abnormally shaped «glomerular»), especially in severe hematuria, are generally clear enough to be identified with certainty, and there is very often a definite correlation between these characteristics and the presence of «urologic» or glomerular lesions. Unfortunately, besides the typical and conclusive cases, there are also atypical or indefinite findigs.

In a few cases of glomerulonephritis there may be a microhematuria in which most cells appear virtually normal [20,40]; this happens most frequently, though not exclusively, in Berger's glomerulonephritis.

Such findings are fairly common even in the terminal phase of chronic glomerulonephritis.

In a few urologic diseases, among them nephrolithiasis and some chronic prostatitis, damaged red cells may be found.

Both in glomerulonephritis and urologic lesions, atypical findings are generally observed when there is only slight microhematuria.

Finally it should be remembered that there may be no clear pattern because of «mixed» erythrocytic populations [20], or a high percentage of cell shapes not easily assigned to the classifications described above.

A critical attitude must, therefore, be applied to the interpretation of hematuria; in some cases the morphological picture of the sediment, albeit highly indicative, can sometimes be interpreted with certainty only in relation to the clinical, anamnestic and other diagnostic findings as a whole.

31 Red cells of «mixed» morphology (phase-contrast) (250x).

32 Red cells more or less normal morphologically in a case of Berger's glomerulonephritis (250x).

33 Damaged red cells (urinary nephrolithiasis) (phase-contrast) (400x).

34 Relatively unchanged and also abnormal red cells (primary IgA nephropathy) (400x).

35 Some abnormally shaped red cells in another case of nephrolithiasis (phase-contrast) (400x).

36 Red cells, mostly relatively normal, with regular edges; granular cast (Berger's glomerulonephritis) (160x).
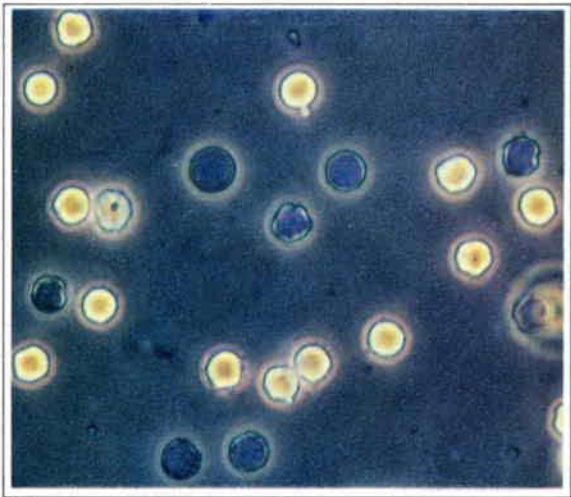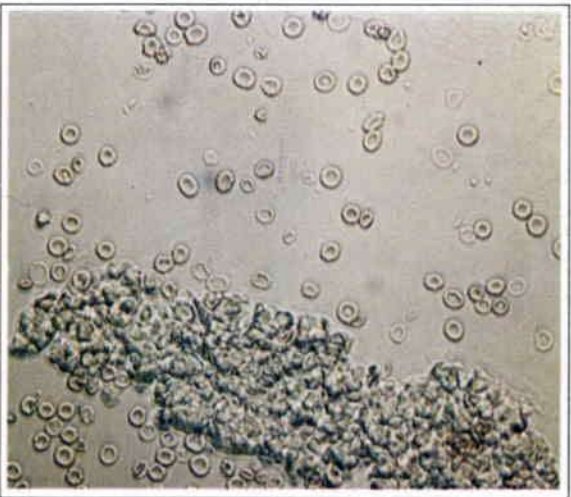
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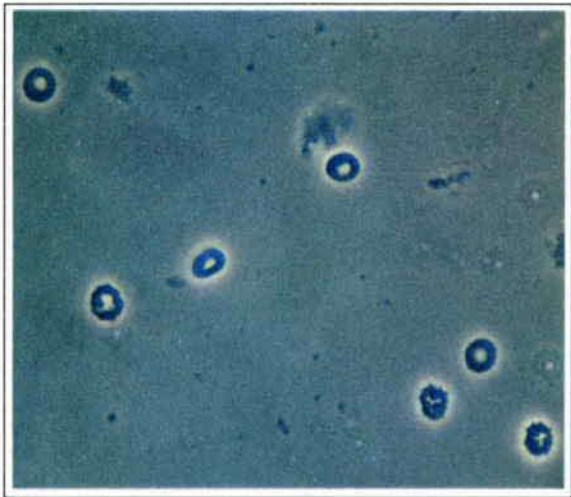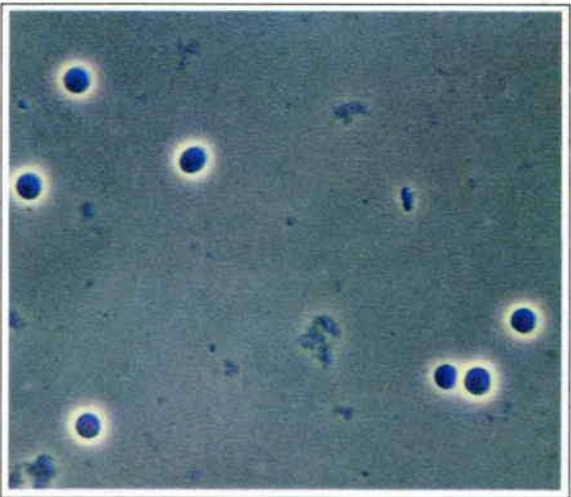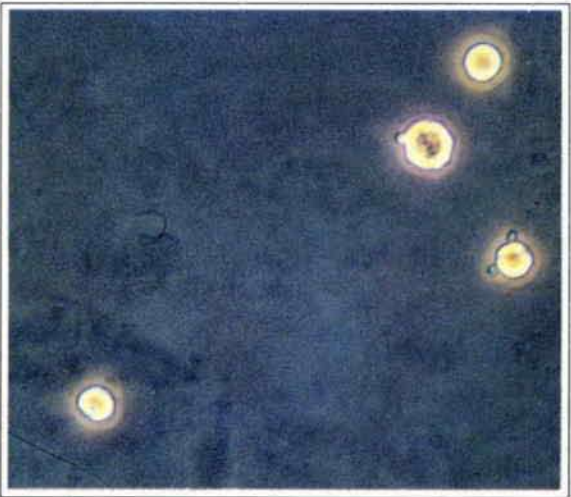
Some elements in the sediment may be mistaken for red cells; the most commonly found are:

— fungi, which have, however, a closer, more compact, sometimes granular appearance, and quite often a typical hyaline-green colour. The frequent variation in size between one element and another, and the presence of small chains, clusters and budding forms are valuable aids in identification and in distinguishing them from the erythrocytes. In doubtful cases the 2% acetic acid test (which destroys the erythrocytes but not the fungi) or eosin staining (which stains the red cells but not the fungi) can be used.

— calcium carbonate crystals (page 146).

37 Fungi. The different sizes of the cells, sometimes showing budding or chain formation, are of considerable help in recognition (400x).

38 Fungi. Considerable variation in the form of the cells. Abundant bacteriuria (400x).

39 Isolated fungi; the granular appearance is clearly evident. In doubtful cases, the absence of hemoglobin in the sediment, the 2% acetic acid test or staining with eosin can ensure conclusive identification (400x).

40 Typical chain formation of monomorphic fungi (400x).

41 Clump of fungi (400x).

42 Calcium carbonate crystals (400x).

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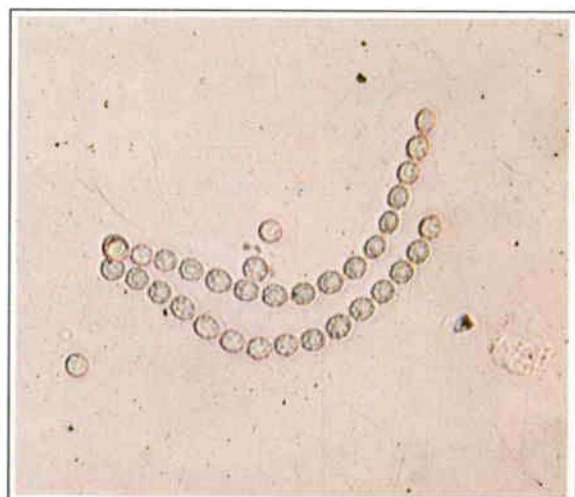
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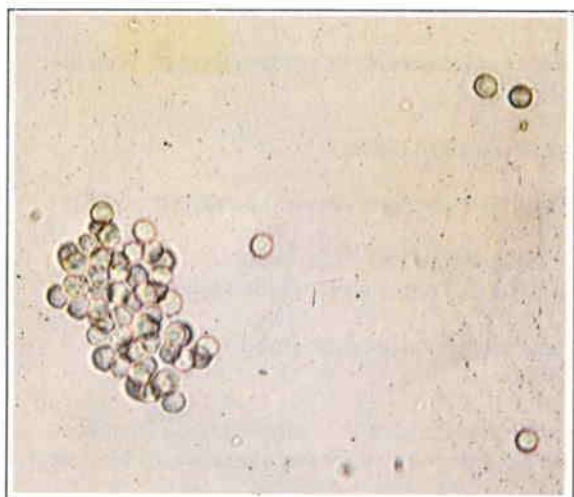
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Other elements that may be mistaken for red cells:

— some calcium oxalate crystals which are denser, more oval and more variable in size than erythrocytes (page 138);

— small air bubbles, easily recognizable as they are highly refractile;

— fat droplets, present on an inadequately cleaned slide, are only vaguely similar to red cells;

— free droplet lipuria: refractile and with more variable dimensions;

— small leukocytes. The 2% acetic acid test, which destroys the red cells but not the granulocytes and also facilitates the identification of their nuclei, can be used in doubtful cases.

43   Oval monohydrate calcium oxalate (whewellite) crystals and envelope-shaped crystals of dihydrate calcium oxalate (weddellite) (400 x).

44   More rounded crystals of monohydrate calcium oxalate (400 x).

45   Small air bubbles trapped under the coverslip; note their refractile appearance (400 x).

46   Droplets of exogenous fat due to inadequate cleaning of the slide. Note their refractile appearance, typical arrangement in rows and the different sizes of the cells (400 x).

47   Endogenous lipuria both with free droplets and in a hyaline cast (membranous glomerulonephritis; nephrotic syndrome) (400 x).

48   Small leukocytes with barely recognizable nucleus. Cells of this kind may be mistaken for erythrocytes. In doubtful cases the acetic acid test or a supravital stain can be used (400 x).

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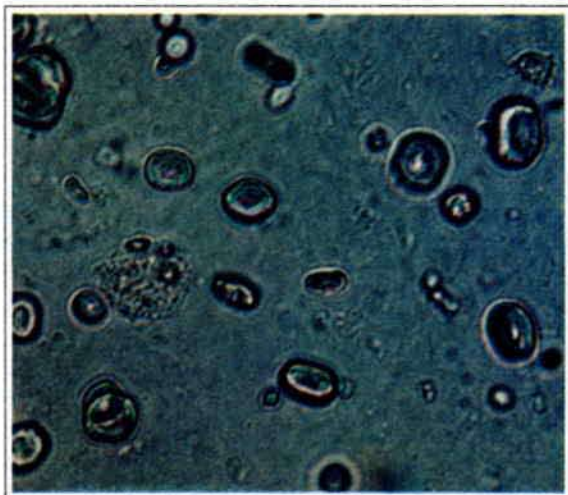
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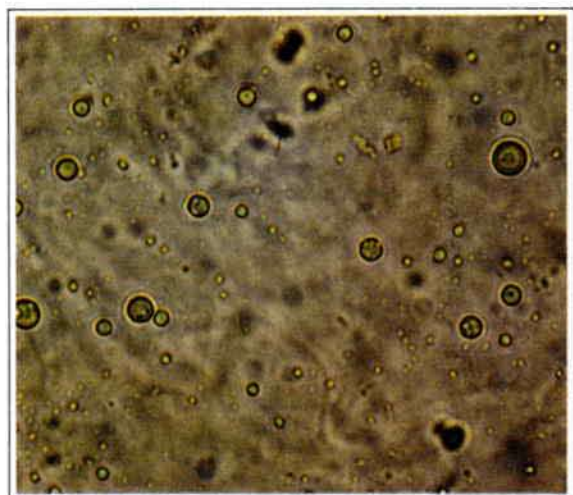
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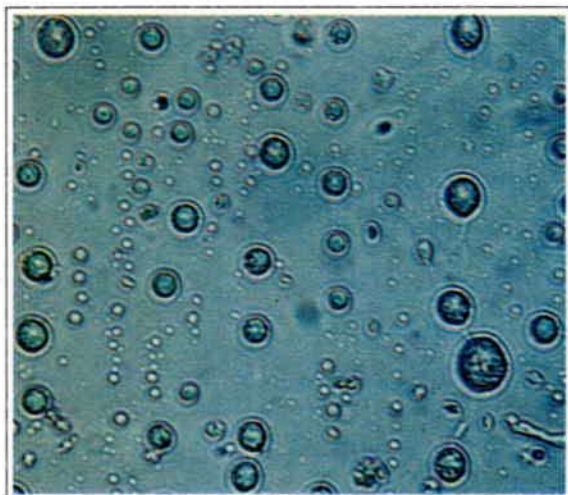
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3.2 GRANULOCYTES

The great majority of granulocytes in urinary sediment are neutrophils. The term leukocyturia or granulocyturia is generally used to indicate the presence of large numbers of these cells in the urine.

They are usually rounded in shape, with a diameter of 14-16 μ. In hypotonic urine they may, however, have a diameter of up to 30 μ, while in hypertonic urine they may be more or less the same size as red cells.

The most reliable method of identifying granulocytes is by studying the morphology of the nucleus, either under the phase-contrast microscope or by staining; the 2% acetic acid test, which brings out the details of the nucleus, may also aid identification. It is not unusual to find eosinophils in the urine; moderately severe eosinophiluria, and concurrent elimination of neutrophils, can be observed in some cases of acute and chronic interstitial nephritis, cystitis (e.g. the cystitis in schistosomiasis) and in some transplant rejections [24,32,33]. Eosinophiluria can be detected by the Papanicolaou or the Wright-Giemsa stain [54] as they stain selectively the eosinophil granules, which tend to mask the usually bilobed nucleus of these cells.

49   Granulocyturia. The nuclear morphology is very clear (400x).

50   Same field. Phase-contrast examination (400x).

51   Granulocytes with nucleus less clearly defined (400x).

52   Granulocyturia: phase-contrast examination (400x).

53   Granulocyturia (Papanicolaou) (400x).

54   Granulocyturia (Kova stain) (400x).
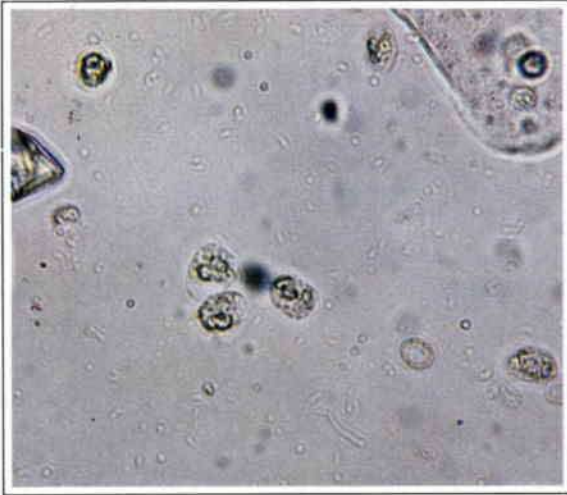
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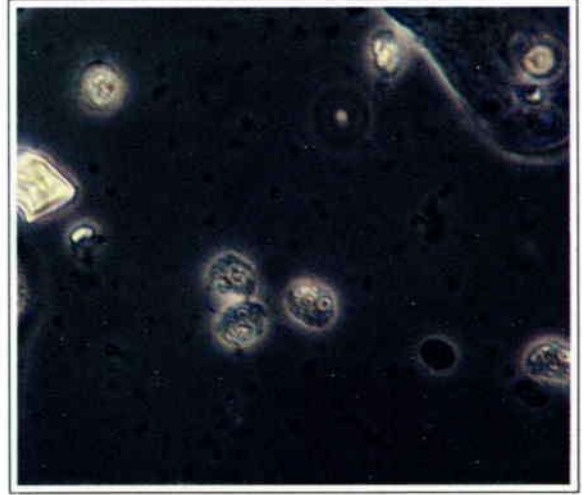
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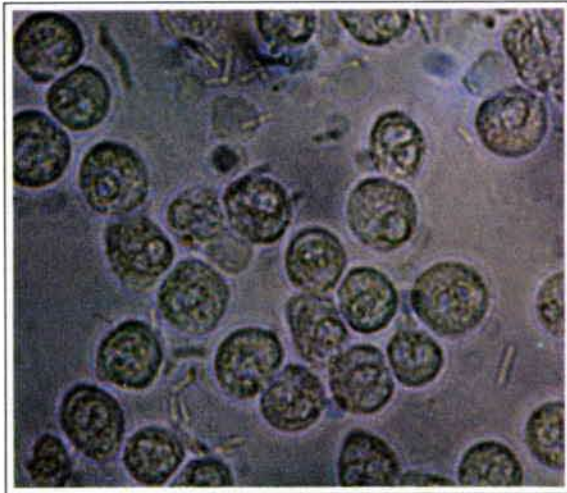
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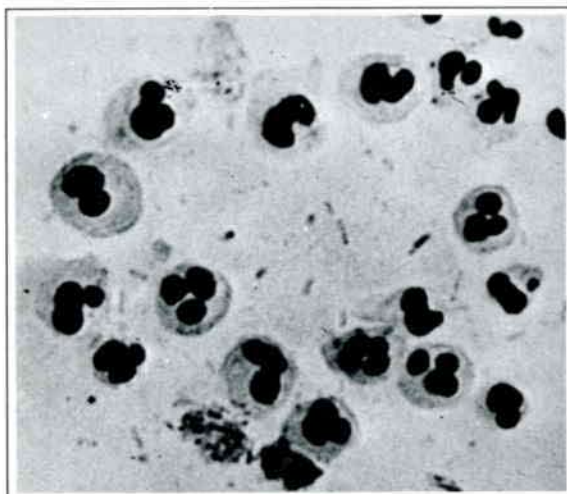
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A few years ago Sternheimer and Malbin [58] reported that in chronic pyelonephritis, the staining of sediment with gentian violet and safranin very frequently reveals swollen neutrophilic granulocytes with pale blue nuclei; their cytoplasm is often rich in fine granules exhibiting Brownian movement. These «pale» cells were then recognized as important in the diagnosis of pyelonephritis. At the same time, a second kind of granulocyte was described, in which the cytoplasm contained numerous rough granules, with no Brownian movement and the nucleus took on a dark red colour, tending to violet; its presence was not considered indicative of pyelonephritis.

Further investigation has shown that, contrary to what was claimed initially, the «pale» cells are not degenerate cells, and that their reaction when stained is very similar to that of the leukocytes in the blood stream.

Hence it is now thought that the presence of «pale» cells in the sediment has no differential diagnostic value, and only indicates the rapid elimination of granulocytes in the urine [64,67,5].

Brownian movement is caused by urinary hypotonicity and may be found in any pyuric condition, although it may be absent in pyelonephritis when the urine is of high osmolality.

In practice, the observation of Brownian movement may make it easier to identify unstained granulocytes.

55 Granulocyturia (400x).

56 Swollen granulocytes with degenerative changes. The morphology of the nucleus is unrecognizable (400x).

57 Granulocyturia and microhematuria. Compare the sizes of the granulocytes and the red cells (400x).

58 Granulocytes «of compact appearance». The morphology of the nucleus is not readily recognizable. These cells may be mistaken for red cells (400x).

59 Swollen, degenerating granulocytes (400x).

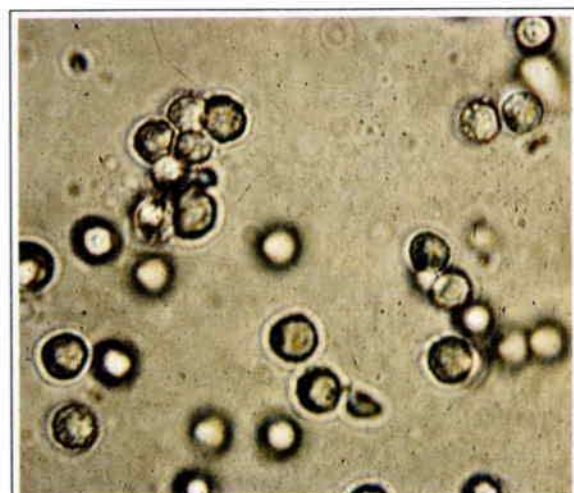60 Granulocytes with even more accentuated swelling and degeneration (400x).
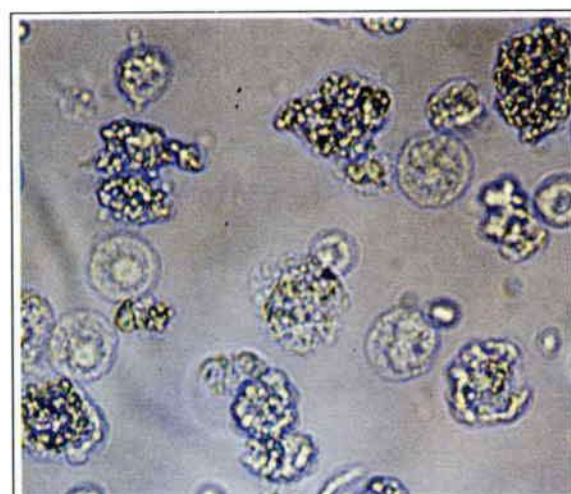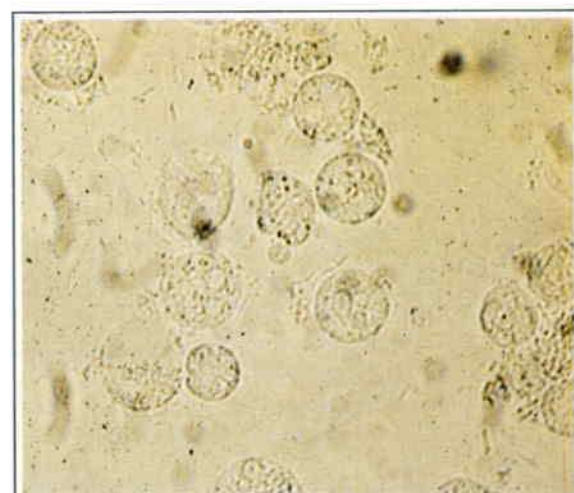
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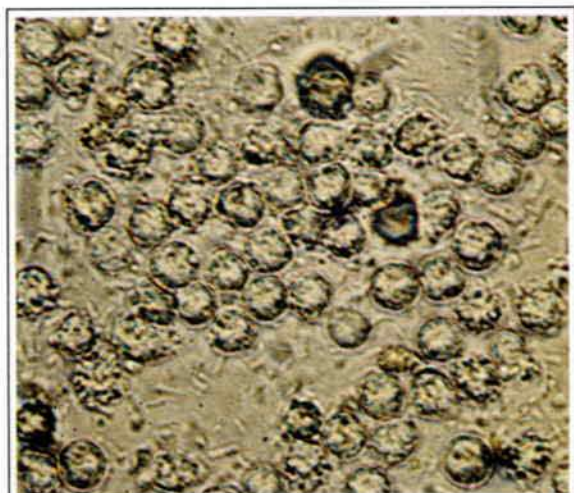
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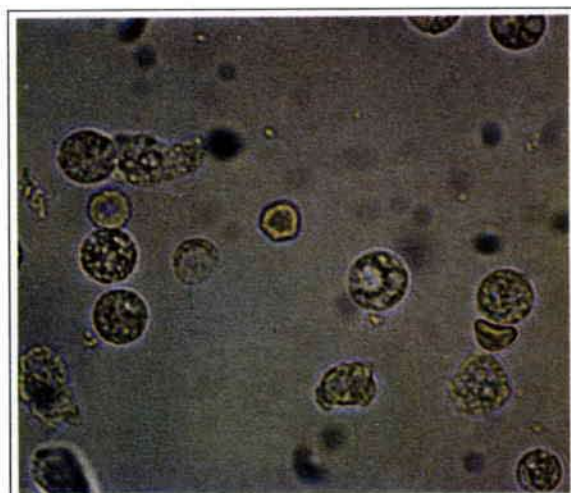
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When the cell degeneration is particularly marked, the granulocytes sometimes assume unusual shapes and dimensions, which may render their identification more difficult. Phase-contrast examination may be useful in such cases.

61 Granulocyturia with cells in an advanced state of degeneration (400x).

62 The characteristics of these granulocytes can be detected more readily by phase-contrast examination (400x).

63 Relatively normal red cells, very badly degenerated leukocytes (cystitis) (400x).

64 The same field in phase-contrast (400x).

65 Numerous leukocytes so badly deteriorated as to be barely recognizable; some of the other cells are probably histiocytes (400x).

66 The same field in phase-contrast (400x).

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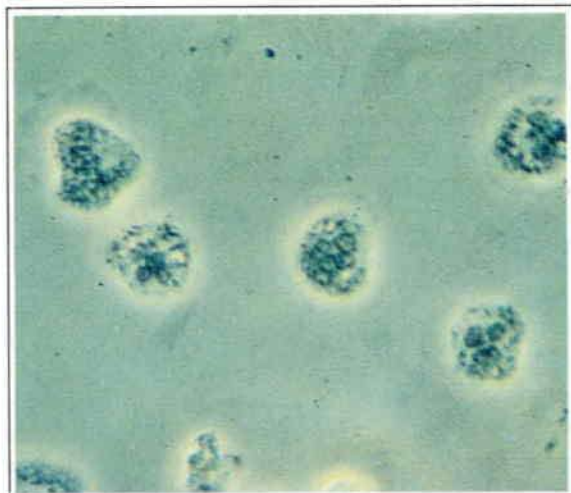
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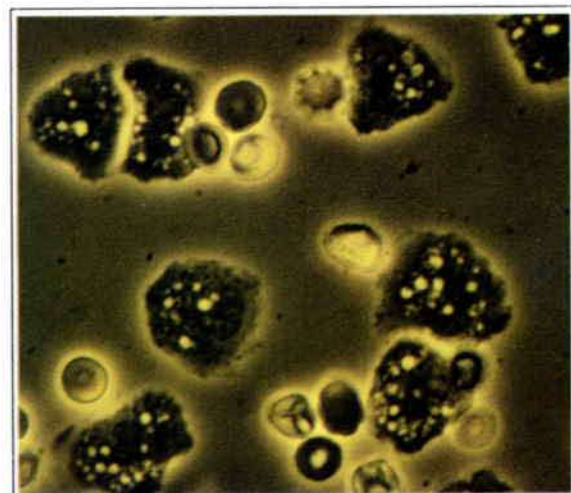
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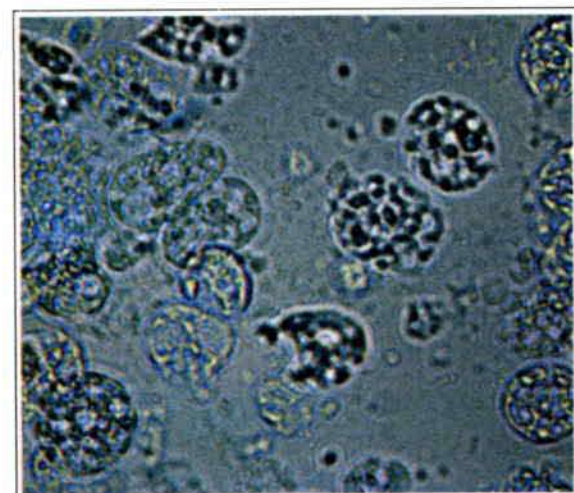
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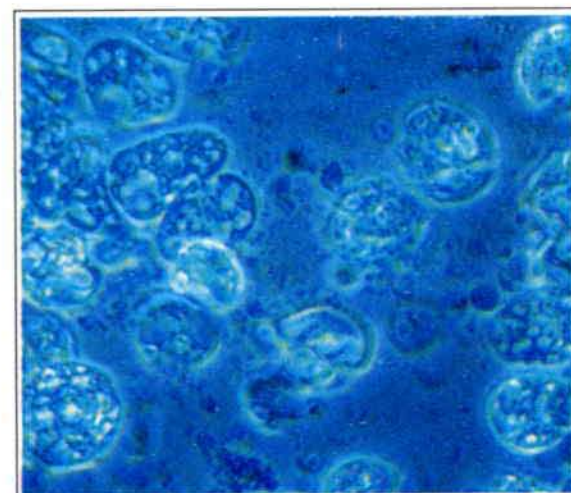
66

3.3 LYMPHOCYTES - PLASMA CELLS - MONOCYTES

Lymphocytes were reported in the urinary sediment many years ago [15,56], but only during the last few years has more attention been paid to them, particularly in kidney transplant cases. Lymphocytes in the urine may be small (8-12 μ in diameter), like those in the blood, and have large nuclei, and narrow, peripheral, non-granular cytoplasm.
With nuclear staining, even the least deteriorated cells often tend to be overstained.
The large lymphocytes are more difficult to recognize as they can be mistaken for other mononuclear cells (renal tubular cells, monocytes). Lymphocyturia is common in some cases of glomerulonephritis [64] and in the course of some kidney transplant rejections.

Plasma cells. It is usually only in fixed specimens that these can be recognized clearly, when suitable staining can be used to bring out the marked basophilia in the cytoplasm, and the wheel-shaped arrangement of the chromatin in the nucleus, which is generally eccentric.

Monocytes. It has been known for some time that urinary sediment may contain monocytes with morphological features similar to those of the cells found in the blood. Greater interest in them has been aroused since they have been found in the glomerulus in some forms of glomerulonephritis.
In many cases they can be clearly recognized only after staining of fixed material; in practice, however, they can very easily be confused with large lymphocytes or renal tubular cells.

The possible significance of the presence of lymphocytes, monocytes and plasma cells in the urinary sediment is still not clear, and thus the practical value of identifying them precisely may be questioned.
At present it would not seem absolutely necessary for diagnosis to identify them systematically and accurately by staining techniques, and the general term «mononuclear cells», which, at least for some of them, can be given without staining, would generally seem sufficient.

67   A lymphocyte (arrow) beside a granulocyte (400x).

68   The same field in phase-contrast (400x).

69   Small lymphocyte (red arrow) beside a larger lymphocyte (black arrow); the other two cells cannot be identified with certainty in this field (phase-contrast) (400x).

70   A lymphocyte (arrow) beside other nucleated cells of uncertain identity: phase-contrast (400x).

71   Small lymphocytes (red arrows) and what is probably a monocyte (black arrow). (Kova stain) (400x).
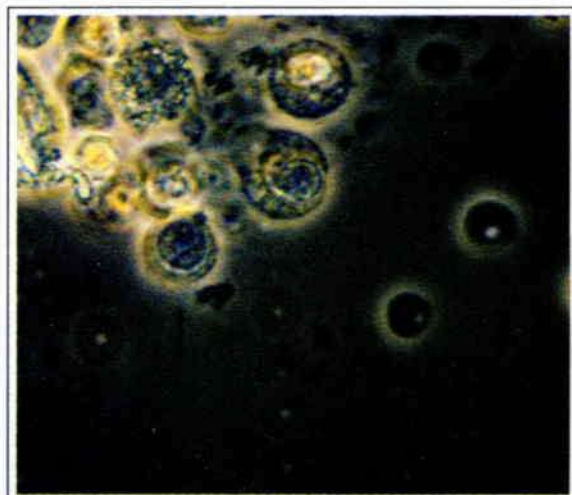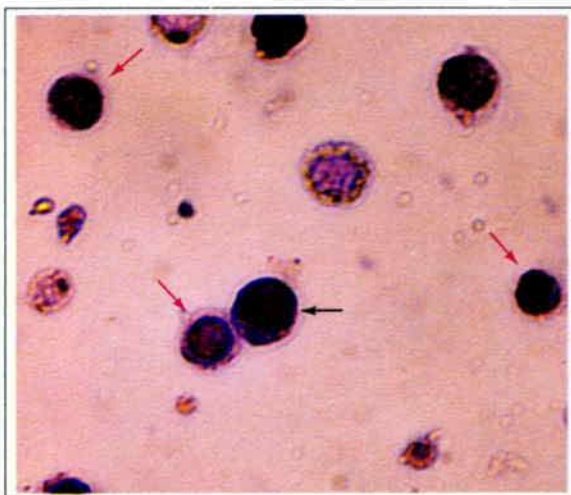
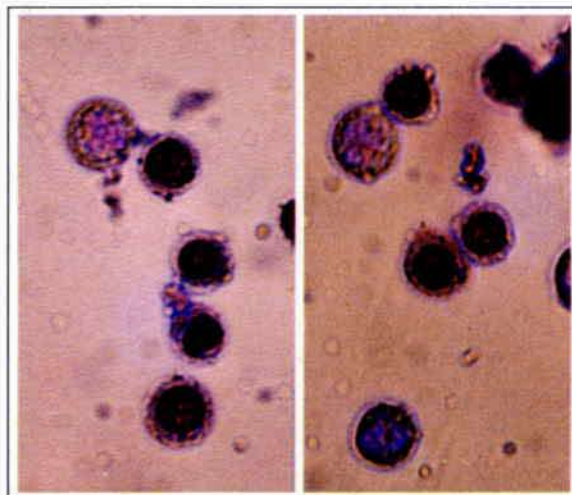72 a-b   Numerous lymphocytes. (Kova stain) (400x).
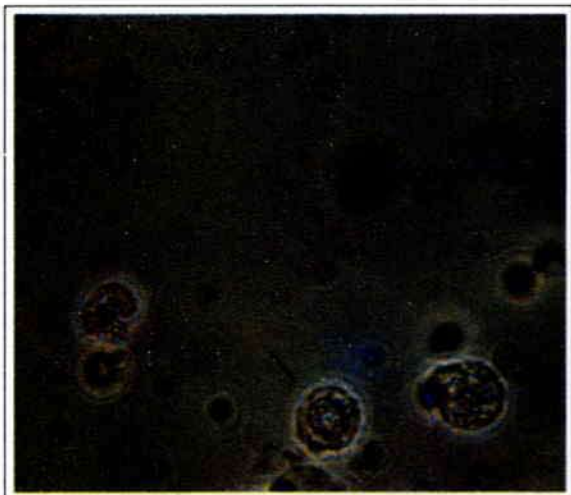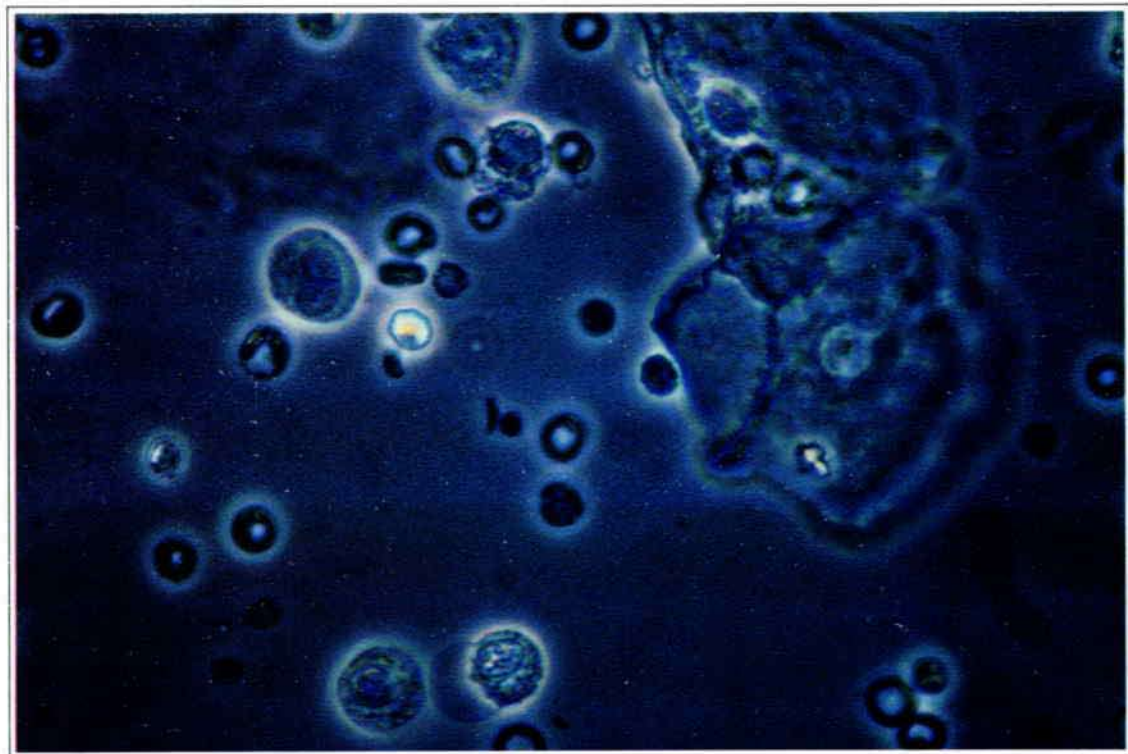
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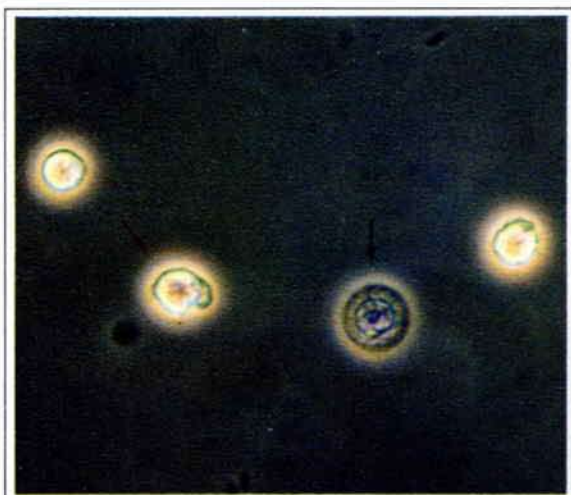
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72   a   b

73 Monocyte (arrow); some nucleated cells of uncertain identity (400x).

74 The same field in phase-contrast (400x).

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3.4 MACROPHAGES

The cells in the urinary sediment can also include macrophages as well as monocytes. Macrophages are mono- or polynuclear, with the nucleus (or nuclei) being frequently kidney-shaped, often situated peripherally, and measuring 10 to 100 μ [54].
The shape of the macrophages is also rather varied; they may be round, oval or irregular with pseudopods [54,13].
It has been claimed that pseudopods (often very clear on phase-contrast examination) [13], or the presence of phagocytosed material, are helpful in their identification. However, also granulocytes may assume a bizarre appearance, and the presence of inclusions is not in itself sufficient evidence for claiming cells to be macrophages, since the same phenomenon can be found in renal tubular epithelial cells.
Here again, Papanicolaou staining may be useful.
At present, no great interest is being shown in the accurate identification of these cells. They are taken simply as a non-specific indication of an inflammatory process in the urinary tract [13].

75 Granulocytes and cells of bizarre appearance, possibly histiocytes, with many pseudo-pod-like projections (sediment of a kidney transplant patient) (250x).

76 Phase-contrast brings out the characteristics of these cells; thread-like prolongations are also clearly visible (250x).

77 The same field examined under lower power shows an abundance of these cells here (100x).

78 In a comparable unstained sample, the positive reaction for esterases (ANAE, α - naphthylacetate esterase) that is very marked in some of the cells, suggests that some of them are possibly histiocytes. In the same sample, however, a strong positive reaction for Naphthol AS-D Chloroacetate esterase, indicated the presence of a large number of granulocytes (160x).

79-80 Cells with inclusions of hemosiderin granules stained with Prussian blue. It is uncertain what kind of cells these are (macrophages or renal tubular epithelial cells) (400x), (400x).
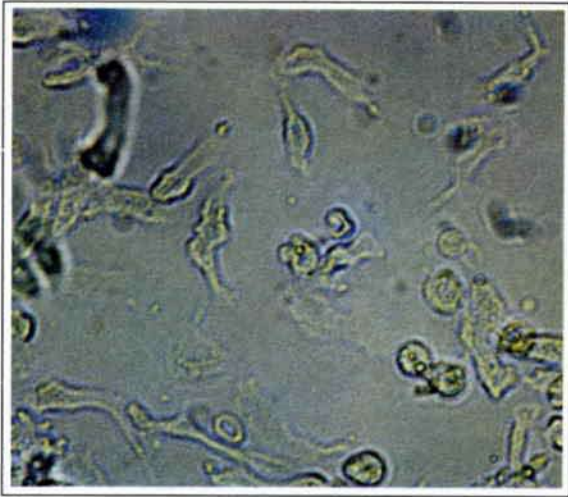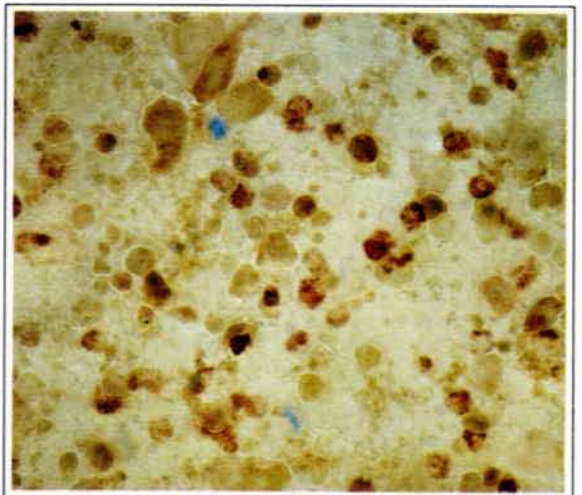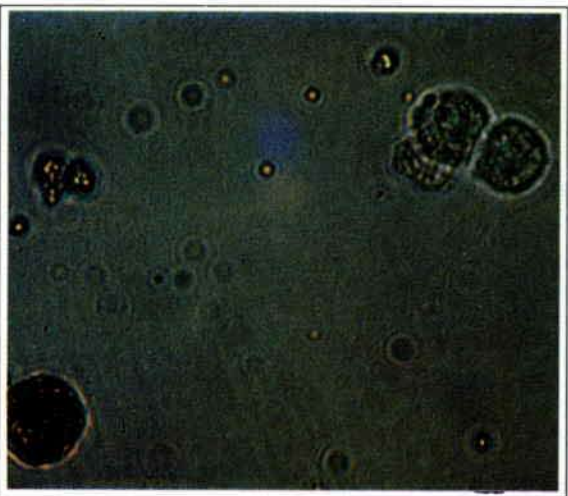
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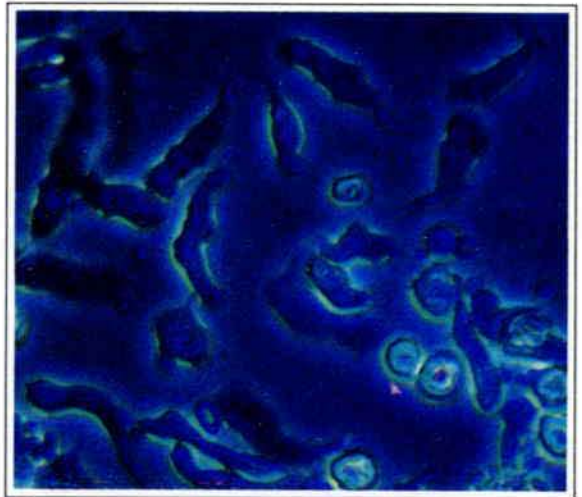
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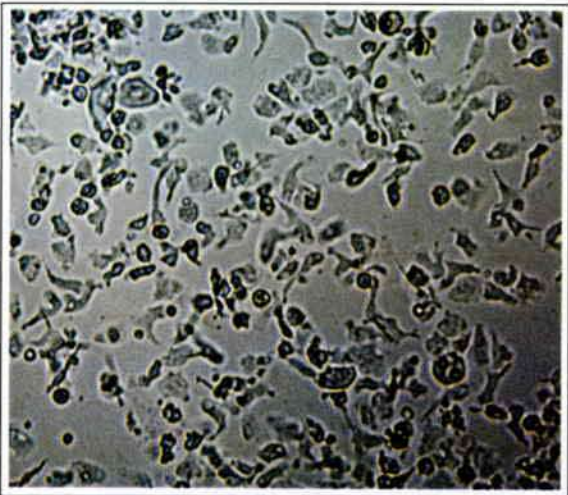
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3.5 RENAL TUBULAR EPITHELIAL CELLS

The morphology of renal tubular epithelial cells has been mainly described from the study of cells (both free and in inclusion casts) in sediment samples, and from examination of tissue imprints [64]; more recently these cells have been studied in tissue sections and by exfoliation techniques [54-55].
It is often difficult to identify tubular epithelial cells with certainty, because some morphological changes, which may already have taken place in the tubules, generally become more pronounced after the cells have been in the urine for some time.

81 Tubular epithelial cast with some cells of normal appearance. The size of these cells is a little larger than that of granulocytes; they have a single nucleus with quite finely granular chromatin (Papanicolaou) (400x).

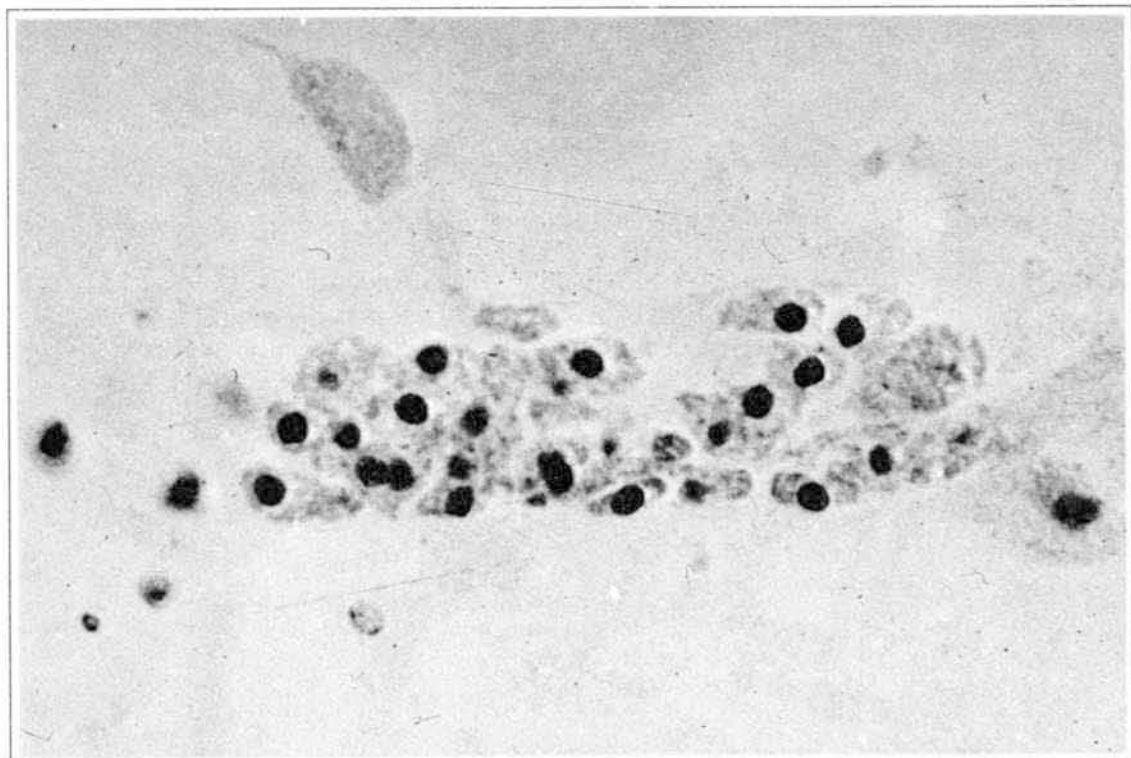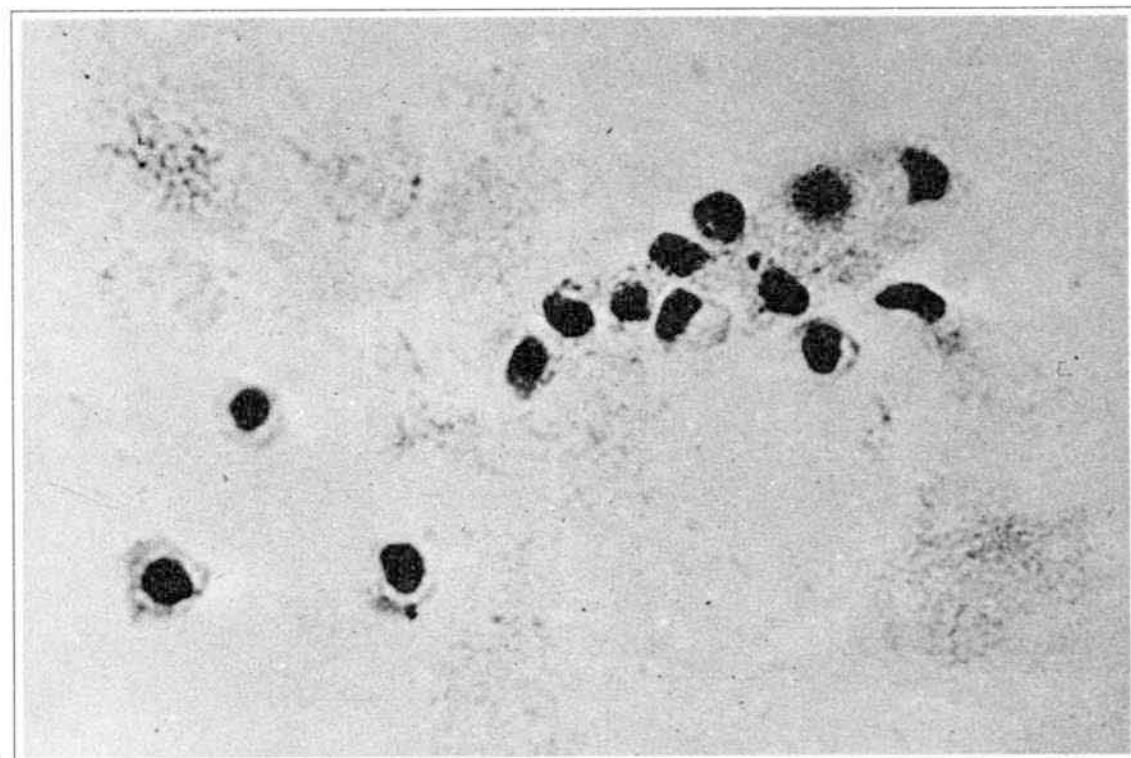82 Epithelial cast with severely degenerating cells (Papanicolaou) (400x).
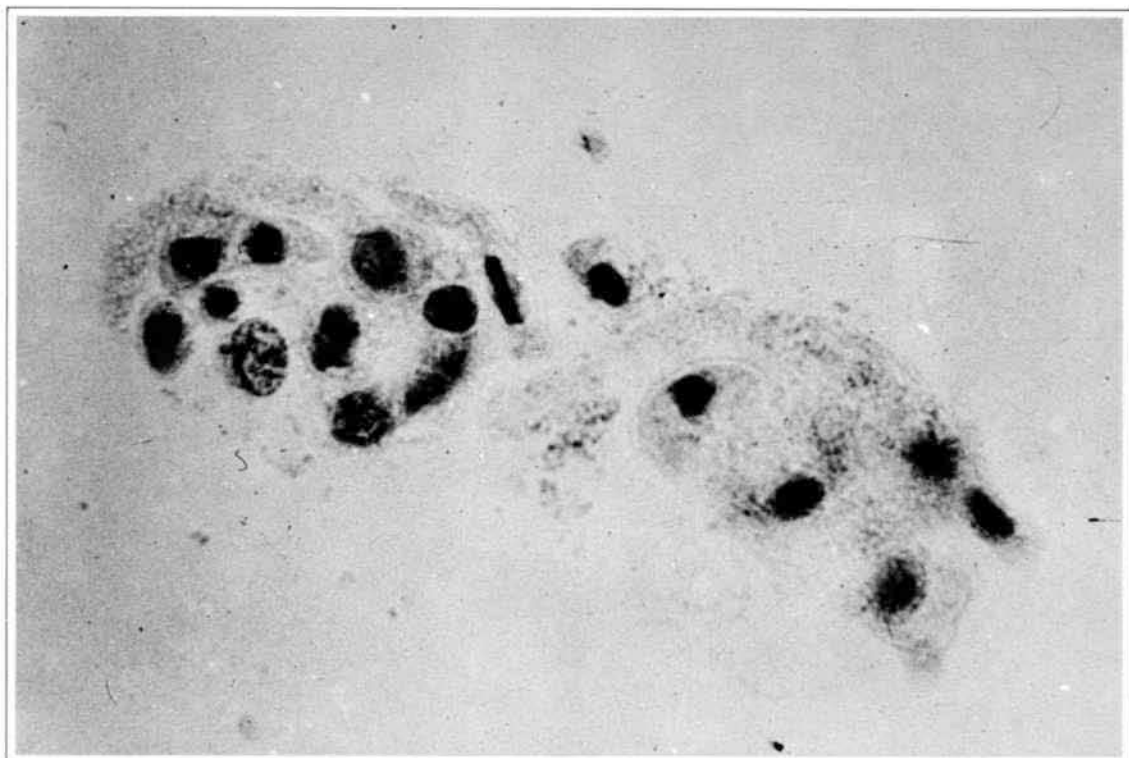
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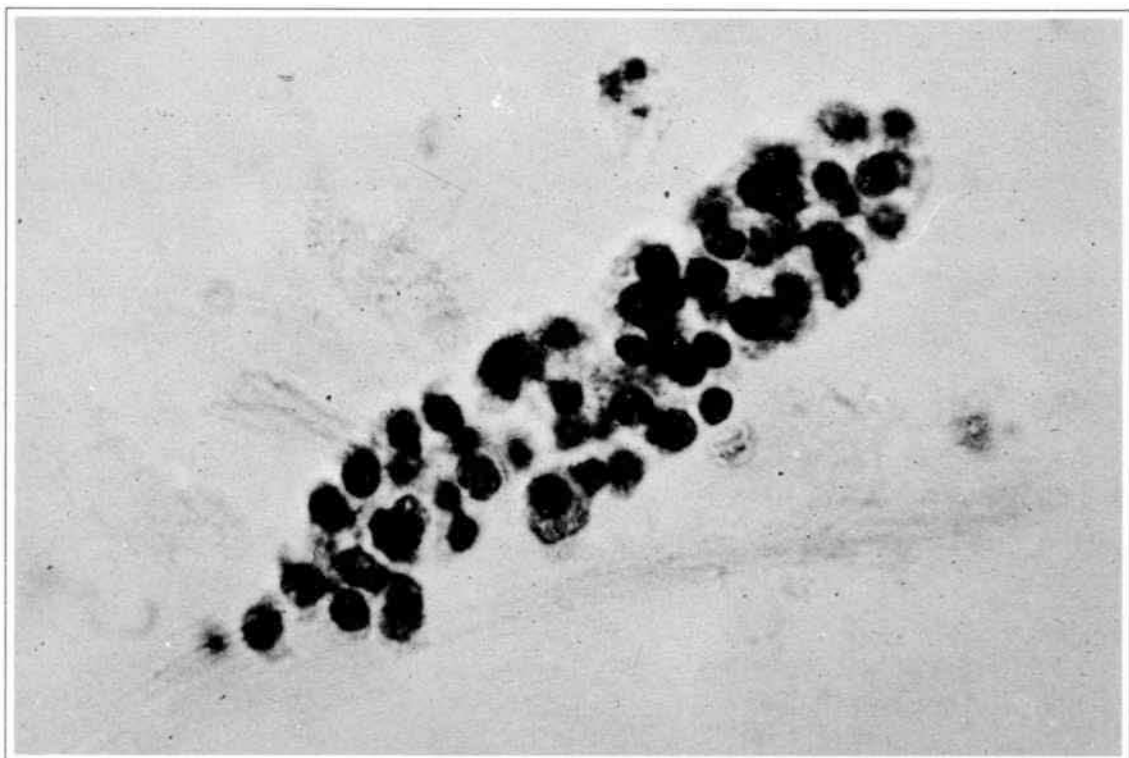
82

83 Epithelial cast with markedly pyknotic nuclei (Papanicolaou) (400 x).

84 Fragment of a cast with rounded cells of uncertain identity; free cells (Papanicolaou) (400 x).
These plates illustrate the frequent difficulties found in recognizing the cells in the urinary sediment.
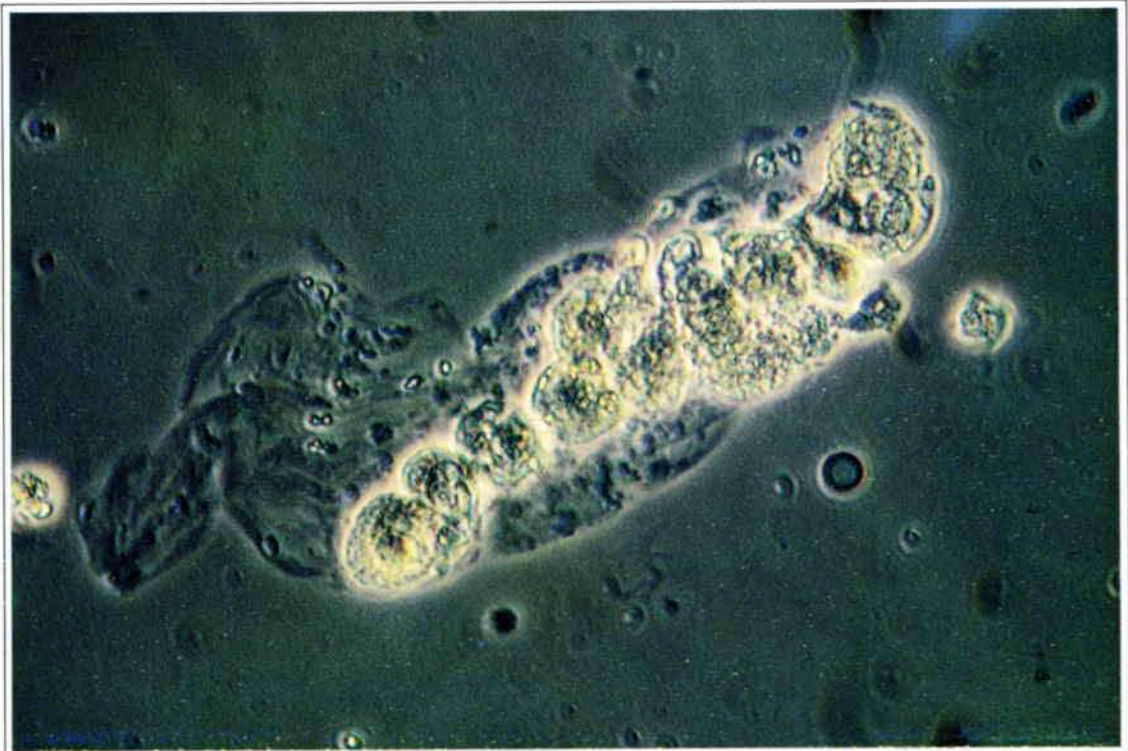
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85 Tubular epithelial cells included in a hyaline cast. The morphological features here are clearly recognizable without staining (400 x).

86 The same field in phase-contrast (400x).

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Various types of renal epithelial cells have been identified in tissue imprints or mechanical exfoliation preparations [54]. However, degenerative changes often make it difficult to identify accurately the renal cells in the urinary sediment. This may also explain the differences found in the descriptions given by various authors.

Well-preserved tubular cells usually have the following features:

— they are irregularly polygonal, cuboid, rounded or faceted;

— their dimensions are slightly greater than those of granulocytes;

— the nucleus is generally large, with finely granular chromatin;

— the cytoplasm is not very dense and tends to be basophilic.

Cells of this kind are found in varying numbers in 90% of glomerular diseases, and in these disorders may constitute as much as 20% of all the nucleated cells of the sediment [64]. Identification of unstained tubular epithelial cells is rather difficult because of degenerative factors, the only notable exception being fatty degeneration, which makes recognition easier. Phase-contrast examination may be helpful.

Some stains also facilitate the identification of tubular epithelial cells. Papanicolaou stain is often satisfactory to study the characteristics of the nucleus and cytoplasm; a supravital stain, such as Sternheimer-Malbin, can also be helpful, when applied to fresh urine.

87   Tubular epithelial cell with a nucleus to cytoplasm ratio of about 1:1 and with finely granular chromatin in the nucleus. Smaller granulocytes (Papanicolaou) (300x).

88   Quite well-preserved renal epithelial cells. Typically these are larger than granulocytes. When no urinary infection has been superimposed on the original nephropathy, the occurrence of granulocytes and renal epithelial cells together is generally indicative of active glomerulonephritis with exudation. In active pyelonephritis there are usually fewer renal epithelial cells in the urinary sediment (Papanicolaou) (400x).

89   Sediment of acute glomerulonephritis in the initial stage; besides granulocytes, clearly distinguishable by their multilobular nucleus, there are also cells with a round nucleus, some of which are probably tubular epithelial cells (Papanicolaou) (300x).

90   Unclassifiable mononuclear cells with broad, rather foamy cytoplasm; as they were also present in casts they may have come from the kidney (300x).

91   Tubular cells obtained from a tissue imprint (Papanicolaou) (300x).

92   Mononuclear cells; some (arrows) are almost certainly epithelial cells (Papanicolaou) (400x).
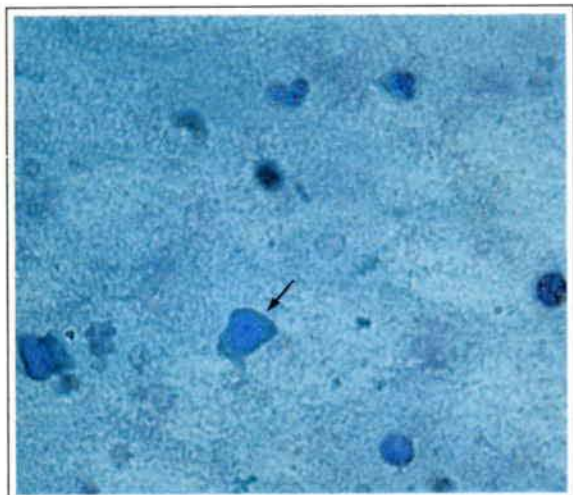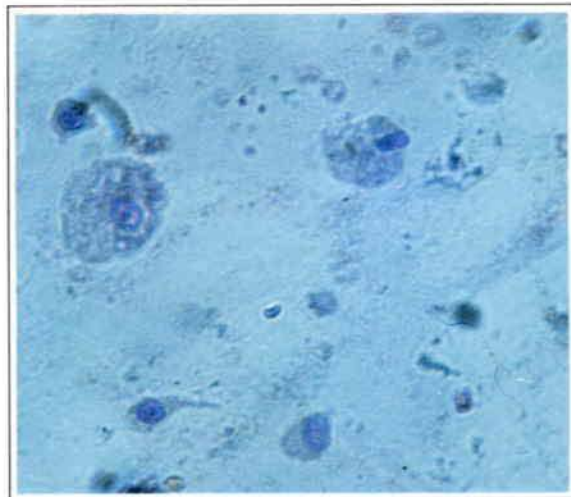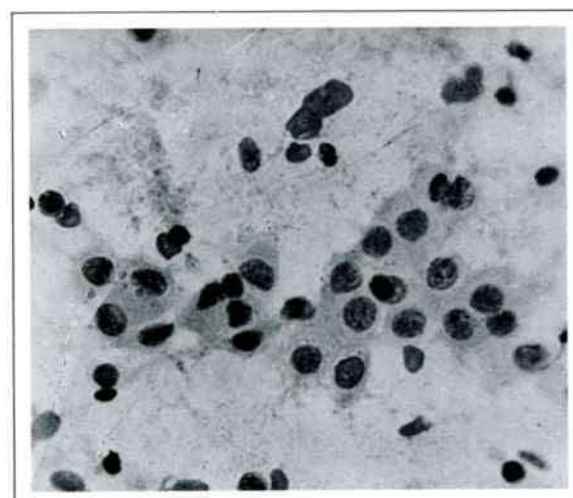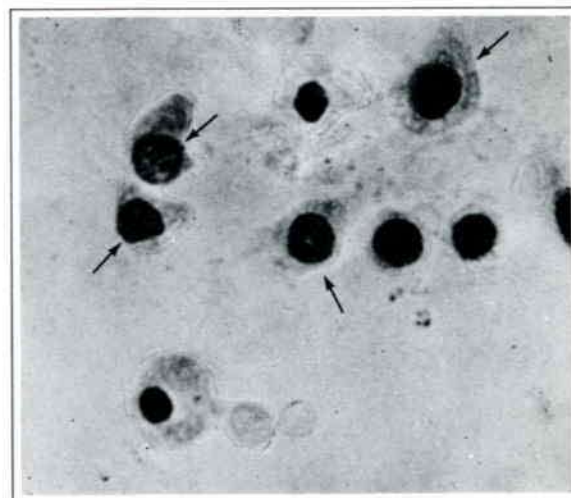
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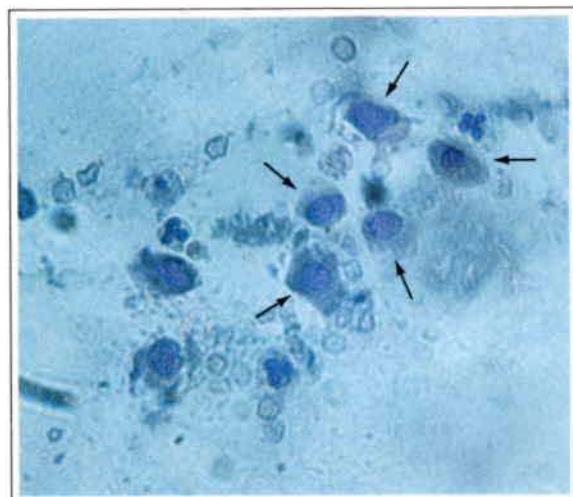
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Several interesting cytoplasmic changes, such as the presence of fat droplets or eosinophilic protein granules, may appear in tubular epithelial cells. The cells with the most noticeable accumulation of fat droplets have been called «oval fat bodies».

Quinn and Zimmermann [44] have identified three types of these cells on the basis of the quantity and size of the fat droplets in the cytoplasm. The fat content is very small in the first type of cell, and the nucleus, although regressing, is still clearly recognizable.

The second and third types are of greater diagnostic value and have a higher fat content; indeed, the cytoplasm of cells of the third type is packed so full of fat droplets that the nucleus is obscured. When the fat droplets are medium and large in size, the cells take on a typical mulberry shape.

Fatty inclusions are easily recognizable if they have a diameter of at least 2 μ and contain cholesterol esters. In these cases polarized light brings out the so-called «Maltese crosses», which are not visible if the droplets are smaller.

Staining with Sudan III may reveal the presence of neutral fats. Oval fat bodies arise from the tubular epithelium, and any appreciable amount of fatty degeneration, particularly when there are cholesterol esters, may be taken to be indicative of a nephrotic syndrome.

The fact that histiocytes with phagocytosed lipids (foam cells) have been described in the renal parenchyma does not change the diagnostic significance of the finding.

Granulocytes may also contain fatty droplets. In certain cases it may be difficult to distinguish them from oval fat bodies, but fat-filled granulocytes never take on a typical mulberry-shaped appearance; in doubtful cases supravital staining of the nucleus may be helpful.
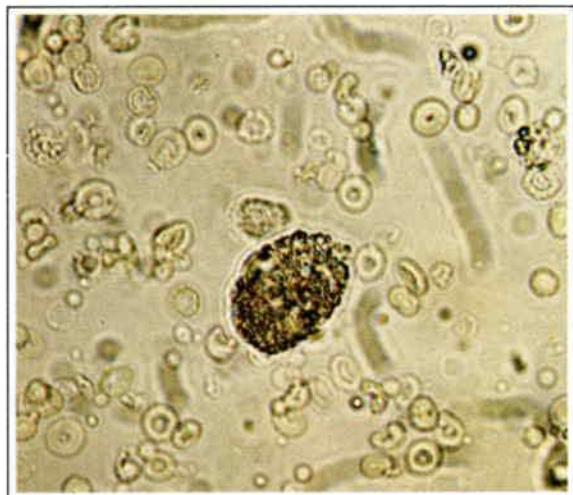
93-94   Oval fat bodies (400x), (400x).

95   Oval fat bodies in a hyaline cast (160x).

96   Cellular hyaline cast containing oval fat bodies (160x).

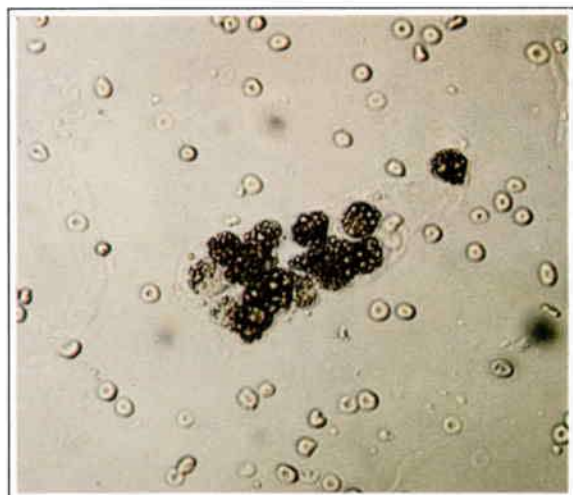97   Staining with Sudan III demonstrates the fat droplets in a cell (400x).
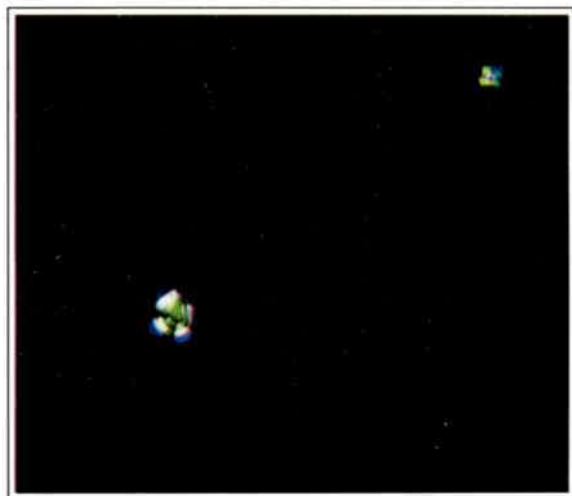
98   «Maltese crosses» (400x).

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99 Free tubular epithelial cell. Red cell granular cast. Degenerating, partially depigmented red cells (acute glomerulonephritis) (400x).

100 Probably tubular epithelial cells (red arrows). Other cells with round nuclei (black arrow) of uncertain identity. Granulocytes. Many erythrocytes, partially depigmented and fragmented. Sediment of this kind suggests a nephropathy in the active phase: primary or secondary glomerulonephritis or, although less likely, malignant hypertension (160x).

101 A large number of erythrocytes and at least one tubular epithelial cell (250x).

102 A similar field to that in 101.

103 Similar field (250x).

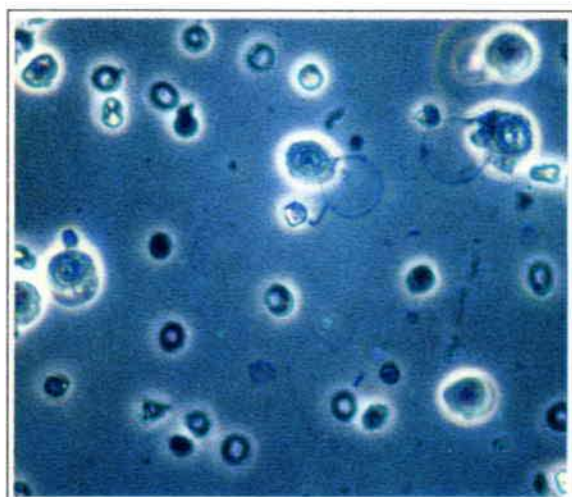**104** The same field in phase-contrast (250x).

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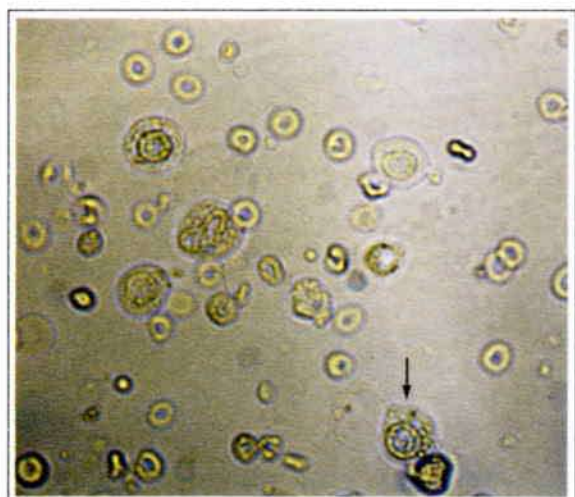
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3.6 EPITHELIAL CELLS FROM THE URINARY TRACT SQUAMOUS EPITHELIAL CELLS

Epithelial cells from the urinary tract used to be defined as «cells from the upper, middle or lower tract», according to their morphology. However, except for the male urethra and the female trigone area and urethra, the excretory system is lined with an epithelium of several layers (transitional or urothelial epithelium). The characteristics of these layers are common throughout, so that the morphological differences depend on the layers from which they come and not on the part of the tract where exfoliation took place.

The deep basal cells are small, rounded or polyhedral, mostly mononuclear, the nucleus having rather coarsely granular chromatin and relatively little cytoplasm.

The cells of the middle layers vary considerably in size and form: they may be rounded, raquet-shaped, oval, polyhedral, etc.

The cells from the surface layers are large, flat, umbrella-shaped, oval or rhomboid. Quite often they have several nuclei and the chromatin network is quite well preserved.

A few of these cells, particularly those from the «surface layers», can be found even in the urine of healthy people; a definite increase, especially of those from the middle and deep layers, is a sign that normal exfoliation has been accentuated, mostly as a result of irritant factors. This finding is difficult to interpret. It is usually, but not necessarily seen in acute inflammation or during relapse, and is of only limited interest in chronic pyelonephritis.

The trigone area and almost all of the female urethra, and the last 0,5-1 cm of the male urethra are lined with squamous epithelial cells. These cells have a rather smaller nucleus and a lower nucleus to cytoplasm ratio than the transitional epithelial cells.

By using the Sternheimer-Malbin staining technique the nuclei of the epithelial cells appear mauve and the cytoplasm purple. With Papanicolaou stain the nuclei take on a deep purple shade and the cytoplasm becomes red or blue-grey.

Keratinized squamous cells, clearly eosinophilic, are often a sign of prolonged irritation of the bladder wall; they are clearly visible with Papanicolaou stain. Cells from the vulva may have similar characteristics.

105   Two transitional cells from the deep layers of the epithelium of the urinary tract (400x).

106   Epithelial cells from the middle layers (400x).

107   Surface cell from the transitional epithelium (250x).

108   Same field in phase-contrast (250x).

109   Cells from the surface and middle layers of the transitional epithelium (160x).

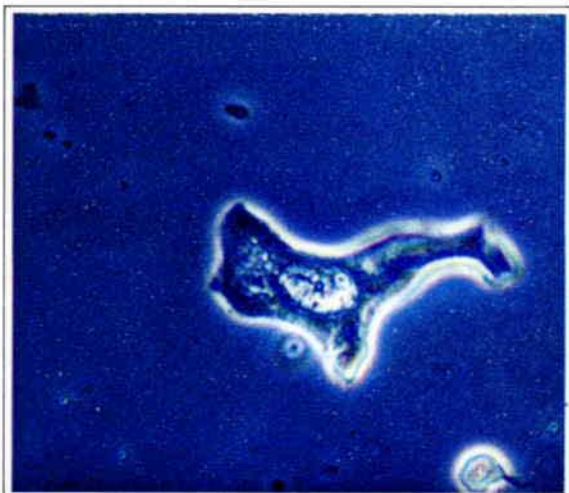110   Vaginal cells. Leukocytes. An example of a urinary sediment sample that was not properly collected (100x).
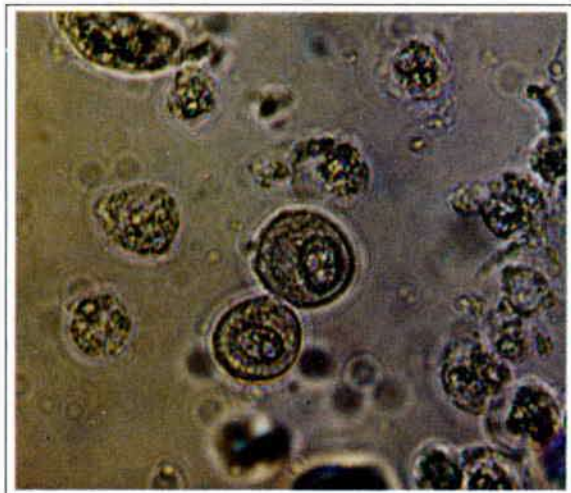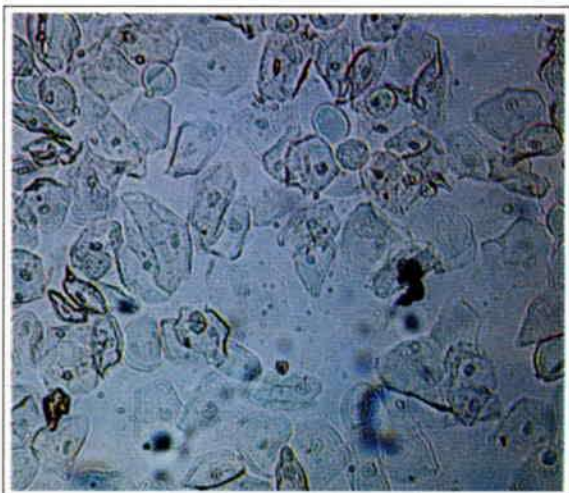
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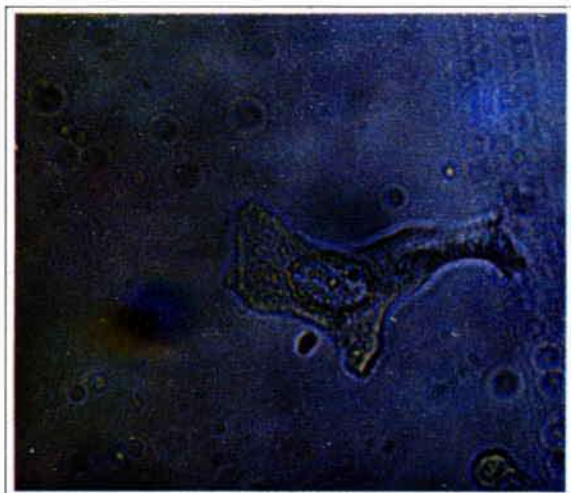
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3.7 EPITHELIAL CELLS WITH EOSINOPHILIC INCLUSIONS

«Epithelial cells with eosinophilic inclusions» were first described by Bizzozzero in 1898, in a patient with pregnancy toxemia. The dimensions of these cells vary considerably, ranging from cells smaller than granulocytes to giant multinuclear cells.

They are generally roundish or oval, more rarely cylindrical or rectangular; the cytoplasm often shows degenerative changes; usually there are one or more nuclei, almost always pyknotic, but the nuclear residue may even have disappeared. The presence of compact, distinctly acidophilic, hyaline droplets in varying number and size is characteristic of these cells. It is not unusual to see free droplets, which may be mistaken for fungi or red cells when unstained. Sometimes a number of droplets coalesce to form one droplet that is even larger than the erythrocytes.

Some of these structures may be derived from renal epithelial cells and they may, though rarely, be found in casts, whereas others appear to be histiocytes.

Since some of them have been found in patients with lesions in the lower urinary tract, it has been suggested that they might be derived from urothelial cells.

On 100 patients with various kinds of nephropathy we found cells of this type in over half of the cases. They were particularly numerous in acute glomerulonephritis and in toxic or post-ischemic acute renal failure in the early polyuric phase, but occurred only rarely in nephrotic syndromes [65]. It has now been established that these cells are a general indication of disease in the urinary system (kidneys and urinary tract) [37]. Without being specifically associated with any particular etiology, they are a possible response common to several diseases.

111   Granulocytes. Mononuclear cells. Clearly visible hyaline droplet in a large cell (Papanicolaou) (250x).

112-116   Intracytoplasmic hyaline droplets. The largest cell in 115 has three nuclei. In 116 there is no nucleus (Papanicolaou) (250x), (250x), (250x), (250x), (250x).
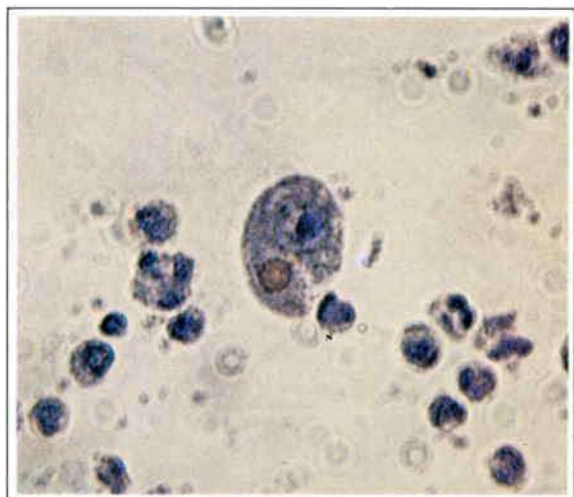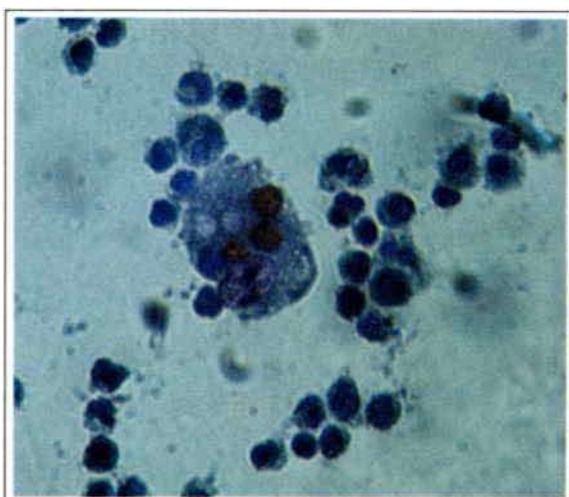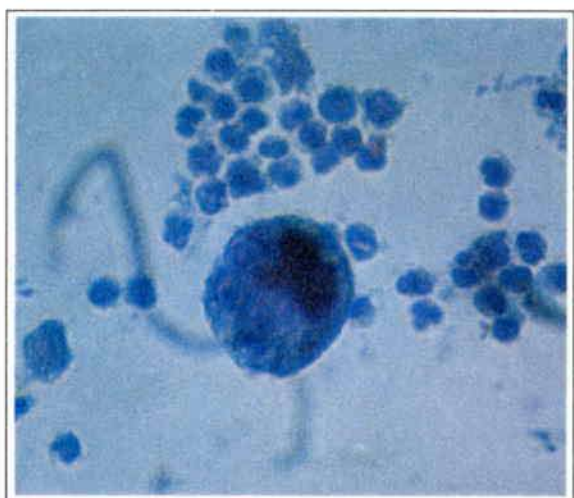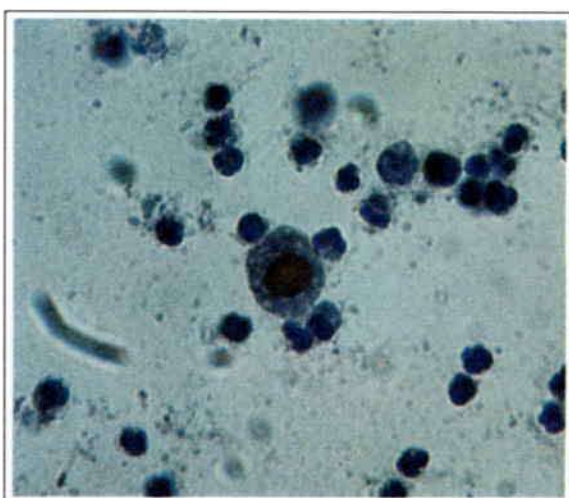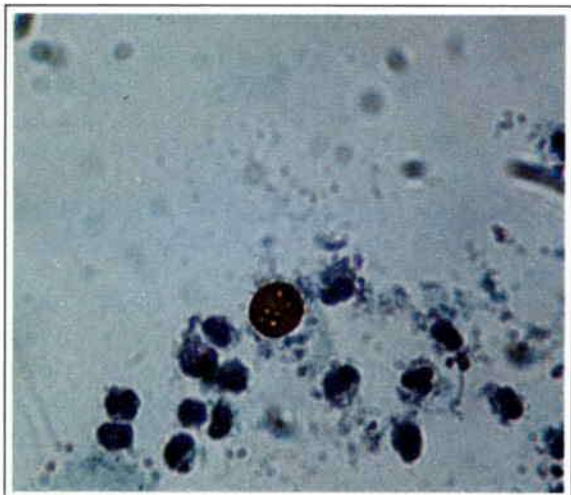
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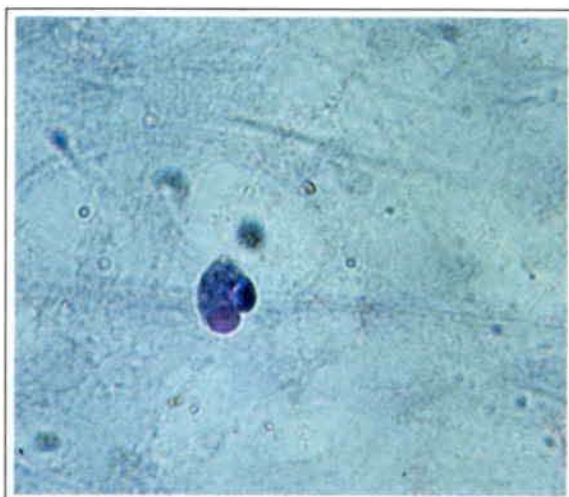
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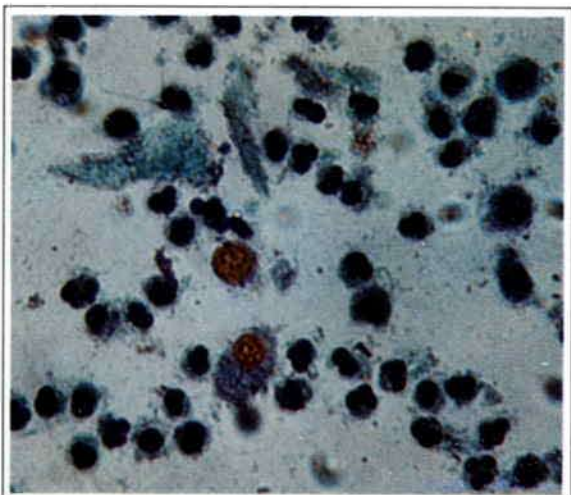

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117   Hyaline droplet surrounded by a residue of cytoplasm (Papanicolaou) (250x).

118   Hyaline droplets, one free and one in a cellular structure with no nucleus
(Papanicolaou) (250x).

119-122   Hyaline degeneration. Unstained samples. The droplets can be distinguished from the
nuclei by their hyaline appearance; Papanicolaou stain ensures correct identification
(400x), (400x), (400x), (400x).

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