Urological Research 1, 113-119 (1973)

Rapid Urinary Cytology by Phase Contrast Microscopy
A Preliminary Report

H. J. de Voogt

Department of Urology, University Hospital Leiden, The Netherlands

Summary. Phase contrast microscopy offers a method for very rapid cytological examination of urine deposits. To test its reliability, results obtained by phase contrast microscopy were compared with those of standard cytological methods using Papanicolaou or Giemsa-stained smears. As only 1.4% false positives and 5.2% false negatives were obtained, phase contrast microscopy can be recommended as a routine office procedure.

Key words: Phase contrast microscopy, urine cytology.

Although several recent publications (1, 2, 13, 19) have favoured phase contrast microscopy for examination of the urinary sediment, only one reference has been made to its value for cytological screening (12). There have been few reports of its value for cytological studies in other circumstances (20). In an attempt to make urine cytology a routine office procedure for all urologists, the value of phase contrast microscopy became apparent (16).

Phase contrast techniques depend upon separation of light that has been transmitted through transparent objects, e. g. unstained cells, from light that has been diffracted by the same objects and has deliberately been retarded by about a quarter of its wavelength.

The two waves are allowed to interfere with each other to produce visible changes in wave amplitude (24, 25). The phase contrast method can be applied readily and inexpensively to light microscope urinalysis. Cellular and nuclear structural details such as vacuoles, granular contents, nucleoli and thickened nuclear membranes become clearly visible, and it is possible, therefore, to distinguish atypical and malignant cells from normal cells of the transitional eptihelium of the urinary tract.

To test the reliability of this method, the results of phase contrast microscopy have been compared with cytological diagnoses made on conventional Papanicolaou and Giemsa-stained smears and verified by biopsy.

Material and Methods

285 fresh urine samples were obtained from 157 patients, attending the Department of Urology. The entire urine specimen was centrifuged, one drop of the sediment was placed on a glass slide and a cover slip placed over it. The specimen was then examined immediately by bright field and phase contrast microscopy. If atypical or probable malignant cells were seen, they were photographed for documentation.

From the remainder of the same sediment, smears were made on normal and frosted glass slides (22) for dry and wet fixation and subsequent staining by the Giemsa or Papanicolaou techniques.

All the slides were screened by a cytotechnician and the final cytological diagnosis checked by a cytopathologist. In all cases of benign and malignant tumours of the urinary tract, biopsies or other surgical procedures were done so that diagnoses could be verified histologically.

All bladder tumours were graded according to the T. N. M. -system, as this is in almost universal use amongst urologists and it corresponds quite well to the histological classification suggested by Dukes (5). To simplify matters, all papillomatous carcinomas without invasive growth (Tx), or with only superficial invasion of the submucosa (T_1), were placed in one group, as there is no cytological difference between them.

The cytological diagnoses were given in a code suggested previously (22). During the comparison of the two methods it was decided to mark as negative all cytological diagnoses of normal eptihelial cells or slight atypia, as well as those in which no cells were detected, and as

positive those in which the findings were suggestive of a benign or malignant tumour.

Results

Table 1 lists the diagnoses of all the patients. 76 had some type of urothelial cancer, of whom 37 patients were diagnosed for the first time by cytology and confirmed by biopsy. In 39 patients the diagnosis had been made before and they were being followed after treatment; in 37 of them the cytological diagnosis was negative and no recurrence was detected by cystoscopy or other means; in 2 a positive cytological diagnosis was confirmed as being due to a recurrent tumour (Table 2).

Of the 285 cytological examinations, 171 were negative by all 3 methods and 95 were positive by all of the three. Phase contrast microscopy gave a false negative on 15 occasions (5.2%), i.e. either Papanicolaou or Giemsa-stained smears were considered suspicious of a tumour. Phase contrast microscopy gave a false positive result on 4 occasions (1.4%); Table 3.

In analysing the false positive cases it was found that 2 specimens came from the same case of medullary sponge kidneys with multiple small stones. Phase contrast microscopy showed atypical urothelial cells that suggested malignancy, but repeated Papanicolaou and Giemsa smears only revealed the atypia which is often found in patients with stones (6, 9, 15). In the other 2 cases, atypical cells were seen by phase contrast microscopy but no cells at all were found in Papanicolaou and Giemsa smears. In the examples of false negatives, the presence of many pus cells or erythrocytes was the main cause of failure to detect epithelial cells (Table 4).

Table 1. Final Diagnoses in the 157 patients studied

Benign papilloma bladder	22
Bladder carcinoma Tx +T_1	26
Bladder carcinoma T_2	14
Bladder carcinoma T_3	14
Bladder carcinoma T_4	6
Carcinoma in situ bladder	4
Squamous cell carcinoma of bladder	2
Transitional cell carcinoma renal pelvis and ureter	10
Ingrowth of other pelvic carcinomas into bladder	5
Prostatic carcinoma	2
Infections of genito-urinary tract	13
Haematuria (stones, trauma, a. o.)	26
Miscellaneous	13
total:	157

Table 2. Analysis of 76 patients with urothelial carcinomas

	Initial diagnosis		Control after therapy		
	Cyt. neg.	Cyt. pos.	Cyt. neg.	Cyt. pos.	Total number of cases
Transitional cell ca. bladder Tx + T_1	-	9	16	1	26
Transitional cell ca. bladder T_2	-	6	8	-	14
Transitional cell ca. bladder T_3	-	9	5	-	14
Transitional cell ca. bladder T_4	-	5	1	-	6
C.I.S. bladder	-	3	1	-	4
Squamous cell ca. bladder	-	2	-	-	2
Tumours of renal pelvis and ureter	-	3	6	1	10
Total:	-	37	37	2	76

Table 3. Comparison of Cytological Diagnoses by the Different Methods

Cytological diagnosis phase-contrast and Pap/Giemsa negative	171
Cytological diagnosis phase-contrast and Pap/Giemsa positive	95
Cytological diagnosis phase-contrast positive but Pap/Giemsa negative	4
Cytological diagnosis phase-contrast negative but Pap/Giemsa positive	15
total:	285

Table 4. Causes of false negative results of phase-contrast cytology

Too many pus cells	4
Too many erythrocytes	7
No epithelial cells discovered	3
False interpreted atypia	1
total:	15

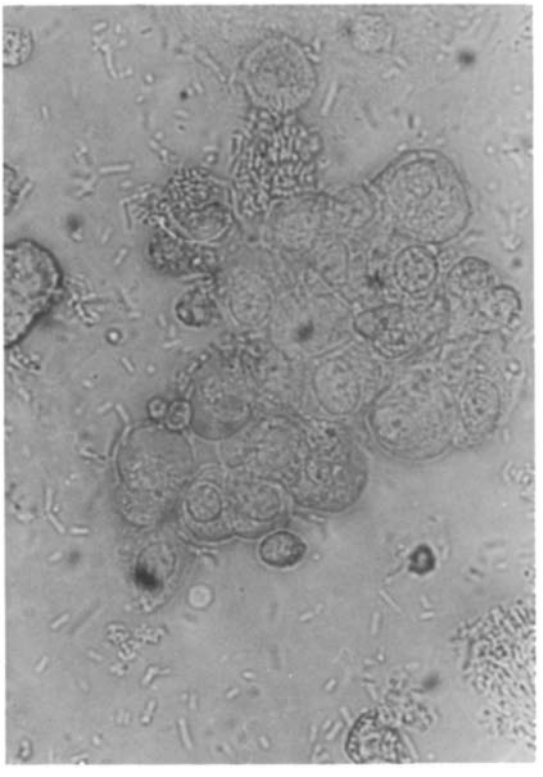


Fig. 1. Group of urothelial cells in urine, seen by bright field microscopy. The outlines are only just visible.

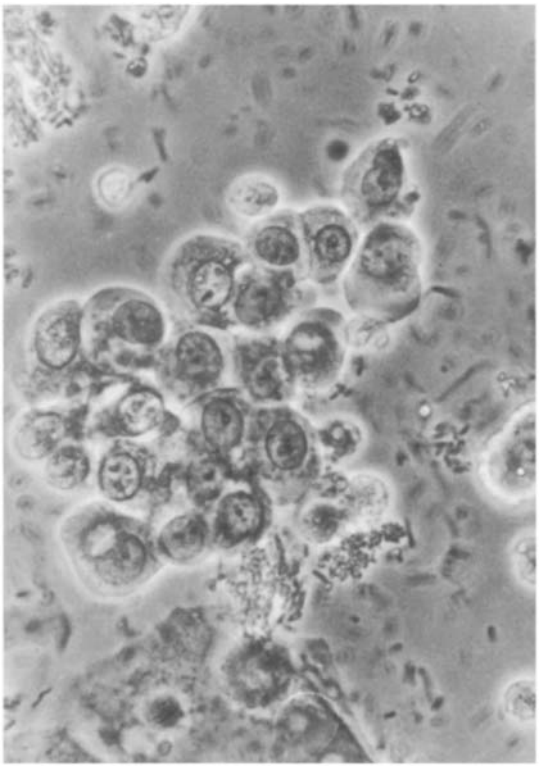


Fig. 2. The same cell group seen by phase contrast microscopy. Nuclear outlines are clearly visible, as well as many nucleoli. The irregular arrangement and the early polymorphism are suggestive of a papillomatous growth with atypia. From a patient with recurrent T1 bladder tumour.

Discussion

As shown by the detailed study of Tyrkkö (21) it seems impossible to over emphasize the importance of urine cytology, and yet many urologists either are not familiar with this diagnostic method, or are not convinced of its importance in the early diagnosis and follow-up of patients with urothelial neoplasms. For these reasons the attempt has been made to introduce urinary cytology as a routine office procedure by simplifying the technique.

Although simple staining techniques have been devised for routine office use (16, 17), phase contrast microscopy has the advantage of enabling the clinician to make a rapid diagnosis, or at least to obtain information about how to direct his diagnostic investigations, whilst the patient is still present. An urologist who is accustomed to looking for erythrocytes, pus cells or bacteria in urinary sediments with ordinary bright field microscopy, can, with simple and inexpensive additional equipment, employ the phase contrast method as well to detect normal, atypical or malignant cells desquamated from transitional epithelium, and with a reliability that meets

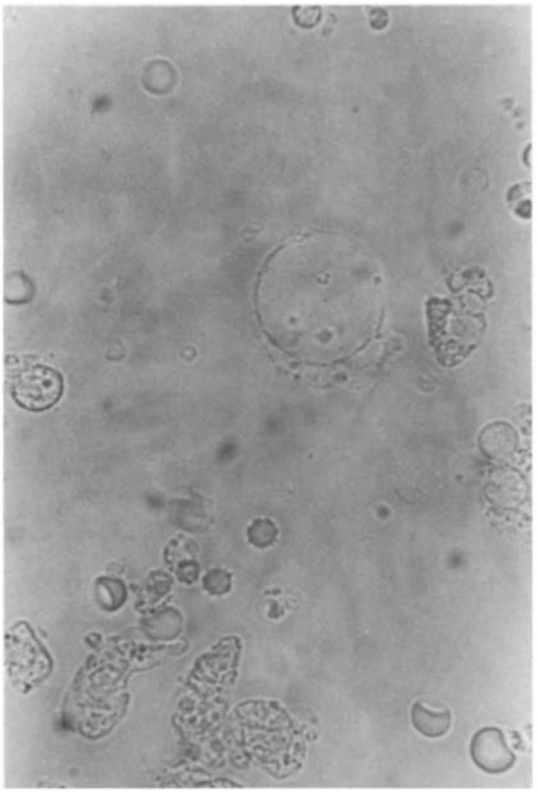


Fig. 3. The structure in the middle could easily be overlooked in bright field scanning of urinary sediment.

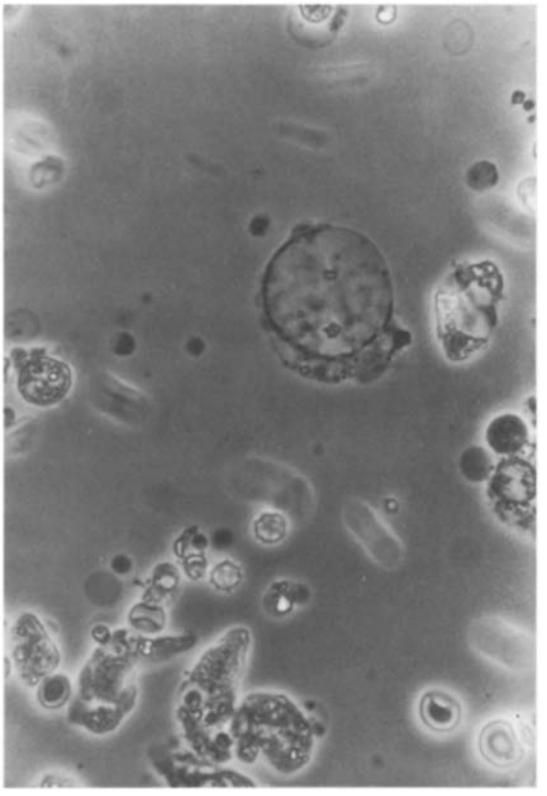


Fig. 4. Under phase contrast this was shown to be a malignant epithelial cell with a giant nucleus. From a patient with a T2 bladder carcinoma.

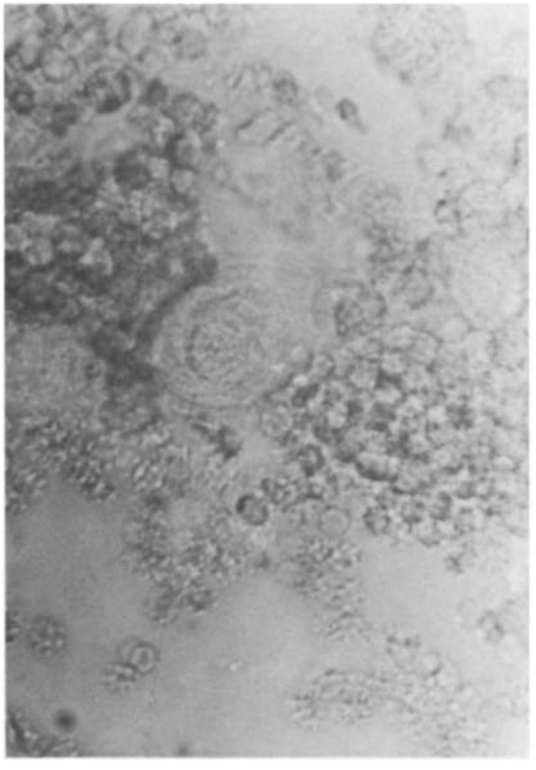


Fig. 5. Between a mass of leucocytes a non-descript structure is seen by bright field microscopy.

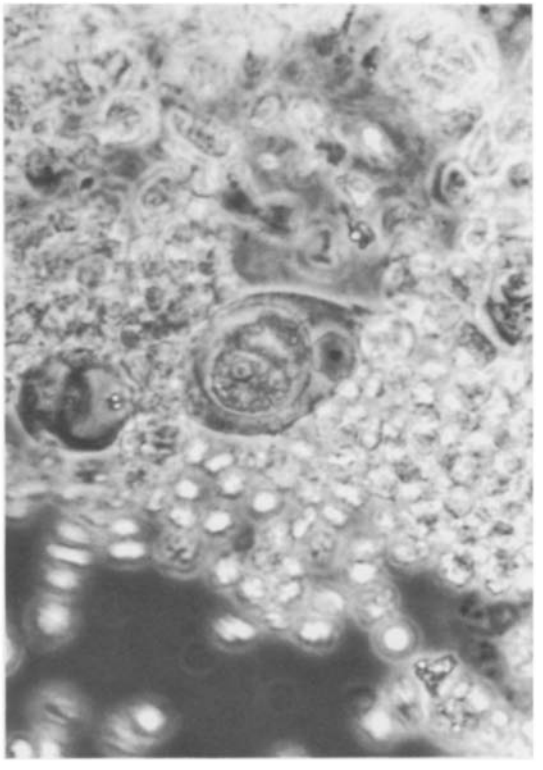
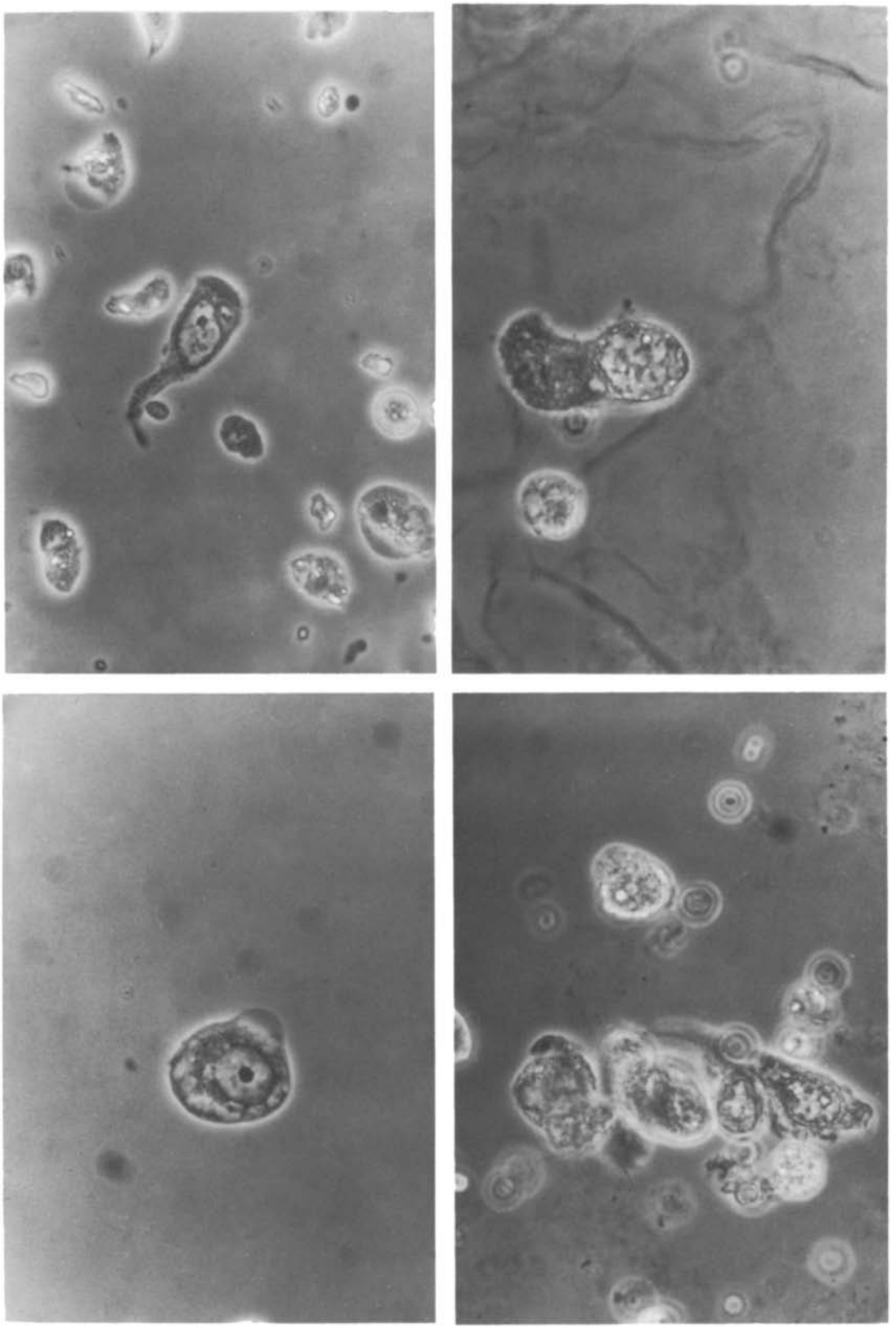


Fig. 6. By phase-contrast it is seen to be a giant binucleated cell with thickened nuclear membrane and large nucleoli. From a patient with A T3 bladder carcinoma who subsequently underwent radical cystectomy.



Figs. 7, 8, 9, and 10. Three single cells and one group of cells are with definite features of malignancy (large nucleoli, irregular nuclear shape, thickened nuclear membranes, polymorphism and altered nuclear-cytoplasmic ratio). Phase contrast view.
All the photographs were taken at a magnification of X400.

standards previously set for urinary cytology. (15, 17, 21); if a tumour is suspected, portions of the same sediment can be smeared for Papanicolaou or Giemsa staining and subsequent confirmation of the diagnosis by a cytopathologist. This overcomes one of the principal disadvantages of phase contrast microscopy, namely that the preparations cannot be preserved, so that rechecking is impossible. Documentation is only possible by photomicrography which requires much more expensive equipment.

A second disadvantage may be that the diagnostic accuracy is probably less than by conventional staining methods. Not all degrees of atypia seen in Papanicolaou-smears, can be detected by phase contrast microscopy. It is possible to see clearly the nucleus-cytoplasmic ratio, irregularity and polymorphism of the nuclei and the presence of large nucleoli; as Kalnins (9) has pointed out, these are important indications of malignancy. However, fine changes in the chromatin pattern can only be seen in stained preparations. Phase contrast microscopy will provide an impression of the numbers of transitional epithelial cells and whether they are solitary or lie in sheets or clumps (suggesting papillomatous growth); slight or marked atypia can be detected and so can cells that are definitely suspicious of malignancy. Thus, phase contrast microscopy provides a means of rapid urinary cytology as a guide to further diagnosis and therapy.

The number of examinations in the present study is too small to permit final conclusions about the value of urinary cytology, although others have confirmed its value, (7, 14, 15, 16, 20, 22). It was very interesting that, in using the three methods simultaneously, none of the first time diagnoses of urothelial cancers were missed, and that more pelvi-ureteric tumours and carcinomas-in-situ of the bladder were detected at an earlier stage than in previous years. Poor results were obtained in the cases of benign papillomas, an observation made by several other workers (6, 15, 21). However, this disappointing aspect has not prevented the establishment of a large scale survey to confirm the value of urinary cytology for the diagnosis of tumour recurrence after therapy (10 and 11), and to examine further the correlation between cytological diagnosis and the histological and clinical staging of urothelial neoplasms, using phase contrast as an initial rapid screening technique.

Acknowledgments. We are grateful to Miss J. Brussee, cytotechnician, and Mrs. Beyer-Boon, cytopathologist, without whom this study could never have been completed.

References

1. Brody, L., Webster, M.C., Kark, R.M.: Identification of elements of urinary Sediment with phase contrast microscopy. J. Amer. med. Ass. 206, 1777 (1968)
2. Brody, L.H., Salladay, J.R., Ambruster, K.: Urinalysis and the urinary sediment. Med. Clin. N. Amer. 55, 243 (1971)
3. Constantian, H.M., de Girolanni, E.: Urothelial tumours detected by cytology: New cell block technique. J. Urol. 109, 304 (1973)
4. Crabbe, J.G.S.: "Cornet" or "Decoy" cells found in urinary sediment smears. Acta Cytol. 15, 303 (1971)
5. Dukes, C.E.: The Institute of Urology Scheme for the histological classification of epithelial tumours of the bladder. In: Tumours of the bladder , p. 105 (D. M. Wallace, ed.) Edinburgh, London: Livingstone 1959
6. Esposti, P.L., Moberger, G., Zajuek, J.: The cytologic diagnosis of transitional cell tumours of the urinary bladder and its histologic basis. Acta Cytol. 14, 145 (1970)
7. Esposti, P.L., Zajuek, J.: Grading of transitional cell neoplasms of the urinary bladder from smears of bladder washings. Acta Cytol. 16, 529 (1972)
8. Forni, A., Getti, G., Armeli, G.: Urinary cytology in workers exposed to carcinogenic aromatic amines: a six-year study. Acta Cytol. 16, 142 (1972)
9. Kalnins, Z.A., Rhyne, A.L., Morehead, R. P., Carter, B.J.: Comparison of cytologic findings in patients with transitional cell carcinoma and benign urologic diseases. Acta Cytol. 14, 243 (1970)
10. Malmgren, R.A., Soloway, M.S., Chu, E. W., Del Vecchio, P.S., Ketcham, A.S.: Cytology of ileal conduit urine. Acta Cytol. 15, 506 (1971)
11. Reichborn-Kjennerud, S., Høeg, K.: The value of urine cytology in the diagnosis of recurrent bladder tumours: a preliminary report Acta Cytol. 16, 269 (1972)
12. Richter, A., Sülldorf, P.: Die Bedeutung der Zytodiagnostik als differential diagnostisches Hilfsmittel zur Früherkennung von Blasentumoren Z. Urol. 65, 573 (1972)
13. Ross, K.F.A.: Phase-Contrast and interference microscopy for cell-biologists. London: Edward Arnold Ltd. 1967
14. Russo, M.A., Cockett, A.T.K.: Microscopic urinalysis with phase contrast microscopy. J. Urol. 107, 843 (1972)
15. Sarnacki, C.T., Cormack, L.J.M., Kiser, W. S., Hazard, J.B., Mc. Laughlin, Th.C., Belovick, D.: Urinary cytology and the clinical diagnosis of urinary tract malignancy: a clinicopathologic study of 1400 patients. J. Urol. 106, 761 (1971)

16. Schiffer, A., Lymberopoulos, S., Charvat, A.: Vergleichende Zytodiagnostik in der Urologie. Z. Urol. 6, 367 (1968)
17. Schoonees, R., Gamarra, M.G., Moore, R., Murphy, G.P.: The diagnostic value of urinary cytology in patients with bladder carcinoma. J. Urol. 106, 693 (1971)
18. Schulte, J.W., King, Ch.D., King, E.B., MacDonald, D.A., Jassie, M.: A simple technique for recognizing abormal epithelial cells in urinary sediment. J. Urol. 89, 615 (1963)
19. Spencer, E.S., Petersen, I.: Hand-atlas of the urinary sediment. Copenhagen: Munksgaard 1971
20. Stoll, P.: Gynaecological vital cytology Berlin, Heidelberg, New York: Springer 1969
21. Tyrkkö, J.: Exfoliative cytology in the diagnosis and follow-up of urothelial neoplasms. Scand. J. Urol. Nephrol. Suppl. 19, 1972
22. de Voogt, H.J., Wielenga, G.: Clinical aspects of urinary cytology. Acta Cytol. 16, 349 (1972)
23. Wiggishoff, C.C., Mc. Donald, J.: Urinary Cytology in the Diagnosis of Bladder Tumours. Acta Cytol. 16, 139 (1972)
24. Zernike, F.: Roy. Astron. Soc. M.N. 94, 377 (1934)
25. Zernike, F.: How I discovered phase contrast. Science 121, 345 (1955)

Dr. H.J. de Voogt
Afdeling Urologie
Academisch Ziekenhuis
Rijnsburgerweg 10
Leiden
The Netherlands