A comparison of thiomersal and 50% alcohol as preservatives in urinary cytology

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SUMMARY The efficacy of 50% ethyl alcohol and of thiomersal as preservatives in urinary cytology were compared. In both methods over 80% of the cells were sufficiently well preserved after three days to allow cytomorphological evaluation, and over 50% on the seventh day. In the specimens without preservative, only 54% and 28% were intact after the same time intervals. In contrast with 50% ethyl alcohol, thiomersal is a more effective bactericide, it does not increase the volume of the sample, it is cheaper, and it does not affect the cytomorphology.

With the increasing incidence of carcinoma of the urinary bladder in industrialised countries the use of cytology has become more important. For a mass screening project a centralised cytology service is preferable, but, as this may lead to a delay in the processing of specimens, preservation of the urine is desirable. The maintenance of life-like features of the cells is not essential, only the retention of morphological and diagnostic features in the fixed and stained cells (Bahr, 1974). This can be achieved either by initial fixation of the whole specimen, for instance with 50% ethyl alcohol (Durfee, 1968), or by using a bactericide such as thiomersal (Merthiolate), the sodium salt of ethyl mercuric thiosalicylic acid (Jamieson and Powell, 1931; O'Connor, 1939).

The present study compares the efficiency of thiomersal and of 50% ethyl alcohol as preservatives for urinary cytology specimens.

Material and methods

The study was carried out on specimens of freshly voided urine obtained from 31 female and 9 male patients attending the Urological Clinic of the University Hospital, Leyden. Six patients had proved carcinoma of the urinary bladder and 34 had non-malignant diseases of the urinary tract. All the patients with carcinomas were males.

Each urine sample was divided into four equal portions. The first portion (sample 1) was used for culture and bacterial counts, and for performing the trypan blue exclusion test to determine the percentage of living cells present. No preservative was added to the second portion (sample 2); 50 mg thiomersal per 100 ml was added to the third portion (sample 3), and an equal quantity of 50% ethyl alcohol was added to the fourth portion (sample 4). The samples were kept at room temperature. The trypan blue exclusion test was also performed on sample 2 on the third and fifth days.

Smears were made from centrifuged aliquots of sample 1 immediately and from all four samples on the third, fifth, and seventh days. The smears were fixed with spray fixative and stained by the Papanicolaou method.

The proportion of intact cells in the stained smears was determined as a percentage of the total number of intact and degenerate epithelial cells present, using the following criteria for an intact cell: well-circumscribed cell border, presence of intact cytoplasm, a well-defined nuclear border, and a clear-cut unblurred nuclear pattern. The presence of bacteria (graded +, ++, or +++), crystals, and background material was also evaluated in each smear.

Results

The quantitative bacteriological examination carried out on the freshly voided urine (sample 1) showed that there was a significant difference in the number of viable bacteria per millilitre cultured from urines with pathogenic and non-pathogenic bacteria. In seven cases, pathogenic bacteria (citrobacter, entero-
bacter or pseudomonas) were present. The number of bacteria in these specimens was $10^6$/ml or more. In the other 33 specimens no pathogens were found and the bacterial count was less than $10^3$/ml.

Bacterial growth took place within 24 hours in those urine samples kept at room temperature to which no preservatives had been added, and most of the urines without preservative became cloudy after three days because of the numerous bacteria present. In the male urines containing no or few bacteria, the cells were still morphologically intact up to the fifth day provided that there was no bacterial contamination from the environment. The urines from the tumour cases all contained blood, and the urines from males with cystitis and from all females contained mucus or protein. In these cases the multiplication of the bacteria was visible by the third day, and most cells had lost their normal morphology by the fifth day.

There was neither increased bacterial growth nor accelerated cellular degeneration in those cases with pathogenic bacteria. On the day of taking the specimen, seven of the cases showed significant bacterial contamination (graded $+++/+++$), and an additional 21 of the specimens without preservative contained numerous bacteria by the third day. In contrast to this there was no increase in bacterial contamination when thiomersal or ethyl alcohol were used. By the seventh day, 38 of the samples without preservative contained numerous bacteria as compared with an additional two of the cases treated with ethyl alcohol and none with thiomersal. One of the ethyl alcohol treated samples was overgrown with fungi on day 7.

In the freshly voided urine of the six patients with urothelial carcinomas, $46\%$ ($\pm 4$) of the cells were vital and most of these cells appeared to be malignant: but only $26\%$ ($\pm 5$) of the cells from cases with non-malignant diseases were vital. After three days almost all the cells in the cases with positive and negative cytology were dead.

There was a difference in the rate of degeneration of epithelial cells in samples with and without added preservative (Figure). In the urine without added preservative, $93\%$ ($\pm 9$) of the epithelial cells were intact on the day of taking the specimens, $54\%$ ($\pm 15$) on the third day, $28\%$ ($\pm 6$) on the fifth day, and $19\%$ ($\pm 5$) on the seventh day. In contrast to this, $81\%$ ($\pm 7$) of the cells in the samples treated with thiomersal and ethyl alcohol were intact on the third day, and by the seventh day $51\%$ ($\pm 12$) were still satisfactory for cytodiagnostic purposes. The difference in the percentages of intact cells when thiomersal and ethyl alcohol were used is not statistically significant.

The cell yield in the smears made from the ethyl alcohol-treated specimens was approximately one-third of that obtained from the thiomersal treated specimens. Crystals were often present on the fifth and seventh days.

**Discussion**

Any increase in the interval between the time of collection of the sample and the preparation of the smear results in an exponential growth of bacteria in the urine if no preservative is added, and degenerative changes occur in the epithelial cells as a result of bacterial toxins. Thus, if there is little or no bacterial contamination (as frequently occurs in healthy males) intact cells can be harvested after three days, while in the majority of urine specimens from female controls (with heavy contamination by vaginal bacteria) the cells show degenerative changes after the same time interval (Beyer-Boon et al., 1977). When thiomersal is added to freshly voided urine bacterial growth is stopped. Thiomersal has these advantages: its low toxicity, its high degree of solubility in water, and, of particular importance in cytology, its devitalising properties (Jamieson and Powell, 1931). Thiomersal has been
proved to be a potent bactericide for pathogenic bacteria (Powell and Jamieson, 1930) and effective against both anaerobic and aerobic bacteria. A high concentration of thiomersal is required, however, to stop all bacterial growth (Elliott et al., 1940; Arden, 1956). When 50% ethyl alcohol is added to freshly voided urine, bacterial growth is slowed down in the majority of cases, but the efficacy of 50% ethyl alcohol as a safe bactericidal agent should be viewed with caution as some mycobacteria and fungi can still grow in specimens treated with this concentration of alcohol (Mitchell, 1977; Tucker, 1977). We also noted fungal proliferation in one alcohol-treated specimen. In the light of these observations, the use of thiomersal is recommended in preference to ethyl alcohol as a safer bactericide in the handling of infectious cytological material, such as sputum, especially if the technical procedures used involve blenders, which are particularly liable to produce potentially infectious aerosols (Harris, 1977). 50% ethyl alcohol prefixes the cells immediately. However, in specimens containing large quantities of protein the resultant precipitate makes the preparation of smears difficult. This problem does not occur in thiomersal treated specimens where the cells are not fixed until after the smear has been made. An additional drawback to using prefixed cells is the reduced adherence of the cells to the slides, which causes loss of cells and a greatly reduced yield (Beyer-Boon, 1977).

Our results show that when bacterial growth is prevented, although almost all cells are dead by the third day, over 80% are sufficiently well preserved to allow cytological evaluation. After this time there is a marked reduction in the percentage of intact cells which may be due partly to the effect of toxic substances released by dead cells (Mohr, 1969). Thiomersal and 50% ethyl alcohol are equally effective in preserving cells for the first three days and a delay of up to three days is therefore possible before cytological preparations need to be made. We prefer the use of thiomersal because it is cheaper, it does not increase the volume of the specimen, it does not affect cytopreparation, it permits a higher cell yield, and it is a safer bactericidal agent.

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References


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