Survival of urinary leucocytes

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SYNOPSIS   The survival of white cells under 15 μ in diameter (95% polymorphonuclear leucocytes), obtained from the urine of patients with urinary tract infections, was examined *in vitro* under different conditions of osmolarity, pH, and temperature of incubation.

The rate at which urinary white cells disintegrate was found to be accelerated by raising the pH, decreasing the osmolarity, and by increasing the temperature at which suspensions were kept. The most rapid disappearance of white cells occurred in hypotonic alkaline suspension at 37°C. There was little change in cell suspensions kept in acid (pH 3·0) or in hypertonic (1,200 mOsmoles/l.) conditions over a period of 24 hours.

The findings indicate that in interpreting the significance of the number of leucocytes in urine it is necessary to take into account the condition of the urine and the time and temperature at which it has been kept before examination.

Estimation of the number of white cells in urine is often used as a guide to the presence of infection in the urinary tract. Occasionally, however, patients with acute urinary tract infection are found to have only small numbers of white cells in the urine, particularly when the infecting organism is a *Proteus* strain (Brumfitt, 1965; Edebo and Laurell, 1958; Gnarpe and Edebo, 1965; Little, 1965). It is well known that leucocytes may disintegrate in urine (*e.g.*, Dukes, 1939), and that this may occur despite refrigeration, particularly in alkaline specimens (McIntyre and Mou, 1965). The absence of white cells in certain infected specimens might therefore be due to their rapid disappearance in the interval between collection and microscopy. In order to investigate this possibility a study was made of the effect of various conditions on the survival of white cells obtained from infected urine.

METHODS

URINE SPECIMENS   These were taken from patients who had indwelling catheters and in whom a coliform infection of the urinary tract had developed. All specimens contained more than 10^5 bacteria and between 10^4 and 10^6 white cells per ml. The bladder was drained as completely as possible through the catheter, which was then closed for one and a half hours; the total amount of accumulated urine was then collected and the specimen taken immediately to the laboratory.

CELL SUSPENSIONS   These were prepared by centrifuging 10 ml. samples of urine at 200 *g* for approximately seven

minutes to deposit the cells. The supernatant urine was discarded and the cells washed once and resuspended in saline containing one tenth its volume of citric acid/phophate buffer. A cell count was made and the suspension diluted to a concentration of approximately 10^4 cells per ml. The concentration of the saline and the pH of the buffer was varied in the different experiments to provide the conditions of pH and osmolarity required. Cephaloridine, 250 μg./ml., was added to prevent bacterial multiplication. The suspension of white cells was held at 37°C. in the water bath, at room temperature (21°C.), or in the refrigerator at 4°C.

CELL COUNTS   Counts were made in a Fuchs-Rosenthal counting chamber after dilution 1/20 in acetic acid-gentian violet diluting fluid. In addition, the nature of the white cells was determined by examination of smears stained with May-Grünwald-Giemsa stain.

During the process of degeneration which could be observed in the white cells they changed from easily-identifiable leucocytes to become apparently structureless bodies before disappearing altogether. It was therefore necessary to establish an arbitrary end-point in this process, beyond which cells could no longer be considered as morphologically identifiable leucocytes when examined in the counting chamber with a magnification of 400. Thus leucocytes were defined as cells of less than 15 μ in diameter, possessing a recognizable nucleus and cytoplasm, and an apparently intact cell outline. Cells larger than 15 μ in diameter were disregarded; they formed less than 5% of the cell populations and appeared to be squamous and transitional epithelial cells (Rofe, 1955).

EXPERIMENTS AND RESULTS

EFFECT OF pH   White cells from a single specimen of urine were suspended in isotonic saline

(300 mOsmoles/l.) buffered at *p*H 3·0, *p*H 7·0, and *p*H 8·0. The suspensions were kept at room temperature (21°C.) and cell counts made at intervals. The results of three separate experiments using cells from different patients are given in Figure 1. It can be seen that at *p*H 3·0 the count changed very little over a period of about 20 hours. At *p*H 8·0 the cell count had fallen by 50% within two to three hours and after 20 hours the count had fallen by approximately 90%. At *p*H 7·0 the cell count fell at an intermediate rate.

EFFECT OF OSMOLARITY   White cells were suspended in solutions of 50, 300, and 1,200 mOsmoles/l. at *p*H 7·0 and kept at room temperature. Cell counts were made at intervals and the results of three separate experiments are shown in Figure 2. White cells were found to degenerate and disappear most rapidly in hypotonic solution. At 50 mOsmoles/l. the count had fallen by 60% within two hours, and by 90% within 22 hours. When the suspending fluid was hypertonic (1,200 mOsmoles/l.) very little fall in the cell count was observed over a period of 22 hours.
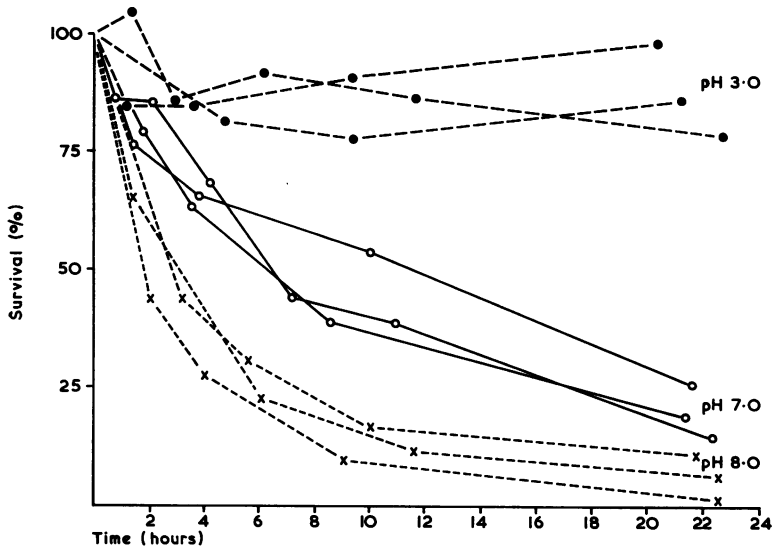

FIG. 1.   *Effect of pH: results on leucocytes from three different patients. Cells suspended in isotonic (300 mOsmoles/l.) saline at 21°C. and buffered at three different pH values.*
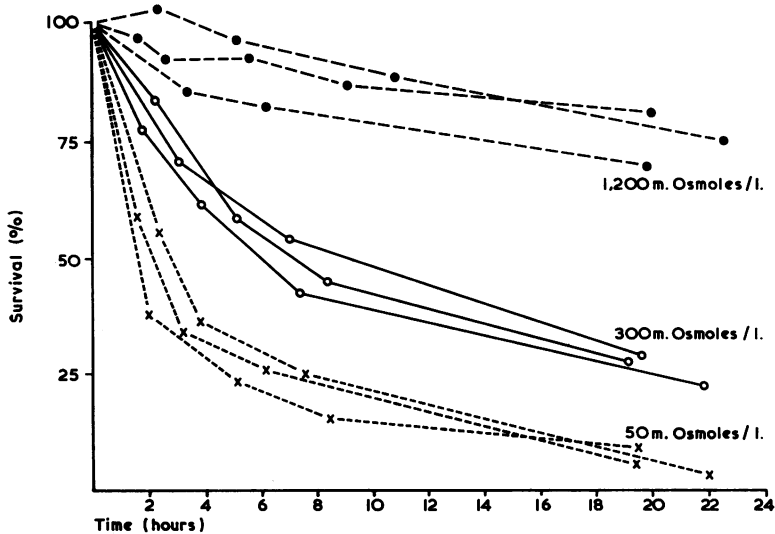

FIG. 2.   *Effect of osmolarity: results on leucocytes from three different patients. Cells suspended at pH 7·0 in buffered saline at 21°C. and at three different osmolarities.*

In isotonic solution (300 mOsmoles/l.) the cell count fell at an intermediate rate.

EFFECT OF TEMPERATURE White cells suspended in isotonic solution at *p*H 7·0 were kept at 4, 21, and 37°C. and cell counts made at intervals. The results of three separate experiments, given in Fig. 3, show that the rate at which the leucocyte count fell was slowed when the suspensions were kept in the cold, and was fastest when they were incubated at 37°C.

EFFECT OF COMBINED ADVERSE CONDITIONS The effect of combining the most adverse of the condi-

tions tested was examined using urinary white cells suspended in hypotonic solution (50 mOsmoles/l.) at *p*H 8·0, and incubated at 37°C. The effect of keeping these suspensions at 4°C. was also examined. The results of three separate experiments are given in Fig. 4, which also shows, for purposes of comparison, the results obtained in experiments at *p*H 7·0 and 21°C. using a hypertonic suspending solution. It is evident that the cells can disappear very rapidly when suspended in dilute alkaline solution at 37°C. Thus the cell count may fall by 90% within two and a half hours. The rate of fall was found to be slower when the suspensions were placed in the refrigerator
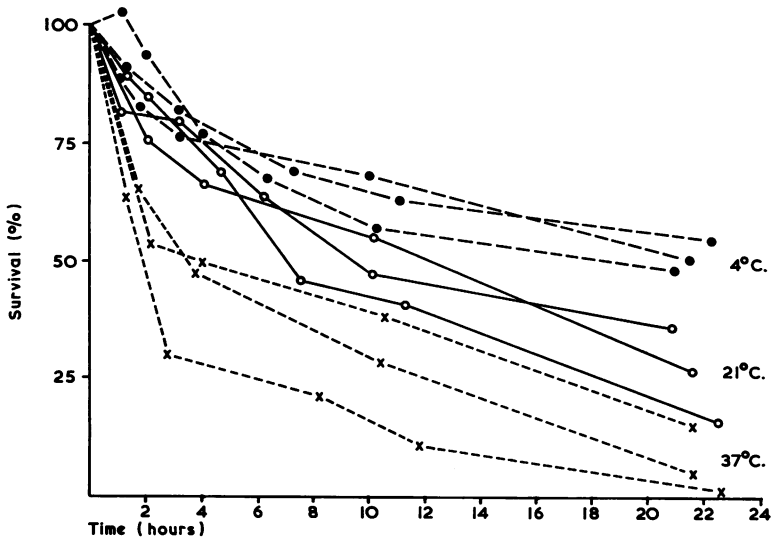

FIG. 3. *Effect of temperature: results on leucocytes from three different patients. Cells suspended at pH 7·0 in buffered isotonic saline at three different temperatures.*
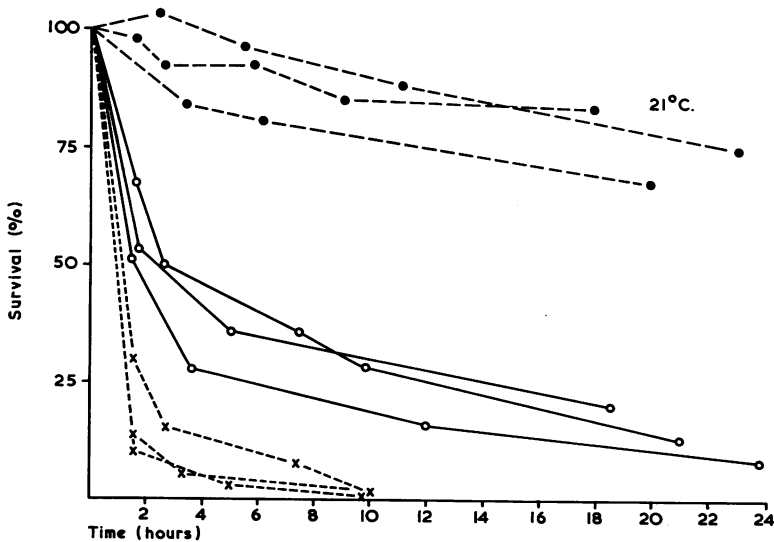

FIG. 4. *Effect of combined adverse conditions: results on leucocytes from three different patients suspended at pH 8·0 in hypotonic (50 mOsmoles/l.) saline at 37°C., ✕ - - - ✕, and also at 4°C., ○——○. The results obtained with cells suspended in hypotonic (1,200 mOsmoles/l.) saline at pH 7·0 at 21°C. are also shown,* ● - - ●.

but nevertheless after two and a half hours at 4°C. the cell count had fallen by about 50%.

STABILITY OF CELLS IN URINE   To test whether the results obtained with the cell suspensions could be used as a guide to what occurs naturally in urine an examination was made of the stability of cells allowed to remain in the urine in which they were passed. The pH of urine samples was measured with a pH meter and the osmolarity determined by measuring the freezing point depression with a Beckmann thermometer. Three suitable specimens were taken in which the values were, respectively: pH 4·8 and 380 mOsmoles/l.; pH 5·5 and 530 mOsmoles/l.; pH 6·6 and 800 mOsmoles/l. Cephaloridine was added and 10 ml. samples of each urine were incubated at 0, 21, and 37°C. and cell counts made at intervals for 20 hours. In each case the rate of fall in the cell count was similar to that which would be expected from the experiments using washed cells suspended in buffered saline.

NATURE OF THE WHITE CELLS   Stained preparations of the freshly-prepared cell suspensions showed that 95% of the cells less than 15 μ in diameter were polymorphonuclear leucocytes. About 4% had the characteristics of renal tubular epithelial cells (Rofe, 1955), and approximately 1% could not be clearly identified. However, when cell degeneration occurred, as the count fell the proportion of cells resembling renal tubular epithelial cells increased. Whilst it is possible that tubular epithelial cells are less liable to lysis than neutrophils, the finding is probably accounted for by the observation of Montgomerie and North (1963) that degenerating pus cells may become morphologically indistinguishable from renal tubular epithelial cells.

An attempt was made to distinguish between neutrophils and renal tubular epithelial cells in the counting chamber by the staining technique of Prescott and Brodie (1964) in which neutrophils are identified from their peroxidase activity. However, this technique was found to give higher counts of tubular cells than was obtained by conventional staining and when the cell suspensions were maintained *in vitro* the disproportion between the two counts increased. Thus in one experiment the proportion of peroxidase-positive cells fell by 89% within four hours, whilst the proportion of neutrophils detectable in preparations stained with May-Grünwald-Giemsa stain fell by only 5% in the same time. During the four hours the total cell count fell by 20%. The requirement for fresh urine when employing a peroxidase-staining technique was noted by Kaye (1958) although he reported that some

staining still occurred several hours after the cells had been collected.

DISCUSSION

It is clear that the conditions in which urinary white cells are suspended can influence their survival considerably and it is probable that the findings obtained experimentally reflect what occurs naturally in urine. The results indicate that a count of the number of white cells in urine may be reduced in specimens not examined shortly after collection, particularly if the urine is alkaline or hypotonic. The low cell counts observed in specimens infected with *Proteus* strains are probably due to the ability of these organisms to produce ammonia from urea (Gnarpe and Edebo, 1965). If conditions are both alkaline and hypotonic the count may fall by 50% within an hour of collection. Cell degeneration in urine which is not unusually alkaline or dilute may also be appreciable; in isotonic suspension at pH 7·0 and at room temperature the white cell count may fall by 50% within five hours of collection.

It is of course well known that urine should be examined shortly after collection to avoid both bacterial multiplication and cell degeneration (Addis, 1925; Dukes, 1939). In recent years the appreciation of the importance of counting the bacteria in urine has led to the suggestion that refrigeration is an acceptable alternative to early examination since multiplication of bacteria is thereby inhibited (Brumfitt and Percival, 1964; Little, 1964). Although refrigeration does slow the degeneration of white cells, nevertheless, in alkaline, hypotonic urine a 50% fall in the cell count can occur in two and a half hours at 4°C. In refrigerated isotonic urine at pH 7·0 a decline of almost 50% in the white cell count may be expected within 24 hours. In interpreting the significance of the white cell count in urine it is clearly necessary to take into account the condition of the urine and the time and temperature of its storage.

The possibility should also be considered that white cell disintegration may proceed rapidly *in vivo* while the urine remains in the bladder and, in order to obtain the highest urine cell count from patients with suspected renal tract disease, it may be advisable to examine urine after it has been lying in the bladder for only a short period of time. To minimize this possible effect the urine used in the experiments reported was collected one and a half hours after the bladder had been previously emptied; this was the shortest time in which a sufficient quantity of urine regularly accumulated.

A further possible factor which may affect the survival of white cells in urine is the infecting bacterium. Thus staphylococci produce a toxin, the

δ lysin, which lyses white blood cells (Gladstone and van Heyningen, 1957) and it is possible that other strains of bacteria elaborate similar substances. In our experience, however, culture filtrates of *Escherichia coli* and *Proteus mirabilis* had no visible effect on urinary cells maintained in isotonic buffer at *p*H 7·0.

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