Effects of delayed culture on semiquantitative urinary bacteriology results

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SYNOPSIS Urine specimens received in the laboratory were cultured at intervals of two hours on three successive occasions. During the intervening period the specimens were left at room temperature. After 24 hours' incubation counts were done, and it was observed that the significance of bacterial growth was not altered by delay in culture.

It has been accepted that urine specimens should be cultured within one hour of collection. If this is not possible they may be stored at 4°C for up to 48 hours (Kass, 1956). The purpose of this is to stop the multiplication of contaminating bacteria which would give a false positive quantitative result as they multiply in normal urine at room temperature. In busy hospital laboratories urine samples seldom reach the department within an hour of collection for various administrative reasons, and they are seldom refrigerated before they reach the laboratory. This short study was carried out to see if this unavoidable delay in transit could alter the picture of urinary tract infection sufficiently to result in misleading reports being issued.

Methods and Materials

As soon as the urine specimens were received in the laboratory, a portion of the specimen was transferred to a sterile bottle. The specimens were diluted 1 in 10 with 1% peptone saline. After thorough mixing, one drop of the sample was dropped onto each of two duplicate Oxoid MacConkey plates using a standard dropping pipette. This inoculum was evenly spread using a wire spreader. Specimens were left on the laboratory bench. After two hours a second duplicate subculture was performed as before, and the process was repeated after a further interval of two hours, making three duplicated cultures over a four-hour period. All the culture plates were incubated at 37°C for 18-24 hours. They were read next day and the colonies were counted by eye or using a mechanical counter as appropriate.

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In addition a few randomly chosen urine samples from the study were observed to see if any urine contained antibiotics which might have accounted for the absence of bacterial growth (Kass, 1955; Gould, 1965). Sterile blotting paper discs of about 5 mm diameter were prepared. Laked blood plates were seeded with one standard Oxford staphylococcus. A disc was dipped into a well-mixed urine sample and was then put on the seeded sensitivity plate. The plate was incubated overnight at 37°C. The diameter zones of inhibition were measured by a calliper.

Results

Table I shows that 44% of specimens had significant growth of bacteria in all three cultures and 34% grew no bacteria at all in any of the three culture plates. Fourteen samples (13%) had increasing colony counts in the first and second culture plates but never reached 10⁵/ml of bacteria in the last plates, that is, after four hours. It is interesting to note that 2.8% of the urine samples had rather

Type of Bacterial Growth	Number	%
Significant growth in all three cultures	48	44
No growth in all three cultures	37	34
Increase in colonies in successive plates but all growth insignificant	14	12.8
Decrease in colonies in successive plates	3	2.8
Static counts of insignificant numbers (no increase or decrease over 4 hr)	4	3.7
Unexplained growth	3	2.8
Total	109	

Table I *Effect of delay of culture on bacterial growth*

No.	Organism	Growth	Zone of Inhibition (mm)	Antibiotic given if known
1	<i>E. coli</i>	Significant	16	Co-trimoxazole
2	<i>Strep. faecalis</i>	Significant	22	Nil
3	<i>Pseudomonas</i>	Significant	23	Gentamicin
4	Coliform	Insignificant	22	Gentamicin
5	<i>Klebsiella</i>	Insignificant	40	Nil
6	<i>Staph. albus</i>	Insignificant	24	??
7	<i>E. coli</i>	Insignificant	25	Nil
8	O	Nil	10	Co-trimoxazole
9	O	Nil	43	Gentamicin and cloxacillin
10	O	Nil	40	Ampicillin
11	O	Nil	15	??
12	O	Nil	34	Nil

Table II Randomly chosen urine samples which contained antibiotics

'Significant' growth means the bacterial concentration is 10^5 /ml or more, and 'insignificant' growth means the concentration is 10^4 /ml or less

decreasing numbers of bacteria growing in successive culture plates. There were 3.7% of specimens which grew insignificant numbers of bacteria and the counts were nearly the same on all three culture plates. Lastly, three specimens could not be explained on any logical basis and could be the result of technical errors.

Table II shows that of three specimens which had significant growth of bacteria two contained antibiotics. Four samples grew insignificant numbers of bacteria, of which only one sample contained antibiotic for certain.

Discussion

The present study is a follow-up of an observation during a previous study on the assessment of usefulness of dip slides (Shrestha and Richardson, 1973). It was noted that colony counts on dip slides were similar in number to those on MacConkey plates, though culture was made on plates two or three hours after collection of the urine. These urine samples were not refrigerated before culture.

The results in table I are interesting. 'Significant' growth of bacteria from an infected urine remains 'significant' even if culture is delayed up to four hours. Similarly, if a sample does not grow any organism in the first culture, there is no growth in subsequent cultures. Hence delay in transit and in the laboratory did not in this study influence the significance of bacterial growth of urine samples. This does not imply that urine samples must not be refrigerated if delay in transit is anticipated. If there are enough refrigerators for storage of urine samples,

it is a good practice. But the results show that if they are not refrigerated it does not make any difference to the proportion of samples with significant bacterial growth. Out of 109, 14 specimens grew an increased number of bacteria in successive cultures but never reached a significant level, that is, 10^5 /ml. The most interesting observation is that an insignificant growth of bacteria did not change to a significant one. However, in reporting on these urines it must be remembered that the white and red cells when present do suffer from delay and may be reduced in numbers (Triger and Smith, 1966).

Finally, it may not be out of place to stress the importance of noting whether or not a patient is receiving antibiotics even if they are not being given for a urinary infection.

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