Radiorespirometric Testing of Antibiotic Sensitivity in Urinary Tract Infections: Concise Communication

K. T. Singh, R. D. Ganatra, D. H. Shah, M. Shanta, Y. S. Nimbkar, B. B. Gaitonde, R. A. Dudani, S. K. Jadav, and V. N. Acharya

Tata Memorial Hospital, Haffkine Institute, and K. E. M. Hospital, Bombay, India

A radiometric method, based on inhibition of $^{14}CO_2$ release from bacterial metabolism of C-14-labeled glucose, was applied to test the susceptibility of urinary organisms to antibiotics. The testing was also carried out by the routine disc diffusion method after isolation of the organisms. Results of susceptibility to antibiotics could be obtained within 2 to 4 hr by the radiometric technique, compared with the 48 hr required for the disc method.

J Nucl Med 21: 135–138, 1980

The effective chemotherapy of urinary infections demands identification of organisms and testing for their susceptibility to antibacterial agents. Normally this kind of investigation takes 2 or 3 days, and often the clinician initiates treatment without waiting for the results.

Practical methods used for this kind of testing are based on dilution techniques or diffusion methods (*1–3*). Although, the tube-dilution method is the standard against which other methods are compared, the single paper-disc diffusion method, because of its ease and inexpensiveness, is used more often in most hospital laboratories.

De Blanc et al. (*4*) used the radiometric method involving C-14-labeled substrates to test susceptibility in standard strains of *Staphylococcus aureus, Escherichia coli,* and *Pseudomonas;* they then compared their results with tube-dilution method. The radiometric method has recently been made more sensitive and simple by the introduction of a two-chambered liquid-scintillation vial, as described originally by Buddemeyer (*5*). We have used a slightly modified version of this technique for our radiometric studies (*6*).

In a series of 25 patients suffering from urinary-tract infection, we have used the radiometric method to test the organism's sensitivity to drugs or antibotics. The aim has been to avoid the delay involved when urinary cultures are used.

MATERIALS AND METHODS

Drug susceptibility was tested by radiometric and disc methods simultaneously by two independent groups of workers. Aliquots of the same urine sample were taken for the two methods. A constant inoculum size was difficult to specify, however, since the results of the colony counts were not available before the susceptibility tests began.

Bacteriological culture of urine sample. The diagnosis of urinary-tract infection was made when three consecutive urine samples showed more than 10^5 organisms per milliliter of urine from a mid-stream sample, or 10^3–10^4 organisms per milliliter for samples obtained by bladder puncture or catheter. Mid-stream collection was used in seven patients, suprapubic bladder aspiration in nine, and a sterile disposable catheter in nine. Colony counts were determined separately by the dip-slide inoculum method (*7*), the results of which were available after 24 hr. A loopful of urine sample was incubated on MacConkey's agar, and one on blood agar, at 37°C for 24 hr. Identification of microorganisms was done by standard biochemical tests (*8*).

Radiometric method. The method for detection of $^{14}CO_2$ was similar to that described by Buddemeyer (*5*).

Received Sept. 1, 1978; revision accepted Sept. 12, 1979.

For reprints contact: R. D. Ganatra, Radiation Medicine Ctr., Tata Memorial Hospital, Annexe, Jerbai Wadia Rd., Parei, Bombay 4000 012 India.

It used a liquid-scintillation vial with an inner glass cylindrical tube having holes at its upper end. The outer vial was lined with freshly prepared Whatman No. 40 filter paper impregnated with alkali and scintillation fluor (PPO 10 g, POPOP 0.125 g, dioxane 100 ml, 1 *N* NaOH 20 ml). The inner vial contained glucose-free tryptic soy broth (TSB), 1 μCi [U-^{14}C]glucose (74 mCi/mmole), and a 0.1 ml urine sample. The vials were incubated at 37°C in a shaking water bath. The length of the inner vial and its small quantity of medium left little scope for direct splash of radioactivity on the paper lining the outer vial. Radiocarbon dioxide released from the inner vial was gradually trapped on this paper. The vial was counted in a liquid-scintillation counter at various times. This method provides a continuous and cumulative detection of $^{14}CO_2$.

Drug susceptibility testing. *Radiometric method.* A suspension containing 1 ml of urine sample in 10 ml of dextrose-free TSB and 1 μCi [U-^{14}C]glucose per ml of medium was prepared. One milliliter of this suspension was added to a number of vials so that each vial contained an amount of microbial inoculum equivalent to that present in 0.1 ml urine sample. Two vials without antibiotic served as controls, while a standard paper disc containing a known amount of antibiotic was added to each of the other vials. The antibiotics used were: ampicillin (100 μg), carbenicillin (25 μg), cephalaxin (25 μg), cephoran (25 μg), chloramphenicol (25 μg), colistin (75 μg), gentamycin (10 μg), Mandelamine (3 mg), penicillin (10 units), sulfonamide trimethoprim (25 μg), streptomycin (10 μg), sulphadiazine (300 μg), tetracycline (25 μg), nitrofurantoin (300 μg), and erythromycin (15 μg). The amount of each drug added was the same as that used on the disc in disc method. The vials were incubated at 37°C in a metabolic shaking waterbath. The $^{14}CO_2$ released was counted at 2, 4, and 6 hr.

Disc method. Sensitivity was determined by the impregnated disc diffusion method (*3*), by inoculating 2-3 colonies into 2-3 ml peptone water. This was incubated at 37°C for 1 to 2 hr, then diluted to make the turbidity comparable with that of a $BaSO_4$ standard. The culture was then streaked evenly with a cotton swab on tryptic soy agar plates. Discs with the aforementioned concentrations were placed on the surface of the agar. Petri dishes were kept in the refrigerator for half an hour and then incubated at 37°C for 18-24 hr. Depending on the zone of inhibition developed around a disc, the results were reported as follows:

+ = zone 8-10 mm in diameter; ++ = zone 11-14 mm in diameter; +++ = zone 15-20 mm in diameter; and ++++ = zone > 20 mm in diameter.

RESULTS

Twenty-five patients suspected of urinary-tract infection were investigated by both radiometric and disc methods. The disc cultures showed that 17 were positive and eight negative. All positive samples were studied at the same time by conventional techniques to exclude contamination and to establish the nature of the infective organism. Among the positive samples, four were obtained by mid-stream collection, six by bladder puncture, and seven by catheter. Further identification of 17 positive cultures showed seven *E. coli*, five *Klebsiella*, two *Pseudomonas aerugenosa*, two *Proteus mirabilis*, and one *Staphylococcus epidermidis*.

The radiometric technique also showed 17 samples positive for the presence of microorganisms, and eight negative. The results of radiometry are represented in graphical form as $^{14}CO_2$ production with respect to time. A typical graph for one urine sample is shown in Fig. 1, where radiometric and disc methods correlated well. The results of the disc method are given in the legend, and in parentheses in the figure, as +4, +3, +2, indicating the diameters of the zones of inhibition.

The radiometric results could be obtained within 2 to 4 hr in all cases except one, which was thought to be negative at 6 hr but subsequent counting at 20 hr showed the presence of microbial growth. No counting was done in this case between 6 and 20 hr. The present urine sample showed *Pseudomonas aerugenosa* in culture,

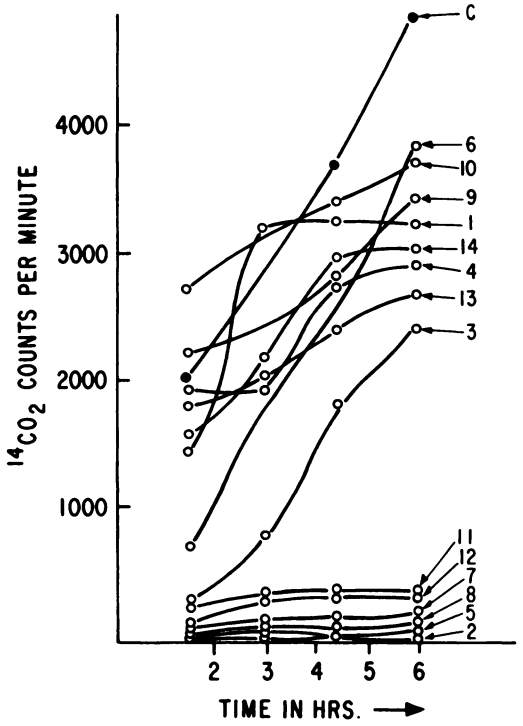

FIG. 1. Representative case showing good correlation between the two methods. Results of sensitivity testing by radiometric method are represented by rising $^{14}CO_2$ count rate. C = control with no antibacterial agent. Results of disc test: 1 ampicillin −ve, 2 cephalaxin +4, 3 cephoran +2, 4 chloramphenicol −ve, 5 colistin +3, 6 nitrofurantoin −ve, 7 gentamycin +4, 8 methenamine mandelate +4, 9 penicillin −ve, 10 carbenicillin −ve, 11 sulfonamide trimethoprim +3, 12 streptomycin +3, 13 sulphadiazine −ve, 14 tetracycline −ve.

although 2 mo earlier the patient had shown *Alcaligenes faecalis* twice in her urine. Inoculum size was also small in this patient, containing on the order of 10^3 organisms per ml.

The results obtained by the disc and radiometric techniques in 17 positive cases are compared in Table 1. Seven samples showed good correlation, meaning that a sensitivity of +4 and +3 by the disc method corresponded with complete inhibition of $^{14}CO_2$ production by radiometry. Drugs that showed susceptibility of +2 and +1 produced comparatively less inhibition of $^{14}CO_2$ production, whereas a control without any antibiotic, or an ineffective antibiotic, showed gradual increase with time in $^{14}CO_2$ count rate.

Fair correlation was observed in six patients. The drugs that showed a susceptibility of +4 and +3 by disc inhibited $^{14}CO_2$ release completely. However, a disc-method susceptibility of +2 and +1 either produced complete inhibition (e.g., +4 or +3) or showed increased production of $^{14}CO_2$ as in control vials. Colistin in two cases, Mandelamine in two, and streptomycin in one sample produced complete inhibition of $^{14}CO_2$ release, whereas the disc showed sensitivities of +2 or +1. In most of these six samples, the organisms showing susceptibility of +2 or +1 produced an increase in $^{14}CO_2$ count rate like that of the control.

Correlation between the two methods was poor in four samples. It was observed that some of the antibacterial agents, which showed susceptibility of +4, +3 by the disc method, did not inhibit $^{14}CO_2$ release. In all these cases an initial inhibition of $^{14}CO_2$ evolution was observed, but this was later (after 3 hr) overcome and $^{14}CO_2$ then increased as in the controls. One such case is represented in Fig. 2. The antibiotics cephalaxin and sulfonamine trimethoprime, which gave a susceptibility of +4 by disc, showed 3 hr of $^{14}CO_2$ inhibition, after which the CO_2 production was similar to that of the control vial.

TABLE 1. CORRELATION BETWEEN THE DISC AND RADIOMETRIC METHODS

No. of patients	Organism isolated	Correlation* Good	Fair	Poor
5	*Klebsiella*	3	1	1
2	*Pseudomonas*	2	—	—
6	*E. coli*	1	2	3
3	*Proteus*	1	2	—
1	*S. epidermidis*	—	1	—

* Good = +4, +3, +2, and +1 all correlate with radiometric results; fair = +4 and +3 correlate with radiometry; poor = +4 and +3 do not correlate with radiometry.

DISCUSSION

In the conventional microbiological method, an initial waiting period of 24 hr is necessary for isolation of an organism before susceptibility to antibiotics can be tested. In contrast, the radiometric method as used by us, which permits direct inoculation of the urine, is quicker. Although the organisms are not identified, the results of susceptibility to drugs can be known within 2–3 hr, which makes it possible to start antibiotic therapy earlier.

In most of the cases studied, the results of the disc method and radiometric methods were comparable. The antibiotics found most active (+4, +3) by disc usually caused complete inhibition of CO_2 release. Susceptibilities of +2, +1 did not correlate well with radiometry. However, +2 or +1 represents inadequate susceptibility, and thus has only minor significance in the selection of the proper antibiotic for treatment.

In the few cases in which correlation between the two methods was poor, an initial inhibition of CO_2 release was seen, in agreement with the disc, but later the inhibition was overcome and an increase in count rate was observed. The explanation for this difference could be that radiometry provides a true representation of the action of antibiotics in a rapidly growing culture with a mixed population of sensitive and resistant organisms. With time, sensitive organisms are destroyed, leaving the resistant organisms to multiply rapidly and raise the

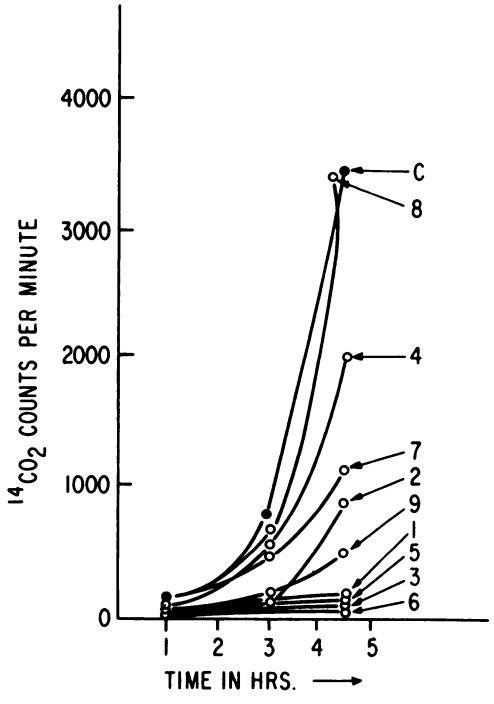

FIG. 2. Typical graph showing poor correlation between disc and radiometry. C = control. Results of disc test: 1 cephoran +4, 2 cephalaxin +4, 3 chloramphenicol +4, 4 Cloxacilin +2, 5 colistin +4, 6 gentamycin +4, 7 nitrofurantoin +2, 8 carbenicillin −ve, 9 sulfonamide trimethoprim +4.

count rate. Another reason could be that number of organisms in 0.1 ml of urine is not always constant, and there may be times when the number inoculated is so high that the amount of antibiotic on the disc is inadequate for total inhibition. Moreover, the comparison was between the inhibitory action of antibiotics in liquid against solid media. In the latter there is a concentration gradient of the drug, which is not so in a liquid medium. In radiometry the effect of drugs is measured by metabolism, whereas with the disc the end point is the density or rather paucity of growth. It was reported by DeLand (*9*) that minimum inhibitory concentration for different antibiotics varies with the method used—i.e., they differ between the radiometric and tube-dilution methods.

The limitation of the radiometric test is that it cannot identify the organism. This may be an advantage in cases of mixed infection, when a drug inhibitory to all the organisms can be prescribed without waiting for isolation and culture of each species. The method can also be of help in the early specific drug therapy of a urinary infection. In the present studies the patients were not treated on the basis of the radiometric test, but our encouraging, though limited, experience suggests that this test should be given a comprehensive clinical trial regarding its ability to indicate therapy.

REFERENCES

1. PETERSDORF RG, SHERRIS JC: Methods and significance of in vitro testing of bacterial sensitivity to drugs. *Am J Med* 39: 766-779, 1965.
2. STAMEY TA, GOVAN DE, PALMER JM: The localization and treatment or urinary tract infections: the role of bactericidal urine levels as opposed to serum levels. *Medicine* 44: 1-36, 1965
3. BAUER SW, KIRBY WM, SHERRIS JC, et al: Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 45: 493-496, 1966
4. DEBLANC HJ, Jr, CHARACHE P, WAGNER HN, JR: Automated radiometric measurement of antibiotic effect on bacterial growth. *Antimicrob Ag Chemother* 2: 360-366, 1972
5. BUDDEMEYER EU: Liquid scintillation vial for cumulative and continuous radiometric measurement of in vitro metabolism. *Appl Microbiol* 28: 177-180, 1974
6. SINGH KT, GANATRA RD, SHANTA MS, et al: Thyroid hormones and [^{14}C] glucose metabolism in bacteria. *J Nucl Med* 18: 736-739, 1977
7. JADAV S, PATEL KC, JAIN U, et al: Dip slide method in the diagnosis of urinary tract infection. *J Assoc Phys. India* 22: 255-259, 1974
8. COWAN ST: *Manual for the Identification of Medical Bacteria.* 2nd Ed. Cambridge, Cambridge University Press, 1965
9. DELAND FH: Metabolic inhibition as an index of bacterial susceptibility to drugs. *Antimicrob Ag Chemother* 2: 405-412, 1972