Use of Technetium ($^{99m}$Tc) as a Bacterial Label in Lung Clearance Studies

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The suitability of technetium ($^{99m}$Tc), a gamma emitter, for labeling of Diplococcus pneumoniae in studies of lung bacterial clearance was examined. A killed bacterial slurry with high specific activity was obtained with a ferric ascorbate reducing system. Approximately 5.5% of radioactive counts dissociated from labeled bacteria in 6 h. Rats were exposed to a uniformly mixed aerosol of untagged, viable pneumococci and killed, $^{99m}$Tc-tagged pneumococci. The aerodynamic behavior of labeled and unlabeled pneumococci was similar. Viable bacterial counts and radioactive counts were determined in lung homogenates at intervals following exposure, and rates of bacterial killing and disappearance of radioactive counts were plotted. Radioactive counts did not increase in the liver during the period of observation, suggesting that the decrease in lung radioactivity represents mucociliary clearance and not release of isotope to the systemic circulation. The use of $^{99m}$Tc for bacterial labeling provides advantages of technical simplicity and personnel safety compared to the use of beta-emitting isotopes.

The antibacterial defenses of the lung consist of two major mechanisms: physical removal via the airways by mucociliary action and bacterial killing in the lung, presumably by alveolar macrophages (5, 6, 8). Lung bacterial "clearance," estimated in experimental animals by determining the numbers of viable bacteria remaining in the lungs at intervals following exposure to airborne bacteria, represents the net activity of these mechanisms. Incorporation of a radioactive particle which has aerodynamic properties similar to that of the viable bacteria in the exposure aerosol affords the opportunity to determine simultaneously the rates of mucociliary removal and bacterial killing (3, 8). Furthermore, such a radioactive tracer permits the determination of bacterial killing in individual animals rather than only in groups of animals, thereby reducing the statistical variability of the technique (3).

Labeled bacteria have been used as the radioactive particles in lung clearance studies to insure that the aerodynamic behavior of the labeled particles is similar to that of the viable bacteria. Labeling of bacteria in vivo has been accomplished by cultivation in media containing phosphorus-32 ($^{32}$P) (3). Alternatively, killed organisms have been labeled with sulfur-35 ($^{35}$S) and added to a suspension of viable bacteria before aerosolization (8). Since these isotopes have predominantly beta emissions, liquid scintillation counting or radioautography is required for quantitation. As a result, the amount of lung tissue which can be examined is limited. Since particles may be nonuniformly deposited in the lungs during aerosol exposure, it would be desirable to homogenize all of both lungs for bacteriological studies and isolate quantitation. Furthermore, use of a gamma-emitting isotope would eliminate the time-consuming steps in solubilization of tissue for liquid scintillation counting. In the present study we report the use of technetium ($^{99m}$Tc), an isotope with only low-energy gamma emissions, as an isotopic bacterial label for lung clearance studies.

MATERIALS AND METHODS

Diplococcus pneumoniae type 3 was grown overnight in Todd-Hewitt broth, centrifuged, washed, and suspended in distilled water. The labeling technique employed was modified from that of McAfee et al. for serum albumin (7). Five milligrams of FeCl$_3$·6H$_2$O and 10 mg of ascorbic acid were added to 4 μCi of $^{99m}$Tc (sodium pertechnetate; BioNuclear, Inc., Dallas, Tex.) in 0.15 M saline. The pH was lowered to
4.5 to 4.0 with 1 N HCl. Bacteria were slowly added with continuous stirring, and the pH was further lowered to 2.0 to 2.5. The mixture was incubated for 30 min at 25 C, and the pH was raised to 5.5 to 6.5. To separate unbound \(^{99m}\text{Tc}\), the mixture was centrifuged, washed three times, and suspended in distilled water.

The concentration of viable bacteria in samples of the mixture was determined before and after labeling by plating serial 10-fold dilutions on 5% sheep blood-agar (BAP). Radioactivity of 1-ml samples of appropriate dilutions was determined in a well counter (Packard series 5000 auto-gamma spectrometer, Packard Instrument Co., Inc., Downers Grove, Ill.) and corrected for physical decay. Stability of the binding of \(^{99m}\text{Tc}\) to bacteria was evaluated by dialysis of the labeled bacterial slurry against distilled water at 25 C. Samples were removed for counting from the dialysis tubing and from the dialysate at 30-min intervals.

For aerosol studies 100 ml of the labeled and washed bacterial suspension was added to 100 ml of a similarly washed and suspended but unlabeled suspension of viable \(D.\ pneumoniae\). This combined slurry was aerosolized from a Collison nebulizer into a recirculating Henderson exposure chamber. The concentration of viable bacteria and radioactivity in the aerosol was determined from samples collected with glass impingers with calibrated rates of air flow. The distribution of particle size for both viable and radioactive particles was determined by drawing the aerosol from the chamber through an Andersen air sampler (1).

Rats were exposed to the combined aerosol for 30 min in groups of four with as many as eight groups being exposed on a single day. Predesignated animals were sacrificed immediately upon termination of exposure and at 1, 2, and 4 h thereafter. The lungs were removed aseptically at the hilus, weighed, and homogenized in 4 ml of distilled water. A total of 1.1 ml of the homogenate was removed for bacterial quantitation and plated in serial 10-fold dilutions on BAP. The remainder of the lung homogenate was transferred to a plastic test tube (150 by 10 mm) for determination of radioactivity. All radioactive counting, including that of the initial slurry, was performed at the termination of the study to simplify the calculation of physical decay. Counts of viable bacteria and radioactive counts were corrected for dilution (the latter was further corrected for physical decay) and expressed as counts per both lungs.

**RESULTS**

In seven studies, the specific activity of labeled bacteria ranged from 0.01 to 5.0 counts per min per bacterium (mean 1.5), when radioactive counts obtained shortly after labeling were compared with prelabeling bacterial counts. Viable bacterial counts decreased during the labeling procedure from a mean of 9.0 \(\times\) 10\(^4\)/ml to 6.4 \(\times\) 10\(^3\)/ml. Dialysis of labeled bacteria against distilled water for 6 h showed that approximately 5.5% of counts were dialyzable.

A comparison of the particle sizes of the radioactive and viable particles in the aerosol is shown in Table 1: 89% of viable particles and 97% of radioactive counts were 2 \(\mu\)m or less in diameter.

Results obtained from the nebulizer and impinger samples of a representative study are shown in Table 2. The bacterial and radioactive counts from the lungs of animals sacrificed up to 4 h after termination of exposure in the same study are shown in Fig. 1. Radioactive particles were slowly cleared from the lungs; 40% of the initial counts remained at 4 h. In contrast, the number of viable bacteria decreased rapidly and only 1.8% of the initial counts remained at 4 h. Radioactive counts in the liver did not change significantly over the 4-h period.

### Table 1. Percentage of viable bacterial and radioactive particle counts on the stages of an Andersen air sampler

| Stage | Total counts (%) | Viable bacteria | \(^{99m}\text{Tc}\) |
|-------|------------------|-----------------|------------------|
| 1     | 0.8              | 0.6             |
| 2     | 1.6              | 0.7             |
| 3     | 3.9              | 0.6             |
| 4     | 4.5              | 1.2             |
| 5     | 58.4             | 46.7            |
| 6     | 29.6             | 50.3            |

* Counts on stages 5 and 6 indicate particles of 2-\(\mu\)m size or less.

### Table 2. Viable bacterial and radioactive counts from a nebulizer slurry and from serial impinger samples in one lung clearance study

| Determination | Viable bacteria (per ml) | \(^{99m}\text{Tc}\) (counts per min per ml) | Ratio (bacteria per counts per min) |
|---------------|--------------------------|------------------------------------------|-----------------------------------|
| Slurry        | 3.33 \(\times\) 10\(^4\) | 7.33 \(\times\) 10\(^4\)                  | 4.5                               |
| Impinger samples* |                         |                                          |                                   |
| 1*            | 1.2 \(\times\) 10\(^4\)  | 5.6 \(\times\) 10\(^3\)                  | 2.1                               |
| 2             | 1.4 \(\times\) 10\(^4\)  | 6.0 \(\times\) 10\(^3\)                  | 2.3                               |
| 3             | 1.0 \(\times\) 10\(^4\)  | 6.4 \(\times\) 10\(^3\)                  | 1.6                               |
| 4             | 1.7 \(\times\) 10\(^4\)  | 5.4 \(\times\) 10\(^3\)                  | 3.1                               |
| 5             | 1.0 \(\times\) 10\(^4\)  | 8.0 \(\times\) 10\(^3\)                  | 2.2                               |
| 6             | 1.5 \(\times\) 10\(^4\)  | 8.0 \(\times\) 10\(^3\)                  | 2.1                               |
| 7             | 1.7 \(\times\) 10\(^4\)  | 8.1 \(\times\) 10\(^3\)                  | 1.1                               |
| 8             | 1.2 \(\times\) 10\(^4\)  | 7.0 \(\times\) 10\(^3\)                  | 2.1                               |

* Using 30-s collection periods with approximately 40 min between samples.
* Exposure periods.
DISCUSSION

The radionuclide \(^{99m}\text{Tc}\) has been widely used in clinical medicine because its short half-life of 6 h and low-energy gamma emission allow the administration of comparatively large doses of radioactivity, thus facilitating external counting. Furthermore, the high reactivity of the pertechnetate ion has permitted labeling of a variety of carrier molecules including albumin, macroalbumin, and sulfur colloids (2). The present study demonstrates that the pneumococcus may be readily labeled with \(^{99m}\text{Tc}\) and that insignificant amounts of \(^{99m}\text{Tc}\) dissociate from labeled organisms for at least 6 h.

For some applications it may be desirable to employ viable radioactively labeled bacteria (4). However, if the labeled and unlabeled particles are uniformly mixed and are aerodynamically similar, this is not required for most uses. The technique of \(^{99m}\text{Tc}\) labeling employed in the present study did not alter the aerodynamic properties of the pneumococcus, and the similar ratios of radioactive counts to viable bacterial counts obtained in serial impinger samples indicate uniform mixing of the two in the aerosol.

Accumulation of \(^{99m}\text{Tc}\) in the liver, as an index of clearance into the systemic circulation, did not occur, suggesting that decreasing radioactive counts in the lungs with time reflect physical removal via the airways, or the rate of mucociliary clearance. We cannot be certain that killed \(^{99m}\text{Tc}\)-labeled pneumococci are removed by mucociliary action similar to viable pneumococci, but this constraint also applies to bacteria labeled with other isotopes. Mucociliary clearance removed 40% of \(^{99m}\text{Tc}\) counts from the lungs in 4 h in the present study. A similar rate of mucociliary clearance was reported in guinea pigs by Rylander using \(^{35}\text{S}\)-labeled bacteria (8). Studies in mice with \(^{35}\text{P}\)-labeled bacteria have shown somewhat slower rates of clearance (3). \(^{99m}\text{Tc}\) is a useful isotope for bacterial labeling in studies of lung clearance. Specimen preparation is minimized, and counts obtained can be easily corrected for physical decay. The short half-life of the isotope and its low-energy gamma emission provide greater safety for personnel.

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