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The lung deposition of salbutamol, directly labelled with technetium-99m, delivered by pressurised metered dose and dry powder inhalers

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Summary

A method is described for the radiolabelling of the β_2-agonist, salbutamol, with the radionuclide ^{99m}Tc. The technique was used to prepare metered-dose inhalers and dry powder inhalers for inhalation by six normal subjects and the deposition of drug within the lungs was measured. In vitro data are presented from studies using an Andersen cascade impactor which show that salbutamol and ^{99m}Tc in the aerosol discharged by the metered dose inhaler, or drawn through the instrument from a dry powder inhaler, have a closely matched particle size distribution. Data from inhalers containing unlabelled salbutamol showed that the addition of the radiolabel had not significantly altered its distribution. Using a dual headed gamma camera (Siemens Rota Camera), we studied six normal volunteers and measured a mean (±SD) lung deposition of 11.3 (2.2)% of the dose discharged from a dry powder inhaler and 24.1 (8.5)% from the metered dose inhaler. The deposition values from the metered dose inhaler are considerably greater than those observed using indirect labelling techniques.

Introduction

Radiolabelling has been an important technique for obtaining information concerning the deposition of bronchodilator aerosols in the lungs. Until recently most data have been obtained from indirect labelling techniques because of the inability to attach a gamma-emitting radionuclide to the drug itself. An inert substance, usually teflon or polystyrene, with size characteristics similar to the drug in question is labelled and used either as a substitute for the drug itself (Newman et al., 1981), or mixed in with the drug in reconstituted metered dose inhalers (MDI) (Zainudin et al., 1989). The latter technique had the advantage of allowing bronchodilator responses to be measured at the same time as the distribution pattern of the radiolabelled particles. However, these techniques made the basic as-

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sumption that the radiolabelled carrier and the unlabelled drug particles have similar physical properties and distribution when inhaled into the thorax.

Until recently, there has been scanty information on deposition patterns of directly labelled drug deposition. Short et al. (1981) radiolabelled ipratropium bromide with Bromine-77, and a new method for directly labelling β_2-agonists has been recently described (Köhler et al., 1988). A similar technique to Köhler has been applied by Newman et al (1989) to sodium cromoglycate and to salbutamol (Newman et al., 1991) using ^{99m}Tc. We describe a modification of Köhler's technique for the preparation of an MDI and a dry powder inhaler (DPI) containing salbutamol that is directly labelled with ^{99m}Tc.

The technique, and results of validation, are presented here including data on the deposition within the thorax in six normal subjects.

Materials and Methods

Labelling of salbutamol in a metered dose inhaler

On each study day, three canisters containing labelled salbutamol and propellants were prepared. The radionuclide ^{99m}Tc was present as the sodium salt of the pertechnetate ion (TcO_4^-).

First, a small volume of sodium pertechnetate, eluted from a molybdenum-^{99m}Tc generator and containing 11 000 Mbq of radioactivity, was placed into a 20 ml separating funnel together with 4 ml of sterile water and 5 ml of butanone. Because of the high activity involved, large amounts of shielding were required.

The funnel was shaken for 3 min so that the contents were well mixed. During this process approx. 60% of the $^{99m}TcO_4^-$ was transferred to the butanone phase. After allowing the two phases to separate, the lower aqueous phase was discarded and the organic phase collected into a 25 ml screw top glass vial.

The open vial was then placed on a hot plate at 100°C and evaporated to dryness, assisted by a stream of warm air, leaving the $^{99m}TcO_4^-$ dried onto the base of the vial. After cooling, a mixture of surfactant (oleic acid) and trichlorofluoromethane (propellant 11) was added to the vial together with micronised salbutamol. The lid was firmly screwed onto the glass vial which was then placed in an ultrasonic bath for 20 min. During this process the TcO_4^- was adsorbed on to the salbutamol. Further propellant 11 was added to ensure that the correct weight of suspension was achieved.

The suspension was then transferred to a 250 ml separating funnel before being carefully weighed into empty MDI cans. Liquid dichlorodifluoromethane (propellant 12) was dispensed from a vacuum flask into the canisters. Finally a metering valve was crimped on to each canister, which was then placed in the ultrasonic bath for 5 min before use.

Labelling of salbutamol as a dry powder

For use as a dry powder, salbutamol was in the sulphate form, and for this delivery method surfactant was not required. The salbutamol sulphate was mixed with propellant 11 in a glass vial containing dried $^{99m}TcO_4^-$ prepared as above. This was placed in the ultrasonic bath for 20 min as before. The propellant 11 was evaporated to dryness by passing warm air over the vial. The dried labelled salbutamol sulphate was then recovered from the vial and a carefully weighed amount was blended with lactose carrier and then dispensed into unit doses, each containing 200 μg of salbutamol.

In vitro validation

Initially, the association of radionuclide and salbutamol was evaluated by taking a sample of the radiolabelled micronised salbutamol in suspension, and placing it into two test-tubes. After centrifuging at 2000 rpm for 20 min, the salbutamol visibly creamed to the surface leaving the clear propellant in the body of the test tube. Each test tube was then imaged close to the collimator of a gamma camera (Siemens Rota camera). Three regions of interest were drawn over the acquired image of the test-tube dividing it into top, middle and bottom.

In a separate experiment no salbutamol was added to the preparation, but every other step in the process was the same as before. The propel-

lant 11/oleic acid solution was transferred to two test tubes and centrifuged again at 2000 rpm. for 20 min. They were then imaged and the activity measured.

Aerodynamic particle size distribution of radiolabelle salbutamol

Metered dose inhalers

To evaluate how closely the radionuclide and the salbutamol particles follow each other within a range of aerodynamic particle sizes, the aerosols generated by 30 actuations from each of four MDIs, containing labelled salbutamol, were sampled into an Andersen MkII cascade impactor. The Andersen cascade impactor is an eight stage instrument modelled to the particle collecting characteristics of the human respiratory tract. Both solid and liquid airborne particles are classified according to their aerodynamic particle size by the amount of radioactivity and salbutamol measured at each of the calibrated stages. Air was drawn through the device at a standardised flow rate of 28.3 l/min measured at the inlet to the impactor. In addition to the radiolabelled inhalers, the aerosol generated by 30 actuations from each of four MDIs containing unlabelled salbutamol were sampled into the Andersen impactor.

After dismantling the instrument, the throat, actuator, filter and the eight stages were individually washed with methanol to dissolve and remove the salbutamol, which was collected into separate samples. The number of radioactive counts in each sample was measured with the gamma camera. This process was performed for each of the four canisters containing labelled salbutamol.

The amount of salbutamol in each set of samples from each of the eight MDIs was determined using ultraviolet spectrophotometry at a wavelength of 246 nm. For the radioactive samples, measurement was performed several days later to allow the radioactivity to decay, and to eliminate any potential hazard during handling of the samples.

Dry powder inhalers

The radiolabelled contents of unit doses from three DPIs were drawn into the Andersen cascade impactor at a flow rate of 60 l/min measured at the inlet of the impactor. The configuration was different to that used for the MDIs and included a pre-separator stage for collecting the large particles. The particle size cut-off for each stage of the impactor was recalculated using Eqn 1 (Hinds 1982), rearranged to give Eqn 2. It is assumed that the Stokes number for each stage of the impactor is constant for the two different flow rates.

$$\mathrm{Stk} = \frac{\rho_p d_p^2 U C_c}{9\mu D_j} \tag{1}$$

where Stk represents the Stokes number, ρ_p the particle density, d_p the particle diameter, U the jet velocity, C_c the slip correction factor, μ the viscosity of air an D_j the jet diameter.

$$d_2 = \sqrt{\frac{d_1^2 U_1}{U_2}} \tag{2}$$

where d_1 is the cut off diameter at the original velocity U_1 an d_2 denotes the corresponding value for the stage at velocity U_2.

The radionuclide content of each stage was measured with the gamma camera in a similar fashion to the MDI samples, and the drug content was measured using high-pressure liquid chromatography (HPLC). The contents of three inhalers containing unlabelled salbutamol were also sampled by the impactor and the drug deposited at each stage similarly measured.

Quality control of MDI and DPI

A Twin Impinger apparatus (Apparatus A, Appendix XVII C, p. 204; British Pharmacopoeia, 1988) in which the proportion of small droplets/particles with an aerodynamic diameter of 6.4 μm has a 50% probability of progressing and depositing in the second stage, has been used throughout the studies to ensure that the technetium labelled inhalers prepared on each study day were consistent in their performance.

Administration of salbutamol to normal volunteers

To evaluate the effectiveness of the labelling method, six normal volunteers were studied on 2

separate days at least 1 week apart. Each subject gave informed written consent and the study was approved by the local ethics committee. The subjects were considered normal if their FEV_1 and FVC were > 80% predicted for their age, sex and height, and the values did not increase by more than 10% after receiving 200 μg of salbutamol from a commercial MDI.

Each subject inhaled from the two different types of inhaler and in addition underwent a ^{81m}Kr scan to provide a clear outline of their lungs. When using the MDI, subjects inhaled 200 μg of salbutamol containing 6–18 Mbq as two actuations, each containing 100 μg. For the DPI study, 200 μg of salbutamol was inhaled from a single unit dose containing 6–18 Mbq of radioactivity.

Each subject was given simple instructions on how to use the inhaler, and allowed some practice runs with placebo inhalers. For the MDI, subjects inhaled steadily and deeply from residual volume actuating the device soon after starting the manoeuvre, followed by a 10 s breath hold at the end of inspiration before gently exhaling into a collecting bag. For the DPI, subjects inhaled from residual volume as quickly and as deeply as

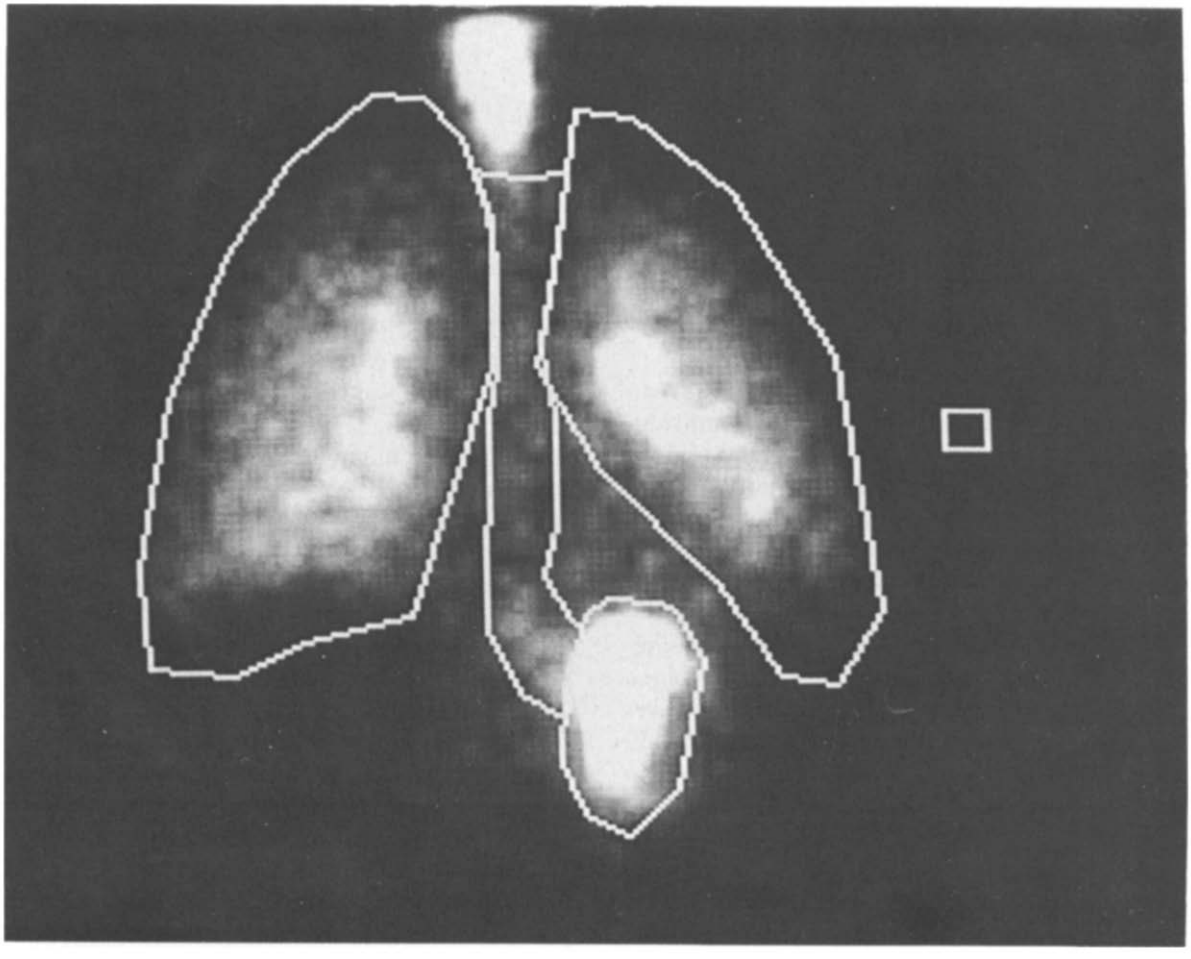

Fig. 1. Gamma camera view showing a typical deposition pattern of the anterior chest after inhalation by a normal subject from a dry powder inhaler. Regions of interest have been drawn around the lungs, mediastinum and stomach.

possible followed by a 10 s breath hold before gently exhaling into a collecting bag, and then repeated the manoeuvre.

Image collection by the gamma camera commenced within 2 min of administration and continued for 5 min.

Imaging procedures

All radionuclide images were acquired using a Siemens Dual headed Rota Camera on line to a DPS-3300 Nuclear Medicine Computer System (ADAC Laboratories). This system is able to acquire simultaneous anterior and posterior views. Each subject was seated between the heads of the camera with the mid point of their chest at the centre of the field of view.

Data collection was split into five 60-s time frames so that movement of the label could be followed as well as measuring the initial deposition. Then, the subject was repositioned and the throat and stomach imaged separately for 120 s each.

All data analysis was carried out with the DPS-3300 computer system. Regions of interest (ROI) were drawn on the lung image using a light pen (Fig. 1), the krypton image acting as a template to provide the lung outline. The anterior and posterior nett counts obtained for each region were multiplied together and their square root taken to give the geometric mean (GM) counts. This was then corrected for radioactive decay and attenuation within the body. The potential activity delivered from two actuations of each MDI used was calculated by actuating the MDI five times into a bag and measuring the count rate. This result was then divided by a factor of 2.5 to give the activity for two actuations. For the DPI, the activity was measured by counting the single dry powder unit dose before administration.

Attenuation correction

Gamma-ray photons emitted by the ^{99m}Tc located within the body are attenuated by body tissues before reaching the gamma camera detectors. This means that the counts detected are less than would be observed if the source were in air alone. A method to correct for this attenuation using gamma-ray transmission, and based on the principles outlined by Tothill and Galt (1971) for quantitative profile scanning, has been adopted.

A source of known radioactivity was sandwiched between different thicknesses of tissue equivalent perspex and between the heads of the camera. The geometric mean (GM) of the counts obtained is not dependent on the depth of the

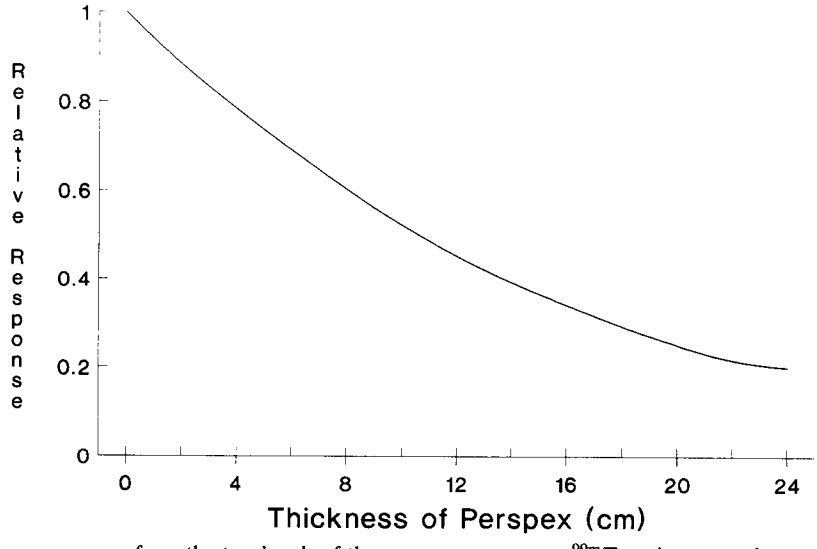

Fig. 2. The geometric mean response from the two heads of the gamma camera to a ^{99m}Tc point source between layers of perspex, expressed relative to the same source in air.

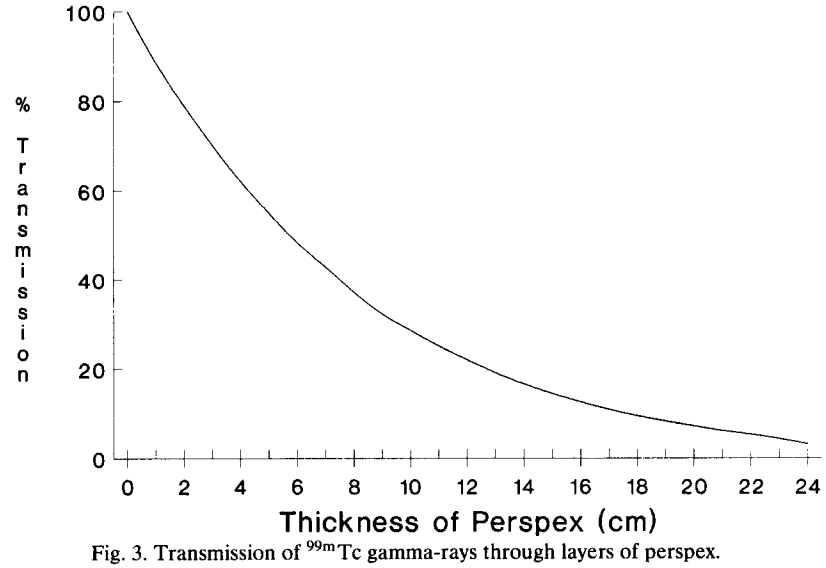

Fig. 3. Transmission of ^{99m}Tc gamma-rays through layers of perspex.

source within the perspex but only on the thickness of perspex that lies between the two heads (Tothill and Galt, 1971). The GM counts for several thicknesses of perspex were obtained and then divided by the GM counts gathered when the source was in air alone to give relative response factors for each thickness of perspex. The relative response factors were then plotted as a function of perspex thickness (Fig. 2).

For ^{99m}Tc deposited in the abdomen or pelvis these factors are very similar to the actual attenuation of the tissue overlying the source and can be directly applied to correct for the attenuation by measuring the patient with callipers. However,

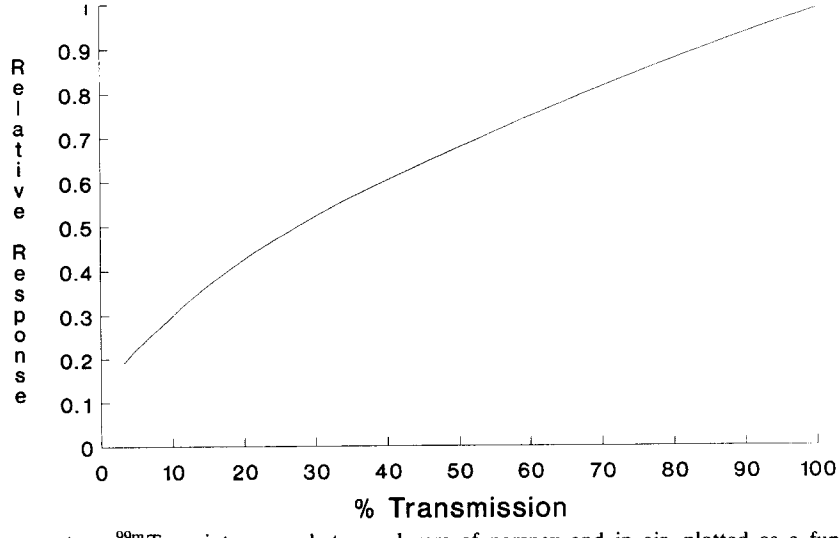

Fig. 4. Relative response to a ^{99m}Tc point source between layers of perspex and in air, plotted as a function of percentage gamma-ray transmission through perspex.

the thorax has a much lower and less uniform density and these factors over-estimate the amount of ^{99m}Tc within the lungs. Therefore, an effective tissue thickness for the throat and thorax must be obtained.

To do this the percentage of gamma-ray photons transmitted through known thicknesses of perspex was determined. Different thicknesses of perspex were placed between a flood source of ^{99m}Tc and one of the heads of the Rota camera. The percentage transmission for each thickness of perspex was obtained by comparing the counts acquired for a given thickness with those obtained when no perspex was present. The percentage transmission was plotted as a function of perspex thickness (Fig. 3). The two plots were then combined to obtain a third plot of relative response as a function of percentage transmission (Fig. 4).

A transmission image of the chest, throat and abdomen of each subject was obtained using the 99m-technetium flood source, with the patient seated between the source and one of the imaging heads. Regions of interest were drawn over the lungs, throat and stomach, and counted. The image was compared to the 'in air image' of the flood source alone to derive the percentage transmission for each region. This was then used to obtain the modified relative response factors to correct for attenuation in each separate region. The attenuation correction factors (inverse of the relative response factors) for the lung of each individual tested is summarised in Table 1.

TABLE 1

Attenuation factors (inverse of relative response factors) used to correct for the attenuation in body tissues in the thorax of the six subjects studied

Subject	Attenuation factor
1	1.90
2	2.03
3	1.66
4	1.88
5	1.92
6	2.10

In order to assess the accuracy of this method, a phantom (Alderson Research Labs Inc.) of the upper torso was used. Water was placed in the chest/head compartment and a transmission scan was obtained. Small amounts of activity comparable with that expected in the lungs during a study were counted first in air and then in the lung compartment of the phantom. Relative response factors were derived as explained above. A mean

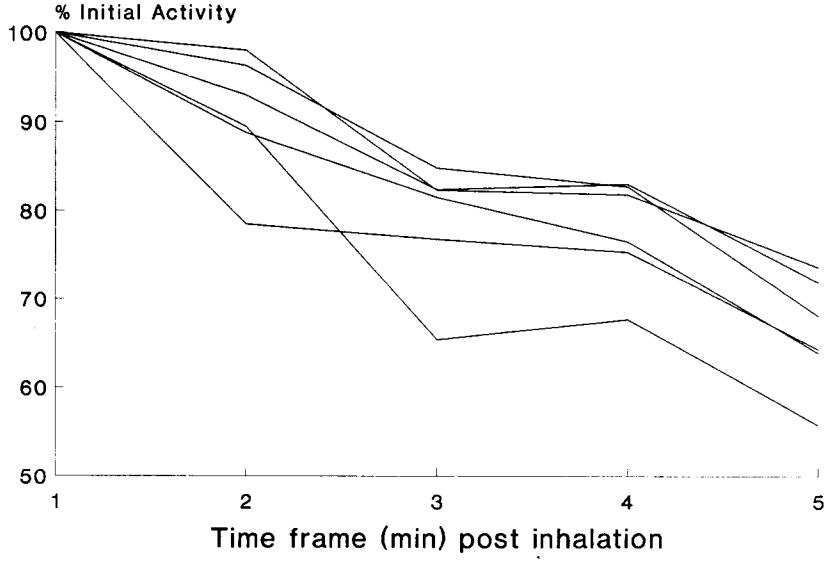

Fig. 5. The dissolution of ^{99m}Tc from the lungs of six normal subjects after inhalation from an MDI.

discrepancy between the in air counts and the corrected phantom counts of 3% (range −1.3% to 10%) was obtained.

Dissolution of radiolabel

The lung counts in each successive one minute time frame after inhalation progressively decreased. There was a substantial decrease during the 5 min of counting (i.e., 2–7 min after inhalation) of the radio-aerosol. Some subjects were imaged over a longer period in order to follow this further. The half-life of clearance of lung radioactivity was about 10 min. This was compatible with that observed when $Na^{99m}TcO_4^-$ alone is absorbed from the lungs (Rinderknecht et al., 1980), and suggests that the pertechnetate dissolves in the lung mucosa after deposition, with subsequent systemic absorption and distribution in the blood. Fig. 5 shows the dissolution effect of the ^{99m}Tc from the lungs in the six normal subjects.

Results

Distribution of activity in labelled drug suspension

Fig. 6a shows the gamma camera image of the radiolabelled salbutamol suspension after centrifugation. Over 90% of counts were in the top region of interest. Visual inspection of the test tube showed that most of the salbutamol was in a layer on the surface of the propellant. Repeating the experiment for six further preparations of suspension gave a mean (±SE) of 85 (3.2)% of the radioactivity found in the top layer of the test tubes.

When the same process was performed without salbutamol, the distribution of the radioactivity was quite different (Fig. 6b), with most of the activity in the bottom of the test tube. In addition, only 12% of the $^{99m}TcO_4^-$ that was available in the glass vial during the preparation was transferred to the propellant/surfactant alone, which was in contrast to more than 80% that was transferred when salbutamol was present. This suggests that the radionuclide binds mainly to salbutamol, and not to propellant nor surfactant.

Results from Andersen impactor

Figs. 7a and b shows the relative percentage of radioactive counts and salbutamol content of each of the stages of the Andersen impactor expressed as a percentage of the total for all the stages, for the MDI and DPI respectively. The actuator and throat counts were not included. These results show a close similarity between the radioactivity and the drug dose for each stage, and indicate that the two are closely associated over a wide range of particle sizes. The close association between the unlabelled and labelled drug indicated that the particle size distribution had not been significantly altered by the addition of the ra-

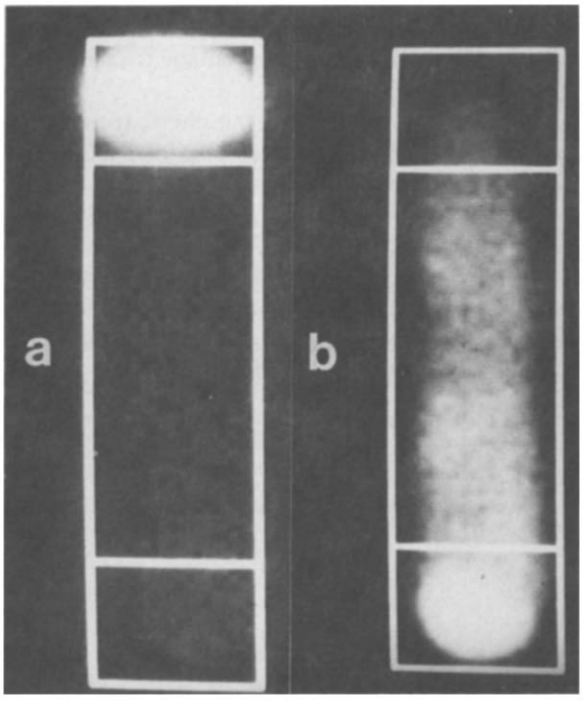

Fig. 6. (a) Gamma camera image showing a sample of salbutamol labelled with ^{99m}Tc suspended in propellant 11 and oleic acid in a test tube. Regions of interest have been drawn to obtain counts in the top, middle and bottom portions. The test tube has been centrifuged at 2000 rpm. for 20 min. The majority of salbutamol has visibly creamed to the surface of the suspension. Over 90% of counts are seen in the top region of the test tube. (b) Gamma camera image showing a test tube containing propellant 11 and oleic acid and ^{99m}Tc. Unlike panel (a), no salbutamol has been added. Every other condition is the same. Few counts are recorded in the top region although there is higher activity at the bottom.

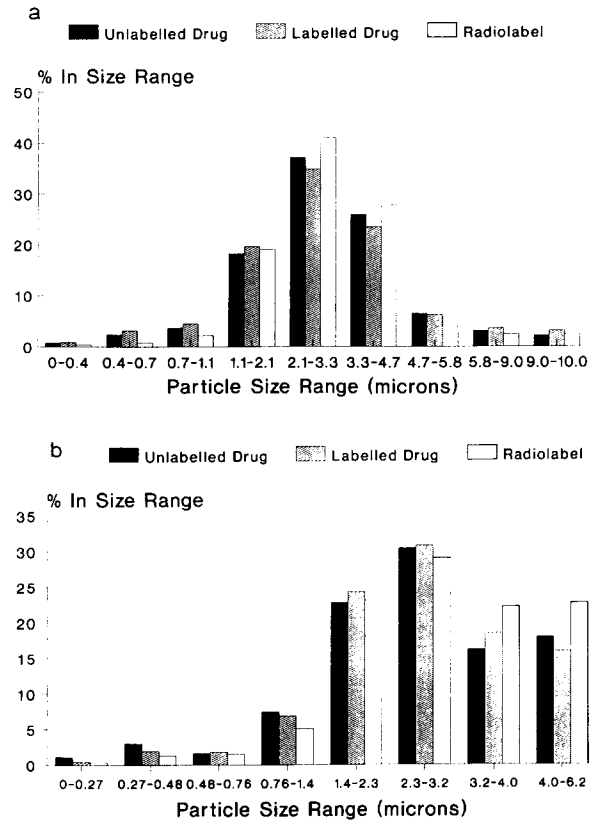

Fig. 7. (a) Mean distribution of radiolabel and salbutamol for four MDIs actuated into an Anderson MkII cascade impactor (operated at 28.3 l/min), shown as a percentage of the total recovery for eight stages plus filter. Also shown is the mean percentage drug distribution for four MDIs containing unlabelled salbutamol. (b) Mean distribution of radiolabel and salbutamol from the contents of three DPIs sampled by an Anderson MkII cascade impactor (operated at 60 l/min), shown as a percentage of the total recovery for seven stages plus filter. Also shown is the mean percentage drug distribution for three DPIs containing unlabelled salbutamol.

dionuclide. For the MDIs the mass median aerodynamic diameter (MMAD) for the unlabelled and labelled drug was 2.9 (GSD 1.5) and 2.8 (1.6) μm, respectively, while the MMAD for the radionuclide was 2.8 (1.5) μm. For the DPIs, the MMAD for the unlabelled drug was 2.7 (1.6) μm, whilst the MMADs for the labelled drug and radionuclide were 2.7 (1.5) and 3.0 (1.5) μm, respectively.

Lung deposition in normal volunteers

The results obtained from the six volunteers studied are listed in Table 2 and an example of a lung image is shown in Fig. 1. Mean ($\pm$SD) deposition of radionuclide in the lungs using the MDI was 24.1 (8.5) and 11.3 (2.2)% for the DPI, and the difference between the two delivery devices was statistically significant (paired t-test, $P < 0.05$). The small difference between the deposition in the throat, stomach and mediastinum for the two techniques was not significant. After using the MDI, the mean dose of radioactivity not accounted for was 14.1% of the total administered, and 17.5% for the DPI.

Discussion

This study has modified an original method of directly labelling β_2-agonists (Köhler et al., 1988), in which commercial MDIs were used. After cooling to -60°C, they pierced the canisters and added further propellant and surface active agent, previously labelled with radionuclide. The radionuclide was reported to transfer to the bronchodilator drug within the canister.

In our present study, the MDI is prepared from its basic constituents so as to produce a canister as close as possible in configuration and performance to the commercially available product. Also, the method of labelling employed for the MDI has been developed into a novel technique of labelling salbutamol sulphate for use in dry powder inhalers.

The validation tests show a close association between the properties of the radionuclide and the drug. The percentages of radionuclide and drug on each stage of the Andersen impactor were similar, although for the DPI the particle size distribution of the radionuclide was slightly coarser than that observed from the drug. Particles of the size reported are suitable for inhalation to all parts of the lungs (Morrow, 1974; Newman and Clarke, 1983).

The absorption of the radiolabel from the lungs into the systemic circulation limited the counting period for the lungs to 5 min; 7 min after inhala-

TABLE 2

Percentage deposition of radiolabelled aerosol following inhalation by six normal volunteers from a metered dose inhaler and dry powder inhaler; less than 1% was exhaled in each study

Subject	MDI			DPI		
	Lungs	Throat/media-stinum/stomach	Actuator	Lungs	Throat/media-stinum/stomach	Device
1	21.4	37.1	27.7	8.5	67.0	7.3
2	17.0	45.2	14.3	10.8	62.0	9.0
3	15.0	31.6	17.7	14.0	52.6	11.7
4	31.6	48.2	13.5	9.7	63.7	13.4
5	37.0	46.6	15.0	10.8	52.5	28.0
6	22.6	58.1	15.4	13.8	48.0	12.1
Mean	24.1	44.5	17.3	11.3	57.6	13.6
S.D.	8.5	9.2	5.3	2.2	7.6	7.4

tion. This phenomenon was also noted by Köhler et al. (1988). The rates of clearance from the lungs of the six subjects were similar (Fig. 5). Whilst counting over the lungs was for 5 min, only the first 3 min of data were used in assessing deposition. It was not possible to reduce count time further because the low count rate within the lungs gave inadequate count statistics.

The percentage total deposition in the lungs in the normal subjects in this study is higher than those previously reported both in this laboratory using inhalers containing radiolabelled teflon spheres and by other workers. A mean of 11.2% was deposited in the lung from a reconstituted MDI and a mean of 9.1% deposited from a dry powder inhaler device in patients with airflow obstruction (Zainudin et al., 1990). The values presented here are also higher than those obtained by groups using either a labelled substitute (Newman et al., 1981) or with directly labelled sodium cromoglycate (Newman et al., 1989). However, Matthys has recorded a mean of 26% lung deposition with directly labelled salbutamol in an MDI in normal subjects (Matthys et al., 1988). They also measured a lung deposition of 18.7% when inhaling from residual volume and 33% when inhaling from 50% of vital capacity (Köhler et al., 1988). Also, Newman et al. (1991), using directly labelled salbutamol, measured a mean total deposition of 22.8% in the lungs of asthmatic patients using their inhalers in the correct fashion. Workers using metered dose inhalers containing propellant soluble drug have reported lung depositions of over 39% (Ashworth et al., 1991).

The finely milled salbutamol particles are smaller and less dense than teflon particles or other drugs that have been studied. This may account for the increased lung deposition of this study compared to those reported by other workers.

The portion of the activity released from the DPI and MDI that is unaccounted for may be partially explained by the dissolution of the activity within the lungs, with a substantial portion being redistributed systemically and therefore not counted. This may imply an even higher lung deposition than has been reported. Very little is expired by the subjects after breath holding indicating that most of the particles inhaled are deposited during this manoeuvre.

The advantages of the current method are that the production of the labelled drug is relatively easy to perform and requires no special equipment. It is able to give an accurate indication of the distribution of the drug and, apart from the technetium, does not require substantial modification of inhaler formulation. Pulmonary function can be studied simultaneously enabling it to provide a simple and effective means of observing the effects of a known distribution of drug on patients suffering from respiratory disease.

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