Inhalation of Single vs Multiple Metered-Dose Bronchodilator Actuations from Reservoir Devices*  
An In Vitro Study  
Joseph L. Rau, PhD, RRT; Ruben D. Restrepo, MD, RRT; and Vijay Deshpande, MS, RRT

Differences in inhalation technique with reservoir or spacer devices may affect metered-dose inhaler (MDI) dose availability to a patient.  
Purpose: This study examined the effect of single vs multiple actuations of an MDI into reservoir devices on dose delivery of albuterol, with three clinically available reservoir brands.  
Methods: An in vitro lung model simulated inspiration from the MDI reservoir system. Albuterol (Proventil; Schering) was delivered by MDI, with the Monaghan Aerochamber, the Diemolding Healthcare Division (DHD) aerosol cloud enhancer (ACE), and the Schering InspirEase, using standardized volumes and inspiratory flows of 30 L min⁻¹. The MDI was actuated into each brand of reservoir 1, 2, or 3 times in rapid succession, followed by a single inhalation. Aerosol dose at the reservoir mouthpiece was captured on a cotton filter, dissolved in ethanol, and measured with a spectrophotometer at 278 nm.  
Results: For all three brands of reservoir, less accumulated dose of drug is delivered with multiple actuations than with multiple single actuations each followed by inhalation. The total dose in milligrams increased significantly with two multiple actuations compared with one actuation in the Aerochamber and ACE (p<0.01), but not in the InspirEase (p>0.05). The Aerochamber, ACE, and InspirEase delivered a mean total dose (SD) of 0.0264 mg (0.012), 0.0271 mg (0.007), and 0.0136 mg (0.006), respectively, with one actuation compared to 0.0485 mg (0.011), 0.0453 mg (0.013), and 0.0218 mg (0.009) with two multiple actuations. The increase in total dose with three multiple actuations was not significant compared to two actuations for any of the brands tested (p>0.05). Although total dose increased with multiple actuations, a decline in efficiency was seen with two and three multiple actuations, compared to single actuation. The dose delivered per actuation decreased for the Aerochamber, ACE, and InspirEase from 0.0264 mg (0.012), 0.0271 mg (0.007), and 0.0136 mg (0.006) with one actuation, to 0.0243 mg (0.006), 0.0226 mg (0.006), and 0.0109 mg (0.005), respectively, with two multiple actuations, for losses of 8.0%, 16.6%, and 19.9% in dose per actuation for each brand. A further decline in delivery per actuation to 0.0164 mg (0.001), 0.0184 mg (0.004), and 0.0097 mg (0.005) for the 3 brands, respectively, was found with 3 multiple actuations before inhalation. This was a loss of 37.9%, 32.1%, and 28.7% of the dose per single actuation in each brand. There was no significant difference between the Aerochamber and the ACE in dose availability with 1, 2, or 3 actuations, but both of these brands provided significantly more drug than the InspirEase.  
Conclusion: Maximal aerosol bronchodilator from an MDI reservoir was given by single actuations each followed by a breath. Two rapid actuations followed by a breath will give a significant accumulation of dose with some loss when compared to two single actuations each followed by inhalation. Three multiple actuations led to a loss of approximately one third of the drug dose obtainable with three single actuations each followed by inhalation, for all three brands.  

(CHEST 1996; 109:969-74)

ANOVA=analysis of variance; MDI=metered-dose inhaler; MSLI=multistage liquid impinger

Key words: aerosol delivery; beta agonist; bronchodilator; metered-dose inhaler (MDI); multiple actuations; reservoir; spacer

The use of metered-dose inhalers (MDIs) to deliver inhaled bronchoactive aerosols provides a convenient and highly portable method of drug delivery for individuals with pulmonary disease. Although this method of drug delivery has come to be widely used by such individuals, there can be several problems with use of MDIs. Two of the most common include lack of patient coordination in synchronizing actuation of the MDI with inspiration1,2 and a relatively large amount of oropharyngeal deposition and drug loss.3,4 The use of add-on or extension reservoir devices, commonly termed spacers, with MDIs can minimize

---

*From the Cardiopulmonary Care Sciences, Georgia State University (Dr. Rau and Mr. Deshpande), and the Pediatric ICU, Egleston Children's Hospital of Emory University (Dr. Restrepo), Atlanta.  
This study was funded by a research grant from Diemolding Healthcare Division, Canastota, NY. The authors have no financial or other interest in the products investigated.  
Manuscript received March 20, 1995; revision accepted October 6.
both of the problems seen with MDI use, while offering the possibility of increasing lung deposition. Although reservoir devices have proved beneficial in facilitating the delivery of aerosolized drugs from MDIs, there are questions concerning the effect of changes in technique with their use. Subjects who use reservoir devices with MDI aerosol delivery are usually instructed to separately inhale each actuation of the MDI. Multiple actuations of a MDI followed by inhalation from a reservoir device further facilitates ease of MDI use and may increase patient compliance with prescribed aerosol medications. Previous and current British Thoracic Society guidelines suggest multiple actuations of MDI bronchodilator into a reservoir device prior to a single inhalation during acute asthma episodes. Several studies have examined the effect of such use on dose delivery from reservoir devices. However, none of the previous studies found in our review of the literature examined the effect of multiple actuations with a single inhalation using a β-agonist and currently available, commonly used reservoir devices in the United States, such as the Aerochamber, the aerosol cloud enhancer (ACE), and the InspirEase. In addition, with the exception of a 1984 in vitro study by Newman and associates, none of the in vitro studies referenced used a lung model to simulate patient inspiration in a clinically realistic fashion.

This study examined the effect of single vs multiple actuations from an MDI on dose delivery of the adrenergic agonist albuterol, using three different commercially available reservoir devices, with simulated inhalation from a lung model.

**Materials and Methods**

**In Vitro Lung Model**

An in vitro model of patient use of the MDI spacer assembly was used to assess the amount of β-agonist drug available with single vs multiple actuations of an MDI (Fig 1). Inspiratory breathing was simulated by connecting the mouthpiece of the MDI spacer assembly to one side of a dual-chambered test lung, with a one-way Rudolph valve to route the gas inspired from the reservoir out to the room on exhalation. The other side of the test lung was inflated by a ventilator (Nellcor-Puritan Bennett Corp MA-2, Pleasanton, Calif), and the two test lung chambers were connected by a bar, thereby creating an active inspiration from the lung connected to the reservoir mouthpiece.

**Study Design**

Aerosolized albuterol was delivered by MDI using three clinically available reservoir devices: the ACE (Diemolding Healthcare Division, Canastota, NY), the Aerochamber (Monaghan Medical Corp, Plattsburgh, NY), and the InspirEase (Schering Corp, Kenilworth, NJ). The ACE has a volume of approximately 175 mL, the Aerochamber 130 mL, and the InspirEase 650 mL. Both the ACE and Aerochamber contain a one-way valve between the reservoir chamber and the mouthpiece. The InspirEase does not contain a valve, but it has a mesh assembly in the mouthpiece. Assembly and use of the reservoir devices with the MDI and simulated inspirations followed manufacturer’s guidelines. For the ACE and the Aerochamber reservoirs, the inspired volume was approximately 1,500 mL, at 30 L min⁻¹, measured at ambient temperature and pressure, with normal room humidity. For the InspirEase, an inspired volume of approximately 700 mL was used, as limited by the size of the collapsible reservoir bag, at the same 30 L min⁻¹ flow rate. All one-way valves or other structures in the reservoir mouthpiece were retained during testing. All inspiratory volumes and flows generated by the test lung were determined by means of a 13.5-L spirometer (Collins) connected directly to the test lung chamber that simulated inspiration.

Full canisters of albuterol (Proventil; Schering) providing a nominal dose of 90 μg per actuation were used, with the same canister assigned to each set of three sample reservoirs from the three brands tested to minimize dose variability. Each new canister was shaken to mix contents, and then four actuations wasted before the any drug measurement was made. If a canister was not used for more than 4 h prior to a dose measurement, one actuation was also wasted to prime the metering valve. In a series of previous trials to develop the method for this investigation, we examined the effect of interval times for MDI actuation on drug availability.
from the canister. A series of six canisters was discharged twice, at intervals of 2, 3, 5, 10, 15, 20, 25, and 30 s, with the dose delivered measured as described below for this study. There was no difference among the intervals when tested with a randomized block, repeated measures analysis of variance (ANOVA) (p=0.1912).13 Based on these results, we used a 2-s pause between multiple actuations of the MDI, because longer time intervals were noted to reduce drug delivery from the reservoir in our pilot measures. A single, double, and triple MDI actuation was used, followed by a single inhalation. Simulated inspirations occurred approximately 1 s after the last actuation of the MDI using a manual count, and were the same for all trials.

A sample of six devices of each brand of reservoir was tested. Each sample reservoir was tested with 1, 2, and 3 actuations per inspiration. The number of actuations per inspiration, whether 1, 2, or 3, was randomized to eliminate an order effect on dose delivery. The order in which samples of each brand of reservoir were tested was also rotated. A total of 18 MDI spacer assemblies were tested. Temperature and humidity were measured at the time of all test trials and recorded.

**Measurement**

Cotton wadding of approximately 0.23 g was placed at the reservoir mouthpiece to capture aerosolized drug, using a specially designed filter holder attached to the reservoir mouthpiece. The quantity of available drug was determined using a spectrophotometer (Beckman DU 640; Beckman Instruments Inc; Fullerton, Calif). A standard solution of the drug having a concentration of 0.0502 mg/mL was prepared by dissolving albuterol base (Sigma Chemical Co; St Louis, Mo) in ethanol. The absorbance of this solution was measured at 278 nm, a characteristic absorbance peak from which unknown concentrations of the drug were calculated. Following MDI actuation and simulated inspiration by the test lung, the cotton filter containing trapped drug particles was removed from its adapter using tweezers, and placed in a 28-mL glass vial. Eight milliliters of ethanol was added by pipette to the vial, the cotton was thoroughly immersed in the solvent using a glass stirring rod, and the vial was covered. After 1 min of agitation, the resulting solution of drug was drawn out of the vial using a glass syringe. A 0.2-μm nylon syringe filter (Acrodisc; Gelman Sciences; Ann Arbor, Mich) was used to remove any cotton particulate matter as the solution was expressed into a 10-mm UV spectrophotometer cell. The absorbance of each sample solution was analyzed at 278 nm and the concentration of drug was calculated from the absorbance of the standard solution. Prior to each measure of drug solution, the spectrophotometer was calibrated to zero using solvent alone. All sample measurements were corrected by the average baseline absorbance measured from a sample of cotton filters, with no drug present. A second cotton filter placed in series to test for efficiency of drug capture during preliminary measurements revealed no loss of aerosol.

**Data Analysis**

Descriptive statistics (means, SDs, ranges) for each condition of multiple actuation and each of the three brands of devices tested were used to report the amount of drug delivered at the mouthpiece of the spacer device. Differences among the types of device and among the various multiple actuations employed were tested using a split-plot repeated measures ANOVA, with follow-up comparisons using Scheffé's ratio for pairwise comparisons among brands and numbers of multiple actuations.13 A significance level of 0.05 was used for the ANOVA and follow-up comparisons.

**RESULTS**

For all devices tested, ambient temperature averaged 19.9°C with an SD of 0.21, and a range of 19.6 to 20.1°C. Relative humidity was 62.4%, with an SD of 6.42% and a range of 54 to 69.7%.

Table 1 summarizes the total doses measured at the reservoir mouthpiece for all three brands of reservoir, with each of the single and multiple actuation techniques. There were significant differences overall across brands of reservoir (p=0.001) and across numbers of actuations (p<0.001) with respect to dose availability using a split-plot repeated measures ANOVA. Both the Aerocamber and the ACE reservoir provided significantly more aerosol drug at the mouthpiece than the InspireEase, with 1, 2, or 3 actuations per inhalation (p<0.01) using a Scheffé ratio for pairwise comparisons between brands. There was no significant difference between doses provided by the Aerocamber and the ACE reservoir, the inhalation of two actuations with a single breath gave significantly more drug than one actuation, when compared using the Scheffé ratio (p<0.01). The increase in dose with two actuations compared to one was not significant for the InspireEase (p>0.05). The actuation of three metered doses into the reservoir did not significantly increase the amount of available drug with any of the three brands tested, over that seen with two actuations, using the Scheffé ratio test (p>0.05).

Figure 2 indicates the efficiency of multiple actuations, expressing the dose per actuation in milligrams for 1, 2, and 3 multiple actuations. The dose per actuation with two actuations is similar for the Aerocamber and the ACE, and less than with one actuation followed by inhalation. The dose per actuation declines further with three actuations for all three brands. The dose per actuation declines almost linearly with the ACE and InspireEase, using 1, 2, and 3 multiple actuations.

**Table 1—Comparison of Aerocamber, ACE, and InspireEase for MDI Albuterol Dose Delivery for Single, Double, and Triple Actuations Followed by a Single Inhalation**

<table>
<thead>
<tr>
<th>No. of Actuations</th>
<th>Mean, mg</th>
<th>SD, mg</th>
<th>Range, mg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aerocamber</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.0254</td>
<td>0.012</td>
<td>0.0115-0.0424</td>
</tr>
<tr>
<td>2</td>
<td>0.0485</td>
<td>0.011</td>
<td>0.0257-0.0595</td>
</tr>
<tr>
<td>3</td>
<td>0.0491</td>
<td>0.003</td>
<td>0.0455-0.0526</td>
</tr>
<tr>
<td><strong>ACE</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.0271</td>
<td>0.007</td>
<td>0.0166-0.0356</td>
</tr>
<tr>
<td>2</td>
<td>0.0453</td>
<td>0.013</td>
<td>0.0305-0.0670</td>
</tr>
<tr>
<td>3</td>
<td>0.0551</td>
<td>0.013</td>
<td>0.0402-0.0714</td>
</tr>
<tr>
<td><strong>InspireEase</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.0136</td>
<td>0.006</td>
<td>0.0024-0.0207</td>
</tr>
<tr>
<td>2</td>
<td>0.0218</td>
<td>0.009</td>
<td>0.0053-0.0343</td>
</tr>
<tr>
<td>3</td>
<td>0.0290</td>
<td>0.014</td>
<td>0.0147-0.0526</td>
</tr>
</tbody>
</table>

*Data show total dose in milligrams measured at the reservoir mouthpiece. N=6 for each brand.*
assessments. Table 2 indicates the loss in efficiency of dose delivery with double and triple multiple actuations, giving the dose per actuation for each delivery method as a percent of the dose obtained with one actuation followed by inhalation.

**Discussion**

Although the availability and use of reservoir devices offers the possibility of simplifying MDI use for subjects as far as hand-breathing coordination is concerned, the use of such devices also introduces further possible permutations in the aerosol delivery system. One such variation is the introduction of multiple actuations into a reservoir device, followed by a single inhalation from the device, to obtain the dose to the lung. Our investigation of this technique found that the use of two or three MDI actuations of albuterol followed by a single inspiration does increase the cumulative amount of drug available from the mouthpiece of the reservoir device. However, for all three brands tested, there is a decrease in relative efficiency. Efficiency for multiple actuations is defined herein as a measure of available dose per individual actuation. As both Figure 2 and Table 2 indicate, the amount of drug available from the reservoir system calculated as a dose per actuation declines for all three brands with the double and triple actuation techniques. For example, in our data, the cumulative dose of 0.0485 mg with the two-actuation technique using the Aerochamber would have an efficiency of 0.0243 mg per individual actuation of the MDI. This is less than the 0.0264-mg dose delivery with a single actuation followed by inspiration (92% of the 0.0243-mg dose in Table 2). The decrease in efficiency with three actuations followed by a single inhalation was greater than that seen with the two-actuation technique in all three brands of reservoir tested. Using the Aerochamber as an example, the three-actuation technique decreased the dose per actuation to 0.0164 mg, which is 62% of the dose of 0.0264 mg with a single actuation (Table 2). Where three single actuations, each inhaled from the Aerochamber, would be estimated to provide a total dose of 0.0792 mg, the triple actuation technique followed by inhalation would give only 0.0491 mg on average, a 38% loss in total delivered drug.

Clearly, a single MDI actuation followed by inhalation delivers the greatest amount of drug on a per actuation basis when compared with double or triple actuation in the protocol described in our study. Although a double actuation technique did show a statistically significant increase in cumulative total dose compared with a single actuation for the Aerochamber and ACE, the triple actuation technique did not significantly increase the total dose over the double actuation technique for any of the three brands. The clinical significance of these differences in total dose remains to be investigated. If patients used the same protocol of actuation and inhalation described herein, would the percent loss with either the double or triple actuation technique be clinically discernible or significant? An *in vitro* study could also examine lung distribution of the aerosol drug, with multiple actuations, thereby giving information on the effect of this technique on particle size. The present study collected all

![Figure 2. The dose in milligrams per actuation for all three brands of reservoir, for single-, double-, and triple-metered-dose actuations. Mean values with SE bars are shown. Aero=Aerochamber; Inspir=InspirEase.](image-url)
of the aerosol inline as a closed system between the reservoir mouthpiece and the test lung during simulated inspiration, measuring the total amount of drug rather than particle size distribution. The use of the Bennett MA-2 in our study, which produces a constant, or square wave, flow pattern, may also differ from a sinusoidal or variable inspiratory flow pattern seen in vivo. The effect of differing inspiratory flow patterns on dose delivery with multiple actuations remains to be investigated. It also remains to be determined if similar results in delivery efficiency with multiple actuations would be found with MDI drugs other than albuterol, such as ipratropium bromide, corticosteroids, or the asthma prophylactics, cromolyn sodium and nedocromil sodium.

The InspirEase delivered less drug with both single and multiple MDI actuations than the Aerocahamber or ACE. The cause of this was not clear. The InspirEase has the largest reservoir volume (650 mL) of the three brands tested. The InspirEase also has a mesh gate in the mouthpiece rather than a one-way valve, allowing rebreathing into the reservoir. We used a single inhalation from the InspirEase for all trials to allow comparison with the other two brands. However, the instructions with the InspirEase do suggest exhaling back into the reservoir and then inhaling again to obtain all of the aerosol drug. Therefore, we added two more trials using two breaths from the InspirEase following both a single and double MDI actuation. With two inhalations from the reservoir, the InspirEase delivered 0.0142 mg and 0.0266 mg for single and double MDI actuations into the reservoir, respectively. This was similar to the 0.0136-mg and 0.0218-mg dose availability averaged with single inhalations from the InspirEase during the trials. Due to its design, the InspirEase limited inspired volume to approximately 700 mL compared with the 1,500 mL used in our study with the Aerocahamber and the ACE. These findings suggest that inspired volume may also influence dose availability from a reservoir device.

Our results differ from but do not contradict other in vitro studies of dose availability with multiple actuations in a reservoir device. Barry and O’Callaghan examined the effect of multiple actuations of salbutamol using a reservoir device (Volumatic; Glaxo Labo-

ratories, U.K.), and found that 62.1 μg of a 100-μg nominal dose was available to the patient with one actuation. This is a 62% efficiency for one actuation followed by one breath. For particles less than 5 μm, the dose was slightly less, at 54.3 μg, or a 54% efficiency. This is much higher than the 29% of the nominal 90-μg dose found in our study with the Aerocahamber or the ACE, using one actuation. However, the reservoir (Volumatic) differed, and the attachment of the reservoir to a multistage liquid impinger (MSLI) for measurement of drug dose differed from our use of a test lung simulating inspiration. The technique of Bell and associates in use of the MSLI was cited by Barry and coworkers. If Barry et al. used the same flowrates as Bell et al. their method employed a 60-L min⁻¹ flow with the MDI reservoir connected to the MSLI, while our study used a 30-L min⁻¹ inspiratory flow through the reservoirs. In addition, there is no control of volume, as with an actual inspiration, such as used in our study. In the study by Barry et al., two actuations decreased drug recovery per 100 μg of actuation by 22%, and five actuations, by 62%. In another study examining delivery of nedocromil sodium with a reservoir (Fisonsair), Barry and associates found that placing two actuations into the reservoir decreased drug delivery in the respirable range of less than 5 μg by 47%. The efficiency of dose delivery per single actuation in that study was 24.9%, which is similar to our efficiency with albuterol using the Aerocahamber or the ACE. Similar results were found by O’Callaghan and others with multiple actuations of cromolyn sodium into the Fisonair reservoir (Fisons; U.K.). In that study, two actuations resulted in a 31% decrease in dose, and a 56% loss after three actuations.

A study by Clark and colleagues examined multiple actuations from an MDI of cromolyn, and with a two MDI combination delivery of cromolyn and albuterol, using an Aerocahamber and an InspirEase reservoir. There was no direct comparison of multiple actuations of cromolyn, because the one actuation technique was used with the InspirEase, and the two-actuation technique was used with the Aerocahamber. However, the combination technique, with two actuations of cromolyn sodium followed by two actuations of albuterol, with subsequent attachment to a MSLI for measurement of dose and particle size, revealed a reduction of 75% of the total dose to the “patient” with the Aerocahamber, and 80% with the InspirEase. It is probably significant that such multiple actuation combination techniques will require substantial time delays before patient inspiration can occur. The study by Clark and others reported a 5- to 10-s time delay for each multiple actuation sequence, and a delay of 1 s for extraction/delivery to the MSLI. These time delays between first and last actuations and ultimately inhalation are considerably greater than those in our study, where the

### Table 2—The Amount of Drug Available per Actuation for Two and Three Multiple Actuations, Expressed as a Percent of the Dose Obtained With a Single Actuation Followed by Inhalation

<table>
<thead>
<tr>
<th>No. of Actuations</th>
<th>Single, %</th>
<th>Double, %</th>
<th>Triple, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aerochamber</td>
<td>100 (0.0264mg)</td>
<td>92.0</td>
<td>62.1</td>
</tr>
<tr>
<td>ACE</td>
<td>100 (0.0271mg)</td>
<td>83.4</td>
<td>67.9</td>
</tr>
<tr>
<td>InspirEase</td>
<td>100 (0.0136mg)</td>
<td>80.1</td>
<td>71.3</td>
</tr>
</tbody>
</table>
interval between two actuations of the MDI was always limited to 2 s, and the breath occurred within 1 s following the last actuation. The effect of such delays was reported in the study by Barry and others\textsuperscript{10} in which a delay of 5 s resulted in a 43\% reduction in drug availability below 5 \( \mu \)m, and a delay of 20 s, in an 81\% loss. This was corroborated in the 1993 study of O’Callaghan and others\textsuperscript{11} in which a 20-s delay after one actuation into the reservoir caused a 67\% decrease in drug delivery from the device.

The results of our study and the differences in the multiple actuation studies cited are consistent with the findings on delay of inhalation following MDI actuation. These differences further emphasize how much difference in technique of use, including possibly inspiratory flow rate, can affect aerosol drug delivery with reservoir devices. Other differences may be due to design variation among the reservoirs tested in other studies as well. Our results are consistent with the in vivo study reported by Newman and associates\textsuperscript{5} in which four actuations given in rapid succession resulted in a decline of aerosol dose to the lungs from 20.9\% with one actuation, to 15.9\% per actuation.

**CONCLUSION**

Based on the results obtained in our study, we conclude that the maximal amount of aerosol albuterol from an MDI reservoir combination will be obtained using a single actuation followed by inhalation. However, the use of two actuations in rapid succession (ie, within seconds) followed by inhalation can give an increase in total dose, with some loss of efficiency. Three actuations followed by inhalation gives less increase in total dose compared to the double actuation, with greater loss in efficiency of dose per actuation. Clinical studies are needed to assess the significance of these in vitro findings for patient effect. Additional studies would be useful to further elucidate the effect of other variables such as volume and flows, type of medication, and brand differences among reservoirs on aerosol drug delivery with MDI reservoir combinations.

**REFERENCES**

2. Crompton GK. Problems patients have using pressurized aerosol inhalers. Eur J Respir Dis 1982; 63(suppl 119):101
12. Barry PW, O’Callaghan C. Multiple actuations of salbutamol MDI into a spacer device reduce the amount of drug recovered in the respirable range. Eur Respir J 1994; 7:1707-09