

# Respiratory pathophysiologic responses

## The efficacy of slow versus faster inhalation of cromolyn sodium in protecting against allergen challenge in patients with asthma

Beth L. Laube, PhD,<sup>a</sup> Alan M. Edwards, MB, BChir,<sup>b</sup> Richard N. Dalby, PhD,<sup>c</sup>  
Peter S. Creticos, MD,<sup>a</sup> and Philip S. Norman, MD<sup>a</sup>

Baltimore, Md., and Southampton, United Kingdom

**Background:** Approximately one third of patients with allergy-induced asthma who are treated with aerosolized cromolyn sodium (CS) fail to achieve a full therapeutic effect. This lack of effectiveness could involve nonhomogeneous distribution of drug in the lung as a result of high inspiratory flow rates.

**Objective:** We sought to determine the efficacy of slow versus faster inhalation of CS in protecting against allergen challenge in patients with asthma.

**Methods:** Eight patients with asthma underwent two allergen challenges 30 minutes after pretreatment with CS that was inhaled from a large holding chamber at ~30 L/min or ~70 L/min. Percent decreases in FEV<sub>1</sub> at a common dose of allergen on the two challenge days were compared. Values of skew (an indicator of aerosol distribution homogeneity) obtained from gamma camera lung images after slow and faster inhalation of radiolabeled CS were also compared.

**Results:** Mean ( $\pm$  SD) allergen-induced decrease in FEV<sub>1</sub> was 5.4%  $\pm$  4.2% after slow inspiration of CS, which was significantly less than the allergen-induced decrease in FEV<sub>1</sub> after faster inhalation of CS with 12.6%  $\pm$  11% ( $p < 0.05$ ). Mean skew values were also significantly decreased after slow inspiration of CS, and differences in decreases in allergen FEV<sub>1</sub> and skew values for the two breathing maneuvers were significantly correlated.

**Conclusion:** These data indicate that protection against allergen-induced asthma can be optimized by slowly inspiring CS from a large holding chamber compared with faster inhalation of the drug. These results appear to be related to enhanced distribution homogeneity of CS within the lungs. (*J Allergy Clin Immunol* 1998;101:475-83.)

**Key words:** Asthma, cromolyn sodium, aerosol deposition

The primary objectives in the treatment of asthma are to minimize symptoms, reduce airway inflammation, maximize airflow rates, and prevent severe exacerbations.<sup>1</sup>

### Abbreviations used

|       |                                        |
|-------|----------------------------------------|
| CS:   | Cromolyn sodium                        |
| HPLC: | High-performance liquid chromatography |
| I:O:  | Inner:outer                            |
| MDI:  | Metered-dose inhaler                   |
| MMAD: | Mass median aerodynamic diameter       |
| P:C:  | Peripheral:central                     |

Although avoidance of factors that may precipitate or aggravate the disease is recommended, medications are also generally required to achieve these goals. The administration of medications by the aerosol route is now preferred because this leads to the reversal or prevention of asthma symptoms by topical action in the lower respiratory tract. This local effect requires smaller doses than when given systemically and usually results in fewer side effects.

Cromolyn sodium (CS) is an antiinflammatory drug that suppresses airway inflammation. When given as an aerosol, it is effective in preventing or blunting symptoms in patients with mild-to-moderate allergy-induced and exercise-induced asthma. In many patients a full therapeutic effect is achieved after treatment of 1 month's duration.<sup>2</sup> However, in most studies approximately one third of patients fail to achieve a therapeutic effect. For example, in two studies with adults who were treated with 20 mg capsules of CS given by inhalation, failure to protect against asthma symptoms occurred in approximately 30% of patients,<sup>3,4</sup> and a study in children demonstrated a similar percentage of treatment failures.<sup>5</sup> In two other studies, a similar proportion of adult patients treated with 1 mg CS administered by metered-dose inhaler (MDI) were classified as nonresponders.<sup>6,7</sup>

One explanation for this lack of effectiveness in some patients may involve nonhomogeneous distribution of the drug within the lungs, resulting in inadequate delivery to potential sites of airflow limitation. Nonuniformity in drug deposition could result from incorrect breathing maneuvers, including rapid inspiration, which could lead to focal deposition in larger central airways and less deposition in the lung periphery. The likely mechanism

From <sup>a</sup>Johns Hopkins University Medical Institutions, Baltimore;

<sup>b</sup>Southampton General Hospital, Southampton; and <sup>c</sup>University of Maryland School of Pharmacy, Baltimore.

Supported in part by the National Institutes of Health Immunologic Disease Cooperative Research Center Grant 1 U01 AI3 1867-04 and by Center Grant ES-03819 from the National Institute of Environmental Health Sciences.

Received for publication July 30, 1997; accepted for publication Nov. 19, 1997.

Reprint requests: Beth L. Laube, PhD, Johns Hopkins University, School of Hygiene and Public Health, Room 2001, 615 North Wolfe Street, Baltimore, MD 21205.

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0091-6749/98 \$5.00 + 0 1/1/87711

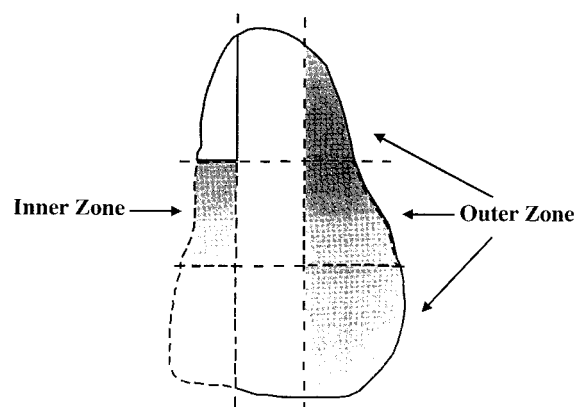


**FIG. 1.** Delineation of inner and outer zones of right lung for analysis of distribution of CS in larger central airways (*inner zone*) and lung periphery (*outer zone*).

by which this could occur is enhanced inertial impaction at higher inhalation flow rates.

Newman et al.<sup>8,9</sup> have examined the effect of varying inspiratory flow rates, in conjunction with the use of a spacer, on the fraction of CS that deposits in the lungs of normal subjects. In one study<sup>8</sup> they compared the amount of CS (1 mg/actuation) deposited in the lungs with MDI alone and a slow inspiration (~26 L/min) to that deposited with MDI attached to a Syncroner spacer and a faster inspiration (~105 L/min) or MDI attached to the spacer and a slow (~24 L/min) inspiration. They reported that the dose to the lungs was 11% with MDI alone and a slow inspiration, 13% with MDI/Syncroner and faster inhalation, and 16% with MDI/Syncroner and slow inspiration.

In a second study Newman et al.<sup>9</sup> compared the dose of CS (5 mg/actuation) deposited in the lungs with MDI alone and a slow inspiration (~28 L/min) to that deposited with MDI attached to an Inhalertube spacer and a faster inspiration (~94 L/min) or MDI attached to the spacer and a slow (~29 L/min) inspiration. They reported that the dose to the lungs was ~9% with MDI alone and a slow inspiration, 7% with MDI/Inhalertube and faster inhalation, and 11% with MDI/Inhalertube and slow inspiration. Although these findings indicated that the deposited fraction of CS within the lungs of normal subjects could be enhanced by inspiring slowly from a spacer device or holding chamber, the investigators did not examine the effect of such alterations in inspiratory flow rate and the use of a spacer on the effectiveness of CS in preventing or blunting an asthma attack.

In this study we examined the biologic consequences of varying inspiratory flow rate during the administration of aerosolized CS from a large volume holding chamber on subsequent allergen-extract inhalation challenges in patients with asthma. Thirty minutes before undergoing two allergen challenges, patients inhaled CS (1 mg/actuation) from the holding chamber during a fast (~70

L/min) or slower (~30 L/min) inspiration. Percent decreases in FEV<sub>1</sub> at a common dose of allergen on the two challenge visits were compared to determine whether the protective effect of CS against allergen was enhanced with one of the inspiratory maneuvers. The fraction of CS deposited in the lungs and the distribution of the deposited fraction were also quantified to determine whether changes in the pulmonary response to allergen challenge were related to changes in the deposited dose and/or the distribution of the dose after fast or slower inspiration.

## METHODS

### Patient population

There were two men and six women in the study, averaging  $34 \pm 8$  years of age. All were nonsmokers and had a history of allergy-induced asthma. Their baseline FEV<sub>1</sub>, as a percent of predicted value, averaged  $84\% \pm 13\%$ . Their FEV<sub>1</sub>/forced vital capacity averaged  $76\% \pm 6\%$ . Five of eight patients had positive skin test responses to ragweed, two had positive skin test responses to cat dander, and one had a positive skin test response to dust mites.

### Use of medications

Study subjects who were being medicated with inhaled bronchodilators discontinued their use 24 hours before each study visit. Oral bronchodilators were discontinued 48 hours before each study visit. Patients who were being treated with CS or inhaled steroids were excluded from the study.

### Study design

This was a clinical trial in which patients underwent a screening pulmonary function test and skin test, a screening allergen inhalation challenge, and two allergen inhalation challenges with CS pretreatment. This study was approved by the Johns Hopkins University Joint Committee on Clinical Investigations. No one participated without signed consent.

### Screening pulmonary function test and skin test

FEV<sub>1</sub> was measured by a spirometer that was incorporated into an Auto Box 2800 body plethysmograph (Sensormedics, Inc., Anaheim, Calif.). Patients with baseline FEV<sub>1</sub> greater than 60% of predicted values underwent an intradermal skin test to increasing concentrations of ragweed extract (Greer Laboratories, Lenoir, N.C.), cat extract (ALK Laboratories, Milford, Conn.), or dust mite extract (Miles, Inc., Elkhart, Ind.). Skin tests were modeled after that described by Chai et al.<sup>10</sup> for ragweed. The concentration of antigen that produced a 10 mm × 10 mm wheal and a 20 mm × 20 mm flare was designated as the concentration that produced a 2+ skin test response. Patients who demonstrated a 2+ response to any of these antigens returned to the laboratory and underwent a screening allergen inhalation challenge with that antigen extract.

### Screening allergen challenge

Patients underwent a screening inhalation challenge with increasing concentrations of aerosolized ragweed, cat, or dust mite extract. Ragweed extract concentrations increased in doubling doses from 0.08 AgE units/ml to 20.48 AgE units/ml. Cat extract concentrations increased in doubling doses from 39 BAU/ml to 5000 BAU/ml. Dust mite extract concentrations increased in tripling doses from 10 AU/ml to 5000 AU/ml. All

inhalation challenges were modeled after that of Chai et al.<sup>10</sup> for ragweed and were performed to be sure that all participants in the study were capable of demonstrating at least a 15% decrease in FEV<sub>1</sub> during allergen challenge.

The method for generating and delivering aerosolized allergen extract during the challenge has been described previously.<sup>11</sup> Patients inspired at approximately 30 L/min from residual volume to 50% of their vital capacity. When they achieved 50% vital capacity, aerosolized allergen extract was generated by a #646 nebulizer (DeVilbiss, Somerset, Pa.) as a bolus for 0.6 seconds. The nebulizer was connected to a compressed air source set at 20 psi through a dosimeter (Rosenthal-French, Baltimore, Md.), which regulated the timing of each pulse of compressed air. After aerosolization, patients continued to inhale at 30 L/min until they reached total lung capacity. Then they exhaled normally with no breath holding. This procedure was repeated five times for each concentration of allergen extract. Ten minutes after inhaling each allergen concentration, patients underwent pulmonary function testing to determine percent decrease in FEV<sub>1</sub>. The challenge was terminated when patients demonstrated a 15% decrease in their FEV<sub>1</sub> from prechallenge values. At the end of the challenge, patients were treated with two inhalations of albuterol to reverse their bronchospasm. Patients who demonstrated a 15% decrease in FEV<sub>1</sub> during this challenge returned to the laboratory for two additional visits, during which they were pretreated with slow or faster inhalations of CS followed by the same allergen inhalation challenge.

### Pretreatment with CS before two additional allergen inhalation challenges

Patients inhaled CS (Intal) aerosol (1 mg/actuation) (Fisons Pharmaceuticals, Loughborough, England) 30 minutes before two additional allergen inhalation challenges. On both occasions, CS was generated by MDI into a large volume holding chamber (~700 ml) that we developed for these laboratory experiments. The chamber was plastic and rectangular in shape. Its dimensions were 11.2 cm in length, 7.9 cm wide, and 7.9 cm in height. Each CS/MDI was primed to waste, shaken, and actuated four times in quick succession into the chamber. The chamber itself was not primed before these four actuations. Three seconds later on one study visit, patients inhaled the entire contents of the chamber during a single slow (~30 L/min) inhalation maneuver. On another visit, they inhaled the contents of the chamber during a single faster (~70 L/min) inhalation maneuver. There was no breath holding on either visit.

Two different holding chambers were used for these experiments. One chamber regulated inspiratory flow rate at ~30 L/min (slow chamber). The other regulated flow at ~70 L/min (fast chamber). Flow was regulated by flow rate-limiting orifices on the back panel of each of the chambers. Flow rates during each fast or slower inhalation procedure were quantified by connecting the chamber to a spirometer and having patients inhale from the chamber as fast as possible. The spirometer pen was positioned at the top of the chart paper and fell with the inspiration. Rate of inspiration was quantified from the slope of the pen recording. Inspiratory flow rate measured in this way averaged  $32.6 \pm 10.1$  L/min from the slow chamber and  $74.5 \pm 12.6$  L/min from the fast chamber. Three of eight patients inhaled CS rapidly on the first visit and slowly on their second visit. The other five patients inhaled CS slowly on their first visit.

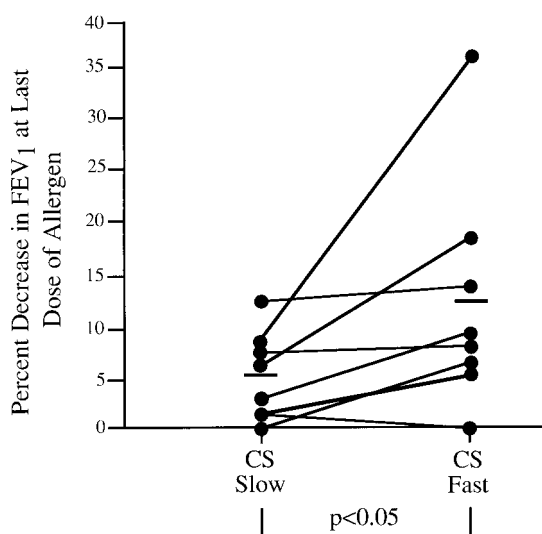


**FIG. 2.** Mean percent decrease in FEV<sub>1</sub> at last dose of allergen, after pretreatment with slow inspiration of CS, was significantly less ( $5.4\% \pm 4.2\%$ ) than with fast inhalation of CS ( $12.6\% \pm 11\%$ ) ( $p < 0.05$ ).

### Allergen inhalation challenges after CS pretreatment

Thirty minutes after slow or faster inhalation of CS as pretreatment, patients inhaled the same concentrations of allergen extract as during the screening challenge and in the same way. After inhaling each allergen concentration, patients underwent pulmonary function testing to determine percent decrease in FEV<sub>1</sub>. At the end of the challenge, patients were treated with two inhalations of albuterol to reverse any bronchospasm. Percent decrease in FEV<sub>1</sub> at the same last dose of allergen on both challenge visits were compared.

Six of eight patients underwent both allergen challenges with CS pretreatment within a 2-week period. One patient underwent both challenges during a 1-month period, and one patient completed both challenges over a 6-month period. To avoid possible alterations in airway responsiveness to inhaled allergen as a result of significant differences in the magnitude of baseline airway function, baseline FEV<sub>1</sub> on each of these two visits was within  $\pm 10\%$  of the value obtained on the screening challenge visit. All ragweed-allergen challenges, including the screening challenge, were performed out of season for ragweed.

### Radiolabeled CS

To quantify the distribution of CS in the lungs, patients inspired radiolabeled CS during a slow (~30 L/min) or faster (~70 L/min) inspiratory maneuver. Four of eight patients inhaled radiolabeled CS 30 minutes before the two allergen challenges described above. Three of eight patients inhaled radiolabeled CS on two occasions when no allergen challenge was performed. One patient did not inhale radiolabeled CS. Interpatient differences in the administration of radiolabeled CS were due to scheduling difficulties for some patients.

CS was radiolabeled with technetium-99m (Tc-99m) according to the method of Kohler et al.<sup>12</sup> Tc-99m pertechnetate (Syncor Inc., Baltimore, Md.) was extracted from its saline vehicle in methyl ethyl ketone, and the methyl ethyl ketone was evaporated by means of a heat lamp. One drop of the surfactant

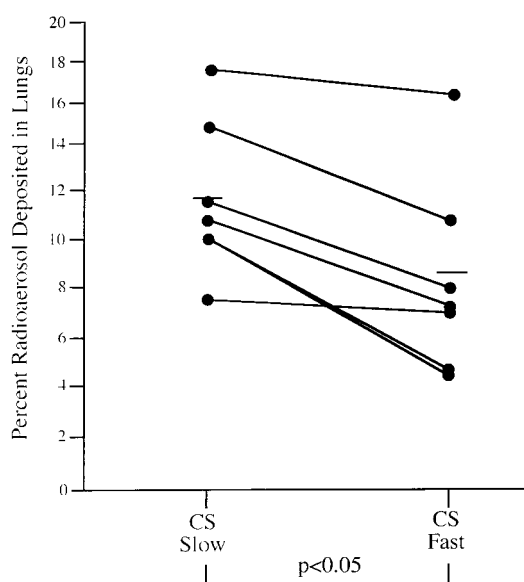


**FIG. 3.** The average deposition fraction of radioaerosol, expressed as percent of radiolabel delivered into holding chamber, was significantly higher with slow inspiration ( $11.8\% \pm 3.5\%$ ) compared with faster inspiration ( $8.6\% \pm 3.9\%$ ) ( $p < 0.05$ ).

sorbitan trioleate and 2 ml of Freon 11 (Penwalt Corp, Philadelphia, Pa.) was then added to the radiolabel. Portions of this mixture (0.5 to 1 ml) were transferred into a CS/MDI canister that had been cooled to  $-60^{\circ}\text{C}$ . This transfer occurred through a small opening in the base of the canister. The canister was resealed and allowed to warm to room temperature. Then it was shaken, primed to waste, and actuated 20 times. Aerosol that was generated during each actuation was collected on a cotton ball. The amount of radioactivity on each cotton ball was quantified in a radioisotope dose calibrator (Capintec, Inc., Ramsey, N.J.). Mean activity/actuation for each of the 20 actuations was calculated. Mean activity/actuation was multiplied times 4 to obtain an estimate of total activity delivered into the holding chamber before patient use.

After fast and slow inspiration of radiolabeled CS, patients sat with their backs to a large field-of-view gamma camera (GE Maxicamera 400; St. Albans, Hertfordshire, England) equipped with an all-purpose, parallel-hole collimator for 4 minutes. This posterior lung image was stored on computer (SMV, Twinsburg, Ohio) for later processing. The fraction of radioaerosol deposited in the lungs and the distribution within the lungs were quantified from analyses of this gamma camera image.

### Quantitative assessments of radioaerosol in the lungs

The primary analyses of the gamma camera images after inhalation of radiolabeled CS were the quantification of the fraction of aerosol that deposited in the lungs and the distribution of aerosol within the right lung. The left lung image was not analyzed in these assessments because radioactivity that deposited in the oropharynx during inhalation often appeared as stomach activity in the left lung image after swallowing. Because the appearance of stomach activity overlapped the left lung image, accurate demarcation of the left lung border was not possible.

*Fraction of radioaerosol deposited in the lungs.* Counts per minute (cpm) within the right lung were multiplied times 2 to obtain total posterior lung cpm. Total cpm for the posterior view were converted to microcuries by using the equation shown below, which was derived from Macey and Marshall<sup>13</sup> and incorporated a lung transmission value and a camera sensitivity calculation. Total lung cpm were squared to account for cpm in the anterior view, which were not acquired but were estimated to be the same as for the posterior view.

$$A(\mu\text{Ci}) = \sqrt{\frac{\text{Total lung cpm}^2}{\text{transmission value}}} \times \frac{1}{\text{camera sensitivity}}$$

Total  $\mu\text{Ci}$  were expressed as a percent of total  $\mu\text{Ci}$  delivered into the holding chamber during four actuations of radiolabeled CS.

To obtain the lung transmission value, patients sat with their backs to the gamma camera for 5 minutes while a plastic phantom containing water and Tc-99m was placed directly in front of their lungs. Counts from the phantom were recorded for 5 minutes by computer. Then the counting procedure was repeated without the patient. A ratio of counts from a region in the posterior lung for the two images was calculated. This ratio was designated as the transmission value and represented the extent to which the patient's thorax attenuated gamma radiation.

*Regional distribution of the radioaerosol.* The regional distribution of radiolabeled CS within the lungs was quantified in terms of radioactivity deposited in an inner and outer region of the aerosol lung image as shown in Fig. 1. Inner and outer zones were first delineated on a ventilation image. To acquire the ventilation image, patients inhaled xenon-133 gas (Syncor, Inc.) from a Pulmonex Xenon System (Biodex Medical Systems, Shirley, N.Y.) while sitting with their back to the gamma camera. Patients breathed the radiogas for 90 seconds to promote penetration throughout the lung. Then a posterior lung image was acquired and stored on computer for processing.

The width of the ventilation lung image was divided into three vertical regions, as shown in Fig. 1, by using computer programs we developed. Similarly, the height of the image was divided into three horizontal regions. This procedure resulted in the lung image being divided into a total of nine smaller regions. During automated computer processing, the two radioaerosol images (one for slow and fast inspiration, respectively) were registered with the ventilation scan. All nine regions delineated in the ventilation scan were then superimposed on the two registered radioaerosol images. In this way, inner and outer zones were standardized on all three images. Mean counts per pixel in the inner and outer zones of the ventilation scan and the radioaerosol scan were calculated. An inner:outer (I:O) ratio was derived for both the ventilation and the aerosol scans. The aerosol I:O ratio was divided by the ventilation I:O ratio to correct for differences in lung volume within the two regions.

Anatomically, it was assumed that the inner zone was composed predominantly of larger airways and the outer zone was composed of peripheral airways. Therefore lower I:O ratios indicated enhanced deposition in the peripheral airways. Because these images are a two-dimensional representation of deposition in a three-dimensional organ, deposition in some smaller airways and alveoli also appear in the inner zone region of the image. However, because of the lung's anatomy, we assumed that the number of smaller airways and alveoli comprising our inner region was significantly less than that found in our outer region. For this reason, we assumed that the two

zones provided us with substantially different regional deposition information.

**Distribution homogeneity of the radioaerosol.** We also analyzed the two radioaerosol lung images in terms of distribution homogeneity. For this assessment, we generated frequency distribution histograms from radioactivity recorded in the right lung of the two aerosol images on a per-pixel basis. The methods for generating frequency distribution histograms from gamma camera lung images have been described previously.<sup>14</sup> Distribution homogeneity was quantified in terms of skew. Lower skew values indicated an increase in distribution homogeneity.

### In vitro comparisons of radiolabeled versus unlabeled CS aerosol

We made two in vitro comparisons of labeled and unlabeled CS aerosol. First, we compared the particle size distribution of labeled versus unlabeled CS aerosol emitted from each chamber at flow rates that approximated the fast and slower inspiratory flows achieved by patients. This comparison was carried out to determine whether adding radiolabel to CS altered the particle size distribution of drug emitted from either chamber compared with unlabeled CS. Secondly, we compared micrograms of labeled versus unlabeled drug that entered the two chambers per actuation. This comparison was performed to determine whether radiolabeling CS altered the amount of drug that was generated into the chamber compared with unlabeled CS.

**Determination of particle size distribution for the slow chamber.** Each of nine unlabeled and seven labeled CS canisters were primed to waste, shaken, and actuated four times into the slow chamber. After a 3-second delay, the contents of the chamber were drawn into an 8-stage Andersen cascade impactor (Andersen Instruments Inc., Atlanta, Ga.) at a flow rate of 28.3 L/min. In this way, the flow rate during particle sizing closely matched the flow rate achieved by the patients during inspiration (30 L/min). Drug deposited in the slow chamber, throat, inlet cone, and each of the stages and filter of the impactor was extracted by washing each component with deionized water. The amount of drug deposited at each location was determined by high-performance liquid chromatography (HPLC). The mass median aerodynamic diameter (MMAD) for labeled and unlabeled drug was estimated by fitting a log-normal distribution to the amount of drug deposited at each location using a validated spreadsheet.

The HPLC assay used a mobile phase consisting of 60:40 0.01 mol/L ammonium dihydrogen phosphate:methanol pumped through a C<sub>18</sub> Phenomenex Prodigy 5  $\mu$ m ODS, 150  $\times$  3.20 mm column (Phenomenex, Torrance, Calif.) at a flow rate of 1 ml/min using a Hitachi L-6200 Intelligent Pump (Hitachi Ltd., Tokyo, Japan). Duplicate injections (50  $\mu$ l) of test solution and standards were made with a Hitachi 655A-40 Autosampler. CS was detected by ultraviolet detection (Hitachi L-3000 Multi-Channel Photo-Detector) at 326 nm. Peak areas were integrated with the Hitachi D-2000 Chromato-Integrator.

**Determination of particle size distribution for the fast chamber.** For this determination, we wanted to simulate the faster breathing maneuver of the patients. However, we could not use the Andersen cascade impactor to quantify particle size distribution under such conditions because the Andersen impactor is calibrated under 28.3 L/min flow conditions, and inspiration from the fast chamber was at rates significantly above this. For this reason, particle size distribution of aerosol emitted from the fast chamber was characterized in terms of percent of

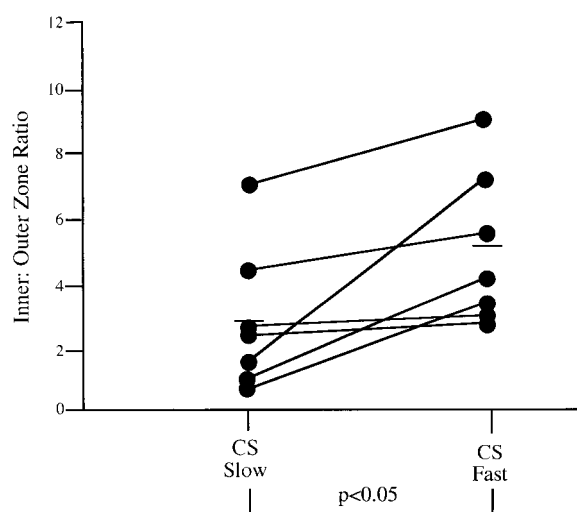


**FIG. 4.** Mean I:O zone ratio of radioaerosol was significantly less after slow inspiration ( $3.07 \pm 2.18$ ) compared with faster inspiration ( $5.30 \pm 2.30$ ) ( $p < 0.05$ ).

particles with aerodynamic diameters less than 10  $\mu$ m for labeled and unlabeled CS by using a Single Stage Liquid Impinger (Fisons Pharmaceuticals, Loughborough, England). Although the Single Stage Liquid Impinger functions under the higher flow rate conditions, it only provides information as to percent particles less than 10  $\mu$ m. It does not provide an MMAD determination.

Each of two unlabeled and labeled CS canisters was primed to waste, shaken, and actuated four times into the fast chamber. After a 3-second delay, the contents of the chamber were drawn through the impinger at a flow rate of 60 L/min by using a vacuum pump. Drug deposited in the fast chamber, the throat, the single stage, and the filter of the impinger was extracted by washing with deionized water. The amount of drug deposited at each location was determined by HPLC. Percent drug collected on the filter represented the fraction of drug with particles less than 10  $\mu$ m.

**Determination of amount of labeled versus unlabeled drug generated into the two chambers.** The amount of labeled or unlabeled drug deposited in the slow chamber, the throat, inlet cone, and each of the stages and filter of the cascade impactor was summed. The amount of unlabeled or labeled drug deposited in the fast chamber, the throat, the single stage, and the filter of the impinger was also summed. These totals represented the amount of labeled or unlabeled drug that entered the chambers during four actuations. Dividing each total by four yielded micrograms per actuation that entered the chambers for labeled and unlabeled drug.

### Data analyses

All data are presented as mean  $\pm$  SD. Mean percent decreases in FEV<sub>1</sub> at the last dose of allergen with slow and faster CS pretreatment were compared by a Wilcoxon signed ranks test. A similar statistical test was used to compare mean fraction of radioaerosol deposited in the lungs, I:O ratios, and values of skew after slow and faster inhalation of radiolabeled CS. A Wilcoxon-Mann-Whitney test was used to compare the MMAD of aerosol emitted from the slow chamber with labeled and unlabeled canisters and to compare micrograms per actuation of labeled and unlabeled drug that entered the two

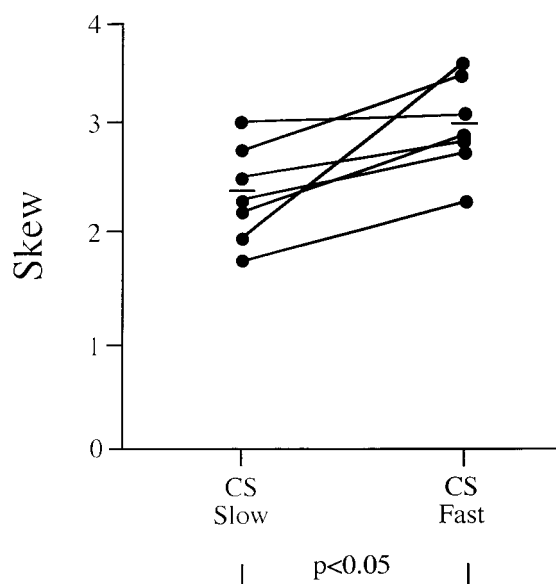


**FIG. 5.** Mean skew value for radioaerosol was significantly lower after slow inspiration ( $2.36 \pm 0.44$ ) compared with faster inhalation ( $3.00 \pm 0.44$ ) ( $p < 0.05$ ).

chambers. Linear regression analyses were performed to determine possible relationships between the differences in percent decrease in  $FEV_1$  allergen and differences in the fraction of radiolabeled CS deposited in the lungs and the distribution of CS, expressed as I:O ratio and skew, for the two inspiratory flow rates. Probability ( $p$ ) values less than 0.05 indicated statistically significant differences.

## RESULTS

### Response to allergen on the screening visit

At the last dose of allergen, the mean decrease in  $FEV_1$  on the screening allergen challenge was  $22.5\% \pm 4.8\%$ .

### Effect of slow and faster inspirations of CS on allergen challenge

Fig. 2 shows percent decrease in  $FEV_1$  at the same last dose of allergen with slow or faster CS pretreatment for each of the eight patients. Percent decrease in  $FEV_1$  after pretreatment with slow inspiration of CS averaged  $5.4\% \pm 4.2\%$ , which was significantly less than that after faster inhalation of CS, which averaged a  $12.6\% \pm 11.0\%$  decrease in  $FEV_1$  ( $p < 0.05$ ).

### Effect of slow and faster inspiration on fraction of radioaerosol deposited in lungs

Fig. 3 shows the fraction of radioaerosol that deposited in the lungs of seven of eight patients after slow or faster inhalation of radiolabeled CS. Fraction deposited is expressed as a percent of radiolabel delivered into the holding chamber. The average deposition fraction was significantly higher with slow inspiration ( $11.8\% \pm 3.5\%$ ) compared with faster inhalation ( $8.6\% \pm 3.9\%$ ) ( $p < 0.05$ ).

### Effect of slow and faster inspiration on regional distribution of radioaerosol

Fig. 4 shows the I:O ratios for seven of eight patients after slow or faster inhalation of radiolabeled CS. Mean I:O ratio was significantly less after slow inspiration ( $3.07 \pm 2.18$ ) compared with faster inspiration ( $5.30 \pm 2.30$ ), indicating an increase in penetration of drug into the lung periphery (the outer zone) with the slower inspiratory maneuver ( $p < 0.05$ ).

### Effect of slow and faster inspiration on distribution homogeneity of radioaerosol

Fig. 5 shows the skew values for seven of eight patients after slow or faster inhalation of radiolabeled CS. Mean skew was significantly lower when CS was inhaled slowly ( $2.36 \pm 0.44$ ) compared with faster inhalation ( $3.00 \pm 0.44$ ), indicating an increase in overall deposition homogeneity with the slower inspiratory maneuver ( $p < 0.05$ ).

### Relationship between protective effect of slow inspiration of CS and distribution homogeneity

Fig. 6 shows the best-fit straight line derived from a linear regression analysis of percent change in decreases in  $FEV_1$  with slow versus faster inspiration of CS and percent change in skew values for slow versus faster inspiration of CS. The analysis revealed that percent change in decreases in  $FEV_1$  for the two inspiratory maneuvers was significantly related to percent change in skew values, such that increases in the protective effect of CS as a result of slow inspiration were associated with increases in distribution homogeneity ( $p < 0.02$ ). There were no significant correlations between the protective effect of slow inspiration of CS against allergen challenges and increased deposition fraction in the lungs or decreased I:O ratio.

### In vitro comparisons of radiolabeled and unlabeled CS aerosol

*Particle size distribution from slow chamber.* Mean MMADs of aerosol exiting the slow chamber after actuation of the seven labeled and nine unlabeled canisters were not significantly different, with  $4.33 \pm 0.25 \mu\text{m}$  (geometric standard deviation =  $1.70 \pm 0.18$ ) and  $4.34 \pm 0.15 \mu\text{m}$  (geometric standard deviation =  $1.64 \pm 0.22$ ), respectively. This finding indicated that the particle size distribution for radiolabeled CS aerosol emitted from the slow box was the same as that for unlabeled CS aerosol.

*Particle size distribution from fast chamber.* The fraction of aerosol particles emitted from the fast chamber and collected on the filter of the Single Stage Liquid Impinger averaged  $11.40\% \pm 0.44\%$  for the unlabeled canisters and  $12.97\% \pm 0.58\%$  for the labeled canisters. The fraction of particles less than  $10 \mu\text{m}$  was therefore equivalent for the radiolabeled and unlabeled CS aerosol emitted from the fast chamber.

*Amount of labeled and unlabeled drug generated into the chambers.* The average amount of unlabeled drug that was generated into the two chambers per actuation

was  $1150.2 \pm 166.8 \mu\text{g}$ . This was not significantly different from the amount of labeled drug that entered the two chambers per actuation, which averaged  $1175.8 \pm 304.8 \mu\text{g}$ .

## DISCUSSION

Results from this study confirm the findings of other investigators who have shown a significant protective effect from CS generated by MDI and inhaled before allergen challenge.<sup>15, 16</sup> However, our study extends these previous findings by examining how the protective effect can be optimized. To answer this question, we compared percent decrease in FEV<sub>1</sub> at a common dose of allergen in patients with asthma who were pretreated with rapid or slow inhalation of CS from a large holding chamber. The average percent decrease in FEV<sub>1</sub> after pretreatment with slow inspiration of CS was  $5.4\% \pm 4.2\%$ . This decrease in FEV<sub>1</sub> was significantly less than that after pretreatment with faster inspiration of CS, which averaged a fall of  $12.6\% \pm 11.0\%$ . These findings indicate that the protective effect of CS against allergen-induced asthma is optimized by inhaling slowly from a large holding chamber compared with faster inhalation of CS.

Quantification of gamma camera images of the lungs after inhalation of radiolabeled CS indicated that the fraction of labeled drug that deposited in the lungs was increased from  $8.6\% \pm 3.9\%$  to  $11.8\% \pm 3.5\%$  by inhaling at the slower rate. In addition, I:O ratio decreased from  $5.30 \pm 2.30$  to  $3.07 \pm 2.18$  with slower inspiration, indicating that more drug was depositing beyond the conducting airways. Skew values decreased from  $3.00 \pm 0.44$  to  $2.36 \pm 0.44$  with slower inspiration, indicating that the overall distribution of CS within the lungs was more homogeneous. This decrease in skew represented an average change of 21%, which was less than the change observed in another study with asthmatic subjects who inhaled methacholine rapidly or slowly. In that study<sup>17</sup> we observed an average decrease in skew values of 32% with slower inspiration. Nevertheless, in this study percent changes in skew were significantly associated with the magnitude of the change in the protective effect of slow inhalation of CS compared with faster inhalation. This association indicated that increases in the protective effect of CS with slow inhalation of the drug were related to increases in distribution homogeneity and suggested that uniform distribution of drug throughout the lungs is an important determinant in maximizing the protective effect of CS.

It is unclear why uniform distribution of drug is an important determinant in optimizing the protective effect of CS. One explanation may involve maximizing delivery of drug to all potential sites of bronchoconstriction by the slow inspiratory maneuver. Another explanation may involve enhanced deposition at the site of action of allergen. Results from in vitro studies suggest that the smaller airways contain a higher density of mast cells than the larger central airways.<sup>18</sup> Theoretically, if mast cells are involved in the development of acute

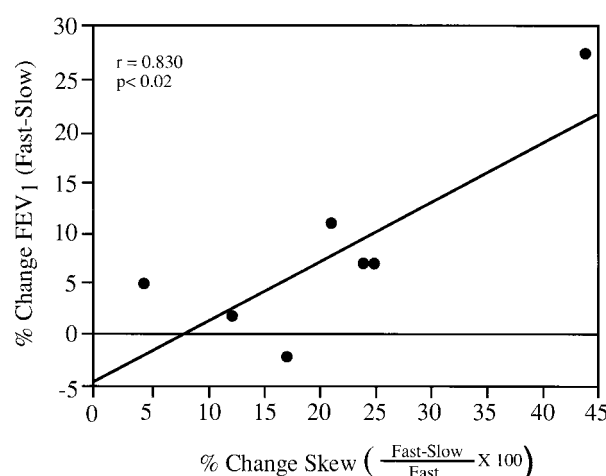


**FIG. 6.** Linear regression analysis showed that percent change in decreases in FEV<sub>1</sub> with slow versus faster inhalation of CS was significantly related to percent change in skew values for the two inspiratory maneuvers ( $p < 0.02$ ).

bronchoconstriction induced by allergen stimulation, targeted deposition of CS to the smaller airways should lead to greater protection in allergen challenges. Additional studies are needed to confirm this hypothesis.

Although quantification of the deposited fraction and distribution of CS was based on analyses of radiolabeled CS within the lungs, we believe these results extend to unlabeled CS inhaled at similar inspiratory rates from this large volume holding chamber. Support for this assumption is found in the results of our comparisons of aerosol particle size distribution with labeled and unlabeled CS and in the amount of drug generated into the two chambers per actuation. Those comparisons showed that the aerosol particle size characteristics were the same whether the canister contained radiolabel or was unlabeled. Since particle size is the major determinant of aerosol distribution in the lung, it is likely that the distribution associated with radiolabeled CS was similar to that of unlabeled CS. Moreover, micrograms of drug actuated into the two chambers was similar for labeled and unlabeled CS. This means that calculations of the deposited fraction, based on the amount of radiolabeled drug administered into the chamber, should also represent the deposited fraction of unlabeled CS.

Newman et al.<sup>8</sup> used an approach that was similar to ours to alter the fraction of CS deposited in the lungs of normal subjects. They delivered a single inhalation of 1 mg CS from an MDI through a 10 cm extension tube. Results from that study demonstrated that slowing the inhalation rate from 100 L/min to 25 L/min increased the fraction of drug that deposited in the lung from 13% to 16%, respectively. They also reported that the peripheral to central distribution (P:C ratio) increased from  $0.81 \pm 0.13$  to  $1.34 \pm 0.11$  with the slower inspiratory maneuver, indicating greater penetration of the drug into the lung periphery with the slower inspiratory maneuver.

Because Newman et al. did not investigate the biologic

consequences of these alterations, we cannot compare our results in terms of optimizing the protective effect of CS against inhalation challenge. However, we can compare our findings with respect to the fraction of drug that deposited in the lungs and the distribution of that fraction within the lungs. We found that slowing the inspiratory maneuver from  $\sim 70$  L/min to  $\sim 30$  L/min increased the fraction of drug that deposited in the lung from  $8.6\% \pm 3.9\%$  to  $11.8\% \pm 3.5\%$ , respectively. This increase in deposition fraction at the lower flow rate was similar to what Newman reported.

When we converted our I:O ratios to P:C ratios, we found that the peripheral-to-central distribution of aerosol increased from an average of  $0.36 \pm 0.12$  to  $0.63 \pm 0.28$  with the slower inspiratory maneuver. This increase was also similar to the findings of Newman et al.,<sup>8</sup> although the two studies differ in the magnitude of the reported P:C ratios. Although this could be due to methodological differences in how the P:C ratios were computed, it is more likely that the differences were due to the populations being studied. Newman et al.<sup>8</sup> looked at normal subjects, whereas we studied patients with mild-to-moderate asthma. It is well known that patients with obstructive airways disease deposit aerosol predominantly in the larger central airways, whereas normal subjects deposit aerosol more uniformly throughout the lung field.<sup>19-21</sup> It is likely that the observed variations in P:C ratio in these two studies reflect differences in the aerosol deposition pattern for the two populations.

Other investigators have estimated the dose of CS that deposits in the lungs from measurements of peak plasma levels. However, these measurements do not appear to reflect the distribution of the drug within the lungs. This is evident from the results of a study by Richards et al.<sup>22</sup> who compared the lung distribution of radiolabeled CS and the plasma levels of CS before and after bronchoconstriction induced by methacholine. Using radioimaging techniques, they found that the peripheral distribution of the drug was reduced after bronchoconstriction, but the pharmacokinetic profile based on plasma levels did not change.

In summary, previous studies of CS have demonstrated that the dose of drug that deposits in the lung is dependent on inhalation technique. Our study adds to these earlier findings by providing new information regarding the relationship between the technique of inhalation and the biologic consequences of varying the technique in terms of protection against allergen-induced asthma. These findings emphasize the importance of assessing the effect of this variable to maximize the efficacy of new formulations and new inhaler systems in the future. In addition, if one assumes that protection against relevant bronchial challenge is a good indicator of likely clinical efficacy in long-term treatment, these results also provide important information as to how to optimize the clinical effects of this antiinflammatory drug both in patients who have never been treated with CS and in patients who were previously thought to be

nonresponsive. The large volume holding chambers we used to deliver CS during slow or faster inhalations were specially constructed for this study. Similar results should be expected with commercially available large volume holding chambers if inspiratory flow is regulated at approximately 30 L/min or patients are trained and motivated to inhale at these low rates without regulation.

We thank Myrna Dolovich, PEng, for teaching us the technique for radiolabeling MDIs; David Velasquez, PhD, and 3M Pharmaceuticals for providing us with sorbitan trioleate for radiolabeling CS; and Rajkumari Jashnani, PhD, D. Todd Loffert, MHS, and Shailaja Somaraju for performing the particle sizing experiments.

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