We studied the effects of nedocromil sodium and cromolyn sodium on early and late bronchial responses to inhaled *Ascaris suum* antigen in allergic sheep in vivo, and the antigen-induced contractile responses of sheep tracheal smooth muscle in vitro. For the in vivo studies the sheep were pretreated with aerosols of placebo (buffered saline solution), 20 mg of nedocromil sodium, or 20 mg of cromolyn sodium (both dissolved in 3 ml of buffered saline solution) and then challenged with aerosol antigen. Specific pulmonary resistance (SRL) was measured before and after challenge to document the responses of the airways. In the trial with placebo, challenge with antigen resulted in significant early and late increases in SRL. Treatment with nedocromil sodium significantly reduced the early response to antigen and blocked the late response. Cromolyn sodium gave the same results; there were no statistical differences between the responses of the airways for the two drugs. In vitro nedocromil sodium at doses of $10^{-5}$M and $10^{-7}$M inhibited significantly the contractile responses of sheep tracheal smooth muscle to *A. suum*. Cromolyn sodium only showed efficacy at $10^{-5}$M. These results suggest that nedocromil sodium may be potentially useful in the treatment of reversible allergic disease of the airways.

Allergic sheep have many pathophysiologic traits analogous to human bronchial asthma, including the development of both early and late bronchial responses after inhalation challenge with specific antigen. Furthermore, the antigen-induced early and late responses in sheep are sensitive to pharmacologic agents which are active in man (eg, cromolyn sodium and glucocorticosteroids), suggesting that this animal model can be used as a preclinical predictor for active agents.

Nedocromil sodium (Tilade) is the disodium salt of a novel pyraquinoquine dicarboxylic acid which has demonstrated promising activity in early clinical trials in asthmatic subjects. Using rat mast cell models of immediate hypersensitivity, the compound demonstrated classic antiallergic activity similar to that of cromolyn sodium. In models having a stronger inflammatory component, including various isolated inflammatory cell systems, the activity of nedocromil sodium was superior to that of cromolyn sodium.

Nedocromil sodium was also found to be active in a primate model of asthma in which cromolyn sodium was ineffective.

It was of interest therefore to compare the efficacy of nedocromil sodium and cromolyn sodium in modifying antigen-induced early and late responses in allergic sheep. In this study, we examined the effects of nedocromil sodium on the antigen-induced bronchial responses in allergic sheep in vivo and the antigen-induced contraction of sheep tracheal smooth muscle in vitro. In addition, the efficacy of nedocromil sodium in these two systems was compared to that of equal doses of cromolyn sodium.

**Materials and Methods**

**In Vivo Studies**

Animal Preparation. Five adult sheep (32 to 52 kg) were used to study the effects of nedocromil sodium and cromolyn sodium on antigen-induced early and late bronchial responses. All animals met the following two criteria: (1) they had a natural cutaneous reaction to 1:1,000 or 1:10,000 dilutions of *Ascaris suum* extract (Greer Diagnostics); and (2) they all demonstrated hypersensitivity of the airways to inhalation challenge with *A. suum* antigen.

Measurement of Airway Mechanics. The unsedated sheep were restrained in a cart in the prone position with their heads immobilized. After topical anesthesia of the nasal passages with a 2 percent solution of lidocaine, a balloon catheter was advanced through one

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nostril into the lower esophagus. The animals were intubated with a cuffed nasotracheal tube through the other nostril using a flexible fiberoptic bronchoscope as a guide. (The cuff of the endotracheal tube was inflated only for the measurement of airway mechanics and during aerosol challenge to prevent undue discomfort. This procedure has no effect on airway mechanics.) Pleural pressure was estimated with the esophageal balloon catheter (filled with 1 ml of air), which was positioned 5 to 10 cm from the gastroesophageal junction. In this position the end-expiratory pleural pressure ranged between −2 and −5 cm H₂O. Once the balloon was placed, it was secured so that it remained in position for the duration of the experiment. Lateral pressure in the trachea was measured with a sidehole catheter (inner dimension, 2.5 mm) advanced through and positioned distal to the top of the endotracheal tube. Transpulmonary pressure, the difference between tracheal and pleural pressure, was measured with a differential pressure transducer (type P-7D; Pace Engineering). Testing of the pressure transducer-catheter system revealed no phase shift between pressure and flow to a frequency of 9 Hz. For the measurement of total pulmonary resistance (RL), the proximal end of the endotracheal tube was connected to a pneumotachygraph (Fleisch; Dyna Sciences). The signals of flow and transpulmonary pressure were recorded on an oscillographic recorder (Electronics for Medicine model DR-12), which was linked to a digital computer (Digital Equipment model PDP-11) for on-line calculation of RL from transpulmonary pressure, respiratory volume (obtained by integration), and flow. Analysis of five to ten breaths was used for the determination of RL.

Immediately after the measurement of RL, thoracic gas volume (Vtg) was measured in a constant-volume body plethysmograph to obtain SRL (S.RL - RL Vtg).

**Aerosol Delivery Systems.** Aerosols of A suum extract (1:20 dilution; 82,000 PNU/ml) were generated using a disposable medical nebulizer (Raindrop; Puritan-Bennett), which produced an aerosol with a mass median aerodynamic diameter of 3.2 μ (geometric standard deviation, 1.9) as determined by a seven-stage Andersen cascade impactor. The output from the nebulizer was directed into a plastic T-piece, one end of which was attached to the endotracheal tube and the other end of which was connected to the inspiratory port of a Harvard respirator. The aerosol was delivered at a tidal volume of 500 ml and a rate of 20 ml/min, so that each sheep received equivalent doses (400 breaths) of antigen for all trials.

**Experimental Protocol**

Baseline measurements of SRL were obtained, and the sheep were given either placebo (buffered saline solution) or 20 mg of nedocromil sodium or cromolyn sodium dissolved in 3 ml of buffered saline solution in a crossover fashion. The sheep were then challenged with A suum antigen. Measurements of SRL were repeated immediately (ie, 0 to 10 minutes) after antigen challenge and subsequently at 1, 2, 3, 4, 5, 6, 6.5, 7, 7.5, and 8 hours after challenge.

**In Vitro Studies**

Adult sheep were killed with a rapid intravenous injection of pentobarbital sodium (25 mg/kg), and their tracheas were immediately removed and placed in cold Krebs-Henseleit solution (composition in mM: Na⁺, 143.9; K⁺, 5.9; Mg²⁺, 1.2; Ca²⁺, 2.5; Cl⁻, 120.0; HCO₃⁻, 25.0; H₂PO₄⁻, 1.3; SO₄²⁻, 1.2; and glucose, 5.6). Tracheas were used immediately or stored for 24 hours at 4°C in this solution until the time of the experiment. Storing the muscle for one day in this manner did not alter the response to agonists or antagonists, as has been shown previously for airway smooth muscle.³¹,³³ Tracheal strips were obtained by cutting single tracheal rings from the middle of the trachea, removing the connective tissue and fat, and then transecting the ventral portion of the cartilage. Each strip was suspended by sutures, in a 40-ml organ bath containing Krebs-Henseleit solution (pH 7.50) maintained at 39°C (sheep body temperature) and bubbled with a gas mixture of 95 percent oxygen and 5 percent carbon dioxide. One suture was connected to a force-displacement transducer (Grass model FT03) which was linked to a physiologic polygraph (Grass model 7D) to record isometric tension. The resting tension of 5 g was placed on the muscle, and the muscle was allowed to equilibrate for at least one hour. During the period of equilibration, the Krebs-Henseleit bathing solution was changed three to four times. The testing tension after equilibration varied between 1 and 4 g.

**Protocol**

Paired tissues (from the same animal) were untreated (controls) or pretreated with nedocromil sodium or cromolyn sodium at 10⁻¹⁰ M, 10⁻⁹ M, or 10⁻⁸ M for 15 minutes prior to addition of purified A suum antigen (100 μl to 300 μl). Contractile responses to A suum were expressed as a percentage of the contractile response to acetylcholine (10⁻⁴ M) obtained prior to the addition of antigen for each tissue. We also determined if nedocromil had any nonspecific effects on contractile responses of airway smooth muscle to acetylcholine. To do this, we performed dose-response curves to acetylcholine in tissues pretreated with 10⁻⁴ M nedocromil (n = 4) and untreated tissues (n = 4) from the same animal (paired tissues) and computed the dose of acetylcholine necessary to produce a 50 percent increase in tension (EC₅₀).

**Statistical Analysis**

For the in vivo studies a two-factor (drug treatment and time) analysis of variance with repeated measures was used to determine statistical differences within each trial (ie, over time) and among trials (ie, drug treatments). If the null hypothesis was rejected, then post hoc pairwise comparisons were performed to determine which values differed within and among the trials. For the in vitro studies, Wilcoxon’s paired t analysis was used to compare the control and treated tissues. Significance was accepted when p < 0.05 using a two-tailed test.

**Results**

**In Vivo**

Inhalation of A suum antigen resulted in both early and late bronchial responses characteristic for this model when the sheep were pretreated with placebo (Table 1 and Fig 1). Mean SRL (± SE) increased 430 ± 151 percent (p < 0.05) over baseline immediately after challenge; SRL remained significantly elevated one hour after challenge but then returned to prechallenge values until 7.0 hours, when mean SRL had increased to 168 ± 19 percent (p < 0.05) over baseline. The SRL remained elevated until eight hours after challenge. In the placebo trial the early response to antigen was more than 100 percent (range, 173 percent to 1,006 percent) over baseline in all sheep; the peak increase in SRL during the late response (ie, the greatest value of SRL between 6.5 and 8 hours after challenge) was also more than 100 percent (range, 118 percent to 203 percent) over baseline in all sheep.

The changes observed in SRL in the placebo trial were significantly reduced by pretreatment with a drug (Table 1 and Fig 1). When these same sheep were pretreated with nedocromil sodium, both the early and late bronchial responses were significantly reduced when compared to the placebo trial. Immediately after
The results with cromolyn sodium were similar to those obtained with nedocromil sodium; both the early and late increases in SRL were significantly reduced with respect to the placebo trial (Table 1 and Fig 1). There were no statistical differences in the response of the airways to antigen between the two drug trials. The same animal which was unprotected by nedocromil sodium was also unprotected by cromolyn sodium; this animal (No. 94) plus one other (No. 119) showed more than 100 percent increases in SRL (485 percent and 290 percent, respectively) immediately after antigen challenge; however, none of the cromolyn-treated animals showed a peak increase in SRL of more than 100 percent during the late response.

In Vitro

Table 2 shows that at 10^{-3}M, both cromolyn sodium and nedocromil sodium provided significant protection against antigen-induced contraction of sheep tracheal smooth muscle. Nedocromil sodium was also effective at 10^{-4}M; however, at this dose, cromolyn sodium lost its protective effect. Nedocromil sodium was ineffective at a dose of 10^{-7}M (Table 2).

To ensure that the protective effect of nedocromil sodium against antigen-induced contractile responses was not the result of nonspecific effects of the drug on smooth muscle contractility, we determined the EC_{50} for acetylcholine in tissues treated with nedocromil sodium and compared them to untreated tissues from the same animal. In untreated tissues (n = 4), mean EC_{50} (± SE) was 1.49 ± 0.60 × 10^{-7}M; this value was not different from the EC_{50} (1.15 ± 0.32 × 10^{-7}M) of the tissues (n = 4) treated with 10^{-5}M nedocromil sodium.

### DISCUSSION

The results of the in vivo study indicated that nedocromil sodium and cromolyn sodium are effective in reducing the early allergic reaction and preventing

**Table 1—Effects of Pretreatment with Nedocromil Sodium and Cromolyn Sodium on Antigen-Induced Changes in SRL in Allergic Sheep**

<table>
<thead>
<tr>
<th>Time after Ascaris</th>
<th>SRL, L/cm H2O/L/sec*</th>
<th>Data</th>
<th>Nedocromil Sodium</th>
<th>Cromolyn Sodium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediately</td>
<td>4.49 ± 1.48†</td>
<td>Baseline</td>
<td>0.80 ± 0.11</td>
<td>0.71 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>2.55 ± 1.50‡</td>
<td>After placebo</td>
<td>0.81 ± 0.11</td>
<td>0.86 ± 0.07</td>
</tr>
<tr>
<td>1 hr</td>
<td>2.18 ± 0.50†</td>
<td>or drug</td>
<td>0.82 ± 0.04</td>
<td>0.84 ± 0.04</td>
</tr>
<tr>
<td>2 hr</td>
<td>1.25 ± 0.22</td>
<td></td>
<td>1.15 ± 0.03</td>
<td>1.02 ± 0.05</td>
</tr>
<tr>
<td>3 hr</td>
<td>1.18 ± 0.29</td>
<td></td>
<td>0.74 ± 0.07</td>
<td>0.82 ± 0.15</td>
</tr>
<tr>
<td>4 hr</td>
<td>0.98 ± 0.19</td>
<td></td>
<td>0.85 ± 0.12</td>
<td>0.90 ± 0.10</td>
</tr>
<tr>
<td>5 hr</td>
<td>1.16 ± 0.22</td>
<td></td>
<td>0.72 ± 0.07</td>
<td>0.77 ± 0.08</td>
</tr>
<tr>
<td>6 hr</td>
<td>1.35 ± 0.23</td>
<td></td>
<td>0.85 ± 0.12</td>
<td>0.80 ± 0.02</td>
</tr>
<tr>
<td>6.5 hr</td>
<td>1.81 ± 0.21</td>
<td></td>
<td>0.75 ± 0.56‡</td>
<td>0.80 ± 0.08‡</td>
</tr>
<tr>
<td>7 hr</td>
<td>2.13 ± 0.32†</td>
<td></td>
<td>1.12 ± 0.22‡</td>
<td>0.88 ± 0.07†</td>
</tr>
<tr>
<td>7.5 hr</td>
<td>2.06 ± 0.37†</td>
<td></td>
<td>1.24 ± 0.43‡</td>
<td>0.75 ± 0.10‡</td>
</tr>
<tr>
<td>8 hr</td>
<td>1.73 ± 0.41</td>
<td></td>
<td>1.30 ± 0.32</td>
<td>0.86 ± 0.05‡</td>
</tr>
</tbody>
</table>

*Values are means ± SE for same five sheep.
†p<0.05 vs placebo.
‡p<0.05 vs baseline.

<table>
<thead>
<tr>
<th>Time</th>
<th>SPECIFIC LUNG RESISTANCE (± FROM BASELINE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BL</td>
<td>PLACEBO</td>
</tr>
<tr>
<td>PL/ PD</td>
<td>NEDOCROMIL SODIUM</td>
</tr>
<tr>
<td>ASC</td>
<td>CROMOLYN SODIUM</td>
</tr>
</tbody>
</table>

**Figure 1.** Mean percentage changes (± SE) in SRL induced by antigen challenge in five sheep when they were untreated or treated with nedocromil sodium or cromolyn sodium. Both nedocromil sodium and cromolyn sodium reduced the magnitude of early antigen-induced response and shortened its duration. Both drugs blocked the late response. There were no statistical differences between two drug trials. Asterisks indicate p<0.05 vs placebo. BL, baseline; PL/PD, post placebo/post drug; and ASC, after antigen.
Table 2—Effect of Nedocromil Sodium and Cromolyn Sodium on Antigen-Induced Contractions of Sheep Tracheal Smooth Muscle in Vitro

<table>
<thead>
<tr>
<th>Drug and Concentration</th>
<th>Percent of Response to Acetylcholine*</th>
<th>Drug</th>
<th>Control</th>
<th>n†</th>
<th>p Value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nedocromil sodium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁻⁷ M</td>
<td>33 ± 4</td>
<td>39 ± 5</td>
<td>6</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>10⁻⁶ M</td>
<td>26 ± 7</td>
<td>46 ± 11</td>
<td>7</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>10⁻⁵ M</td>
<td>18 ± 12</td>
<td>37 ± 16</td>
<td>7</td>
<td>&lt;0.02</td>
<td></td>
</tr>
<tr>
<td>Cromolyn sodium</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10⁻⁷ M</td>
<td>46 ± 11</td>
<td>48 ± 14</td>
<td>6</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>10⁻⁶ M</td>
<td>16 ± 8</td>
<td>36 ± 14</td>
<td>8</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

*Values are means ± SE and are expressed as percent of contractile response to 10⁻⁴ M acetylcholine. Drug-treated and control (untreated) tissues were paired to each experiment.
†n, Number of animals used to each experiment.
‡Statistical analysis by Wilcoxon's signed t-test. NS, Not significant.

It seems probable, therefore, that both nedocromil sodium and cromolyn sodium prevent the immediate response by inhibiting the allergen-induced release of anaphylactic mediators. If this is the case, then the protective actions of these compounds on the late response can be easily explained. We have previously suggested that the late response in allergic sheep is dependent on mechanisms initiated by mediators released during the acute allergic reaction.¹²⁻¹⁴ Thus, pre-treatment with inhaled cromolyn sodium at a dose of 1 mg/kg completely prevented the early and late responses to both inhaled antigen¹ and aerosols of compound 45/80, a nonimmunologic mast cell degranulating agent.¹⁵ These previous results are consistent with the current studies, except that in the present experiments, some sheep were not protected by either drug. This lack of protection may have resulted from the lower doses used in this study (20 mg) when compared to the approximately 30-mg dose used in the previous studies. The rationale to use lower doses of both drugs in the current study was based on preliminary evidence which indicated nedocromil sodium to be more potent than cromolyn sodium. Thus, we expected that the 20-mg dose would be sufficient to separate the effects of the two compounds in vivo; however, this was not the case.

There was some discrepancy between the findings in vivo and those obtained in vitro. In vivo, despite the appearance of a trend in favor of cromolyn sodium against the late response, there were no significant differences in the activity of the two compounds; however, in vitro, nedocromil sodium was effective at doses tenfold lower than cromolyn sodium in modifying the antigen-induced contractile responses. This apparent discrepancy may have resulted either from differences in the in vivo pharmacokinetics of the two compounds or from our failure to perform in vivo dose-response curves against antigen (or both). Thus, it is possible that if a series of experiments were conducted at lower doses, differences between nedocromil sodium and cromolyn sodium may have been seen.

In conclusion, these findings indicate that nedocromil sodium is effective in this model of reversible obstructive disease of the airway, and suggest the potential usefulness of this new compound in the treatment of airway diseases in man. ACKNOWLEDGMENTS: We thank Ms. Yvonne Ortiz for typing the manuscript and Ms. Sharon Manley for her artwork.

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