7.1. Responses of RARS

7.1.1. Group 1: Control Animals

The experimental protocol of this group was performed on 6 RARs recorded from 6 guinea pigs (one fiber in each guinea pig). At the commencement of the experimental protocols, the arterial blood pH, PCO$_2$ and PO$_2$ values in these 6 animals were $7.38 \pm 0.02$, $38.0 \pm 2.0$ mmHg and $110 \pm 5.0$ mmHg respectively. The mean values of tracheal pressure, dynamic compliance and airway resistance were $6.85 \pm 0.48$ cmH$_2$O, $1.503 \pm 0.091$ ml/cmH$_2$O and $0.0416 \pm 0.0027$ cmH$_2$O/s/ml respectively.

The RARs isolated exhibited sparse irregular resting discharge. All of them adapted rapidly to a maintained hyperinflation of the lung and the adaptation index was found to be between 90 and 100 %. In each of the RAR, the resting discharge increased when the lungs were allowed to collapse (by disconnecting the animal from the ventilator) or on removal of positive end expiratory pressure (PEEP).

Of these six fibers, five of them were located within 0.5 cm from the hilum of the lung and one fiber was located within 1 cm from the hilum of the lung.

In each animal, before administering the drug, the receptor activity was counted for the first 10 breaths, averaged and expressed as impulses/breath. This averaged value was taken as the basal activity in the control period. After starting the inhalation (drug/saline), the receptor activity was counted for 100 breaths, averaged and expressed as impulses/breath. This served as the activity during the experimental period. Airway mechanics was analyzed in a similar fashion.

The changes in the RAR activity and airway mechanics to the protocols performed in Groups 2, 3 and 4 were also analyzed in a similar fashion.
7.1.1.1. Effect of Histamine on RARs

The basal RAR activity in this group was 0.45 ± 0.26 impulses/breath. After inhalation with normal saline, there was no change in RAR activity and it remained as 0.45 ± 0.25 impulses/breath. When histamine was given as inhalation in doubling doses, there was significant stimulation of RARs with the higher doses.

**Fig. 4:** Effects of inhalation of normal saline and different doses of histamine on RAR activity in Group 1. TP- tracheal pressure, AP action potential.
The basal RAR activities during the control period before inhalation of 0.04, 0.08, 0.16 and 0.32 mg/ml of histamine were $0.46 \pm 0.18$, $0.38 \pm 0.16$, $0.47 \pm 0.28$ and $0.43 \pm 0.11$ impulses/breath respectively. The corresponding RAR activities after histamine inhalation were $0.54 \pm 0.31$, $0.49 \pm 0.24$, $0.87 \pm 0.44$ and $4.37 \pm 1.47$ impulses/breath respectively. An interval of 15 min was given between two successive doses to avoid tachyphylaxis and for allowing the recovery of RARs. An example is shown in Fig. 4 and the results are summarized in Fig. 5.

In this Group, the 50 % increase in airway resistance ($ED_{50}$) was observed at the histamine dose of 0.32 mg/ml in 4 animals and the airway resistance increased from $0.0433 \pm 0.0024$ to $0.0625 \pm 0.0038$ cmH$_2$O/s/ml. At this dose alone, there was a significant increase in the RAR activity compared to its corresponding control value (Fig. 5, $p<0.05$). In the remaining 2 animals, $ED_{50}$ was achieved at the histamine dose of 0.64 mg/ml. In these 2, the airway resistance increased from $0.0475 \pm 0.0075$ to $0.0800 \pm 0.0200$ cmH$_2$O/s/ml and the RAR activity increased from $0.57 \pm 0.28$ to $3.56 \pm 2.30$ impulses/breath (Fig. 5).

![Fig. 5](image_url): Changes in the rapidly adapting receptor (RAR) activity with successive doses of histamine in Group 1. (The effect of 0.64 mg/ml of histamine was examined on 2 RARs only. Please see text for details). C = Control, H = Histamine dose (mg/ml), $* = p<0.05$, compared to the corresponding control.

7.1.1.2. Changes in Airway Mechanics

Histamine inhalation produced an increase in tracheal pressure, a decrease in dynamic compliance and an increase in airway resistance. The basal values of tracheal
Results

Tracheal pressure, dynamic compliance and airway resistance were 6.85 ± 0.48 cmH$_2$O, 1.503 ± 0.091 ml/cmH$_2$O and 0.0416 ± 0.0027 cmH$_2$O/s/ml respectively. After saline inhalation, these parameters did not change and the respective values were 6.95± 0.48 cmH$_2$O, 1.487±0.087 ml/cm H$_2$O and 0.0416 ± 0.0027 cmH$_2$O/s/ml. Subsequently, when histamine inhalation was given in doubling doses, the tracheal pressure and airway resistance increased significantly at the dose of 0.32 mg/ml only (p<0.05). There was a significant fall in the dynamic compliance also, at this dose. The results are presented in Table 2.

Table 2: Effects of histamine on tracheal pressure, dynamic compliance and airway resistance in Group 1.

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<th>Parameters</th>
<th>Histamine (mg/ml)</th>
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<th>Experimental Period</th>
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<td>Dynamic compliance (ml/cmH$_2$O)</td>
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<tr>
<td>0.32</td>
<td>1.430±0.092</td>
<td>1.267± 0.088**</td>
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<td>Airway Resistance (cmH$_2$O/s/ml)</td>
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<td>0.64</td>
<td>0.0475±0.0075</td>
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<td></td>
</tr>
</tbody>
</table>

n= 6 for all the doses and n=2 for the dose of 0.64 mg/ml (please see text for details).

*= p<0.05, **= p<0.01, compared to the corresponding control.

There was a positive correlation between RAR activity and tracheal pressure with increasing doses of histamine. However, it was not significant (Pearson r = 0.8473, p>0.05). There was a negative correlation between RAR activity and dynamic compliance with increasing doses of histamine. However, it was not significant (Pearson r = -0.8003, p>0.05). There was a positive correlation between RAR activity...
and airway resistance with increasing doses of histamine. However, it was not significant (Pearson $r = 0.9028$, $p > 0.05$).

### 7.1.2. Group 2a (Early Asthmatic Response) – Animals Sensitized and Challenged with Ovalbumin

The experimental protocol of this group was performed on 6 RARs recorded from 6 guinea pigs (one fiber in each guinea pig). At the commencement of the experimental protocols, the arterial blood pH, PCO$_2$ and PO$_2$ values in these 6 animals were $7.39 \pm 0.01$, $39.0 \pm 1.0$ mmHg and $114 \pm 4.0$ mmHg respectively. The mean values of tracheal pressure, dynamic compliance and airway resistance were $9.3 \pm 0.60$ cmH$_2$O, $1.258 \pm 0.124$ ml/cmH$_2$O and $0.0525 \pm 0.0044$ cmH$_2$O/s/ml respectively.

Of these six fibers, four of them were located within $0.5$ cm from the hilum of the lung and two fibers were located within $2$ cm from the hilum of the lung.

#### 7.1.2.1. Effect of Histamine on RARs

The basal RAR activity before ovalbumin challenge was $4.91 \pm 1.27$ impulses/breath. After ovalbumin challenge, the RAR activity increased significantly to $25.36 \pm 5.01$ impulses/breath ($p < 0.01$). After 30 minutes, there was complete recovery and the RAR activity returned back to basal value. When histamine was given as inhalation in doubling doses, there was significant stimulation of RARs. The basal RAR activities during the control period before inhalation of 0.04 and 0.08 of histamine were $4.63 \pm 0.71$ and $5.05 \pm 0.60$ impulses/breath respectively. The corresponding RAR activities after histamine inhalation were $8.87 \pm 1.66$ and $13.71 \pm 3.64$ impulses/breath respectively. An interval of 15 min was given between two successive doses to avoid tachyphylaxis and for allowing the recovery of RARs. An example is shown in Fig. 6 and the results are summarized in Fig. 7.

In this group, following ovalbumin challenge, the airway resistance increased significantly from $0.0525 \pm 0.0044$ to $0.0850 \pm 0.0071$ cmH$_2$O/s/ml ($p < 0.001$), an increase by more than $50\%$, suggesting the early asthmatic response. Simultaneously, there was a significant increase in RAR activity. After 30 minutes, there was recovery and the airway resistance was $0.0541 \pm 0.0037$ cmH$_2$O/s/ml. In this background, the
50% rise in the airway resistance to histamine (ED$_{50}$) was achieved at the histamine dose of 0.08 mg/ml in 4 animals (Fig. 7). At this dose, there was a significant increase in the RAR activity (p<0.05).

In the remaining 2 animals, the ED$_{50}$ was achieved at the histamine dose of 0.16 mg/ml. In these 2, the airway resistance increased from 0.0600 ± 0.02 to 0.100 ± 0.02 cmH$_2$O/s/ml and the RAR activity increased from 4.34 ± 0.05 to 10.54± 1.25 impulses/breath (Fig. 7). In this group, even a lower dose of 0.04 mg/ml produced a significant increase in the activity of all the six RARs.

**Fig. 6:** Effects of ovalbumin challenge and inhalation of different doses of histamine on RAR activity in Group 2a. TP- tracheal pressure, AP action potential, Ova- Ovalbumin challenge.
Results

Fig. 7: Changes in the rapidly adapting receptor (RAR) activity with successive doses of histamine in Group 2a. (The effect of 0.16 mg/ml of histamine was examined on 2 RARs only. Please see text for details). C = Control, H = Histamine dose (mg/ml), Ova = Ovalbumin, *= p<0.05, **= p<0.01, compared to the corresponding control.

7.1.2.2. Changes in Airway Mechanics

Histamine inhalation produced an increase in tracheal pressure, a decrease in dynamic compliance and an increase in airway resistance. The basal values of tracheal pressure, dynamic compliance and airway resistance were 9.3 ± 0.60 cmH$_2$O, 1.258 ± 0.124 ml/cmH$_2$O and 0.0525 ± 0.0044 cmH$_2$O/s/ml respectively. After ovalbumin challenge, tracheal pressure increased significantly to 11.47 ± 0.87 cmH$_2$O (p<0.01), dynamic compliance decreased significantly to 0.956 ± 0.054 ml/cm H$_2$O (p<0.05) and airway resistance increased significantly to 0.0850 ± 0.0071 cmH$_2$O/s/ml (p<0.001). After 30 minutes, there was complete recovery. Subsequently, when histamine inhalation was given in doubling doses, the tracheal pressure and airway resistance increased significantly at 0.04 and 0.08 mg/ml doses of histamine. There was a significant fall in the dynamic compliance also, at these doses. The results are presented in Table 3.

The RAR activity showed a positive correlation with the tracheal pressure with increasing doses of histamine and the correlation was significant (Pearson r= 0.9989, p<0.05).
Results

Table 3: Effects of histamine on tracheal pressure, dynamic compliance and airway resistance in Group 2a.

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<thead>
<tr>
<th>Parameters</th>
<th>Histamine (mg/ml)</th>
<th>Control Period</th>
<th>Experimental Period</th>
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<td>Tracheal pressure (cmH₂O)</td>
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<td>0.04</td>
<td>9.41± 0.63</td>
<td>10.07± 0.65**</td>
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<td>0.08</td>
<td>9.50± 0.64</td>
<td>11.13± 0.84***</td>
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<td>0.16</td>
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<td>10.40± 0.70</td>
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<tr>
<td>Dynamic compliance (ml/cmH₂O)</td>
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<td>0.08</td>
<td>1.170±0.1021</td>
<td>0.995±0.0671**</td>
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<td>Airway resistance (cmH₂O/s/ml)</td>
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<td>0.16</td>
<td>0.0600±0.02</td>
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</table>

n= 6 for all the doses and n=2 for the dose of 0.16 mg/ml (please see text for details). *= p<0.05, ** = p<0.01, *** = p<0.001, compared to the corresponding control.

There was a significant negative correlation between RAR activity and dynamic compliance with increasing doses of histamine (Pearson r = -0.9808, p<0.05). Additionally, there was a significant positive correlation between RAR activity and airway resistance with increasing doses of histamine (Pearson r = 0.9842, p<0.05).

7.1.2.3. Comparison between Group 1 and Group 2a

7.1.2.3.1. Responses of RARs

After sensitization with ovalbumin, the basal RAR activity in Group 2a was 4.91 ± 1.27 impulses/breath which was significantly higher than that in Group 1 (0.45 ± 0.26 impulses/breath) (p<0.01). After ovalbumin challenge, the RAR activity increased to 25.36 ± 5.01 impulses/breath in Group 2a, which was significantly higher (p<0.001) than the RAR activity after saline inhalation in Group 1 (0.45 ± 0.25 impulses/breath) (Fig. 8).
Results

![Graph](https://via.placeholder.com/150)

**Fig. 8:** Changes in rapidly adapting receptor (RAR) activity to saline inhalation and ovalbumin challenge. Ova - ovalbumin challenge, ***= p<0.001, compared to Group 1.

The ED$_{50}$ was found to be reduced significantly in Group 2a as compared to that in Group 1, the respective doses of histamine being 0.10 ± 0.01 mg/ml and 0.42 ± 0.06 mg/ml (p<0.001) (Fig. 9).

![Graph](https://via.placeholder.com/150)

**Fig. 9:** Effective histamine dose required to increase airway resistance by 50 % (ED$_{50}$) in Groups 1 and 2a. ***= p<0.001, compared to that in Group 1.

At the ED$_{50}$ dose, even though the RARs were stimulated in both the groups, the stimulation in Group 2a was significantly higher compared to that in Group 1 (14.40 ± 3.47 vs. 5.21 ± 1.30 impulses/breath, p<0.05) (Fig. 10).
Fig. 10: Rapidly adapting receptor (RAR) activity at ED_{50} histamine doses in Groups 1 and 2a. *= p<0.05, compared to Group 1.

### 7.1.2.3.2. Changes in Airway Mechanics

After sensitization with ovalbumin, the basal tracheal pressure in Group 2a was 9.30 ± 0.60 cmH\textsubscript{2}O, which was significantly higher than the basal tracheal pressure of 6.85 ± 0.48 cmH\textsubscript{2}O in Group 1 (p<0.05). After ovalbumin challenge, the tracheal pressure increased to 11.47 ± 0.87 cmH\textsubscript{2}O in Group 2a, which was significantly higher than that after saline inhalation (6.95 ± 0.48 cmH\textsubscript{2}O) in Group 1 (p<0.01).

At the ED_{50} histamine dose (0.10 ± 0.01 mg/ml) in Group 2a, the tracheal pressure increased to 11.28 ± 0.81 cmH\textsubscript{2}O which was significantly higher than the increase in tracheal pressure to 8.63 ± 0.57 cmH\textsubscript{2}O for the ED_{50} histamine dose (0.42 ± 0.06 mg/ml) in Group 1 (p<0.05).

After sensitization with ovalbumin, the basal dynamic compliance in Group 2a was 1.258 ± 0.124 ml/cmH\textsubscript{2}O. Even though less, it was not significantly different when compared to that (1.503 ± 0.091 ml/cmH\textsubscript{2}O) in Group 1. After ovalbumin challenge, the dynamic compliance decreased to 0.956 ± 0.054 ml/cmH\textsubscript{2}O in Group 2a, which was significantly lower (p<0.001) than the fall in dynamic compliance after saline inhalation in Group 1, i.e., 1.487 ± 0.087 ml/cmH\textsubscript{2}O.

At the ED_{50} histamine dose (0.10 ± 0.01 mg/ml) in Group 2a, the dynamic compliance decreased to 0.969 ± 0.059 ml/cmH\textsubscript{2}O which was significantly lower than
the decrease in dynamic compliance to $1.220 \pm 0.074$ ml/cmH$_2$O for the ED$_{50}$ histamine dose ($0.42 \pm 0.06$ mg/ml) in Group 1 (p<0.05).

After sensitization with ovalbumin, the basal airway resistance in Group 2a was $0.0525 \pm 0.0044$ cmH$_2$O/s/ml. Even though increased it was not significantly different when compared to that ($0.0416 \pm 0.0027$ cmH$_2$O/s/ml) in Group 1. After ovalbumin challenge, the airway resistance increased to $0.0850 \pm 0.0071$ cmH$_2$O/s/ml in Group 2a, which was significantly higher than that after saline inhalation ($0.0416 \pm 0.0027$ cmH$_2$O) in Group 1 (p<0.01).

At the ED$_{50}$ histamine dose ($0.10 \pm 0.016$ mg/ml) in Group 2a, the airway resistance increased to $0.0841 \pm 0.0084$ cmH$_2$O/s/ml which was significantly higher than the increase in airway resistance to $0.0633 \pm 0.0035$ cmH$_2$O/s/ml for the ED$_{50}$ histamine dose ($0.42 \pm 0.06$ mg/ml) in Group 1 (p<0.05).

**7.1.3. Group 2b (Late Asthmatic Response) – Animals Sensitized and Challenged with Ovalbumin – Response after 24 hr**

The experimental protocol of this group was performed on 6 RARs recorded from 6 guinea pigs (one fiber in each guinea pig). At the commencement of the experimental protocols, the arterial blood pH, PCO$_2$ and PO$_2$ values in these 6 animals were $7.37 \pm 0.02$, $37.0 \pm 2.0$ mmHg and $105 \pm 6.0$ mmHg respectively. The mean values of tracheal pressure, dynamic compliance and airway resistance were $9.26 \pm 1.02$ cmH$_2$O, $1.294 \pm 0.133$ ml/cm H$_2$O and $0.0483 \pm 0.0040$ cmH$_2$O/s/ml respectively.

Of these six fibers, five were located within 0.5 cm from the hilum of the lung and one fiber was located within 2 cm from the hilum of the lung.

**7.1.3.1. Effect of Histamine on RARs**

The basal RAR activities during the control period before inhalation of 0.04, 0.08 and 0.16 mg/ml of histamine were $3.66 \pm 1.81$, $3.64 \pm 1.95$ and $3.94 \pm 2.68$ impulses/breath respectively. The corresponding RAR activities after histamine inhalation were $5.17 \pm 2.20$, $6.64 \pm 2.42$ and $11.69 \pm 2.61$ impulses/breath respectively. An interval of 15 min was given between two successive doses to avoid tachyphylaxis.
and for allowing the recovery of RARs. An example is shown in Fig. 11 and the results are summarized in Fig. 12.

**Fig. 11:** Effects of inhalation of different doses of histamine on RAR activity in Group 2b. TP - tracheal pressure, AP - action potential.

In this Group, the 50% rise in the airway resistance to histamine (ED$_{50}$) was achieved at the histamine dose of 0.16 mg/ml in 4 animals and the airway resistance increased from 0.0483±0.0040 to 0.0717±0.0074 cmH$_2$O/s/ml (p<0.001). At this dose, there was a significant increase in the RAR activity (Fig. 12, p<0.05). In the remaining 2 animals, the ED$_{50}$ was achieved at the dose of 0.32 mg/ml. In these 2, the airway resistance increased from 0.0500±0.0100 to 0.0800 ± 0.0200 cmH$_2$O/s/ml and the RAR activity increased from 4.00 ± 2.70 to 14.94± 7.60 impulses/breath (Fig. 12). In this group, even lower doses of 0.04 and 0.08 mg/ml produced a significant rise in the activity of all the six RARs.
Fig. 12: Changes in the rapidly adapting receptor (RAR) activity with successive doses of histamine in Group 2b. (The effect of 0.32 mg/ml of histamine was examined on 2 RARs only. Please see text for details). C = Control, H = Histamine dose (mg/ml), * = p<0.05, compared to the corresponding control.

7.1.3.2. Changes in Airway Mechanics

Histamine inhalation produced an increase in tracheal pressure, a decrease in dynamic compliance and an increase in airway resistance. The tracheal pressure increased significantly at 0.04, 0.08 and 0.16 mg/ml, and airway resistance increased significantly at 0.08 and 0.16 mg/ml doses of histamine. There was a significant fall in the dynamic compliance also, at these three doses. The results are presented in Table 4.

Table 4: Effects of histamine on tracheal pressure, dynamic compliance and airway resistance in Group 2b.

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</table>

n = 6 for all the doses and n = 2 for the dose of 0.32 mg/ml (please see text for details). * = p<0.05, ** = p<0.01, compared to the corresponding controls.
The RAR activity showed a positive correlation with the tracheal pressure with increasing doses of histamine and the correlation was highly significant (Pearson r = 0.9999, p<0.001). There was a significant negative correlation between RAR activity and dynamic compliance with increasing doses of histamine (Pearson r = -0.9552, p<0.05). Additionally, there was a significant positive correlation between RAR activity and airway resistance with increasing doses of histamine (Pearson r = 0.9505, p<0.05).

**7.1.3.3. Comparison between Group 1 and Group 2b**

**7.1.3.3.1. Responses of RARs**

Twenty four hours after challenge with ovalbumin in guinea pigs sensitized with ovalbumin, the basal RAR activity in Group 2b was 3.66 ± 1.81 impulses/breath which was significantly higher (p<0.05) than that in Group 1 (0.45 ±0.26 impulses/breath). The ED$_{50}$ was found to be reduced significantly in Group 2b as compared to that in Group 1, the respective doses of histamine being 0.20 ± 0.04 mg/ml and 0.42 ± 0.06 mg/ml (p<0.05) (Fig. 13).

![Histamine concentration](image)

**Fig. 13:** Effective histamine dose required to increase airway resistance by 50 % (ED$_{50}$) in Groups 1 and 2b. *= p<0.05 as compared to that in Group 1

At the ED$_{50}$ dose, even though the RAR activity was stimulated in both the groups, the stimulation in Group 2b was significantly higher as compared to that in Group 1 (11.15 ± 2.30 vs. 5.21±1.30 impulses/breath)(p<0.05) (Fig. 14).
Results

Fig. 14: Rapidly adapting receptor (RAR) activity at ED₅₀ histamine doses in Groups 1 and 2b. * = p < 0.05, compared to Group 1

7.1.3.3.2. Changes in Airway Mechanics

Twenty four hours after challenge with ovalbumin, the basal tracheal pressure in Group 2b was 9.26 ± 1.02 cm H₂O, which was significantly higher than the basal tracheal pressure of 6.85 ± 0.48 cm H₂O in Group 1 (p < 0.05). At the ED₅₀ histamine dose (0.20 ± 0.04 mg/ml) in Group 2b, the tracheal pressure increased to 11.55 ± 1.15 cm H₂O which was significantly higher than the increase in tracheal pressure to 8.63 ± 0.57 cm H₂O for the ED₅₀ histamine dose (0.42 ± 0.06 mg/ml) in Group 1 (p < 0.05).

Twenty four hours after challenge with ovalbumin, the basal dynamic compliance in Group 2b was 1.294 ± 0.133 ml/cmH₂O. Even though less, it was not significantly different when compared to that (1.503 ± 0.091 ml/cmH₂O) in Group 1. At the ED₅₀ histamine dose (0.20 ± 0.04 mg/ml) in Group 2b, the dynamic compliance decreased to 1.023 ± 0.106 ml/cmH₂O which was not significantly different than the decrease in dynamic compliance to 1.220 ± 0.074 ml/cmH₂O for the ED₅₀ histamine dose (0.42 ± 0.06 mg/ml) in Group 1.

Twenty four hours after challenge with ovalbumin, the basal airway resistance in Group 2b was 0.0483 ± 0.0040 cmH₂O/s/ml. Even though increased, it was not significantly different when compared to that (0.0416 ± 0.0027 cmH₂O/s/ml) in Group
Results

1. At the ED$_{50}$ histamine dose (0.20 ± 0.04 mg/ml) in Group 2b, the airway resistance increased to 0.0660 ± 0.0767 cmH$_2$O/s/ml which was not significantly different than the increase in airway resistance to 0.0633±0.0035 cmH$_2$O/s/ml for the ED$_{50}$ histamine dose (0.42±0.06 mg/ml) in Group 1.

7.1.4. Group 4a (Antioxidant Supplementation and Early Asthmatic Response) – Animals Fed with Antioxidants, Sensitized and Challenged with Ovalbumin

The experimental protocol of this group was performed on 6 RARs recorded from 6 guinea pigs (one fiber in each guinea pig). At the commencement of the experimental protocols, the arterial blood pH, PCO$_2$ and PO$_2$ values in these 6 animals were 7.35 ± 0.02, 36.0 ± 2.0 mmHg and 114 ± 7.0 mmHg respectively. The mean values of tracheal pressure, dynamic compliance and airway resistance were 7.31 ± 0.64 cmH$_2$O, 1.422 ± 0.140 ml/cmH$_2$O and 0.0416 ± 0.0040 cmH$_2$O/s/ml respectively.

Of these six fibers, four were located within 0.5 cm from the hilum of the lung and two fibers were located within 1 cm from the hilum of the lung.

7.1.4.1. Effect of histamine on RARs

The basal RAR activity before ovalbumin challenge was 2.76 ± 0.64 impulses/breath. After ovalbumin challenge, the RAR activity increased significantly to 4.60 ± 0.62 impulses/breath (p<0.01). After 30 minutes, there was complete recovery and the RAR activity returned back to basal value. When histamine was given as inhalation in doubling doses, there was significant stimulation of RARs with the higher doses. The basal RAR activities during the control period before inhalation of 0.04, 0.08, 0.16 and 0.32 mg/ml of histamine were 2.33 ± 0.47, 2.63 ± 0.55, 2.84 ± 0.46 and 2.61 ± 0.71 impulses/breath respectively. The corresponding RAR activities after histamine inhalation were 2.88 ± 0.43, 3.42 ± 0.51, 3.76 ± 0.63 and 4.93 ± 0.51 impulses/breath respectively. An interval of 15 min was given between two successive doses to avoid tachyphylaxis and for allowing the recovery of RARs. An example is shown in Fig. 15 and the results are summarized in Fig. 16.
**Fig. 15:** Effects of ovalbumin challenge and inhalation of different doses of histamine on RAR activity in Group 4a. TP - tracheal pressure, AP - action potential, Ova - Ovalbumin challenge.
Results

In this group, after ovalbumin challenge, the airway resistance increased significantly from $0.0416 \pm 0.0040$ to $0.0633 \pm 0.0055$ cmH$_2$O/s/ml ($p<0.01$), an increase by more than 50%, suggesting the early asthmatic response. Simultaneously, there was a significant increase in RAR activity. After 30 minutes, there was complete recovery and the airway resistance was $0.0433 \pm 0.0042$ cmH$_2$O/s/ml. In this background, the 50% rise in the airway resistance to histamine ($ED_{50}$) was achieved at the histamine dose of 0.32 mg/ml in all the 6 animals (Fig. 16). At this dose, there was a significant increase in the RAR activity. In this group, even a lower dose of 0.16 mg/ml produced a significant increase in the activity of all the six RARs.

7.1.4.2. Changes in Airway Mechanics

Histamine inhalation produced an increase in tracheal pressure, a decrease in dynamic compliance and an increase in airway resistance. The basal values of tracheal pressure, dynamic compliance and airway resistance were $7.31 \pm 0.64$ cmH$_2$O, $1.422 \pm 0.140$ ml/cm H$_2$O and $0.0416 \pm 0.0040$ cmH$_2$O/s/ml respectively. After ovalbumin challenge, tracheal pressure increased significantly to $8.65 \pm 0.70$ cmH$_2$O ($p<0.01$), dynamic compliance decreased significantly to $1.228 \pm 0.136$ ml/cmH$_2$O ($p<0.05$) and airway resistance increased significantly to $0.0633 \pm 0.0055$ cmH$_2$O/s/ml ($p<0.001$). After 30 minutes, there was complete recovery. Subsequently, when histamine
Results

Inhalation was given in doubling doses, the tracheal pressure increased significantly at 0.16 and 0.32 mg/ml doses of histamine and airway resistance increased significantly at 0.32 dose of histamine. There was a significant fall in the dynamic compliance also, at these doses. The results are presented in Table 5.

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<td>0.0620±0.0048***</td>
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</table>

n = 6 for all the doses. * = p<0.05, ** = p<0.01, *** = p<0.001, compared to the corresponding control.

The RAR activity showed a positive correlation with the tracheal pressure with increasing doses of histamine and the correlation was significant (Pearson r= 0.9549, p<0.05). There was a significant negative correlation between RAR activity and dynamic compliance with increasing doses of histamine (Pearson r = -0.9741, p<0.01). Additionally, there was a significant positive correlation between RAR activity and airway resistance with increasing doses of histamine (Pearson r = 0.9864, p<0.01).

7.1.4.3. Comparison between Group 1 and Group 4a

7.1.4.3.1. Responses of RARs

After sensitization with ovalbumin, the basal RAR activity in Group 4a was 2.76 ± 0.64 impulses/ breath which was significantly higher than that in Group 1 (0.45 ± 0.26 impulses/breath) (p<0.01). After ovalbumin challenge, the RAR activity increased to
4.60 ± 0.62 impulses/breath in Group 4a, which was significantly higher (p<0.001) than the RAR activity after saline inhalation in Group 1 (0.45 ± 0.25 impulses/breath) (Fig. 17).

![Fig. 17: Changes in rapidly adapting receptor (RAR) activity to saline inhalation and ovalbumin challenge (Ova). ***= p<0.001, compared to Group 1.]

Even though less, the ED\textsubscript{50} was not significantly different in Group 4a as compared to that in Group 1, the respective doses of histamine being 0.29 ± 0.02 mg/ml and 0.42 ± 0.06 mg/ml (Fig. 18).

![Fig. 18: Changes in histamine dose required to increase airway resistance by 50 % (ED\textsubscript{50}) in Groups 1 and 4a.]

At the ED\textsubscript{50} dose, even though the RARs were stimulated in both the groups, the stimulation in Group 4a was not significantly different compared to that in Group 1 (5.10 ± 0.45 vs. 5.21 ± 1.30 impulses/breath) (Fig. 19).
7.1.4.3.2. Changes in Airway Mechanics

After sensitization with ovalbumin, the basal tracheal pressure in Group 4a was 7.31 ± 0.64 cmH$_2$O, which was not significantly different from the basal tracheal pressure of 6.85 ± 0.48 cmH$_2$O in Group 1. After ovalbumin challenge, the tracheal pressure increased to 8.81 ± 0.67 cmH$_2$O in Group 4a, which was significantly higher than that after saline inhalation (6.95 ± 0.48 cmH$_2$O) in Group 1 (p<0.05).

At the ED$_{50}$ histamine dose (0.29 ± 0.02 mg/ml) in Group 4a, the tracheal pressure increased to 8.53 ± 0.75 cmH$_2$O which was not significantly different from the increase in tracheal pressure to 8.63±0.57 cmH$_2$O for the ED$_{50}$ histamine dose (0.42±0.06 mg/ml) in Group 1.

After sensitization with ovalbumin, the basal dynamic compliance in Group 4a was 1.422 ± 0.140 ml/cmH$_2$O. Even though less, it was not significantly different when compared to that (1.503 ± 0.0912 ml/cmH$_2$O) in Group 1. After ovalbumin challenge, the dynamic compliance decreased to 1.228 ± 0.136 ml/cmH$_2$O in Group 4a, which was not significantly different from the change in dynamic compliance after saline inhalation in Group 1, i.e., 1.477 ± 0.087 ml/cmH$_2$O.

At the ED$_{50}$ histamine dose (0.29 ± 0.02 mg/ml) in Group 4a, the dynamic compliance decreased to 1.200 ± 0.134 ml/cmH$_2$O which was not significantly different
from the decrease in dynamic compliance to $1.220 \pm 0.074$ ml/cmH$_2$O for the ED$_{50}$ histamine dose ($0.42 \pm 0.06$ mg/ml) in Group 1.

After sensitization with ovalbumin, the basal airway resistance in Group 4a was $0.0416 \pm 0.0040$ cmH$_2$O/s/ml, which was not significantly different from the basal airway resistance of $0.0416 \pm 0.0027$ cmH$_2$O/s/ml in Group 1. After ovalbumin challenge, the airway resistance increased to $0.0633 \pm 0.0055$ cmH$_2$O/s/ml in Group 4a, which was significantly higher than that after saline inhalation ($0.0416 \pm 0.0027$ cmH$_2$O/s/ml) in Group 1 ($p<0.01$).

At the ED$_{50}$ histamine dose ($0.29 \pm 0.02$ mg/ml) in Group 4a, the airway resistance increased to $0.0666 \pm 0.0061$ cmH$_2$O/s/ml which was not significantly different from the increase in airway resistance to $0.0633 \pm 0.0035$ cmH$_2$O/s/ml for the ED$_{50}$ histamine dose ($0.42 \pm 0.06$ mg/ml) in Group 1.

### 7.1.4.4. Comparison between Group 2a and Group 4a

#### 7.1.4.4.1. Responses of RARs

After sensitization with ovalbumin, the basal RAR activity in Group 2a was $4.91 \pm 1.27$ impulses/breath which was not significantly different than that in Group 4a ($2.76 \pm 0.64$ impulses/breath). After ovalbumin challenge, the RAR activity increased to $25.36 \pm 5.01$ impulses/breath in Group 2a, which was significantly higher ($p<0.01$) than the increase in RAR activity to $4.60 \pm 0.62$ impulses/breath in Group 4a (Fig. 20).

![Fig. 20: Changes in rapidly adapting receptor (RAR) activity following ovalbumin challenge in Group 2a and 4a. **= p<0.01, compared to Group 4a.](image-url)
The ED$_{50}$ was found to be reduced significantly in Group 2a as compared to that in Group 4a (p<0.01), the respective doses of histamine being 0.10 ± 0.01 mg/ml and 0.29 ± 0.02 mg/ml (Fig. 21).

**Fig. 21:** Changes in histamine dose required to increase airway resistance by 50 % (ED$_{50}$) in Groups 2a and 4a. **= p<0.01, compared to Group 4a

At the ED$_{50}$ dose, even though the RARs were stimulated in both the groups, the stimulation in Group 2a was significantly higher compared to that in Group 4a (14.40 ± 3.47 vs., 5.10 ± 0.45 impulses/breath, p<0.05) (Fig. 22).

**Fig. 22:** Rapidly adapting receptor (RAR) activity at histamine doses required to increase airway resistance by 50 % (ED$_{50}$) in Groups 2a and 4a. * = p<0.05, compared to Group 4a.
7.1.4.4.2. Changes in Airway Mechanics

After sensitization with ovalbumin, the basal tracheal pressure in Group 2a was 9.30 ± 0.60 cmH₂O, which was significantly higher from the basal tracheal pressure of 7.31 ± 0.64 cmH₂O in Group 4a (p<0.05). After ovalbumin challenge, the tracheal pressure increased to 11.47 ± 0.87 cmH₂O in Group 2a, which was significantly higher than the increase in tracheal pressure (8.65 ± 0.70 cmH₂O) in Group 4a (p<0.05).

At the ED₅₀ histamine dose (0.10 ± 0.01 mg/ml) in Group 2a, tracheal pressure increased to 11.28 ± 0.81 cmH₂O which was significantly higher than the increase in tracheal pressure to 8.53 ± 0.75 cmH₂O for the ED₅₀ histamine dose (0.29 ± 0.02 mg/ml) in Group 4a (p<0.05).

After sensitization with ovalbumin, the basal dynamic compliance in Group 2a was 1.258 ± 0.124 ml/cmH₂O. Even though less, it was not significantly different when compared to that (1.422 ± 0.140 ml/cmH₂O) in Group 4a. After ovalbumin challenge, the dynamic compliance decreased to 0.956 ± 0.054 ml/cmH₂O in Group 2a, which was not significantly different from the change in dynamic compliance in Group 4a, i.e., 1.228 ± 0.136 ml/cmH₂O.

At the ED₅₀ histamine dose (0.10 ± 0.01 mg/ml) in Group 2a, the dynamic compliance decreased to 0.969 ± 0.059 ml/cmH₂O which was not significantly different from the decrease in dynamic compliance to 1.200 ± 0.1340 ml/cmH₂O for the ED₅₀ histamine dose (0.29 ± 0.02 mg/ml) in Group 4a.

After sensitization with ovalbumin, the basal airway resistance in Group 2a was 0.0525 ± 0.0044 cmH₂O/s/ml. Even though increased it was not significantly different when compared to that (0.0416 ± 0.0040) cmH₂O/s/ml in Group 4a. After ovalbumin challenge, the airway resistance increased to 0.0850 ± 0.0071 cmH₂O/s/ml in Group 2a, which was significantly higher than that (0.0633 ± 0.0055 cmH₂O/s/ml) in Group 4a(p<0.05).

At the ED₅₀ histamine dose (0.10 ± 0.01 mg/ml) in Group 2a, the airway resistance increased to 0.0841 ± 0.0084 cmH₂O/s/ml which was not significantly different from the increase in airway resistance to 0.0666 ± 0.0061 cmH₂O/s/ml for the ED₅₀ histamine dose (0.29 ± 0.02 mg/ml) in Group 4a.
7.1.5. **Group 4b (Antioxidant Supplementation and Late Asthmatic Response) – Animals Fed with Antioxidants, Sensitized and Challenged with Ovalbumin**

The experimental protocol of this group was performed on 6 RARs recorded from 6 guinea pigs (one fiber in each guinea pig). At the commencement of the experimental protocols, the arterial blood pH, PCO$_2$ and PO$_2$ values in these 6 animals were $7.38 \pm 0.02$, $38.0 \pm 1.0$ mmHg and $108 \pm 5.0$ mmHg respectively. The mean values of tracheal pressure, dynamic compliance and airway resistance were $7.93 \pm 0.53$ cmH$_2$O, $1.342 \pm 0.109$ ml/cmH$_2$O and $0.0433 \pm 0.0024$ cmH$_2$O/s/ml respectively.

Of these six fibers, five were located within 0.5 cm from the hilum of the lung and one fiber was located within 2 cm from the hilum of the lung.

**7.1.5.1. Effect of Histamine on RARs**

The basal RAR activities during the control period before inhalation of 0.04, 0.08, 0.16 and 0.32 mg/ml of histamine were $1.36 \pm 1.01$, $1.41 \pm 1.06$, $1.51 \pm 1.07$ and $1.65 \pm 1.21$ impulses/breath respectively. The corresponding RAR activities after histamine inhalation were $1.78 \pm 1.30$, $2.13 \pm 1.47$, $2.81 \pm 1.49$ and $3.99 \pm 1.58$ impulses/breath respectively. An interval of 15 min was given between two successive doses to avoid tachyphylaxis and for allowing the recovery of RARs. An example is shown in Fig. 23 and the results are summarized in Fig. 24.

In this Group, the 50% rise in the airway resistance to histamine (ED$_{50}$) was achieved at the histamine dose of 0.32 mg/ml in 4 animals and the airway resistance increased from $0.0442 \pm 0.0023$ to $0.0633 \pm 0.0051$ cmH$_2$O/s/ml in them. At this dose, there was a significant increase in the RAR activity ($p<0.05$). In the remaining 2 animals, the ED$_{50}$ was achieved at the dose of 0.64 mg/ml. In these 2, the airway resistance increased from $0.0400$ to $0.0600$ cmH$_2$O/s/ml and the RAR activity increased from $4.35 \pm 3.50$ to $7.98 \pm 4.84$ impulses/breath (Fig. 24). In this group, even lower dose of 0.16 mg/ml produced a significant increase in the activity of all the six RARs.
Histamine inhalation produced an increase in tracheal pressure, a decrease in dynamic compliance and an increase in airway resistance. The tracheal pressure increased significantly at 0.08, 0.16 and 0.32 mg/ml, and airway resistance increased significantly at 0.16 and 0.32 mg/ml doses of histamine. There was a significant fall in the dynamic compliance also, at these three doses. The results are presented in Table 6.
Results

Fig. 24: Changes in the rapidly adapting receptor (RAR) activity with successive doses of histamine in Group 4b. (The effect of 0.64 mg/ml of histamine was examined on 2 RARs only. Please see text for details). C = Control, H = Histamine dose (mg/ml), * = p<0.05, compared to the corresponding control.

Table 6: Effects of histamine on tracheal pressure, dynamic compliance and airway resistance in Group 4b.

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<th>Parameters</th>
<th>Histamine (mg/ml)</th>
<th>Control Period</th>
<th>Experimental Period</th>
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n= 6 for all the doses and n=2 for the dose of 0.64 mg/ml (please see text for details). * = p<0.05, **= p<0.01, compared to the corresponding control.
The RAR activity showed a positive correlation with the tracheal pressure with increasing doses of histamine and the correlation was significant (Pearson $r = 0.9884$, $p<0.05$). There was a significant negative correlation between RAR activity and dynamic compliance with increasing doses of histamine (Pearson $r = -0.9851$, $p<0.01$). Additionally, there was a positive correlation between RAR activity and airway resistance with increasing doses of histamine (Pearson $r = 0.9840$, $p<0.01$).

### 7.1.5.2. Comparison between Group 1 and Group 4b

#### 7.1.5.2.1. Responses of RARs

Twenty four hours after challenge with ovalbumin in guinea pigs previously sensitized with ovalbumin and fed with antioxidants, the basal RAR activity in Group 4b was $1.36 \pm 1.01$ impulses/breath which was not significantly different from that in Group 1 ($0.45 \pm 0.26$ impulses/breath). The $ED_{50}$ in Group 4b was $0.42 \pm 0.06$ mg/ml which was similar to that in Group 1 ($0.42 \pm 0.06$ mg/ml) (Fig. 25).

**Fig. 25:** Effective histamine dose required to increase airway resistance by 50% ($ED_{50}$) in Groups 1 and 4b.

At the $ED_{50}$ dose, even though the RARs were stimulated in both the groups, the stimulation in Group 4b was not significantly different compared to that in Group 1 ($4.58 \pm 1.74$ vs. $5.21 \pm 1.30$ impulses/breath, $p > 0.05$) (Fig. 26).
Results

Fig. 26: Rapidly adapting receptor (RAR) activity at ED$_{50}$ doses in Groups 1 and 4b.

7.1.5.2.2. Changes in Airway Mechanics

Twenty four hours after challenge with ovalbumin, the basal tracheal pressure in Group 4b was 7.93 ± 0.53 cmH$_2$O, which was not significantly different from the basal tracheal pressure of 6.85 ± 0.48 cmH$_2$O in Group 1. At the ED$_{50}$ histamine dose (0.42 ± 0.06 mg/ml) in Group 4b, tracheal pressure increased to 9.26 ± 0.58 cmH$_2$O which was not significantly different from the increase in tracheal pressure to 8.63 ± 0.57 cmH$_2$O for the ED$_{50}$ histamine dose (0.42 ± 0.06 mg/ml) in Group 1.

Twenty four hours after challenge with ovalbumin, the basal dynamic compliance in Group 4b was 1.342 ± 0.109 ml/cmH$_2$O. Even though less, it was not significantly different when compared to that (1.503 ± 0.091 ml/cmH$_2$O) in Group 1. At the ED$_{50}$ histamine dose (0.42 ± 0.06 mg/ml) in Group 4b, the dynamic compliance decreased to 1.147 ± 0.085 ml/cmH$_2$O which was not significantly different from the decrease in dynamic compliance to 1.220 ± 0.074 ml/cmH$_2$O for the ED$_{50}$ histamine dose (0.42 ± 0.06 mg/ml) in Group 1.

Twenty four hours after challenge with ovalbumin, the basal airway resistance in Group 4b was 0.0433 ± 0.0024 cmH$_2$O/s/ml. Even though increased it was not significantly different when compared to that (0.0416 ± 0.0027 cmH$_2$O/s/ml) in Group 1. At the ED$_{50}$ histamine dose (0.42 ± 0.06 mg/ml) in Group 4b, the airway resistance
increased to 0.0666 ± 0.0035 cmH\(_2\)O/s/ml which was not significantly different than the increase in airway resistance to 0.0633 ± 0.0035 cmH\(_2\)O/s/ml for the ED\(_{50}\) histamine dose (0.42 ± 0.06 mg/ml) in Group 1.

7.1.5.3. Comparison between Group 2b and Group 4b

7.1.5.3.1. Responses of RARs

Twenty four hours after challenge with ovalbumin in guinea pigs previously sensitized with ovalbumin and fed with normal diet, the basal RAR activity in Group 2b was 3.66 ± 1.81 impulses/breath which was significantly higher from that (1.36 ± 1.01 impulses/breath, p<0.05) in guinea pigs previously sensitized with ovalbumin and fed with antioxidants (Group 4b). The ED\(_{50}\) was found to be reduced significantly in Group 2b as compared to that in Group 4b, the respective doses of histamine being 0.20 ± 0.04 mg/ml and 0.42 ± 0.06 mg/ml (p<0.05) (Fig. 27).

![Graph showing histamine dose vs RAR activity]

**Fig. 27:** Changes in histamine dose required to increase airway resistance by 50 % (ED\(_{50}\)) in Groups 2b and 4b. *=p<0.05, compared to Group 4b.

At the ED\(_{50}\) dose, even though the RARs were stimulated in both the groups, the stimulation in Group 2b was significantly higher as compared to that in Group 4b (11.15 ± 2.30 vs. 4.58 ± 1.74 impulses/breath, p<0.05) (Fig. 28).
Fig. 28: Rapidly adapting receptor (RAR) activity at ED$_{50}$ doses in Groups 2b and 4b. * = p<0.05, compared to Group 4b.

7.1.5.3.2. Changes in Airway Mechanics

Twenty four hours after challenge with ovalbumin, the basal tracheal pressure in Group 2b was 9.66 ± 1.02 cmH$_2$O, which was not significantly different from the basal tracheal pressure of 7.93 ± 0.53 cm H$_2$O in Group 4b. At the ED$_{50}$ histamine dose (0.20 ± 0.04 mg/ml) in Group 2b, tracheal pressure increased to 11.55 ±1.15 cmH$_2$O which was not significantly different than the increase in tracheal pressure to 9.26 ± 0.58 cm H$_2$O for the ED$_{50}$ histamine dose (0.42 ± 0.06 mg/ml) in Group 4b.

Twenty four hours after challenge with ovalbumin, the basal dynamic compliance in Group 2b was 1.294 ± 0.123 ml/cmH$_2$O, which was not significantly different when compared to that (1.342 ± 0.100 ml/cmH$_2$O) in Group 4b. At the ED$_{50}$ histamine dose (0.20 ± 0.04 mg/ml) in Group 2b, the dynamic compliance decreased to 1.023 ± 0.106 ml/cmH$_2$O which was not significantly different from the decrease in dynamic compliance to 1.147 ± 0.085 ml/cmH$_2$O for the ED$_{50}$ histamine dose (0.42 ± 0.06 mg/ml) in Group 4b.

Twenty four hours after challenge with ovalbumin, the basal airway resistance in Group 2b was 0.0483 ± 0.0040 cmH$_2$O/s/ml, which was not significantly different from the basal airway resistance of 0.0433 ± 0.0024 cmH$_2$O/s/ml in Group 4b. At the
ED$_{50}$ histamine dose (0.20 ± 0.04 mg/ml) in Group 2b, the airway resistance increased to 0.0766 ± 0.0080 cmH$_2$O/s/ml which was not significantly different from the increase in airway resistance to 0.0666 ± 0.0035 cmH$_2$O/s/ml for the ED$_{50}$ histamine dose (0.42 ± 0.06 mg/ml) in Group 4b.

7.1.6. Group 3 (Xanthine-Xanthine Oxidase Inhalation) – in vivo Generation of Oxidants

The experimental protocol of this group was performed on 6 RARs recorded from 6 guinea pigs (one fiber in each guinea pig). At the commencement of the experimental protocols, the arterial blood pH, PCO$_2$ and PO$_2$ values in these 6 animals were 7.36 ± 0.01, 36.0 ± 2.0 mmHg and 106 ± 4.0 mmHg respectively. The mean values of tracheal pressure, dynamic compliance and airway resistance were 7.60 ± 0.91 cmH$_2$O, 1.322 ± 0.059 ml/cmH$_2$O and 0.0400 ± 0.0012 cmH$_2$O/s/ml respectively.

Of these six fibers, five were located within 0.5 cm from the hilum of the lung and one fiber was located within 2 cm from the hilum of the lung.

7.1.6.1. Effect of Histamine on RARs

The basal RAR activity before xanthine-xanthine oxidase inhalation was 0.62 ± 0.15 impulses/breath. After xanthine-xanthine oxidase inhalation, the RAR activity increased significantly to 1.93 ± 0.28 impulses/breath (p<0.05). After 30 minutes, there was complete recovery and the RAR activity returned back to basal value. When histamine was given as inhalation in doubling doses, there was significant stimulation of RARs with the higher doses. The basal RAR activities during the control period before inhalation of 0.04, 0.08 and 0.16 mg/ml of histamine were 0.63 ± 0.23, 0.71 ± 0.27 and 0.79 ± 0.35 impulses/breath respectively. The corresponding RAR activities after histamine inhalation were 1.01 ± 0.33, 1.25 ± 0.38 and 2.17 ± 0.60 impulses/breath respectively. An interval of 15 min was given between two successive doses to avoid tachyphylaxis and for allowing the recovery of RARs. An example is shown in Fig. 29 and the results are summarized in Fig. 30.
Results

Fig. 29: Effects of inhalation of xanthine - xanthine oxidase and histamine on RAR activity in Group 3. TP- tracheal pressure, AP action potential, XO – Xanthine xanthine oxidase

In this group, after xanthine-xanthine oxidase inhalation, the airway resistance increased significantly from $0.0400 \pm 0.0012$ to $0.0608 \pm 0.0020$ cmH$_2$O/s/ml ($p<0.001$). Simultaneously, there was a significant increase in RAR activity. After 30 minutes, there was complete recovery and the airway resistance was $0.0400 \pm 0.0013$ cmH$_2$O/s/ml. In this background, the 50% rise in the airway resistance to histamine ($ED_{50}$) was achieved at the dose of $0.016$ mg/ml in 4 animals. At this dose, there was a significant increase in the RAR activity. In the remaining 2 animals, the $ED_{50}$ was
achieved at the dose of 0.32 mg/ml. In these 2, the airway resistance increased from 0.0375±0.0025 to 0.0575±0.0025 cmH\textsubscript{2}O/s/ml and the RAR activity increased from 1.17±0.53 to 4.71±1.68 impulses/breath (Fig. 30).

**Fig. 30:** Changes in the rapidly adapting receptor (RAR) activity with successive doses of histamine in Group 3. (The effect of 0.32 mg/ml of histamine was examined on 2 RARs only. Please see text for details). C = Control, H = Histamine dose (mg/ml), XO = Xanthine-xanthine oxidase, * = p<0.05, compared to the corresponding control.

### 7.1.6.2. Changes in Airway Mechanics

Histamine inhalation produced an increase in tracheal pressure, a decrease in dynamic compliance and an increase in airway resistance. The basal values of tracheal pressure, dynamic compliance and airway resistance were 7.60 ± 0.91 cmH\textsubscript{2}O, 1.322 ± 0.059 ml/cmH\textsubscript{2}O and 0.0400 ± 0.0012 cmH\textsubscript{2}O/s/ml respectively. After xanthine-xanthine oxidase inhalation, tracheal pressure increased significantly to 9.60 ± 1.04 cmH\textsubscript{2}O (p<0.001), dynamic compliance decreased significantly to 0.976 ± 0.049 ml/cmH\textsubscript{2}O (p<0.001) and airway resistance increased significantly to 0.0608 ± 0.0020 cmH\textsubscript{2}O/s/ml (p<0.001). After 30 minutes, there was complete recovery. Subsequently, when histamine inhalation was given in doubling doses, the tracheal pressure and airway resistance increased significantly at the dose of 0.16 mg/ml only (p<0.05). There was a significant fall in the dynamic compliance also at this dose. The results are presented in Table 7.
Table 7: Effects of histamine on tracheal pressure, dynamic compliance and airway resistance in Group 3.

<table>
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<tr>
<th>Parameters</th>
<th>Histamine (mg/ml)</th>
<th>Control Period</th>
<th>Experimental Period</th>
</tr>
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<tbody>
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<td>0.04</td>
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<td>0.08</td>
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<td>9.27±1.0</td>
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<td></td>
<td>0.16</td>
<td>8.60±0.91</td>
<td>9.82±1.10**</td>
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<td>8.90±2.90</td>
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<td>0.04</td>
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<td>0.16</td>
<td>1.224±0.075</td>
<td>1.014±0.059**</td>
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<td>0.32</td>
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<td>Dynamic compliance (ml/cmH₂O)</td>
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<td>0.0575±0.0025</td>
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<tr>
<td>Resistance (cmH₂O/s/ml)</td>
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</tbody>
</table>

n= 6 for all the doses and n = 2 for the dose of 0.32 mg/ml (please see text for details). * - p<0.05, ** - p<0.01, compared to the corresponding control.

The RAR activity showed a positive correlation with the tracheal pressure with increasing doses of histamine. However, the correlation was not significant (Pearson r = 0.9514, p<0.05). The RAR activity showed a negative correlation with the dynamic compliance with increasing doses of histamine. However, the correlation was not significant (Pearson r = -0.9235, p>0.05). The RAR activity showed a significant positive correlation with the airway resistance with increasing doses of histamine (Pearson r = 0.9892, p<0.05).

7.1.6.3. Comparison between Group 1 and Group 3

7.1.6.3.1. Responses of RARs

The basal RAR activity in Group 3 was 0.62 ± 0.15 impulses/breath which was not significantly different from that in Group 1 (0.45 ± 0.26 impulses/breath). After xanthine-xanthine oxidase inhalation, the RAR activity increased to 1.93 ± 0.28 impulses/breath in Group 3, which was significantly higher (p<0.01) than the RAR activity after saline inhalation in Group 1 (0.45 ± 0.25 impulses/breath) (Fig. 31).
The $ED_{50}$ was found to be reduced significantly in Group 3 as compared to that in Group 1, the respective doses of histamine being $0.19 \pm 0.04$ mg/ml and $0.42 \pm 0.06$ mg/ml ($p<0.05$) (Fig. 32).

At the $ED_{50}$ dose, even though the RARs were stimulated in both the groups, the stimulation in Group 3 was not significantly different (impulses/breath) compared to that in Group 1 ($4.27 \pm 1.13$ vs. $5.21 \pm 1.30$ impulses/breath) (Fig. 33).
Fig. 33: Rapidly adapting receptor (RAR) activity at histamine doses required to increase airway resistance by 50% ($ED_{50}$) in Groups 1 and 3.

7.1.6.3.2. Changes in Airway Mechanics

The basal tracheal pressure in Group 3 was 7.60 ± 0.91 cmH$_2$O, which was not significantly different from the basal tracheal pressure of 6.85 ± 0.48 cmH$_2$O in Group 1. After xanthine-xanthine oxidase inhalation, the tracheal pressure increased to 9.60 ± 1.04 cmH$_2$O in Group 3, which was significantly higher than that after saline inhalation (6.95 ± 0.48 cmH$_2$O) in Group 1 ($p<0.05$).

At the $ED_{50}$ histamine dose (0.19 ± 0.04 mg/ml) in Group 3, the tracheal pressure increased to 10.67 ± 1.04 cmH$_2$O which was not significantly different from the increase in tracheal pressure to 8.63 ± 0.57 cmH$_2$O for the $ED_{50}$ histamine dose (0.42 ± 0.06 mg/ml) in Group 1.

The basal dynamic compliance in Group 3 was 1.322 ± 0.059 ml/cmH$_2$O. Even though less, it was not significantly different when compared to that (1.503 ± 0.091 ml/cmH$_2$O) in Group 1. After xanthine-xanthine oxidase inhalation, the dynamic compliance decreased to 0.976 ± 0.049 ml/cmH$_2$O in Group 3, which was significantly lower ($p<0.001$) than the change in dynamic compliance after saline inhalation in Group 1, i.e., 1.477 ± 0.087 ml/cmH$_2$O.

At the $ED_{50}$ histamine dose (0.19 ± 0.04 mg/ml) in Group 3, the dynamic compliance decreased to 0.978 ± 0.045 ml/cmH$_2$O which was significantly lower than
the decrease in dynamic compliance to \(1.220 \pm 0.074 \text{ ml/cmH}_2\text{O}\) for the \(ED_{50}\) histamine dose \((0.42\pm0.06 \text{ mg/ml})\) in Group 1 \((p<0.05)\).

The basal airway resistance in Group 3 was \(0.0400 \pm 0.0012 \text{ cmH}_2\text{O}\), which was not significantly different from the basal airway resistance of \(0.0416 \pm 0.0027 \text{ cmH}_2\text{O}\) in Group 1. After xanthine-xanthine oxidase inhalation, the airway resistance increased to \(0.0608 \pm 0.0020 \text{ cmH}_2\text{O}\) in Group 3, which was significantly higher than that after saline inhalation \((0.0416 \pm 0.0027 \text{ cmH}_2\text{O})\) in Group 1 \((p<0.001)\).

At the \(ED_{50}\) histamine dose \((0.19 \pm 0.04 \text{ mg/ml})\) in Group 3, the airway resistance increased to \(0.0590 \pm 0.0008 \text{ cmH}_2\text{O/s/ml}\) which was not significantly different from the increase in airway resistance to \(0.0633 \pm 0.0035 \text{ cmH}_2\text{O/s/ml}\) for the \(ED_{50}\) histamine dose \((0.42 \pm 0.06 \text{ mg/ml})\) in Group 1.

### 7.2. Responses of SAR

#### 7.2.1. Group 1 – Control Animals

The experimental protocol of this group was performed on 5 SARs recorded from 5 guinea pigs (one fiber in each guinea pig). At the commencement of the experimental protocols, the arterial blood pH, \(PCO_2\) and \(PO_2\) values in these 5 animals were \(7.39 \pm 0.01, 39.0 \pm 1.0 \text{ mmHg}\) and \(108 \pm 4.0 \text{ mmHg}\) respectively. The mean values of tracheal pressure, dynamic compliance and airway resistance were \(6.41 \pm 0.38 \text{ cmH}_2\text{O}, 1.516 \pm 0.182 \text{ ml/cmH}_2\text{O}\) and \(0.0390 \pm 0.0024 \text{ cmH}_2\text{O/s/ml}\) respectively.

The SARs isolated exhibited regular respiratory rhythmic resting discharge. All of them adapted slowly to a maintained hyperinflation of the lung. Of these five fibers, three were located within 1 cm from the hilum of the lung and two were located within 2 cm from the hilum of the lung.

In each animal, before administering the drug, the receptor activity was counted for the first 10 breaths, averaged and expressed as impulses/breath. This averaged value was taken as the basal activity in the control period. After starting the inhalation (drug/saline), the receptor activity was counted for 100 breaths, averaged and expressed as impulses/breath. This served as the activity during the experimental period. Airway mechanics was also analyzed similarly.
The changes in the SAR activity and airway mechanics to the protocols performed in Groups 2, 3 and 4 were also analyzed in a similar fashion.

7.2.1.1. Effects of Histamine on SARs

The basal SAR activity in this group was 50.02 ± 3.20 impulses/breath. After inhalation with normal saline, there was no change in SAR activity and it remained as 50.35 ± 3.19 impulses/breath. When histamine was given as inhalation in doubling doses, there was significant stimulation of SARs only with the highest dose administered. The basal SAR activities during the control period before inhalation of 0.04, 0.08, 0.16 and 0.32 mg/ml of histamine were 50.18 ± 3.18, 50.26 ± 3.19, 50.42 ± 3.13 and 50.62 ± 3.08 impulses/breath respectively. The corresponding SAR activities after histamine inhalation were 51.02 ± 3.15, 51.56 ± 3.13, 52.78 ± 3.15 and 55.29 ± 3.23 impulses/breath respectively. An interval of 15 min was given between two successive doses to avoid tachyphylaxis and for allowing the recovery of SARs. The results are summarized in Fig. 34.

Fig. 34: Changes in the slowly adapting receptor (SAR) activity with successive doses of histamine in Group 1. C = Control, H = Histamine dose (mg/ml), ** = p<0.01, compared to the corresponding control.

In this Group, the 50 % increase in airway resistance (ED$_{50}$) was observed at the histamine dose of 0.32 mg/ml in all the 5 animals and the airway resistance increased
from \(0.0410 \pm 0.0040\) to \(0.060 \pm 0.0031\) cmH\(_2\)O/s/ml. At this dose alone, there was a significant increase in the SAR activity compared to its corresponding control value (Fig. 34, \(p<0.01\)).

### 7.2.1.2. Changes in Airway Mechanics

Histamine inhalation produced an increase in tracheal pressure, a decrease in dynamic compliance and an increase in airway resistance. The basal values of tracheal pressure, dynamic compliance and airway resistance were \(6.41 \pm 0.38\) cmH\(_2\)O, \(1.516 \pm 0.182\) ml/cmH\(_2\)O and \(0.0390 \pm 0.0024\) cmH\(_2\)O/s/ml respectively. After saline inhalation, these parameters did not change and the respective values were \(6.44 \pm 0.39\) cmH\(_2\)O, \(1.488 \pm 0.180\) ml/cm H\(_2\)O and \(0.0390 \pm 0.0024\) cmH\(_2\)O/s/ml.

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<th>Parameters</th>
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<th>Control Period</th>
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<td>6.58(\pm) 0.38</td>
<td>7.52(\pm) 0.32*</td>
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<td></td>
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<td>0.0410(\pm) 0.0040</td>
<td>0.0600(\pm) 0.0031**</td>
</tr>
</tbody>
</table>

\(n=5\) for all the doses. \(*= p<0.05, **= p<0.01,\) compared to the corresponding control.

Subsequently, when histamine inhalation was given in doubling doses, the tracheal pressure and airway resistance increased significantly at the dose of \(0.32\) mg/ml only. There was a significant fall in the dynamic compliance also, at this dose. The results are presented in Table 8.
Results

There was a significant positive correlation between SAR activity and tracheal pressure with increasing doses of histamine (Pearson $r = 0.9878$, $p<0.01$). There was a significant negative correlation between SAR activity and dynamic compliance with increasing doses of histamine (Pearson $r = -0.9526$, $p<0.05$). There was a significant positive correlation between SAR activity and airway resistance with increasing doses of histamine (Pearson $r = 0.9809$, $p<0.01$).

7.2.2. Group 2a (Early Asthmatic Response) – Animals Sensitized and Challenged with Ovalbumin

The experimental protocol of this group was performed on 5 SARs recorded from 5 guinea pigs (one fiber in each guinea pig). At the commencement of the experimental protocols, the arterial blood pH, PCO$_2$ and PO$_2$ values in these 5 animals were $7.38 \pm 0.01$, $39.0 \pm 1.0$ mmHg and $110 \pm 3.0$ mmHg respectively. The mean values of tracheal pressure, dynamic compliance and airway resistance were $9.02 \pm 0.73$ cmH$_2$O, $1.182 \pm 0.203$ ml/cmH$_2$O and $0.0520 \pm 0.0068$ cmH$_2$O/s/ml respectively.

Of these five fibers, three were located within 1 cm from the hilum of the lung and two were located within 2 cm from the hilum of the lung.

7.2.2.1. Effect of Histamine on SARs

The basal SAR activity before ovalbumin challenge was $51.07 \pm 5.99$ impulses/breath. Following ovalbumin challenge, the SAR activity increased significantly to $59.69 \pm 6.28$ impulses/breath ($p<0.05$). After 30 minutes, there was complete recovery and the SAR activity returned back to basal value. When histamine was given as inhalation in doubling doses, there was significant stimulation of SARs at the higher doses. The basal SAR activities during the control period before inhalation of 0.04 and 0.08 of histamine were $51.49 \pm 6.04$ and $51.82 \pm 6.07$ impulses/breath respectively. The corresponding SAR activities after histamine inhalation were $53.20 \pm 6.14$ and $57.17 \pm 6.40$ impulses/breath respectively. An interval of 15 min was given between two successive doses to avoid tachyphylaxis and for allowing the recovery of SARs. The results are summarized in Fig. 35.
Fig. 35: Changes in the slowly adapting receptor (SAR) activity with successive doses of histamine in Group 2a. (The effect of 0.16 mg/ml of histamine was examined on 1 SAR only. Please see text for details. C = Control, H = Histamine dose (mg/ml), Ova = Ovalbumin, * = p<0.05, compared to the corresponding control.

In this group, following ovalbumin challenge, the airway resistance increased significantly from 0.0520 ± 0.0068 to 0.0870 ± 0.0099 cmH₂O/s/ml (p<0.001), an increase by more than 50%, suggesting the early asthmatic response. Simultaneously, there was a significant increase in SAR activity. After 30 minutes, there was complete recovery and the airway resistance was 0.0530 ± 0.0076 cmH₂O/s/ml. In this background, the 50% rise in the airway resistance to histamine (ED₅₀) was achieved at the histamine dose of 0.08 mg/ml in 4 animals (Fig. 35). At this dose, there was a significant increase in the SAR activity (p<0.05). In the remaining 1 animal, the ED₅₀ was achieved at the histamine dose of 0.16 mg/ml. In this one, the airway resistance increased from 0.0800 to 0.1200 cmH₂O/s/ml and the SAR activity increased from 45.0 to 49.95 impulses/breath (Fig. 35, Table 9).

7.2.2.2. Changes in Airway Mechanics

Histamine inhalation produced an increase in tracheal pressure, a decrease in dynamic compliance and an increase in airway resistance. The basal values of tracheal pressure, dynamic compliance and airway resistance were 9.02 ± 0.73 cmH₂O, 1.182 ±
Results

0.203 ml/cmH\textsubscript{2}O and 0.0520 \pm 0.0068 cmH\textsubscript{2}O/s/ml respectively. After ovalbumin challenge, tracheal pressure increased significantly to 11.06 \pm 0.88 cmH\textsubscript{2}O (p<0.01), dynamic compliance decreased significantly to 0.858 \pm 0.196 ml/cmH\textsubscript{2}O (p<0.001) and airway resistance increased significantly to 0.0870 \pm 0.0099 cmH\textsubscript{2}O/s/ml (p<0.001). After 30 minutes, there was complete recovery. Subsequently, when histamine inhalation was given in doubling doses, the tracheal pressure and airway resistance increased significantly at 0.04 and 0.08 mg/ml doses of histamine. There was a significant fall in the dynamic compliance also, at these doses. The results are presented in Table 9.

**Table 9:** Effects of histamine on tracheal pressure, dynamic compliance and airway resistance in Group 2a.

<table>
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<tr>
<th>Parameters</th>
<th>Histamine (mg/ml)</th>
<th>Control Period</th>
<th>Experimental Period</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tracheal pressure</strong> (cm H\textsubscript{2}O)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.04</td>
<td>9.14\pm 0.75</td>
<td>9.74\pm 0.75**</td>
<td></td>
</tr>
<tr>
<td>0.08</td>
<td>9.26\pm 0.75</td>
<td>10.5\pm 0.78***</td>
<td></td>
</tr>
<tr>
<td>0.16</td>
<td>9.20</td>
<td>11.80</td>
<td></td>
</tr>
<tr>
<td><strong>Dynamic compliance</strong> (ml/cmH\textsubscript{2}O)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.04</td>
<td>1.151\pm 0.191</td>
<td>1.053\pm 0.201**</td>
<td></td>
</tr>
<tr>
<td>0.08</td>
<td>1.144\pm 0.199</td>
<td>0.942\pm 0.207**</td>
<td></td>
</tr>
<tr>
<td>0.16</td>
<td>1.650</td>
<td>1.300</td>
<td></td>
</tr>
<tr>
<td><strong>Airway resistance</strong> (cmH\textsubscript{2}O/s/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.04</td>
<td>0.0530\pm 0.0076</td>
<td>0.0620 \pm 0.0068**</td>
<td></td>
</tr>
<tr>
<td>0.08</td>
<td>0.0520\pm 0.0068</td>
<td>0.0780 \pm 0.0048**</td>
<td></td>
</tr>
<tr>
<td>0.16</td>
<td>0.0800</td>
<td>0.1200</td>
<td></td>
</tr>
</tbody>
</table>

n = 5 for all the doses and n = 1 for the dose of 0.16 mg/ml. (please see text for details) ** = p<0.01, *** = p<0.001, compared to the corresponding control.

The SAR activity showed a positive correlation with the tracheal pressure with increasing doses of histamine and the correlation was significant (Pearson r = 0.9843, p<0.05). There was a significant negative correlation between SAR activity and dynamic compliance with increasing doses of histamine (Pearson r = -0.9808, p<0.05). Additionally, there was a significant positive correlation between SAR activity and airway resistance with increasing doses of histamine (Pearson r = 0.9854, p<0.01).
7.2.2.3. Comparison between Group 1 and Group 2a

7.2.2.3.1. Responses of SARs

After sensitization with ovalbumin, the basal SAR activity in Group 2a was 51.07 ± 5.99 impulses/breath which was not significantly different from that in Group 1 (50.02 ± 3.20 impulses/breath). After ovalbumin challenge, the SAR activity increased to 59.69 ± 6.28 impulses/breath in Group 2a, which was not significantly different from the SAR activity after saline inhalation in Group 1 (50.35 ± 3.19 impulses/breath) (Fig. 36).

![Fig. 36](image)

**Fig. 36:** Changes in slowly adapting receptor (SAR) activity to saline inhalation and ovalbumin challenge. Ova - ovalbumin challenge

The ED$_{50}$ was found to be reduced significantly in Group 2a as compared to that in Group 1, the respective doses of histamine being 0.09 ± 0.01 mg/ml and 0.32 ± 0.0 mg/ml (p<0.001) (Fig. 37).

![Fig. 37](image)

**Fig. 37:** Effective histamine dose required to increase airway resistance by 50% (ED$_{50}$) in Groups 1 and 2a. In Group 1, there is no standard error as the ED$_{50}$ histamine dose was similar in all the five animals (Please see results). *** = p<0.001, compared to that in Group 1.
At the ED$_{50}$ dose, the SARs were stimulated in both the groups, the stimulation in Group 2a was similar to that in Group 1 (55.29 ± 3.23 vs. 57.45 ± 6.31 impulses/breath, p>0.05) (Fig. 38).

Fig. 38: Slowly adapting receptor (SAR) activity at ED$_{50}$ histamine doses in Groups 1 and 2a.

### 7.2.2.3.2. Changes in Airway Mechanics

After sensitization with ovalbumin, the basal tracheal pressure in Group 2a was 9.02 ± 0.73 cmH$_2$O, which was significantly higher than the basal tracheal pressure of 6.41 ± 0.38 cmH$_2$O in Group 1 (p<0.05). After ovalbumin challenge, the tracheal pressure increased to 11.06 ± 0.88 cmH$_2$O in Group 2a, which was significantly higher than that after saline inhalation (6.44 ± 0.39 cmH$_2$O) in Group 1 (p<0.01).

At the ED$_{50}$ histamine dose (0.09 ± 0.01mg/ml) in Group 2a, the tracheal pressure increased to 10.80 ± 0.82 cmH$_2$O which was significantly higher than the increase in tracheal pressure to 7.52 ± 0.32 cmH$_2$O for the ED$_{50}$ histamine dose (0.32 ± 0.0 mg/ml) in Group 1 (p<0.01).

After sensitization with ovalbumin, the basal dynamic compliance in Group 2a was 1.182 ± 0.203 ml/cmH$_2$O. Even though less, it was not significantly different when compared to that (1.516 ± 0.182 ml/cmH$_2$O) in Group 1. After ovalbumin challenge, the dynamic compliance decreased to 0.858 ± 0.196 ml/cmH$_2$O in Group 2a, which was significantly lower (p<0.05) than the change in dynamic compliance after saline inhalation in Group 1, i.e., 1.478 ± 0.180 ml/cmH$_2$O.
At the ED$_{50}$ histamine dose (0.09 ± 0.01 mg/ml) in Group 2a, the dynamic compliance decreased to 0.898 ± 0.179 ml/cmH$_2$O which was not significantly different than the decrease in dynamic compliance to 1.260 ± 0.170 ml/cmH$_2$O for the ED$_{50}$ histamine dose (0.32 ± 0.0 mg/ml) in Group 1.

After sensitization with ovalbumin, the basal airway resistance in Group 2a was 0.0520 ± 0.0068 cmH$_2$O/s/ml. Even though increased it was not significantly different when compared to that (0.0390 ± 0.0024 cmH$_2$O/s/ml) in Group 1. After ovalbumin challenge, the airway resistance increased to 0.0870 ± 0.0099 cmH$_2$O/s/ml in Group 2a, which was significantly higher than that after saline inhalation (0.0390 ± 0.0024 cmH$_2$O) in Group 1 (p<0.01).

At the ED$_{50}$ histamine dose (0.09 ± 0.01 mg/ml) in Group 2a, the airway resistance increased to 0.0840 ± 0.0097 cmH$_2$O/s/ml which was significantly higher than the increase in airway resistance to 0.0600 ± 0.0031 cmH$_2$O/s/ml for the ED$_{50}$ histamine dose (0.32 ± 0.0 mg/ml) in Group 1 (p<0.05).

### 7.2.3. Group 2b (Late Asthmatic Response) – Animals Sensitized and Challenged with Ovalbumin - Response after 24 h

The experimental protocol of this group was performed on 5 SARs recorded from 5 guinea pigs (one fiber in each guinea pig). At the commencement of the experimental protocols, the arterial blood pH, PCO$_2$ and PO$_2$ values in these 5 animals were 7.38 ± 0.02, 38.0 ± 2.0 mmHg and 110 ± 6.0 mmHg respectively. The mean values of tracheal pressure, dynamic compliance and airway resistance were 8.86 ± 0.97 cmH$_2$O, 1.322 ± 0.137 ml/cmH$_2$O and 0.0420 ± 0.0058 cmH$_2$O/s/ml respectively.

Of these five fibers, four were located within 0.5 cm from the hilum of the lung and one was located within 2 cm from the hilum of the lung.

### 7.2.3.1. Effect of Histamine on SARs

The basal SAR activities during the control period before inhalation of 0.04, 0.08 and 0.16 mg/ml of histamine were 51.31 ± 5.25, 51.49 ± 5.26 and 51.84 ± 5.28
impulses/breath respectively. The corresponding SAR activities after histamine inhalation were $51.89 \pm 5.09$, $53.42 \pm 4.98$ and $58.56 \pm 6.28$ impulses/breath respectively. An interval of 15 min was given between two successive doses to avoid tachyphylaxis and for allowing the recovery of SARs. The results are summarized in Fig. 39.

In this Group, the 50% rise in the airway resistance to histamine ($ED_{50}$) was achieved at the histamine dose of 0.16 mg/ml in all the 5 animals and the airway resistance increased from $0.0440 \pm 0.0058$ to $0.0680 \pm 0.0096$ cmH$_2$O/s/ml ($p<0.01$). At this dose, there was a significant increase in the SAR activity (Fig. 39, $p<0.01$).

![Fig. 39](image_url)

**Fig. 39:** Changes in the slowly adapting receptor (SAR) activity with successive doses of histamine in Group 2b. C = Control, H = Histamine dose (mg/ml), ** = $p<0.01$, compared to the corresponding control.

### 7.2.3.2. Changes in Airway Mechanics

Histamine inhalation produced an increase in tracheal pressure, a decrease in dynamic compliance and an increase in airway resistance. The tracheal pressure increased significantly at 0.04, 0.08 and 0.16 mg/ml, and airway resistance increased significantly at 0.08 and 0.16 mg/ml doses of histamine. There was a significant fall in the dynamic compliance also, at the three histamine doses. The results are presented in Table 10.
Table 10: Effects of histamine on tracheal pressure, dynamic compliance and airway resistance in Group 2b.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Histamine (mg/ml)</th>
<th>Control Period</th>
<th>Experimental Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracheal pressure (cm H$_2$O)</td>
<td>0.04</td>
<td>8.86±0.97</td>
<td>9.28±0.94*</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>8.94±0.97</td>
<td>9.80±0.95*</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>9.04±0.94</td>
<td>10.70±0.87**</td>
</tr>
<tr>
<td>Dynamic compliance (ml/cmH$_2$O)</td>
<td>0.04</td>
<td>1.322±0.137</td>
<td>1.246±0.133**</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>1.298±0.140</td>
<td>1.160±0.132**</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>1.274±0.146</td>
<td>1.026±0.136**</td>
</tr>
<tr>
<td>Airway resistance (cmH$_2$O/s/ml)</td>
<td>0.04</td>
<td>0.0420±0.0058</td>
<td>0.0460±0.0067</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>0.0420±0.0058</td>
<td>0.0540±0.0074*</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>0.0440±0.0058</td>
<td>0.0680±0.0096**</td>
</tr>
</tbody>
</table>

n=5 for all the doses. * = p<0.05, ** = p<0.01, compared to the corresponding control.

The SAR activity showed a positive correlation with the tracheal pressure with increasing doses of histamine and the correlation was significant (Pearson r = 0.9531, p<0.05). There was a significant negative correlation between SAR activity and dynamic compliance with increasing doses of histamine (Pearson r = -0.9670, p<0.05). Additionally, there was a significant positive correlation between SAR activity and airway resistance with increasing doses of histamine (Pearson r = 0.9627, p<0.05).

7.2.3.3. Comparison between Group 1 and Group 2b

7.2.3.3.1. Responses of SARs

Twenty four hours after challenge with ovalbumin in guinea pigs previously sensitized with ovalbumin, the basal SAR activity in Group 2b was 51.31 ± 5.25 impulses/breath which was not significantly different compared to that in Group 1 (50.02 ± 3.20 impulses/breath). The ED$_{50}$ was found to be reduced significantly in Group 2b as compared to that in Group 1, the respective doses of histamine being 0.16 ± 0.0 mg/ml and 0.32 ± 0.0 mg/ml (p<0.001) (Fig. 40).
**Results**

![Histamine concentration bar graph](image)

**Fig. 40:** Effective histamine dose required to increase airway resistance by 50 % (ED$_{50}$) in Groups 1 and 2b. In Groups 1 and 2b, there is no standard error as the ED$_{50}$ histamine dose was similar in all the five animals (Please see results). ***= p<0.001 as compared to that in Group 1

At the ED$_{50}$ dose, the SARs were stimulated in both the groups, and the stimulation in Group 2b was similar to that in Group 1 (58.56 ± 6.28 vs. 55.29 ± 3.23 impulses/breath, p>0.05) (Fig. 41).

![SAR activity bar graph](image)

**Fig. 41:** Slowly adapting receptor (SAR) activity at ED$_{50}$ histamine doses in Groups 1 and 2b.

### 7.2.3.3.2. Changes in Airway Mechanics

Twenty four hours after challenge with ovalbumin, the basal tracheal pressure in Group 2b was 8.86 ± 0.97 cmH$_2$O, which was significantly different than the basal tracheal pressure of 6.41 ± 0.38 cm H$_2$O in Group 1 (p<0.05). At the ED$_{50}$ histamine
dose (0.16 ± 0.0 mg/ml) in Group 2b, the tracheal pressure increased to 10.70 ± 0.87 cmH₂O which was significantly higher than the increase in tracheal pressure to 7.52 ± 0.32 cmH₂O for the ED₅₀ histamine dose (0.32 ± 0.0 mg/ml) in Group 1 (p<0.01).

Twenty four hours after challenge with ovalbumin, the basal dynamic compliance in Group 2b was 1.322 ± 0.137 ml/cmH₂O. Even though less, it was not significantly different when compared to that (1.516 ± 0.182 ml/cmH₂O) in Group 1. At the ED₅₀ histamine dose (0.16 ± 0.0 mg/ml) in Group 2b, the dynamic compliance decreased to 1.026 ± 0.136 ml/cmH₂O which was not significantly different from the decrease in dynamic compliance to 1.260 ± 0.170 ml/cmH₂O for the ED₅₀ histamine dose (0.32 ± 0.00 mg/ml) in Group 1.

Twenty four hours after challenge with ovalbumin, the basal airway resistance in Group 2b was 0.0420 ± 0.0058 cmH₂O/s/ml. Even though increased it was not significantly different when compared to that (0.0390 ± 0.0024 cmH₂O/s/ml) in Group 1. At the ED₅₀ histamine dose (0.16 ± 0.0 mg/ml) in Group 2b, the airway resistance increased to 0.0680 ± 0.0096 cmH₂O/s/ml which was not significantly different than the increase in airway resistance to 0.0600 ± 0.0031 cmH₂O/s/ml for the ED₅₀ histamine dose (0.32 ± 0.0 mg/ml) in Group 1.

7.2.4. Group 4a (Antioxidant Supplementation and Early Asthmatic Response) – Animals Fed with Antioxidants, Sensitized and Challenged with Ovalbumin

The experimental protocol of this group was performed on 5 SARs recorded from 5 guinea pigs (one fiber in each guinea pig). At the commencement of the experimental protocols, the arterial blood pH, PCO₂ and PO₂ values in these five animals were 7.37 ± 0.01, 38.0 ± 1.0 mm Hg and 116 ± 6.0 mm Hg, respectively. The mean values of tracheal pressure, dynamic compliance and airway resistance were 6.85 ± 0.45 cmH₂O, 1.420 ± 0.208 ml/cmH₂O and 0.0400 ± 0.0070 cmH₂O/s/ml respectively.

Of these five fibers, three were located within 1 cm from the hilum of the lung and two were located within 2 cm from the hilum of the lung.
7.2.4.1. Effect of Histamine on SARs

The basal SAR activity before ovalbumin challenge was 50.24 ± 4.09 impulses/breath. Following ovalbumin challenge, the SAR activity increased significantly to 55.02 ± 4.45 impulses/breath (p<0.05). After 30 minutes, there was complete recovery and the SAR activity returned back to basal value. When histamine was given as inhalation in doubling doses, there was significant stimulation of SARs with the highest dose administered. The basal SAR activities during the control period before inhalation of 0.04, 0.08, 0.16 and 0.32 mg/ml of histamine were 50.32 ± 4.12, 50.39 ± 4.08, 50.45 ± 4.11 and 50.53 ± 4.09 impulses/breath respectively. The corresponding SAR activities after histamine inhalation were 50.63 ± 4.03, 51.54 ± 4.05, 52.75 ± 4.32 and 55.07 ± 4.75 impulses/breath respectively. An interval of 15 min was given between two successive doses to avoid tachyphylaxis and for allowing the recovery of SARs. The results are summarized in Fig. 42.

![Graph showing SAR activity changes](image)

**Fig. 42:** Changes in the slowly adapting receptor (SAR) activity with successive doses of histamine in Group 4a. C = Control, H = Histamine dose (mg/ml), Ova = Ovalbumin, * = p<0.05, compared to the corresponding control.

In this group, following ovalbumin challenge, the airway resistance increased significantly from 0.0400 ± 0.0070 to 0.0640 ± 0.0092 cmH$_2$O/s/ml (p<0.01), an increase by more than 50%, suggesting the early asthmatic response. Simultaneously, there was a significant increase in SAR activity. After 30 minutes, there was complete
recovery and the airway resistance was $0.0400 \pm 0.0070 \text{ cmH}_2\text{O/s/ml}$. In this background, the 50% rise in the airway resistance to histamine (ED$_{50}$) was achieved at the histamine dose of 0.32 mg/ml in all the 5 animals. At this dose, there was a significant increase in the SAR activity ($p<0.05$).

### 7.2.4.2. Changes in Airway Mechanics

Histamine inhalation produced an increase in tracheal pressure, a decrease in dynamic compliance and an increase in airway resistance. The basal values of tracheal pressure, dynamic compliance and airway resistance were $6.85 \pm 0.45 \text{ cmH}_2\text{O}$, $1.420 \pm 0.208 \text{ ml/cmH}_2\text{O}$ and $0.0400 \pm 0.0070 \text{ cmH}_2\text{O/s/ml}$ respectively. Following ovalbumin challenge, tracheal pressure increased significantly to $8.48 \pm 0.66 \text{ cmH}_2\text{O}$ ($p<0.05$), dynamic compliance decreased significantly to $1.213 \pm 0.186 \text{ ml/cmH}_2\text{O}$ ($p<0.01$) and airway resistance increased significantly to $0.0640 \pm 0.0092 \text{ cmH}_2\text{O/s/ml}$ ($p<0.01$). After 30 minutes, there was complete recovery. Subsequently, when histamine inhalation was given in doubling doses, the tracheal pressure increased significantly at 0.16 and 0.32 mg/ml doses of histamine and airway resistance increased significantly at 0.32 mg/ml dose of histamine. There was a significant fall in the dynamic compliance also, at the last two doses. The results are presented in Table 11.

### Table 11: Effects of histamine on tracheal pressure, dynamic compliance and airway resistance in Group 4a.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Histamine (mg/ml)</th>
<th>Control Period</th>
<th>Experimental Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracheal pressure (cmH$_2$O)</td>
<td>0.04</td>
<td>6.94$\pm$0.46</td>
<td>7.27$\pm$0.48</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>6.97$\pm$0.43</td>
<td>7.63$\pm$0.51</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>7.03$\pm$0.47</td>
<td>7.97$\pm$0.57*</td>
</tr>
<tr>
<td></td>
<td>0.32</td>
<td>7.18$\pm$0.52</td>
<td>8.38$\pm$0.64*</td>
</tr>
<tr>
<td>Dynamic compliance (ml/cmH$_2$O)</td>
<td>0.04</td>
<td>1.391$\pm$0.198</td>
<td>1.334$\pm$0.186</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>1.389$\pm$0.197</td>
<td>1.292$\pm$0.183</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>1.369$\pm$0.185</td>
<td>1.255$\pm$0.176*</td>
</tr>
<tr>
<td></td>
<td>0.32</td>
<td>1.366$\pm$0.185</td>
<td>1.208$\pm$0.173*</td>
</tr>
<tr>
<td>Airway resistance (cmH$_2$O/s/ml)</td>
<td>0.04</td>
<td>0.0400$\pm$0.0070</td>
<td>0.0400$\pm$0.0070</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>0.0400$\pm$0.0070</td>
<td>0.0460$\pm$0.0087</td>
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<tr>
<td></td>
<td>0.16</td>
<td>0.0400$\pm$0.0070</td>
<td>0.0520$\pm$0.010</td>
</tr>
<tr>
<td></td>
<td>0.32</td>
<td>0.0420$\pm$0.0080</td>
<td>0.0620$\pm$0.0106**</td>
</tr>
</tbody>
</table>

$n=5$ for all the doses. $* = p<0.05$, $** = p<0.01$, compared to the corresponding control.
The SAR activity showed a positive correlation with the tracheal pressure with increasing doses of histamine and the correlation was significant (Pearson r = 0.9824, p<0.01). There was a significant negative correlation between SAR activity and dynamic compliance with increasing doses of histamine (Pearson r = -0.9725, p<0.01). Additionally, there was a significant positive correlation between SAR activity and airway resistance with increasing doses of histamine (Pearson r = 0.9921, p<0.001).

7.2.4.3. Comparison between Group 1 and Group 4a

7.2.4.3.1. Responses of SARs

After sensitization with ovalbumin, the basal SAR activity in Group 4a was 50.24 ± 4.09 impulses/breath which was not significantly different than that in Group 1 (50.02 ± 3.20 impulses/breath). After ovalbumin challenge, the SAR activity increased to 55.02 ± 4.45 impulses/breath in Group 4a, which was not significantly different from the SAR activity after saline inhalation in Group 1 (50.35 ± 3.19 impulses/breath) (Fig. 43).

![Fig. 43: Changes in slowly adapting receptor (SAR) activity to saline inhalation and ovalbumin challenge.](image)

The ED$_{50}$ histamine dose was the same in Group 4a as in Group 1, the respective doses of histamine being 0.32 ± 0.0 mg/ml and 0.32 ± 0.0 mg/ml (Fig. 44).
Fig. 44: Changes in histamine dose required to increase airway resistance by 50 % (ED$_{50}$) in Groups 1 and 4a. In Groups 1 and 4a, there is no standard error as the ED$_{50}$ histamine dose was similar in all the five animals (Please see results).

At the ED$_{50}$ dose, even though the SARs were stimulated in both the groups, and the stimulation in Group 4a was similar to that in Group 1 (55.29 ± 3.23 vs. 55.07 ± 4.75 impulses/breath) (Fig. 45).

Fig. 45: Slowly adapting receptor (SAR) activity at histamine doses required to increase airway resistance by 50 % (ED$_{50}$) in Groups 1 and 4a.

7.2.4.3.2. Changes in Airway Mechanics

After sensitization with ovalbumin, the basal tracheal pressure in Group 4a was 6.85 ± 0.45 cmH$_2$O, which was not significantly different from the basal tracheal pressure of 6.41 ± 0.38 cmH$_2$O in Group 1. Following ovalbumin challenge, the
tracheal pressure increased to 8.48 ± 0.66 cmH₂O in Group 4a, which was significantly higher than that after saline inhalation (6.44 ± 0.39 cmH₂O) in Group 1 (p<0.05).

At the ED₅₀ histamine dose (0.32 ± 0.0 mg/ml) in Group 4a, the tracheal pressure increased to 8.38 ± 0.64 cmH₂O which was not significantly different from the increase in tracheal pressure to 7.52 ± 0.32 cmH₂O for the ED₅₀ histamine dose (0.32 ± 0.0 mg/ml) in Group 1.

After sensitization with ovalbumin, the basal dynamic compliance in Group 4a was 1.420 ± 0.208 ml/cmH₂O. Even though less, it was not significantly different when compared to that (1.516 ± 0.182 ml/cmH₂O) in Group 1. Following ovalbumin challenge, the dynamic compliance decreased to 1.213 ± 0.186 ml/cmH₂O in Group 4a, which was not significantly different from the change in dynamic compliance after saline inhalation in Group 1, i.e., 1.478 ± 0.180 ml/cmH₂O.

At the ED₅₀ histamine dose (0.32 ± 0.0 mg/ml) in Group 4a, the dynamic compliance decreased to 1.208 ± 0.173 ml/cmH₂O which was not significantly different from the decrease in dynamic compliance to 1.260 ± 0.170 ml/cmH₂O for the ED₅₀ histamine dose (0.32± 0.0 mg/ml) in Group 1.

After sensitization with ovalbumin, the basal airway resistance in Group 4a was 0.0400 ± 0.0070 cmH₂O/s/ml, which was not significantly different from the basal airway resistance of 0.0390 ± 0.0024 cmH₂O/s/ml in Group 1. Following ovalbumin challenge, the airway resistance increased to 0.0640 ± 0.0092 cmH₂O/s/ml in Group 4a, which was significantly higher than that after saline inhalation (0.0390 ± 0.0024 cmH₂O/s/ml) in Group 1 (p<0.05).

At the ED₅₀ histamine dose (0.32 ± 0.0 mg/ml) in Group 4a, the airway resistance increased to 0.0620 ± 0.0106 cmH₂O/s/ml which was not significantly different from the increase in airway resistance to 0.0600 ± 0.0031 cmH₂O/s/ml for the ED₅₀ histamine dose (0.32 ± 0.0 mg/ml) in Group 1.

7.2.4.4. Comparison between Group 2a and Group 4a

7.2.4.4.1. Responses of SARs

After sensitization with ovalbumin, the basal SAR activity in Group 2a was 51.07 ± 5.99 impulses/breath which was not significantly different than that in Group 4a (50.24
**Results**

± 4.09 impulses/breath). Following ovalbumin challenge, the SAR activity increased to 59.69 ± 6.28 impulses/breath in Group 2a, which was not significantly different than the increase in SAR activity to 55.02 ± 4.45 impulses/breath in Group 4a (Fig. 46).

![Fig. 46: Changes in slowly adapting receptor (SAR) activity following ovalbumin challenge in Groups 2a and 4a.](image)

The ED\textsubscript{50} was found to be reduced significantly in Group 2a as compared to that in Group 4a (p<0.001), the respective doses of histamine being 0.09 ± 0.02 mg/ml and 0.32± 0.0 mg/ml (Fig. 47).

![Fig. 47: Changes in histamine dose required to increase airway resistance by 50 % (ED\textsubscript{50}) in Groups 2a and 4a. In Group 4a, there is no standard error as the ED\textsubscript{50} histamine dose was similar in all the five animals (Please see results). ***= p<0.001, compared to Group 4a.](image)
At the ED$_{50}$ dose, the SARs were stimulated in both the groups, and the stimulation in Group 2a was similar to that in Group 4a (57.45 ± 6.31 vs., 55.07 ± 4.75 impulses/breath) (Fig. 48).

![Graph showing SAR activity at histamine doses required to increase airway resistance by 50% (ED$_{50}$) in Groups 2a and 4a.]

**Fig. 48:** Slowly adapting receptor (SAR) activity at histamine doses required to increase airway resistance by 50% (ED$_{50}$) in Groups 2a and 4a.

### 7.2.4.4.2. Changes in Airway Mechanics

After sensitization with ovalbumin, the basal tracheal pressure in Group 2a was 9.02 ± 0.73 cmH$_2$O, which was significantly different from the basal tracheal pressure of 6.85 ± 0.45 cmH$_2$O in Group 4a (p<0.05). After ovalbumin challenge, the tracheal pressure increased to 11.06 ± 0.88 cmH$_2$O in Group 2a, which was significantly different than the tracheal pressure (8.48 ± 0.66 cmH$_2$O) in Group 4a (p<0.05).

At the ED$_{50}$ histamine dose (0.09 ±0.01 mg/ml) in Group 2a, tracheal pressure increased to 10.80 ± 0.82 cmH$_2$O which was significantly different than the increase in tracheal pressure to 8.38 ± 0.64 cmH$_2$O for the ED$_{50}$ histamine dose (0.32 ± 0.0 mg/ml) in Group 4a (p<0.05).

After sensitization with ovalbumin, the basal dynamic compliance in Group 2a was 1.182 ± 0.203 ml/cmH$_2$O. Even though less, it was not significantly different when compared to that (1.420 ± 0.208 ml/cmH$_2$O) in Group 4a. After ovalbumin challenge, the dynamic compliance decreased to 0.858 ± 0.196 ml/cmH$_2$O in Group 2a, which was
Results

not significantly different from the change in dynamic compliance in Group 4a, i.e., $1.213 \pm 0.1868 \text{ ml/cmH}_2\text{O}$.

At the ED$_{50}$ histamine dose ($0.09 \pm 0.01 \text{ mg/ml}$) in Group 2a, the dynamic compliance decreased to $0.898 \pm 0.179 \text{ ml/cmH}_2\text{O}$ which was not significantly different from the decrease in dynamic compliance to $1.208 \pm 0.173 \text{ ml/cmH}_2\text{O}$ for the ED$_{50}$ histamine dose ($0.32 \pm 0.0 \text{ mg/ml}$) in Group 4a.

After sensitization with ovalbumin, the basal airway resistance in Group 2a was $0.0520 \pm 0.0068 \text{ cmH}_2\text{O/s/ml}$. Even though increased it was not significantly different when compared to that ($0.0400 \pm 0.0070 \text{ cmH}_2\text{O/s/ml}$) in Group 4a. Following ovalbumin challenge, the airway resistance increased to $0.0870 \pm 0.0099 \text{ cmH}_2\text{O/s/ml}$ in Group 2a, which was significantly different than that ($0.0640 \pm 0.0092 \text{ cmH}_2\text{O/s/ml}$) in Group 4a ($p<0.05$).

At the ED$_{50}$ histamine dose ($0.09 \pm 0.01 \text{ mg/ml}$) in Group 2a, the airway resistance increased to $0.0840 \pm 0.0097 \text{ cmH}_2\text{O/s/ml}$ which was not significantly different from the increase in airway resistance to $0.0620 \pm 0.010 \text{ cmH}_2\text{O/s/ml}$ for the ED$_{50}$ histamine dose ($0.32 \pm 0.0 \text{ mg/ml}$) in Group 4a.

7.2.5. Group 4b (Antioxidant Supplementation and Late Asthmatic Response): Animals Fed with Antioxidants, Sensitized and Challenged with Ovalbumin- Response after 24 hr

The experimental protocol of this group was performed on 5 SARs recorded from 5 guinea pigs (one fiber in each guinea pig). At the commencement of the experimental protocols, the arterial blood pH, PCO$_2$ and PO$_2$ values in these 4 animals were $7.39 \pm 0.01$, $38.0 \pm 1.0 \text{ mmHg}$ and $112 \pm 5.0 \text{ mmHg}$ respectively. The mean values of tracheal pressure, dynamic compliance and airway resistance were $7.12 \pm 0.61 \text{ cmH}_2\text{O}$, $1.318 \pm 0.108 \text{ ml/cmH}_2\text{O}$ and $0.0420 \pm 0.0020 \text{ cmH}_2\text{O/s/ml}$ respectively.

Of these five fibers, four were located within 1 cm from the hilum of the lung and one was located within 2 cm from the hilum of the lung.
7.2.5.1. Effect of Histamine on SARs

The basal SAR activities during the control period before inhalation of 0.04, 0.08, 0.16 and 0.32 mg/ml of histamine were 51.09 ± 3.47, 51.19 ± 3.47, 51.33 ± 3.49 and 51.34 ± 3.32 impulses/breath respectively. The corresponding SAR activities after histamine inhalation were 51.43 ± 3.56, 52.48 ± 3.64, 53.94 ± 3.86 and 56.56 ± 3.54 impulses/breath respectively. An interval of 15 min was given between two successive doses to avoid tachyphylaxis and for allowing the recovery of SARs. The results are summarized in Fig. 49.

![Fig. 49](image)

**Fig. 49:** Changes in the slowly adapting receptor (SAR) activity with successive doses of histamine in Group 4b. C = Control, H = Histamine dose (mg/ml), * = p<0.05, compared to the corresponding control.

In this Group, the 50% rise in the airway resistance to histamine (ED\textsubscript{50}) was achieved at the histamine dose of 0.32 mg/ml in all the 5 animals and the airway resistance increased from 0.0430 ± 0.0020 to 0.0670 ± 0.0037 cmH\textsubscript{2}O/s/ml in them. At this dose, there was a significant increase in the SAR activity (p<0.05).

7.2.5.2. Changes in Airway Mechanics

Histamine inhalation produced an increase in tracheal pressure, a decrease in dynamic compliance and an increase in airway resistance. The tracheal pressure increased significantly at 0.08, 0.16 and 0.32 mg/ml, and airway resistance increased significantly at 0.16 and 0.32 mg/ml doses of histamine. There was a significant fall in the dynamic compliance also, at these three doses. The results are presented in Table 12.
Table 12: Effects of histamine on tracheal pressure, dynamic compliance and airway resistance in Group 4b.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Histamine (mg/ml)</th>
<th>Control Period</th>
<th>Experimental Period</th>
</tr>
</thead>
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<tr>
<td>Tracheal pressure</td>
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<td></td>
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<tr>
<td>(cmH2O)</td>
<td>0.04</td>
<td>7.12±0.61</td>
<td>7.33±0.62</td>
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<td></td>
<td>0.08</td>
<td>7.14±0.61</td>
<td>7.58±0.65**</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>7.22±0.61</td>
<td>7.86±0.67**</td>
</tr>
<tr>
<td></td>
<td>0.32</td>
<td>7.22±0.61</td>
<td>8.37±0.64**</td>
</tr>
<tr>
<td>Dynamic compliance</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>(ml/cmH2O)</td>
<td>0.04</td>
<td>1.318±0.108</td>
<td>1.270±0.105</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>1.316±0.106</td>
<td>1.224±0.101**</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>1.290±0.104</td>
<td>1.144±0.106**</td>
</tr>
<tr>
<td></td>
<td>0.32</td>
<td>1.254±0.111</td>
<td>1.084±0.111**</td>
</tr>
<tr>
<td>Airway resistance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(cmH2O/s/ml)</td>
<td>0.04</td>
<td>0.0420±0.0020</td>
<td>0.0420±0.0020</td>
</tr>
<tr>
<td></td>
<td>0.08</td>
<td>0.0420±0.0020</td>
<td>0.0490±0.0033</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>0.0420±0.0020</td>
<td>0.0520±0.0020**</td>
</tr>
<tr>
<td></td>
<td>0.32</td>
<td>0.0430±0.0020</td>
<td>0.0670±0.0037**</td>
</tr>
</tbody>
</table>

n= 5 for all the doses. **= p<0.01, compared to the corresponding control.

The SAR activity showed a positive correlation with the tracheal pressure with increasing doses of histamine and the correlation was significant (Pearson r = 0.9916, p<0.001). There was a significant negative correlation between SAR activity and dynamic compliance with increasing doses of histamine (Pearson r = -0.9911, p<0.01). Additionally, there was a positive correlation between SAR activity and airway resistance with increasing doses of histamine (Pearson r = 0.9800, p<0.01).

7.2.5.3. Comparison between Group 1 and Group 4b

7.2.5.3.1. Responses of SARs

Twenty four hours after challenge with ovalbumin in guinea pigs previously sensitized with ovalbumin and fed with antioxidants, the basal SAR activity in Group 4b was 51.09 ± 3.47 impulses/breath which was not significantly different from that in Group 1 (50.02 ± 3.20 impulses/breath). The ED50 in Group 4b was 0.32 ± 0.0 mg/ml which was similar to that in Group 1 (0.32 ± 0.0 mg/ml) (Fig. 50).
**Fig. 50:** Effective histamine dose required to increase airway resistance by 50% (ED$_{50}$) in Groups 1 and 4b. In Groups 1 and 4b, there is no standard error as the ED$_{50}$ histamine dose was similar in all the five animals (Please see results).

At the ED$_{50}$ dose, the SARs were stimulated in both the groups, and the stimulation in Group 4b was similar to that in Group 1 (56.56 ± 3.54 vs. 55.29 ± 3.23 impulses/breath) (Fig. 51).

**Fig. 51:** Slowly adapting receptor (SAR) activity at ED$_{50}$ doses in Groups 1 and 4b.

### 7.2.5.3.2. Changes in Airway Mechanics

Twenty four hours after challenge with ovalbumin, the basal tracheal pressure in Group 4b was 7.12 ± 0.61 cmH$_2$O, which was not significantly different from the basal tracheal pressure of 6.41 ± 0.38 cmH$_2$O in Group 1. At the ED$_{50}$ histamine dose (0.32 ±
0.00 mg/ml) in Group 4b, tracheal pressure increased to $8.37 \pm 0.64$ cmH$_2$O which was not significantly different from the increase in tracheal pressure to $7.52 \pm 0.32$ cmH$_2$O for the ED$_{50}$ histamine dose (0.32 ± 0.00 mg/ml) in Group 1.

Twenty four hours after challenge with ovalbumin, the basal dynamic compliance in Group 4b was $1.318 \pm 0.108$ ml/cmH$_2$O. Even though less, it was not significantly different when compared to that ($1.516 \pm 0.182$ ml/cmH$_2$O) in Group 1. At the ED$_{50}$ histamine dose (0.32 ± 0.0 mg/ml) in Group 4b, the dynamic compliance decreased to $1.084 \pm 0.111$ ml/cmH$_2$O which was not significantly different than the decrease in dynamic compliance to $1.260 \pm 0.170$ ml/cmH$_2$O for the ED$_{50}$ histamine dose (0.32 ± 0.0 mg/ml) in Group 1.

Twenty four hours after challenge with ovalbumin, the basal airway resistance in Group 4b was $0.0420 \pm 0.0020$ cmH$_2$O/s/ml. Even though increased it was not significantly different when compared to that ($0.0390 \pm 0.0024$ cmH$_2$O/s/ml) in Group 1. At the ED$_{50}$ histamine dose (0.32 ± 0.00 mg/ml) in Group 4b, the airway resistance increased to $0.0670 \pm 0.0037$ cmH$_2$O/s/ml which was not significantly different than the increase in airway resistance to $0.0600 \pm 0.0031$ cmH$_2$O/s/ml for the ED$_{50}$ histamine dose (0.32 ± 0.00 mg/ml) in Group 1.

**7.2.5.4. Comparison between Group 2b and Group 4b**

**7.2.5.4.1. Responses of SARs**

Twenty four hours after challenge with ovalbumin in guinea pigs previously sensitized with ovalbumin and fed with normal diet, the basal SAR activity in Group 2b was $51.31 \pm 5.25$ impulses/breath which was not significantly different from that in Group 4b ($51.09 \pm 3.47$ impulses/breath) in guinea pigs previously sensitized with ovalbumin and fed with antioxidants. The ED$_{50}$ was found to be reduced significantly in Group 2b as compared to that in Group 4b, the respective doses of histamine being $0.16 \pm 0.0$ mg/ml and $0.32 \pm 0.0$ mg/ml (p<0.001) (Fig. 52).
Results

Fig. 52: Changes in histamine dose required to increase airway resistance by 50% (ED\textsubscript{50}) in Groups 2b and 4b. In Groups 2b and 4b, there is no standard error as the ED\textsubscript{50} histamine dose was similar in all the five animals (Please see results). ***=p<0.001, compared to Group 4b.

At the ED\textsubscript{50} dose, the SARs were stimulated in both the groups, and the stimulation in Group 2b was similar to that in Group 4b (58.56 ± 6.28 vs. 56.56 ± 3.54 impulses/breath) (Fig. 53).

Fig. 53: Slowly adapting receptor (SAR) activity at ED\textsubscript{50} doses in Groups 2b and 4b.

7.2.5.4.2. Changes in Airway Mechanics

Twenty four hours after challenge with ovalbumin, the basal tracheal pressure in Group 2b was 8.40 ± 1.16 cmH\textsubscript{2}O, which was not significantly different from the basal tracheal pressure of 6.95 ± 0.75 cmH\textsubscript{2}O in Group 4b. At the ED\textsubscript{50} histamine dose (0.16 ± 0.0 mg/ml) in Group 2b, tracheal pressure increased to 10.48 ± 1.11 cm H\textsubscript{2}O
which was not significantly different than the increase in tracheal pressure to 8.18 ± 0.79 cm H₂O for the ED₅₀ histamine dose (0.32 ± 0.0 mg/ml) in Group 4b.

Twenty four hours after challenge with ovalbumin, the basal dynamic compliance in Group 2b was 1.322 ± 0.137 ml/cmH₂O, which was not significantly different when compared to that (1.318 ± 0.108 ml/cmH₂O) in Group 4b. At the ED₅₀ histamine dose (0.16 ± 0.0 mg/ml) in Group 2b, the dynamic compliance decreased to 1.026 ± 0.136 ml/cmH₂O which was not significantly different from the decrease in dynamic compliance to 1.084 ± 0.111 ml/cmH₂O for the ED₅₀ histamine dose (0.32 ± 0.0 mg/ml) in Group 4b.

Twenty four hours after challenge with ovalbumin, the basal airway resistance in Group 2b was 0.0420 ± 0.0058 cmH₂O/s/ml, which was not significantly different from the basal airway resistance of 0.0420 ± 0.0020 cmH₂O/s/ml in Group 4b. At the ED₅₀ histamine dose (0.16 ± 0.0 mg/ml) in Group 2b, the airway resistance increased to 0.0680 ± 0.0096 cmH₂O/s/ml which was not significantly different from the increase in airway resistance to 0.06700 ± 0.0037 cmH₂O/s/ml for the ED₅₀ histamine dose (0.32 ± 0.0 mg/ml) in Group 4b.

**7.2.6. Group 3 (Xanthine-Xanthine Oxidase Inhalation) – *In vivo* Generation of Oxidants**

The experimental protocol of this group was performed on 5 SARs recorded from 5 guinea pigs (one fiber in each guinea pig). At the commencement of the experimental protocols, the arterial blood pH, PCO₂ and PO₂ values in these 5 animals were 7.38 ± 0.01, 38.0 ± 1.0 mmHg and 110 ± 5.0 mmHg respectively. The mean values of tracheal pressure, dynamic compliance and airway resistance were 6.58 ± 0.55 cmH₂O, 1.371 ± 0.085 ml/cm H₂O and 0.0390 ± 0.0040 cmH₂O/s/ml respectively.

Of these five fibers, three were located within 1 cm from the hilum of the lung and two were located within 2 cm from the hilum of the lung.

**7.2.6.1. Effect of Histamine on SARs**

The basal SAR activity before xanthine-xanthine oxidase inhalation was 49.29 ± 4.21 impulses/breath. After xanthine-xanthine oxidase inhalation, the SAR activity
increased significantly to $54.30 \pm 4.03$ impulses/breath ($p<0.05$). After 30 minutes, there was complete recovery and the SAR activity returned back to basal value. When histamine was given as inhalation in doubling doses, there was significant stimulation of SARs with the highest dose. The basal SAR activities during the control period before inhalation of 0.04, 0.08 and 0.16 mg/ml of histamine were $49.35 \pm 4.20$, $49.45 \pm 4.22$ and $51.31 \pm 5.00$ impulses/breath respectively. The corresponding SAR activities after histamine inhalation were $50.21 \pm 4.18$, $52.02 \pm 4.36$ and $56.86 \pm 5.44$ impulses/breath respectively. An interval of 15 min was given between two successive doses to avoid tachyphylaxis and for allowing the recovery of SARs. The results are summarized in Fig. 54.

In this group, after xanthine-xanthine oxidase inhalation, the airway resistance increased significantly from $0.0390 \pm 0.0040$ to $0.0610 \pm 0.0055$ cmH$_2$O/s/ml ($p<0.05$). Simultaneously, there was a significant increase in SAR activity. After 30 minutes, there was complete recovery and the airway resistance was $0.0390 \pm 0.0040$ cmH$_2$O/s/ml. In this background, the 50% rise in the airway resistance to histamine ($ED_{50}$) was achieved at the dose of 0.16 mg/ml in all the 5 animals. At this dose, there was a significant increase in the SAR activity ($p<0.05$).

**Fig. 54:** Changes in the slowly adapting receptor (SAR) activity with successive doses of histamine in Group 3. C = Control, H = Histamine dose (mg/ml), XO = Xanthine-xanthine oxidase, * = $p<0.05$, compared to the corresponding control.
7.2.6.2. Changes in Airway Mechanics

Histamine inhalation produced an increase in tracheal pressure, a decrease in dynamic compliance and an increase in airway resistance. The basal values of tracheal pressure, dynamic compliance and airway resistance were $6.58 \pm 0.55 \text{ cmH}_2\text{O}$, $1.371 \pm 0.085 \text{ ml/cmH}_2\text{O}$ and $0.0390 \pm 0.0040 \text{ cmH}_2\text{O}/\text{s/ml}$ respectively. After xanthine-xanthine oxidase inhalation, tracheal pressure increased significantly to $7.98 \pm 0.51 \text{ cmH}_2\text{O}$ ($p<0.01$), dynamic compliance decreased significantly to $1.016 \pm 0.086 \text{ ml/cmH}_2\text{O}$ ($p<0.01$) and airway resistance increased significantly to $0.0610 \pm 0.0055 \text{ cmH}_2\text{O}/\text{s/ml}$ ($p<0.01$). After 30 minutes, there was complete recovery. Subsequently, when histamine inhalation was given in doubling doses, the tracheal pressure and airway resistance increased significantly at the dose of $0.16 \text{ mg/ml}$ only ($p<0.05$). There was a significant fall in the dynamic compliance also at this dose. The results are presented in Table 13.

**Table 13:** Effects of histamine on tracheal pressure, dynamic compliance and airway resistance in Group 3.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Histamine (mg/ml)</th>
<th>Control Period</th>
<th>Experimental Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracheal pressure (cmH\textsubscript{2}O)</td>
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<td>6.69±0.53</td>
<td>7.04±0.52</td>
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<td></td>
<td>0.08</td>
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<td>7.32±0.52</td>
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<tr>
<td></td>
<td>0.16</td>
<td>6.71±0.55</td>
<td>7.80±0.53**</td>
</tr>
<tr>
<td>Dynamic compliance (ml/cmH\textsubscript{2}O)</td>
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<tr>
<td></td>
<td>0.16</td>
<td>1.312±0.085</td>
<td>1.060±0.090**</td>
</tr>
<tr>
<td>Airway resistance (cmH\textsubscript{2}O/s/ml)</td>
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<td>0.0390±0.0040</td>
<td>0.0510±0.0055</td>
</tr>
<tr>
<td></td>
<td>0.16</td>
<td>0.0410±0.0050</td>
<td>0.0620±0.0058*</td>
</tr>
</tbody>
</table>

n= 5 for all the doses. * - $p<0.05$, ** - $p<0.01$, compared to the corresponding control.
The SAR activity showed a positive correlation with the tracheal pressure with increasing doses of histamine and the correlation was significant (Pearson r = 0.9513, p<0.05). The SAR activity showed a significant negative correlation with the dynamic compliance with increasing doses of histamine and the correlation was significant (Pearson r = -0.9633, p<0.05). The SAR activity showed a significant positive correlation with the airway resistance with increasing doses of histamine (Pearson r = 0.9928, p<0.01).

7.2.6.3. Comparison between Group 1 and Group 3

7.2.6.3.1. Responses of SARs

The basal SAR activity in Group 3 was 49.29 ± 4.21 impulses/breath which was not significantly different from that in Group 1 (50.02 ± 3.20 impulses/breath). After xanthine-xanthine oxidase inhalation, the SAR activity increased to 54.30 ± 4.03 impulses/breath in Group 3, which was not significantly different than the SAR activity after saline inhalation in Group 1 (50.35 ± 3.19 impulses/breath) (Fig. 55).

![Fig. 55: Changes in slowly adapting receptor (SAR) activity during saline and xanthine xanthine oxidase (XO) inhalation in Groups 1 and 3.](image-url)

The ED$_{50}$ was found to be reduced significantly in Group 3 as compared to that in Group 1, the respective doses of histamine being 0.14 ± 0.01 mg/ml and 0.32 ± 0.0 mg/ml (p<0.001) (Fig. 56).
**Fig. 56**: Changes in histamine dose required to increase airway resistance by 50 % (ED$_{50}$) in Groups 1 and 3. ***=p<0.001, compared to Group 1. In Group 1, there is no standard error as the ED$_{50}$ histamine dose was similar in all the five animals (Please see results).

At the ED$_{50}$ dose, the SARs were stimulated in both the groups, and the stimulation in Group 3 similar to that in Group 1 (55.29 ± 3.23 vs., 55.38 ± 4.47 impulses/breath) (Fig. 57).

**Fig. 57**: Slowly adapting receptor (SAR) activity at histamine doses required to increase airway resistance by 50 % (ED$_{50}$) in Groups 1 and 3.

### 7.2.6.3.2. Changes in Airway Mechanics

The basal tracheal pressure in Group 3 was 6.58 ± 0.55 cmH$_{2}$O, which was not significantly different from the basal tracheal pressure of 6.41 ± 0.38 cmH$_{2}$O in Group
1. After xanthine-xanthine oxidase inhalation, the tracheal pressure increased to 7.98 ± 0.51 cm H$_2$O in Group 3, which was significantly higher than that after saline inhalation (6.44 ± 0.39 cm H$_2$O) in Group 1 (p<0.05).

At the ED$_{50}$ histamine dose (0.14± 0.01 mg/ml) in Group 3, the tracheal pressure increased to 7.80 ± 0.53 cmH$_2$O which was not significantly different from the increase in tracheal pressure to 7.52 ± 0.32 cmH$_2$O for the ED$_{50}$ histamine dose (0.32 ± 0.0 mg/ml) in Group 1.

The basal dynamic compliance in Group 3 was 1.371 ± 0.085 ml/cmH$_2$O. Even though less, it was not significantly different when compared to that (1.516 ± 0.182 ml/cmH$_2$O) in Group 1. After xanthine-xanthine oxidase inhalation, the dynamic compliance decreased to 1.016 ± 0.086 ml/cmH$_2$O in Group 3, which was significantly lower (p<0.05) than the change in dynamic compliance after saline inhalation in Group 1, i.e., 1.478 ± 0.180 ml/cmH$_2$O.

At the ED$_{50}$ histamine dose (0.14 ± 0.01 mg/ml) in Group 3, the dynamic compliance decreased to 1.060 ± 0.090 ml/cmH$_2$O which was not significantly different than the decrease in dynamic compliance to 1.260 ± 0.170 ml/cmH$_2$O for the ED$_{50}$ histamine dose (0.32 ± 0.0 mg/ml) in Group 1.

The basal airway resistance in Group 3 was 0.0390 ± 0.0040 cmH$_2$O, which was not significantly different from the basal airway resistance of 0.0390 ± 0.0024 cmH$_2$O in Group 1. After xanthine-xanthine oxidase inhalation, the airway resistance increased to 0.0610 ± 0.0055 cmH$_2$O in Group 3, which was significantly higher than that after saline inhalation (0.0390 ± 0.0024 cmH$_2$O) in Group 1 (p<0.01).

At the ED$_{50}$ histamine dose (0.14 ± 0.02 mg/ml) in Group 3, the airway resistance increased to 0.0620 ± 0.0058 cmH$_2$O/s/ml which was not significantly different from the increase in airway resistance to 0.0600 ± 0.003162 cmH$_2$O/s/ml for the ED$_{50}$ histamine dose (0.32 ± 0.0 mg/ml) in Group 1.
7.3. Biochemical Estimations

7.3.1. Measurement of Oxidative Stress

The parameters of oxidant-oxidant status were measured in the plasma, red blood corpuscles and white blood corpuscles. The results were expressed as mean ± SEM.

7.3.1.1. Plasma Lipid Peroxides (LPO)

Plasma LPO was measured as thiobarbituric acid reactive substances (TBARS) formed. In Group 1, 2a, 2b, 3, 4a and 4b the lipid peroxide levels measured were 5.42 ± 0.37, 9.94 ± 0.91, 8.36 ± 0.18, 7.96 ± 0.34, 6.55 ± 0.15 and 6.45 ± 0.19 nM TBARS/ml respectively (Fig. 58). The LPO levels in Group 2a, 2b, 3, 4a and 4b were significantly different from that of Group 1 (p<0.001, p<0.001, p<0.001, p<0.05, p<0.05 respectively).

![Fig. 58: Plasma lipid peroxide (LPO) levels. * = p<0.05, *** = p <0.001; Compared with Group I. ## = p<0.01, Group 4a compared with compared with Group 2a and Group 4b compared with Group 2b.](image)

However after dietary antioxidant supplementation, the lipid peroxide levels were significantly reduced in Groups 4a and 4b as compared to the respective lipid peroxide levels in Groups 2a and 2b (p<0.01).
7.3.1.2. Superoxide Generation by Peripheral Blood Leucocytes

Superoxide generation was measured as nM O$_2$ produced /15min/10$^6$ cells). In Groups 1, 2a, 2b, 3, 4a and 4b, the superoxide generation measured were 1.72 ± 0.23, 5.16 ± 0.40, 3.93 ± 0.18, 3.78 ± 0.32, 2.51 ± 0.15 and 2.44 ± 0.11 nM O$_2$ produced /15min/10$^6$ cells respectively (Fig. 59). The superoxide generation measured in Group 2a, 2b and 3 were significantly different from that of Group 1 (p<0.001, p<0.001 and p<0.001 respectively).

![Fig. 59: Superoxide generation by peripheral blood leucocytes. *** = p <0.001; Compared with Group I. ## = p<0.01, Group 4a compared with compared with Group 2a and Group 4b comparedwith Group 2b.]

However after dietary antioxidant supplementation, the superoxide generation in Groups 4a and 4b were significantly reduced when compared to the corresponding values in Groups 2a and 2b (p<0.01).

7.3.2. Measures of Antioxidant Status

7.3.2.1. Red Blood Corpuscle Catalase Activity

Red blood corpuscle catalase activity was measured as k/g Hb. The specific activity of catalase in Groups 1, 2a, 2b, 3, 4a and 4b were 0.51 ± 0.02, 0.23 ± 0.02, 0.31 ± 0.01, 0.28 ± 0.02, 0.40 ± 0.03 and 0.45 ± 0.03 k/g Hb respectively (Fig. 60). The
catalase activity in Group 2a, 2b and 3 were significantly different from that of Group 1 (p<0.001, p<0.001 and p<0.001 respectively).

![Graph showing catalase activity](image)

**Fig. 60:** Red blood corpuscle catalase activity. *** = p <0.001; Compared with Group I. ## = p<0.01, ### = p<0.001, Group 4a compared with compared with Group 2a and Group 4b compared with Group 2b.

However after dietary antioxidant supplementation, the catalase activity was increased significantly in Groups 4a and 4b as compared to the corresponding values in Groups 2a and 2b (p<0.001, and p<0.01).

### 7.3.2.2. Red Blood Corpuscle Glutathione Peroxidase Activity

Red blood corpuscle glutathione peroxidase activity was measured as µM NADPH oxidized/min/g Hb. The glutathione peroxidase activity in Groups 1, 2a, 2b, 3, 4a and 4b were 0.51 ± 0.02, 0.23 ± 0.02, 0.31 ± 0.01, 0.28 ± 0.02, 0.40 ± 0.03 and 0.45 ± 0.03 µM NADPH oxidized/min/g Hb respectively (Fig. 61). The glutathione peroxidase in Group 2a, 2b and 3 were significantly different from that of Group 1 (p<0.001, p<0.001 and p<0.001 respectively).

However after dietary antioxidant supplementation, the glutathione peroxidase activity was increased significantly in Groups 4a and 4b as compared to the corresponding values in Groups 2a and 2b (p<0.001).
Results

Fig. 61: Red blood corpuscle glutathione peroxidase activity. *** = p < 0.001; Compared with Group I. ### = p<0.001, Group 4a compared with Group 2a and Group 4b compared with Group 2b.

7.3.2.3. Plasma Total Antioxidant Capacity

Total antioxidant capacity of plasma was measured as ferric reducing ability of plasma (FRAP) value. In Groups 1, 2a, 2b, 3, 4a and 4b the FRAP values were 497.2 ± 63.26, 271.8 ± 46.01, 256.1 ± 41.54, 212.3 ± 32.16, 409.2 ± 42.17 and 391.0 ± 36.06 μM/l respectively (Fig. 62). The FRAP values in Groups 2a, 2b and 3 were significantly different from that in Group I (p<0.01).

Fig. 62: Plasma total antioxidant capacity. ** = p<0.01, *** = p <0.001; Compared with Group I. # = p<0.05, Group 4a compared with Group 2a and Group 4b compared with Group 2b.
However after dietary antioxidant supplementation, the FRAP values was increased significantly in Groups 4a and 4b as compared to the corresponding values in Groups 2a and 2b (p<0.05).

7.3.3. Nitrosative Stress

7.3.3.1. Plasma Nitrate and Nitrite Levels

Plasma nitrate and nitrite level was measured in Group 1, 2a, 2b, 3, 4a and 4b and they were 38.50 ± 3.45, 96.37 ± 7.60, 90.79 ± 3.45, 87.50 ± 6.01, 61.91 ± 2.88 and 60.16 ± 2.53 µM/L respectively (Fig. 63). The nitrate and nitrite level in Group 2a, 2b, 3, 4a and 4b were significantly different from that of Group 1 (p<0.001, p<0.001, p<0.001, p<0.05, p<0.05 respectively).

**Fig. 63:** Plasma nitrate and nitrite levels. * = p<0.05, *** = p <0.001; Compared with Group I. ### = p<0.001, Group 4a compared with Group 2a and Group 4b compared with Group 2b.

However after dietary antioxidant supplementation, the nitrate and nitrite levels were significantly reduced in Groups 4a and 4b as compared to the corresponding values in Groups 2a and 2b (p<0.001).
7.3.3.2. Plasma Nitrotyrosine Level

Plasma nitrotyrosine levels measured in Group 1, 2a, 2b, 3, 4a and 4b were 3.37±0.45, 33.79± 2.19, 29.90± 1.12, 28.53± 0.90, 15.06± 0.82 and 14.24 ± 0.70 nM respectively (Fig. 64). The nitrotyrosine level in Group 2a, 2b, 3, 4a and 4b were significantly different from that of Group 1 (p<0.001, p<0.001, p<0.001, p<0.001, p<0.001 respectively).

![Fig. 64: Plasma nitrotyrosine levels. *** = p <0.001; Compared with Group I. ### = p<0.001, Group 4a compared with compared with Group 2a and Group 4b compared with Group 2b.](image)

However after dietary antioxidant supplementation, the nitrotyrosine levels were significantly reduced in Groups 4a and 4b as compared to the corresponding values in Groups 2a and 2b (p<0.001).

7.3.4. Plasma Vitamin C Level

In Groups 1, 2a, 2b, 3, 4a and 4b, the plasma vitamin C levels measured were 11.39 ± 1.45, 6.67 ± 0.61, 6.32 ± 0.77, 7.11 ± 0.86, 9.03 ± 0.63 and 11.13 ± 1.43 µg/ml respectively (Fig. 65). The vitamin C levels in Groups 2a, 2b and 3 were significantly different from that of Group 1 (p<0.01, p<0.01, p<0.05 respectively). However, the vitamin C level in Group 4a and 4b were not significantly different as compared to that in Group 1.
Fig. 65: Plasma vitamin C levels. * = p<0.05, ** = p <0.01; Compared with Group I. # = p<0.05, ## = p<0.01, Group 4a compared with compared with Group 2a and Group 4b compared with Group 2b.

However after dietary antioxidant supplementation, the vitamin C levels were increased significantly in Groups 4a and 4b as compared to the corresponding values in Groups 2a and 2b (p<0.05 and p<0.01 respectively).

7.3.5. Plasma Vitamin E Levels

Plasma vitamin E level was measured in Group 1, 2a, 2b, 3, 4a and 4b and they were 0.87 ± 0.08, 0.24 ± 0.03, 0.35 ± 0.03, 0.31 ± 0.02, 0.53 ± 0.03 and 0.56 ± 0.02 mg/100 ml respectively (Fig. 66). The vitamin E levels in Group 2a, 2b, 3, 4a and 4b were significantly different from that of Group 1 (p<0.001, p<0.001, p<0.001, p<0.05, p<0.05 respectively).

However after dietary antioxidant supplementation, the vitamin E levels were increased significantly in Groups 4a and 4b as compared to the corresponding values in Groups 2a and 2b (p<0.01 and p<0.05).
7.4. Histopathology

Histopathological studies were carried out in lung sections taken from upper lobe and lower lobe in all the Groups. All sections were stained with Hematoxylin and eosin and were examined under light microscope. The observations were classified as described by (Underwood et al., 1995) (see Table 1). Examples are shown in Figs. 67-72 and the results are summarized in Table 14.

7.4.1. Group 1

Histopathological sections revealed normal lung architecture without any abnormality. The bronchial epithelium was intact and there was no inflammation in the interstitium, interalveolar spaces and peribronchiolar areas (Fig. 67, Table 14).

7.4.2. Group 2a

In Group 2a, there was moderate infiltration of neutrophils, macrophages, lymphocytes and monocytes in the peribronchial and perivascular regions. Mild to moderate edema was observed in the alveoli. Damage to the epithelial cells was mild (Fig. 68, Table 14).
7.4.3. Group 2b

In Group 2b, moderate to high infiltration of eosinophils along with macrophages, lymphocytes and monocytes was observed in the peribronchial and perivascular regions. This was associated with moderate edema in the alveoli with extensive damage to the epithelial cells (Fig. 69, Table 14).

7.4.4. Group 4a

In Group 4a following antioxidant supplementation, there was reduction in the infiltration of inflammatory cells, edema and epithelial cell damage (Fig. 70, Table 14). When compared to the pathological changes in Group 2a, these changes were termed as mild (Table 14). The lungs showed a near normal architecture.

7.4.5. Group 4b

In Group 4b also, following antioxidant supplementation, there was reduction in the infiltration of inflammatory cells, edema and epithelial cell damage (Fig. 71, Table 14). When compared with the pathological changes in Group 2b, these changes were less.

7.4.6. Group 3

In Group 3 also there was mild infiltration of inflammatory cells in the peribronchiolar and perivascular regions. This was associated with mild epithelial cell damage (Fig. 72, table 14).

These results are summarized in Table 14.

**Table 14**: Inflammatory changes in lung sections in different groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Peribronchiolar eosinophilia and neutrophilia</th>
<th>Edema</th>
<th>Epithelial damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Group 2a</td>
<td>3 (2-3)</td>
<td>2</td>
<td>1(1-2)</td>
</tr>
<tr>
<td>Group 2b</td>
<td>4 (4-5)</td>
<td>3 (3-4)</td>
<td>4 (3-4)</td>
</tr>
<tr>
<td>Group 4a</td>
<td>1 (1-2)</td>
<td>1</td>
<td>1 (0-1)</td>
</tr>
<tr>
<td>Group 4b</td>
<td>2 (2-3)</td>
<td>2 (1-2)</td>
<td>2 (2-3)</td>
</tr>
<tr>
<td>Group 3</td>
<td>2 (1-2)</td>
<td>1 (0-1)</td>
<td>1 (1-2)</td>
</tr>
</tbody>
</table>

Inflammatory changes were graded by histopathological assessment using a semiquantitative scale of 0-5. Results are presented as median, with range in parenthesis. Group size was 2 for each Group.
Fig. 67: Lung histopathology of Group 1 showing distal airway with peribronchiolar arteriole showing normal alveolar architecture. Haematoxylin and Eosin (H&E) stain x 10.

Fig. 68: Lung histopathology of Group 2a showing distal airway with peribronchiolar arteriole showing moderate cell infiltration and edema. Haematoxylin and Eosin (H&E) stain x 10.
Results

Fig. 69: Lung histopathology of Group 2b showing distal airway with peribronchiolar arteriole showing moderate to high cell infiltration and edema. Haematoxylin and Eosin (H&E) stain x 10.

Fig. 70: Lung histopathology of Group 4a showing distal airway with peribronchiolar arteriole showing mild cell infiltration and edema. Haematoxylin and Eosin (H&E) stain x 10.
Fig. 71: Lung histopathology of Group 4b showing distal airway with peribronchiolar arteriole showing mild to moderate cell infiltration and edema. Haematoxylin and Eosin (H&E) stain x 10.

Fig. 72: Lung histopathology of Group 3 showing distal airway with peribronchiolar arteriole showing mild moderate cell infiltration. Haematoxylin and Eosin (H&E) stain x 10.