**Discussion**

**Dr. Karr:** In the transfer with serum, when those recipient animals were challenged, no lesions developed. Is that correct?

**Dr. Moore:** That's not true; I should have mentioned that to you. After immune serum transfer and one aerosol challenge, there was a mononuclear cell infiltrate in the lungs at 6 hrs after challenge. This was not the acute type of inflammation expected if the lesions are caused by immune complex disease. At three weeks, even with continued aerosol challenge, these lung lesions had resolved. We have not produced acute hemorrhagic pneumonitis in rabbits by aerosol challenge in these experiments. However, we have been able to produce lesions consistent with immune complex disease by aerosol challenge of immunized guinea pigs.

**Influence of Immunity on the Absorption of Inhaled Antigen***

Christopher A. Dawson, Ph.D.; Janet F. Braley, Ph.D.;** and Vernon L. Moore, Ph.D.

Previously we observed that following intratracheal administration of antigen, the concentration of antigen found in the blood was lower in immunized than in nonimmunized rabbits.1 To determine whether differences in the rate of absorption through the alveolocapillary barrier could contribute to these differences in blood levels, we examined the absorption of inhaled soluble antigens through isolated blood perfused rabbit lungs.2 In this preparation, the only route by which antigen can enter the blood is via the alveolo-capillary barrier, and the rate of antigen removal from the blood by other organs of the body is not a factor influencing blood levels. We observed that when isolated lungs from normal non-immunized rabbits inhaled radiolabeled ovalbumin (OA) or human serum albumin (HSA), the label appeared in the blood in two fractions. One was precipitable with 5 percent trichloroacetic acid (TCA) or antiserum; the other fraction was TCA soluble and dialyzable. Thin layer chromatography of the TCA soluble fraction revealed radioactivity in several peaks, indicating that this fraction probably represented protein breakdown products. Immune mechanisms resulted in a decrease in the amounts of intact antigen and an increase in the TCA soluble fraction appearing in the blood after the aerosol exposure.

In the present study we have begun to examine the mechanisms whereby immunization alters the absorption of inhaled soluble antigen through the alveolo-capillary barrier.

**Methods**

New Zealand white rabbits were immunized by subcutaneous injection of human serum albumin (HSA) or ovalbumin (OA) in complete Freund’s adjuvant, and the isolated lungs were prepared as previously described.2 The lungs were removed from normal and immunized rabbits, and the pulmonary artery, the left atrium and the trachea of each of the lungs were cannulated. They were then mounted in a chamber and ventilated by negative pressure. The lungs were perfused at a constant flow rate (180 ml/min) with autotransduced blood at 37°C while breathing a mixture of respiratory gases which maintained normal blood gas composition. Arterial and venous blood pressures and the volume of blood in the perfusion circuit were monitored throughout the experiments.

The radiolabeled antigens (125I-HSA and/or 125I-OA in physiologic saline solution) were introduced into the isolated lung as an aerosol using an ultrasonic nebulizer (DeVilbiss 65, Somerset, Pa.) which was operated to produce vapor particles from 2 to 10 μm in diameter. The lungs rebreathed the aerosol for 15 minutes. The nebulizer was then removed and the lungs ventilated and perfused for an additional four hours, during which time blood samples were taken for analysis of radioactivity in TCA-soluble and TCA-precipitable fractions as previously described.2

**Results and Discussion**

Various experimental groups were studied. In order to determine whether the decrease in protein absorption caused by immunization was due to a specific mechanism directed at the antigen or to a nonspecific change initiated by antigen-antibody interaction, such as a decrease in permeability to protein in general, we immunized rabbits with either HSA or OA and instilled their isolated lungs with an aerosol containing both 125I-HSA and 125I-OA. Lungs from normal (non-immunized) rabbits were insufflated with 125I-HSA and 125I-OA for comparison.

Table 1 shows the results. The percentage of intact radiolabeled protein appearing in the blood after four hours of perfusion was reduced for the antigen but not for the simultaneously inhaled nonspecific protein. This indicates that only absorption of the specific antigen was inhibited by immunization. There was also a tendency for a larger fraction of the inhaled proteins to appear in the blood as metabolites in immunized lung preparations than in non-immunized controls. This was true for both of the inhaled proteins, suggesting that an immunologic reaction to the antigen may alter protein metabolism and/or permeability to metabolites in a nonspecific manner.

To determine whether humoral immune mechanisms were involved in the inhibition of antigen absorption, normal rabbits were passively immunized by intravenous injection of rabbit anti-HSA serum 18 hours prior to isolation of the lung. Table 2 shows the results, which indicate that inhibition of intact HSA uptake could be passively transferred with serum.

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*From the Research Service, Wood Veterans Administration Center, and the Departments of Physiology, Medicine and Pathology, The Medical College of Wisconsin, Milwaukee. This investigation was supported by Grant HL 19733 and Specialized Center of Research (SCOR) Grant HL 15389 from the National Heart, Lung and Blood Institute, and by the Medical Research Service of the Veterans Administration.

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To begin to evaluate the role of circulating and tissue antibodies, we perfused isolated lungs from HSA immunized rabbits with blood from normal rabbits, and lungs from normal rabbits with blood to which anti-HSA serum was added. HSA absorption was inhibited under both conditions (Table 2), indicating that antibodies in both lung and blood may contribute to the inhibition of antigen absorption. Tissue antibodies in the lung may explain the reduced protein absorption from lungs of actively immunized rabbits, perhaps by formation of antigen-antibody complexes within the alveolo-capillary barrier. Inhibition of antigen absorption into the blood of the normal lungs perfused with anti-HSA serum may be the result of rapid antibody uptake by lung tissue with antigen-antibody complex formation within the tissue, or it may be due to the formation of antigen-antibody complexes in the blood which are rapidly removed from the circulation by reuptake into the lung. The latter possibility is supported by the observation that antigen, but not an unrelated protein, added directly to the blood perfusing the lungs of immunized rabbits was taken up by the lung. The concentration used was similar to that found in blood perfusing normal lungs after inhalation of the antigen.

Further studies into the mechanisms of antigen absorption and the influence of immunization will help us to better understand the distribution of inhaled antigens. This information may be important in regard to human diseases such as hypersensitivity pneumonitis, in which the lung is the important route of antigen access to the body and the target organ for the immunologic disease as well.

### Table 1—Percentage (±SE) of Inhaled Dose of Radioactivity Present in Blood Perfusing the Isolated Lungs Four Hours after Introduction of the Aerosol Containing Both 131I-HSA and 131I-OA

<table>
<thead>
<tr>
<th>Condition</th>
<th>Normal (n=11)</th>
<th>HSA Immune (n=11)</th>
<th>OA Immune (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCA precipitable</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>131I-HSA</td>
<td>5.1±0.7</td>
<td>1.4±0.3*</td>
<td>5.7±1.2</td>
</tr>
<tr>
<td>131I-OA</td>
<td>8.3±1.2</td>
<td>8.7±0.8</td>
<td>2.4±0.5**</td>
</tr>
<tr>
<td>TCA soluble</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>131I-HSA</td>
<td>5.4±0.5</td>
<td>7.5±0.7†</td>
<td>7.8±0.7‡</td>
</tr>
<tr>
<td>131I-OA</td>
<td>28.8±4.4</td>
<td>40.4±4.1§</td>
<td>48.1±2.8$</td>
</tr>
</tbody>
</table>

*Values significantly lower than normal and OA immune lungs; P<0.01.
**Values significantly lower than normal and HSA immune lungs; P<0.001.
†Values significantly higher than normal lungs; P<0.05.
‡Values significantly higher than normal lungs; P<0.02.
§Values significantly higher than normal lungs; P<0.01.
$Values significantly higher than normal lungs; P<0.005.

### Table 2—Percentage (±SE) of Inhaled Dose of 131I Present in Blood Perfusing the Isolated Lung Four Hours after Introduction of the 131I-HSA Containing Aerosol

<table>
<thead>
<tr>
<th>Condition</th>
<th>TCA Precipitable</th>
<th>TCA Soluble</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n=11)</td>
<td>5.1±0.7</td>
<td>5.4±0.5</td>
</tr>
<tr>
<td>Passively immunized* (n=9)</td>
<td>2.0±0.5</td>
<td>7.0±0.4 NS</td>
</tr>
<tr>
<td>Immune serum** (n=6)</td>
<td>2.0±0.4</td>
<td>9.7±1.0 0.001</td>
</tr>
<tr>
<td>Immune lung (n=5)</td>
<td>1.6±0.6</td>
<td>10.9±3.0 0.025</td>
</tr>
</tbody>
</table>

P values are for t-test comparison with normal lungs.
*Passively immunized refers to lungs obtained from rabbits passively immunized with rabbit anti-HSA serum 18 hours prior to removal of the lung.
**Immune serum refers to lungs from normal rabbits to which rabbit anti-HSA serum was added to the blood in the isolated perfusion system.
†Immune lung refers to lungs from actively immunized rabbits which were perfused with blood from normal rabbits. Residual blood was removed by saline perfusion prior to normal blood perfusion.

### REFERENCES


### DISCUSSION

**Dr. Bice:** Is sufficient antigen absorbed into the blood to end up immunizing a normal previously unexposed animal?

**Dr. Dawson:** Apparently enough intact antigen can be absorbed through the rabbit respiratory tract to induce hormonal immunity during chronic aerosol exposure.

**Dr. Brooks:** What flow rates were used in your lung perfusion model?

**Dr. Dawson:** About 160 ml/min.

**Dr. Brooks:** Did you look at alveolar macrophage function in the immunized animals, because they could be a cause for the increased metabolism noted? Our own studies have demonstrated the metabolic capability of alveolar macrophages.

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Immunology of the Lung 277
Airway Cellular and Immune Response after Exposure to Inhaled Endotoxins

Ragnar Rylander, M.D.; Inger Matsby, B.Sc.; and Marie-Claire Snella, B.Sc.

Airborne Gram-negative bacteria can be found in a variety of general and working environments such as cotton mills, sewage treatment plants and contaminated air from water in humidifiers. From data from epidemiologic studies demonstrate a relation between the presence of pulmonary symptoms such as byssinosis, and the number of airborne Gram-negative organisms.

Little information is available on the local response in lungs after inhalation of Gram-negative bacteria and endotoxins. To elucidate this problem, animals were exposed to Gram-negative bacteria or different endotoxin preparations in acute and subacute exposures. The free lung cell response in the airways was determined by differentiating cells from lung lavage preparations in pulmonary alveolar macrophages (PAM) and polymorphonuclear leukocytes (PMN).

An acute exposure caused an increase of PMN with a peak at 24 hours after the exposure. At repeated exposures, the level decreased after the initial peak, but remained higher than in controls. If the exposure was terminated, the levels fell rapidly toward control values. When the exposure was recommenced, the number of PMNs increased within 12-24 hours.

Measurements were made of the antibody levels in animals exposed to inhaled endotoxin. IgG, IgM and IgA antibodies against LPS were found in serum after ten days' exposure. IgA and IgG antibodies were present in the bronchial fluid. The antibody levels remained unchanged after cessation of exposure after ten days and when this was resumed three days thereafter.

A relation was found between the number of leukocytes in bronchial fluid and the ratio of IgG and IgA anti-LPS antibody titers in the bronchial fluid.

The data suggest that subjective symptoms encountered after a break in exposure to Gram-negative bacteria, for instance among cotton workers, are not due to fluctuations in the LPS antibody level but rather to a change in the size of the leukocyte population in the airways.

References
1 Cinkotai FF, Lockwood MG, Rylander, R: Airborne microorganisms and prevalence of byssinotic symptoms in cotton mills. Am Ind Hyg Assoc J 38:554-559, 1977

Discussion

Dr. Fruhmann: Do you really think endotoxin plays an important role in the pathogenesis of byssinosis? And if so, how do you explain the pressure symptoms? Remember the papers that suggested that the liberation of histamine is the most important thing.

Dr. Rylander: Yes, I believe endotoxin is important. The data also show that the changes in the leukocyte population is important. When endotoxin antibodies aggregate in the lung and are taken up by macrophages, you may get a release of histamine. The symptom of chest tightness is not of asthmatic origin, but could rather be explained by increased pressure in pulmonary veins.

Dr. McCallum: Your exposure protocol mimics one of endotoxin tolerance, and would you envision a similar