ALLERGIC BRONCHOCONSTRICTION IN CONSCIOUS GUINEA PIGS.

THE PATHOPHYSIOLOGY OF ALLERGIC BRONCHOCONSTRICTION IN CONSCIOUS GUINEA PIGS.

by

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ABSTRACT

Pulmonary resistance was measured in conscious, spontaneously breathing guinea pigs sensitized to horseradish peroxidase before and during 2 aerosolized challenges with this antigen. The first challenge was administered to ensure that all animals were sensitized. A second challenge was administered 10-30 minutes later, with the animals having received either atropine or indomethacin. The increase in resistance during the second challenge was similar to that of the first challenge in the indomethacin treated group, but decreased significantly in the atropine treated group. These results show that vagal reflexes are important in allergic bronchoconstriction and that indomethacin in doses large enough to block the synthesis of prostaglandins had no effect on this model of allergic airway disease.

Finally, the effect of a non-specific irritant on airway resistance was compared in both sensitized and non-sensitized animals. It was found that all animals responded to this irritant but that the degree of response was slightly greater in the sensitized animals.

RESUME

Les résistances pulmonaires de cobayes sensibilisés à la peroxidase de raifort ont été mesurées avant et pendant deux expositions à un aérosol de cet antigen. Les animaux étaient éveillée et respiraient spontanément. L'aérosol était administré une première fois pour s'assurer de la sensibilisation des animaux. 30-40 minutes plus tard, les cobayes étaient exposés une seconde fois à un aérosol de l'antigen après avoir reçu une injection d'indomethacin ou d'atropine.
Lors de ces deux expositions, l'augmentation des résistances était identique chez les cobayes prétraités à l'indomethacin, mais décrut de facon significative lors de la seconde exposition chez les animaux atropinisés. Ces résultat démontrent que les réflexes vagaux jouent un role important dans la bronchoconstriction allergique et que l'indomethacin en concentration suffisante pour blocker la synthèse des prostaglandines n'a aucun effet sur ce modèle de maladie des voies respiratoires.

Finalement, l'effet d'un irritant sur la sensibilité des voies aériennes a été comparé chez des cobayes normaux et des cobayes sensibilisés. Une augmentation des résistances fut observée chez tous les animaux mais le degré de la réponse était légèrement plus élevé chez les animaux sensibilisés.

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CHAPTER I

INTRODUCTION

In humans and in animals, the lung is a prime target organ for several immunologically mediated diseases such as: bronchial asthma, allergic alveolitis, pulmonary aspergillosis and maybe, fibrosing alveolitis and pulmonary tuberculosis. Asthma affects primarily the bronchial tree while the other diseases cause damage to the lung parenchyma. Although some insight has been gained during the last few years, the immunological mechanisms underlying these disorders are not yet completely understood and are still under active investigation.

Asthma is a manifestation of allergy or hypersensitivity and is characterized by intermittent attacks of bronchospasm caused by allergic or irritant stimuli (101). Despite extensive studies carried out on both humans and animals for more than 70 years, the relative importance of the humoral mediators and of the autonomic nervous system in the pathogenesis of this disorder remains unclear.

Anaphylaxis denotes a severe hypersensitivity reaction to a foreign protein to which a person or an animal had been previously exposed artificially or naturally. The symptoms of anaphylaxis differ from species to species, but a general feature is smooth muscle constriction (100). In the guinea pig, the target organ is the lung and anaphylaxis is characterized by an intense bronchoconstriction resembling the symptoms of asthma. Thus, the study of anaphylaxis in this animal represents a good model for the study of allergic bronchoconstriction. Many experiments have been conducted on sensitized guinea pigs. However, most of this work has been carried out on deeply anesthetized, artificially ventilated animals.

The aim of the present study was to evaluate the influence of one of the chemical mediators, prostaglandins (86) and of the vagus nerves in allergic bronchoconstriction under more physiological conditions, i.e. in conscious, spontaneously breathing animals.

Finally, we studied the effects of a non-specific irritant: cigarette smoke on the lung function of sensitized versus non-sensitized guinea pigs. The purpose of this experiment was to find out if the well known airway hypersensitivity of asthmatic patients is due to the process of immunization or if it is an unrelated event. As in the preceding experiments, these measurements were carried out on conscious guinea pigs.

In this study, we have used pulmonary mechanics measurements to investigate an immunological reaction. Therefore, a brief review of the pulmonary mechanics theory, as well as a review of the literature concerning the mechanism of allergic bronchoconstriction will be presented.

CHAPTER II

MECHANICS OF THE LUNGS

A. INTRODUCTION

Although the mechanics of breathing was studied by Galen 2000 years ago (40), the principles governing the motion of air in and out of the lungs were explained only centuries later. Rohrer, in 1919, was the first to apply Newtonian mechanics to the lung, particularly the third law of motion which states that any force applied to a body is opposed by an equal force developed by that body (103). In order to use this principle, Rohrer made the assumption that the respiratory system behaves as a rectilinear system with one degree of freedom in which the elements are non-linear. For a system to have one degree of freedom means to have only one way in which to move. For example, a sliding door can move only sideways. On the other hand, a fish, moving in a three dimensional space has three degrees of freedom. For a system to have one degree of freedom also means that the internal parts of this system must behave in a fixed relationship to the motion of the system as a whole. Since it has been shown (102, 80) that the distribution of the ventilation does not change when the respiratory frequency is varied, we can assume that the motion of the different lung units is fixed in relationship with the motion of the lung as a whole and that the lungs can be considered as a system with one degree of freedom.

Rohrer chose the lung volume as the variable in his derived equation of motion and stated that when a force is applied to the lungs, the changes in volume, flow (the first derivative of volume) and acceleration (the second derivative of volume) are proportional to the pulmonary elastic, resistive

and inertial forces; that is:

$$F_{appl.} = K_1V + K_2\dot{V} + K_3\ddot{V} \qquad \text{Eq. 1}$$

where K_1, K_2 and K_3 are constants, V = volume, $\dot{V}$ = flow and $\ddot{V}$ = acceleration. The force applied may be equaled to the transpulmonary pressure and the equation may be restated:

$$P_L = P_{CL} + P_{RL} + P_{IL} \qquad \text{Eq. 2}$$

where P_L represents the difference in pressure between the airway opening and the pulmonary surface (transpulmonary pressure), P_{CL} is the difference in pressure due to the elastic properties of the lung, P_{RL} the difference in pressure due to the resistive properties and P_{IL} the pressure difference due to the inertia of the lung (67).

Fig. 1 shows the variations of volume, flow and acceleration during several normal breathing cycles. It can be seen that there is a 90° phase difference between flow and volume and between flow and acceleration and a 180° difference between volume and acceleration. Rohrer calculated that the inertia of the respiratory system is negligible at normal respiratory frequencies. This was later confirmed by J. Mead (66). However, as the frequency increases, the pressure due to inertia increases more or less proportionately. In the case where the tidal volume (and hence elastic forces) is maintained constant and the frequency increased, the pressure due to inertia will increase and eventually reach a magnitude equivalent, but opposite in sign to the pressure due to elasticity. The frequency at which the two forces cancel each other is called the resonant frequency (see fig.1).

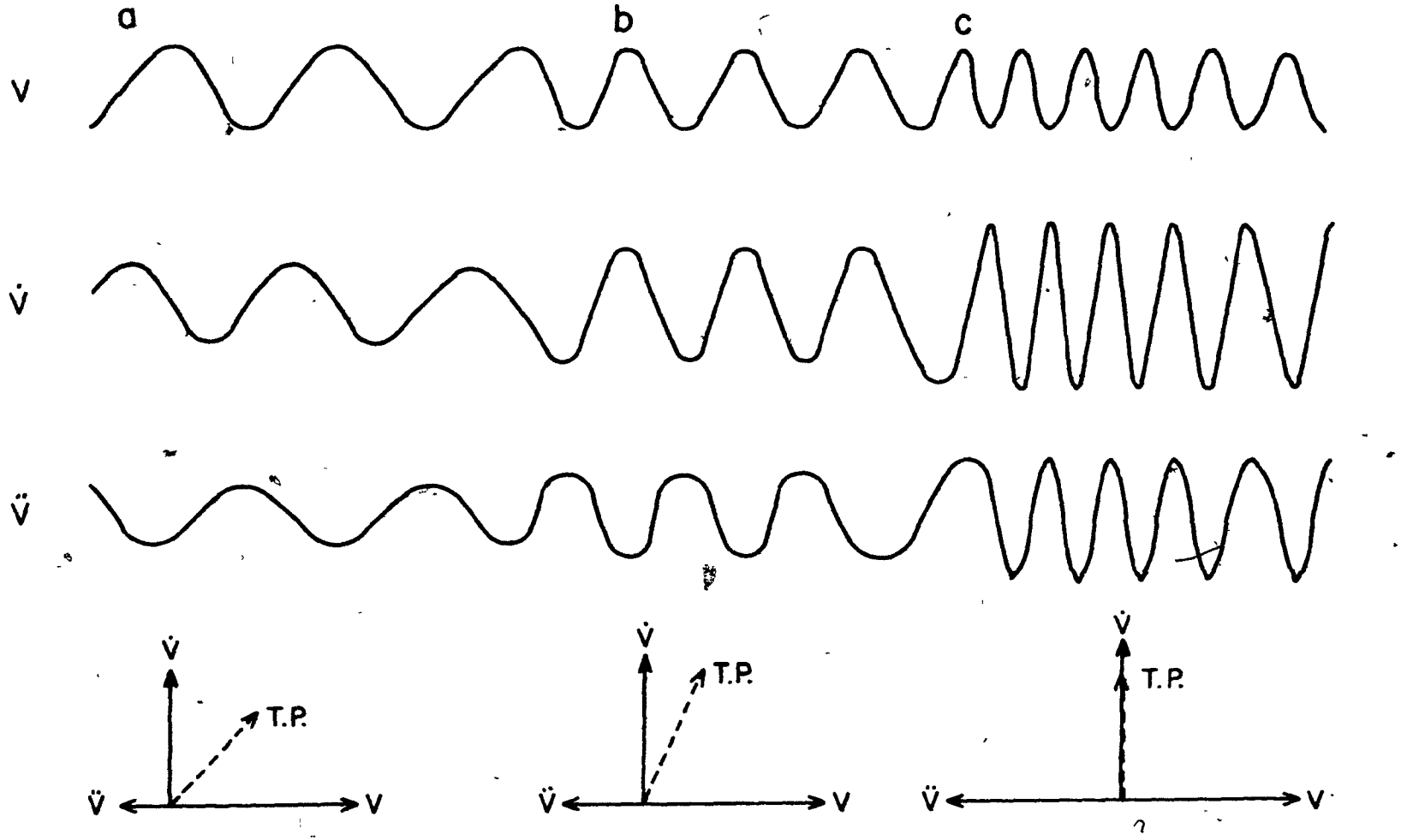

Figure 1 shows that volume is 90° out of phase with flow and 180° out of phase with acceleration when the breathing pattern is a sine wave (a). When frequency is increased and volume is maintained constant, inertial forces increase (b) and at resonant frequency inertial and elastic forces are equal and as they are 180° out of phase, they cancel each other (c).

To summarize: the motion of air in and out of the lungs depends on the elastic, flow resistive and inertial properties of this organ. Since the inertia of the respiratory system is negligible at normal respiratory frequencies, it will be ignored in the rest of this work.

B. STATIC PROPERTIES OF THE LUNGS

Elasticity: the elasticity of an object refers to the tendency of this object to resume its original shape after a deforming force has been applied to it. The term is used here to describe the static mechanical properties of the lungs. This is not strictly correct since, in vivo, gravity and surface tension forces also influence the retractive forces of the lungs. However, the term elasticity is used interchangeably with static properties in respiratory physiology terminology (95).

1. Elasticity of the lungs

For a perfectly elastic object, Hook's law states that the change in length is directly proportional to the applied force. To measure the lung elastic recoil, volume change is interpreted as a change in length and pressure is substituted for force. The pressure-volume relationship of the lungs is linear for most of the vital capacity range and the slope $\Delta V/\Delta P$, termed compliance, is used to express the degree of lung elasticity.

Working on dead cats, Carson (40), in 1820, measured elastic recoil for the first time by observing the increase in tracheal pressure upon opening the thorax. More exact pressure-volume curves were obtained at the beginning of this century by Jaquet, Bernoulli and Rohrer (40). The latter produced pressure-volume curves both in relaxation and with maximum inspiratory and expiratory efforts. Unfortunately his pioneer work was not immediately understood and it was not until 1946 when Rahn and his colleagues "remeasured" pressure-volume curves that the nature of pulmonary elastic properties began to be generally appreciated (40). It is now believed that the elasticity

of the lungs can be described as due primarily to a combination of surface tension and tissue forces.

Surface tension forces: The fact that curves obtained during inflation of collapsed lungs differ from those obtained during deflation was pointed out rather recently, in 1956, by McIlroy et al. (63) and Radford (94). Radford attributed this phenomenon called hysteresis to the fact that during inflation there are both units opening and units expanding, whereas during deflation all units contract. Since the pressure required to open a lung unit is considerably greater than that required to expand it or to keep it open, one would therefore expect inflation and deflation curves to differ. This theory, however, was not completely satisfactory as hysteresis still occurs at pressures where all units are opened. An alternative explanation was offered by Clements and Brown (19,21) who showed that the fluid lining the alveoli, called surfactant, has a surface tension coefficient dependent upon lung volume and that surface forces developing during inflation are greater than surface forces developing during deflation.

The importance of surface forces was first pointed out by von Neegaard (74) who showed that the elastic recoil pressure decreased markedly when the lungs were fluid-filled. Subsequently Radford (94) noticed that, after washing the lungs several times to remove the mucus, the pressure-volume curves of fluid-filled lungs exhibited very little hysteresis. He concluded that surface tension in the lungs was the major cause of hysteresis and an important component of the elastic recoil pressure. At the same time, however, Pattle (82) measured the surface tension coefficient of fluid extracted from the lungs and found it to be low. A controversy followed, with von Neegaard

and Radford claiming that surface forces are a major component of the elastic recoil pressure and Pattle maintaining that these surface forces contributed very little. The difference of opinion was solved when Clements and Brown showed that the surface tension coefficient is not fixed but varies with lung volume.

Tissue forces: The elastic properties of the lungs themselves are largely due to elastin, a protein which has a length-tension curve linear up to 70% extension (50). The other major connective tissue fiber, collagen, is relatively inextensible and is believed to function as a supportive framework, preventing the elastic fibers from stretching too much and rupturing (95). The contribution of the other lung tissues to elastic recoil has not been fully established. Radford (95) showed that the elastic properties of excised lungs refrigerated for several days did not change markedly although it is known that this treatment damages the delicate epithelial cells lining the airways and alveoli.

It has been postulated that smooth muscle may play an indirect role in tissue elasticity. If the smooth muscles lining the alveolar ducts and alveolar sacs contract, alveolar constriction follows. This modification of the lung's structure provokes changes in surface tension forces and therefore changes in the pressure-volume curve itself (95).

The direct contribution of blood vessels to the elastic recoil is unknown. It has been shown that the compliance is decreased when the pulmonary vascular volume is increased. It is likely that this decrease in compliance is not due to a change in the intrinsic elastic properties of the tissue but, as with smooth muscle, due to modification of the surface tension secondary to an alteration of the lung's geometry (95).

2. Measurement of a pressure-volume curve

Pressure-volume curve of the total respiratory system: If the equation of motion is applied in condition of no flow, resistive and inertial forces are nil, and the pressure applied will be equal to the pressure developed by the elastic forces of the lungs and chest wall at a given volume.

$$P_{appl.} = P_L + P_W \qquad \text{eq. 3}$$

where P_L is the pressure developed by the lungs and P_W the pressure developed by the thorax. The applied pressure is defined as the pressure at the airway opening (Pao) - the pressure at the body surface (Pbs) (1). A simple way to establish a pressure-volume curve was developed by Rohrer (104) and later by Rahn et al.(96): the subject inspires a known volume of air and then relaxes against an obstructed airway. The total pressure developed by the lungs and the thorax is measured with a manometer combined to a side arm of the mouth piece. This manoeuvre is repeated several times at different lung volumes so that the pressure-volume relationship can be established over the total lung capacity range.

It should be noticed that with this technique, Pbs is atmospheric and also that this way of establishing a pressure-volume curve is valid only if the subject is able to relax his respiratory muscles completely.

Other ways of measuring the elasticity of the respiratory system have been devised where, instead of varying Pao, Pbs is varied by applying positive pressures around the body (1).

Pressure-volume curve of the lungs and chest wall: A partitioning of the elastic recoil of the respiratory system into a component due to the lung

and that due to the chest wall can be made if the pleural pressure (Ppl) is known. In this instance, the lung's elastic recoil is equal to the alveolar pressure (Palv) - Ppl and the elastic recoil of the thorax is equal to Ppl - Pbs.

A direct measurement of the pleural pressure is extremely difficult, if not impossible, to obtain in humans and in large animals. It is possible, however, to get an approximation of Ppl by measuring the pressure inside the oesophagus (the wall of the oesophagus being flexible, it is assumed that the pressure inside the oesophagus is a reflection of the pressure inside the thorax). This measurement is made by placing in the oesophagus an air-filled latex balloon sealed over a catheter which in turn transmits the balloon pressure to a manometer. In small animals, like guinea pigs, it is possible to record directly the pleural pressure by introducing a catheter in the pleural cavity.

At a constant lung volume and with the airways unobstructed, the alveolar pressure will be equal to the Pao and a pressure volume curve of the lungs can be obtained by measuring Pao - Ppl at different lung volumes.
The elastic recoil of the thorax may be established with the subject relaxed against an obstructed airway. In this case:

$$Pao - Pbs = P_L + P_W \qquad \text{Eq. 4}$$

and since $P_L = Palv - Ppl$, and $Pao = Palv$ we obtain, simplifying:

$$Ppl - Pbs = P_W \qquad \text{Eq. 5}$$

It is worth noticing that the end of a normal expiration represents the resting position of the respiratory system. At this point, the elastic recoil

of the lungs and chest wall are opposite in direction and equal in force so that the static pressure developed by the respiratory system is zero (1).

$Pao - Ppl = 5 \text{ cm } H_2O$

$Ppl - Pbs = -5 \text{ cm } H_2O$

Measurement of pressure-volume relationship during breathing: Von Neegaard and Wirz first noted that at two points along the respiratory cycle (end inspiration and end expiration) there is no flow and therefore the difference in pressure between these two points is due only to a difference in volume (73).

At end expiration, we have Pel = Px, and

at end inspiration: $Pel = Px + \frac{1}{Cdyn} \times \Delta V$ Eq. 6

where ΔV is the tidal volume and Cdyn is the slope of the pressure curve.

Subtracting, we obtain: $\Delta Pel = \frac{1}{Cdyn} \cdot \Delta V$ or

$C \text{ dyn} = \frac{\Delta V}{\Delta Pel}$ (67) Eq. 7

Because it is measured under conditions of continuous breathing instead of breath holding, the relationship pressure-volume is called dynamic compliance. This technique is valid only if the thousands of units composing the lungs fill and empty synchronously, i.e. if their time constants are the same. The time constant is the product of resistance and compliance and represents the time taken by a particular lung unit to equilibrate to a pressure change. Indirect evidence i.e. that dynamic compliance is independent of respiratory frequency indicates that this assumption regarding the lung unit behaviour is correct for normal mammalian lungs (80).

This particular method for measuring compliance is used in the present study and a graphic demonstration is shown on fig. 2.

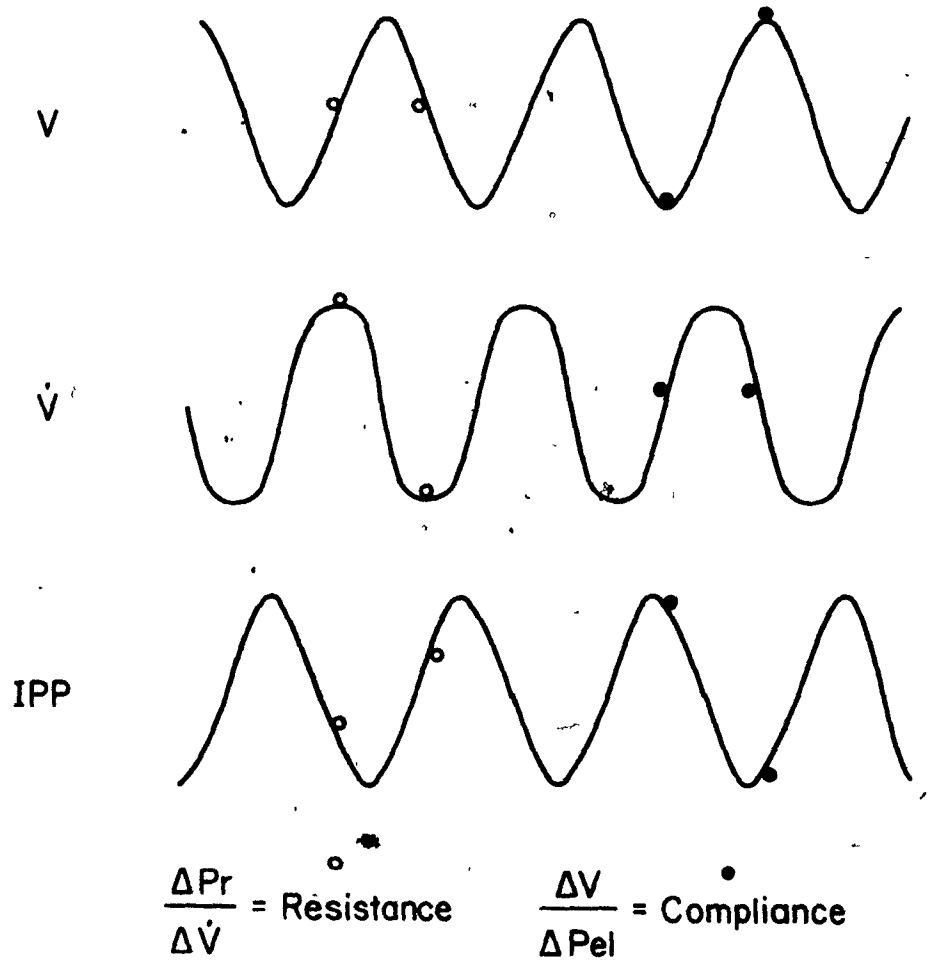

Figure 2: Method of measuring pulmonary resistance and pulmonary compliance.

C. DYNAMIC PROPERTIES OF THE LUNGS.

1. Introduction

In 1842, Poiseuille, a French physician investigating the relationships between pressure and flow through a tube, reported that flow was proportional to the difference in pressure between the ends of the tube, the fourth power of the radius of the tube and to the reciprocal of the length of the tube. The coefficient of viscosity was later defined by E. Hagenbach who worked out the equation known as Hagen-Poiseuille law (89).

$$\Delta P = \frac{\dot{V}.8\eta l}{\pi R^4} \qquad \text{Eq. 8}$$

where $\dot{V}$ = flow, r = radius of the tube, μ the viscosity of the fluid in poises, l = the length of the tube, ΔP = the difference in pressure between the ends of the tube.

This equation is valid only when flow is laminar, i.e.: in certain conditions, fluid flows through à tube as a series of concentric layers whose velocity is maximal at the center and decreases parabolically to the edge of the tube where flow becomes zero. Such flow is termed laminar and its occurrence depends on a number of factors as defined by the following equation:

RN = Reynold's number = $2\rho\dot{v}/\pi r\mu$

where $\dot{V}$ = flow, r = radius of the tube, μ = viscosity in poises, and ρ the density in g/cm^3.

If the RN is less than about 2000, laminar flow occurs. In this case, the only interaction between concentric fluid layers is frictional and thus, all

other conditions being equal, flow will depend on the viscosity of the fluid. In turbulent flow, however, (RN greater than 2000) the flow of concentric layers past each other is not smooth, and molecules are transfered from one fluid layer to another. This results in constant accelerative and decelerative forces being applied at various layers and so density rather than viscosity becomes the important flow determining factor. In addition to changes in flow, radius etc., turbulent flow is also produced at points of tube branching. At these points, the laminar profile of the fluid is disturbed and there is formation of eddy currents, so producing turbulent flow. To reestablish a laminar profile, energy will now be required to overcome inertial as well as viscous forces. Consequently, the pressure difference required to regain laminar flow will be greater than the pressure that would have been necessary to continue the flow if branching had not occurred. The length of the tube required to reestablish a laminar profile is called the entrance length (50).

Recently, Pedley et al. (83,84) have developed sophisticated equations taking into account changes in kinetic energy and viscous energy dissipation downstream of a branching to predict the pressure drop within the airways. However, the development of these complex equations involves modern concepts of aerodynamics which are beyond the understanding of the author and therefore this will not be discussed in any further detail.

2. Pulmonary resistance

The study of the dynamic properties of the respiratory system is concerned with the measurement of the different forces driving air in and out of the lung. One of the most important parameters measured is resistance. This is

defined as the pressure difference required to move a certain volume of air in and out of the lungs in a certain time period. That is:

$$R = \Delta P / V/T = \Delta P/\dot{V}. \qquad \text{Eq. 9}$$

Where P = pressure, V = volume, T = time, $\dot{V}$ = flow.

Rohrer calculated the resistance to air flow in the lungs by measuring the airway size in excised lungs and subsequently determining the P drop through tubes of the same size. He developed the following equation:

$$Pres = K_1\dot{V} + K_2\dot{V}^2 \qquad \text{Eq. 10}$$

where K_1 is a constant including viscosity and K_2 a constant including density (103).

Using this equation, Rohrer determined with a surprising accuracy that nasal resistance accounted for 50% of the total resistance during quiet breathing and that resistance due to the upper airway would be about 25% of the total during mouth breathing (103). These values have since been confirmed by Butler (20), Opie et al. (77) and Hyatt and Wilcox (51).

Rohrer also calculated that 70% of the remaining resistance was to be found in small airways. Recently, however, Macklem et al. (61) measured the resistance of the small airways (2.5 mm or smaller) and lung tissue using a retrograde catheter, and found it to be only 10% of the total pulmonary resistance. In addition, Olson et al. (76) and Pedley et al. (84) calculated on the basis of morphological data that the large airways are responsible for most of lower airway resistance. A great part of this resistance is due to an increased pressure drop along airways due to entrance length phenomena.

Summarizing therefore, we may roughly partition pulmonary resistance into 50% upper airways, 40% large lower airways and 10% small airways and lung tissue.

3. Measurement of pulmonary resistance

During inspiration or expiration, there is gas flow, consequently the transpulmonary pressure will have an elastic and resistive component (neglecting the small inertial component). The resistive component may then be obtained by subtracting from the total transpulmonary the elastic component derived in equation 6.

$$Pres = P_L - \left[Px + \frac{1}{Cdyn} \times \Delta V \right] \qquad \text{Eq. 11}$$

Different methods to accomplish this subtraction have been devised. Neegaard and Wirz, who were the first to suggest it, employed a graphical technique using time plots (73) while Mead and Wittenberger developed an electrical method (65).

Another technique was derived by DuBois et al. (37) who utilized the fact that the respiratory system has a resonant frequency of 4-6 cycles/sec. At this frequency, elastic and inertial forces cancel each other (see p.4), so that the total transpulmonary pressure measured represents only the flow resistive component.

The present study utilized a technique developed by Amdur and Mead (3). Since elastic forces are equal at any two points of equal volume during a respiratory cycle, the pressure difference between these two points must be related to flow resistive forces. Resistance may therefore be obtained by dividing the pressure difference by the flow difference at points of equal volume. This technique neglects the hysteresis factor but this is justified because of its small magnitude in the tidal volume range (67). A graphic demonstration of the method is shown on fig. 2.

4. Measurement of airway resistance

Airway resistance is equal to the difference between alveolar pressure and mouth pressure, divided by flow. The measurements of flow and mouth pressure present no problem but an estimation of the alveolar pressure is more difficult to obtain.

In one method developed by DuBois and his associates (38), the subject is placed in a body plethysmograph and the movement of air in and out of his chest is measured as fluctuations of pressure in the chamber. DuBois amplified these pressure changes by having his subject pant. The pressure in the chamber is then calibrated by having the subject pant against an occluded airway. In this case, mouth pressure equaled the alveolar pressure and the simultaneous pressure change in the box could be equaled to alveolar pressure.

McIlroy (63) developed a technique for measuring airway resistance which did not necessitate estimation of the alveolar pressure. He first measured the tissue component of resistance by using gases of different viscosity. By extrapolation, he obtained resistance at zero viscosity, i.e. lung tissue resistance, and subtracting the latter from pulmonary resistance, he obtained airway resistance.

5. Measurement of tissue resistance

Attempts to evaluate tissue viscous resistance (TVR) have yielded somewhat varying results. Subtracting airway resistance from pulmonary resistance, DuBois found the TVR to be about 20% of the total resistance. Utilizing gases of different viscosities as described above, McIlroy et al. (63) found that about 30-40% of the pulmonary resistance was due to the tissue component,

while Macklem and Mead (61), using a retrograde catheter estimated the small airway+tissue resistance to be only 10%.

This wide range of values has been attributed by J. Mead (67) to differences in breathing patterns during the measurements.

6. Factors affecting pulmonary resistance

Lung volume: The relationship of pulmonary resistance to lung volume was thought to be hyperbolic, with the lowest resistance being at high lung volume (39). However, Macklem and Mead (61) recently found that the lowest resistance occurs at about 70% of the vital capacity. These authors suggest that the increase in R at higher lung volumes may be due to a decrease in the diameter of the airways secondary to their lengthening.

Lung size: The relationship of airway resistance to lung size has been shown to be hyperbolic (15). This indicates that changes in lung and in airway dimensions are linear.

Bronchomotor tone: The smooth muscles lining the airways are innervated by both the sympathetic and the parasympathetic nervous systems. An increase in parasympathetic outflow, or the blockage of the sympathetic, causes constriction while an increase in sympathetic activity or blockage of the vagus nerves causes dilatation of the airways. In man, the normal bronchomotor tone seems to be the balance between the actions of these two systems (39).

Besides this regulation by the autonomic nervous system, a number of endogenous substances such as histamine or exogenous substances such as irritants can induce smooth muscle constriction while adrenalin and sympathomimetic drugs will induce dilatation.

CHAPTER III

IMMUNOLOGY

A. INTRODUCTION.

The immune system of the body (immune means protection) has three main functions (8):

1. Defense: protection of the organism against the invasion of foreign bodies such as bacteria, viruses, fungi.
2. Homeostasis: the removal of dead cells and residues from the organism.
3. Surveillance: concerned with the destruction of malignant cells or cells in mutation.

In the present study, we investigated one aspect of the defense mechanisms. Consequently, the two other functions of the immune system, namely homeostasis and surveillance will not be discussed in any further detail.

B. ANATOMY OF THE IMMUNE SYSTEM.

The immune system consists of several different types of specialized cells. In some locations, these cells have clustered together and, held by reticular fibers, form organs such as the thymus, the lymph nodes, the lymphatic nodules and the spleen.

The different cells composing the immune system have been divided into two categories depending on whether their action is specific or non specific. The non specific category include cells capable of destroying a foreign body by phagocytosis or by releasing various substances directly or indirectly noxious to the foreign body, while the specific cells are characterized by

their ability to produce special proteins (antibodies) reacting with a particular foreign body (antigen). These latter cells are also characterized by their immunological memory, i.e. their faculty of remembering an antigen, and, upon subsequent exposure to it, their response will be faster, greater in amplitude and last longer.

The non specific cells are: macrophages, neutrophils, eosinophils and basophils and the specific cells are: the lymphocytes and plasma cells.

The lymphocytes have been in turn divided into two categories:

1. The T Lymphocyte (Thymus-derived lymphocyte): These cells are responsible for the so-called "cell mediated immune response" and also, probably, for immunologic memory (43). They react to an antigen (Ag) by releasing different substances which increase vascular permeability, attract eosinophils and monocytes and prevent macrophages from migrating. Although it has not been studied in man, experimental evidence from animals shows that the thymus is an essential organ for the development of immunologic competence during embryonic life and that not only the cell mediated immune response, but also the antibody production are impaired following neonatal thymectomy (43).

The mode of action of the thymus on the lymphocytes has not yet been clearly established. One current hypothesis is that it might secrete a hormone.

2. The B Lymphocyte: These cells are characterized by their production and release of antibodies. Their site of origin is unknown in man. The letter B stands for Bursa of Fabricius, their site of origin in the chicken where they were first isolated and their role studied. In mammals, it is generally believed that they arise from the bone marrow.

Both types of lymphocytes are found in the spleen, the lymph nodes, the Peyer's patches and the peripheral blood.

C. DEFENSE MECHANISMS.

1. Introduction

Upon entry of a foreign body in the organism, three different types of reactions may be encountered:

a) the foreign body is met by a macrophage and phagocytosed. The reaction however does not elicit a response from the specific cells.

b) a foreign body - generally a protein or carbohydrate with a molecular weight greater than 10,000 - enters the body. The subsequent phagocytosis by a macrophage triggers a series of complex events which ultimately leads either to the proliferation and differentiation of B lymphocytes into plasma cells and the production by the latter of antibodies, or to the sensitization of T lymphocytes.

Both the cells of the B and T system seem to be able to recognize an antigen by an antibody-like molecule on their surface (43).

c) in some very specific conditions an antigen does not elicit an immune response. This happens when 1) the organism has been exposed to the antigen during its fetal life, 2) the foreign body is poorly antigenic, 3) the Ag is administered in a dose either too high or too low which leads to what is known as high or low dose paralysis. This non responsive state, as well as the ability of recognizing "self" from "non self" is called tolerance (43).

2. Immunoglobulins

In humans, five basic types of immunoglobulins have been found: IgG, IgA, IgM, IgD and IgE. The basic chemical structure is the same for each class.

It consists of four polypeptide chains held together by disulfide bonds (see fig. 3). Two of the chains are long with a molecular weight of 55,000 and are known as the heavy chains, while the two others are shorter with a molecular weight of 22,000 and are called the light chains. Each immunoglobulin posesses two identical light chains and two identical heavy chains. The chemical configuration of the heavy chain is different for each class of immunoglobulins ($\gamma, \partial, \mu, \delta, \varepsilon$). There are also two types of light chains k and λ. Each type of light chain can be found in any class of immunoglobulin (43).

The functional structure of the immunoglobulin has been studied by splitting the molecules into three fragments with the proteolytic enzyme papain (see fig. 3). Two of the fragments, each formed of one light chain and one segment of the heavy chain retained their ability to bind to an antigen and were called the FAB fragments while the third one, called FC, formed of two segments of the heavy chains, was found to confer to the molecule's biological properties such as fixing the complement, crossing the placenta, and fixing to tissue cells (43).

The IgG molecule is the most abundant. It represents 70% of the total amount of immunoglobulins and is found both vascularly and extravascularly. This immunoglobulin is responsible for the immunity to bacteria, viruses, parasites and fungi (75).

The IgA molecule is found as a monomer in the serum and as a dimer held by a secretory piece in the secretions lining the gastrointestinal, respiratory and urogenital tracts.

Its function is not clearly known, it is thought however, to represent a first line of defense against respiratory infections and to play some role

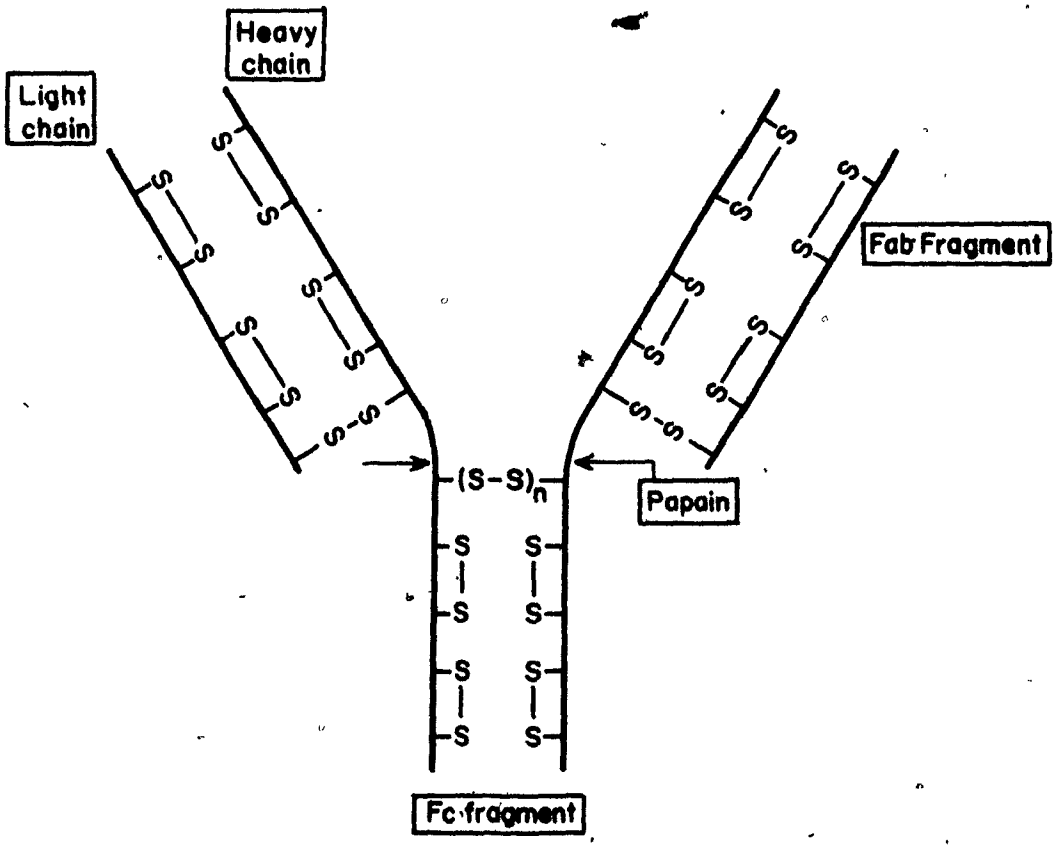

Figure 3: Diagrammatic representation of an immunoglobulin structure. (After S.O. Freedman).(43)

in regulating the flora of the gastrointestinal and respiratory tracts (75).

The IgE molecule is found in very small amounts in the linings of the respiratory and intestinal tracts. It has the unique property of adhering to cells through its FC fragment. This immunoglobulin, also called reaginic antibody, is responsible for the allergic reactions such as asthma, hay fever, etc.

Its normal function is unknown, but it is speculated that it could be somewhat similar to that of IgA (55).

IgM is a pentamer of the four chains type found in the vascular bed. As the concentration of this immunoglobulin is increased early after exposure to an antigen, it is believed that IgM represents the first line of defense (75).

IgD is found in very small amounts in the serum, and its role is completely unknown (9).

3. The immune response

The action of the immune system is generally beneficial to the host. However, it may sometimes be harmful. This is the case when the system overacts to substances generally inocuous, like in allergies, or as a secondary effect when Ag-Ab interaction leads to tissue injury, as in tuberculosis or glomerulonephritis.

This type of harmful response has been divided into four categories:

Type I or immediate hypersensitivity or homocytotropic reaction: In this reaction, the antibody is bound to a target cell (generally a mast cell or a basophil) and the antigen is circulating. The binding of the antibody with

the antigen triggers an intricate chain of reactions which ultimately leads to the release by the target cell of chemical mediators producing bronchoconstriction and intense vasodilatation. This reaction is responsible for the various types of allergies and for allergic asthma.

The role of some of the mediators of this reaction being the subject of the present study, the mechanism of immediate hypersensitivity is discussed in detail in section D.

Type II or cytotoxic reaction: In this reaction, the Ag is either a component of the cell or has become bound to the cell surface and the Ab is circulating. The subsequent coating of the cell surface by the Ag-Ab binding renders the cells more susceptible to intravascular and extravascular destruction. The Ag-Ab interaction also activates the complement system. This system is composed of a series of nine plasma proteins which act in cascade following their activation. This reaction leads to the release of chemical mediators enhancing lysis and phagocytosis of the target cells and ultimately leading to tissue damage and necrosis (10).

Type III or immune complex mechanism: Circulating Ag-Ab complexes of intermediate size with a slight excess of antigen are not removed from the organism and tend to localise in the walls of a vessel or the kidney. Evidence shows that the complement system is activated, releasing - among others - a factor chemotactic for neutrophils. These cells are responsible for phagocytosing the immune complexes and it has been shown that they also release proteolytic enzymes capable of destroying elastin, collagen and cartilage, therefore producing tissue injury (10).

Type IV or delayed hypersensitivity: This reaction seems to be due to a direct interaction of the Ag with a sensitized T lymphocyte. The subsequent release

of mediators result in mononuclear cells invasion, vascular damage and necrosis. Classical examples of delayed hypersensitivity are the Tuberculin test and the rejection of grafts (10).

D. TYPE I: HOMOCYTOTROPIC REACTION: IMMEDIATE HYPERSENSITIVITY.

1. Introduction

In 1839, Magendie (99) noticed that a substance, apparently inocuous in itself, could trigger a fatal reaction when injected for the second time in the same animal. During the second half of the 19th century, several other investigators - studying the mechanism of immunity - noticed the same phenomenon without attaching much importance to it, considering it as "un accident de parcours" (99), and it was only in 1902 that the first systemic study of this reaction was published by Portier and Richet (91). These authors called it anaphylaxis (meaning lack of protection) and stated that the substance albuminoid (antigen), insufficient to kill, or even to make a normal animal sick, provoked an overwhelming and often fatal reaction in an animal, which, a long time ago, had received this same substance. In a book published some years later, Richet (99) mentions that the symptoms of anaphylaxis vary from species to species and Auer and Lewis (4) showed that in the guinea pig, the respiratory system is primarily affected, the animals dying from asphyxia due to intense bronchiolar constriction and acute emphysema. Richet attributed these symptoms to the action of a toxin on the central nervous system while Auer thought that they were due to a paralysis of the peripheral vasomotor system.

At about the same time von Pirquet (88) observed the same phenomenon and called it allergy, meaning a state of altered reactivity which resulted from exposure to an antigen and was harmful to the host. Since then, allergy and

hypersensitivity have been used interchangeably to describe an altered reactive state while the meaning of anaphylaxis has extended and is used to describe a severe, immediate reaction which can be due to a cytotropic, cytolitic, or immune complex mechanism (6). In the present study, however, anaphylaxis will be used synonymously with allergy.

In 1907, two German scientists, Otto and Friedmann (81,44) independently discovered that anaphylaxis could be passively transfered i.e. they showed that if they injected a normal animal with the blood of a sensitized (rendered allergic) one, and after an appropriate latent time injected the recipient with the antigen, the recipient died in anaphylactic shock. This important discovery proved that exposure to an antigen induced in the donor animal the production of substances (later called antibodies) circulating in the blood stream.

Otto was also the first to notice that if an animal survived the anaphylactic shock, it developed a state of tolerance to the antigen (81) and Besredka and Steinhardt (12) showed that this same state of tolerance could be induced by repeated small injections of the antigen and called this reaction "desensitization". This procedure is still used for the clinical treatment of hay fever and other types of allergies.

Besredka (13) also suggested that antigen-antibody reaction was taking place at the surface of the cell and that the latent period was the time taken by the antibody to fix on a cell. This hypothesis was later confirmed by Schultz (107) and by Dale (31) in a now classic experiment: These authors showed that an isolated strip of smooth muscle obtained from a sensitized animal contracted when antigen was added to the fluid bathing it, and this in complete absence of blood.

Some years later, Manwaring and Kusama (62) developed a similar technique to study anaphylaxis in isolated, artifically perfused lungs of sensitized guinea pigs.

Another important step in the understanding of allergy was taken in 1921 when Prausnitz and Kustner (92) investigating the passive transfer of anaphylaxis, showed that a local reaction could be produced if the serum of an allergic person was injected intracutaneously into a normal person. After an appropriate latent time, the antigen, injected via the same route provoked an inflammatory lesion at the site of injection of the serum. Although it has since been given up in humans because of the potential danger of transmitting hepatitis, this classic experiment is still used nowadays in laboratory animals as a semi-quantitative measurement of their sensitization and is known as passive cutaneous anaphylaxis or PCA.

Some years later, Alexander et al. (2) reported that it was possible to sensitize an animal to an antigen by repeated inhalations and Ratner et al. (97), using this procedure, sensitized guinea pigs to horse dander and suggested this as a model of experimental asthma. Since then, numerous studies have been conducted in sensitized guinea pigs, both in vivo and in vitro, in order to elucidate the mechanism of allergies.

Quite recently, the antibodies causing this reaction have been isolated and classified as IgE (53,57). In vitro experiments by Mota (70) and by Ishizaka et al. (56) have shown that these antibodies bind on at least two types of cells: the mast cells and the leukocytes and that in the presence of the antigen these cells degranulate, releasing histamine.

Guinea pigs, as well as rats and mice have two types of homocytotropic antibodies. In rats, the two types of immunoglobulins have been identified as

being $\gamma 1$ and IgGa (54). In the guinea pig, Dobson et al. (35) recently isolated by physicochemical techniques and by skin sensitizing tests, two types of homocytotropic antibodies: one belongs to the IgG class and is termed Igγ1, the other one is analogous to the human reaginic antibody of the IgE class.

2. Humoral mediators

That anaphylaxis could be due to humoral mediators had been recognized very early (99), and the similarity between anaphylaxis and the reaction to histamine had been noticed by Dale and Laidlaw in 1910 (30). In 1932, Bartosh et al. (7) found that a histamine like substance was liberated during anaphylactic shock in vitro and some years later, Code (22) identified this substance as being histamine. Histamine causes smooth muscle constriction, local dilatation of small blood vessels and an increase in vascular permeability (6).

In 1940, Kellaway and Trethewie (60) discovered that, during anaphylactic shock, isolated lungs secreted a substance different from histamine in that it caused a slower contraction of the guinea pig ileum than histamine. Brocklehurst distinguished this substance from bradykinin, serotonin and substance P and called it the slow reacting substance of anaphylaxis or SRS-A (16). The chemical nature of SRS-A is not yet known, nor is its site of synthesis. It seems to be an acid lipid and might be released by mast cells and/or leukocytes (6). The release of SRS-A during anaphylaxis has been demonstrated in vitro and up until now in vivo in only two species: the rat (6) and the guinea pig (108).

In vitro experiments showed that mast cells and leukocytes released histamine (6,56). The first step of this reaction is thought to be the binding of the antigen to at least two antibodies (93) this in turn activates an esterase

which modifies the cyclic AMP of the target cell and, in presence of calcium triggers a chain reaction which ultimately leads to the release of histamine (78).

Brocklehurst and Lahiri showed that bradykinin concentration was increased in the blood during anaphylaxis in vitro and found a bradykinin forming enzyme in the lungs perfusate of guinea pigs (17,18). More recently, it was shown by Piper et al. (85,86) that adrenaline and at least two types of prostaglandins ($F2\alpha$ and E2) were released during anaphylaxis in vitro. The mechanism of the liberation of prostaglandins is not yet completely understood: Piper and Vane found that smooth muscle liberates prostaglandins when it contracts, and think that almost any kind of stimulus, chemical or mechanical will lead to the release of prostaglandins. These authors also established that there is no storage of prostaglandins in the tissue, but that this substance is synthetized on demand (87).

$PGF2\alpha$ causes smooth muscle contraction while the role of PGE2 in the guinea pig is still unclear. Vane found that it sometimes constricted smooth muscle, while some other authors think it is a bronchodilator (105).

The mechanism of the release of adrenaline is also unclear. However, each of the bronchoconstrictor agents described above is also an adrenaline releaser; it has been proposed that bradykinin may be especially important in this regard (26).

3. The role of the parasympathetic nervous system

The role of the vagus nerves in anaphylaxis was questioned as early as 1910 (5) and is still under active investigation. At the beginning of this century, Auer (5) tried to inhibit the anaphylactic reaction in the guinea pig by unilateral or bilateral vagotomy. This procedure did not prevent the animals

from dying in anaphylactic shock, but the author also noticed that animals pretreated with atropine were somewhat protected. This apparent illogical observation could be due to the fact that guinea pigs very often die spontaneously following vagotomy (69). Recently, Collier and James (25) working on anesthetized, artificially ventilated guinea pigs were unable to measure any change in lung resistance to inflation during anaphylaxis, before and after destruction of the central nervous system. These results contradict those of Mills and Widdicombe (69) in the guinea pig and those of Gold et al. in humans and in dogs (46,116). Mills and Widdicombe (69) showed that the increase in airway resistance during anaphylaxis was significantly reduced following vagotomy. A few years ago, Mills, Sellick and Widdicombe (68) found nervous endings responding to chemical and mechanical stimuli located in the bronchial epithelium of the guinea pig which they called the irritant receptors. These authors also showed that the efferent pathways of these receptors are in the vagus nerves and that their response to a stimulus is a reflex bronchoconstriction. They, therefore, suggest that a reflex of this kind, triggered by a local release of mediators, is partly responsible for the bronchoconstriction in anaphylaxis. Their results were confirmed by those of Gold et al. (46) who suppressed part of the allergic bronchoconstriction in the dog by vagotomy, cooling of the vagi or inhalation of atropine.

Information concerning the possible role of the sympathetic nervous system is less. Adrenalectomy or β Blockade intensifies the airway resistance to inflation during anaphylaxis in the guinea pig (25) but there seems to be great individual differences in sympathetic tone from animal to animal (34).

4. Inhibition of the humoral mediators

In order to clarify the relative importance of the humoral factors, several attempts have been made in vitro and in vivo to antagonize either their release or their action.

Administration of propranolol, a β receptor blocking agent, or adrenalectomy intensifies the allergic bronchoconstriction.

The increase in lung resistance to inflation being greater following propranolol administration than after adrenalectomy suggests that not only adrenaline but noradrenaline, moderate the anaphylactic bronchoconstriction by their bronchodilator actions (34).

Collier and his colleagues (24,25,27) found that aspirin and other non-steroidal anti-inflammatory drugs antagonized the bronchoconstriction induced by kinins and SRS-A and also that the degree of allergic bronchoconstriction was decreased following administration of mepyramine, an antihistaminic agent.

In vitro experiments conducted by Tethrewie showed that very high doses of aspirin can also inhibit the release of histamine from sensitized guinea pigs lungs during anaphylaxis (112).

More recently, Vane (113) has demonstrated that aspirin, and more particularly indomethacin, inhibited prostaglandin synthesis in vitro and these results were confirmed by Hamberg and Samuelsson (47) who inhibited 98% of prostaglandin synthesis in guinea pigs by administering indomethacin.

5. Inhibition of the vagus nerves

To inhibit the action of the vagi during allergic bronchoconstriction, two possibilities are offered to the experimenter:

a) vagotomy

b) atropine

Vagotomy implies cutting afferent and efferent fibers running along with the vagi, and therefore is not a selective inhibition. The side effects of this drastic measure are difficult to control and therefore difficult to assess. For these reasons, vagotomy is not a particularly good method. In small doses, atropine sulfate, an alcaloid derived from Atropa belladonna, blocks the parasympathetic postganglionic synapses only and therefore interferes less with the rest of the autonomic nervous system traffic (52).

6. Effect of a non-specific irritant

It is known that patients with bronchial asthma have airways more sensitive than normal people to acetylcholine, histamine, β adrenergic blocking agents and inhaled dusts (29, 42, 64).

Popa et al. (90) studying the airway response to inhaled histamine, acetylcholine and propranolol in immunized and non-immunized guinea pigs were unable to detect any difference in airway sensitivity between the two groups, except immediately following an antigen challenge. These authors think that the enhanced reactivity of the airways of the sensitized animals may be due to some residual chemical mediators of hypersensitivity like histamine or SRS-A which would potentiate the effects of propranolol or histamine and that the airways hyperreactivity of asthmatics is not due to the formation of circulating antibodies but to some other unrelated event.

In the present study, we compared the acute effect of a non-specific irritant, i.e. cigarette smoke, on the airway resistance and dynamic compliance of sensitized versus non-sensitized animals. Although the literature concerning the effects of cigarette smoke on the respiratory system is extensive, there is, to my knowledge, no study comparing the effects of this irritant on the

on the pulmonary function of asthmatics and normals.

Nadel and Comroe (71) showed that in humans, the airway resistance is increased immediately following the inhalation of smoke and that this effect is transient, lasting 10-80 minutes, and Davis et al. (32) showed in the guinea pig that the increase in airway resistance due to inhaled irritants was abolished following tracheotomy and attributed the observed changes in lung function in the intact animal to receptors located in the nasopharynx and larynx.

CHAPTER IV

METHODS AND TECHNIQUES

A. PHYSIOLOGICAL MEASUREMENTS

The measurements of the pleural pressure, the tidal volume and the gas flow were carried out according to a technique developed by M. Amdur and J. Mead (3).

1. Measurement of the pleural pressure

A thin polyethylene tube (20 inches long, 0.03" internal diameter and 0.048" external diameter) was introduced into the pleural cavity under local anaesthesia (Xylocaine 1%, 0.1 - 0.2 ml s.c.). The catheter, fed over a metal wire, was introduced through the right side of the animal - to one side of the vertebral column below the scapula - pushed through the ribs into the pleural cavity and brought out to the side of the sternum, generally at the level of the 6th intercostal space. It was then positioned in such a way that small holes in its middle were in the pleural cavity and filled with saline and heparin. Rinsing of the catheter could be done without filling the pleural cavity by having a syringe connected at each extremity and by pushing the fluid at one extremity and withdrawing the same volume at the other end. By means of a needle and a stopcock the catheter was connected to a Sanborn pressure transducer 267 and changes in pleural pressure were recorded on a Honeywell ink recorder.

The transducer was calibrated with a water manometer and the calibration electrically stored. The electrical calibration was checked regularly but showed practically no variation during 12 months period during which the

experiments were conducted. Before and after each experiment, the transducer was calibrated using the electrical calibration.

2. Measurement of the volume

Volume displacements were measured by a pressure sensitive body plethysmograph. The plethysmograph was so designed that the animal could sit comfortably in its natural position (Fig. 4). A rubber collar shaped as the "head" of the plethysmograph was fitted around the head of the guinea pig and sealed with plasticine to insure that the system was leak-proof. The plethysmograph was connected to a reservoir bottle filled with copper wire in order to maintain a temperature as constant as possible in the system. The bottle itself was connected to a Hewlett Packard 270 pressure transducer and the signal recorded on a Honeywell ink recorder.

Calibration of a set-up was made in the following manner: A 350 ml bottle simulating the guinea pig was placed in the body plethysmograph. Known volume of air was introduced into the system by means of a syringe and the subsequent calibration electrically stored. Before and after each experiment the set-up was calibrated by means of the electrical calibration.

The frequency response of the system was also checked by having a pneumotachograph (Fleisch no. 00) connected at one extremity to the plethysmograph and at the other to a loud-speaker coupled with a sine wave generator. The flow signal recorded from the pneumotachograph and the flow signal obtained from the electrical differentiation of the volume (see page 40) were displayed on the X and Y axis of an oscilloscope (Tectronix type R564B). Air flow was then introduced and withdrawn from the system with an approximate sine wave at different frequencies by activating the loud-speaker. As long as both signals are in phase, a straight line will be seen on the oscilloscope.

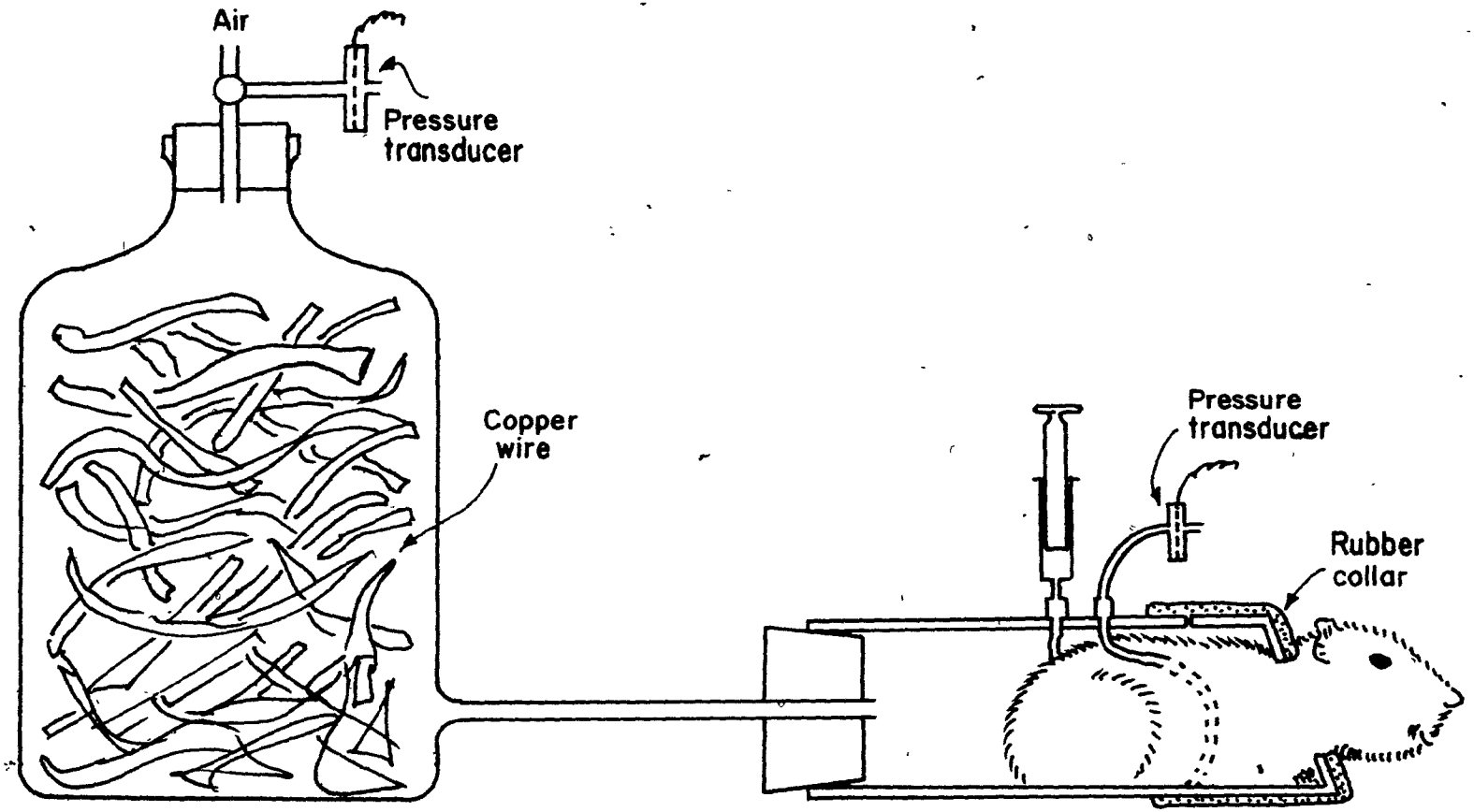

Figure 4: Schematic representation of the apparatus used to measure volume and pleural pressure changes in the conscious guinea pig.

If, on the other hand, one signal is out of phase with the other, a loop will be formed. The frequency response of the system was good, i.e. both signals remained in phase up to 240 cycles/min.

The frequency response of the pleural catheter was also checked by putting the catheter in the plethysmograph and oscillating the system at different frequencies as described above. Pressure and volume changes were recorded at a high paper speed and no phase lag in the catheter response in relationship with the volume signal could be detected up to frequencies of 180/min.

3. Measurement of the flow

The flow of air in and out of the respiratory tract was measured by electrical differentiation of the volume signal with respect to time and recorded on a Honeywell ink writing recorder.

The differenciator was built by an engineer of the Meakins Christie research laboratories at McGill University, Mr. B. Murphy.

Before each experiment, a calibration of the flow was made, using a sine wave generator coupled with a loud-speaker, the loud-speaker itself being connected to the body plethysmograph. The slope of the volume sinusoidal wave was measured, giving a $\Delta V/time$ and the flow corresponding to the particular point of the tracing where the slope had been measured (usually at mid volume) was assigned the calculated ratio $\Delta V/time$.

Fig. 5 shows a guinea pig sitting in the body plethysmograph during control measurements and on Fig. 6 is shown a typical recording of volume, flow and pleural pressure of a guinea pig at rest.

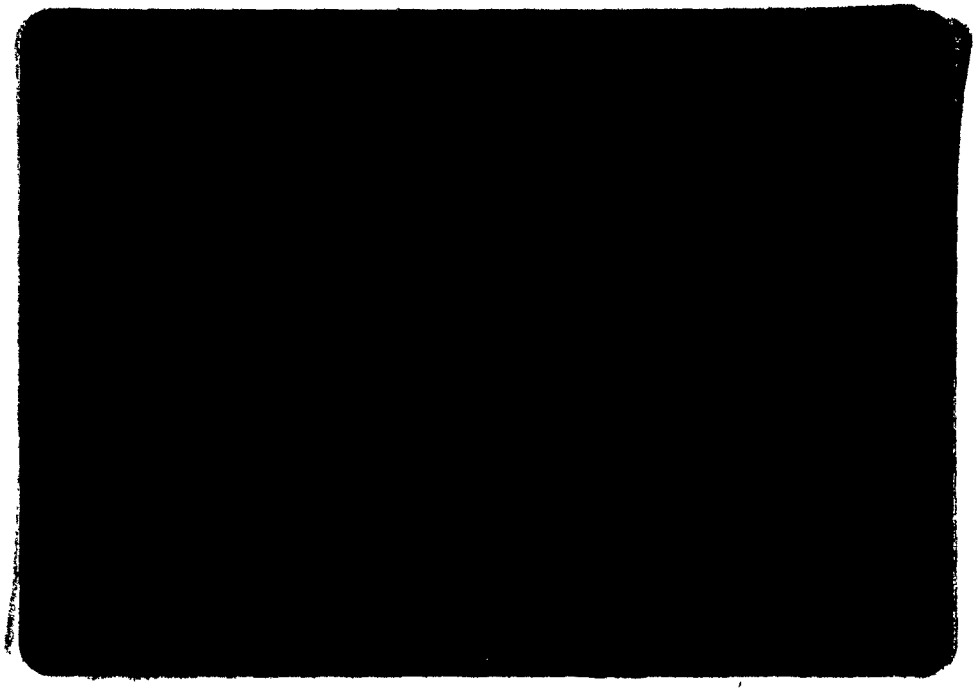

Figure 5: Guinea pig resting in the body plethysmograph during control measurements.

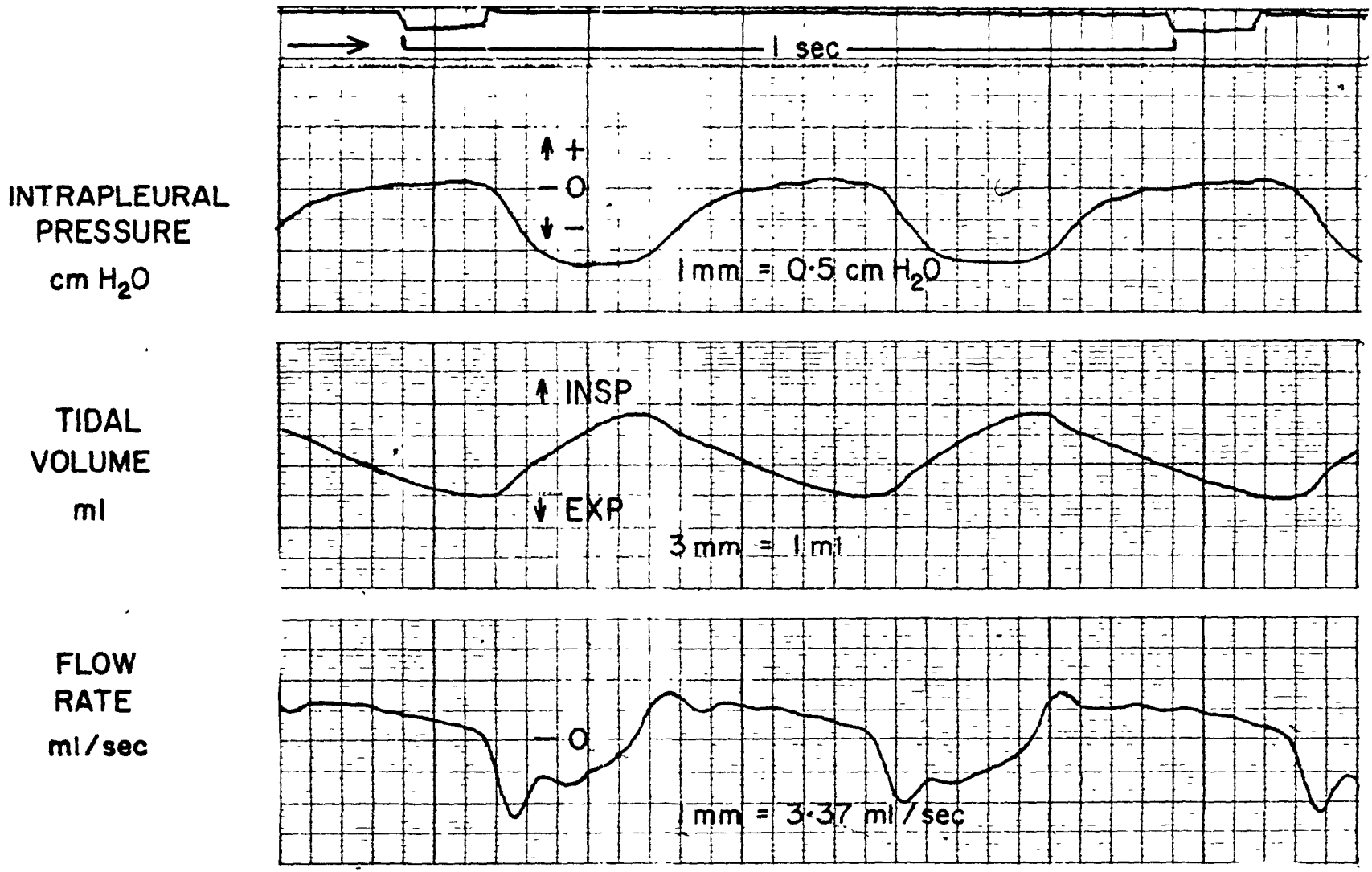

Figure 6: Sample tracing of the respiration of a guinea pig during a control period.

B. IMMUNIZATION

For two series of experiments, namely the one concerning the effect of prostaglandins and the one concerning the role of the vagi, the animals were immunized with an intraperitoneal injection of 1 mg horseradish peroxidase (Nutrition Biochemical, Cleveland, Ohio) dissolved in 1.0 ml of saline and 1.0 ml of pertussis vaccine acting as an adjuvant. A PCA test was performed on another set of 4 animals immunized in the same manner and 3 showed positive skin reactions, indicating that this method of sensitization was adequate.

However, as a positive skin reaction is not an absolute criterion that an animal will respond to the inhaled antigen with an allergic bronchoconstriction, each animal was challenged with the antigen aerosol and their degree of bronchoconstriction measured prior to treatment with the drug.

A previous series of experiments conducted in our laboratory (98) showed that non-sensitized animals did not respond to an aerosol of horseradish peroxidase by an increase in pulmonary resistance and that consequently the increase in resistance measured in the sensitized ones is related to the immunization and not due to a non-specific irritation by the aerosol.

For the third series of experiments, namely the one concerned with the effects of a non-specific irritant, the animals were immunized with an intraperitoneal injection of 2 mg egg albumin dissolved in 1.0 ml saline and studied 10 days later.

C. PROTOCOLS OF THE EXPERIMENTS

1. Effect of indomethacin

Five guinea pigs, weighing 397-440 g., were studied 10-15 days after immunization.

After the pleural catheter had been introduced and positioned adequately, the animal was placed in the body plethysmograph. A latent period of 10-15 minutes was allowed for the animals to calm down and reach a steady state. The data were then collected in the following manner: control measurements of pulmonary resistance and dynamic compliance over a period of 10 minutes (n=12). Antigen exposure was then begun. The antigen was administered as an aerosol spray (20mg HRP/100 ml saline) generated by a Devilbiss ultrasonic nebulizer (model 2-100) for 10 minutes (Fig. 7). During the exposure, further measurements of pulmonary resistance and compliance were obtained (n=12). Then the animals received an intraperitoneal injection of 10 mg/kg of indomethacin diluted in saline to a concentration of 1%. A period of 30-40 minutes was allowed for the absorption of the drug. Control measurements were then repeated over a period of 10 minutes (n=12) and, after that, the animals were re-exposed to the antigen aerosol for a period of 10 minutes and their airway response measured (n=12).

After the experiment, the animals were sacrificed and an autopsy performed in order to check the location of the pleural catheter. In about 50% of the cases, we found that the catheter had perforated the right lower lobe. However, according to Amdur and Mead (3), and it is also our observation, this did not interfere with the measurements of resistance and compliance.

The possible effects of indomethacin on pulmonary resistance and compliance were checked on two non sensitized animals using the same protocol, but without antigen exposure.

2. Effect of atropine

Five animals, weighing 320-450g were studied 10-15 days after immunization. The data were collected in the same manner as for the preceding series of experiments, except that, instead of indomethacin, an intraperitoneal injection of 0.2 mg/kg atropine sulfate was given.

The effect of atropine on pulmonary resistance was checked on 3 non sensitized animals, using the same protocol, but without antigen exposure.

3. Effect of a non specific irritant

Four sensitized and four non sensitized animals, weighing 370-570g were studied. As for the first two series of experiments, control measurements were obtained after the animals had been resting in the body plethysmograph for at least 10 minutes. They were then exposed to the smoke of two cigarettes and further measurements of airway resistance and compliance were obtained (n=6). The procedure used to expose the animals to smoke is shown on fig. 8. The "smoking machine" was built in our department by Mr. H. DeHeer. It drew smoke into a 20ml syringe from which it was exhausted in a 200 ml airspace around the head of the guinea pig.

The animals were exposed at a rate of 2 puffs/minute so that at their normal breathing frequency (80-100/min) they had to inhale the smoke with each tidal breath. After this period of smoke, the animals were allowed to breathe room air for 10 minutes and were then reexposed to the smoke of two cigarettes.

Measurements of resistance and compliance were again obtained (n=6) during the resting period and the second period of smoke.

Figure 7: Guinea pig exposed to an aerosol of HRP.

Figure 8: Guinea pig exposed to cigarette smoke.

CHAPTER V

RESULTS

A. COMPARATIVE VALUE OF RESISTANCE AND COMPLIANCE IN GUINEA PIGS

Table I shows comparative values of pulmonary resistance and compliance in guinea pigs. The large discrepancy seen between measurements can be attributed to the different methods used, the conditions of anesthesia, to whether or not the animals had a tracheal canula and to differences in body weight. The data of Amdur and Mead showed that there is a rather large interindividual variability and Denis et al. measured a considerable intraindividual variability.

Our values of resistance are relatively low compared to those of some of the other authors (3, 109) using the same technique. This can be attributed to differences in body weight of the animals and to the fact that these authors used ether anesthesia, a procedure which is known to increase mucus production in the airways (115) and hence might have increased airway resistance.

B. INDOMETHACIN TREATMENT

1. Resistance

Table II and Fig. 9A show the increase in resistance of 5 sensitized animals during the first exposure to horseradish peroxidase, prior to indomethacin treatment, while Fig. 9B shows the increase in resistance in the same animals during the second antigen exposure, following treatment with indomethacin. An analysis of variance showed that there were differences ($p<0.01$) in individual

guinea pig's resistance profiles before and after treatment with the drug, but that the indomethacin had no statistically significant effect in reducing the response of the group as a whole to the second challenge with peroxidase ($p>0.05$).

Control experiments regarding the effect of indomethacin were carried out in 2 non-sensitized animals. Their airway resistance decreased slightly after the administration of the drug (82% and 85% of control).

2. Compliance

Table III and Fig. 10A and B show the effect of aerosol challenge on the compliance values of animals before and after indomethacin administration. An analysis of variance showed that neither the aerosol challenge, nor the drug treatment had any effect on compliance measurements for the group as a whole ($p>0.05$), although there were some individual variations. Control experiments regarding the effects of indomethacin were carried out in two non-sensitized animals. After the administration of the drug, the compliance increased slightly in one of the animals (125%) and decreased in the other one (86%).

C. ATROPINE TREATMENT

1. Resistance

Table IV and Fig. 11 show the effect of aerosol challenge on airways resistance of 5 sensitized guinea pigs before and after atropine treatment. An analysis of variance showed that there were differences ($p<0.01$) in individual guinea pig's resistance profiles before and after treatment with the drug and that

The increase in resistance during challenge was significantly reduced by the atropine treatment for the group as a whole ($p < 0.05$).

Control experiments regarding the effect of atropine were carried out in 3 non-sensitized animals. After the administration of the drug the mean airway resistance was 98% of the mean control value.

2. Compliance

Table V and Fig. 12 show the effect of aerosol challenge on the compliance values of the animals before and after atropine administration. An analysis of variance showed that neither the aerosol challenge, nor the drug treatment had any effect on compliance measurements for the group as a whole ($p > 0.05$), although there were some individual variations.

Control experiments regarding the effect of atropine were carried out in 3 non-sensitized animals. Their compliance decreased slightly after the administration of the drug (88% of control).

D. EFFECT OF CIGARETTE SMOKE

1. Resistance

Table VI and Fig. 13 show the increase in resistance of sensitized and non-sensitized animals during exposure to cigarette smoke. An analysis of variance was carried out on the square roots of the resistance measurements. The square roots had to be used in this analysis, instead of the raw data as in the other two series, because of larger interindividual differences

which made the population tested not homogenous enough.

The analysis yielded the following results:

a) There was a significant increase in resistance during the smoke periods averaged over all animals ($p<0.01$).

b) There was a significant interindividual difference in response to cigarette smoke ($p\leq0.001$).

c) Exposure to cigarette smoke had a different effect on the pulmonary resistance of sensitized versus non - sensitized animals ($p<0.03$).

2. Compliance

Table VII and Fig. 14 show changes in compliance during exposure to cigarette smoke. An analysis of variance conducted on the raw data showed that:

a) There was no significant effect of cigarette smoke on dynamic compliance averaged over all animals ($p>0.05$).

b) There was significant interindividual differences in response to cigarette smoke ($p<0.001$).

c) The effect of cigarette smoke on dynamic compliance was not different in sensitized versus non - sensitized animals ($p>0.05$).

TABLE I

COMPARATIVE VALUES OF PULMONARY RESISTANCE AND COMPLIANCE IN GUINEA PIGS.

REFERENCES	NO. OF ANIMALS	BODY WEIGHT GRAMS	PULMONARY RESISTANCE cm H_2O/ml/sec	COMPLIANCE ml/cm H_2O	CONDITIONS OF ANESTHESIA
AMDUR AND MEAD (3)	200	219±32*	0.73±0.21*	0.20±0.05*	ether°
AMDUR AND MEAD (3)	20	192±25*	0.38±0.16*	0.24±0.04*	not indicated†
CROSSFILL ET AL (28)	not shown	430-1050	0.059	0.94	Pentobarbitone sodium†
DAVIS ET AL (32)	6	250	~0.22	~0.32	ether°
DAVIS ET AL (32)	6	250	~0.12	~0.35	ether†
DENNIS ET AL (33)	9	250-350	0.34±.03+	0.16±.01+	ether°
MICHOUD	23	426±70*	0.20±.02+	0.46±0.03+	Xilocaine s.c.°
MILLS ET AL (69)	22	not shown	0.19±.02+	0.51±0.04+	Pentobarbitone sodium†
RICHARDSON ET AL (98)	12	395±102*	0.42±.06+	0.54±0.16+	Xilocaine s.c.°
STEIN ET AL (109)	10	210-270	0.63±0.02+	0.23±0.01+	ether°

*STANDARD DEVIATION

+STANDARD ERROR

°INTACT ANIMALS

†HACHEOTOMIZED ANIMALS

TABLE II

		I		II	
	ANIMAL NO.	PULMONARY RESISTANCE cm H_2O/ml/sec		PULMONARY RESISTANCE cm H_2O/ml/sec	
		CONTROL	HRP	CONTROL	HRP
IMMUNIZED	I	.162±.012	.300±.024	.178±.010	.260±.020
	II	.202±.021	.369±.031	.299±.043	.340±.053
	III	.206±.008	.293±.016	.235±.009	.378±.025
	IV	.130±.005	.290±.017	.097±.006	.285±.017
	V	.314±.013	.538±.017	.288±.010	.544±.029
	X	.203±.031	.358±.047	.219±.037	.361±.050
NON IMMUNIZED	I	.094±.034	----	.087±.020	----
	II	.170±.028	----	.124±.011	----
	X	.132±.038	----	.106±.019	----

Pulmonary resistance values ± standard error (n=12 measurements) of immunized and non-immunized guinea pigs during control and during challenge with HRP before (I) and after treatment with indomethacin (II).

TABLE III

		I		II	
	ANIMAL NO.	COMPLIANCE ml/cm H_2O		COMPLIANCE ml/cm H_2O	
		CONTROL	HRP	REST	HRP
IMMUNIZED	I	.460±.022	.486±.038	.336±.009	.421±.035
	II	.290±.009	.323±.016	.269±.013	.243±.011
	III	.559±.016	.543±.014	.483±.015	.475±.023
	IV	.734±.022	.609±.024	.416±.022	.473±.020
	V	.336±.007	.390±.009	.377±.006	.511±.020
	X	.476±.080	.470±.051	.376±.036	.425±.048
NON IMMUNIZED	I	.220±.012	----	.189±.006	----
	II	.358±.017	----	.446±.017	----
	X	.289±.069	----	.318±.128	----

Compliance values ± standard error (n=12 measurements) of immunized and non-immunized guinea pigs during control and during challenge with HRP before (I) and after treatment with indomethacin (II).

TABLE IV

		I		II	
	ANIMAL NO.	PULMONARY RESISTANCE cm H_2O/ml/sec		PULMONARY RESISTANCE cm H_2O/ml/sec	
		CONTROL	HRP	REST	HRP
IMMUNIZED	I	.136±.003	.234±.030	.174±.014	.292±.010
	II	.223±.016	.524±.066	.152±.003	.275±.012
	III	.146±.009	.606±.048	.088±.008	.287±.016
	IV	.303±.016	.454±.032	.349±.031	.324±.028
	V	.371±.013	.460±.038	.338±.010	.304±.027
	X	.236±.045	.456±.062	.220±.052	.296±.008
NON IMMUNIZED	I	.320±.031	----	.309±.008	----
	II	.242±.016	----	.236±.020	----
	III	.186±.006	----	.185±.043	----
	X	.249±.039	----	.243±.036	----

Pulmonary resistance values ± standard error (n=12 measurements) of immunized and non-immunized guinea pigs during control and during challenge with HRP before (I) and after treatment with atropine (II).

TABLE $\overline{V}$

		$\overline{I}$		$\overline{II}$	
	ANIMAL NO.	COMPLIANCE ml/cm H_2O		COMPLIANCE ml/cm H_2O	
		CONTROL	HRP	REST	HRP
IMMUNIZED	$\overline{I}$	.551±.007	.517±.037	.372±.007	.471±.019
	$\overline{II}$	.288±.012	.327±.029	.381±.008	.369±.017
	$\overline{III}$	.623±.032	.297±.028	.543±.031	.522±.041
	$\overline{IV}$	.412±.022	.373±.016	.339±.018	.365±.038
	$\overline{V}$	.407±.017	.375±.014	.387±.013	.340±.027
	$\overline{X}$	.456±.059	.378±.038	.404±.036	.413±.035
NON IMMUNIZED	$\overline{I}$	.383±.007	----	.455±.029	----
	$\overline{II}$	.484±.019	----	.452±.029	----
	$\overline{III}$	.460±.018	----	.260±.019	----
	$\overline{X}$	.442±.030	----	.389±.065	----

Compliance values ± standard error (n=12 measurements) of immunized and non-immunized guinea pigs during control and during challenge with HRP before ($\overline{I}$) and after treatment with atropine ($\overline{II}$).

TABLE VI

	ANIMAL NO.	PULMONARY RESISTANCE cm H_2O/ml/sec			
		CONTROL	SMOKE	REST	SMOKE
IMMUNIZED	I	.063±.021	.399±.142	.127±.033	.251±.036
	II	.163±.009	.090±.017	.171±.033	2.27 ±.50
	III	.033±.012	2.43 ±.35	2.68 ±.23	2.81 ±.07
	IV	.256±.031	.619±.094	.329±.052	.820±.156
	X	.129±.051	.885±.527	.827±.619	1.54 ±.60
NON IMMUNIZED	I	.143±.007	.652±.082	.356±.021	.262±.077
	II	.217±.007	.252±.097	.282±.027	.285±.030
	III	.359±.023	.322±.006	.401±.015	.296±.017
	IV	.036±.000	.274±.039	.204±.027	.251±.037
	X	.189±.068	.375±.093	.311±.043	.274±.010

Pulmonary resistance values ± standard error (n=6 measurements) of immunized and non-immunized guinea pigs during rest and during exposure to cigarette smoke.

TABLE VII

	ANIMAL NO.	COMPLIANCE ml/cm H_2O			
		CONTROL	SMOKE	REST	SMOKE
IMMUNIZED	I	.802±.100	.572±.193	.736±.126	.330±.061
	II	.182±.002	.345±.057	.344±.024	.103±.031
	III	.194±.006	.103±.018	.070±.003	.074±.003
	IV	.313±.008	.270±.025	.295±.021	.153±.004
	$\bar{X}$	.373±.146	.322±.097	.361±.138	.165±.057
NON IMMUNIZED	I	.272±.034	.316±.019	.563±.011	.617±.103
	II	.607±.037	.497±.068	.558±.043	1.091±.079
	III	.428±.007	.304±.014	.407±.027	.331±.023
	IV	.549±.005	.311±.003	.515±.088	.309±.019
	$\bar{X}$	.464±.073	.357±.047	.511±.036	.587±.182

Compliance values ± standard error (n=6 measurements) of immunized and non-immunized guinea pigs during rest and during exposure to cigarette smoke.

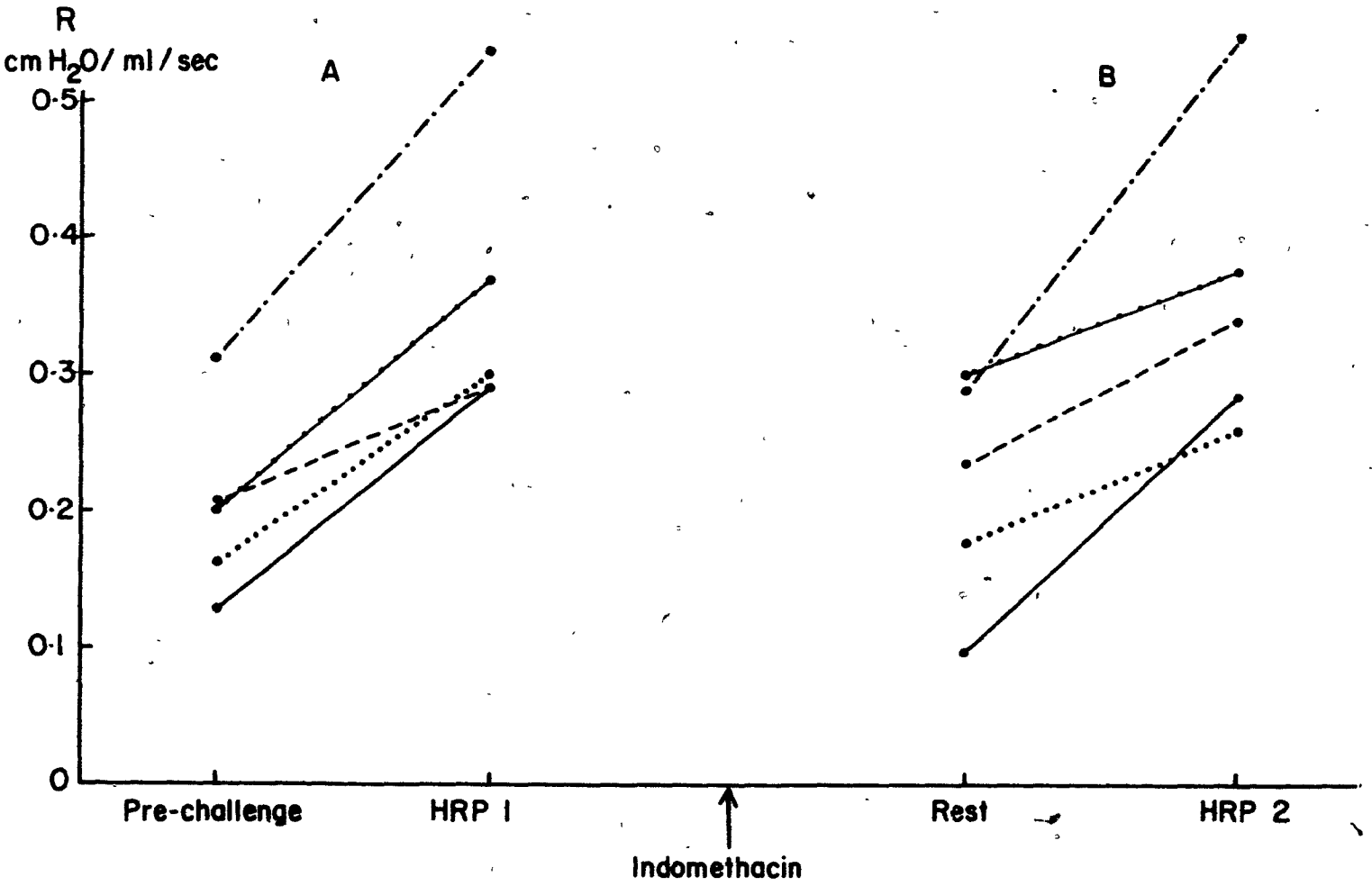

Figure 9: Effect of antigen aerosol on the pulmonary resistance of 5 sensitized guinea pigs, before and after administration of indomethacin (10 mg/kg i.p.). Each point represents the mean of 12 measurements.

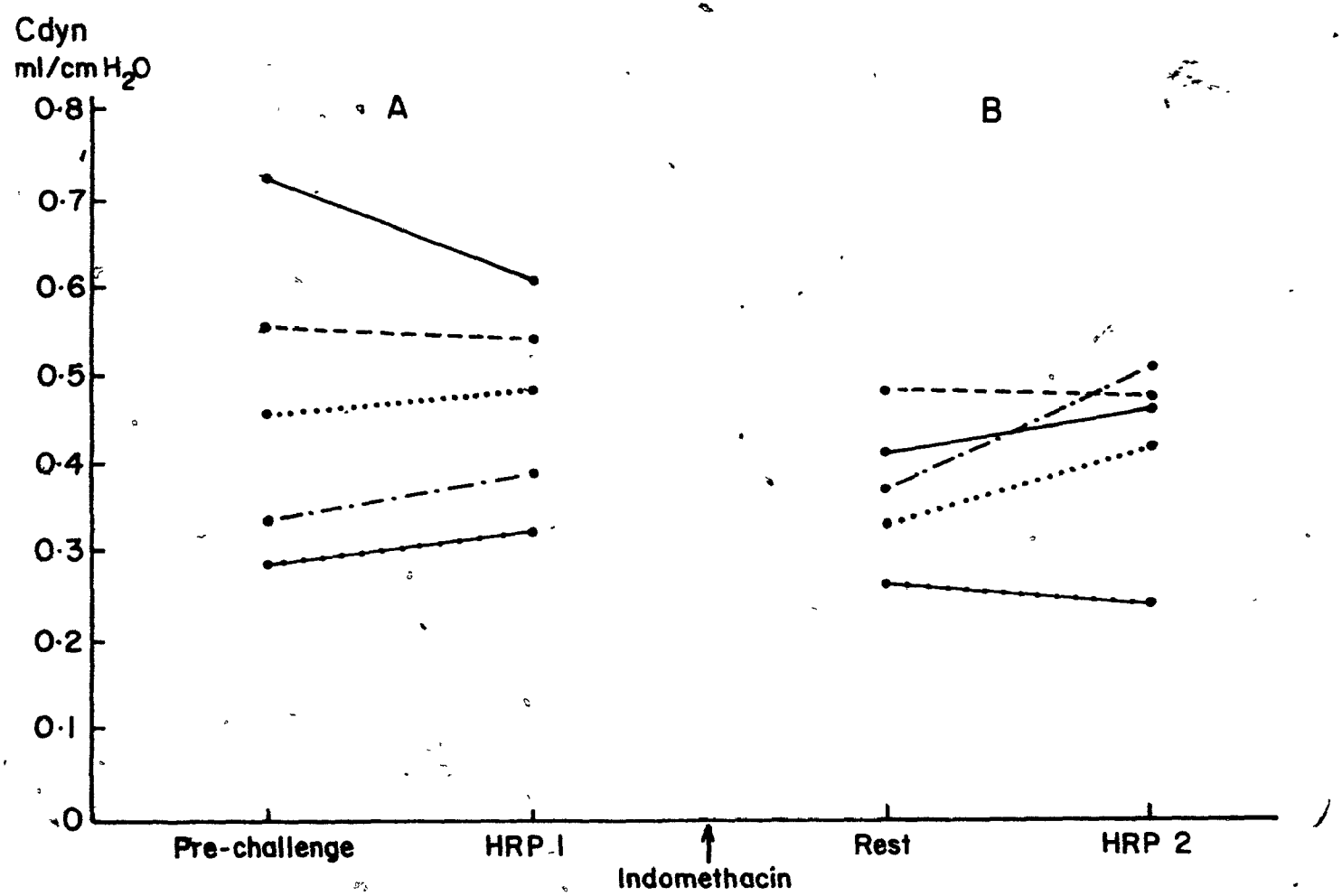

Figure 10: Effect of antigen aerosol on the dynamic compliance of 5 sensitized animals before and after administration of indomethacin (10 mg/kg i.p.). Each point represents the mean of 12 measurements.

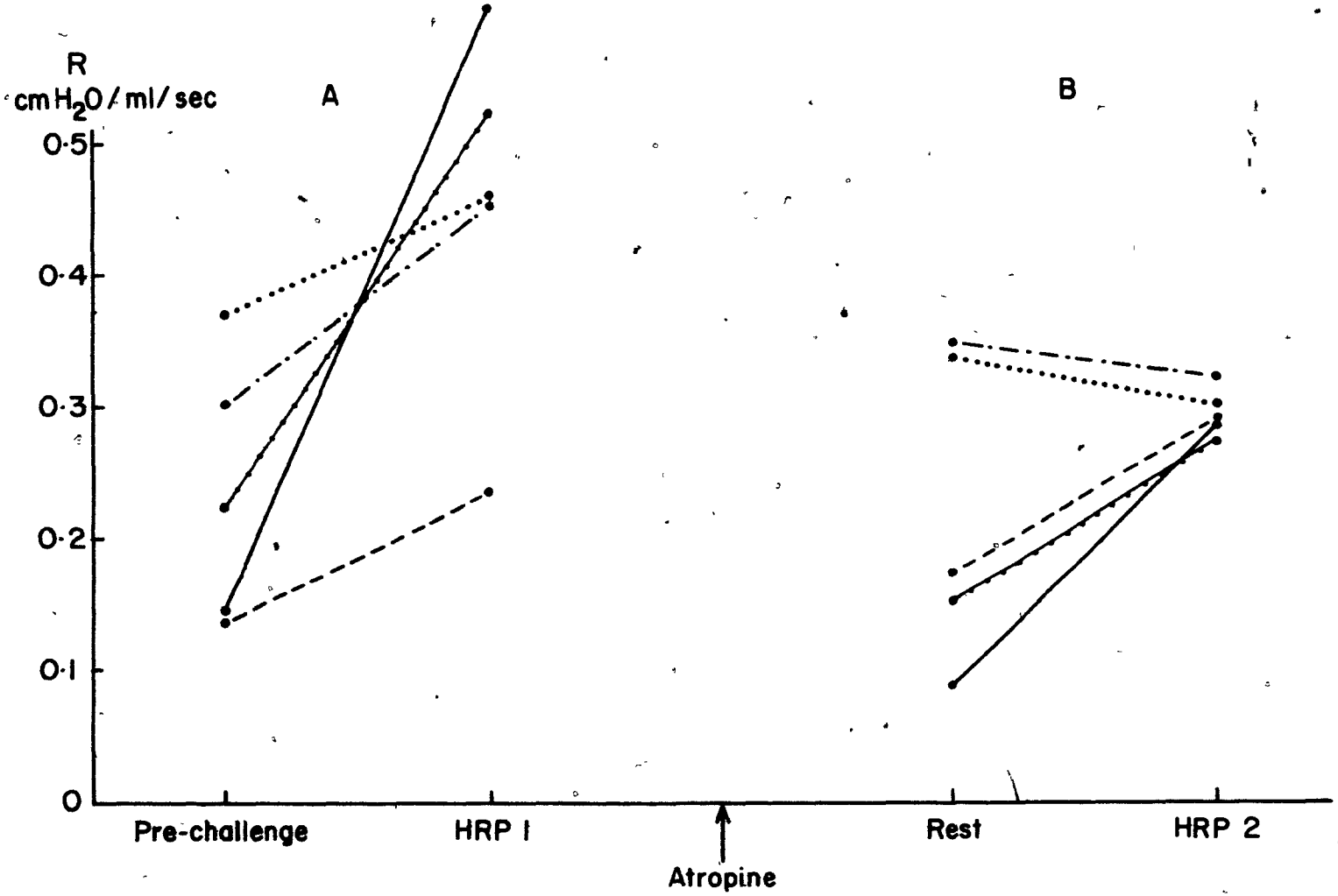

Figure 11: Effect of antigen aerosol on the pulmonary resistance of 5 sensitized animals, before and after administration of atropine (0.2 mg/kg i.p.). Each point represents the mean of 12 measurements.

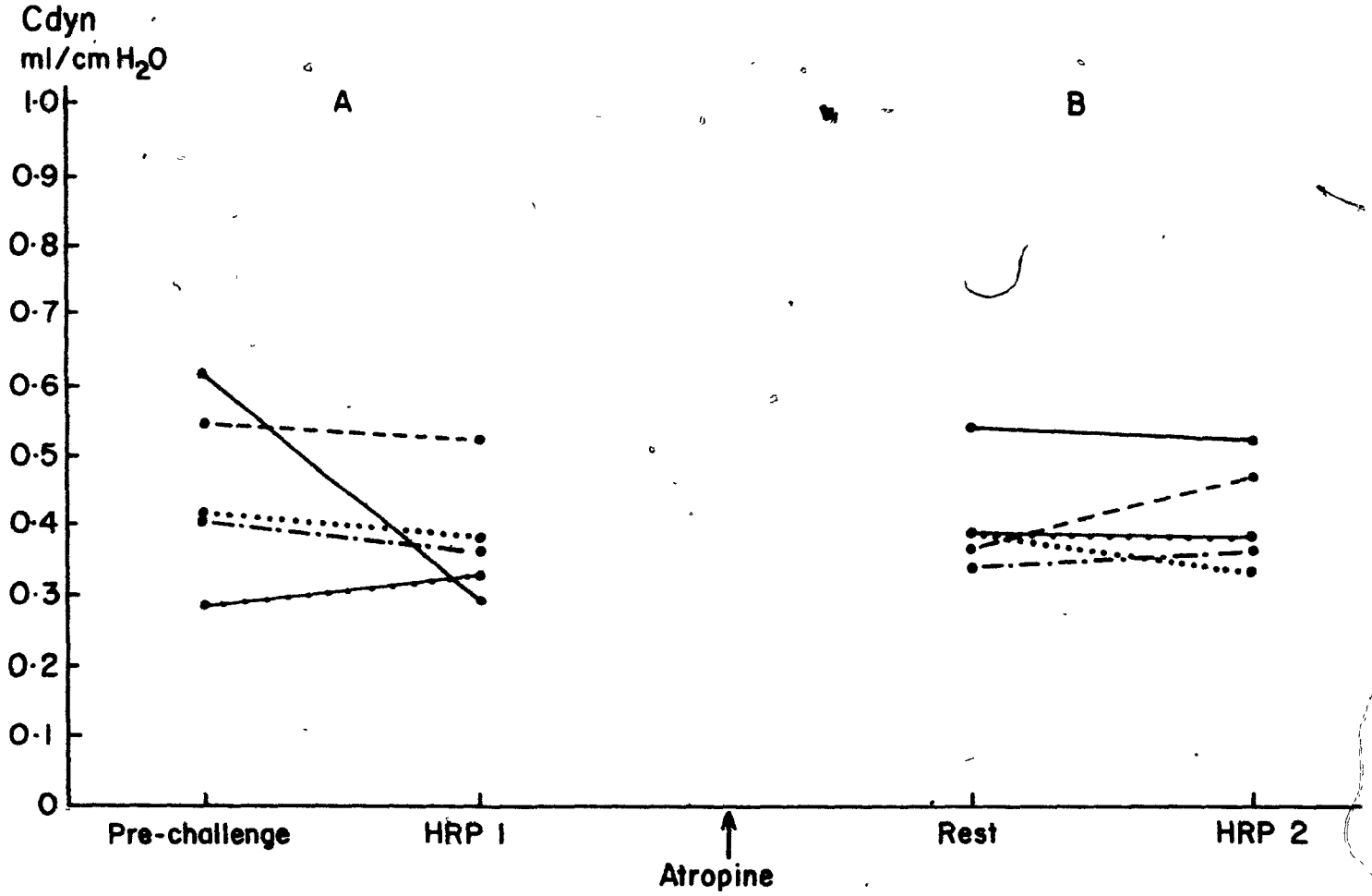

Figure 12: Effect of antigen aerosol on the dynamic compliance of 5 sensitized animals before and after administration of atropine (0.2 mg/kg i.p.). Each point represents the mean of 12 measurements.

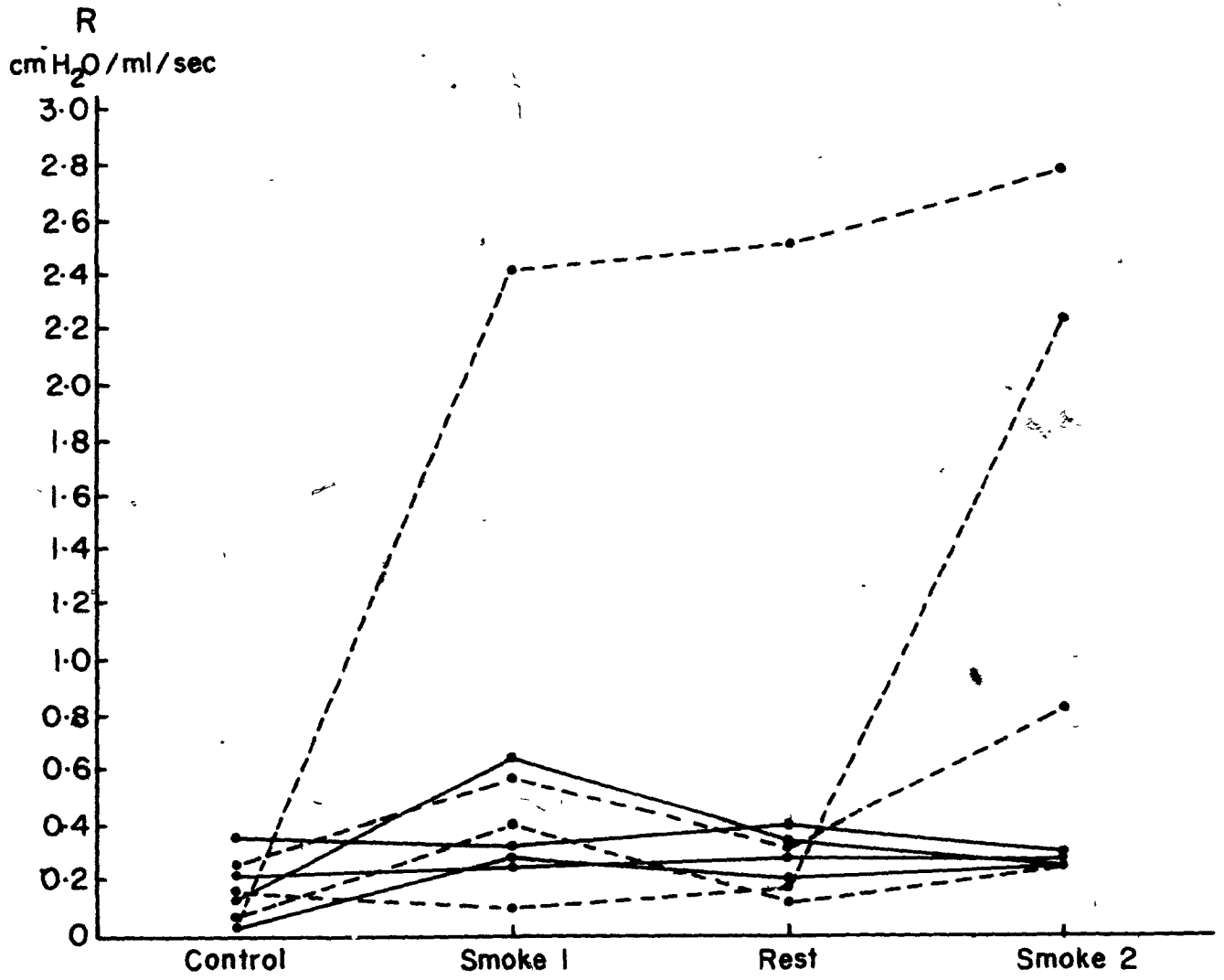

Figure 13: Effect of cigarette smoke on the pulmonary resistance of 4 sensitized (---) and 4 non-sensitized (——) guinea pigs. Each point represents the mean of 6 measurements.

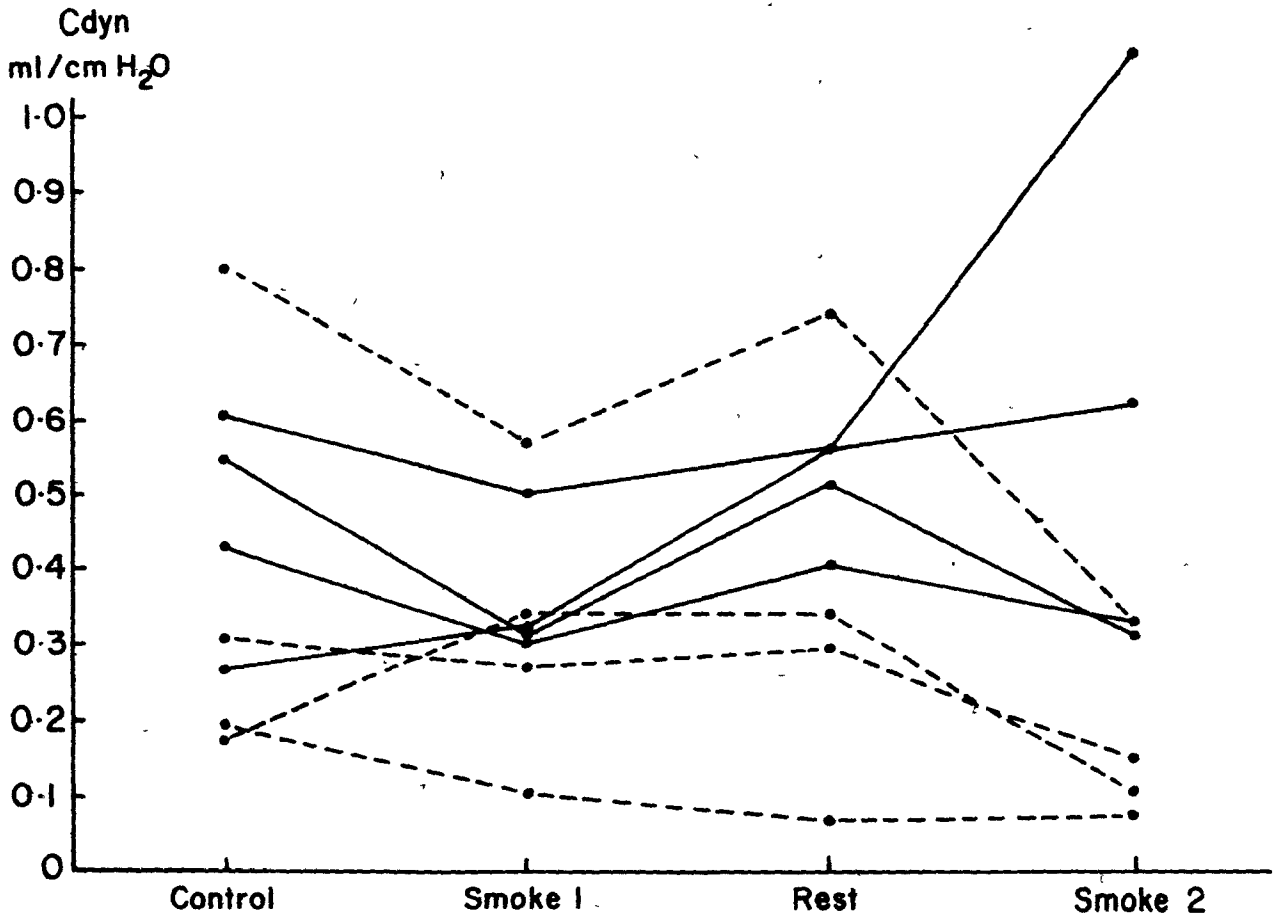

Figure 14: Effect of cigarette smoke on the dynamic compliance of 4 sensitized (---) and 4 non-sensitized (——) guinea pigs. Each point represents the mean of 6 measurements.

CHAPTER VI

DISCUSSION

A. INDOMETHACIN TREATMENT

1. Resistance

Non-steroid anti-inflammatory drugs have been shown to inhibit the synthesis of prostaglandins and to diminish the bronchoconstrictor effect of SRS-A and bradykinin (11,23,25,27,113). The purpose of our experiments was to examine the overall effect of such a drug, indomethacin, on the degree of allergic bronchoconstriction in conscious spontaneously breathing animals.

Comparing our dosage of indomethacin with the ones used by other authors (41,47), it seems reasonable to assume that 10 mg/kg was sufficient to block the synthesis of prostaglandins.

It has been shown that prostaglandins not only have an action of their own on airway smooth muscle, but also affect the release of histamine and SRS-A by influencing the level of cyclic AMP in the target cells. Low levels of prostaglandins (PGE_1 and $PGF_{2\alpha}$) are associated with low levels of cyclic AMP and enhancement of mediator release (111).

In long-term experiments, Junstad et al. (58) measured an increase in excretion of noradrenaline following indomethacin treatment. These authors attributed the increase in noradrenaline excretion to an increased release of the transmitter at the adrenergic nerve endings, suggesting that prostaglandins may also mediate the release of the bronchodilator noradrenaline.

Finally, it was shown that aspirin and indomethacin reduced the resting muscle tone, decreased the effect of small doses of acetylcholine or histamine but increased the effects of the largest doses of these two agents in isolated guinea pig tracheal smooth muscle (79). It is well-known that patients with reaginic asthma have airways hypersensitive to chemical stimuli such as propranolol, acetylcholine and histamine (29, 64). In the guinea pig on the other hand, it was shown by Popa et al. (90) that immunization does not lead to airway hypersensitivity to these mediators. Indeed, the only evidence of hyperreactivity was a temporary enhancement in the response to acetylcholine, histamine and propranolol immediately after antigen challenge. The effect seemed to be more apparent on the compliance and lasted for only 15-30 minutes following small doses of the antigen. In our experiments, we found it necessary to challenge the guinea pig prior to drug treatment to ensure that they have been adequately sensitized, as it is well-known that in humans (14) and in monkeys (Hogg et al. work in progress) a positive skin test does not necessarily indicate bronchial sensitization. We cannot completely rule out the possibility that some of the increase in resistance observed during the second challenge was due to a non-specific irritation, although it seems unlikely since the time interval between the first and the second challenge was at least 45 minutes. Therefore we conclude that the increase in airway resistance during both challenges was immunologically mediated and that indomethacin had no effect on this allergic response.

This conclusion is at variance with the results of Collier et al. (23,24,25,27) who showed, in the guinea pig, a significant decrease in sensitivity to bradykinin and SRS-A after treatment with non-steroid anti-inflammatory drugs.

However, their animals showed a great individual variability regarding their response to aspirin (24). Moreover, several differences in their technique and ours might explain the discrepancy. Their animals were deeply anesthetized, tracheotomized and artificially ventilated, and the Konzett-Rossler technique, which measures components of both resistance and compliance was used. Our animals were conscious, breathed spontaneously and airway resistance and compliance were measured directly. Furthermore, allergic bronchoconstriction was induced by antigen inhalation rather than by the intravenous injection of the antigen as in their experiments.

2. Compliance

Fig. 10 shows that dynamic compliance did not change significantly during antigen exposure before and after treatment with indomethacin. Mills and Widdicome (69) measured a slight non-significant decrease in compliance during allergic bronchoconstriction in the anesthetized guinea pig, and Gold et al. (46) measured a decrease in compliance in allergic dogs although this decrease was sometimes corrected by a single large inflation of the lungs. Conscious guinea pigs sigh frequently and this could account for our inability to measure a change in compliance. Moreover, in our experiments, the antigen most likely deposited in the large conducting airways because of the droplet size of the aerosol (mass mean diameter 5 microns) (49) so that the mediators would be released in highest concentrations in these airways causing bronchoconstriction preferentially to alveolar duct constriction. Stein et al. (109) who also studied pulmonary mechanics on conscious guinea pigs during allergic bronchoconstriction found a marked decrease in compliance during antigen exposure and the only explanation we can offer for this discrepancy is that the aerosols particles in their experiments might

have been smaller so that they reached the lung parenchyma where the released mediators would cause alveolar duct constriction and a decrease in compliance. This explanation is consistent with the findings of Drazen et al. (36) who measured a decrease in compliance following an intravenous injection of SRS-A. With an i.v. injection SRS-A would perfuse the pulmonary circulation and affect the periphery of the lungs (72). Therefore we would expect alveolar duct constriction and a change in lung compliance in Drazen's experiments and bronchoconstriction in ours. Thus, changes in compliance in allergic bronchoconstriction might depend on the site of antigen deposition.

B. ATROPINE TREATMENT

1. Resistance

Our results corroborate those of Mills and Widdicombe (69) in the guinea pig and are consistent with those of Gold et al. in humans (116) and dogs (46). These two groups found that vagotomy, cooling of the vagi or atropine reduced the degree of allergic bronchoconstriction in anesthetized artificially ventilated animals. According to Dennis and Douglas (34) atropine abolished the bronchoconstriction due to an aerosol of histamine. However, these authors used a very high dose of atropine (5mg/kg) which might have affected the central nervous system (52).

The surprisingly slight decrease in airway resistance of the control animals as well as the sensitized ones following atropine treatment is in agreement with the measurements of airway conductance of Mills and Widdicombe (69) on vagotomized, anesthetized, spontaneously breathing animals and indicates that guinea pigs are different from humans and dogs in that they have very little resting parasympathetic tone.

Our experiments support the hypothesis of Mills and Widdicombe that an important part of the immediate bronchoconstriction in the guinea pig is due to a vagal reflex. The failure of Collier and James (25) to see any change in bronchoconstriction after pithing and decerebration may be due to the fact that the anaesthetic used prior to pithing and decerebration abolished all reflexes.

It is possible that the reflex is started by an antigen antibody reaction on the mast cell surface which induces vagally mediated bronchoconstriction as Gold et al. have suggested (46). However, it also seems possible that nerve endings in the epithelium could be responsible for the degranulation of sensitized mast cells as these cells are commonly found around nerves, and acetylcholine increases mediator release from the target cells (59). Both these hypotheses await further experimentation.

2. Compliance

Fig. 12A shows that dynamic compliance did not change significantly during the first exposure to HRP prior to atropine treatment. These results are consistent with the compliance measurements made on the animals treated with indomethacin. As the conditions of the 2 series of experiments are the same, we attribute the lack of change in compliance to the factors that have already been discussed in detail in the previous section. (p. 67).

Fig. 12B shows that atropine treatment affected neither the control values nor the values obtained during the second period of antigen exposure. As it appears from the resistance measurements that guinea pigs have very little parasympathetic tone, it is not surprising that the compliance did not change after treatment with atropine. Moreover, Woolcock et al. (114), measuring the influence of the autonomic nervous system in dogs, concluded that in this

species, the effect of the parasympathetic nervous system is blocked in the peripheral airways by the sympathetic nervous system so that changes in vagal tone would not affect the elastic properties of the lungs.

These results therefore support the hypothesis that in this model of bronchoconstriction, antigen deposition occurs in the large airways so that the vagal reflex and the release of mediators cause bronchoconstriction in preference to alveolar duct constriction.

C. EFFECT OF CIGARETTE SMOKE

1. Resistance

The purpose of this experiment was to determine if sensitization with a specific antigen could be responsible for the induction of airway hyper-sensitivity to non-specific irritants. Unfortunately, cigarette smoke is a strong irritant and the guinea pigs became very restless during the period of exposure, making difficult the collection of the data and this most certainly explains the large standard errors seen on tables VI and VII.

The increase in airway resistance that we observed during exposure to smoke is consistent with the results of Davis et al. (32) in the guinea pig and those of Nadel et al. (71) and of Sterling (110) in humans. These authors found that the increase in resistance due to smoke was only short lived and Sterling showed that it could be abolished by atropine, suggesting that this effect of smoke is due to a reflex bronchoconstriction. Davis et al., however, did not measure any change in airway resistance in tracheotomized guinea pigs exposed to cigarette smoke. These authors therefore attributed the increase in resistance seen in intact guinea pigs during smoke exposure to a reflex affecting the upper airways above the larynx.

The analysis of variance carried out on our results showed that smoke had a different effect on sensitized versus non-sensitized animals. These results are at variance with those of Popa et al. (90) who found that immunized guinea pigs did not show an increase in airway sensitivity to acetylcholine, propranolol and histamine compared to non-immunized ones. These authors however did not study the effect of a non-specific irritant and reported data primarily concerned with compliance rather than resistance.

Our findings indicate that immunized guinea pigs have either more sensitive irritant receptors or that the reflex resulting from the stimulation of these receptors is stronger. As mast cells are often found around nerves and possess cholinergic receptors on their surface, (59), an interesting hypothesis would be that sensitized animals respond more readily than non-sensitized ones because stimulation of the irritant receptors induces a release of mediators from mast cells in the sensitized animals.

The small number of animals in the present study does not allow us to draw any definitive conclusion concerning the effect of immunization on airway sensitivity but suggests that further experimentation into the effect of non-specific irritants on specifically sensitized animals is warranted.

2. Compliance

A slight but non-significant decrease in compliance was generally observed in both sensitized and non-sensitized animals during exposure to cigarette smoke. These results are consistent with those of Davis et al. (32) who measured a decrease in compliance in both intact and tracheotomized animals. As the frequency of breathing fell slightly both in Davis' and in our experiments, this decrease in compliance cannot be attributed to a frequency

dependance phenomenon but is probably due to alveolar duct constriction. This hypothesis is confirmed by the fact that smoke particles are small enough (0.1-1.0 microns (45, 48) to reach the lung parenchyma (49) and also by the observation of Davis et al., that compliance was also decreased in tracheotomized animals, although the increase in resistance was abolished. Again, our finding that cigarette smoke had no different effect on immunized versus non-immunized guinea pigs must be interpreted cautiously in view of the few number of animals and of the wide scatter of the data. They suggest that immunization might in some unknown way modify the sensitivity of the irritant receptors in the upper but not the lower airways. Further experimentation will be required before this hypothesis can be either supported or rejected.

CONCLUSIONS

Our experiments on unanesthetized guinea pigs confirm the results of Mills and Widdicombe (69) concerning the importance of the vagi in allergic bronchoconstriction in this species. These experiments also showed that indomethacin had little effect on the degree of allergic bronchoconstriction even though the doses given were large enough to inhibit prostaglandin synthesis and interfere with the release of the humoral mediators.

It was also shown that, in this model of allergic bronchoconstriction produced by aerosol inhalation, the smooth muscle constriction occurs in the large airways preferentially to the lung parenchyma. This observation indicates that the studies made on the lung function of sensitized animals following intravenous injection of the antigen, which induces release of the mediators in the lung parenchyma, represent another type of anaphylaxis and that experiments carried out with these two different techniques are not exactly comparable.

Finally, we showed that inhalation of cigarette smoke affects pulmonary resistance and, to a lesser extent, compliance, suggesting that smoke particles might affect both the upper and the lower airways. The effect of smoke on pulmonary resistance was slightly different in sensitized versus non-sensitized guinea pigs. This finding suggests that the immunization procedure might affect the sensitivity of the upper airways. The exact mechanism of this latter finding is not clear at present and requires further investigation.

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