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STUDIES OF AIRWAY SMOOTH MUSCLE IN A CANINE MODEL OF ALLERGIC ASTHMA

BY

L.A. ANTONISSEN

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STUDIES OF AIRWAY SMOOTH MUSCLE IN A CANINE MODEL OF ALLERGIC ASTHMA

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LUDOVIEUS ANTHONY STANLEY ANTONISSEN

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ABSTRACT

Asthma is primarily characterized by the airway's extreme sensitivity to a variety of physical, chemical and pharmacological stimuli. The postulation that there may be a single primary defect to explain the pathophysiology of asthma has led to considerable investigation of the immunological, genetic, neurologic and pharmacologic aspects of the disease. Evidence implicating a single factor is lacking. Despite the fact that airway smooth muscle (ASM) is the tissue ultimately mobilized during an asthmatic bronchospasm, the ASM, with a few exceptions, has not been studied in any great depth. It was felt that an alteration in the contractile function of ASM, and its sensitivity to anaphylactic mediators may provide the underlying mechanism involved in asthmatic bronchospasm. Therefore, the present studies were undertaken to determine whether the defect in asthma lies in airway smooth muscle.

Experiments designed to test whether the muscle contractility is altered, were carried out by studying tracheal smooth muscle (TSM) function - and the mechanism by which control of that function is effected - in a canine model of allergic asthma. Previous work in our laboratory has shown canine TSM to be ideally suited to systematic mechanical studies and to be a valid model for the study of ASM in general. Mongrel dogs were sensitized to ovalbumin (OA); induction of high titers of IgE antibody production was achieved by repeated intraperitoneal immunizations with a conjugate of DNP and OA, beginning from the neonatal period and

using Al $(OH)_3$ as adjuvant. These sensitized dogs previously had been shown to respond to antigenic bronchoprovocation with marked increases in airflow resistance. Control animals were littermates which had been immunized with $Al(OH)_3$, but without antigen. TSM's were isolated from sensitized and control dogs, and subjected to studies of their mechanical function under various conditions and different stimuli.

Isotonic mechanical studies using electrical stimuli were conducted; the force-velocity relation of the sensitized TSM displayed a 48% increase in V_{max} and the $\underline{b}$ constant, with absence of change in the P_o and $\underline{a}$ constant, when compared to their littermate control values. The increased velocity of shortening and $\underline{b}$ constant suggest that the actomyosin ATPase activity of ASM is increased in the asthmatic condition. In addition, the isotonic shortening capacity of the sensitized TSM is significantly greater than that of control. These data suggest that the first component of the asthmatic attack may be a sudden accelerated shortening of ASM resulting in a rapid rise in airway resistance, followed by further shortening to markedly narrow the airways and further increase airflow resistance.

Isometrically, the sensitized TSM displayed phenomena - such as a prolonged active tension plateau to electrical stimulation, myogenic responses to stretch spontaneously rising resting tension with phasic activity, and a contractile response to cold which are consistent with altered membrane properties. These characteristics would further aggravate the asthmatic bronchospasm. Parameters concerning

the active tension developement and relaxation of TSM after electrical and carbachol stimuli were not different between the sensitized and control preparations. The possibility of nerve dysfunction and alterations in the excitation-contraction coupling, calcium release and sequestration mechanisms of ASM in asthma is therefore unlikely.

The active tension developement - paralleling the in vivo allergic bronchospasm - to challenge by the sensitizing antigen is both specific to the OA and a characteristic of the sensitized TSM only. Histamine is the anaphylactic mediator released during the antigen-antibody reaction in the allergic dog; the sensitized TSM displays a statistically significant hypersensitivity to this agonist when compared to control TSM. Only mepyramine-sensitive H_1 receptors could be demonstrated in the sensitized and control TSM preparations. The asthmatic ASM's altered sensitivity to the anaphylactic mediator, in addition to providing the link between the antigen-antibody reaction and the muscle response, may contribute as well to the increased airway resistance observed during bronchospasm.

The presence of a dysfunction of the ASM in a model of allergic bronchoconstriction has been demonstrated by this study. Whether the alteration of mechanical activity is primary, or a result of changes in other initiator systems remains to be determined.

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DEDICATED TO MY MOTHER AND FATHER

INTRODUCTION

A. GENERAL INTRODUCTION

One of the most characteristic features of asthma is the extreme sensitivity of the airway smooth muscle to physical, chemical and pharmacological stimuli. It is evident in patients with both immunologically and nonimmunologically induced asthma. The resulting reversible obstruction is associated with increased resistance to breathing, disturbances in distribution of ventilation and impairment of gaseous exchange. Four mechanical factors contribute to the airway obstruction: (1) contraction of smooth muscle in the passageways (2) edema of the mucosa which increases the turbulence of airflow (3) secretion of viscid mucous which results in bronchiolar and alveolar occlusion, and (4) reduction of vital capacity while residual volume and functional residual capacity are increased. This overinflation reflects premature airway closure, diminished time for exhalation due to increased airflow resistance and loss of lung elastic recoil (Gold, 1976).

Review of the literature reveals that there are four major theories regarding the causation of asthma. Recognition of the fact that the asthmatic airway is hypersensitive to a variety of immunologic and nonimmunologic stimuli has provided the basis for these ideas.

The classical theory of asthma states that IgE antibodies are manufactured in response to exposure of a specific antigen. These IgE molecules then migrate to mast cells distributed in the various portions of the airways and attach to their surface via their Fc endings. Repeated exposure results in the antigens

diffusing into the airway tissues where they contact the antibodies. The IgE-antigen interaction on the surface activates the mast cells' internal enzyme systems to release chemical mediators. These diffuse to the smooth muscles of the airways and cause bronchoconstriction (Austen, 1973; Gold, 1973; Lichtenstein, 1973). It is sometimes argued that allergens may be too large to penetrate the epithelial wall and gain access to mast cells. The issue has not been resolved at this time, however, examination of pathological specimens has proved confusing, revealing both the presence and absence of the tight cell-to-cell junctions.

The irritant receptor - bronchoconstrictor reflex theory (Nadel, 1965) proposes that bronchial hyperirritability might be due to a lowering of the threshold, or an increase in the response, of certain pulmonary vagal receptors. Their stimulation results in reflex bronchoconstriction through the parasympathetic nervous pathway innervating the tracheobronchial tree. Widdicombe (1977) now suggests that this system interacts with IgE-mediated antibody-sntigen reactions. It is hypothesized that the chemical mediators released by the mast cells act on the nervous receptors in the bronchial epithelium to elicit a reflex bronchoconstriction (Gold et al., 1972 (a); Richardson et al., 1973; Gold, 1975). It is clear from the work of different authors using different animals, that the cholinergic nervous system is involved in the acute bronchomotor response to antigen challenge. Evidence now establishes that vagotomy (Mills and Widdicombe, 1970; Gold, 1975) or the administration of atropine (Gold et al., 1972(b); Drazen and Austen, 1975; Gold, 1975) modified the pulmonary response to

antigen challenge. While claimed to be a primary phenomenon in the dog (Gold et al., 1972(b)), the role of parasympathetic reflexes in human asthma is less well documented. Evidence suggests that similar reflexes are present, but these may be due secondarily to released chemical mediators, hypoxemia, hypotension or abnormal breathing patterns during the asthmatic attack (Gold, 1976).

The adrenergic system had been thought to be the principal inhibitory system of the tracheobronchial smooth muscle, through a direct action on the beta-adrenergic receptors of the tissue. The beta-adrenergic theory (Szentivanyi, 1968) suggested a paucity of dilator beta-receptors or a blockage of $beta_2$-subtype receptors was responsible for the atopic abnormality in bronchial asthma. This would leave the parasympathetic nervous system relatively unopposed. Cyclic AMP levels may also be reduced with subsequent potentiation of bronchoconstriction via reduced inhibitory effects on mediator release. However, the evidence for this hypothesis is not considered convincing (Gold, 1975). Unfortunately, the majority of the experiments performed to substantiate the theory had been carried out in subjects already on treatment with beta-adrenergic agonists (McNeill, 1964). Furthermore, it has been shown that pharmacologic beta-blockade fails to increase histamine sensitivity in healthy human subjects (Zaid et al., 1966). Indications are, as well, that the number of adrenergic nerve endings controlling airway smooth muscle relaxation are less than previously thought. The density and distribution of these fibres seem to be species dependent. In the guinea pig, adrenergic fibres have been demonstrated (Coburn and Tomita, 1973) with the inhibitory effects of

these fibres eliminated with beta-blocking agents or by prior depletion of tissue catecholamines. Coburn and Tomita (1973) also observed a decrease in the adrenergic system in the thoracic portion of the guinea pig trachea. More peripheral airways may have no adrenergic inhibitory fibres. It has therefore been suggested that any effect seen on bronchoconstriction may be due to the level of circulating catecholamines, released by adrenergic nerves found around local blood vessels (Mann, 1971). In the dog it has been demonstrated that the adrenergics reduce bronchoconstriction induced by vagal stimulation (Cabezas et al., 1971), however such studies have not been performed on humans. To summarize then; although adrenergic inhibition can be demonstrated (Cabezas et al., 1971; Coburn and Tomita, 1973), there is little evidence of the presence of adrenergic nerves in the smooth muscle of the airways and if they are present, their exact contribution to the inhibition of the smooth muscle is controversial.

The establishment of a non-adrenergic inhibitory system in the gastrointestinal tract (Crema et al.,1968; Burnstock, 1972) resulted in speculation that it may be present in the airways as well, due to their common embryological origin. Coburn and Tomita (1973) elucidated the existence of these putative "purinergic" nerves in the guinea pig trachea, with Richardson et al. (1976) most recently demonstrating its presence in human airway. Richardson et al. (1975,1976) speculated the "purinergics" may play a more dominant role than the adrenergics as an inhibitory system maintaining normal smooth muscle tone in the lung. Any defect or dysfunction in this "purinergic" inhibitory system would be of great importance; it

has been postulated to result in the disturbance of the musculature and hyperreactive airways observed in asthma.

Apart from these four main avenues of research, a considerable amount of research has been performed to determine the role of genetic factors in the development of asthma. It has been generally concluded that the non-genetic factors discussed above, play a much greater role in the etiology of asthma than genetic factors; these perhaps being responsible more for the observed heterogeneity of the disease (Bias, 1973).

B. DEFINITION OF THE PROBLEM

The postulation that there may be a single primary defect to explain the pathophysiology of asthma has led to considerable investigation of the pathogenic role the immune and nervous systems may play in this disease. Evidence implicating one such factor is lacking, however. While conceding the possibility that the bronchial smooth muscle of asthmatics may be abnormal in the pharmacological sense (Widdicombe, 1977), investigators still concentrate upon the trigger mechanism in allergen induced attacks. The hypotheses just described illustrate this fact.

As a result, very little is known about the airway smooth muscle in asthma - surprising when one considers that it is the tissue ultimately mobilized during an asthmatic bronchospasm. The few in vitro studies of tracheal or bronchial smooth muscle in asthma have dealt with the effects of drugs or other agents - neural and anaphylactic - on the smooth muscle, illustrating the preoccupation with investigation of triggering mechanisms. It therefore becomes important to determine if a specific smooth

muscle defect is present in asthma. In studying the way the pathological condition of asthma affects airway smooth muscle physiology, investigations logically should be conducted in terms of the muscle's function and the mechanism by which the control of that function is normally effected. Muscle function includes, first of all, the ability to stiffen and bear weight, and secondly to shorten and move a load through a distance. Both isometric and isotonic contractile parameters for canine tracheal smooth muscle in these terms have been measured (Stephens et al., 1969; Stephens et al., 1974) as well as isometric parameters in the normal and sensitized guinea pig trachea (Douglas et al., 1976; Souhrada and Dickey, 1976(b)).

Airway smooth muscle contractility depends upon the interrelationships of local chemical and reflex effects. A hypersensitivity of asthmatic airway smooth muscle to various mediators could result from a defect in processes such as membrane excitability, excitation-contraction coupling, the contractile mechanism with its associated energy utilizing reactions, or one of the components of the pharmacologic receptor systems. Various sites of defect or dysfunction are possible. At the membrane level, hyperexcitability could be due to a partially depolarized resting membrane potential resulting from abnormal ion permeabilities, a reduced threshold for the opening of sodium/calcium channels, or a greater number of channels engaged in excitation. Thus a muscle could become hypersensitive and/or hyperreactive to various stimuli. Excitation-contraction coupling changes could result in greater

amounts of calcium released after excitation, such that active tension is increased. Another possibility is that a defect in calcium sequestration systems might result in a muscle which displays delayed relaxation abilities. Abnormalities in the contractile proteins and the associated enzymes may result in altered active tensions or shortening velocities, as well as shortening capacities. For example, an increase in the number of cross-bridges would result in higher active tensions developed and the presence of abnormal enzymes might result in altered velocities of shortening The structure of the contractile element and other cellular aspects associated with contraction may change to affect shortening capacities. These three parameters, namely active tension, velocity of shortening and shortening capacity, are extremely important to the pathology of asthma. They are responsible for both the rapidity with which the bronchospasm is manifested, as well as the magnitude of airway closure.

If all the mediators and other agents which induce bronchoconstriction demonstrate increased activity during an asthmatic attack, the smooth muscle itself must be hyperexcitable. Presumably, if the defect lies in the contractile elements above, then all agents (including anaphylactic mediators) which stimulate, would as well produce a hypersensitive response in the airway smooth muscle. However, the possibility also exists that the defect lies in only one of the systems mentioned and thus exhibits a great deal of specificity (Fleisch, 1973). This might be the case if the abnormality existed in a certain receptor system. One must also keep in mind that an alteration in the mechanical properties of the

muscle could stem from intrinsic properties or variations exhibited in other control systems for bronchoconstriction which affect the muscle. For example, the contractile properties of skeletal muscles are known to be modified when the muscle is subject to a certain pattern of neural activity (Eccles et al., 1962).

The present experiments will attempt to answer the question whether the principle defect in asthma lies in airway smooth muscle.

C. THE MODEL

A canine model of allergic asthma (Kepron et al., 1977) was chosen to study the contractile properties of airway smooth muscle and reveal its possible role in the pathogenesis of asthma. Canine tracheal smooth muscle (TSM) was used as a model for the study of airway smooth muscle in general. Past evidence indicated that it is mechanically similar to smooth muscle down to the sixth generation of bronchi (Hawkins and Schild, 1951; Stephens, 1965). Also, the response of the smooth muscle in the larger airways to inotropic agents appears qualitatively the same as in the resistance units (Permutt, 1971; Nadel, 1973).

Pathophysiologic studies of dogs with natural reaginic hypersensitivity to pollen have also shown these dogs to represent a suitable model for the study of allergic asthma (Patterson, 1969). Spontaneous canine ragweed hypersensitivity has been found to be the only example of a respiratory pollenosis, similar to asthma in humans, that occurs naturally in animals (Patterson, 1960). Furthermore, the immunologic reactivity has been shown to be mediated by a class of immunoglobin similar to human IgE (Kessler

et al., 1974). Dain and Gold (1975) have shown that specific antigen aerosol challenge to allergic dogs resulted in increased airflow resistance similar to that observed in human asthma. Antigen inhalation by these dogs caused degranulation of mast cells and the release of histamine from central bronchi as a result of the immunological reaction (Meyers et al., 1973).

The allergic dog is thus the only animal which possesses pathological, physiological and immunological changes similar to the human disease. Furthermore, studies of canine structure and function in vitro and in vivo are numerous so that results obtained in this study can be easily incorporated into other findings reported in this introduction. While our model deviates from these experiments in that artificial antigens were used to sensitize - an adaptation of a method devised by Pinckard et al. (1972) -, prolonged IgE antibody responses were obtained and anaphylactic sensitivity could be evoked by either bronchial or intravenous challenge with the immunizing antigen (Kepron et al., 1977). Active immunization of small animals, such as guinea pigs, with purified protein is possible as well, but technical difficulties are encountered during pulmonary physiologic and isolated tissue studies. Most recently, Pare et al. (1977) have conclusively demonstrated that neither the acute nor the chronic lung volume changes in allergic guinea pigs reproduce changes observed in human asthma. The immunologic class of antibodies involved may differ as well - it has not been adequately documented.

While this model at best could serve as one for extrinsic asthma, if the fundamental defect is proven to lie at the

muscle level and if the different manifestations of clinical asthma are merely due to differences in precipitating stimuli, then the canine tracheal smooth muscle model will be of more general application. Eventually the airway smooth muscle in the smaller passageways (<2mm) will have to be studied. New techniques enable investigation of in vitro muscle contractile function in airways from 2-3mm in diameter to under 500μm (Evans et al., 1977).

D. IMMUNOLOGIC FACTOR IN ALLERGIC ASTHMA

(i) Mediator Release

In allergic asthma, the immune response is mediated by IgE, an immunoglobin which is synthesized - by the systemic immune system, as well as in the mucosa of the neopharynx and airways - in response to appropriate introduction of a sensitizing antigen to the body (Gold, 1976). In vitro studies (Ishizaka and Ishizaka, 1971) have established that IgE molecules have an affinity for tissue mast cells and basophil granulocytes, and combine with these target cells through the Fc portion of the molecule. When the appropriate antigen is introduced, the antibody - antigen interaction triggers the biochemical secretion of anaphylactic mediators from the sensitized target cells. Mast cells in the bronchial epithelium of human asthmatics, for example, have been shown to degranulate in the active phase of the disease (Salvato, 1976).

A variety of low molecular weight chemical mediators are released by the antigen-antibody interaction, including histamine, SRS-A (slow reacting substance of anaphylaxis), ECF-A

(eosinophil chemotactic factor of anaphylaxis), prostaglandins, thromboxanes, various kinins, and others (Wilson and Galant, 1974). The secretory process has been shown to involve a calcium dependent phase in which a diisopropylfluorophosphate sensitive esterase is activated, followed by a glycolytic energy dependent stage,which is perhaps necessary for microtubular contractile proteins to extrude mediators. Exocytolysis of the chemical containing granules occurs in the final, calcium dependent phase (Gold, 1976). The chemicals undergo ion exchange during release into the surrounding fluid.

The release of mediators, particularily histamine and SRS-A from mast cells and basophils, appears to be modulated by the level of cyclic nucleotides. Orange et al., (1971) showed that increased levels of cyclic adenosyl-monophosphate (cAMP) suppressed IgE-mediated release of both histamine and SRS-S from sensitized monkey lung, while beta-adrenergic blockade (decreases cAMP levels) enhanced the release of both mediators. Indirect evidence also suggests that increased cyclic guanosyl-monophosphate (cGMP) augments antigen-induced secretion of mediators. Observations are, in general, consistent with the view that relaxation of smooth muscle is associated with an elevation of the cyclic AMP content of the tissue and a relationship between cyclic GMP and contraction has been suggested (Schultz et al., 1973). Smooth muscle contraction and relaxation in the airways may be initiated and/or modulated by altered concentrations of these cyclic nucleotides.

(ii) Role of the Nervous System

Opposing cholinergic and adrenergic mechanisms have been shown

to modulate both the release of mediators from lung tissue during IgE-mediated reactions (Austen and Orange, 1975) and normal bronchomotor tone (Cabezas et al., 1971). Alterations in these functions have often been used to explain the airway hyperirritability observed in allergic bronchial asthma.

Adrenergic beta-stimulating substances catalyze the conversion of adenosine triphosphate (ATP) to cyclic AMP; phosphodisterase can then degrade this cAMP to the inactive 5'AMP. Accumulation of intracellular cyclic AMP leads to bronchial smooth muscle relaxation and inhibition of the mast cell's manufacture and/or release of mediators, including histamine and SRS-A (Austen and Orange, 1975). Inhibition of mediator release by catecholamines is postulated (Lichtenstein, 1973) to be based upon increased cAMP levels leading to the phosphorylation of microtubular proteins via a protein kinase. A disaggregation of the tubules might result, with a consequent failure to extrude mediator from the sensitized cell. Szyntivanyi (1968) has proposed the fundamental abnormality in asthma to be a defect in the beta-adrenergics supplying the airway smooth muscle. The resulting lower levels of cAMP would be associated with failure of the microtubules to disrupt and facilitation of anaphylactic mediator release. Alpha-adrenergic stimulating agents have been hypothesized to cause a similar effect by stimulating phosphodiesterase to degrade cAMP. Mast cells release of mediators would be facilitated, resulting in bronchoconstriction (Bardana, 1976). The value of

these observations is now questionable (Richardson and Bouchard, 1975; Richardson and Béland, 1976) since the role the adrenergic inhibitory system plays may be extremely small compared to the purinergic neural inputs, especially in humans. It is now suggested (Barnett and Gold, 1976) that circulating catecholamines acting on beta receptors may play the dominant role in regulating cyclic AMP levels.

Cholinergic-stimulating agents result in an increase of cyclic GMP - increased cyclic GMP and decreased cAMP levels together augmenting the release of mediators from human lungs following antigen-antibody reactions (Yu et al., 1972). Atropine effectively blocks increased cGMP levels, and along with other observations, supports a fundamental role for the vagal cholinergic system in the pathogenesis of bronchial asthma. A hyperactive cholinergic system would thus potentiate mediator release.

(iii) Chemical Mediators of Anaphylaxis

Various chemical mediators are released from sensitized lung tissues by antigen. They include prostaglandins - synthesized, released and inactivated by the lung - which have been recovered from lung perfusate from challenged, sensitized guinea pigs (Piper and Vane, 1969), and it seems both PGE and PGF types are released (Gold, 1973). PGE's generally dilate blood vessels and airways while PGF's constrict, however, PGE_2 has also been shown to be a bronchoconstrictor, particularily in asthmatic patients (Smith, 1974). Because of this discrepancy, it is now suggested that prostaglandins may play a messenger role between cells,

the level of PG being related to cGMP levels, as any concommitant rise in cAMP (observed by Stoner et al., 1977) can be blocked by indomethacin (Farmer et al., 1974).

PAF (platelet activating factor) is released from lung tissue during anaphylaxis in experimental animals and from passively sensitized human lung in vitro (Gold, 1976). PAF causes platelets to aggregate and release serotonin, an airway smooth muscle constrictor which is also capable of activating reflex bronchoconstriction. Another chemotactic factor, NCF-A (neutrophil chemotactic factor of anaphylaxis) augments local inflammation, due to the release of lysosomal enzymes as neutrophils are attracted to the antibody-antigen reaction site. There is some evidence as well for the release of PG endoperoxides or thromboxane A_2, both potent bronchoconstrictors, from anaphylactic lungs (Gryglewski et al., 1976).

Local eosinophil infiltration is common in asthmatic lungs, the cells being attracted to the site of allergic response by a substance called ECF-A (eosinophil chemotactic factor of anaphylaxis). Release upon IgE interaction with antigen (Kay et al., 1971) occurs as a preformed mediator, like histamine, from the target cells (Wasserman et al., 1973). Eosinophilic infiltration may represent a protective measure, since eosinophils contribute substances which eliminate products of the mast cell allergic response. Preliminary data suggest they contain a histaminase which participates in histamine inactivation and an arylsulphatase which inactivates SRS-A (Gold, 1976).

SRS-A (slow reacting substance of anaphylaxis) is a low molecular weight, unsaturated acidic sulphate ester which is capable of contracting bronchial smooth muscle in very low concentrations. Its delayed bronchoconstrictor effect is a result of the fact that it must not only be released by the mast cell but also be manufactured in response to the antigen-antibody reaction (Wasserman et al., 1973). SRS-A is resistant to the effect of antihistamines, studies indicating that the release of SRS-A is modulated by levels of cyclic nucleotides (Orange et al., 1971; Kaliner et al., 1972).

Histamine was the first mediator to be associated with anaphylaxis (Dale and Laidlaw, 1910) and the contraction of airway smooth muscle. Bartosch et al. (1932) demonstrated that isolated perfused lungs of a sensitized guinea pig release histamine in anaphylaxis, with Schild et al. (1951) demonstrating the same phenomena in a human asthmatic lung. The ineffectiveness of antihistamine therapy for asthmatics, however, suggests the role of histamine in the pathogenesis of human asthma may be small. Schild et al. (1951) proposed this may be due to the inability of the antihistamine to penetrate the tissues. While exogenous administration of histamine is effectively countered, the histamine levels in the tissue may be up to 10,000 times higher and inaccessible to the antihistaminic drug. Experiments have shown that mast cells degranulate and release histamine in allergic dogs when challenged by antigen aerosols (Meyers et al., 1973; Gold et al., 1977); however, plasma histamine concentrations were also shown to drop off while airway resistance remained high (Chiesa et al., 1975).

The discrepancy between decreasing plasma histamine concentration and persistent airflow obstruction suggests that other humoral (anaphylactic mediators previously described) or neural mechanisms are triggered by the antibody-antigen reaction.

A series of studies have been carried out to evaluate the role of reflexes in the airway reaction. Mills et al. (1969,1970) showed that antigen-induced bronchoconstriction, in sensitized guinea pigs and rabbits, depended upon stimulation of rapidly adapting vagal sensory endings and resulted in reflex airway constriction. Bronchograms from allergic dogs given aerosolized antigen produced results similar to airway constriction due to electrical vagal stimulation (Kessler et al., 1973), also indirectly suggesting vagal pathways are involved. Gold et al. (1972) administered antigen to one lung only of a sensitized dog and produced bilateral airway constriction, however, vagotomy or vagal cooling were claimed to abolish this. Colebatch et al. (1966) demonstrated atropine-insensitive increased airflow resistance following injection of histamine into the right side of the heart and Nadel et al. (1966) reported similar results using aerosolized histamine sulphate. These results indicated that at least peripheral airway resistance is due to direct action on bronchial smooth muscle.

On the other hand, there is a preponderance of data that the bronchoconstrictor effect of histamine delivered to large airways in vivo can be altered by atropine or vagotomy, suggesting that histamine exerts at least part of its action on airway smooth muscle by a cholinergic mechanism (Karczewski and Widdicombe, 1969).

Histamine has been shown also to increase efferent vagal fibre activity in cat bronchi (Mills et al., 1969). More recently, Drazen and Austen (1975) have demonstrated that antigen-induced bronchoconstriction in conscious, sensitized guinea pigs can be inhibited by cholinergic blockade using atropine.

The persistence of bronchoconstriction after inhibition of vagal reflex mechanisms makes it seem unlikely that vagal reflexes are responsible for the entire sequence of reactions induced in an anaphylactic attack. Mitchell et al. (1976) suggest an interaction of histamine and cholinergic stimuli on airway smooth muscle. This mechanism might explain experiments where vagal blockade diminished or blocked airway smooth muscle response to histamine and other stimuli, simply by interfering with histamine-cholinergic interaction at the smooth muscle level.

Gold et al. (1977) indicated antigen-antibody interaction is essential for initiation of the reaction sequence for mediator-release since degranulation of mast cells cannot be induced by electrical vagal stimulation or methacholine. Furthermore, since large molecules of antigen can induce airway responses and histamine release in less than 30 seconds, it is suggested that the permeability of bronchial epithelium in allergic dogs is abnormally increased or that an IgE-antigen reaction on human cells releases substances which increase the permeability of the bronchial epithelium (Boucher et al., 1977). This would enable the antigen to reach the submucosal mast cells - important for the release of histamine and other mediators - and trigger the anaphylactic bronchospasm.

Ash and Schild (1966) were the first to postulate the existence of more than one receptor for histamine. Eyre and Wells (1973) described H_1 contractile and H_2 relaxant receptors in the pulmonary circulation of the calf, the histamine H_2-receptors were able to modulate the strong H_1 effect in systemic anaphylaxis. Histamine has been demonstrated to induce contractions of airway smooth muscle which are antagonized by H_1 antihistamines (Castillo et al., 1947) and recently Eyre (1973) was able to identify specific relaxant H_2-receptor actions of histamine on the isolated airway smooth muscle of cat and sheep. H_2 receptor blockers have successfully been used as well to potentiate IgE-mediated release of histamine and SRS-A by hindering the operation of an autoregulatory H_2-receptor in the immunologic release of mediators from sensitized cells. The H_2 receptor apparently stimulates adenyl cyclase in its action (Lichtenstein and Gillespie, 1973). Asthmatics may then differ from normals in that their H_1/H_2 receptor ratio may be larger, resulting in increased irritability to histamine stimuli in allergic asthma. The data presented indicate this defect could lie in the receptor fields of both nerves and airway smooth muscle.

E. TRACHEAL SMOOTH MUSCLE (TSM)

The tracheo-bronchial tree is not, as is often assumed, a passive set of conduits through which air enters and then exits from the pulmonary system. Its important regulatory effect on the regional and generalized distribution of air in the lungs is a function of the airway smooth muscles, whose tone depends upon

the interrelationships of local chemical and reflex effects. Macklem and Engel (1975) consider the physiological significance of normal bronchomotor tone to include (a) the production of an optimal balance between anatomical deadspace and airway resistance (Widdicombe and Nadel, 1963). This normally minor role of airway smooth muscle becomes extremely critical in pathological conditions such as asthma, where an alteration in activity is manifested through reduced anatomical deadspace and considerably increased resistance to airflow (b) the stabilizing of cartilage-containing bronchi to render them less compressible (Olsen et al., 1967). They are argued, however, to be predisposed to closure during deflation, with the resultant trapping of gas which increases the regional residual volumes (Macklem and Engel, 1975). This is a characteristic of asthma, perhaps potentiated by the higher airway smooth muscle tone observed in the disease (c) making the lungs behave in a more isotropic manner. An abnormal degree of tone, as occurs in asthma, might result in anisotropic lungs which leads to unequal distribution of stresses, and predisposes alveoli to damage (Macklem and Engel, 1975). An understanding of the mechanism by which the respiratory tree actively effects control of airflow requires study of airway smooth muscle with respect to its physiological properties. An understanding of the derangement of this control in asthma, for example, may elucidate the pathogenic basis of the disease.

Despite considerable early research (Miller, 1947; Widdicombe, 1963; Widdicombe, 1966), the function and regulation of tracheal smooth muscle had been poorly defined until very recently. Indeed,

the trachealis - which traverses the dorsal aspect of the incomplete cartilaginous rings of the cervical trachea - has proven to be an active and responsive tissue (Widdicombe, 1966; Olsen et al., 1967; Stephens et al., 1968).

Airway calibre appears to be modulated largely through the effective control of the autonomic nervous system. Although neural regulation is achieved by sympathetic nerve stimulation which relaxes (Miller, 1947), and parasympathetic (vagal-cholinergic) nerve stimulation (Loofbourrow et al., 1957; Widdicombe, 1966) which contracts the smooth muscle, the parasympathetic system predominates both anatomically and physiologically (Cabezas et al., 1971). The existence of non-cholinergic, non-adrenergic relaxant nerve fibres has recently been demonstrated (Burnstock, 1972); these have been tentatively named purinergic nerves since it is suggested that the transmitter released is a purine nucleotide. The presence of non-cholinergic, non-adrenergic inhibition has been established in the trachea of the guinea pig (Coburn and Tomita, 1973; Richardson and Bouchard, 1975) and in the human (Richardson and Beland, 1976). These authors suggest that adrenergic innervation of the TSM may play a smaller role in airway tone than the purinergics. However, stimulation of beta-adrenoreceptors by circulating catecholamines may be significant. Canine TSM has been shown recently to be a tonic muscle, predominantly activated by cholinergic neural inputs in response to electrical stimulation in normal solution (Stephens and Kroeger, 1970). Neither direct electrical stimulation nor nerve stimulation can evoke an action potential; they do however result in graded

depolarization. Evidence obtained by Suzuki et al. (1976) indicates that a marked rectification of the membrane prevents spike generation, but changes in the membrane potential by cholinergic and adrenergic nerve activity modify the mechanical response, due to a low mechanical threshold of the muscle. These same workers also report the failure to demonstrate the existence of a purinergic nervous system in the dog.

Tonic contraction of TSM can be evoked by chemicals such as barium, potassium chloride and acetylcholine (Stephens et al., 1974; Spilker and Minatoya, 1975) as well as by serotonin (Colebatch et al., 1966; Daley and Hebb, 1966; Spilker and Minatoya, 1975) and histamine (Hawkins and Paton, 1958; Spilker and Minatoya, 1975). Epinephrine and isoproteronol relax the trachea (Hawkins and Paton, 1958); the balance between endogenous prostaglandins may also modulate smooth muscle tone (Yamaguchi et al., 1976). While norepinephrine and high potassium solutions have been reported (Keatinge, 1966) to convert intermediate (Burnstock and Prosser, 1960) type arterial smooth muscle into single unit type preparations, neither resulted in phasic activity or the production of a myogenic response to stretch in canine TSM (Stephens et al., 1975). Such characteristics are consistent with those of multiunit muscles and emphasize the predominant neural control of tracheal smooth muscle.

Recent evidence has shown that TSM possesses an intrinsic ability to be spontaneously active under the action of specific agents or pathological conditions. Kirkpatrick (1975) reported depolarization of bovine trachea with slow oscillations in membrane potential

(E_m) under a histamine stimulus. These were correlated with rhythmic fluctuations in contractile activity, and seemed dependent upon extracellular calcium concentrations. Tracheal preparations isolated from sensitized guinea pigs were shown to exhibit spontaneous mechanical activity, before and after a histamine stimulus or antigen challenge (Souhrada and Dickey, 1976(a)). Akasaka et al. (1975) revealed that during spontaneous and mecholyl-induced asthmatic attacks in humans, both the amplitude and frequency of action potentials were increased over those observed in healthy patients. It was thought this might prove to be the major etiological mechanism involved in an asthmatic attack.

Tetraethylammonium (TEA), which functionally converts TSM into a single unit preparation, has been successfully used to induce spontaneous electrical and mechanical activity in airway smooth muscle (Kroeger and Stephens, 1975) where other common stimulatory agents and conditions failed. Resultant membrane depolarization and the trains of small, decrementally conducted action potentials were thought to be due to the unmasking of the depolarization activation of calcium and/or sodium channels by the reduction of a normally predominant resting potassium permeability. Normally, the marked rectification properties of the TSM membrane prevent spike generation (Suzuki et al., 1976). In addition to spontaneous activity observed with TEA, a previously inelicitable myogenic response was now obtained upon a quick stretch of the TSM (Kroeger and Moorhouse, 1973; Stephens et al., 1975). Various pathological conditions, such as asthma, may have an underlying etiology similar to the alteration

of cellular mechanisms following the administration of TEA.

F. SMOOTH VS. STRIATED MUSCLE

In order for any type of muscle fiber to contract, it is generally recognized that free myoplasmic calcium (Ca^{++}) must reach a critical level to activate the actomyosin filaments. The rapid rise in intracellular calcium is dependent upon Ca^{++} influx across the sarcolemmal membrane and the release of Ca^{++} from intracellular binding sites. In striated muscle, the calcium concentration is normally maintained at a low level ($< 10^{-7}M$) by calcium pumps in the surface membrane, sarcoplasmic reticulum and mitochondria (Bianchi, 1969). The initiation of contraction by a chemical stimulus (acetylcholine) is mediated by surface depolarization which is transmitted to the interior via a transverse tubular system (T-tubules) which is continuous with the sarcolemma (Huxley and Taylor, 1958; Huxley, 1964(b)). The associated sarcoplasmic reticulum is triggered to release calcium raising the intracellular calcium concentration to the threshold level needed for the activation of contraction (Sandow, 1965). The calcium releases the inhibition effected by the troponin-tropomyosin complex which normally prevents the interaction of actin and myosin (Wakabayashi and Ebashi, 1968). Energy is used (Davies, 1963) (ATP is hydrolyzed) as the filaments slide over each other and tension is developed. According to the classical sliding filament theory (Huxley and Niedergerke, 1954; Huxley, 1957; Huxley, 1964(a)), tension development and shortening are brought about by the action of physical cross-links (cross-bridges) attaching and detaching many times per second between the

interdigitating actin and myosin filaments which comprise the contractile apparatus. The elements of this theory are consistent with the basic structure, energetics, biochemistry and mechanics of muscle. Huxley and Simmons (1971) updated the theory to make it compatible with new findings of structural features, assuming that the arm of the cross-bridge is compliant, and that the myosin head, which rotates, can sustain tension. Therefore when activated, and after the myosin head has attached transiently on the actin filament, it rotates clockwise, pulling on the compliant link to develop tension or sliding of the filaments. Support for the mechanism of force generation by physical cross-links is substantial. With the development of sophisticated techniques, most other theories of muscle contraction were found inadequate.

Recently, however, Iwazumi (1970) has proposed an electrostatic theory consistent with those characteristics claimed to be conclusive evidence for cross-bridges. It assumes the myosin-ATP complex forms an electrochemical cell, and positive and negative charges are separated(ie. generation of a dipole) upon hydrolysis of ATP. An electric field is thus established which gives rise to corresponding energy density; hence, a force is exerted on the thin actin filament. This model has been used to successfully explain mechanical events. Whether the theory will come into greater prominence will depend upon more complex ultrastructural and biochemical findings.

The apparent absence of demonstrable sarcomere units in smooth muscle suggests a difference in its contractile properties as compared to striated muscle. The basic mechanism of contraction

and the role of calcium appear similar, however. Smooth muscle actomyosins qualitatively resemble their skeletal muscle counterpart and have an ATPase activity sensitive to calcium. Tropomyosin is present as well (Murphy, 1976(b)) and although various studies suggest a troponin-like component, its exact role if any is unknown. Contractile systems of all vertebrate smooth muscles have common characteristics (Murphy, 1976(a)), however, more information is required to define a contractile unit comparable to the sarcomere of striated muscle.

In contrast to calcium release in twitch muscle fibres (which is mediated by the T-tubules and the sarcoplasmic reticulum (SR)), the surface membranes of tonic skeletal fibres and ventricular fibres (which lack SR) compensate for the function of the missing SR (Bianchi, 1969). Since smooth muscle is similar and possesses a high surface area to volume ratio, it is presumed that the calcium release and uptake mechanisms reside, for the most part, in the surface membrane. Smooth muscle cells do have a variety of calcium stores however, and the fact that multi-unit muscles seem to possess considerable sequestered calcium, suggests at least a decreased dependence of multi-unit fibres on extracellular calcium (Devine et al., 1973). Various stimuli could then effect and mobilize different calcium pools to varying degrees in eliciting contractile responses. The physiological diversity of smooth muscle appears to reflect, therefore, variations in systems controlling intracellular calcium concentration, rather than in the contractile or regulatory proteins.

In summary, then, mechanical, ultrastructural, and biochemical observations reveal a strong similarity between the contractile systems of smooth and striated muscles, based on a sliding filament mechanism (Huxley, H.E., 1973; Somlyo and Somlyo, 1975; Murphy, 1976(a)). Generally, when compared to striated muscle, smooth muscle is capable of a similar force generating potential, has lower shortening velocities and a large physiological shortening capacity. These must be related to the properties and organization of the contractile proteins as well as the mode of force transmission within and between cells (Murphy, 1976(a)). A discussion of the complex and controversial data in this area of research, however, is beyond the scope of this thesis.

G. MUSCLE MECHANICS

Because insufficient data prevent the development of a model for smooth muscle contraction, the tendency has been to interpret its mechanical data in terms of the counterpart skeletal muscle models. For example, the maximum isometric force developed per unit cross-sectional area of muscle is a common and desirable measurement of the contractile function of muscle. It reflects the interaction of contractile proteins and is a parameter for the amount of contractile material (or the number of cross-bridges) acting within the muscle. However, the index assumes that the maximum force is elicited and measured in the axis of force development, that myofilament length and packing are similar, and that extracellular spaces are comparable between muscles. In most smooth muscles this is unknown.

While some argue (Murphy et al., 1974) that the higher force measurements found in some smooth muscles must reflect a contractile system which is specialized for force generation when compared to skeletal muscles, an examination of a constants (Hill, 1938) reveals most smooth muscles fall in the same range as skeletal, when comparing their abilities to develop tension (Stephens, et al., 1969). Limited mechanical data reported thus far (Stephens et al., 1969; Stephens and Kromer, 1971) suggests the smooth muscle model must also consist of a contractile element - characterized by force-velocity relationships qualitatively similar to those for skeletal muscle - which is in series with a series elastic component. This too is qualitatively similar to that for skeletal muscle. Similar length-tension and force-velocity relationships have been described for other smooth muscles, as well as trachealis (Meiss, 1971; Hellestrand and Johansson, 1974; Herlihy and Murphy, 1974). Temperature studies (Stephens et al., 1977) indicate the contractile element of tracheal smooth muscle is supported by active processes while its series elastic component is apparently passive. The similarity of the data to trends found in cardiac and skeletal muscles further suggest striated muscle models may also be applied to smooth muscle, especially the trachealis.

In reference to our model of allergic asthma and airway smooth muscle in general, the properties of the canine tracheal smooth muscle make it well suited to the study of its mechanical properties (Stephens, 1976). It is a parallel-fibred preparation which, under normal circumstances, exhibits no spontaneous rhythmic

contractile or electrical activity and does not manifest a myogenic response (Stephens and Kroeger, 1970). More than 70% of the tissue is muscle, making it very compliant at rest so that at optimal length it has almost negligible resting tension (Stephens et al., 1969). The muscle can be tetanized and the apex of its active tension curve is relatively flat, enabling determination of valid force-velocity curves. The shortening capacity of tracheal smooth muscle is higher than that of most other smooth muscles, being able to contract down to 10-15% of its optimal length (Stephen et al., 1969). This suggests the muscle is capable of controlling airway diameter over a fairly wide range.

In order to investigate fully the contractile properties of a muscle, both the tension developed (isometric and isotonic) and the velocity of shortening should be considered (Katz, 1939). Pharmacologica agents or a number of pathological conditions have been known to alter, for example, shortening velocities while leaving the maximum active tension unaltered. In vivo contraction of the tracheal smooth muscle is probably more isotonic than isometric, since its primary purpose is to regulate the calibre of the airways and thus the distribution of ventilation. What magnitude of load the cartilaginous rings place upon the trachealis remains unknown especially since it becomes increasingly difficult to compress the rings as airway calibre is reduced. This may become more markedly evident in pathological conditions such as asthma which feature altered transmural pressures. Thus, the individual cells may contract in a quasi-isometric mode but the aggregate response may be isotonic. This argument

is perhaps relatively unimportant since it is the airway smooth muscle in smaller airways - where isolated cartilagious plaques exist instead of rings, which contribute more to airway resistance in anaphylactic bronchospasm than the upper airways. Nevertheless, TSM, is a useful and reliable model for smooth muscle in 2 mm airways.

(i) Isometric mechanics

Information about contractile parameters which describe muscle function at the level of the filaments can be elicited effectively isometrically. This includes data such as resting tension (RP), active tension (AP) and total tension (TP), derived when performing length-tension experiments. For example, the maximum active tension (P_o) is a measure of the number of actomyosin bridges formed during contraction and represents the sum of the tensions developed at each cross-bridge. More bridges per unit cross-sectional area result in a higher P_o.

The rate of cycling of these cross-bridges can be effectively approximated by the rate of active tension development $(dP/dt)_{max}$. This provides an indication of the rate of ATP hydrolysis (Buccino et al., 1967) during contraction and is related to the maximum velocity of shortening determined by force-velocity studies (assuming the SEC is unchanged); generally the higher the dP/dt_{max}, the higher the V_{max}. The time to reach P_o (t_{p_o}) indirectly measures the duration of the active state of a contracted muscle (Sonnenblick, 1962(a)). The integral of t_{p_o} as a function of maximum active tension has been shown to be an index of the magnitude of the stable component of the

maintenance energy rate for some muscles (Mommaerts, 1969). Woledge (1971) and Aubert (1956) suggest this can be broken down into labile and stable maintenance heats; the latter is a time dependent function of the tension developed.

The analysis of isometric contractions using these parameters enables determination of length-tension relationships, plus energy utilization under the influence of various agonists and in various pathological conditions. Hargraves and Weiss (1977) argue that the use of length-tension determinations elicited by various agonists under simple isometric conditions, optimize the knowledge of the mechanochemical properties of a muscle, since,in vivo, the muscle is not always at optimum length. While idealistic, the practicality of comparing data obtained at lower lengths between agonists may not be valid, due to membrane stabilizing effects. One can speculate perhaps that the deformation of the muscle may also effect the availability of calcium pool target sites to agonists, and to varying degrees, since agonists differ in their mechanism of action. The l_o length used for studies of the effects of various agents on isometric tetanic tension, however, is the most consistently identifiable muscle length on the length-tension curve. Its advantage is that it provides a working muscle with a maximal contractile response, allowing for a more sensitive preparation to study muscle function under varying conditions.

(ii) Isotonic Mechanics

The relation between the velocity of isotonic shortening (V) and the force developed (P) - the force-velocity relation -

has been termed the most fundamental mechanical property of the contractile element of the muscle during activation (Sonnenblick, 1962(b)). The classical work of A.V. Hill (1938) on striated voluntary muscle and of Csapo (1962) on uterine muscle established this, indicating that muscle function studied in these terms provides an index of power production. This yields a superior account of energy utilization by a working muscle, than that given by any other analysis.

Brady (1965) demonstrated that a muscle must possess certain characteristics before force-velocity curves elicited can be considered valid. These include: (1) the individual muscle fibres must be parallel to each other (2) the major portion of the tissue must be muscle (3) the muscle should be capable of being tetanized and the maximum tension development be relatively independent of length over a wide range of muscle lengths, and (4) the resting tension must be small. The canine trachealis muscle appears to satisfy these criteria (Stephens et al., 1969).

A.V. Hill (1938) derived the classical equation

$$(P + \underline{a})\ (V + \underline{b}) = (P_o + \underline{a})\ \underline{b}$$

to best describe the curve (the force-velocity curve) formed by plotting the velocities of shortening his frog sartorius muscle preparation displayed during contraction against the tension developed while bearing various loads. In this equation, P represents the load, V the velocity of shortening, P_o the maximum load which the muscle is just unable to move, $\underline{a}$ is a constant with units of force and $\underline{b}$ is a constant with units of velocity.

The theoretical maximum velocity at zero load can be obtained by substituting the appropriate constants in the original equation. The ability to fit the Hill equation to the force velocity relationship of the trachealis muscle (Stephens et al., 1969) will eventually permit analysis of the function of the trachealis in terms of a model similar to that proposed for skeletal muscle. It becomes a useful tool for studying airway smooth muscle in general, as discussed previously.

Hill (1938) has shown that both a and P_o are functions of the thickness of the muscle; the thicker the muscle, the greater these values. The constant a reflects the number of force-generating sites formed, the more actomyosin cross-bridges, the greater the value of a. The b constant is dependent upon environmental factors (temperature and pH) which influence enzymatic rates. Kinetics studies on frog sartorius muscle have shown its value to be an index of the energy-liberating reactions during contraction. It has been equated with the rate of ATP hydrolysis, that is, the rate of cycling of cross-bridge attachment and detachment in the isotonically shortening muscle.

Both Hill (1938) and Woledge (1968) demonstrated an equality between the product of the a and b constants (a x b) of the force-velocity equation and the maintenance energy (heat) rate during muscle contraction. This represents a comparison of mechanical correlates with actual heat energy measurements. For other muscles, care must be exercised in extrapolating mechanical data only. This point is illustrated by the finding that the a constant is not really a constant, but rather increases slightly as the load lifted by the muscle increases (Hill, 1964).

The ratio $\underline{a}/P_o$, however, is relatively constant from muscle to muscle and for different states of a given muscle. Woledge (1968) indicated that the $\underline{a}/P_o$ ratio determines the curvature of the force-velocity relation, with an increase in the curvature reflecting an increase in muscle efficiency. For example, a lower $\underline{a}/P_o$ ratio means an increased curvature and therefore an increased efficiency and a decreased maintenance energy rate.

The ability to fit the Hill equation to the force-velocity relationship of the trachealis muscle is of some interest. Not only do the force-velocity constants permit comparison of contractile parameters with other muscle preparations, but also they permit a greater understanding of muscle function at the subcellular level. The strong qualitative mechanical similarity existing between tracheal smooth muscle and striated muscles (length-tension, force-velocity) (Stephens et al., 1969); series elastic component (Stephens and Kromer, 1971); temperature studies (Stephens et al., 1977) suggest force generation in airway smooth muscle may have a mechanism similar to that of striated muscle. Information from force-velocity studies of smooth muscle (TSM) about energy characteristics, tension development, velocities of shortening or extents of isotonic shortening can be used to interpret TSM muscle function, applying various aspects of the striated muscle model. Examination of altered parameters in pathological conditions such as asthma, in similar terms, may help elucidate the underlying etiology of the disease.

H. EXPERIMENTAL PLAN AND SPECIFIC AIMS

With the failure of extensive immunologic, neurogenic and genetic studies to provide a comprehensive theory regarding the underlying etiology of asthma, it becomes important to determine if a specific primary airway smooth muscle defect is present in asthma. It might be that the effects of stimuli known to trigger bronchospasm, are due to alterations at the smooth muscle level. Experiments designed to determine if muscle contractility is altered, were carried out, by studying tracheal smooth muscle function - and the mechanism by which control of that function is effected - in a canine model of allergic asthma.

Mongrel dogs were sensitized to ovalbumin (OA) and trachealis smooth muscle (TSM) isolated from sensitized animals and their littermate controls. The TSM strips were subjected to studies of their mechanical function under various conditions and different stimuli in an attempt:

(1) to determine whether the velocity of shortening, active tension development, or shortening capacity of the working sensitized TSM are altered in allergic asthma. These alterations may provide a mechanism for the events of allergic bronchospasm. Isotonic mechanical studies,using electrical stimuli,were conducted to investigate this aspect.

(2) to determine if the active tensions developed isometrically to electrical and carbachol stimuli were different between sensitized and control TSM. Alterations in airway smooth muscle detected by these studies could stem from primary changes

in the muscle or be secondary to changes in the function of the nerves controlling airway smooth muscle. To this end, studies of "P_o" - maximum active tension - using electrical and carbachol stimuli were undertaken. Possible sites for alteration include muscle membrane excitability, excitation-contraction coupling or the contractile proteins.

(3) to establish that a specific allergen is responsible for the bronchospastic response seen in sensitized TSM. This would validate our model of allergic asthma. To investigate this aspect, experiments were undertaken in which the sensitizing antigen (OA) and a related protein (bovine albumin) were used to challenge, in vitro, control and sensitized TSM's.

(4) to determine the anaphylactic mediators released by the antigen-antibody reaction in our canine model of allergic asthma. To this end antagonists of anaphylactic mediators were used and their ability to eliminate the sensitized TSM's bronchospastic response to the sensitizing antigen, studied. The delineation of which mediator is at work in our model would allow for a better understanding of the events linking the antigen-antibody reaction to airway smooth muscle contraction in asthma.

(5) to examine the sensitivity of control and sensitized TSM to histamine - this study has proven it to be the primary anaphylactic mediator at work in our model. Histamine dose-response curves were elicited for the two preparations. Hypersensitivity of the sensitized muscle to histamine, if shown, might account for the hyperirritability of the airways to antigen challenge and the increased airway smooth muscle tone observed in asthma.

METHODS

A. SENSITIZATION PROCEDURE

An adaptation of the method developed by Pinckard et al. (1972) was used to elicit the production of IgE antibodies and anaphylactic sensitivity to ovalbumin - in preference to hemaglutinating antibodies - in developing our canine model of allergic asthma. For the induction of IgE antibody production, mongrel dogs received intraperitoneal immunization with 10 μg of a conjugate of dinitrophenol and ovalbumin (DNP_2-OA) mixed with 30 mg of $Al(OH)_3$, within 24 hours of birth. Booster injections, consisting of the same dose, were repeated at weekly intervals for eight weeks, thereafter every two weeks. This regimen of immunization has been shown to induce prolonged IgE antibody production of high titers against both the DNP and OA determinants (Kepron et al., 1977). Serum IgE antibody titers were measured by homologous passive cutaneous anaphylaxis (PCA) in non-sensitized dogs (for example, a PCA titer of 64 means serum diluted up to 1/64 results in positive cutaneous anaphylaxis. Further serum dilutions would fail to cause a reaction).

On antigenic bronchoprovocation, sensitized dogs with serum IgE anti-OA antibody titers in excess of 64 developed a marked increase in specific airflow resistance (Kepron et al., 1977). Littermates of sensitized dogs were given injections of the adjuvant, $Al(OH)_3$, only (without DNP_2-OA) using the identical regimen. These animals were used as controls. During the course of this study, tracheal smooth muscle (TSM) was obtained from sensitized dogs whose PCA titers were at or above 256. In vitro sensitivity to OA was

checked by challenging isometrically mounted control and sensitized TSM strips with OA. In all experiments, only sensitized muscles developed active tension when an OA solution was introduced to the Krebs-Henseleit of the muscle baths (final concentration .3 mg/ml). The specificity of the OA antibody-antigen reaction was demonstrated by a lack of cross-reactivity to bovine albumin.

B. DISSECTION

Trachealis muscle was obtained from the cervical tracheae of mongrel dogs anaesthetized with 30 mg/kg sodium pentobarbitol (Nembutal, Abbott) by intravenous injections. An intracardiac injection of saturated potassium chloride solution was used to kill the animal after a section of tracheae was removed. Excision was completed within five minutes, with the tracheae section being immediately immersed in oxygenated Krebs-Henseleit solution. This prevents the muscle tissue from becoming hypoxic as well as reducing equilibration time in the muscle bath.

The trachealis muscle (musculus transversus tracheae) composes the most dorsal layer of the paries membranaceus which traverses the incomplete dorsal ends of the cartilaginous canine tracheal rings (fig. 1). The anterior aspect of a single tracheal ring was bisected and the two cartilaginous ends everted. The trachealis muscle and tunica fibrosa separate easily, and the muscle layer could be dissected out with minimal handling (fig. 2). Parallel fibered strips of the trachealis muscle (TSM) were cut out. Only 10 minutes elapsed between excision of the TSM

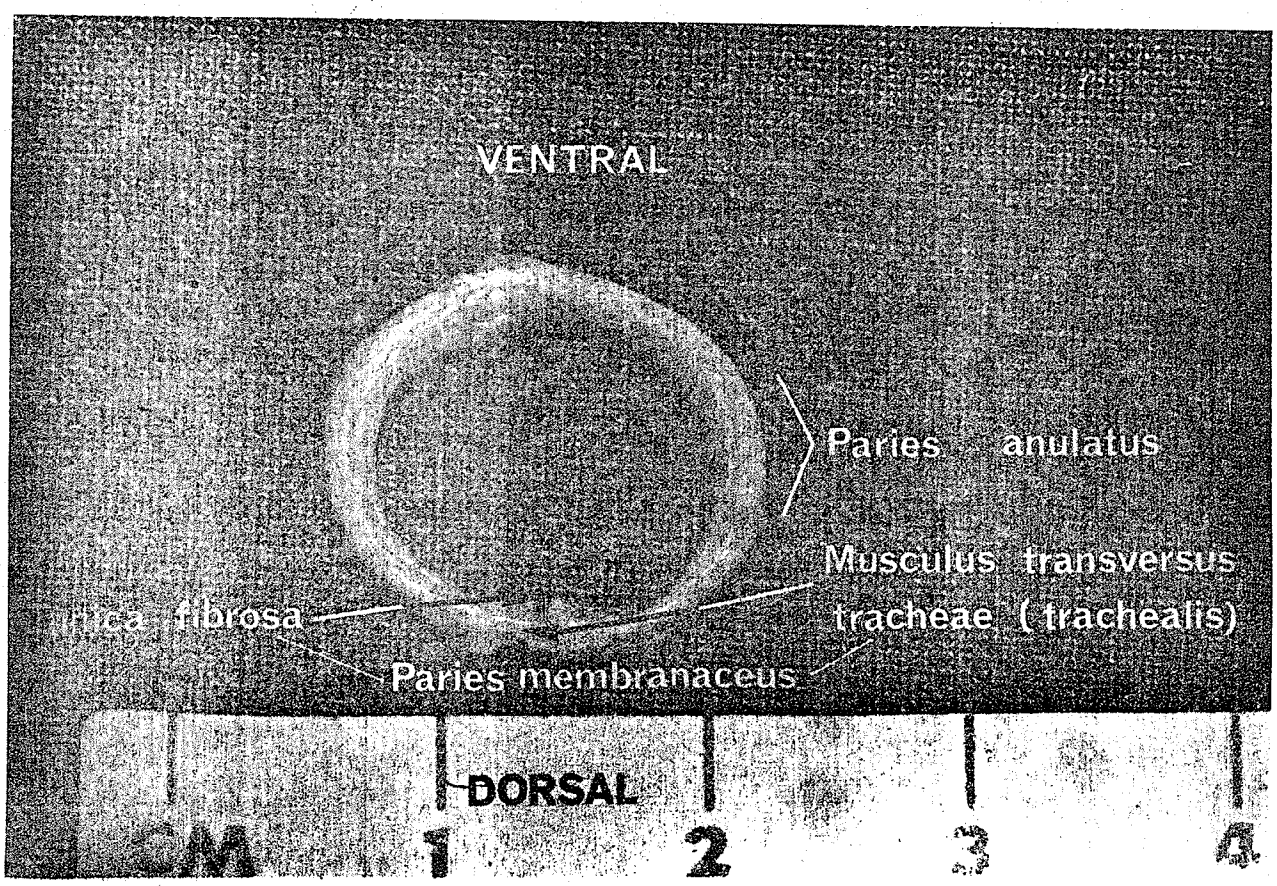

Fig. 1 A single intact canine tracheal ring showing the incomplete cartilaginous ring, closed by the paries membranaceous dorsally.

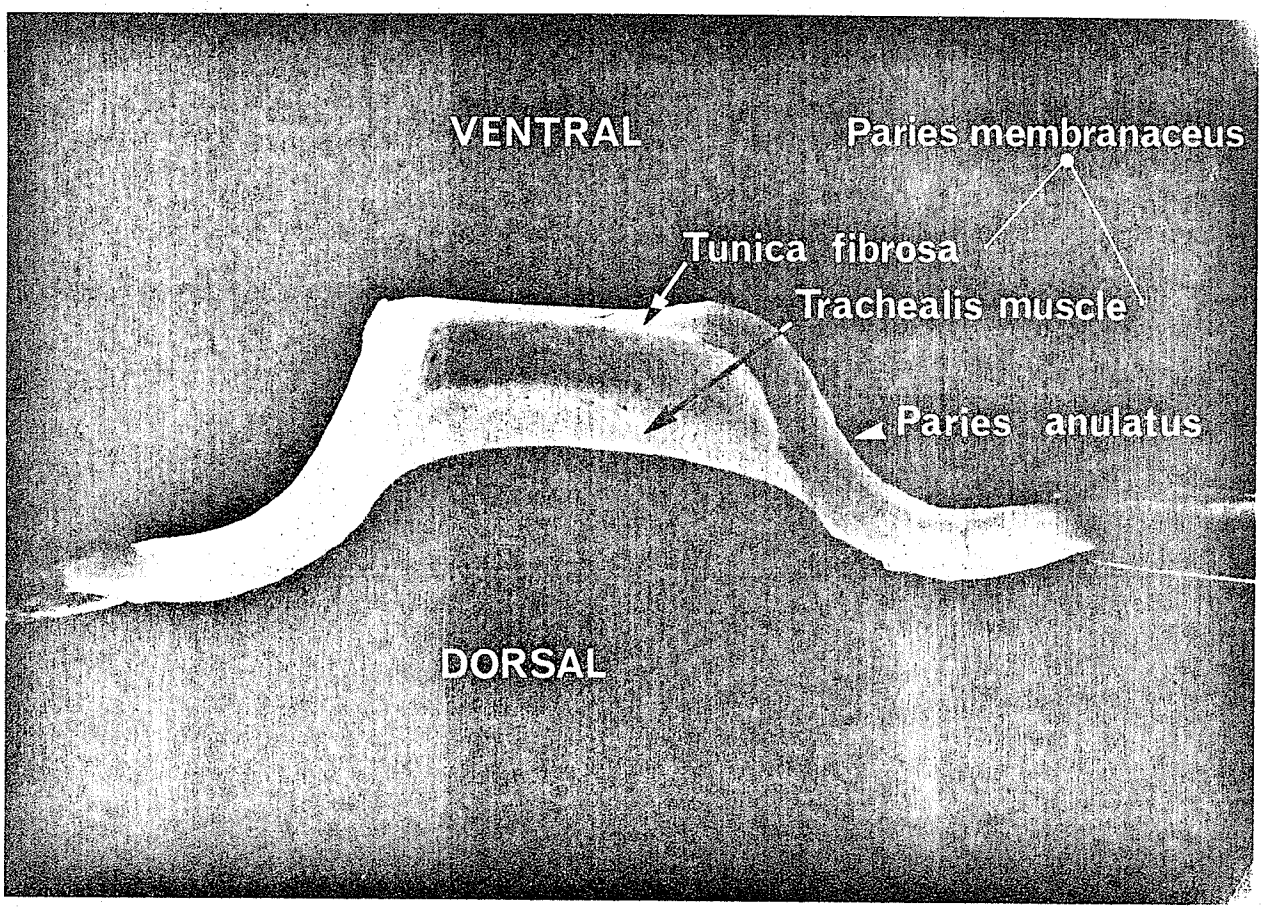

Fig. 2 Photograph of a single tracheal ring shown in the transverse plane. The cut everted tracheal ring is shown. Eversion causes the trachealis and the tunica to separate, and facilitate further dissection of the TSM.

and the mounting of the strips in an appropriate muscle bath containing mammalian Krebs-Henseleit solution, the composition of which is given in Table 1. Where varying potassium concentrations were needed, the KCl content was adjusted accordingly, substituting for NaCl on an equimolar basis. All solutions were equilibrated with a 95% O_2 - 5% CO_2 mixture which maintained a P_{O_2} of 600 Torr, a P_{CO_2} of 40 Torr and a pH of 7.40, at a temperature of 37°C.

C. MECHANICAL STUDIES

Previous work in our laboratory has shown canine TSM to be ideally suited to systematic mechanical studies (Stephens et al., 1969). It is a quiescent tissue consisting of parallel fibers and seventy-five percent muscle, which can be easily tetanized and possesses a low resting tension at optimal length (l_o).

(i) Isometric Studies

For the isometric studies, the lower ends of the muscle strips were attached by a short loop of 000 braided surgical silk to the hook at the end of the rigidly clamped aerating tube of the muscle bath (fig. 3). The upper end was fastened to a Grass FT .03 force transducer mounted on a rack and pinion, enabling the muscle to be stretched to any desired length and held there isometrically. Output from the force transducer was amplified and recorded on a 4-channel Gould 2400 Brush recorder. Compliance of the system was negligible.

For the isometric electrical stimulus- and the dose-response studies, the muscles were stretched to their appropriate l_o - the length at which maximum active tension is elicited - by placing

TABLE 1: Composition of Krebs-Henseleit Solution

	mM	g/L
NaCl	115	6.72
$NaHCO_3$	25	2.10
NaH_2PO_4	1.38	0.167
KCl	2.51	0.187
$MgSO_4 \cdot 7H_2O$	2.46	0.296
$CaCl_2$	1.91	0.145
dextrose	5.56	1.00
osmolarity = 304 mOsM		

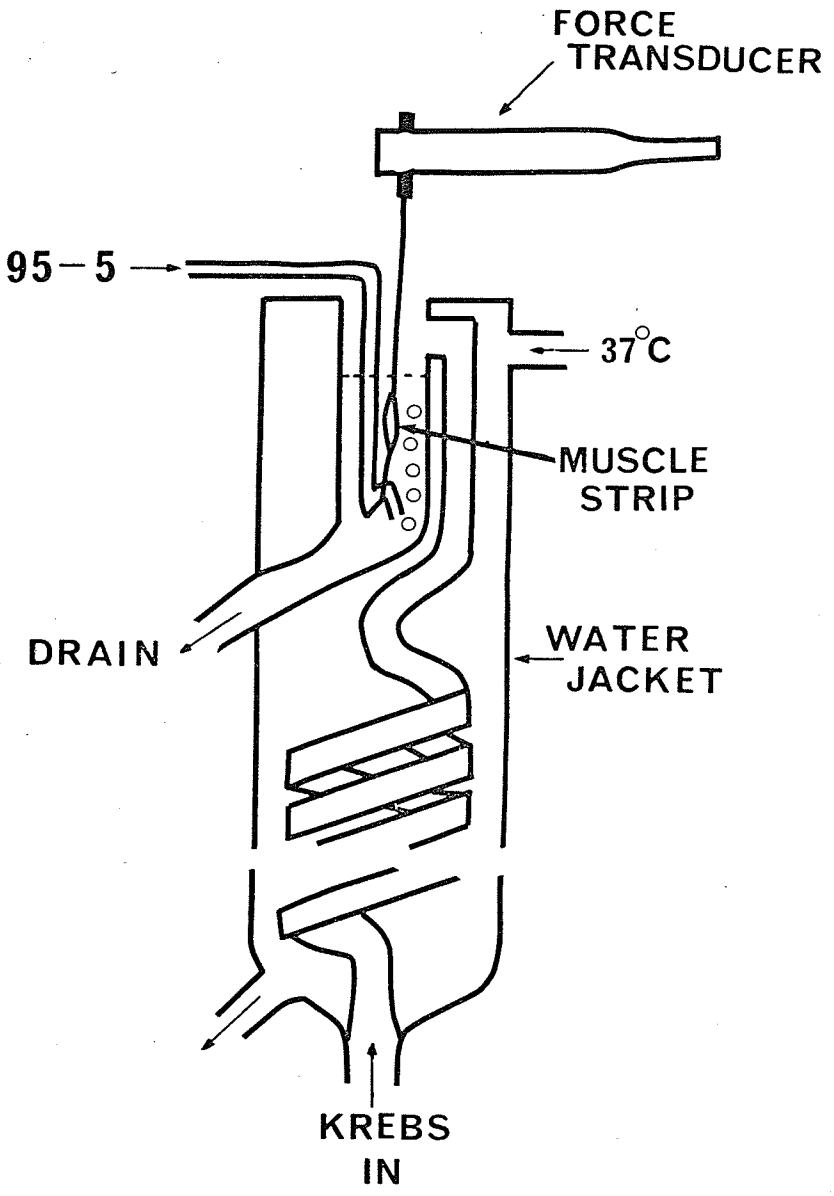

Fig. 3 Simplified schematic diagram of the apparatus used in isometric expeirments, which allows the recording of tension development under various stimuli.

a 0.6 to 0.8 g. resting tension (RP) upon them. Previous experience in our laboratory has shown that for this RP, the muscle is at its optimal length (l_o). This was confirmed in each experiment by changing length slightly around this RP, to gain maximum active tensions (AP), essentially a limited length-tension study.

The tissue baths in which the muscle strips were immersed, contained a fixed volume (15 ml) of Krebs-Henseleit solution at 37°C and aerated with 95% O_2 - 5% CO_2. After mounting, the TSM was allowed to equilibrate for one hour to reestablish ionic equilibrium. During this time, it was stretched periodically until a suitable resting tension was established. Four identical muscle baths were used, with random placement of sensitized and control TSM. The apparatus was rigidly mounted to prevent extraneous vibrations.

For the electrical stimulus-response studies, electrical stimulation was effected from a constant-voltage 60-Hz AC source via rectangular, platinum plate electrodes which provided supra-maximal field stimulation with a current density of approximately 400 mamp/cm^2. The TSM strips were tetanized every five minutes for the duration of the experiment; this rest period enables the muscle to recover and provide maximal, precise responses. Stimulus duration was held to the minimum necessary to elicit the maximal active response (10 - 12 sec.). Stimulus-response curves were determined for a series of sensitized and control tracheal strips by plotting isometric active tension developed (kg/cm^2), converted to a percent of maximum control response against the stimulus voltage. Cross-sectional area of the muscle

was estimated using the length of the tissue and its blotted wet weight, and the density of the tissue to be about 1.

Cumulative dose-response curves were obtained for carbamyl choline (carbachol, see Table 2), in both the absence and presence of atropine. The maximum active tensions developed were recorded for each concentration and plotted after appropriate normalization (maximum isometric tension in kg/cm^2 (converted to a percent of maximum control response) against the log of the dose), to obtain proper dose-response curves.

Histamine dose-response curves for sensitized and control TSM were obtained by administering successively higher half-log doses of histamine to the muscle bath. Three rinses of fresh, 37°C Krebs-Henseleit solution followed the moment active tension reached a plateau for each dose. Contact time with the agonist was thus reduced. This diminished the development of tachyphylaxis. Five minutes elapsed before the introduction of the next dose. The maximum active tensions developed were recorded for each concentration and after appropriate normalization (maximum isometric tension in kg/cm^2 against the log of the dose), plotted to obtain dose-response curves.

Tests to determine histamine receptors and their properties were undertaken using agonists and antagonists listed in Table 3. For example, a dose of pyrilamine maleate would be added to a muscle bath, and after appropriate incubation, its ability to block the isometric tension developed by the TSM under a histamine stimulus would be determined.

TABLE 2: Cholinergic/adrenergic antagonists and agonists.

Drug	Action	Stock Solution	Source
carbachol	cholinergic agonist	10^{-2} M	Sigma
atropine	cholinergic antagonist	.6 mg/ml	BDH
noradrenaline	beta - adrenergic agonist	.2% solution	Winthrop Laboratories
propanolol	beta - adrenergic antagonist	1 mg/ml	Sigma

TABLE 3: Histamine agonists and antagonists.

Compound	Action	Stock Solution	Source
histamine dihydrochloride		10^{-3}, 10^{-2}, 10^{-1}M in .1 N HCl	Sigma
2 - methyl histamine dihydrochloride	H_1 agonist	10^{-2} M in .1 N HCl	+Smith, Kline & French
2-(2-pyridyl)-ethylamine dihydrochloride	H_1 agonist	10^{-3}M in .1 N HCl	+Smith, Kline & French
pyrilamine maleate (mepyramine)	H_1 antagonist	1 mg/ml in DDW*	Sigma
4 - methyl histamine dihydrochloride	H_2 agonist	10^{-1}M in .1 N HCl	+Smith, Kline & French
metiamide	H_2 antagonist	10^{-3} M in .1 N HCl	+Smith, Kline & French

* double distilled deionized water

+generously donated by Smith, Kline and French Laboratories Ltd., England

Statistical means and standard errors were determined for each stimulus - voltage or dose when plotting the response curves. Histamine dose-response data were analyzed using Duncan's (1955) new multiple range test to determine significant differences between sensitized and control preparations. Because the electrical stimulus- and carbachol dose-response data displayed unequal variances between the two preparations, a Chi-square test was used to test the presence or absence of association in a two-way contingency table.

(ii) Isotonic studies

For the isotonic studies, a modified muscle bath was used in which the previously described Krebs-Henseleit solution was constantly circulating. The lower end of the muscle strip was attached firmly to a solid metal rod which passed through a mercury seal in the bottom of the bath as shown in figure 4. The rod was attached to a Grass FT .03 force transducer and the provided springs used to gain optimal sensitivity with minimum extraneous compliance. The upper end of the muscle strip was tied with 000 braided surgical silk to a titanium lever mounted on jewelled bearings. The displacement of the lever during isotonic shortening was measured using a Packard 7-DCDT displacement gauge. The force and displacement gauges were connected to a Tektronics RM 564, two-channel storage oscilloscope which enabled simultaneous measurement of shortening velocities (dL/dt) and active tension development (P). Supramaximal field electrical stimulation was effected from a constant-voltage 60-Hz AC source via platinum plate electrodes, placed as close as possible to the

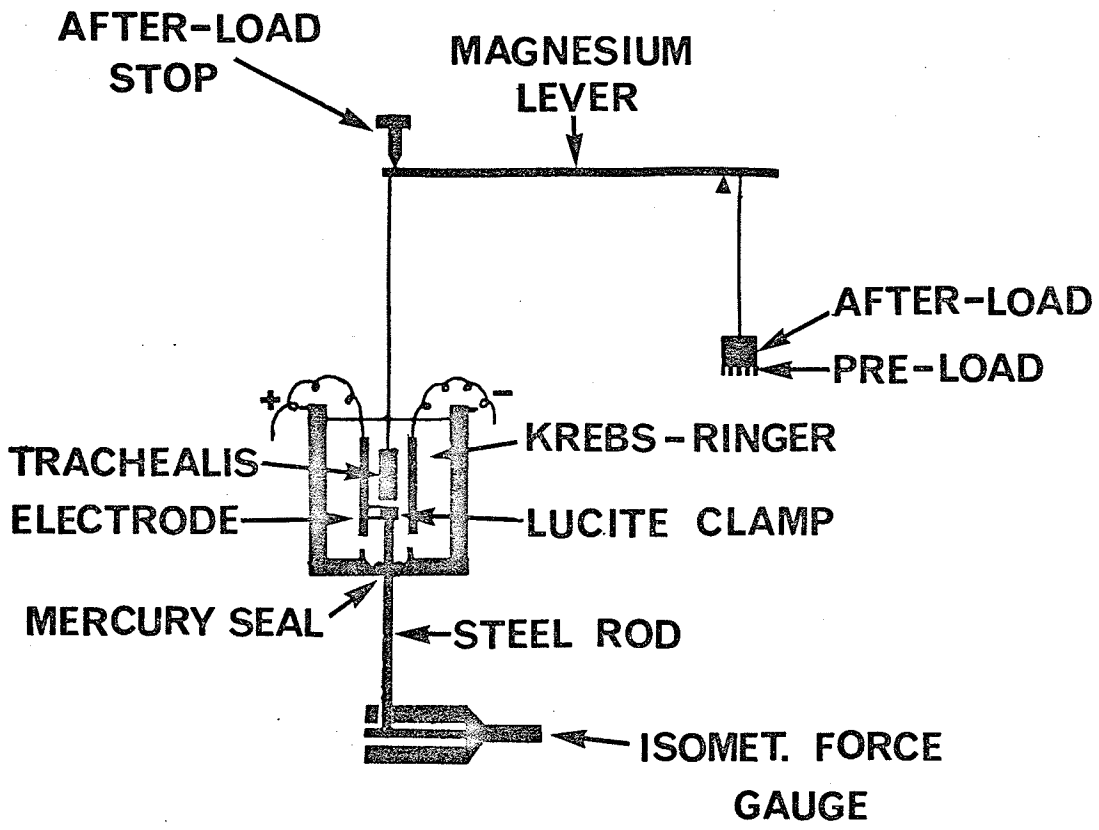

Fig. 4 Schematic diagram of the apparatus used in isotonic experiments, allowing the simultaneous measurement of shortening and active tension development with respect to time. The afterload stop maintains the TSM strip at its optimal length l_o.

tissue. The current density of the plates was approximately 400 $mamp/cm^2$.

Electrical stimulus response and length-tension studies were carried out (after the equilibrium period)to determine the supramaximal tetanizing stimulus and l_o of the TSM for the subsequent force-velocity study. The afterload stop was then set to maintain l_o when afterloads were added. Velocities of shortening for sensitized and control muscle strips were determined and plotted against respective afterloads. The force-velocity curves produced were analyzed according to the classical method of A.V. Hill (1938). The constants derived from the Hill equation analysis were used to examine differences between sensitized and control airway smooth muscles. A t-test was used to determine significance since the means of the groups are normally distributed according to the central limit theorem. Sampling was random, the observations were independent and variance homoscedasticity was demonstrated (Steel and Torrie, 1960).

RESULTS

In order that a logical and sequential interpretation of the data might be expressed in the discussion section, the results of the isotonic mechanical studies are presented first. Although the results of the sensitization procedure are documented later, it is realized an active tension response of sensitized TSM to OA challenge must be established to justify our model of allergic asthma. All sensitized TSM used in the isotonic study revealed reactivity to the sensitizing antigen; controls did not.

I. ISOTONIC STUDIES

It has already been established (A.V. Hill, 1938; Csapo, 1962) that the study of a muscle's force-velocity relation - an index of power production - provides a superior account of energy utilization in a working muscle to that given by any other method. In a pathological condition such as asthma, the energy requirements of airway smooth muscle might be altered in a manner which effects contractility. A second reason for performing these studies is that a muscle's function can be affected with respect to the maximum load it can bear (P_o) and/or its maximum velocity of shortening (V_{max}) - these parameters form the basis of the force-velocity relation. Sonnenblick (1965) has demonstrated for cardiac muscle that these variables can fluctuate independently of one another. In asthmatic airway smooth muscle, a change in either would reflect a variety of possible sites of defect of the contractile state of the muscle. Lastly, the force-velocity experiments allow analysis of muscle shortening capacities, under various loads. This is probably the most significant parameter,

since in asthma, the shortening capacity of the muscle would determine the degree of airway narrowing and thus the magnitude of airway resistance.

A series of force-velocity experiments were carried out on eleven sensitized and nine littermate control dogs, using the apparatus described in figure 4. Figure 5 illustrates the results of a typical paired force-velocity experiment in which a random series of afterloads were lifted by the sensitized and control tracheal smooth muscles. All values were normalized with respect to their velocities of shortening in l_o/sec, and tension developed in kg/cm^2 in order that a more valid comparison of the mechanical data from the TSM strips could be undertaken. Note that the shortening velocity of the sensitized TSM is higher than control for a given load, while their P_o's remain the same.

Hill's (1938) equation was used to describe and analyze the force-velocity curves, since they proved to be rectangular hyperbolas - as demonstrated by a goodness of fit test - similar to that found for frog skeletal muscle; the equation is:

$$(P + \underline{a})\ (V + \underline{b}) = (P_o + \underline{a})\ \underline{b} \qquad (1)$$

where P represents load, V the velocity of shortening, P_o the load which the muscle is just unable to move, $\underline{a}$ a constant with units of force, and $\underline{b}$ a constant with units of velocity. For all experiments, a linearized transform of the equation was used to prove the relationship was hyperbolic, viz:

$$(P_o - P)/V = P/\underline{b} + \underline{a}/\underline{b} \qquad (2)$$

With $(P_o - P)/V$ plotted against P, the data points fall on a straight line (Ostle, 1956; Natrella, 1963). Figure 5 illustrates

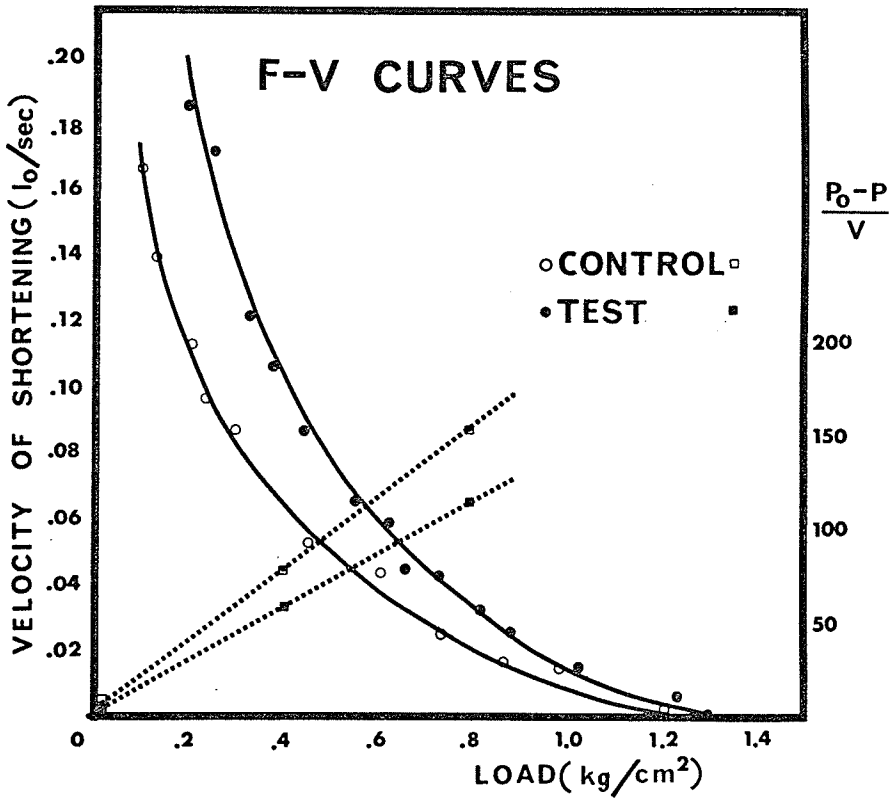

Fig. 5 Typical force velocity curves elicited in a paired experiment. Note the shift to the right (about P_o) by the sensitized TSM curve, indicating that its velocity of shortening is higher without a significant change in P_o, when compared to control values. The linearized transforms prove the relationships are hyperbolic; a goodness of fit test yielded a correlation coefficient r^2 = .95 for both lines.

this analysis; with $(P_o - P)/v$ plotted against P, the data points of the sensitized and control TSM fall on two separate, statistically significant, straight lines with different slopes, proving the relationships to be hyperbolic in both muscles. The difference in slope suggests the velocity constants are different in the two muscles. Calculation of the regression equation (Snedecor, 1946) based on equation 2, yields the slope $1/\underline{b}$ and thus the velocity constant $\underline{b}$. Using the intercept and the value of $\underline{b}$, the value of $\underline{a}$ was computed (intercept = $\underline{a}/\underline{b}$). The theoretical maximum shortening velocity at zero load was calculated by substituting the appropriate constants into the equation:

$$V_{max} = P_o \, \underline{b}/\underline{a} \qquad (3)$$

In table 4, the various force-velocity parameters for the sensitized TSM are compared with those of the control muscles. Hill (1938) has shown that both P_o and $\underline{a}$ are functions of the thickness of the muscle. Since the constant $\underline{a}$ reflects the number of force generating sites and thus P_o, the thicker the muscle is, the greater these values. Both of these constants are expressed in grams force per square centimeter cross-sectional area of muscle. This was estimated using the length of the tissue (cm) and its blotted wet weight, assuming the muscle is a cylinder, and has a specific gravity of about 1. Statistical analysis revealed no significant differences in these parameters when comparing control and sensitized TSM. In addition, the $\underline{a}/P_o$ ratio was not significantly different for the two preparations. All of the control pup's values shown in table 4 compare favourable with results found for adult canine TSM, using a modified apparatus for force-velocity analysis based upon a

TABLE 4: Force-velocity parameters of sensitized and littermate control TSM.

		Control	Sensitized
N		9	11
V_{max}	(l_o/sec)	.234 ± .022	*.346 ± .043
b	(l_o/sec)	.047 ± .003	*.062 ± .004
a	(kg/cm^2)	.263 ± .032	.238 ± .030
P_o	(kg/cm^2)	1.211 ± .071	1.161 ± .056
a/P_o		.230 ± .040	.212 ± .032

*$p < .05$

Force-velocity parameters calculated (using Hill's analysis) from data obtained in force-velocity experiments performed on a series of sensitized and control TSM strips. Note that only V_{max} and b are statistically significantly increased - 48% higher - in the sensitized TSM. All values include the standard errors of the means.

quasi-isotonic spring technique (Stephens et al., 1969).

Standardization of the b constant and V_{max} was achieved by expressing velocity in muscle lengths per second (l_o/sec). Normally the speed of muscle shortening is related to the number of cross-bridges in series, thus a longer muscle will display a greater velocity of shortening (cm/sec). The resting length l_o - the length at which active tension is maximal - was determined by length-tension experiments carried out before the start of each force-velocity study. By standardizing velocities of shortening in l_o/sec, a more valid comparison of muscles can be made. Both the V_{max} and the b constant of the sensitized TSM were significantly higher (t-test $p < .05$) than those values of control. The V_{max} of the sensitized preparation increased from the control level of .234 $\pm$ .022 l_o/sec to .346 $\pm$ .043 l_o/sec; the b constant increased from the control level of .047 $\pm$ .003 l_o/sec to .062 $\pm$ .004 l_o/sec.

In addition to the observed changes in shortening velocities, an analysis of the isotonic shortening trace from the force-velocity experiments indicated that the sensitized trachealis muscles possess a statistically significant, increased capacity to shorten over that of the controls (figure 6). This is in addition to smooth muscle's greater inherent capacity to shorten, more than either skeletal or cardiac muscles. Analysis of shortening using loads less than 15% P_o was not possible since these loads approached the preload needed to maintain the muscles at their respective l_o's and balance the lever. This is a limitation imposed by the type of lever-arm apparatus employed in the force-velocity experiments and the physical properties of the resting muscle.

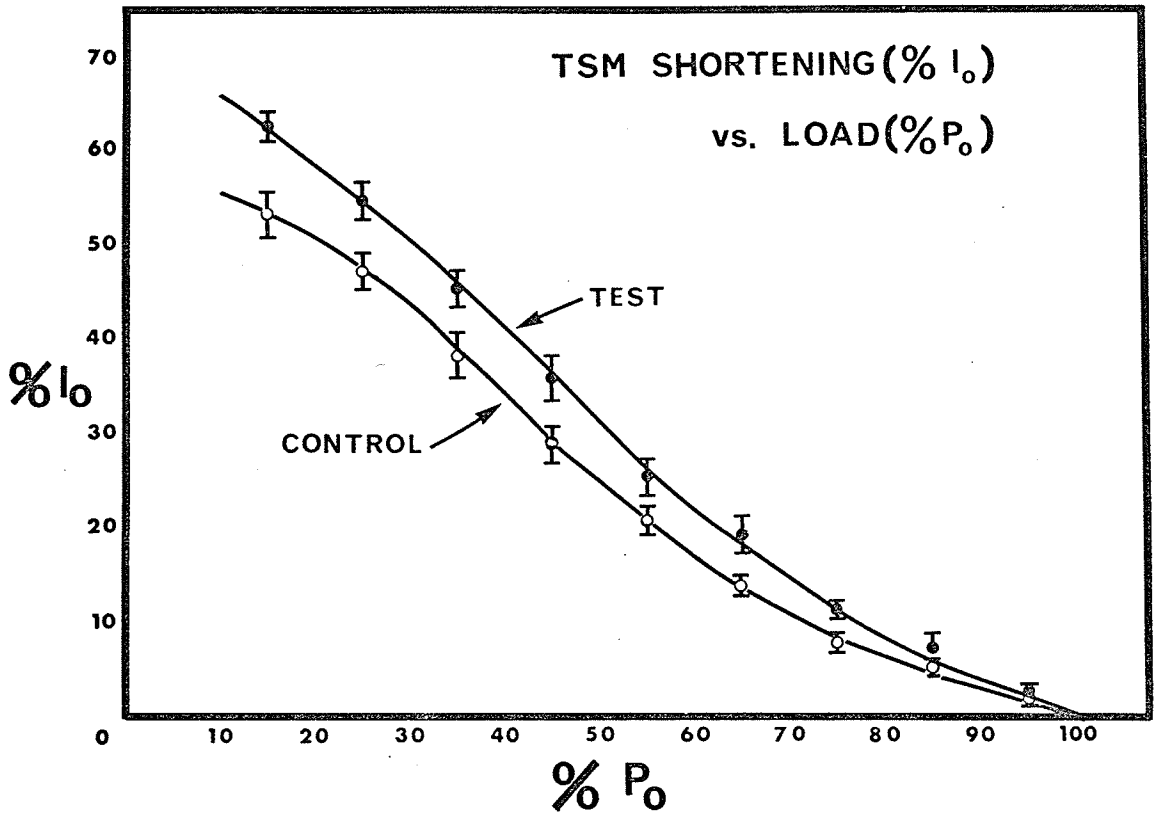

Fig. 6 Sensitized and control TSM shortening under various loads, determined in force-velocity experiments. At almost all loads the sensitized TSM shortened significantly more than the control. Standard error bars are shown; all values are expressed using the l_o and P_o of the respective TSM strips as one hundred percent.

II. ISOMETRIC STUDIES

(a) Electrical stimulus response experiments

The sensitized tracheal smooth muscles failed to exhibit statistically significant hyperexcitability (shift to the left) or hyperreactivity (higher maximal tension) to electrical stimuli when compared to normalized control values (figure 7). All values are expressed as a percentage of the control P_0, the means of the grouped data (N = 12) are plotted. Electrical field stimuli varied randomly in the study from 0-14 volts (measured by a voltmeter) with 14 volts in all cases proving to be supramaximal.

Investigation of the time to reach peak tension (tP_0) (assuming the tetanus is some integral multiple of twitch responses) and the maximum rate of tension development (dP/dt's) as well as relaxation times, failed to establish definable differences between sensitized and control TSM. However, phenomena peculiar to the sensitized muscles were observed in a number of isometric experiments, but not in all. Figure 8 shows that upon electrical stimulation, the isometric tension developed by the sensitized muscle continues to rise slowly, in contrast to the normal control situation (lower panel) where the isometric tetanic tension plateaus and then falls.

The presence of a beta-adrenergic blocker would block the activation by the electrical stimuli of any relaxant beta-adrenergic fibers present. Propanolol failed to alter the trace. Noradrenaline (1.2 μM), however, relaxes both the sensitized and control TSM. Assuming the lack of "purinergic" relaxant fibers in canine TSM (Suzuki et al., 1976), the fade in tension to electrical stimuli

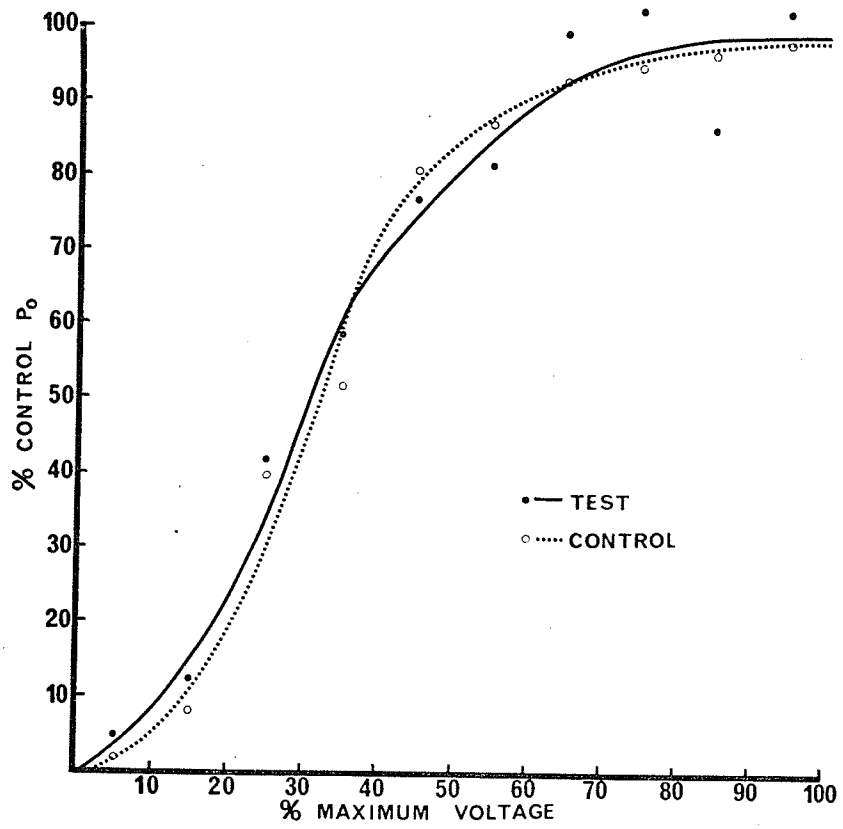

Fig. 7 Electrical stimulus-response curves elicited for both sensitized and control TSM (N = 12). The means of the active tensions developed by the two preparations for each of the voltage strength groups are plotted, the standard errors are not shown (see Table 5). A Chi-squared test ($p < 0.05$) failed to reveal statistically significant differences between the two curves.

TABLE 5: Electrical-stimulus responses of sensitized and control TSM.

	tension (% of control max.)	
% of maximal voltage	sensitized	control
0 - 10	4.47 ± 2.27	1.24 ± .60
10 - 20	12.04 ± 3.56	7.67 ± 2.13
20 - 30	41.90 ± 6.13	39.62 ± 5.29
30 - 40	58.75 ± 11.58	51.69 ± 7.72
40 - 50	77.00 ± 8.40	80.68 ± 3.63
50 - 60	81.55 ± 10.17	87.00 ± 4.00
60 - 70	99.00 ± 8.19	92.58 ± 1.64
70 - 80	102.13 ± 10.97	94.50 ± 1.73
80 - 90	86.25 ± 15.32	96.25 ± 1.25
90 - 100	102.53 ± 5.91	97.26 ± 1.09

mean ± S.E.

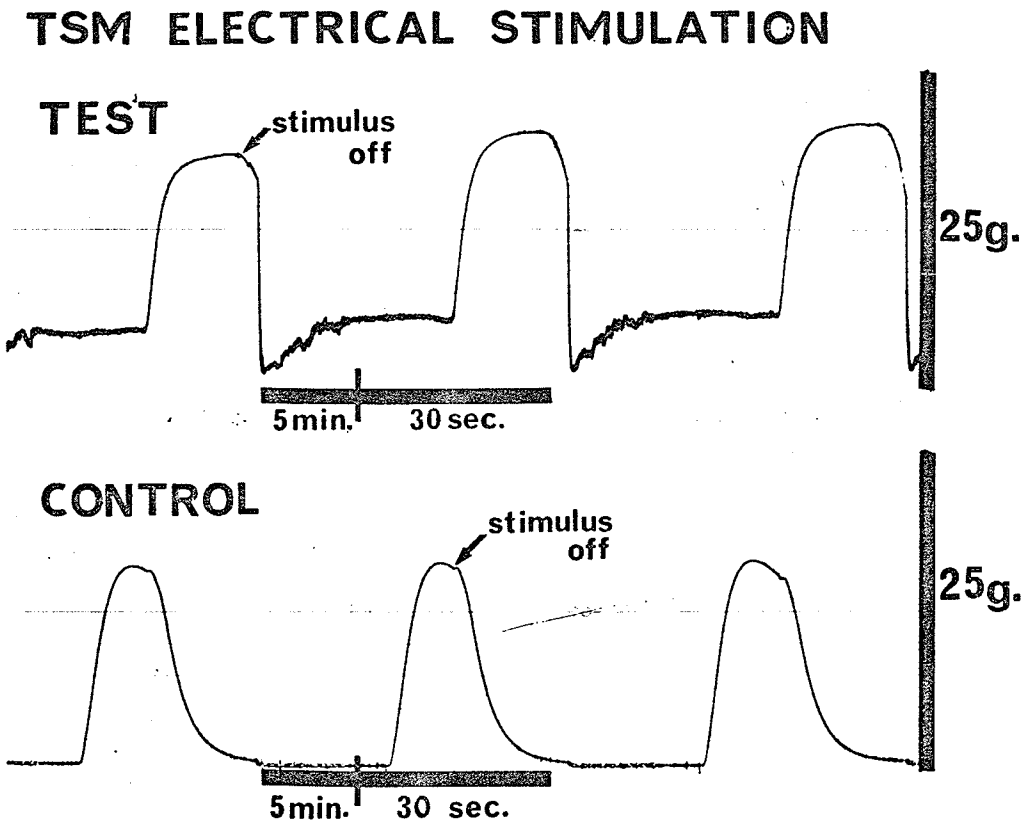

Fig. 8 Isometric tension trace using supramaximal electrical stimulation. Note that in the upper panel a prolonged and increasing active tension plateau to electrical stimulation is observed, as well as spontaneously rising resting tone with phasic activity between stimuli. In the lower panel, the control muscle displays a stable resting tension and a short plateau at peak active tension.

must be due to acetyl choline depletion. Similar isometric tension records (unpublished results) - with the prolonged plateau - are obtained to electrical stimulus when control TSM's are pretreated with a submaximal dose of TEA (.9 mM) (see Stephens et al., 1975).

After cessation of the electrical stimulus the control TSM's returned to their normal resting tensions. The sensitized TSM did so as well, but as illustrated (figure 8), exhibited spontaneously increasing resting tone with superimposed phasic activity within the five minute period between electrical stimuli (One must keep in mind when looking at fig. 8 that the trace is part of a multichannel recording and that the paper speed changes as marked). The spontaneous activity could be abolished by flushing the muscle bath with fresh Krebs-Henseleit. Subsequent electrical stimuli sometimes cause the phenomenon to reappear, suggesting that the sensitized TSM is producing a "factor" which is responsible. Supportive evidence for this hypothesis was gained when Krebs-Henseleit was transferred from the muscle baths containing spontaneously active TSM to other preparations (both control and sensitized) which consequently developed active tension. In control experiments where Krebs-Henseleit was transferred from electrically stimulated bathing medium which contained no TSM, to other preparations, failed to elicit any responses. Obviously then, the factor must be associated with the sensitized TSM.

While both the slowly rising active tension under electrical stimulation and the spontaneously rising resting tone was observed in 4 of 12 experiments, they did not always occur together (2 of 4) suggesting, perhaps, that two different mechanisms may be involved.

A third phenomenon exhibited by sensitized TSM (3 of 4 tested) is illustrated in figure 9. The application of a quick stretch (in this particular case to 112% of l_o) resulted in a myogenic response. At no time was this observed in control tissues similarily stretched. The normal canine tracheal smooth muscle has previously been reported (Stephens and Kroeger, 1972) to be quiescent and not to possess a myogenic response; except in the presence of compounds such as TEA (Stephens et al., 1975) and 4-amino pyridine (Kannan and Daniel, 1977).

(b) Carbamylcholine dose-response experiments

The sensitized TSM failed to exhibit statistically significant hyperexcitability (shift to the left) or hyperreactivity (higher maximal tension) to a carbamylcholine (carbachol) stimulus when compared to normalized control values (figure 10). The dose-response curves were plotted expressing active tension developed for each dose as a percentage of the maximum control P_o. The carbachol log doses ranged from 10^{-10}M to 10^{-2}M. Standard error bars are not shown (Table 6). The addition of a supramaximal dose of atropine (10^{-7}M) shifted the carbachol dose-response curves to the right as expected for a competitive antagonist but again statistically significant differences between the sensitized and control TSM preparations were not established.

(c) Investigation of the mechanism of allergic bronchospasm

(i) antigen-antibody reaction

In order to prove our canine model of allergic asthma valid, the airway smooth muscle must display a "bronchospasm" when challenged, which is specific to the sensitizing antigen. During

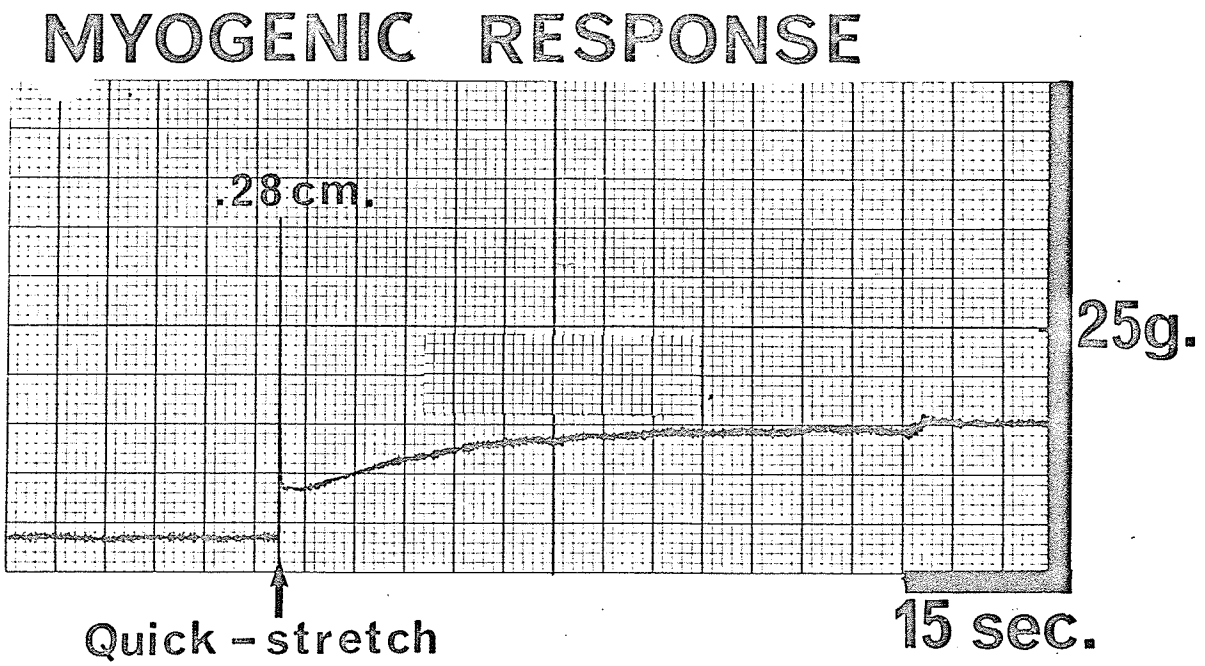

Fig. 9 A myogenic response was observed in some sensitized TSM strips. Quick stretch to 112% of l_o produced this particular isometric active tension trace.

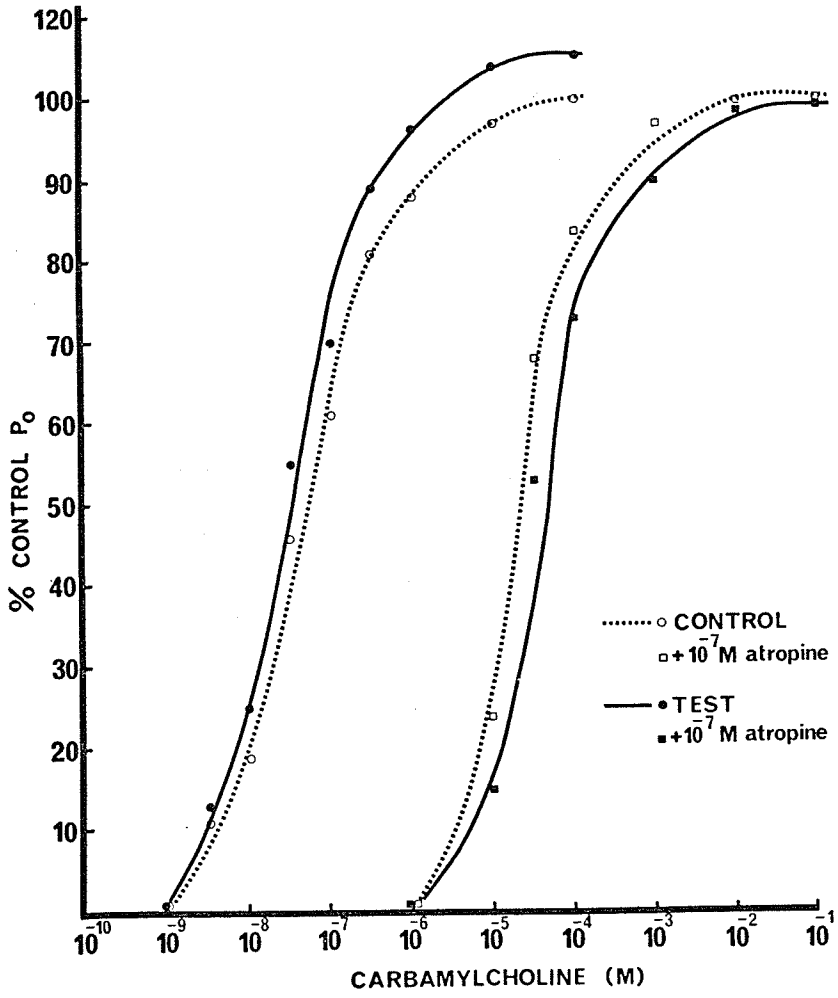

Fig. 10 Carbachol dose-response curves elicited for both sensitized and control TSM. The means of the active tensions developed by the two preparations and the log of each dose are plotted, the standard errors are not shown (see Table 6). The presence of atropine (10^{-7}M) - a competitive cholinergic antagonist - shifted the curves to the right. A Chi-squared test ($p < .05$) failed to reveal statistically significant differences between the two preparations.

TABLE 6: Carbachol dose-responses of canine TSM.

dose carbachol (M)	control		sensitized	
		+ 10^{-7}M atropine		+ 10^{-7}M atropine
10^{-9}	0.50 ± 0.50	-	0.17 ± 0.17	-
5 x 10^{-9}	11.0 ± 7.62	-	13.17 ± 6.29	-
10^{-8}	18.67 ± 11.81	-	25.17 ± 11.41	-
5 x 10^{-8}	49.50 ± 7.46	-	54.33 ± 9.61	-
10^{-7}	61.17 ± 4.98	-	69.83 ± 6.67	-
5 x 10^{-9}	81.17 ± 2.57	-	88.83 ± 5.44	-
10^{-6}	88.00 ± 2.27	0	95.83 ± 5.16	0
10^{-5}	97.00 ± 1.93	23.50 ± 13.59	103.00 ± 5.30	15.33 ± 15.33
5 x 10^{-5}	-	68.00 ± 8.60	-	53.33 ± 15.98
10^{-4}	100.00 ± 2.31	84.25 ± 5.92	105.67 ± 4.52	72.67 ± 9.96
10^{-3}	-	97.00 ± 5.28	-	90.00 ± 14.42
10^{-2}	-	100.00 ± 5.55	-	98.67 ± 9.94
10^{-1}	-	100.00 ± 11.00	-	95.50 ± 10.50

mean ± S.E.

All values are expressed as a percent of control maximum active tensions.

the course of every experiment contained in this thesis, such a study was carried out. Both sensitized and control TSM strips were challenged with ovalbumin (OA) by adding OA to the muscle bath to a final concentration of 0.3 mg/ml of Krebs-Henseleit. In every experiment, only the sensitized TSM developed active tension in response to contact with OA; control tissues were always quiescent (figure 11). The in vitro sensitivity of the test TSM to OA was observed both in animals challenged in vivo prior to killing and those not challenged. The in vitro response to OA could only be elicited once, the sensitized TSM becoming desensitized to further challenges.

The specificity of the antigen-antibody reaction was demonstrated with the inability to produce any in vitro responses to bovine albumin. Also, the in vivo response of the same sensitized preparation (Kepron et al., 1977) is specific to OA and results in increased airway resistance.

Other workers have shown the release of histamine and other mediators from mast cells (or other sensitized cells), to be due to an IgE-antigen reaction on the cell surface. These mediators affect muscle tone (see introduction). Data obtained in this study suggests that histamine is the major anaphylactic mediator responsible for bronchospasm, at least in our canine model of allergic asthma. First of all, preincubation of sensitized TSM with 7×10^{-6}M pyrilamine maleate - a specific H_1 antagonist - prevents the response to OA challenge in vitro (figure 12). The presence of the complete H_1 block could be demonstrated by

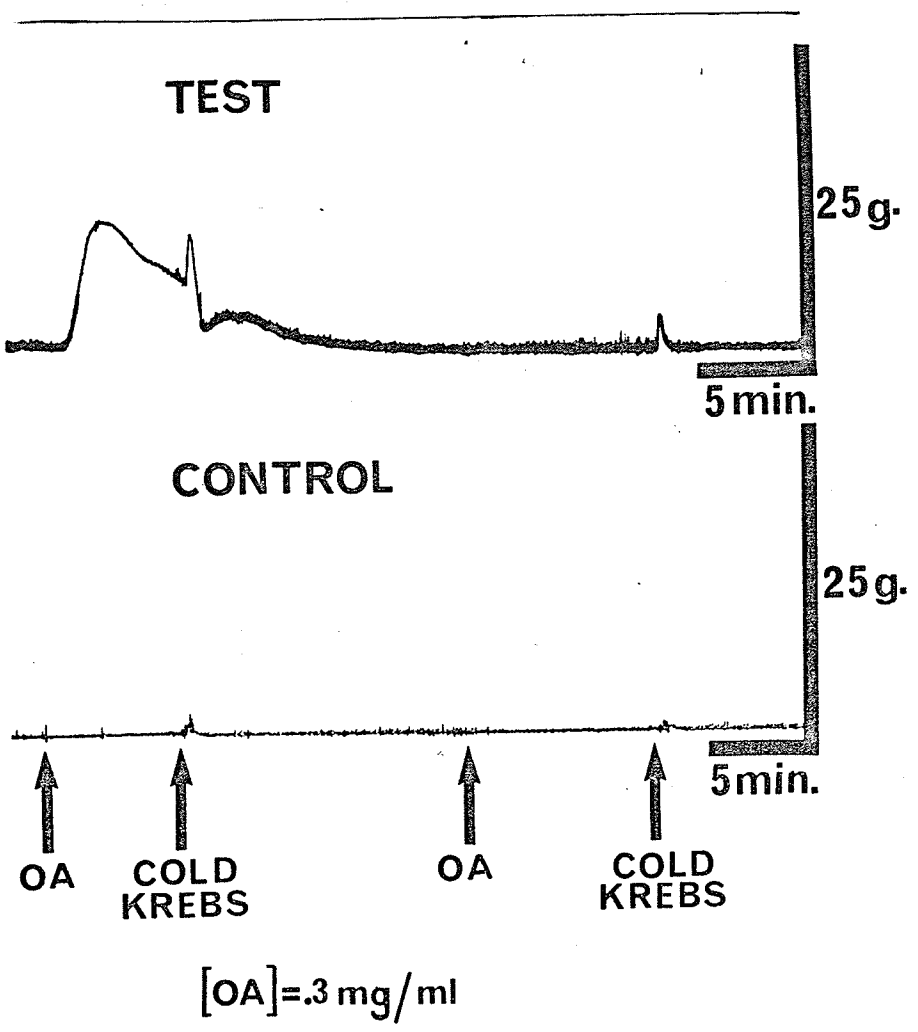

Fig. 11 Active tension development by the sensitized TSM in response to OA challenge. The control tissue displayed no response. Note that a second response to OA in the sensitized TSM could not be elicited. Sensitized TSM displays a cold response - tension development when the muscle bath was flushed with room temperature ($22\ ^{o}C$) Krebs-Henseleit; the control does not.

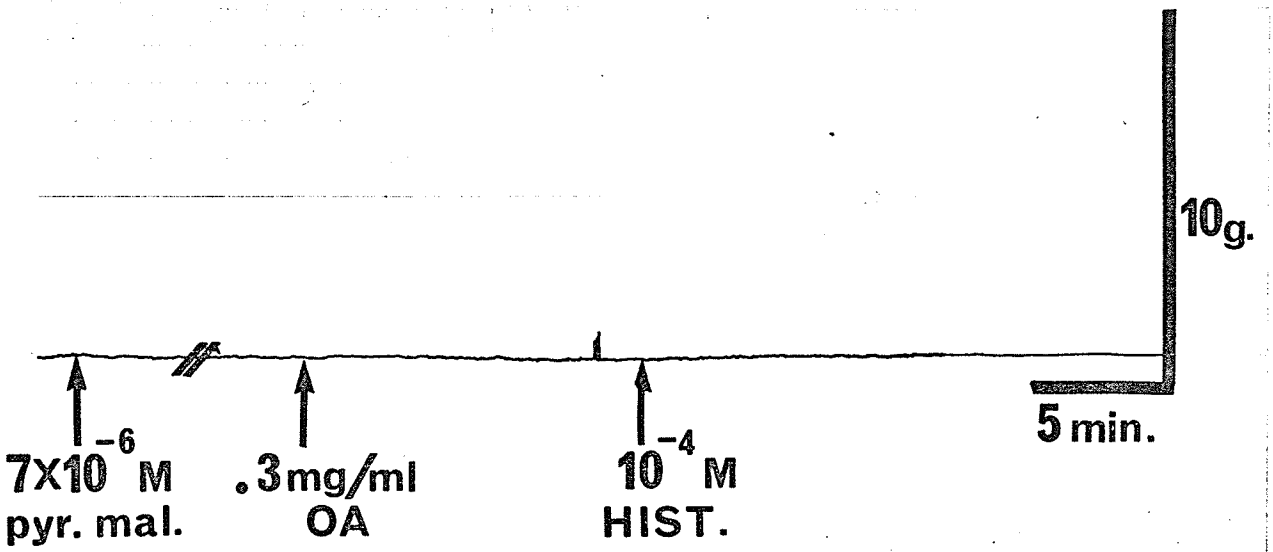

Fig. 12 Preincubation of sensitized TSM with pyrilamine maleate (mepyramine) eliminated the allergic response to OA (c.f. fig. 11) The complete H_1 receptor block was demonstrated by the lack of response of the TSM to a dose of 10^{-4}M histamine.

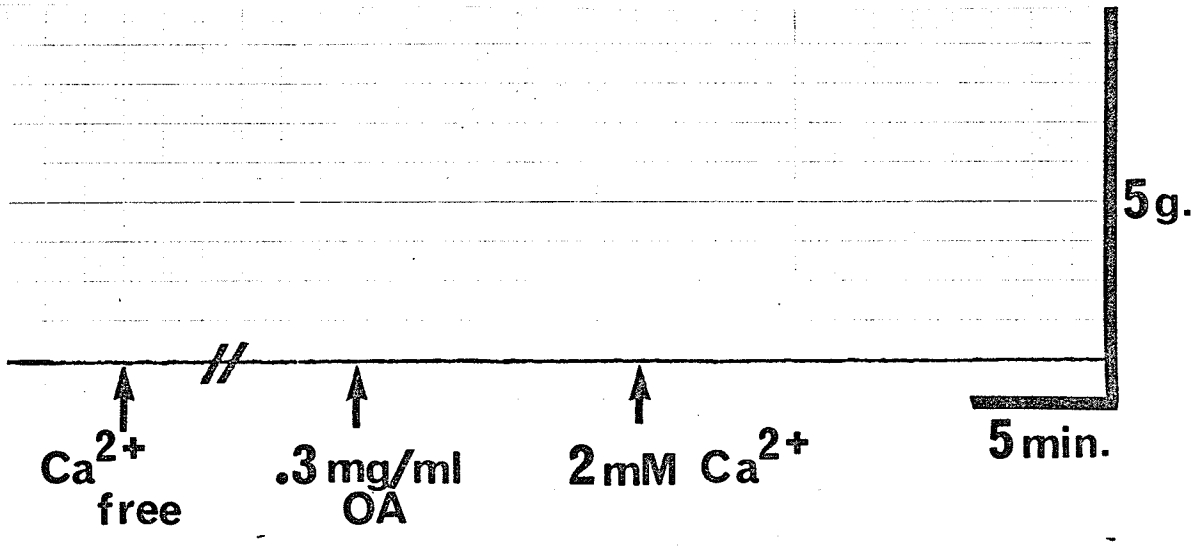

Fig. 13 Sensitized TSM incubated in zero calcium Krebs-Henseleit failed to display tension development to OA challenge (c.f. fig. 11). Incubation times were not sufficiently long to deplete intracellular calcium stores.

the TSM's lack of response to a supramaximal dose of histamine (10^{-4}M). Secondly, histamine is implicated in bronchospasm since the response of the sensitized TSM to OA challenge could be reduced by adding 7×10^{-6}M pyrilamine maleate to the muscle bath (figure 14). Histamine's role is further substantiated by the fact that secondary rises in resting tone - which would suggest another anaphylactic mediator being release, for example, SRS-A, - were not observed after the initial H_1 sensitive response; incubation up to six hours with the OA in the muscle bath produced negative results as well. Lastly, the OA response in sensitized TSM could be abolished in a modified zero calcium Krebs-Henseleit medium (figure 13) - histamine release is known to involve calcium ion dependent steps. Incubation times were not sufficiently long to adversely affect contractile response, due to depletion of intracellular calcium stores. Other muscles incubated for a similar time (figure 16) still display the fast phase to histamine stimuli.

Challenge of sensitized TSM with OA failed to result in active tension development where the tissue had been previously exposed to histamine. This may be related to the OA desensitization documented earlier. Histamine is known to inhibit its own release from mast cells (Gold, 1976); if histamine is the primary mediator released by the OA-IgE interaction, then perhaps this is the mechanism at work.

In all sensitized muscles, an accentuated cold response (active tension development) was observed with the addition of room temperature (22°C) Krebs-Henseleit to the muscle bath (fig. 11).

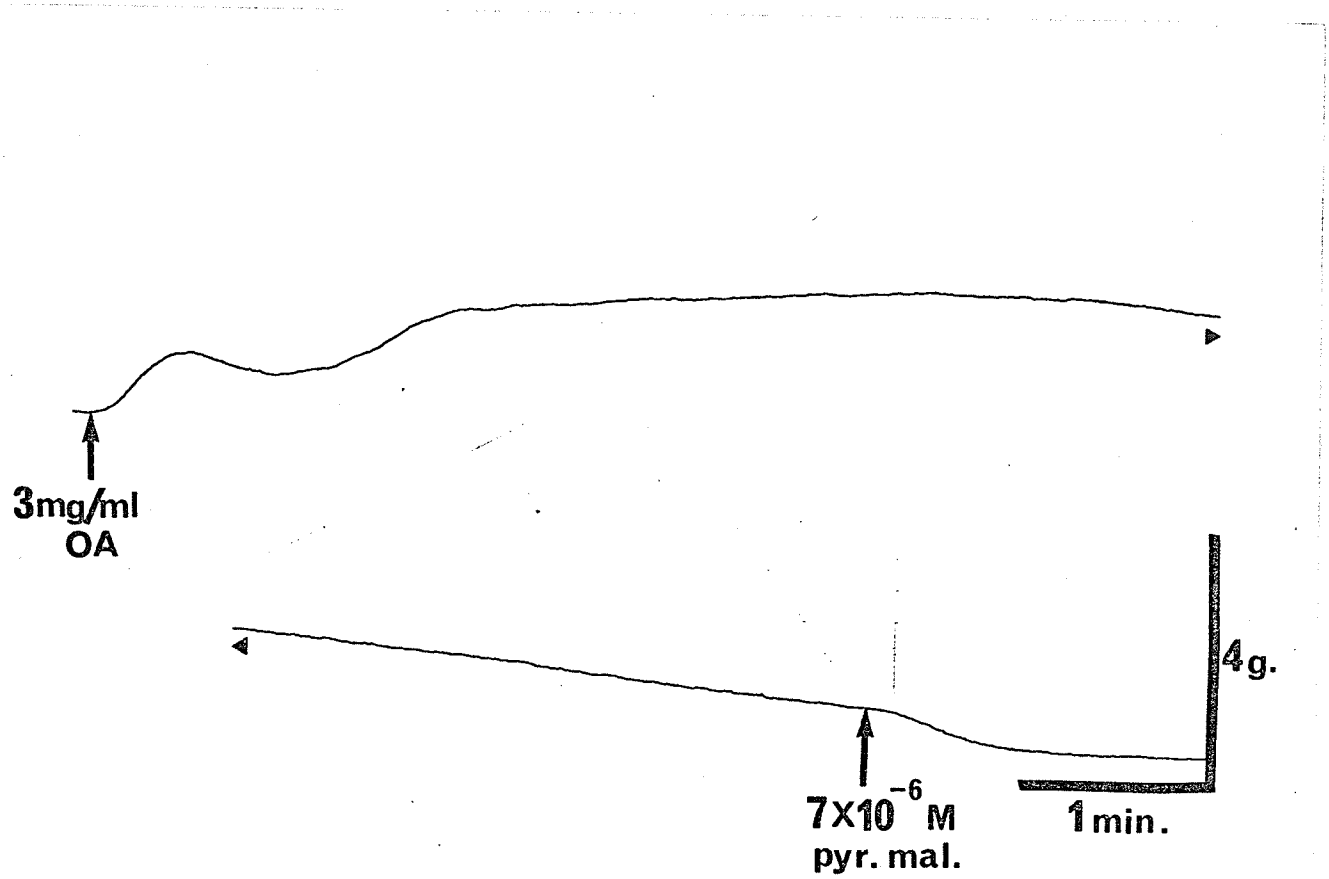

Fig. 14 Pyrilamine maleate added to the muscle bath in the midst of the sensitized TSM's response to OA challenge. The reduction of active tension can be attributed to a histamine H_1 antagonist effect.

(ii) Histamine dose-response studies

With histamine implicated as the primary mediator involved in allergic bronchospasm, dose-response studies were carried out in an attempt to define demonstrable differences between sensitized and control TSM's reactivity to the chemical. The sensitized tracheal smooth muscles exhibited statistically significant hyperexcitability (shift to the left) and hyperreactivity (higher maximal active tension) to a histamine stimulus when compared to normalized control values (fig. 15). Histamine doses administered ranged from 10^{-6}M to 10^{-2}M; the standard errors from the means of the active tensions developed for each dose are shown. Concentrated stock solutions (table 3) of histamine were used so that the pH and the mEq concentrations of ions in the bathing medium were not significantly affected.

Both the sensitized and control TSM developed maximal active tensions to a histamine dose of 5×10^{-4}M; however at this dose the sensitized muscle was hyperreactive, developing a tension of .943 kg/cm^2 compared to the control value of .668 kg/cm^2. The ED_{50} (the dose which gives 50% of the maximal response) of the sensitized TSM was 10^{-5}M; the control 4×10^{-5}M.

A series of histamine dose-response studies were also performed on TSM from control adults. The results are summarized in table 7 and are compared with the data cited above. Statistically significant differences (t-test $p < .05$) were not found between the mean responses of TSM from control adults and control pups to the same dose of histamine. Data are not available for TSM from sensitized adults.

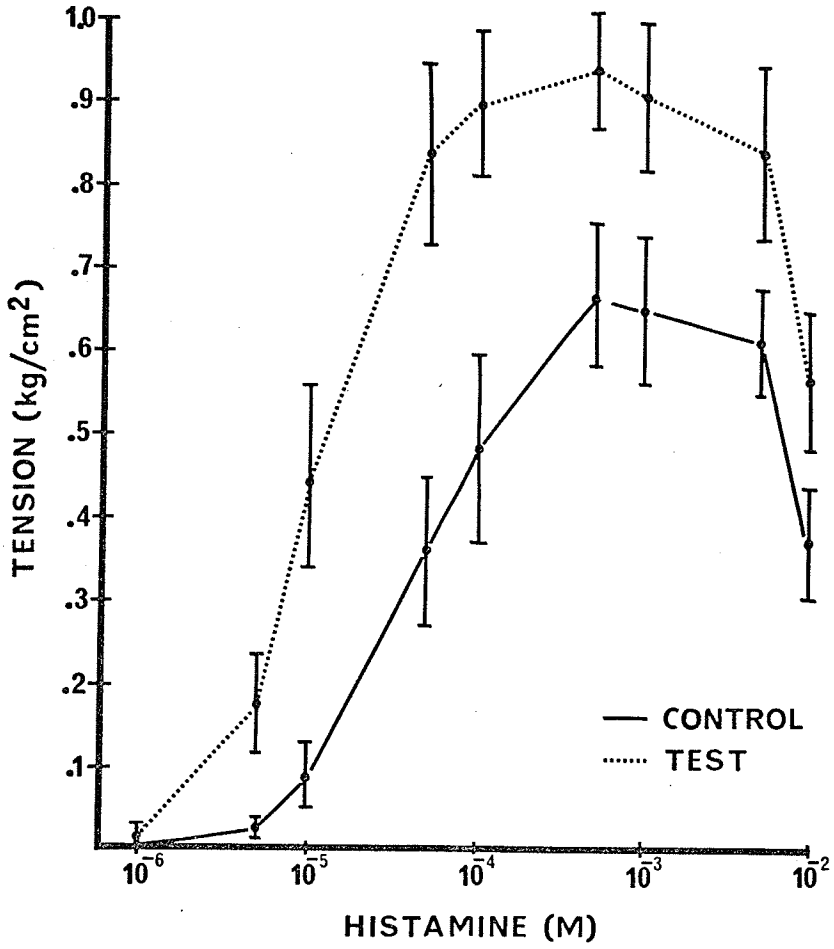

Fig. 15 Histamine dose-response curves elicited for both sensitized and control TSM (N = 7). The test curve showed both a hyperexcitability (shift to the left) and hyperreactivity (higher maximal tension) when compared to control. Standard error bars are shown; a Duncan's multiple range test ($p < .05$) between the mean active tensions developed by the sensitized and control TSM for each dose, established the differences to be statistically significant.

TABLE 7: Histamine dose-responses for canine TSM

dose histamine (M)	Tension (kg/cm^2)* sensitized pup	control pup	control adult
No. of animals	7	7	6
1×10^{-6}	.017 ± .012 (20)	.001 ± .001 (19)	.007 ± .001 (38)
5×10^{-6}	.175 ± .060 (20)	.025 ± .013 (18)	.028 ± .004 (35)
1×10^{-5}	.441 ± .118 (20)	.089 ± .041 (18)	.069 ± .015 (35)
5×10^{-5}	.838 ± .120 (20)	.360 ± .091 (18)	.232 ± .031 (34)
1×10^{-4}	.898 ± .091 (20)	.485 ± .114 (18)	.484 ± .049 (35)
5×10^{-4}	.943 ± .076 (18)	.668 ± .095 (18)	.593 ± .043 (35)
1×10^{-3}	.910 ± .091 (20)	.653 ± .090 (18)	.541 ± .039 (35)
5×10^{-3}	.844 ± .095 (15)	.615 ± .074 (12)	.520 ± .038 (21)
1×10^{-2}	.566 ± .089 (13)	.371 ± .068 (10)	.345 ± .036 (20)

* Histamine tensions ± S.E. developed by the TSM. t-tests ($p < 0.05$) performed on the means showed the active tensions developed by the sensitized TSM displayed statistically significant hyperexcitability and hyperreactivity to doses of histamine when compared to control data. Significant statistical differences could not be established between the responses of the control adults and control pups.

( ) indicate the total number of TSM strips tested.

Histamine contractions consist of a slow and a fast phase, the slow component dependent upon the extracellular calcium (fig. 16). Since the higher maximal response observed in sensitized TSM to a histamine stimulus may be related to mobilization of different calcium pools, future experiments might be profitably directed to analysis of the relative contribution of the slow and fast phases of a histamine contraction to the active tensions developed by the sensitized and control TSM.

Various types of stimuli are known to act on different pools of calcium and to varying degrees in eliciting a contractile response. In figure 17, histamine is shown to have the capability to further contract a muscle in the presence of a high K^+ solution, carbachol can increase this further yet.

(iii) Effect of histamine agonists and antagonists

To further elucidate the mechanism of histamine action upon the tracheal smooth muscle in our model of allergic asthma, experiments involving the use of histamine receptor agonists and antagonists were conducted. Previous work investigating histamine receptors (Eyre 1973; Eyre and Wells, 1973) in the pulmonary system of various animals has indicated the existence of mepyramine sensitive H_1 receptors capable of mediating bronchoconstriction and H_2 receptors capable of bronchorelaxation.

Mepyramine sensitive H_1 receptors in both sensitized (fig. 12) and control TSM were demonstrated in the dog; a contractile response to a maximal dose of histamine is prevented when the TSM is preincubated with pyrilamine maleate. Two histamine H_1 agonists

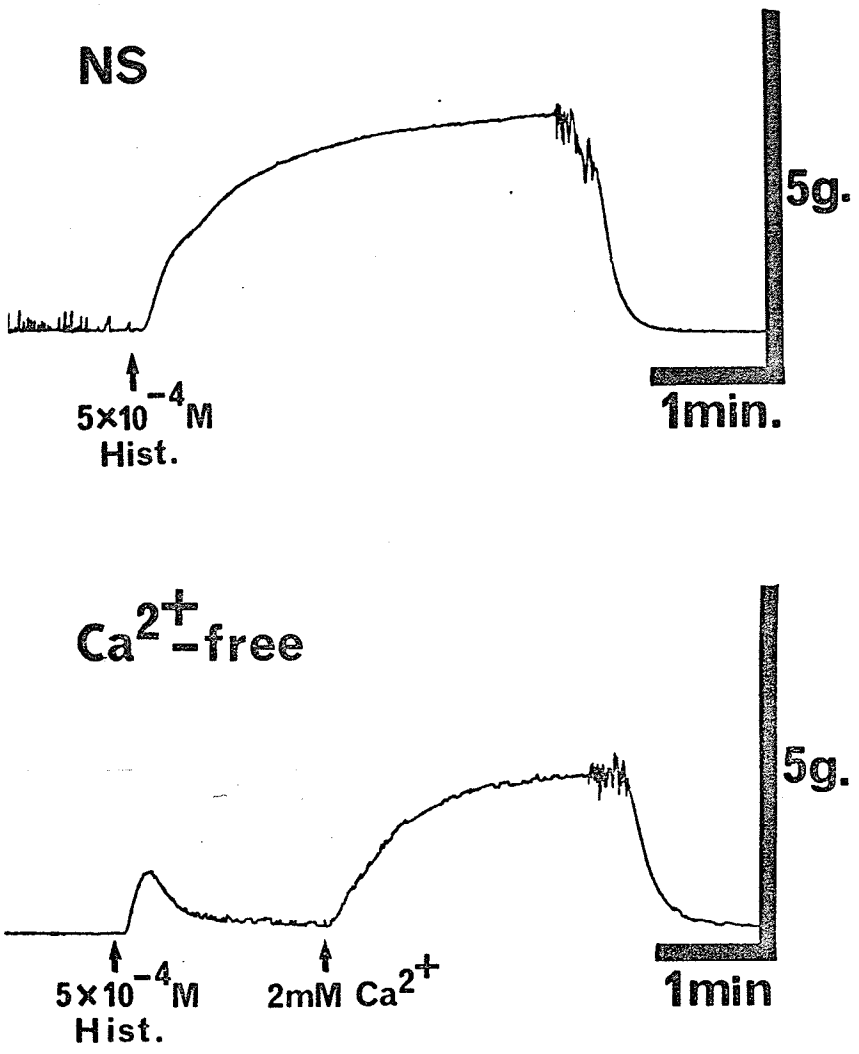

Fig. 16 Histamine contractions in TSM consisted of a fast and slow phase (upper panel). The two components could be separated (lower panel) by preincubating in a calcium free KH solution. Histamine only elicited a fast contractile phase, the return to normal calcium concentrations allowed the slower phase to reappear.

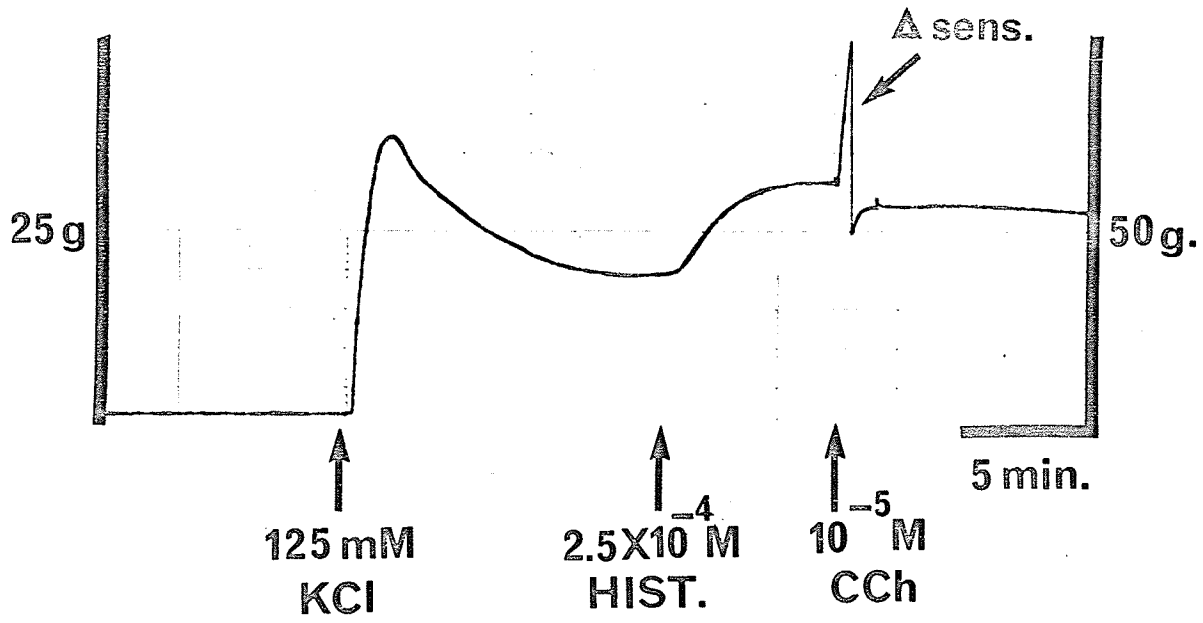

Fig. 17 Histamine was shown to have the capability to further contract a muscle in the presence of a high K^+ solution, carbachol can increase this further yet.

- 2 methylhistamine and 2-(2-pyridyl)-ethylamine dihydrochloride - are capable of contracting TSM in a dose dependent fashion, these responses are also sensitive to the presence of mepyramine. Although mediating strictly an H_1 response, the active tensions developed are far below that elicited by histamine, even with the use of maximal doses. These H_1 agonists have been reported to have a lower intrinsic activity than histamine, a fact with probably explains the decreased responses observed. For the same reason then, proper dose response curves for the two H_1 agonists were not attempted.

Documentation of H_2 receptors in other tissues plus the fact that a histamine contraction relaxes shortly after reaching a plateau suggests an H_2 receptor is present in canine TSM as well. If the sensitized TSM possesses fewer H_2 receptors, this might provide a mechanism for the prolonged muscle tone observed in bronchospasm. This argument proved false in light of data obtained. Preincubation of the TSM with metiamide - a specific H_2 antagonist - in the muscle bath, failed to prevent the TSM relaxation observed after the active tension plateau to a histamine stimulus, establishing that H_2 receptors are absent. As well, 4 methylhistamine - an H_2 agonist - was used in an attempt to relax TSM contractions to various stimuli. However, conflicting results were obtained. 4-methyl histamine reduced the active tension produced by a histamine stimulus, the amount of relaxation is more, as the concentration of 4-MH is increased (fig. 18). However, the muscle never relaxed to the prestimulation resting tension. Addition of the same dose of 4MH to a 25 mM KCl contraction

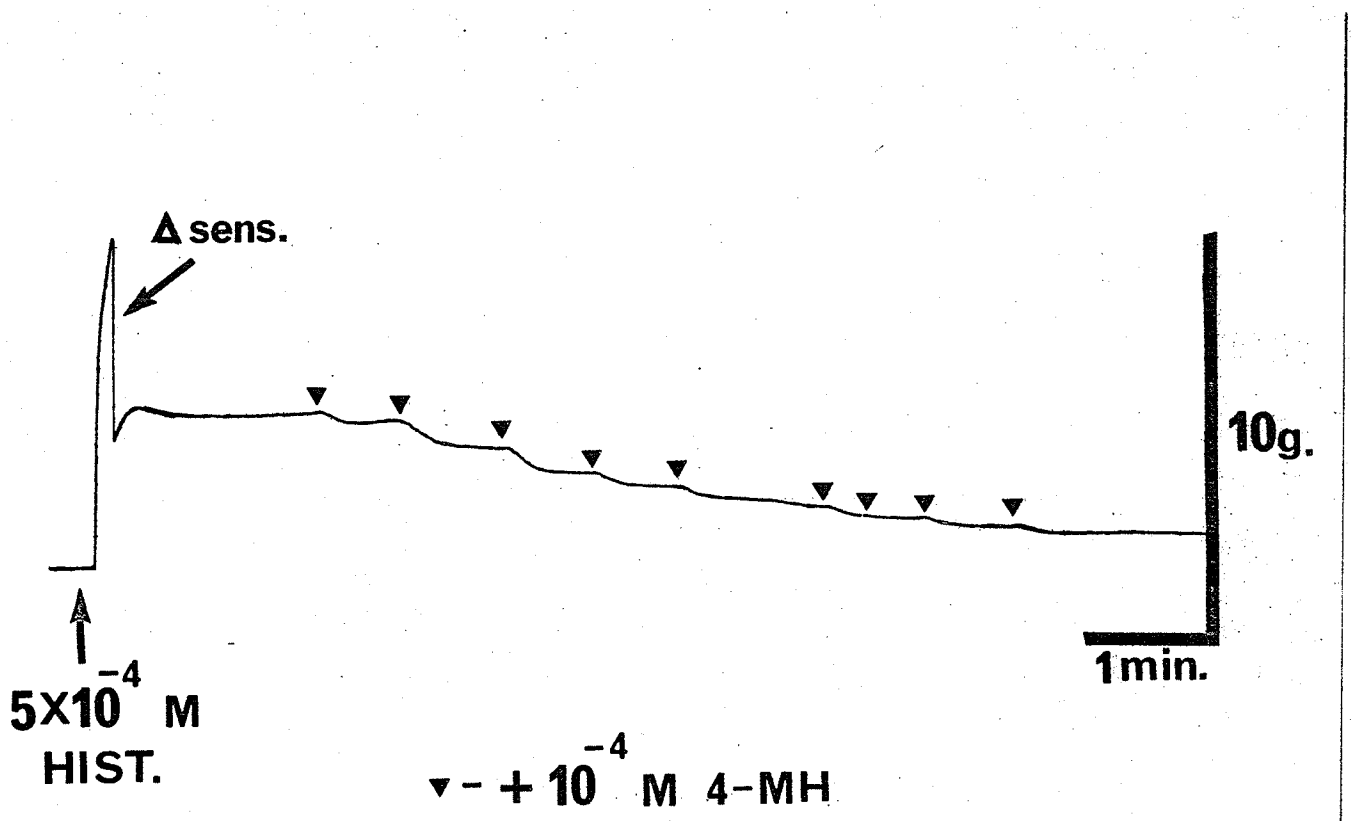

Fig. 18 4-methylhistamine, considered to be an H_2-receptor agonist, reduced the active tension produced by control TSM to a maximal dose of histamine. The amount of relaxation was more as the concentration of 4 MH was increased by the addition of successive 10^{-4}M doses to the tissue bath. The muscle did not relax to the prestimulation resting tension.

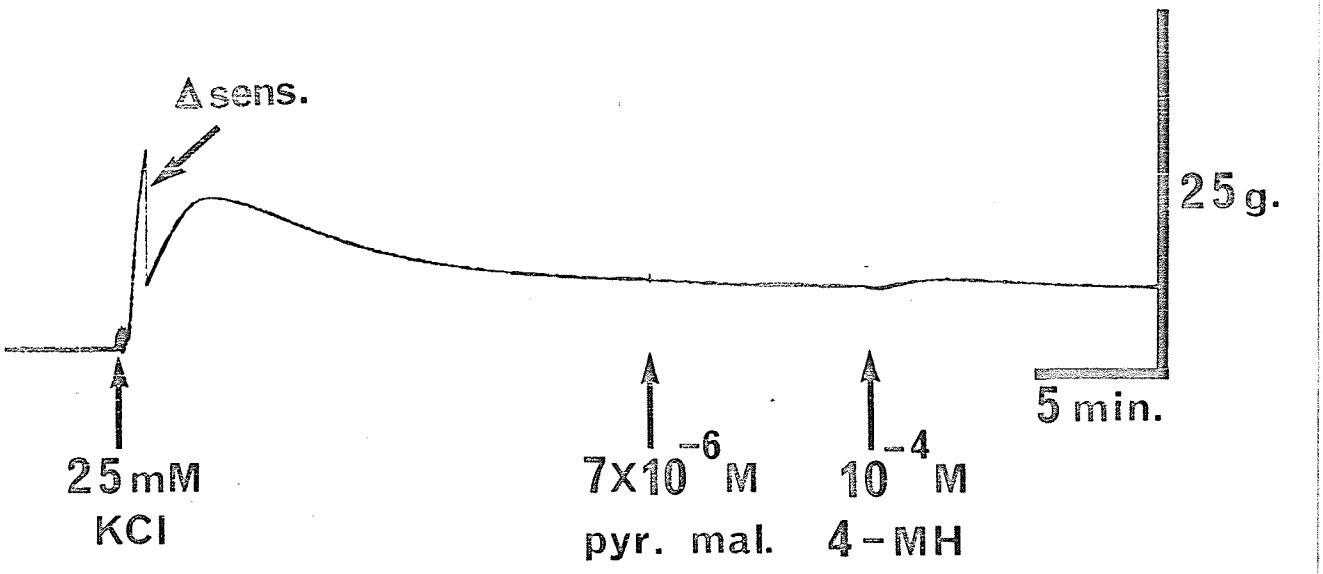

Fig. 19 Addition of 4 MH (10^{-4}M) to a 25 mM K^+ contraction in TSM caused a slight potentiation of tension developed. The inability of pyrilamine maleate to reduce the 25 mM K^+ contraction eliminated the possibility that the 25 mM K^+ had depolarized the mast cells to release histamine which might be adding to the contraction.

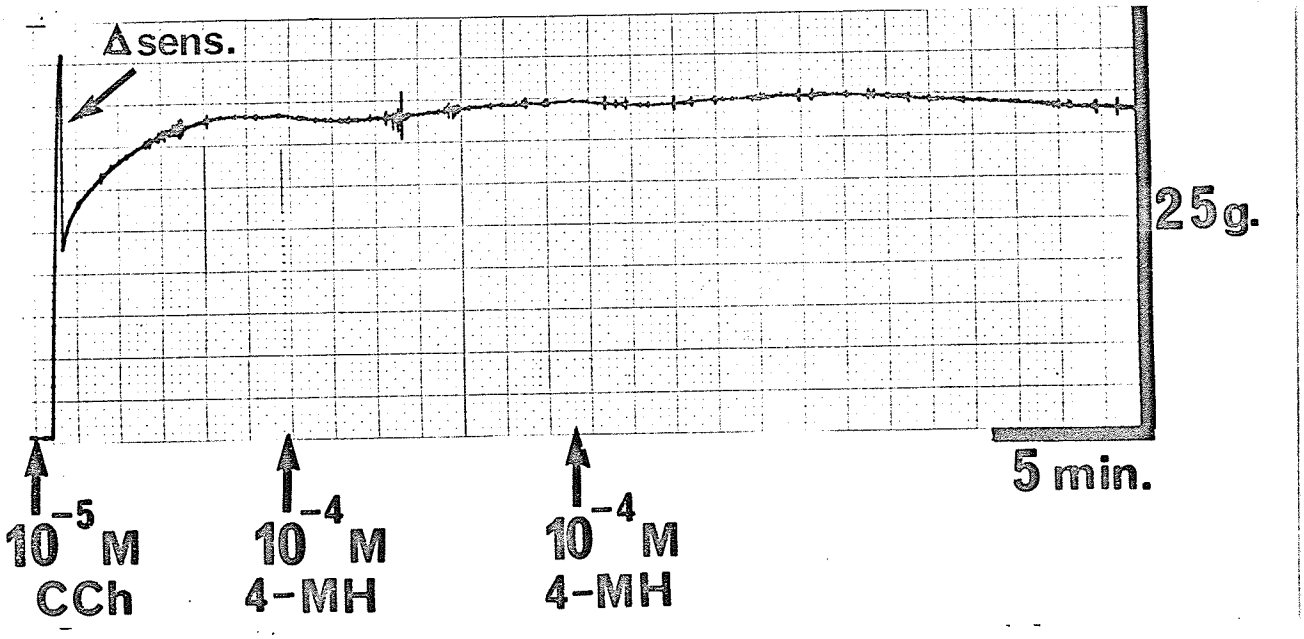

Fig. 20 The contractile response of canine TSM to a 10^{-5}M carbachol stimulus. As the active tension begins to fall slowly after reaching a plateau, a dose of 10^{-4}M 4-methyl histamine was added, resulting in a slight rise in active tension. The addition of a second dose of 10^{-4}M 4MH caused a further slight rise.

(fig. 19) and a 10^{-5}M carbachol contraction (fig. 20) resulted in a slight potentiation of the active tensions.

A maximal dose of pyrilamine maleate (fig. 17) failed to relax the TSM contraction to 25 mM KCl. This eliminates the possibility that mast cells might be depolarized by the potassium to release histamine (and thus add to the K^+ contracture). This result was expected in view of the reported inability of vagal stimulation to cause mast cell degranulation (Gold, 1977) by depolarizing the mast cell membrane in a manner similar to the potassium.

DISCUSSION

A. ISOTONIC STUDIES: their value in the investigation of "asthmatic" airway smooth muscle contractility.

In order to investigate the contractile properties of a muscle more fully, both the tension developed and the velocity of shortening should be considered. The study of isotonic contraction of muscle in terms of the force-velocity relation - the relation between the velocity of isotonic shortening (V) and the force developed (P) when lifting a load - is considered superior to that offered by any other method (Hill, 1938; Csapo, 1962), provided that the so-called active state of the muscle is time-independent. This is easily achieved in those muscles where tetanization is possible, for example, tracheal smooth muscle. The force-velocity relation also provides an index of power production and rate of energy utilization by the working muscle.

The pathological bronchospasm occurring in asthma suggests that if any altered airway smooth muscle function is present, it must manifest itself in increased magnitude of airway closure, with or without increased speed of shortening. Various possibilities for changes exist; it has been established (Sonnenblick, 1965) that the tension development of the muscle and its velocity of shortening may be altered independently of one another. Analysis of shortening velocities and capacities (maximum magnitude of shortening) as well as the maximum active tensions developed by the sensitized TSM represent the best estimation of investigative procedures, since they may reveal whether the primary defect in asthma lies in the airway smooth muscle.

The canine trachealis muscle (TSM) appears to satisfy the criteria needed to undertake a valid force-velocity analysis (Stephens et al., 1969; Stephens, 1976), even though ultrastructural evidence is unable to demonstrate the presence of sarcomere units. Their presence is regarded often by investigators of skeletal muscle as a sine qua non for the comprehensive study of the tissue and understanding of the contractile mechanism at the molecular level. Even so, the similarity in behavior of the various parameters of muscle contraction in the trachealis to those in skeletal and cardiac muscles suggests that striated muscle models may also be applied to the TSM (Stephens et al., 1977).

Results obtained from force-velocity experiments carried out on sensitized and control TSM show the "asthmatic" muscle to have altered contractile properties. Use of the classical Hill (1938) analysis of the force-velocity curves revealed, first of all, that the $\underline{b}$ constant of the sensitized TSM increased significantly from the control value (see Table 4). The control value of $\underline{b}$ compares favourably with data obtained previously for canine trachealis (Stephens et al., 1969). Secondly, a statistically significant increase in the maximum shortening velocity - V_{max} - of sensitized TSM was found. While the velocities of shortening of the sensitized TSM at a given load were higher than those of control, the P_o's of the two preparations were similar. Figure 5 offers a graphical illustration of the findings. The linearized transforms of the two curves revealed a reduced slope ($1/\underline{b}$) for the sensitized TSM; hence the calculated $\underline{b}$ constant and V_{max} were greater than those of control.

The b constant has been equated with the rate at which energy liberating reactions for contractile purposes occur (ATP hydrolysis), which thus determines the rate of the making and unmaking of actomyosin cross-bridges, and the maximum velocity of shortening. Evidence documented by Bárány (1967) establishes that the intrinsic speed of muscle contraction is a characteristic property of the myosin ATPase activity of muscle. While the intrinsic speed of shortening represents the theoretical maximum velocity of shortening at zero load, this speed is related to the velocity of shortening under different loads as well. Wilkie (1954) has demonstrated the force-velocity relation to have the same general form in a number of muscles. The ATPase activity of myosin can be correlated with the speed of shortening, both with and without a load (Bárány, 1967). The work performed when a muscle shortens under a load can be related directly to the free energy of ATP hydrolysis catalyzed by the myosin ATPase.

The findings that the sensitized TSM possesses a higher V_{max} and b constant (48% increase over control) indicates that the intrinsic ATPase activity of the myosin may be increased. Whether the increases in b and V_{max} represent an ATPase with higher enzymatic activity or a greater concentration of the ATPase will have to be determined. Since the ATPase activity resides, in all likelihood, in one of the myosin light chains, study of the altered ATPase in the sensitized muscle is critical. Bose and Stephens (1977) have demonstrated the feasibility of such an approach in beef TSM. Recent evidence suggests (Hartshorne - personal

communication) that phosphorylation and dephosphorylation of the light chain will also have to be considered since these processes are probably related to activation/deactivation of the ATPase.

Various intracellular conditions are known to affect enzymatic rates. Both temperature (Stephens et al., 1977) and pH (Bose and Stephens, 1977) have been demonstrated to alter myosin ATPase activity; it may be that the increased ATPase activity of the sensitized TSM is related to these conditions. Close (1965), when discussing the proportionality of shortening speed and myosin ATPase activity, raised the question of whether the speed is related to the amount of calcium made available during contraction. Sreter and Gergely (1964) had suggested that the speed of calcium uptake, or differences in the distribution of calcium due to the presence of varying amounts of sarcoplasmic reticulum, determines the shortening speed of the muscle. According to Weber and Herz (1963) though, only 1-2 moles of calcium are required per mole of myosin to maximally activate the actomyosin ATPase. This represents approximately 10-20% of the amount of calcium stored in the vesicles of muscle in general, which is available for contractile purposes. Bárány (1967) concluded that the variation in the speed of contraction between different muscles must therefore be determined by the intrinsic rate of their actomyosin ATPases, since the calcium concentration required for maximal ATPase activity is low enough to be saturated during every muscle contraction, in spite of the fact that the total amount of calcium may differ between muscles types.

Experimental results obtained pursuing this question are difficult to interpret. Sonnenblick (1962b) and Grassi et al., (1977)

were able to alter the V_{max} of cardiac muscle by adding calcium to the perfusion medium. Brutsaert et al. (1973) obtained similar results with a papillary muscle preparation. However, the results of Costantin et al. (1967), and Podolsky and Nolan (1971) demonstrated that the maximum shortening velocity of the skinned frog muscle fiber is unaffected by calcium concentrations. Since the degree of activation of the contractile proteins of contracting smooth muscle most closely resembles that of skeletal, the increased V_{max} of the sensitized TSM most probably stems from an increase in actomyosin ATPase activity. As well, the results observed in cardiac muscle are being attributed to increased activator calcium (Brutsaert, 1974), a system characteristic to of cardiac muscle.

Although the magnitude of shortening (Δl) and faster shortening velocity of the sensitized TSM is significantly different statistically, from that of the control, the definition of its exact physiological role is speculation. It remains one of the slowest contracting muscles, much slower than the frog sartorius (Hill, 1938) and the cat heart papillary (Sonnenblick, 1962a). Assuming that TSM is a satisfactory model for airway smooth muscle at the flow limiting segment, then the increased Δl may play a role in the magnitude of airway closure during an asthmatic attack, and thereby provide a possible mechanism for the increased airway resistance observed during bronchospasm. What the role of the increased V_{max} is, is difficult to say.

While ATPase activity is correlated with the speed of shortening, the actin-binding ability is reflected in the maximal tension produced by the muscle - tension generation

controlled by actomyosin bridge formation. Differences in maximal tension development between muscles presumably reflects variations in the number of actomyosin cross-bridges formed per cross-sectional area of muscle. The value of P_o (maximal active tension) is an index of the strength of a muscle; significant statistical differences between the strengths of the sensitized ($1.161 \pm .056$ kg/cm^2) and the control TSM ($1.211 \pm .071$ kg/cm^2) were not found (see Table 4). This is further substantiated by the finding that the $\underline{a}$ constant which reflects the number of force generating sites per cross-sectional area and thus determines P_o, was not found significantly different. As a result, the values of the ratio $\underline{a}/P_o$ for the sensitized and control TSM are similar, as well. The $\underline{a}/P_o$ value is considered to be a universal muscle constant. The ratio for canine TSM of 0.21 to 0.23 compares well with that for cat papillary muscle, 0.22 (Buccino et al., 1967) and frog sartorius 0.25 (Hill, 1938). However $\underline{a}$ has been found to be not completely independent of load as originally postulated. Hill (1964) reinvestigated his original results and concluded that the heat of shortening increases with increasing loads. While it may invalidate the derivation of the Hill equation from thermal measurements, the Hill analysis remains applicable as an empirical description of the force-velocity relation.

Why an alteration in the contractility of the airway smooth muscle is reflected in an increased velocity of shortening and not an increase in strength, may be related to the fact that the in vivo situation of the trachealis is probably more isotonic than isometric (its primary role is to regulate the calibre of the

airways, reduce dead space and improve alveolar ventilation). Since the causal factor in the pathogenesis of asthma is likely to be predominantly a narrowing of the airways, the shortening capacity of the airway smooth muscle becomes the important parameter. Indeed, the shortening capacity of the sensitized TSM is significantly higher than that of control (fig. 6); at a load of 15% P_o, the sensitized muscle strip shortens 6-14% more than the control trachealis. While the amount of tension necessary to overcome the elasticity of the cartilage rings may be important to the in vivo function of TSM, the important narrowing in asthma is in the small, 2-3mm, flow-limiting airways where cartilage rings do not exist. With TSM shown to be a good model for airway smooth muscle in general (see introduction), the increased shortening capacities may account for the increased narrowing of the airways in asthma. TSM is known to exert considerable active tension even at a length of 10% l_o (Stephens et al., 1969), enough perhaps to overcome frictional forces engendered by cartilage plaques in the smaller airways as they imbricate during airway narrowing. The extent to which this increased capacity to shorten plays a role in bronchospasm will have to be determined; the asthmatic individual may differ from normal only in the airway smooth muscle's shortening response to a given stimulus. This might manifest itself as the increased airflow resistance observed during an asthmatic reaction.

B. ISOMETRIC STUDIES

The isotonic experiments just discussed have shown that the sensitized TSM possesses an increased velocity of shortening and an increased capacity to shorten; the maximum active tension, however, does not change when compared to that of control muscles. The first two parameters represent intrinsic contractile properties of the muscle, which would be the same under maximal stimulation by any agonist - the ATPase activity saturates at a characteristic point, and the structural arrangement of the contractile elements limits the maximal amount of shortening. However, it is known that P_o is dependent upon calcium concentrations. Since most drugs, mediators, and chemical transmitters operate at the muscle membrane or intracellular membrane level, calcium supply, and hence P_o, can be altered. Presumably then, the maximal contractile response of a certain muscle type to stimulation by any agonist would have a characteristic V_{max}, while the P_o's produced by different agonists will differ because of the variation in the amount of calcium mobilized. Therefore, the force-velocity curves of the muscle to the various agonists would have different slopes, yet the Hill (1938) analysis will show all constants the same except P_o and $\underline{a}$; the curves will all shift about the same V_{max}. Investigation of a muscle's isometric contractile parameters, such as P_o, during stimulation by neurotransmitters and mediators of anaphylaxis may elucidate mechanical alterations in the sensitized TSM which might result from changes in nerve or muscle membrane excitability, excitation-contraction coupling, calcium sequestration, or alterations in receptors and their

properties - in addition to those already delineated in the isotonic experiments.

(i) Electrical stimulus response experiments

An alteration in the magnitude of the maximum active tensions developed by airway smooth muscle, in response to electrical field stimulation, might originate from a number of sources. Changes could occur in (1) the number of cholinergic fibers present (2) the relative numbers of cholinergic receptors present (3) the relative excitability of the cholinergic fibers (4) the intrinsic contractile properties of the muscle, or (5) the role of the relaxant nervous sytems. Muscle contraction in response to electrical field stimulation is probably mediated via nerves only; there is no direct muscle depolarization since the administration of atropine effectively blocks (95% complete) the contractile responses (Stephens and Kroeger, 1970).

Electrical stimulus-response experiments failed to demonstrate any statistically significant hyperexcitability or hyperreactivity (P_o) of the sensitized TSM when compared to normalized control values (Fig. 7). This result was expected in view of the force-velocity data - an electrical stimulus was also used - which showed a similar P_o in sensitized and control TSM. Investigation of parameters other than P_o, such as the times to reach peak tension (tP_o) and relaxation times - functions of the excitation-contraction coupling mechanism, calcium release and calcium sequestration - failed to establish differences between the sensitized and control TSM. The maximum rate of tension development $(dP/dt)_{max}$, considered an indicator of the velocity of shortening (Buccino et al., 1967)

and hence of actomyosin ATPase activity, was not different - surprising when considering the results of the isotonic experiments. While a 48% increase in isotonic shortening velocity was observed in sensitized TSM, the measurement of dP/dt_{max} may be too insensitive an analysis to detect such changes in the shortening velocity of the contractile element. As well, the correlation between dP/dt's and shortening velocity requires the assumption that the physical properties of the series elastic component are unchanged; perhaps a more compliant SEC in the sensitized TSM prevents the dP/dt of the muscle from being higher than that of the control. Therefore, while the rate of isometric active tensions development (dP/dt) provides an approximation of the rate of crossbridge cycling and hence an indication of the rate of ATP hydrolysis, the magnitude of both cycling and ATP hydrolysis are likely to be different in an isotonic contraction (due to work performed).

While these isometric data failed to delineate neural and mechanical alterations in sensitized TSM, the presence of certain phenomena (figs. 8,9) in isometric traces suggest the isometric methods employed are valuable in detecting altered mechanical properties. Upon electrical stimulation, the isometric tension developed by some sensitized muscles continued to rise slowly, in contrast to the normal control situation (lower panel fig. 8) where the isometric tetanic tension reached a plateau and then fell. The decrease in tension may be due to acetylcholine depletion, since the presence of the adrenergic blocker - propanolol - to eliminate the relaxant action of any beta-adrenergic fibers present - failed to alter the control trace. The purinergic

relaxant system is absent in dogs (Suzuki et al., 1976). The prolonged plateau observed in the sensitized muscle could be due to a change in acetylcholine release and/or degradation, the relative effectiveness of any relaxant fibers present, or calcium release and sequestration systems. To what magnitude the prolonged plateau continues under stimulus is unknown, in all cases the electrical stimulus was terminated to reduce possible damage to the tissues.

A prolonged plateau has also been observed in canine TSM which had been pretreated with a subthreshold dose of tetraethylammonium (TEA) - .9mM - before electrical stimulation, the phenomenon observed in sensitized TSM may be related. TEA is thought to unmask the depolarization activation of calcium and/or sodium channels by the reduction of a normally predominant resting potassium permeability (Kroeger and Stephens, 1975). Perhaps the sensitized TSM possesses calcium channels, not related directly to depolarization, which causes the slow intracellular accumulation of calcium, to result in the slowly climbing active tension. It is interesting to note as well (fig. 8) that the sensitized TSM returned to normal resting tension after the electrical stimulus was discontinued, suggesting - along with the negative data reported on relaxation times - the absence of any defect in calcium sequestration and the muscle relaxant system.

A second phenomenon, consisting of a spontaneously rising resting tone associated with phasic activity, is observed in a number of the sensitized muscles (fig. 8). Similar phasic activity has been reported in sensitized guinea-pig tracheae

(Souhrada and Dickey, 1976(b)). Although phasic activity is sometimes observed in canine TSM in vivo (Loofbourrow et al., 1957), most direct stimulatory agents yield tonic responses without evidence of phasic electrical or mechanical activity in vitro (Stephens and Kroeger, 1970; Stephens, et al., 1975). The use of substances, such as norepinephrine and high potassium solutions, which are reported to convert intermediate type arterial smooth muscle into single unit preparations (Keatinge, 1966), proved unsuccessful in eliciting phasic activity (Stephens et al., 1975). TEA, however, has been used successfully to induce spontaneous electrical and mechanical activity in airway smooth muscle (Kroeger and Stephens, 1975; Suzuki et al., 1976). More recently, Kannan and Daniel (1977) have used procaine dihydrochloride and 4-amino pyridine to produce similar results in a canine TSM preparation.

Spontaneous activity in the sensitized TSM could be reduced by flushing the muscle bath with fresh Krebs-Henseleit solution. Experiments in which the bathing medium was transferred from the baths containing spontaneously active TSM, to others in which the tissues were quiescent, caused the latter to develop tension. These results suggest the muscle may be producing a "factor", responsible for the development of the mechanical tension. An attempt to reproduce these results using Krebs-Henseleit solution in which electrodes had been immersed and charged, failed, establishing the effect was not due to pH or other ion changes consequent to maximal, sustained electrical stimulation.

A third phenomenon, illustrated in figure 9, was displayed by sensitized muscles. A myogenic response, an increase in active tension in response to the muscle being quickly stretched, was noted. Control tissues similarily stretched failed to react; Stephens and Kroeger (1972) report canine TSM to be normally quiescent and not manifest a myogenic response. While Burnstock (1960) and Sparks (1964) have elicited such a response from a number of smooth muscle preparations, these have all uniquely been single-unit types of smooth muscle characterized by spontaneous rhythmic contractile activity associated with action potentials. Treatment of canine TSM with TEA causes biophysical changes such that it resembles the single unit smooth muscle (Kroeger and Moorhouse, 1973; Stephens et al., 1975), perhaps functionally the sensitized TSM has been changed in a similar manner. The isometric trace displayed in fig. 9 may also be interpreted as the development of active tone induced by a muscle which possesses a "leaky" membrane, that is, a membrane which exhibits greater premeability to calcium than normal TSM. Presumably, the mechanical stretching would raise the surface-to-volume ratio such that calcium influx is increased. If the calcium pump cannot compensate, actomyosin crossbridges become activated as time progresses, and the tone of the TSM is increased. This possibility must be considered in view of the fact that the time to the onset of tension development is longer than that normally recorded for myogenic responses (Burnstock and Prosser, 1960(a)).

The phenomena of prolonged plateaus, spontaneous activity, and myogenic respones possessed by the sensitized TSM, suggest, in combination, that the airway smooth muscle in asthma may have an underlying etiology similar to that induced by the administration of drugs such as TEA, procaine dihydrochloride, and 4-amino pyridine. This would be a membrane phenomenon, since the contractile response to these drugs can be blocked effectively by D-600 (Kohlhardt et al., 1972; Kannan and Daniel, 1977), which specifically inhibits the calcium influx associated with membrane depolarization (Kohlhardt et al., 1972; Kroeger et al., 1975). In support, Stephens et al. (1975) established the myogenic response to be dependent upon extracellular calcium. The prospect of membrane alterations in sensitized TSM is interesting in light of the fact that multiunit smooth muscle usually contains a relatively large store of sequestered calcium which is resistant to depletion and which may be mobilized for contraction by certain drugs (Kroeger and Stephens, 1971; Devine et al., 1973). Perhaps sensitized TSM differs in its depolarization related calcium influx (Hinke, 1965) from that of control. This aspect may be increased while the internally sequestered calcium mobilized by stimulatory agonists is similar. It is of interest to note that studying P_o and dP/dt do not exhaust all the possibilities in studying isometric contraction. Detection of the qualitative changes described may be equally as helpful as the previous study of P_o and dP/dt's.

Several future investigations are suggested by this discussion. To date, ultrastructural and electrophysiological properties of

asthmatic airway smooth muscle have not been adequately examined. Electron microscopy has revealed that the asthmatic mucosa seems more permeable to inhaled antigens (Boucher et al., 1977), but these techniques have not been employed to investigate whether the number of nexuses or gap junctions between smooth muscle cells is increased significantly in cases where spontaneous phasic activity has been observed (Akasaka et al., 1975) in the human asthmatic muscle. Certainly, the number of gap junctions between smooth muscle cells has been demonstrated to increase significantly when phasic activity is induced by drugs such as TEA and 4-amino pyridine (Kannan and Daniel, 1977). If asthmatic muscle displays the same characteristics, an etiology for asthmatic muscle similar to the sites of action of these drugs could be proposed. If the presence of a factor can be established - with properties like the compounds mentioned - the changes observed in sensitized TSM, and asthmatic muscle in general, may be a result of its action.

Electrophysiological data of asthmatic airway smooth muscle is lacking. Kirkpatrick (1975) has shown histamine to produce a slow oscillation in membrane potential (E_m) of normal bovine TSM, which can be correlated with phasic mechanical activity. Suzuki et al. (1976) demonstrated histamine depolarizes canine TSM approximately 10mV, without evoking spikes. Electrophysiological examination of sensitized TSM - both resting and under stimulation by anaphylactic mediators - might reveal a slightly depolarized membrane with electrical properties similar to those found for canine TSM under the influence of TEA (Kroeger and

and Stephens, 1975; Kannan and Daniel, 1977). A slightly depolarized membrane, suppressed rectification of the membrane, and increased membrane resistance (Suzuki et al., 1976), if present, may explain the isometric phenomena, as well as the hypersensitivity to anaphylactic stimuli (discussed below) observed in "asthmatic" airway smooth muscle. Electromyographic data of bronchial smooth muscle in asthma (Akasaka et al., 1975) suggest the smaller airways may be similarily afflicted.

Why acetylcholine (electrical stimulus response) or carbachol did not elicit hypersensitivity in the asthmatic muscle is unknown, but it may be that the calcium channels affected are different for the various stimuli. Further investigation is necessary. The presence of a "factor" must be analyzed and its site of action established; it may account for much of the etiology of "asthma" established in this study. Certainly ultrastructural and electrophysiological alterations in asthma need not include the presupposition that a chemical compound is responsible; however, on the evidence presented, such speculation may be valid.

(ii) Carbamylcholine dose-response experiments

When establishing the protocol for this thesis, it was realized that electrical stimulus-response studies might demonstrate a change in sensitized TSM, however, such an alteration could originate from a nerve and/or a muscle defect. For this reason, concurrent experiments were conducted which essentially isolated the muscle component involved, devoid of nervous influences. Examination of these results with those of the electrical stimulus response studies would further define the site of

defect in the "asthmatic" preparation.

Isolation of the muscle component of TSM is achieved through the use of the cholinergic agonist, carbamylcholine (carbachol) to stimulate the muscle directly - using the same receptors as acetylcholine, but without augmenting the release of acetylcholine from cholinergic nerve fibers present (any relaxant nerve fibers present also would not be activated). Cumulative carbachol dose-response curves elicited (fig. 10), failed to reveal statistically significant hyperexcitability (shift to the left) or hyperreactivity (higher maximal tension) of the sensitized TSM to the stimulus when compared to normalized control values. Atropine (10^{-7}M) - a competitive cholinergic anatagonist - caused a parallel shift of the dose-response curves to the right, but failed as well to establish differences between the two TSM preparations. These results correspond well with those found by Souhrada et al. (1977), who reported that sensitized guinea-pig TSM displayed normal reactivity to carbachol.

Evidence from these experiments then, indicates that the sensitized and control TSM strips do not differ in the properties and characteristics of calcium pools mobilized by an acetylcholine or carbachol stimulus. Sensitized TSM displayed neither a hyperexcitability - which might emanate from lower muscle membrane thresholds to the stimulus - nor hyperreactivity, which could occur if more calcium is mobilized by the carbachol, thereby increasing P_o. When the results of the carbachol study are combined with those of the electrical stimuli, mutual exclusion suggests the further conclusion that nerve fibers present in the

TSM preparation (cholinergic, adrenergic or purinergic) must have similar functional properties as well. Although these data are not supportive to the vagal reflex theory (see introduction), the concept cannot be ruled out in our canine model of allergic asthma. The irritant receptors - which form the basis of the theory - may have been distributed in nerves in close proximity to the tracheal mucosa and eliminated during dissection of the TSM. The theory is limited nonetheless - for the vagal reflex hypothesis to hold true, the phenomenon must be due entirely to the number and distribution of irritant receptors. Certainly the excitability and reactivity of the nerve fibers which transmit the actions of these irritant receptors are not changed in our canine model.

Mills et al. (1969) have established however that the number of impulses travelling the vagal nerves were increased during bronchospasm. In light of the data presented, this could arise from irritant receptors stimulated by contact with the allergen or irritant receptors stimulated secondarily by deformation during muscle contraction initiated by anaphylactic mediators, or a third possibility may be that nerves possess receptors to anaphylactic mediators, their interaction might then result in the increased vagal activity recorded during bronchospasm. This neural activity could result in muscle changes, for example, the altered myosin ATPase activity established by the isotonic studies. The contractile properties of muscle are known to be modified when the muscle is subject to a certain pattern of neural activity (Eccles et al., 1962).

Both the electrical and carbachol studies were devoid of information regarding the relaxant nervous systems present. Cabezas et al. (1971), and Coburn and Tomita (1973) have demonstrated adrenergic inhibition, however their exact contribution to the inhibition of airway smooth muscle cannot be agreed upon. Noradrenaline has been demonstrated to relax the canine trachea (Russell, 1977); our experiments corroborate this. Richardson et al. (1975,1976) has suggested the number of adrenergics in the human airways may be less than previously thought, and that the "purinergic" relaxant system might be the important inhibitory system present. Suzuki et al. (1975) reported, however, that canine TSM does not possess a similar purinergic inhibitory nervous system. It may be that in the dog, the adrenergics play a greater inhibitory role (the function of which is not altered in sensitized TSM). This fact may prove important to our model - and certainly illustrates the danger of extrapolating animal studies to the human situation. The data suggest, that this being the case, the nerves do not play a primary role in the etiology of asthmatic bronchospasm. This does not, however, exclude their possible role in either the secondary aspects of bronchospasm or the change in the contractile function of airway smooth muscle.

C. INVESTIGATION OF THE MECHANISM OF ALLERGIC BRONCHOSPASM

(i) Antigen-antibody reaction

It is now generally agreed that the antigen-antibody reaction must provide the trigger for the manifestation of an allergic bronchospasm. Current thinking reflects this, holding that all

forms of asthma - both intrinsic and extrinsic - are dependent upon a triggering mechanism involving either an antigen-antibody reaction, or the presence of anaphylactic mediators (J. Pepys - personal communication). It is felt that the number of intrinsic types of asthma will decrease as their etiologies are identified and they become recognized as being varieties of extrinsic asthma. Sophisticated techniques now being used, for example, have established many cases of so-called intrinsic asthma to be really due to allergens so potent, (eg. toluene diisocyanate) that 100,000 molecules are capable of initiating immediate bronchospasm (J. Pepys - personal communication). Interpretation of findings which demonstrate physiological variations involving the pulmonary system of asthmatics, must bear this in mind. For example, Gold (1977) has modified his presentation of the vagal reflex theory to include the essential antigen-antibody interaction as an initiator of a vagal reflex in anaphylactic bronchospasm.

The validity of our canine model of allergic asthma hinges upon this immunologic concept. The sensitized canine TSM exhibits reactivity to an in vitro ovalbumin (OA) challenge which is characterized by active tension development (figure 11) when in contact with the sensitizing antigen; the control tissue always remains quiescent. This is observed with both in vivo challenge prior to killing the animal and without previous challenge. These in vitro results establish the airway smooth muscle contraction observed in our canine model to be due to an antigen-antibody interaction, a series of events which parallel those of asthmatic bronchospasm observed in vivo. The specificity of the

antigen-antibody reaction was demonstrated by the absence of response of the sensitized TSM to challenge with bovine albumin.

With the proof that an immunological reaction has occurred, it becomes important to know the events linking this to the resulting anaphylactic bronchospasm in allergic dogs. Studies have demonstrated that the mast cells in the bronchial epithelium of human asthmatics (Salvato, 1976) and allergic dogs (Meyers et al., 1973; Gold et al., 1977) degranulate upon challenge with aerosolized antigen, to release mediators of anaphylaxis. A variety of low molecular weight chemical medaitors are known to be released from sensitized mast cells or basophils - including histamine, SRS-A, ECF-A, prostaglandins, various kinins, and others (Wilson and Galant 1974)[1] - however, the evidence is too preliminary to allow conclusions regarding which mediator plays the most important role in the bronchospastic mechanisms. It may, in addition, be species dependent.

In our canine model of allergic asthma, histamine is the primary mediator of anaphylactic bronchospasm. The OA response in sensitized TSM (fig. 11) could be effectively abolished by preincubation with 7×10^{-6}M pyrilamine maleate - an effective H_1 antagonist (fig. 12). The inability of the muscle to respond to a maximal dose of histamine demonstrates the completeness of the H_1 block. Furthermore, the addition of 7×10^{-6}M pyrilamine maleate to the muscle bath in the midst of the sensitized TSM's response to ovalbumin, results in relaxation of the muscle (fig. 14). Secondary rises in resting tone - which would suggest the release

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[1] - see introduction for full dissertation

of another anaphylactic mediator, for example, SRS-A - are absent; SRS-A is known to be resistant to the effect of antihistamines (Orange et al., 1971). Incubation of the sensitized TSM with OA in the muscle bath for up to six hours after the initial H_1 - sensitive response, failed to result in a contractile response. Further substantiating evidence for the role of histamine is the (fig. 13) fact that the OA response in sensitized TSM could be abolished when the muscle was placed in a modified zero calcium Krebs-Henseleit solution - histamine granule release from mast cells is known to involve specific calcium ion dependent steps. Consideration of these data then, leads to the conclusion that histamine is released by the antigen-antibody reaction, and that it is apparently the only anaphylactic mediator of contraction released in the dog.

While many have argued a diminished role for histamine in the anaphylactic bronchospasm, data from this study suggests its effect is primary, at least in the dog. Histamine release during anaphylaxis has been demonstrated in the lungs of both sensitized guinea pigs (Bartosch et al., 1932) and the human asthmatic (Schild et al., 1951) as well. The ineffectiveness of antihistamine treatment in human asthmatics has been traditionally cited to suggest that histamine does not play a significant role in asthma. The recent results of Chiesa et al. (1975) - that plasma histamine concentrations fall while airway resistance re. ins high in challenged allergic dogs - also have been used to support this argument. However, Schild et al. (1951) have demonstrated tissue levels of histamine may be up to 10,000 times

higher than those of plasma. The inaccessibility of this pool to antihistamines might account for the results obtained. A measurement of tissue histamine levels therefore may provide a more definitive answer to the question of mechanisms involved in the anaphylactic reaction.

Electrophysiological analysis of the response of bovine TSM to histamine reveals membrane depolarization, with slow oscillations in membrane potential which can be correlated with rhythmic fluctuations in contractile activity. Suzuki et al. (1976) also report that canine TSM is depolarized in response to histamine. Phasic mechanical activity similar to Kirkpatrick's (1975) findings, was noted in sensitized guinea pig TSM following a histamine stimulus or antigen challenge (Souhrada and Dickey, 1976(a)). These results may be related to in vivo electromyographic data of human asthmatic TSM (Akasaka et al., 1975), and the phasic mechanical activity of airway smooth muscle in anaphylactic bronchospasm.

Sensitized canine TSM displays the common desensitization phenomenon observed in vitro to second challenges of antigen, the preparation responds only once to OA challenge, becoming unresponsive to further challenges, even after six hours of repeated washings of the muscle bath with fresh Krebs-Henseleit solution. Similar observations have been reported by Schild et al. (1951) in isolated human asthmatic bronchial chains, and by Souhrada and Dickey (1976(a)) in the sensitized guinea pig TSM. Interestingly, muscle strips exposed to histamine stimuli first, were desensitized to OA-induced contractions. Presumably

then, histamine release due to the antigen-antibody reaction is responsible for the desensitization phenomenon. Dale and Okpako's (1969) results are the only documentation of smooth muscle resensitization after the Shultz-Dale reaction. While in vitro desensitization is adequately documented, it has no parallel in vivo - a sensitized animal will repeatedly respond with increased airway resistance to aerosolized antigen challenge.

An accentuated cold response - active tension development in response to the addition of cold (22°C) Krebs-Henseleit during wash-out - is a common characteristic of sensitized TSM (fig. 11). The magnitude of such a response in control tissue is extremely small. An increase in muscle tension due to falling temperature has been documented in another smooth muscle - pregnant rat myometrium - by Marshall and Kroeger (1973), and Kroeger and Marshall (1974). Phasic contractions of the myometrium merged into a tonic contracture as the temperature neared 10°C; this was accompanied by a small but insignificant depolarization of the cell membrane. Kroeger and Marshall (1974) speculated that changing transmembrane Na and K gradients were unlikely to be the cause of cold contraction, in light of the facts that depolarization was insignificant and that contracture was apparent at 17-20°C, a temperature at which tissue sodium and potassium does not change (Daniel, 1963). In the sensitized TSM, the tonic contracture was pronounced at 22°C. Daniel (1964) speculated that calcium may be involved - since the electrochemical gradient for calcium is inwardly directed, a decrease in the activity of a membrane calcium pump at low temperature could result in an accumulation

of calcium inside the cell, which then initiates muscle contraction. The isotonic mechanical studies have already suggested the myosin ATPase of the sensitized muscle may be different from that of the control. Similarily, the ATPase of a membrane calcium pump might be altered in the sensitized muscle, rendering its kinetics more susceptible to cold. The problem would become amplified if the sensitized TSM were to have a greater permeability to calcium than normal, intracellular calcium accumulation would be greater and the muscle contraction intensified.

The tonic contracture to low temperature may play a role in the response of an asthmatic airway to cold air; often asthmatics complain of encountering increased airflow resistance during inhalation of cold air. This may be the mechanism responsible, however, its exact mode of action - without cellular depolarization - is obscure.

(ii) Histamine dose-response studies

With histamine demonstrated to be the only anaphylactic mediator released upon the IgE antibody-antigen interaction in our canine model of allergic asthma, it becomes important to determine what effect this compound has upon airway smooth muscle tone in asthma. For this reason dose-response curves for histamine were carried out on a series of sensitized and control TSM, in order that its receptors and cellular action might be analyzed. The experimental data elicited revealed that the sensitized TSM exhibited hypersensitivity to a histamine stimulus when compared to normalized control values (fig. 15). The hypersensitivity

displayed was two fold: first, the sensitized TSM was hyperexcitable - a shift of the dose-response curve to the left, indicated by the approximate ED_{50} of the sensitized TSM ($1 \times 10^{-5}M$) being significantly less than that of control ($ED_{50} = 4 \times 10^{-5}M$ histamine) - and second, the sensitized TSM was hyperreactive, developing an active tension of .943 kg/cm^2 to a maximal dose of histamine ($5 \times 10^{-4}M$) compared to the control value of .668 kg/cm^2. This represents a capability to produce 41% more isometric tension to a supramaximal dose of histamine. These results are in direct conflict with findings most recently reported by Souhrada et al. (1977) in sensitized guinea pig TSM. They found normal reactivity to histamine and carbachol challenge. However, species differences may account for the dissimilar results; sensitized guinea pigs, have proven to be a most unreliable model of allergic asthma (Pare et al., 1977).

In order to interpret our data, a pharmacological approach, involving classic receptor theory, was employed. According to van Rossum (1968), dose response curves reflect both the receptor affinity and the intrinsic activity of a drug. The process of receptor occupancy is reflected by the curve's shape, assuming the concentration of the drug in the direct vicinity of the receptor is directly proportional to the dose, and the intensity of the drug effect is directly proportional to the quantity of occupied receptors. These assumptions are more easily satisfied by in vitro studies than by in vivo studies (van Rossum, 1968).

Dose-response curves elicted for a particular group of receptor agonists which display similar slopes and maximal responses, are classically interpreted to mean the drugs have the same intrinsic activities but differ in their affinities for the receptor sites. Similarily, if two tissues, stimulated by the same drug are compared, and they exhibit displaced curves with equal slopes and maximal responses, then the receptor-agonist binding must be capable of similar cellular effectuation, but the receptor affinities for the agonist molecules must be different. The results obtained in this study do not reveal such a parallel displacement between the sensitized and control TSM histamine dose-response curves. Rather, the shape of the dose-response curves display both altered slopes and a higher maximal active tension for sensitized TSM. In view of the latter, the use of ED_{50} as a valid parameter becomes questionable, since it is dependent upon the intensity of drug action, and hence would not adequately reflect the dissociation constant for the drug receptor complex. van Rossum (1968) discussed the significance of dose-response curve shapes - two theoretical possibilities are consistent with our results. First of all, they coincide with theoretical curves for drugs with the same affinity constant but with different values for their intrinsic activities. Secondly, the shape of the dose-response curves are consistent with agonist action in which one curve represents its action on the intact receptor population, the other its action after inactivation of a certain fraction of these receptors (van Rossum, 1968).

At the tissue level then, since only one drug - histamine - is involved, the results may be interpreted to mean that the sensitized TSM possesses (a) histamine receptors which have the same affinity for the agonist but a higher intrinsic activity (i.e. cellular effectuation) (b) an increased number of histamine receptors with the same affinity and intrinsic activity, so an additive cellular action, that is, higher active tension development, is observed. If the second case is true, the location of the additional receptors in the TSM becomes critical to the understanding of anaphylactic bronchospasm. The histamine response in both sensitized and control TSM appears to be atropine sensitive (as yet unpublished). This suggests a number of histamine receptors could reside on the cholinergic fibers innervating the muscle; therefore histamine would cause TSM contraction via direct stimulation of the muscle histamine receptors and acetylcholine release due to stimulation of the histamine receptors residing on the cholinergic nerves present in the preparation. Karczewski and Widdicombe (1969) also have concluded that histamine exerts at least part of its action on airway smooth muscle by a cholinergic mechanism since the bronchoconstrictor effect of histamine delivered to large airways in vivo can be altered by atropine or vagotomy. Whether it is the histamine receptor population on the nerve which might be increased or those receptors on the muscle is unknown. While it could be argued that the sensitized TSM possesses more acetylcholine receptors - stimulated subsequent to normal histamine stimulation of the nerves - it is unlikely in

light of the absence of demonstrable differences in the electrical stimulus response curves of the sensitized and control TSM (fig. 7).

Alternatively, the sensitized TSM may have cellular mechanisms altered in such a way that the same dose of histamine elicits a contractile response greater than that of control tissue. This might occur if calcium stores available to the histamine stimulus are changed such that more calcium is released by the agonist in the sensitized muscle; more cross-bridges per cross-sectional area are activated and more active tension is developed. Obviously the calcium stores are accessible only to histamine, since the electrical stimulus - and carbachol dose-response studies failed to establish differences between the sensitized and control preparations. It has been shown that agonists are capable of acting on calcium pools independent of those activated by membrane depolarization (Hinke, 1965). One must remember that a P_o of .943 kg/cm^2 does not represent the maximum number of cross-bridges per cross-sectional area of TSM, which can be activated by calcium (a carbachol P_o would range from 2.5 - 3.0 kg/cm^2). Figure 17 demonstrates the ability of histamine to further contract TSM after depolarization by 125 mM KCl; carbachol can potentiate this further; pharmomechanical coupling may be responsible (Somlyo and Somlyo, 1968).

Histamine contractions consist of a fast phase and slow phase, the slow component being dependent upon the extracellular calcium influx (fig. 16). Experiments designed to delineate possible changes in the mobilization of these compartments during the sensitized TSM's contractile response to histamine, will have to

be conducted. Conceivably data from these studies could be used in conjunction with those of the histamine dose-response studies. If more histamine receptors are present, with the same intrinsic activity, then the contribution of the fast and slow phases to the hyperreactivity observed in the sensitized muscle must be increased proportionately. However, if one phase's contribution is increased over the other, then the histamine receptors of the sensitized muscle must not be changed in number; rather they would have similar affinities to those histamine receptors of control muscle, but their altered intrinsic activities would account for the shape of the histamine dose-response curves observed. Various sites of alteration are possible; if the fast phase is changed, examination of intracellular calcium compartments will have to determine whether more calcium is released by the stimulus because the agonist is capable of mobilizing calcium more effectively and/or the calcium pools themselves are larger. If the slower phase is involved, electrophysiological studies will have to be pursued as this component of histamine contraction depends upon the amount of calcium which crosses the membrane into the cell. Perhaps the sensitized TSM exhibits greater depolarization to a histamine stimulus such that more calcium channels are activated, and/or opened to a greater degree. This might be related to membrane changes suggested by the presence in the sensitized muscle of spontaneous activity, myogenic response, prolonged plateau and cold responses discussed earlier.

Why both the control and sensitized TSM histamine dose-response curves demonstrate lower tension development at doses higher than

5×10^{-4}M histamine is unknown. Tachyphylaxis is unlikely because it seems to make little difference in what order the doses are administered. As well, the same dose of histamine elicits a reproducible response, provided that an adequate period of equilibration is allowed between challenges. Since the presence of H_2-relaxant receptors could not be established (see following section), it is possible that larger doses of histamine are deactivating receptors in a manner similar to the desensitization mechanism to OA discussed earlier. A series of histamine dose-response studies carried out on control adult canine TSM, while revealing the absence of statistically significant differences when compared to pup control values, also displayed this phenomenon.

The fact that sensitized airway smooth muscle is hypersensitive to histamine suggests that the mediator release during and antibody-antigen reaction might be responsible both directly and indirectly for the increased airway resistance observed during bronchospasm. Airway smooth muscle is stimulated to contract by a direct action of histamine. Histamine also stimulates the cholinergic innervation of the muscle to result in further bronchoconstriction and a magnification of airway resistance. Stretch receptors in the mucosa of the tracheae may be activated by the contracting TSM to trigger vagal reflexes which add to the airway narrowing. Histamine has been implicated in the activation of vagal efferent pathways also, which in turn cause rapid, shallow breathing. This activation is common during acute attacks of allergic asthma (Cotton et al., 1977), and indicates increased vagal activity which may lead to further histamine release, by inhibiting, through an unknown mechanism, histamine's negative feedback effect upon its own release (Kaliner et al., 1972). Increased

vagal activity alone, however, does not result in depolarization of mast cells and release of histamine (Gold, 1977) - 25 mM K^+ failed to initiate histamine release by mast cell depolarization as well (fig. 19). Finally, Laitenen et al. (1976) concluded that histamine causes a significant decrease in static lung compliance in humans, thereby adding to the difficulty in breathing during an asthmatic bronchospasm. An examination of the location of histamine receptors in the airways becomes necessary in order that the role of histamine in and the overall mechanism of allergic bronchospasm can be properly ascertained.

(iii) Studies of histamine receptor agonists and antagonists

Previous studies regarding histamine receptors in the pulmonary system of various animals have indicated the existence of mepyramine sensitive histamine H_1 receptors capable of mediating bronchoconstriction and H_2 receptors capable of bronchorelaxation (Eyre and Wells, 1973; Eyre, 1973). The possibility exists that asthmatics may differ from normal in possessing a higher H_1/H_2 receptor ratio, or, more simply, possess only H_1 receptors.

Mepyramine sensitive H_1 receptors were established in both sensitized and control TSM. Two specific H_1 agonists - 2-methyl histamine and 2-(2-pyridyl)-ethylamine dihydrochloride - are capable of contracting canine TSM in a dose dependent fashion, and are sensitive, as well, to the presence of mepyramine. Although mediating strictly an H_1 response, the active tensions developed to these two agonists are lower - even at maximal doses - than those of histamine. Black et al. (1972) reported 2-methylhistamine has only 16% the relative agonist activity of histamine.

The documentation of the presence of H_2 receptors in other pulmonary systems plus the fact that a histamine contraction decreases in tension shortly after reaching a plateau, suggested

the presence of such receptors in the canine TSM. However, the presence of metiamide - an H_2 antagonist - failed to prevent or modify the relaxation observed. Bradley and Russell (1977) recently reported identical results for both the extrapulmonary and intrapulmonary airways of the dog. They concluded, as this study does, that the canine TSM does not possess relaxant H_2 receptors.

Bradley and Russell (1977) report 4-methylhistamine - considered an H_2 agonist (Black et al., 1972) - to have insignificant effects, again positive evidence for the lack of H_2 receptors in canine TSM. However, 4-methylhistamine (4 MH) produced conflicting results in this study. It reduced the active tone produced in canine TSM to a histamine stimulus in a step-wise fashion (fig. 18), however, 4 MH slightly potentiated a 10^{-5}M carbachol (fig. 20) and a 25 mM potassium contraction (fig. 19). The action of 4 MH in producing these results is obscure, especially in view of the negative H_2-antagonist data which established the absence of H_2 receptors. Perhaps the 4 MH is acting as a weak, competitive H_1 agonist. In "relaxing" the TSM histamine contraction the 4 MH may be displacing the histamine on the H_1 receptors. Since the former is a very weak agonist, the overall tension is less. The 4 MH may be potentiating the carbachol and K^+ contractions via a weak H_1 agonist effect. Electrophysiological examination of the 4 MH effect, as well as examination of the sensitivity of its effect to the presence of H_1 antagonists and its effect on TSM's histamine response curves should provide a more definitive answer.

With the absence of demonstrable H_2 receptors in sensitized and control TSM, all arguments regarding their histamine receptor

populations must be reassessed in terms of differences involving only H_1 receptors. As a result, the mechanism of 4 MH potentiation may prove important in providing insights into the asthmatic bronchospasm. Preliminary studies (as yet unpublished) establish that a resting TSM preincubated with 4 MH - which has no effect on tone - displays a higher $(dP/dt)_{max}$ to a histamine stimulus when compared to normal. Perhaps the 4 MH has caused a slight depolarization of the TSM membrane, which results in a greater mobilization of calcium when stimulated. Interestingly, these studies also indicate the $(dP/dt)'s_{max}$ of the sensitized TSM to a histamine stimulus to be increased more than the control, in the presence of 4 MH. If small doses of histamine were to act in vivo in a manner similar to 4 MH - either by partially depolarizing the muscle membrane, or inducing slight airway tone - the asthmatic airways may become predisposed to be hypersensitive to a variety of other bronchoconstrictive mediators.

Various studies have suggested (Bouhuys, 1976; Mitchell and Bouhuys, 1976; Benson and Graf, 1977) that interaction between bronchomotor stimuli at the cellular level may create a response greater than the sum of the individual agonist responses (for example, acetylcholine and histamine). Benson and Graf (1977) emphasized that the effect of base-line bronchomotor tone must be considered in evaluating airway reactivity; an increase in the degree of resting bronchomotor tone might contribute to the hyperreactivity observed in asthmatic patients. Newball (1976) stressed that local production of chemical mediators is important; they

may induce a contractile response of effector cells, and/or the environment necessary to produce hypersensitivity of the airways to a variety of stimuli. Such a mechanism might account for the continued airway resistance observed after plasma levels of histamine have fallen (Chiesa et al., 1975) - even though levels have fallen, they remain capable of potentiating airway smooth muscle response to other agonists.

D. GENERAL CONCLUSIONS

In this study of airway smooth muscle's role in a canine model of allergic asthma, the canine tracheal smooth muscle (TSM) isolated from sensitized animals displayed both altered mechanical function and a hypersensitivity to the anaphylactic mediator released during the antigen-antibody interaction of the allergic response. These results focus the asthmatic effect on ASM. Isotonically, the force-velocity relation of the sensitized TSM displayed a 48% increase in V_{max} and the $\underline{b}$ constant, with the absence of change in the P_o and $\underline{a}$ constant, when compared to their littermate control values. The increased velocity of shortening and $\underline{b}$ constant suggested that the actomyosin ATPase activity of airway smooth muscle (ASM) is increased in the asthmatic condition. In addition, the isotonic shortening capacity of the sensitized trachealis - associated with the narrowing and obstruction of the airways in asthma - was significantly greater than that of the control TSM. These data suggest that the first component of the asthmatic attack may be a sudden shortening of ASM resulting in a rapid rise in airway resistance, followed by further shortening to markedly narrow the airways and further increase airflow resistance.

Isometrically, the sensitized TSM displayed phenomena - such as a prolonged active tension plateau to electrical stimulation, the development of a myogenic response to quick stretch, spontaneously rising resting tension with phasic activity, and an accentuated cold response - which are consistent with altered membrane properties. These characteristic activities might superimpose themselves over the isotonic contraction to further complicate the asthmatic bronchospasm. Parameters concerning the active tension development and relaxation of TSM to electrical and carbachol stimuli were not different between the sensitized and control preparations. Thus the possibility of nerve dysfunction and muscle alterations - specifically excitation-contraction coupling, calcium release and sequestration - adding to the asthmatic response are effectively eliminated.

The active tension development - paralleling allergic bronchospasm - to challenge by the sensitizing antigen is both specific to OA and a characteristic of the sensitized TSM only. The anaphylactic mediator released during the antigen-antibody reaction in the allergic dog was shown to be histamine. Only the presence of mepyramine-sensitive H_1 receptors could be established in both the sensitized and control TSM preparations. A proportion of these reside on the cholinergic innervation of the tracheae.

When subjected to a histamine stimulus, the sensitized TSM displays a statistically significant hypersensitivity to the agonist when compared to control. The hyperexcitability

and hyperreactivity exhibited are consistent with the hypotheses that the sensitized TSM preparation possesses a greater number of histamine receptors than the control, or an equal number but with a greater intrinsic activity. The asthmatic ASM's altered sensitivity to the anaphylactic mediator, in addition to providing the link between the antigen-antibody reaction and the muscle response, would contribute as well to the increased airway resistance observed during bronchospasm.

The following model of asthmatic bronchospasm can be proposed from this study of TSM in a canine model of allergic asthma. Upon challenge by the sensitizing antigen, histamine is released from the mast cells in close proximity to the ASM. This stimulates the muscle to contract, the first component of the contraction is the sudden shortening of the ASM which results in a rapid rise in airway resistance. Since the ASM is hyperreactive to histamine, this would be followed by a progressively rising tension and continued shortening to further narrow the flow-limiting airways and increase airway resistance. Superimposed to complicate the bronchospasm would be spontaneous activity, myogenic and cold responses, and rising resting tone in the ASM.

The presence of an alteration in ASM in a model of allergic bronchoconstriction has been demonstrated by this study. Electrophysiological examination of the sensitized TSM will have to follow to elucidate the mechanism of the altered membrane characteristics; study of the location and properties of the histamine receptors will be important as well. If a substantial number of histamine

receptors reside on the cholinergic nerves to the TSM, the increased vagal activity may be responsible for the muscle changes observed. Alternatively the ASM contraction to histamine may be activating a vagal reflex through stretch receptors, which subsequently alters the muscle. Study of the altered actomyosin ATPase also will have to be carried out. Further investigation to determine whether the alteration of mechanical activity is primary or due specifically to changes in other initiator systems will have to be undertaken.

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