Increased Contractility in Antigen-Sensitized Canine Saphenous Vein

by

Zhiping Wang

A thesis presented to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Physiology
University of Manitoba
Winnipeg, Manitoba

ISBN 0-315-54875-4

INCREASED CONTRACTILITY IN ANTIGEN-SENSITIZED CANINE SAPHENOUS VEIN

BY

ZHIPING WANG

A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ABSTRACT

A considerable amount of research has been carried out on the pathogenesis of asthma employing animal models. The focus, naturally, has been on airway smooth muscle. However, it is likely that sensitization is not restricted to airway smooth muscle alone, but probably involves all tissues, including the systemic vein. Sensitization of systemic veins could have important implications in systemic anaphylaxis, an allergic reaction which leads to decrease in the cardiac output. We therefore decided to test the hypothesis that saphenous vein from pollen extract treated dogs is sensitized and has alteration of mechanical properties. The rationale is that such alteration *in vivo* result in reduced venoconstriction and cardiac output failure. To this end we studied responses to specific antigen challenge, the sensitivity and reactivity to histamine and norepinephrine, length-tension relationship, force-velocity relationship and stiffness in sensitized (S) and control (C) saphenous veins (SV). Antigen challenge revealed that the venous smooth muscle was strongly sensitized and developed a Schultz-Dale response whose two main mediators were norepinephrine (NE) and histamine (HIST). Compared with CSV, SSV is hyperreactive as determined from dose-response curve; but it has the same sensitivity to both NE and HIST. Length-tension relationship studies showed that while there is no difference in maximum isometric tension (P_o) development between SSV (P_o=93.95 mN/mm^2) and CSV (P_o=87.86 mN/mm^2), SSV

exhibited a significantly greater maximum shortening capacity (ΔL_{max})-0.762 l_o- than that of CSV (0.656 l_o). Unloaded shortening velocity (V_o), which reflects the crossbridge cycling rate, was determined at given intervals during muscle contraction. Maximum V_o was attained early (5 sec) in the contraction; 15% decline in V_o was observed at the plateau of the contraction (15 sec). At 5 sec, V_o of SSV (0.316 l_o) was significantly higher than that of CSV (0.269 l_o), though V_o's were same at 15 sec (0.249 l_o/s for SSV and 0.237 l_o/s for CSV). The increase in shortening could be partly accounted for by increase in the early crossbridge cycling rate. These findings indicate that mechanical properties of the sensitized saphenous vein are altered when compared to the control and suggest that the haemodynamics of venous circulation could also be altered in the sensitized model. Whether this alteration is specific or non-specific is difficult to determine at this time.

ACKNOWLEDGEMENT

Sincere gratitude is extended to Dr. N.L. Stephens for his patience and guidance through the course of this study. His extensive and profound knowledge in science, his understanding and his kindness makes his laboratory an enjoyable place to stay.

I am also grateful to Drs E.A. Kroeger and D. Bose for their advice.

Special thanks go to all those with whom I have had the opportunity to collaborate: Chun, Andrew, Jiang, Kong and Jing.

I especially wish to thank my wife Ilgar for her love and encouragement.

This project was conducted with the aid of a studentship from the Canadian Heart Foundation and operating grants to Dr. Stephens from the Manitoba Heart Foundation.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

A. General Introduction

While sensitized airway smooth muscle has been well studied, other tissues in the sensitized animal have not. This is surprising because there is no *a priori* reason for believing that they are not also sensitized. The likely explanation is that as the specific antigen, which is commonly air borne, has easy access to the airway, allergic bronchospasm is easily and frequently manifested. On the other hand, as the common antigens have almost no access to the circulation, for example, vascular dysfunction is rare. On the rare occasions when given intravenous injection, anaphylactic shock is seen.

Anaphylaxis is the decrease in cardiac output upon intravenous antigen challenge. The heart has been described as a target organ for mediators released in the lung in systemic reactions (Capurro and Levi, 1975). The consequence includes a rapid and sustained reduction in coronary flow which was suggested to be due to coronary vasoconstriction (Feigen *et al.*, 1960). Systemic anaphylaxis has been detected in various animals including humans (Essex, 1965). The primary manifestation of anaphylactic shock is severe hypotension (Smith *et al.*, 1980). Following *in vivo* intravenous challenges with specific antigen, the earliest change appeared to be severe hepatomegaly resulting from hepatic venous constriction (Essex, 1965). Pulmonary blood vessels taken from asthmatic horses contract *in vitro* to specific antigen demonstrating a Schultz-Dale response (antigen-antibody reaction) (Austen, 1973; Gold, 1973; Lichtenstein, 1973; Eyre, 1972; 1977; Kong

and Stephens, 1981). This indicates that vascular smooth muscle can be directly involved in the anaphylactic reaction.

The classical theory regarding causation of anaphylactic reaction of smooth muscle states that IgE antibodies are manufactured in response to exposure to a specific antigen. These IgE molecules then migrate and attach to mast cells and basophil granulocytes via the Fc portion of the molecules, to trigger, on subsequent antigen challenge, the release of chemical mediators (namely histamine, slow reacting substance of anaphylaxis (SRS-A), now known to be leukotrienes C_4 and D_4). These released mediators then diffuse to smooth muscle and cause constriction (Austen, 1973; Austen *et al.*, 1976; Gold, 1973; Lichtenstein, 1973).

B. Systemic Circulation and Venous Smooth Muscle.

The systemic circulation supplies all the tissues of the body, except the lungs, with blood flow. The veins function as conduits for transport of blood from the tissues back to the heart. The proper function of the heart depends on the adequate return of blood through the systemic veins. The venous system accounts for as much as 80% of the total vascular volume (Wiedeman, 1963). Thus, the veins are a major reservoir, of variable capacity, in the vascular system and subject to hydrostatic and other forces that can redistribute blood within this reservoir in amounts sufficient to alter cardiovascular function; this alteration is offset by appropriate active and passive adjustments of the capacity of

the different components of the systemic venous bed. The passive behaviour is due to the structural components of the wall of the vein which are mainly elastin and collagen fibres. Although the smooth muscle fibres contribute to the passive properties, their major role is to cause active changes in the tension that may stiffen the vessel wall or may lead to constriction or dilatation. Hence, the smooth muscle cell is the vital link in any venomotor response; without it, the vein would behave as a passive tube.

Vertebrate smooth muscle has been divided into two categories (Bozler, 1948). One type consists of morphologically and functionally independent cells that contract only when an external stimulus is applied; the muscle is composed of numerous individual units (multi-unit smooth muscle). In the other type, close appositions exist between the individual cells, cell-to-cell propagation occurs, and automaticity within the tissue creates a basal level of myogenic tone or spontaneous rhythmic activity; the muscle behaves as one entity (single-unit smooth muscle). The mechanical behaviour of isolated veins suggests that most large veins are of multi-unit type and do not possess inherent myogenic tone, exceptions being the portal-mesenteric veins of most species (Shepherd and Vanhoutte, 1975).

Veins are rich in smooth muscle, which responds to neural and humoral stimuli.

Neural Simulation. It has been known for a long time that the veins are innervated by sympathetic nerves (Hooker, 1918;

Donegan, 1921) and that, in the intact organism, changes in the activity of these nerves are the major way in which alterations in venomotor tone are produced. Adrenergic fibres are present in most veins, including caval, cutaneous, iliac, jugular, mesenteric, renal, penile, uterine, and those of skeletal muscle (Shepherd and Vanhoutte, 1975). However, the density of adrenergic innervation varies among the different preparations. The intensity of response to nerve stimulation, both in the intact organism and in isolated veins, correlates well with the morphology and density of innervation (Shepherd and Vanhoutte, 1975). Cutaneous and splanchnic veins constrict vigorously while deeper limb veins respond minimally to nerve stimulation as compared to their ability to constrict in response to exogenous norepinephrine (Vanhoutte, 1974). The electric impulses cause venous contraction by stimulation of sympathetic nerve endings rather than by a direct action on the smooth muscle cells, and catecholamine are the main transmitters (Shepherd and Vanhoutte, 1975).

Norepinephrine. Norepinephrine causes constriction of veins in the intact animal, in various isolated veins from animals, and in isolated human cutaneous veins (Shepherd and Vanhoutte, 1975). The constrictor effect of epinephrine on isolated veins has also been long recognized (Waterman, 1930; Maloff, 1934; Franklin, 1937). The constriction of veins caused by norepinephrine and epinephrine can be inhibited by α-adrenergic blocking agents (Holman *et al.*, 1968; Glover *et al.*, 1958), demonstrating that the former act on α-adrenergic

receptors. Epinephrine and, to a lesser degree, norepinephrine have a weak ß-adrenoceptor mediated dilator action in addition to their predominant -constrictor effect in veins; the former can only be demonstrated after -adrenergic blockade (Sicuteri *et al.*, 1966; Hughes and Vane, 1967; Guimaraes and Oswald, 1969).

5-hydroxytryptamine. Infusion of 5-hydroxytryptamine constricts the smaller capacitance vessels and the large cutaneous veins of the dog limb (Haddy, 1960; Hurwitz *et al.*, 1961; Diana and Masden, 1966). 5-hydroxytryptamine also constricts isolated preparations of cutaneous veins of the dog and man, portal-mesenteric veins of the cat, dog, rabbit, and rat and jugular veins of the dog (Shepherd and Vanhoutte, 1975). Antiserotonin compounds block the contractions caused by 5-hydroxytryptamine in isolated veins, suggesting the presence of serotonergic receptors in veins. However, the contraction effect can be abolished by -adrenergic blocking agents in cutaneous vessels, such as the saphenous vein of man and dog, suggesting that, even in the case where there are specific 5-hydroxytryptamine receptors, they must be very similar to those responding to norepinephrine (Vanhoutte, 1978).

Acetylcholine. Acetylcholine has been shown to dilate the small capacitance vessels of skeletal muscle and of the splanchnic circulation in the cat and the dog, but constricts larger splanchnic veins of the cat and cutaneous veins of the dog (Shepherd and Vanhoutte, 1975). In cutaneous, femoral, jugular and mesenteric veins of the dog preconstricted with

norepinephrine, the only effect of acetylcholine is a further contraction; only in smaller veins draining skeletal muscle has relaxation been noted (Shepherd and Vanhoutte, 1975).

Histamine. The constrictor effect of histamine on peripheral and splanchnic veins and the possible role of this in causing histamine-induced edema are still under discussion (Shepherd and Vanhoutte, 1975; Altura and Halevy, 1977). For most systemic veins, histamine is a relatively weak agonist (Vanhoutte, 1978). In canine cutaneous veins preconstricted with norepinephrine, histamine causes further increase in tension (O'Mahony, 1963); in portal-mesenteric veins, it augments the myogenic activity.

Prostaglandins. In the dog, prostaglandins A_1, A_2, E_1, and E_2 dilate the capacitance vessels of skeletal muscle and the cutaneous veins (Greenberg and Sparks, 1969). In contrast to their *in vivo* effect, prostaglandins A_2 and E_2 have been reported to cause contraction of isolated cutaneous and mesenteric veins of man and dog (Levy, 1972; Greenberg *et al.*, 1973). Prostaglandin F_2 constricts small and large veins in the skin, muscle, and splanchnic bed. The constriction of the larger veins is due to a direct effect on the smooth muscle (Ducharme *et al.*, 1968; Greenberg and Sparks, 1969; Mask *et al.*, 1971). F_2 causes contractions of isolated strips of canine skeletal muscle veins, portal-mesenteric veins of the rabbit, and umbilical veins of the sheep and man but not of the cutaneous or mesenteric veins of the dog (Greenberg and Sparks, 1969; Dyer, 1970; Dyer *et al.*, 1972; Greenberg *et al.*, 1973).

Leukotrienes. Leukotrienes C_4 and D_4 are potent vasoconstrictors of the coronary circulation *in vivo* and *in vitro* (Piper, 1984). A 5-lipoxygenase system is present in porcine coronary and pulmonary arteries, and the vessels and adjacent adventitia generate a leukotriene-like substance when challenged by substance A23187 (a Ca^{2+}-ionophore). The possibility therefore exists that leukotrienes are generated from vascular tissue in pathological conditions and act locally to cause vasoconstriction (Piper, 1984).

Role of the Endothelium.

The contraction of the isolated blood vessels can also be modulated by the endothelium (Furchgott and Zawadzki, 1980; De May and Vanhoutte 1982; 1983; Yanagisawa, *et al.*, 1988). Upon certain stimuli, the endothelium will release relaxing factor(s)(EDRF, possible NO) or contracting factor(s), such as endothelin, to modulate the reactivity and/or responsiveness of the underlying smooth muscle (Furchgutt and Zawadzki, 1980; Yanagisawa, *et al.*, 1988).

C. Smooth and Striated Muscle Contraction Mechanisms.

Muscle contraction is basically an interaction of two proteins, actin and myosin, which is induced by an increase in intracellular calcium ion (Ca^{2+}) concentration greater than 10^{-7} M. The rapid rise in intracellular Ca^{2+} is dependent upon Ca^{2+} influx across the sarcolemmal membrane and the release of Ca^{2+} from intracellular Ca^{2+} storage sites-the sarcoplasmic reticulum (SR). In the relaxed muscle cell, the Ca^{2+}

concentration is maintained at a low level (0.1 M) by Ca^{2+} pumps in the surface membrane, sarcoplasmic reticulum and by Na^{+}-Ca^{2+} exchange across the membrane (Ruegg, 1986).

Excitation-contraction (E-C) coupling is effected by intracellular Ca^{2+}. There are two major forms of E-C coupling, electromechanical and pharmacomechanical coupling. In striated muscle, electromechanical coupling is the mechanism that influences contraction through changes in membrane potential. Membrane depolarization, with or without the production of action potentials, is an event that triggers contraction (Hodgkin and Horowicz, 1960). The transverse (T) tubular system, a continuous network of tubules in striated muscles, that opens to the extracellular space, conducts depolarization waves to account for the inward spread of excitation, which then release Ca^{2+} from the SR (Huxley, 1959; Anderson-Cedergren, 1959). This mechanism is different in smooth muscle where no T-tubule exist, but where membrane depolarization can still result in intracellular Ca^{2+} release. Pharmacomechanical coupling has been defined (Somlyo and Somlyo, 1985) as the stimulation of contraction or relaxation by receptor-operated mechanism(s) independent of changes in membrane potential. It operates through the release of Ca^{2+} from SR mediated by inositol 1,4,5-triphosphate (IP_3) (Somlyo and Somlyo, 1968; 1985). The physiologically most relevant form of pharmacomechanical coupling occurs together with depolarization, when normally polarized smooth muscles are stimulated by neurotransmitters. The interactions between

actin and myosin that lead to tension development and contraction are regulated by the sarcoplasmic Ca^{2+} concentration.

In striated muscle, upon binding to troponin C of thin filament, Ca^{2+} relieves the inhibition effected by filamentous tropomyosin which normally prevents the interaction of actin and myosin (Ebashi *et al.*, 1969; Weber and Murray, 1973; Gergely, 1976). In smooth muscle, the regulation of contraction is not thin (actin) filament linked. Instead, an increase in the intracellular Ca^{2+} concentration results in the formation of a Ca^{2+}-calmodulin complex, which then interacts with the myosin light chain kinase (MLCK) to form the active ternary complex of the kinase. The phosphorylation of the 20,000 dalton myosin light chain by MLCK allows the activation by actin of its Mg^{2+}-ATPase activity, i.e., the formation of the active actomyosin complex. This event initiates the contractile process (Hartshorne, 1984).

In smooth muscle, the absence of a well-defined repeating sarcomeric pattern found in striated muscle has made it difficult to accept the existence of compatible sliding-filament crossbridges in smooth muscle, despite the fact that the mechanical properties of smooth muscle are qualitatively similar to those of skeletal muscle (Bagby and Corey-Kreyling, 1984). Positive evidence, however, has accumulated in recent years. As a consequence of employing optimal conditions of fixation, large number of thick filaments can be seen and they appear highly organized in smooth muscle (Somlyo *et al.*, 1973),

Anti-myosin fluorescent antibody revealed a highly orderly striated pattern similar to that observed in striated muscle (Groschel-Stewart *et al.*, 1975; Chamley *et al.*, 1977a; b; Bagby and Pepe, 1978). As early as 1948, Csapo (1948) isolated actomyosin from the uterus, and subsequently actomyosin was recognized as a component of all the smooth muscles that were examined and the extracted actomyosin showed properties qualitatively similar to those of skeletal muscle-derived actomyosin (Hartshorne *et al.*, 1980). These ultrastructural and biochemical observations, coupled with the mechanical data, reveal a strong similarity between the contractile systems of smooth and striated muscles. This is the basis for applying the sliding filament and crossbridge theory of contraction to smooth muscle also. However, there are differences in the regulation of contraction. In smooth muscle, calcium-sensitive regulation of contraction is not mediated via a troponin-tropomyosin system but by a calmodulin-mediated, myosin-linked light chain phosphorylation mechanism (Bremel, 1974; Bremel *et al.*, 1977; Driska and Hartshorne, 1975). Thus the molecular mechanism to be studied in attempting to understand disorders of smooth muscle relaxation, for example, which occur in allergic bronchospasm (Souhrada and Loader, 1979), may be quite different from those in striated muscle.

Although it is generally accepted that contraction of smooth muscle is, like that of skeletal muscle, brought about by crossbridge cycling and filament sliding, there are some quantitative differences between smooth and skeletal muscle.

One of the striking features of smooth muscle is the relative content of contractile proteins. The weight ratio of actin:myosin in smooth muscle, 1 to 2:1, is the reverse of that (1:3) in rabbit skeletal muscle (Small and Sobieszek, 1980; Potter, 1974; Tregear and Squire 1973). The ratio of actin to myosin filaments is approximately 13:1 compared with 2:1 in vertebrate striated muscle (Somlyo and Somlyo, 1986). The recent discovery (Dillon *et al.*, 1981) that dephosphorylated cross-bridges can remain attached in a non- or slowly cycling (latch) state during stimulation emphasizes a unique mechanism of smooth muscle contraction with a different regulatory mechanism. Furthermore, an observation of the corkscrew-like shortening in single smooth muscle cells suggests, in terms of a structural model, an helically-oriented contractile apparatus or cytoskeleton within cells (Warshaw *et al.*, 1987).

D. Muscle Mechanics.

The contraction of smooth as well as striated muscle involves a protein interaction of organized structures leading to shortening or force development. Thus, measurements of shortening velocity or force development under appropriate conditions can provide a measure of the mechanical output of the contractile system. Such measurements permit quantification of the system leading to inferences on the nature of the chemomechanical transduction process and enable assessment of muscle function under physiological conditions when load and activation can be specified.

Our current understanding of smooth muscle mechanics is largely derived from applying the classic experimental analysis that A.V. Hill and his colleagues developed for skeletal muscle (Hill, 1965; 1970). This approach treats a muscle fibre or group of cells as a black box where mechanical output and behaviour are accessible for measurement by means of attachments at the ends of the tissue. The tension and length changes are discussed in terms of a simple analog model, which simulates the properties of a more complex real system (Simmons and Jewell, 1974).

The simplest useful model, which has been extensively applied to skeletal muscle fibres at a length at which no resting tension is present, consists of two components. The first component is the contractile component (CC) and the second a series elastic component (SEC), which is assumed to be like a non-linear spring. In the resting state, the CC behaves as a viscous element - that is, it resists being stretched with a small force that depends on the velocity of stretch, but it offers no permanent resistance to a change of its length. In its activated state, the CC is capable of shortening and of tension development if shortening is hindered in any way. Contraction of the muscle is brought about by a rapid change of the CC from the resting to the active state. Relaxation results from a slower reversal of this process.

In order to simulate the mechanical properties of muscles at lengths where tension is present , a third element capable

of supporting the resting tension must be in parallel with the CC; it is known as the parallel elastic component (PEC). The PEC can be placed in parallel with the SEC, an arrangement duplicating the Maxwell mechanical element, or it can be placed in series with the SEC, duplicating the arrangement of the Voigt element. A three-component model is also needed to adequately represent the properties of smooth muscle (Stephens and Hoppin, 1986; Murphy, 1980).

To study the contractility of muscle, it is not sufficient just to measure isometric force development. Muscle shortening and shortening velocity should also be considered. Contractility is defined as the force-velocity-length-time interrelation (Brutsaert, 1974), and it can be determined by both appropriate isometric and isotonic contraction studies.

a) *Isometric Contraction.* Information about the contractile parameters, which describe muscle function at the level of the filaments, can be elicited effectively under isometric contraction. This includes data such as resting tension (RP), active (AP) and total tension (TP), obtained from length-tension experiments. The maximum active tension, $P_o(l)$, at any given length (l) reflects the formation of actomyosin bridges during contraction and represents the sum of the tension development at each cross-bridge, more bridges per unit cross sectional area resulting in a higher P_o. The RP curve represents the PEC in inactivated tissue. The length-tension curves also enable us to compute a theoretical maximum shortening capacity (ΔL_{max}) of the muscle (Stephens *et al.*, 1988;

Murphy, 1976). The length at which P_o is developed is termed l_o, which, in striated muscle, represents the maximum effective overlapping of the thin and thick filaments. Maximum activation of muscle is therefore length dependent. In order to delineate the mechanical behaviour of a muscle with maximum activation, studies should be carried out at l_o, which can be determined by eliciting length-tension curves.

b) *Isotonic Contraction.* When a muscle is allowed to shorten isotonically, the most fundamental mechanical characteristic of CC during activation is the force-velocity relation, which was first described by A.V. Hill in 1938 (Hill, 1970) as part of a rectangular hyperbola:

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

where P is the force developed by the muscle pulling a given load during shortening at velocity V, P_o is the maximum force during an isometric tetanus, and $\underline{a}$ and $\underline{b}$ are constants with units of force and velocity respectively. Hill originally thought that $\underline{a}$ and $\underline{b}$ could be obtained from heat measurements as well as from mechanical measurement, and therefore, the equation above was believed to be of fundamental significance. It is now clear that this is not so (Woledge *et al.*, 1985). However, the values of P_o, V_o (V at P =0), and $\underline{a}/P_o$, which actually specify the curve, are of more general interest because they may be interpreted in terms of cross-bridge activity (Woledge *et al.*, 1985). The value of P_o depends on the number of crossbridges that are attached, assuming that the force per attached crossbridge is a constant under isometric

condition. The value of V_o reflects the maximum rate of crossbridge turnover, but is independent of the number of bridges that are operating. A simple counterpart of the constant $\underline{a}/P_o$, which is a measure of the curvature of the force-velocity relation, is not so obvious. In terms of the hypothesis, there are two processes responsible for the decline in force as velocity rises: decreased number of crossbridges exerting positive force and increased number exerting negative force. At high forces the first of these reasons would be more important; it determines the slope of the force-velocity curve near P_o. At high velocity the second process is more important and determines the slope near V_o. The curvature of the force-velocity curve ($\underline{a}/P_o$) is determined by the ratio of these two slopes, and thus its value indicates the relative contribution of these two processes. The value of $\underline{a}/P_o$ is related to the shortening velocity (V/V_o) at which mechanical power output is maximal (Woledge *et al.*, 1985). The term $\underline{a}/P_o$ has also been related to the thermodynamic efficiency (the ratio of work rate to (work + heat) rate) of the muscle. With a low $\underline{a}/P_o$ ratio, a muscle is faster but less efficient (Woledge, 1968).

The strong qualitative mechanical similarities existing between smooth muscle and striated muscle length-tension and force-velocity curves (Stephens *et al.*, 1969), series elastic component (Stephens and Kromer, 1971) and responses of these parameters to change in temperature (Stephens *et al.*, 1977) suggest force generation in smooth muscle may evince a

mechanism similar to that of striated muscle. Information from force-velocity studies of smooth muscle about energy characteristics, tension development, velocities of shortening or extent of isotonic shortening can be used to interpret smooth muscle function. Examination of altered parameters in pathological conditions, in similar terms, may help elucidate the underlying pathogenesis of the disease.

E. Statement of the Problem.

While asthma research has focused on airway smooth muscle (ASM) and study of the role of allergy in producing ASM hyperreactivity, little work has been carried out on vascular smooth muscle of asthmatic objects. There is no *a priori* reason for believing that the sensitization process is restricted to the airways alone, but could, in fact, involve all tissues of the body. In working with *in vitro* ASM from ragweed pollen-sensitized dogs in our laboratory (Kong and Stephens, 1981), it was found that the pulmonary veins were also sensitized. Following *in vivo* intravenous challenges with specific antigen, it was observed by us (Kepron and Stephens, unpublished observations) and others (Essex, 1965) that the earliest change appeared to be severe hepatomegaly resulting from hepatic venous constriction. This led us therefore to more closely examine the hypothesis that sensitization was more widespread. We found that the sensitized canine saphenous vein (SSV) demonstrated a marked Schultz-Dale response, with development of rhythmic spontaneous activity. These were not

present in control saphenous vein (CSV). We also found that SSV was hyperreactive to norepinephrine.

These findings might have important implication for the elucidation of anaphylaxis, in which one major manifestation is the systemic vascular collapse due to hypotension (Essex, 1965). Wagner *et al.* (1986) reported that the primary circulatory mechanisms responsible for anaphylactic shock are an increase in resistance to venous return, which appears to result from constriction of the larger veins, and a shift of the systemic pressure volume curve and not an acute loss of plasma volume. In addition, immunological mediators released from sensitized mast cells and basophils could cause vasodilation and increased permeability of capillaries (Levy *et al.*, 1986) and postcapillary venules (Gabbiani *et al.*, 1970; Levy *et al.*, 1986). These processes could lead to the loss of fluid. The consequence of all the above effects would be decreased venous return. These manifestations of alterations in the properties of the venous system and the sensitization of saphenous vein, point out the importance of the venous circulation in the hypotension developing during anaphylactic shock. The hyperreactivity of the SSV indicates that the haemodynamics of the venous return in such animals could be altered and that their response to exercise, for example, could differ from that of controls. Therefore, mechanical study of the contractile properties of SSV was carried out by comparing SSV with CSV.

F. Choice and Justification of Model.

A canine model of allergic asthma (Kepron *et al.*, 1977) was chosen to study the contractility of systemic vascular smooth muscle and reveal its possible role in the systemic anaphylaxis. Our laboratory has been working with a canine model of hypersensitivity and has reported results of completed studies of the mechanical (Antonissen *et al.*, 1979; Kong and Stephens, 1983; Stephens *et al.*, 1988) and pharmacological (Antonissen *et al.*, 1980; Kong and Stephens, 1981) properties of airway and pulmonary smooth muscles in the model. Pathophysiologically, dogs are suitable models for the study of allergic asthma (Patterson, 1969). Spontaneous canine ragweed hypersensitivity has been found to be the only example of a respiratory pollinosis, similar to asthma in human, that occurs naturally in animals (Patterson, 1960). Furthermore, the immunologic reactivity has been shown to be mediated by a class of immunoglobulin similar to human IgE (Kessler *et al.*, 1974). Anaphylaxis has been shown in sensitized dogs following *in vivo* intravenous challenge with specific antigen. The earliest changes appeared to be severe hepatomegaly resulting from hepatic venous constriction (Essex, 1965). The allergic dog thus demonstrates pathophysiological and immunological changes very similar to those seen in human patients.

Severe hypotension is the primary manifestation of anaphylactic shock. This indicates that the haemodynamics of the venous return might have been changed, since the venous system accounts for as much as 80% of the total volume. Our

study should therefore be focused on the systemic veins and their role in the venous return. The saphenous vein was employed as a reasonable model for these. Furthermore, it is a feasible preparation for *in vitro* as it responds very well to electrical and pharmacological stimuli.

G. Experimental Plans and Specific Aims.

Mongrel dogs were sensitized to ragweed pollen extract and saphenous veins (SV) isolated from hindlimbs of sensitized dogs and their litter-mate controls. The following studies were carried out on SV strips.

1) Determination of whether the Schultz-Dale reaction occurs in SSV.

2) Elucidation of mediator(s) released during the antigen-antibody reaction.

3) Examination of the sensitivity and reactivity of SSV and CSV to histamine (HIST) and norepinephrine (NE).

4) Determination of whether latch cross bridges are present during contraction in the saphenous vein.

5) Determination of which type of crossbridge is altered with respect to function, in the SSV.

6) Finally, and most importantly, whether the maximum capacity of shortening is increased in the SSV.

The following are the experiments that were carried out in this study.

1) Length-tension curves for SSV and CSV. Derivation of maximum shortening capacity.

2) Time course of unloaded shortening velocity (V_o) for SSV and CSV. From these the presence of latch bridges will be determined.

3) Force-velocity curves for SSV and CSV. Direct measurement of maximum, isotonic shortening capacity.

4) Stiffness of SSV and CSV during contraction.

Methods

a) *in vivo* Sensitization Procedure.

Mongrel puppies were sensitized to ragweed pollen extract using an adaptation of the method developed by Pinckard *et al.* (1972) to elicit the production of immunoglobin (IgE) antibodies and anaphylactic sensitivity (Kepron *et al.*, 1977) to ragweed pollen extract. For the induction of IgE antibodies, the pups received intraperitoneal immunization with 50 mg ragweed pollen extract mixed with 30 mg of AL(OH)3 within 24 h of birth. Booster injections consisting of the same dose were repeated at weekly intervals for 8 weeks, and every 2 weeks thereafter. This regime of immunization has been shown to induce prolonged IgE antibody production of high titers against ragweed. Littermates of sensitized animals were given injections of the $AL(OH)_3$ alone, using the identical regime and served as controls. Serum IgE anti-ragweed antibody titers were measured in sera of both test and control dogs by passive cutaneous anaphylaxis (PCA) in unrelated mongrel dogs. For the present study, PCA titers of sensitized dogs were at least 256 dilutions. That is, serum samples of these sensitized dogs when diluted 256 times still produced PCA (inflammation and discolouration of the injection site). Meanwhile, controls showed no detectable PCA reactivity.

b) Tissue Preparation.

The canine saphenous veins of hindlimbs was used in the experiments. The dog was anaesthetized by an intravenous injection of 30 mg/kg wt. of pentobarbital sodium. The

saphenous veins were quickly removed and placed in ice-cold Krebs-Henseleit solution of the following composition (in mM): NaCl, 115; $NaHCO_3$, 25; NaH_2PO_4, 1.38; KCl, 2.51; $MgSO_4 \cdot H_2O$, 2.46; $CaCl_2$, 19.1; dextrose, 5.56. The connective tissue of the vessel was carefully dissected away under a binocular dissecting microscope. Transverse strips (2 mm wide) were cut from the vessel, as such strips produced greater isometric forces compared with helical strips cut over a wide range of angles. The lower end of the strip was fixed by a clamp attached to the bottom of a muscle bath. The upper end was tied firmly by 7-zero surgical silk thread to the lever. The bath contained Krebs-Henseleit solution, which was aerated with a 95% O_2 - 5% CO_2 gas mixture that maintained a PO_2 of 600 Torr, a PCO_2 of 40 Torr and pH of 7.40 at a temperature of 34°C. The determined optimal bath temperature of 34°C was chosen following the comparison of the temperature effect on isometric tetanic tension developed. The strips were equilibrated in the bath for 1 hour. The average of the optimal lengths (l_o's) of the strips was about 6 mm.

c) Data Acquisition.

i) For the studies of antigen-antibody reaction, dose-response, as well as temperature effect, isometric force was measured. The upper end was fastened to a Gould-Statham UC-3 force transducer suspended above the bath on a rack-and-pinion, thus enabling the muscle to be stretched to its optimal length and held there isometrically. The signal from

the force transducer was amplified and recorded on a 4-channel Gould Brush 2400 recorder. The compliance of the system was negligible over the range of forces applied to it.

ii) For the length-tension and force-velocity studies, the instantaneous force and displacement produced by the muscle were recorded with an electromagnetic lever system. The apparatus was originally developed by Brutsaert *et al.* (1971) and adapted for use with the slower smooth muscle. The voltage signal was fed into a HP9836 computer that analyzed and plotted out the data in graphic form. The total compliance of the lever system was 0.2 μm/mN and the total equivalent moving mass was 225 mg.

d) Electrical Stimulation.

Electrical stimulation of SV preparation was effected with a constant voltage 60-Hz AC source via rectangular platinum plate electrodes. The supramaximal voltage was determined by varying the stimulus voltages and used for further studies. The stimulus was applied at an interval of 6 minutes and was held for a train duration of 15-17 second, the time necessary to elicit the maximum response. By varying the preload (hence the length) and measuring the isometric tetanic tension, the optimal length (l_o) was determined. The latter was defined as that length at which maximum isometric tension (P_o) developed.

e) Optimal Temperature.

The optimal muscle bath temperature was chosen following the comparison of the temperature effect on the isometric tetanic tensions developed at 23°C, 30°C, 34°C, 37°C and 43°C.

f) Schultz-Dale Response.

To test the antigen-antibody reaction, both SSV and CSV were challenged with ragweed pollen extract by adding it into the muscle bath to a final concentration of 0.3 mg/ml. The same concentration of bovine albumin was used to demonstrate the specificity of the response to ragweed pollen. To study the mediator(s) released in the Schultz-Dale response, the effects of pyrilamine, phentolamine, methysergide, atropine, indomethacin and 5,8,11,14,-eicosatetraynoic acid (ETYA) were tested. The muscle strips were incubated with drugs 15 to 30 minutes before being challenged with the pollen extract. The inhibitory effect was detected by comparison of the Schultz-Dale responses between strips from the same vein with and without drug incubation.

g) Dose-response Curve.

Dose-response relations to norepinephrine (NE) and histamine (Hist) were studied in both SSV and CSV. The log dose-response curve was used in analysis of data. The curves were obtained by cumulatively adding drug doses to higher concentration until the muscle reached its maximal force development.

h) Length-tension (L-T) Relation.

The effect of length on the tension developed was analyzed by altering the length of the muscle through imposing different preloads and measuring the resultant isometric tetanic tension. After l_o was determined, the muscle was set at different lengths at $0.1Xl_o$ intervals ranging between 0.2 l_o and 1.2 l_o. Tensions were normalized by the cross-sectional areas of preparation and plotted against muscle length to obtain total, resting and active (as a difference of former two) tension curves. By drawing a horizontal line parallel to length axis and through resting tension at l_o, ΔL_{max} was obtained by measuring the distance between intersections of the line with active and resting curves.

i) Force-velocity (F-V) Relation.

During an isometric contraction, the muscle, set at l_o, was allowed to shorten isotonically after abrupt release to a series of critically-damped abrupt load clamps. The response consisted of a very rapid elastic recoil followed by a much slower shortening of the contractile element (CE). The initial CE shortening velocity was estimated by drawing a tangent to the displacement curve at 0.2 seconds after the quick release (as shown by Fig. 1). The F-V relation was obtained by applying, in a random order, a series of such load clamps, and the data were fitted with the hyperbolic Hill equation $V = \underline{b}(P_o-P)/(P+\underline{a})$, where V = velocity of shortening, P = load on the muscle, P_o = maximum isometric tension, and $\underline{a}$ and $\underline{b}$ are constants with units of force and velocity respectively. The

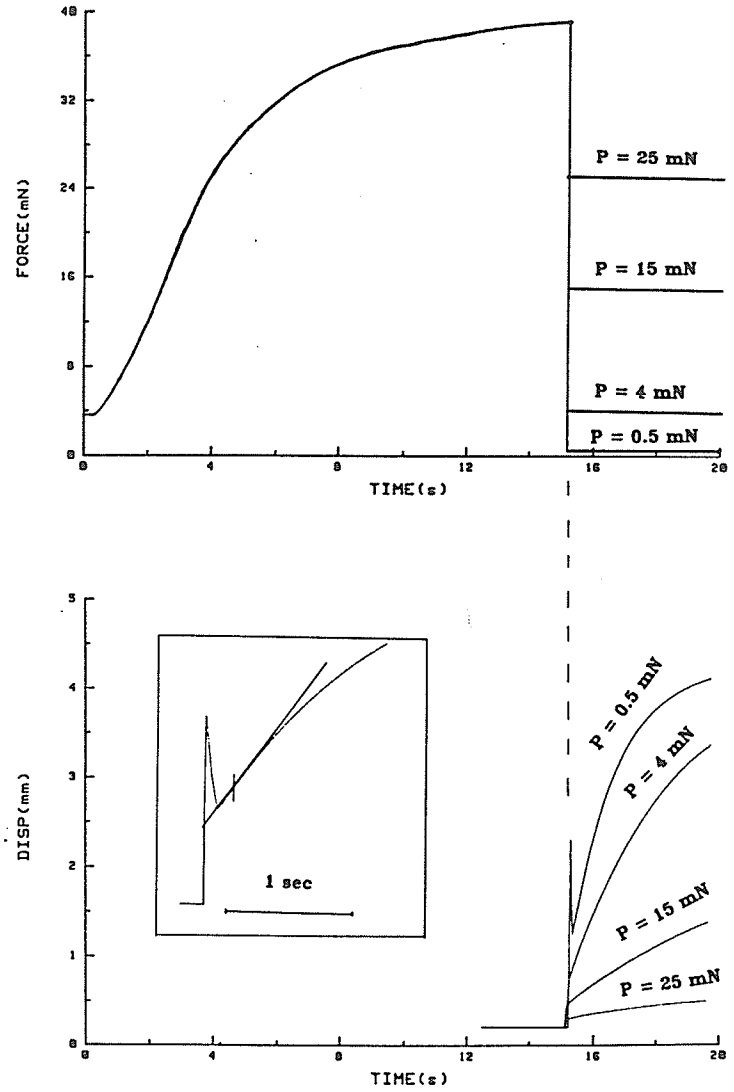

Figure 1. Quick release to various loads at specific time in a contraction by electrical stimulation. Shortening velovity after a quick release was obtained by measuring slope of curve at 0.2 sec, this (see inserted window) was achieved in the amplified graph by changing the time scale. The SEC length was obtained by measuring the upstroke of the dissplacement as shown. P was the constant afterload after quick release.

Hill equation was linearized and the least-square best fit to the experimental curve was determined, from which a and b were identified. Two F-V curves were obtained from the same preparation at 5 sec and 15 sec respectively after onset of stimulation.

j) Time-velocity Relation.

The shortening velocity at zero external load (V_o) was an estimate of the mean crossbridge cycling rate (Dillon *et al.*, 1981). The shortening velocity obtained after quick-release to a quasi-zero afterload was proportional to the true value of V_o. Velocity at an afterload of 0.5 mN ($V_{0.5\,mN}$) was measured by quick-release method at compartment times in the course of isometric tetanic contraction. Velocity was plotted against time to show the change in the cycling rates as a function of time during a contraction.

k) Stiffness.

The stress strain relation for the series elastic component (SEC) was obtained by analyzing the rapid transients from the same experiments as for measuring the F-V relation. The amount of the elastic recoil was taken as the change in length of the SEC caused by the change in force (from isometric tension to isotonic afterload). The SEC length was measured by determining the distance between the point of the intersection of the tangent drawn for estimating the CE shortening (as shown in Fig. 1). The extension of the SEC by a given

afterload was determined as the difference between the magnitude of the initial recoil under zero afterload and that obtained with a given afterload. The data were fitted with the following equation by the least-square best fit method.

$$\sigma = B\ (\ \exp(\ A\varepsilon\ )\ -\ 1\ ) \qquad (1)$$

Where σ is stress, ε is strain. The goodness of fit of the data with equation 1 indicates the stiffness is linear with load (Herlihy and Murphy, 1974; Seow and Stephens, 1987). That is, $d\sigma/d\varepsilon = A(\sigma+B)$, where $d\sigma/d\varepsilon$ is stiffness.

RESULTS

a) Effect of Temperature.

Fig. 2 is a plot of active tension vs bath temperature, which demonstrates that at 30-34°c the preparation of SV developed the maximum isometric tension. The temperature 34°C was therefore chosen for this study.

b) Optimal Stimulus Voltage.

Fig. 3 is an active tension vs stimulus voltage plot, which shows that the preparation generates increasing active tension as the stimulus voltage is increased and the active tension reaches its plateau at voltage of 15 V. Fifteen volts, at which the current density is about 200 mA/cm^2, was therefore considered to be the supramaximal voltage and used for the subsequent experiments with the electrical stimulation. To what extent the stimulus is neurogenic or myogenic is difficult to say, but 50% of the contractile response could be blocked by phentolamine.

c) Antigen-antibody Reaction.

The antigen-antibody reaction was studied for two reasons: to prove the SSV was indeed sensitized and to determine the transmitters involved. The results showed that there was a strong Schultz-Dale response (Fig. 4) in SSV, which was not seen in CSV. The maximum active tension developed during the Schultz-Dale response was 0.73 P_o ± 0.09 (SE), (n=5). The reaction was specific since the preparation did not respond to bovine albumin. Only 34.5% ± 8.9% (SE), (n=3) of the

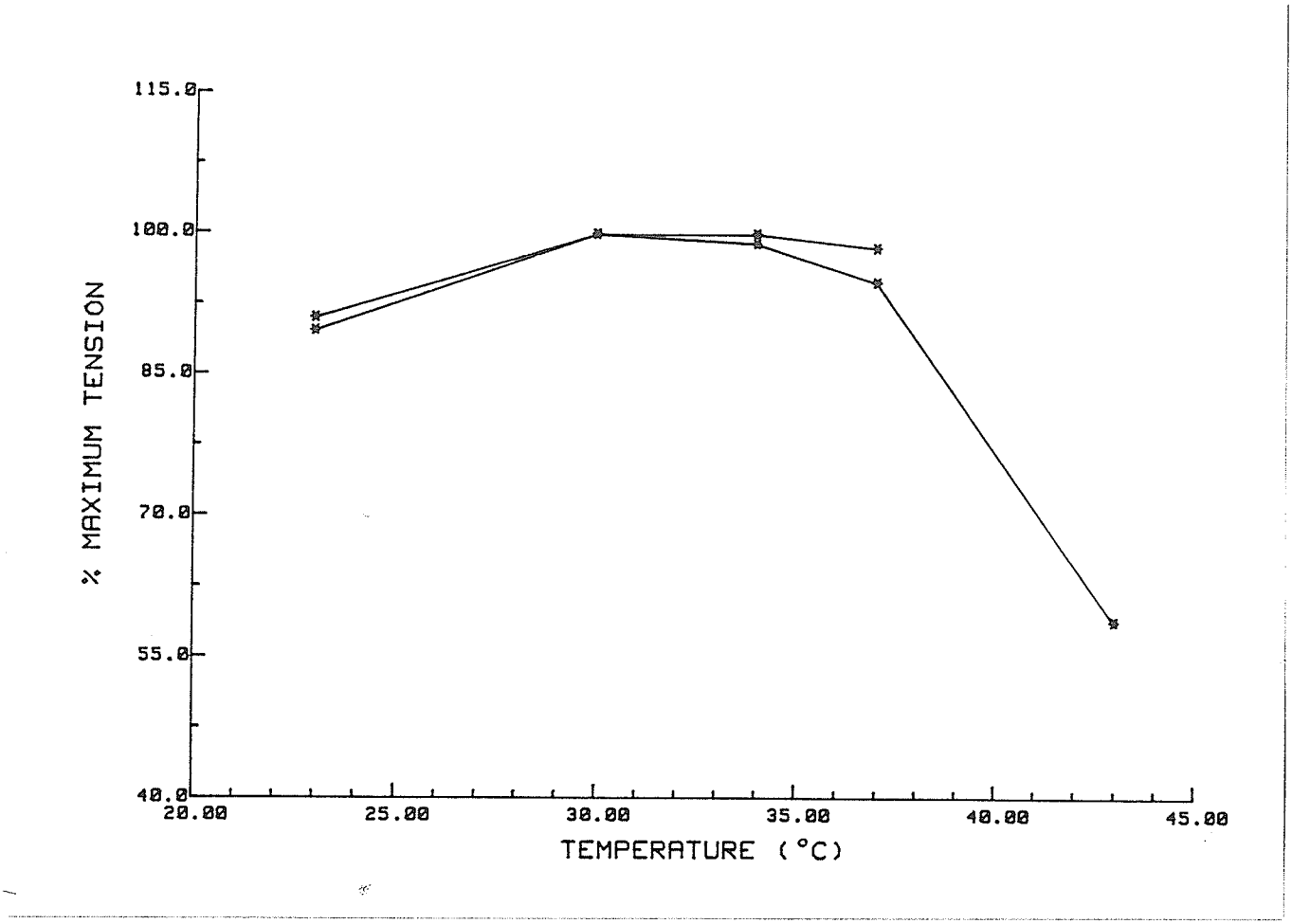

Figure 2. The optimal bath temperature. The ralation between tension development by the saphenous vein vs bath temperature was obtained by varying the bath temperature and measuring the isometric tension upon the electrical stimulation. The plot of active tension vs bath temperature (n=2) demonstrates that at 30-34°C the SV preparation developes maximum isometric tension. The temperature 34°C was chosen for this study.

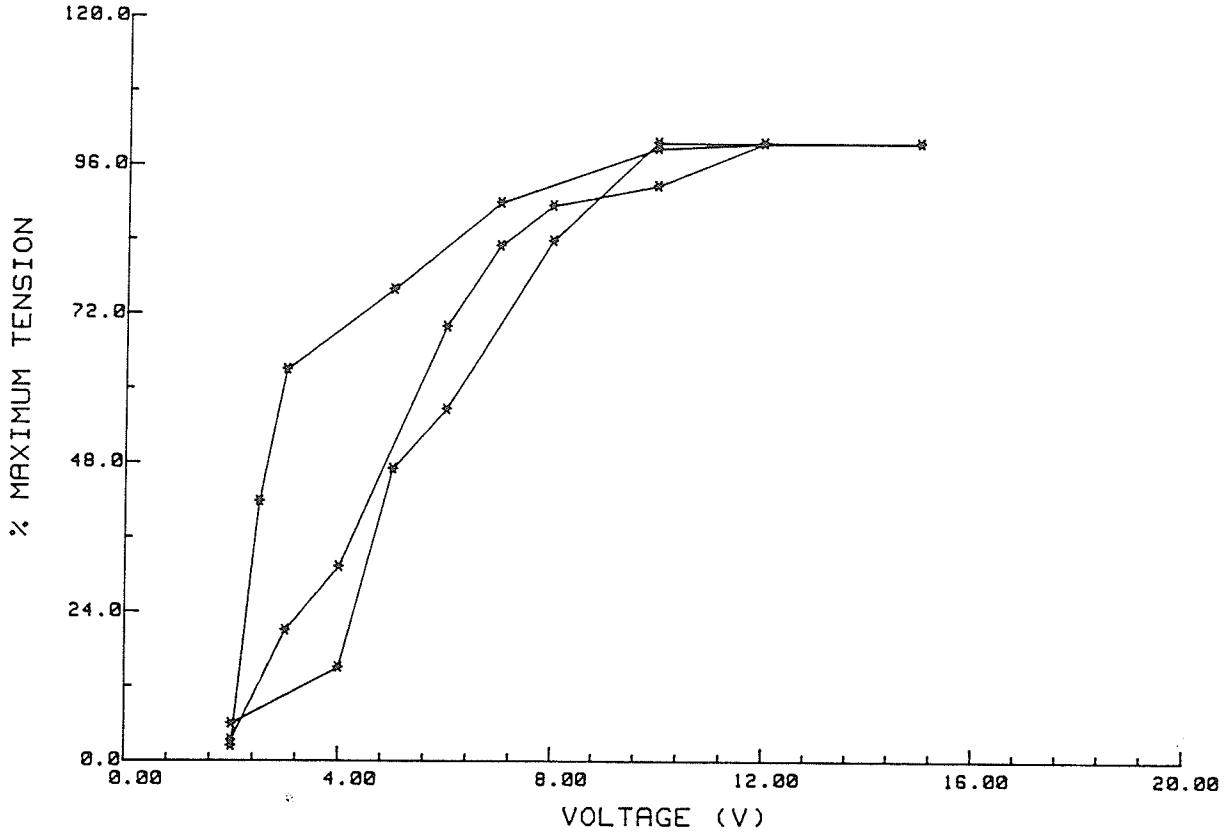

Figure 3. The optimal stimulus voltage. The SV strip generates increasing active tension as the stimulus voltage in increased and the active tension reaches its plateau at voltage of 15 V. Fifteen volts was therefore considered to be supramaximal voltage and used for the subsequent experiments with electrical stimulation. The figure shows the curves of tension vs stimulus voltage from three different SV strips.

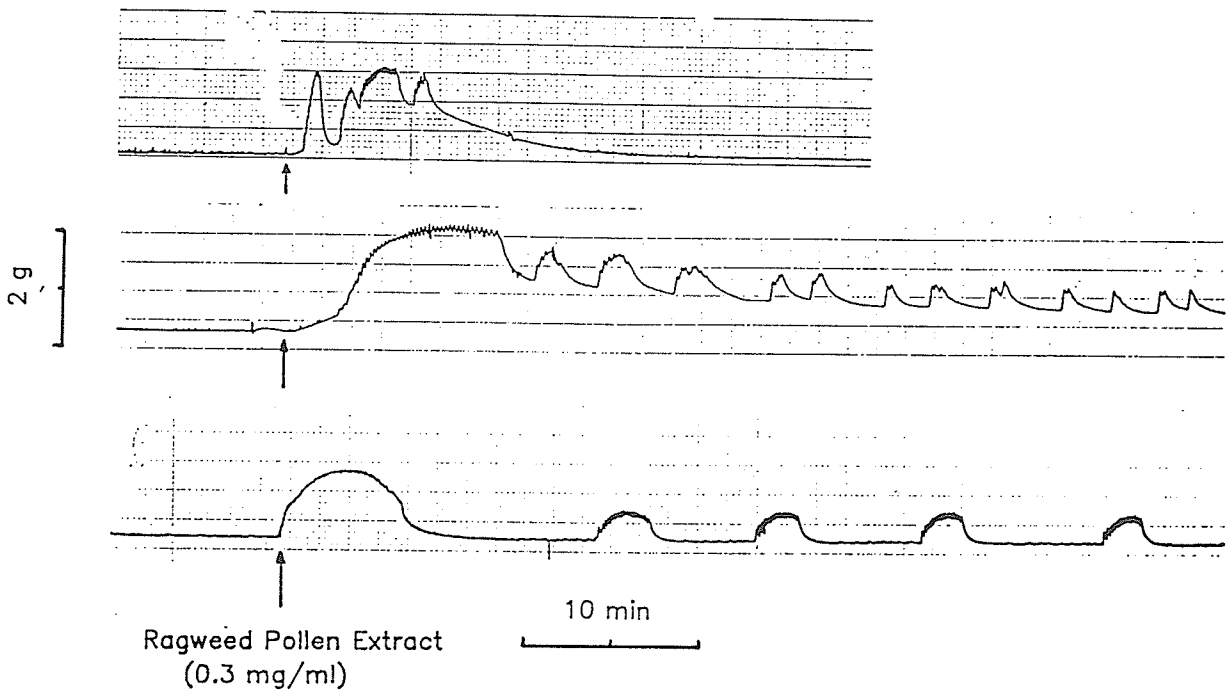

Figure 4. Schultz-Dale response developed in SSV with different shapes of force trace and the rhythmic spontaneous activities observed in SSV. The figure shows records of the time course of force developed by the three SSV strips. Ragweed pollen extract was used as an antigen to stimulate the antigen-antibody reaction. The final concentration of the extract in the muscle bath was 0.3 mg/ml.

Schultz-Dale response could be blocked by phentolamine (10^{-6} M) and 25.4% ± 2.1% (SE), (n=3) by pyrilamine (10^{-7}M); in a few experiments the effect of combined phentolamine and pyrilamine was found to be additive. Methysergide (10^{-6}M), atropine (10^{-6}M), indomethacin (10^{-5}M) and ETYA (10^{-6}-10^{-5}M) exerted no blocking effect at all.

d) Dose-response curves of Histamine and Norepinephrine.

The histamine dose-response curves (Fig. 5 and Table 1) did not show significant difference between SSV and CSV at doses below 10^{-3}M, but showed difference at doses from 10^{-3}M to 10^{-2}M. The active tension increased with higher doses. ED_{50}'s were $10^{-3.46}$M and $10^{-3.94}$M for SSV and CSV respectively. They were not significantly different. Thus, compared with CSV, the reactivity of SSV to histamine is increased but the sensitivity remains the same.

The norepinephrine (NE) dose-response curve for SSV was shifted upward (Fig. 6 and Table 2). ED_{50}'s were $10^{-5.47}$ M and $10^{-5.40}$ M for SSV and CSV respectively, which were not significantly different. The maximal response of SSV to NE was 128.85 (mM/mm^2), which was significantly ($p < 0.05$) higher than that of CSV (91.11 mN/mm^2). Thus again, compared with CSV, the reactivity of SSV to norepinephrine is increased but the sensitivity remains the same.

e) Determination of Presence of Pre-synaptic histamine receptors in SV.

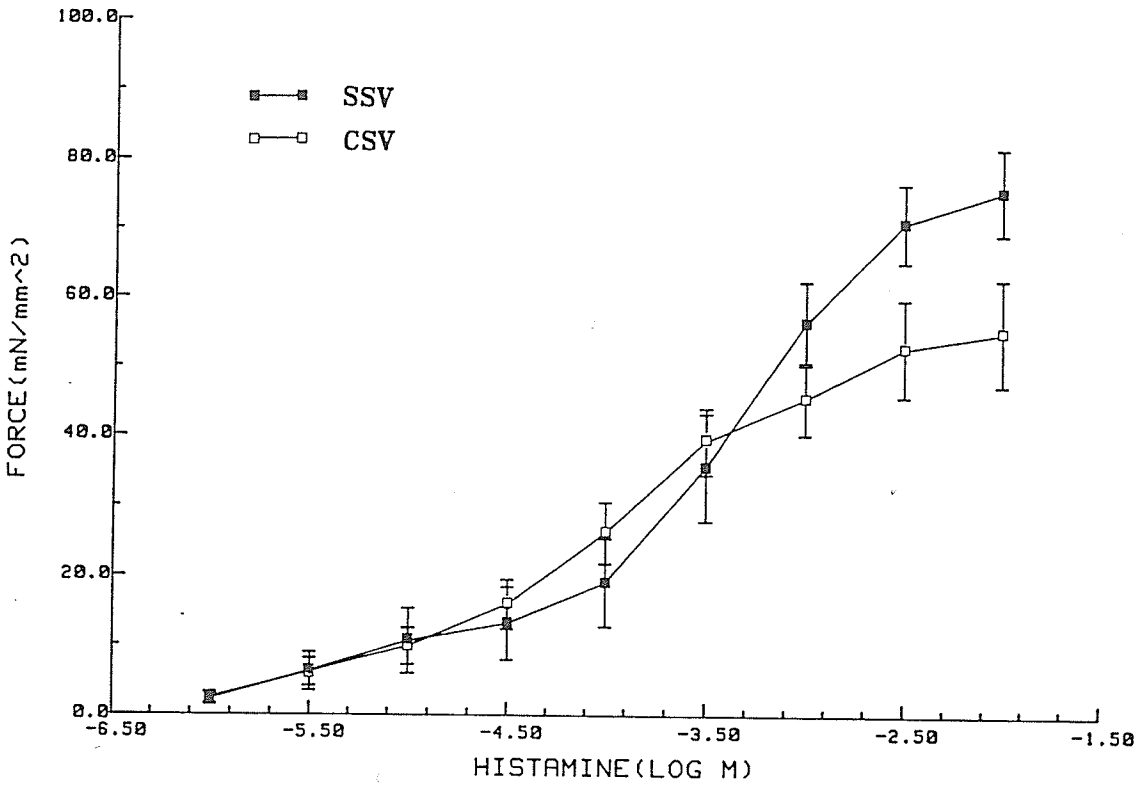

Figure 5. Cumulative dose-responses of SVs to histamine. The curves were obtained from SSV (n=11) and CSV (n=17) respectively by cumulatively adding drug doses to higher concentration until muscle reaches its maximal force developement. The isometric tension was plotted against log dose of histamine. The ED_{50}'s were $10^{-3.46}$ M and $10^{-3.94}$ M for SSV and CSV respectively, which were not significantly different. However SSV developed higher forces than CSV at higher drug doses.

Table 1. Histamine (HIST) dose-response data.

Log M dose of HIST	-6	-5.5	-5	-4.5	-4	-3.5	-3	-2.5	-2
SSV (n=11):									
Mean Isometric Force (mN/mm^2)	2.28	6.23	10.60	13.14	19.06	35.06	56.43	71.87	75.23
±S.E.	0.84	2.73	4.69	5.28	6.37	7.66	6.07	6.03	6.30
CSV (n=17):									
Mean Isometric Force (mN/mm^2)	2.42	6.14	9.82	15.91	26.22	37.20	45.43	51.15	55.79
±S.E.	0.85	1.97	2.66	3.57	4.36	4.93	5.3	5.67	6.06
t-test	NS	NS	NS	NS	NS	NS	$P<.05$	$P<.025$	$P<.025$

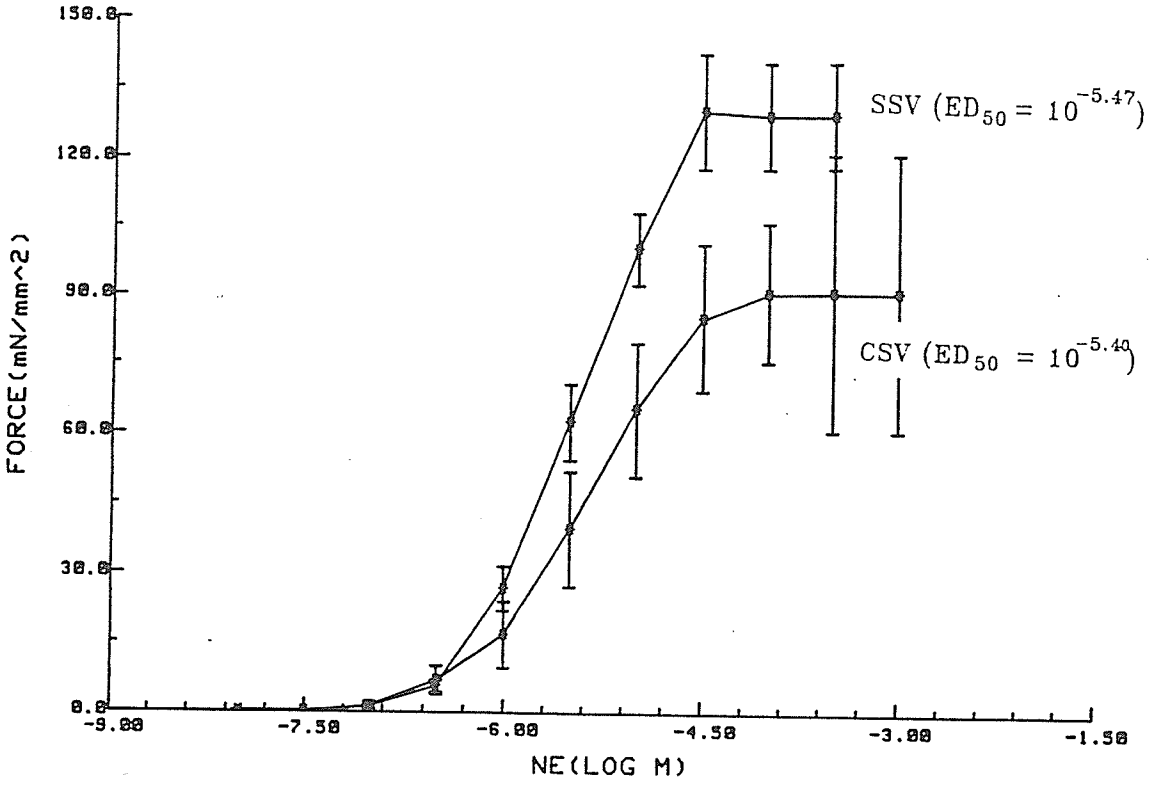

Figure 6. Cumulative dose-responses of SVs to norepinephrine. The curves were obtained from SSV (n=12) and CSV (n=9) respectively by cumulatively adding higher drug dose until muscle tension reached its maximum. The isometric tension was plotted against log dose of histamine. The ED_{50}'s were $10^{-5.47}$M and $10^{-5.40}$M respectively, which were not significantly different. However, SSV developed higher forces than CSV at high dose (>$10^{-5.5}$M).

Table 2. Norepinephrine (NE) dose-response data.

Log M dose of NE	-7	-6.5	-6	-5.5	-5	-4.5	-4	-3.5	-3
SSV:									
Mean Isometric Force (mN/mm^2)	1.22	5.65	26.74	62.55	100.44	130.55	129.68	129.85	-
±S.E.	0.50	1.27	4.80	8.14	7.88	12.36	11.48	11.43	-
n	12	10	12	10	12	10	12	12	
CSV:									
Mean Isometric Force (mN/mm^2)	1.29	5.12	16.74	39.60	65.33	85.40	90.90	91.11	91.11
±S.E.	1.00	2.81	7.19	12.39	14.46	16.16	15.35	30.37	30.37
n	9	8	9	8	9	9	9	9	9
t-test	NS	NS	NS	NS	P<.025	P<.025	P<.05	P<.05	-

As histamine and norepinephrine are among the mediators released during a positive Schultz-Dale response observed in the sensitized saphenous vein, it became necessary to determine whether pre-synaptic histamine receptors existed in the vein. Such receptors have been shown to exist in control (Kroeger and Bergen, 1980) and sensitized airway smooth muscle (Mitchell *et al.*, 1980). Fig. 7 shows that (10^{-3} M) histamine caused a two-phase contraction of the saphenous vein. The second phase could be totally blocked by phentolamine (10^{-6} M) added either before histamine, or at the plateau of the second phase. However, the second phase diminished at a lower dose of histamine (10^{-4} M). These findings suggest that, in addition to direct stimulation of the muscle cell histamine receptors, histamine at high dose could cause norepinephrine release due to stimulation of histamine pre-synaptic receptors on adrenergic terminals.

f) Spontaneous Activity and Myogenic Response.

Spontaneously rising tone associated with phasic activity was observed in almost all the sensitized saphenous veins studied (Fig. 8). Frequent stimulation of the sensitized tissue with norepinephrine, histamine, high potassium or electricity induced spontaneous activity. Even in the absence of any stimulation, the sensitized tissue would generate spontaneous activity after incubating in the bath for about 6 hours. The spontaneous activity was not sensitive to temperature as it could not be blocked by reducing the bath

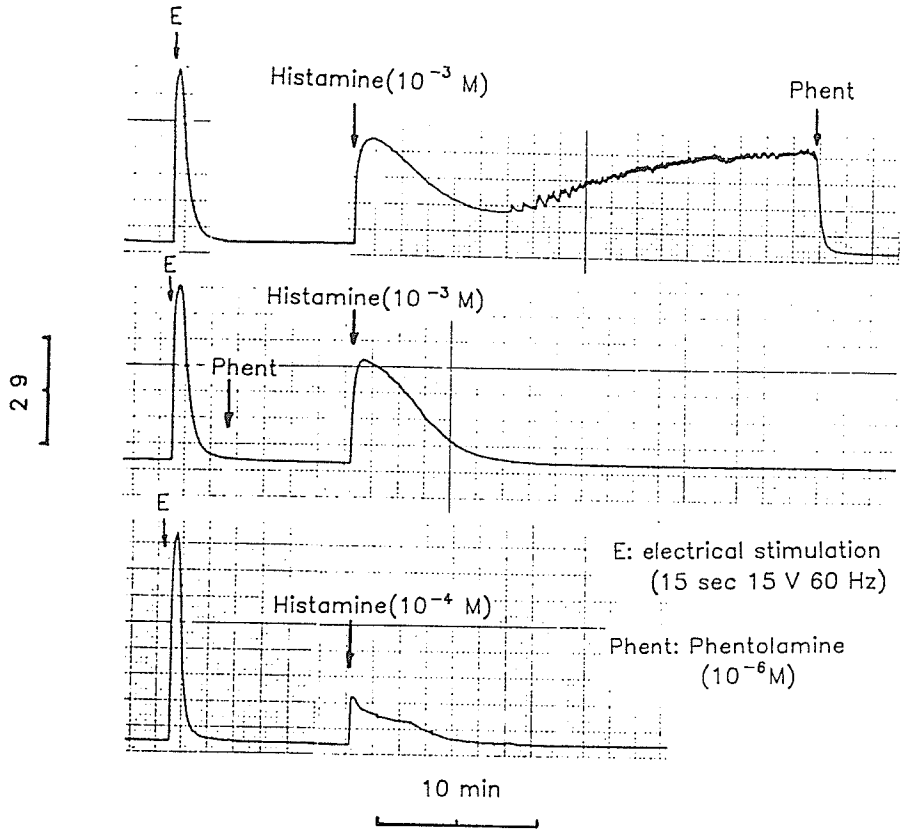

Figure 7. Effect of phentolamine on the contraction of SV induced by histamine (HIST). HIST at dose of 10^{-3}M causes a two-phase contraction of saphenous vein. The second phase was totally blocked by phentolamine (10^{-6}M) added either at the plateau of the second phase or before HIST (shown in middle panel). However, the second phase diminished at a lower dose of HIST (10^{-4}M). The isometric contraction to the electrical stimulation (15 sec 15 V 60 Hz) were elicited and compared with the histamine contraction.

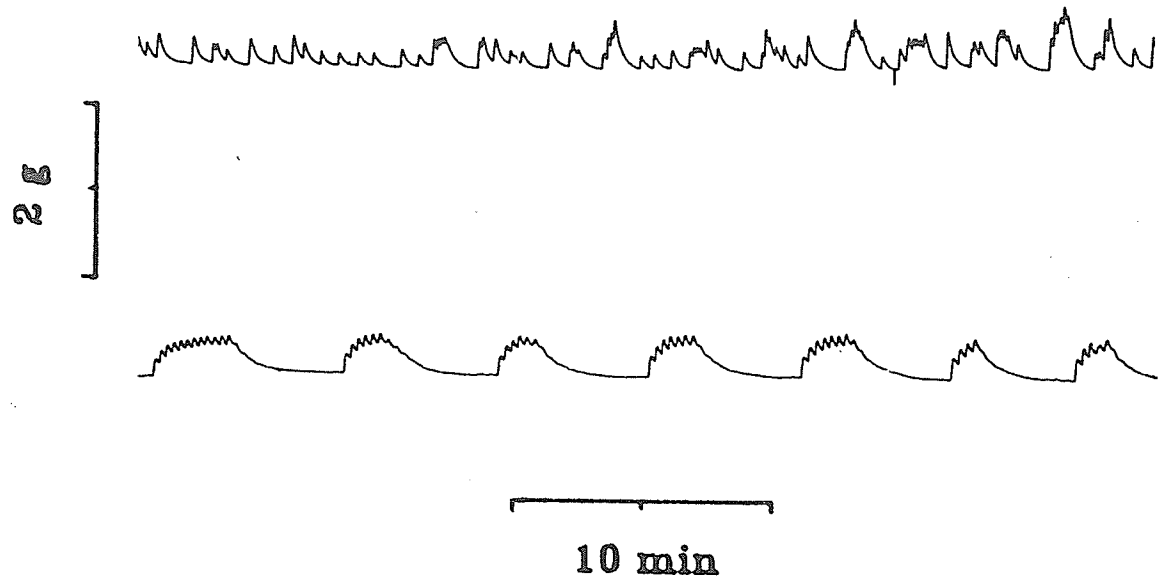

Figure 8. Spontaneous activity. Even in the absence of any stimulation, the SSV would generate spontanous activity after incubating in the muscle bath for about 6 hours. Two such traces are shown above.

temperature to room temperature (22°C). It could be abolished temporarily by flushing the muscle bath with fresh Krebs-Henseleit solution. However, spontaneous activity was not detected in saphenous veins from control dogs. The bathing medium from sensitized SV could not induce phasic activity in control SV.

Another interesting phenomenon exhibited by several sensitized SV is illustrated in Fig. 9. The application of aquick stretch and release back to initial length resulted in a myogenic response. The normal canine saphenous vein has previously been reported (Vanhoutte, 1978) and confirmed by us to be quiescent and not possess a myogenic response to stretch. Interestingly too, spontaneous mechanical activity was superimposed on the myogenic response.

g) Length-tension (L-T) Curves.

L-T experiments were conducted on SVs from both sensitized and control dogs. Mean values and standard errors are plotted in Fig. 10 and Fig. 11 respectively for SSV and CSV. Active tension for SSV was recorded at shorter length (0.2 l_o) than for CSV (0.3 l_o), however, there was no significant difference ($P > 0.05$) in P_o's at l_o, between CSV (87.86 mN/mm^2) and SSV (93.95 mN/mm^2). The L-T plots derived showed that the maximum afterloaded isotonic shortening capacity (ΔL_{max}) of CSV is 0.638 l_o, and the ΔL_{max} of SSV is 0.711 l_o. However, ΔL_{max} estimated from a L-T curve is a theoretic and maximal value; more direct and reliable measurements of actual shortening can

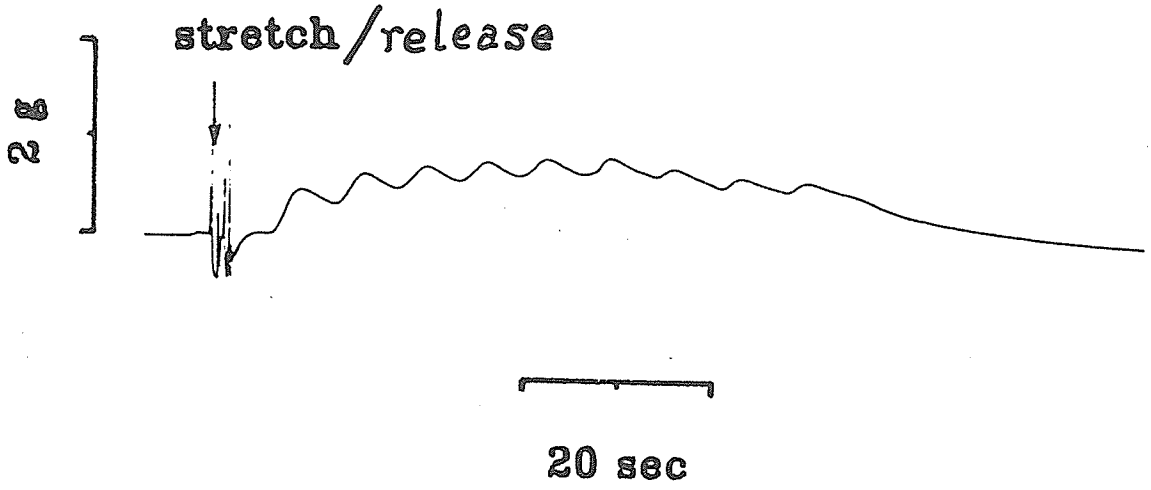

Figure 9. Myogenic response. The application of a quick stretch and release back to initial length resulted in a myogenic response.

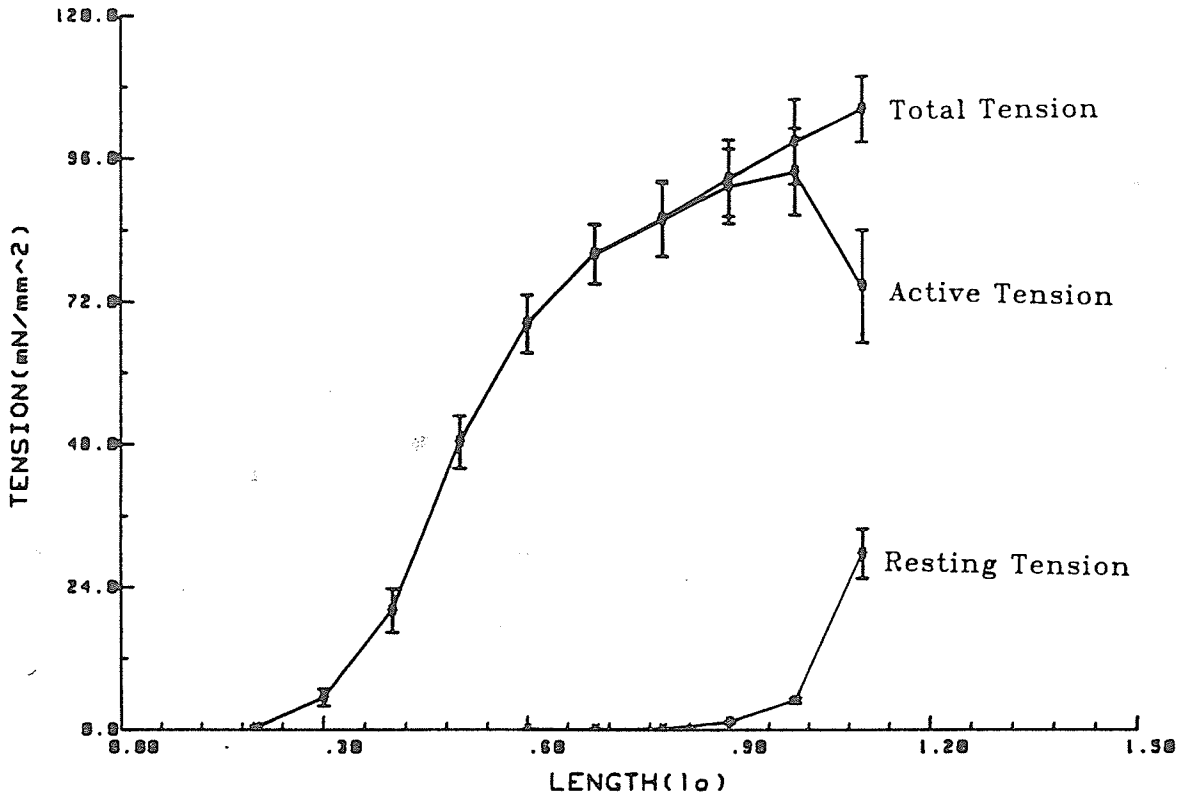

Figure 10. Length-tension curves for SSV. At difference muscle lengths, the total tension, the resting tension and the active tension, which is the difference of the former two, were measured. The statistical data (in mean ± SE) were plotted with 6 experiments. The vertical bars indicates standard errors of means.

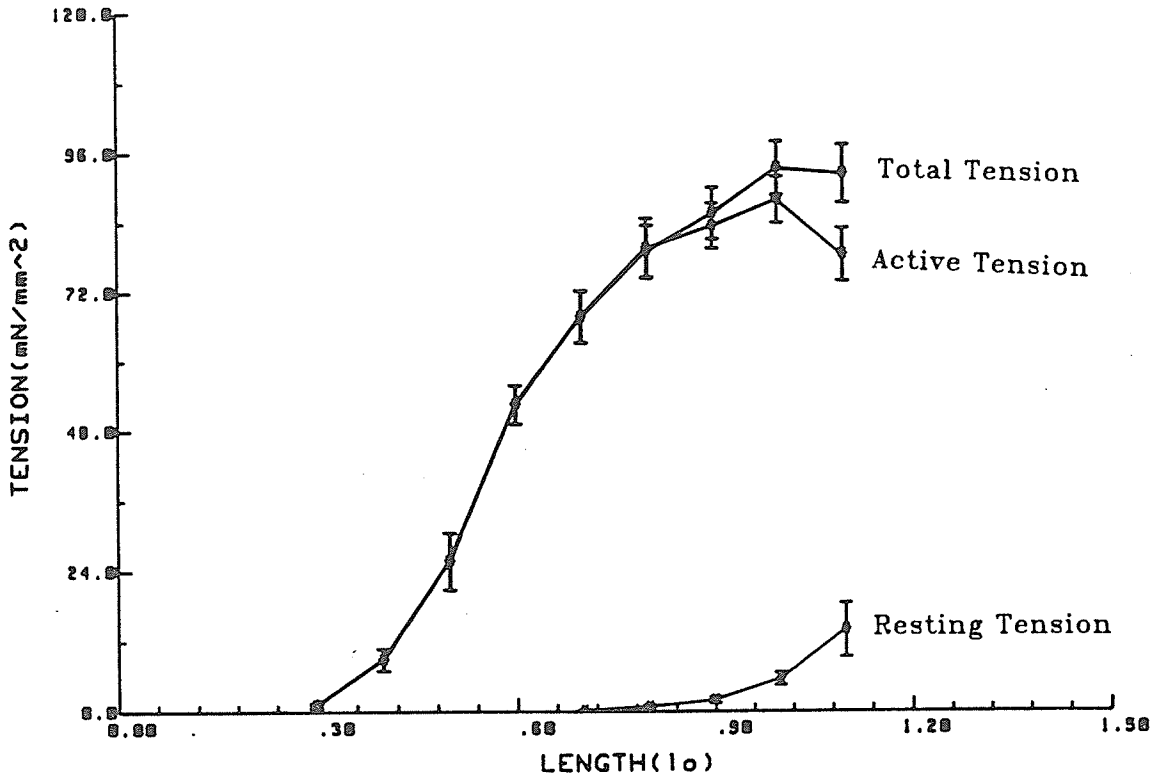

Figure 11. Length-tension curves for CSV (n=6). The vertical bars stands for standard errors.

be obtained by studying isotonic shortening from l_o; ΔL_{max} obtained from isotonic shortening was 0.578 l_o ± 0.012 for CSV (n=4), and 0.613 l_o ± 0.009 for SSV (n=4). In both cases, the shortening that developed within 5 seconds contributed 67% of the ΔL_{max}. The increase of ΔL_{max} in SSV is significant ($p < 0.05$ by paired t-test); and, by Poiseuille's law, this increment represented theoretically, a 41% increase in resistance of the vessel to blood flow.

h) Time Course of Shortening Velocity.

The shortening velocity at a quasi-zero load is proportional to the true value of V_o, the shortening velocity at zero external load. The time course of shortening velocity ($V_{0.5\,mN}$) at light load (0.5 mN) is shown in Fig. 12. $V_{0.5\,mN}$ rose to a maximum within 5 seconds of stimulus onset and then fell to lower values even though the isometric tetanus was still increasing. This indicates the existence, in smooth muscle, of two types of crossbridges, namely normally cycling crossbridges that are active early in contraction and slowly cycling or noncycling latch bridges operative late in contraction.

i) Force-velocity (F-V) curves.

Two particular points of time, 5 seconds at which the maximum shortening velocity was attained and 15 seconds at which the maximum isometric tetanic was developed, were selected for measurement of the force-velocity properties of

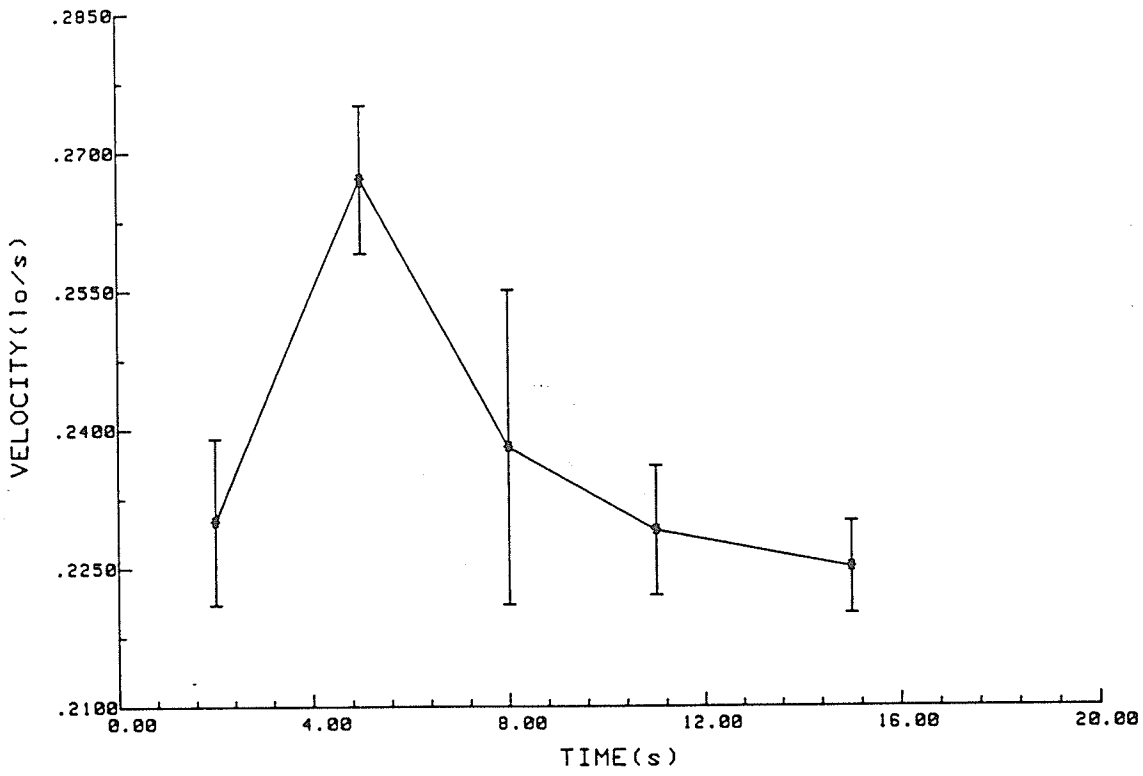

Figure 12. Time course of velocity. As an estimate of the crossbridge cycling rate, the shortening velocity ($V_{0.5\,mN}$) at a small after load (0.5 mN) was obtained at different times after the electrical stimulation. The statistical data (in mean ± SE) were plotted for 5 experiments. The vertical bars indicate standard errors of means.

SSV and CSV. A typical pair of force-velocity curves of CSV is shown in Fig. 13. Table 3 lists the values of P_o, V_o, $\underline{a}/P_o$ and $\underline{b}$ obtained from both SSV and CSV. The data were analyzed by paired t-tests. V_o dropped significantly (paired t-test, $p<0.05$) by 11.9% in CSV and 21.2% in SSV between 5 second and 15 second values during a contraction. Comparing the velocities of SSV with those of CSV, we found that at 5 seconds V_o of SSV is significantly greater than that of CSV. while at 15 second no significant difference is showed. There is no significant difference in P_o's between CSV and SSV at either 5 seconds or 15 seconds. At 5 seconds, $\underline{a}/P_o$, which reflects the curvature of F-V curve, is smaller for SSV.

j) Stiffness.

The stress-strain relation of the SEC was studied at 5 seconds and 15 seconds. The values of parameters are listed in Table 4 for CSV and Table 5 for SSV. A pair of typical curves for CSV are shown in Fig. 14. In both the control and sensitized saphenous veins stiffness increase with time. However, the value of the constant A (obtained from equation 1 and shown in Table 4) at 5 seconds was significantly larger than that at 15 seconds for either CSV or SSV. The SEC elongation at the maximum tension developed in an isometric contraction was 7% ± 0.3% of l_o for CSV and 8.1% ± 0.9% of l_o for SSV. There were no significant differences ($P > 0.05$, by t-test) between CSV and SSV in regard to the parameters A, B, P_o, stiffness and SEC.

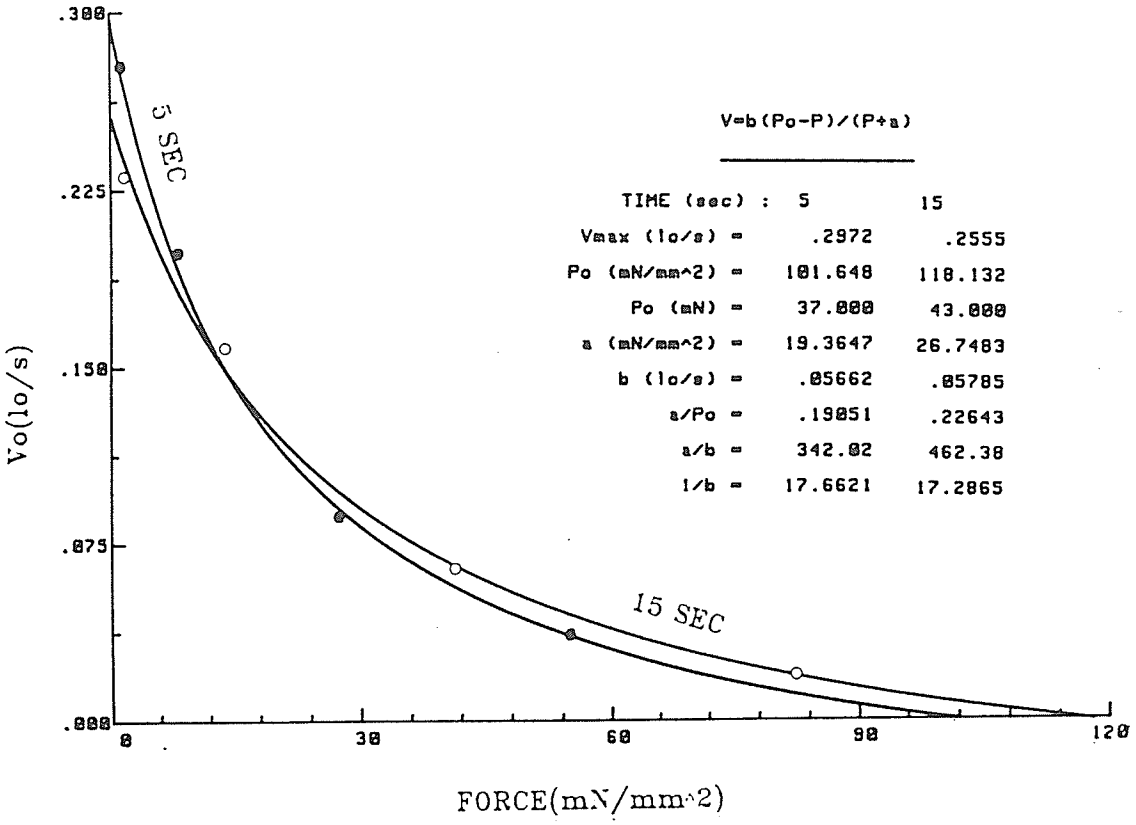

Figure 13. A typical pair of F-V curves elicited at 5 sec and 15 sec during an isometric contraction in the same preparation. The hyperbolic Hill equation was used to fit the experimental data.

Table 3. Force-velocity parameters

	sensitized(n=7)		control(n=7)	
time(s)	5	15	5	15
Vo(lo/s)	.316±.019*	.249±.021	.269±.018*	.237±.019
Po(mN/mm^2)	90.25±10.05	105.60±9.96	84.65±11.77	103.79±13.11
a/Po	.190±.015$	.186±.013	.269±.025$	.211±.016
b(lo/s)	.060±.006	.046±.005	.072±.008	.050±.006

Values are means±SE.
* significantly different($p<.025$).
$ significantly different($p<.05$).

Table 4. SEC parameters for CSV.

	5 (sec)	15 (sec)	paired t-test
A ($1/l_o$)	78.51 ± 3.67	62.89 ± 2.63	$P < 0.005$
B (mN/mm^2)	2.52 ± 0.73	1.41 ± 0.44	NS
P_o (mN/mm^2)	70.53 ± 9.80	96.03 ± 12.13	$P < 0.005$
stiffness ($mN/mm^2/l_o$)	5578.3 ± 863.2	5808.6 ± 1340.8	$P < 0.01$
SEC (l_o)	0.045 ± 0.002	0.070 ± 0.003	$P < 0.005$

Data are means ± S.E.s

Table 5. SEC parameters for SSV.

	5 (sec)	15 (sec)	paired t-test
A ($1/l_o$)	76.42 ± 16.41	65.57 ± 13.96	$P < 0.025$
B (mN/mm^2)	4.38 ± 3.40	4.19 ± 2.73	NS
P_o (mN/mm^2)	74.47 ± 13.64	107.62 ± 18.34	$P < 0.005$
stiffness ($mN/mm^2/l_o$)	5367.8 ± 1180.2	7107.3 ± 1526.9	$P < 0.005$
SEC (l_o)	0.061 ± 0.006	0.081 ± 0.009	$P < 0.005$

Data are means ± S.E.s

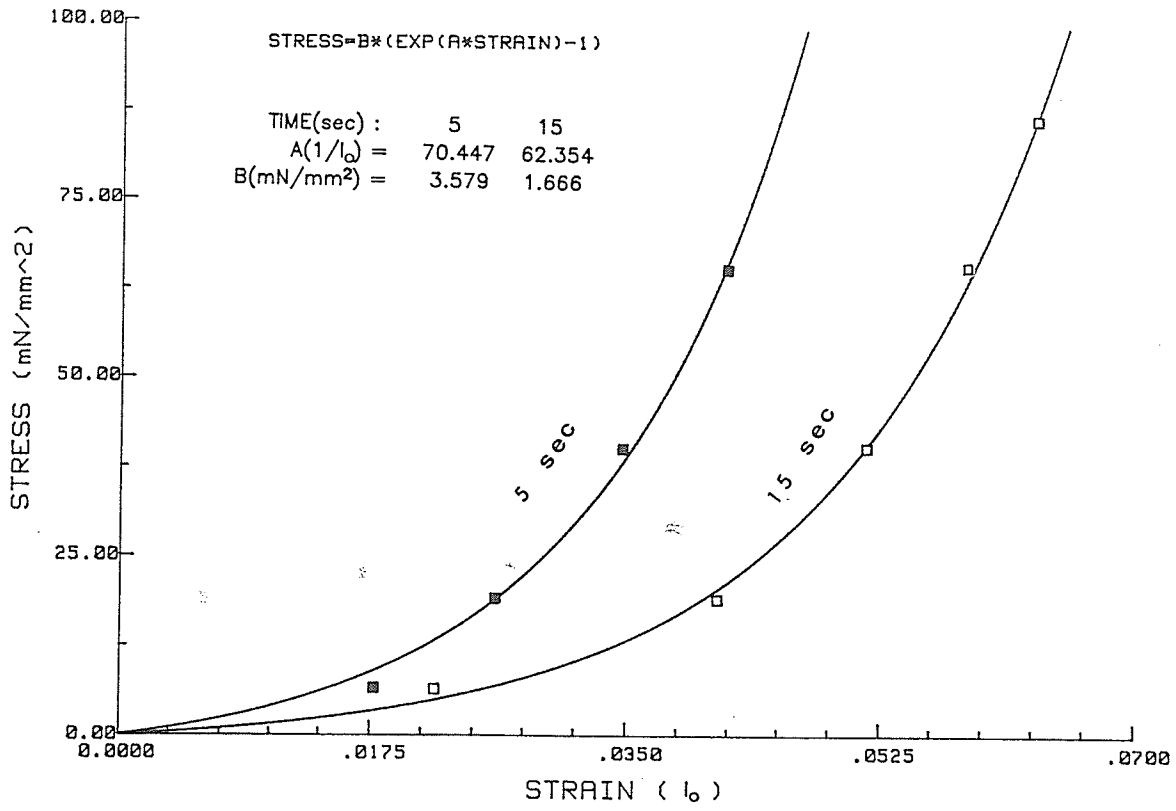

Figure 14. A typical pair of stress-strain curves of the SEC at 5 sec and 15 sec in an isometric contraction from the same preparation. The experimental equation ($\sigma=B(\exp(A\varepsilon)-1)$) was used to fit the experimental data.

DISCUSSION

a) Antigen-antibody Reaction.

The Schultz-Dale response, as seen *in vitro*, has not been reported before from systemic vessels. Our finding of the *in vitro* sensitization of canine saphenous vein indicates a possible role for the vascular smooth muscle in the phenomenon of systemic anaphylaxis in allergic subjects. The Schultz-Dale response of the sensitized SV results from the release of IgE-mediated mediator(s) from mast cells and basophils which then act on the smooth muscle. This is supported by the fact that it can be partially blocked by pyrilamine and phentolamine. In sensitized tracheal smooth muscle, the Schultz-Dale response can be totally blocked by histamine blocker (H_1 blocker), however, in sensitized saphenous vein, the Schultz-Dale response can only be blocked 25.4% by pyrilamine (H_1 blocker) and 34.5% by phentolamine. Some, as yet unidentified transmitter(s) other than histamine and catecholamine must be involved; our current experiments indicate these are not serotonin, acetylcholine, prostaglandins and leukotrienes. This partial inhibition in venous smooth muscle is similar to the partial inhibition produced by pyrilamine (25-45% block) and phentolamine (5-15% block) in sensitized pulmonary vein (Kong and Stephens, 1981), in which the cause for the remaining 40-70% remains unknown.

b) Norepinephrine Dose-Response Studies.

The saphenous vein is richly innervated with sympathetic nerves (Shepherd and Vanhoutte, 1975), which release

norepinephrine during stimulation by the nervous impulse. In addition, circulatory catecholamine are among the important modulators of vascular tone (Shepherd and Vanhoutte, 1975; Bulbring and Tomita, 1987). These, together with our finding that the Schultz-Dale response can be blocked by an α-adrenergic blocker by 35% indicate the importance of α-adrenergic agonists in determining vascular tone in sensitized subjects. To this end, dose-response curves for NE were elicited from a series of SV strips from ragweed sensitized and littermate control dogs.

Fig. 6 and Table 2 show that, compared with CSV, SSV is hyperreactive to NE, which is indicated by an upward shift of the curve. No significant difference in ED_{50} values between SSV and CSV indicates the sensitivity of SSV to NE is not altered. These findings are consistent with interpretation that the same numbers of agonist receptors of equal affinities are present but with increased efficacy or intrinsic activity. The efficacy in SSV may have been increased by reduced neuronal or extraneuronal uptake of NE (Bevan *et al.*, 1980), or by reduced ability of monoamine (MAO) or catechol-O-methyltransferase (COMT) to hydrolyse the NE to its inactive metabolites. It is also possible that alterations in excitation-contraction coupling via Ca^{2+} concentration, or in the contractile proteins of SSV may manifest themselves by an apparent increase in the efficacy of pharmacological agents.

c) Histamine Dose-response Studies.

With histamine (released upon antigen challenge) indicated to be a major mediator of anaphylaxis in sensitized SV, it became important to determine its effect upon saphenous venous tone. Dose-response curves for histamine were therefore elicited from sensitized and control SVs. The results (Fig. 5 and Table 1) revealed that the responses to histamine were the same for sensitized and control SVs at doses below 10^{-3} M. At higher doses (10^{-3} to 10^{-1} M), sensitized SV developed significantly higher isometric forces. However, at high doses of histamine, the contraction of saphenous venous smooth muscle was also partly due to norepinephrine. This was clearly shown in Fig. 7, where 10^{-3} M histamine induced a profound, second, tonic contraction, which was totally blocked by phentolamine (10^{-6} M). Interesting as this phenomenon is, its physiological relevance must be questioned *in vivo* of the high concentrations of histamine used. The second phase of contraction diminished at a lower dose (10^{-4} M). Since we have known that SSV is hyperreactive to norepinephrine, the increased response of sensitized SV at high doses of histamine can be well explained by the action of norepinephrine released from presynaptic nerve ending upon histamine binding to the presynaptic histamine receptors.

The above arguments suggest that the sensitized SV has the same sensitivity as, but higher reactivity to histamine than control SV. Thus it is tentatively concluded that the numbers of histamine receptors remains the same. The increase in reactivity might be due to either the presynaptic release of

norepinephrine or increased intrinsic activity, or both.

d) Pre-synaptic Histamine Receptor.

Histamine is known to be a powerful stimulant of "irritant" pulmonary receptors (Karczewski and Widdicombe, 1969) which give rise to vagal reflex bronchomotor effects, in addition to direct tracheobronchoconstriction. Histamine has not only a direct action on smooth muscle cells, but can also modify transmitter release from the adrenergic nerves and thus potentiate (Bevan *et al.*, 1975; Kroeger and Bergen, 1980) or suppress (McGrath and Shepherd, 1976; Powell, 1979) the muscle contractions produced by perivascular nerve stimulation. The action at dose of 0.9×10^{-6} M, histamine depressed the release of norepinephrine during contractions caused by electric stimulation (McGrath and Shepherd, 1975). Fig. 7 demonstrated the existence of presynaptic histamine receptors, which upon stimulation by histamine at dose of 10^{-3} M, cause release of norepinephrine from the presynaptic terminal leading to contraction of the smooth muscle. Norepinephrine was responsible for the second phase of the contraction elicited by histamine (Fig. 7), which could be totally blocked by phentolamine, an α-adrenergic blocker. The opposite actions of histamine to presynaptic norepinephrine release by different doses might suggest that histamine has a dual effect upon the presynaptic norepinephrine release depending on concentration and the specificity of the action phentolamine.

e) Spontaneous Activity and Myogenic Response.

Normal canine saphenous vein is quiescent and does not manifest a myogenic response. While such a response can be elicited from a number of smooth muscle preparations (Burnstock, 1972; Sparks, 1964), these have all uniquely been so-called single-unit types of smooth muscles characterized by spontaneous rhythmic contractile activity associated with action potentials. Treatment of canine tracheal smooth muscle with tetraethylammonium (TEA) causes biophysical change such that it resembles single unit smooth muscle (Kroeger and Stephens, 1975). Perhaps functionally, during the development of anaphylaxis, the normally quiescent multi-unit type of venous smooth muscle has been changed into a single unit type of muscle.

f) Length-tension Studies.

The results of the length-tension experiments indicate the canine saphenous vein has static properties qualitatively similar to skeletal and other smooth muscle (Murphy, 1980; Stephens and Hoppin, 1986). The overall shape of the active length-tension relation indicates the sliding filament mechanism, existing in skeletal muscle, is probably also operative in the saphenous vein.

Canine SV can develop substantial active tension which is comparable to that of other smooth muscles (Table 6). Maximum shortening capacity (ΔL_{max}) of canine SV, like that of canine trachealis, is very high, which implies an important *in vivo* role

Table 6. Dynamic smooth muscle parameters

Tissue	P_o (Kg/cm^2)	V_o (l_o/s)	ΔL_{max} (l_o)	SEC ($\%l_o$)	Stimulus
Hog carotid media*	3.78	0.12	0.6	7.2	K^+
Dog trachealis**	1.17	0.30	0.9	7.5	electrical
Dog SV	0.90	0.27	0.64	7.0	electrical

Values are delineated from L-T curves.

* Herlihy & Murphy, 1973; 1974.

** Stephens and Kromer, 1971; Stephens and Hoppin, 1976.

of SV in regulating blood flow. This is in marked contrast to systemic arteries whose capacity is very limited. The physiologic significance of this is not inconsiderable. The arteries are quite stiff at rest and considerable work is done in distending them with blood in systole. In diastole the considerable elastic recoil could aid in the delivery of blood to the periphery. In the veins where capacitance is about 19 times greater than that of arteries and which are highly compliant at rest, increased shortening capacity is required to facilitate venous return to the heart. The maximum shortening velocity (V_o) and SEC are again similar to those of canine trachealis (Table 6). These mechanical parameters of the SV indicate that it is a good preparation for studying smooth muscle mechanics.

Sensitized saphenous vein developed length-tension curves qualitatively similar to those of control SV. The maximum active tension development at l_o (P_o) of SSV was not significantly different from P_o of CSV. However, the maximum shortening capacity (ΔL_{max}) of SSV was increased by 11.4%. Measurement of ΔL_{max} by isotonic shortening also showed an increase of 6.1%, which by Poiseuille's law represents, theoretically, a 41% increase in resistance of the vessel to blood flow. Interestingly, such mechanical alterations have also been found in the sensitized tracheal smooth muscle (TSM), of which ΔL_{max} is increased while P_o remains the same (Antonissen, *et al.*, 1979). SSV provides another example of the inadequate of using P_o to detect changes in mechanical

properties. In our hands, both in the TSM and the SV changes in ΔL_{max} and V_o precede any change in P_o.

g) Time Course of Shortening Velocity and Force-velocity.

It is generally accepted that V_o reflects the cycling rate of the crossbridges. The shortening velocity ($V_{0.5\,mN}$) at light load (0.5 mN) is a good index of the true value of V_o. Velocity-time plot (Fig. 12) shows that, in the saphenous vein, the crossbridges active early in contraction are cycling at a much faster rate than those recruited later. V_o attains its peak at 5 seconds and then declines gradually even though the maximum tension is progressively increasing. This suggests that the two types of crossbridges operative in other smooth muscles (Dillon *et al.*, 1981) are also functioning in the saphenous vein and could be well explained by the theory of Dillon *et al.* (Dillon *et al.*, 1981; Dillon and Murphy, 1982) who postulated that calcium-calmodulin-dependent myosin light chain phosphorylation is responsible for the mobilization of early cycling crossbridges, whereas dephosphorylation is responsible for the later slowly cycling or noncycling, latch bridges. Another explanation for the drop in velocity could be the length dependent deactivation of the muscle, since the length of the CE decreased (caused by lengthening of the SEC) as isometric tetanic force increased. However, the SEC was lengthened only 2.5% of l_o from 5 to 15 seconds (Table 4). Our velocity versus time data showed that varying muscle length (through varying the preload) by 5% of l_o did not exert

any significant influence on the V_o at 15 seconds. Therefore the entire drop in V_o of 12% for CSV and 21% for SSV from 5 to 15 seconds can not be accounted for by the reduced-activation-at-short-length effect alone. Yet another explanation for a reduction in V_o with time is based on the presence of a so-called internal resistance to shortening; this has, so far, not been investigated by us.

For further elucidation of the behaviour of the two types of crossbridges, force-velocity experiments were carried out at 5 seconds at which the early crossbridges are maximally active and at 15 seconds at which the maximum tension is developed and maintained by late recruited crossbridges. It is worth noting that the value of V_o can be obtained by extrapolation of the force-velocity curve to zero force. Force-velocity curves (Fig. 13) are hyperbolic and can be fitted by Hill equation $V=b(P_o-P)/(P+a)$. The curvature of the force-velocity curve, and thus the efficiency of muscle contraction, is determined by the parameter a/P_o. As shown in Table 3, the parameter was not significantly different for the two F-V curves obtained at 5 and 15 seconds. In accordance with the velocity-time data, V_o at 5 second is shown again to be significantly higher than V_o at 15 sec.

In previous studies on the alterations of contractile properties of the sensitized tracheal smooth muscle (TSM), Antonissen, *et al.* (1979) have shown that, in TSM from the sensitized dog, both maximal ability to shorten (ΔL_{max}) and the maximal velocity of shortening are increased (Stephens, *et al.*,

1988) and that the increased velocity of shortening seen in the sensitized TSM is due to an increase in the cycling rate of crossbridges active early in contraction. These findings may represent a basic alteration in tracheal smooth muscle function that could contribute to the general bronchoconstriction seen in allergic asthma. Mechanical studies of sensitized pulmonary vessels (Kong and Stephens, 1983) also showed that both the magnitude and velocity of shortening were increased in the sensitized pulmonary artery.

The above findings in sensitized TSM and pulmonary artery led us to determine whether similar alterations existed in the SSV. Our study provides a positive answer. The ΔL_{max} of SSV is increased by 6.1%. Like sensitized TSM, in which maximal velocity is only increased in the early contraction phase (3 seconds), the SSV shows an increase in maximal velocity of shortening at early (5 seconds) phase but no difference at late (15 seconds) phase. In isotonic shortening, 67% of the total shortening is attained within 5 seconds. Therefore the increased shortening of SSV could be accounted for by increase in early rapidly cycling, but not in late, slowly cycling crossbridges. However, as the early bridges appear to be responsible for more than 70% of the total shortening in TSM (Stephens *et al.*, 1988), it is evident that the activity of these bridges is paramount in elucidating the mechanism underlying 'allergic' bronchospasm. Furthermore, as at this time, the regulatory myosin light chains are in a phosphorylated state, elucidation of the mechanism must be

focused on the properties of the phosphorylated light chain or the factors regulating this phosphorylation, such as Ca^{2+}-calmodulin and myosin light chain kinase.

As the myosin ATPase of smooth muscle differs radically from that of striated, inasmuch as calcium stimulates activity in the former while inhibiting it the latter, any study of induced myosin ATPase activity must include studies of calcium metabolism. These are to be conducted at a later date. Of course, the most directly related parameter is the myofibrillar ATPase activity itself. It has been shown that in canine tracheal smooth muscle the myofibrillar ATPase activity of sensitized muscle is faster than that of control (Stephens *et al.*, 1988). This could account for the increased V_o of sensitized muscle. Preliminary studies in our laboratory have shown (Stephens, unpublished observations) a similar increase in actomyosin ATPase activity in the sensitized saphenous vein.

The curvature of the force-velocity relation is measured by a/P_o. The value of a/P_o has been related to the thermodynamic efficiency of the muscle (Woledge, 1968) such that the more efficient the muscle , the lower the value of a/P_o. As shown in Table 3, a/P_o was significantly different at 5 sec, but not different at 15 sec between SSV and CSV. These tend to suggest that the early normal cycling crossbridges of SSV are more efficient than those of CSV, whereas the efficiencies of the later latch bridges of SSV and CSV are similar.

In summary, length-tension relationship studies showed that while there was no difference in maximum isometric tension (P_o) development between SSV and CSV, SSV exhibited a significant greater maximum shortening capacity (ΔL_{max}) than that of CSV. Unloaded shortening velocity (V_o), which reflects the crossbridge cycling rate, was determined at compartmental time. Maximum V_o was attained early (5 sec) in the contraction; 15% decline in V_o was observed at the plateau of the contraction (15 sec). At 5 sec, V_o of SSV was higher than that of CSV, though V_o's were same at 15 sec. The increase in shortening could be accounted for by increase in the early normal crossbridge cycling rate. The latter could be due to increased myosin light chain phosphorylation. However, this needs to be confirmed in future studies of myosin light chain phosphorylation in the sensitized saphenous vein.

h) Stiffness

As in other smooth muscles (Stephens and Kromer, 1971; Warshaw and Fay, 1983), the stiffness found in SV is a linear function of the tension. In both SSV and CSV, the stiffness increases from 5 to 15 seconds during an isometric contraction (Table 4 and Table 5). This seems to suggest that the number of attached crossbridges increases from 5 to 15 seconds. However, as the tension at 15 seconds is higher than that at 5 sec during an isometric contraction and stiffness is proportional to tension, the increased stiffness from 5 to 15 sec might simply be due to the increased tension. A possible

criterion has been suggested (Seow and Stephens, 1987) to determine the number of attached crossbridges by examining the stiffness at a constant tension, which can also be achieved by evaluating the constant A of equation 1, where A represents the magnitude of the stiffness. By this criterion, the decrease of A from 5 to 15 seconds in SV tends to suggest that attached number of crossbridges in SV actually declined during the latch maintenance phase of isometric tension. No significant differences between the corresponding parameters of SECs of control and sensitized saphenous veins suggests the SEC of sensitized muscle is normal.

i) Physiological Significance of the Alteration of Mechanical Properties.

The systemic venous system accounts for as much as 80% of the total vascular volume. Thus, the veins are a reservoir of considerable capacity in the vascular system and are subject to hydrostatic and other physical forces that can cause redistribution of blood within this reservoir in an amount sufficient to alter cardiovascular function through altering venous return (Shepherd and Vanhoutte, 1975). The hypotension developed during anaphylactic shock indicates that the venous circulatory mechanism must have failed. In studies of the mechanics of anaphylactic shock, Essex *et al.* (1965) demonstrated constriction of hepatic veins sphincters and engorgement of the liver. Wagner *et al.* (1986) suggested the primary circulatory mechanisms are the increased systemic

resistance to venous return and decreased mean systemic pressure for venous return. Our finding of increased shortening capacity *in vitro* of the sensitized saphenous vein provides a basis for the increased *in vivo* constriction. Venoconstriction would increase venous resistance, which, together with decrease of mean systemic venous pressure, would eventually lead to the decreased venous return and the ultimate development of shock.

REFERENCES

Altura, B.M., and S. Halevy. (1977).Cardiovascular actions of histamine. In: Handbook of Experimental Pharmacology, ed. M. Recha, E. Silva. vol 18, part 2: Histamine and antihistaminics. Springer-Verlag, Heideberg, pp. 1-39.

Anderson-Cedergren, E. (1959). Ultrastructure of motor end plate and sarcoplasmic components of mouse skeletal muscle fiber as revealed by three-dimensional reconstructions from serial sections. J. Ultrastruct. Res. Suppl. 1:5-91.

Antonissen, L.A., R.W. Mitchell, E.A. Kroeger, W. Kepron, K.S. Tse and, N.L. Stephens. (1979). Mechanical alterations of airway smooth muscle in a canine asthmatic model. J. Appl. Physiol. 46: 681-687.

Antonissen, L.A., R.W. Mitchell, E.A. Kroeger, W. Kepron, N.L. Stephens, and J. Bergen. (1980). Histamine pharmacology in airway smooth muscle from a canine model of asthma. J. Pharmacol. Exp. Ther. 213: 150-155.

Austen, K.F. (1973). Ch. 8 A Review of Immunological, Biochemical and Pharmacological Factors in the Release of Chemical Mediators from Human Lung. In: Asthma: Physiology, Immunopharmacology and Treatment. ed. K.F. Austen and L.M. Lichtenstein. Academic Press, New York, pp. 109-22.

Austen, K.F., S.I. Wasserman, and E.J. Goetzl. (1976). Mast cell-derived mediators: Structural and functional diversity and regulation of expression. In: Molecular and Biological Aspects of the Acute Allergic Reactions. ed.

S.G.O. Johansson, K. Strandberg and B. Uvnas. Plenum, New York, pp. 293-318.

Bagby, R.M., and M.D. Corey-Kreyling. (1984). Structural aspects of the contractile machinery of smooth muscle: "Is the organization of contractile elements compatible with a sliding filament mechanism?". In: Smooth Muscle Contraction. (ed.) N.L. Stephens. Dekker, New York.

Bevan, J.A., R.D. Bevan, and S.P. Duckles. (1980). Ch 18. Adrenergic regulation of vascular smooth muscle . In: The Cardiovascular System. vol II. ed. D.F. Bohr *et al.*, Am. Physiol. Society. Bethesda. pp. 515-56.

Bulbring, E., and T. Tomita. (1987). Catecholamine action on smooth muscle. Pharmacol. Rev. 39(1): 49-96.

Bozler, E. (1948). Conduction, automaticity, and tonus of visceral muscles. Experientia 4:213.

Bremel, R.D. (1974). Myosin linked calcium regulation in vertebrate smooth muscle. Nature 252: 405-7.

Bremel, R.D., A. Sobieszek, J.V. Small. (1977). Regulation of actin-myosin interaction in vertebrate smooth muscle. In: The Biochemistry of Smooth Muscle. ed. N.L. Stephens. pp. 533-49.

Brutsaert, D.L., V.A. Claes, and E.H. Sonnenblick. (1971). Effects of abrupt load alterations on force-velocity-length and time relation during isometric contractions of heart muscle: load clamping. J. Physiol. (Lond.) 216: 319-30.

Brutsaert, D.L. (1974). The force-velocity-length-time

interrelation of cardiac muscle. The Physiological Basis of Starling's Law of the Heart. Ciba Foundation Symposium 24, pp. 155-186.

Burnstock, G. Purinergic nerves. Pharmacol. Rev. 24:509-581, 1972.

Capurro, N., and R. Levi. (1975). The heart as a target organ in systemic allergic reactions: Comparison of cardiac anaphylaxis *in vivo* and *in vitro*. Circ. Res. 36: 520-8.

Chamley, J.H., G.R. Campbell, J.D. McConnell, and U. Groschel-Stewart. (1977a). Comparison of vascular smooth muscle cells from adult human, monkey and rabbit in primary culture and in subculture. Cell Tiss. Res. 177:503.

Chamley, J.H., U.L. Groschel-Stewart, . R. Campbell, and G. Burnstock. (1977b). Distinction between smooth muscle fibroblasts and endothelial cells in culture by the use of fluoresceinated antibodies against smooth muscle actin. Cell. Tiss. Res. 177-445.

Csapo, A. (1948). Actomyosin content of the uterus. Nature 162:218-9.

De May, J.G., and P.M. Vanhoutte. (1982). Heterogeneous behaviour of the canine arterial and venous wall: importance of the endothelium. Circ. Res. 51:439-47.

De May, J.G., and P.M. Vanhoutte. (1983). Anoxia and endothelium-dependent reactivity of the canine femoral artery. J. Physiol. 335:65-74.

Dillon, P.F., M.O. Aksoy, S.P. Driska, and R.A. Murphy. (1981). Myosin phosphorylation and the crossbridge

cycling in arterial smooth muscle. Science Wash. DC 211:495-7.

Dillon, P.F., R.A. Murphy, and V.A. Claes. (1982). Tonic force maintenance with reduced shortening velocity in arterial smooth muscle. Am. J. Physiol. 242:C102.

Donegan, J.F. (1921). The physiology of veins. J. Physiol. (Lond.) 55:226.

Driska, S., and D.J. Hartshorne. (1975). The contractile proteins of smooth muscle. Properties and components of a Ca^{2+}-sensitive actomyosin from chicken gizzard. Arch. Biochem. Biophys. 167: 203-12.

Ducharme, D.W., .R. Weeks, and R.G. Montgomery. (1968). Studies on the mechanism of the hypertensive effect of prostaglandin F2 . J. Pharmacol. Exp. Ther. 160:1.

Dyer, D.C. (1970). Comparison of the constricting actions produced by serotonin and prostaglandins on isolated sheep umbilical arteries and veins. Gynecol. Invest. 1:204.

Dyer, D.C., K. Ueland, and M. Enf. (1972). Responses of isolated monkey umbilical veins to biogenic amines and polypeptides. Arch. Int. Pharmacodyn. Ther. 200:213.

Ebashi, S., M. Endo, and I. Ohtsuki. (1969). Control of muscle contraction. Q. Rev. Biophys. 2:351

Essex, H.E. (1965). Anaphylactic and anaphylatoid reaction. In: Handbook of Physiology. Wash. D.C.:2391-2408.

Eyre, P. (1972). Release of 5-hydroxyptamine from sensitized calf lung by antigen and compound 48/80. Arch. Int.

Pharmacodyn. 199:245-52.

Eyre, P. (1977). Pulmonary histamine H1 and H2 receptor studies. In: Asthma, Physiology, Immunopharmacology, and Treatment. ed. L.M. Lichtenstein and K.F. Austen. Academic Press. pp. 169-80.

Feigen, G.A., E.M. Vaugham Williams, J.K. Peterson, and L.B. Nielson. (1960). Histamine release and intracellular potentials during anaphylaxis in the isolated heart. Circ. Res. 8: 713-23.

Franklin, K.L. (1937). A Monograph on Veins. Springfield, Illinois, Charles C Thomas, Publisher. p1.

Furchgott, R.F., and J.V. Zawadzki. (1980). Obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288:373-6.

Gabbiani, G., M.C. Badonnel, and G. Majno. (1970). Intra-arterial injections of histamine, serotonin, or bradykinin: a topographic study of vascular leakage. P.S.E.B.M., vol 135.

Galant, S.P., L. Durisetti, S. Underwood, S. Allred, and P.A. Insel. (1980). Beta-adrenergic receptors of polymorphonuclear particulates in bronchial asthma. J. Clin. Invest. 65:577-85.

Gergely, J. (1976). Troponin-tropomyosin-dependent regulation of muscle contraction by calcium. In: Cell Motility. ed. R. Goldman, T. Pollard and J. Rosenbaum. Cold Spring Harbor Laboratory, Book A:137-49.

Glover, W.E., A.D.M. Greenfield, B.S.I. Kidd, and R.F. Whelan. (1958). The reactions of the capacity blood vessels of the human hand and forearm to vaso-active substances infused intraarterially. J. Physiol. (Lond.) 140:113.

Gold, W.M. (1973). Ch 11 Cholinergic Pharmacology in Asthma. In: Asthma: Physiology, Immunopharmacology, and Treatment. ed K.F. Austen and L.M. Lichtenstein. pp. 169-184.

Greenberg, R.A., and H.V. Sparks. (1969). Prostaglandins and consecutive vascular segments of the canine hindlimb. Am. J. Physiol., 216:567.

Greenberg, S., J.P. Long, and F.P. Diecke. (1973). Effect of prostaglandins on arterial and venous tone and calcium transport. Arch. Int. Pharmacodyn. Ther. 204:373.

Groschel-Stewart, U., J.H. Chamley, J.D. McConnell, and G. Burnstock. (1975). Comparison of the reaction of cultured smooth muscle and cardiac muscle cells and fibroblasts to specific antibodies to myosin. Histochemie 43: 215-24.

Guimaraes, S., and W. Osswald. (1969). Adrenergic receptors in the veins of the dog. Eur. J. Pharmacol. 5:133.

Haddy, F.J. (1960). Serotonin and the vascular system. Angiology 11:21.

Hartshorne, D.J. (1984). Regulatory mechanisms in smooth muscle: the role of myosin phosphorylation. In: Smooth Muscle Contraction. ed. N.L. Stephens. New York: Dekker, pp.271-82.

Hartshorne, D.J., and A. Gorechka. (1980). Ch4 Biochemistry of the contractile proteins of smooth muscle. In: The Cardiovascular System. vol 2. American Physiological Society, Bethesda. pp. 93-120.

Herlihy, J.T., and R.A. Murphy. (1974). Force-velocity and series elastic characteristics of smooth muscle from the hog carotid artery. Circ. Res. 34: 461-6.

Hill, A.V. (1965). Trails and Trails in Physiology. London: Arnold. Hill, A.V. (1970). First and last Experiments in Muscles Mechanics. London: Cambridge Univ. Press.

Hodgkin, A.L., P. Horowicz. (1960). The effect of sudden changes in ionic concentrations on the membrane potential of single muscle fibres. J. Physiol. (Lond.) 153:370-85.

Holman, M.E., C.B. Kasby, M.B. Suthers, and J.A.F. Wilson. (1968). Some properties of the smooth muscle of rabbit portal vein. J. Physiol. (Lond.) 196:111.

Hooker, D.R. (1918). The veno-pressor mechanism. Am. J. Physiol. 46:591.

Hughes, J., and J.R. Vane. (1967). An analysis of the responses of the isolated portal vein of the rabbit to electrical stimulation and to drugs. Br. J. Pharmacol. Chemother. 30:46.

Hurwitz, R., R.W. Cambell, P. Gordon, and F.J. Haddy. (1961). Interaction of serotonin with vasoconstrictor agents in the vascular bed of the denervated dog forelimb. J. Pharmacol. Exp. Ther. 133:57.

Huxley, A.F. (1959). Local activation in muscle. Ann. NY Acad.

Sci. 81:446-52.

Karczewski, W., and J.G. Widdicombe. (1969). The role of the vagus nerves in the respiratory and circulatory reaction to anaphylaxis in rabbits. J. Physiol. Lond. 201:293-304.

Kepron, W., J.M. James, B. Kirk, A.H. Sehon, and K.S. Tse. (1977). A canine model for reaginic hypersensitivity and allergic bronchoconstriction. J. Allergy Clin. Immunol. 59: 46-9.

Kong, S.K., and N.L. Stephens. (1981). Pharmacological studies of sensitized canine pulmonary blood vessels. J. Pharmacol. Exp. Ther. 219:551-7.

Kong, S.K., and N.L. Stephens. (1983). Mechanical properties of pulmonary arteries from sensitized dogs. J. Appl. Physiol. 55: 1669-1673.

Kroeger, E.A., and J. Bergen. (1980). Presynaptic regulation of cholinergic and adrenergic neurotransmitter release by histamine and serotonin in airway smooth muscle. (abstract). Fed. Proc. 39:1176.

Kroeger, E.A., and Stephens. (1975). Effect of tetraethylammonium on tonic airway smooth muscle: initiation of phasic electrical activity. Am. J. Physiol. 228(2): 633-36.

Levy, J.H., M.F. Roizen, and J.M. Morris. (1986). Anaphylactic and anaphylatoid reactions. Spine 11(3): 282-91.

Levy, J.V. (1972). Effect of synthetic bradykinin on contractile tension of human saphenous vein stripes. Br. J. Pharmacol., 46:517.

Lichtenstein, L.M. (1973). Ch 7 The Control of IgE-Mediated Histamine Release: Implications for the Study of Asthma. In: Asthma: Physiology, Immunopharmacology, and Treatment. ed K.F. Austen and L.M. Lichtenstein. Academic Press, New York, pp. 91-107.

Maloff, G.A. (1934). Zur Pharmakologie der Venen: Über selbständige Venekontraktionen. Arch. Int. Pharmacodyn. Ther. 48:333.

Mask, A.C., P.G. Schmid, J.W. Eckstein, and M.G. Wendling. (1971). Venous responses to prostagandin F2 . Am. J. Physiol. 220:222.

Murphy, R.A. (1976). Contractile system function in mammalian smooth muscle. Blood Vessels 13: 1-23.

Murphy, R.A. (1980). Ch 13. Mechanics of vascular smooth muscle. In: Handbook of Physiology. The Cardiovascular System. vol. II. Bethesda, Am. Physiol. Society, pp. 325-51.

O'Mahony, D.P. (1963). The pharmacology of isolated venous muscle. Ir. J. Med. Sci., Series 6:401.

Patterson, R. (1960). Investigation of spontaneous hypersensitivity of the dog. J. Allergy 31: 351-363.

Patterson, R. (1969). Laboratory models of reaginic allergy. Prog. Allergy 13: 322.

Pinckard, R.N., M. Halonen, and A.L. Meng. (1972). Preferential expression of anti-bovine serum albumin IgE homocytotropic antibody synthesis and anaphylactic sensitivity in the neonatal rabbit. J. Allergy Clin.

Immunol. 49: 301.

Piper, P.J. (1984). Formation and actions of leukotrienes. Physiol. Rev. vol 164:744-761.

Potter, J.D. (1974). The content of troponin, tropomyosin, actin and myosin in rabbit skeletal muscle myofibrils. Arch. Biochem. Biophys. 162:436.

Ruegg, J.C. (1986). Calcium in Muscle Activation. Spinger-Verlag. Said, S.I. (1982). Vasoactive peptides in the lung, with special reference to vasoactive intestinal peptide. Exp. Lung Res. 3:343-8.

Said, S.I. (1982). Vasoactive peptides in the lung, with special reference to vasoactive intestinal peptide. Exp. Lung Res. 3: 343-8.

Seow, C.Y., N.L. Stephens. (1987). Time dependence of series elasticity in tracheal smooth muscle. J. Appl. Physiol. 62(4): 1556-61.

Shepherd, J.T., and P.M. Vanhoutte. (1975). Vein and their Control. Publ: Saunders.

Sicuteri, F., M. Anciullacci, and P.L. Del Bianco. (1966). Alpha and beta adrenergic receptors in human veins: activity of a new beta-blocking agent. Med. Pharmacol. Exp. 15:73.

Simmons, R.M., and B.R. Jewell. (1974). Mechanics and models of muscular contraction. In: Recent Advances in Physiology. ed. R.J. Linden. Edinburgh: Churchill Livingstone. pp. 87-147.

Small, J.V., and A. Sobieszek. (1980). The contractile apparatus of smooth muscle. Int. Rev. Cytol. 64:241.

Smith, P.L., A. Kagey-Sobotka, E.R. Bleecker, R. Traystman, A.P. Kaplan, H. Gralnick, M.D. Valentine, S. Permutt, and L.M. Lichtenstein. (1980). Physiologic manifestations of human anaphylaxis. J. Clin. Invest. 66:1072-80.

Somlyo, A.P., C.E. Devine, A.V. Somlyo, and R.V. Rice. (1973). Filament organization in vertebrate smooth muscle. Phil. Trans. Roy. Soc. London Ser. B265: 223-9.

Somlyo, A.V., and A.P. Somlyo. (1968). Electromechanical and pharmacomechanical coupling in vascular smooth muscle. J. Pharmacol. Exp. THer. 159:129-45.

Somlyo, A.P., and A.V. Somlyo. (1985). Excitation-contraction coupling and the ultrastructure of smooth muscle. Circ. Res. 57:497-507.

Somlyo, A.P., and A.V. Somlyo. (1986). Smooth muscle structure and function. In: The Heart and Cardiovascular System. ed. H.A. Fozzard *et al.*, Raven Press, New York.

Souhrada, J.F., and J. Loader. (1979). Changes of airway smooth muscle in experimental asthma. In: Mechanisms of Airway Obstruction in Human Respiratory Disease, ed. M.A.

Stephens, N.L., R. Cardinal, and B. Simmons. (1977). Mechanical properties of tracheal smooth muscle: effect of temperature. Am. J. Physiol. 233(3): C92-98.

Stephens, N.L., and F.G. Hoppin, JR. (1986). Ch 17. Mechanical properties of airway smooth muscle. In: Handbook of Physiology. The Respiratory System. vol. III. pp. 263-

276.

Stephens, N.L., S.K. Kong, C.Y. Seow. (1988). Mechanisms of increased shortening of sensitized airway smooth muscle. Asthma: basic mechanisms and clinical management. ed. P.J. Barnes. Academic Press. pp. 231-54.

Stephens, N.L., E.A. Kroeger, and J.A. Mehta. (1969). Force-velocity characteristics of respiratory airway smooth muscle. J. Appl. Physiol. 26 :685-92.

Stephens, N.L., and U. Kromer. (1971). Series elastic component of tracheal smooth muscle. Am. J. Physiol. 220: 1890-95.

Tregear, R.T., and J.M. Squire. (1973). Myosin content and filament structure in smooth muscle and striated muscle. J. Mol. Biol. 77: 279-90.

Vanhoutte, P.M. (1974). Inhibition by acetylcholine of adrenergic neurotransmission in vascular smooth muscle. Circ. Res. 34: 317-26.

Vanhoutte, P.M. (1978). Heterogeneity in Vascular Smooth Muscle. in Microcirculation vol 2. ed. G. Kaley and B.M. Altura. pp. 181-309. Univ. Park Press, Baltimore.

Wagner, E.M., W.A. Mitzner, and E.R. Bleecker. (1986). Peripheral circulatory alterations in canine anaphylactic shock. Am. J. Physiol. 251: H934-H940.

Warshaw, D.M., and F.S. Fay. (1983). Cross-bridge elasticity in single smooth muscle cells. J. Gen. Physiol. 82: 157-99.

Warshaw, D.M., W.J. McBride, and S.S. Work. (1987). Corkscrew-like shortening in single smooth muscle cells. Science 236: 1457-9.

Waterman, L. (1930). Sur l'action des poisons sympathiques et parasympathiques sur les veins isolées. Arch. Neerl. Physiol. 155:545.

Weber, A., and J.M. Murray. (1973). Molecular control mechanisms in muscle contraction. Physiol. Rev. 53:612.

Wiedeman, M.P. (1963). Dimensions of blood vessels from distributing artery to collecting vein. Circ. Res. 12:375.

Woledge, R.C. (1968). The energetics of tortoise muscle. J. Physiol. (Lond.) 197:685-707.

Woledge, R.C., N.A. Curtain, and E. Homsher. (1985). Energetic Aspects of Muscle Contraction. Academic Press.

Yanagisawa, M., H. Kurihara, S. Kimura, Y. Tomobe, M. Kobayashi, Y. Mitsui, Y. Yazaki, K. Goto, and T. Masaki. (1988). A novel potent vasoconstrictor peptide produced by vascular endothelial cells. Nature 332: 411-5.