Effect of Temperature on the Responsiveness of Human
Digital Arteries with Special Reference to Raynaud's
Phenomenon

by

Elizabeth Dean

A thesis
presented to the University of Manitoba
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

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EFFECT OF TEMPERATURE ON THE RESPONSIVENESS OF HUMAN

DIGITAL ARTERIES WITH SPECIAL REFERENCE TO RAYNAUD'S PHENOMENON

BY

ELIZABETH DEAN

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

In this study in vivo and in vitro experiments were con-
ducted to try and elucidate the mechanisms which may under-
lie normal and abnormal responses of human digital arteries
to cold including local and indirect reflex effects.   Meas-
urements of finger systolic pressure in response to changes
in local temperature during reflex induced changes in vaso-
motor tone were utilized to study physiologic mechanisms in
vivo and to assess the potential value of the measurements
as a clinical diagnostic tool. Since in vivo studies do not
allow assessment of basic cellular mechanisms, in vitro ex-
periments were carried out in human digital arteries.   Pre-
vious techniques to measure cold sensitivity in vivo have
not proven reliable.   Nielsen and Lassen (1977) described a
procedure to quantify cold sensitivity by measurements of
finger systolic pressure after a period of local cooling
during arterial occlusion. The reductions in finger systol-
ic pressure (FSP) were used as an index of arterial tone.
They found marked reduction or loss of FSP in patients with
Raynaud's phenomenon.

In preliminary studies,   we used the Nielsen and Lassen
method to compare changes in FSP in healthy subjects and a
small group of Raynaud's patients at three local finger

temperatures during vasoconstriction and vasodilatation induced by body cooling or heating, respectively. A slow stepwise deflation of the blood pressure cuffs, a technique previously shown to give reliable measurements of systolic pressures in the digits, was used. Compared with healthy subjects, Raynaud's patients exhibited lower FSP in response to finger cooling and this response was augmented in both groups during body cooling. Also, the Raynaud's patients had lower pressures during body cooling even without local cooling. However, no vessel closure (loss of measurable pressure) was observed in either group. Our body cooling procedure was more stringent than those used previously by others. However, possible warming of the finger during stepwise deflation of the blood pressure cuff may have been responsible for the absence of closure during these experiments. This led to the study of the effect of various deflation rates on the measurements and of other aspects of the technique of measurements which have not been investigated previously. These included examination of the type of distal sensor used to detect the beginning of blood flow during deflation of the blood pressure cuff, the effect of a period of arterial occlusion and comparison of measurements in one finger versus two fingers of the same hand simultaneously. The findings indicated that the mercury-in-rubber strain gauge was a reliable sensor for cold sensitivity testing. The use of a short period (6 minutes) of arterial occlusion during local cooling did not appreciably affect finger pres-

sures. Negligible differences were found between systolic pressures taken individually and on two fingers. A deflation rate of 2 mmHg/sec was found to be preferable to either a slower or faster rate which likely overestimated or underestimated respectively the measured pressure.

Also in contrast to previous studies, we controlled vasomotor state during measurements by continuously monitoring the skin temperature of the tips of the adjacent fingers.

With these modifications we used the method to examine 37 subjects with and 20 without Raynaud's phenomenon. During body cooling, a positive test, i.e., vessel closure (zero pressure) in a locally cooled finger ($10^{\circ}C$) was found in two-thirds of Raynaud's patients and in 2 of 20 subjects without Raynaud's phenomenon. Thus, vessel closure appears to be a good indicator of the presence of Raynaud's phenomenon but its absence does not rule it out. Also, during body cooling closure occurred in control fingers which were not locally cooled in 13 of 37 patients and in no subjects without Raynaud's phenomenon. This latter finding, which was not previously reported, provides evidence for the importance of sympathetic vasoconstriction in the precipitation of Raynaud's phenomenon and may have special diagnostic potential. It also raises question about the use of the difference between pressure in cooled and noncooled fingers by other investigators in clinical assessment of Raynaud's phenomenon. Although there were some differences among sub-

groups of patients with Raynaud's phenonemon of various etiologies, these were not statistically significant perhaps due in part to the relatively small numbers of patients.

Elucidation of the underlying pathophysiology of Raynaud's phenomenon will likely require in vitro study of cellular mechanisms. To date no model exists for the in vitro study of digital arteries implicated in the phenomenon. Therefore, we examined the use of human digital arteries removed from limbs amputated as a result of arterial disease, neoplastic disease or trauma. Helically-cut muscles were used to measure the development of isometric tension in response to a variety of stimuli. This preparation was found to be a satisfactory model for study of the responsiveness of the smooth muscle of human digital arteries as the muscles were viable and gave consistently reproducible responses.

Several hypotheses were tested regarding the effect of cold. First, normal and abnormal reactivity to cold of digital arteries could reflect altered adrenoceptor-mediated function. Alpha adrenoceptor-mediated activity was found to be depressed with cooling down to $10^{\circ}C$. β adrenoceptor blockade failed to augment α adrenoceptor responses, and the β adrenoceptor agonist, isoproterenol, failed to elicit relaxation in most cases. Thus, the presence of β adrenoceptors and their role need further investigation.

Good indirect evidence existed for a Na/K pump with an electrogenic function in human digital arteries. Following pre-incubation with K deficient solution and reintroduction of normal K, the response threshold to K was increased. This observation suggested the muscles had become hyporesponsive secondary to an apparent transient hyperpolarization corresponding to a maximal activation of the Na/K pump. However, cold induced contractures secondary to a presumed inhibition of these mechanisms did not consistently occur as the majority of muscles tested did not show increased tone with cooling. However, increase in basal tone in 30 percent of muscles suggests that this mechanism may contribute to exaggerated responses in Raynaud's phenomenon. Delayed relaxation with cooling however was observed in all muscles and this latter finding could also contribute to the exaggerated responses to cold. The absence of some of these findings in other muscles may be due to a variety of factors both technical and clinical (drugs, severe ischemia) which might not have been controlled despite attention to such details. Alternatively some other mechanisms or modulating factors (e.g. endothelium-derived relaxing factor, prostaglandins etc.) rather than hyperreactivity in response to cooling could be responsible. Further studies are required to resolve these questions and to explain some apparent differences betwen the results of the _in vivo_ and _in vitro_ experiments.

CHAPTER I

INTRODUCTION

The effect of temperature on the responsiveness of human digital arteries is not well understood. Cooling the digits locally or the body generally is known to elicit peripheral vasoconstriction. In patients with Raynaud's phenomenon, abnormal responses to cooling elicit vasospasm associated with virtual cessation of blood flow to the digits and white or cyanotic discoloration. Study of centrally and locally mediated responses to cooling are needed to enhance our understanding of both normal and abnormal reactivity of human digital arteries implicated in Raynaud's phenomenon.

Although there is evidence that digital arteries close during vasospastic attacks, the mechanisms of these phenomena are not well understood. Also, Raynaud's phenomenon may be associated with several disease states or conditions. Based on our current understanding however, two broad categories of Raynaud's phenomenon are observed clinically. Raynaud's phenomenon which exists in the absence of any underlying disease state is considered to be the primary form of the disorder and is termed Raynaud's disease. Raynaud's phenomenon associated with underlying conditions which may result in irreversible, organic damage to the blood vessels, is termed the secondary form of the disorder. Conditions

that may be associated with Raynaud's phenomenon include arterial occlusive disease due to atherosclerosis, Buerger's disease, occupational trauma, connective tissue disease, abnormal blood constituents and certain drugs. Where there is obstruction, blood flow and blood pressure may be reduced even at comfortable ambient temperatures. In such cases, abnormal responses may be produced with even mild to moderate cooling of the fingers and normal contraction of the smooth muscle can result in vessel spasm and closure. Therefore, in Raynaud's phenomenon various abnormalities may contribute or be responsible for vasospastic episodes whereas the category of primary Raynaud's disease implies that there is some functional abnormality of vascular smooth muscle, its control systems or both.

Marked color changes are associated with the vasospastic attacks in Raynaud's phenomenon and consist of pallor, and/or cyanosis of one or more fingers, on one or both hands. An unmistakable feature is the clear demarcation of the area of color change, e.g., part of or the entire digit. Toes are less often affected, and occasionally parts of the face may be involved.

The management of Raynaud's phenomenon is not based on a clear understanding of its mechanism which remains to be elucidated. Therefore, treatment in most cases has been palliative rather than rationally based on a reversal of a pathologic mechanism. Patients with either type of Ray-

naud's phenomenon are usually cautioned to avoid situations in which the symptoms are aggravated. This may entail having to give up an occupation, recreational hobbies or a specific geographic place of residence, in addition to learning how to dress appropriately and taking medication. Only in a small proportion of cases can the underlying causes be eliminated with treatment, for example, surgery for cervical rib removal.

The etiology of peripheral vasospasm is also of considerable interest because it may bear some relationship to the occurrence and management of vasospasm in other vascular beds. Patients with Raynaud's disease have been reported to demonstrate a higher incidence of such disorders as migraine headache and variant angina compared with the normal population, suggesting some indirect evidence for a generalized vasospastic tendency (Miller et al., 1981).

Diagnostic testing and objective assessment of cold sensitivity in the laboratory have not been found reliable. A noninvasive test described by Nielsen and Lassen in 1977 was of particular interest. Apparent finger systolic pressure changes in response to a cold challenge provided an index of digital artery tone. Apparent reductions in finger systolic pressure reflected increased digital artery tone. Thus, the lower the pressure following local cold stimulation of the fingers reflected an increased reactivity of the digital arteries.

In the past, various cold sensitivity tests have been found to be unreliable. Most of these are based on measurements of blood flow which depend on peripheral resistance and specifically of the arterioles. The method of Nielsen and Lassen (1977) is of interest because for the first time it has provided a method that appears to allow for assessment of tone and closure of the digital arteries. Reports (Krähenbühl et al., 1977; Nielsen and Lassen, 1977; Hirai, 1979; Nielsen, 1978; Nielsen et al, 1980) of this method showed differences in methodology, and results, and did not appear to control for vasomotor state. Therefore, because of these discrepancies and because the method allows for assessment of the digital arteries implicated in the phenomenon, detailed study of various aspects of the methodology and its application constituted one major phase of this work. If the test could be shown to be a valid and reliable measure of changes of arterial tone with cooling, this non-invasive procedure could be used to characterize Raynaud's phenomenon, and shed some light on its underlying pathophysiology and management. For example, the method could be applied to elucidate the relative contribution of neural and local reactivity to the production of vasospasm and to the study of decreased transmural pressure resulting from organic arterial obstruction. Previously, study of the effect of these factors on the major digital arteries was not possible.

However, because there are limitations to _in vivo_ studies and specific details of mechanisms at the cellular level cannot be ascertained, the effect of temperature on vascular reactivity was examined in an _in vitro_ human model utilizing digital arteries from amputated limbs. Such a model using the arteries from human fingers and toes has not, to our knowledge, been previously reported in the literature. _In vitro_ studies were therefore designed to investigate vascular responses of these vessels.

Cold potentiation of vascular smooth muscle contractility as the underlying cause of Raynaud's disease could be secondary to a variety of mechanisms at the cellular level. Increased α adrenoceptor affinity with moderate cooling to 20°C in superficial canine veins (Janssens and Vanhoutte, 1978) has suggested a possible mechanism for the disorder.

The presence of β adrenoceptors which may relax the tone of the peripheral arteries is controversial. Should β receptors be normally present in human digital arteries, this might suggest that in vasospastic disease, β receptors are absent, diminished in number or rendered inactive in some unexplained way.

Normally the function of the Na/K pump subserves a number of distinct ionic processes, each of whose net result is to promote relaxation. In importing K, intracellular K is diminished and, as E_M thus becomes negative, the resting mem-

brane potential is established. The latter is important to tone in that depolarization, by whatever mechanism, results in activation of Ca channels and a tendency toward increased tone. A second function of the Na/K pump, the export of Na, results in an inward electrochemical gradient for Na which, in turn, provides the energy for the forward operation of the Na/Ca exchange mechanism. The importance of this prominent mechanism of relaxation of the tone of human digital arteries has not yet been determined but its possible contribution must be considered whenever an alteration of Na/K pump function is effected. Finally, as a result of its unequal exchange of ions (3Na:2K), the pump directly produces a current which contributes to the normal polarization of the membrane. Thus, a decrease in Na/K pump function and the concomitant electrogenicity leads to an immediate partial depolarization of E_M whose effect is isotropic. With respect to the temporal relation of these effects, loss of electrogenicity results in instantaneous depolarization compared with the relatively longer time that would be required for dissipation of the Na and K gradients and thus, loss of Na and K exchange and E_M. Therefore, accurate recording of the time course of the effects of cooling on prescribed parameters could potentially provide indirect evidence for differentiating between mechanisms related to electrogenic pumping and redistribution of ionic gradients.

Identification of adrenergic receptor populations and the presence of an electrogenic Na-K pump and its sequelae in digital arteries is needed to better understand normal and abnormal responses including the effect of temperature in these vessels.

A. ANATOMY OF THE DIGITAL ARTERIES

In order to understand the physiology of normal responses
and the pathophysiology of abnormal responses to cold in the
digital arteries of the fingers and toes, a brief review of
the relevant anatomy is presented. The gross structure and
function of the blood vessels appear to be comparable in the
hand and foot (Williams and Warwick, 1980). The common
aspects of the vasculature of the digits will be presented
with some reference to what is known about structural dif-
ferences between the arterial arrangement in the hands and
feet.

1. Gross anatomy

The following outline of the anatomy of the blood supply
to the hand is based upon the descriptions in Gray's Anatomy
(Williams and Warwick, 1980) and by Basmajian (1970).

The blood supply to the hand is dependent upon the super-
ficial and deep palmar arches (Fig. 1). Immediately distal
to the elbow joint, the brachial artery bifurcates into its
two terminal branches, the radial artery and the ulnar ar-
tery. The radial artery courses down the lateral side of
the anterior forearm. It plunges through the small intrin-
sic muscles of the hand and enters the palm in a very deep
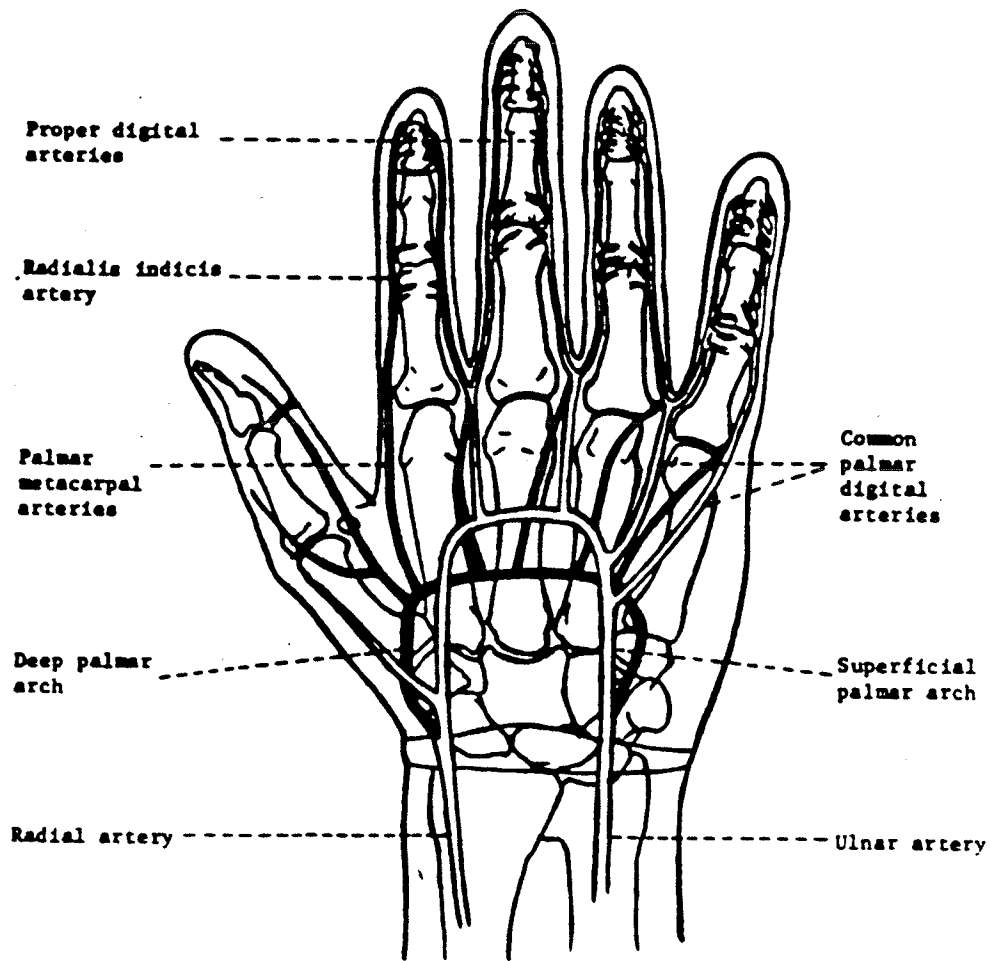
Fig. 1. Major arteries of the anterior aspect of the hand.

position.   It supplies blood to the thumb and index finger
and forms the deep palmar arch which is completed on the me-
dial side by a deep branch of the ulnar artery.   The ulnar
artery courses deeply down the medial surface of the anteri-
or forearm.   It enters the hand more superficially than the
radial artery protected only by skin and fascia. The super-
ficial palmar arch is derived from the ulnar artery, and is
completed on the lateral side by the radial artery.   It is
the larger of the two arches.   The deep palmar arch runs
more proximally and deeper than the superficial arch.   Each
arch gives rise to the palmar digital arteries that unite at
the clefts between the fingers (Fig. 1).   These then divide
and give rise to the proper digital arteries to two adjoin-
ing adjacent anterolateral sides of the fingers.   Therefore
adjacent fingers share a common palmar digital artery aris-
ing from the superficial palmar arch which unites with a
palmar metacarpal artery arising from the deep palmar arch.
They unite for only a short distance (about 0.5 cm) before
bifurcating into digital arteries supplying adjacent sides
of two adjacent fingers. These vessels course distally giv-
ing rise to anastomoses around the joints and finger pulp.
Each finger has these two main anterior vessels (proper di-
gital arteries) which form the main blood supply to the fin-
gers because the dorsal digital arteries are smaller in cal-
iber and less extensive.

The blood supply to the dorsal surface of the hand arises proximally from a short branch of the ulnar artery near its origin, the common interosseus artery. In turn the common interossous artery gives rise to the anterior and posterior interosseus arteries. These vessels unite with the dorsal carpal branches of the ulnar and radial arteries to give rise to the dorsal metacarpal arteries which run superficial to the deep interosseus muscles lying between the metacarpal bones in the hand. The dorsal metacarpal arteries bifurcate at the finger clefts and give rise to small dorsal digital arteries which have multiple anastomoses along their length particularly around the interphalangeal joints, connecting them with palmar anastomoses. The dorsal digital arteries do not extend along the full length of the finger (Fig. 2).

Variable, but numerous and free anastomoses occur between the radial and ulnar arteries, between the anterior and posterior aspects of the wrist, through the palmar and dorsal carpal arteries, between the superficial and deep palmar arches and their digital and metacarpal branches, between the proper digital arteries and small branches coming off the dorsal digital arteries; and between two digital arteries and the two dorsal arteries of the same digit. The degree to which these anastomoses are able to develop into collateral pathways in response to occlusion or persistent arterial spasm may determine whether sufficient blood flow can be provided to the digits. The vascular architecture of the hand can exhibit several variations specifically with
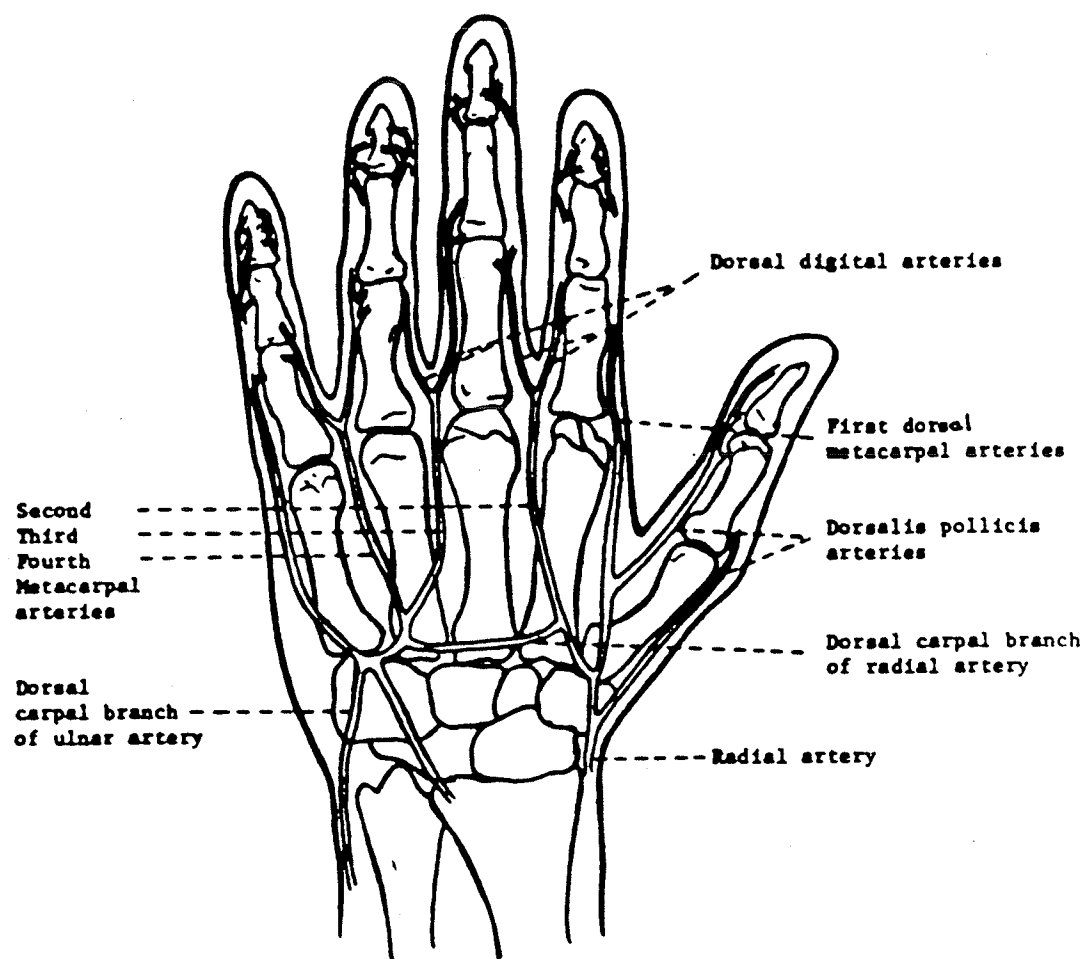
Fig. 2. Major arteries of the posterior aspect of the hand.

respect to the organization of the superficial arch within the palm. Karlsson and Niechajev (1982) found that in 139 patients, 64% of the superficial arches were predominantly supplied by the ulnar artery, 32% by a superficial branch of the radial artery, and in 4% of the hands the median artery was involved in the formation of the arch. The number and size of the common (palmar) digital arteries that branch off the superficial palmar arch can also vary. The deep palmar arch was shown to be structurally less variable, and the number and size of the metacarpal arteries projecting from it tended to be more constant. Usually three or four pass distally to anastomose with the corresponding common digital arteries at the interdigital webs. The most consistent of these are those supplying the thumb and second finger. A large common digital artery tends to be paired with a small corresponding metacarpal artery and vice versa. Of those vessels supplying the dorsum in the same study, the most consistently occurring (91%) was the dorsal carpal branch of the radial artery. This vessel unites with a comparable branch of the ulnar artery to give rise to the dorsal meta-carpal arteries which run superficial to the deep interosseus muscles lying between the metacarpal bones in the palm. Although some variability in the vascular architecture of the palm may be common, the arrangement of the large vessels in the digits is more fixed. Therefore, anatomic variation of the digital arteries is not likely to be a significant factor among the mechanisms of Raynaud's phenomenon. In

summary, each finger is normally supplied by two larger an-
terolateral digital arteries, and two rudimentary, smaller
dorsal digital arteries.

Comparable to the hand, the blood supply to the foot is
supplied by two arches; the plantar and the arcuate arch
(Fig. 3 and Fig. 4). These arise from the posterial tibial
and the dorsal pedis arteries respectively. Each arch simi-
lar to the hand, gives rise to the plantar and dorsal meta-
tarsal arteries. These anastomose proximal to the webs of
the toes for a short distance and again bifurcate to supply
small superficial digital branches to the toes. Like in the
hand the main arteries supplying the toes originate on the
plantar surface. There are dorsal digital arteries which
are smaller and generally less significant.

2. Histology

The walls of blood vessels with the exception of capil-
laries are composed of three morphologically distinct lay-
ers: tunica intima, tunica media and tunica adventitia. The
intimal layer adjacent to the vessel lumen consists of a
layer of endothelial cells and a thin, 80 nm thick basal la-
mina.

The endothelium consists of a continuous single layer of
squamous cells. Endothelial cells are flat and elongated
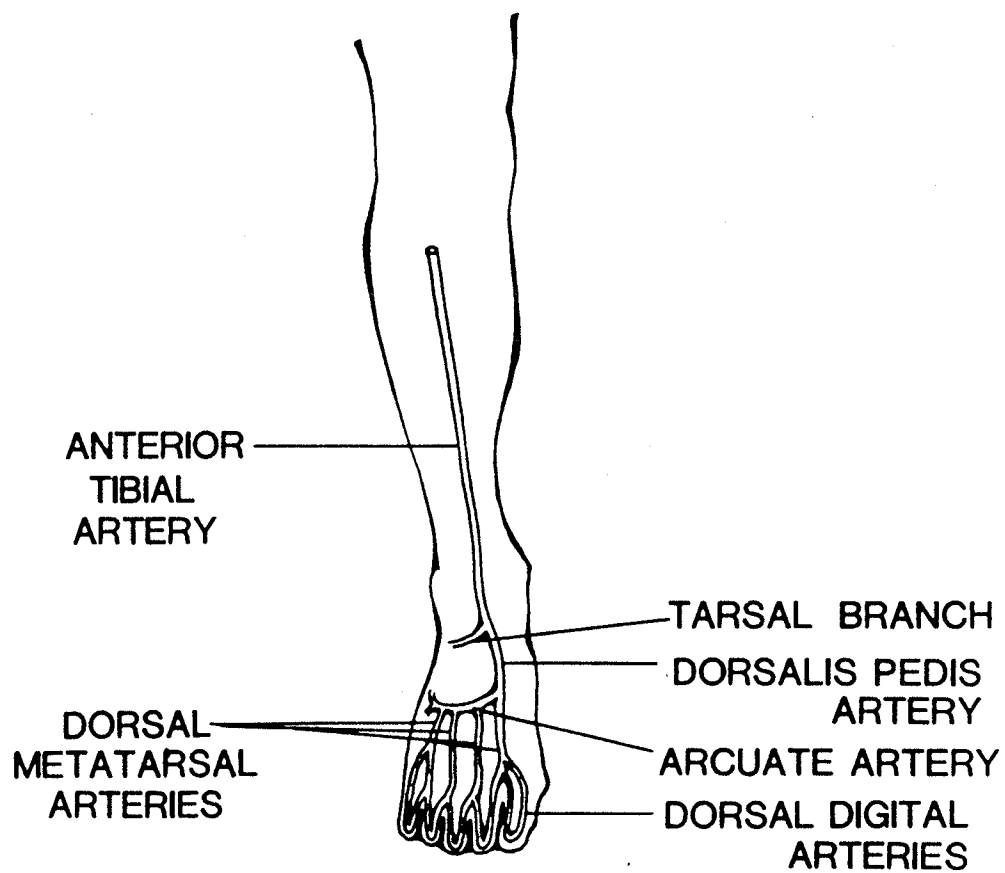
Fig. 3.   Blood supply to the foot.   Dorsal view.

PLANTAR
DIGITAL
ARTERIES

PLANTAR
META
TARSAL
ARTERIES

PLANTAR
ARCH

LATERAL
PLANTAR
ARCH

MEDIAL
PLANTAR
ARCH

PLANTAR
ARTERIES
DIGITAL

Deep
Dissection

Superficial
Dissection
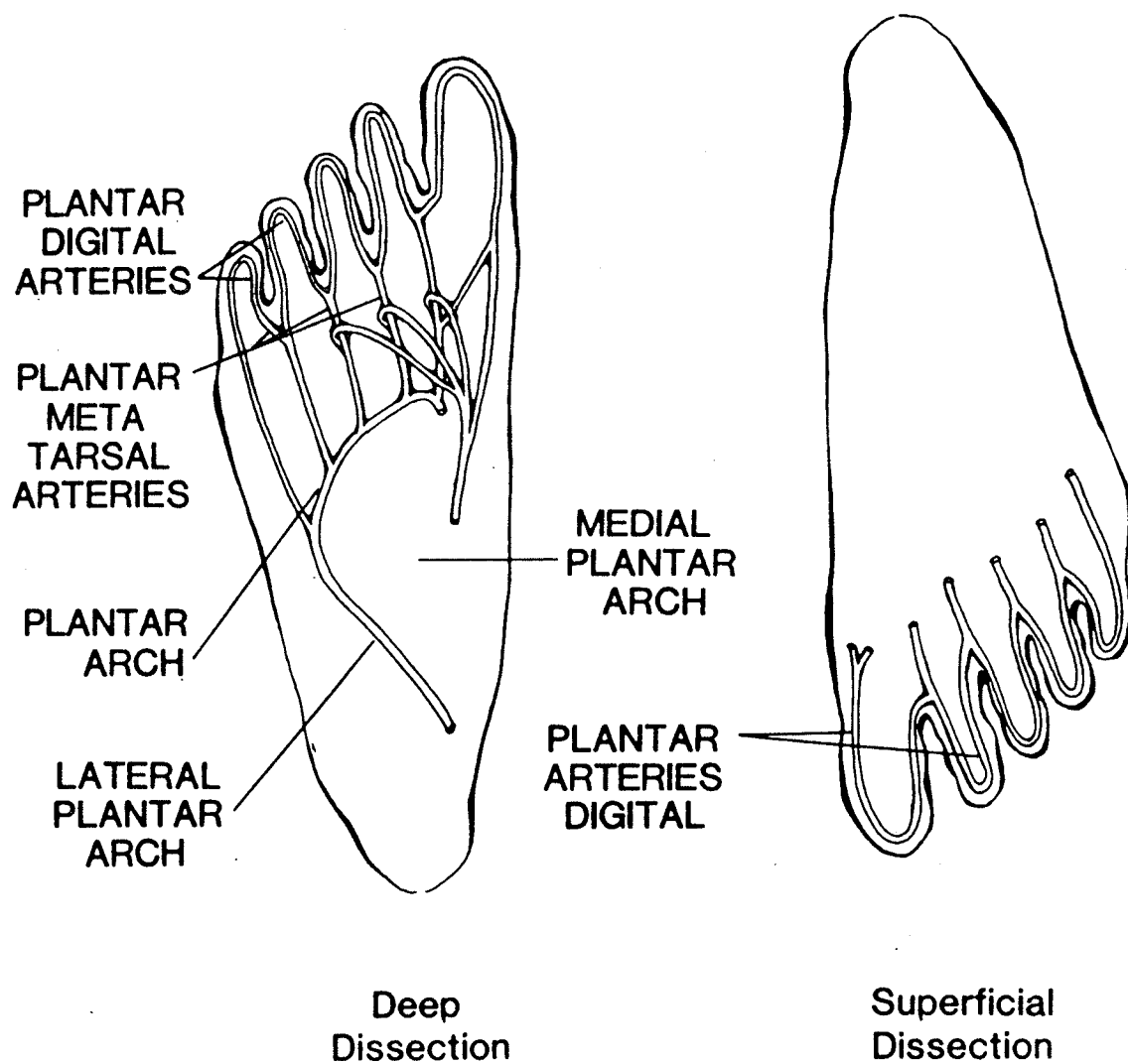
Fig. 4.   Blood supply to the foot.   Plantar aspect.

such that their long axis is parallel to the blood vessel. The 200 Å gap between cells consists of amorphous ground substance. The centrally-placed nucleus tends to protrude slightly and results in an increase in cell thickness in this area from the usual 0.2 to 0.5 μm.

Arterial endothelium is characterized by two types of junctions; tight occluding junctions and communicating gap junctions. The occluding junctions represent apparent fusion between adjacent endothelial cell membranes. The gap junctions are distinguished by patches of adjacent cell membrane consisting of a lattice of membrane subunits that provide areas for cell-to-cell transfer of ions and metabolites (ionic, electrotonic and metabolic couplings). These endothelial cell junctions have been considered important for transendothelial transport between cells. Surface pinocytotic vesicles have been implicated in this process. Thirty percent of vesicles which average 65-70 nm in diameter, are connected with the luminal cell membrane, 40% to the basal cell membrane and the remaining 30% are free in the cytoplasm of the endothelial cell. Together several vesicles have the capacity to fuse with one another forming small channels across the endothelium. Vesicular transport contributes to the transendothelial transport of the blood vessel.

The second part of the tunica just outside the endothelium intima is the basal lamina which is seen as a moderately

dense band 500-800 Å thick. It is characterized by an amorphous or finely filamentous texture and is composed of collagen and a carbohydrate component. The basal lamina and endothelium communicate by means of fine channels between them.

The tunica media as the names implies is the middle layer of blood vessels including the digital arteries. It lies between the intima and adventitial layers, and is composed of smooth muscle cells, elastic sheets, bundles of collagen fibrils and an elastic fibril network. In arteries the tunica media is proportionately thicker compared with that in veins. A highly fenestrated internal elastic lamina separates the intima and media. The fenestrations are believed to facilitate metabolic processes and diffusion. As the vascular wall contracts the internal elastic lamina becomes undulated and some of the fenestration or gaps close. Smooth muscle function is dependent on this elastic support.

A circumferential or helical arrangement of smooth muscle cells is characteristic of blood vessels. The larger vessels have a helical arrangement which becomes more circular distally (Fox and Hugh, 1966). Between the elastic laminae the smooth muscle cells lie obliquely; and in alternate layers, they may lie in the opposite direction or longitudinally (Wilens, 1951). The angle of the cells becomes more acute to the long axis of the vessel in the inner layers. Depending on whether smooth muscle cells are relaxed or con-

tracted their shape and size vary. These cells are more spindle-shaped when relaxed and more cylindrically shaped when contracted (Stehbens, 1960). Cell size also diminishes proximally (Wolinsky and Glagow, 1969).

The number of muscle layers depends on the size of the vessel. The precise orientation of the muscle layers in vascular smooth muscle has been controversial and found to differ in different vessels, in different segments of the same vessel and in different layers of the same segment (Fischer, 1965).

The outer layer of the blood vessel is a connective tissue sheath called the adventitia consisting of dense collagen. The external elastic lamella separates the media from the adventitia. Externally the adventitia gradually merges with the connective tissue of the surrounding structures. The role of the adventitia is primarily passive providing a structural support to the vessel protecting it from excessive distension.

3. Cytology

Of vital importance to both the structure and function of vascular smooth muscle in the blood vessel wall is the relationship it shares with postganglionic nerve endings and adrenergic varicosities. This section therefore describes the ultrastructure of both the vascular smooth muscle cell

and the adrenergic nerve terminal which innervates it. Because the detailed ultrastructure of the digital arteries however, has not apparently been studied in detail, a description of the common features of the ultrastructure of blood vessels that have undergone study will be presented.

a) Smooth muscle cell ultrastructure.

Electron optical technique has elucidated the ultrastructural morphology of vascular smooth muscle. With refinement of microscopy techniques however, smooth muscle was found to have a myofilament arrangement consistent with the cross-bridge theory for contraction (Somlyo et al., 1973). Dense bodies functionally comparable to the Z lines of skeletal muscle appear in vascular smooth muscle in association with both the cell membrane and free within the cytosol. From these structures project actin filaments which are considerably longer and more numerous than those of skeletal muscle. These characteristics have been thought to compensate for the relative paucity of myosin in smooth muscle and thereby maintain comparable maximal tensions.

Three types of filaments can be identified ultrastructurally in vascular smooth muscle. The filamentous organization of two fiber types, actin and myosin, and the characteristic length-tension curve of vascular smooth muscle support a sliding filament mechanism of contraction (Small, 1977). The characteristics of these three fiber types are

described below, however, the function of the well-distributed intermediate filaments is less well understood.

Biochemical reviews of the contractile proteins of vertebrate smooth muscle describe many similarities to those of striated muscle (Somlyo and Somlyo, 1968; Weber and Ruegg, 1966). The myosin molecules assemble into filaments in a tail-to-tail bipolar arrangement resulting in tapering ends. A central bare zone, M protein, and M bridges in the center of the A band have not been confirmed for smooth muscle. The precise arrangement of the crossbridges has not been described, however they have been clearly observed on the thick filaments of smooth muscle (Somlyo et al., 1973). Artifacts in the cell isolation and preparation procedure have to be reduced before conclusive evidence can be derived (Somlyo et al., 1977).

Myosin distribution in smooth muscle appears to be less organized and less abundant than in skeletal muscle. Smooth muscle has about one fifth the myosin content of skeletal muscle. Murphy and his colleagues (1974) however have reported that smooth muscle has the capacity to generate at least as much force as striated muscle. Since maximal force generated is dependent upon the length of the sarcomere or the number of bridges acting in parallel, this may be explained by the increased length of the myosin filament and the increased ratio of thin to thick filaments in smooth muscle.

Actin filaments are composed of G-actin monomers, that form a double stranded helical filament with an axial repeat of about 37 nm. Each monomer is a single-chain globular protein with a molecular weight of 42,000 and a diameter of 5.5 nm. F-actin or filamentous actin is found in both muscle and non-muscle cells. The length of the actin filament is typically longer than that of skeletal muscle.

Regulation of vertebrate smooth muscle appears to be myosin-linked. The S-1 subfragment of the heavy meromyosin is the enzymatically active globular portion of the molecule (Lowey and Holt, 1972). Phosphorylation of the myosin light chain is closely linked to direct calcium activation. Smooth muscle generally lacks the actin-linked regulatory proteins characteristic of skeletal muscle which lends support for the phosphorylation of the myosin molecule (Sobieszek and Bremel, 1975).

The regulatory protein tropomyosin has been identified in smooth muscle but varies greatly from that in striated muscle. Structurally tropomyosin consists of two chains in smooth muscle but these are not strictly analogous to the chains of skeletal muscle tropomyosin. Other differences include different amino acid composition, electrophoretic behavior and peptide maps and immunochemical properties (Cummins and Perry, 1974).

Current evidence suggests a Ca binding regulatory site that is associated with myosin (Weeds and McLachlan, 1974). Ca regulation of smooth muscle contraction is modulated by calmodulin (Walsh et al., 1980; Adelstein et al., 1982). Ca binds to calmodulin which then activates myosin light chain kinase leading to phosphorylation of the regulatory light chain of myosin. Myosin then hydrolyzes ATP and the muscle contracts. Relaxation reflects dephosphorylation of the light chain by a phosphatase. The existence of a dual regulatory system both myosin and actin linked, has not been totally ruled out (Murphy and Megerman, 1977).

The third type of intracellular filament, intermediate filaments, about 100 $\overset{o}{A}$ in diameter, are clearly distinct from actin and myosin. Their regular arrangement within the cell and their close association with the dense bodies suggest their major role is structural. Their arrangement may contribute to optimal force generation (Cooke and Fay, 1972). A network of dense bodies attached to the plasma membrane and free-floating cytoplasmic dense bodies appear to provide attachment points for actin during contraction. Functional differentiation between these however, has not been established. Cytoplasmic dense bodies are typically surrounded by intermediate fibers. Some investigators have suggested that such filaments represent anchor fibers which may maintain cell structural organization during the contraction cycle (Junker and Sommer, 1977). Others have ob-

served actin filaments directly entering into, and their profiles have been observed within, dense bodies which would implicate them directly in the contractile mechanism (Ashton et al., 1975).

Alpha actinin, the major constituent of the Z line in skeletal muscle, has been identified in dense bodies of smooth muscle (Schollmeyer et al., 1973). This finding supports an analogous role for α actinin within these muscles.

Connective tissue elements are synthesized within the arterial wall. In addition to the contractile proteins, collagen, elastin, and glycosaminoglycans also contribute to optimal force generation within the vessel wall. In part, the transmission of force in series or in parallel is determined either by side-to-side or end-to-end connections of smooth muscle cells (Somlyo et al., 1977). It is possible that intracellular actin filaments attach to membrane dense bodies and in turn connect with connective tissue to provide a force transmitting system.

Supporting elastic-like microfibers can be identified between the cell membrane and fibrillar collagen. These consist of a glycoprotein without amino acid cross-links, desmosine or hydroxyproline characteristic of pure elastin. Extracellular proteoglycan granules connect collagen fiber bands by adjoining elastic fibers. Glycoprotein granules are capable of binding smooth muscle cells to the surround-

ing connective tissue (Wight and Ross, 1975). These matrix granules probably arise from the chondroitin sulfate and hyaluronic acid synthesized by the arterial smooth muscle cells. These granules associated with the basement membrane behave anionically since cations have been shown to bind strongly to them. This observation may implicate the specific binding and exchange of cations such as Ca at these sites (Somlyo and Somlyo, 1968).

Unlike striated muscle, smooth muscle has a comparatively poorly developed system of intracellular channels, or the sarcoplasmic reticulum (SR), believed to contribute to the uptake and storage of Ca. This is a self-contained system which is not penetrable by commonly used extracellular markers such as ferritin and colloidal lanthanum (Somlyo and Somlyo, 1971). Experimentally when strontium is used as an electron-opaque Ca substitute, electron probe analysis shows the SR can be both a source and sink for Ca (Somlyo and Somlyo, 1971).

Smooth muscle fibers, being smaller in diameter than skeletal muscle fibers in general, are not as dependent on a regular interconnecting system of tubules to facilitate the speed of depolarization and consequent Ca release from adjacent rudimentary SR. Coupling has been observed between reticulum tubules and the surface membrane in the form of bridging structures across a 10 nm gap between the two membranes (Somlyo, 1978). Whether these couplings produce a

depolarization-sensitive Ca release is a matter of debate. Calsequestrin and other Ca binding proteins that are prevalent in the well-developed SR of skeletal muscle have not been identified in smooth muscle. The volume of SR varies for each blood vessel. The more elastic arteries may have 5 to 10% SR of the volume of the smooth muscle cell, whereas myogenically-active veins may have less than 5% SR. The SR volume is directly proportional to the ability of the cell to contract in a Ca-free solution; that is, reduced SR implies reduced Ca stores and greater dependence on extra-cellular Ca for contraction. This volume may also depend on the ability of the cell to synthesize proteins. Increased connective tissue is characteristic of the large elastic arteries which are known to have an increased SR volume. Uterine smooth muscle in pregnancy increases its ability to synthesize protein and is identified as having a greater amount of SR (Bo et al., 1968). This is also true for proliferating and developing cells (Somlyo and Somlyo, 1968).

The surface membrane area of smooth muscle cells is effectively increased by some 25 to 75% by surface vesicles. These characteristic flask-shaped invaginations of the cell membrane or caveolae (50 to 80 nm in diameter) are longitudinally oriented within the smooth muscle cell. The SR forms a fenestrated network around these surface vesicles which are also in close apposition with the mitochondria. This physical arrangement would enable the caveolae to pro-

vide active sites for ion binding and pumping; however this has been disputed since membrane particles are not always present at these sites (Gabella and Blundell, 1978).

Golgi bodies are arranged in lamellae around the perinuclear region of the vascular smooth muscle cell. They are closely associated with the rough endoplasmic reticulum and their polysome structures are responsible for the synthesis of required cellular protein. The Golgi complex is responsible for formation of glycolipids, formation of glycosaminoglycans from inorganic sulfate, the hydroxylation of lysyl and prolyl residues of collagen, and possibly may be involved in the manufacture of receptor sites on plasma membranes (Whaley et al., 1972).

Microtubules have been identified in vascular smooth muscle. Thirteen protofilaments composed of α and β tubulin protein form the wall of the microtubules. They function largely in intracellular transport, cell motility and serve as the backbone of the cytoskeleton.

Smooth muscle cells are characterized by having a wide range of cell junctions from small gap junctions (2 to 4 nm) to the large clefts for example, at desmosome attachments. These structures are important in ionic and metabolic communication between cells. The sites of fusion of the outer membranes of smooth muscle cells have been proposed as a low resistance area for electrotonic coupling between cells

(Dewey and Barr, 1962). The three-dimensional lattice structure of these junctions has been obtained from freeze-fractures procedure and X-ray diffraction studies. Although the basic structure of an isolated gap junction is quite uniform, the size of the region of the membrane involved has been found to vary considerably (Bennett, 1973). Gap junctions of smooth muscle are not usually associated with increased cytoplasmic density.

Gap junctions are dynamic structures which in response to physiologic stimuli are capable of assembling and disassembling as the need arises. Hormonal change (Garfield and Daniel, 1977), decreased extracellular Ca (Asada and Bennett, 1971) and temperature (Payton et al., 1969) have been reported to alter cell electrical conductivity and the density of intramembranous gap junctions.

Strong evidence exists for the involvement of gap junctions in ionic coupling. In electrically excitable cells, gap junctions facilitiate transmission of electrical signals with a resistance estimated to be considerably less than the plasma membrane (Jongsma and Van Rijn, 1972). In cardiac muscle and single-unit smooth muscle, gap junctions are responsible for their syncytial behavior. Tetraethylammonium (TEA) which blocks the potassium channels, can induce phasic activity in multi-unit smooth muscle with a concomitant increase in the number of gap junctions (Kannan and Daniel, 1977). This may suggest a role for the gap junctions in the electrical excitability of the cell.

Desmosomelike attachments or intermediate contacts are commonly seen in vascular smooth muscle. Increased cyto-plasmic density is found around the parallel interfacing cell membranes. Because of their similarity to membrane structures in cardiac muscle, they may serve as a low resis-tance pathway for electrotonic conduction or as a cellular binding structure within the tissue. The presence of mem-brane particles are not necessarily indicative of a cell coupling mechanism but may for example, represent intramem-branous binding sites for antibodies, lectins, hormones and drugs (Albertini et al., 1975).

b) Neuroeffector structure.

The principal means by which the circulatory system can respond and adapt to a wide range of conditions, including temperature change is reflected in the structure and func-tion of the sympathetic innervation to the blood vessels. A comprehensive understanding of the neuroeffector unit in the blood vessel wall may shed light on the possible underlying mechanisms of digital vasospasm, and how this may be exacer-bated with either general body cooling or local finger cool-ing.

i. Postganglionic sympathetic adrenergic neurons.

The sympathetic nervous system is the primary source of innervation of the vasculature. Two plexuses are formed within the tunica adventitia. The fibers of the outer plex-

us ramify in the middle to outer one third of the adventitia. The inner plexus is limited to the adventitiomedial border. Vascular smooth muscle tone is affected by transmitter release from this inner plexus. The plexus consists of nonmyelinated axons about 0.25 to 0.50 μm in diameter surrounded by a Schwann cell. At 3 to 10 μm intervals along the axon are varicosities ranging from 1.5 to 2.0 μm in diameter (Bevan et al., 1980). These contain a large number of norepinephrine (NE) storage vesicles which can be released when appropriately stimulated. Predominantly the vesicles range from 35 to 60 μm in diameter, however larger vesicles up to 60-120 μm are not uncommon (Burnstock, 1975).

Generally the gap width between the axon terminal and the smooth muscle is proportional to the size of the blood vessel. In small vessels, the narrow cleft is filled with basement membrane. When greater than 100 nm, the basement membrane cannot be continuous between the two cells and the space becomes filled with extracellular substances (Bevan et al., 1980).

The primary function of the adrenergic nerve terminal is to regulate autonomic function by means of its ability to synthesize, store, release and take up NE. Extracellular tyrosine is the precursor for NE synthesis. Biosynthesis of NE from tyrosine is comparable to the sequence of steps occurring in other tissues (Vanhoutte et al, 1981). The rate limiting step in the chain is the initial hydroxylation of

tyrosine to DOPA. Decarboxylation produces dopamine which is converted to NE by dopamine ß-hydroxylase which, like NE, becomes incorporated into the storage granules. Virtually all of the newly synthesized NE becomes complexed with ATP and stored in the vesicles, thereby protecting it from degradation from the neuronal monoamine oxidases (MAO). Two distinct vesicle populations have been identified by density gradient centrifugation (Kupferman et al., 1970). Reserpine pretreatment differentially blocks NE uptake into these two types of vesicles. The low density peak on a density gradient showed a large decrease in NE content, whereas the high density peak showed little change in content (Nelson and Molinoff, 1976). Different vesicle populations may have different mechanisms for transmitter regulation. Thus the net concentration of transmitter may reflect release and uptake mechanisms of these populations.

Depolarization of the prejunctional membrane is the usual stimulus for calcium (Ca) influx and release of NE from the storage vesicles. The vesicular membrane fuses with the nerve terminal membrane. Large molecules, NE, ATP, dopamine hydroxylase and chromogranin A are then removed from the nerve terminal by exocytosis (Vanhoutte et al., 1981). Another process for release has been implicated to explain the observation that the ratio of protein to NE in the storage vesicles is not proportional to the ratio measured on release (Smith and Winkler, 1972). Another explanation how-

ever may simply be that the measured vesicles were not representative of those stimulated to release, or that different pools of NE are handled in different ways. For example, newly synthesized NE is released preferentially during nerve stimulation (Kopin et al., 1968). Differentially labeled NE stores and different stimulus train length provided evidence for more than one NE pool.

A discrepancy exists between the amount of NE stored per vesicle and the amount released from a vesicle on stimulation. That is, the amount of transmitter released by an action potential from a varicosity is the sum of fractional amounts of the total content of the vesicles involved. Two concepts of the mode of release have been considered to explain this discrepancy. Folkow et al. (1967) preferred the idea of controlled partial release and Bevan (1978) described a periodic release of the vesicular contents on stimulation.

Neurosecretion is subject to a variety of local modulators. Transmitter release can be locally regulated by receptors on the prejunctional membrane. Alpha adrenergic antagonists, angiotensin and nicotinic drugs act through specific prejunctional receptors to facilitate NE release (Vanhoutte et al., 1981). Alpha adrenergic agonists, prostaglandins, dopamine, and morphine all can reduce transmitter release by also acting upon specific prejunctional receptors (Vanhoutte et al., 1981).

Most experimental evidence in vascular smooth muscle supports a physiological role for NE and prostaglandins in negative feedback inhibition of adrenergic neurotransmitter release (Farnebo and Hamberger, 1971; Hedqvist, 1970). The NE prejunctional inhibitory mechanism is considered to be of greater physiologic significance. Although β receptors have been identified on the prejunctional membranes of vascular smooth muscle, these have not been implicated in any regulatory feedback mechanism. Prostaglandins have been shown to partially depolarize the neurolemma (Sjostrand, 1972). The amplitude of the action potential and the release of transmitter have been shown to be depressed. Whether NE prejunctional inhibition operates similarly is not yet known.

Differences in pre- and post-junctional potency ratio of different agonists support distinct structural differences between receptors at these sites (Starke and Endo, 1976). Similar NE sensitivity of pre- and post-junctional receptors, i.e., an effective dose, ED_{50} of about $10^{-7}M$, is consistent with a tonic physiologic role of the negative feedback system. Such negative feedback systems only alter the amount of transmitter released by nerve stimulation or a high exogenous concentration of potassium ions (Starke and Montel, 1974). Chemical modulation of NE release is not affected by this feedback regulatory mechanism. Depolarization-induced transmitter release is dependent on Ca ions.

Distribution of the transmitter released into the cleft is dependent upon diffusion and the width of the cleft. Narrow clefts limit transmitter diffusion to within the cleft. The diffusion and distribution of moving transmitter into the adventitia and media may depend on their relative thickness and their physical resistance to transmitter movement (Bevan et al., 1980). Adventitial overflow of transmitter is removed via the adventitial nerve plexus.

At the intimal surface excess transmitter enters the circulation directly. When NE is applied to the adventitial surface its effectiveness is dependent on neuronal uptake unlike NE entering from the intimal surface. The sensitivity of the ear artery to NE appears to be increased 10-fold at the intraluminal surface compared with at the extraluminal surface (De LaLande et al., 1974). In the presence of cocaine which blocks the neuronal uptake of NE however, this phenomenon persists (Kalsner, 1972). This could be explained as a consequence of uneven distribution of NE within the wall. Another possibility is that myogenic activity is preferentially initiated in response to agonists applied on the intimal surface rather than to adventitia. This may reflect an inherent morphological difference between the inner and outer surface of the vessel.

Estimating transmitter concentrations during neural activity has been attempted through two basic procedures involving calculations of kinetics and transmitter concentra-

tion, and the derivation of concentrations in terms of a steady-state equivalent concentration of exogenous NE. These experimental approaches have shown comparable results and internal agreement to support their validity but each has its peculiar limitations and deficiencies. In comparing steady-state NE concentrations equivalents in vessels with a sizeable neuromuscular separation at 10 Hz both intra and perisynaptic thresholds are approximately 10^{-7} M. In the rabbit pulmonary artery, the intra synaptic peak transmitter concentration was lower than for the ear artery. Innervation density of the pulmonary artery is one-third that of the ear artery and synaptic diffusion distances are greater (Bevan et al., 1980). Qualitatively, transmitter estimates were comparable at corresponding sites. Lowest concentrations were detected at the outer lamella smooth muscle, intermediate concentrations were observed at the intrasynaptic receptor sites, and the highest concentrations occurred at the adrenergic plexuses, i.e., the perisynaptic region.

ii. Routes for transmitter disposal.

Means of transmitter disposal vary according to differences in neuroeffector organization, e.g., density and pattern of innervation, synaptic cleft width, distance between smooth muscle cells and adrenergic nerve terminals. Diffusion of NE away from the neuromuscular site to the adventitial capillary plexus and the plasma is an on-going process. NE in the adventitia leaves via the outer surface, NE in the

media leaves via the intima. Transmitter in the outer media probably does not exit through the adventitia in an innervated vessel because it is taken up in the neuronal plexus. Diffusional loss of NE from the extracellular space would tend to drain NE from the extraneuronal compartment with which it is in equilibrium.

The neuronal membrane carrier responsible for active transport of transmitter into the axoplasm of the adrenergic nerves has certain common characteristics, e.g., stereospecificity, temperature-sensitivity, Na-dependency, and saturability that obeys Michaelis-Menten kinetics (Bevan et al., 1980). The proportion of NE removed by this process varies with cleft width, species and vessel type.

NE is also taken up by all elements of the blood vessel wall. This extraneuronal uptake is temperature-sensitive, somewhat Na dependent, is governed by Michaelis- Menten kinetics, yet is not stereospecific. When extraneuronal sites are saturated at high NE concentrations, they may serve as a NE source (Kalsner, 1976b). Binding of NE to extracellular elements serves a relatively limited role in NE adrenergic transmitter inactivation. Tetracycline, a drug that binds to connective tissue, may prevent the binding of NE to collagen and elastin (Powis, 1973). This may be of physiologic significance in those vessels with a sizeable collagen and elastin content.

Chemical inactivation of NE is controlled by two enzymes. COMT is found at extraneuronal intracellular sites and deactivates NE through O-methylation to normetanephrine, the major extraneuronal metabolite. MAO is found intracellularly in both the smooth muscle cell (MAO B) and nerve terminal (MAO A) and metabolizes NE by means of an oxidative deamination process.

The various transmitter disposal mechanisms have been well described. Their relative importance within a given blood vessel has been investigated (Trendelenburg, 1974; Su and Bevan, 1971). The significance of these findings in normal vascular responsiveness has yet to be clarified.

iii. α and β adrenoceptor mediated responses.

Alpha and beta adrenoceptor mediated responses are characteristic of vascular smooth muscle, however, α adrenoceptor receptor-mediated responses predominate (Folkow and Neil, 1971). Stimulation of the α receptors produces smooth muscle contracture resulting in vasoconstriction. β-receptors are believed to produce relaxation in some vascular smooth muscles resulting in dilatation. The net effect will depend on the relative numbers and contribution of these two primary receptor populations. In the majority of blood vessels, the NE concentration necessary to produce a contraction 50% of maximal adrenergic response, i.e., ED_{50}, is approximately 10^{-7} to $10^{-8}M$. The possibility that α

adrenergic receptors of vascular smooth muscle differ from those of other organs has been examined but it remains unknown whether substantial differences exist (Furchgott, 1972).

Distribution of receptors is dependent on the condition and age of the organism, on location within the vessel, and vessel size. Inhomogeneity of adrenergic receptors at different levels of the vessel wall has been suggested (Bevan and Verity, 1967). Alpha adrenergic receptors have been thought to be limited to the smooth muscle cells close to the nerve terminal (Ljung et al. 1973). Spatial separation of α and β adrenoceptors has been reported in other tissues (Rosell and Belfrage, 1975). Depending on whether β adrenoceptors are stimulated by circulating or neurogenically-released catecholamines, different vessels will exhibit different sensitivities to these agents. For example, innervated arterioles are less likely to be affected by circulating catecholamines compared with non-innervated precapillary sphincters.

Human arterial plasma at rest has a NE level of $2 \times 10^{-9} M$ whereas epinephrine is 20% to 30% of this. Therefore at rest, little effect is exerted by these catecholamines on the vasculature. These values can be dramatically altered in physiological or pathological conditions. In exercise, the plasma NE level can increase to $7 \times 10^{-8} M$ probably as a direct result of increased sympathetic activity.

4. Summary

In summary, the anatomy of digital arteries and of the neuroeffector interaction underlying vascular smooth muscle activity is described. This provides a basis for understanding subsequent discussions on the physiology of smooth muscle responsiveness in conjunction with alterations in temperature, and possible mechanisms involved in the pathology of arterial vasospasm underlying Raynaud's phenomena.

B. PHYSIOLOGY OF THE DIGITAL ARTERIES

1. Cellular Physiology

The effect of temperature on the responses of normal digital artery smooth muscle and on those of patients with Raynaud's disease is not well understood. Normally, it appears that the degree to which digital arteries constrict in response to central vasomotor influences and to local factors reflects the summation of both excitatory and inhibitory factors acting upon the smooth muscle cells. Raynaud's disease may in turn reflect an imbalance of these opposing forces: that is, between the excitatory effects leading to constriction of the vessel wall, and the inhibitory effects producing relaxation and dilatation of the vessel. In Raynaud's disease the excitatory influences override the inhibitory ones in some way to produce abnormal contraction of the smooth muscle and excessive constriction of the digital arteries.

Although the cooling of vascular smooth muscle from other vessels has shown inhibition and depression of some cellular processes, cooling has also been shown to augment vessel constriction through cold-induced facilitation of other cellular activities. For example, an increase in the concen-

tration of neuro-transmitter in the junctional gap as a result of increased release, decreased uptake or depressed inactivation could help to explain the augmented response of the digital arteries observed with cooling. Cooling may exert an effect on the voltage-dependent Ca channels indirectly as a result of inhibition of the Na-K pump, or by directly inhibiting the active removal of Ca from the cell. To examine in greater detail the contribution of these potential mechanisms of increased smooth muscle responsiveness with cooling in digital arteries, this section is divided into two major parts. First is a discussion of the autonomic nerve supply since this is the principal regulator of vascular tone and the effect of temperature on autonomic function. This is followed by a discussion of the effect of temperature on membrane and intracellular processes. An analysis of the effect of temperature on the components of the contractile behavior of vascular smooth muscle could provide some insight into the possible underlying mechanism(s) of pathologic vasospasm in the digital arteries.

a) Postganglionic nerves.

The function of adrenergic nerves is depressed with progressive decreases in temperature from a physiologic temperature of 37°C. The biosynthesis of NE is directly reduced by cold-induced inhibition of the activity of tyrosine hydroxylase (Vanhoutte et al, 1981). Cooling has been shown to reduce neuronal transmitter reuptake which may increase

the concentration of transmitter in the junctional cleft and facilitate excitation postjunctionally (Vanhoutte et al., 1981). Some of the neuronally stored transmitter continuously diffuses from the storage vesicle into the neuroplasm where most becomes deaminated by neuronal monoamine oxidase A. Temperature reduction tends to have a stabilizing effect on the vesicular membrane and thereby could reduce leakage of transmitter (Vanhoutte et al. 1981). Similarly, cooling limits pharmacologic displacement of neurotransmitter by such agents as tyramine and guanethidine, both of which have a high affinity for the storage proteins within the vesicles (Lai and Hudgins, 1975). Cold-induced stabilization of the neurolemma inhibits the entry of Ca which is required for the exocytosis of neurotransmitter (Potter, 1966). Thus, cooling results in a generalized inhibition of cellular processes some of which contribute to potentiation, and others contribute to the depression of smooth muscle contraction.

The effect of temperature on the release of transmitter is controversial. Perfused mesenteric artery shows a potentiated response to nerve stimulation and a depressed response to exogenous NE with cooling (Malik, 1969). Such cold-induced potentiation has been observed in superficial canine veins in the presence of a reduced release of transmitter (Vanhoutte and Verbeuren, 1976b). The apparent inconsistency of these findings has yet to be resolved.

Since cooling has an inhibitory effect on the neuronal uptake of endogenous and exogenous NE, it increases junctional concentration of NE upon stimulation. In the presence of the neuronal NE uptake inhibitor cocaine, cooling does not significantly affect the uptake of (^{3}H)NE in superficial canine veins (Janssens and Vanhoutte, 1978). Cold-induced potentiation persists in surgically-denervated tissue and cocaine-pretreated veins exposed to NE (Janssens and Vanhoutte, 1977). This evidence suggests that the cold-potentiation observed in the superficial canine vein does not result from inhibition of neuronal uptake of the neurotransmitter. The contractility of canine vein pretreated with the α adrenoceptor blocker, phentolamine, and exposed to potassium is significantly depressed by cold. Extraneuronal uptake inhibitors such as β-estradiol, and normetanephrine which block NE uptake by the vascular smooth muscle do not contribute to the augmented response observed with cooling either in response to nerve stimulation or exogenous NE (Janssens and Vanhoutte, 1978). Thus, this evidence does not support the possibility that cold potentiation of smooth muscle contraction results from blockade of extraneural transmitter uptake.

Specific binding of transmitter to periarterial connective tissue accounts for a minimal amount of NE removal and is not a temperature-sensitive process (Gillespie and Towart, 1973). Oxytetracycline, which inhibits such binding

in connective tissue has no demonstrable effect on smooth muscle responsiveness to nerve stimulation or exogenous NE (Kalsner, 1976a).

Although the NE degradation enzymes COMT and MAO have significant roles in neuroeffector interaction in vascular smooth muscle, inhibition of their actions appears to have an insignificant effect on cold-induced potentiation observed in some vessels (Vanhoutte and Shepherd, 1969). Inactivation of these enzymes however, might play some role in the stable prolonged adrenergic responses observed at reduced temperatures.

The adrenergic innervation to the blood vessel produces tetanic contractions and maintained shortening which is instrumental in resisting intravascular pressure. Both neural and myogenic responses can be modulated and counteracted by potent negative feedback arising from accumulated local humoral agents, tissue metabolites and reduced tissue pO_2 (Folkow and Oberg, 1961).

b) Prejunctional influences.

Prejunctional modulation can have either a net inhibitory or excitatory effect on the smooth muscle response in the vessel. Adrenergic receptors on the neuronal membrane exert a negative or inhibitory effect on further exocytotic release of transmitter from the terminal by some unknown mechanism (Westfall, 1977). Naturally occurring exogenous

catecholamines and synthetic adrenergic agonists can also activate prejunctional α receptors. Such stimulation produces a decrease in the release of endogenous $(^3H)NE$ during sympathetic nerve stimulation. Conversely inhibitory adrenolytic drugs greatly enhance the evoked release of transmitter from the nerve terminal. Facilitated transmitter release exerted prejunctionally has been reported in response to low concentrations of β agonists. Circulating epinephrine is capable of stimulating these receptors to produce selective vasoconstriction and redistribution of blood flow. The finding of methyltransferase in vasoconstrictor nerves suggests an inherent ability to synthesize epinephrine, which can be released in combination with NE. Epinephrine can then act selectively on the prejunctional β receptors since these receptors are hardly affected by NE (Dahlof, et al., 1980). Prejunctional negative feedback systems may explain the effects of PGE_2 and adenosine.

The effect of tonic sympathetic discharge to blood vessels is generally to enhance vasomotor tone. Denervation of the vascular bed fails to produce maximal dilatation; therefore, it is likely that non-neurogenic factors contribute significantly to vascular basal tone (Folkow, 1964). These intrinsic factors are discussed below.

c) Membrane potential.

Changes in temperature have been reported to have an effect on the membrane potential of vascular smooth muscle cells. Vascular smooth muscle from the rat tail artery exhibited a less negative E_M at 16°C compared with 36°C over a range of K concentrations from 3 to 150 mM (Hermsmeyer, 1976). Also, the conduction of action potentials and the spread of electrotonic potentials have been reported to be lower at 25° than 38°C for portal-mesenteric vessels (Ljung and Stage, 1970). Cooling the sheep carotid artery progressively inhibits NE from producing its normal electrical and mechanical responses (Keatinge, 1964). At 4°C adrenoceptor-mediated responses were abolished in this vessel whereas K-induced contractures persisted. Receptor-mediated events therefore appeared more thermosensitive. This observation may have some importance in local peripheral vascular control in humans.

d) Na-K pump.

A Na-K pump is required to offset the inward diffusion of Na and outward diffusion of K down their electrochemical gradients. The pump thus exports Na and imports K as a coupled phenomenon. If Na and K are exchanged in equal proportion, the pump is electroneutral. When more Na is extruded than K brought in, an electrogenic current is produced. The coupling ratio of the pump has been reported to

be 3Na to 2K (Thomas, 1972; Casteels et al., 1973) and thus contributes directly to E_M as an electrogenic pump. The function of the pump is dependent upon intracellular Na and extracellular K. In addition, because the pump is a protein carrier in a lipid matrix its activity is sensitive to temperature and pump inhibitors such as ouabain. Na-K ATPase in mammalian non-vascular smooth muscle is inactivated at a critical temperature of 18-20° C (Charnock et al., 1971; Taylor et al., 1979).

Different means by which to investigate the presence of electrogenic ion transport have been employed. Exposing the rat caudal artery to a K-free solution for example, results in the smooth muscle cells becoming Na-rich. Thus, in the absence of K in the bathing medium, the Na-K pump is inhibited, K diffuses out of the cell down its concentration gradient and Na influx is unopposed (Hermsmeyer, 1976). On K replacement, a transient hyperpolarization appears which is more negative than the K equilibrium potential. Interestingly, in hypertensive animals the ouabain-sensitive hyperpolarization was significantly more pronounced, which might suggest the Na-K pump was more active in these animals or R_M was greater. At 16° and 37°C spontaneously hypertensive rats compared with normotensive rats showed a less negative membrane potential in the smooth muscles of the caudal artery over a range of K concentrations. A lowered K was postulated to be offset by a compensatory increased pump ac-

tivity, and be responsible for the increased reactivity of arteries to NE in hypertension. In tissues with intact adrenergic nerve terminals, the K-free stimulated contractures have been attributed to prejunctional NE release. These contractures have been reported to be sensitive to α receptor blockers and chemical sympathectomy (Bonaccorsi et al., 1977). These various factors have not been studied in human digital arteries.

e) Adrenergic receptor affinity.

The affinity of adrenergic receptors has been reported to be differentially affected by temperature change in cutaneous and deep blood vessels. Dose response relationships under conditions of cooling to 24°C have shown a reproducible shift to the left for cutaneous vessels and to the right for deep vessels (Janssens and Vanhoutte, 1977). The postjunctional adrenoceptor affinity for NE and the α-adrenergic antagonist, phentolamine, was reported to be increased in cutaneous canine veins. In contrast, responses to NE in deep vessels were depressed although the affinity was not significantly affected (Janssens and Vanhoutte, 1978). Responses to other adrenergic agents, ACh and histamine have been augmented in a similar cutaneous preparation with cooling (Vanhoutte and Verbeuren, 1976a).

f) Intracellular contractile processes.

Intracellular processes have been reported to be affected by alteration in temperature. There is no available evidence for the response to cooling of skinned smooth muscle cells from cutaneous arteries, however those from the mammalian carotid artery showed a depressed rate of tension development and a maximal response at 20°C. This maximal response did not change significantly with either warming or cooling (Bohr et al., 1962). Peiper et al (1975) conducted a similar analysis of mechanical behavior in rat portal vein. At moderate temperatures down to 20° C, rate parameters such as maximum velocity of shortening and rate of tension increase were depressed whereas no change was reported for the extent of isotonic shortening or extent of peak force generation when compared with physiologic temperature. The depressed effect that cooling exerts on the mechanical behavior of blood vessels may reflect increased viscosity at the level of the myofilaments of the contractile proteins or depressed actomyosin ATPase activity. These factors could explain the cold-induced depression of wall tension and general inhibitory effect reported in deep veins (Vanhoutte and Lorenz, 1970).

g) Oxygen consumption.

Most energy-dependent reactions within the cell are known to be depressed with cooling. Oxygen consumption related to

ATP synthesis is reduced with cooling as a result of reduced metabolic demand. This observation has been confirmed in vascular smooth muscle (Brecht and Gebert, 1971). Similarly in isolated superficial canine veins, responses to catecholamines are depressed with anoxia and glucose depletion with cooling (Vanhoutte, 1976). On the basis of studies comparing metabolic activity, mechanical calcium-dependent responses to NE, K-induced depolarization and myogenic response to stretch, cooling appears to have the greatest inhibitory effect on myogenic activity than receptor-mediated activity which in turn is greater than K-mediated activity (Peiper et al., 1971; Regnat et al., 1975; Vanhoutte and Lorenz, 1970).

h) Intracellular Ca metabolism.

Factors affecting calcium influx and efflux are essential determinants of activator Ca within the smooth muscle cell available to trigger contraction. Influx of Ca from the extracellular space is thought to supplement the small amount of Ca released from intracellular stores following depolarization of the cell membrane (van Breemen et al., 1972). Saida and van Breemen (1984) have recently reported the presence of an intracellular Ca store, the sarcoplasma reticulum, that may contribute substantially to smooth muscle contraction. Intracellular calcium is removed by both active reuptake of Ca within these intracellular sites and active extrusion from the cell which may be effected by a Ca pump and a Na-Ca exchange mechanism which is likely to be

temperature sensitive. Active Ca extrusion into the extra-
cellular space and the sequestration of Ca by intracellular
stores are likely to be temperature-sensitive similar to the
Na:K pump. The net contractile response observed in vascu-
lar smooth muscle with cooling may therefore reflect to a
large extent temperature-sensitivity of the energy-dependent
processes associated with Ca metabolism.

There are differential effects of cooling on the phasic
(fast) and tonic (slow) components of the contractile pro-
cess. Phasic contraction which is relatively less signifi-
cant in total contribution is dependent upon the release of
Ca from intracellular stores. This process is more de-
pressed with cooling compared with the slower more prolonged
tonic component which is dependent upon membrane permeabili-
ty and Ca influx (Sitrin and Bohr, 1971). These components
have not been studied in digital arteries. Such an analysis
however, might help to determine whether the translocation
of Ca is somehow interfered with or altered with cooling in
patients with Raynaud's disease. Altered Ca metabolism as a
result of changes in the membrane characteristics of the
smooth muscle in the digital arteries of Raynaud's patients
could provide one explanation for the disorder.

i) **Myogenic activity.**

Myogenic activity is believed to contribute to normal vascular tone (Johanssen and Mellander, 1975). It has long been known that increasing intravascular pressure within a blood vessel in situ stimulates active shortening and contributes to an increased basal tension in vascular smooth muscle cells (Bayliss, 1902). The mechanism of this response is still not entirely understood, however it appears to be an inherent property of the smooth muscle and not mediated by some neurogenic mechanism. A reduction in the negativity of the membrane potential and increase in spike activity in response to stretch has been a consistent finding in myogenically-active vessels (Burnstock and Prosser, 1960). Based on such observations of stretch-induced altered electrical properties of the membrane, stretch may have a direct effect in changing the membrane ionic permeability. Increased Na permeability has been thought responsible in the case of guinea pig taenia coli (Bulbring and Kuriyama, 1963). Complete depolarization of the membrane however did not abolish the myogenic response. Others have attributed this phenomenon to a slow Ca influx resulting in activation of the contractile elements (Bohr and Verrier 1971) and to a Ca mobilization that is associated with depolarization (Stephens et al., 1973). The effect of cooling on myogenic activity has been largely one of depression (Bandick and Sparks, 1970; Regnat et al., 1975), and therefore not likely to participate in vasospasm.

j) Possible role for cold-induced prostaglandin synthesis.

Endogenous prostaglandins may contribute to the local control of the circulation (Hadhazy et al., 1985; Hadhazy et al., 1986; Hassman et al., 1981). Hadhazy et al. (1986), for example, showed that human femoral arteries dilate in response to PGI_2, PGE_2 and cyclooxygenase inhibition increased vessel tone. Thus, these investigators concluded that these prostaglandins are significant modulators of smooth muscle tone in these vessels.

The bovine tibial artery at temperatures below 33°C has been shown to exhibit a prolonged stable contracture without external stimulation. That prostaglandin B_2 which enhances the release of NE may be the endogenous substance released on cooling has been speculated (Greenberg et al., 1974). Given that heat-induced vasodilation is mediated by inhibition of sympathetic activity to the cutaneous circulation, PGB_2 could delay or prevent the inhibition of NE release. Greenberg et al. (1974) concluded that PGB_2-induced cutaneous vasoconstriction in the canine hind paw is mediated partially by NE release.

Cooling the rabbit aorta to 27°C stimulates the production of prostaglandin E_1 which is associated with dilatation (Bevan and Purdy, 1973) and cooling canine basilar artery depresses the production of prostaglandin F_2 which is associated with constriction (Ljung, 1970). The direct effect

of cold on prostaglandin mediated-activity of the peripheral arterial circulation in humans is unknown.

h) **Summary**.

In summary, the effect of temperature on the net response of vascular smooth muscle is the sum of inhibitory and facilitatory effects of temperature on the function of the nerve terminal, membrane-mediated activity and intracellular processes. With reduced temperatures, transmitter synthesis and release are generally inhibited. Cold-induced depolarization of the rat tail was reported, however, other vessels show reduced responses to K. Carrier-mediated processes such as Ca extrusion, neuronal transmitter uptake and Na-K pump activity are all inhibited. Increased post-junctional α adrenoceptor affinity has been reported to be potentiated in some vessels and reduced in others. In turn, these events affect the degree of membrane depolarization, the stimulation of the adrenoceptors, the concentration of activator calcium within the cells and the overall tissue response. Studies of the role of cooling on prostaglandin-mediated responses may also provide insight into normal and abnormal smooth muscle responses in the peripheral circulation of humans.

2. Vessel Physiology

An overview of nervous system control of the autonomic innervation supplying blood vessels will be discussed briefly. This is followed by a discussion of the effect of temperature on these neurogenic factors as well as non-neurogenic factors that affect the dynamic function of the blood vessel. Although muscle mechanics are not a focus of this review, these are briefly discussed to help provide a more complete framework in which to view the problem of peripheral arterial vasospasm.

a) Neurogenic aspects.

The following outline of the neurogenic aspects of thermoregulation as it relates to blood vesssel physiology is based upon the descriptions of Folkow and Neil (1971) and Daube and Sandok (1978).

Temperature detection and regulation within the body are the result of a complex interaction between thermosensitive receptors on the skin, the central vasomotor center in the medulla of the midbrain which is closely linked with the thermoregulatory center in the hypothalamus and smooth muscle function of the arterioles in particular.

The cutaneous thermosensitive receptors are thought to be of two types; those that are sensitive to cold and those that are sensitive to warmth. Generator potentials are be-

lieved to be initiated by stimulation of the cutaneous thermal receptors to a certain threshold level. These afferent impulses are conveyed to the central nervous system via the lateral spinothalamic tract. The primary neuron cell body is located in the dorsal root ganlion. Second order neurons terminate in the ventral posterolateral nucleus of the thalamus. Third order neurons give rise to thalamocortical fibers which establish the final link with the post central gyrus of the parietal lobe and specific topographic localization of the stimulus. Third order neurons also give rise to afferent impulses to the hypothalamic thermoregulatory centre. In addition, the temperature of the blood can stimulate the thermoregulatory centre which in turn interconnects with the vasomotor centre of the medulla. An increase in efferent impulses leads to vasoconstriction whereas their absence is associated with vasodilatation.

Mass sympathetic discharge of the medullary cardiovascular center results in stimulation of the sympathetically-innervated adrenal glands. Even with sympathetic denervation, cutaneous vessels are able to constrict in response to increased levels of circulating catecholamines resulting from release by the adrenal glands. The contribution of these glands to the control of blood flow within the body however, is considered to be less significant than that of the vasomotor nerves (Folkow and Neil, 1971). The blood flow of any organ-system including muscle beds reflects vasomotor tone

as well as the metabolic needs of the area. The metabolic needs of the skin are minimal compared with other organs whose metabolic demands can override central control when needed.

Tonic discharge of the sympathetic nerve fibers contributes to the basal tone characteristic of many blood vessels particularly the arteriolar resistance vessels. The range of discharge frequency varies from less than 1 impulse to 8 impulses/sec. Although a seemingly narrow range, these frequencies span practically the full range of effector responses. There may be minimal additional contribution to flow from non-neurogenic factors.

Vessels differ with respect to the temperature at which they are capable of generating their maximal contractile response. This might reflect different functional roles among vessels depending on their location within the body. Maximal responsiveness to exogenous catecholamines has been reported to occur below physiologic temperature for a variety of superficial vessels studied in vitro. Maximal response around 20° C occurs in the ear arteries of the rabbit (Martin and Wallace, 1970), ox and sheep (Surgeon and Wallace, 1975). The rat caudal artery exhibits maximal responsiveness at 30°C (Wade and Beilin, 1970). Both superficial arteries and veins in birds exhibit response potentiation to catecholamines at 27°C (Millard and Reite, 1975). Vanhoutte et al. (1981) have been proponents of the functional differ-

entiation of superficial and deep blood vessels based upon in vitro observations of their responses to cooling. Some peripheral vessels respond maximally at temperatures below 37°C. Therefore 37°C may not be the temperature at which human cutaneous vessels respond maximally, but rather at some temperature closer to environmental temperature.

Species differences with respect to vascular reactivity have also been reported. In the dog, for example, cooling to 20°C potentiates adrenergic responsiveness of mesenteric arteries (Vanhoutte and Lorenz, 1970). However in the rat, ox, and sheep the responses of the mesenteric arteries to NE are markedly depressed with cooling (Bohr and Verrier, 1971; Malik, 1969; Surgeon and Wallace, 1975). Although mesenteric arteries appear to differ in their response to temperature depending on the species, other deeply situated mammalian blood vessels have been reported to exhibit depressed responsiveness to catecholamines (Vanhoutte et al, 1981). At a few degrees above 0°C progressive depression of contractile responsiveness has been reported to be agonist-specific for femoral artery of the rabbit (Glover et al., 1968), the rat (Peiper et al., 1971), and the femoral vein of the dog (Vanhoutte and Lorenz, 1970).

Augmented α adrenergic receptor activity with cooling has been explained by an increase in α adrenoceptor affinity to NE (Vanhoutte and Lorenz, 1970). Beta adrenoceptor-mediated relaxation has also been reported to be enhanced in some

vessels at reduced temperatures (29°C). Vanhoutte and Shepherd (1970) showed that the effect of the ß receptor-mediated relaxation mechanism in superficial canine vein was more pronounced at reduced (20°C) than at higher temperatures (29°C). These observations fail to support cold-induced inhibition of ß adrenergic effects to explain the potentiation of the α adrenoceptor-mediated response with cooling.

b) Effect of active and passive factors other than nerves.

In the unstimulated vessel, passive wall tension has been reported to decrease with reduced temperature in conjunction with changes in the viscoelastic characteristics of the vessel wall (Apter, 1967). Cooling to 20°C in the absence of external stimulation has little effect on the measured perfusion pressure in the isolated canine saphenous vein perfused at constant flow (Vanhoutte and Shepherd, 1970). These passive effects are believed to be relatively unimportant compared with the effect that temperature change has on the active properties of vascular smooth muscle.

c) Muscle mechanics.

The mechanical properties of vascular smooth muscle can be described according to Hill's classic equation (Hill, 1938). Although vascular smooth muscle mechanics were not a primary focus of this thesis, a knowledge of how temperature affects the mechanical behavior of muscle and contributes to

vessel closure is important in elucidating the mechanism relevant to Raynaud's disease.

In his work of 1938, Hill described a physical model of active muscle consisting of a contractile element (CE) arranged in series with one elastic component (SEC) and in parallel with another (PE). In this model the development of force depends upon the force-velocity relation of the CE and the stiffness of the SE. That is, the rate of tension development by the muscle is a product of these factors.

The literature on the effect of temperature on SE and CE in smooth muscle has been scant. One exception is work on canine tracheal smooth muscle. For this muscle Stephens et al. (1977) reported that at $17^{\circ}C$, P_0 (maximal isometric tetanic tension) was reduced. The Vmax (maximal velocity of shortening at zero load) and the "b" constant (constant in Hill's force-velocity equation which represents units of shortening or relative enzymatic activity) were both reduced significantly at $17^{\circ}C$ compared with $37^{\circ}C$. Although the SEC was found to increase in stiffness with cooling, changes in dp/dt were attributed to effects on the CE rather than the SEC since changes in metabolism suggested that active processes were involved. The "a" constant of Hill's equation represented the number of active force generating sites which were not found to change with a reduction in temperature.

A physical and mechanical analysis of the blood vessel wall reveals that it is a viscoelastic system. Its viscous nature is manifested by the time dependency of its behavior. The existence of elastic components enables the wall to modify the response of the viscous component to deforming forces. The parallel elastic component is in parallel with the contractile element. Vessel closure and opening in patients with Raynaud's phenomenon secondary to connective tissue disease may reflect viscoelastic changes as a result of histologic changes of the vascular smooth muscle.

Stress relaxation and creep phenomena in smooth muscle can be explained on the basis of viscoelastic properties (Meiss, 1977). In stress relaxation, stretch results from potential energy being stored in the elastic component. This results from a sudden extension of the elastic component which is proportional to the applied force or stretch. The viscous element deforms at a constant rate also proportional to this force. The force decreases in a time-independent exponential manner; unlike that in a pure elastic system where the deformation is directly proportional to the applied force. Creep is the reverse of stress relaxation, and results from the exposure of the tissue to sudden drop in tension. The physiologic significance of stress relaxation and creep in normal vascular physiology is not known.

The nonlinearity of force-length behavior characteristic of the resting tension of smooth muscle reflects its viscoe-

lastic properties in characteristic hysteresis loops. That is, on the ascending limb of the curve, extending the tissue to a given length is associated with a greater tension compared with the same length on the descending limb. The viscous component resists change in length as the tissue is stretched out. On release the elastic component is shorter, and therefore there is less energy within the system compared with the stretching phase.

Kimoto and Goto (1967) compared hysteresis loops (L-T) of different blood vessels at different temperatures. The curves from the aorta, a vessel of elastin and collagen composition, and vena abdominalis, a muscular vessel from the toad, illustrated greater tension development at extremely low and high temperatures (near freezing temperatures and above 40°C) over a range of muscle length compared with more physiologic temperatures. At temperatures between 6° and 38°C. the aponeurosis plantaris, a collagenous tendon, exhibited hysteresis loops that resembled those of the aorta pretreated with formic acid. Formic acid was used to digest collagen in the aorta, an elastin dominated tissue. Pretreated aortic tissue showed no temperature dependence of its length tension relationships and no hysteresis effect over a range from 6°C to 53°C. These results suggest that both elastin and collagen in blood vessels play a rather minor role in changing the hysteresis characteristics in response to temperature.

This section has dealt with the physiology of the vascular smooth muscle. Mechanisms of modulation of neuroeffector interaction responsible for vessel function and how these may be affected by alterations in temperature are described. Physical characteristics of vascular smooth muscle are discussed in terms of muscle mechanics and viscoelastic properties. The relationship of temperature-induced altered muscle mechanics to the pathophysiology of Raynaud's phenomenon awaits clarification. However, structural changes of the digital vessels may account in part for changes in wall stiffness which may predispose the vessel to closure with cooling and prevent vessel relaxation. Much work remains to be done in correlating altered muscle mechanics with the symptomatology of Raynaud's phenomenon.

a) Cold vasodilatation.

According to Folkow and Neil (1971), cold vasodilatation is a protective local vascular response which is usually preceded by several minutes of intense vasoconstriction in the skin resulting from local and neurogenic factors. Cold vasodilatation involves the A-V anastomoses preferentially. Collateral axons from nociceptive fibers to arterioles liberate a mediator which induces a period of vasodilatation. In addition, cold depresses vascular smooth muscle activity (Keatinge, 1964) and the sensitivity of the vessels to sympathetic discharge (Vanhoutte et al., 1981). It is possible that the normal protective response of the peripheral circu-

lation is compromised in Raynaud's phenomenon (Davies, 1981).

3. Hemodynamics

This section first describes the basic principles of pressure-flow relationships in blood vessels, and the dynamics of resistance modulation effected through passive and active elements of the blood vessel wall. The effect of temperature on normal hemodynamic function and how under certain conditions vessel closure may result, are described.

Over 200 years ago, Poiseuille verified experimentally in cylindrical tubes the relationship between laminar flow and pressure gradient, and derived the following equation:

$$Q = \frac{\pi r^4 (P_1 - P_2)}{8L\eta}$$

where Q is the flow, $(P_1 - P_2)$ is the difference in driving pressure between two points, r is the internal radius, η is the viscosity coefficient and L is the length of the vessel (Folkow and Neil, 1971). The apparent viscosity of blood greatly depends on hematocrit, vessel caliber and temperature. From 37° to $17^\circ C$ viscosity increases, however, by about 10 percent. Attempts have been made to measure viscosity in Raynaud's patients. These are described below. Although Poiseuille's law does not apply precisely to the circulation, it is a practical way to assess changes.

Blood viscosity, a contributor to the resistance term of Poiseuille's relationship, is relatively constant under physiologic conditions (Baeckstrom et al., 1971). In vessels less than 200 μ, viscosity progressively decreases with decreasing vessel calibre (Folkow and Neil, 1971).

Unlike homogeneous materials, arteries do not obey classical elastic theory. Young's Modulus, the ratio of stress to strain, was derived as an index of elasticity of the wall. The non-linearity of the elastic behavior of the blood vessel wall reflects its non-homogeneous composition (Burton, 1954). The two connective tissue elements elastin and collagen have different Young's Moduli. Elastin has a modulus of about 3×10^6 dynes/cm^2 compared with 1×10^9 dynes/cm^2 for collagen. Elastin contributes chiefly to the resistance to stretch of the blood vessel at low transmural pressures, whereas collagen contributes significantly at higher pressures. Over the range of physiologic pressures, resistance to stretch is provided by both the elastin and collagen elements. At high pressures collagen forms a relatively non distensible jacket around the vessel. Inactive smooth muscle has a comparatively low elastic modulus 6×10^4 dynes/cm^2 (Burton, 1954).

The physical properties of blood vessels differ from one vessel to the next depending on its location in the vascular tree, on the age of the individual and on pathology. With aging, vessels are less extensible due to morphologic chang-

es in elastin and collagen. This reduction in elasticity may result from increased collagen, fragmentation of the elastic lamellae, formation of extensive crosslinks between collagen fibers, and calcium deposits which interfere with collagen unfolding. In older adults these changes become superimposed on the normal tendency of vessels to become stiffer towards the periphery (Harkness et al., 1957).

Normally, the principal function of the elastic elements collagen and elastin, is to hold the wall in equilibrium in the face of vessel distension secondary to an increase in transmural pressure, and to store energy within the vessel wall during systole. This energy is returned to the circulation by promoting flow during diastole. This function reflects the passive properties of the elastic tissue in the sense that this function is not energy-dependent. The role of the smooth muscle is to develop active tension; thereby altering vessel calibre and distribution of blood flow. According to Burton (1954), the specific role of the elastic tissue is to grade active tension generated by the smooth muscle in the wall of blood vessels. Despite the presence of elastic tissue however, vessel closure may result from either increased smooth muscle activity or reduced transmural pressure or both. With increased vasomotor tone the physical equilibrium of the blood vessel wall may be stabilized by the interplay of the passive elastic component and the active tension generated by the smooth muscle.

The physical properties of active blood vessels are discussed with respect to the effect that vasomotor tone has on pressure-flow relationships. For example, dilated ear arteries of the rabbit with minimal vasomotor tone indicate an almost linear relationship between pressure and flow (Girling, 1952). With increasing vasomotor tone through stimulation of the superior cervical ganglion, the curves become progressively nonlinear with correspondingly lower flows resulting from the same pressures. In addition closing pressure as described below, increased with sympathetic stimulation indicated by the higher intercept on the pressure axis. Similar pressure-flow relationships have been observed in the human forearm during body warming and cooling which correspond to low and high sympathetic tone respectively (Burton and Yamada, 1951).

Burton's classical analysis defined critical closing pressure as that pressure at which a blood vessel closes due to instability in the equilibrium between two opposing forces; the transmural pressure distending the wall and the active tension generated by the smooth muscle which opposes this distending pressure force. Transmural pressure sustained by the vessel wall can be computed from the Law of LaPlace, i.e., T=P x r; where T is wall tension, P is transmural pressure and r is the radius. The application of this law to explain physical disequilibrium or critical closure holds for vessels with an infinitesmal wall thickness.

Burton's original notion of critical closure in blood vessels has been challenged on the grounds that his analysis may be too narrow because it excludes certain factors. Metabolic and myogenic factors for example, have been suggested to have a role. Infolding of the endothelium has also been observed either to partially or completely close a small vessel (Johnson, 1974).

Cellular orientation in vascular smooth muscle is another factor believed to affect the ability of a vessel to close. The helical orientation of vascular smooth muscle cells according to Alexander (1977) can provide mechanical advantage to the vessel in producing complete closure. For example, closure was observed in a thin-walled vessel with fibers oriented at a 45° angle to the long axis of the vessel.

4. Summary

Normal hemodynamics in blood vessels as described by Poiseuille have been largely understood for two centuries. How these are precisely altered with cooling under normal conditions and under less physiologic conditions to produce the serious hemodynamic consequences in Raynaud's phenomenon is not clear. Organic changes may occur in Raynaud's phenomenon such as fibromucinous intimal vascular changes. These may also occur in response to pharmacologic agents and vibration (Winkelmann et al., 1977). Altered physiologic responses such as increased transmitter release with or

without increased sympathetic nerve activity, decreased transmitter uptake or degradation can result in an increased concentration of NE and an exaggerated vessel tone. Cold-induced changes in the sensitivity of the receptor mechanisms for the transmitter on the post junctional membrane of the smooth muscle cell may produce the same net effect. Alternatively indigenous dilating mechanisms that effectively check and balance normal degrees of constriction of the vessel may be malfunctioning or absent in individuals with Raynaud's disease. Such mechanisms might include dilating mechanism related to β receptors or prostaglandins. Investigation of these various possibilities may help elucidate the mechanisms underlying the disorder. Treatments may then be more rationally administered to help avert the consequences of impaired hemodynamic function and its sequelae to the digits.

C. PATHOPHYSIOLOGY OF RAYNAUD'S PHENOMENA

To date Sir Thomas Lewis has probably made the single most significant contribution to our current understanding of the pathophysiology of Raynaud's phenomena. In the 1920s and 30s he completed an extensive series of clinical studies with Raynaud's patients in which he meticulously described color and skin temperature changes on exposing affected digits to local cold challenges, in addition to manipulating centrally-mediated vasomotor activity by altering room temperature. Lewis was the first to suggest that the fundamental disorder in Raynaud's disease was local not central in origin. He hypothesized vasospastic attacks resulted from smooth muscle hyperreactivity and contraction of the blood vessel wall. Therefore, this section will examine possible cellular and vessel-related mechanisms which might be responsible for the underlying pathophysiology. Because of the relative importance of Lewis' contribution to the field, some aspects of his work will be detailed in order to provide a clearer understanding of the hemodynamic changes associated with the disorder. A knowledge of cellular and vessel responses may help to provide greater insight into the basic mechanism of the disease.

1. Pathophysiology and drug effects

The precise mechanism triggering prolonged spasm of the digital arteries of the fingers in Raynaud's patients in response to a cold stimulus and occasionally emotional upset is unknown. Local cooling can exert localized effects on cellular activity within the vessel wall and theoretically could augment or override centrally-mediated responses. In the section on Physiology, normal physiological responses of vascular smooth muscle to cold were presented. In Raynaud's disease it is probable that one or more of these local effects are altered in some way with changes in temperature resulting in the overall potentiation of smooth muscle contraction observed in vivo. The various possible mechanisms will be reviewed briefly.

Cooling has an effect on both the function of the adrenergic nerves and the vascular smooth muscle in cutaneous blood vessels. Transmitter release is generally unaffected down to 20°C whereas this temperature depresses neuronal and extraneuronal uptake, and enzymatic degradation. The net result is an increased NE concentration in the vicinity of effector smooth muscle cells which potentiates contraction. With moderate cooling to 20°C cutaneous canine veins have been reported to have an increased affinity for the transmitter at the α receptor site (Janssens and Vanhoutte, 1978). This action explained cold-induced potentiation of smooth muscle responsiveness, and alterations in vessel cal-

iber. Adrenoceptor responses have been similarly reported to be potentiated in the canine saphenous vein. This tended to offset the potentiated β responses since β receptors mediate relaxation and α receptors mediate constriction responses. The hypothesis that an increase in α adrenoceptor affinity may be responsible for digital vasospasm may explain the effectiveness of α adrenoceptor blocking agents in the treatment of Raynaud's disease (Coffman and Davies, 1975).

Drug studies in Raynaud's patients may help elucidate what mechanisms may be affected by the disorder, and thereby contribute to a more rational basis for management. A brief overview of drug therapy in the treatment of Raynaud's phenomenon therefore is presented. Based on the fact that the blood vessels are affected by sympathetic adrenergic innervation, drugs that result in the interruption of adrenergic nerve transmission, neurotransmitter depletion or α adrenoceptor blockade have been logical choices for the potential treatment of digital vasospasm. Alpha blocking agents represent the single largest group of drugs that has been applied in the treatment of Raynaud's phenomenon. Carter (1981) has treated 23 patients with severe Raynaud's phenomena with phenoxybenzamine (POB), a non-specific α adrenoceptor antagonist and reported a beneficial effect on the healing of finger lesions.

Another α receptor blocking drug Prazosin has more selective α receptor blocking properties. It has gained some popularity as an effective antihypertensive agent, and has also been reported to be of some benefit for some Raynaud's patients (Karlsberg et al., 1980). On the basis of these findings the role of α receptors in the etiology of vasospastic disease warrants further attention.

Reserpine which depletes the NE stores within the nerve terminal has been reported to relieve symptoms in some patients, however the effect tended to be transitory (Parks, et al., 1961). When objective assessment of cold sensitivity was compared before and after reserpine treatment in six patients with Raynaud's disease an acute initial improvement was observed (Nobin et. al., 1978). One week after the drug was discontinued, however, cold sensitivity had essentially returned to the pretreatment levels.

More recently interest has been directed to the angiotensin-converting enzyme inhibitor, captopril which has an antihypertensive effect. It decreases angiotensin II levels and peripheral resistance without concomitant changes in heart rate, cardiac output and circulating levels of NE. One report on the administration of this drug in a patient with Raynaud's phenomenon claimed acute and long term success based on both subjective and objective improvement (Miyazaki, 1982).

An interesting feature that has been described in patients with variant angina is that the incidence of Raynaud's symptoms, and migraine headaches is significantly increased (Miller et al., 1981). This has suggested that a disorder such as Raynaud's disease might be related to the existence of some generalized vasospastic disorder. Since calcium antagonists have been used successfully in the treatment of variant angina, this therapy may have some value in the management of other vasospastic conditions including Raynaud's disease. A recent report demonstrated that nifedipine was effective in the treatment of Raynaud's phenomenon and produced a significant reduction in digital vasospastic attacks (Kahan et al., 1981).

The relative importance and contribution of ß adrenoceptors in the control of peripheral blood flow in the human have not been clear. There are reports that Raynaud's symptoms occur in patients taking ß blockers (Thulesius, 1976), and are relieved with ß receptor agonists (Thune and Fyrand, 1976). The action of these drugs which tend to decrease blood pressure could decrease transmural pressure in digital arteries, but resulting increased sympathetic tone could bring about excess tone and vasospasm with and without decrease in local blood pressure. Evidence for a ß adrenergic dilating mechanism in human fingers in vivo is inconclusive (Cohen and Coffman, 1981) and awaits verification.

Although some evidence exists for a decreased PGI_2 (prostacyclin) in vascular disease, the role of PGI_2 and its interaction with thromboxane (TXA_2) have not been elucidated in Raynaud's disease. PGI_2 and TXA_2 are essential for blood coaguability and local blood flow (McGiff, 1981). PGI_2 is synthesized and released by the blood vessel endothelium. It is associated with potent inhibition of platelet aggregation and vessel dilatation via a cAMP mechanism.

A role for prostaglandins in peripheral vascular regulation is suggested by the subjective symptomatic relief provided by PGE_2. A series of 26 patients with Raynaud's phenomenon received PGE_2 intravenous infusions over 72 hours. This resulted in marked dilatation of the peripheral circulation indicated by increased skin temperatures, increased amplitude of the finger pulse volumes and peripheral pulsatility index derived by Doppler ultrasound (Clifford et al., 1980). A role for prostaglandins in the etiology of Raynaud's disease has not been elucidated.

Kahan et al. (1981) recently evaluated the therapeutic effect of the Ca blocker, nifedipine in patients with Raynaud's phenomenon secondary to connective tissue disease in ten cases and six cases were idiopathic. Nifedipine protection against vasospasm was claimed in 14 of the patients.

Disturbances within the immune system in Raynaud's phenomenon have been suggested by the presence of cold reactive

serum proteins which have been documented in some patients. In connective tissue disease, IgM which is associated with anti-IgG activity has been identified as a prevalent cryoglobulin (Levo, 1982). Cold sensitive cryoglobulins are believed to form a reversible precipitate in the venous plexuses resulting in reduced blood flow and vascular stasis. Reports of symptomatic relief from vasospastic attacks following plasmaphoresis support some role for some plasma factor such as cryoglobulin in the etiology of Raynaud's phenomenon (O'Reilly et al., 1979).

A role for histamine (H) in the phenomenon has also been suggested. When normal subjects were exposed to H_1 and H_2 blockers, blood flow in response to warming and cooling of the hands resembled that observed in patients with Raynaud's disease who were not exposed to histamine blockers (Lafferty et al., 1983). On exposure of the hand to a cold stimulus, vasoconstriction occurred and vasodilatation was delayed in patients until a hot stimulus was reapplied. This response was mimicked by the healthy subjects on histamine blockers. Since histamine stimulates prostacyclin synthesis from vascular endothelium, these findings according to Lafferty et al. (1983) may point to a prostaglandin deficiency, hence reduced dilatory influence, secondary to a local fault of the histaminergic system of the blood vessels.

This section has examined those physiologic processes occurring in the vascular smooth muscle cell which, if affect-

ed by cooling could result in an augmented contractile response and thereby help to explain vasospasm in the digits. To further elucidate a mechanism for the disorder, a review of drug therapy is outlined. Although no causality can be directly implied when a drug is effective in reducing symptoms, this information in combination with other information may contribute to our present understanding of Raynaud's disease.

2. Vessel Pathophysiology

Information on vessel pathology and pathophysiology of digital arteries in Raynaud's phenomenon is scant. The histology of normal control vessels has also not been studied in detail. In the digital arteries removed from normal subjects ranging in age from 19 to 76, whose limbs were amputated due to trauma intimal fibrosis with associated luminal narrowing has been reported (Rodnan et al., 1980). Although only nine subjects were studied in this series, intimal fibrosis appeared to be correlated with age. The frequency of severe narrowing in normal vessels, was significantly less than in vessels from 16 subjects with scleroderma and Raynaud's phenomenon.

Obtaining biopsy material or complete vessel segments from patients with Raynaud's phenomena is very difficult. Digital arteries are not routinely dissected at autopsy. Also, it is not always known whether an individual has the

disorder since it is seldom a prominent feature related to the death of the individual. Therefore, due to the paucity of available tissue, information on vessel pathology is minimal especially for primary Raynaud's disease. Relatively more data are available on vessels from individuals with secondary Raynaud's phenomenon. Traditionally, Raynaud's disease has been considered a functional disorder with no underlying organic disease. This however has not been conclusively established with anatomic or histologic evidence. Until such time, some knowledge of the pathophysiology in vessels with secondary disease may be helpful in contributing to an understanding of primary disease. Because the phenomenon has been reported frequently to precede the appearance of other manifestation of collagen disease it might represent a very early sign of underlying disease which manifests itself later.

Biomicroscopy has revealed differences between the nailfold capillaries in Raynaud's patients and normals. In primary disease the majority of vessels are generally normal in appearance between attacks, although some capillaries appear narrowed (Conrad, 1968). This pathologic narrowing appears more pronounced during an attack. Severe forms of Raynaud's phenomena, particularly if secondary to connective tissue disease, exhibit distended and distorted capillaries along side these narrower capillaries (Conrad, 1968).

Advanced disease is characterized by such anatomical and organic changes as intimal thickening, severe intimal fibrosis and intraarterial thrombus formation. These have been more commonly observed in cases resulting in marked luminal narrowing leading frequently to frank tissue ischemia and ulceration (Rodnan et al., 1980). Organic abnormality may precipitate critical closure with lesser degree of spasm or even with a normal force of contraction due to the reduced pressure distal to the site of occlusion. Whether a physiologic abnormality occurs first resulting in abnormal constriction, and the intimal thickening and thrombus formation follow, has not been substantiated.

Lewis (1938) described intimal fibromucinous changes in digital arteries in patients with Raynaud's phenomenon which have been reported more recently to resemble the changes observed in scleroderma (Norton and Nardo, 1970). Winkelmann et al (1977) have also reported intimal thickening with associated acid mucopolysaccharides. The internal elastic lamina, media and adventitia were normal. The intimal changes may not be a direct result of connective tissue disease because they have also been associated with the use of vibrating tools (Winkelmann et al., 1977), normal ageing (Spittell, 1980); hormonal contraceptives and pregnancy (Spittell, 1980). The latter two examples suggest that fibromucinous changes in blood vessels are related in some way to the presence of female sex hormones, which might account for the predilection of Raynaud's disease in women.

Although there are few detailed histologic studies of both normal digital arteries and digital arteries from subjects with Raynaud's phenomenon, structural differences are apparent. The most prevalent distinction is the intimal hyperplasia or fibrosis in the diseased arteries. The intima is characterized by a preponderance of collagen with little clearly distinguishable ground substance. Medial thickness is however, comparable in the two groups. In some instances Raynaud's phenomenon may therefore, reflect primary abnormality in collagen metabolism resulting in wall thickening of the digital arteries similar to the fibrosis and thickening observed in scleroderma. Further studies are warranted to describe and distinguish the structural differences between the digital arteries from subjects with primary and secondary Raynaud's phenomena and to identify how these differ from normal digital arteries taking into consideration histologic changes over the life cycle.

3. Hemodynamics

The cause of episodic digital ischemia in Raynaud's disease has been a matter of controversy for many decades. Maurice Raynaud who first described the disorder, attributed it to an increased reactivity of the autonomic nervous system supplying the blood vessels (Juergens et al., 1980). This was thought to be responsible for eliciting the exaggerated response to cold. The exact role of the nervous

system in producing Raynaud's symptoms needs to be elucidated.

a) Local fault. The early classical studies by Lewis published in a single volume of "Heart" in 1929 have contributed significantly to our present understanding of vasospasm in the digits. This work has provided the foundation for much of the work on Raynaud's phenomenon that is described in this chapter. Lewis concluded that the abnormal spasm of Raynaud's attacks is evoked locally and does not result from abnormal vasomotor impulses which was commonly held up to Lewis' time. He based this conclusion on two lines of evidence. First, blocking sympathetic vasoconstrictor fibers in the ulnar nerve with xylocaine did not eliminate onset of vasospasm of the digital arteries. Secondly, vasomotor paralysis did not greatly affect the degree of vessel spasm during a vasospastic attack. The relaxation of vessel tone that may occur was found to be insufficient to raise the skin temperature. Lewis described several cases in which sympathetic denervation of the limbs of Raynaud's patients failed to prevent cold from triggering digital vasospasm although the release of normal constrictor tone tended to decrease the tendency for spasm to occur within one to two months after surgery. Some doubt may exist regarding whether the sympathectomy was sufficiently extensive to eliminate all sympathetic innervation to the limb. Lewis argued that if sympathetic fibers should bypass

the sympathectomized area it would be doubtful that these remaining fibers would totally innervate the hand to provide the extensive symptoms observed in patients post operatively. Axonal regrowth, being relatively slow, was also not likely to be responsible. The role of denervation hypersensitivity might have explained some of Lewis' findings. The conclusion drawn, however, was that sympathectomy was unsuccessful for the same reason that vasospastic symptoms persisted following localized nerve block. Therefore, the fundamental cause of the disorder was thought to be a local fault within the digital artery itself and not a centrally-induced phenomenon. This, Lewis believed, did not suggest that generalized increased vasomotor was unimportant, but rather the degree of constriction of the vessel depended upon both vasomotor tone and local factors. He carefully pointed out that although the vessels could readily be blocked to produce some degree of relaxation this did not suggest the vasomotor impulses were in any way abnormal. He concluded therefore that the cause of Raynaud's disease is a local one, which apparently could be modified by vasomotor influences. In some cases the latter can determine entirely whether the digital circulation will be arrested or not. Lewis subjected Raynaud's patients to a cold water challenge to the fingers in room temperatures ranging from 21° to 11°C. At warmer room temperatures cyanosis developed within about 10 minutes compared with a couple of minutes when patients were tested in a cooler draughty room. At the cooler

ambient temperatures, wrist pulses were less perceptible compared with the warm ambient temperatures. Likewise, restoration of color in the tested hand was much more prolonged. The general conclusion from this series of studies was that digital spasm could be more easily induced if the patient was cool. Furthermore, exposure to general body cooling without local cooling of the finger could elicit digital spasm alone. However, if the fingers were kept warm and the room cooled, cyanosis or spasm was never observed. Cyanotic fingers would redden in warm water even in a cooled room. This suggested that the nerve impulses were not likely to be abnormal in strength or frequency because they were insufficient to induce closure on their own.

Based on Lewis' detailed clinical accounts, it can be concluded that an abnormality does exist in the digital arteries of Raynaud's patients which is exhibited by an observable hypersensitivity or hyperreactivity of the affected blood vessels to cold temperatures. The mechanism was not identified.

b) Localization of the fault.

Lewis (1929) was the first to provide evidence localizing vasospasm to the digital arteries. He excluded any involvement of the veins in the hand by observing the effect of simply raising and lowering the patient's hand during an attack. Raising and then lowering the hand produced an in-

crease in the depth of cyanosis of the fingers resulting from inflowing blood from the veins draining into the minute vessels. Reddening would have resulted if blood was flowing from the arteries. This was further substantiated by placing a pressure capsule over a digit and thereby observing the pressure at which blanching of the cyanotic skin appears. This was performed in conjunction with venous occlusion of the upper arm. This latter maneuver transmitted the pressure in that region to the minute vessels of the finger. A pressure in the capsule of 30 mmHg for example, did not produce blanching when the upper arm cuff was 60 mmHg. These demonstrations indicated that connections do exist between the veins and the smaller vessels in some of Lewis' patients. But participation of the veins in spasm together with the arterial vessels is not ruled out by these observations.

By conducting studies to observe the relief of digital spasm with warming, Lewis localized the origin of vasospastic episodes to the digital arteries in the fingers. One study involved placing the discolored fingers of a Raynaud's patient in which the circulation had completely stopped, into a water bath of 40°C up to the midphalangeal region of the fingers. In one subject the discoloration reached the bases of the digits. After five minutes of warming the distal portions of the fingers the color had darkened with no evidence of spasm release. During this test, the other hand

was similarly discolored at skin temperatures of 13°C. The palm of the hand was immersed up to the webs of the fingers into water of 40°C. The mid, distal and most of the proximal phalanges were exposed. Within four minutes there was widespread recovery and complete resolution after 12 minutes. The thumb had not been exposed to warming and remained blue throughout, suggesting that dilatation was not being reflexly mediated. This demonstration clearly showed the effect of local warming on the blood vessel in the absence of any effect secondary to reflex body warming. Similar studies revealed that recovery always began proximally and proceeded distally in a gradual yet not necessarily uniform manner. Collectively these studies confirmed that when only the finger tips underwent warming no recovery was observed because the spasm persisted proximally. On warming the hand proximally leaving the fingers exposed however, the spasm resolved quickly and the fingers reddened within a few minutes followed by increases in skin temperatures.

On the basis of these observations, evidence favored the site of the local fault within the digital arteries. Lewis did not totally exclude simultaneous involvement however, of other small arteries or arterioles. To further localize the fault, Lewis attempted to induce vessel spasm directly by exposing the digits to local cooling. One study involved warming both hands to 30°C for a 10 minute period. The right hand was then immersed into 15°C water such that the

fourth and fifth digits and the ulnar border of the hand
were below water level. The distal phalanges of the fourth
and fifth digits of the left hand were also immersed for a
15 minute period. Deep cyanosis was observed bilaterally
over the parts of the fingers exposed to the cold water.
Immersion of both hands in 30°C water produced recovery in 2
1/2 minutes in the left hand, and in 6 minutes in the right
hand. Recovery occurred rapidly following the onset of pre-
warming in both cases. Similar results were obtained when
the test was repeated on other occasions. In both the two
cooling conditions the portion of digit which was cooled de-
termined the degree of blood flow cessation and vasospasm.
For example, cooling of the two distal phalanges resulted in
vasospasm in the distal phalanx, whereas cooling the whole
hand produced vasospasm to the bases of the fingers. This
was indicated in both situations by a distinct demarcation
of the affected discolored area suggesting further the pri-
mary involvement of the digital arteries as opposed to some
other vessel.

Difficulties are often encountered when one wishes to in-
duce a Raynaud's attack. As a means of overcoming these,
Lewis designed a limb cooling system in order to standardize
cold sensitivity testing. This consisted of an arm bath
partitioned into two compartments such that the fingers and
the forearm could be exposed to different temperatures si-
multaneously. Lewis found that symptoms could be induced

quite reliably when the affected digits were exposed to 15°C in the bath. Some results using the compartmentalized limb bath are reported. In one case, the hand was positioned upwards to the wrist in 15°C water. Within three minutes, two of the warm digits (at 30°C) were cyanotic, and the third one became so in 7 1/2 minutes. Thus, cooling the palm including the bases of the fingers served as an adequate stimulus for producing spasm in warmed digital arteries. This observation provided further support for the involvement of the larger arteries beyond the metacarpal joints. This test was repeated such that the partition in the bath was positioned one centimeter proximal to the bases of the fingers, thereby permitting the proximal portion of the limb to be warmed to that point and to be cooled distal to that point, after the 15 minute cooling period no change in color was noted. Distinct cyanosis was detected however under comparable conditions except that the partition was now positioned one centimeter proximally, such that the finger bases were now exposed to the cooling side and not to the warming side. Within two minutes, two fingers exhibited discoloration and by the fourth minute a third finger had become discolored. Symptoms were totally reversed in a few minutes by immersion of the digits in a 30°C bath. These tests were also repeatable. They confirmed that vasospastic symptoms could be induced in warmed digits provided that the bases of the digits were included in the portion of the proximal limb subjected to cooling. These results were confirmed using a

cold box as the cooling device rather than the water bath. In normal subjects cold challenge to the digits produced minimal cyanosis on cooling the fingers to 15°C. Cyonosis increased in the digits when the room was cooled to 13° or 15°C.

Another method was devised to further localize the induction of spasm to one finger or to a part of one finger. A brass capsule through which water of a desired temperature could be circulated was fitted around the part of the finger to be tested. For example, water at 10°C could be circulated around one finger while the other fingers and the remainder of the hand and arm were immersed in water at 30°C or above. Cooling therefore could be selectively applied and effected around the base of an individual finger. It was concluded that digital blood flow could be arrested by the occurrence of spasm anywhere along the digit implying that cold sensitivity of the vessel was not specific along the length of the digit.

c) Further considerations of Lewis.

Lewis' important contribution to the understanding of Raynaud's phenomenon is the notion of the local fault situated within the digital arteries. He provided substantial evidence from a series of meticulous case studies, that local cooling of the fingers alone could increase vessel tone sufficiently to produce signs of a vasospastic attack with

or without the vasomotor nerve supply intact. Lewis monitored skin temperatures to assess the effect of cooling and rewarming on blow flow and he indicated he studied patients with primary disease. A diagnosis of underlying disease was made if the disease signs and symptoms were apparent. Some of Lewis' patients may have had arterial occlusion judging by the skin temperatures although a disease of primary diagnosis was made.

Studies of Raynaud's phenomenon need to distinguish clearly between two pathophysiologic mechanisms which can effect vessel closure with cooling; one, cold-induced abnormal increase in digital artery smooth muscle tone and two, closure due to low intravascular pressure distal to an arterial occlusion in the presence of normal vascular tone. The contribution of each of these mechanisms to vessel closure both individually and in combination is of interest in order to determine their relative importance.

Lewis did not provide a possible explanation for the local fault. Although he demonstrated that functioning vasomotor nerves were not necessary to induce vessel closure and symptoms of a vasospastic attack, how they might contribute to excessive vessel reactivity was not studied. More recent evidence on normal neuroeffector interaction suggests that this depends to some degree on appropriate nerve and muscle function (Bevan et al., 1980). Endogenous substances may be released by each component of the neuroeffector interaction

which in turn help to nourish and maintain the function of the adjacent structure. The role of such trophic factors has only been recognized in recent years (Vanhoutte et al., 1981). Physiologically, the local fault may represent the absence or excess of chemical mediators that contributes to the overall dilatation or constriction of the digital artery respectively. Such mediators may act directly on the blood vessel or indirectly by sensitizing the blood vessel to constricting chemical mediators such as norepinephrine (NE) (Vanhoutte et al., 1981). A sensitization of the blood vessel could reflect some anaphylactic response observed in allergic reactions. In asthma, for example, SRS-A (slow reacting substance of anaphylaxis) is thought to be released and produces prolonged pharmacologic responsiveness of airway smooth muscle (Orange, 1977). The leukotrienes, byproducts of arachidonic acid metabolism, rather than the prostaglandins have been reported to be implicated in this reaction in the asthmatic model of spasm in the airways (Dahlen et al., 1983).

Vessel histology on the digital arteries from patients with primary and secondary Raynaud's phenomenon suggests that structural changes, e.g., intimal thickening and fibromucinous changes, are likely to be responsible in part for changes observed in vessel function (Winkelmann et al., 1977; Juergens et al., 1980). Structural changes observed in patients with Raynaud's phenomenon secondary to connec-

tive tissue disease are well defined in terms of the fibrosis and fibromucinous changes observed throughout the layers of the blood vessel wall. These structural changes are likely to significantly impair the behaviour of vascular smooth muscle particularly when cooling is superimposed.

d) Blood flow.

Studies of the blood flow in the hands of Raynaud's patients have been conducted in an attempt to examine in detail the interaction between the role of sympathetic nervous stimuli and local cold stimuli in altering vascular hemodynamics that directly or indirectly affect the digits. Hand blood flow is commonly determined by the venous occlusion technique described by Hewlett and Van Zwaluwenberg (1911) using a water-filled plethysomograph. Hillestad (1970) initially compared hand blood flow in normal subjects at local plethysmograph temperatures of 6, 15, 20, 30 and 40°C at three different ambient room temperatures, 10, 23 and 32°C. At all three room temperatures, blood flow determined by the venous occlusion technique in a water-filled plethysmograph, was at a minimum (2.5 ml/100 ml/min) at a local hand temperature of 15°C. At all higher local temperatures, dilatation of the vessels occurred and blood flow was correspondingly greater the higher the room temperature. A maximal hand flow of 15 ml/100 ml/min was observed at a room temperature of 32°C and local temperature of 40°C. The ambient temperature was a significant determinant of whether cold vasodila-

tation appeared when the hand was cooled to 6°C in the plethysmograph. At a room temperature of 10°C, no increased flow was observed, whereas at room temperatures of 32° and 23°C hand flows were approximately 6 ml/100 ml/min which exceeded that observed at local temperatures of 15° and 20°C at the same room temperatures. At local temperatures greater than 15°C peripheral vascular effects appeared to be mediated through the vasomotor system. With progressive reduction to 15° C temperature, hand blood flows became less variable for a given local temperature. Flow changes in response to changes in sympathetic tone mediated by different room temperatures dramatically declined with reduced local temperatures and were negligible at a hand temperature at 15°C. These findings suggested that the local cold stimulus was the principal determinant of tone of the cutaneous vessels on exposure to cold down to 6°C. Local heating was observed to reverse the effect of sympathetic constriction. Conversely abolishing sympathetic activity did not appear to prevent cold-induced peripheral vasoconstriction and marked decreases in hand blood flow.

Similar studies were conducted with patients with vasospastic disease and obliterative arterial disease (Hillestad, 1970). Ambient temperature however, was restricted to 32°C. The hands were considered to be largely free from vasoconstrictor influence at this temperature. Two females with vasospastic disease had no evidence of underlying organic

disease, had normal arteriograms and both patients had had symptoms in excess of 10 years' duration. At all local temperatures including 40°C, the Raynaud's subjects and patients with occlusive disease of the hand, systematically exhibited reduced hand blood flows compared with healthy subjects. Subnormal flows were observed in the hands with occlusive disease at 40°C, and at 20° and 15°C reflecting cold sensitivity at these reduced temperatures. This observation at 40°C may suggest increased vessel tone in patients with primary Raynaud's. Although the cold-sensitive mechanism may be different in the two conditions, absence of vasoconstrictor tone did not alleviate the abnormal constriction. Although significantly less than normal in the Raynaud's patients, cold vasodilatation was observed at 6°C in all groups of subjects.

Peacock (1960) performed a similar study to compare hand blood flow with plethysmograph temperatures beginning at about 18°C and increasing by increments of 2° to 42°C. He compared flows between normals and Raynaud's patients graded by severity into three groups. All measurements were performed at a room temperature of 20 ±0.5°C. Raynaud's patients were categorized as Grade 1 having no evidence of lesions; and normal capacity of the hand circulation to respond to reflex dilatation. At local hand temperatures of 34°C, blood flow was 24% less than that for the normal subjects. This increased dramatically to 70% below normal at a

plethysmograph temperature of 27°C.   Blood flow ceased com-
pletely at 22°C in all three subjects.   Peacock concluded
that although a neural influence could not be totally ruled
out, the local effect of plethysmograph temperature played a
significant role in potentiating the arterial cold sensitiv-
ity of the hand. The effect of structural disease (grades 2
and 3) was observed not to increase vessel sensitivity at
reduced temperatures. Rather, the more severe symptoms seen
in patients with occlusion were explained by a reduced abil-
ity of the vessel to relax following contraction and produce
reactive hyperemia.   Peacock concluded generally that Ray-
naud's patients have reduced blood flow compared with nor-
mals at all ambient temperatures.

Downey and Frewin (1973) observed that both the percent-
age and absolute falls in hand blood flows were similar in
normal subjects and patients with Raynaud's phenomena. In a
room about 30°C in temperature, eight normal subjects had a
mean initial flow of 10.7 ml/100 ml/min and the Raynaud's
patients had a mean initial value of 7.2 ml/100 ml/min
(p<0.01). No difference was observed in terms of recovery
time and the time to return to normal resting flow following
a cold stimulus.   In a room near 20°C the normal average
initial flow was 6.5 ml/100 ml/min and for the patients was
2.85 ml/100 ml/min.   These values were 55 and 62% lower re-
spectively when compared with the 30°C room temperature.
Temperatures of 4°C in the plethysmograph produced marked

constriction in the hands of normal subjects. No apparent
difference was observed in the amount or the duration of the
fall in flow in the uncooled hand between the two groups.
This reflex constriction was attributed to afferent sensory
stimulation and also by the return of cooled blood to the
central thermoregulatory system resulting in a thermoregula-
tory reflex constriction as described by Pickering and Hess
(1933). Downey and Frewin interpreted their results, which
had been carefully corrected for spontaneous fluctuations
with reference to the normal hand, to support a direct ac-
tion of cold on peripheral blood vessels without mediation
via neurogenic impulses. The absolute fall and duration
were not different between the normal and patient subjects.
Given that this was observed in both cooled and uncooled
control hands, the investigators ruled out abnormal sensi-
tivity of the blood vessels of the hands of Raynaud's sub-
jects. They inferred from their findings that the therm-
oregulatory control and mediation of vasoconstriction
peripherally are not altered in Raynaud's subjects. Consid-
ering Peacock's observation (1960) that blood flow was sys-
tematically reduced in a variety of ambient temperatures,
Downey and Frewin favored the hypothesis that the mechanism
of Raynaud's phenomenon is a quantitatively normal constric-
tor tone superimposed on a reduced initial flow. This re-
sults in a diminished circulation to the digits responsible
for the characteristic signs of vasospastic attack. It was
suggested that normal constriction in response to cold in

the presence of low initial flow can reduce circulation to the fingers to a critical point and produce vasospastic symptoms.

In conclusion, these studies showed that blood flow was significantly reduced in the hands of Raynaud's subjects prior to and during cooling. Therefore, low flow in the digital arteries with normal vasoconstrictor tone may predispose the vessels to closure.

e) Viscosity.

Studies on blood viscosity in Raynaud's phenomenon have been difficult to interpret because the methods of measuring viscosity have differed and the types of patients have been too diverse or have been inadequately categorized. Recently, Dintefass (1982) reported that viscosity factors were similar in subjects with Raynaud's phenomenon and normals, however in some patients blood and plasma viscosity, red cell aggregation and rigidity were increased.

Based on a clinical suspicion that patients with Raynaud's disease had "thicker" blood, Pringle and his colleagues (1965) measured viscosity in 22 patients and in 22 normal subjects at a room temperature of 20°C. The procedure consisted of inserting a calibrated needle which was connected into a saline solution manometer, into a large superficial vein in the antecubital fossa. The vein pressure was raised to approximately 30 cm of saline solution, by

lowering the arm below heart level. When the pressure had stabilized at the needle site, blood was permitted to flow into a graduated heparinized tube for exactly 30 seconds. Pressure was remeasured. Thus blood flow was measured for a standard venous pressure. The viscosity coefficient was then derived from Poiseuille's equation. Using this technique normal viscosity values ranged from 2.30 to 2.75 cPoise (cP) with a packed cell volume between 40 to 45%. The mean value of the Raynaud's subjects was 5.2 cP and for the controls 2.5 cP. This was a statistically significant difference (p<.05). Plasma fibrinogen level was also significantly higher in the patients (p<0.001), but this was not consistently elevated in those patients having an increased blood viscosity. Slit-lamp microscopy of the conjunctival vessels revealed a greater tendency for sludging of red cells and aggregation in the patients. Generally normal values were observed in both groups for hematocrit, hemoglobin, white cell count, platelet count, serum protein levels and erythrocyte sedimentation rate. The significance of these apparent viscosity changes in the patient group with respect to the mechanism of vasospastic attacks is not known. It is likely that reduced temperature may further raise blood viscosity which may explain regional distribution of the disease. Jahnsen et al. (1977) examined viscosity at 7 shear rates in 5 young females with Raynaud's phenomenon. No abnormal increase in whole blood or plasma viscosity changes was noted down to 10°C. At 27°C whole

blood viscosity appeared to be generally increased but this was only significant at a shear rate of 11.5s^{-1}. Objective cold sensitivity measures were then performed using a method described by Nielsen and Lassen (1977). Following finger cooling, increased arterial tone was indicated by a correspondingly reduced finger systolic pressure. Zero pressure demonstrated complete closure of the digital arteries. Despite the minimal increase in viscosity, finger pressures were reduced at 20°C in the Raynaud's subjects, and zero pressure or vessel closure occurred between 14° and 18°C in all patients. Results were compared with those two days after a venesection of 500 ml of blood. Predictably hematocrit, whole blood and plasma viscosity were decreased, but this did not alter local reaction to cold gauged by finger systolic pressures. On the basis of these findings viscosity was thought to contribute little, if at all to symptoms associated with primary Raynaud's phenomenon. These different results probably reflect differences in the patients' diagnoses, disease severity, and techniques for measuring viscosity.

f) Changes in circulating catecholamine levels in the blood.

Circulating catecholamines have been suggested as a factor responsible for Raynaud's phenomenon. Following Von Euler's (1946) demonstration that NE was contained within the sympathetic adrenergic nerve terminals and subsequent demon-

strations that NE was released from these nerves on stimula-
tion, it was postulated that some neuronal aberration was
responsible for the exaggerated vessel constriction observed
in Raynaud's disease. Peacock (1959) looked specifically at
NE and epinephrine (EPI) levels in venous blood from the
wrist in 11 patients and 6 healthy subjects. The patients
were selected on the basis of Allen and Brown's criteria for
primary Raynaud's disease (1932). Measurements were taken
when the subject had rested, covered with blankets, in the
recumbent position at a room temperature of $26 \pm 1.0^{o}C$, with
one hand immersed in a water plethysmograph at $32 \pm 0.5^{o}C$.
An indwelling venous canula was positioned on the dorsum of
the opposite hand. Blood flows were recorded with simulta-
neous sampling of venous blood. This procedure was repeated
several times for each subject in this warm condition and
also in a relatively cool condition. The latter condition
resembled the warm condition with the exception of a small
area over the abdomen exposed to an air conditioning duct
(20 m.p.h. blast of cold air at $4^{o}C$). Hand blood flows were
greater in the normals in both warm and cool states; normals
11.4 and 3.1 ml/100 ml/min and Raynaud's 4.9 and 0.6 ml/100
ml/ min. These results suggested that in the patients ab-
normal peripheral constriction prevailed in both vasomotor
conditions. Both catecholamine levels were above normal in
the Raynaud's patients and this seemed to be related to the
severity of disease in the cold condition. This observation
was explained by the effect of cooling on the arterial wall

and inhibition of the degradation of both catecholamines. Peacock (1959) found elevated amine oxidase activity of two digital arteries removed from two fingers of a patient suffering from primary Raynaud's disease. Peacock speculated that high catecholamine levels resulted from a metabolic abnormality of these substances. Subsequent studies by Kontos and Wasserman (1969), unlike Peacock's observation, showed no evidence of increased catecholamine levels in the brachial artery, venous plasma, or arteriovenous gradients of catecholamines across the hand in patients with Raynaud's phenomenon. It is not clear why different results were obtained and further work in this area is warranted.

4. Possible Mechanisms in Raynaud's Phenomenon

This section deals with smooth muscle mechanisms which might be implicated and disturbances in which might be responsible for abnormal responses observed in Raynaud's phenomenon.

Potentiation of smooth muscle responses with moderate cooling has been reported in some in vitro studies in such vessels as the ear arteries of the ox and sheep (Surgeon and Wallace, 1975), the rat tail artery (Wade and Beilin, 1970) and the canine saphenous vein (Vanhoutte and Shepherd, 1969). An increased reactivity with cold in superficial arteries is an interesting hypothesis to explain arterial spasm of the fingers of Raynaud's patients. Response poten-

tiation with cooling may be effected by an increased sensitivity of the cell membrane (receptor sites) to vasoactive agonists, reduced transmitter disposal, inhibition of an electrogenic pump and decreased removal of sarcoplasmic Ca. Janssens and Vanhoutte (1978) found that cold-induced potentiation in isolated superficial canine veins was produced by a change in the receptor affinity of the postjunctional α adrenoceptors and could not be explained on the basis of cold-induced changes in transmitter overflow, uptake or degradation, or altered Ca metabolism per se.

In some mammalian blood vessels, contraction and relaxation are mediated by α and β adrenoceptor function. Changes in vasomotor tone of the vessels supplying human skin are thought to be mainly due to changes in the number of sympathetic impulses which innervate α adrenoceptors (Shepherd, 1963). β receptors whose stimulation causes relaxation appear to play little or no part in the control of skin circulation. This has been supported by evidence in human palmar arteries which did not show direct β adrenergic-mediated relaxation (Moulds et al., 1978). However patients on β receptor blocking drugs tend to have increased cold sensitivity of the hands (Thulesius, 1976). Cohen and Coffman (1981) reported that β receptors are present in arteriovenous shunts of human finger tips, however this work thus far has not been confirmed. Such data led to the suggestion that primary Raynaud's disease is related to an absence or de-

creased activity of ß adrenoceptors (Vanhoutte and Janssens, 1980). However, there is no evidence for the presence or absence of ß adrenoceptors in human digital arteries. Studies are needed to establish the effect of stimulation of adrenoceptors in these vessels.

A reduction in temperature has been reported to affect the excitability of vascular smooth muscle cells. For example, the portal vein of the guinea pig has been shown to exhibit an immediate depolarization with cooling, and a ouabain-sensitive hyperpolarization following rewarming (Kuriyama et al., 1971). These findings suggest that an electrogenic pump contributes significantly to the E_M in this smooth muscle preparation. Hermsmeyer (1976) reported a rapid cold-induced depolarization in the rat caudal artery. The effect of cold-induced changes in membrane polarization of superficial blood vessels in humans needs investigating in both normal and diseased arteries because the effect of cooling on partially depolarized vascular smooth muscle may augment contraction and peripheral vasoconstriction.

The activity of mammalian Na-K ATPase, the enzyme effecting active sodium and potassium transport through the membrane, is known to be inhibited between 18° and 20° C (Charnock et al., 1971). The contribution of this temperature-dependent depressant effect on Na-K ATPase and any consequent augmented responsiveness of the vascular

smooth muscle of digital arteries in normal and exaggerated pathologic reactions to cold is not known. Based on previous observations with other vessels however, cold may potentiate contraction by means of cell depolarization through the inhibition of pumping and decrease in E_M as well as the loss of the Na gradient and Na/Ca exchange, to produce smooth muscle activation and vasospasm of the digital arteries in Raynaud's disease.

D. INDIRECT SYSTOLIC BLOOD PRESSURE MEASUREMENTS WITH SPECIAL REFERENCE TO THE DIGITS

1. Historical Perspectives

Indirect pressure measurement techniques were first applied to the fingers by Gaertner in 1899. Gaertner's method consisted of placing a rigid pneumatic capsule at the base of the finger, blanching the finger, inflating the cuff lining the capsule and watching for a visual flush indicating the resumption of blood flow to the finger.

Transition from the rigid Gaertner cuff was brought about by Weaver and Bohr (1950) who used a rubber bladder within a cloth cuff. Rubber tubing from the bladder connected with a mercury manometer and hand bulb to control pressure inflation and deflation of the cuff. To improve the quality of the visible return of blood to the finger, a piece of rubber was stretched over the digit to produce blanching prior to cuff inflation.

From the 1950s onward, attention has focussed on the development of more sophisticated distal sensors, and the control of certain cuff parameters in the measurement of blood pressure in the digits (Gaskell and Krisman, 1958a; Lezack and Carter, 1970). Both the distal sensor applied and arti-

facts introduced by the cuff or its application were recog-
nized more recently as being potential sources of variation
in digital pressure measurements (Gundersen, 1972; Gaskell
and Krisman, 1958a; Lezack and Carter, 1970). Over the last
30 years, the oscillometric method (Wishart, 1933) requiring
elaborate equipment has been found to be too cumbersome to
apply routinely.   Nailfold visualization of flow resumption
although readily performed was reported to be of limited use
in patients with pigmented skin or with skin thickening in
the nailfold area.   Gaskell and Krisman (1958b) compared
auscultatory and plethysmographic methods simultaneously and
found good agreement between the digital pressures measured
in both vasodilated and vasoconstricted states.   Both these
methods however involved somewhat cumbersome procedures
which also made them unsuitable for routine use.   Gaskell
(1965) has reported successful use of the spectroscope tech-
nique which was found to give essentially the same results
as the auscultatory method. By reflecting a light beam from
the blanched finger tip and viewing the area through a spec-
troscope the return of the absorption band associated with
oxyhemoglobin in the skin vessels proved to be a reliable
measure under normal laboratory conditions.   Gaskell (1965)
successfully applied this technique to detect the disappear-
ance and reappearance of the oxyhemoglobin band in measuring
the critical closing and opening pressures of small arteries
in the finger tip.

Over the past decade plethysmographic techniques have been used most frequently for routine digital pressure measurement. The technique involves the recording of volume changes in a limb or part of a limb. One of the earliest reports of applying the principle of using a volume signal to measure repeatedly arterial blood pressure was that of Doupe et al. (1939b). Each pulse beat generated a current which activated an electromagnet and opened or closed a valve supplying air to the blood pressure cuff. Therefore when blood pressure was greater than systolic, cuff pressure would decrease; and when it was less than systolic pressure, air would be added to the cuff. In this way, by electronically automating the plethysmographic signal cuff pressure was maintained within a few millimeters of systolic pressure. This system was less sensitive however if the fingers were cold.

Essentially all plethysmographic devices reflect changes in volume but differ with respect to their sensitivity and ease of application. The most commonly used plethysmographic sensor is the mercury strain gauge.

The so-called photoelectric plethysmograph is one of the more recent innovations to detect blood content of the skin. Pulse tracings are remarkably similar to such plethysmographic devices as mercury strain gauge making photoelectric plethysmography suitable for detecting pulse resumption in clinical investigation (Holmgren et al., 1981).

The ultrasonic flow detector using the Doppler effect has also been advanced in more recent years as being technically easy to apply, providing quantitatively reliable results in blood pressure measurement in the limbs, giving good agreement with other techniques and therefore has been recommended for routine clinical use (Carter, 1969). However, use of small pencil-like probes in the fingers for detecting vessel patency and blood pressure has been found less reliable, particularly during vasoconstriction.

Various indirect methods to detect the beginning of blood flow in the measurement of blood pressure agree with each other and are reliable tools (Carter and Lezack, 1971; Downs et al., 1975; Lezack and Carter, 1970).

Several variables have been found to influence blood pressure and these appear to exert a greater effect on pressures measured distally. These factors include both variables intrinsic to the subject such as vasomotor state, skin temperature, deep breaths, digital circumference, and body size and external variables which include body and limb position, sudden noises, movements, lights, cold or warm air, momentary ice application to the skin, and the distal sensing device used. Sudden noises and sudden application of local cold stimuli to the body elicit transient increases in vasomotor tone. Considering that all these factors may exert an effect on the digital pressure measured, each of these variables and their effects need to be fully under-

stood when undertaking digital blood pressure recording. This emphasizes the importance of procedural standardization to ensure that the pressures are as valid and reliable as possible. The control of this variability to provide more valid and reliable measures warrants further study.

2. Determinants of Peripheral Blood Pressure

Digital pressures have been found to be generally less reproducible than pressures taken more centrally (Gundersen, 1972; Gundersen, 1973; Nielsen, 1978). To ensure that one estimates intraarterial pressure as closely as possible through noninvasive means requires a thorough knowledge of the determinants of digital blood pressure. This section describes those factors which influence peripheral pressures. Important technical considerations are also discussed in this section.

a) Vasomotor tone.

Changes in vasomotor tone are known to alter blood flow and pressure considerably in the extremities. Doupe et al (1939a) conducted a series of elegant studies in which reflex vasodilatation was induced by immersion of an indifferent limb into water of 45°C. This reflex vasodilation resulted in reduction of finger pressures of 20 mm Hg below brachial pressure. Reflex vasoconstriction of an indifferent limb induced by immersion into water of 20° to 25°C led

to finger pressures increasing to values equal to brachial pressure. These observations have been confirmed more recently by others (Gaskell and Hoeppner, 1967; Lezack and Carter, 1970), and explained by the corresponding changes produced reflexly in total peripheral resistance with either body warming or body cooling.

Transient vasoconstriction of the digits during body vasoconstriction and vasodilatation was induced with stimuli such as ice or a pin prick to the skin. With subjects cold and vasoconstricted as indicated by the decreased pulse amplitude on the plethysmographic record, little additional change in flow was noted following exposure to the noxious stimuli. Following warming and dilatation of the digital vessels evidenced by increased pulsations on the plethysmographic tracing, ice or pin prick resulted in a sudden transient diminution in pulse amplitude and simultaneous increase in finger systolic pressure. Finger systolic pressure transiently approached brachial pressure which otherwise had remained stable throughout testing. Thus the transient digital pressure increase was apparently independent of a generalized systemic blood pressure increase. Marked reductions of digital blood flow occurred concurrently with increased digital pressure.

Similar reflex vasoconstriction resulted after a subject took a deep breath. On initially taking a deep inspiration the volume of the digits increased transiently and on expi-

ration finger volume decreased to a much lower level. Other workers have concurred with Doupe that this was an autonomic reflex since with surgical sympathectomy or peripheral neuropathy secondary to diabetes this inspiratory reflex was abolished (Strandness et al., 1964). However, unlike transient vasoconstrictions induced with ice application or pin prick, the brachial pressure in the sympathectomized limb tended to fall slightly (Doupe et al, 1939a).

The vasculature of the skin has a considerable capacity to accommodate a wide range of blood flows to fulfill its role in thermoregulation. Minimal flows of 1 ml/100 ml of tissue/min or less occur with intense discharge of sympathetic fibers. With complete inhibition of sympathetic tone during body heating superimposed with an application of heat to the hand, (40° to 43°C), blood flow increases one hundred fold to about 150 ml/100 ml of tissue/min (Folkow and Neil, 1971). At these high flow rates, skin temperature is particularly an insensitive index of flow because in this range of skin temperatures large changes in blood flow are associated with small changes in skin temperature (Felder et al., 1954).

Digital arterial pressure is more constant when performed with the subject either vasoconstricted or vasodilated because blood flow is likely to be less variable in the limbs of subjects who are vasodilated or vasoconstricted and therefore pressure energy is more constant. Many studies

have reported digital pressures without reporting the vaso-
motor state of the subject or performing them when the sub-
jects were in a "thermoneutral" vasomotor state. Skin temp-
eratures and blood flows, hence pressure, fluctuate sponta-
neously when the subject is thermoneutral.

The transient vasoconstrictions induced by cold stimula-
tion, pin prick, solving a mental problem or deep inspira-
tion can be elicited when a subject is warmed, as was previ-
ously described. These sympathetically-mediated
constrictions occur too rapidly for skin temperatures to be
significantly affected, however, blood flow changes can be
recorded. Doupe et al. (1939a) reported that by the sixth
second after such a stimulus, blood flow was at its lowest
rate and by the tenth second flow approached pre-stimulus
levels. He also observed that locally warming one digit
while the subject was vasoconstricted markedly increased the
flow to the warmed digit, however the flow was less than
during body warming.

Doupe et al. (1939a) demonstrated that the effects of pe-
ripheral vasoconstriction on finger pressures could be mim-
icked by decreasing hand blood flow. When blood pressure
cuffs were inflated on four fingers of one hand, the blood
pressure in the remaining digit increased and approached
brachial pressure. Krähenbühl et al. (1977) reported simi-
lar findings. Hemodynamically these phenomena can be ex-
plained in the same way. If blood flow is dramatically re-

duced in the hand, less energy is expended in producing flow resulting in greater pressure energy distally and in increased finger systolic pressure. These findings support the fact that finger systolic pressure is dependent on hand blood flow, and is independent of blood flow through the digital arteries on the measured finger.

In the fingers and toes, blood temperature can be up to several degrees less than core temperature. The effect of such a temperature variation on clinical blood pressure determinations in the digit has recently been thought to be an important factor warranting further investigation (Krähenbühl et al., 1977). Local cooling in the periphery appears to have a paradoxical effect on digital blood pressure. Reducing the temperature of the finger arteries by means of locally cooling the skin and cutaneous arteries results in an apparent reduction in finger systolic pressure. The observation that finger systolic pressure was apparently reduced with local cooling (Krähenbühl et al., 1977) provided the basis for the method described by Nielsen and Lassen (1977) for detecting and assessing the severity of cold sensitivity in the fingers. Increased tone in the digital arteries was reported to be associated with a reduction in apparent systolic blood pressure in the fingers. Finger systolic pressures of zero in cold sensitive individuals were indicative of excessive vessel constriction, capable of overcoming intravascular pressure, resulting in vessel clo-

sure. This was generally not true for healthy subjects. A local decrease in apparent finger systolic pressure appears to reflect abnormal cold sensitivity of the digits. Typically generalized vasoconstriction results in increased finger systolic and systemic pressures.

b) Systemic blood pressure.

Moment-to-moment changes in systemic blood pressure are transmitted to the periphery. Therefore, a recorded increase or decrease in the finger pressure may represent a momentary systemic pressure change. To detect this, a brachial pressure is frequently taken concurrently with or immediately after each digital pressure and may be used to make corrections for changes in systemic pressure (Carter and Lezack, 1971).

c) Skin temperature.

The temperature of the skin provides a gross index of the blood flow as discussed. In health, blood flow and pressure of an extremity are largely a function of vasomotor tone. Monitoring skin temperatures can be useful when carrying out digital pressures to grossly assess the status of blood flow.

d) Limb position.

Normally digital blood flow decreases with elevation or dependency of the limb from the supine position (Gaskell and

Becker, 1971). With elevation, decrease in the hydrostatic pressure exerted from the weight of the column of blood in the digit or limb, results in reduction in systolic pressure. In dependency of the limb, this reduction is due to the stimulation of the myogenic venovasomotor reflex (Gaskell and Becker, 1971).

Limb dependency has been a means of relieving an occluded limb from ischemic pain for many patients. In arterially occluded limbs, dependency has been shown to increase rather than decrease blood flow (Dahn et al., 1966). Dependency in this situation increases the hydrostatic pressure in the limb which passively distends the collateral vessels. This reduces resistance and increases blood flow to the dependent limb with arterial occlusion.

Greater increases in ankle and toe pressures than would have been predicted by hydrostatic considerations alone have been observed in patients with arterial occlusive disease on changing position from supine to sitting and standing (Gaskell and Becker, 1971; Lezack and Carter, 1969). This finding suggests that distention of collateral vessels is responsible for the smaller pressure drop across the obstruction; hence reduction of signs and symptoms of ischemia. Because of the effect of hydrostatic pressure, it is critically important in routine measurement that the subject's position is standardized. For example, blood pressure of the fingers should be taken with the subject lying

comfortably in the supine position. The arm should be ideally at heart level with the forearm supinated and fingers minimally curled.

Lezack and Carter (1970) investigated whether higher pressure measured in the fingers of normal subjects compared with the toes could be explained by differences in hydrostatic pressures and related to posture since pressures were routinely measured with the subject in the supine position with the toes about 10 cm above finger level. Elevation reduced the observed systolic pressure of both fingers and toes. Pressures in the toes however, were consistently lower than in the fingers even when they were at the same level during measurements. These investigators concluded that the hydrostatic difference is not solely accountable for the discrepancy. Vessels of the feet may be geometrically different than from those of the hand and offer greater resistance. Therefore greater utilization of pressure energy is required to maintain flow and consequently a reduced systolic pressure is measured distally.

e) Age and sex.

The effects of sex and age on digital systolic pressure have not been well documented, but may be important considerations. There is a tendency for older persons to have higher systolic pressures than younger persons. With age, blood vessels become less elastic which increases wall

stiffness and thereby decreases wall compliance. This is known to affect circulatory hemodynamics due to a general increase in systemic and peripheral pressure. Digital pressures expressed as a percentage of brachial pressure compare favorably, in older and younger adults without peripheral vascular disease (Carter and Lezack, 1971).

The effect of sex on digital pressure has not been established. There is some evidence that sex hormones may affect blood flow. The rationale for this was based on the observations that digital vasospasm was notably exacerbated during the menstrual period and menopause and remitted during pregnancy (Spittell, 1980). The role of age and sex has not been systematically studied in relation to vasospastic disease.

A role for sex hormones in the regulation of blood flow, however has been demonstrated experimentally in the resistance vessels of female rats (Altura, 1972). Dose-response curves in response to epinephrine and norepinephrine were found to be consistently shifted to the left compared with vessels from male rats, suggesting arterioles in the female rats were more sensitive to catecholamine concentrations. Webb et al. (1981) have reported in humans minimal increases in peripheral blood flow in females compared with males at rest, and flow as statistically significantly increased after 3 to 5 minutes of arterial occlusion. Such sex-linked differences may be important in the physiopathology of va-

sospastic disorders considering that females are afflicted four times as frequently as men. Further in vivo study into the effects of both age and sex are indicated to establish digital pressure ranges for the normal population in addition to elucidating on the mechanisms of cold-sensitive disorders which might explain their predilection for women.

f) Arterial occlusion.

Over the last twenty years, segmental noninvasive blood pressure measurements in the extremities have been reported to provide a reliable and valid index of arterial occlusion (Carter, 1968; Carter, 1972a; Downs et al., 1975). A significant reduction in blood pressure along the limb reflects reduced pressure energy as a result of greater total energy being lost in blood flow across the stenosis or through narrow collateral vessels. Based on the correlation of segmental blood pressures in about 150 limbs with angiographic evidence of arterial occlusion, guidelines for interpretation of segmental blood pressures have been provided by Carter (1968). Mild or questionable occlusion has been more difficult to detect on the basis of systolic blood pressure measurements alone. However, when the measurements follow limb exercise, mild occlusion can frequently be unmasked (Carter, 1972b). In assessing arterial occlusion the absolute pressure is important as well as the pressure gradient or the percentage of the pressure proximal to the obstruction (often taken as brachial pressure). In assessing digital pres-

sures however, greater emphasis is placed on absolute pressure and pressure difference between adjacent fingers (Downs et al., 1975). Absolute pressure of the digits is important as an index of tissue perfusion. However in patients with higher central pressure and occlusion, systolic pressure may be reduced to an apparently adequate perfusion pressure, e.g., 70 to 80 mm Hg. In this situation absolute pressure may not be a good index of occlusion. A difference of more than 15 mmHg between corresponding fingers of the two hands has been shown by Downs et al. (1975) to provide a good criterion to rule out digital occlusion.

Measurements of digital pressures as a clinical tool can be of additional value when compared to pressure at a proximal site and/or expressed as a pressure difference or percentage of the proximal pressure. The severity of a stenosis in the digits for example, has been reported to be effectively assessed by comparing the systolic pressure of finger or toe with the corresponding ipsilateral wrist (Downs et al., 1975); or the ankle (Lezack and Carter, 1970) respectively. The degree of pressure drop along the limb helps to establish the presence and severity of stenoses and occlusions. The use of noninvasive blood pressure measurements has therefore been found to be of significant clinical value in the assessment of arterial occlusive disease. A greater appreciation of the determinants of blood pressure in the limbs will further contribute to the usefulness and

versatility of this clinical tool, and possibly enhance the usefulness of pressure measurements in conjunction with other clinical procedures such as determination of digital cold sensitivity.

3. Distal Sensors

A variety of distal sensors have been used over the past fifty years for blood pressure measurement in the digits. A summary of these sensors is presented and some of the various advantages and disadvantages associated with them are described.

a) Oscillometric method.

Wishart (1933) introduced the oscillometric method for detection of blood flow return following digital occlusion. The oscillometer detects movement of the arterial wall by detecting the amplitude of pulse oscillations transmitted from a pneumatic cuff. This method is not currently used for routine blood pressure measurement in the digits because it is cumbersome, and is inaccurate in low flow states.

b) Capillaroscopy.

Gaskell and Krisman (1958c) reported good results with direct visualization of the return of blood flow to the nailfold capillary bed. A cover slip was sealed over the nailfold and the finger under observation was enclosed in a plethysmographic box. A blood pressure cuff was attached over the proximal phalanx and deflated gradually as the nailfold area was viewed through a microscope. This technique is also awkward for routine use and has limited application in cases where there is thickening or dark pigmentation of the nailfold region.

c) Auscultatory method.

Gaskell and Krisman (1958b) reported performing digital blood pressure measurements by direct auscultation of the digital systolic and diastolic pressures using a conventional stethoscope bell attached to the cuff. Cold fingers would result in muffling of the Korotkoff sounds thus limiting the usefulness of this method to relatively high flow states.

d) Spectroscope.

Oxyhemoglobin in the blood of the skin capillaries can be detected as an absorption band of reflected light viewed through a hand spectroscope. By reflecting a light beam over the finger tip, the reappearance of the band provided a

visual end point for return of digital flow during blood pressure measurement (Gaskell, 1965). When applied carefully, this sensor can be used clinically for peripheral blood pressure measurement.

e) Strain gauge plethysmography.

Silastic strain gauges are constructed of fine bore silicone rubber tubing filled with mercury. Copper electrodes inserted into each end of the tubing establishes an electrical contact. The gauges form a complete circle for fitting around a digit. Current enters the gauge through a set of lead wires, the voltage drop is detected by a bridge circuit. With each heart beat a detectable change in the circumference of the gauge is manifested by volume change of the digit. The electrical resistance of the gauge is increased when it is stretched. The gauge is balanced on a wheatstone bridge, and with the appropriate amplification digital pulses can be readily detected. In measuring pressures of the digits, an occluding cuff is placed proximal to the gauge. This cuff is initially inflated to above the systolic pressure, which obliterates the pulse signal. Systolic pressure is recorded as the pressure at which the pulse returns during cuff deflation and as an increase in volume of the digit because flow has resumed to the digit. The basic theory therefore, underlying strain gauge plethysmography is the relationship between relative changes in gauge resistance and relative changes in gauge length which

is a function of limb volume. The strain gauge technique has been found useful as a diagnostic and assessment tool (Strandness and Bell, 1964).

f) Photoelectric plethysmography.

Photoelectric plethysmography is the latest in a series of distal sensors used to measure peripheral blood pressures and has been reported to be as valid and reliable as other methods (Vollrath et al., 1980). The small photocell can be easily positioned on a finger tip of an adult. It contains a diode capable of both transmitting a light source onto the skin and receiving the reflected light. The amount of light reflected represents the amount of blood in the small vessels of the skin. Also changes are detected with changes in volume of blood observed with the pulse. As pressure in the occluding cuff is reduced, blood flow returns to the digit and increased light is reflected back to the photocell. Depending on whether an AC or DC mode is used, pulse and/or volume changes can be detected.

g) Doppler ultrasonic techniques.

Doppler ultrasound has been found to be an excellent sensing device for detecting flow resumption for blood pressure measurements (Strandness, 1978). The technique is based on the principle that a moving object in the path of the transmitted sound beam shifts the frequency of the transmitted signal. Thus as pulsatile flow resumes as cuff

pressure falls below systolic pressure, sounds are detected through earphones attached to the control box, which reflect the motion of the blood which can be easily detected in superficial distal arteries especially when flow velocity is low as in vasoconstriction. The use of Doppler techniques is however, less reliable for digital pressure measurements than for more proximal measures because the vessels are small, and particularly because of very low blood flow with low temperature.

4. Cuff Characteristics

a) Cuff material.

Digital blood pressure cuffs for routine measurements are commercially available. Because these may be expensive, difficult to obtain, and poorly fitting, some investigators have reported constructing their own (Gundersen, 1972; Downs et al., 1975). The same principles governing the manufacturing of larger cuffs have been applied: non-elastic external coating, a more flexible bladder which allows for even pressure transmission and a fine adjusting velcro strap or tape if the cuff is not of the cylindrical type.

Krähenbühl et al (1977) have reported that commercially available rubber cuffs may be too thick-walled and give erroneously high values. In normal subjects they compared a thin walled plastic foil bladder cuff (0.1 mm thick, of pli-

able polyvinyl chloride and measuring 1.5 cm in width and 10 cm in length), a commercially available rubber cuff, and an occluding cuff without the conventional bladder. Keeping the bladder-free cuff air tight posed a problem and consequently was not recommended for routine use.

Krähenbühl et al (1977) defined the value of a digital cuff on the basis of how closely digital pressure approximated branchial pressure. No rationale was reported for this criterion. Based on the results from one subject in a cool-body state, the least gradient between auscultatory brachial systolic pressure and finger systolic pressure (FSP) was observed in the plastic cuff (0.2 mm Hg, SD 4.4). This same gradient was -7.8 mm Hg (SD 8.8) for the rubber cuff and 2.2 mm Hg (SD 1.9) for the bladder free cuff. Although these results were the average of multiple measurements their variability makes the use of this criterion questionable. Because of the awkwardness of the application of the bladder-free cuff, the plastic cuff was preferred by this group.

Downs et al. (1975) and Gundersen (1972) have constructed thin rubber bladders from latex Penrose tubing. The tubing is commercially available in several different widths. In the middle of the bladder tubing an elastic band was inserted to maintain an open passage around the inside of the cuff. Gundersen closed the tubing at both ends with glue. The cuff was fastened to the digit by an adjustable Velcro

strap. Ensuring that the bladder is smooth and even is imperative if reliable pressures are to be expected. Downs and his associates attempted to overcome this potential problem by using a cylindrically fitting cuff. Cuffs of this construction are non-adjustable and are made for a digit of a given diameter. Cuffs applied too loosely may promote wrinkling of the inner surface of the bladder and permit an inadvertent stream of blood through prematurely, because of the uneven pressure transmission that results. To prevent this the cuff should fit the digit as exactly as possible (being neither too loose nor too tight). Downs et al. (1975) used the criterion of just being able to rotate the cuff with the application of talcum power to prevent sticking to the skin.

b) Cuff width.

The established standard for blood pressure cuff widths is that the cuff be 20% greater than the diameter of the limb or digit to which it is to be applied (Kirkendall et al. 1967). This standard has become known as the "20% rule". In the digits this rule has not been routinely applied; and whether in fact this rule is relevant to them has been the subject of debate.

The logical question is which cuff width for a given sized extremity or digit will produce an indirect blood pressure measurement which corresponds to intraarterial

measures of the underlying major arteries. This question although investigated has not been definitively answered. Nielsen et al. (1973) have found in normal subjects a slight increase in pressure at the wrist compared with brachial, and higher yet in the fingers when measured indirectly. These investigators also showed by direct arterial puncture that in fact the pressure gradient was in the opposite direction; the more distal pressure being lower. Gaskell (1965) has attributed this discrepancy to the cuff width. The cuff used by Hirai et al. (1976) which measured 2.4 cm in width overestimated the pressures by several millimeters of mercury in Gaskell's study in which 3.8 cm wide cuffs were used. Direct versus indirect blood pressure measurements is discussed in Section D5. For the present discussion, the rule of thumb for the suitability of cuff width in the digits is that it gives systolic pressures approximately less than or equal to brachial systolic pressure in the normal digit depending on various relevant factors previously described. Downs et al. (1975) have shown that a very narrow cuff tends to give values much above arm systolic pressures. Alternatively an excessively wide cuff may erroneously underestimate the systolic pressure that is much below arm systolic pressure.

Hirai et al. (1976) examined the effect of different cuff widths in all digits except the second, on the measurement of systolic blood pressures using the strain gauge tech-

nique. Twenty-four subjects with no history of arterial insufficiency participated in the study. Pressures were measured at both the proximal and intermediate phalanges in the four medial fingers, and on the proximal phalanx in the thumb. The criterion for accepting a cuff width for a digit was that the resulting systolic pressure approximated brachial systolic pressure. Of the four cuff widths tested the 16 mm wide cuff applied around the proximal phalanx chronically overestimated the pressures of all fingers. Brachial pressure was best approximated in finger V with the 20 mm wide cuff, and in digits I, III and IV with either the 24 mm or 27 mm wide cuff. On comparing the variation of pressures taken with the last two sized cuffs, the 27 mm wide cuff was found to be more variable, therefore the 24 mm wide cuff was considered ideal for the 15 normal subjects studied. Measured in this way no significant differences were apparent between fingers. These results are in agreement with the cuff widths predicted for these subjects by the "20% rule".

The criterion that digital pressures should approximate brachial pressure was not qualified by Hirai. Given that vasomotor state and other factors have a pronounced effect on digital pressures, some guidelines need to be established to optimize the validity and reliability of these measures.

c) Cuff length.

The general rule for both arm cuffs and finger cuffs is that the cuff should fully encircle the limb or digit (Simpson et al., 1965, Gundersen, 1972). Gundersen examined the effect of 3 different cuff lengths (6, 9 and 11 cm) on thumb blood pressure in one normal healthy male. The cuff width remained constant at 24 mm. Using a short cuff (6 cm), tended to overestimate the recorded blood pressure. Accuracy seemed less affected by using a long cuff. The blood pressure measured was not significantly different between the 9 cm and 11 cm long cuffs.

d) Cuff tension.

Gundersen (1972) has examined the effect of cuff tension on the digital blood pressure. This is an important consideration when taking pressure measurements in the digits since technically achieving ideal tension is more difficult than in an arm or an ankle which are larger and less tapered. In one healthy male, Gundersen applied a cuff that was 24 mm wide and 9 cm long to a thumb, and measured the effect on blood pressure of three standardized tensions; tight, loose and very loose. A tight fit was obtained by applying the cuff "firmly". A loose fit was obtained by applying the cuff around the digit and a metal bar 4 mm in diameter, and removing the bar after cuff application. A very loose fit was similarly obtained by using a metal bar 7 mm

in diameter. Gundersen concluded that a tight fit did not have a significant effect whereas with a loose or very loose fit the blood pressure was overestimated. A too tightly fitting cuff should be avoided however since resulting stasis and engorgement distal to the cuff will interfere with changes in recorded finger volume. If the cuff is too tight, it could press the artery and thus less pressure would be required to close the vessel and measured pressure could be underestimated. For standard cuff applications Gundersen concluded that after talcum powder application to the cuff and digit the cuff should just be able to be rotated by the examiner.

Downs et al. (1975) used cylindrical cuffs which have certain advantages. A set of sizes have to be available for different sizes of digits. The tension was deemed appropriate if it was neither too difficult nor too easy to rotate the uninflated cuff on the digit.

e) Cuff placement.

Comparisons have been made of the blood pressures in fingers and toes when the cuff is placed proximally or distally along the digit. Hirai et al. (1976) conducted such a comparison in all five fingers and found that the blood pressure of the proximal phalanx was significantly greater than that of the intermediate phalanx in each finger except the fifth by about 4 to 7 mm Hg. One advantage perhaps of plac-

ing the cuff at the proximal phalanx is that the anatomic variation appears less when compared with the more tapered shape of the intermediate phalanx. This factor which may differ from one individual to the next should be considered when applying the cuffs. On inflation, pressure applied at the wider portion of the digit will reflect both cuff and inflation pressure; hence uneven pressure may be exerted along the cuff. However, for a thorough assessment of suspected arterial insufficiency in the digits, measurement of the intermediate phalanx is likely to be more valuable and if necessary can be compared.

Changes in pressures between proximal and more distal sites along the fingers and toes in two vasomotor states have been studied by Lezack and Carter (1970). Although no significant differences were noted in the toe during either vasoconstriction or vasodilatation nor in the finger following dilatation, the distal pressure in the finger during vasoconstriction was significantly less (10 mmHg) than the proximal pressure. This suggested that cooling produced sufficient vessel constriction to apparently reduce the distal pressure. Krähenbühl's work later described a technique to harness this observation as a means of testing the cold sensitivity of the fingers (Krähenbühl et al, 1977).

These studies generally indicate that standardized cuff position and placement are essential in digital pressure measurement, and that consistency from one application to

the next must be ensured when studying the same patient or making comparisons between individuals.

f) Deflation rate.

The importance of cuff deflation rate has been largely ignored as a possible contributing factor to measurement error. Cuff deflation rate has not been considered of sufficient significance in studies to warrant reporting by investigators. Gundersen (1972), however, was among the first to draw attention to cuff deflation rate and to systematically investigate this factor. He analyzed the amount of measurement error (the difference between direct pressure and indirect pressure measurements) in 8 subjects using five different deflation rates in 187 indirect blood pressure measurements in the thumb. These measures were compared with pressures measured directly at the radial artery. Pressures taken at deflation rates between 4 and 6 mm Hg per second were reported to be comparable and error was least. At higher rates of deflation (greater than 8 mm Hg) indirect pressures were underestimated and the measurement error increased. The shape of finger volume recordings were also changed with different deflation rates. That is, low rates showed more gradual volume increases and smaller amplitudes of initial pulses. Although these observations have only been made in the thumb, they should be repeated and extended to other digits.

5. Comparison with Direct Blood Pressure Measurements

Comparison of indirect and direct blood pressure measurement is essential both for determining the validity of non-invasive techniques and for extrapolating what cuff characteristics will best approximate the directly measured blood pressure in a limb or digit. Intraarterial brachial pressure for example, has been found to agree well with indirect measurements using a 12 cm wide cuff and a stethoscope. A 12 cm wide cuff is approximately 20% greater than the average adult upper arm. From this observation cuff width in general has been taken to be 20% greater than the limb or digit it encircles.

Gundersen and Lassen (1970) have found good agreement between indirect thumb pressures taken with a 24 mm wide cuff and brachial pressure. Whether these measurements should directly correspond was not addressed. Gundersen (1972) however measured thumb pressures in patients who also had intraarterial measurements made in the radial artery. The radial artery was selected since intraarterial pressures were already being monitored during surgery. Two men and three women with no history of peripheral vascular disease were studied. Two additional women were given a hypotensive drug for control of bleeding during surgery. The correlation between direct and indirect measurements in both conditions, i.e., with and without the administration of a hypotensive drug, was greater than 0.9. Althought one cannot

deduce from this that the absolute blood pressure in the digital artery of the thumb is that determined by indirect means, Gundersen argued that since cuff width conformed quite closely to the 20% rule and that there was good correspondence between the indirect thumb pressure and the pressure in the radial artery, then indirect digital pressures should agree closely with direct digital pressures.

Indirect measurements generally support the findings from direct measurements and amplification of the systolic pressure in the arteries of the arm (Kroeker and Wood, 1955; Kroeker and Wood, 1956). Intraarterial blood pressures have been measured in brachial and posterior tibial arteries in 13 normal sujects and compared with indirect pressure measurements using the strain gauge technique (Nielsen et al., 1973). Intraarterial systolic brachial and ankle pressures were 128 and 154 mmHg respectively. By auscultation, the brachial pressure was 123 mmHg and the ankle pressure was 142 mmHg using the strain gauge technique. Intraarterial measures tended to be 10 mm Hg greater than corresponding indirect pressure measurements. For reasons previously cited, there are problems with direct pressure measurements in vessels as small as the digital arteries in the fingers and toes. Lezack and Carter (1970) have reported that systolic pressures distal to the ankles decrease suggesting that these vessels offer greater resistance to flow. Others (Doupe et al., 1939a; Gaskell and Krisman, 1958a, and Downs

et al., 1975) have made a similar observation with indirect pressure measurements in the digits of the upper extremity where systolic pressures similarly decreased distal to the wrist.

Lezack and Carter (1970) observed that systolic pressure decreases distal to the ankle. Sigiura and Freis (1962) had reported similar findings in arteries of comparable size in dogs. This suggested that vessels distal to the ankle of comparable size in dogs offer greater resistance to blood flow.

Comparative studies between direct and indirect blood pressure determinations involving the digits need to be conducted if indirect pressure measurements are to provide valid estimates of the absolute blood pressure within the digital arteries of fingers and toes. There are certain problems with direct measurements in digital arteries. First of all, the digital arteries are only about one mm in diameter and difficult to puncture. Secondly, thrombo-embolic complications could arise from such a procedure. However, indirectly measured pressures are reproducible, and with a thorough knowledge of their application they can be a valuable tool for assessing patients with vascular disease.

6. Comparison with Angiography

An important means of assessing arterial obstruction and localizing stenoses and sites of occlusion in the limbs is measuring noninvasive segmental blood pressure along the limb. To determine the sensitivity and specificity of this laboratory procedure in identifying the presence of a stenosis or occlusion, correlation of the pressures with angiographic evidence of stenosis and/or occlusion must be performed since this constitutes the definitive direct test of the presence and degree of arterial occlusions.

Systolic blood pressures measured in the wrists and fingers using the spectroscope were correlated with angiographic evidence of stenoses in the same limbs of 29 patients (Downs et al., 1975). A digit was found to exhibit a normal pressure as long as one digital artery was patent. Angiograms of 68 fingers predicted normal pressures in those fingers, and 55 normal pressures were observed. Thus 13 false positive results suggested false evidence for obstruction. Angiograms of 67 fingers suggested occlusion to both sides of each digit. Abnormally low pressures were observed in 63 digits, and the remaining four constituted false negative outcomes. Despite some false conclusions, generally the results were accurate when assessing the entire hand. Of the 26 hands showing angiographic obstructions, all but one had correspondingly low pressure measurements. Normal pressures were observed in five hands with no angiographic evidence of

occlusion. Based on their results, Downs et al. (1975) found a difference of more than 15 mmHg between corresponding digits of two hands to indicate an occlusive process rather than Gaskell's (1965) recommendation of 10 mmHg. Wrist-to-digital pressure gradient is normally not more than 30 mmHg.

Similar comparisons have been made in the toes of patients with arterial occlusive disease (Carter and Lezack, 1971). Systolic pressures were abnormally low in 54 of 56 limbs showing complete occlusion with angiography, in 21 of 25 limbs with severe stenosis and in 13 of 21 limbs exhibiting mild stenosis.

Clinically it is of interest to know how well digital pressures correlate with the existence of ischemic changes. Hirai (1978) using the photoplethysmographic technique observed only one instance of ischemia in 184 digits with normal blood pressures. The criteria for ischemia were not defined. In 203 digits with arteriographic evidence of arterial occlusion, at/or proximal to the digit, 173 digits had abnormally low pressures, 132 of those showing "ischemic" changes. Of the 30 digits with normal pressures, two were ischemic. No significant differences were recorded between the fingers in 80 normal subjects. No probability value was given.

Clearly arteriography has provided valuable information with respect to the location and degree of obstruction of the arteries if surgery is being considered for severe disease. Others have used it to distinguish arteriosclerosis from other vascular disorders by close examination of the integrity and smoothness of the vessel lumen. Arteriography however is time-consuming, expensive and can be risky for the patient. On the other hand, the noninvasive determination of digital blood pressure is simple to perform. Hirai (1978) showed that with pressure measurements alone, digital arterial insufficiency due to arterial occlusive disease could be determined with a high degree of accuracy when using arteriographic findings as a standard. This accuracy is increased by conducting measurements on the intermediate phalanx as well as the proximal phalanx to examine the pressure gradient along the digit.

Angiographic findings have shown that blood pressure measurements should be made in all fingers when obstruction is suspected. If only performed in one or two fingers obstruction can be missed completely in other fingers.

7. Reproducibility

When noninvasive blood pressures are measured distally, they have been reported to be more variable and less reproducible than pressures measured more centrally. Nielsen (1978) estimated the degree of variation in arm and finger

systolic pressures both within and between days of measurement using the strain gauge as a distal sensor. Brachial pressure was measured generally with higher accuracy than finger pressures. He measured the variability between blood pressures taken on one day in ten young females and compared these with pressures taken ten minutes and three to five days later. When finger blood pressures however, were taken on different days their variability increased. Mean difference within day measurements were less than the mean difference between measurements taken on different days. Variability of the measurements were higher between measures on different days.

Reproducibility of distal blood pressures was measured at both the ankle and toe in 20 patients with arterial occlusive disease (Nielsen et al., 1973). Standard deviations of the differences between two measurements progressively increased from measurements performed on the same day with the same cuff (4 mmHg), to measurements performed on the same day but with cuff reapplication (7 mmHg) and measurements performed on separate days (7.5 mmHg).

Carter and Lezack (1971) compared the reproducibility of brachial pressures and toe pressures. Pressures were repeated within an interval of 6 to 246 days in 48 limbs of 34 patients. Toe pressures measured with the strain gauge and visual flush technique were just as reproducible over time as the brachial pressures measured by auscultation. How-

ever, the mean differences and their standard deviations were relatively large (15 ± 22 mmHg for the systolic toe pressure and 15 ± 14 mmHg for the brachial systolic pressure). The variability was thought to be due to the prolonged time intervals betweeen measurements in some cases and the residual effect of unrelated surgery on blood pressure in others.

Lezack and Carter (1970) examined the reproducibility of the spectroscopic technique by comparing 107 duplicate measurements at intervals ranging from 5 to 20 minutes. In 90% of the cases differences were within 10 mm Hg. No difference in reproducibility was observed between fingers and toes nor between vasodilated or vasoconstricted states. Similar results were found for the strain gauge phethysmographic technique. When measurements were repeated at longer intervals from 4 to 18 months, the mean difference was 12 mm Hg for 6 out of 7 cases tested. All measurements were performed at a constant room temperature, with the subject in a standard supine position wearing a hospital gown, and in a given vasomotor state with skin temperatures in prescribed ranges as a prerequisite for testing. In addition, eating and smoking restrictions were imposed for several hours prior to testing. Variability in Lezack and Carter's series was probably significantly reduced by careful standardization of the testing procedure.

When pressures were retaken after removing and reapplying the cuff in 5 patients, Nielsen (1976b) noted that the SD difference between measurements was 6 and 7 mm Hg for the ankle and toe respectively. Similar differences have been confirmed (Gundersen, 1972).

Digital blood pressure measurements are acceptably reproducible for routine clinical testing. Measurement reproducibility can be optimized by careful standardization of the protocol with particular attention given to the vasomotor state of the subject. Cuff application should be performed in a uniform way to further limit potential variability. These are important considerations if digital blood pressures are to provide estimates of intraarterial blood pressure and to serve as an indicator of change over time. Furthermore, the considerations discussed have important implications for the use of indirect blood pressure measurement in the assessment of finger cold sensitivity.

E. INDIRECT BLOOD PRESSURE MEASUREMENTS IN THE ASSESSMENT OF COLD SENSITIVITY IN THE FINGERS

1. The Development of the Technique

Tests for assessing vasospasm in Raynaud's patients, for assessing sensitivity change over time, and for evaluating the effect of peripheral dilating drugs have been criticized for poor reproducibility. None of the previously-used procedures has been shown to be a particularly suitable instrument for the routine measurement of cold sensitivity. Up to now methods combined with provocation tests have been used. These have been semiquantitative at best and have included such methods as measurement of finger blood flow (Coffman and Cohen, 1971), skin temperature of the digits (Veradi and Lawrence, 1969), rewarming after cold provocation (Juul and Nielsen, 1981), and characteristics of the pulse wave (Hyvaninen et al, 1973).

Krähenbühl et al. (1977) have described an innovative technique using finger blood pressure measurements as an index of cold sensitivity. These investigators made use of the observation made by Burton (1951) that closing pressure could serve as an estimate of vascular tone. They observed that systolic pressure could be underestimated under certain circumstances. Systolic blood pressure in the digits is re-

corded when the transmural pressure is sufficient to open the artery during cuff deflation. This pressure is a function of vessel tone. These investigators examined digital blood pressures during states of high vascular tone induced by body cooling, mental or physical discomfort, and intraarterial norepinephrine infusion. Vascular tone was further increased by locally cooling one finger in $5^{\circ}C$ water for seven minutes, with the circulation to the finger arrested with a tourniquet. After the tourniquet was released, pressures were measured immediately in the cooled and a noncooled finger. In the cooled finger, systolic pressure "sometimes" decreased to zero indicating complete vessel closure, and in the control finger no systematic change in pressure was detected. Closure or apparent pressure of zero was observed in the cooled fingers of normal subjects but the details of this observation and the number of subjects were not given. Vessel closure was abolished when the vasodilating agent, nicotinic acid, was injected subcutaneously at the base of the proximal phalanx.

When patients with Raynaud's phenomena were tested, vessel closure was reported at local temperatures of 15° to $20^{\circ}C$ which suggested that a temperature threshold may affect vascular smooth muscle function of these patients. Since closure was dependent on arterial tone, Krähenbühl et al. (1977) reasoned that other factors which increase vessel tone could also contribute to vessel closure. To demon-

strate this, norepinephrine (NE) was infused intraarterially at the rate of up to 10 μg/min. Closing pressure was estimated by subtracting the finger systolic pressure (FSP) of the finger exposed to infused NE, from the finger systolic pressure measured in a control finger whose circulation had been arrested, and therefore unexposed to the drug. Closing pressure derived in this way, served as a measure of the tone of the digital arteries. This pressure dropped in a dose-dependent fashion from approximately 55 mm Hg with 10 μg/min NE infusion, to a pressure of zero with concentrations of NE less than 1μg/min. Because these closing pressures represented pressure differences, zero closing pressure referred to equal pressures measured in the test finger exposed to NE and in the unexposed finger and was therefore indicative of no change in tone. Vascular tone in the fingers of Raynaud's subjects was reported to increase sharply with cooling suggesting the role of a temperature threshold in vessel closure. In two Raynaud's patients studied such a threshold was reported at 18° and 20°C. From the observations of this study the effect of cooling was concluded to exert a direct effect on the vascular smooth muscle of the digital arteries in a comparable fashion to exogenous NE.

Krähenbühl's original work was extended a year later for the potential development of a standardized and reproducible cold sensitivity test for Raynaud's phenomenon (Nielsen and Lassen, 1977). Nielsen and Lassen's method consisted also

of the simultaneous measurement of finger systolic pressure in two fingers. One finger served as a control for changes in systemic arterial pressure. The test finger was cooled by means of a specially designed double inlet cuff. Cuff dimensions were those of a suitable pneumatic plastic cuff for the digits, 24 mm x 80 mm. Water of any given temperature could be perfused through the double-inlet cuff. Prior to cooling, a smaller plastic cuff (10 mm x 80 mm) placed at the base of the test digit served as a tourniquet to arrest the circulation to the finger during the cooling period and to ensure the arterial wall equilibrated with the temperature of the water perfusing through the double inlet cooling cuff before blood pressure measurement. The time period for temperature equilibration in the digit has been determined both by direct and indirect means (Nielsen and Lassen, 1977). Subcutaneous temperatures were measured directly with thermocouples inserted via cannula into normal fingers. Following a five minute cooling period of the finger during circulatory occlusion, subcutaneous temperatures were within $1^{\circ}C$ of the surface temperature. Some increase did occur during conversion of the water circulation to the air system, however this was within $0.5^{\circ}C$. Following the cooling period, the pump supplying water to the perfusion cuff was turned off. The water system was converted to a pneumatic system through a series of stopcock adjustments for blood pressure measurement. The finger systolic pressure was taken in the conventional way using the double inlet cuff with

the outlet hose clamped and a mercury-in-rubber strain gauge placed over the distal phalanx. The digital pressure was taken as that cuff pressure at which a volume increase and/ or return of pulse in the distal phalanx was recorded.

Subcutaneous temperatures and skin temperatures were not reported to increase significantly until blood flow resumed to the finger. Indirectly, temperature equilibration was examined in a model of a finger with polyvinyl chloride cylinder filled with a gel. A double inlet cuff was applied, and subcutaneous and skin temperatures were recorded. Temperature change occurred quickly even in the center of the model and leveled off to the temperature of the circulated water within five to seven minutes. Because the finger arteries are known to lie superficially, this cooling period was considered sufficient to attain temperature equilibration between the arterial wall and the water temperature in the cuff during arterial occlusion.

Because strain gauges were used as distal sensors on the finger tips to detect the first inflow of blood distal to the cuff, care was taken by the original investigators to lightly compress and drain the finger of blood prior to cuff inflation, and to heat the finger tip prior to blood pressure measurement. These precautions were observed in an attempt to optimize the DC volume signal obtained with strain gauges. Nielsen and Lassen (1977) examined normal responses to local cooling of the finger. Eleven normal young women

were tested in a room 22 to 25°C so they felt thermally comfortable. Percent drops in pressure between a test and a control finger were 0.2 for a finger temperature of 30° C, 1.5 at 25°C, 8.5 at 20°C, 11.4 at 15°C and 15.5 at 10°C. This translated into apparent finger systolic pressures of about 100 mmHg at 30° and 25°C, 90 mmHg at 20° and 15°C and down to about 85 mmHg at 10°C.

Nielsen and Lassen's method is an innovative and important contribution to noninvasive vascular diagnostic procedures. Other methods have not been found to be reliable. For the first time, a procedure for assessing vasospasm has been developed that has promise for assessing digital arteries, those arteries that have been directly implicated in vasospastic attacks. Previous methods have largely been based on blood flow which is regulated at the arteriolar level. Although Nielsen and Lassen's method appears to have potential, various aspects of the application of the technique and its limitations need to be investigated.

2. Cold Sensitivity Testing of Patients with Raynaud's Phenomenon

The work just described has led to further studies based on its clinical applications. Preliminary evidence in Raynaud's subjects generally showed significantly greater decreases in FSPs with local cooling compared with non-Raynaud's subjects. These responses were variable which

probably reflected differences in subject populations, disease severity and methodology.

One early report on four patients with vascular disease examined abnormal cold responses in a young woman with Raynaud's phenomenon, a middle-aged woman with radial artery thrombosis, a middle-aged woman complaining of cold cyanotic fingers on exposure to cold and a young man with thromboangiitis obliterans (Nielsen and Lassen, 1977). The woman with cold hands did show a more pronounced decrease in finger systolic pressure (FSP) than the normals, which was less than the Raynaud's patient whose pressure dropped to zero at temperatures under 20°C in the cuff. The other two patients also showed abnormal reduction in FSP at temperatures between 15° and 20°C.

The first systematic study of group differences in cold sensitivity in normal subjects and patients with Raynaud's phenomenon was reported by Nielsen (1978). He compared the cold sensitivity of 22 normal females and 18 females with primary Raynaud's disease. Finger cooling to 20°, 15° and 10°C progressively reduced finger systolic pressures in the Raynaud's group significantly more than in the normal group (p<.05). Eleven of the 18 patients exhibited arterial closure, i.e. zero finger systolic pressure. Responses were further exaggerated with standardized body cooling for 20 minutes in both groups. Finger pressures were taken in seven normal subjects and seven patients with Raynaud's disease

at a room temperature of 22°C and with body cooling for 20 minutes at local temperatures of 20, 15 and 10°C. Digital artery closure occurred in six of the seven patients who had not demonstrated closure with local cooling alone. No closure was observed in the normal subjects. Such a test involving local, or local and body cooling may have considerable potential as a diagnostic tool for Raynaud's phenomena. Ranges of normal variation have yet to be determined. The difficulty of good reproducibility in peripheral pressures is more pronounced in digits subjected to local temperature changes and this also applies to finger blood pressure measurements used in cold sensitivity testing (Nielsen, 1978).

To obtain a clearer picture of the variation of cold sensitivity in normal subjects and in patients with Raynaud's phenomena, Nielsen and his colleagues (1980) examined FSPs in three groups of normal subjects; young females who worked indoors, young males who worked indoors, young males who worked outdoors and 18 females with Raynaud's phenomenon. No other details of the work environments were given. To increase sympathetic discharge to the digits, subjects were exposed to a perfused water blanket at approximately 10°C for ten minutes before cold sensitivity testing of the fingers. The effect of temperatures of 30°, 15°, 10° or 6°C was investigated.

Pressures dropped about 20 percent of pressure at 30°C adjusted for changes in pressure of a control finger, for

both male and female indoor workers at 10°C, and outdoor male workers at 6°C. Warm handed female subjects (n=17), were compared with cold handed female subjects (n=17) and females with Raynaud's phenomenon (n=18). The criteria for assigning subjects to the warm or cold handed group were not given. The difference between the three groups in FSP percent at 15°C was statistically significant (p<0.01). Only two of the Raynaud's patients did not exhibit complete vessel closure when the finger was cooled to 15°C. Median FSP percents of a reference pressure (at 30°C) were 85 for the warm handed groups, 69 for the cold handed group and 0 for the Raynaud's subjects. Finger systolic pressures ranged from 104 to 71 mmHg in the warm handed group, from 92 to 20 mmHg in the cold handed group and from 54 to 0 mmHg in the Raynaud's group. Nielsen and his associates concluded from this study that normal reactions to local cooling were independent of sex, age and working conditions. Further, the differential responses between the three female groups, warm handed, cold handed and Raynaud's phenomenon suggested that such a test could be developed to aid diagnosis and assessment of disease severity.

Hoare et al. (1982) found no overlap in percent pressure changes of FSPs after exposure to 10°C water in the cuff. (1982) between healthy volunteers (n=25) and subjects with Raynaud's phenomenon (n=25). In the Raynaud's group, seven patients had systemic sclerosis, two had Buerger's disease,

a specific diagnosis was not established in the remaining fourteen patients and two other subjects were not accounted for. Clear separation between the FSPs for the two groups was also apparent following cooling. This study was unique in that unlike previous work, a photoplethysmographic probe was used as a distal sensor. Also body vasoconstriction was effected by reducing the room temperature to 18°C (for 20 minutes with subjects fully clothed) rather than using a water blanket for inducing a state of high vasomotor tone. Whether or how much vasoconstriction this procedure achieved is not known.

Thulesius et al. (1981) have reported that with consecutive sequence of descending temperatures from 35° to 5°C in 5°C decrements, percent pressure change in 18 normal controls was only three or four percent which was considerably lower than that reported previously (Krähenbühl et al, 1977; Nielsen, 1978). At a local finger temperature of 20°C the pressure (mmHg) was 99 ± 4 percent of that at 30°C (mean ± SD); at 15°C, 98 ± 8 percent and at 10°C, 97 ± 10 percent. In the Raynaud's subjects which included 32 primary cases and 74 secondary vibration-induced cases, pressures were generally lower and more variable. The pressures at temperatures of 15° were 95 ± 19 percent of that at 30°C (mean ± SD); 10°C, 48 ± 40 percent; and 5°C, 38 ± 41 percent. These results provided good evidence for the usefulness of this procedure in evaluating degree of cold sensitivity. Differ-

ences in percent pressure changes between the normal subjects and patients with Raynaud's phenomena were not statistically significant from local temperatures of 35° to 20°C. At local temperatures of 15°, 10° and 5°C, differences in finger pressure changes were highly statistically significant (p<.005 or less). In Nielsen's study, 11 out of 18 Raynaud's subjects showed vessel closure with local cooling only down to 10°C (61%). In Thulesius et al's study, 37 out of 107 patients demonstrated vessel closure under comparable conditions (35%). Down to 5°C, 72 of the patients demonstrated closure (68%). The differences reported between these studies may reflect the different patient pools with respect to disease etiologies and severity and methodologic differences such as the inclusion of body cooling by Nielsen.

3. Cold Sensitivity Testing in Patients with Arterial Occlusion

The effect of arterial obstruction on cold sensitivity of the digits is of interest since a reduced transmural pressure in a vessel with even normal tone may produce Raynaud's phenomenon.

Using a different cooling technique Hirai (1979) compared finger systolic pressure responses between Raynaud's patients, and patients with digital arterial occlusive disease due to Buerger's disease or arteriosclerosis obliterans as a

means of investigating differences in the pathophysiologic mechanisms of responses to cold. The procedure involved the subject placing a gloved hand through an opening in the side of a cold water (0° to 4°C) bath and projecting the finger to be tested out through an opening on the other side. Sufficient length of the finger was exposed from the bath to apply a 24 mm wide cuff at the base of the thumb. Finger pressures were taken every minute for a 10 minute period using a photocell as a sensor. The bath was drained as soon as the apparent systolic pressure in the patient's fingers was zero and the recovery rate of blood pressure was monitored. The results of 11 normal subjects showed a maximal decrease in blood pressure 8 ± 9.9% (mean ±SD).

Patients with arterial occlusion had marked brachial-finger pressure differences at rest in a room at 23° to 26°C, whereas the Raynaud's patients were much closer to normal values. The Raynaud's group showed a more dramatic response to the hand cooling than the arterial occlusive disease group. That is, the Raynaud's group exhibited vessel closure in one to six minutes, whereas the arterial occlusive group closed in 5 to eight minutes. The Raynaud's group also took longer time to recover from the cold exposure (20 to 100 minutes) than the occlusion group (5 to 40 minutes). These different reactions suggested some difference in the pathophysiologic mechanism associated with vessel closure in the two groups of patients. However, overlap of the respon-

ses of the two groups was observed and may suggest a limitation of the procedure for diagnostic purposes.

The occlusions themselves were responsible for cold sensitivity in the arterial occlusive group, however diminished finger pressures were not requisite for cold sensitivity in the Raynaud's group. Hirai attributed this latter finding to sympathetic hyperreactivity, based partially on the observation that sympathectomy has greater benefit in patients with Raynaud's symptoms without arterial obstruction. Hirai failed to discuss his findings in terms of the local effect of cooling on the vascular smooth muscle. His method appeared to discriminate Raynaud's patients and patients with arterial occlusive disease to some degree but there was considerable overlap. Results may have been influenced by the fact that Raynaud's phenomenon has numerous etiologies causes (i.e., eleven of the 17 fulfilled Allen and Brown's criteria for Raynaud's disease, two had rheumatoid arthritis, one had systemic lupus and two had no established diagnoses). Also, a major disadvantage of this test's being used routinely is the severity and unpleasantness of the cold challenge using temperatures of 0° to $4^\circ C$.

Arterial obstruction may contribute to Raynaud's phenomena in its secondary form. Therefore a better knowledge of the effect of temperature both in normal subjects and patients with Raynaud's phenomenon with and without arterial occlusion is needed to assess the importance of these factors in the pathophysiology of Raynaud's phenomena.

4. Potential Sources of Measurement Error

Indirect measurement of finger systolic pressure has been reported to be a potentially useful and reliable method for assessment of patients with arterial disease of the hands. As described in Section D however, both physiologic and technical variables can affect these measurements. A knowledge of the potential sources of measurement error is necessary to help reduce their effects. Conventional finger systolic pressure measurement is known to be subject to an array of variables which must be taken into account to ensure meaningful and reproducible measures. Similarly, FSP used as an index of arterial tone in cold sensitivity measurement, is also prone to the effects of various physiologic and technical variables. These factors may have a greater effect on FSP because of its dependence on arterial tone. The effects of these physiologic and technical variables are described next.

a) Physiologic variables.

Like other physiologic functions, hemodynamic parameters such as skin temperature, peripheral blood flow, digital systolic pressures and cold sensitivity can vary widely within the normal population. One attempt to assess normal human variation of peripheral cold sensitivity was described by Nielsen et al. (1980). The specific criteria however, for differentiating normal young women into warm handed and

cold handed groups were not described although there was evidence to show the results of cold sensitivity testing was different between the two groups.

Other studies have neglected to specify the sex and age of their normal healthy controls (Krähenbühl et al., 1977; Nielsen and Lassen, 1977; Nielsen, 1978). These parameters may be important considering that hand blood flow in normal young females has been reported to be less than normal young males; and that the hand blood flow of post menopausal women is closer to that of the normal young males (Bollinger and Schlumpf, 1976). Furthermore, the incidence of Raynaud's phenomenon is four to five times greater in women than in men (Spittell, 1980). Therefore closer examination of normal hemodynamic responses to cold in both sexes over an age range is warranted.

The literature on Raynaud's phenomenon has been difficult to interpret because of the confusion surrounding its precise definition. The term Raynaud's disease has been adopted for the primary form of the disease with no underlying organic pathology or disease state. However, several studies have included patients who also had arterial occlusion in groups referred to as Raynaud's disease in the subject pool (Nielsen et al., 1978). In some cases including Maurice Raynaud's original work, Raynaud's disease and secondary Raynaud's phenomenon were not distinguished (Hirai, 1979). Such a distinction however, is important

considering that abnormal responses to cold observed in individuals with and without arterial obstruction may have different pathophysiologic mechanisms (Nielsen et al., 1978). There is evidence to support the notion that closure may occur in arteries distal to an obstruction with normal vasomotor tone, whereas in arteries without obstruction this tone may be unusually increased to produce closure (Mendlowitz and Naftchi, 1959).

b) Vasomotor tone.

Change in vasomotor tone is the most important factor affecting changes in peripheral blood flow. The evidence for altered vasomotor tone in Raynaud's patients is not conclusive. Hillestad (1970) and Bollinger and Schlumpf (1976) provided some evidence that may suggest increased vasomotor tone in these patients, however Lottenbach (1968) showed that heat release from the forefinger at 30°C was not different in Raynaud's patients and normals when vasomotor state was standardized.

Skin temperature measurements of the fingers can provide a useful index of vasomotor state under some conditions. Because of the relationship between digital systolic pressure, and low and high flow states of the finger, appropriate control of flow to the fingers is a crucial consideration in the finger pressure measurement protocol. Between the temperatures of 20° to 30°C small changes in temperature

can reflect large alterations in blood flow; therefore for optimal control of this variable, skin temperatures should be outside this range during measurement. Such rigid control of peripheral blood flow through manipulation of the vasomotor state has not been uniformly practised. Krähenbühl et al, (1977), Nielsen and Lassen (1977) and Thulesius (1981) studied all subjects lying supine, lightly dressed and thermally comfortable in a room between 22° and 25°C. In a subsequent study the room temperature was limited to 21° to 22°C to obtain a thermoneutral vasomotor state in the subjects prior to cold sensitivity testing (Nielsen, 1978). To promote vessel closure in seven subjects who failed to close under these conditions and local finger cooling to 10°C, Nielsen implemented a body cooling procedure to effect increased vasomotor tone. This was achieved with a cooling blanket which covered the anterior surface of the body. Tap water at 15°C was circulated for 20 minutes. No other criteria for the cooling period were reported. Body cooling did produce statistically significant differences in FSPs at local temperatures of 15° and 10°C both in normals and Raynaud's patients when compared with the thermoneutral state which was not specifically defined. Stabilization of skin temperatures in this study was not attempted. The temperature range of the fingers of the Raynaud's patients with and without body cooling was 28° to 32°C, and 28° to 35°C. Based on these ranges, subjects cannot be classified as being vasoconstricted. Greater attention to skin temperature

may increase pressure reproducibility, sensitivity, and test validity.

c) Peripheral skin temperature.

The fundamental feature of Nielsen and Lassen's method was equilibration of the temperature of the arterial wall with the temperature of the water in the cooling cuff during five to seven minute periods of water circulation around the finger in combination with arrest of the arterial circulation. Using direct measurement (tissue thermistors), Nielsen and Lassen (1977) showed that the subcutaneous tissue temperature equilibrated to cuff temperature within five minutes, and this temperature changed less than $0.5^{\circ}C$ when exchanging the water in the cuff to air in preparation for blood pressure measurement. Large changes in temperature were reported only as flow resumed to the finger during cuff deflation. Routine skin temperatures under the cuff should therefore provide an estimate of the temperature of the digital artery.

Subsequent studies that have applied this procedure have not always reported the time allowed for temperature equilibration to occur (Nielsen et al., 1980; Thulesius, 1981). Comparison with modifications of this technique in which the finger is cooled without digital occlusion is difficult because the arterial wall temperature cannot be estimated, and in addition, the vasomotor state of the subjects was uncertain (Hirai, 1979).

d) Cuff deflation rate.

A small initial volume increase of blood in the finger pulp during cooling may not easily be detected. This is due to low flow state and negligible initial flow increase when the cuff is deflated. Krähenbühl et al. (1977) compensated for this problem by using a slower deflation rate than the standard 2 mmHg/sec. The details of this adjustment and any associated changes in skin temperature were not reported. It is possible that at some faster cuff deflation rate the vessel wall stiffened by the cold challenge and failed to open correspondingly. Opening of the vessel therefore, tended to lag behind. Although not explicitly stated, Nielsen and Lassen (1977) apparently used a deflation rate closer to 4 mmHg/sec. Unfortunately the results of this faster deflation can not be compared with the slower rate used in the work of Krähenbühl et al (1977). The FSPs of normal subjects at 10°C however, in Nielsen and Lassen's study showed comparable 15% pressure decreases at both the standard and faster rate. Since many studies do not report the deflation rate used (Hirai, 1978; Olsen and Nielsen, 1978; Nielsen et al., 1980), the effect of this variable on the recorded pressure cannot be assessed. Fast deflation rates may obscure the endpoint, i.e., the resumption of blood flow to the finger. A very slow deflation rate may allow sufficient time for warming of the finger hence the temperature of the vessel wall increases. The use of different deflation rates

in the measurement of finger pressures under cooling conditions may contribute to a significant measurement error. However, if applied in a controlled way, changes in digital pressures as a function of deflation rate may yield important information about the effect of cold on blood pressure determinations in the digits. In addition, altered apparent finger systolic pressures with different cuff deflation rates may help distinguish different pathophysiologic categories of cold sensitive individuals.

e) Physical properties of the cuff.

Technically routine noninvasive digital pressures can be directly affected by such factors as cuff material, length, width and fit. A description of these factors was presented in Section E4. These factors are also important for cold sensitivity testing using blood pressure measurements.

f) Distal sensors.

End-point determinations of the systolic pressure depend on the application and sensitivity of the distal sensor, as well as their sensitivity to temperature. The choice of a distal sensor must be therefore considered carefully in cold sensitivity testing which uses a local finger cooling procedure. Hemodynamic changes occur normally with local cooling and with increased vascular tone such as decreased pulse amplitude, decreased blood flow, and relatively small volume increases with flow resumption during cuff deflation. These

normal changes are further enhanced in cold sensitive persons. Pulse amplitude for example, may be abolished completely at pressures less than 40 mmHg. Therefore, distal sensors must be sufficiently sensitive to pick up minimal volume or pulse with a low signal to noise ratio.

Most commonly used sensors for digital pressure measurements are the mercury-in-rubber strain gauge (Krähenbühl et al., 1977; Nielsen, 1976a; Nielsen, 1978) and the photosensitive probe (Hirai, 1978; Hirai and Kawai, 1977; Holmgren et al, 1981). Sensors which depend on a volume increase of the finger tip have been used in the original studies on cold sensitivity testing. To enhance the quality of the end points, warming of the finger tip during local cooling, and light external compression and blanching of the finger before cuff inflation have been recommended (Nielsen and Lassen, 1977). Conceivably different results among reported studies may be attributed at least to some extent to methodologic differences. To what extent the precautions described by Nielsen and Lassen (1977) optimize detection of the end-point is not known. Since the clarity of the end point and its detection are critical further investigation of these parameters is needed to identify limitations associated with them.

q) **Replication and sequence of measurements.**

Most of the pioneering studies of cold sensitivity testing report their results in terms of the best estimate of FSP, i.e., as the mean of a number of determinations for each subject. However, the details of how these repeated measures were performed have not always been clearly stated. It is known that local cooling superimposed over varying degrees of vasomotor tone may result in significant constriction of the vascular smooth muscle and prolonged rewarming or relaxation time especially in cold sensitive individuals (Porter and Reiney 1975; Juul and Nielsen, 1981). Therefore, if one temperature has a residual long lasting effect on the arterial wall, how much time should separate one blood pressure determination from the next? Should the digit be warmed or cooled to some predetermined baseline temperature between local cooling? Should local temperatures be investigated in a descending order as described by Thulesius et al. (1981)? Could a gradual reduction in temperature desensitize the smooth muscle compared with a more sudden temperature drop? These questions, and how measurement reproducibility can be affected by these variables remain to be investigated.

5. Reproducibility and Validity

Nielsen (1978) evaluated the reproducibility of FSP measurements in a group of ten healthy females. Blood pressure determinations were estimated twice within three to five days. As described in Section D brachial pressures were measured with greater accuracy than FSPs within and between days. The variation coefficient for within day measurements was 10% and for between day measurements was 20%. Although reproducibility data have not been systematically reported for cold sensitive testing, studies using this technique commonly report the mean FSP for a given finger temperature based upon repeated measurements in order to arrive at a single best estimate for each subject. Data on reproducibility of FSP at different finger temperatures do not appear to have been reported for patients with Raynaud's phenomenon. These data are needed in order to establish the ultimate value of this technique as a clinical measure of cold sensitivity.

The validity of the cold sensitivity test has been assessed by comparing test results with disease symptoms. Thulesius et al. (1981) measured FSPs by strain gauge at temperatures between 35° and 5°C at 5° C intervals. On comparing severity of symptoms of the disease (rated according to Taylor and Pelmear, 1976) with observed FSPs, a significant difference was observed between stage 0-1 (mild symptoms) and stage 3-4 (severe symptoms) at 10°C. At a finger

temperature of 5°C however, three distinct groups including stage 2 (moderate symptoms) were significantly distinguishable (Thulesius et al, 1981). This study provided some support for the use of this tool for assessment of the severity of cold sensitivity. The authors stressed that complete pressure-temperature curves are needed on each patient however, to obtain a full picture of the vascular responsiveness because evidence of cold vasodilatation and apparent increase in FSP was observed in one instance below 15° C.

F. AIMS

Although Raynaud's phenomenon was first described over 100 years ago, little is known about the underlying mechanisms of the disorder as reviewed in detail in Section C. Without a clear understanding of the pathophysiology, both adequate assessment and treatment have not been feasible.

There appear to be two main reasons why there has been little progress in advancing the understanding of these disorders. First, until recently, there has been no method to study the behavior of the main digital arteries in man in vivo. Second, there have been no studies of cellular mechanisms of these vessels that can only be studied in vitro.

Concerning the study of digital arteries in vivo, previous methods for assessing peripheral vasospasm have utilized measurement or indices of blood flow. In 1977, Nielsen and Lassen described a method for assessment of cold sensitivity in the fingers. This method allows for the first time the examination of the digital arteries which are those directly implicated in vasospastic attacks. Specifically, their method provides an index of tone of the digital arteries and constitutes a promising approach to the quantitative evaluation of Raynaud's phenomenon.

Although there have been a number of reports using Nielsen and Lassen's method, serious methodological problems exist and need to be resolved to further establish the value and limitations of this method. This thesis attempts to deal with these concerns. For example, distal sensors selected for measurement, vasomotor state, and method of cuff deflation often have not been taken into account. In addition, because occlusion of blood flow in the remaining digits is known to affect the measurement of pressure in an adjacent digit, the effect of cuff occlusion which is part of the cold sensitivity test needs to be studied. Once such methodological issues have been addressed, Nielsen and Lassen's method could be applied to elucidate the relative importance of local cold reactivity of the digital arteries and sympathetic vasoconstrictor discharge, to study the effect of proximal arterial obstruction on sensitivity to cold and assessment of the reactivity of these vessels in patients with Raynaud's phenomenon of various etiologies.

Concerning elucidation of cellular mechanisms which may be involved in responses of vascular smooth muscle to cold, there have been extensive studies of experimental animal preparations and a number of human vessels from certain vascular beds, (previously reviewed in detail in Sections A, B, and C) but no studies dealing with the arteries of the human digits whose spasm is thought to be responsible for vasospasm in Raynaud's phenomenon.

In many vessels adrenergic function has been shown to be modified in some way by temperature. It seemed reasonable therefore to postulate that a similar adrenergic mechanism might exist in arterial smooth muscle of the human finger. The specific questions addressed concerned the role of adrenergic mechanisms in cooling and whether normal responses were exaggerated in Raynaud's individuals. Studies were therefore carried out to establish the functionally predominant adrenoceptor types in human peripheral arteries, and to find out whether adrenergically-mediated reactivity i.e. threshold and maximal response, was altered at temperatures down to 10°C. Such studies would also identify whether α adrenoceptor-mediated contractions were maximal at temperatures less than 37°C.

The first general aim of the planned in vitro studies was to determine qualitatively, whether both α and β adrenoceptors were indigenous to human digital arteries. Because adrenergic responses predominate in other vessels to effect changes in vasomotor tone and these have been shown to be modified by changes in temperature, it was anticipated that adrenergic receptors would predominate in human digital arteries. The present literature on the responsiveness of superficial vessels has shown some evidence for augmented responses with cooling as previously described. We therefore hypothesized that a cold induced increase in α adrenoceptor affinity for norepinephrine may be responsible for the lat-

ter phenomenon. There is little knowledge about the presence of ß adrenoceptors in human digital arteries. Therefore pharmacologic studies were carried out to examine the effect of the ß antagonist agent propranolol on the responses of digital artery smooth muscle. Because this drug has a membrane stabilizing effect, the direct effect of the ß agonist, isoproterenol, was also studied. Specifically, an examination of receptor-mediated function and depolarizing function in this preparation could help elucidate their respective roles at normal temperatures and in response to cooling.

The presence of an electrogenic Na pump, and implications of its sequelae were studied indirectly to examine whether potentiation of mechanical responses could be explained by a temperature-dependent inhibition of the pump. Vessel strips were therefore pre-equilibrated with a potassium-deficient solution to inactivate the pump and render the preparation Na rich. The subsequent addition of a normal potassium concentration re-activates the pump strongly and the membrane may become hyperpolarized. Hyperpolarization would lead to transient hypoexcitability until the ionic gradients are reestablished and pump function returns to normal. Thus, the demonstration of rapidly depressed activation during this period should provide strong evidence for the presence of an electrogenic Na pump as distinct from Na-Ca exchange. Temporal distinctions between these two means of inducing

relaxation would shed light on the possible contribution of each.

Although cold may directly affect the response of vascular smooth muscle to active stimulation, it seemed important to establish whether the response to cold is associated with changes in resting tension and spontaneous activity, and whether different degrees of cooling produce differential effects on these events. Smaller vessels are known to function more autonomously and are more characteristic of single unit smooth muscle, whereas larger vessels are more dependent on external stimulation and their innervation for their function; thus are more characteristic of the so-called multi-unit type of smooth muscle. Characterizing the smooth muscle of the digital arteries in terms of its intrinsic contractile properties could provide additional information regarding normal responses to cold as well as the presence of a "local fault" mechanism in Raynaud's phenomenon. In preliminary studies, spontaneous activity of digital artery strips was observed over several hours from a temperature of 37°C down to 10°C. In summary, the proposed in vitro studies were designed to focus primarily on the effect of temperature on adrenergic function, likely results of altered Na/K pumping and spontaneous activity of digital artery smooth muscle and thereby provide some insight into normal and abnormal responses to cold.

The work described in this thesis has examined cold-induced responsiveness of human digital artery smooth muscle (DASM), and the mechanisms which might mediate cold-induced vasospasm. Specifically, a series of _in vivo_ studies were carried out: 1) to examine certain methodologic aspects of Nielsen and Lassen's method for cold sensitivity testing that may affect the validity and reliability of the pressure measurements, e.g., the effect of cuff occlusion, vasomotor state, simultaneous pressure measurements of fingers on one hand, choice of distal sensor, and cuff deflation rate, 2) to define the contribution of myogenic and neurogenic factors to digital artery tone in normal subjects and in patients with primary and secondary Raynaud's phenomena with and without arterial occlusion. Specifically, a series of _in vitro_ studies were done: 1) to examine changes in basal tension of the human digital arteries with changes in temperature, 2) to describe qualitatively α and β adrenoceptor populations in these vessels and the effect of temperature on their function, 3) using indirect methods to evaluate the role of Na/K pump and its sequalae in the regulation of digital artery smooth muscle tone at normal and reduced temperature.

CHAPTER II

IN VIVO STUDIES

A. Introduction

The in vivo studies are divided into three parts. First, preliminary studies were conducted comparing cold sensitivity, using Nielsen and Lassen's procedure, of healthy subjects and patients with Raynaud's phenomenon. The effect of local cooling with body cooling and body heating was studied. Because our results failed to show the marked effect of local cooling with body cooling on apparent finger systolic pressure of Raynaud's subjects, a second series of studies was indicated to examine several important aspects of the methodology of the cold sensitivity test. These studies included the effect of four distal sensors on apparent finger systolic pressure, effect of cuff occlusion on finger pressures taken simultaneously and effect of method of cuff deflation on finger systolic pressure. Once these methodologic issues had been examined, a third series of studies was conducted using a refined cold sensitivity testing procedure in Raynaud's patients. Thus, the methodologic aspects studied in Section D (Chapter I) led to the adoption of the modified method presented in Section E (Chapter I) which described the cold sensitivity of healthy subjects and patients with Raynaud's phenomenon.

B. Preliminary Studies of Cold Sensitivity in Healthy Subjects and Patients with Raynaud's Phenomenon

Studies using the procedure of Nielsen and Lassen have reported variability in the sensitivity of the fingers of normal individuals to cold (Nielsen and Lassen, 1977; Nielsen, 1978, and Thulesius et al., 1981). Previous studies have confirmed the prominent influence of the local thermal state on the sensitivity of the digital arteries to cold (Hertzmann and Roth, 1942; Gaskell and Diosy, 1959). Also in the presence of increased general vasoconstriction, vasoconstriction to local cold in the fingers of subjects was increased compared with that during vasodilatation.

This section describes a preliminary study evaluating cold sensitivity in the fingers of healthy subjects and patients with Raynaud's phenomenon using a modification of the Nielsen and Lassen method. The degree of vasoconstriction was altered by using a modification of the Gibbon and Landis procedure (Gibbon and Landis, 1932). This was done for two important reasons. Vasomotor state was standardized because it was thought that it may reduce some of the variability observed in previous studies. Second, we compared the effects of local cooling during vasoconstriction and vasodilatation, in order to shed some light on the relative importance of local and central factors in the responsiveness of digital arteries.

We also attempted to confirm the results of previous studies on cold sensitivity testing in subjects with Raynaud's phenomenon and thereby help assess the feasibility of its use in differentiating categories of Raynaud's phenomenon on the basis of such an objective test. The degree of reproducibility of changes of finger systolic pressure in response to cooling was also assessed in cold sensitive subjects with a history of Raynaud's vasospastic episodes.

Finger temperature was changed locally in subjects in two vasomotor states achieved by body cooling and body heating. Changes in finger systolic pressure (FSP) in each condition were taken as an index of corresponding changes in arterial tone. Comparison of cold sensitivity of the fingers in these two vasomotor states provided a means of distinguishing the relative contributions of local cold and centrally-mediated responses. Cold sensitivity measured with a subject in the vasodilated state would primarily reflect a local effect of cold on smooth muscle arterial tone. In the vasoconstricted state, a centrally-mediated sympathetic effect in addition to the local effect of cold on digital artery smooth muscle would contribute jointly to changes in arterial tone with finger cooling. Therefore, as predicted from other studies, arterial tone should be significantly greater during sympathetic stimulation with body cooling and show a corresponding lower FSP. The use of an adjacent control finger which was not subjected to local temperature

changes should reflect the effect of the sympathetic stimulation alone on finger systolic pressure. If degree of cold sensitivity could be satisfactorily assessed on the basis of the Nielsen and Lassen method applied in two vasomotor states, then theoretically these modifications might not only provide a more sensitive tool with which to evaluate cold sensitivity and its progression and treatment in disorders such as Raynaud's phenomenon, but they might also provide some insight into the fundamental mechanism underlying cold sensitivity in patients with Raynaud's phenomenon.

a) Methods.

i) Subjects.

Cold sensitivity of the fingers in response to local temperature change was assessed in normal subjects; nine female and six males (age 24 to 73). Five subjects with histories of Raynaud's phenomenon of at least three years duration were studied. General information on these subjects appears in Table 1.

All subjects were tested on two different days once during body heating and once during cooling. Testing was performed at the same time on each of the two test days, and lasted about three hours. The two sessions were usually performed within a few days of each other. On the first

TABLE 1

Profile of Five Patients with Raynaud's Phenomenon

	SUBJECT'S INITIALS	SEX	AGE	RAYNAUD'S PHENOMENA 1°	2°	OCCLUSION
1.	JD	F	23	1°		NIL
2.	BM	F	53	1°		NIL
3.	YD	M	40		Occupational	NIL
4.	WdeL	F	44	1°		NIL
5.	LK	F	51	1°		NIL

visit, a general history was obtained from each subject which included status of general health, history of any vasospastic disorder, a subjective report of sensitivity of the fingers or toes to cold, occupation, and medication or drug usage. Healthy subjects denied any unusual cold sensitivity of the fingers. All were nonsmokers and were right hand dominant. Prior to cold sensitivity measurement of the fingers in the Raynaud's subjects, routine blood pressure measurements were performed on all fingers to identify the presence of any organic obstruction of the digital arteries. Pressure of less than 70 mm Hg in a digit or a difference of 15 mmHg or greater between adjacent fingers was used as an index of arterial occlusion (Downs et al., 1975). None of the five patients had evidence of occlusion.

ii) Protocol.

Subjects were asked to refrain from smoking, alcohol, eating, caffeinated beverages, and heavy exercise for at least two hours prior to the test. On arrival at the laboratory, the subject wore a hospital gown, and lay supine in bed for at least 30 to 45 minutes until skin temperatures reached the specified criterion for the specific vasomotor state being tested (see next section). Copper-constantan thermocouples were attached to the thumb, middle and little fingers of the hand to be tested. Temperatures were monitored every 40 seconds on a Honeywell recorder throughout the test. Additional thermocouples recorded room tempera-

ture maintained at 20 ± 1.0°C and the temperature of a thermos bottle containing water of a known temperature which provided a calibration check for the recorder. Prior to testing, skin temperatures of the second and fourth digits were recorded to ensure that they were comparable with the other three digits.

iii) Vasomotor State.

Skin temperatures of the finger tips were used as an index of vasomotor tone. Vasodilatation was achieved by body warming which involved immersing a leg into a tub of water at 44°C and by covering the subject with an electric blanket. The tub was situated at bedside in order that the subject could continue to lie comfortably during testing with the limb immersed. The criterion for the vasodilated state required that skin temperatures had stabilized at a temperature at least 12°C above room. Temperatures were usually 35°C or greater. Testing commenced after temperatures had been stable in this range for at least 20 minutes. Sweating was always present when the subject had achieved the dilated state. Temperatures were usually well maintained over the three hour testing period. Rarely skin temperatures were observed to fluctuate out of the designated range in which case testing was discontinued until the skin temperatures returned to criterion.

Vasoconstriction was achieved with body cooling which involved immersion of a leg in a tub of water at 17° to 19^oC, with the subject covered only by a single flannelette sheet. The criterion for vasoconstriction required that temperatures of the finger tips were reduced to at least within 4^oC of room temperature, and remained there for a minimum of 20 minutes before the test was begun. Usually these cool skin temperatures were readily maintained over the three hour testing period.

iv) Blood Pressure Measurement.

Cylindrical cuffs which served both as a blood pressure cuff and a cooling, water perfusion cuff for cold sensitivity testing were made in the lab according to certain technical specifications (see Appendix). Cuffs were constructed to meet the requirements for noninvasive blood pressure determinations (see Section E4), and to withstand the circulation of water at different temperatures through them. They were cylindrical, 29 mm wide, and were constructed from Penrose drainage tubing. Two small inlet hoses were positioned tangentially on opposite sides of the cuff and sealed to it. Wrinkling of the inner surface was avoided during construction, because this could result in uneven pressure transmission through the cuff and overestimation of pressure measurements. Because cylindrical cuffs cannot be adjusted in size, cuffs of several diameters were made to ensure that appropriately fitting cuffs were available for a wide range

of finger girths.   A cuff was considered to fit properly if it could be just rotated when positioned on the midphalanx. In the warmed state talcum powder was applied to the finger to avoid sticking.

Mercury-in-rubber strain gauges have been used success-fully as distal sensors to detect blood flow resumption at the finger tip in Nielsen and Lassen's method (1977).   The strain gauge is considered as a plethysmographic measure since it measures a volume change of the distal phalanx. With pulse-to-pulse changes in volume of the finger tip, the mercury column of the gauge is stretched longitudinally and the radius of the column decreases resulting in an increased resistance to current flow across a Wheatstone bridge.   The increased finger tip volume is recorded as a positive shift or deflection of the recorder pen from the baseline (multi-channel R600 Beckman recorder).   Normally without cuff oc-clusion the gauge monitors continuous pulse waves.   After cuff inflation to a suprasystolic pressure using a standard mercury sphygmomanometer, pulse is abolished.   As cuff pres-sure was decreased to systolic pressure, pulses reappeared with an upward deflection of the baseline which indicated increased blood volume distal to the occluding cuff.   To augment the volume changes, finger blanching prior to cuff inflation was used routinely in pressure measurements of both the test and control fingers.

A gauge was selected and fitted to the finger so that it was neither too tight nor too loose when it encircled the finger tip at the base of the nail.

The Beckman recorder (R600) and the transducer for pressure measurements were equilibrated at room temperature (20.0 $\pm$ 1.0°C) at least one hour prior to testing. Two mercury gauges were fitted, one for the test finger and one for the control finger. Each gauge was connected to a control box which was connected by another cable to the Beckman recorder.

A pneumatic hose system to link the pressure cuff, transducer and sphygmomanometer through a series of stopcocks was constructed for blood pressure measurements and water circulation through the cuff on the test finger (Fig. 5). This system allowed for simultaneous pressure measurements of two fingers on the same hand, and circulation of water through one of the cuffs by means of connection with a pump circulating water at a given temperature. The mercury manometer was controlled with a hand bulb. A one-litre air bottle container was connected into the pneumatic system to aid finger pressure regulation in the system during pressure measurements.

v) Cold sensitivity testing procedure.

The cooling system is also shown in Fig 5. In this study, the right index finger was used as the test finger
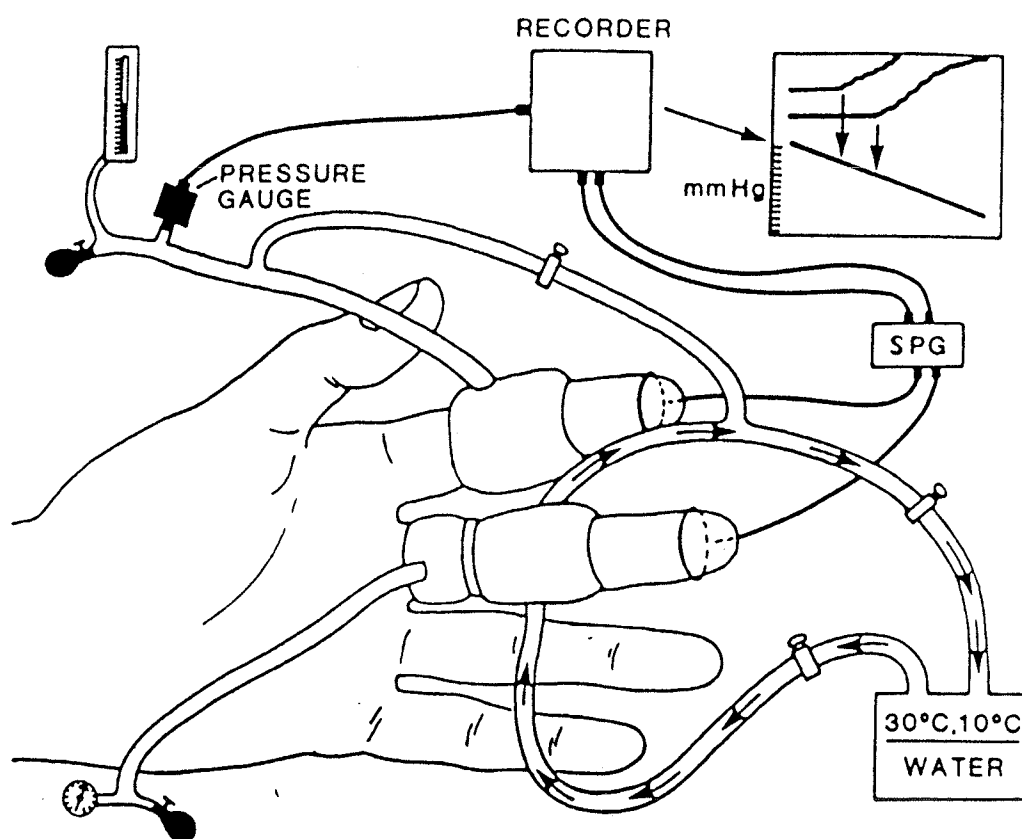
Fig. 5. Diagram of pneumatic hose system and water
circulating system used in measurement of
cold sensitivity of the finger.

and the fourth finger as the control finger. A 1 cm occlusion cuff was attached to the base of the test finger. Double inlet cuffs of the appropriate size were placed over the midphalanges of the test and control fingers. The general cold sensitivity testing protocol was the same for both vasomotor states. After the subject had achieved criterion for finger skin temperatures for the vasomotor state being tested, at least three baseline pressures were recorded. Following this, two repeated pressures were done on the test finger after water at 30°, 20° and $10^\circ C$ was circulated through the cuff as prescribed for temperature sensitivity testing (Nielsen and Lassen, 1977). Each of these measurements took 15 to 20 minutes including cooling, pressure recording and 4 or 5 minute recovery. Prior to any temperature change of the finger, light external compression of the test finger was applied to drain the subcutaneous venous plexuses. Compression was not released until a small occlusion cuff at the base of the digit was inflated to a suprasystolic pressure of 180 to 200 mmHg. Cylindrical cuffs were then placed over the midphalanx of the occluded test finger and the control finger. One of the inlet hoses on the cuff of the test finger was attached to a long outflow hose which returned the water circulated by the pump back to the reservoir. Water was circulated through the cuff for a six minute period. Throughout this period, the inflowing and outflowing temperature of the water was monitored to ensure the correct finger temperature was being maintained.

This cooling method had previously been reported to equilibrate effectively the temperature of the finger arteries to cuff temperature during a five minute period (Nielsen and Lassen, 1977). After the cooling period, the pump was turned off, water was drained out of the system, the pump hose was clamped; and the outlet hose was clamped. Both the test and control fingers were blanched with a rubber dam, the pressure cuffs were inflated simultaneously to a suprasystolic pressure, and the strain gauges positioned and electronically balanced in preparation for blood pressure measurement and detection of pulse or volume change by the gauge. The occlusion cuff on the base of the test finger was then released. The cuff pressure was deflated by 4 mm Hg every 8 to 10 seconds. This stepwise deflation rate was initially selected to allow sufficient time for blood flow to return to the finger following cooling as it was shown to be acceptable in finger pressure measurements previously (Lezack and Carter, 1970). A four minute period separated consecutive pressure measurements to allow flow to the finger to stabilize before the onset of the next cold challenge.

The data were analyzed using analysis of variance and a significance level of $p < .05$.

b) Results

The reproducibility of FSPs for the healthy subjects was assessed in different ways. First, in the analysis of variance, trials, i.e., repeated measures of FSPs were statistically significantly different (Table 2). However, when the data were expressed as a percentage of brachial pressure, there was no signficant effect (Table 2). Also, when the data were transformed using Nielsen's equation (Nielsen, 1978), which is intended to correct for changes in vasomotor tone and systemic blood pressure, there was no trial effect (Tables 3 and 4). Therefore, the apparent trial effect or difference between repeated pressure measurements in absolute terms is likely due to moment-to-moment changes in the general hemodynamic state as reflected by changes in brachial pressure.

Repeated measures were expressed as differences between duplicate measurements for purposes of analysis of reproducibility. Table 5 shows the analysis of the relative differences between repeated blood pressure. Table 6 shows the analysis of the absolute differences between repeated blood pressure measurements. No significant differences in reproducibility and between test and control fingers were found among measurements for three local temperatures. A significant difference in reproducibility was observed, however, for vasomotor state in the analysis of absolute differences of repeated measures. This difference can be explained by the

TABLE 2

Comparison of p values for Brachial Pressure; and Finger Systolic Pressure (FSP) With and Without Being Expressed as a Brachial Index, i.e., Percent of Brachial Pressure in Healthy Subjects and Raynaud's Subjects

SOURCE OF VARIATION	FSPs*	FSPs as % BRACHIAL	BRACHIAL PRESSURE
Group (Normals vs. Raynaud's)	p<.0756	p<.7375	p<.3178
Vasomotor State (VMS)	p<.5253	p<.0295	p<.0472
Local Temperature (LT)	p<.0007	p<.0005	p<.4638
VMS x LT	p<.0285	p<.0138	p<.9740
Trial	p<.0369	p<.8414	p<.0273
VMS x Trial	p<.6046	p<.6073	p<.4603
LT x Trial	p<.2952	p<.4163	p<.9248

*Means appear in Table 9.

TABLE 3

ANOVA for FSP Data Adjusted for Pressure in a Control Finger for Healthy Subjects

SOURCE*	df	SS	MS	F
Vasomotor State (VMS)	1	128.478	128.478	13.86**
Local Temperature (LT)	1	175.582	175.582	18.94**
VMS x LT	1	31.232	31.232	3.37
Trials (T)	1	27.068	27.068	2.92
VMS x T	1	3.580	3.580	0.39
LT x T	1	0.287	0.287	0.03
VMS x LT x T	1	13708.849	139.560	

* Means appear in Table 4.

** $p < .01$

TABLE 4

Descriptive Statistics for FSP Data Adjusted for Pressure in a Control Finger for Healthy Subjects (in mmHg)

	Mean of Adjusted Pressure	SD	N
VC-20°C Trial 1	91.6	6.88	14
VC-20°C Trial 2	98.1	10.35	14
VC-10°C Trial 1	79.7	8.66	15
VC-10°C Trial 2	83.3	15.50	15
VD - 20°C Trial 1	98.1	10.87	13
VD-20°C Trial 2	99.7	13.59	13
VD-10°C Trail 1	91.9	13.99	15
VD-10°C Trail 2	95.0	8.38	14

Legend: VC - vasoconstriction
         VC - vasodilatation
         20°C & 10°C - finger temperatures

TABLE 5

Reproducibility of Finger Pressures (mmHg). Analysis of Relative Differences of Pressures (Trial 1 - Trial 2) Between Two Repeated Measures in Healthy Subjects

	MEAN	SD	N
VC - 30°C - TEST	-1.8	11.02	15
VC - 30°C - CONTROL	-4.3	6.53	15
VC - 20°C - TEST	-5.3	4.72	15
VC - 20°C - CONTROL	-1.9	8.04	15
VC - 10°C - TEST	1.2	10.69	15
VC - 10°C - CONTROL	2.0	6.09	15
VD - 30°C - TEST	-2.0	6.87	14
VD - 30°C - CONTROL	-1.9	4.05	14
VD - 20°C - TEST	-0.7	6.60	13
VD - 20°C - CONTROL	-1.2	7.69	13
VD - 10°C - TEST	-0.4	8.62	14
VD - 10°C - CONTROL	-1.5	9.33	14

SOURCE	df	F
Finger	1	0.0 NS
Local Temperature	2	2.31 NS
Vasomotor State	1	0.11 NS

LEGEND: VC - vasoconstriction
VD - vasodilatation
30°, 20°, 10°C - temperature of test finger
TEST - test finger
CONTROL - control finger
NS - non significant

TABLE 6

Reproducibility of Finger Pressures (mmHg). Analysis of Absolute
Differences of Pressure Between Two Repeated Measures in Healthy Subjects

	MEAN	SD	N
VC - 30°C - TEST	9.0	6.12	15
VC - 30°C - CONTROL	6.1	4.67	15
VC - 20°C - TEST	6.3	3.14	15
VC - 20°C - CONTROL	7.3	3.33	15
VC - 10°C - TEST	8.2	6.61	15
VC - 10°C - CONTROL	5.0	3.79	15
VD - 30°C - TEST	5.4	4.45	14
VD - 30°C - CONTROL	3.6	2.50	14
VD - 20° - TEST	5.5	3.29	13
VD - 20°C - CONTROL	4.8	6.01	13
VD - 10°C - TEST	6.5	5.33	14
VD - 10°C - CONTROL	6.5	6.63	14

SOURCE	df	F
Finger	1	2.96 NS
Local Temperature	2	0.25 NS
Vasomotor State	1	4.71*

LEGEND: VC - vasoconstriction
          VD - vasodilatation
          30°, 20°, 10°C - temperature of test finger
          TEST - test finger
          CONTROL - control finger
          NS - nonsignificant
          * - $p < .05$

reduction of the magnitude and variability of the effect of local cooling during vasodilatation compared with vasoconstriction.

Coefficients of variation for this clinical trial were calculated to be less than 15 percent for all experimental conditions. This finding was consistent with the findings of others (Nielsen, 1978).

Reproducibility of repeated pressure measurements for Raynaud's subjects on the same day is shown in Table 7. The average absolute difference between repeated measures when corrected for brachial pressure at 20°C was 7 and 6 mmHg and at 10°C was 18 and 11 mmHg with the patient vasodilated and vasoconstricted respectively.

Averaged data (mmHg) for each trial for healthy subjects are shown in Figure 6. Means, standard errors and ranges of the FSPs measured during two vasomotor states, at three finger temperatures and for two duplicate measures (trials) are shown in Table 8. The results of the analysis are summarized in Tables 2 and 9. Local temperature, vasomotor state, and the local temperature-vasomotor state interaction were all found to affect FSPs significantly. The direct effect of cold on the arterial smooth muscle of the finger, when sympathetic activity was inhibited by body warming, reduced FSP less than in the high vasomotor tone state. Cooling the test finger decreased FSP to all three local

TABLE 7

Repeated Finger Systolic Pressures During Finger Cooling in Subjects with
Raynaud's Phenomenon.   Pressures Corrected for Changes in Systemic Blood Pressure.

	VASODILATATION				VASOCONSTRICTION			
	LOCAL TEMPERATURES				LOCAL TEMPERATURES			
	20°C		10°C		20°C		10°C	
	Trial				Trial			
SUBJECT	1	2	1	2	1	2	1	2
LK	104	100	79	102	111	98	62	72
WdeL	104	-	94	-	93	85	67	82
YD	82	96	73	94	84	86	68	25
JD	98	98	93	105	106	102	103	98
BM	104	93	97	83	79	84	73	85
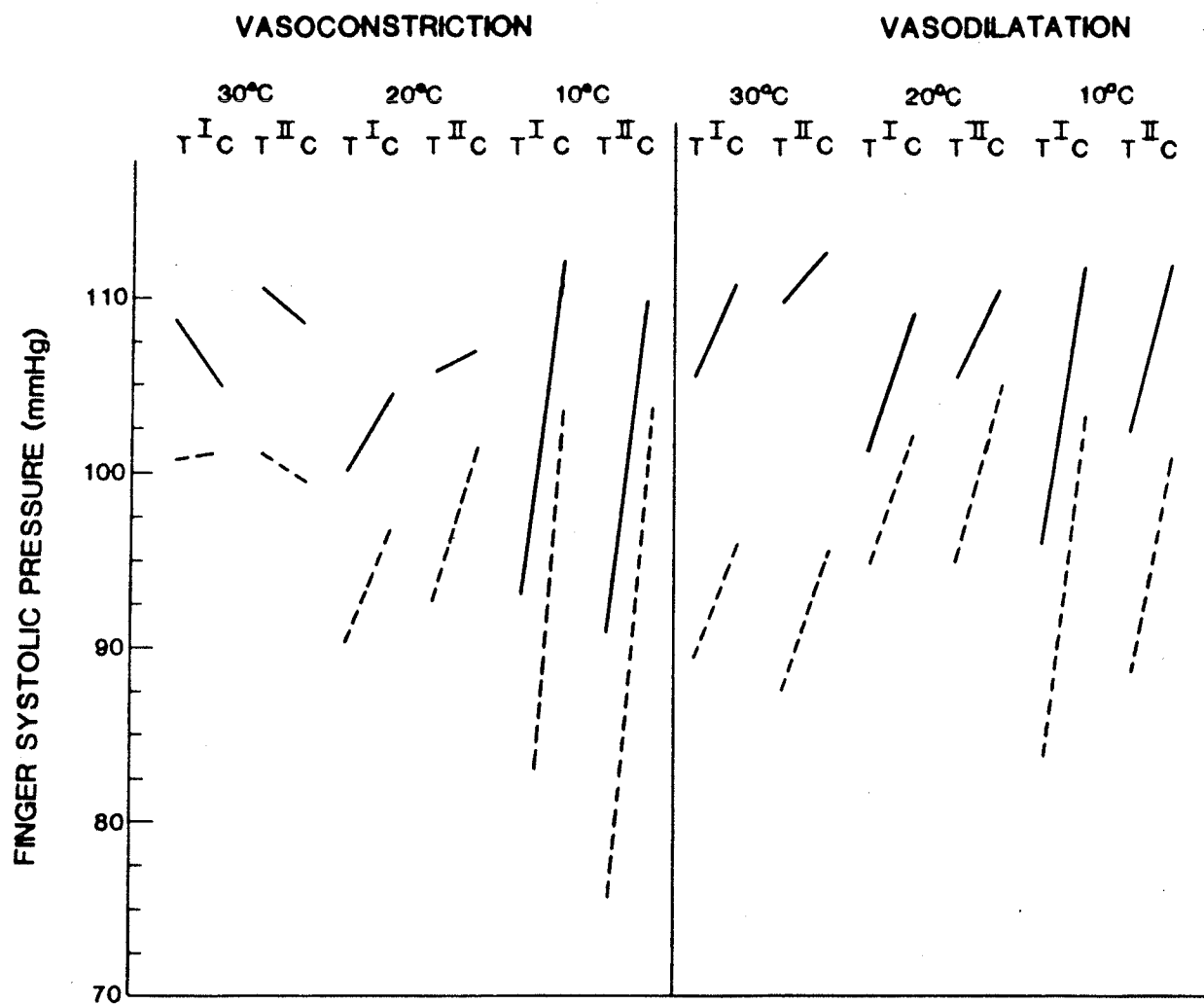
Fig. 6. Finger systolic pressure measurements in 15 healthy subjects (———) and 5 Raynaud's subjects (-----). The figures show pressures recorded in the two vasomotor states, for 3 different finger temperatures of the test finger (T) and a control finger (C). Measurements were repeated (I & II).

TABLE 8   195

Finger Blood Pressure Measurements for Healthy Subjects (in mmHg)*

		VASOCONSTRICTION		VASODILATATION	
Finger Temperature		Test Finger	Control Finger	Test Finger	Control Finger
30°C					
	N	15	15	15	15
	$\overline{X}$	109.9	107.1	107.2	111.4
	SEM	3.9	4.3	3.0	3.5
	Range	93–145	84–138	92–134	94–135
20°C					
	N	15	15	14	14
	$\overline{X}$	103.1	106.7	103.2	108.5
	SEM	3.6	3.9	3.1	4.3
	Range	83–134	81–133	87–130	90–147
10°C					
	N	15	15	15	15
	$\overline{X}$	92.5	111.1	98.6	111.0
	SEM	4.4	4.0	3.4	4.0
	Range	70–122	91–140	81–119	86–144

* Trials were averaged for each subject

TABLE 9

Finger Systolic Pressures ($\overline{X}$ mmHg ± SD) of Test and Control Fingers for Healthy
Subjects and Subjects with Raynaud's Phenomenon for Two Vasomotor States,
Three Local Finger Temperatures and Two Trials

		HEALTHY SUBJECTS	SUBJECTS WITH RAYNAUD'S PHENOMENON
VASOCONSTRICTION			
30°C			
Trial 1-FT		108.92 ± 13.58	100.80 ± 11.67
FC		105.08 ± 16.69	101.40 ± 8.96
Trial 2-FT		110.31 ± 16.58	100.80 ± 10.08
FC		108.31 ± 15.55	99.60 ± 8.56
20°C			
Trial 1-FT		100.15 ± 14.87	90.40 ± 4.22
FC		104.46 ± 14.06	96.80 ± 5.36
Trial 2-FT		105.77 ± 12.74	92.80 ± 3.56
FC		107.00 ± 14.38	101.40 ± 6.91
10°C			
Trial 1-FT		93.15 ± 15.77	76.00 ± 10.98
FC		112.08 ± 14.16	103.80 ± 7.22
Trial 2-FT		90.85 ± 16.74	74.00 ± 28.25
FC		109.85 ± 16.40	102.00 ± 11.73
VASODILATATION			
30°C			
Trial 1-FT		105.46 ± 10.69	89.60 ± 5.55
FC		110.69 ± 13.57	96.00 ± 8.28
Trial 2-FT		109.46 ± 13.92	87.40 ± 5.64
FC		112.62 ± 14.33	95.40 ± 12.14
20°C			
Trial 1-FT		101.38 ± 12.37	94.00 ± 13.45
FC		109.00 ± 17.21	102.00 ± 7.97
Trial 2-FT		105.23 ± 13.18	94.80 ± 5.40
FC		110.38 ± 16.22	105.00 ± 8.66
10°C			
Trial 1-FT		96.08 ± 15.06	83.80 ± 7.92
FC		111.46 ± 15.28	103.40 ± 9.29
Trial 2-FT		102.15 ± 13.70	88.40 ± 6.11
FC		111.85 ± 18.39	101.00 ± 7.38

LEGEND: FT - test finger
         FC - control finger
         30°, 20°, 10°C - temperature of test finger

temperatures suggesting increased vascular tone. Since the skin temperatures were maintained at approximately 35°C in this condition, a local temperature of 30°C was observed to exert a small but direct constrictiong effect. At 20° and 10°C this local response on the test finger was correspondingly more pronounced. In the vasoconstricted state, FSPs were not different from those in the vasodilated state at local finger cooling of 20°C. FSP was significantly lower at a local temperature of 10°C. The predominant contributor to a decreased FSP therefore was the local direct effect of cold on the finger. A local finger temperature of 30°C in the vasoconstricted state tended to produce an increase in FSP (Table 9) suggesting decreased vessel tone and a warming-induced relaxation of the vessel wall.

To compare the present results with those of other investigators, FSPs were transformed to percents of a control pressure at 30°C according to an equation defined by Nielsen (1978). This equation adjusted the reference pressure of the test finger at 30°C for any change in systemic pressure observed in the same period of time in the control finger. The data were transformed and reanalyzed (Tables 3 and 4). Percentage decreases were averaged over two pressure determinations for the analysis. The results compared favorably with the analysis of nonadjusted FSPs in which local temperature and vasomotor state showed a statistically significant effect on FSP (p < .05).

The results for the Raynaud's subjects are shown in Tables 2, 9 and 10. In the vasodilated state, the FSP of the test finger compared with the control finger was reduced at local finger temperatures of 20° and 10°C. At 30°C, no difference between the test and control fingers when averaged over two trials was observed. In vasoconstriction local cooling to 10°C produced significantly lower pressure than for the normal subjects (p < .05). The FSPs for the Raynaud's patients tended to be lower than for the healthy subjects (p < .10). FSPs were statistically different for local temperatures on the test finger (p < .01) and for an interaction between local temperatures and vasomotor states (p < .05). The apparent relaxation of vessel tone at a local temperature of 30°C during vasoconstriction observed in the normal subjects was not observed in the five subjects with Raynaud's phenomenon.

Changes in FSPs in healthy subjects and Raynaud's patients could be separated statistically on the basis of local finger temperature during vasoconstriction (p < .01). Although not statistically significant the two groups showed a similar difference (p approached .05 level) during vasodilatation.

TABLE 10

Descriptive Statistics on Finger Systolic Pressures (mmHg)* of Data for Five
Patients With Raynaud's Phenomenon

Finger Temperature	VASOCONSTRICTION		VASODILATATION	
	Test Finger	Control Finger	Test Finger	Control Finger
30°C				
N	5	5	5	5
$\overline{X}$	100.7	100.4	88.3	95.6
SEM	4.8	3.6	2.3	4.3
Range	89-118	91-113	84-95	85-108
20°C				
$\overline{X}$	91.5	99.0	94.2	103.3
SEM	1.7	2.6	4.0	3.6
Range	88-98	91-106	85-105	90-109
10°C				
$\overline{X}$	74.8	102.7	86.1	102.0
SEM	7.1	4.0	2.7	3.5
Range	51-91	88-111	80-94	92-112

* Trials were averaged for each patient

c) Discussion

Our data showed that large differences can occur in repeated measurements of apparent finger systolic pressure in cold sensitivity testing. The reproducibility in our study was not as good as that reported by others (Nielsen and Lassen, 1977; Nielsen, 1978). Various explanations were considered. First, the use of stepwise deflation rate may have contributed to warming of the finger, hence reduced arterial tone and greater apparent finger systolic pressure. Also the rate of recovery of the arterial temperature may vary in repeated measures. It is known that the vasospastic phenomenon is elusive in the laboratory.

The skin temperatures of subjects during the body cooling procedure would suggest the subjects in Nielsen's study and the present study were in a different vasomotor state. Nielsen reported skin temperatures between 24° and 30°C. which would be more characteristic of a thermoneutral vasomotor state, whereas in the present study skin temperatures averaged 22°C and were within a few degrees of room temperature. Nielsen used a room temperature of 22°C. Why the present results agreed quite so well with Nielsen's results was of interest since the cooling procedure used in the present study was relatively more severe and the criterion for its use more stringent; that is distal skin temperatures had to be within 4°C of room prior to cold sensitivity testing. One explanation might be that in the present study

greater time was permitted for flow to resume particularly at the lower temperatures. This is discussed next in relation to methodologic concerns of the test procedure.

Several methodologic issues related to the cold sensitivity test procedure were considered in this study. First, Nielsen and Lassen (1977) had reported good success with their temperature equilibration procedure in which water of a desired temperature was circulated through a cuff with a double inlet hose while the circulation to the finger was occluded. They reported rapid temperature equilibration of the arterial wall to cuff temperature, which remained stable until flow resumed to the finger. Measurement of temperature under the cuff in the present series revealed that at especially low temperatures such as $10^\circ C$ in the cuff, skin temperature would equilibrate to within a few degrees of $10^\circ C$ but did not consistently reach that temperature. This discrepancy appeared to be more pronounced in the vasodilated state. This suggested that the vessels may have equilibrated to a slightly warmer temperature. This may explain in part the generally good agreement between our results and those of Nielsen and Lassen. Although the evoked sympathetic drive was seemingly greater in the present study the local temperature may have been warmer. Furthermore, the skin temperature under the cuff of the cooled finger was not found to remain stable. Rather temperature was observed to very gradually increase up to a few degrees from the time

the pump was stopped for pressure measurement. It was doubtful that this primarily reflected the changeover from the water to the pneumatic system because of the relatively gradual change in temperature. With the use of the slow stepwise deflation rate, a pressure could take up to a couple of minutes to record. In this time, the skin temperature under the cuff could reach up to 14°C during vasodilatation when water at 10°C was circulated through the cuff. Although the temperature of the underlying artery was not directly measured in this study, it was assumed that until blood flow resumed skin temperature was a reasonable estimate, considering that the digital arteries lie within 1 to 2 mm of the surface of the skin and the flow to the finger was occluded during the critical skin temperature measurement. These observations suggested that in future studies, temperature under the cuff should be routinely monitored and that the water reservoir temperature should be adjusted to maintain skin temperature at the desired temperature. In addition, temperature might be more stable if during the change from a water to a pressure system, water was not drained from the cuff prior to pressure measurement. Krähenbühl et al. (1977) favored a slower deflation rate to allow flow sufficient time to be picked up by the sensor. This may impose a potential source of variability on the FSPs measured. When a finger is exposed to local cooling, vessel tone is increased and therefore, less pressure is needed in the cuff to occlude the flow. The existing vessel

tone may also impede the reopening of the vessel to some extent. At faster deflation rates, vessel opening may lag behind decreasing cuff pressure (Gundersen, 1972). Faster deflation rates could produce lower FSPs if cooling were to exert such an effect on peripheral finger arteries. This may be an especially important consideration in cold sensitive individuals.

The absence of an increase in FSP in Raynaud's patients during body cooling and a local finger temperature of $30^\circ C$ suggested increased basal tension in the digital arteries of these patients. Another explanation might be the presence of organic changes in the digital arteries of these patients which were not detectable on routine digital pressure measurement. FSPs expressed as a percent of a control pressure were reduced by about 9 percent at $10^\circ C$ in vasodilatation and by about 26 percent during vasoconstriction (Table 10).

Nielsen (1978) reported that a local temperature of $20^\circ C$ and body cooling using a cooling blanket elicited an FSP of zero or vessel closure in 17 of 18 patients with Raynaud's phenomenon. None of the five Raynaud's subjects exhibited vessel closure in these experiments.

A possible explanation for the different results may be that the present group of subjects was both small and may have been different with respect to subjects reported by others. Also, the methodologic considerations previously

described (Sections D and E) may have resulted in slightly warmer temperature of the arterial wall in this study, thus resulting in higher FSP. The cuff was deflated in a step-wise manner in the present study. During the time elapsed until the end point was observed, the temperature often rose under the cuff. Thus the end point was not always observed at the temperature to which the water bath was set and to which the digital arteries were intended to be equilibrated. Hence these results are not comparable to previous work. Nielsen and Lassen (1977) did not specify what deflation rate was used.

Another explanation for the different results may be related to differences in the vasomotor states of the subjects. Nielsen and others used a water blanket over the anterior body to achieve increased vasomotor tone (Nielsen, 1978). The skin temperatures reported with use of the blanket, however, would suggest that a thermoneutral or even a vasodilated state was present. The skin temperatures in the present studies were within 4°C of room temperature prior to cold sensitivity testing to meet the criterion for vasoconstriction. This indicates that in the present study the sympathetic vasoconstrictor discharge was greater. However, the local temperatures were somewhat higher than in the Nielsen study. As observed in the healthy subjects, the temperature under the cuff in the subjects with Raynaud's phenomenon was more variable than that reported by Krähen-

bühl et al. (1977). The temperature tended to be warmer in our series, which may have offset the effect of the apparently greater vasomotor tone.

These preliminary results in Raynaud's subjects, however, support an exaggerated local response to cold which is further augmented with increased sympathetic tone. The local response was quantitatively greater than that observed in normal subjects. FSPs for both fingers were generally lower than normals in both vasomotor states.

In summary, cold sensitivity of normal fingers and those with Raynaud's phenomenon was studied in terms of cold induced changes in FSP as measured according to a method described by Nielsen and Lassen (1977). In healthy fingers cooled to 20° and $10^\circ C$, FSPs were decreased in both a warm and cool body state. By inhibiting sympathetic activity with body warming, the observed cold-induced decreases in FSPs in response to finger cooling suggested that cold exerted a direct constricting influence on the smooth muscle of the digital arteries. By increasing sympathetic activity with body cooling, decreases in FSP were elicited both by a direct effect of cold on arterial smooth muscle and a neurogenic constrictor effect. This led to greater increase than when cooling was applied during body heating.

The five subjects with histories of Raynaud's symptoms failed to demonstrate vessel closure reported by others un-

der conditions of increased vasomotor tone and finger cooling to 10°C. The primary explanation for the differences observed between our findings and others was the incomplete temperature equilibration achieved in our studies. Despite the differences compared with the findings of other studies, these results suggest that cold has a more pronounced direct effect on the vascular smooth muscle of the finger arteries of Raynaud's subjects, and that this effect is further augmented with increased vasomotor tone. This effect was greater than that observed in healthy subjects studied in our lab (Section A2).

Because of the methodologic considerations discussed above, another series of studies was carried out to examine these and other methodologic issues.

C. Studies of Methodology
1. Comparison of Four Distal Sensors

Mercury-in-rubber strain gauge has been used extensively but other sensors have also been used in measuring systolic pressures of the fingers. Comparisons were made between four commonly used sensors to detect resumption of flow for blood pressure measurement in the digits of normal subjects during body and local cooling. Methods used were strain gauge (SG), photoplethysmograph (PPG), spectroscope (SP) and the visual flush technique (FL). The reproducibility of these methods in routine digital blood pressure determination has been previously described (Section D). Previously

no comparison has been made between distal sensors when applied simultaneously, nor has their relative suitability been assessed for accurate measurement of FSP in cold sensitivity testing in the fingers.

a) Methods

i) Subjects.

Two male and three female subjects whose ages ranged from 29 to 53 years participated in the study. All subjects were normotensive, in good general health, nonsmokers, and had no symptoms related to Raynaud's phenomenon.

ii) Protocol.

Subjects were prepared for cold sensitivity testing as described in Section A2. Prior to testing, each subject was stabilized in a vasoconstricted state according to a modified Gibbon and Landis procedure (1932) with finger tip temperatures within $4^{\circ}C$ of room temperature for at least 45 minutes. Finger systolic pressures were measured in duplicate following local cooling of the test finger to $10^{\circ}C$ with a double inlet cuff as described in Section A2. This was performed for each of the four distal sensors. At least four minutes separated the end of each pressure measurement and the onset of the subsequent finger cooling period.

To ensure that the inflow of blood to the finger tip could be readily detected by each sensor a double "blanching" technique was applied to render the digit relatively bloodless. This involved wrapping the finger tightly with the rubber dam prior to the inflation of the proximal occlusion cuff. In addition, following the six minute cooling period, the distal phalanx of the finger was again blanched prior to the application of sensors and deflation of the blood pressure cuff. Warming the tip of the cooled finger was carried out with a small hot water bottle during the last 90 seconds of cooling of the middle portion of the finger just prior to pressure measurement.

Previous work has shown that blood pressure in the digits measured with SG has acceptable reproducibility (Lezack and Carter, 1970; Gunderson and Lassen, 1970; Gundersen, 1972), therefore each of the other three sensors was evaluated in combination with the SG on the right forefinger of each subject. Thus SG measurement was taken simultaneously with each of the other methods on the same digit.

Application of the sensors was performed in the standard way. Strain gauge application on the right forefinger and blood pressure measurement was performed for each pressure determination as described in Section A2.

In the PPG method, the photocell was applied and attached with double-sided adhesive tape. Secure attachment was

critical to ensure the light sensitive cell was not activated by inadvertent exposure to room light. Similar to the SG, PPG tracings of flow resumption during deflation of the blood pressure cuff were recorded on the Beckman recorder. With the use of a light source beamed on the finger tip in a darkened room the spectroscope displayed a brown band as blood flow of oxygenated blood returned to the finger and oxyhemoglobin appeared in the skin vessels. Visual flush technique is the most subjective of the four methods. However, it has been reported to have good reproducibility when performed by a trained observer (Carter and Lezack, 1971). Following the double blanching procedure, the observer recorded the pressure at which the first sign of increased redness appeared in the finger.

b) Results and Discussion.

The averages of duplicate finger systolic pressure determinations for each distal sensor for each of the five normal subjects tested, were graphed against FSPs obtained with SG. Comparing the identity lines of PPG, SP and FL methods with SG, PPG and FL agreed well (within 10 mmHg) with the mercury gauge (Figures 7,8). Measurements with the spectroscope (Fig. 9) consistently underestimated the apparent finger systolic pressure (p < .05). This may be related to the difficulty of detecting the oxyhemoglobin band with local cooling and slow resumption of blood flow.
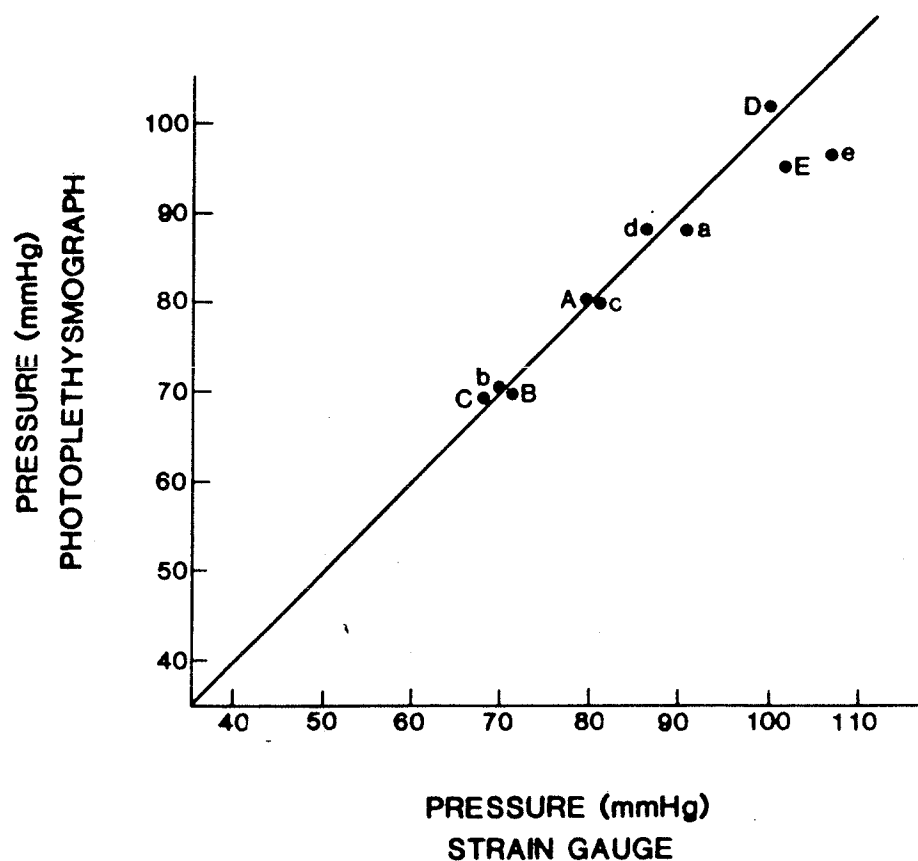
Fig. 7. Relationship of finger systolic pressures deter-
mined by photoplethysmography and strain gauge
with the subject vasoconstricted and a local
finger temperature of 10°C. Upper and lower
case matching letters represent two trials for
each subject.
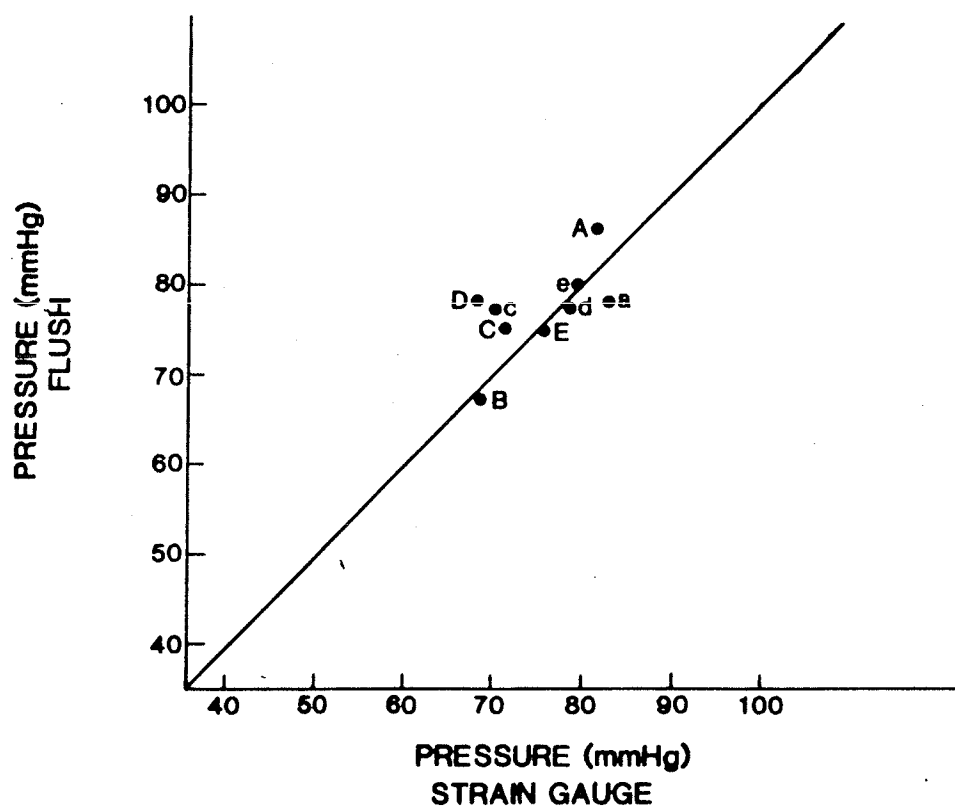
Fig. 8. Relationship of finger systolic pressures deter-
mined by flush technique and strain gauge with
the subject vasoconstricted and a local finger
temperature of 10°C. Upper and lower case
matching letters represent two trials for each
subject. Second trial for B is missing.
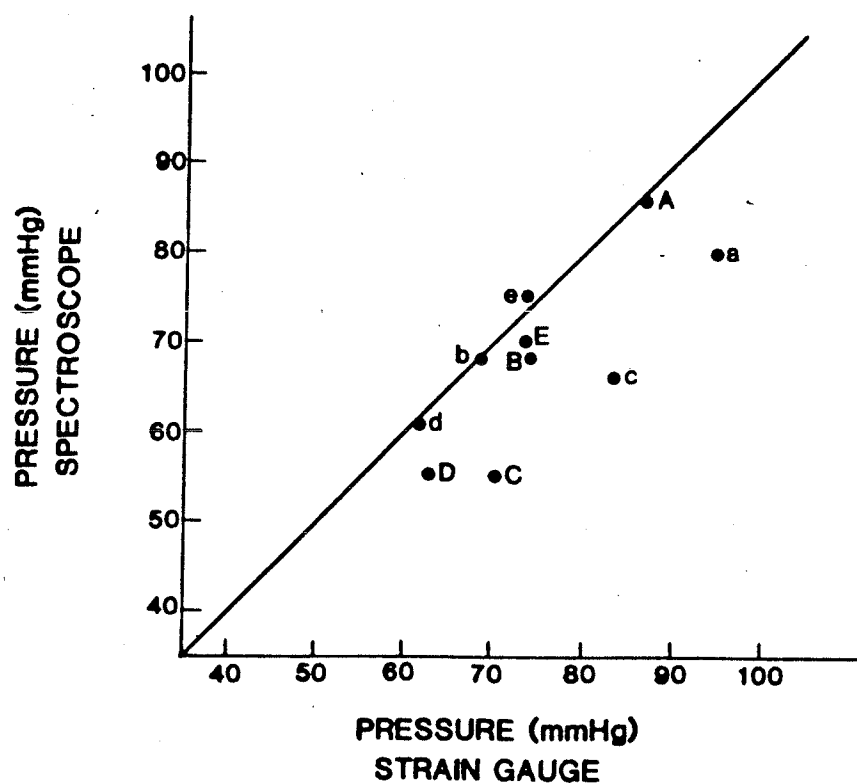
Fig. 9. Relationship of finger systolic pressures
determined by spectroscope and strain gauge
with the subject vasoçonstricted and a local
finger temperature of 10°C.

Although the PPG compared favorably with the SG, signal artifact was more problematic with the PPG. Using the PPG in the AC mode limited end point detection to pulse only. Because pulse amplitude is reduced with peripheral and body cooling, the gain was set high to maximize end point detection under these conditions. Thus electronic noise was correspondingly amplified. Changes in systemic blood pressures in subjects were not significantly different during pressure measurements.

SG and PPG showed generally good reproducibility which compared favorably with that reported for measurements in noncooled conditions (Lezack and Carter, 1970; Hirai and Kawai, 1977). Flush technique also demonstrated good reproducibility. Spectroscope appeared to be generally less reproducible and agreed less well with the SG measurements for the possible reasons described.

On the basis of these findings, SG appeared to be a suitable distal sensor for future studies using a version of Nielsen and Lassen's method for cold sensitivity testing in the fingers. The use of DC mode in the application of the PPG may help to overcome some of the problems experienced with the AC mode because with the DC mode both pulse and volume change can be monitored. Study of such use of the PPG is warranted in further studies, however, in this thesis, the strain gauge was used.

2. Effect of Cuff Occlusion

The effect of a transient occlusion of digital blood sup-
ply prior to taking finger blood pressure has not previously
been thought to influence the recorded blood pressure in a
significant way (Gaskell, 1965). Noninvasive cold sensitiv-
ity measurement in the fingers as described by Nielsen and
Lassen (1977) requires at least a five minute period of ar-
terial occlusion during which time water of a given tempera-
ture circulates through a double inlet cuff placed distal to
an occlusion cuff. As previously discussed (Section E),
Nielsen and Lassen believed that the transient occlusion of
finger circulation during the test had no effect, although
this has not been systematically investigated.

Krähenbühl et al. (1977) were the first to report the ap-
pearance of an apparent decrease in finger systolic pressure
with previous finger cooling while the arterial blood supply
was occluded. The decreased pressure observed under these
conditions has been attributed to a cold induced increase in
digital artery tone, which in turn results in an apparent
diminution in arterial pressure. As the arterial tone was
decreased with radiating warmth or the resumption of flow,
the pressure returned to the pressure observed prior to
cooling of the finger. This suggested that the pressure
that was recorded locally at that site was directly related
to the state of arterial tone. This phenomenon served as
the basis of Nielsen and Lassen's procedure (1977), in which

an apparent change in FSP (as a result of local temperature change of the finger) served as an index of arterial tone for a given local finger temperature.

A combination of decreased tissue pO_2 (Barcroft, 1972) and of increased tissue pCO_2 (Dougherty et al. 1967) and metabolites result in a dilatation of the vascular bed distal to the occlusion (Barcroft, 1972). Nielsen and Lassen's cold sensitivity test involved cooling a finger locally. The change in tone is usually superimposed on some degree of sympathetic vasomotor tone in the digital arteries. In the same way that cooling can physiologically increase tone of the digital arteries, reactive hyperemia might counteract this effect and reduce the tone and the apparent effect of cooling.

The effect of arterial occlusion was therefore investigated by comparing FSPs in two fingers of the same hand of normal subjects. Blood supply to one of the fingers was occluded for six minutes prior to simultaneous blood pressure measurements of the two fingers. Because skin temperature was observed to decrease in the occluded finger during circulatory arrest, water set at the preocclusion temperature of the finger was circulated in the water perfusion cuff to avoid such a temperature drop. In this way, the effect of occlusion could be studied with and without the effect of a decreased skin temperature secondary to arterial occlusion.

a) Methods.

i) Subjects.

Three normal subjects were studied; two females, aged 29 and 31, and one male aged 38. Subjects were nonsmokers and had no symptoms of cold sensitivity.

ii) Protocol.

Blood pressure measurements were carried out as described in Section A2 except that no local cooling was applied. A dilated state was used for the male subject and one female. In the event that dilatation from reactive hyperemia and its effect on finger systolic pressure were masked in the vasodilated state, the effect of arterial occlusion was also studied in both females in a thermoneural vasomotor state. Skin temperatures during vasodilatation were above $35^{\circ}C$, and in the thermoneutral state between 26° and $29^{\circ}C$. The room temperature was $20 \pm 1^{\circ}C$.

In another series of studies, water was circulated through the blood pressure cuff to maintain the skin temperature of the occluded finger at the temperature recorded during the pre-occlusion period. This was done to help rule out any effects of finger cooling secondary to circulatory arrest.

b) Results and Discussion

Prior to arterial occlusion, simultaneous pressure measurements on the second and fourth fingers were similar except for one subject who showed a 6 mmHg difference (Fig. 10). After a six minute period of occlusion of the digital arteries in the test fingers pressure of the occluded test finger was lower in four of six measurements than in an adjacent control finger both with and without rewarming the finger to its baseline temperature (Fig. 10 and Table 11).

By rewarming the finger to its baseline temperature, differences in pressure between the two fingers was reduced (Fig. 10). Temperature compensation seemed to reduce the variability of the pressure measurements, however pressures were consistently reduced in the occluded finger. Reduced vascular resistance as a result of the reactive hyperemic
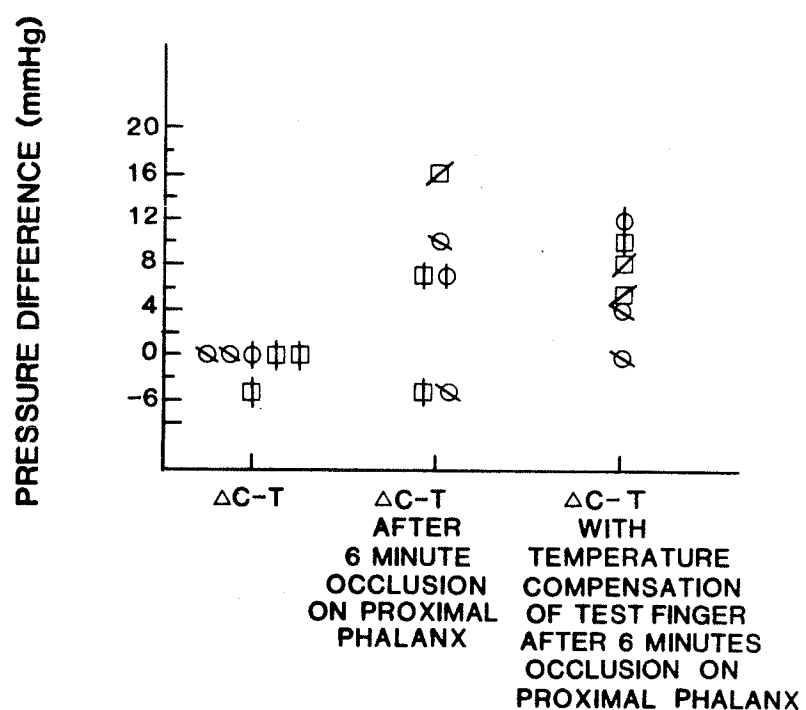
Fig. 10. The effect of proximal finger occlusion
on subsequent finger systolic pressure
(FSP).
C - control finger FSP
T - test finger FSP

Subjects: DP:\ EP:| LW: /

○ - vasodilated
▢ - vasoconstricted

TABLE 11

The Effect of Occlusion on Subsequent Finger Systolic Pressure (mmHg)

SUBJECT	SEX	AGE	VASOMOTOR	6 min PERIOD OF OCCLUSION on F_2			6 min OCCLUSION with FINGER TEMPERATURE COMPENSATION on F_2		
				F_2 (mmHg)	F_4 (mmHg)	Brachial (mmHg)	F_2 (mmHg)	F_4 (mmHg)	Brachial (mmHg)
DP	M	37	Vasodilated skin Temperatures $\geq 35^\circ C$	94	105	104	99	102	110
EP	F	ew	Vasodilated Skin Temperatures $\geq 35^\circ C$	90	97	97	97	109	93
			Thermoneutral Skin Temperatures $26-29^\circ C$	94	101	94	90	101	88
LW	F	29	Thermoneutral Skin Temperatures $26-29^\circ C$	115	113	112	107	116	108
				106	123	116	115	120	118

LEGEND: F_2 - test finger

F_4 - control finger

response due to vascular occlusion might have been responsible for the decreased pressure despite temperature compensation.

These results suggest that a period of occlusion of 6 minutes reduces the tone of the digital arteries at thermoneutral and high temperatures. However, since these effects were small averaging less than 10 mmHg, they may not be important when local cooling results in loss of measurable pressure in patients with Raynaud's phenomenon. Furthermore, reactive hyperemia observed in healthy subjects following circulatory arrest is absent or less pronounced in Raynaud's patients (Davis, 1981).

3. Comparison of Pressures Measured in a Single Digit with Simultaneous Measurement in Two Fingers

One of the features of the cold sensitivity test described by Nielsen and Lassen (1977) is that blood pressure is measured on a test finger and on a control finger of the same hand simultaneously. In this way, pressure of the test finger can be adjusted for hemodynamic state of the subject as gauged by a pressure change in the adjacent control finger. Doupe et al (1939a) and Krähenbühl et al. (1977) reported that occluding flow to four digits of one hand increased the recorded pressure in the remaining digit. This can be explained physiologically by the fact that the reduced flow through the hand results in less energy loss,

thus greater remaining pressure at the digit. Thus, similar to the increased digital pressures observed with body cooling, occlusion of four digits of one hand results in a higher pressure in the fifth digit. Therefore it was important to determine if this mechanism could affect blood pressure measurements when pressures are measured in two fingers of the same hand during cold sensitivity testing.

a) Methods.

i) Subjects.

One male (34 years) and two female (27 and 29 years) healthy subjects (non-smokers and no reports of cold sensitivity) were studied.

ii) Protocol.

The methods were described in Section A2. Blood pressure measurements of two fingers on the same hand were taken simultaneously and individually. Pressures were studied with subjects in two vasomotor states; vasodilatation (see Section A1) and thermoneutrality. A thermoneutral state was defined as skin temperatures 7 to 9°C above room at 20 $\pm$1°C.

b) Results and Discussion.

The results are shown in Table 12 and Figure 11. The data do not indicate that measurements taken simultaneously on two fingers overestimate the pressure in the finger in

TABLE 12

Comparison of Finger Systolic Pressures (mmHg) Measured Individually or Simultaneously on the Same Hand

SUBJECT	SEX	AGE	VASOMOTOR STATE	TEST FINGER PRESSURE (mmHg)	CONTROL FINGER PRESSURE (mmHg)	BRACHIAL PRESSURE (mmHg)
DP	M	37	Vasodilatation Skin Temperature $\geq$ 35°C	110	110	108
				103	103	106
				110	–	108
				102	–	108
				–	99	107
				–	108	106
EP	F	32	Day 1 Vasodilatation Skin Temperature $\geq$35°C	90	88	97
				92	–	95
				–	97	97
			Day 2 Vasodilatation Skin Temperature $\geq$ 35°C	93	93	98
				95	98	102
				94	–	96
				–	99	99
			Day 3 Thermoneutral Skin Temperature 26-29°C	101	104	96
				99	–	95
				–	97	91
LW	F	29	Thermoneutral Skin Temperature 26-29°C	120	120	110
				119	119	109
				119	–	114
				118	–	110
				–	124	108

Fig. 11. The effect of taking finger blood pressure
measurements individually or simultaneously
shown by brachial-finger pressure gradients.

Legend: B - brachial pressure, F_2 - pressure
of test finger, F_4 - pressure of control
finger.

Subjects: \ DP, | EP, / LW

O - vasodilated
□ - thermoneutral

which flow resumption is detected first. When blood pressure is being measured in only two fingers normal blood flow is likely occurring in the remaining three fingers. The change in pressure related to the absent flow in one occluded finger was not sufficient to change significantly the pressure recorded in the finger in which the flow resumed first. This observation has important implications for the validity of the Nielsen and Lassen cold sensitivity procedure in which simultaneous finger blood presssure measurements are performed on the same hand routinely. Individually measured pressures corresponded with those finger pressures measured simultaneously. These findings do not rule out, however, such a bias if cold sensitivity was measured simultaneously in more than one finger and a control finger. In fact it is reasonable to suspect that this complicating effect would be increasingly more pronounced should more than two finger pressures be measured simultaneously in modified versions of this cold sensitivity testing procedure.

4. Effect of Method of Cuff Deflation on the Apparent Finger Systolic Pressure Measured in Cooled Fingers

Previous studies have revealed quantitative differences between the FSPs of subjects in our lab in response to local cooling, and those reported by others (Nielsen and Lassen, 1977; Nielsen, 1978). This observation may be explained by the fact that the step-wise deflation rate used in our stud-

ies may have resulted in rewarming of the digital arteries.
This deflation method was used because it has been found to
be accurate in our lab in the past. Although a deflation
rate of 2 mmHg/sec is recommended for routine brachial blood
pressure measurement (Kirkendall et al, 1977), Krähenbühl et
al. (1977), and Nielsen and Lassen (1977) alluded to using
an unspecified slower rate of deflation to allow for the
slow return of blood to the finger tip when the finger was
cooled. Since blood pressure was measured using a strain
gauge on the finger tip, Nielsen and Lassen reasoned that
unless time was allowed for blood flow to resume, the ob-
served changes in apparent finger systolic pressure could be
underestimated. Others used faster deflation rates (such as
5 mmHg/sec) in cold sensitivity testing but how this com-
pared with other rates was not reported (Nielsen and Lassen,
1983). Considering that finger systolic pressure may be in-
fluenced by the deflation rate (Gundersen, 1972), it was im-
portant to examine the effect of deflation rate on the re-
corded pressure and to determine whether this effect is
accentuated in cooled fingers. Lack of standardization of
cuff deflation rate when taking the blood pressure of the
cooled digit might explain the differences between our find-
ings (see Section B) and those of other investigators.

a) Methods.

i) Subjects.

Six healthy subjects (age 23 to 34 years) participated in the study. All were non-smokers and had no unusual cold sensitivity.

ii) Protocol.

The procedure used as described in Section A2. The effects of three deflation rates on the FSPs were compared resulting at temperatures of 30° and $10^\circ C$ both in a vasoconstricted and a vasodilated state. Deflation rates included two continuous rates of 5 mmHg/sec and 2 mmHg/sec, and a stepwise deflation of 6 mmHg every 6 seconds.

Temperature under the cuff was measured using a thermistor (Yellow Springs, Inc.) positioned over the skin under the midportion of the cuff. Temperature under the cuff was observed to increase somewhat with the slower deflation rates in the normal subjects. Therefore additional comparisons were made between FSPs before and after compensation of the cuff temperature, if warming had occurred especially during the slower rates. Compensation was achieved by circulating water in the cuff around the finger, colder than that required for the test to offset any increase in finger temperature as quickly as possible.

b) Results and Discussion.

The results of the deflation rate comparisons on the observed FSP in cold sensitivity testing with and without compensation of the temperature of the test finger for six normal subjects appear in Table 13. As Gundersen found (1972), we observed that low deflation rate resulted in gradual volume increases of the finger tip and small amplitudes of initial pulses, in addition to higher FSPs compared with a faster rate.

Table 14 shows the mean FSP of both the compensated and non-compensated temperature conditions. When the finger was locally exposed to a temperature of $30^\circ C$ in either the vasoconstricted or vasodilated state, little difference existed between the control and test fingers for the three deflation rates. Larger differences were apparent for local finger temperatures of $10^\circ C$ in both vasomotor states, and were more pronounced in the vasoconstricted state. Differences between the control and test fingers were least for the stepwise deflation rate, greater for the slow continuous rate (2 mmHg/sec), and most for the fast continuous rate (5 mmHg/ sec). During the fast deflation 5 to 10 mmHg drop in cuff pressure may occur between heart beats, thus resulting in a systematic underestimation of digital blood pressure, particularly in individuals with low heart rates. This must have occurred in this study since the pressures were consistently lower at the fast deflation rate. The effect was

TABLE 13

Effect of Compensatory Finger Warming on Finger Temperature and Finger Pressures Taken with 3 Different Deflation Rates. Pressure (mmHg) and Temperature (°C) Means for Healthy Subjects (N=6)

	DEFLATION RATE: 5 mmHg/sec			DEFLATION RATE: 2 mmHg/sec			DEFLATION RATE: STEP WISE		
	TF-P (mmHg)	TF-T (°C)	CF-P (mmHg)	TF-P (mmHg)	TF-T (°C)	CF-P (mmHg)	TF-P (mmHg)	TF-T (°C)	CF-P (mmHg)
	NC C	NC C	NC C	NC C	NC C	NC C	NC C	NC C	NC C
30°C									
Vasodilatation	87 76	30 29	93 85	92 78	28 29	103 85	95 78	28 30	103 83
Vasoconstriction	88 89	29 31	98 82	82 86	29 30	90 77	95 89	27 30	96 90
10°C									
Vasodilation	77 75	13 11	88 93	89 80	13 11	104 93	89 90	14 11	99 93
Vasoconstriction	25* 48	12 11	99 93	42 64	13 10	84 84	75 74	13 12	97 90

LEGEND:

TF-P test finger pressure

TF-T test finger temperature

CF-P control finger pressure

NC   no compensation

C   compensation

10°, 30°C test finger temperature

*   mean includes a score of zero

TABLE 14

Effect of Deflation Rate on Finger Systolic Pressures in Cold Sensitivity
Testing in Healthy Subjects

CONDITION	DEFLATION RATE COMPARISON	LEVEL OF SIGNIFICANCE
VC - 30°C	SS vs 2 mmHg/sec	p<.10
VD - 30°C	"	p<.10
VC - 10°C	"	p<.05
VD - 10°C	"	NS
VC - 30°C	SS vs 5 mmHg/sec	NS
VD - 30°C	"	NS
VC - 10°C	"	p<.05
VD - 10°C	"	p<.10
VC - 30°C	2 mmHg/sec vs 5 mmHg/sec	NS
VD - 30°C	"	NS
VC - 10°C	"	NS
VD - 10°C	"	p<.10

LEGEND: VC - vasoconstriction
        VD - vasodilatation
        10° & 30°C local finger temperatures
        NS - nonsignificant

DEFLATION RATES: SS - short step; 6 mmHg/6 sec
                   2 mmHg/sec - slow continuous
                   5 mmHg/sec - fast continuous

greater in the cooled test finger. This observation might be explained by the effect of cold on the mechanical behavior of the blood vessel wall. With a fast deflation rate the opening of the blood vessel deformed by the cuff pressure may lag further behind the release of pressure in the cuff. Therefore, a fast deflation rate results in a reduced FSP. At a slower rate the opening of the vessel occurs closer in time to the occurrence of pressure at which the vessels first open.

The question arises as to which deflation rate is optimal for cold sensitivity testing. Two subjects who had low heart rates and systemic blood pressure, exhibited vessel closure at the fast deflation rate at a local finger temperature of 10°C during vasoconstriction. This suggested that the fast deflation rate may be unsuitable for routine use to detect decreases in FSP with local finger cooling and thus to discriminate between normal subjects and patients with Raynaud's phenomenon. The stepwise deflation did not show any vessel closure. However, a greater discrepancy tended to result between the temperature under the cuff and the desired temperature. It was thought that warming of the finger resulted from the longer time needed for this deflation method. Because the essence of the cold sensitivity testing procedure rests upon the temperature of the arterial wall equilibrating to cuff temperature, compensation of the water temperature needed to be instituted for the slower deflation

rates. Thus it appears that the optimal deflation rate for this test is the intermediate rate of 2 mmHg/sec. The pressure could be measured reasonably quickly with less opportunity for finger warming. This rate provided greater range of FSPs compared with the other deflation rates suggesting a greater sensitivity. Thus, 2 mmHg was chosen as a deflation rate to study patients with Raynaud's phenomenon.

D. Cold Sensitivity in the Fingers of Patients with Raynaud's Phenomenon

The modification of the method of Nielsen and Lassen as described in Section B of this Chapter was used in this study of patients with Raynaud's phenomenon. We used this modified procedure to study the responses of digital arteries thought to be involved in Raynaud's phenomenon.

Although the Nielsen and Lassen method has been used by others in experimental clinical trials since its development, studies reported earlier have suggested that the validity and reproducibility of the test could be improved by more stringent control of specific methodologic parameters (Section A). For example, vasomotor state, temperature under the cuff of the test finger and the deflation rate are especially important factors which should be more rigidly controlled than described in the original reports of the test. Without taking these precautions, we have previously observed that finger systolic pressures can vary considerably and hence the value of the test is questionable.

The present studies were undertaken to investigate the underlying pathophysiology of Raynaud's phenomenon. In the past, identifying the precise mechanism(s) involved has been hampered for several reasons. First, as previously discussed, the lack of a reliable test for evaluating cold sensitivity in the hand has hindered diagnosis, assessment of the disease, its natural history, and response to treatment and prognosis. Comparison of results from different laboratories has been hampered by these shortcomings. A second difficulty has been the disagreement on the criteria for the differential diagnosis of Raynaud's phenomena, and classifying its primary and secondary forms. Consistent and universally accepted nomenclature is essential if Raynaud's phenomenon is to be meaningfully studied. Standardized assessment techniques and the application of the refined cold sensitivity test developed in previous studies was therefore undertaken to investigate both normal and abnormal responses of the digital arteries to cold.

1. Methods

a) Subjects.

Patients were categorized as primary or secondary Raynaud's phenomenon, or Raynaud's phenomenon of uncertain diagnosis. If the history, physical examination, X-rays, hematologic studies and routine finger blood pressure provided no evidence of an underlying disease state or arterial oc-

clusion, a diagnosis of primary Raynaud's phenomenon was made. However, because symptoms may not become apparent for a number of years, patients with a short duration of symptoms (less than four years) were classified as Raynaud's phenomenon of uncertain etiology.

Patients with secondary Raynaud's phenomenon included those with connective tissue disease and exposure to vibrating tools with and without occlusion. Criteria for obstruction included a finger pressure under 70 mmHg and/or a difference of pressure between adjacent fingers of 15 mmHg or more (Downs et al., 1975).

Thirty-seven patients were included in the study. There were 22 patients without obstruction consisting of 4 with primary disease, 7 with uncertain diagnosis and 11 with secondary phenomenon including vibrating tool syndrome, and connective tissue disease. Fifteen patients had evidence of obstruction; including 11 with Raynaud's phenomenon secondary to connective tissue disease and to vibrating tool syndrome. Four had uncertain diagnosis.

Twenty subjects with no history of peripheral vascular disease or cold-induced symptoms constituted the healthy control groups. Ten of these subjects had no complaints of cold extremities and numbness. Ten subjects reported some problem with cold extremities and numbness but did not have the classic signs of Raynaud's phenomenon.

b) Protocol.

The basic procedure followed for cold sensitivity testing was described in Section B (pp 178 to 185). The basic procedure which was followed and the modifications introduced are dealt with in Sections B and C. The strain gauge was used as a distal sensor and the cuff deflation rate was 2 mmHg/sec. A vasoconstricted vasomotor state was used. The test finger was tested at 30° and 10°C, and the control finger was exposed only to the room air. The temperature under the cuff, i.e., of the skin of the test finger was readily maintained at a given temperature and monitored by an insulated thermistor placed under the cuff in order to achieve the desired temperatures.

The effect of local and body cooling on the pressures in the test and control fingers were examined. Differences between the FSPs at local temperatures of 30°C and 10°C on a test finger were analyzed between categories of subjects using two sample t tests (p<.05).

2. Results

Differences in FSPs taken following exposure of the test finger to 30° and 10°C for the various groups of subjects studied are shown in Table 15. The results of statistical analysis are shown in Table 16. The difference in FSPs between the two local finger temperatures was significantly

TABLE 15

Descriptive Statistics on Differences in Finger Systolic Pressure When Test Finger Equilibrated to 30° and 10°C

SUBJECTS	N	MEAN AFSP (mmHg)	SEM
Non Raynaud's			
Normals	10	29.9	7.30
Cold Sensitive	10	16.1	4.49
Raynaud's			
No Obstruction			
Primary	4	55.8	19.00
Secondary	11	84.2	13.98
Uncertain Diagnosis	7	89.1	12.66
Obstruction	15	73.4	9.44

TABLE 16

Analysis (2 sample t tests) of Differences in Finger Pressures When
Test Finger Equilibrated to 30°C and 10°C

SUBJECTS	t value	df
Raynaud's:		
Obstruction vs nonobstruction	-0.58^{ns}	35
Obstruction vs non Raynaud's	5.70^{**}	40
Nonobstruction vs non Raynaud's	5.23^{**}	33
Primary vs Secondary	-1.09^{ns}	13
Raynaud's vs non Raynaud's	6.23^{**}	55
Non Raynaud's:		
Normals vs Cold sensitive	1.50^{ns}	18

** p .01

ns nonsignificant

greater in subjects with than those without Raynaud's phe-
nomenon (p<.05).

The refined cold sensitivity test also showed significant
differences in FSP between Raynaud's subjects with and with-
out obstruction and non-Raynaud's subjects (p<.01).

Two-thirds of all the subjects with Raynaud's phenomenon
and two (10%) of non-Raynaud's subjects showed vessel clo-
sure of the test finger (Table 17). In addition, one-third
of the Raynaud's subjects showed closure of the digital ar-
teries of the control finger. Closure of the digital ar-
teries on the control finger was always associated with clo-
sure of the arteries on the test finger, however the reverse
was not true.

3. Discussion

This study examined first the use of a modified cold sen-
sitivity test in subjects with and without Raynaud's phenom-
enon and second applied the test to elucidate the pathophy-
siology of the disorder and help differentiate subjects with
and without Raynaud's phenomenon. Marked differences were
observed between FSPs taken after exposure of a test finger
to 30° and 10°C when subjects with and without Raynaud's
phenomenon were compared. Pressures in both the test and
control fingers fell significantly more in patients with
than subjects without Raynaud's phenomenon. This finding
indicates that the modified version of Nielsen and Lassen's

TABLE 17

Number of Vessel Closures on Test and Control Fingers in Healthy and
Raynaud's Subjects

SUBJECTS	N	TEST FINGER CLOSURE	CONTROL FINGER CLOSURE
Non Raynaud's			
No cold sensitivity	10	2	0
Cold Sensitivity	10	0	0
Raynaud's			
Nonobstruction -			
Primary	4	1	1
Secondary	11	6	2
Uncertain Diagnosis	7	5	2
Obstruction	15	12	8

procedure may be useful in the study of Raynaud's phenomenon to distinguish these subjects and should be further explored. However, without taking closure of the control finger into account, one could mistakenly conclude that cold sensitivity was normal. Thus, interpreting differences of FSP between the control and test fingers must be done cautiously.

Closure in subjects not reporting cold sensitivity and closure of the control finger in Raynaud's subjects typically have not been reported by other investigators. We observed that our test for cold sensitivity can yield information with respect to vessel closure in the test finger and the control finger.

Regardless of whether vessel closure occurs, the degree of drop in apparent finger systolic pressure can be used to assess severity of vasospasm or to establish a potential cut-off pressure to use as an index of disease severity. We observed that closure is likely to occur in patients with connective tissue disease. It is difficult to explain why the Raynaud's patients without connective tissue disease did not reliably exhibit closure when the same severity of symptoms existed in patients with connective tissue disease whose vessels closed. This may suggest a limitation of the procedure. We observed closure in two out of 20 healthy subjects. Both of these subjects were female, in their early thirties and had systolic brachial pressures under 100

mmHg. One was on the birth control pill, which has been reported to reduce blood flow, and thus may have contributed to vessel closure (Webb et al., 1981), and the other was habitually exposed to cold environments. Thus, the closure observed in the two healthy subjects may have occurred due to a predisposition to cold sensitivity of the digits superimposed on low systolic finger pressures.

However, 13 of 37 Raynaud's subjects did not exhibit closure, and 2 normal subjects did exhibit closure; thus there may be a limit to the usefulness of this test. These observations could not be fully explained. There was no attempt to quantitate disease severity in this study. Closure observed in healthy subjects may reflect adaptations to climatic conditions and seasonal variations. One-third of patients with arterial occlusion had a diagnosis of connective tissue disease, whereas two of eleven subjects had connective tissue disease with no arterial occlusion. These two groups might be distinguished on the basis of cold sensitivity testing with larger sample sizes.

The fact that in our trial only two-thirds of Raynaud's subjects exhibited closure of the test finger suggested that a local cold challenge of 10°C may not be ideal to elicit this response clinically. We have observed that cooling the test finger below 10°C can increase the probability of closure. Future studies should be undertaken to establish whether local temperatures down to 5°C are prefer-

able. This would have certain practical advantages over testing a series of decreasing local temperatures as suggested by others (Thulesius et al. 1981). However comparison of these different approaches is needed.

Differentiation of the responses of different subgroups with Raynaud's phenomenon may become more apparent with larger sample sizes.

A new and previously unexpected finding of the study was that by using standardized vasomotor state and vasoconstriction, closure of the control finger was found to occur in patients with Raynaud's phenomenon. This finding indicates the importance of sympathetic vasoconstrictor discharge in the production of vasospastic Raynaud's phenomenon, which was appreciated by Lewis, and fits with clinical observations that patients exhibit the phenomenon when the body is chilled in daily life. Raynaud's patients are known to have lower blood flows, such that sympathetic vasoconstriction is associated with low skin temperatures and likely to promote closure of the digital arteries at low environmental temperatures.

It appears that sympathetic vasoconstriction together with reduced local temperatures (skin and arteries), usually is needed to produce vasospasm. With local temperatures of 30°C, sympathetic vasoconstriction resulted in closure in only one patient. Closure of the control finger in one-

third of patients may have diagnostic implications of clinical value. In addition, this observation raises serious questions about the use of a control finger to obtain a reference pressure. Furthermore, using formulae which incorporate the use of differences in FSPs between test and control fingers used by others may be misleading.

This study showed large differences between the responses of healthy subjects and subjects with Raynaud's phenomenon to a refined cold sensitivity test. Our findings regarding closure of the arteries of the control finger raised some interesting questions regarding its diagnostic potential, as well as limitation of the use of a control finger, in cold sensitivity studies. Further study is needed to examine these issues.

CHAPTER III

IN VITRO STUDIES

A. Introduction

The precise etiology of vasospasm observed in the finger digital arteries in patients with Raynaud's phenomenon is unknown. A series of in vitro investigations based on an appropriate in vitro model was proposed for the present studies to help elucidate the possible mechanism(s) of Raynaud's phenomenon. No in vitro model for the study of human digital arteries supplying the fingers or toes has previously been described. A few studies have been reported, however, using palmar digital arteries (Moulds et al. 1980; Rittinghausen and Moulds, 1980). The presence of NE and 5-HT receptor populations and the existence of inhibitory prejunctional receptors were demonstrated in palmar digital arteries. These studies used an in vitro model based on human palmar digital arteries that were removed from older individuals up to 72 h after death. Vascular tissue was reported to remain viable over this period of time, with little or no change in the reactivity of the vascular smooth muscle. The effect of temperature on smooth muscle reactivity was not studied in this tissue preparation.

For the present studies examining the effect of temperature on cutaneous arterial smooth muscle, we investigated directly the vessels involved in vasospasm. The inherent diversity of vascular smooth muscle - not only among species, but within the same species and even along the length of the same vessel-is well known (Fischer, 1965) and provides physiologic justification for the direct study of cold sensitivity in the digital arteries of humans.

The use of digital arteries removed from amputated hands and feet was investigated to determine the suitability of these vessels in pharmacologic experiments involving the peripheral circulation. This model could provide a method of directly studying changes in the responsiveness of digital artery smooth muscle in response to cold, and thereby aid in the formulation of hypotheses to explain the mechanism of vasospasm in Raynaud's phenomenon. Thus, studies were designed to assess smooth muscle reactivity with changes in temperature.

The most common type of surgery performed in which digital arteries can be made available for research purposes is the amputation of part of the lower extremity due to vascular insufficiency. Limbs having a history of vascular occlusive disease with consequent diminution of pulses, skin nutrition, limb temperature, segmental blood pressures along the limb as well as ischemia and frank gangrene are prime candidates for orthopedic surgical amputation. History of

vascular disease over at least a few years is common in patients for whom amputation is indicated, however, acute occlusion of the major arteries especially in the lower limb may also occur.

Although vascular disease in the lower extremity is the principal indicator for limb amputation, this is less common in the upper extremity. Amputations of the upper extremity are principally due to accidental trauma usually in a younger population of individuals. Older persons are more prone to vascular disease and thus to surgical amputation of the lower extremities. In the present studies digital arteries from limbs amputated because of neoplastic tumors without frank arterial disease served as the control. Comparison of the responsiveness and sensitivity of this material with that of tissue from limbs with vascular disease was considered imperative for several reasons:

1. Raynaud's phenomenon occurs more commonly in the fingers than in the toes.

2. Patients with Raynaud's phenomenon do not routinely have arterial occlusive disease.

3. Comparison of the classical anatomical arrangement of human toe arteries and finger arteries indicate that many similarities exist; however, whether this is true at a pharmacologic level has not been determined.

Thus, comparisons of responses were made between digital arteries from vascular diseased lower limbs, and nonvascular diseased upper and lower limbs.

Preliminary testing of the viability of this tissue in our laboratory showed that the smooth muscle within the vessels could remain viable over several hours in a muscle bath containing physiologic solution maintained at 37°C.

The primary purpose of the present studies was to identify the contribution of α and β adrenergic receptors in smooth muscle contraction of human digital arteries, and to determine the effect of temperature on adrenergic responsiveness. Hypotheses considered included one, α adrenoceptor-mediated responses are augmented with cooling, and two, if β adrenoceptors are present their responsiveness may be diminished or altered in some way such that their presumed dilating effect does not occur.

A knowledge of the effect of temperature on the reactivity of vascular smooth muscle is important for understanding the physiologic and pharmacologic responses of normal peripheral arteries. Normal responses may become exaggerated and result in the vasospastic episodes associated with Raynaud's phenomenon (Spittel, 1980; Lewis, 1929). Better understanding of the responsiveness of human digital arteries and investigation of the possible cellular mechanisms of Raynaud's phenomenon will improve medical management of the disorder.

Specifically, digital arteries removed from amputated limbs were used to examine the effect of cold on the adrenergic mechanisms of these vessels and whether abnormal cold-induced constrictor responses observed clinically could reflect a greater α adrenoceptor-mediated response, reduced or absent β receptor-mediated response or an imbalance of α and β adrenoceptor-mediated responses.

Na/K pump function and its sequelae have not been previously characterized in the smooth muscle of human digital arteries. A greater knowledge of pump activity and Na-Ca exchange mechanisms in this tissue preparation however, may provide more insight into both normal responses of peripheral blood vessels to cooling and the mechanism of digital arterial vasospasm in patients with Raynaud's phenomenon.

Another purpose of the present studies using indirect methods, was first to establish the contribution of an electrogenic function of the Na/K pump in a vascular preparation of the human digital artery. Second, it was of interest to examine the effect of cooling on pump function and its sequelae in human digital arteries.

There are a number of indirect methods that can be used to detect electrogenic pumping in a smooth muscle preparation (Hendrickx and Casteels, 1974; Taylor et al., 1969; Taylor et al., 1979). Experimentally, Na loading can be achieved by decreasing the concentration of K_0, by reducing

the temperature, and by exposing the preparation to a pump inhibitor.

Reintroduction of K to the surrounding medium results in a marked transient increase in the activity of the Na-K pump. If increased Na-K pumping in response to the reintroduction of K produces a transient hyperpolarization and tissue hyporesponsiveness, the presence of an electrogenic pump can be assumed. A reduction in temperature also produces an increase in the concentration of Na. Instantaneous warming of the preparation should similarly result in a rapid and transient stimulation of the pump and consequent hyperpolarization. Both of these methods, namely the use of K-deficient solution and reduction of the temperature, should render the preparation transiently hyporesponsive during the period of hyperpolarization following introduction of K. Thus, response of the preparation to stimulation during this period will be depressed.

The third common method used to establish the presence of an electrogenic pump is the use of a sodium pump inhibitor.

The quantification of the electrogenic component of the membrane potential with inhibiton of the Na/K pump is associated with several problems. First, E_M is progressively decreased due to loss of intracellular K, possible accumulation of K on the outside of the membrane, changes in the permeability of Na and K secondary to depolarization and the

transient repolarizing effect of chloride influx with membrane depolarization. Despite these difficulties, it is generally accepted that within a few minutes of pump inhibition, the loss of the electrogenic component will be evidenced by depolarization prior to marked changes in ion redistribution may require 15 minutes to occur. Therefore, if depolarization is observed under these conditions, it can be concluded that there is an electrogenic contribution to the resting membrane potential (Thomas, 1972).

Conceivably, decreased electrogenic pumping causes partial depolarization, increased vessel tone, and altered sensitivity to stimulating agonists. Postjunctional supersensitivity is one response that has been associated with partial depolarization. This may be an adaptive response in which excitable cells become more sensitive to agonists during chronic suppression of normal physiologic stimuli. Postjunctional sensitivity may therefore contribute to a variety of pathological problems. The phenomenon is demonstrated as a shift to the left of the dose-response curve in response to a variety of agonists usually without a change in the maximum response.

In the present studies, characterization of the Na-K pump was undertaken by the indirect approaches described. Specifically, human arterial tissue was exposed to a K-deficient solution followed by a low dose of K. Muscles were exposed to increasing doses of NE and K at 37° and 20°C.

Secondly, potassium-induced relaxation was assessed by introducing K on a stabilized submaximal NE-induced contracture following preincubation of the tissue with K-deficient solution. A rapid response to the reintroduction of low K would provide support for an electrogenic function of the pump whereas a slow response would suggest a primary role of the redistribtuion of ionic gradients. Thirdly, ouabain was introduced after muscle tissue pretreatment with phentolamine to block the neural component. Response potentiation would provide some support for the presence of an electrogenic pump.

Since Na-Ca exchange is diminished following inhibition of the Na/K pump and this could conceivably affect the results in each of the approaches described, this mechanism could provide an alternative interpretation to the results.

B. Methods

Amputated limbs provided the source of _in vitro_ material used in the study. Limbs were immediately cooled to 4°C after surgery and transported to the pathology department for macrodissection of the digital arteries.

Gross dissection of the digital arteries was begun by exposing the area to be dissected and keeping it cool with frequent dousing of pre-cooled physiologic solution. Human physiologic salt solution was prepared in advance and cooled

to 4°C.   The composition and the concentrations used are
given in Table 18. Usually one long cylindrical vessel seg-
ment could be removed from each skin flap, that is from the
soft tissue removed from one side of a digit.   Fine periar-
terial tissue was then cut away with an extra fine pair of
microsurgical scissors.   As much surrounding tissue as pos-
sible was removed from the vessel segments under a dissect-
ing microscope.

Helically-cut muscles were obtained from each vessel seg-
ment.   From initial reports of the practical advantages of
the use of helically-cut muscle strips (Speden, 1960), this
technique has since been used routinely in assessing the re-
sponsiveness of various smooth muscle preparations in vitro.
A tapered, fine bore glass tube with a polished tip was used
to insert through the vessel cylinder and anchor it during
helical cutting of the vessel. The tube was inserted at the
proximal end of the cylinder.   Based on tissue histology,
the helical strip was routinely cut at a 45° angle. Usually
one cylindrical segment from the larger digits furnished two
muscle strips, and from the smaller digits one strip.   Most
strips used were from the mid-shaft area of the digit. Mus-
cles averaged 10mm in length, 1.5 mm in width and 3 mg in
weight.

Mounting each muscle in an organ bath necessitated that
each end of the muscle could be secured to the apparatus
within the bath for the measurement of the isometric tension

TABLE 18

Composition of Physiologic Salt Solutions

CONSTITUENT	HUMAN PSS		HIGH − K (127mM)		0 − K	
	g/L	mM	g/L	mM	g/L	mM
NaCl	6.92	118.4	−	−	6.9	118.1
NaHCO$_3$	2.10	24.9	2.0	23.7	2.0	23.7
KH$_2$PO$_4$	0.150	1.1	0.16	1.2	−	−
KCl	0.246	3.3	9.5	127.4	−	−
MgSO$_4$•H$_2$O	0.108	0.9	−	−	0.288	2.4
CaCl$_2$•H$_2$O	0.278	2.5	0.3	2.7	0.278	2.5
Dextrose	2	11.1	1.0	5.6	−	−
MgCl$_2$	−	−	0.1	.5	−	−
NaHPO$_4$	−	−	−	−	0.163	1.2

development. For this purpose the lower end of the muscle was attached by a short loop of 5-0 braided surgical silk to the hook at the end of a rigidly clamped aerating tube immersed in the bath (Fig. 12). The upper end of the muscle was attached by surgical silk to a Grass FT .03 force transducer mounted on a rack and pinion. The apparatus was rigidly mounted to reduce extraneous vibrations and allowed the muscle to be stretched to and maintained at any desired length. Output from the force transducer was amplified and recorded on a 4-channel Gould Brush 2400 recorder. This arrangement for measuring isometric tension development was used for all in vitro studies.

For all studies, muscles were stretched to their estimated Lo. the length at which maximum active tension is elicited, using a preload of 0.3-0.5 g resting tension (Rp). Preliminary length tension studies in our laboratory have shown that at this Rp, the muscle in this preparation is at its optimal length (L_0).

The double-jacketed tissue baths in which the muscles were immersed contained a fixed volume (15 ml) of Kreb's physiologic solution. The latter was maintained at 37°C by an external central thermostatically-controlled heater-circulator (Neslab, TX.9). The solution was aerated with 95% O_2 - 5% CO_2 mixture. After mounting, the digital artery smooth muscles were allowed to equilibrate for 90 to 120 minutes to reestablish ionic steady state which was lost
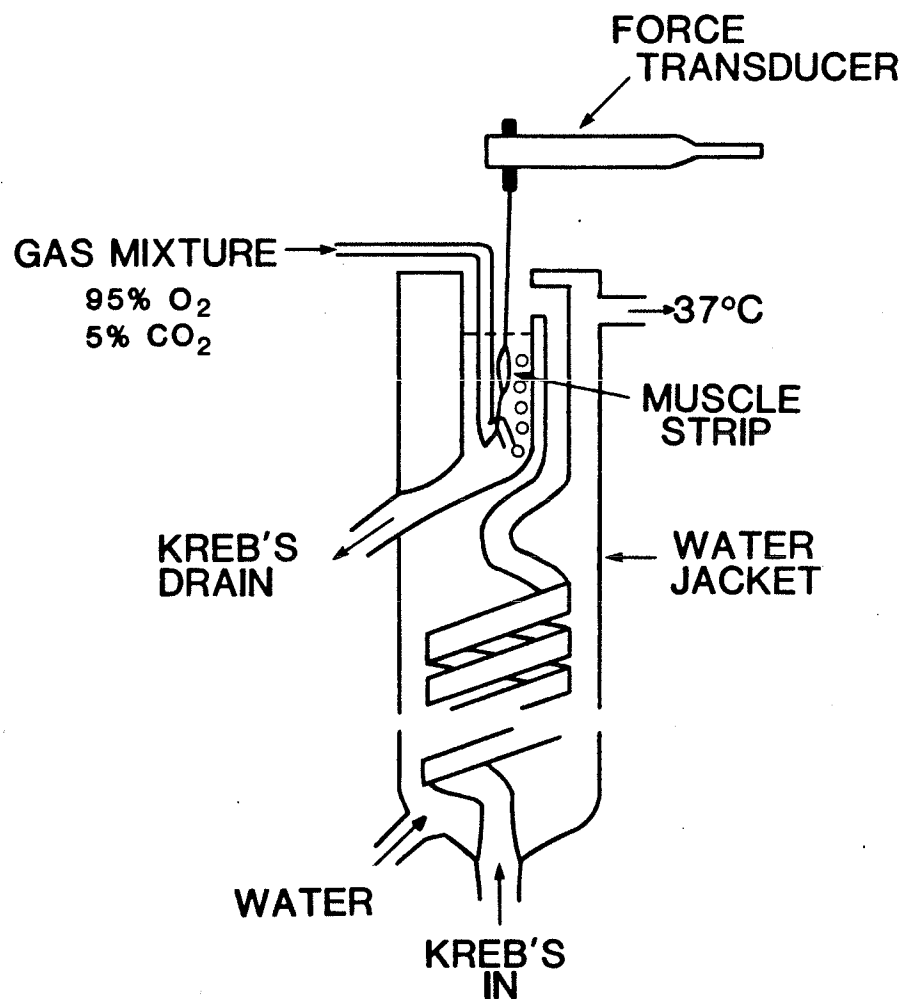
Fig.12. Schematic diagram of the apparatus used in isometric experiments, which allows for recording of muscle tension development.

across the smooth muscle cell membranes with cooling and dissection.

Contractile responses of muscles were normalized as a percentage of their maximal contraction to high-K and as force generated per unit of cross sectional area (g/cm^2). Force generated by strips in response to high-K in arterial diseased lower limbs was 345 ± 40 g/cm^2, in non-vascular diseased lower limbs 406 ± 53 g/cm^2, and in non-vascular diseased upper limbs 297 80 g/cm^2. Percentage force of the standard high K-induced contracture at $37^\circ C$ was used in the analysis. In cases where experiments were repeated on muscles from the same limb, the results were averaged.

All drugs used in the studies namely norepinephrine, serotonin, isoproterenol, phentolamine, propranolol, and ouabain were supplied by Sigma Chemical Co. (St. Louis, MO).

The NE dose response relationshps were examined with a minimum of 3 concentrations usually 10^{-8}, 10^{-7}, $10^{-5}M$ respectively so as to extend the viability of the preparation through the experimental period. NE was selected for study because it is the predominant endogenous chemical neurotransmitter regulating circulatory function and exerts its action primarily via the α adrenoceptors. Cumulative dose response studies were performed at 37°, and at stepwise temperature decrements which included 30°, 25°, 20°, 15°, 10° and rewarming to $37^\circ C$.

NE dose response studies were similarly conducted with a 15 min pretreatment of muscles with the β adrenergic antagonist propranolol (PROP, 10^{-5}M). As in the above studies, NE was selected for study because it is the predominant endogenous mediator in the circulation and exerts its action primarily via the α adrenoceptors. In addition, effects were examined directly with the β adrenoceptor agonist isoproterenol (ISO). Dose response studies were carried out at 3 concentrations of ISO (10^{-8}, 10^{-7}, and 10^{-5}M) after 15 min of muscle pretreatment with the α receptor antagonist phentolamine (PHEN, 10^{-6}M) and after stabilization of a contracture following a low dose of 5-HT (10^{-6}M).

To examine changes in NE and K threshold when the Na-K pump was inactivated, NE and K dose response experiments were conducted with and without O-K pre-incubation. The composition of the zero potassium solution appears in Table 18. Those muscles undergoing pre-incubation with O-K for 45 min were first exposed to normal K and then to increasing doses of NE and K. Similar dose response curves were repeated at 20°C. In addition, five muscles pre-incubated with O-K were exposed to a submaximal dose of NE followed by 3 mM K. Up to 8 muscles from each of 4 amputated lower limbs were studied in each experiment. All but one limb were surgically removed due to complications of arterial occlusive disease.

Lastly, the effect of ouabain (10^{-6}M) (20 min) was examined on the responsiveness of the preparation.

Because of the characteristics of the recording experiment and for purposes of presentation, the mechanograms have been retouched.

C. Results

1. Characterization of the Behavior of Human Digital Artery Smooth Muscle used in an In Vitro Model

a) Viability.

Viability of the dissected smooth muscle tissue was satisfactory for the purposes of the proposed studies. For inclusion into the proposed studies, muscles had to fulfill two criteria of viability. First, maximal tension in response to a depolarizing solution of potassium (127 mM K) was equal to or greater than 100g/cm^2. This tension was selected on the basis that of the majority of muscles tested initially (N=24), those that remained viable (N=21) over several hours of testing, exhibited pre and post test contractures greater than 100g/cm^2. Second, the response to a high-K solution at the end of any experiment was within 10% of a baseline contracture. These criteria eliminated the use of data for seven muscles in the data analysis.

b) Typical initial responses to stretch during equilibration.

During the equilibration period, the responses of muscles to pre-stretch were comparable. This was observed in muscles from all three vessel types studied, namely vessels from lower limbs with arterial disease, and vessels from upper and lower limbs without vascular disease. An example is shown in Fig. 13. Typically after the preload tension or equilibration tension was set, muscle tension continued to rise for about five to ten minutes and then slowly declined to some basal level usually within 30 minutes. Thereafter basal tension would usually remain constant over several hours if unstimulated.

c) Spontaneous phasic activity. Spontaneous phasic activity of smooth muscle has been described as a characteristic of single unit or unitary smooth muscle. Although the control of the function of digital artery smooth muscle is believed to be predominantly neurogenically-mediated, the digital artery muscles under study did exhibit spontaneous phasic activity in several conditions. Phasic activity frequently resulted from an experimentally imposed stimulus; however, a few muscles did exhibit phasic activity intermittently throughout an experiment with no exogenous stimulus. This may have resulted from the equilibration of ionic gradients and metabolism. Spontaneous phasic activity was most commonly observed during the equilibration period apparently
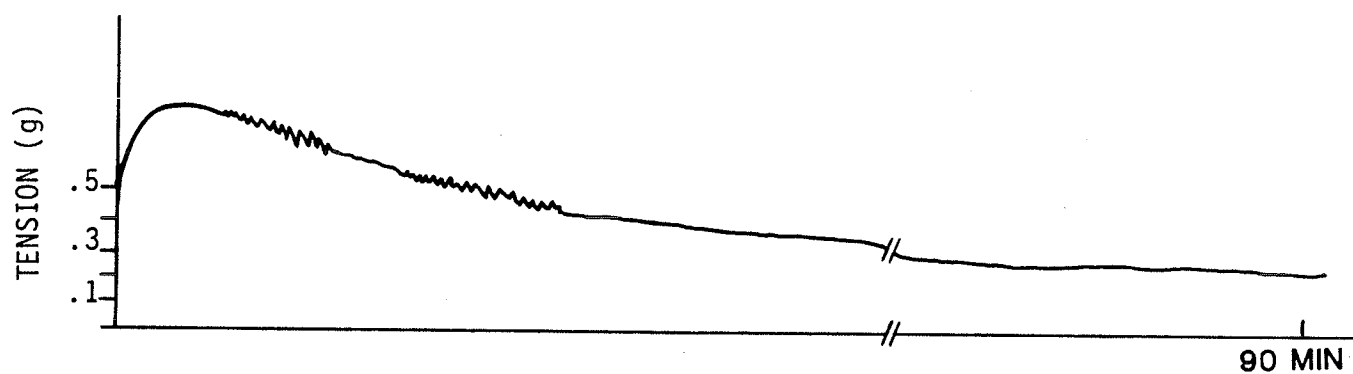
Fig. 13. Typical mechanical response upon initial equilibration
of human digital artery. Note interruption of the
record represents 30 minutes.

in response to the stretch imposed by the preload (Fig. 13).
This tended to be a transient phenomenon, and in most in-
stances was absent once a stable resting tension had been
obtained. Phasic activity was also associated with cooling
(Fig. 14) and rewarming of a muscle, and in response to the
introduction of a variety of agonists, e.g., α adrenergic
agonists and potassium chloride solution. In response to
cooling, amplitude of the phasic activity appeared little
affected whereas frequency was substantially reduced.

d) Basal tension.

Basal tension of muscles was observed to change with
cooling and warming of the physiologic solution in the mus-
cle bath. The effect of temperature on basal tension of the
arterial muscles is summarized in Table 19. In addition,
when considering limbs rather than strips of muscles, in 22
vascular diseased limbs, in which at least one muscle was
tested and exposed to temperatures down to 10°C, muscles
from 10 limbs (45%) showed increased tension in response to
cooling alone. In terms of the number of strips cut from
the digital arteries of the 10 limbs, 27 out of 100 showed
this response. Of those remaining 66 showed a reduction in
basal tension and 7 showed no change. A similar proportion
of muscles from nonvascular diseased limbs showed augmented
responsiveness with cooling. Any contribution of altered
vessel geometry, hence biophysical properties, with in-
creased smooth muscle reactivity with cooling and changes
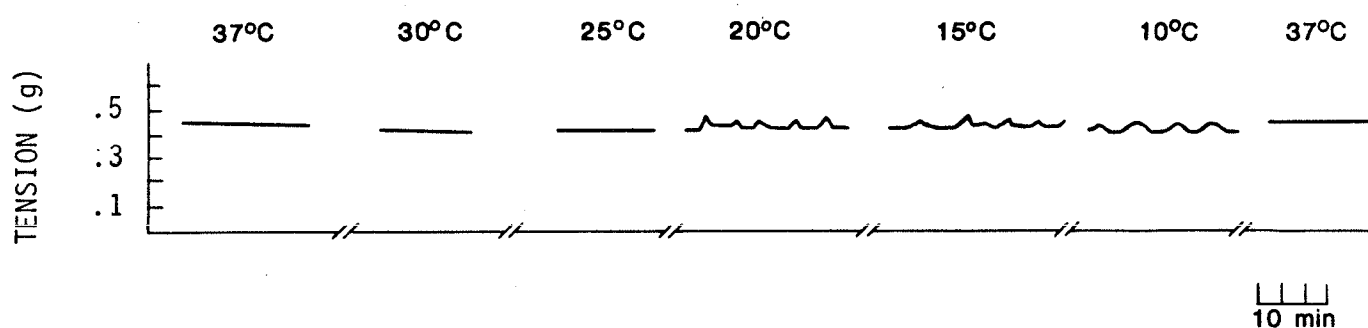
Fig. 14. Effect of cooling on basal, unstimulated tone. Note
that the preparation exhibited phasic activity
beginning at 20°C down to 10°C and rewarming at 37°C.
Breaks in record represent 50 minute periods. Typical
of 3 muscles tested.

TABLE 19

Effect of Temperature on Basal Tension

LIMBS	NO.	NO. OF MUSCLES	DIRECTIONAL CHANGES IN BASAL TENSION NUMBER OF MUSCLES(%)		
			Increase	None	Decrea
With Vascular Disease	22	100	27(27)	7(7)	66(66
Absence of Vascular Disease	8	51	16(31)	6(12)	29(57

induced by altered blood viscosity were ruled out by using the helically cut preparation. Therefore reduced passive wall tension with cooling and increased wall tension with rewarming reflected effects of temperature change on the intrinsic properties of the smooth muscle itself.

Among muscles in seven upper or lower limbs without vascular disease, at least one muscle tested for each limb showed increased basal tension with cooling down to 10°C. Five muscles of 28 (18%) removed from upper limbs and 11 muscles removed from 24 (46%) lower limbs showed increased basal tension. Among the remaining muscles five showed no change in basal tension and the other 31 showed cold-induced depression of the tension. Different responses of muscles from the same limbs could be due to differences in severity of obstruction upstream in the arterial supply of individual digits or different effects of the preparation techniques in individual muscles which were not clearly apparent.

Fig. 15 shows the typical depressed response with cooling with and without exposure to NE. Fig. 16 shows increased basal tension with cooling and represents the maximal increase observed in any instance with cooling. This example was observed to have phasic activity superimposed during cooling. Cold-induced phasic activity was only observed occasionally in most of the other muscles studied. This contracture was insensitive to phentolamine and propranolol at 20°C.
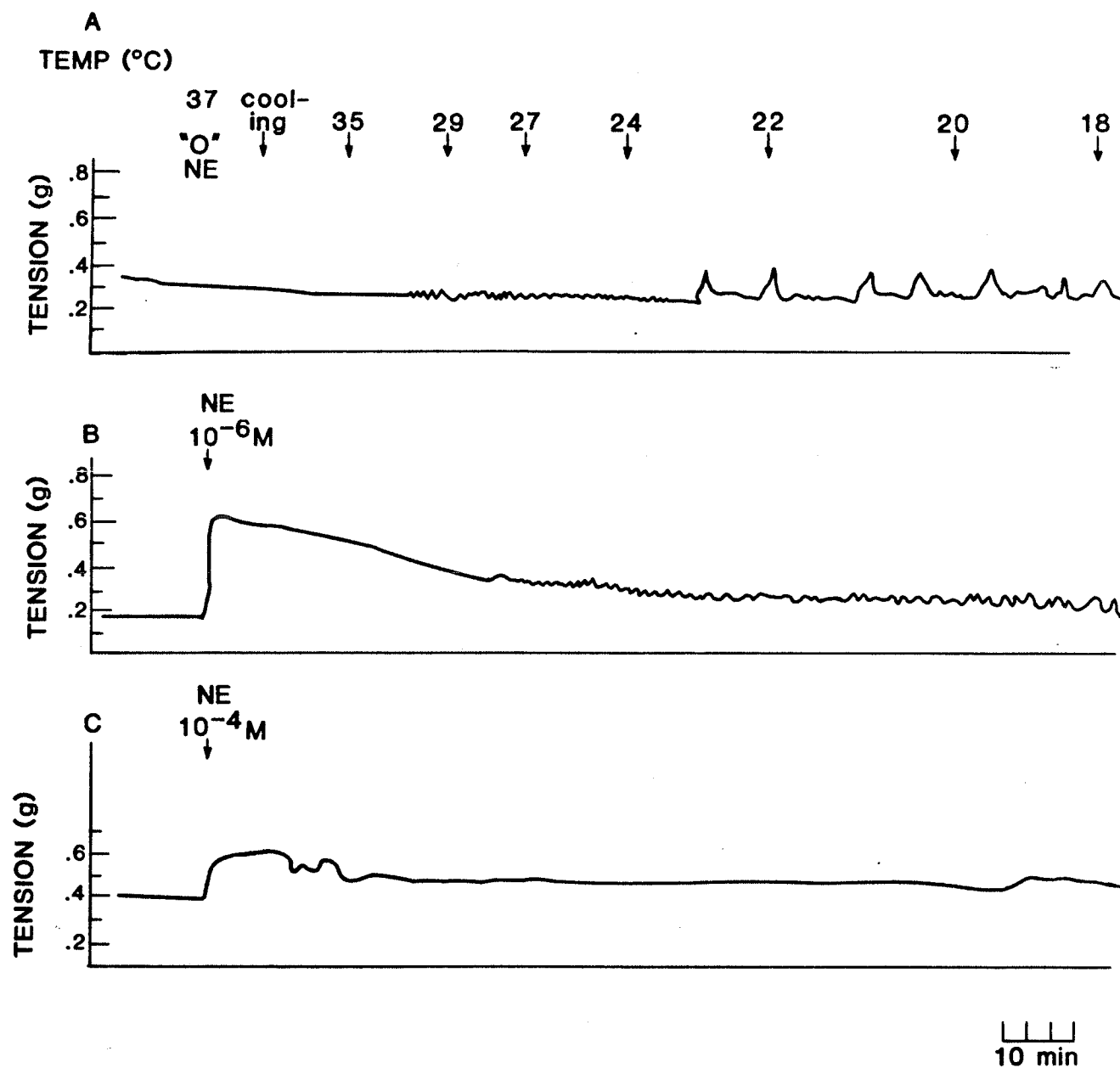
Fig. 15.   A.   Effect of cooling on basal tone in absence of NE.
           B.   Effect of cooling on basal tone and NE-induced
                contracture with a submaximal dose of NE (10^{-6}M) and
           C.   A maximal dose of NE (10^{-4}M)

           Responses are typical of 3 muscles tested.

Fig. 16. Effect of adrenergic antagonists on the cold-induced increase in basal tension and phasic activity. No changes were noted following phentolamine (PHEN 10^{-5} M) and propranolol (PROP 10^{-6} M).

2. Alpha and Beta adrenoceptor Mediated Responses in Human Digital Arteries with Cooling

Cooling of digital arteries removed from lower limbs with vascular disease, and upper and lower limbs without vascular disease depres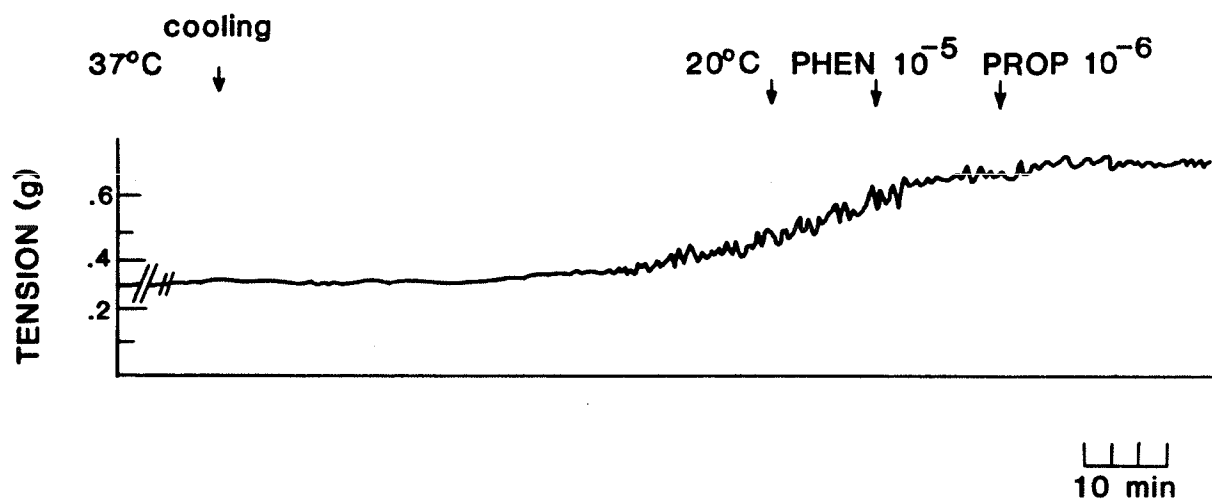sed isometric tension development in response to increasing concentrations of NE (Figs. 17, 18 and 19). This was observed in all cases by a shift to the right and downward of the NE dose response curve when the temperature was reduced from 37° to 10°C. Incomplete relaxation was observed in the cooling condition after wash-out compared with physiologic temperature. One explanation is that Ca efflux was inhibited. This could implicate the effect of cooling on the Na/K pump, and, thus E_M, the Na-Ca exchange mechanism or both.

Paired data for muscles tested at 37° and 20°C are shown in Fig. 20. Cooling reduced the sensitivity of the preparation by increasing NE threshold one log dose, in addition to reducing the maximal reactivity of the muscles by one-half.

At 37°C, resting tension averaged about 40% of a high-K contracture for the three vessel types studied. Response threshold was consistently between 10^{-9} and 10^{-8}M NE. Maximal responses to NE (10^{-5}M) were not statistically significantly different from 100% of the reference contracture for muscles from upper limbs without and lower limbs with vascular disease. Muscles from lower limbs without vascular disease generated 80 percent the tension of the reference

Fig. 17. Effect of temperature on the responses of digital arteries from lower limbs with arterial occlusive disease (means of 4-17 experiments are shown; vertical lines represent SEM). R_{p1} and R_{p2} represent resting tension before and after NE dose response experiments respectively. Response (%) is the response expressed as percent of maximal response to high K (127 mM).
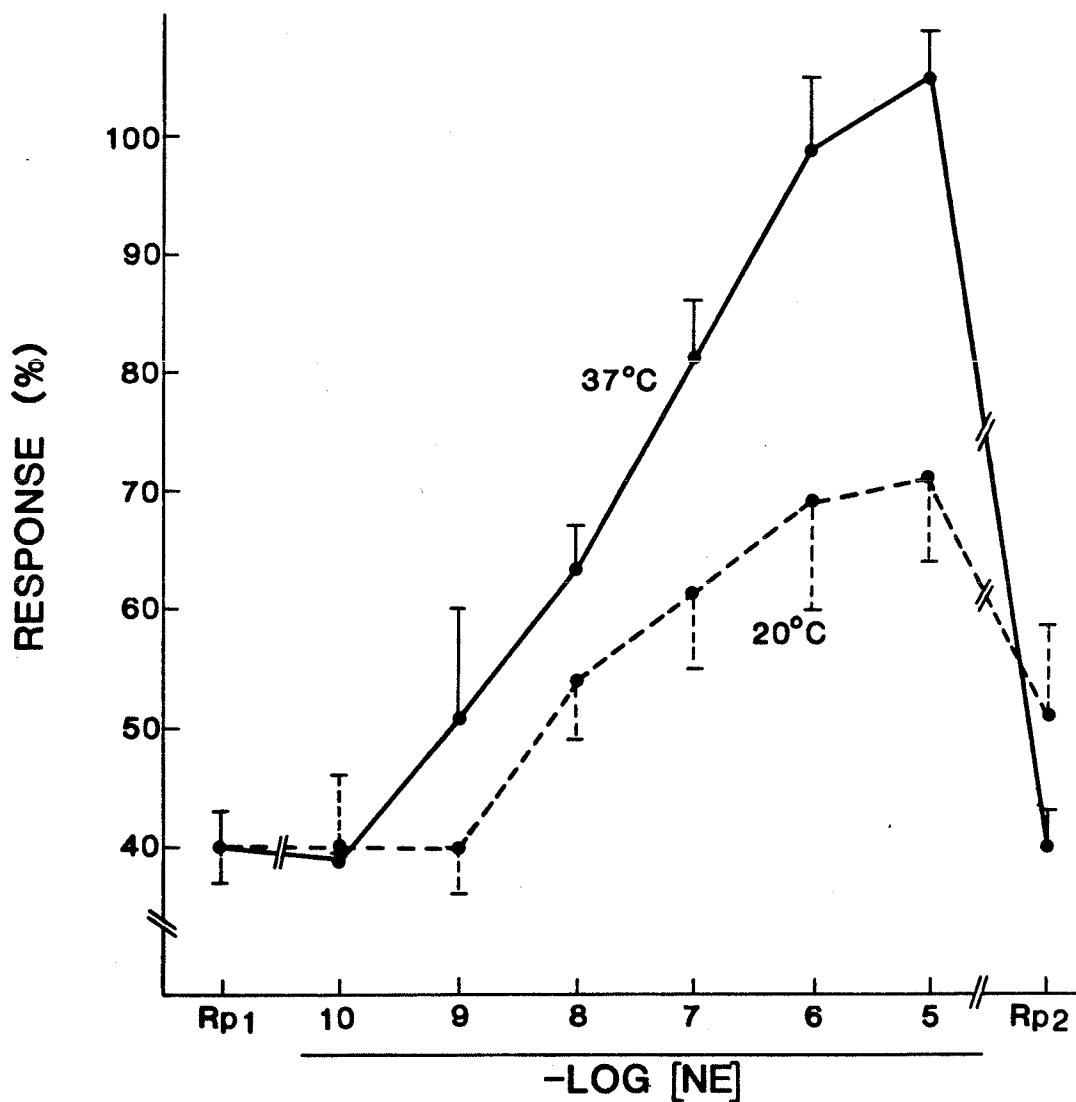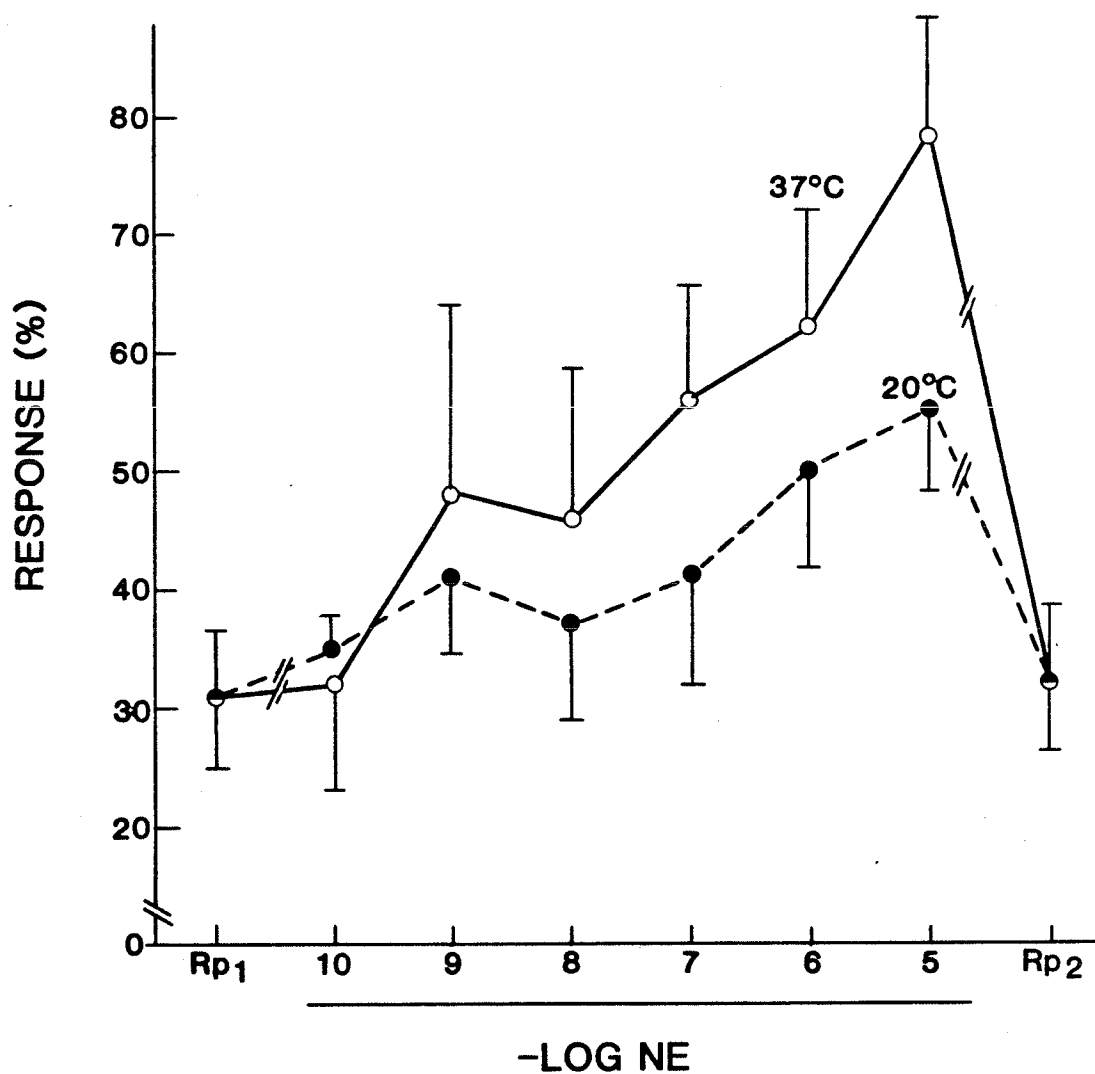
Fig. 18. Effect of temperature on responses of digital arteries from lower limbs without arterial occlusive disease (means of 2-3 experiments are shown). R_{p1} and R_{p2} represent resting tension before and after NE dose response experiments respectively. Response (%) is the response expressed as percent of maximal response to high K (127 mM).
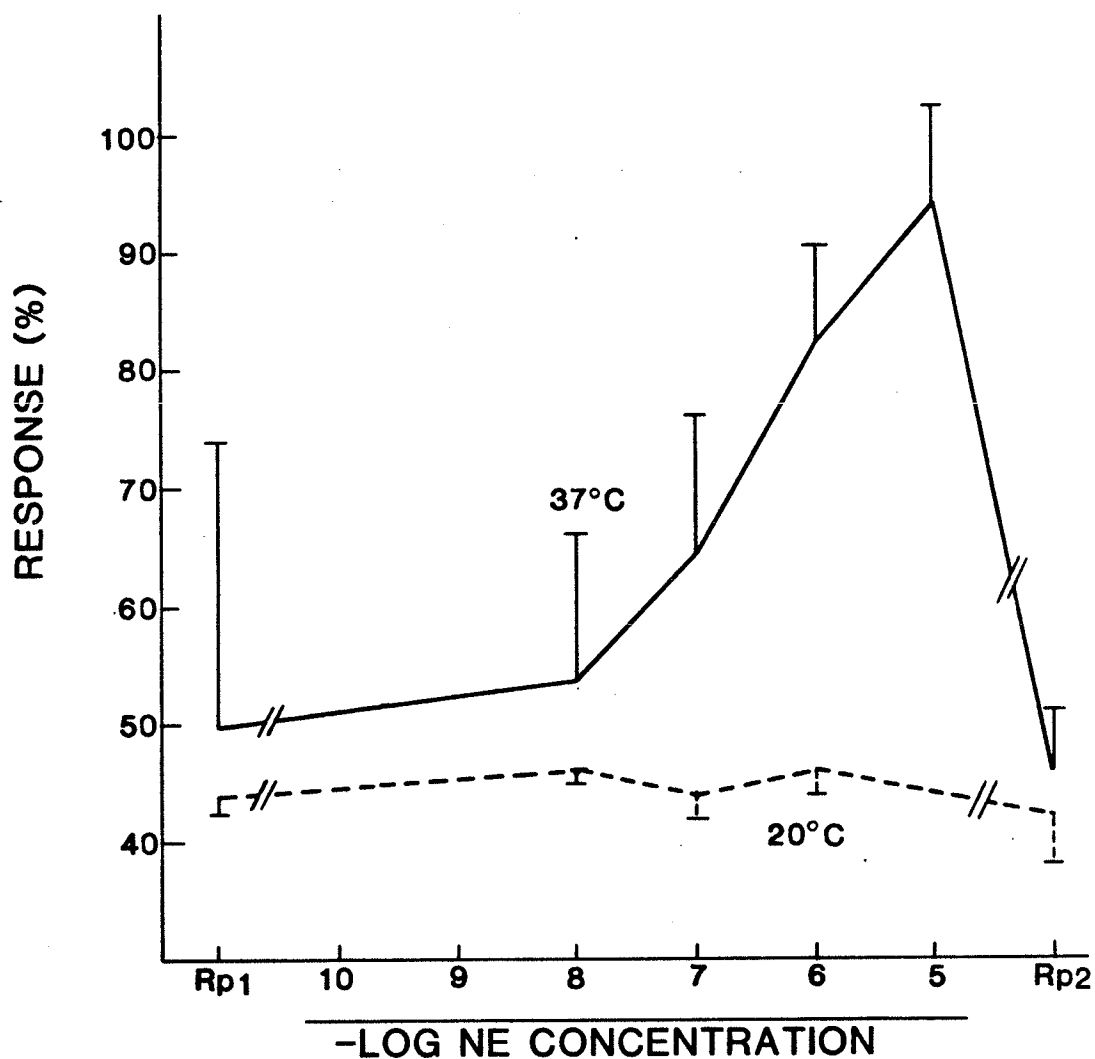
Fig. 19. Effect of temperature on responses of digital arteries from upper limbs without vascular disease (means of 2-3 experiments are shown; vertical lines represent SEM). R_{p1} and R_{p2} represent resting tension before and after NE dose response experiments respectively. Response (%) is the response expressed as percent of maximal response to high K (127 mM).
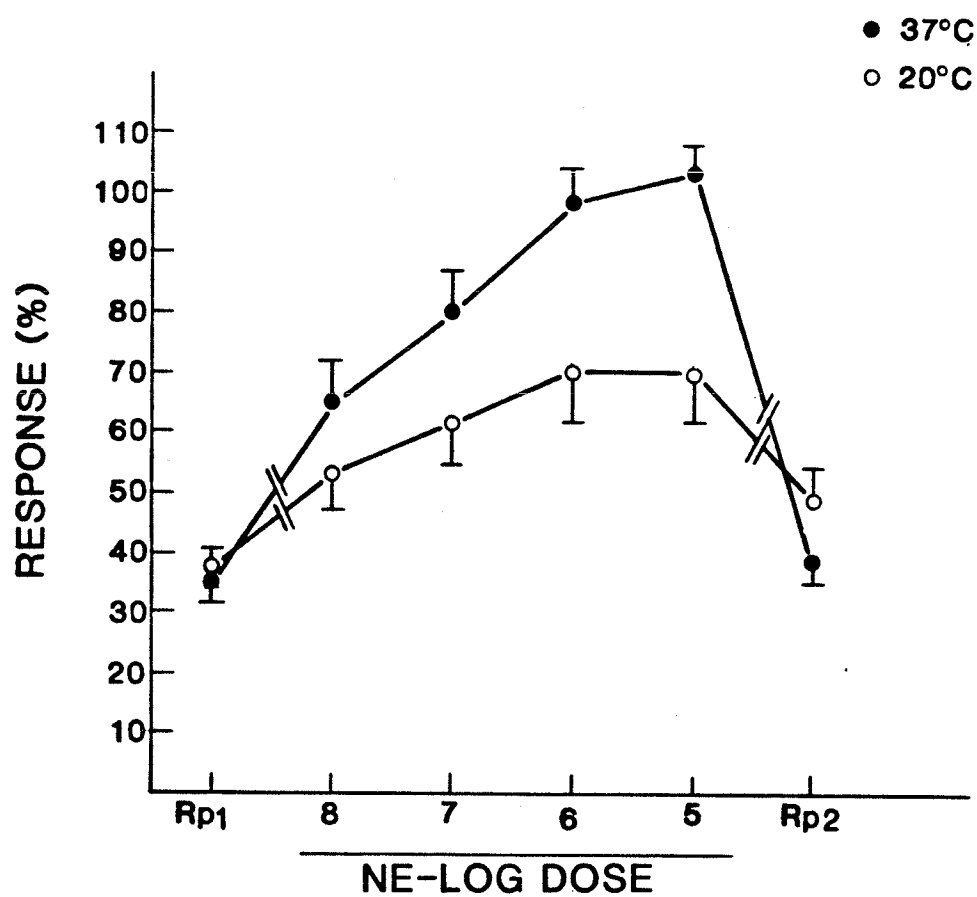
Fig. 20. Effect of temperature on the responses of muscles from
digital arteries taken from limbs with vascular disease
(means of 7 paired experiments on the same vessels are
shown; vertical lines represent SEM). R_{p1} and R_{p2} represent
resting tension before and after NE dose response experiments
respectively. Response (%) is the response expressed as
percent of maximal response to high K (127 mM).

contracture when NE 10^{-5}M was introduced. At 20°C, resting tension was reduced in all vessel types. A few muscles from upper limbs showed complete loss of responsiveness. Progressive inhibition of responsiveness generally for all muscles was consistently observed at intermediate temperatures of 30°, 25°, 15° and 10°C for the three vessel types. Responses of four limbs for all temperatures tested appear in Figure 21. Mean Rp substantially increased with cooling, e.g., increase of 10 percent of reference tension when temperature decreased to 10°C from 30°C. At a NE concentration of 10^{-5}M the response at 30°C was greater for all four muscles than at 37°C. At lower NE concentrations however, all temperatures below 37°C produced progressively greater depression of responsiveness.

Response to NE was also observed to be augmented with rewarming. This occurred with introduction of a submaximal dose (10^{-6}M) of NE before cooling to 15°C (Fig. 22), and at 15°C (Fig. 23). In both figures, tension peaked between 27° and 33°C during rewarming and proceeded to decrease until physiologic temperature was reached. This fade in response probably reflects the oxidation of NE at higher temperatures. Augmentation of the response to K was also observed with rewarming (Fig. 24).

The similarities in the responses of muscles from upper limbs without and lower limbs with vascular disease observed
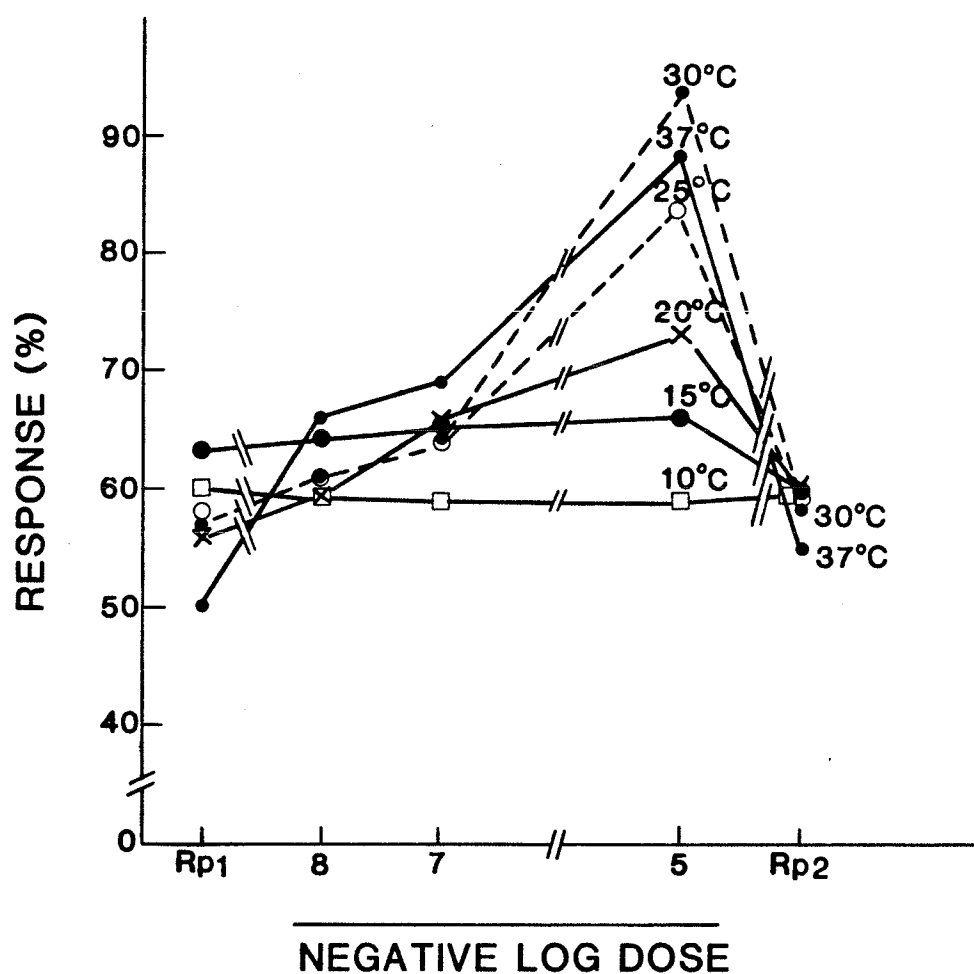
Fig. 21. Effect of temperature on response to NE of 4 arterial muscles from limbs with peripheral vascular disease. Means are shown. R_{p1} and R_{p2} represent resting tension before and after NE dose response experiments respectively. Response (%) is the response expressed as percent of maximal response to high K (127 mM).
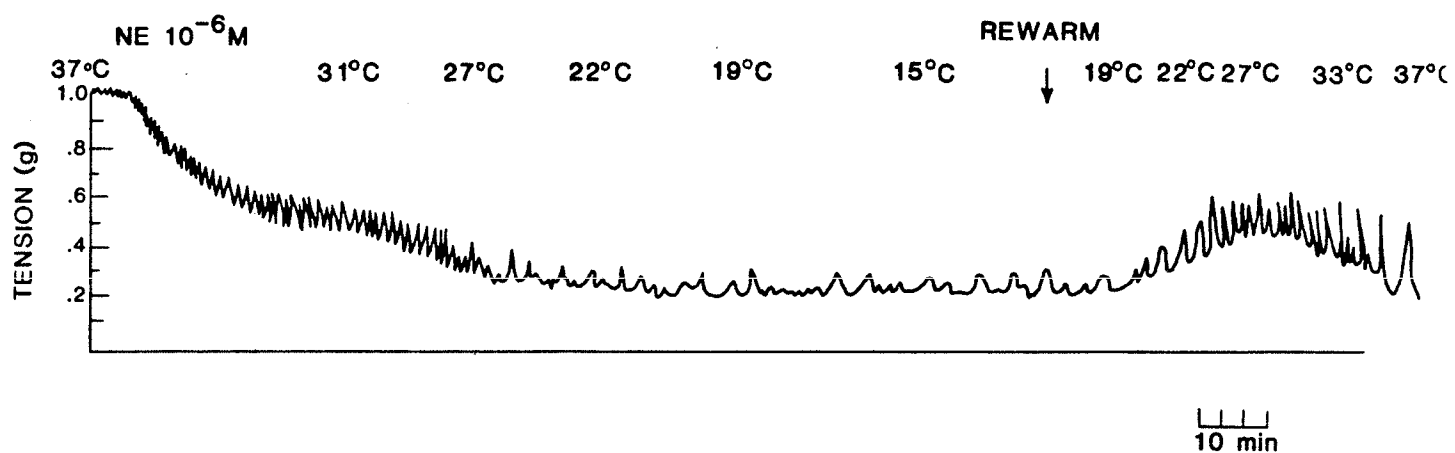
Fig. 22. Effect of cooling and rewarming on response of a muscle to NE (10^{-6}M). Response is typical of 3 muscles tested.
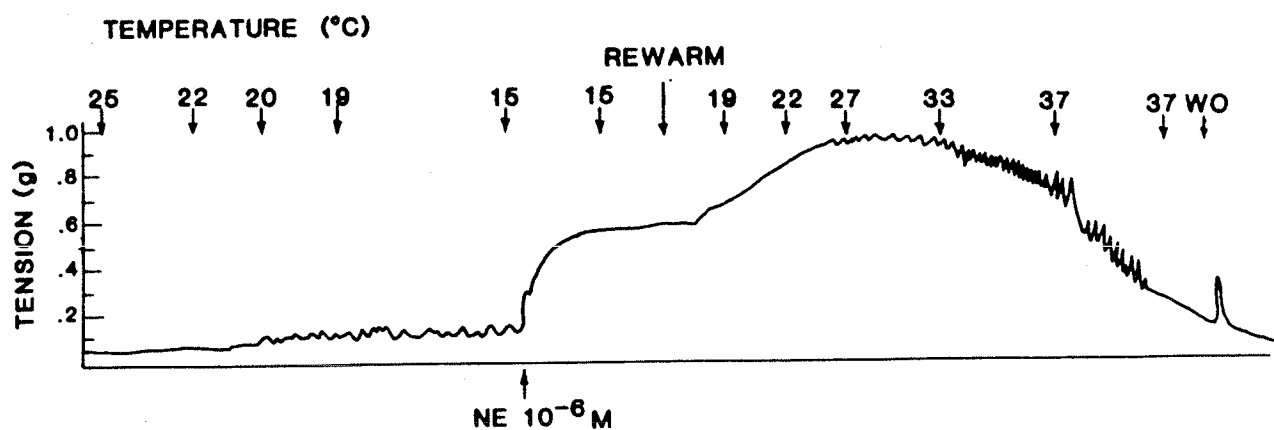
Fig. 23. Effect of NE (10⁻⁶M) introduced during cooling and
subsequent rewarming of the muscle. Upward arrow
indicates introduction of NE (10⁻⁶M). Typical of
3 muscles tested. WO - washout.
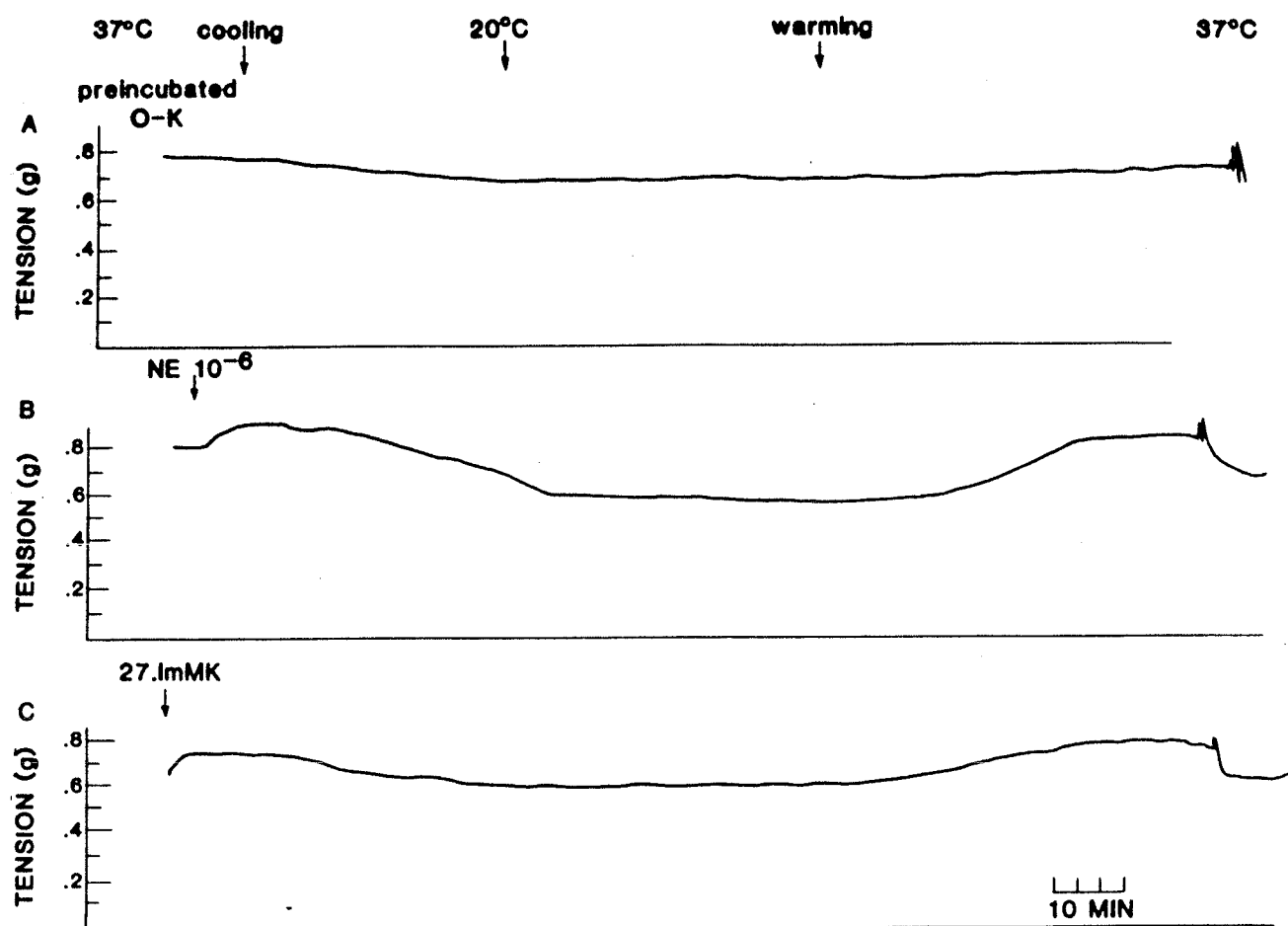
Fig. 24. Myograms of 3 muscles cooled to 20°C and rewarmed.

    A. In a solution of "0" K
    B. Exposed to NE $(10^{-6}M)$
    C. Exposed to K (27.1 mM)

at 37°C and at lower temperatures, suggested that the use of digital arteries removed from limbs with arterial disease could provide a suitable experimental model for the study of the local effect of temperature on the responsiveness of peripheral arterial smooth muscle in humans. Although the number of non-vascular diseased limbs was comparatively smaller, there were persisting similarities in response between these upper and lower limbs with cooling. The muscles from upper limbs tended to show marked response depression at 20°C for all NE concentrations. Since none of the limbs studied was to our knowledge afflicted with vasospastic disease, extrapolation to disease states must be done cautiously.

Response depression with cooling was apparent not only for NE-induced responses, but also to a lesser extent in muscles stimulated by 5-HT and K (Table 20). At 20°C, NE responses were reduced by 54% as compared with 37°C whereas 5-HT and K-induced responses were reduced by only 18 and 15% respectively. This suggested a definite adrenoceptor-specific effect of cooling in these vessels. The effects of cooling and rewarming were compared on muscles pretreated with submaximal doses of NE and K and on a muscle pretreated with a potassium-deficient solution to examine any electrical membrane depolarizing effects under these conditions (Fig. 24). Responses of all three muscles were progressively depressed with cooling, and augmented with rewarming.

TABLE 20

Comparison of Responses to Various Agonists at Two Temperatures. Responses
Expressed as Percent of High-K Contracture at 37°C. ($\bar{X}$ ± SEM)

AGONIST	37°C	20°C	N	%DECREASE
↑K (127 mM)	100	85 ± 1.53	4	(15)
NE (10^{-5} M)	105.3 ± 4.45	51.7 ± 5.12	17	(51)
5-HT (10^{-6}M)	84.9 ± 12.8	69.8 ± 11.8	5	(18)

By blocking possible ß adrenoceptor-mediated effects in this muscle preparation with PROP, and then exposing the muscles to NE, the presence of a ß adrenoceptor-mediated mechanism should be manifested by a potentiated response to NE. Such a potentiation of the vascular response was not observed at 37°, 20°C (Fig. 25) nor at four other intermediate temperatures to 10°C. On comparing Fig. 25 two groups of muscle with and without PROP pre-treatment (Figs. 25 and 17 respectively) there was no suggestion of potentiation with PROP at either 37° or 20°C. Most limbs we studied were amputated due to vascular disease. Many patients undergoing surgery were on medications and it is possible these may have included ß blockers. Although medications are usually discontinued some hours prior to surgery it is possible some effect persisted.

Certain inherent problems with PROP, such as its local anesthetic and membrane stabilizing effects, suggested another series of studies using the agonist isoproterenol (ISO) to investigate ß adrenoceptor function directly in these vessels. ISO was therefore applied in a dose-dependent manner to muscles pretreated with PHEN and submaximally contracted with 5-HT (10^{-6}M).

Typically at 37°C, 5-HT initiated an immediate contraction followed by a gradual diminution of tension (Fig. 26). Consistent with a lack of apparent potentiation of an α adrenoceptor-mediated contraction with PROP blockade, little or no direct ISO-dependent relaxation was observed at 37°
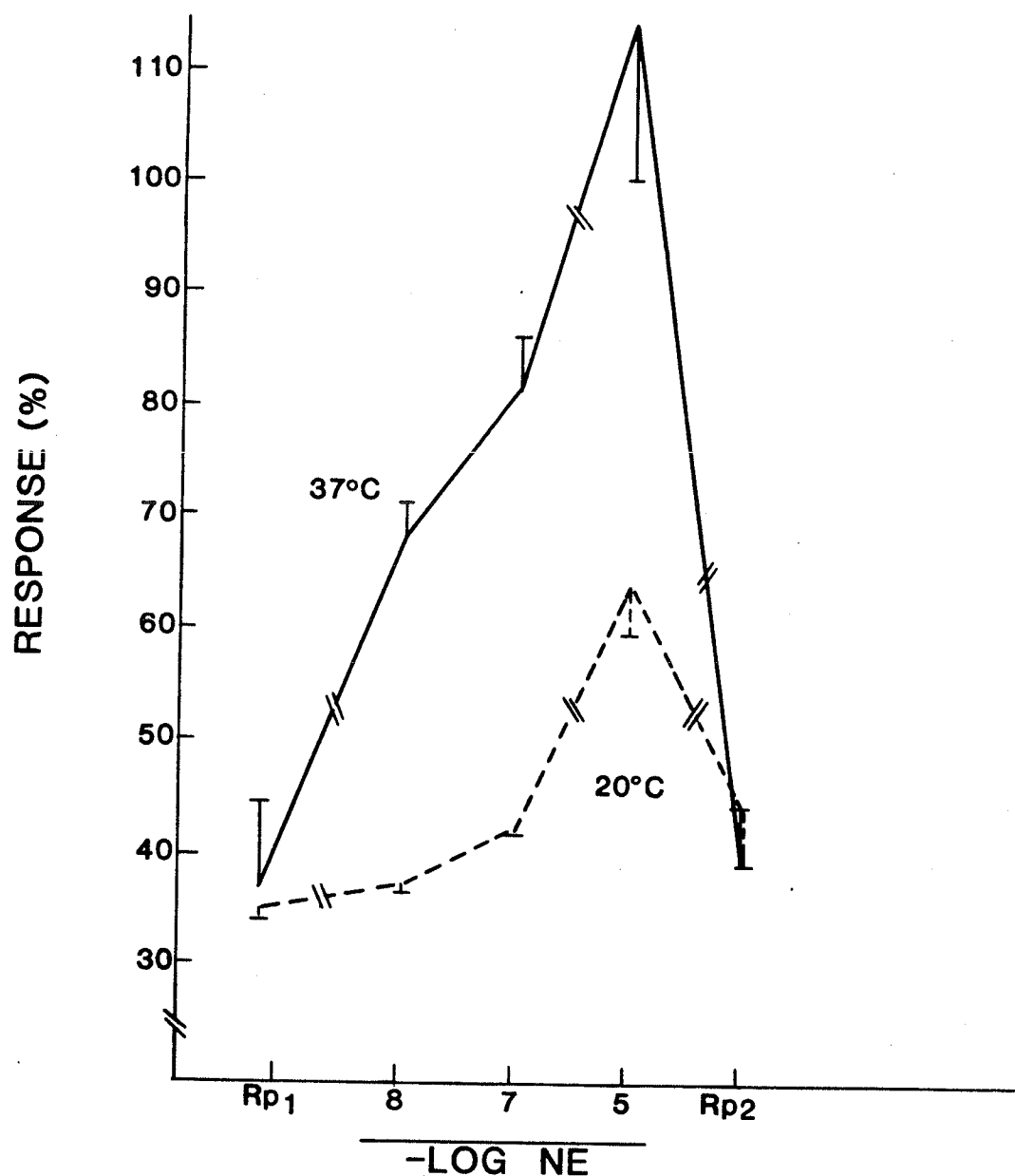
Fig. 25. Effect of temperature on NE-induced responses of PROP-pretreated
digital artery muscles from limbs with arterial disease
(means of 4 experiments are shown, vertical lines represent
SEM). R_{p1} and R_{p2} represent resting tension before and after
NE dose response experiments respectively. Response (%) is
the response expressed as percent of maximal response to
high K (127 mM).
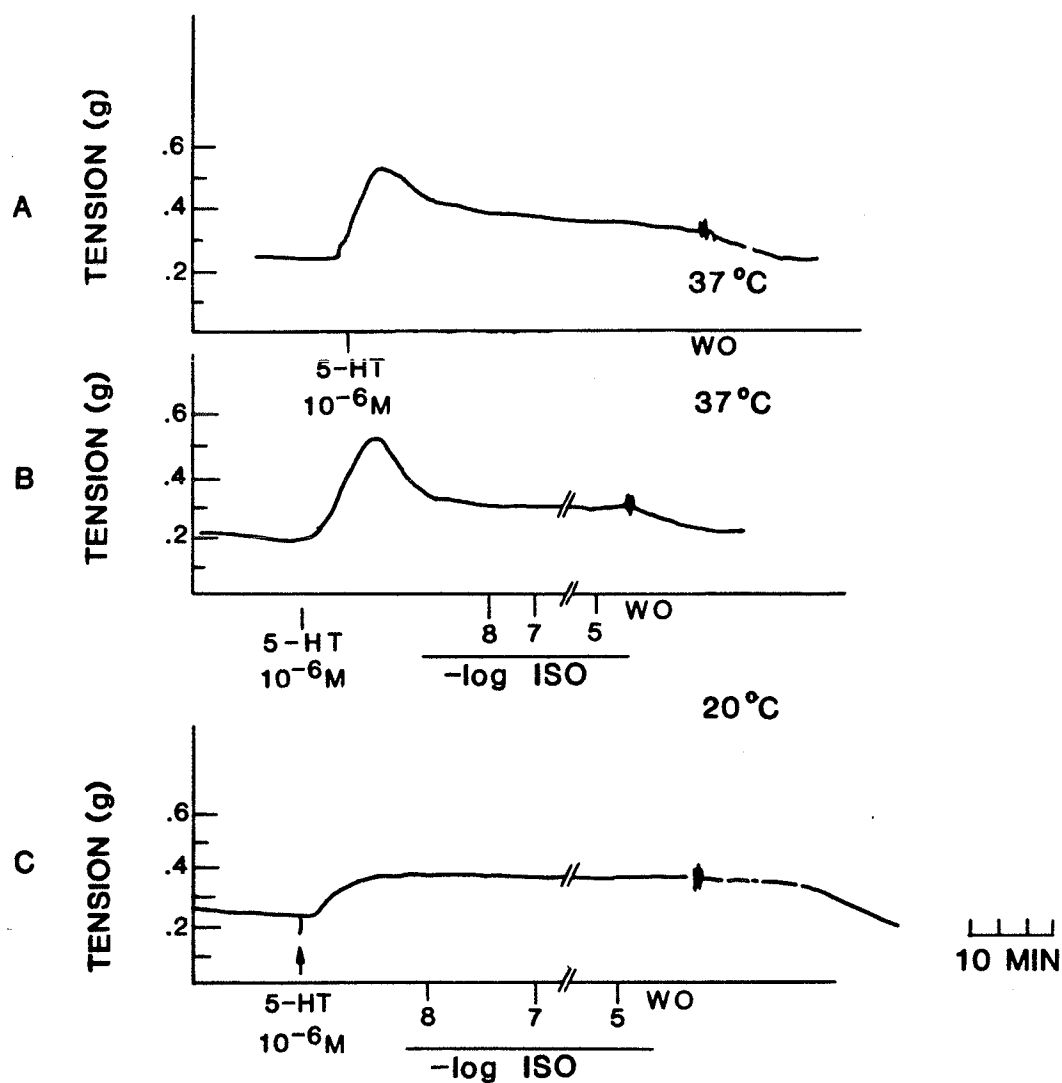
Fig. 26. A. Effect of time (10 min) on 5-HT (10^{-7}M) induced contracture.

B. Effect of ISO on muscle pretreated with PHEN and after exposure to a submaximal dose of 5-HT (10^{-6}M) at 37°C.

C. Effect of ISO on muscle pretreated with PHEN and after exposure to a submaximal dose of 5-HT (10^{-6}M) at 20°C.

WO - washout

and 20° (Fig. 27), nor at four other intermediate temperatures down to 10°C (Fig. 28).

ß adrenoceptor-mediated relaxation was not observed in the majority of muscles studied. At 37°C ISO-induced relaxation had apparently occurred (Fig. 27). At this temperature, however, we found that the response to 5-HT exhibited a tachyphylaxis-like relaxation. Although ISO was not introduced into the organ bath until stabilization of the contracture had occurred, the preparation continued to relax slightly with time. This trend may be reflected in Fig. 27 since the introduction of ISO and the gradual time-dependent decrease in tension were indistinguishable. The only exceptions to this trend were observed in 2 of 9 muscles in which ISO induced an immediate, discernible 16% decrease in tension. A myogram is shown in Fig. 29. Interestingly, the tachyphylaxis-like relaxation seen with 5-HT (Fig. 27) was not seen in cooled muscles suggesting a possible inhibition of Ca extrusion.

Response to electrical field stimulation was also depressed with cooling down to 17°C (Fig. 30). Time-to-peak tension and relaxation times were increased and peak tension decreased at 17°C compared with 37°C. This probably reflects cold-induced depression of the nerve terminal in addition to depressed α adrenoceptor sensitivity.
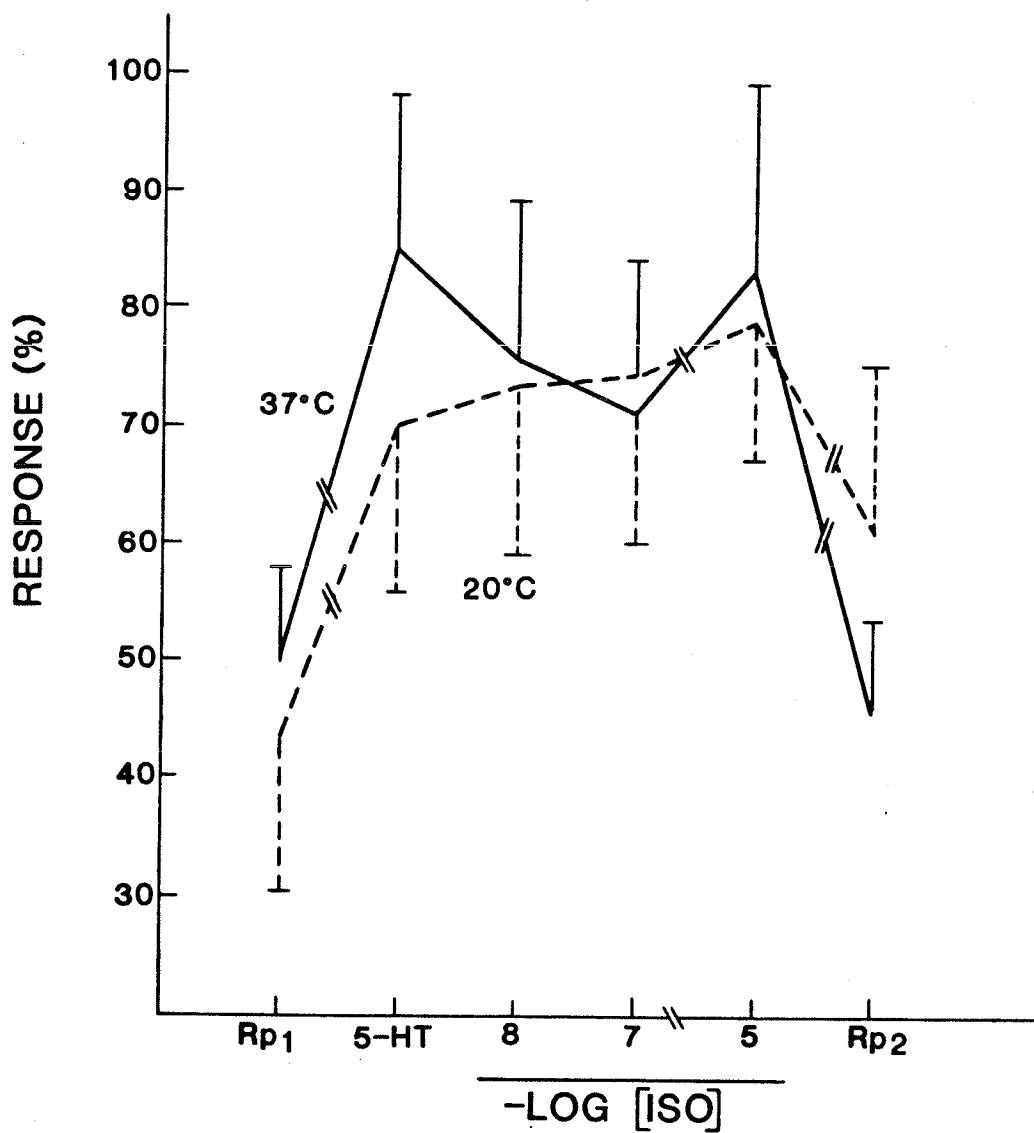
Fig. 27. Effect of temperature on ISO-induced responses of PHEN-
pretreated digital artery muscles precontracted submaximally
with 5-HT (10^{-6}M) (means of 6 experiments are shown, vertical
lines represent SEM). R_{p1} and R_{p2} represent resting tension
before and after NE does response experiments respectively.
Response (%) is the response expressed as percent of maximal
response to high K (127 mM).
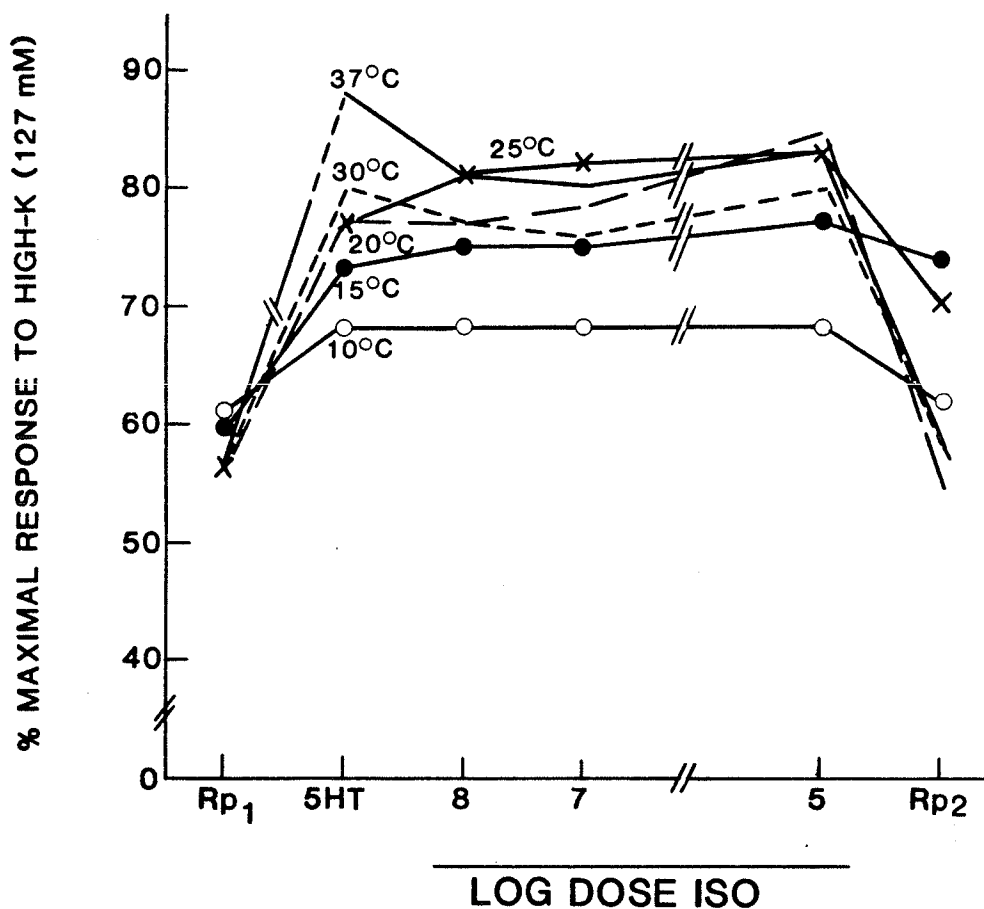
Fig. 28. Effect of temperature on ISO-induced responses of PHEN-pretreated digital artery muscles, submaximally precontracted with 5-HT $(10^{-6}M)$ (means of 3 experiments). R_{p1} and R_{p2} represent resting tension before exposure to 5-HT and WO (washout) respectively.

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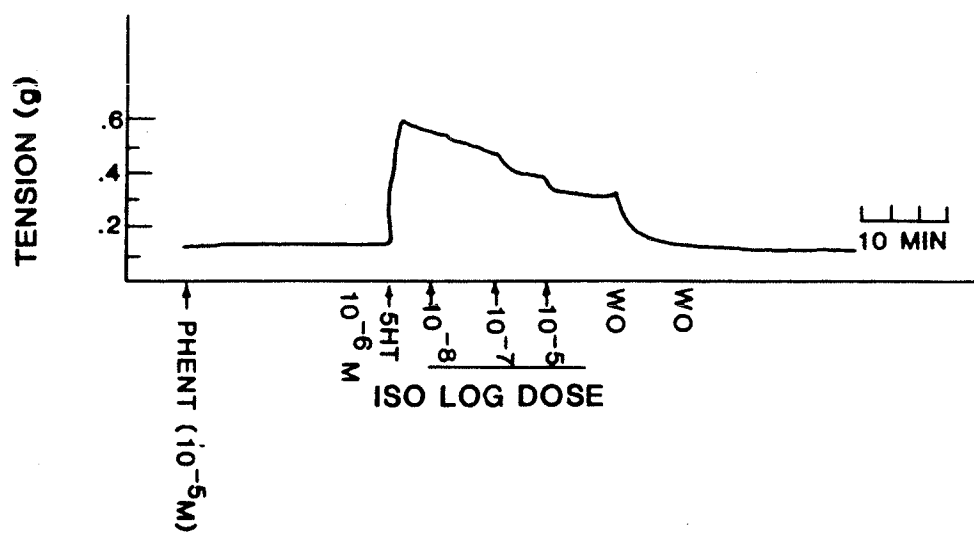
Fig. 29. Effect of ISO on 5-HT induced contracture
of muscle pretreated with PHEN.
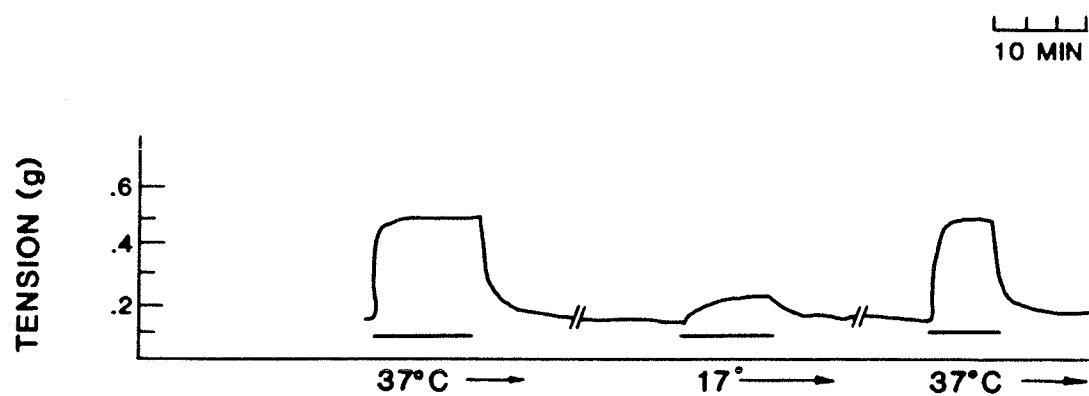
Fig. 30. Effect of cooling on response of a digital artery
muscle to electrical field stimulation (60 Hz) at
·37°C, 17°C and on rewarming to 37°C. Lines beneath
contractures represent duration of electrical
stimulation. Responses are typical of 3 muscles
tested.

3. Physiologic Consequences of Na/K Pump Function in Human Digital Arteries

The results of NE dose response studies performed before and after O-K solution incubation and reintroduction of normal physiologic solution appear in Table 21. Apparent K-induced transient relaxation of the preparation following incubation with O-K solution was manifested by an increased response threshold in two of 5 muscles. When the procedure was repeated at 20°C the response threshold was increased hence we did not observe cold-induced potentiation. At 37°C, ouabain exposure in three muscles failed to elicit contraction, but it did increase the sensitivity of one muscle to NE.

The results of K dose-response studies performed before and after O-K incubation (for 45 min), and repeated at different temperatures appear in Table 22. All nine muscles showed reduced responsiveness after exposure to O-K solution. An example at 37° and 20°C is shown in Fig. 31. Dose response curves are shown with and without pretreatment with O-K solution. The rapidity of the response to 3mM K of muscles pretreated with O-K (Fig. 31) and of the response to ouabain in muscles without O-K pretreatment (Fig. 32) support an electrogenic function of the pump in addition to its ion gradient-related effects. It is expected that the electrogenic pump activity is instantaneous whereas the dissipation/regeneration of ionic gradients takes time.

TABLE 21

Threshold Doses of NE (M) Under Different Experimental Conditions*

MUSCLE	37°C	20°C	After O-K		OUABAIN
			37°C	20°C	
1	10^{-7}		10^{-7}		
2	10^{-9}	10^{-7}	10^{-9}	10^{-8}	
3	10^{-9}		10^{-7}	10^{-6}	10^{-9}
4	10^{-9}	10^{-8}			
5	10^{-9}				10^{-10}
6	10^{-10}				10^{-10}
7	10^{-9}	10^{-5}			
8	10^{-8}		10^{-7}		
9	10^{-9}		10^{-9}		
10	10^{-9}				

* Experiments were carried out over a 6 hr period including an equilibration period of at least 45 min and sufficient time between experimental interventions for wash-out and return of muscle to resting tension for at least a 20 min period. Muscles were exposed to ouabain for a 20 minute period.

TABLE 22

Threshold Doses of K (mM) Under Different Experimental Conditions*

JSCLE	37°C	20°C	After O-K Pre-Incubation	
			37°C	20°C
1	27	27	35	
2	27	27		35
3	27			
4	17	17		
5	17	17		
6	12	27	17	
7	17	27	27	17
8	17			
9	17		27	
.0	27	27		
.1	27	35	35	35
.2	17	17	27	27
.3	27			
.4	27			
.5	27	35	42	35
.6	17		35	27
.7	17	17		
.8	27	27		
.9	27	27	35	35

* Experiments were carried out over a 6 hr period including an equilibration
period of at least 45 min and sufficient time between experimental interventions
for wash-out and return of muscle to resting tension for at least a 20 min period.

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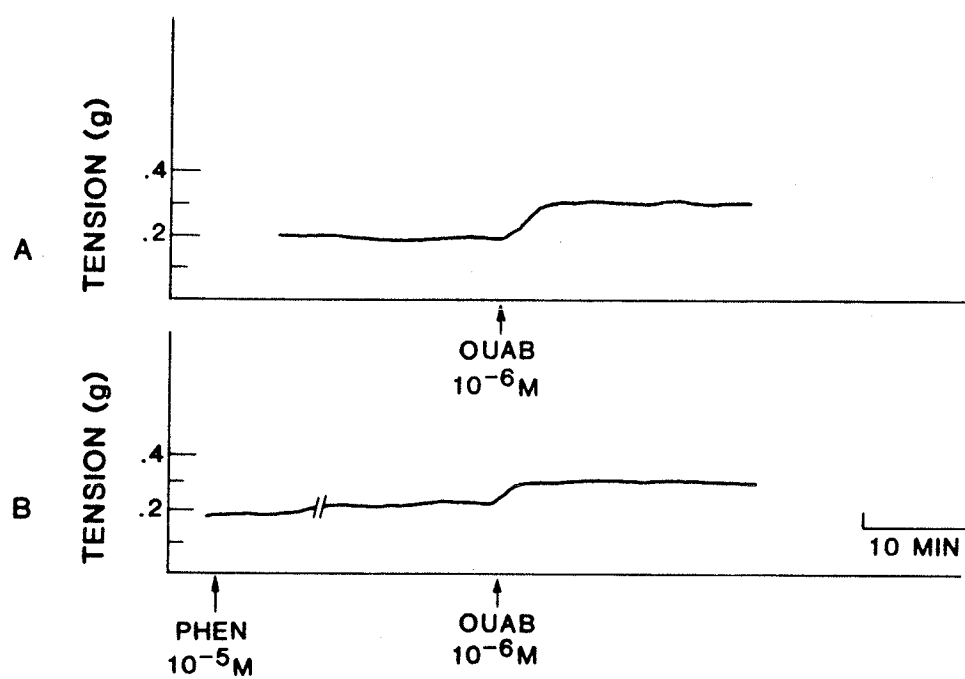
Fig. 32. A. Effect of ouabain (10^{-6} M) without PHEN
pretreatment.
B. Effect of PHEN on mechanical response to
ouabain.

At 37°C the re-introduction of a small dose of K to a muscle pre-incubated with O-K resulted in an immediate and pronounced decrease in tension when superimposed on an intermediate contracture to NE (10^{-6}M). This was observed in three muscles tested (Table 21).

The presence of an electrogenic pump predicts that ouabain will have a depolarizing effect due to Na-K pump inhibition and thereby potentiate muscle responsiveness (Fig. 32 and 33). Ouabain (10^{-6}M) produced contracture in phentolamine-pretreated muscle (N=2). At temperatures of 20°C, ouabain had no apparent effect. In muscles (pretreated with phentolamine), the effect of cooling potentially may be explained on the basis that cooling inhibited the pump and depolarized the cell membrane such that no further depolarization could occur.

D. Discussion

The use of digital arteries removed from amputated human limbs proved to be a useful and practical means of studying the effect of temperature on their physiological and pharmacological function. This in vitro model is the first attempt that has been described to study directly those arteries implicated in Raynaud's phenomenon. Helically-cut strips of digital artery muscle mounted in organ baths were found to remain viable for several hours and maintained satisfactory reproducibility of their responses over this time
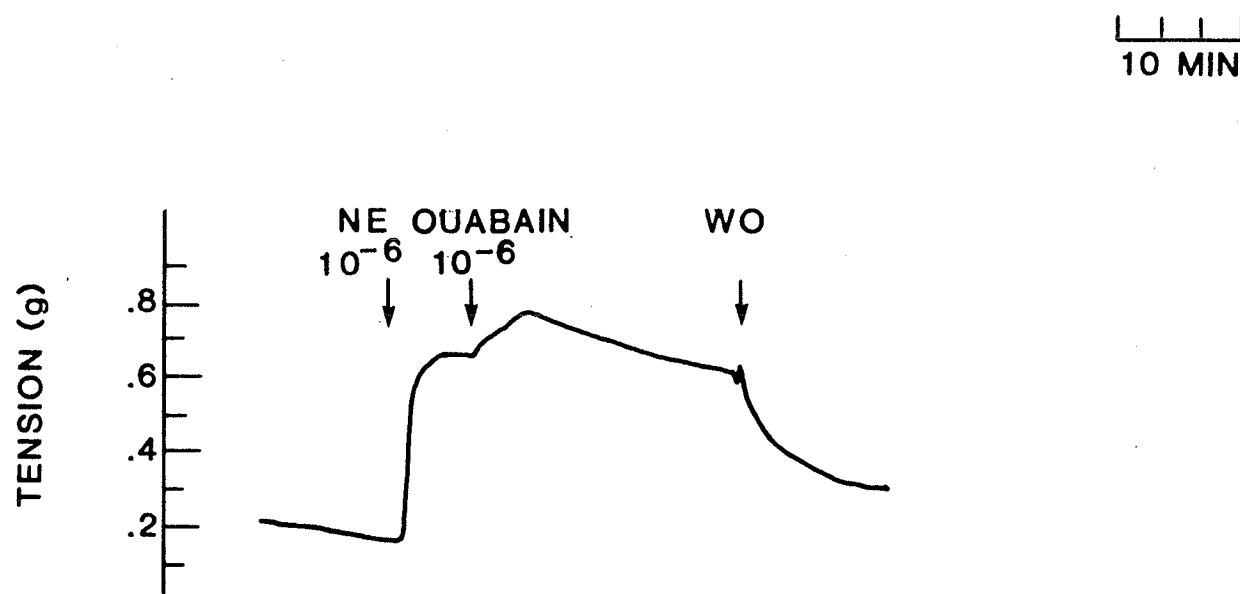
Fig. 33. Effect of ouabain (10^{-6}M) on a muscle precontracted with NE (10^{-6}M). WO - washout.

period. Responses of the three types of vessels used, name-
ly lower limbs with arterial occlusive disease and upper and
lower limbs with neoplasms or removed due to trauma, were
qualitatively similar, i.e., responses with cooling were
systematically diminished. However, cooling to 30°C pro-
duced a somewhat greater NE-induced contracture than at
37°C. Muscles from upper limbs, however, showed absence of
responses at 20°C whereas the responses of the muscles from
lower limbs were diminished. Future studies are warranted
examining the responses of muscles from upper limbs down to
temperatures of 20°C which can be considered to be a physio-
logical range for human digital arteries.

Our results contrast with those of Janssens and Vanhoutte
(1978), who observed cold-induced potentiation of smooth
muscle responsiveness in canine cutaneous blood vessels. In
the present studies cold-induced depression, particularly of
α adrenoceptor mediated contraction and to a lesser extent
of contractures produced by 5-HT and KCl, was observed in
both diseased and normal human digital arteries in vitro.
These observations suggested that adrenoceptor-mediated
function is more affected by cooling than some other types
of mediated function characteristic of vascular smooth mus-
cle. This response pattern was characteristic of that re-
ported for deep vessels, for example, the rabbit femoral ar-
tery (Glover et al., 1968), the aorta of the rat and rabbit
(Godfraind and Kaba, 1972; Devine et al., 1973), and canine
femoral veins (Vanhoutte and Lorenz, 1970). Our results

suggest that local cold-induced vasoconstriction of the digital arteries is not likely explained on the basis of changes in adrenoceptor-mediated function. An alternative hypothesis, however, is that cold reduces the sensitivity of the adrenoceptors in healthy human digital arteries, thereby compensating for an increased blood viscosity by passive vasodilatation. It is possible this compensation is lacking in patients with Raynaud's phenomenon.

Direct comparison of our results with those of others has not been possible since human finger arteries have not, to our knowledge, been previously studied in vitro. However, examination of more proximal human arteries has shown predominantly an inhibition of arterial smooth muscle contraction with cooling (Sams and Winkelmann, 1969; Winkelmann et al., 1977). Some studies (Winkelmann et al, 1977) showed evidence of maximal responses at temperatures below 37°C in some peripheral vessels. None of these vessels, however, have been implicated in vasospastic disease per se.

We observed increased basal tension with cooling in about 30% of muscles studied. Responses to various agonists were not potentiated with cold in these muscles, i.e., these muscles exhibited a typical cold-induced depression of responsiveness. Increased basal tension with cooling probably reflects Na/K pump inhibition and its sequelae. With rewarming, basal tension returned to pre-cooling levels.

The effect of cooling on the mechanical responses of smooth muscle in any given vascular bed probably reflects the net effect of inhibitory and excitatory influences on neuroeffector interaction. Although we did not study specifically the effect of temperature on nerve function, some investigators have reported facilitated neurotransmission in the vas deferens (Illes et al., 1973), and in the rat mesenteric artery (Malik and McGiff, 1974), while others have reported depressed activity of adrenergic nerves with a resulting decrease in neuronal transmitter release in addition to stabilization of neuronal membranes (Potter, 1966). Neuroeffector transmission was depressed in human digital arteries with cooling as indicated by the diminution of response to electrical field stimulation with cooling.

Cold-induced inhibition of vascular smooth muscle responsiveness could reflect altered membrane potential, depressed receptor function, depression of actomyosin ATPase and interference with the movement of the contractile proteins. Alternatively augmentation of smooth muscle contraction could result directly from the inhibition Na-K pumping, resulting in membrane depolarization and depression of Ca extrusion and transmitter reuptake, hence in increased intracellular concentration of activator Ca. These mechanisms may explain the cold-induced increase in basal tension we have observed in 30% of muscles studied, and the longer time courses for time-to-peak contraction and for relaxation at reduced temperatures. The precise contribution and interre-

lationship of these factors in normal and abnormal responses of human digital arteries to cold have yet to be elucidated. Although cooling has been shown to depress contractile behavior of smooth muscle, it is not likely to occur primarily at the level of the contractile proteins or beyond, since skinned vascular smooth muscle fibers have shown little change in reactivity with cooling compared with physiologic temperatures (Bohr et al., 1962).

The present in vitro results do not support the hypothesis of cold-induced augmentation of vascular smooth muscle responsiveness to explain either normal or exaggerated pathologic peripheral responses to cold in Raynaud's phenomenon. Considering that the vessels studied were not vasospastic, however, our observations do not preclude the existence of such a mechanism in these vessels. Further, it is doubtful that a β adrenoceptor-mediated vasodilating mechanism contributes significantly to peripheral vascular control in digital arteries and that vasospasm is secondary to an imbalance of α and β adrenoceptors.

The results of the present studies are consistent with that an electrogenic Na-K pump in an arterial smooth muscle preparation from human digital arteries. Evidence exists to support the contribution of an electrogenic Na/K pump to E_M in a variety of other vascular smooth muscle preparations (Casteels et al., 1977; Matthews and Sutter, 1967; Kuriyama et al., 1971). It is generally accepted that vasodilation

and vasoconstriction are associated with hypokalemia and hyperkalemia respectively (Anderson, 1976). The presence of an electrogenic pump in vascular smooth muscle can explain these observations. It appears that at physiologic concentrations of K_0 an electrogenic pump can be identified in vascular smooth muscle. Hermsmeyer (1983) stimulated electrogenic pumping in rat caudal artery, however, which has anatomic and physiologic similarity to the human digital artery.

To evaluate the role of an electrogenic pump, two lines of indirect evidence were employed. First in the presence of a Na/K pump, pre-incubation of the muscle with O-K likely resulted in disruption of the Na and K gradients and a decrease in E_K and E_M; on reintroduction of normal K. Hyporesponsiveness of muscles was observed suggesting hyperpolarization had occurred. Second, the introduction of ouabain to a phentolamine-pretreated muscle produced a consistent, rapid contracture suggesting depolarization due to inactivation of an electrogenic Na/K pump rather than nerve stimulation.

Exposure of the preparation to O-K followed by the re-introduction of 3 mM K resulted in hyporesponsiveness suggesting transient hyperpolarization had occurred. Exposure to ouabain resulted in potentiation of response presumably resulting from depolarization. We observed rapid relaxation and contraction in response to the reintroduction of low-K

and ouabain respectively supporting a primary electrogenic function of the Na/K pump in human digital arteries rather than these responses primarily being explained on the basis of ionic redistribution.

Cooling may potentiate vasoconstriction by Na/K pump inactivation and its consequences as discussed above. Thirty percent of muscles did show increased basal tension with cooling but no instance of response potentiation was observed. However, the nonspecific effect of cooling on depressing other pumps, interference with mobilization of Ca and steps involved with excitation-coupling processes, probably masked the effect of cooling on the Na/K pump.

The thermosensitivity of human digital arteries used in the present studies appears to resemble that of canine femoral veins reported by Janssens and Vanhoutte (1978). Evidence of depolarization with cooling was apparently masked and no response potentiation, i.e., either increased sensitivity or greater maximal response was observable.

Following O-K and subsequent immediate exposure to increasing doses of K, muscles were hyporesponsive. This effect could be explained by hyperpolarization resulting from the reintroduction of K. Decreased sensitivity was manifested by a shift to the right of the NE dose-response curve. Future studies need to examine the effect of smaller doses of NE following O-K in order to detect more subtle threshold changes.

With respect to transmembrane Ca movements, Ca influx and efflux, cooling inhibits Ca pumping to the extracellular space (Kurihara et al, 1974; Magaribuchi et al, 1973). Furthermore, this may also explain the greater depression of the slow component of a contracture seen in the rat and rabbit aorta (Brodie and Bohr, 1959) compared with the fast component, which represents release of intracellular Ca stores. The inhibition of Ca efflux with cooling could explain the characteristic prolonged relaxation times associated with the cooled preparation.

Na-Ca exchange has been reported to be a potentially important means of regulating Ca in vascular smooth muscle (Blaustein, 1982). Our studies suggest that this secondary effect of the Na/K pump in human digital arteries is not likely to contribute primarily to cold potentiated contractures.

Although the phenomenon of calcium leak has not been well described, an increase in calcium permeability has been reported when vessels are exposed to 4°C for several hours (Bohr and Verrier, 1971). This mechanism is not likely to underlie the observed effects of cooling on Na/K pump activity, and thereby serve as a mechanism for vasospasm, since this phenomenon is believed to reflect irreversible changes and damage within the membrane.

Future studies are needed to examine changes in membrane potential specifically to Na, K and Ca in human digital artery smooth muscle to help explain the different responses of this _in vitro_ preparation to O-K, ouabain and cooling.

GENERAL DISCUSSION AND CONCLUSIONS

The research for this thesis was undertaken for two main reasons. First, to date there has been no in vivo method for studying the effect of local versus neurogenic influences on the finger digital arteries (those implicated in Raynaud's phenomenon). Second, the basic understanding of the nature of the local fault in Raynaud's phenomenon will have to come from the study of digital arteries at the cellular level in vitro. In vitro investigation has not been previously attempted in finger digital arteries. This led to two approaches to the problem. The Nielsen and Lassen method provided a possible in vivo method of studying the arteries implicated in the phenomenon. To understand smooth muscle responsiveness at the cellular level, digital arteries from amputated limbs were investigated for potential use in an in vitro model.

In Vivo Studies

With respect to in vivo study of cold sensitivity, apparent finger systolic pressure had been reported earlier by Nielsen and Lassen (1977) to provide an index of arterial tone. Following cooling of the digits, finger systolic pressure was observed to fall progresssively with decreases in temperature in both healthy subjects and patients with

Raynaud's phenomenon. The effect was more marked in the Raynaud's group with pressures near or equal to zero being recorded in some patients reflecting closure of the digital arteries.

Nielsen and Lassen's method has been used by others since it was first reported; however, interpretation and comparison of the results have been difficult because of procedural modifications instituted by various investigators. Procedural differences included unspecified criteria for standardizing vasomotor tone, duration of imposed arterial occlusion, choice of distal sensor, skin temperature and the cuff deflation rate. We identified all these as variables that could affect pressure measurements, thus requiring stringent control. Also, the effect of simultaneous pressure measurement on two fingers of the same hand which is required in Nielsen and Lassen's method, may affect the results. This effect has not been previously examined.

The present studies were designed to investigate and control the methodological details of this cold sensitivity testing procedure and thereby enhance its sensitivity and specificity as a clinical tool. Once these had been examined, studies were designed to investigate the normal physiologic response to cold of the digital arteries in healthy subjects and the pathophysiological responses of Raynaud's phenomenon. To achieve this goal, we planned to use our refined cold sensitivity test.

Methodologic Implications

Our results contrasted with those of the original work by Krahenbuhl et al (1977) and Nielsen and Lassen (1977) in a number of respects. Vasomotor state was much more rigidly controlled in the present studies and was directly monitored in an ongoing fashion by thermocouples attached to the finger tips throughout the testing procedure. Without measuring sympathetic activity invasively, the only conservative means of ensuring reasonably steady sympathetic activity was by either vasodilating or vasoconstricting the subject by body heating or cooling respectively. At thermoneutral skin temperatures, skin temperatures varied suggesting variation of the intensity of sympathetic discharge. In most previous studies skin temperatures were not reported to have been controlled. Rather, skin temperatre was merely reported to be in the thermoneutral range or the subject was thermally comfortable. How this was measured was not always described.

Another factor that may account for the dissimilarity in our results and previous reports of the Nielsen and Lassen method is that we observed an increase in finger temperature under the cuff following the equilibration period immediately before pressure measurement and flow resumption in the test finger. Increases in finger temperature were more marked during finger cooling in the vasodilated state than in the vasoconstricted state probably because body heat was

conducted along the digit even though blood flow to the digit was occluded. In further trials the temperature under the cuff was rigidly controlled and monitored because the test depends on maintaining the temperature of the walls of the digital arteries at a predetermined stable temperature for the recording of the systolic pressure at that given temperature.

The present studies compared the apparent finger systolic pressure of digits, measured at cuff temperatures of 30°, 20° or $10^\circ C$. The desired temperature was achieved by undershooting the temperature in the water bath by 2° to $4^\circ C$ to counteract any warming effect that might occur from the time the circulating pump was turned off to the time the strain gauge detected a pulsation in the finger, hence resumption of flow distal to the cuff.

The method of deflation rate was reported to be a significant factor in routine blood pressure recording in the digits (Gundersen, 1972). Earlier studies have not systematically standardized this parameter, resulting in a possible source of extraneous variability. The present studies examined the effect of three different deflation rates during finger systolic pressure measurements in cold sensitivity testing. Of significance was the finding that the cold may have affected the distensibility of the digital arteries. By making the vessels stiffer, it appeared as if there was a latency period that interfered with the mechanics of vessel

opening. This appeared to contribute to even lower pressures. Even when finger warming was compensated, a fast continuous deflation rate of 5 mmHg/sec was found to yield the lowest pressures compared with continuous 2 mmHg/sec and a step-wise 5 mmHg/6 seconds deflation methods.

The role of circulatory occlusion in the Nielsen and Lassen cold sensitivity test was studied because arterial occlusion was an essential part of the procedure for cold sensitivity testing and because it is known to result in relaxation of vascular smooth muscle distally (reactive hyperemia). Our results indicate that transient periods of arterial occlusion do not have a marked effect on vascular smooth muscle and agree with the findings of Gaskell (1965).

The most commonly used distal sensors in cold sensitivity testing are mercury in rubber strain gauge and photoplethysmograph. The suitability of these devices and other commonly used sensors for routine digital pressure measurement was of interest. Of the four sensing techniques studied, spectroscopy revealed systematically lower pressures and greater variability among these techniques. The strain gauge used in some other studies of cold sensitivity showed good results with respect to the agreement of pressures measured using this technique and those measured with the flush and photoplethysmograph.

Cold Sensitivity Testing in Raynaud's Phenomenon

Local exposure of the finger to cooling does not appear to be solely responsible for the exaggerated vascular constriction seen in Raynaud's phenomenon. Although in some instances, during vasodilatation and negligible vasomotor tone, finger pressures were reduced, no instance of vessel closure was found elicited in patients with Raynaud's phenomenon tested with finger temperatures of 10°C. However during body cooling and associated sympathetic vasoconstrictor discharge, a similar finger temperature of 10°C produced marked pressure decrease or closure. These findings indicated the importance of vasomotor state. Also, increased vasomotor state during body cooling tended to constrict the vessel and reduce finger systolic pressure even without local cooling.

Classification of patients with respect to primary or secondary type and the presence of arterial occlusion may be important for interpretation of the test results because arterial occlusion itself leads to low local blood pressure distally. Blood pressure measurements of the fingers prior to cold sensitivity testing are, therefore, essential to distinguish the direct effect of cooling and effect of arterial occlusion on finger systolic pressure.

The use of our refined method showed that an abnormal response to combined local and body cooling was found to be a

good indicator of Raynaud's phenomenon. However, a number of negative responses among patients indicated that a negative test does not rule out the presence of Raynaud's phenomenon.   Also, the finding of closure during body cooling on the control finger not locally cooled may be useful as a diagnostic aid.

In Vitro Studies

A primary difficulty in studying the pathophysiology of Raynaud's disease and characterizing possible mechanism(s) in vitro is that it is difficult to obtain specimens of normal human digital arteries and arteries from patients with Raynaud's phenomenon.   Previous studies in examining the physiology and pharmacology of human peripheral arteries have isolated skin vessels during biopsy (Winkelmann et al., 1977) and post-mortem palmar digital arteries (Moulds et al, 1978).   Neither of these types of vessels, however, have been directly implicated in Raynaud's phenomenon. It was of interest in the present studies to develop a suitable in vitro model in which to study the effect of temperature on those arteries directly implicated in Raynaud's phenomenon. Lewis (1929) established that hyperresponsiveness and closure of the finger digital arteries during cooling were responsible for the vasospastic attacks associated with Raynaud's phenomenon.   We determined from pilot studies that digital arteries removed from amputated human limbs provided a good source for study of their responsiveness to tempera-

ture. We observed qualitatively comparable responses be-
tween digital arteries removed from upper and lower limbs
and from limbs with and without vascular disease.

Response of In Vitro Preparation to Cold

Several hypotheses were investigated using a human digi-
tal artery in vitro model to explain cold-induced potentia-
tion of digital artery smooth muscle. The hypothesis of in-
creased vessel sensitivity to cold resulting in
hyperreactivity of the smooth muscle was attractive in that
the etiology of Raynaud's phenomenon could be attributed to
a normal but exaggerated physiologic response. With respect
to the role of local versus neurogenic factors underlying
digital artery responses to cold, sympathectomy does little
in the long run to ameliorate Raynaud's symptoms. Degranu-
lation of the sympathetic nerve endings contributes to den-
ervation hypersensitivity post-junctionally, and increases
sensitivity to circulating catecholamines. This has been
suggested previously as the mechanism for the return of va-
sospastic symptoms following sympathectomy (Lewis, 1929).

The fundamental basis of both the in vivo and in vitro
investigations was that increased vascular responsiveness
can originate at two levels, central and peripheral. Exces-
sive sympathetic activity could augment locally-induced
smooth muscle contraction. Peripherally, cold-induced cel-
lular changes that may produce changes in the responsiveness
of vascular smooth muscle include inhibition of the Na-K

pump, hence altered Na/Ca exchange and an increased sensitivity of receptors on the smooth muscle membrane. These factors could amplify the response of the smooth muscle to normal concentrations of catecholamines.

Therefore, in vitro studies were designed to examine first α and β adrenoceptor-mediated responses at reduced temperatures by selecting a preparation that effectively eliminated sympathetic nerve activity. In vitro evidence has been accumulating in a variety of cutaneous mammalian blood vessels in support of Lewis' local fault hypothesis, suggesting increased vessel reactivity with cooling. Janssens and Vanhoutte (1978) have shown in the cutaneous veins of dogs that cold results in an increased α adrenoceptor affinity for norepinephrine. Our data did not support the hypothesis that this mechanism was responsible for the vasospastic attacks observed in the fingers and toes of patients with Raynaud's phenomenon. Arterial muscle strips from vessels removed from amputated limbs, with and without vascular disease, were observed to respond qualitatively the same to catecholamines when cooled down to 10°C, i.e., contractures decreased progressively as temperature was lowered.

At the level of the smooth muscle cells, cold-induced augmentation by altered α adrenoceptor activity can occur in one of two ways. First, as proposed by Janssens and Vanhoutte (1978) cooling may produce a spontaneous increase in α adrenoceptor affinity for norepinephrine. Secondly in

combination or alternatively, the response of ß receptors present on the smooth muscle cell membranes of digital arteries may be diminished. In vivo evidence has shown that patients on ß blocking drugs are prone to cold hands. This suggests a possible mechanism for Raynaud's disease in which ß receptors in the digital arteries of these individuals are absent or reduced in number compared with normal individuals. Other studies show, in support of the presence of ß receptors in the digital arteries, that the use of ß agonists in cold sensitive individuals can successfully alleviate vasospastic symptoms. Cohen and Coffman (1981) have also provided some support for a ß adrenoceptor mediated vasodilating mechanism in the hands. These reports contrast with the conventional belief that ß adrenoceptors do not play a significant role in the peripheral regulation of blood flow in the hands and feet.

Membrane properties may be altered with progressive cooling resulting in disrupted channel function and depressed affinity of NE for the α adrenoceptors. Consistent with the finding of cold-induced depression of responsiveness, apparent membrane depolarization in response to low K failed to produce augmented responsiveness to NE (data not included in thesis). Responses to K were depressed with cooling in a qualitatively similar manner to receptor-mediated contractions. This response to K, however, was quantitatively less depressed compared with receptor-mediated contractures such as NE and 5-HT.

It was hypothesized that if ß adrenoceptors were present and blocked in human digital arteries, then α adrenoceptor mediated responses would be augmented. Alternatively, response to ß agonists such as ISO should result in diminished vessel responsiveness. The majority of muscles did not show evidence supporting a ß adrenoceptor-mediated dilating mechanism in the finger artery preparation examined in our study. Furthermore, this finding was consistent over a range of temperatures from 37° to 10°C. Two muscles, however, did show a relaxation response in response to ISO. This suggested that a ß adrenoceptor dilating mechanism may contribute to normal and possibly abnormal regulation of the circulation to the fingers.

The present data do not support the hypothesis of a cold-induced increase in α adrenoceptor affinity for catecholamines. It is not clear whether the absence or reduced number of ß adrenoceptors contribute to the occurrence of digital cold sensitivity and vasospasm.

Other explanations for cold-induced hyperresponsiveness that were considered at the smooth muscle level were increased basal tension and inhibition of the Na-K pump that might result in potentiated muscle countracture with cooling. In 30 percent of muscles studied basal tension increased with cooling, and reversed to pre-cooling levels with rewarming. This may reflect cold-induced inhibition of the Na/K pump thus Na-Ca exchange and E_M. Basal tension was

generally observed, however, to be progressively reduced with reduced temperature in the majority of muscles. Depressed basal tension in 70 percent of cases could reflect technical differences in the preparation of the muscles, clinical differences with respect to the quality of the limb from which muscles were removed or differences in the patients themselves. Depressed basal tension was reversed on rewarming. Possible explanations for cold potentiated basal tension include inhibition of Ca extrusion and/or depolarization-activated Ca influx. The effect of temperature on basal tension warrants further study to explain the differences observed.

Three lines of investigation corroborated the physiological importance of Na/K pumping and its sequelae to the control of in human digital arteries. First, reintroduction of K in 0-K treated muscles resulted in an increased K threshold. Hence an electrogenic effect of Na-K pumping was supported by the observed hyporesponsiveness, presumably secondary to hyperpolarization of the preparation. Addition of an intermediate dose of NE, i.e., $10^{-6}M$, produced a diminished response to NE, which is both a receptor- and a depolarization-mediated response. Second, normal K produced a consistent, instantaneous step decrease in tension in a NE-induced contracture in 0-K solution. This response was too rapid to have been accounted for by Na-Ca exchange since the latter would require a primary redistribution of Na. These

findings were corroborated by a third line of evidence. Ouabain produced a phentolamine-insensitive contractile response in the majority of muscles. This suggested ouabain inactivated the pump, hence resulted in loss of electrogenicity and decrease in E_M.

Conclusions

The results of our _in vivo_ and _in vitro_ studies lead us to conclude that:

1. Normal responses to cold and exaggerated responses of digital artery smooth muscle as observed in Raynaud's phenomenon, reflect both central and local factors. The present observations support the existence of at least two pathophysiologically distinct mechanisms of Raynaud's phenomenon. One involves increased vessel responsiveness; and the other mechanism involves the effect of reduced finger pressure in the digital arteries as a result of proximal arterial obstruction.

2. Standardization of the cold sensitivity testing technique and procedure must be rigidly controlled to reduce the effect of extraneous variables on the finger systolic pressure after body and finger cooling. These factors include distal sensor used for recording pressure measurements, cuff deflation rate, the vessel occlusion procedure, skin temperature, the cooling procedure, the duration of finger occlusion and vasomotor state.

3. The degree of sympathetic discharge may be more important in producing digital vasospasm than is currently believed. Some patients who reported severe symptoms failed to produce a positive response in the cold sensitivity test procedure. Changes in central effect are more likely to occur than changes in local finger sensitivity, given that vasomotor tone reflects thermoregulatory control and input from higher and lower central nervous system centres. It is well known for example that Raynaud's attacks can occur in the absence of cold stimulus, and in the presence of psychologic anxiety.

4. Arterial closure in the control finger in Raynaud's subjects has not been reported previously. Such a finding may have additional diagnostic potential in cold sensitivity testing. Also, it has to be taken into account in reporting the results of the test.

5. A positive cold sensitivity test was a good indicator of Raynaud's phenomenon, however, a negative test was less good at predicting the absence of disease. Differentiation of subgroups of patients with Raynaud's phenomenon could not be reliably achieved on the basis of a modified cold sensitivity test. Sample sizes were small and this may have accounted for this finding. Another limitation may have been the heterogeneity of disease severity within groups.

6. An _in vitro_ model using digital arteries from amputated limbs was found to be a satisfactory model for the study of human peripheral vascular smooth muscle with respect to tissue viability, response reproducibility and predictability of certain aspects of its behavior.

7. α adrenoceptor-mediated responses were not augmented with cooling in dissected human digital arteries, but rather showed progressive depression of both force generated and sensitivity to NE. The study of β adrenoceptors indicated their presence in some but not other preparations. It is not known whether medications could affect the results. More data and further studies are needed to elucidate the role of β adrenoceptors.

8. Response to depolarizing solutions of K was also depressed, but this was less marked compared with receptor-mediated function such as that mediated by NE and 5-HT.

9. Resting tension was depressed in the majority of muscles with cooling; but 30% of muscles tested showed an increase which reversed with rewarming.

10. A contribution of electrogenic NA pump activity to the control of tone was inferred on the basis of three lines of evidence. First an increase in response threshold to K followed O-K incubation. Second, hyporesponsiveness was observed in response to

introduction of low K following pre-incubation with K-deficient solution, which suggested relaxation mediated via hyperpolarization had occurred; and ouabain-potentiated contractures in muscles pretreated with phentolamine. Although Na-Ca exchange was not likely to contribute significantly to the effects of cooling on digital artery smooth muscle because of the rapidity of the responses observed to the above interventions, a role for Na-Ca exchange cannot be ruled out with respect to cold-induced increase in basal tone and warrants further study. A third line of evidence supporting the presence of an electrogenic pump in human digital artery smooth muscle was the fact that ouabain could elicit contractures in the muscles.

Future Studies

Future in vivo studies are needed to further refine Nielsen and Lassen's cold sensitivity procedure. For routine clinical use in the diagnosis of pathological cold sensitivity, a test protocol needs to be developed that is time and labor efficient. A definitive reliable test to differentiate subgroups of patients with Raynaud's phenomenon with varying etiologies awaits further development.

Categorizing patients according to the classification for disease severity described by Taylor and Pelmear (1975) may

be useful in assessing the results of the cold sensitivity test among subgroups of patients. Correlating classification and objective test results of patients may result in significant differences among subgroups.

The present in vitro data did not support cold potentiation of α adrenergic responses to account for increased smooth muscle responsiveness of the digital arteries. Further study of the role of ß adrenoceptors in human digital arteries is needed. Our results may have been affected by the fact patients may have been taking ß blockers prior to surgery.

Physiologic studies examining Ca metabolism are needed. Cooling likely results in greater availability of activator Ca in muscle and an increase in vascular tone. An increase in activator Ca would result if Ca influx was facilitated, and Ca extrusion from vascular smooth muscle cells and Ca uptake into the intracellular stores were inhibited. Altered Ca metabolism with cooling likely has a net facilitatory effect on vascular smooth muscle tone. The components contributing to this effect warrant further study.

Cooling may also release from inhibitory nerve endings a variety of regulatory chemical mediators that are responsible for buffering normal constrictor action. Possible relaxing factors that may contribute to regulating vessel constriction have yet to be identified. It is possible that

prostaglandins and leukotrienes, in addition to endothelial-dependent responses are involved in some way (McGiff, 1981; Piper, 1983). Studies are needed to investigate the role of these potential mechanisms of normal and abnormal responses of smooth muscle in peripheral blood vessels of humans.

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Appendix A

CONSTRUCTION OF CYLINDRICAL CUFFS FOR COLD SENSITIVITY TESTING OF THE FINGERS

CONSTRUCTION OF CYLINDRICAL CUFFS FOR COLD SENSITIVITY

TESTING OF THE FINGERS

List of Materials

- 18 x 3/4" latex penrose tubing

- 1/8 I.D. x 1/16] (Wall) lastex hosing (tubing) or 1/8 I.D.

x 1/32" (Wall)

- Carter's rubber cement

- small, thin, glue applicators (toothpicks, sticks)

- baby powder

- small sharp manicure scissors

- tweezers

- adhesive tape (1")

- metric ruler

Cut pieces of hose approximately 10 cm long (2 for each cuff). From the 3/4" tubing cut squares approximately 2 cm x 2 cm. In the middle of these squares make a tiny incision. Run a hose through this slit and pull the rubber up the hose about 2 cm (this should be a firm fit).

Apply glue around the base of the rubber square and pull down over the top of the glue to produce a sealed gasket (this must be allowed to dry for a sufficient period due to the potential stress on this area). Leave about 1/2 cm of the hose extended past the rubber square.

When sealed gasket is dry, trim the square into a circle or oval (the larger the better). With the small scissors

trim the excess hose as close as possible to the rubber gasket. The hoses are now ready to be mounted on the cuff.

Using a metric ruler, choose the circumference of the cuffs to be made. Leave an overlap of .5 cm. This will be used as a seam. Cut as many pieces of tubing as the number of cuffs needed (3/4" latex). Ink the .5 cm seam as it will be needed as a guide in linking up the two ends.

Insert the ends into each other and tack one side with rubber cement. Keep the seam even. Apply in the centre basically (be sure not to get glue on the inside of the tubing as it will cause problems).

Four separate applications of glues are needed to secure the entire circumference of the tubing (go on to other tubings so as to allow each application to dry). A fine dusting of baby powder on the finger tips prevents them from sticking to the rubber. After the glue has set turn the tubing inside out so as to expose the other side. Apply glue as was done earlier (keep the seam flat and neat). Make sure you get an even application of glue so there are no links and allow enough time to dry. Return the tubing back to its original position by turning it inside out. Now glue the two remaining corners.

At this point cut two holes in the outer wall of the tubing where the hoses will enter. Mark the spot with an ink mark. The holes should be on opposite sides of each other.

Stay at least 1 cm away from the seam. Lift the outside
surface with the tweezers and snip (small scissors) a slit
in the rubber being careful not to penetrate the inner wall
of the tube. Cut holes approximately 1/2 cm in diameter.
Sprinkle powder into the inside of the tubing in order to
free any surfaces that may have adhered together accidently
(use the tweezers through the holes to work areas free).

You should now be able to roll the tubing back and forth
on the tips of the fingers. Roll it so the remaining un-
glued parts of the seam are made accessible. Apply glue.
Press firmly. Let dry. Roll the tubing back to the origi-
nal position. You should now have a completely sealed tube
(cuff) except for the two inlet, outlet holes. We can now
refer to the tubes as cuffs. The hosing to the cuff can now
be applied.

Apply glue around one of the holes. Cover a wide area,
more than is necessary. Position the hose and gasket over
the hole and join. Press firmly until adhesion occurs.
Proceed to the other hole. Use the same routine. You
should now have a round cuff with an inlet and outlet hose
attached.

Reinforce the joint between the hose and the cuff. Cut
four pieces of adhesive tape 1 1/2 cm by 2 1/2 cm. Position
them around the base of the hose using the small scissors to
trim. Do the same to the other hose. Be sure the applica-
tion is firm.

Cut 2 pieces of 1" tape into 10 cms lengths. Bring the
tape right to the edge of the cuff. Trim the tape so it
surrounds the hoses. Press down firmly.

N.B. Blow dry the cuffs with air after every use to
avoid rotting of the rubber.