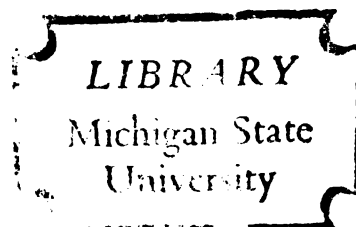


COLD-INDUCED VASODILATION IN
THE ANESTHETIZED CAT

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
LARRY R. KLEVANS
1968

THESIS



ABSTRACT

COLD-INDUCED VASODILATION IN THE ANESTHETIZED CAT

by Larry R. Klevans

Immediately following local cold exposure of the human digit in a water bath less than 15°C, skin surface temperature falls rapidly until it approaches bath temperature. Eight to ten minutes after cold immersion, skin surface temperature increases to a level which is sustained throughout the exposure (1). This increase in temperature (called "cold-induced vasodilation") supposedly provides protection against local cold injury.

The mechanism of the cold-induced vasodilation (CIVD) response has been attributed to activation of axon reflexes by histamine (1). The effect of sensory nerve degeneration and histamine blockade (10 mg/kg. Benadryl, I.V.) on CIVD was studied in anesthetized cats to assess the validity of this hypothesis. Copper-constantan thermocouple wires, attached to both hindlimb footpads, recorded alterations in emf produced by changes in surface temperatures during local cold exposure of the extremities in a stirred ice-water bath. Immediately following immersion footpad surface temperature

fell to approximate bath temperature, then increased and decreased in an oscillatory manner for 10-15 minutes. The first cold-induced vasodilation phase was characterized by its "peak temperature," "rewarming rate," "onset time," and "time to peak." Degeneration of the sciatic and tibial nerves delayed the onset of dilation and time to reach peak dilation, but failed to change the peak temperature and rate of rewarming. Benadryl, administered in a dose (10 mg/kg.) that antagonized the hypotensive response to injected histamine, failed to alter the CIVD response ("peak temperature," "rewarming rate," "onset time," "time to peak"). It was concluded that cold vasodilation does not depend on an axon reflex mechanism activated by histamine.

1. Lewis, T. (1930). Observations upon the reactions of the vessels of the human skin to cold. Heart 15: 177.

COLD-INDUCED VASODILATION IN
THE ANESTHETIZED CAT

By
Larry R. Klevans

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Physiology

1968

G 50131
7-11-65

ACKNOWLEDGMENTS

The author is indebted to the able assistance of Dr. T. Adams and Dr. J. Schwinghamer who provided funds for this research project and contributed to the preparation of the manuscript.

TABLE OF CONTENTS

CHAPTER	Page
ACKNOWLEDGMENTS	ii
LIST OF FIGURES	v
I. INTRODUCTION	1
II. PROPOSED MECHANISMS OF CIVD	6
III. METHODS AND MATERIALS	14
A. Preparation of the Animal	14
B. Quantification of the CIVD Response	16
C. Nerve Section Studies	16
D. Antihistamine Studies	17
IV. RESULTS	24
A. CIVD in the Human Finger and Cat's Footpad	24
B. Nerve Section Studies	25
C. Antihistamine Studies	25
V. DISCUSSION	53
A. Skin Surface Temperature Measurements as an Index of Blood Flow	53
B. CIVD in the Anesthetized Cat	57
C. CIVD After Sensory Nerve Degeneration	58
D. Benadryl as a Histamine Antagonist	60
E. CIVD After Administration of Benadryl	62
F. The Possible Role of a Vasodilator Sub- stance for CIVD in the Anesthetized Cat	63
VI. SUMMARY	66
BIBLIOGRAPHY	68
APPENDICES	74

LIST OF FIGURES

FIGURE	Page
1. Position of footpad in stirred insulated water bath.	23
2. CIVID response of human finger and cat's footpad .	29
3. CIVID responses prior to nerve section	31
4. CIVID responses after nerve section.	34
5. Statistical analysis of CIVID responses in the control and denervated footpads	36
6. The effect of histamine on mean arterial blood pressure before and after the systemic administration of Pyribenzamine.	38
7. The effect of histamine on mean arterial blood pressure before and after the systemic administration of Chlor-trimeton	40
8. The effect of histamine on mean arterial blood pressure before and after the systemic administration of Benadryl	42
9. Changes in mean blood pressure as a function of systemic administration of histamine.	44
10. The effect of Pyribenzamine, Chlor-trimeton, and Benadryl on control footpad temperature	46
11. The effect of histamine on footpad temperature before and after administration of Benadryl . . .	47
12. CIVID responses before and after administration of Benadryl.	50
13. Statistical analysis of CIVID responses in the control and Benadryl pretreated animals	52

LIST OF APPENDICES

APPENDIX	Page
I. Chronological List of Proposed CIVD Mechanisms.	75
II. Tabulation of Data from the Cold-Induced Vasodilation Response in the Intact vs. the Chronically Denervated Hindfoot	76
III. Tabulation of Data from the Cold-Induced Vasodilation Responses in the Control vs. the Benadryl Pretreated Animals	77
IV. Calibration for Strip Chart Recorder.	79

CHAPTER I

INTRODUCTION

Exposure of an animal to a cold environment involves potentially greater heat loss to the surroundings than heat gain or production by the organism. For the homeotherm, thermoregulatory processes must re-establish a dynamic equilibrium between heat gain and heat loss to maintain internal body temperature within narrow limits (36,52). The rate at which heat is lost through conduction, convection, evaporation, and radiation has to be balanced by heat gain from metabolism, the specific dynamic action of foods, shivering, exercise, hormonal, or drug effects (14,26,34,35, 37). Maintenance of a thermal steady state during whole body cold exposure also includes decreasing heat loss to the environment by changing two thermal gradients: (i) the gradient from the viscera ("core") through the insulating "shell" to the skin surface, and (ii) the gradient from the skin surface to the environment. The former may be altered by changes in vasomotor tone, and the latter by variations in external insulation (i.e., clothing and/or body hair). The net result of increasing heat gain and decreasing heat loss during whole body cold exposure is hopefully the

restoration of a thermal steady state at a normothermic level (36,52).

Vascular responses to whole body cold exposure conserve heat in two ways. Sympathetic activity increases vasoconstrictor tone to the cutaneous circulation, thereby providing an increase in the thermal insulating "shell" (23,55). With greater tissue insulation thermal flux is reduced during vasoconstriction through the relatively large distance from the core to the skin surface (36). Heat may also be conserved by a counter-current exchange of thermal energy between the arteries and veins of the extremities. This exchange involves shunting blood from superficial to deep veins, located in juxtaposition with the deep arteries. As a result arterial blood is precooled by conductive heat transfer from the arteries to the deep veins, reducing the capillary to skin-surface temperature gradient, and thus conserving heat (3,53). The net effect of vascular responses during whole body cold exposure is to conserve heat and to maintain a constant internal body temperature at the expense of intense cooling of the extremities (7).

Numerous investigators have examined the effect of local cooling on regional circulation in man (22,30,42). Generally, local cold exposure of any extremity initiates vasoconstriction, and a subsequent decrease in blood flow in the exposed limb (27). For some peripheral tissues, however, the reduction in blood flow is not maintained

throughout a period of local cold exposure. For example, seven to ten minutes after the index finger is immersed in an ice-water bath, blood flow to the exposed digit increases rather than remaining at the low level of flow attained immediately after immersion (25,42). An increase in blood flow during local cold exposure (called "cold-induced vasodilation") supposedly provides protection against local cold injury (22,23,29,30,42).

Characteristics of human cold-induced vasodilation were originally reported by Lewis (42) who described changes in skin surface temperature during exposure of the digit to an unstirred water bath less than 15°C. Immediately following immersion, he observed that finger surface temperature decreased rapidly to approximate bath temperature, then increased and decreased in an oscillatory fashion for 5-10 minutes after the initial exposure. Lewis called this series of cyclic temperature changes the "hunting phenomenon," referring to a single phase as the cold-induced vasodilation (CIVD) response. After the finger was removed from the water bath, Lewis noted a rise in digital temperature above the pre-immersion level, identified as the "vascular after-reaction." When he recorded the response of the finger to local cold exposure, three components were consistently identified--initial vasoconstriction, the "hunting phenomenon" (or a series of cold vasodilations) and the "vascular after-reaction." Similar responses have also been recorded

from other extremities in man (25), and from the extremities of a variety of laboratory animals (10,17,28).

Numerous mechanisms have been implicated in the control of the digital response to local cold exposure. The initial decrease in skin temperature is primarily due to a reduction in blood flow caused by vasoconstriction from a sympathetic discharge and from a direct effect of cold on vascular smooth muscle (38). The mechanisms controlling the increase in finger temperature 5-10 minutes after local cold exposure (the first cold-induced vasodilation phase) have not been elucidated; however, a variety of hypotheses have been suggested (see Proposed Mechanisms of CIVD). Cold-induced vasodilation apparently results from an increase in blood flow initiated by shunting the blood from the capillary network through arteriovenous anastomoses (10,11,17,28), thereby creating a thermal flux from the core of the finger to its skin surface. The subsequent increase in skin surface temperature is not dependent on an intact sympathetic nerve supply (42), but may be influenced by the thermal state of the entire body (50,58,60). Hence, CIVD is neither entirely a local phenomenon, nor strictly centrally controlled, but may result from an unknown combination of both local and whole body circulatory control. The "vascular after-reaction" has been reported to result from axon reflex vasodilation (42). This phase of the response, however, cannot be adequately reviewed due to limited studies of its

mechanism. Most workers investigating the vascular response to local cold exposure have confined their studies to the first cold-induced vasodilation cycle, and usually avoided investigating the mechanisms involved in the "hunting phenomenon" and "vascular after-reaction."

The simplicity of human CIVD testing lends itself to a detailed characterization of the digital response to local cold exposure. Cold vasodilation has previously been characterized by its vasodilator subcomponents (time to re-warm," "rate of rewarming," "peak temperature," and "time to peak temperature") to predict cold injury susceptibility of the extremities (61) and to identify local cold acclimitization (1,19,48). The subcomponents of cold vasodilation may also be used to characterize the CIVD response recorded from the extremities of a suitable laboratory animal in a variety of acute and chronic experimental conditions. By quantifying the response recorded from the extremities of an experimental animal, tests may be designed to examine the mechanisms involved in cold-induced vasodilation.

CHAPTER II

PROPOSED MECHANISMS OF COLD-INDUCED VASODILATION

Mechanisms for cold-induced vasodilation were originally postulated by Lewis (42). He used copper-constantan thermocouple wires attached to the index finger to record changes in skin surface temperature during local cold exposure. Cold vasodilation was observed after section and degeneration of the sympathetic nerves innervating the hand. The response was also maintained after section of the somatic nerves, but disappeared as the peripheral nerves degenerated. Lewis suggested from these data that the cold-induced vasodilation response was in part dependent upon an axon reflex mechanism. This concept of axon reflex or antidromic vasodilation was supported by other investigators who failed to elicit a CIVD response after degeneration of axon reflexes (8).

Lewis also reported that cold-induced vasodilation was initiated by a vasoactive substance released from cells injured during cold exposure (42,43). The subsequent release of this vasodilator material allegedly induced changes in skin color--the "red line," "flare," and "wheal"--which Lewis collectively called the "triple response" to injury.

He deduced that histamine was the substance released during potential injury of the skin, since intradermal injections of the agent and thermal or mechanical stimulation could elicit the "triple response."

Lewis determined the site of action of histamine during cold-induced vasodilation by demonstrating the genesis of the "red line," "flare," and "wheal" (43). The "red line" was produced by firmly stroking the epidermis with a blunt instrument. Lewis contended that the subsequent change in skin color was due to an increase in blood volume in the physiological capillary bed or minute vessels (anatomical, capillaries plus minute venule plexuses) resulting from active dilation of these vessels. Since the reaction appeared after the circulation was arrested above the point of stimulation, Lewis reasoned that the increase in blood volume in the minute vessels was not due to passive dilation resulting from opening of arterioles; such a passive effect could not happen when the circulation to the skin is occluded and the pressure in the arteries and veins equalized. Furthermore, Lewis reported that the "red line" was not a result of axon reflex vasodilation, since the reaction occurred after the skin was chronically denervated. These data indicated that the "red line" reaction resulted from non-neurogenic active vasodilation of minute vessels. With repeated or strong stimuli, a bright, scarlet, red "flare" appeared on the skin, concealing the "red line." Lewis reported that the "flare" was

apparently due to axon reflex vasodilation of arterioles, since chronic denervation of the skin, or arresting the circulation above the point of stimulation abolished the reaction. Finally, a "wheal" developed if the skin was pricked repeatedly with a needle. This was supposedly due to increased permeability of the vessel wall, a change independent of vasodilation. Lewis could reproduce the "triple response" by injecting histamine into the skin, and delay the response until the circulation was released by administering histamine above the occluded vessels. As a result he suggested that after histamine or a histamine-like substance ("H-substance") was released from cells damaged by an injurious stimulus, it dilated minute vessels directly to produce the "red line" and activated axon reflexes innervating arterioles to produce the "flare." In addition, Lewis reported that cold-induced vasodilation results from activation of axon reflexes by "H-substance," released from damaged cells during local cold exposure (42).

Greenfield and Shepherd (31), using a calorimetric technique to measure heat output from the fingertip during local cold exposure, demonstrated weak CIVD responses after sensory nerve degeneration or after the application of a local anesthetic to the tip of the finger (32). They suggested that an intact nerve was not necessary to elicit a response, but its presence enhanced cold vasodilation. This suggestion was supported by Shepherd and Thompson (56)

who noted an augmented dilator response to local cold exposure following regeneration of sensory nerves concomitant with the return of sensation. Calorimetric measurement of the CIVD response indicated that axon reflex vasodilation may be involved in CIVD, but was not the sole mechanism.

The direct action of a locally released vasoactive substance on cutaneous vessels was considered as a potential mechanism of cold vasodilation (15). Since intradermal injections of histamine can produce the skin surface changes observed immediately following cold injury (43), this agent has been tested for its involvement in the control of cold-induced vasodilation. Duff et al. (15) used the calorimetric technique employed by Greenfield and colleagues to record cold-induced vasodilation responses during intraarterial injection of histamine. Heat output from the index fingers was measured during simultaneous immersion of both digits in separate water calorimeters at 0°C. After local cold exposure, histamine was either intraarterially injected or electrophoretically applied to one of the fingers. Histamine had no effect on cold vasodilation, however, these data were difficult to interpret since histamine was applied during the initial minutes of cold exposure when intense vasoconstriction may have prevented the drug from reaching cutaneous vessels.

Although many other vasoactive substances have been tested to determine their action on skin vessels, few have

been examined in reference to altering peripheral vascular responses during local cold exposure. The effects of bradykinin (24), serotonin (51), and ATP (16) on the cutaneous circulation, for example, have been studied extensively in non-thermally stressed laboratory animals, but little work has been done to test their action on the cold-induced vasodilation response.

It has been suggested that the direct effect of cold on vascular smooth muscle can decrease the vessel's sensitivity to catecholamine, and initiate cold vasodilation. Using isolated strips of ulnar arteries, Keatinge (40) demonstrated a gradual decrease in sensitivity of the vessel to a 50 μ g dose of adrenaline when the artery was cooled in a 36°C to 5.8°C range. Adrenaline, which normally produced peripheral vascular constriction was ineffective when the vessel was cooled below 6.5°C (40). Keatinge also demonstrated that iontophoresis of adrenaline or noradrenaline during local cold exposure of the finger at 30°C constricted the vessels maximally, but failed to keep them constricted during exposure at 0°C (41). These studies suggest that blood vessels may passively dilate during local cold exposure due to a thermally induced decreased sensitivity of the vascular smooth muscle to catecholamines.

Cold-induced vasodilation has been recorded from body surface areas containing skeletal muscle (13). Mercury-in-rubber strain gauges, placed near the wrist and elbow, have

been used to measure skin and muscle blood flow respectively during local cold exposure of the forearm in a water plethysmograph at 1°C (12). Since flow in the lower forearm did not reach as high a level as the upper arm during CIVD, muscle blood supply was presumed to be involved in cold vasodilation (55). Folkow proposed a mechanism for CIVD responses recorded from extremities containing skeletal muscle. Knowing that skeletal muscle is susceptible to the vascular reflex that opposes stretch of the smooth muscle ("Bayliss response" or "myogenic reflex"), he suggested that an increase in flow during local cold exposure of the forearm may result from abolition of the myogenic reflex induced by extreme cold (20, 21). Folkow believed that inhibition of myogenic reflexes was one of many factors contributing to cold-induced vasodilation.

An increase in blood viscosity at low temperatures may be a potentially important factor in the control of cold-induced vasodilation. Nichol (49) reported that the increase in resistance to flow during cold exposure of the isolated perfused rabbit ear is entirely due to the change in viscosity of the perfusion fluid, and that blood viscosity changes partly explain the increase in resistance to flow in the cold exposed intact innervated ear. Burton (5) suggested that an increase in the viscosity of blood supplying an intact cold exposed extremity may initiate a chain of events ultimately resulting in cold vasodilation. Theoretically,

these events occur in the following order:

(i) increased resistance to flow between arterioles and venules across the capillary bed, resulting from an increase in blood viscosity.

(ii) increased pressure gradient between terminal arterioles and A-V shunts across the constricted sphinctors (assuming arterial pressure and vasoconstrictor tone constant).

(iii) blood forced through the constricted sphinctors, increasing flow through arterio-venous anastomoses (cold-induced vasodilation).

(iv) conductive heat transfer from the warm blood in A-V shunts to the capillary vessels.

(v) decreased blood viscosity in the capillary vessels.

(vi) reduced pressure gradient between terminal arterioles and A-V shunts.

(vii) sphinctors close under prevailing constrictor tone, reducing blood flow in the exposed extremity. The above changes in blood viscosity produce a series of events which can be repeated, thereby creating oscillatory changes in blood flow. Continued repetition of these events provides an explanation for the "hunting phenomenon" as well as cold-induced vasodilation.

The Appendix (Table I) lists in chronological order the mechanisms of cold-induced vasodilation proposed from human testing. Since the CIVD response may vary with sex (62),

diet (63), or emotional state of the individual (i.e., stress of examinations may increase the time to dilation (47) or verbally induced emotional stress may alter the rate of re-warming (1)), more rigorous standards are needed to examine the controlling mechanisms of CIVD.

Recently a CIVD response has been demonstrated for the footpads of anesthetized cats similar to the response observed for conscious human subjects (54), suggesting that this animal may be used for acute and chronic experiments to test the CIVD mechanisms. The study reported in this paper was undertaken to re-examine CIVD mechanisms by recording responses from the footpads of anesthetized cats after nerve degeneration and during histamine blockade with high levels of antihistamine. These data may be used for further interpretation of the mechanism originally advanced by Lewis.

CHAPTER III

METHODS AND MATERIALS

A. Preparation of the Animal

Male or female adult cats weighing between 2 and 4 kg. were anesthetized with sodium pentobarbital (Nembutal; 30 mg/kg.; I.P.). Areas surrounding both hindlimb footpads were clipped, and the calloused pads debrided with a lanolin base cleaner ("Pretty-Feet," Chemway Corp., Wayne, N.J.), and washed with soap and warm water. The cats were placed prone on a canvass hammock lined with a heating pad which maintained internal body temperature between 36 and 38°C. A thermistor probe (#401, Yellow Springs Instrument Co., Yellow Springs, Ohio) attached to a bridge unit (Telethermometer model #47, Yellow Springs Instrument Co., Yellow Springs, Ohio) was inserted 10 cm. in the lower colon to record rectal temperature. All experiments were performed at an ambient temperature of $25 \pm 2^{\circ}\text{C}$.

A 2 mm, 36g copper-constantan thermocouple was referenced to an ice water bath (maintained in a vacuum flask) and attached by a single layer of plastic tape (Surgical tape, Minnesota Mining and Manufacturing Co., St. Paul, Minn.) to each hindlimb footpad previously sprayed with a resin base

adherent (Ace adherent, Becton-Dickinson & Co., Rutherford, N.J.). Changes in emf produced by alterations in footpad surface temperature were continuously recorded on a multi-point strip chart instrument (Speedomax W "AZAR," Leeds and Northrup, Philadelphia, Pa.) calibrated (see Appendix, Table IV) from 0-40°C against a National Bureau of Standards thermometer. Calibration indicated that temperature could be recorded accurately to the nearest 0.1 of a degree Centigrade. Footpad surface temperatures were recorded for 2 minutes after they were stabilized between 33-36°C by adjusting rectal temperature within a 36-38°C range with a heating pad. After constant pre-immersion footpad temperatures were recorded, the distal 6 cm. of both extremities were simultaneously exposed to a constantly stirred, insulated water bath (Lab-line instruments, Inc.; Melrose Park, Ill.) maintained at 0°C with crushed ice trapped at the bottom of the bath. An air driven stirring motor (Precision Scientific Co.; Chicago, Ill.) circulated water for the duration of the cold exposure (about 20 minutes). The position of the hindlimb in ice water is shown in Figure 1. After removal of the extremity from the ice bath, footpad surface temperature was recorded until it returned to the initial pre-exposure level.

B. Quantification of the Cold-Induced Vasodilation Response

The cold-induced vasodilation response was characterized by four criteria: (i) time from initial immersion to the onset of the first dilation phase, (ii) time of initial immersion to peak dilation, (iii) temperature at peak dilation, and (iv) rate of rewarming. These criteria were determined by a standard method: "Rewarming rate" was established from the slope of a line connecting the point of "onset of dilation" and the point at which "peak temperature" was first reached. The time from initial immersion to the first point of "peak temperature" was computed as the "time to peak." A line was drawn tangent to the lowest footpad temperature before dilation to intersect the slope line. The time from initial immersion to the point of intersection was designated the "onset time."

C. Nerve Section Studies

This study was undertaken to evaluate the role of axon reflex dilation in the control of cold-induced vasodilation. Seventeen cats were tested as described in "Methods," (section A). Five of these animals demonstrated similar bilateral CIVD patterns during simultaneous local cold exposure; both extremities consistently rewarmed at the same time and at the same rate, and both reached the same peak temperatures at approximately the same time. The animals demonstrating

similar responses in both hindlimbs were used for the nerve section studies described below.

Animals were anesthetized with sodium pentobarbital (36 mg/kg.; I.V.). Incisions (5cm.) were made on the dorsal thigh and medial calf to expose the sciatic and tibial nerves in the right hindleg. The sciatic nerve was sectioned at mid-thigh level and the tibial nerve at the level of the ankle joint. The proximal ends of the nerves were turned cranially and the distal ends ligated to prevent possible regeneration. The skin incisions were sutured and the animals given 150,000 units of penicillin I.M. (Crysticillin, E.R. Squibb & Sons, N.Y.).

Cold exposures began fourteen days after nerve section to allow time for peak degeneration (63). CIVD responses were compared in the right and left hindlimb footpads over a period of three months. At least five days elapsed before an experiment was repeated on the same cat. A total of 23 experiments performed on five cats were evaluated by a standard paired comparison test (Student's t-test) in the intact-versus-denervated extremities.

D. Antihistamine Studies

An antihistamine was administered prior to local cold exposure in order to evaluate the role of histamine in the control of CIVD.

1. Selection of Drugs

Three drugs representing three distinct structural classes of antihistamines were chosen. They are: (i) an ethanolamine, diphenhydramine hydrochloride (Benadryl, Parke, Davis & Co.; Detroit, Michigan.); (ii) an ethylenediamine, tripeleennamine hydrochloride (Pyribenzamine, CIBA Pharmaceutical Co.; Summit, N.J.) and (iii) an alkylamine, chlorpheniramine (Chlor-trimeton, Shering Corp.; Bloomfield, N.J.). These particular agents were selected since they have been shown to be potent histamine antagonists with relatively low toxicities (44).

2. Characteristics of the Selected Antihistamines

Experiments were conducted to determine which antihistamine would be most effective in blocking the peripheral vascular activity of histamine without changing the normal cutaneous blood flow pattern.

Since antihistamines have been shown to exhibit different potencies in antagonizing the hypotensive response to injected histamine (44), Benadryl, Chlor-trimeton, and Pyribenzamine were tested to determine the most effective histaminic blocking agent. Nine male or female cats weighing between 2 and 4kg. were anesthetized with sodium pentobarbital (36 mg/kg.; I.P.). Heparin Sodium (Upjohn, Kalamazoo, Mich.) was used as anticoagulant in doses of 10 mg/kg. Both

carotid arteries were exposed and cannulated with polyethylene tubing (#90 P.E. tubing). The catheter in the left carotid artery was connected through a high pressure transducer (Model P23 Statham Laboratories Inc., Los Angeles, Calif.) to a strain guage preamplifier (350-100C Sanborn Co., Waltham, Mass.) and Sanborn recorder (7714-04A Sanborn Co., Waltham, Mass.) to record blood pressure. The cannula in the right carotid was directed into the abdominal aorta and used for rapid systemic injections of histamine (2,4,6,8 μ g) before and after a single dose of antihistamine (3.3 mg/kg. of Chlor-trimeton, 10 mg/kg. of Benadryl, or 10 mg/kg. of Pyribenzamine), slowly infused over a period of 1-3 minutes. Three experiments were conducted on separate cats for each drug.

Since previous studies have shown that antihistamine, either systemically or topically applied, may cause vasoconstriction in the microcirculation (2,23), experiments were conducted to determine whether systemically injected antihistamine could alter a control footpad temperature. Nine male or female cats were anesthetized with sodium pentobarbital (36 mg/kg.; I.P.). The animals were placed on a heating pad (except the hindlimbs), to maintain the internal body temperature constant, while footpad surface temperatures were recorded in the manner previously described (see Methods, section A). Temperature measurements were made before, during and twenty minutes after a single intra-venous dose of

3.3 mg/kg. of Chlor-trimeton or 10 mg/kg. of Benadryl or 10 mg/kg. of Pyribenzamine. Three separate experiments were conducted for each drug.

The method used to record cold-induced vasodilation involved measuring temperature changes from the cat's footpad. As a result the antihistamines were tested to determine whether they could block the effect of systemically injected histamine on footpad temperature.

Copper-constantan thermocouples, attached to each hindlimb footpad by a single piece of plastic tape, were used to record footpad surface temperatures (see Methods, section A). A single carotid artery was cannulated and used for drug injection. The animal was placed on a heating pad (except the hindlimbs) to maintain the internal body temperature and footpad surface temperature between 36-38°C and 33-36°C respectively. After thermal stability was achieved within these ranges, histamine (2,4,6µg) was systemically injected through the cannula before and after a single dose of antihistamine (Benadryl, 10 mg/kg.; Pyribenzamine, 10 mg/kg.; Chlor-trimeton, 3.3 mg/kg.).

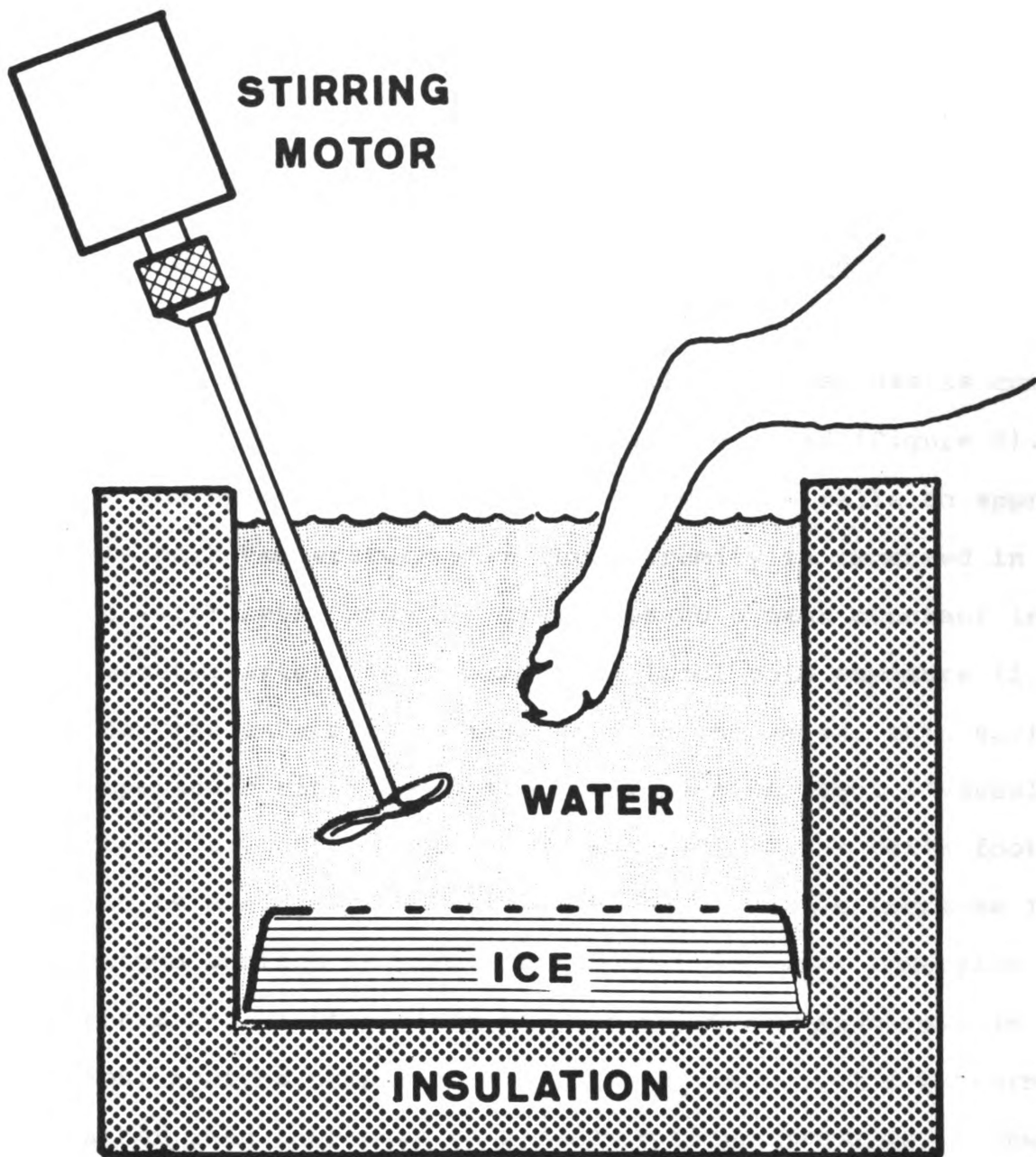
3. Effect of Benadryl on CIVD

The hypothesis that cold-induced vasodilation is dependent on the release of histamine was tested by administering Benadryl (10 mg/kg.; I.V.) prior to local cold exposure. Control CIVD responses were measured on nine cats

by the method previously described (see Methods, section A). At least five days elapsed before a CIVD response was tested on Benadryl pretreated animals. Control and experimental values were obtained for both hindlimbs in each cat, providing data for eighteen comparisons. These data were analyzed statistically by the Student's t-test.

Figure 1

Position of hindlimb footpad immersed in
stirred insulated ice-water bath.



CHAPTER IV

RESULTS

A. CIVD in the Human Finger and Cat's Footpad

A typical cold-induced vasodilation response is compared for the human finger and cat's footpad (Figure 2). For the human, digital surface temperature falls to approximate bath temperature when the extremity is immersed in an ice water bath, and then increases to a near constant level 9 minutes after the beginning of local cold exposure (1,31). After the extremity is removed from the water, skin surface temperature rises above the pre-immersion level ("vascular after-reaction"). Local cold exposure of the cat's footpad elicits a similar CIVD response, however, the increase in footpad surface temperature during cold water immersion occurs 3-5 minutes after the beginning of exposure. In addition, footpad temperature fluctuates in a cyclic pattern rather than increases to a near constant level as in the human response. After the extremity is removed from the ice water, footpad surface temperature rises above the pre-immersion level, similar to the "vascular after-reaction" observed by Lewis (42).

B. Nerve Section Studies

Five of seventeen control cats demonstrated similar bilateral cold-induced vasodilation responses prior to nerve section (Figure 3). Table I represents a quantitative comparison of the CIVD pattern for the right and left hindfootpads in each of these animals before denervation. Figure 4 illustrates these changes in cold vasodilation produced by unilateral nerve section and subsequent nerve degeneration. These tracings show that the denervated footpads dilate later than the controls, but warm to nearly the same peak temperatures as the controls. The results of 23 cold exposures (see Appendix, Table II) are statistically compared (Student's t-test) in the intact and chronically denervated hindlimbs (Figure 5).

C. Antihistamine Studies

1. Characteristics of the Antihistamines

Figures 6, 7, and 8 report recordings of mean arterial blood pressure during "bolus" injections of histamine (2-8 μ g) before and after administration of antihistamine (see Methods, section D-2). These data show that injections of 10 mg/kg. of Pyribenzamine (Figure 6) and 3.3 mg/kg. of Chlor-trimeton (Figure 7) do not block the hypotensive response to systemically injected histamine, but that 10 mg/kg. of Benadryl (Figure 8) effectively antagonizes the action of histamine on

the peripheral circulation. Data reported in Figure 9 indicate changes in mean arterial blood pressure (ordinate) as a function of increasing doses of histamine (abscissa).

After administration of Pyribenzamine or Chlor-trimeton, histamine still reduces mean blood pressure by as much as 30 mm Hg and 35 mm Hg respectively; however, histamine does not alter blood pressure in the Benadryl pretreated cats.

The results of three experiments demonstrating the effect of the selected antihistamines on control footpad temperature are shown in Figure 10. Pyribenzamine (10 mg/kg.) and Chlor-trimeton (3.3 mg/kg.) cause large decreases in footpad temperature (3.4 and 6.8°C respectively over a period of 20 minutes), while Benadryl (10 mg/kg.) has essentially no effect (fall of 0.8°C over a period of 20 minutes) on footpad temperature.

Data reported in Figure 11 imply that Benadryl blocks vascular changes in the footpad produced by histamine. Extremity temperature is decreased during injection of histamine (2-6µg), but this response is blocked after systemic administration of Benadryl (10 mg/kg.). This experiment was omitted for Chlor-trimeton and Pyribenzamine, since these agents could not compare with the effectiveness of Benadryl in antagonizing the depressor response of injected histamine (Figure 8) without changing the normal cutaneous blood flow pattern (Figure 10).

2. Effect of Benadryl on CIVD

The recordings in Figure 12 compare typical CIVD responses of both hindlimb footpads before and after an intravenous injection of Benadryl (10 mg/kg.). These data show that cold vasodilation is not blocked by this dose of Benadryl which completely antagonizes the vascular action of injected histamine (Figure 8). The results of nine experiments (see Appendix, Table III) are statistically compared (Student's t-test) in the control and antihistamine pretreated footpads (Figure 13) to evaluate the effect of Benadryl (10 mg/kg.; I.V.) on the cold-induced vasodilation response.

Figure 2

Cold-induced vasodilation response of human finger (T_h) and cat's footpad (T_c). The extremities were immersed and removed from the stirred ice-water bath at "IN" and "OUT" respectively.

$$T_c = \text{---}$$

$$T_h = \text{---}$$

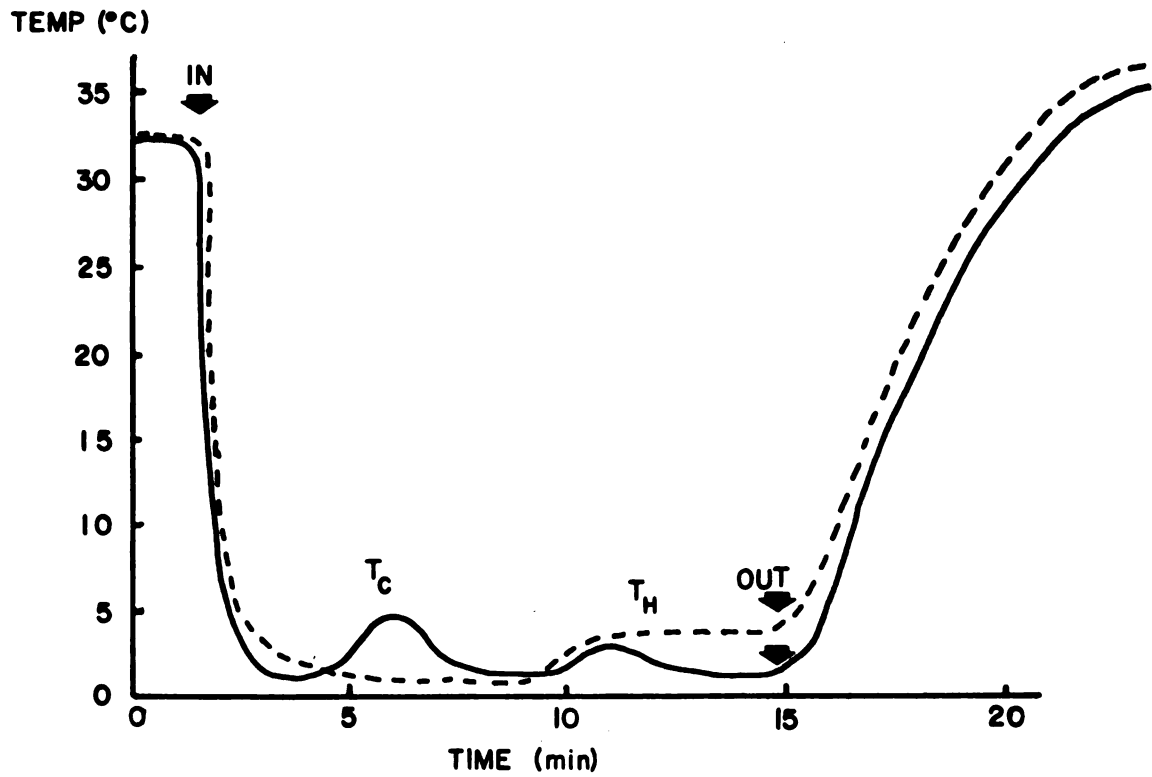


Figure 3

CIVD responses prior to nerve section (Cats A-E).
The time of immersion is at $t=0$, and the initial
footpad temperature is indicated by "IFT."

R_t = rectal temperature
---- = right footpad
—— = left footpad

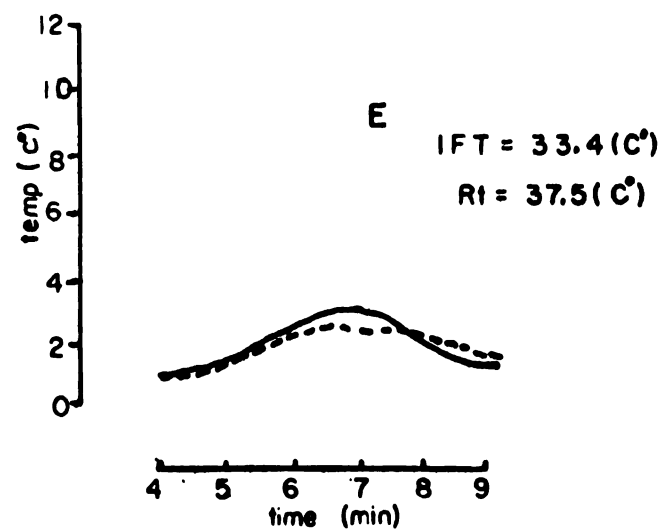
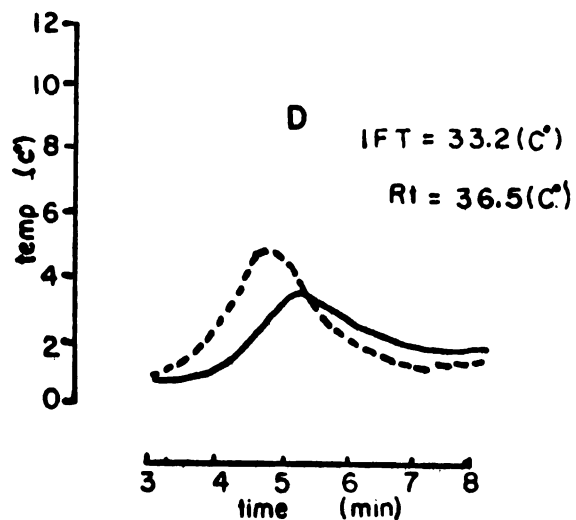
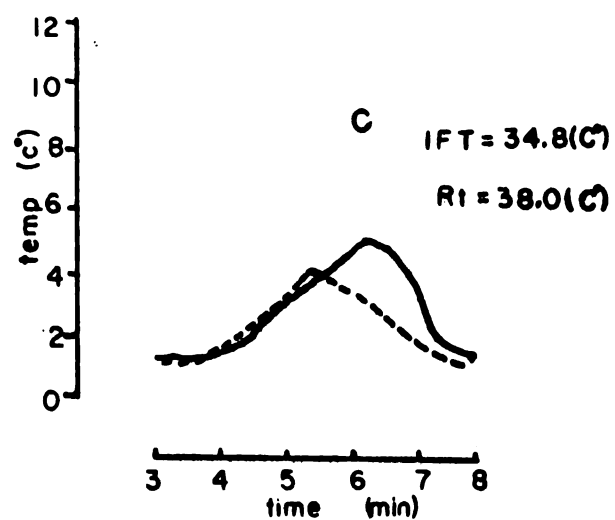
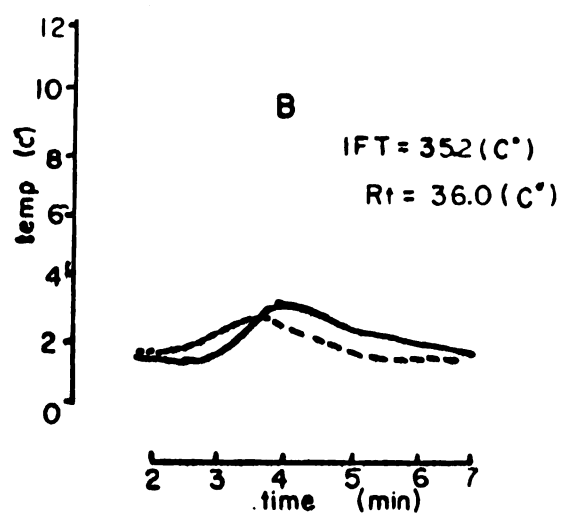
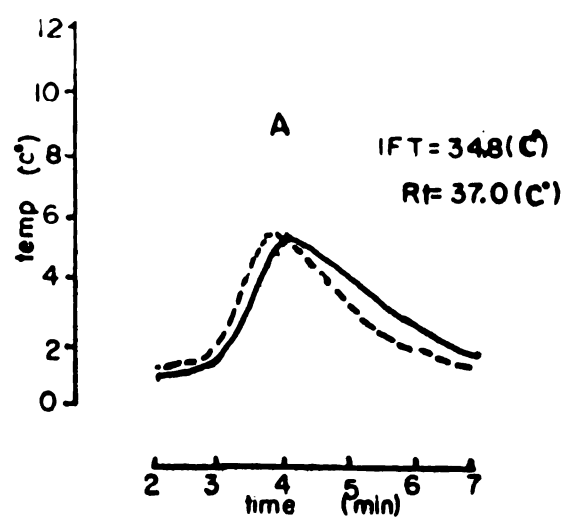


Table I. CIVD Responses Prior to Nerve Section

Cat	Temp. Peak (°C)		Rewarming Rate (°C/min)		Onset Time (min)		Time to Peak (min)	
	LF	RF	LF	RF	LF	RF	LF	RF
A	5.2	5.6	7.2	9.2	3.2	3.2	4.0	3.9
B	3.6	3.2	3.2	2.4	3.4	3.1	4.2	3.8
C	5.4	4.0	2.4	2.4	3.9	3.6	6.1	5.1
D	3.6	4.4	3.6	5.6	4.7	4.3	5.5	4.9
E	3.6	2.8	1.4	0.6	4.5	4.5	6.5	6.7

Figure 4

CIVD responses after section of the right sciatic and tibial nerves. The time of immersion is at $t = 0$, and the initial footpad temperature is at "IFT."

R_t = rectal temperature

T_d = days after nerve section

---- = right footpad

—— = left footpad

IFTR = initial footpad temperature
of right footpad

IFTL = initial footpad temperature
of left footpad

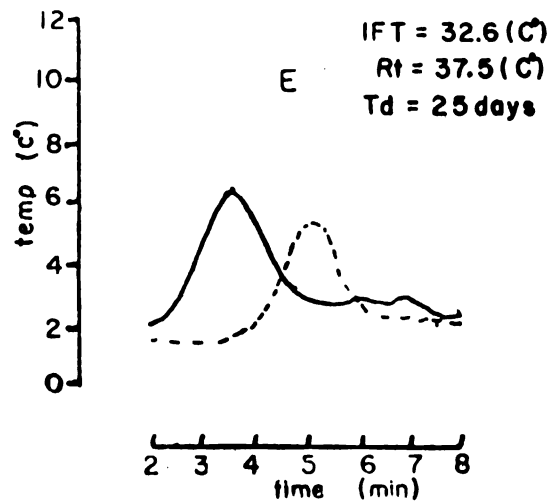
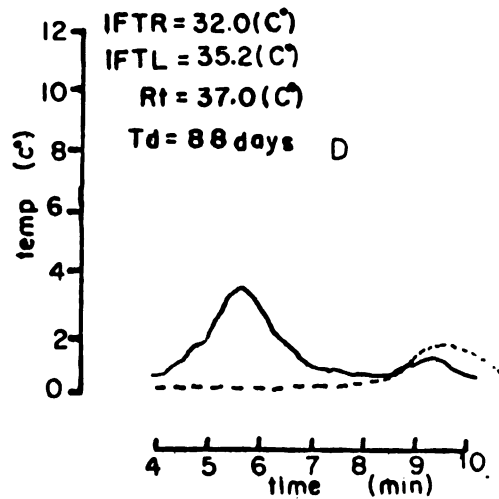
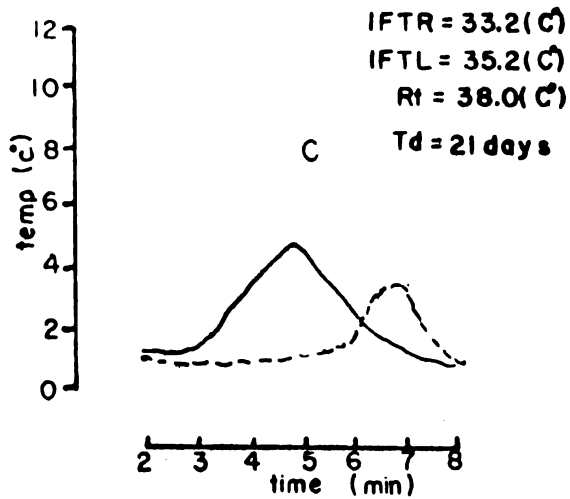
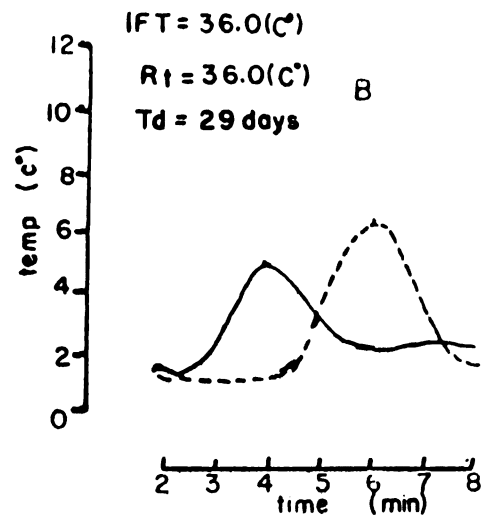
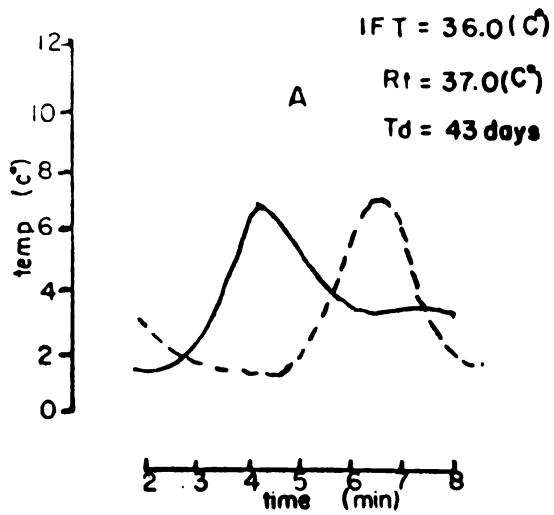


Figure 5

Statistical analysis of CIVD responses in the control and denervated hindlimb footpads.



= control footpad illustrating mean value and standard error



= denervated footpad illustrating mean value and standard error

N = 23

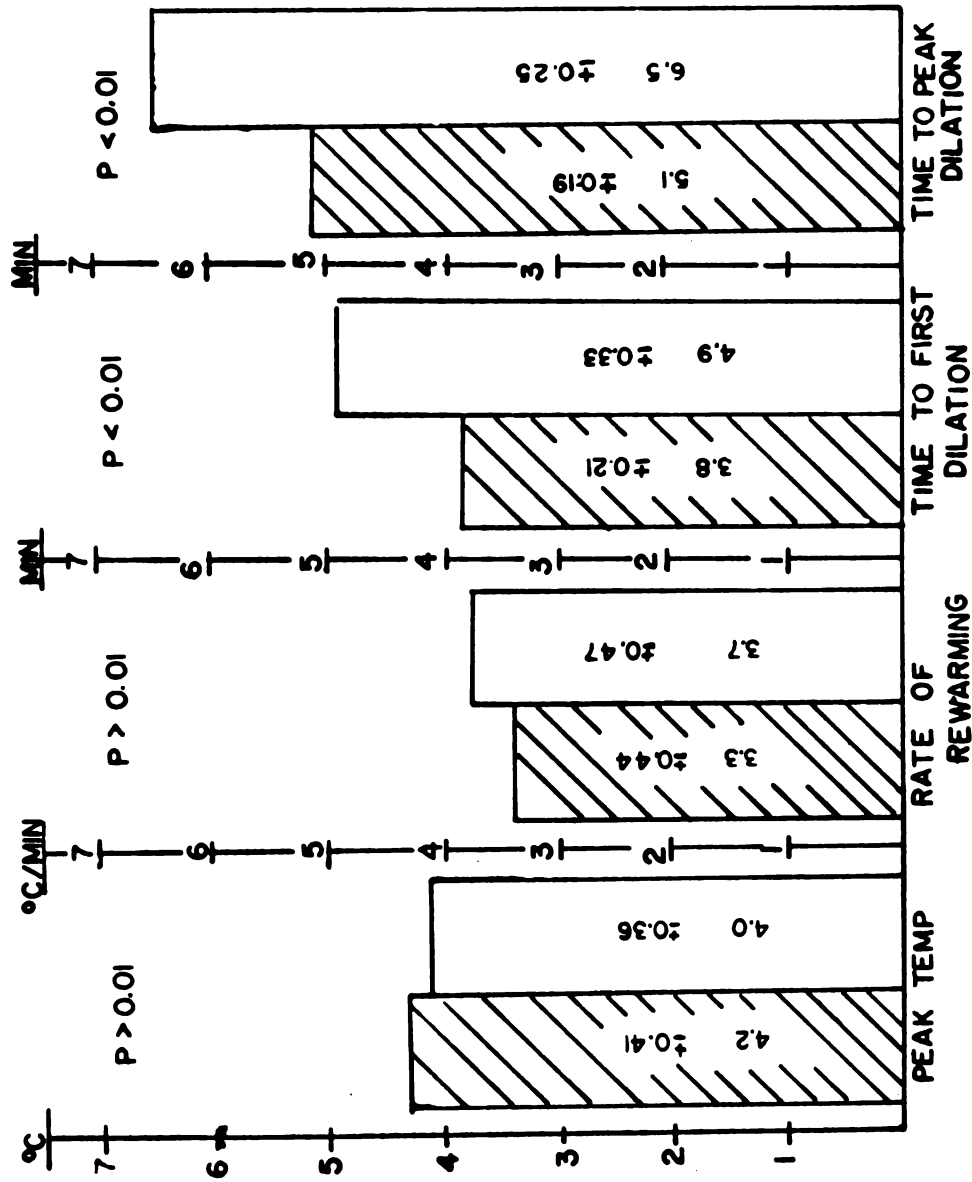


Figure 6

The effect of histamine on mean arterial blood pressure (ABP) before and after the systemic administration of Pyribenzamine (10 mg/kg.). Histamine and Pyribenzamine injected into the carotid artery (see Methods, p. 18).

1,2,3 = effect of histamine on mean arterial blood pressure before Pyribenzamine

4,5,6 = effect of histamine on mean arterial blood pressure after Pyribenzamine

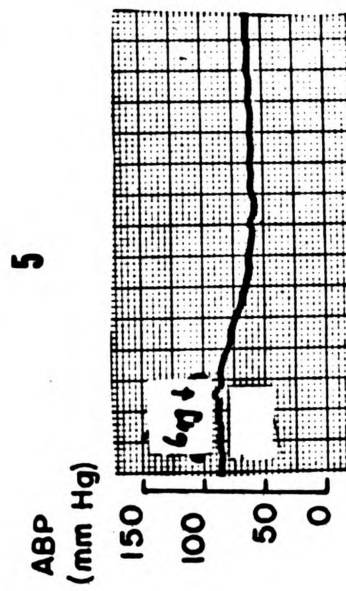
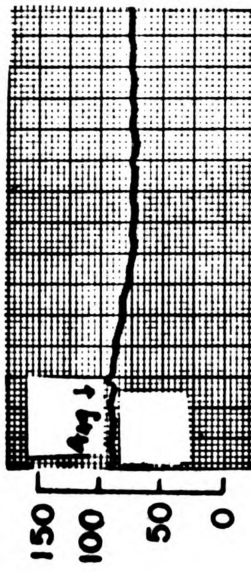
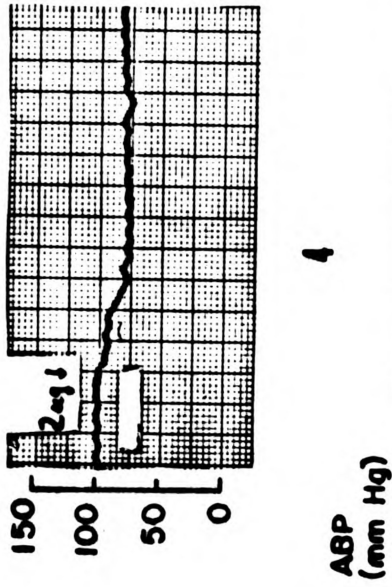
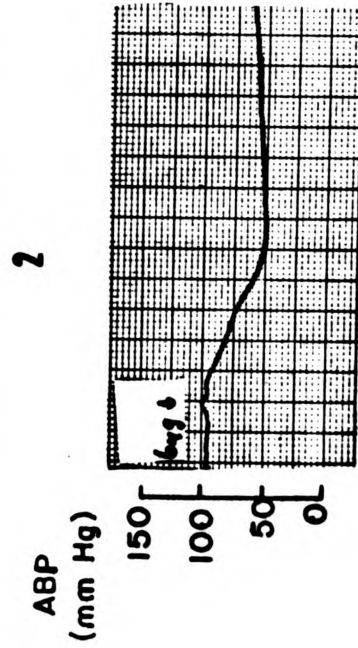
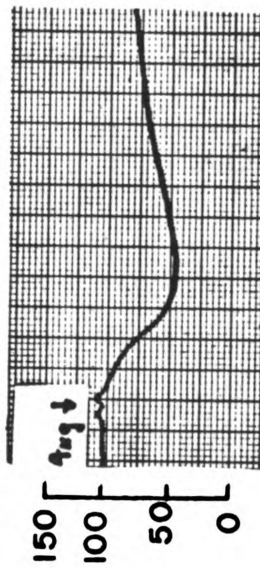
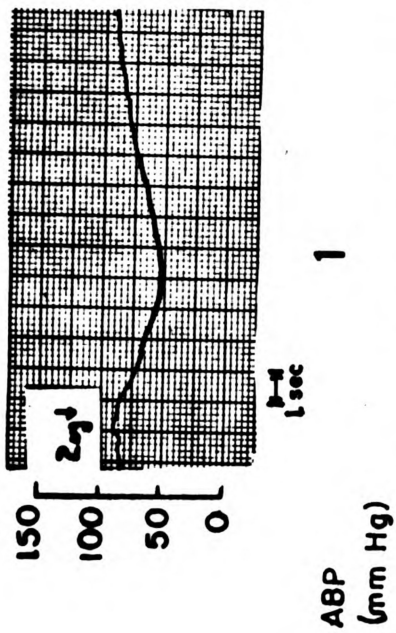


Figure 7

The effect of histamine on mean arterial blood pressure (ABP) before and after the systemic administration of Chlor-trimeton (3.3 mg/kg.). Histamine and Chlor-trimeton injected into the carotid artery (see Methods, p. 18).

1,2,3 = effect of histamine on mean arterial blood pressure before Chlor-trimeton
4,5,6 = effect of histamine on mean arterial blood pressure after Chlor-trimeton

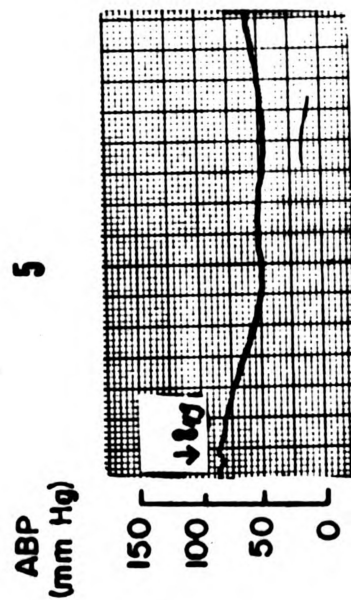
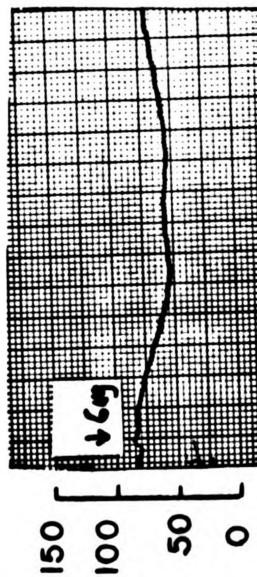
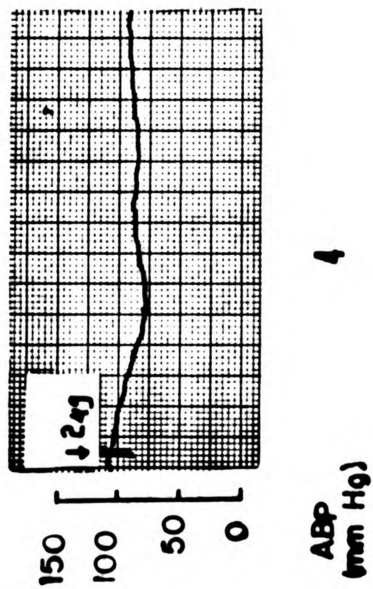
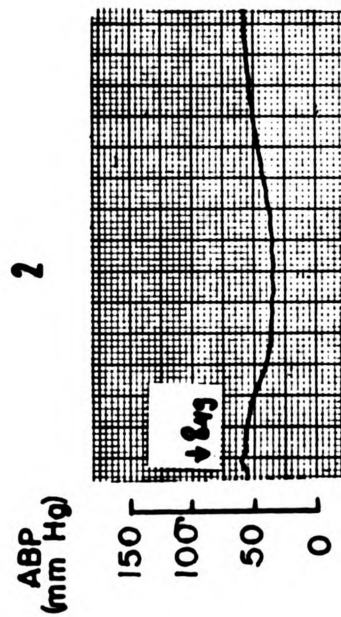
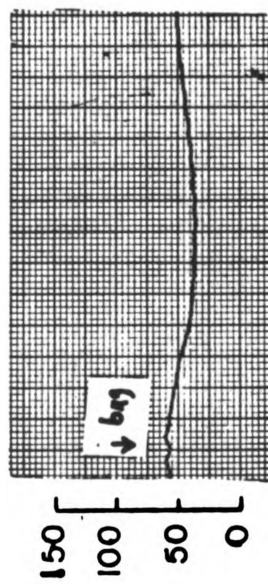
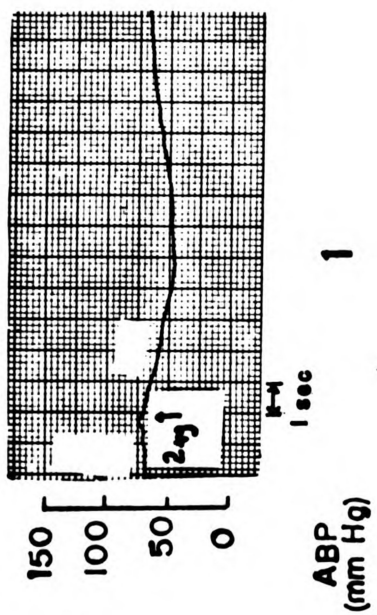


Figure 8

The effect of histamine on mean arterial blood pressure (ABP) before and after the systemic administration of Benadryl (10 mg/kg.). Histamine and Benadryl injected into the carotid artery (see Methods, p. 18).

1,2,3 = effect of histamine on mean arterial blood pressure before Benadryl

4,5,6 = effect of histamine on mean arterial blood pressure after Benadryl

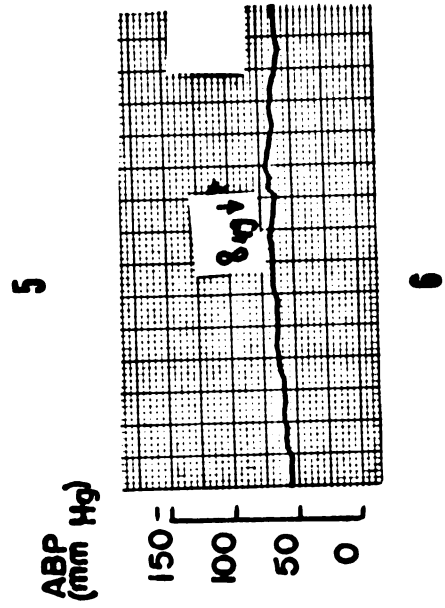
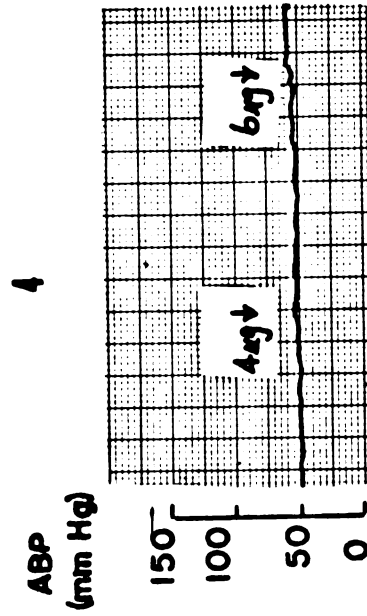
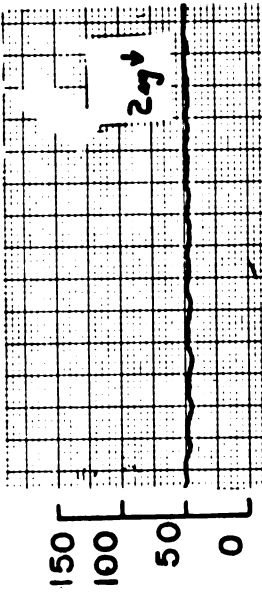
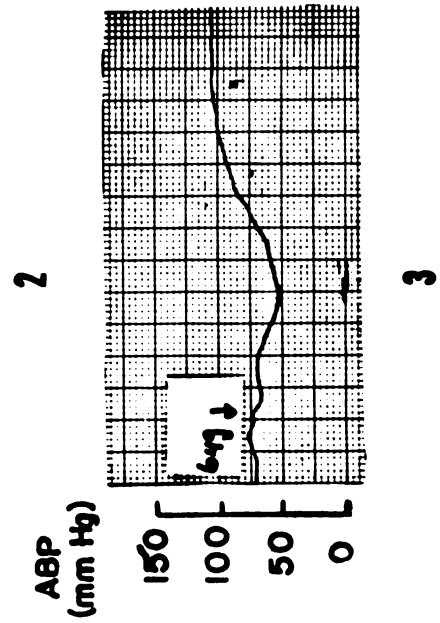
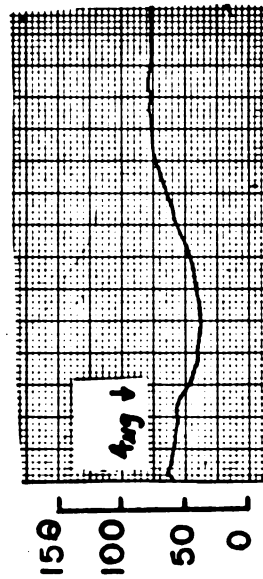
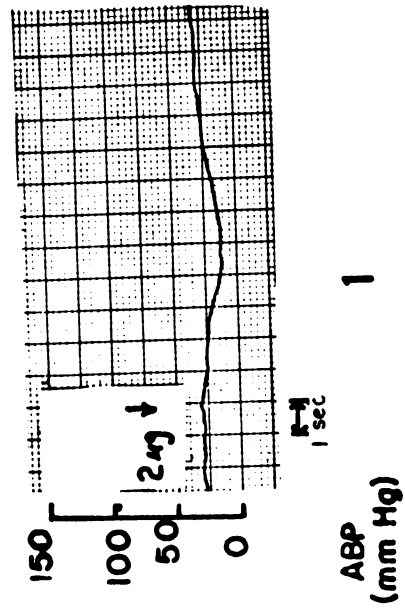


Figure 9

Changes in mean blood pressure as a function of systemic administration of histamine.

—— = before antihistamine

---- = after antihistamine

A = Pyribenzamine (10 mg/kg.)

B = Chlor-trimeton (3.3 mg/kg.)

C = Benadryl (10 mg/kg.)

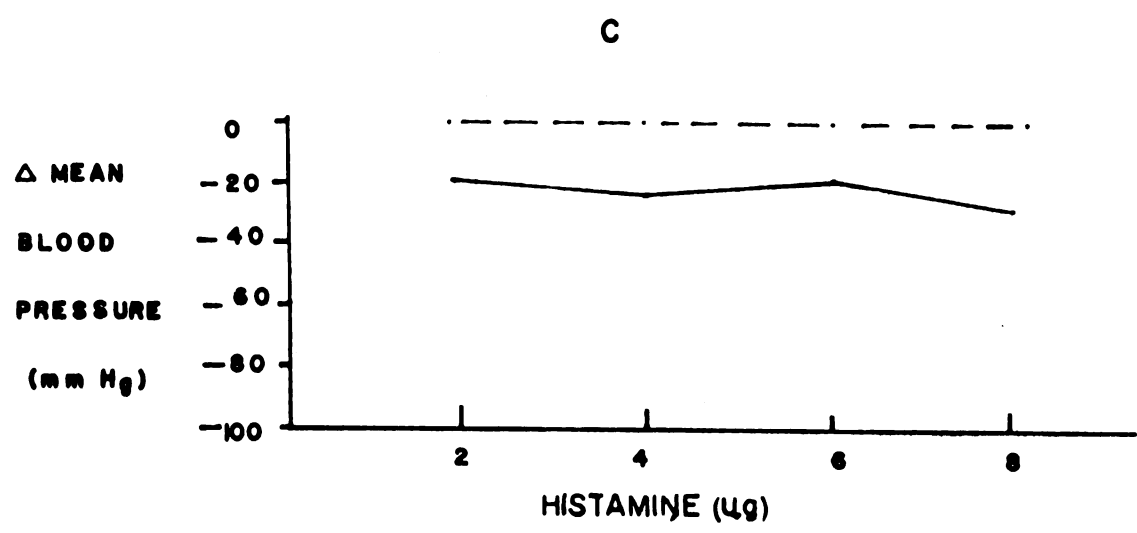
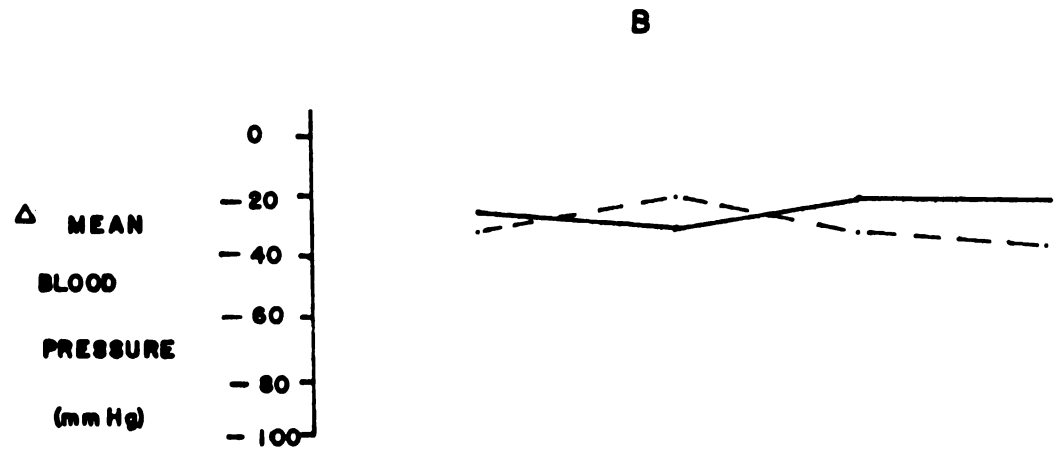
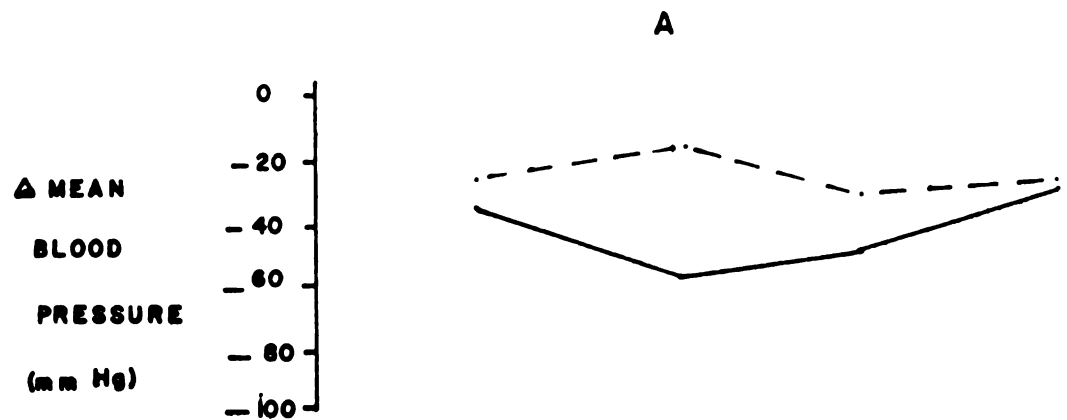


Figure 10

Effect of Pyribenzamine, Chlor-trimeton, and
Benadryl on control footpad temperature.

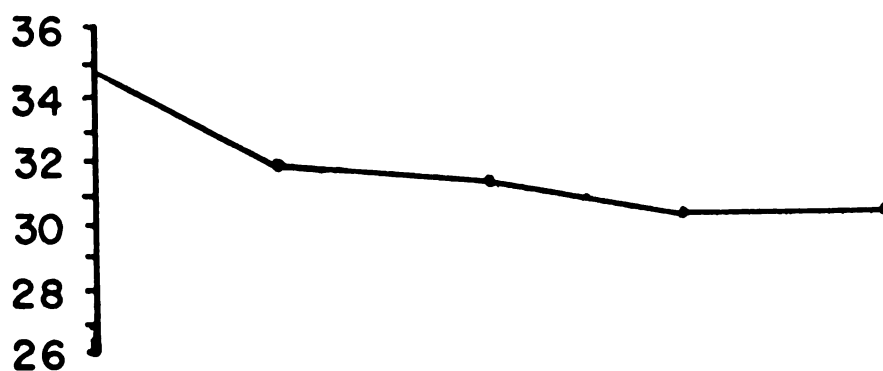
A = Pyribenzamine (10 mg/kg.; I.V.)

B = Chlor-trimeton (3.3 mg/kg.; I.V.)

C = Benadryl (10 mg/kg.; I.V.)

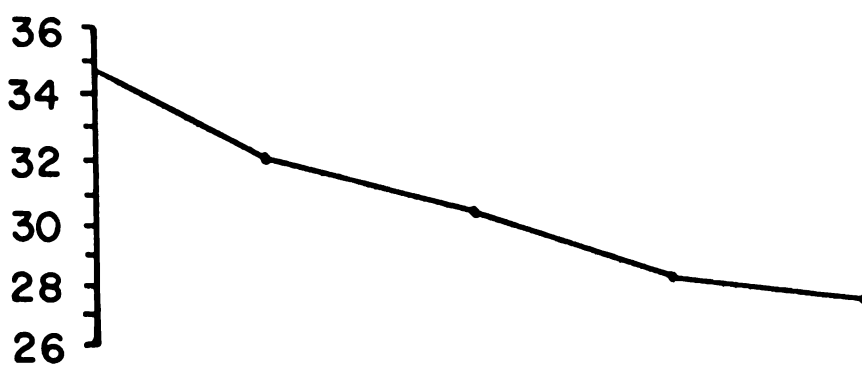
TEMP. (°C)

A



TEMP. (°C)

B



TEMP. (°C)

C

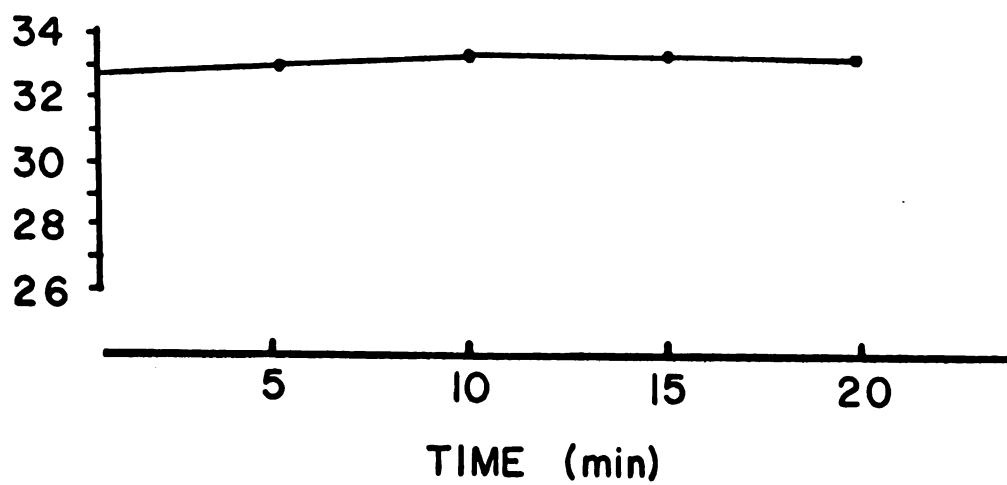


Figure 11

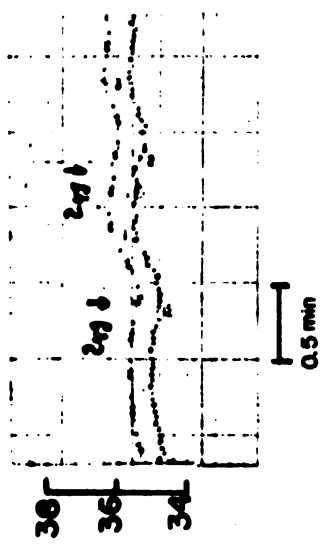
The effect of Histamine of footpad temperature before and after the administration of Benadryl. Histamine and Benadryl (10 mg/kg.- injected into the carotid artery (see Methods, p. 20).

Even numbered data points = left footpad

Odd numbered data points = right footpad

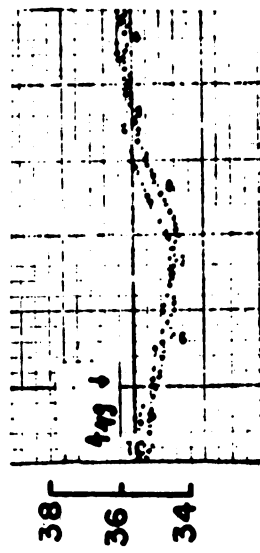
1,2,3 = before Benadryl

4,5,6 = after Benadryl



TEMP (°C)

1

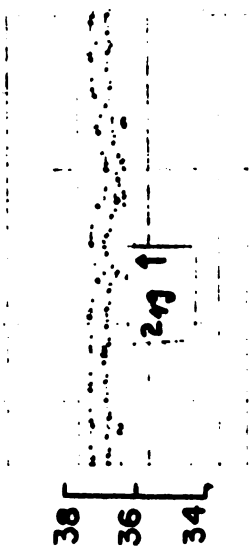


TEMP (°C)

2



3



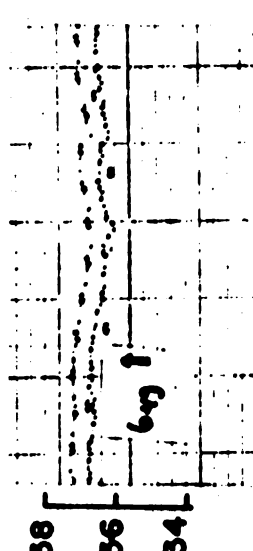
TEMP (°C)

4



TEMP (°C)

5



6

Figure 12

Typical CIVD responses before and after administration of Benadryl. The time of immersion is at $t=0$, and the initial footpad temperature is indicated by "IFT."

A = CIVD response during simultaneous local cold exposure of both hindlimb footpads

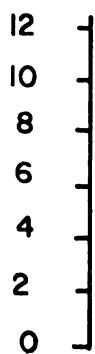
B = CIVD response of the same cat after Benadryl (10 mg/kg.; I.V.)

R_t = rectal temperature

----- = right footpad

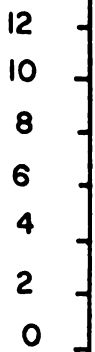
———— = left footpad

TEMP. (°C)



A

IFT = 34.0 (c°)
Tr = 37.0 (c°)



B

IFT = 34.8 (c°)
Tr = 37.0 (c°)

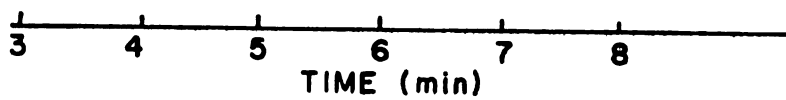
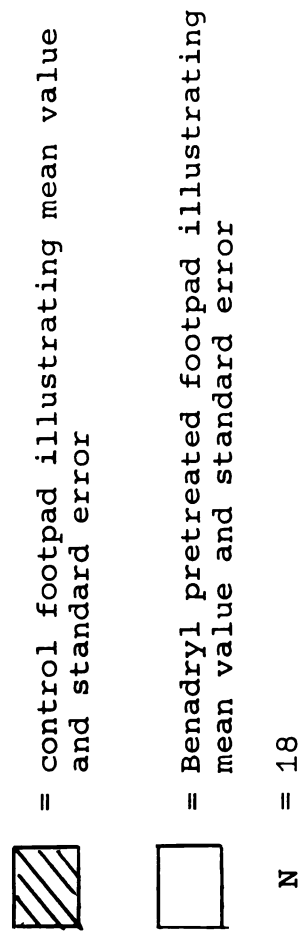
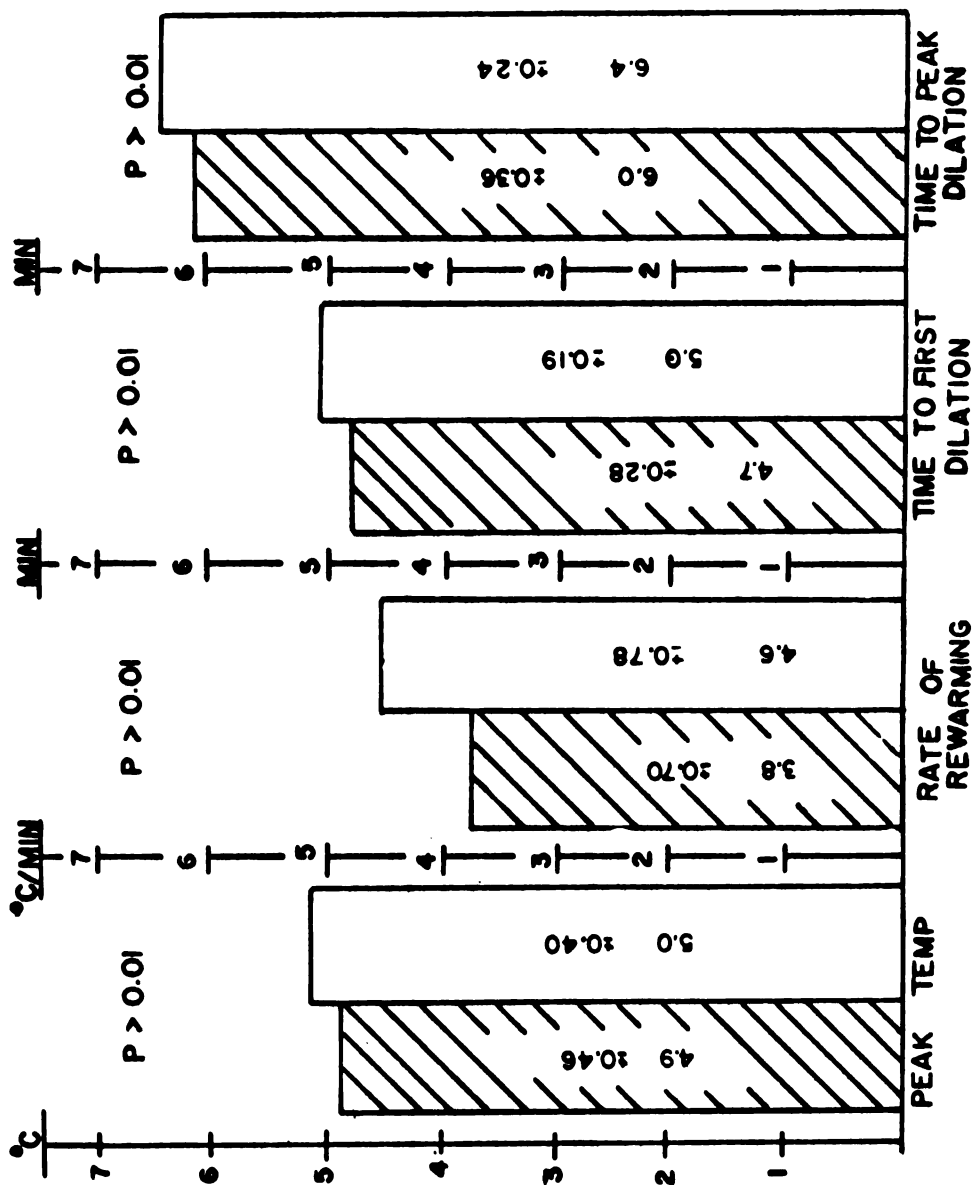


Figure 13

Graphic illustration of the statistical analysis of CIVD responses in the control and Benadryl pretreated animals.





CHAPTER V

DISCUSSION

A. Skin Surface Temperature Measurements as an Index of Blood Flow

A change in extremity blood flow during local cold exposure of the hindlimb footpad was indexed by a change in footpad surface temperature (see Methods, section A). Skin surface temperature depends upon the composite effects of the heat flow rate from the body core to the skin surface, the heat flow rate from the skin surface to the surroundings, and the rate at which heat is stored in the tissues. As described by Burton and Edholm (7), the rates of heat flow through the tissue to the skin surface, and from the skin surface to the environment are represented in two thermal gradients: (i) internal temperature drop (the thermal gradient between the body core and skin surface) $T_c - T_s$, and (ii) an external temperature drop (the thermal gradient between the skin surface and surroundings), $T_s - T_a$.

At a thermal steady state (heat production is equal to heat loss and total body heat content is constant) the internal temperature gradient, and consequently heat flow to the skin surface, is dependent upon the tissue thermal

insulation. The relationship between heat flow rate (H_1 ; Kcal sq.m.⁻¹hr.⁻¹), internal temperature gradient ($T_c - T_s$; °C), and tissue insulation (I_t ; °C Kcal⁻¹ sq.m.⁻¹ hr.⁻¹) is expressed by (7):

$$H_1 = T_c - T_s / I_t \quad (1)$$

Tissue insulation is a function of tissue thermal conductivity and thermal convection, the former varying with tissue composition and the latter with peripheral blood flow. Since experiments reported for the cat's footpad were conducted over periods too short to allow changes in tissue composition, it can be argued that I_t varies primarily as a function of tissue perfusion. With a constant internal temperature (T_c), and the animal in a thermal steady state, the internal temperature gradient and change in I_t is a function only of T_s .

When the environmental temperature (T_a) is constant, the external thermal gradient ($T_s - T_a$; °C) and rate of non-evaporative heat loss from the skin surface to the surroundings (H_2 ; Kcal sq.m.⁻¹ hr.⁻¹) is a function of the environmental insulation (I_a ; °C Kcal⁻¹ sq.m.⁻¹ hr.⁻¹). This relationship may be expressed by (7):

$$H_2 = T_s - T_a / I_a \quad (2)$$

The environmental insulation of a bare-skinned body exposed to a fluid is affected primarily by the temperature of a thin

layer of fluid next to the skin surface. If the integrity of the thin fluid layer is modified by convection in the external environment, the thermal "insulation zone" is reduced. By immersing an extremity in a constantly stirred water bath maintained at 0°C (see Methods, Section A), the external thermal gradient and subsequently heat loss to the environment is defined solely as a function of skin surface temperature.

If the heat delivered to the peripheral tissues is greater than the heat lost from the skin surface (i.e., cold vasodilation), the excess heat is stored and the average temperature of the tissue increases. The rate of heat storage is a function of the mass, specific heat, and surface area of the tissue. A change in any of these parameters will affect the rate of tissue temperature rise. During local cold exposure of the cat's footpad the tissue composition presumably did not change; thus, the rate of heat storage is constant. As a result footpad surface temperature is only affected by the heat flow rates from the core to the skin surface, and from the skin surface to the environment.

At a thermal steady state ($H_1 = H_2$ and total body heat content as measured by T_c constant) where external insulation and environmental temperature is stabilized (see Methods, section A), skin surface temperatures reflects the physiological condition of tissue insulation. Using this argument,

Burton (6) devised a "thermal circulatory index" (TCI) which expresses the ratio of external insulation to tissue insulation and described a linear index of tissue perfusion.

$$TCI = I_a/I_t = (T_s - T_a)/(T_c - T_s) \times k \quad (3)$$

where k represents a constant that corrects for heat loss by evaporation ("insensible perspiration") from the bare skin surface of the human ($H_1 = 1.21 H_2$) (7). Since rates of water evaporation from the furred skin can be expected to be less than from bare human skin, and since exposure conditions in the present study (see Methods, section A), precludes evaporative losses from the footpad, k (equation 3) is assumed to be 1. An increase in the TCI represented by a change in T_s when T_c , T_a , k are constant and I_a is stabilized would result from a decrease in tissue insulation brought about by a matched increase in peripheral circulation.

If the TCI is used as an index of blood flow during local cold exposure of an extremity, the inflowing arterial blood must approach deep body temperature and outflowing venous blood approximate skin surface temperature to insure that counter-current heat exchange does not occur in the cooled limb (4). Edwards and Burton (18) have shown that during local cold exposure of the human finger (0°C), the temperature of the inflowing arterial blood and outflowing venous blood is nearly equal to deep body and skin surface

temperatures respectively as long as the individual is in "warm surroundings and in a general state of vasodilation." Variability in footpad surface temperature due to counter-current heat exchange can presumably be eliminated by maintaining a constant high internal body temperature (see Methods, section A) during local cold exposure of the cat's footpad.

In summary, surface temperatures measured from the hairless footpad can be an index of blood flow during controlled conditions described in "Methods" (section A) (i.e., environmental temperature maintained constant by rapidly stirring the ice water bath, internal body temperature held constant by external heating, counter-current heat exchange limited (18), mass, specific heat and surface area of extremity constant).

B. CIVD in the Anesthetized Cat

Numerous investigators have described the local blood flow changes (initial vasoconstriction, cold vasodilation, "vascular after-reaction") which occur in the extremities of unanesthetized human subjects (25,42,30,25,42). Similar changes in the initial vasoconstriction and "vascular after-reaction" patterns have been demonstrated in the footpad of the anesthetized cat during local cold exposure; however, cold vasodilation of the cat's footpad differs from the human response in several respects. First, restoration of

blood flow to the footpad usually begins 2-3 minutes after cold exposure, while the finger typically remains vasoconstricted throughout the first 8-9 minutes of exposure (Figure 2). This pattern of human response has been reported earlier (38,42). The temporal difference may be explained by a more rapid fall of internal footpad temperature due to a larger surface area to mass ratio for the cat's footpad (54). Secondly, cold vasodilation in the human digit is a sustained response which persists for the duration of the exposure. In contrast, the footpad usually exhibits alternating periods of vasoconstriction and vasodilation (Figure 2). This variation does not appear to be primarily species related, since the cat's ear responds to cold with a sustained dilation similar to that of the finger (54).

C. CIVD After Sensory Nerve Degeneration

The primary objective of the experiments reported in "Results" (section B), was to determine the role of axon reflexes in cold-induced vasodilation. In order to examine Lewis' hypothesis related to neural influences on vascular reactivity to cold, CIVD responses were tested in the cat after sciatic and tibial nerve degeneration. As indicated in Figure 5, the magnitude of cold vasodilation (represented by "peak temperature" and "rewarming rate") was not significantly altered following sensory nerve degeneration ($P > 0.01$). These data do not support Lewis' hypothesis regarding axon

reflex involvement in cold vasodilation, and suggest that CIVD is primarily dependent upon mechanisms other than vasodilation triggered by antidromic nerve conduction. Data from the nerve section study, however, cannot exclude axon reflex vasodilation as a possible component of the CIVD response, since "onset time" and "time to peak" were significantly delayed in the denervated footpads ($P < 0.01$) (Figure 5) when compared to control values. If axon reflexes are involved in cold vasodilation, they apparently play a minor role in the magnitude of the response, if not in the entire pattern of reactivity.

The concept that axon reflex vasodilation may be involved in the CIVD response, but not as the primary mechanism controlling this response has been supported by several investigators. Greenfield et al. have shown that in the human finger, cold vasodilation occurs in the absence of axon reflexes. They demonstrated that "weak" CIVD responses could be recorded following section and degeneration of nerves innervating the finger (31), and following infiltration of the finger tip with an anesthetic solution (32). Shepherd and Thompson (56) reported that axon reflexes were not necessary to elicit CIVD, but enhanced cold vasodilation in the human digit. They demonstrated an augmented dilator response to local cold exposure following regeneration of sensory nerves concomitant with the return of sensation. In contrast, Celander and Folkow (8) have shown that the

CIVD response during local cold exposure of the cat's footpad is abolished in the sympathectomized, deafferentated, antihistamine pretreated hindlimb. Since histamine apparently does not participate in cold vasodilation (see Discussion, section E), and sympathectomy does not abolish the response (30,42), the change in cold vasodilation described by these investigators may be due to axon reflex degeneration.

By measuring changes in venous outflow during local cold exposure of the cat's footpad, Celander and Folkow failed to record a CIVD response after sensory nerve degeneration (8); however, cold vasodilation has been demonstrated in the denervated cat's footpad when skin surface temperatures were used to record the response (Figure 4). It is not known why there is a discrepancy in results using different recording techniques, although failure to stabilize internal body temperature between 36-38°C during venous outflow measurements must be considered as an uncontrolled variable.

D. Benadryl as a Histamine Antagonist

Since it has been reported that locally released histamine is involved in cold vasodilation (42), three potent and nontoxic antihistamines were selected (see Methods, p. 17) to block the vascular effect of histamine during local cold exposure of the cat's footpad. Benadryl (10 mg/kg.) appears to be the most affective of the three agents in antagonizing histaminic vascular receptor sites in the anesthetized cat.

Data reported in Figure 9 show that Benadryl is a more potent blocking agent compared with Pyribenzamine and Chlor-trimeton. Benadryl completely antagonizes the hypotensive effect of histamine on the systemic circulation (Figure 8) as well as its vascular effect on the cutaneous circulation (Figure 11).

Previous studies have shown that systemically injected antihistamines have vascular effects other than those attributed to the suppression of endogenous or exogenous histamine (2,45,57). Treatment with Benadryl diminishes the depressor response to injected acetylcholine and histamine, suggesting that this drug is not specific for histamine vascular receptor sites (45). It has also been demonstrated that an enhanced and prolonged pressor response to epinephrine occurs in anesthetized dogs treated with Benadryl (57). Finally, intravenous injections of Benadryl may cause vasoconstriction of microvessels in the mesoecum of rats (2). It is evident that systemic administration of Benadryl in anesthetized cats might alter blood flow to the peripheral circulation prior to local cold exposure, thereby possibly changing the normal cold-induced vasodilation response. These non-specific properties of Benadryl apparently do not affect blood flow to the cutaneous circulation, since the drug failed to alter footpad temperature for at least 20 minutes after its systemic administration (Figure 10).

Data reported in "Results" (section C-1) suggest that for anesthetized cats, a high dose of systemically injected Benadryl (10 mg/kg.) effectively antagonizes the vascular action of histamine on the peripheral circulation without greatly reducing its blood flow. As a result, this anti-histamine may be used with reliability to block the vascular action of histamine on the cutaneous blood vessels in anesthetized cats.

E. CIVD in Benadryl Pretreated Cats

Lewis reported that histamine, released by an injurious stimulus (e.g., cold), dilates arterioles by activation of axon reflexes innervating these vessels, and dilates minute vessels by its direct action (see Proposed Mechanisms of CIVD, p. 7). Vascular responses of footpad in Benadryl pretreated cats were examined during cold exposure to test the role of histamine in CIVD. According to Lewis' hypothesis (42), complete abolition of the response would indicate that histamine is involved by its direct action as well as by activation of axon reflexes. Local cold exposure of Benadryl pretreated footpads failed to change significantly the normal CIVD response ($P > 0.01$) (Figure 13), indicating that locally released histamine is not involved in cold vasodilation. These observations support the earlier reports of Duff et al. (15) who showed that electrophoretic application of anti-histamine to the human finger failed to delay cold vasodilation

or reduce the magnitude of the response, and Whittow (59) who reported that the human CIVD response was unaltered by oral administration of Benadryl.

F. The Possible Role of a Vasodilator Substance
for CIVD in the Anesthetized Cat

The cold-induced vasodilation response is apparently controlled through a number of coexistent mechanisms (see Appendix, Table I). There are reasons to believe that one such mechanism, the accumulation of a vasodilator substance, may have a predominant affect on the CIVD responses in the anesthetized cat.

Lewis (42) reported that cold vasodilation is dependent upon the integrity of axon reflexes. It is apparent, however, that other factors must be influencing CIVD, since the response has been recorded from the cat's footpad (Figure 4) and human finger (31) after sensory nerve degeneration.

Hertzman and Roth (38) suggested that the phenomenon of cold-induced vasodilation may be explained by the failure of impulse conduction in vasoconstrictor fibers. There is an apparent discrepancy in this hypothesis, however, since nonmyelinated nerve fibers have been shown to conduct impulses at 0°C (9,46). In addition, Keatinge (41) has reported that cold vasodilation is not due to the paralysis of vasoconstrictor fibers or inhibition of transmitter released at the nerve terminals, but results from a decreased

sensitivity of vascular smooth muscle to catecholamines (see Proposed Mechanisms, p. 10). The studies of Hertzman and Roth and Keatinge still do not satisfactorily explain cold vasodilation, since Folkow (20) was able to elicit a CIVD response from the cat's footpad after sympathectomy and extirpation of the adrenals.

The direct effect of a vasodilator metabolite on skin vessels may play a prevalent role in cold vasodilation, since vasoconstrictor nerve paralysis, decreased sensitivity of vascular smooth muscle to catecholamines, or axon reflex vasodilation are apparently minor influences on CIVD in the anesthetized cat. Histamine (see Discussion, section E) and acetylcholine (15) apparently do not influence cold vasodilation, however, many other vasoactive substances remain to be tested. The participation of ATP in cold-induced vasodilation should be investigated, since it has been shown that this dilator substance is released from axon reflex nerve endings during sensory nerve stimulation (39). Final elucidation of a possible role of ATP in cold vasodilation will have to await the development of specific inhibitors of its vasodilator action.

The effect of a vasodilator substance on skin vessels might explain the phasic CIVD response (Figure 2) recorded from the footpad of the anesthetized cat. During the initial vasoconstriction period of local cold exposure (Figure 2), the released metabolite would accumulate in an amount

sufficient to cause cold vasodilation. Since the reactivity of the vessels to vasoactive substances is markedly depressed at low temperatures (40), other mechanisms might influence the onset of cold vasodilation (i.e., axon reflex vasodilation). As blood flow increases to the footpad, the vasodilator substance would become progressively more effective. The increase in flow would "wash" the substance from its site of action, and subsequently restore the mechanisms responsible for the decrease in blood flow (see Introduction, p. 4). Although there is still no satisfactory explanation for the mechanism of cold-induced vasodilation for the human or in the anesthetized cat, it is probable that the response is influenced in part by the direct action of a specific vasodilator substance on the cutaneous vessels. Future studies should be concerned with identifying this substance.

CHAPTER VI

SUMMARY

Experiments were designed to evaluate the hypotheses relating CIVD to local neural control by axon reflexes activated by histamine. Accordingly, cold vasodilation was measured from the footpad of the anesthetized cat after sensory nerve degeneration or antihistamine pretreatment. Copper-constantan thermocouples measured temperature changes from the surface of hindlimbs footpads simultaneously immersed in a constantly stirred water bath maintained at 0°C. The data from 23 experiments on chronically denervated cats and 9 experiments on antihistamine pretreated cats are summarized as:

- 1) The footpads of anesthetized cats elicit a cold-induced vasodilation response.

- 2) CIVD is not dependent upon the integrity of axon reflexes in the anesthetized cat.

- 3) Histamine apparently is not involved in cold-induced vasodilation, since a response was elicited after injecting a drug (Benadryl, 10 mg/kg.; I.V.) that blocked the vascular action of histamine without affecting cutaneous blood flow.

The data presented in this study has led to the following questions concerning CIVD:

- 1) What is the nature of the vasodilator metabolite?
- 2) What is the effect of various blocking agents on CIVD in the denervated footpad?
- 3) What is the effect of histamine releasing compounds on CIVD?
- 4) What are the causes for the different CIVD responses observed in the cat's ear and footpad?
- 5) How does the CIVD response change in the denervated hindlimb after acclimitization?

BIBLIOGRAPHY

1. Adams, T. and R. E. Smith (1962). Effect of chronic local cold exposure on finger temperature responses. J. Appl. Physiol. 17(2): 317-322.
2. Altura, B. M. (1965). Pharmacologic properties of anti-histamines in relation to vascular reactivity. Am. J. Physiol. 209: 550-555.
3. Bazett, H. C., Love, L., Newton, M., Eisenberg, L., Day, R., and R. Foster, II. (1948). Temperature changes in blood flowing in arteries and veins in man. J. Appl. Physiol. 1: 3-24.
4. Bazett, H. C., Mendelson, E. S., Love, L. and B. Libet. (1948). Precooling of blood in the arteries, effective capacity and evaporative cooling as factors modifying cooling of the extremities. J. Appl. Physiol. 1: 169-182.
5. Burton, A. C. (1965). Physiology and Biophysics of Circulation. Year Book Medical Publishers Inc., Chicago. p. 208.
6. Burton, A. C. (1934). The application of the theory of heat flow to the study of energy metabolism. J. Nutrition. 7: 481-495.
7. Burton, A. C. and A. C. Edholm (1955). Man in a cold environment. W. & J. Mackay & Co., Ltd., Chatham. p. 90-106.
8. Celander, O. and B. Folkow (1953). Nature and distribution of afferent fibers provided with the axon reflex arrangement. ACTA Physiol. Scand. 29: 359-370.
9. Chapman, R. A. (1957). Dependency on temperature of the conduction velocity of the action potential of giant squid axon. Nature 213: 1143-4.
10. Clark, E. R. (1938). Arteriovenous anastomoses. Physiol. Rev. 18: 229-245.

11. Clark, E. R. and E. L. Clark. (1934). Observations on living arteriovenous anastomoses as seen in transparent chambers introduced into the rabbit's ear. Am. J. Physiol. 54: 229-286.
12. Clarke, R. S. J., Ginsburg, J., and R. F. Hellon. (1958). Use of the strain gauge plethysmograph in assessing the effect of certain drugs on the blood flow through the skin and muscle of the forearm. J. Physiol. 140: 318.
13. Clarke, R. S. J., Hellon, R. F. and A. R. Lind. (1958). Vascular reactions of the forearm to cold. Cli. Sci. 17: 165-177.
14. Davison, M. E., Jasper, R. L., Heistand, W. A., and M. X. Zarrow. (1956). Effects of castration, testosterone propionate and exposure to cold on oxygen uptake. Am. J. Physiol. 186: 471-474.
15. Duff, F., Greenfield, A. D. M., Shepherd, J. T., Thompson, I. D., and R. F. Whelan (1953). The response to vasodilator substances of the blood vessels in fingers immersed in cold water. J. Physiol. 121: 46-54.
16. Duff, F., Patterson, G. C., and J. T. Shepherd. (1954). A quantitative study of the response to ATP in blood vessels of human hand and forearm. J. Physiol. 125: 581-589.
17. Edwards, M. A. (1965). The role of arteriovenous anastomoses in cold-induced vasodilation, rewarming, and reactive hyperemia as determined by ^{24}Na clearance. Canad. J. Physiol. and Pharmacol. 45: 39-48.
18. Edwards, M. and A. C. Burton. (1960). Correlation of heat output and blood flow in the finger, especially in cold-induced vasodilation. J. Appl. Physiol. 15: 201-208.
19. Elsner, R. W., Helms, J. D., and L. Irving. (1960). Circulation of heat to the hands of Arctic Indians. J. Appl. Physiol. 15(4): 662-666.
20. Folkow, B., Fox, R. H., Krog, J., Odelram, H. and O. Thoren. (1963). Studies on the reactions of cutaneous vessels to cold exposure. ACTA Physiol. Scand. 58: 342-354.

21. Folkow, B. and B. Oberg (1961). Autoregulation and basal tone in consecutive vascular sections of the skeletal muscle in reserpine treated cats. ACTA Physiol. Scand. 53: 105-113.
22. Fox, R. H. (1961). Local cooling in man. British Med. Bulletin. 17(1): 14-18.
23. Fox R. H. and O. G. Edholm. (1963). Nervous control of cutaneous circulation. British Med. Bulletin 19(2): 110-114.
24. Fox, R. H., Goldsmith, R., Kidd, D. J., and G. P. Lewis, (1960). Bradykinin as a vasodilator in man. J. Physiol. 154: 16P.
25. Fox, R. H., and H. T. Wyatt. (1962). Cold-induced vasodilation in various areas of the body surface of man. J. Physiol. 162: 289-297.
26. Froese, G. (1958). Effect of breathing O₂ at one atmosphere on the response to cold in human subjects. J. Appl. Physiol. 13: 66-74.
27. Froese, G. and A. C. Burton. (1957). Heat losses from the human hand. J. Appl. Physiol. 10: 235-241.
28. Grant, R. T. and E. F. Bland. (1931). Observations on arteriovenous anastomoses in human skin and bird's foot with special reference to reaction to cold. Heart 15: 385.
29. Greenfield, A. D. M., Kernoham, G. A., Marshall, R. F., Shepherd, J. T., and R. F. Whelan. (1957). Heat losses from toes and fore-feet during immersion in cold water. J. Appl. Physiol. 4: 37-45.
30. Greenfield, A. D. M., and J. T. Shepherd. (1950). A quantitative study of the response to cold of the circulation through the fingers of normal subjects. Cli. Sci. 9: 342-347.
31. Greenfield, A. D. M., Shepherd, J. T., and R. F. Whelan. (1951). The part played by the nervous system in the response to cold of the circulation through the fingertip. Cli. Sci. 10: 347-360.
32. Greenfield, A. D. M., Shepherd, J. T., and R. F. Whelan. (1952). Circulatory response to cold in fingers infiltrated with anesthetic solution. J. Appl. Physiol. 4: 785-788.

33. Haley, T. J. and H. Harris. (1949). The effect of topically applied antihistaminic drugs on the mammalian capillary bed. J. Pharmacol. Exptl. Therap. 95: 293-302.
34. Hall, V. E., Crismon, J. M. and P. E. Chamberlin. (1937). The influence of cold on the calorogenic action of dinitrophenol. J. Pharmacol. Exptl. Therap. 59: 193-205.
35. Hammel, H. T., Wyndham, C. H., and J. D. Hardy. (1958). Heat production and heat loss in the dog at 8-36C environmental temperature. Am. J. Physiol. 194: 99-108.
36. Hardy, J. D. (1961). Physiology of temperature regulation. Physiol. Rev. 41: 521-606.
37. Herrington, L. P. (1960). The heat regulation of small laboratory animals at various environmental temperatures. Am. J. Physiol. 129: 123-139.
38. Hertzman, A. B. and L. W. Roth. (1942). Vasomotor components in the vascular reaction in the finger to cold. Am. J. Physiol. 136: 669-689.
39. Holton, P. (1959). The liberation of adenosine triphosphate on antidromic stimulation of sensory nerves. J. Physiol. 145: 494-504.
40. Keatinge, W. R. (1958). The effect of low temperature on the response of arteries to vasoconstrictor drugs. J. Physiol. 142: 395-405.
41. Keatinge, W. R. (1961). The return of blood flow to fingers in ice-water after suppression by adrenalin and noradrenalin. J. Physiol. 159: 101.
42. Lewis, T. (1930). Observations upon the reactions of the vessels of the human skin to cold. Heart 15: 177-192.
43. Lewis, T. (1945). Exercises in human physiology. Macmillan and Co., Ltd., London. pp. 48-55.
44. Loew, E. R. (1947). Pharmacology of antihistamine compounds. Physiol. Rev. 27: 542-573.
45. Loew, E. R., MacMillan, R. and M. E. Kaiser. (1946). The antihistamine properties of Benadryl, beta-dimethylaminethyl ether hydrochloride. J. Pharm. Exptl. Therap. 86: 22-238.

46. Lundberg, A. (1948). Potassium and the differential thermosensitivity of membrane potential, spike and negative after potential in mammalian A and C fibers. ACTA Physiol. Scand. 15: Suppl. 50. 1.
47. Meehan, J. P. (1957). General body cooling and hand cooling. In F. Fisher(Ed.), Protection and functioning of hands in cold climates. Natl. Acad. Sci. NRC., Washington.
48. Nelms, J. D. and D. J. G. Soper. (1962). Cold vasodilation and cold acclimatization in the hands of British fish filleters. J. Appl. Physiol. 17(3): 444-448.
49. Nichol, J. T. (1952). The effect of temperature on the circulation of the isolated perfused rabbit ear. Canad. J. of Med. Sci. 30: 340-352.
50. Rapaport, S. I., Fetchner, E. S., Shaub, H. G. and J. F. Hall. (1949). Control of blood flow to the extremities at low ambient temperatures. J. Appl. Physiol. 2: 61-71.
51. Roddie, I. C., Shepherd, J. T. and R. F. Whelan. (1955). The action of 5-hydroxytryptamine on the blood vessels of the human hand and forearm. British Journ. of Pharmacol. 10: 445-452.
52. Ruch, T. C. and H. D. Patton (1965). Physiology and Biophysics. 19th edition. W. B. Saunders Co., Philadelphia, pp. 1045-1055.
53. Scholander, P. F. (1958). Countercurrent exchange--a principle in biology. Publ. no. 44, Inst. Zoophysiol. Univ. of Oslo, Norway.
54. Schwinghammer, J., Adams, T. and L. R. Klevans. (1967). Peripheral vascular responses to acute cold exposure in the anesthetized cat. Physiologist 10(3): p. 303.
55. Shepherd, J. T. (1963). Local effects of cold on limb vessels, p. 101-126. In J. T. Shepherd Physiology of the Circulation in Human Limbs in Health and Disease. W. B. Saunders Co., New York.
56. Shepherd, J. T. and I. D. Thompson. (1953). The response to cold of the blood vessels of denervated fingers during regeneration of the nerves. Irish J. Med. Sci. 6th Ser., p. 208.

57. Sherrod, T. R., Loew, E. R., and H. F. Schloemer (1947). Pharmacological properties of antihistaminic drugs, Benadryl, Pyribenzamine and Neoantergan. J. Pharm. Exptl Therap. 89: 247-255.
58. Spealman, C. R. (1945). Effect of ambient air temperature on blood flow in hands. Am. J. Physiol. 145: 218-222.
59. Whittow, G. C. (1955). Effect of antihistamine on cold vasodilation in the finger. Nature 176: 511.
60. Wyndham, C. H. and W. G. Wilson-Dickson (1951). Physiological responses of hands and feet to cold in relation to body temperature. J. Appl. Physiol. 4: 199-207.
61. Yoshimura, H. and T. Iida (1952). Studies on the reactivity of skin vessels to extreme cold. Part II Factor governing individual differences against frostbite. Jap. J. Physiol. 2: 177-185.
62. Yoshimura, H., Lida, T. and H. Koishi (1952). Studies on the reactivity of skin vessels to extreme cold. Part III Effects of diets on the reactivity of skin vessels to cold. Jap. J. Physiol. 2: 310-316.
63. Young, J. Z. (1942). Functional repair of nervous tissue. Physiol. Rev. p. 318-371.

APPENDICES

APPENDIX I

Chronological List of Proposed CIVD Mechanisms

AUTHOR	MECHANISM
Lewis (1930)	Activation of axon reflexes by histamine
Duff et al. (1953)	Direct effect of vasodilator metabolites
Keatinge (1961)	Decreased sensitivity of vascular smooth muscle to catecholamines
Folkow et al. (1963)	Inhibition of the myogenic reflex
Burton (1963)	Changes in blood viscosity

APPENDIX II

CIVD in the Intact vs. Chronically Denervated Footpads

Cat	Days after Sec.	Temp. Peak ($^{\circ}\text{C}$)		Rewarming Rate($^{\circ}\text{C}/\text{min}$)		Onset Time (min.)		Time to Peak(min.)	
		cont.	dener.	cont.	dener.	cont.	dener.	cont.	dener.
A	14	5.6	3.6	5.8	4.4	3.2	3.4	4.2	4.6
	23	4.8	3.6	3.6	2.4	5.2	5.5	6.2	6.7
	29	10.0	6.4	4.8	4.4	3.8	4.2	4.7	4.9
	43	7.4	7.4	3.0	5.0	3.0	5.0	4.0	6.0
	99	4.8	4.0	3.4	5.0	4.1	5.1	5.9	6.3
B	14	3.8	5.0	4.8	6.4	3.7	4.6	4.5	5.7
	23	3.6	6.0	2.4	5.6	3.5	5.4	5.1	6.6
	29	5.6	8.0	10.4	9.6	3.0	5.0	3.5	5.6
	48	3.2	4.4	4.0	5.3	4.0	5.3	5.3	6.5
	63	3.0	1.2	2.4	0.6	4.5	5.5	5.5	7.0
C	14	5.2	3.0	2.8	2.0	3.0	3.5	4.8	5.2
	21	4.4	4.0	2.0	2.0	3.1	5.1	5.0	6.8
	27	4.0	4.0	2.0	2.2	2.8	4.1	4.4	5.4
	42	1.6	2.4	4.3	6.6	4.3	6.6	6.6	8.1
	54	1.6	1.6	0.4	0.4	2.8	5.3	4.0	7.3
D	14	2.0	2.8	1.0	3.2	3.3	3.2	6.2	6.7
	21	4.0	3.6	2.8	2.6	6.5	7.0	5.1	7.8
	27	4.8	5.6	4.0	4.0	5.0	6.2	6.0	5.4
	46	1.4	3.2	5.5	6.2	5.5	6.2	6.2	7.9
	88	2.8	2.0	1.1	1.9	4.2	8.5	5.2	9.5
E	14	5.4	2.8	1.6	1.4	2.5	3.5	6.5	6.7
	19	2.4	3.2	1.2	1.2	3.5	0.5	4.7	7.5
	25	6.0	5.4	2.8	2.8	2.4	4.0	3.2	4.9
$\bar{X}$		4.2	4.0	3.3	3.7	3.8	4.9	5.1	6.5
$\pm\text{SE}$		0.41	0.36	0.44	0.47	0.21	0.33	0.19	0.25

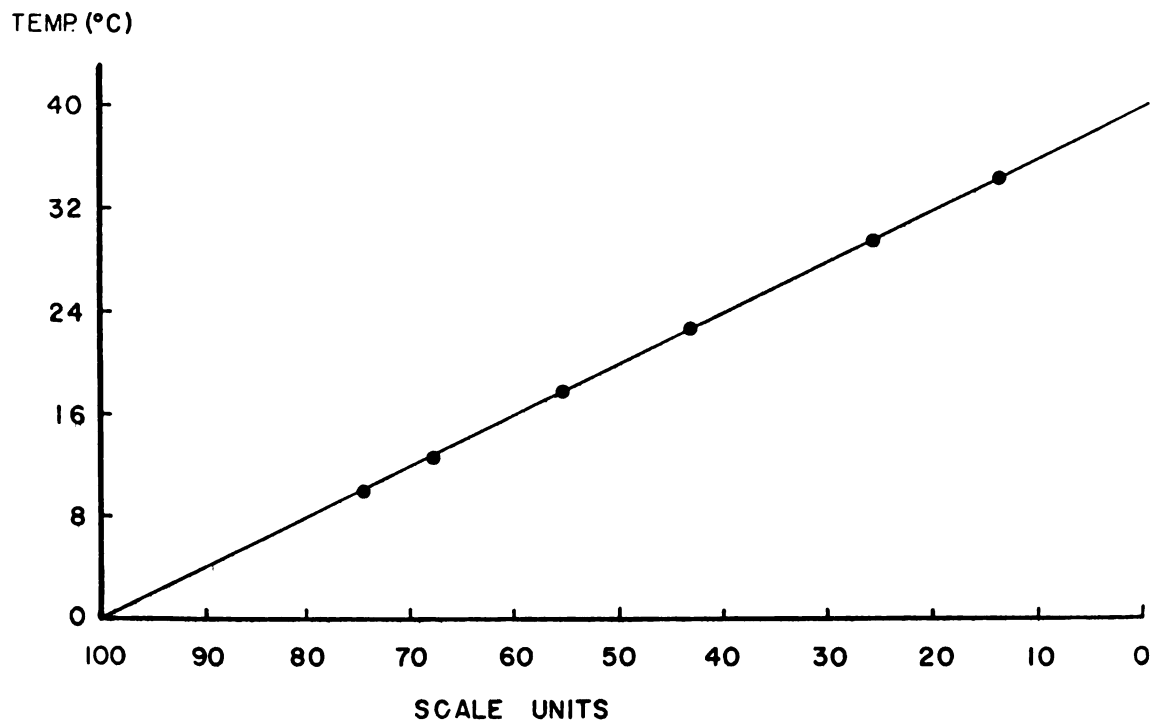
APPENDIX III

CIVD in the Control vs. Benadryl Pretreated Footpads

Temp. Peak ($^{\circ}\text{C}$)		Rewarming Rate($^{\circ}\text{C}/\text{min}$)		Onset Time (min)		Time to Peak (min)	
cont.	Benad.	cont.	Benad.	cont.	Benad.	cont.	Benad.
2.8	4.4	1.2	2.5	3.2	3.2	4.4	4.4
6.0	3.6	2.4	2.4	2.7	3.2	4.0	4.2
3.0	4.0	1.7	3.0	5.5	4.9	7.1	6.0
3.4	3.0	2.4	1.2	6.1	5.0	7.5	7.0
2.0	4.0	0.8	2.5	6.4	5.9	8.0	7.4
2.8	4.4	1.6	5.6	5.5	5.9	6.7	7.2
2.6	4.0	1.2	1.6	6.1	6.0	8.3	8.0
2.8	2.8	1.6	0.6	7.1	4.9	9.6	7.7
4.0	6.6	2.4	7.4	4.3	5.6	5.6	6.9
4.4	4.8	4.0	7.3	4.0	5.6	4.8	6.6
5.2	4.8	3.6	5.1	3.6	4.4	4.7	5.8
6.2	5.6	3.4	6.4	4.9	4.4	6.3	5.6
7.2	10.0	8.8	14.7	3.8	5.0	4.8	5.7
5.2	7.2	8.8	7.0	3.8	4.4	4.6	5.4
7.6	6.4	4.2	6.4	4.5	5.0	5.4	6.0
6.0	5.6	2.0	3.2	4.8	5.5	6.0	6.9
6.6	5.2	7.2	3.2	4.3	5.7	5.9	6.9
8.4	4.4	10.4	3.2	4.0	5.7	5.2	6.9
$\bar{X}$	4.8	3.8	4.6	4.7	5.0	6.0	6.4
$\pm\text{SE}$	0.46	0.70	0.78	0.28	0.19	0.36	0.24

APPENDIX IV

Calibration for Strip Chart Recorder



MICHIGAN STATE UNIVERSITY LIBRARIES



3 1293 03144 9774