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FACTORS AFFECTING GINGIVAL BLOOD FLOW

A project report submitted in partial fulfilment

for the degree of Master of Dental Surgery

by

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SUMMARY

The present study was undertaken to investigate the effect of nicotine and adrenaline on gingival blood flow. An animal model was used to obtain the controlled data but supporting human studies were also conducted.

The gingival blood flow was monitored by a microelectronic device using a thermal diffusion method. The drugs examined had a profound effect upon gingival blood flow: in combination they approximated the effect of total carotid occlusion.

The hypothesis of Kardachi and Clarke (1974) that stress and smoking could be significant factors in the initiation of Acute Necrotizing Ulcerative Gingivitis (A.N.U.G.), by combining to compromise the gingival blood supply, resulting in epithelial necrosis, is supported by the experimental evidence recorded in this research report.

SIGNED STATEMENT

This project report is submitted in partial fulfilment of the requirements of the Degree of Master of Dental Surgery in The University of Adelaide.

This report contains no material which has been accepted for the award of any other degree or diploma in any University. To the best of my knowledge and belief, it contains no material previously published or written by another person except where due reference is made in the text of the report.

BRIAN C. SHEPHARD

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CHAPTER I

INTRODUCTION

In 1896 Vincent postulated that the aetiology of Acute Necrotizing Ulcerative Gingivitis (A.N.U.G.) was due to fuso-spirochaetal invasion of the gingival tissues. His theory received general acceptance although many workers were concerned that the disease could not fulfil Koch's postulates. It is now generally accepted that A.N.U.G. has a multi-factorial aetiology. The causation of A.N.U.G. can be hypothetically constructed on the basis of a triad of factors, with lesser roles being played by other agents.

It has been stated that smoking (Goldhaber, 1957), stress (Shannon, Kilgore and O'Leary, 1969), and sepsis (Pindborg, 1951) form a triad of inter-related predisposing factors in the aetiology of A.N.U.G. These three factors have one systemic factor in common: the ability to powerfully influence the circulatory efficiency of the gingivae. Recently Kardachi and Clarke (1974) have suggested that these factors could operate in the aetiology of A.N.U.G. by reducing blood flow levels in the end-arteries of the gingival crest for prolonged periods, resulting in ischaemic necrosis of the crestal gingivae. This hypothesis explains the absence of prodromal signs for A.N.U.G. and why the predominant fuso-spirochaetal organisms consistently found on the ulcerated surface of the gingiva are unable to fulfil Koch's postulates. Fuso-spirochaetes are always present in the gingival crevice and are well adapted or ideally suited for superinfection within

necrotic tissue.

The evidence for Kardachi and Clarke's hypothesis is substantial as virtually all A.N.U.G. patients have established chronic marginal gingivitis which causes stasis in the gingival circulation (Spector and Willoughby, 1968). Under conditions of prolonged emotional stress the sympathetic nervous system is stimulated, resulting in the central release of adrenaline (Burn, 1960) and the local production of noradrenaline from the walls of gingival arterioles (Watts, 1960). The two cortico-amines have well known vasoconstrictive properties which further decrease the circulatory deficiency in a chronically inflamed site.

Some of the pharmacological effects of smoking are similar to those of stress. Smoking supplies the potent alkaloid nicotine to the systemic circulation and decreases the peripheral circulation by releasing amines similar to those released in the stressed state. Nicotine activates the release of noradrenaline in or near vessel walls, and promotes the release of adrenaline from the adrenal bodies, potentiating the effects of stress in reducing the gingival circulation.

This study was designed to investigate the relationship between crestal gingival blood flow and the presence of circulating nicotine and adrenaline in healthy gingival tissue unaffected by inflammation or stress and to determine whether flow rates are reduced more than when nicotine is acting alone. The rabbit was chosen as a suitable experimental animal for this study.

It is significant that the lesion of A.N.U.G. is contained within avascular epithelial tissue, which is entirely dependent upon diffusion from the connective tissue ground substance for its oxygen and nutrient supply. Severe reduction of blood flow induced by marginal inflammation, stress and smoking could cause loss of vitality to the most vulnerable regions of the gingival epithelium. These areas are located at the tips of the gingival papillae and the col crests where the circulation is end-arterial and without collateral support.

Kindlova (1965) described the vasculature of the gingiva and showed that the two capillary networks present were arterio-venous anastomoses which if sphincter operated, could be separated from the circulation. She conjectured that if the blood flow in either of these two capillary networks was severely reduced then a necrotic lesion could result. MacPhee and Beagrie (1962) felt that the separate sources of gingival blood supply were significant for the onset of acute ulcerative gingival lesions.

Many of the accepted concepts regarding vascularization of the gingival tissues have been obtained from anatomical and histological studies. Such studies do not provide useful data concerning the volume of blood flow through a system that is modified by external factors.

The historic technique of measuring blood flow by severing a vessel and timing the accumulation of blood in a graduated container has obvious theoretical and practical limitations. Measurement of the actual blood

flow into a tissue without disturbing the physiological status of the tissue, thereby altering the flow by the measuring device, presents a very difficult problem.

An appropriate method for measuring blood flow is the thermal diffusion technique. A constant current was used to heat an electronic device whose core temperature was dependent upon heat dissipation, a function assumed to be dependent upon blood flow. One of the major problems solved was the miniaturisation of the electronic device to allow insertion of the assembly within the potential space of the gingival crevice.

The second major problem solved was the direct measurement of blood pressure by cannulation and its relationship to gingival blood flow rates without artificially reducing systemic blood flow. A portion of this report is concerned with the development of techniques to overcome these two major problems, while the remainder is concerned with the effects of intravenous and intra-arterial administration of saline, nicotine and adrenaline on the physiological parameters of gingival blood flow, blood pressure and peripheral body temperature.

Finally, the object of this study was to determine whether the triad of predisposing factors for A.N.U.G. could initiate the disease by acting together to cause an ischaemic necrosis of gingival crest epithelium.

CHAPTER II

REVIEW OF THE LITERATURE

THE BLOOD SUPPLY TO THE PERIODONTIUM

There have been relatively few studies of the vascularity of the periodontal ligament. Wedl (1881) considered that the blood supply was "abundant", while Strubell (1904) agreed and observed that the apical region of the ligament was especially vascular. Schweitzer (1909) described the course of the vessels in the periodontal ligament as entering the tissue from perforations in the alveolar wall, and to a lesser extent from the gingiva.

A thorough study of the blood supply to the periodontium was reported by Hayashi (1932). His investigation was based on serial sections of the jaws of a cadaver injected with Berlin blue dye, and showed that the main blood supply to the periodontal ligament was via the dental artery. The interalveolar branches of the dental artery were shown by Hayashi to give rise to four or five perforating alveolar branches that pass through foraminae in the alveolar wall to enter the periodontal ligament that also received a collateral blood supply from the gingiva. Steinhardt (1935) believed the blood supply to be greater to the gingival and apical parts of the periodontal ligament than to the central region. Birn (1966) studied the vascular supply to the periodontium in 84 teeth and showed that there was no significant

difference in the blood supply to the centro-alveolar and peri-alveolar surfaces of the molar teeth. He also showed that the blood supply to the periodontal ligament increased gradually from tooth to tooth towards the posterior teeth with a high degree of similarity between the upper and lower jaws in the distribution of the blood vessels. Fröhlich (1964) suggested that there was "diagonal symmetry" in the vascularity of the periodontal ligament; i.e., if there were many vessels lingually in the apical region, then there were also many facially in the gingival area.

Turner, Ruben, Frankl, Sheff and Silberstein (1969) considered a knowledge of the basic details of periodontal vascularization to be important because the microcirculation may be altered in distribution, structure and behaviour in many disease processes (e.g. inflammation, diabetes).

The functional anatomy of the capillary circulation has been determined for some cold blooded animals through both anatomical and microscopic observations of vessels *in vivo* (Gregg, 1966). Under normal conditions blood flows from the arterioles directly into a metarteriole and then into capillaries. The metarterioles lead directly into channels which are main thoroughfares from the capillary bed to the venules. The true capillaries concerned with interchange between blood and tissues are inter-anastomosing side branches of the main channels through the vascular bed. At the ostia of each capillary is a small pre-capillary sphincter of smooth muscle which is controlled by sympathetic nerves. These

nerves also control the arterioles and metarterioles. The metarterioles and the precapillary sphincters undergo periodic contractions at intervals ranging from 15 seconds to 3 minutes. When the tissue is in a resting state, the constrictor phase of this rhythm predominates and the precapillary sphincters may be completely closed. When the tissue becomes active, the dilator phase of the metarterioles predominates and the precapillary sphincters are open. Gregg (1966) believes that the local conditions in the tissue, coupled with nervous and humoral stimuli, affect the degree of constriction and relaxation of the metarterioles and the precapillary sphincters during vasomotion, and that these same factors affect arteriolar diameters. Sympathetic stimuli and adrenaline in the blood intensify the constrictor phase of vasomotion in most areas of the body in the same manner that they constrict arterioles. Conversely, vasodilator substances decrease the vasoconstrictor phase. The significance and value of this arrangement to capillary beds and to overall circulatory dynamics remains to be established.

The distribution of blood via the capillary circulation should be considered independently from the systemic circulation. The peripheral circulatory system consists of terminal arterioles, precapillaries, capillaries, and venules.

According to Zweifach (1961), the capillary bed demonstrates a definite pattern of muscular and non-muscular vessels. The capillary bed is barely wider than the red blood cell that it contains. Blood flow is regulated by

widening or narrowing of centrally located muscular thoroughfare channels. Flow is further regulated by the design of the bed itself, with abrupt angles which create valve or sluice gates at points of exit. A vasoconstrictor such as adrenaline or nicotine will narrow these thoroughfare channels, while an increase in intravascular pressure or the action of the vasoactive kinins (Bradykinin) will cause an increase in their diameter. During periods of relative inactivity, the basal metabolic rate of the tissues is low and blood is confined to the central channels. With increased activity the side channels of the capillary network open to increase the amount of blood flow. Under normal conditions the capillary bed functions at about 1/50th of its capacity. Zweifach (1961) considered the most important features of the capillary network to be the precapillary sphincter and its side branches because these components of the micro-circulation regulate the channelling and flow of blood into the capillary bed. Zweifach (1961) calculated the diameter of the side branches of the microcirculation to range from 15 - 20 microns. The abrupt angle of branching and small amounts of arteriolar muscle restricted to the area of the bifurcation assists in the regulation of blood flow. The regulation is apparently independent of control by the systemic circulation.

The periodontium has undergone many and varied types of investigation and examination. Turner *et al.* (1969) have maintained that routine histologic preparations do not permit complete visualization of the vascular supply

of these tissues. They consider that in thinly cut sections the vascular pathways are vague and difficult to reveal because the microvessels are only seen in crossection. To overcome this problem, perfusion and injection techniques utilizing dye solutions, silicone or colloid materials, gelatine preparations, and radiopaque substances have been employed to demonstrate vascular pathways.

Kindlova and Matena (1959) studied the detailed vascular pattern of the tooth and perodontium of the rat by injecting live animals with latex. They claimed that the functional arrangement of the blood supply within the periodontal ligament would appear to be of multi-faceted significance. The perpendicular projection of vessels from the cribriform plate into the periodontal ligament paralleling the Sharpey's fibres was found to be consistent in tracings of the arterial blood supply. This route would appear to enable blood to pass via the more protected intrabony vessels into any level of the periodontal ligament, with very little prior vascular compression. Later investigations by Castelli and Dempster (1965), Folke and Stallard (1967), Birn (1966), and Carranza, Itoiz, Cabrini and Dotto (1966), support these findings.

Both Keller and Cohen (1955) and Quintarelli (1959) perfused India ink solutions through the vessels of the periodontal ligament to study anatomical and functional considerations of the periodontal structures. Boyer and Neptune (1962) employed the intravascular

precipitation of lead chromate, followed by dehydration and clearing, to demonstrate patterns of blood supply.

Most perfusion methods by which blood vascular patterns have been delineated *in situ*, have involved the replacement of blood by injection of a known mass circulated under pressure, or by pressure produced by a pump to overcome back-flow pressure within the artery.

Angiology of the jaws may also be studied by injection of a radiopaque mass into the vessels to permit visualization. Schuback and Goldman (1957) perfused the gingiva and attachment apparatus vessels of the teeth with a mercury-gelatin mass in order to study them. Saunders (1966) utilized a fine particle radiopaque solution to demonstrate pulpal and periodontal vessels in both monkey and man.

Cohen (1960) used multiple techniques in his investigation of vascular architecture. His investigations included injection into the common carotid artery of radiopaque carmine-gelatin and India ink as well as perfusion of a suspension of barium sulphate into cancellous bone.

The limitations of some perfusion techniques are overcome by the use of plastic microspheres that permit *in vivo* perfusion without significantly interfering with tissue integrity. Folke and Stallard (1967) demonstrated the microvasculature of the periodontium in Rhesus monkeys by

this technique. They found that the vessels of the periodontal ligament branch and form a polyhedric plexiform pattern which is located closer to the bone than to the cementum. The microspheres trapped in the periodontal ligament were of the same average diameter, but were comparatively fewer in number. Folke and Stallard (1967) suggested that the microvasculature either consists of a greater number of preferential channels or an overall increase in vessel diameter. This arrangement may allow the blood to flow in several directions and provide a hydraulic cushion during function while still maintaining nutritional demands (Castelli and Dempster, 1965).

The microspheres present in the periodontal ligament associated primarily with the glomeruli-like structures might add further evidence to the work by Gasparini (1959) and Provenza, Biddington and Cheng (1959) which suggests that these structures serve as a mechanism to regulate flow through the periodontal ligament, and which may serve as a shunt between an arteriole and a venule.

Folke and Stallard (1967) noted also that the major vessels on the periosteal side of the alveolar process were arranged in pallisades parallel to the bone. Direct communication was occasionally found between the periodontal ligament and the periosteum of the alveolar bone. Above the crest of the alveolar bone, communications were seen between the vessels of the periodontal ligament and those originating on the periosteal side of the

alveolar process. Microspheres were lodged primarily within arterioles closely associated with the periosteal side of the alveolar process. By combining the information derived from both the direct histologic preparations and three dimensional glass-plate reconstructions, Folke and Stallard concluded:-

1. the arterial pattern of the periodontium conforms to the configuration of the connective tissue - epithelial or basement membrane;
2. the arterioles supplying the periodontal ligament arise from the alveolar bone and branch to form a network within the ligament; and
3. it is from the alveolar bone and its periosteum that the microvasculature of the periodontium arises.

THE BLOOD SUPPLY TO THE GINGIVA

Investigations using carbon particle perfusion (Keller and Cohen, 1955), latex casts (Kindlova, 1965), vital microscopy (Staple and Copley, 1959), and microspheres (Folke and Stallard, 1967) have all contributed to an understanding of the microcirculation in the gingiva. From these investigations there is general agreement that the blood supply to the gingiva is derived from three sources:-

1. supraperiosteal arterioles along the facial and lingual surfaces of the alveolar bone, from which capillaries extend along the sulcular epithelium and between the rete pegs of the external gingival surface;

2. vessels of the periodontal ligament which extend into the gingiva and anastomose with capillaries in the sulcus area; and
3. arterioles which emerge from the crest of the interdental septa and extend parallel to the crest of the bone to anastomose with vessels of the periodontal ligament, gingival sulcus area, and with vessels which run over the alveolar crest.

Karring and Löe (1969) showed capillaries beneath the epithelium on the outer gingival surface extending into the papillary connective tissue between the epithelial rete pegs in the form of terminal hair pin loops with efferent and afferent branches, spirals, and varices. Egelberg (1966) found that the terminal capillaries of the sulcular epithelium were arranged in a flat anastomosing plexus which extended from the gingival margin to the apical extension of the junctional epithelium. The "col" area of the gingiva was shown by Folke and Stallard (1967) to be a combination of the vascular capillary patterns described above. The arterial vessels derived from the crestal area of the interdental alveolar bone approach the col epithelium and deviate sharply to run parallel to the basement membrane near the surface.

Kindlova (1967) described the vasculature of the gingiva and showed that two groups of capillary networks were present. The first group gave rise to slender loops extending into the apex of the papillae from the facial

and lingual surfaces of the gingiva. The main vessels of the second group gave rise to capillaries resembling renal glomeruli which appeared to be arteriovenous anastomoses. The interdental area below the col was supplied by only this type of capillary. *THE CIRCULATION AT THE TIPS OF THE GINGIVAL PAPILLAE AND THE COL CRESTS IS END-ARTERIAL AND WITHOUT COLLATERAL SUPPORT*.

Folke and Stallard (1967) noted that the col area of the interdental gingiva was the most common site for inflammation. Kindlova (1965) showed that the capillary configuration of the sulcus in diseased states is replaced by a dense network of enlarged varicose capillaries. Egelberg (1966) also observed changes in the vessels of the sulcular region when sections of healthy and inflamed gingiva were compared histologically. A comparison of the inflamed areas with healthy areas showed that the normal vascular pattern had been replaced by a random orientation of vessels.

One of the implications of the flat arrangement of the crevicular plexus in healthy gingiva is that the various components of the terminal vascular bed - the arterioles, capillaries, and venules - are located in a more superficial position in relation to the surface of the epithelium than all other gingival vasculature (Egelberg, 1966).

The diameter of the vessels constituting the crevicular plexus was found by Egelberg (1966) to vary from 7 to 40 microns. The plexus was dominated by

vessels having a diameter larger than 7 microns. In recent years it has been suggested that vessels of the terminal circulation with diameters greater than 7 - 8 microns (with the exception of arterioles) should not be considered true capillaries, but should be classified as postcapillary venules, small venules, and venules (Majno and Palade, 1961). It has been shown that such postcapillary venules and venules have a number of functional characteristics not present in true capillaries and arterioles (Majno, 1965). An important difference is revealed in the finding that venules have a greater disposition towards increased permeability than true capillaries and arterioles (Majno and Palade, 1961; Zweifach, 1964, 1965). There is a general impression that the crevicular plexus is very rich in vessels of venular type. Due to the architecture of the crevicular epithelium, these venules are more superficially situated than the venules under the oral epithelium of the gingiva. The venules of the crevicular plexus are thus more susceptible to injury, haemorrhage, thrombosis (Fulton, 1957; Lee and Stetson, 1965), and allergic injury (Movat and Fernando, 1963).

METHODS FOR INVESTIGATION OF BLOOD FLOW

Measurement of actual blood flow into a tissue without disturbing the physiological status of the tissue is a difficult problem. The historical technique of measuring blood flow by severing a vessel and timing the

accumulation of blood in a graduated container has obvious theoretical and practical limitations.

In recent years rotameters, hot wire anemometers, thermostromuhrs, pitot tubes, orifice meters, flowmeters (bubble, bristle, ultrasonic), tissue impedence changes (Nyboer, 1944, 1959), calorimetry (Stewart, 1911), heat-disk thermocouples (Fox, Goldsmith, Kidd and Lewis, 1961) and venous occlusion plethysmography (Edholm, Fox and MacPherson, 1956) have all provided measurement of blood flow, but none of these methods provide a simple and accurate technique. Recent developments in semiconductor technology, optics, and instrumentation have allowed the study of vascular reactions in a single gingival papilla (Brown, Giddon and Dean, 1965). Difficulty in calibration, cardiac ballistics, transient light, and position of light source in relationship to the photo-electric cell and the underlying vascular system imposes limitations on this method.

The theoretical basis for calculating capillary blood flow through any small homogeneous segment of a tissue area using an isotope has been carefully delineated (Kety, 1960). Critical evaluation and assumptions involved in peripheral blood flow measurements by tissue clearance have been presented by Hyman (1960).

It is also necessary that blood flow be measured within intact blood vessels and that the subject should have some freedom of movement to carry on normal activities.

Ideally, the measuring instrument should record backflow as well as forward flow.

A most significant breakthrough in blood flowmeter design occurred in 1932 with the independent development of the electromagnetic flowmeter by Kolin in the United States and by Wetterer in Germany. This meter used the principle of magnetic induction and provides most of the essential features necessary for experimental and clinical blood flow determination. These features include a linear relation between rate of blood flow and the instrument reading, rapid instrument response to velocity change, and stability of the calibration.

Spencer and Denison (1960) developed a square wave, electromagnetic blood flowmeter that was capable of recording continuously the mean flow through surgically exposed but intact blood vessels. Direct cannulating electromagnetic blood flowmeters offer several advantages for experimental procedures: calibration can be achieved in terms of volume blood flow; there is no obstruction placed in the blood flowpath; and simultaneous blood pressure and blood flow recordings from the same vessel permit calculation of peripheral resistance units. Cannulation involves introduction of a foreign structure through which the blood must flow and heparinization is required; and surgery, anaesthesia, and anticoagulants render blood flow measurements at low flow rates of little practical value for human studies.

Perfusion of blood into an isolated organ or into regions of the body at a measured rate, while the lateral pressure required to drive the blood inward is also measured, yields valuable fundamental information (Green, 1948). Selection of the perfusion pressures and flow rates involves the investigation of many parameters. These include the peripheral resistance, critical pressure, vasoactive drug assays, vascular patterns by dyes, and clearance of radioactive compounds and nondiffusible substances.

Various adaptations of perfusion systems were detailed by Green (1944, 1948, 1950). Perfusion studies were used (Redden, Bishop, Mathews and Dorman, 1961) to determine the critical closing pressure in the mandibular region. Using the perfusion method, Miller (1965) studied the vasomotor activity of vessels of the periodontal ligament, dental pulp, and supporting bone of the teeth. Green, Lewis, Nickerson and Heller (1944) in response to several physiological variables, obtained data which permitted them to calculate the peripheral resistance in terms of the peripheral resistance unit.

Plastic microspheres (Meyer and Tschetter, 1966) and radioactive microspheres (Stone, Bishop and Guyton, 1963) can be utilized in determining the blood flow to an area. Microspheres of sufficient diameter to be trapped in capillary beds are injected into the aorta, with the percent trapped in a given tissue being proportional to the percent of the cardiac output which was received by the tissue during the test period.

Thermal dilution was introduced as a method for the measurement of volumetric blood flow rate by Fegler (1953). He called this method "thermodilution". Fegler found that following the intravenous injection of cool blood or Ringer's solution he could record temperature-time curves by means of thermocouples and these thermodilution curves bore a close resemblance to dye-dilution curves. Following his original work on measurement of cardiac output, Fegler extended his method to the measurement of flow in single blood vessels and made *in vivo* studies in the superior and inferior venae cavae. In 1960 Fronek and Ganz obtained results from single blood vessels in which the points of injection and temperature measurement were separated by less than one centimetre. They found no systematic difference between the calculated flows and the standards of comparison.

All thermal methods for the study of the circulation depend upon the induction of a change in the heat content of the blood stream. This change may be achieved in several ways: by generation of heat within the blood itself (diathermy-thermostromuhr); by conduction of heat across a vessel wall; by an intravascular heating or cooling device; or by the injection into the blood stream of a mass which is miscible with the blood and at a different temperature. The latter method most closely resembles other common indicator-dilution methods. The measurement of temperature is fundamental to the thermodilution method. Two types of temperature sensitive devices suitable for measurement and recording of intravascular temperature are thermocouples and thermistors.

Thermocouples produce small electromotive forces and consequently are used in low resistance circuits with sensitive galvanometers of long period unless suitable amplification can be arranged. Thermistors have a high negative temperature coefficient of resistance and have an almost logarithmic temperature-resistance characteristic. The smaller the thermistor, the more easily it can dissipate the heat it generates, and its thermal inertia to temperature change decreases.

The thermodilution method of measuring blood flow is simple from both technical and instrumental aspects; it requires neither blood sampling nor the introduction of any foreign material, and frequent measurements may be made. Its technical simplicity and satisfactory comparison with longer established methods for quantification of blood flow make thermodilution a method of considerable promise for the investigation of the circulation.

GINGIVAL BLOOD FLOW

The blood flow of the gingiva has not been studied as extensively as other more readily accessible tissues because of the delicate and easily traumatized anatomy of the area and because of the physical size of most conventional forms of blood flow measuring devices.

A microscopic technique was developed by King (1947) to observe capillary blood flow in gingival tissues. Staple and Copley (1959) also used a capillaroscopy technique to observe gingival blood flow

and concluded that it was influenced by three mechanisms:-

1. changes in the calibre of arterioles,
2. inactive capillary beds opening to allow inflow while others shut down, and
3. opening and closing of arterio-venous shunts.

They concluded that the gingival circulation was primarily regulated by changes in the diameter of arterioles, which in turn increased the blood volume of the gingival area. However, the basic mechanism involved in the regulation of gingival blood flow is not known.

Forsslund (1953) used a stereoscopic microscope to photograph peripheral blood vessels in human and animal studies. He noted that adrenaline caused a vaso-constriction while histamine caused a vasodilation of the terminal gingival capillaries. In a later report Forsslund (1964) noted that the vessels of the gingiva are formed into loops, anastomoses, and central canals, with a preponderance of arterio-venous anastomoses. Also, he failed to find capillaries near the gingival surface devoid of erythrocytes, which may indicate that this part of the gingival circulation is in service maximally at all times. However there appears to be instances in which capillaries contain stationary red blood cells (Staple, 1957). This efficient circulation may form a portion of the local tissue resistance not only to infection, but also against the constant mechanical stresses to which the gingiva is frequently subjected (Forsslund, 1959).

Other investigators have shown that gingival oxygen tension requirements regulate capillary flow

(Dorman and Bishop, 1964). The oxygen tension in the extracellular fluid of the gingiva should provide an excellent criteria for the adequacy of the local circulation, and techniques for measuring this tension have recently become available. The oxygen electrode, which operates on the basis of the polarographic principle and measures oxygen tension in terms of current carried between two polarized electrodes, has been miniaturized to permit the electrode assembly to fit inside a 19 gauge hypodermic needle. The normal oxygen tension in the gingiva of dogs has been found to average 56 mm Hg (Dorman and Bishop, 1964). Intravascular injection of adrenaline in dogs caused a temporary reduction of the oxygen tension in the gingiva, whereas intravascular injection of acetylcholine in the same species produced a temporary increase in the oxygen tension of the gingiva.

A photo-electric method that continuously monitored gingival vascular reactions to vasoactive drugs in the gingivae of dogs was used by Giddon, Kushnir and Gustafson (1963). By this method, both local and systemic administration of small amounts of adrenaline were shown to reduce the vascular activity in the gingivae.

Using a double thermocouple technique, gingival blood flow was indirectly measured by Ito, Matsukawa, Takahashi and Cho (1973), who showed that a reduction of gingival blood flow in dogs followed intra-carotid administration of adrenaline and noradrenaline. The

gingival circulation was apparently susceptible to these vasoconstrictive agents because the effects of adrenaline and noradrenaline on the gingival circulation were longer lasting than their effects on the carotid arteries. The response to drug administration obtained by Ito *et al.* (1973) indicated that a complex regulatory mechanism controlled gingival blood flow.

CONTROL OF BLOOD FLOW

A. CENTRAL NERVOUS SYSTEM MECHANISMS

Control of the calibre of the arterioles and the precapillary sphincters regulates the quantity of blood flowing into various tissues and organs. The early work of Bernard (1949) and later Cannon (1939, 1953) established the important role of the autonomic nervous system in control of blood circulation.

1. PERIPHERAL VASOMOTOR CONTROL

a. VASOCONSTRICTION - Nerve fibres that produce vasoconstriction are supplied by the sympathetic division of the autonomic nervous system. Vasoconstrictor fibres have been found to have an exceptionally low frequency of nerve impulses (Folkow, 1952). The function of these nerves is to induce vasoconstriction; they are continually discharging and are "in tone". Vasomotor activity may be enhanced by increasing the frequency of discharge or reduced by decreasing the firing rate.

b. VASODILATION - The existence of sympathetic vasodilator fibres was not well accepted prior to reviews by Folkow (1955) and Uvnäs (1954, 1960). Sympathetic vasoconstrictor endings can be blocked selectively by pharmacological agents, they degenerate more rapidly following nerve section, and they are more susceptible to cold than vasodilator fibres. These techniques using pharmacological agents have been employed to delineate, by stimulation, sympathetic vasodilator action in nerves containing both types of fibre. Stimulation of the sympathetic nerves to a tissue which contains both constrictor and dilator fibres may result in constriction because the constrictor effects usually predominate.

2. MEDULLARY VASOMOTOR CENTRE

The impulse discharge rate, or "tone", of the spinal vasoconstrictor neurons is profoundly influenced by activity of the vasoconstricting centre located in the medulla oblongata. The specific location and function of this centre has been comprehensively reviewed by Uvnäs (1960) who suggests that the tonicity of the vasoconstricting centre is influenced by a host of afferent inputs, such as:-

1) chemoreceptors, located in the carotid and aortic bodies;
2) pressoreceptors, located in the carotid sinus and aortic arch;
3) afferent fibres that convey pain information and also send collaterals to vasoconstricting centres; and

4) descending fibres that originate in the hypothalamus and cortex sending collaterals to the vasomotor centre.

Rhythmicity of vasomotor discharge is influenced by

a) chemical composition of the blood,

b) temperature of the blood, and

c) pressure on the vasomotor centre.

The frequency of discharge of the vasomotor centre at any one time is the algebraic sum of all factors imparting tonicity to the centre.

B. SYSTEMIC MECHANISMS

Chemical mediators are synthesized in the post-ganglionic nerve endings of both sympathetic and para-sympathetic fibres, and the precursors and enzymes which are essential for their formation are present (Triggle, 1965). The newly formed chemical mediators are stored in presynaptic vesicles and are later released by a nerve impulse. They diffuse across the synaptic space and combine with the post-synaptic protein receptor.

Many tissues have an abundant, rich, perivascular nerve supply, and vasomotor responses have been elicited by stimulation of components of the nerve plexus (Fulton, Lutz and Callahan, 1960). No tissue that contains blood vessels is entirely devoid of vasomotor fibres, but their influence is countered by the local production of vasomotor metabolites that provide protection from the possible adverse effects of vasoconstrictor nerve-induced ischaemia (Folkow,

1955). Although circulating catecholamines, autoregulation, and central cardiac effects have received major attention, it is reasonable to view the extensive nerve plexus around the vessels of the microcirculation as functionally significant.

C. LOCAL FACTORS

The smallest blood vessels have an inherent muscular activity that is independent of blood-borne substances or nervous influences but which can be altered by stimulation of nerve fibres, local electrical stimulation, or by changing the physical and chemical environment. The mechanism of this inherent vasomotion is unknown; it may arise from locally produced metabolites (Lutz and Fulton, 1958). Locally produced metabolites such as the products of nuclear metabolisms, 5-hydroxytryptamine, noradrenaline, histamine, CO_2 and lactic acid are released which may act in different ways on arterioles, precapillaries and capillaries. The extent of such basal vasomotion has been evaluated in sympathectomized vascular areas where neurohumoral constrictor influences are eliminated (Folkow, 1949). Although large quantitative differences exist in different parts of the vascular tree, there is an inverse relationship between the local inherent activity of smooth muscle cells and the extent of neurohumoral control in any given vascular bed (Brodie, Beaven, Erjavec and Johnson, 1966). Evidence suggests that reactivity to local metabolic effects is stronger in the metarterioles and precapillary sphincters, whereas constrictor fibres predominantly affect the arterioles. This would indicate that a difference in type of control

may exist within the same vascular bed. Presumably, in all beds there is some locally produced vasodilation which counteracts the centrally induced reduction in blood flow by constrictor nerves and protects the tissue against ischaemia.

The vascular system of the oral tissues appears to be functionally similar to that found in most other body regions, but a few exceptions are noted (Bishop and Dorman, 1968). Smooth muscle sphincters guard the opening of capillaries in the majority of tissues, including those in the oral region. Characteristically, these vessels are intermittently patent. Capillaries of the marginal gingiva may be an exception to this general rule because the capillaries that loop toward the outer edge of the gingiva always contain red blood cells. It has been suggested by Forsslund (1959) that this represents a high blood flow and may be a factor in the resistance of the gingiva to infection and trauma.

Generalizations concerning the innervation of blood vessels in the oral tissues must be made with considerable caution. Some vascular smooth muscle is innervated by both constrictor and dilator fibres; others may have only constrictor fibres; whereas still others have only dilator fibres. Vasoactive drugs can be used to obtain pertinent information on the reactiveness of oral blood vessels, but lack of specificity and the presence of different smooth muscle receptor types limit the usefulness of these data.

ACUTE NECROTIZING ULCERATIVE GINGIVITIS

The term "Acute Necrotizing Ulcerative Gingivitis" (A.N.U.G.) describes an acute inflammatory destructive disease of the gingiva. Other terms by which this condition is known are Vincent's infection, Trench mouth, acute ulcerative gingivitis, fuso-spirillary gingivitis and spirochaetal gingivitis.

History

The disease was recognized as early as the fourth century B.C. by Xenophen who noted that many Greek soldiers were affected with sore mouths and foul smelling breath (Prinz and Greenbaum, 1935). During the seventeenth century, the presence of noma was noted and described by many physicians. Many of the writers on the subject were able to recognize the early ulcerative gingivitis but in most instances the dramatic appearance of noma allowed the physician to overlook the less spectacular forms of A.N.U.G. which occurred in the mouth.

The disease was described in modern times by John Hunter in 1778. He observed that any portion of the gingival tissue in both jaws could become infected causing inflammation and ulceration of the gingival papillae. Hunter also noted that the disease may occur in people who are in all other respects healthy, as well as in children (Hirschfeld, Beube and Siegel, 1940).

Descriptions of the acute condition in groups of soldiers and debilitated children during the nineteenth century were made by many French medical clinicians. It remained for Bergeron (1859) to elicit the fact that the disease exists not only in an acute form, but that untreated cases may lapse into a chronic condition which may or may not have acute exacerbations. He deserves credit for being the first to bring out these clinical variations of the disease. He also pointed out that the acute condition may recur in persons who were apparently cured of the disease previously.

An excellent review article summarising the writings of the Nineteenth Century clinicians, was made by Hirsch in 1886. His account included the diagnostic features of drooling ropy saliva, enlarged lymph nodes, fever and general malaise. These were given as concomitant symptoms with fetid breath, bleeding painful gums and pseudo-membranous ulcers.

At the turn of this century, Plaut (1894) and Vincent (1896) investigated the cause of the disease and attempted to explain the aetiology in microbiological terms. In their studies, two predominant anaerobic organisms were found to be constantly present: spirochaetes trepanaema Vincentii and fusiform bacilli. Vincent was the first to emphasize the constant association of the spirochaetes and the fusiforms with the disease process.

Smith (1930) and Proske and Sayers (1934) investigated the pathogenic properties of bacteria in experimental animals and found that the typical A.N.U.G. lesion was produced by the symbiotic action of four organisms: an oral spirochaete, a fusiform bacillus, a vibrio and a streptococcus.

With the advent of bacteriologic investigation, smears from the A.N.U.G. lesion have been widely used as a diagnostic aid. It was found by MacDonald, Sutton, Knoll, Madlener and Grainger (1956) that a typical "fusospirochaetal" lesion was produced by a combination of four organisms: two bacteroides, a motile Gram-negative anaerobe and a facultative diphtheroid. Paradoxically, none of these organisms was a spirochaete or fusiform bacillus. Rosebury and Sonnenwirth (1958) stated that fuso-spirochaetal organisms, commonly seen in A.N.U.G. lesions, always appeared to be superimposed on tissue damage induced by other agents. Burnett and Scherp (1962) maintained that the overgrowth of a particular bacterial species indigenous to an area did not necessarily indicate responsibility for that disease. Many investigators have studied the involvement of bacteria in A.N.U.G., but because the bacteria are unable to fulfill all of Koch's postulates, the relationship between these microorganisms and the aetiology of the disease has remained uncertain.

PREDISPOSING FACTORS

Numerous predisposing factors have been suggested for A.N.U.G. but there is little data to indicate their relative importance. Stammers (1944) considered that poor oral hygiene, calculus, mouthbreathing and smoking were of paramount importance while the systemic factors of vitamin deficiencies, illness, overwork and lack of exercise were considered contributory to the local factors.

In a study of serving members of the United States army, Schluger (1949) noted a high incidence of A.N.U.G. which he attributed to fatigue and local trauma. He also found that 7.5 percent of A.N.U.G. cases occurred in patients who were hospitalized for long periods after operations, fractures or other non-oral problems. In 1951 Pindborg considered a pre-existing gingivitis to be an important predisposing factor in A.N.U.G. In a study of 91 A.N.U.G. cases 87 were considered to have developed from a pre-existing chronic gingivitis.

Environmental or emotional stress may be an important factor in the aetiology of A.N.U.G. by altering intrinsic factors and by influencing the manner in which the oral tissues respond to altered environmental conditions. Stress causes the production of adrenaline from the adrenal medulla and noradrenaline from the sympathetic nerve endings in the vascular bed (Silverman and Cohen, 1960). Adrenaline exerts its pressor action through cardiac stimulation and cutaneous vasoconstriction, whereas noradrenaline acts as an overall vasoconstrictor.

According to Raab, Humphreys and Lepeschkin (1950) the mineralocorticoids participate in the maintenance of the pressor efficiency of adrenaline and noradrenaline and enhance the pressor effect when present in excess. The glucocorticoids have a similar, but more rapid effect, in maintaining vascular constrictor responsiveness to noradrenaline. Urinary excretion of certain adreno-cortical hormones, in healthy persons rises during emotional stress (Jensen and Ek, 1962), however similar studies of A.N.U.G. patients have been limited. Shannon, Kilgore and O'Leary (1969) showed an increased adrenocortical activity associated with A.N.U.G. patients as measured by free hydroxycorticosteroids in the urine. Since stress results in an increased output of adreno-cortical hormones, the body fluid steroid levels provide an accurate indicator of stress. Although the data from this study is not conclusive, it demonstrates an objective indication of increased adrenocortical activity in the presence of A.N.U.G.

In addition, emotional stress is frequently accompanied by poor oral hygiene, improper diet, excessive smoking, fatigue and other changes in habits. Such habit changes may also be related to the disease process in A.N.U.G. The possible relationship of emotional stress and A.N.U.G. was first introduced into the dental literature by means of scattered case reports, including those of Miller and Firestone (1947), and Roth and Weiss (1951). Evidence from controlled studies suggests an association of emotional factors and A.N.U.G. Psychological tests have demonstrated a correlation between emotional disturbances and A.N.U.G. The study

of Moulton, Ewen and Thieman (1952), supported this concept, and was particularly significant since the senior author was a psychiatrist. In this study, the most outstanding feature of the A.N.U.G. group was the apparent precipitation of the disease "by acute anxiety arising from a life situation about dependency and/or sexual needs". The relationship between emotional factors and A.N.U.G. was further substantiated by Goldberg, Ambinder, Cooper and Abrams (1956) who reported that 22 out of 54 A.N.U.G. cases volunteered some stressful incidents which might be related to the onset of the disease.

Animal studies have confirmed the relationship of stress to periodontal disease. Stahl (1961) demonstrated a delayed repair of tissue injury for local irritants in systemically stressed animals, while Glickman, Stone and Chawla (1953) showed that when animals were treated with cortisone, inflammatory changes resulted in an increased destruction of the interdental papilla. In 1971 Manhold, Doyle and Weisinger, demonstrated that there was less oxygen utilized by the oral tissues of animals who were under a condition of continued social stress compared to a control group. Manhold *et al.* hypothesized that the constriction of blood vessels resulting from long continued or extreme emotions could be a complicating, or even causative factor in pathological periodontal breakdown.

It is interesting to note that the results of the animal studies of Giddon and Goldhaber (1960) somewhat parallel those which Moulton *et al.* (1952) performed on

human subjects. The latter investigators engaged in longitudinal studies on patients with recurrent attacks of A.N.U.G. during remission. They discovered that their subjects psycho-physiologic responses to standardized stress procedures suggested that a peripheral vasomotor defect in A.N.U.G. may be reflected in elevated digital temperatures and general hypotonicity of the digital vasomotor system. Manhold *et al.* (1971) commented that "if such a peripheral hypotonicity is demonstrable in the finger tips, demonstration of the results of improper blood supply to the gingiva certainly are not incredible".

A more recent observation concerning A.N.U.G. was carried out by Giddon, Goldhaber and Dunning (1963) who studied university students at examination time. A positive correlation was found in this survey between A.N.U.G. and stress, while Shannon *et al.* (1969) found increased adreno-cortical activity in patients experiencing A.N.U.G. and concluded that the emotional factor appeared to be one of the most important aetiological factors.

Another common predisposing factor found in the aetiology of A.N.U.G. was tobacco smoking. Pindborg (1951) and Goldhaber (1957) considered tobacco smoking to be an important predisposing factor in A.N.U.G. because their studies both revealed a positive correlation between tobacco smoking and A.N.U.G.; both investigators found that 98% of their A.N.U.G. patients were smokers.

ACTION OF NICOTINE

Tobacco has occupied an important place in the social customs of the Western World for the last 500 years. Chemical analysis reveals that the chief pharmacologically active agent of tobacco condensate is the alkaloid nicotine. Ever since nicotine was first isolated in 1828 it has been regarded as the most toxic substance in tobacco smoke.

The percentage of nicotine in tobacco varies, ranging from 0.5 to 8.0% (Goodman and Gilman, 1970). Nicotine is present in the tobacco leaf as the salt of organic acids; the free base is liberated by heat and passes in varying degrees into smoke. Approximately 90% of the nicotine in inhaled smoke is absorbed while Van Proosdij (1960) estimates that 2.5 mg of nicotine is absorbed from the smoke of one cigarette. Grollman and Grollman (1970) noted that the effects of 2.5 to 3.5 mg of nicotine absorbed from one cigarette are comparable to those noted after intravenous injection of 1 mg of nicotine. The immediately lethal nicotine doses are said to range from 40 mg to 60 mg (Goodman and Gilman, 1970). Smoking 20 cigarettes per day may result in the absorbtion of approximately 50 mg nicotine which is an extremely active pharmacological dose even allowing for a degree of acquired tolerance.

PHARMACOLOGICAL ACTION OF NICOTINE

The complex and often unpredictable changes that occur in the body after administration of nicotine are due

not only to its actions on a variety of neuro-effector junctions but also to the fact that the alkaloid has both stimulant and depressant phases of action. It acts on all autonomic ganglia but also on voluntary muscle and on the central nervous system and releases catecholamines from the adrenal medulla (Goodman and Gilman, 1970). The ultimate response of any one structure or system represents the algebraic summation of the several different and opposing effects of nicotine (Grollman and Grollman, 1970).

1) NERVOUS SYSTEM - nicotine markedly stimulates the central nervous system. Appropriate doses produce tremors in both man and laboratory animals; with larger doses, the tremor is followed by convulsions. The action of nicotine on the spinal functions appears to be one of stimulation, followed by inhibition or paralysis (Grollman and Grollman, 1970).

2) RESPIRATION - following the injection of nicotine, the respiratory centre is first stimulated and then depressed. The failure of the respiration is often due to the curare-like action of nicotine on the nerve endings in the diaphragm which prevents the respiratory muscles from responding (Grollman and Grollman, 1970).

3) CARDIOVASCULAR SYSTEM - the action of nicotine on the circulation is extremely complex, since it is the resultant of the divergent action of the drug on the vasomotor and vagus centres in the medulla, the sympathetic and parasympathetic ganglia, the adrenal medulla, and the chemoreceptors of the carotid sinus and aortic body. The observed rise

in blood pressure is the combined result of stimulation of the medullary vasoconstrictor centres and the ganglia of the vasoconstrictor nerves, and the release of adrenaline and noradrenaline from the adrenal glands, (Grollman and Grollman, 1970). The administration of 2 mg of nicotine to man by injection or through the medium of smoking results in an increase in blood pressure, heart rate, cardiac output and vaso-constriction in the extremities (Roth, McDonald and Sheard, 1944). The question as to whether nicotine causes a constriction of the peripheral vessels has been extensively investigated by clinicians using direct and indirect techniques. The plethysmographical results showed that smoking was capable of reducing the volume of the part of the body examined. Denicotinisation of the smoke annulled this effect almost entirely; sham smoking also was free from the active effect (Van Proosdij, 1960).

Using skin temperature registration Evans and Stewart (1943) found that epidermal temperature dropped as a result of smoking whereas skin temperature was barely affected by the inhalation of denicotinised smoke. Roth and Shick (1958) performed 192 standard smoking tests on 29 normal adults and found that the higher the nicotine content of the cigarette smoked, the greater was the drop in skin temperature. The significance of these results is further underlined by the observations of Moyer and Maddock (1940)

and Roth and Shick (1958) of the effect of intravenous administration of nicotine. Intravenous administration of 1 to 2 mg of nicotine produced drops in temperature in the fingers and toes strikingly similar to those observed accompanying the smoking of cigarettes. Freund and Ward (1960) found that following cigarette smoking there was a significant reduction in digital skin temperature, radio-sodium clearance and venous oxygen saturation, thereby indicating a decrease in peripheral blood flow.

The narrowing of capillaries in the course of smoking, as demonstrated with the help of the capillaroscope, agrees with the findings by indirect means. Wright (1933) saw lag, sometimes even stasis, occurring in the capillary circulation immediately after the first deep inhalations of the cigarette smoke. These results were confirmed by Euzière *et al.* (1936) and Benedittini (1936). Merely taking a deep breath had no effect, nor had the smoking of corn silk cigarettes. Maddock and Coller (1933) and Moyer and Maddock (1940) presented evidence that the vasoconstriction produced by smoking cigarettes was analogous to that produced by intravenous injection of as much nicotine as was contained in the cigarette smoked.

These facts constitute irrefutable evidence that nicotine is the most important factor in the production of vascular effects during smoking.

Almost all A.N.U.G. patients have an established chronic gingivitis which may cause stasis in the gingival

circulation (Spector and Willoughby, 1968). Emotional stress can reduce the blood flow to the gingiva by the central release of adrenaline (Shannon *et al.* 1969) and the peripheral production of noradrenaline in the walls of gingival arterioles (Silverman and Cohen, 1960). Some of the pharmocological effects of smoking are similar to those of stress. Nicotine, the toxic alkaloid of smoking activates the release of noradrenaline in or near vessel walls (Burn, 1960), and promotes the release of adrenaline from the adrenal bodies (Watts, 1960), which further reduces the blood flow to the gingiva.

PHARMACOLOGICAL ACTION OF ADRENALINE

In general, the responses to adrenaline resemble the effects of stimulation of adrenergic nerves. However there are several differences which are due to the differences between adrenaline and the adrenergic mediator, noradrenaline. The effects of adrenaline are particularly prominent upon the heart, the vascular and the smooth muscle (Goodman and Gilman, 1970).

1) BLOOD PRESSURE - Adrenaline is one of the most potent vasopressor drugs known. Given rapidly intravenously to animals, it evokes a characteristic blood pressure response. Blood pressure rises rapidly to a peak that is proportional to the dose. The increase in systolic pressure is greater than in diastolic pressure, so that the pulse pressure increases. The pressure then falls below normal before returning to the control level. After the blood pressure returns to normal,

repeated doses of adrenaline continue to have the same pressor effect. The mechanism of the rise in blood pressure due to adrenaline is effected by increased heart rate and contraction and marked vasoconstriction of the precapillary resistance vessels (Goodman and Gilman, 1970).

2) VASCULAR EFFECTS - The chief vascular action of adrenaline is exerted on the smaller arterioles and precapillary sphincters, although veins and larger arteries also respond to the drug. The blood vessels to skin, mucosa and kidney are constricted by the action of their alpha-receptors whereas the vessels to skeletal muscles are dilated by the action on their beta-receptors, which are sensitive to much lower concentrations of adrenaline than are the alpha-receptors. The overall effect of full activation of both alpha- and beta-receptors is an increase in peripheral resistance and consequently, a rise in blood pressure.

Injected adrenaline markedly reduces cutaneous blood flow constricting precapillary vessels and subpapillary venules. Cutaneous vaso-constriction accounts for a marked decrease in blood flow of the hands and feet.

Blood flow to skeletal muscles is increased by therapeutic doses of adrenaline in man. This is due to a powerful beta-receptor vasodilator action followed by a vasoconstrictor

action on the alpha-receptors that are also present in this vascular bed (Goodman and Gilman, 1970).

3) CORONARY BLOOD FLOW - Coronary blood flow is enhanced by adrenaline in man as well as animals. The increased flow occurs even with doses that do not increase the aortic blood pressure and is the resultant of three factors: increase in mechanical compression of the coronary vessels, direct action of drug on coronary vessels, and a dilator effect due to locally produced metabolites (Goodman and Gilman, 1970).

4) CARDIAC EFFECTS - Adenaline is a powerful cardiac stimulant. It acts directly on the beta-receptors of the myocardium and of the cells of the pacemaker and conducting tissues. This stimulation is independent of alterations in cardiac function secondary to increased venous return and other peripheral vascular effects (Goodman and Gilman, 1970).

5) RESPIRATORY EFFECTS - Adrenaline stimulates respiration but this effect is brief and considerably less striking than the stimulation of the respiratory centre by certain non-catecholamines. The drug has no clinical value as a respiratory stimulant. Adrenaline can effect respiration by its peripheral action on bronchial muscle, causing a powerful broncho-dilation (Goodman and Gilman, 1970).

6) METABOLIC EFFECTS - Adrenaline has a number of important influences on metabolic processes in

laboratory animals and man e.g. carbohydrate metabolism, blood concentration of free fatty acids, plasma protein levels. Body temperature is also elevated, in part as a result of cutaneous vasoconstriction. These are probably the major effects of endogenous adrenaline that are of importance under physiological conditions (Goodman and Gilman, 1970).

Some of the pharmacological effects of smoking are similar to those of stress. Watts (1960) has shown that nicotine is one of the most effective compounds for releasing adrenaline from the adrenal glands, producing a high blood adrenaline level. Burn (1960) demonstrated a peripheral vasoconstrictive action of nicotine which activated the release of noradrenaline from stores near to, or in vessel walls. The combined action of adrenaline and noradrenaline produced a lowered skin temperature, tachycardia, elevated blood pressure and increased metabolic rate (Goldhaber and Giddon, 1964). Wood (1960) discovered that the effects of the two vasoconstrictor stimuli on the peripheral circulation were additive.

Kardachi and Clarke (1974) postulated that the aetiology of A.N.U.G. was due to a powerful and extended vascular constriction resulting in ischaemic necrosis of the tips of the interdental papillae and the col crests, where the circulation is end-arterial and without collateral support. MacPhee and Beagrie (1962) described the tips of the interdental papillae and the col crests as the susceptible zones for the onset of A.N.U.G. lesions. They

felt that the separate sources of gingival blood supply to these two areas were significant.

A fourth predisposing factor in the aetiology of A.N.U.G. was a seasonal influence reported by Pedler and Radden (1957), who noticed an increased incidence of the disease during the winter months. The increased incidence of A.N.U.G. in winter may be explained by the fact that low temperatures induce peripheral vasoconstriction, augmenting the circulatory effects of sepsis, smoking and stress.

In summary, the most conspicuous predisposing factors in A.N.U.G. include:

1) Sepsis (Chronic marginal gingivitis)
2) Emotional stress (Adrenaline)
3) Tobacco smoking (Nicotine)
4) Cold climatic conditions

The mechanism involved in the aetiology of A.N.U.G. has been hypothetically constructed by Kardachi and Clarke (1974) on the basis of inferences from the literature. It has been separately stated that sepsis, smoking and stress form a triad of inter-related predisposing factors in the aetiology of A.N.U.G. Kardachi and Clarke (1974) suggest that these three factors have one systemic effect in common; the ability to markedly influence the circulatory efficiency of the gingivae.

Kardachi and Clarke's hypothesis satisfactorily accounts for the roles of the major predisposing factors

of A.N.U.G. and the inability of previous workers to establish a causal relationship between bacteria and the incidence of the characteristic lesion.

CHAPTER III

MATERIALS AND METHODS

As mentioned earlier in Chapter I one of the major obstacles which had to be overcome was the miniaturisation of an electronic sensing device used for the indirect measurement of gingival blood flow. A microthermistor assembly was designed for this purpose which satisfied the necessary criteria of small physical size, adequate mechanical strength and electrical insulation.

The second major problem was the development of a reliable technique for direct blood pressure measurement in the experimental animal during drug infusion. Accurate and reliable blood pressure measurements were achieved by loop cannulation of the carotid artery. Incorporation of a physiological blood pressure transducer into the cannula loop permitted continuous monitoring of the animal's blood pressure.

The development of the microthermistor assembly and the technique for loop cannulation are described in greater detail later in the chapter.

This chapter is presented in four segments:

1) equipment and associated development,
2) materials,
3) experimental method, and
4) human study

1. EQUIPMENT

1) EXPERIMENTAL ANIMAL. The New Zealand lop-eared rabbit weighing approximately 2.5 kg was used because it satisfied the following criteria:

a) sufficient gingival crevice depth to accommodate the thermistor assembly used in thermal diffusion experiments.

b) cannulation of the carotid artery, a relatively simple procedure.

c) intra-venous infusion of drugs into the marginal ear vein, a straightforward procedure.

A total of 45 animals were used in the study and each animal was used for four series of experiments. There was a minimum time lapse of two weeks between experimentation on the same animal.

2) NEGATIVE TEMPERATURE COEFFICIENT THERMISTOR

a) Thermistor Characteristics.

A negative temperature coefficient thermistor (ITT U23UD) was used as the transducer for the indirect measurement of gingival blood flow. Thermistors are preformed ceramic elements prepared from a carefully controlled mixture of manganese, nickel and copper oxides. They are semi-conducting resistors which possess large temperature coefficients of resistance ($R \alpha {}^{1}/T$). The equation used to determine the thermistor resistance at a temperature other than its reference temperature is as follows:

$$R_2 = R_1 \text{ EXP. } B\ ({}^{1}/T2 - {}^{1}/T1)$$

where R_1 = resistance at temperature T1 (^{O}K)

R_2 = resistance at temperature T2 (^{O}K)

B = characteristic temperature (^{O}K)

Thermistors can be considered to respond immediately to a temperature change at a rate dependent upon the mass of the thermistor bead and the nature of the ambient environment. Additional technical information is shown below:

CODE	R20	R25	Rmin	B
U23UD	2K	1.7	60	2900

where B = characteristic temperature (^{o}K)

R20 = resistance (ohms) at $20^{o}C$

R25 = resistance (ohms) at $25^{o}C$

Rmin = minimum operating resistance (ohms)

K = dissipation constant (87 $\mu W/^{o}C$)

TAmax = maximum ambient temperature ($180^{o}C$)

Pmax = maximum continuous power dissipation (20 mW at $20^{o}C$)

TBmax = maximum bead temperature ($180^{o}C$)

b) Thermistor Assembly.

Microthermistors (ITT U23UD) were used because their small size (0.4 mm x 0.5 mm) enabled them to be inserted into the gingival crevice of the experimental animal with minimal gingival displacement. The fragile platinum - ruthenium leads (0.025 mm diameter) were kept short to minimize fracture. A multi-core (7 x 0.05 mm diameter) teflon coated cable was soldered to the thermistor leads for the connection to the measuring and recording equipment. The soldered joints were made using a low voltage soldering unit* which incorporated a 1/32" width PTH7 soldering tip.

* WELLER (Model W - TCP)

Approximately 1 mm of teflon insulation was carefully stripped from the multi-core cable, taking great care not to damage the wire strands which were to be inserted into the central region of the cable. When the platinum - ruthenium lead wire was in place, the rose was closed and the joint soldered under a dissecting microscope using the PTH7 soldering tip.

The framework for the thermistor assembly required rigidity adequate for insertion into the tight gingival space but the dimension of the frame had to be small for the thermistor bead, insulating material, and framework to be accommodated within the gingival crevice. The contact of the frame and thermistor required that the former be constructed from an insulating material.

A MYLAR cellulose acetate matrix strip (0.02" thickness) was used for the mounting frame, and Araldite* was used to attach the thermistor assembly to the frame and to provide electrical insulation.

c) Thermistor Stabilization.

Stabilization of the framework was necessary to maintain the thermistor bead in a fixed position

* HARDENER HY951, Epoxy resin CY 221 in ratio 8.6:1
CIBA PRODUCTS

within the gingival crevice throughout the entire experimental period. This was achieved by acid etching the labial surface of the lower incisor and bonding the thermistor framework to the tooth using a composite resin restorative material.*

d) Thermistor Operation.

Reference control of the measuring thermistor was achieved by using a decade resistance box** to obtain a null balance with the thermistor. This device ensured that the cause of voltage changes measured across the thermistor were limited to local circulatory changes.

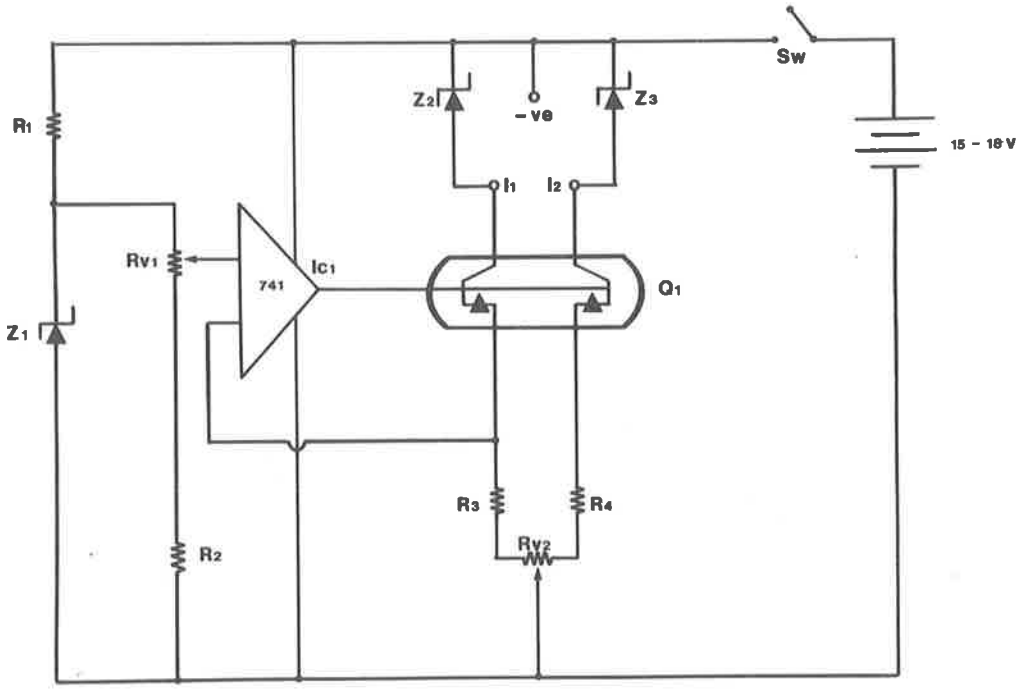
The microthermistors were fragile and sudden current surges were found to destroy the sensing element. The operating life of the thermistor was significantly prolonged by increasing the current through the device by increments to attain the required current level.

3) CONSTANT CURRENT GENERATOR

A constant current generator was used to supply a constant current within the range between 5 - 13 mA to the thermistor assembly. An 18V D.C. power source was used and the circuit is shown in Figure 1.

* CONCISE (3M brand)
** TRITON ENGINEERING MODEL LDB 116

FIGURE 1. Circuit diagram of constant current generator.



Two output terminals supplying the same constant current and a reference terminal were available. The measuring thermistor was connected across the output and reference jacks. A digital voltmeter was used to measure the voltage across the measuring thermistor when operating at a constant current. This voltage was applied to the first channel of a multi-channel pen recorder.

4) PHYSIOLOGICAL PRESSURE TRANSDUCER

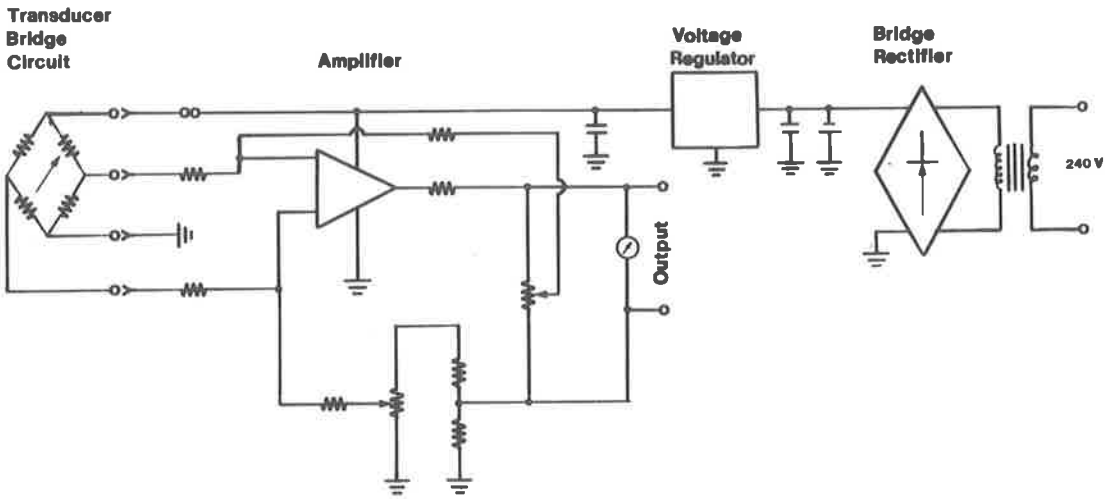
Blood pressure was directly monitored by a physiological pressure transducer* electrically coupled to a blood pressure indicator. (O/P IV = 100 mm). The transducer consisted of a diaphragm-transducer assembly and a transparent pressure dome. Applied pressure displaced the sensing diaphragm and activated the transducing element which consisted of an unbonded strain gauge with the following performance characteristics:

Excitation	7.5 volts
Input resistance	322 ohms
Output resistance	323 ohms
Calibration Factor	48.27 uV per volt per C. Hg.

The pressure transducer was powered by a 6V D.C. source and the transduced signal was relayed to the second channel of the pen recorder. Calibration of the transducer prior to actual experimentation showed it to be accurate (Figure 2).

* STATHAM MODEL P23

FIGURE 2. Circuit diagram of pressure transducer and power supply.



2. MATERIALS

Although the investigation was designed to determine the effects of circulating nicotine and adrenaline upon gingival blood flow using the thermal diffusion technique, infusion of a control chemical was also essential. The drugs used in this study were:

1) PHYSIOLOGICAL SALINE (5 mls)

Physiological saline was used as the control chemical because of its minimal influence on the circulatory system and homeostatic mechanisms of the experimental animal.

2) 10^{-7}M NICOTINE (5 mls)

Nicotine dosage was calculated from the body weight of the animal (2.5 kg average) using VAN PROOSDIJ's (1960) information that 2.5 mg nicotine is absorbed from the smoke of one cigarette in humans (65 kg average).

The complex and often unpredictable changes that occur in the body after administration of nicotine are due to its actions on a variety of neuro-effector junctions and because the alkaloid has both stimulant and depressant phases of action. It acts on all autonomic ganglia, voluntary muscle, the central nervous system and releases cate-cholamines from the adrenal medulla. The ultimate response of any one structure or system represents the algebraic summation of the several different and opposing effects of nicotine (Gro-lman and Grollman, 1970).

Nicotine is a powerful stimulant of the central nervous system. Small doses produce tremors in both man and laboratory animals while larger doses cause tremors and convulsions. The action of nicotine on the spinal functions appears to be one of stimulation, followed by inhibition or paralysis.

Following the injection of nicotine, the respiratory centre is first stimulated and then depressed. Respiration failure is often due to the curare-like action of nicotine on the nerve endings in the diaphragm which prevents the respiratory muscles from responding.

The action of nicotine on the circulation is extremely complex; it is the result of the divergent actions of the drug on the vasomotor and vagus centres in the medulla, the sympathetic and parasympathetic ganglia, the adrenal medulla and the chemo-receptors of the carotid sinus and the aortic body.

3) 10ugm ADRENALINE (5 mls)

Adrenaline dosage was derived from the study by GIDDON et al (1963), who used the same concentration as a "stress procedure" in animal experimentation. Adrenaline is one of the most potent vasopressor drugs known. Particularly prominent are the actions on the heart and on the smooth muscle of the vascular system. The chief vascular action of adrenaline is exerted on the smaller arterioles and precapillary sphincters, although veins and

larger arteries also respond to the drug.

Adrenaline evokes a characteristic effect on blood pressure, causing it to rise rapidly to a peak that is proportional to the dose. The mechanism of the rise in blood pressure due to adrenaline is due to a direct myocardial stimulation that increases the strength of ventricular contraction, an increased heart rate, and vasoconstriction of the vascular beds of the micro-circulation. The pulse rate which is at first accelerated, may be retarded at the height of the blood pressure rise by compensatory vagal discharge.

Adrenaline administration stimulates respiration in both animals and man. However, a brief period of apnea may occur before respiratory stimulation. The apnea is probably due to a transient reflex inhibition of the respiratory centre through the bioreceptors. Adrenaline can also affect respiration by its bronchodilator action on the bronchial muscle.

4) COMBINATION OF 1), 2) and 3) (5 mls)

3. EXPERIMENTAL METHOD

1) ANIMAL SEDATION

Aqueous Ethyl Carbamate (URETHANE: 2.5 gms per 10 mls), injected intra-peritoneally at 1 gm/Kg body weight, was used to obtain a tranquil anaesthesia of long duration without circulatory depression.

2) DRUG ADMINISTRATION

Intra-arterial drug infusion was achieved using a syringe pump* to inject the test drug into the carotid artery of the experimental animal at a rate of one ml per minute for five minutes. Immediately following the drug infusion a further 3 mls of physiological saline was introduced into the circulation via the syringe pump to "flush" the drug fully into the vascular system.

A scalp vein infusion kit was used for intravenous drug administration into the marginal ear vein of the rabbit using the same equipment and method as described earlier for intra-arterial infusion.

3) BLOOD PRESSURE MEASUREMENT

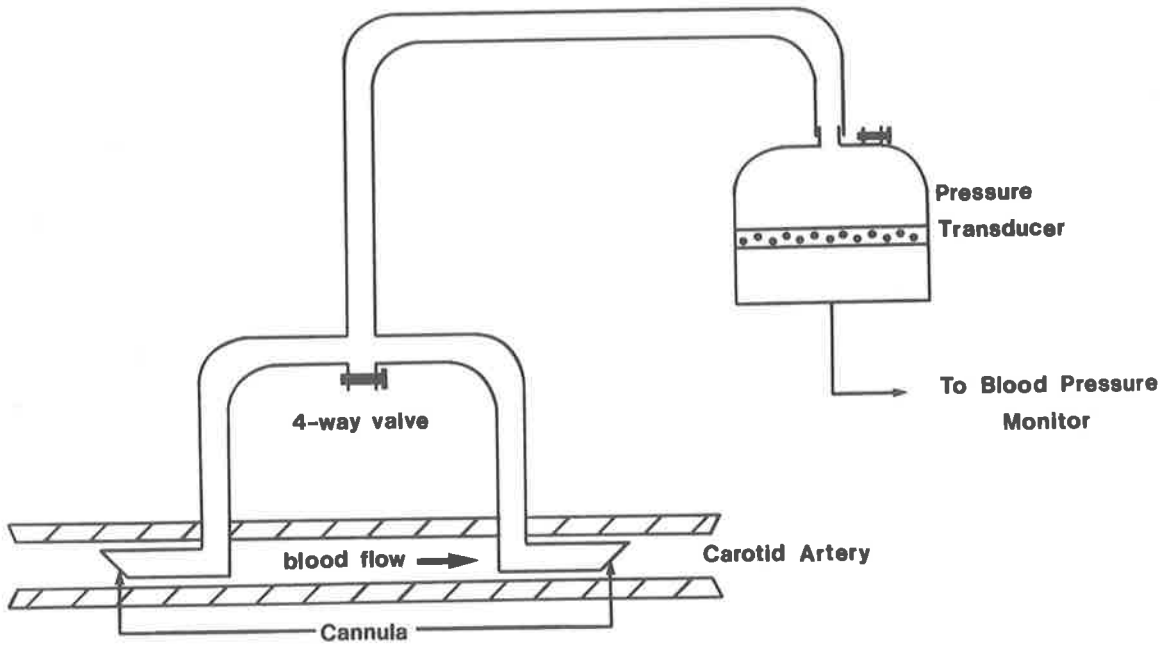
Because the main objective of the study involved the measurement of changes in gingival blood flow, the

* SAGE (MODEL 351)

blood pressure response which paralleled the peripheral circulatory changes was also monitored.

To accurately measure blood pressure, cannulation of the common carotid artery was made on the same side of the body as the thermistor assembly. A three inch incision was made in the midline from larynx to sternum; subepithelial blunt dissection was used to separate the fascial sheath surrounding the infrahyoid and sternocleidomastoid muscles from the major vessels. After the carotid sheath was isolated, the common carotid artery was exposed by careful dissection to the bifurcation of the internal and external carotid branches. The common carotid artery was then temporarily clamped to permit cannulation of the vessel. A closed cannula loop was developed by cannulating the artery close to the base of the incision and returning the blood to the artery close to its division into internal and external branches. Use of the closed cannula loop and four way valve permitted continuous monitoring of the animal's blood pressure while drug infusion was in progress. This system provided optimum blood flow through the carotid artery and thus ensured that the gingiva received adequate circulation. The blood pressure response was directly monitored by a physiological pressure transducer which was electrically coupled to the second channel of the multipen recorder (Figure 3).

FIGURE 3. Diagrammatic representation of cannula loop.



4) GINGIVAL BLOOD FLOW

Gingival blood flow was measured indirectly by the thermal diffusion principle; a negative temperature coefficient (N.T.C.) thermistor heated by a constant current achieved a stable resistance and temperature under constant conditions. If the conditions varied then the thermal diffusion from the thermistor bead altered, resulting in a changed bead resistance. The variable parameter measured was voltage, but with the constant current known, a simple Ohm's Law calculation enabled the resistance to be calculated.

OHM'S LAW: $E = IR$

where E = voltage in volts (measured)
I = current in amps (measured)
R = resistance in ohms (by calculation)

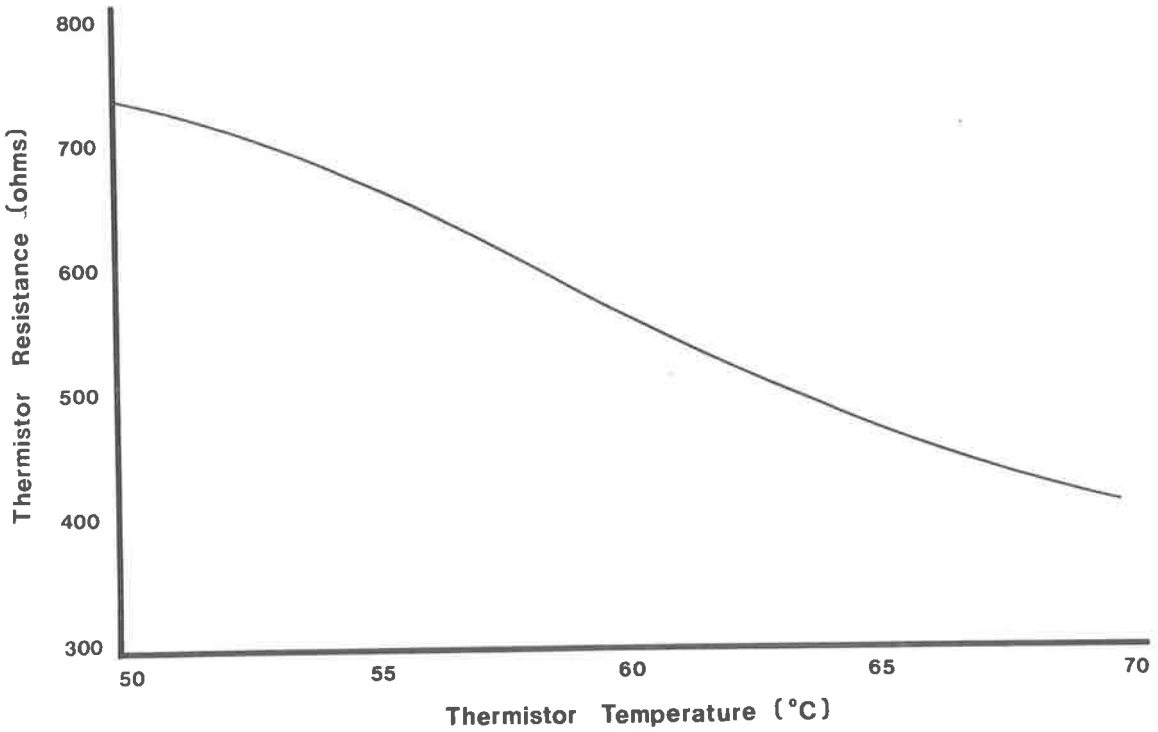
The voltage across the thermistor was monitored by a digital voltmeter, the constant current was delivered by a current generator, and the thermistor bead temperature was calculated from manufacturer's data of resistance plotted against temperature (Figure 4).

Temperature change within the gingival crevice was assumed to be dependent on circulatory changes within the gingival vessels. An increase in circulatory efficiency provided for increased heat dissipation, a cooler thermistor bead, and increased resistance and observed voltage.

FIGURE 4.

MANUFACTURER'S SPECIFICATIONS FOR

THERMISTOR RESISTANCE vs TEMPERATURE



Conversely, a reduced blood flow within the gingival vessels resulted in an increased bead temperature, and reduced resistance and voltage.

With the thermistor assembly firmly bonded to the lower incisor tooth of the experimental animal, the micro-assembly was balanced to "null" balance using the decade resistance box. A stable baseline voltage across the measuring thermistor was achieved for 15 minutes prior to drug infusion. Following infusion, any change in blood flow rates through the gingival capillaries adjacent to the device were reflected by changes in the temperature of the thermistor bead. As previously mentioned the millivoltage changes of the thermistor assembly were recorded on the first channel of the pen recorder.

The body temperature of the animal was maintained at a constant 78 ± 1^{o}F with thermostatically controlled equipment to avoid constriction of peripheral vessels that occurs with a drop in body temperature (Coffman, 1969).

5) ADMINISTRATION OF MICROSPHERES

Black plastic microspheres which were physically inert and caused no tissue reaction nor demonstrated adsorption or absorption of blood were utilized in the histological study. Random sampling of the

FIGURE 5. Diagrammatic representation of thermistor assembly.

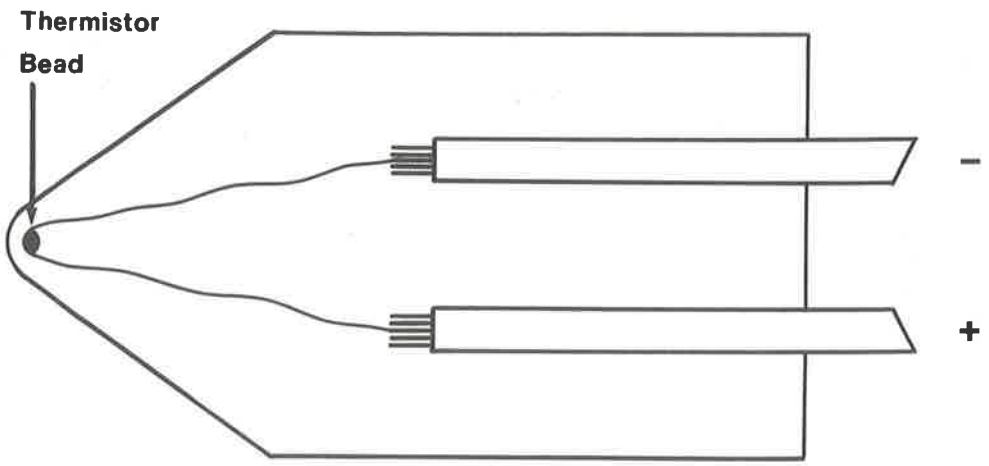
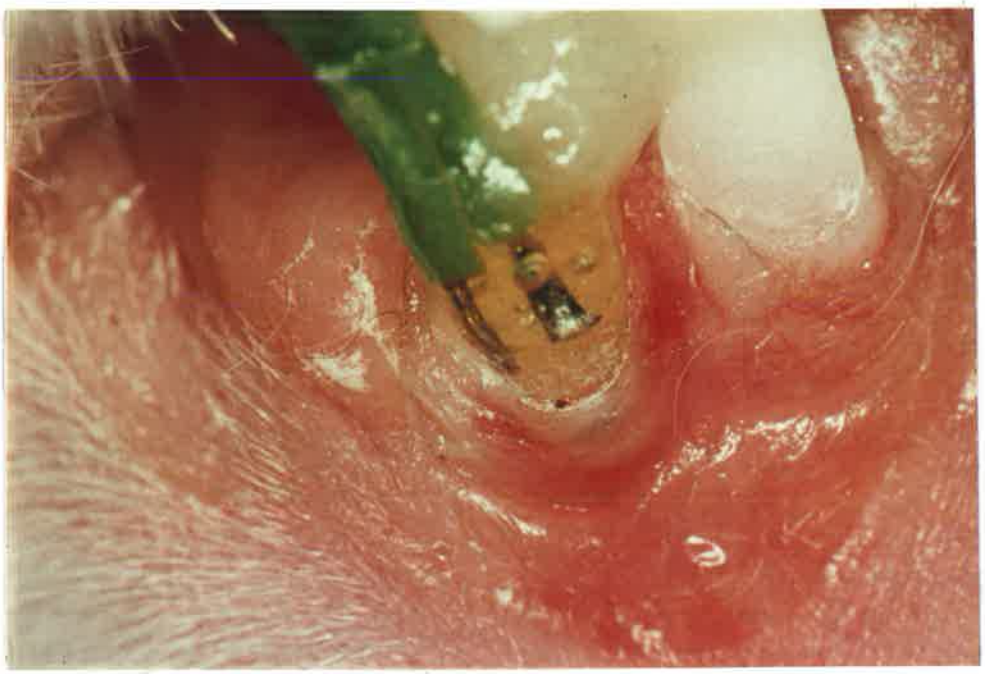


FIGURE 6. Thermistor assembly in the gingival crevice of the experimental animal.



microspheres revealed that more than 90% of the spheres were within 15 ± 5 microns. The specific gravity of the microspheres was approximately 1.4 and a long lasting suspension was made using 15% sterile Dextran as a vehicle.

A 2 ml suspension of microspheres was slowly introduced into the common carotid artery of the experimental animal using a 5 ml syringe connected to the second branch of the cannula loop. Microsphere infusion was followed by a 3 ml physiological saline "flush". It was possible to maintain infusion pressure only slightly exceeding the intra-arterial pressure by observing the direction of flow of the microspheres in the clear polyethylene cannula loop. Using the correct infusion pressure the microspheres were transported by the blood to the site of impaction within the gingival vessels of the peripheral circulation where the vessels and the microspheres were of the same diameter. Histologic comparison of the location of impaction of the spheres was made under the influence of the tested drugs.

6) SACRIFICE

Following completion of each experiment, the animal was sacrificed with an intra-peritoneal overdose of sodium pentobarbitol (130 mg/Kg body weight).

7) HISTOLOGIC PREPARATION (See Appendix I)

4. HUMAN STUDY

A study using five female volunteers was also undertaken to ascertain the effects of smoking on gingival blood flow and peripheral temperature. The subjects used in this study were randomly selected from volunteer nursing staff at the Royal Adelaide Dental Hospital. The criteria for their selection were as follows:

a) clinically normal periodontal tissues

b) age range 17 - 20 years

c) all subjects smoked an average of 10 cigarettes/ day.

Circulating nicotine levels were reduced to a minimum by asking the subjects to refrain from smoking for a period of 12 hours before an experiment.

1) MATERIALS

A cellulose acetate crown form with an attached pre-soldered thermistor was inserted into the gingival crevice of the subject and cemented to the upper left lateral incisor tooth with a temporary filling material*.

An acrylic stent was also made for each subject for left and right sides as a method of isolating the lip from the thermistor assembly and gingiva. The stents passed in a horseshoe shape around each lateral incisor tooth, covering their gingival

* CAVIT-W., ESPE Co., Germany

margins. They were approximately 2 mm in thickness, were well tolerated by the subjects, were stable and reduced mechanical stimulation of the thermistor by lip movement.

Peripheral temperature was monitored at the same time as thermistor voltage by an electronic thermometer (Figure 7). The sensor of the thermometer was taped to the tip of the inside surface of the subject's third finger of the left hand. The instrument was calibrated before each experiment by immersion into a water bath of known temperature.

2) EXPERIMENTAL METHOD

The subject was seated in a cubicle adjacent to the measuring and recording equipment. This cubicle eliminated external distracting auditory and visual stimuli as far as possible. The electronic thermometer was calibrated and the sensor probe taped to the finger of the subject's left hand which was then placed under a protective covering to insulate from air currents. A crown form thermistor assembly was cemented onto the subject's upper left lateral incisor and the acrylic stent was then fitted to separate the lip from the thermistor assembly. The subject was instructed to keep lip movements and swallowing to a minimum but was allowed to read or sleep during the experiment.

FIGURE 7. Circuit diagram of electronic thermometer.

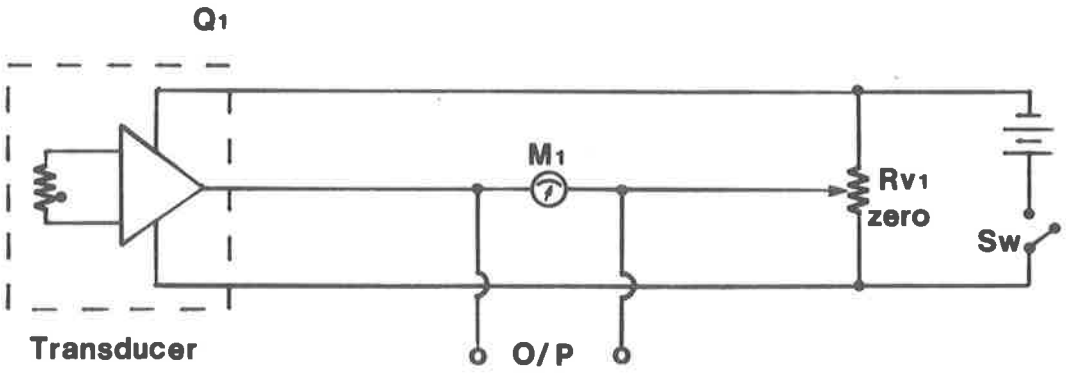


FIGURE 8. Diagrammatic representation of crown form thermistor assembly.

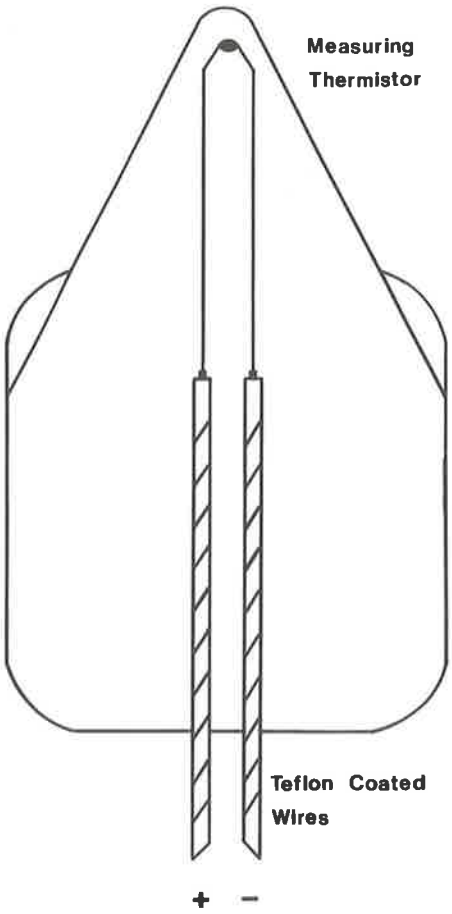
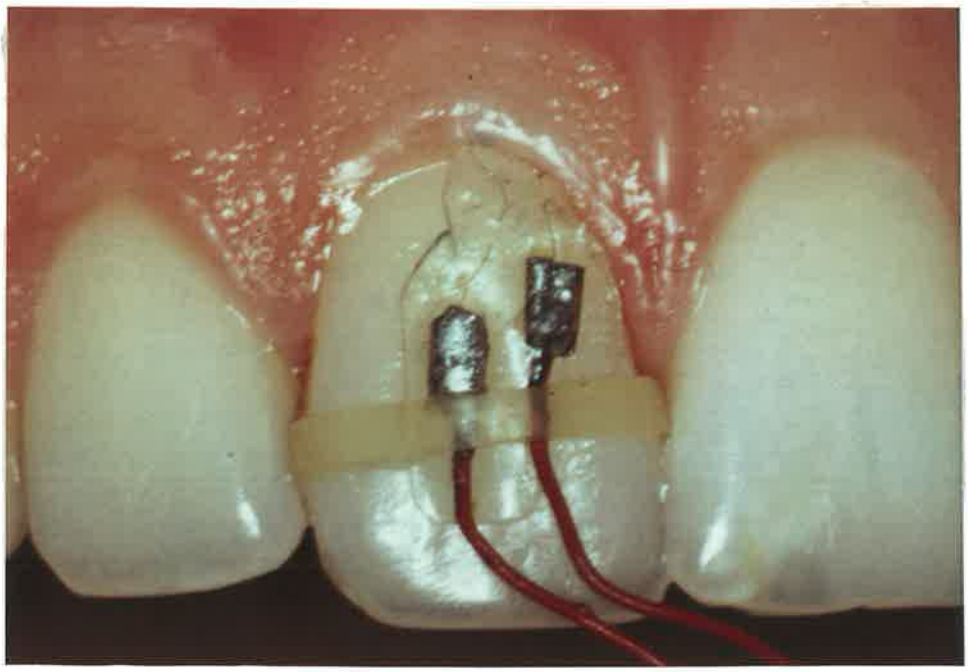


FIGURE 9. Initial thermistor assembly in situ, stabilised by an orthodontic elastic. The thermistor bead can be seen through the gingival crest.



When both thermistor voltage and digital temperature reached a stable level, the subject smoked one cigarette. All subjects smoked either:

	TAR CONTENT (mg)	NICOTINE CONTENT (mg)
MARLBORO	22.4	1.24
or ALPINE	26.4	1.52

(Moore, Bross, Shamberger and Bock, 1967). Recording continued until voltages returned to pre-experimental levels.

CHAPTER IV

RESULTS

INTRODUCTION

The present study was designed to investigate the effects of smoking and stress on gingival blood flow. For that reason the experimental agents used were nicotine, the principal drug derived from smoking, and adrenaline, the principal catecholamine associated with physiological stress. Saline was used as a control to record the mechanical effect of the employed technique and so provide baseline data against which the effects of the other drugs could be compared.

The relationship between blood flow, capillary radius, blood pressure and viscosity was determined from Poiseuille's Law. The radius of the vessel is the critical parameter determining blood flow, hence any factor changing capillary bore must significantly influence the rate of blood flow in gingival tissues.

The gingival blood flow data reported in this chapter was derived from experimentation employing a thermal diffusion technique. In addition blood pressure and peripheral temperature of the experimental animals was also recorded. Ten experiments were completed for each drug or combination and the data obtained is tabulated in Appendix II.

RESULTS

I. SALINE

A. INTRAVENOUS ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

Saline infusion initially caused a small rise in millivoltage values equivalent to a 0.2^{o}C decrease in thermistor bead temperature after approximately 2 minutes. The small rise in millivoltage values and the associated decrease in thermistor bead temperature was a constant finding during all drug infusions. Three minutes later the minimum millivoltage value was recorded which corresponded to an increase in the bead temperature of 0.1^{o}C (Table 1). The resting value was quickly regained and maintained for the remainder of the experimental period (Figure 10).

2) BLOOD PRESSURE

The typical blood pressure of the anaesthetised rabbit was 55 mm Hg (mean pulsatile pressure) prior to administration of saline. Experimental infusion of saline caused slight changes in blood pressure values as shown in Figure 10. An early hypotension was followed by a mild hypertension with pre-experimental values restored approximately nine minutes after infusion began.

FIGURE 10.

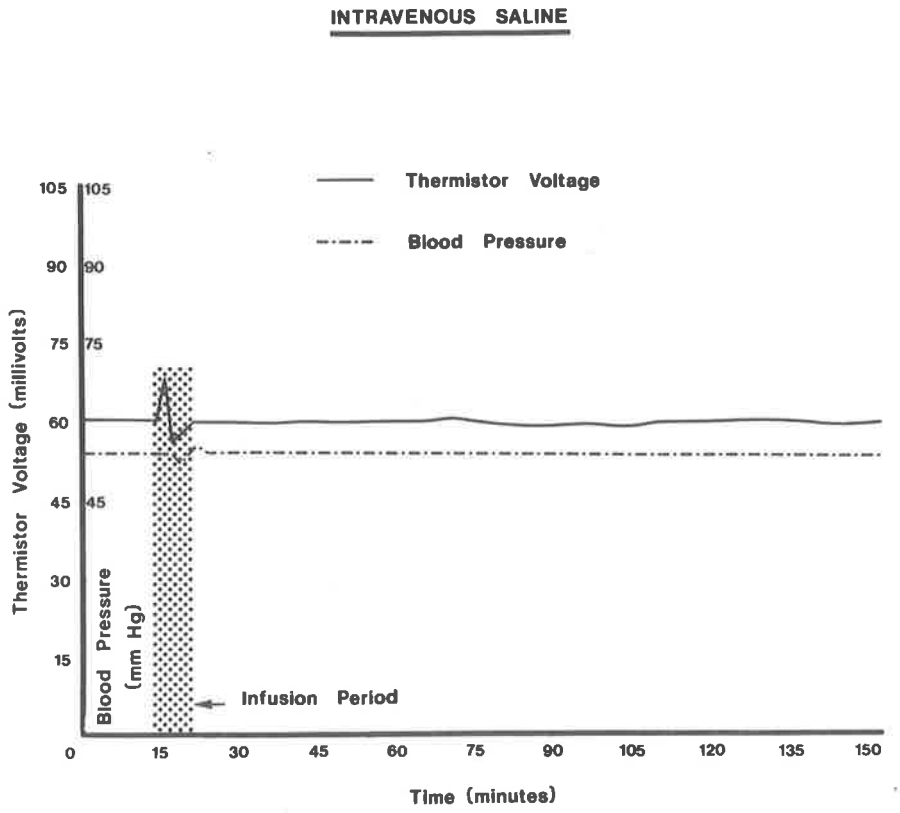
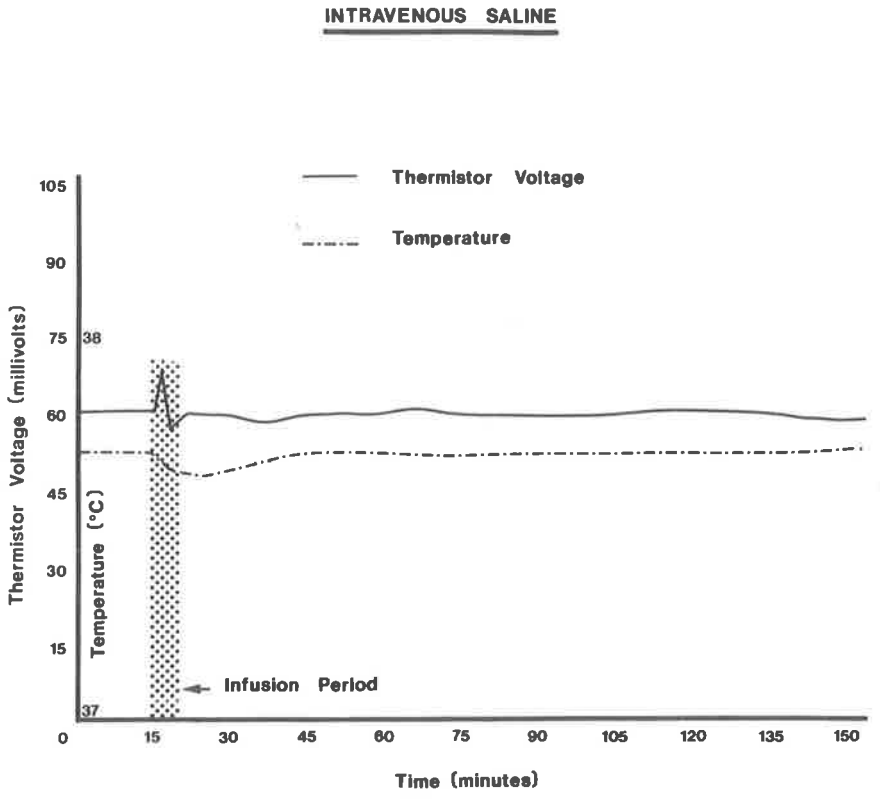


FIGURE 11.



3) PERIPHERAL TEMPERATURE

Saline infusion caused a maximum change of 0.1^{o}C which occurred 12 minutes after infusion ceased. The pre-experimental temperature was regained after 20 minutes (Figure 11).

B. CONTINUOUS HALF HOURLY INTRAVENOUS ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

The multiple infusion experiment gave essentially the same result following each infusion as the single dose experiment (Figure 12). The values for the thermistor assembly operative are shown in Table 2.

2) BLOOD PRESSURE

Multiple infusions of saline at 30 minute intervals over a period of 6 hours gave results comparable with the single dose experiment following each infusion (Figure 12).

3) PERIPHERAL TEMPERATURE

For four infusions the peripheral temperature varied in a manner that closely followed the results obtained from a single dose procedure. Virtually no change in peripheral temperature was recorded for the remainder of the experimental period (Figure 13).

FIGURE 12.

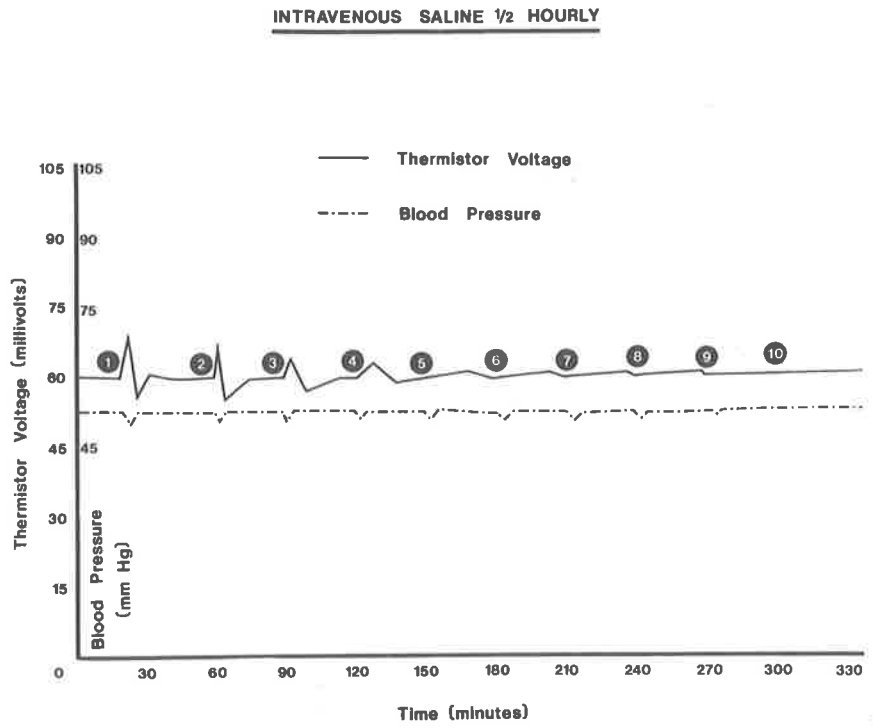
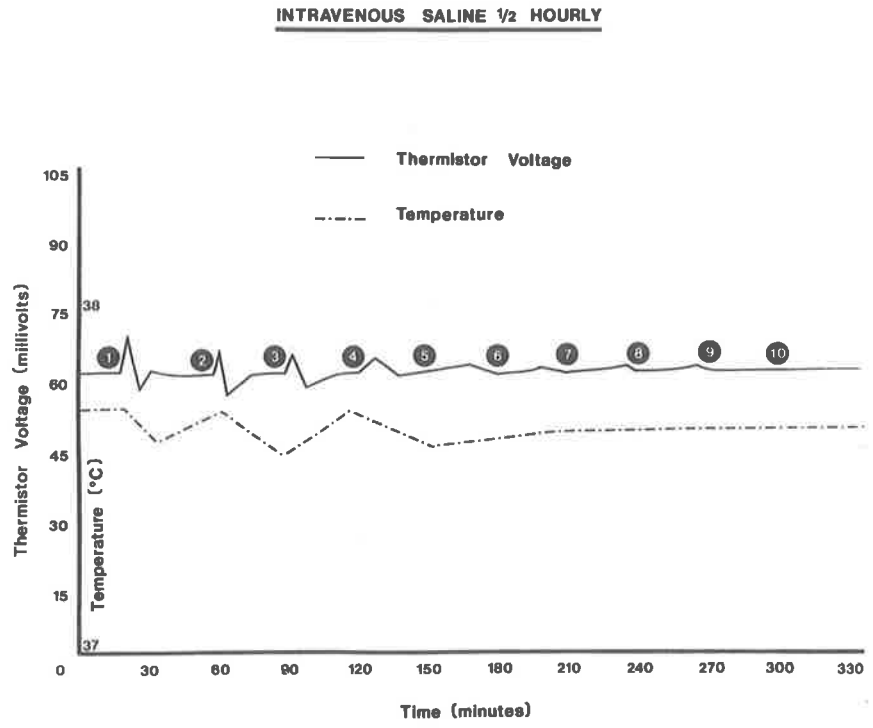


FIGURE 13.



C. INTRA-ARTERIAL ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

Intra-arterial saline infusion had little effect upon thermistor temperature; a variation of 0.2°C was observed approximately 3½ minutes after infusion began (Figure 14). The values for the thermistor assembly operative are shown in Table 3.

2) BLOOD PRESSURE

Intra-arterial saline caused a fall of 8 mm Hg in blood pressure values immediately following infusion. Stable pressures were regained within 15 minutes of infusion cessation and were maintained for the duration of the experimental period (Figure 14).

3) PERIPHERAL TEMPERATURE

The maximum temperature variation recorded in the experimental period was 0.1°C above the resting value, eight minutes after infusion began (Figure 15).

D. CONTINUOUS HALF HOURLY INTRA-ARTERIAL ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

The first intra-arterial infusion of saline caused the thermistor temperature to fall 0.2°C at the end of infusion. The thermistor temperature returned to the resting level after 3 minutes and remained stable until the next infusion produced a similar response (Figure 16).

FIGURE 14.

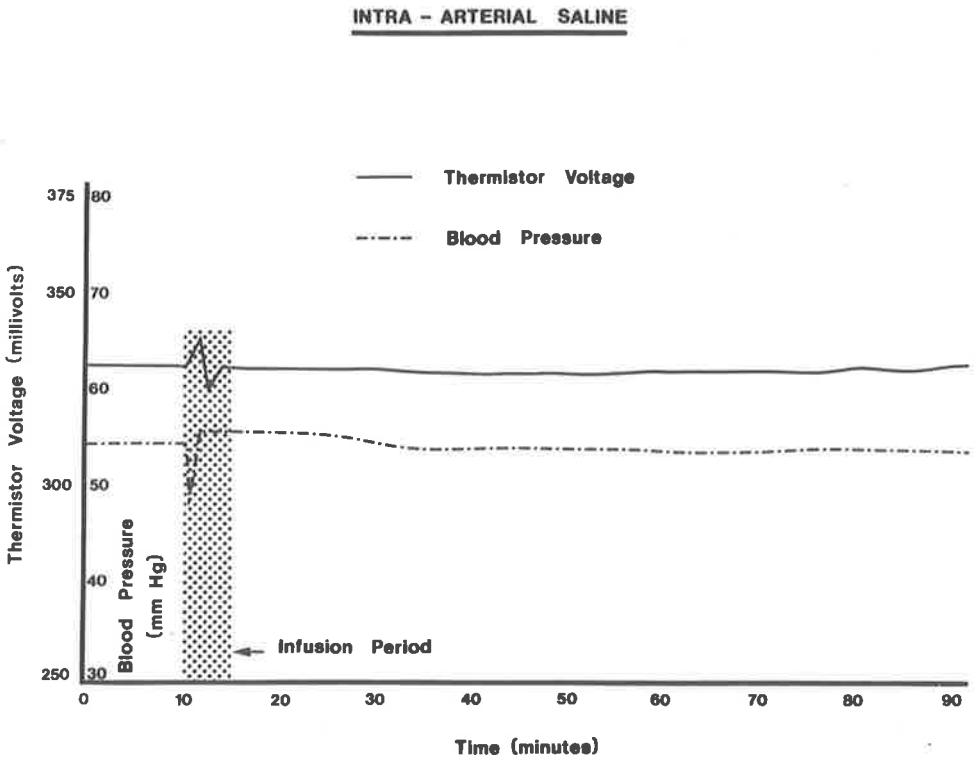
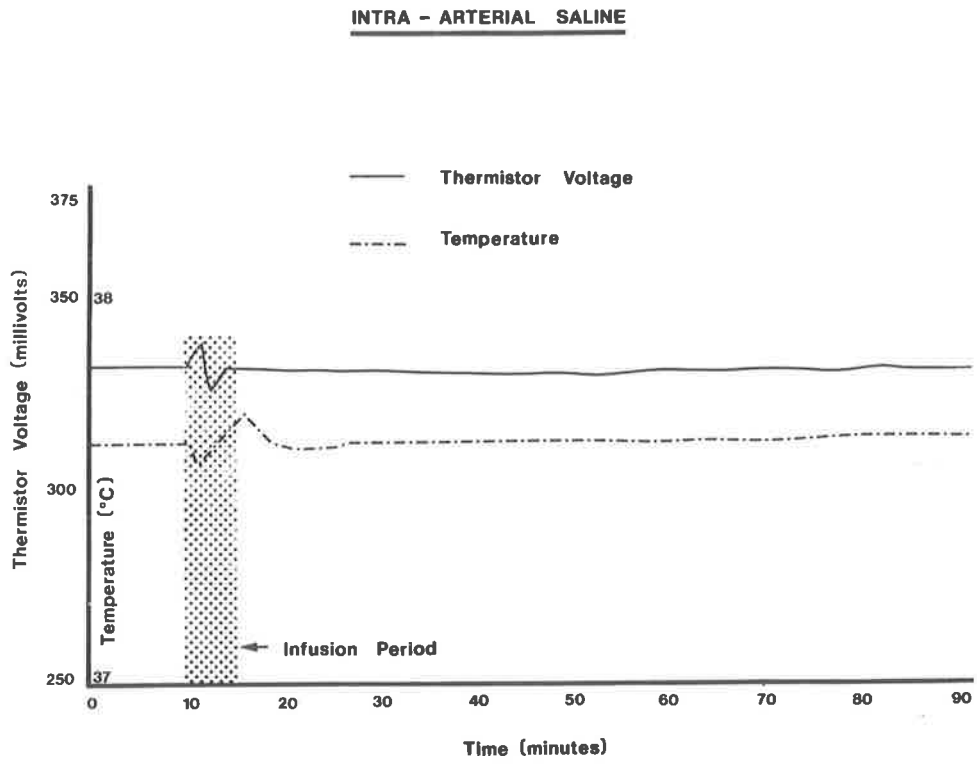


FIGURE 15.



The values for the thermistor assembly operative in this experiment are shown in Table 4.

2) BLOOD PRESSURE

The results obtained for the first four presentations were very similar to the results obtained for the single dose experiment.

The blood pressure response to the remaining saline infusions registered a slow but steady fall in values for the remainder of the experimental period (Figure 16).

3) PERIPHERAL TEMPERATURE

Initial infusions caused an early fall in peripheral temperature followed by a slight rise when the values were compared with resting levels.

Later infusions caused a slow but gradual decrease in peripheral body temperature which remained relatively constant for the final 90 minutes of the experimental period (Figure 17).

E. SUMMARY

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

Intravenous (IV) and intra-arterial (IA) saline administration caused only minimal changes to

FIGURE 16.

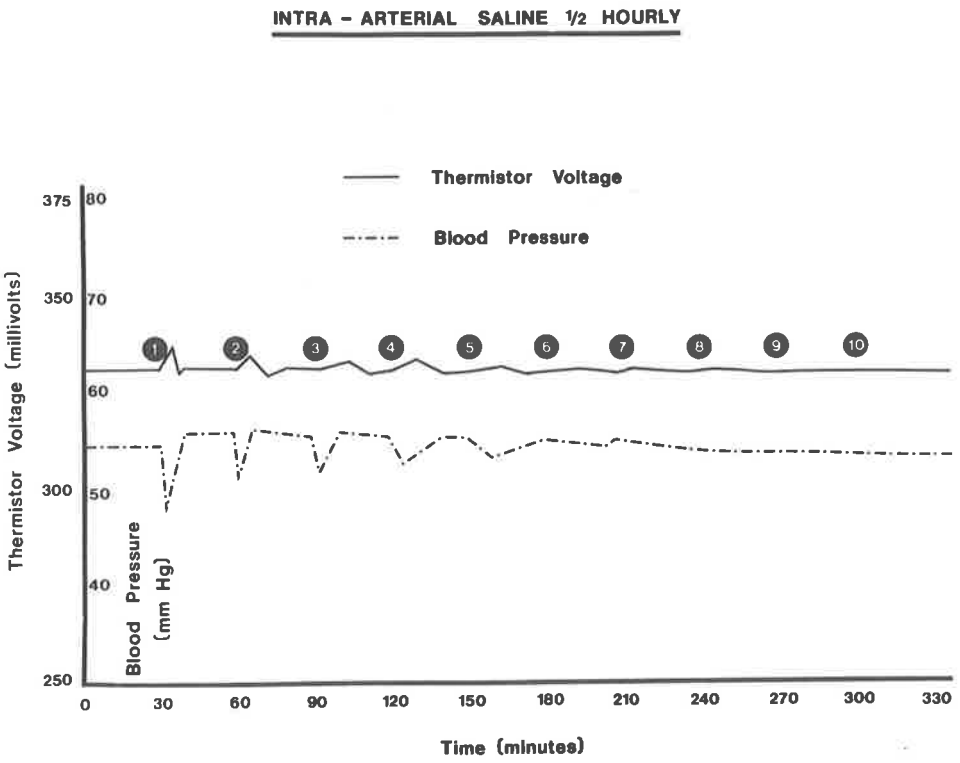
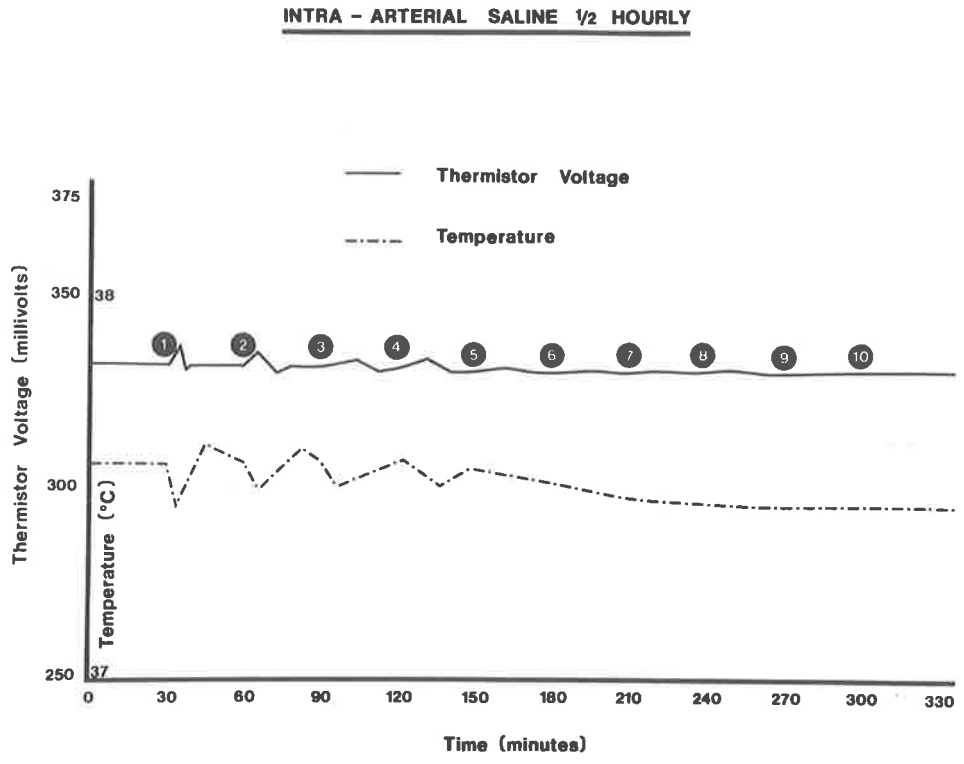


FIGURE 17.



the resting millivoltage values of the microthermistor assembly. The only differentiating feature in millivoltage values occurred following IA saline presentation when millivoltage values demonstrated an exceptionally quick response.

2) BLOOD PRESSURE

IV or IA saline infusion had minimal effect upon the blood pressure of the experimental animal (Figures 1 and 5). Continuous half hourly IV or IA saline infusion exhibited the same degree of response throughout the experimental period as the single dose experiment.

3) PERIPHERAL TEMPERATURE

IV and IA saline infusion resulted in a similar temperature fall but the time required to regain the resting value was significantly longer following IV infusion. Continuous half hourly infusions exhibited similar characteristics to the single dose experiments except that repeated infusions resulted in gradual diminution of temperature response.

F. DISCUSSION

IV saline infusion appeared to exhibit a greater millivoltage response than IA administration. This phenomenon was possibly due to local environmental factors

active in relation to the marginal ear vein used in this experiment.

The progressive decrease in millivoltage response with multiple infusion was possibly due to fatigue within the controlling mechanisms.

Blood pressure and flow within the circulatory system are most sensitive to minute changes in blood volume of the arterioles. The arterioles are the final or end branches of the circulatory system operating as valves controlling the entry of blood into the capillaries. The lumen of the arterioles is controlled by the state of tone of the involuntary circular muscle within the vessel wall and is influenced by autonomic nervous system output as well as by circulating metabolites and local products of muscle metabolism.

The different temperature response to single dose saline infusion via IV and IA routes could be due to local factors related to the marginal ear vein. IA infusions effected a quicker return to equilibrium temperature values than IV infusion because one of the physiological functions of the central circulation is to maintain a constant core body temperature.

Saline had an insignificant effect upon blood pressure and peripheral temperature although minor fluctuations were recorded. The effect of saline upon thermistor voltage was minimal but demonstrated the utility of the device in responding to small changes in blood flow.

The data obtained from the effects of saline infusion upon thermistor voltage, blood pressure and peripheral temperature was used to compare the data obtained from the pharmacologically active drugs subsequently investigated.

2. NICOTINE

INTRODUCTION

The pharmacological actions of nicotine on the cardiovascular system are complex and often unpredictable because the alkaloid has both stimulant and depressant phases of activity. The overall response of infusion represents the algebraic summation of the several different and opposing effects of nicotine. The cardiovascular responses to nicotine parallel those that accompany stimulation of the sympathetic nervous system. Nicotine stimulation of the sympathetic ganglia and the adrenal medulla, together with the discharge of catecholamines from sympathetic nerve endings accounts for this similarity. Also contributing to the sympathomimetic response of nicotine is the activation of chemoreceptors of the carotid and aortic bodies causing vasoconstriction, tachycardia and elevated blood pressure (Goodman and Gilman, 1970). The action of nicotine on the peripheral circulation was shown by Kottegoda (1953) and Burn (1960), to produce effects similar to sympathetic responses by constriction of the peripheral vessels and liberation of noradrenaline from stores near or in the vessel walls.

RESULTS

A. INTRAVENOUS ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

During nicotine infusion the millivoltage across the

thermistor assembly rose to a peak value after 1½ minutes, which was calculated to represent a fall in thermistor bead temperature of 0.2°C (Table 5). Forty minutes later a minimum value was recorded which corresponded to an increase in bead temperature of 0.6°C above the resting value. The pre-experimental value of the assembly was regained after a further 50 minutes (Figure 18).

2) BLOOD PRESSURE

Nicotine infusion caused an initial hypotensive episode of 15 mm Hg within 2 minutes of drug infusion before exhibiting a hypertensive peak to 62 mm Hg 8 minutes later. Equilibrium values were regained and stabilized after a further period of 40 minutes (Figure 18).

3) PERIPHERAL TEMPERATURE

Nicotine infusion caused a 0.2°C decrease in peripheral temperature which was registered approximately 25 minutes after infusion. A stable temperature value was regained after 45 minutes which was slightly higher than the pre-experimental value (Figure 19).

B. CONTINUOUS HALF HOURLY INTRAVENOUS ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

The first nicotine infusion exhibited a response which was almost identical to the single dose experiment. The decrease in thermistor voltage

FIGURE 18.

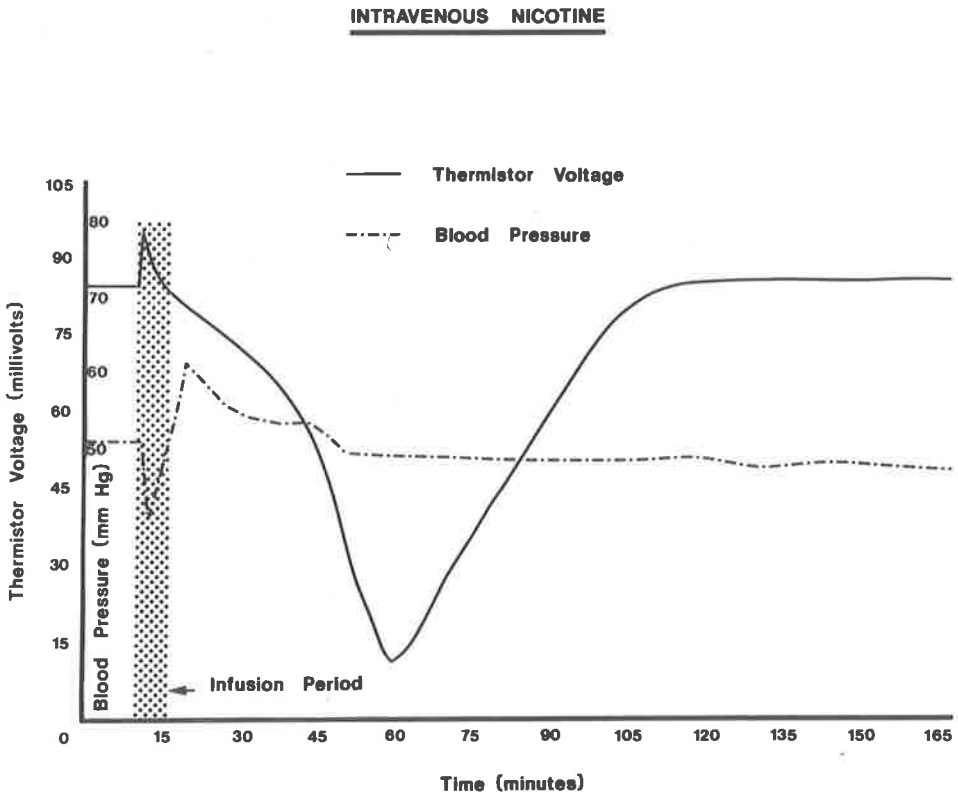
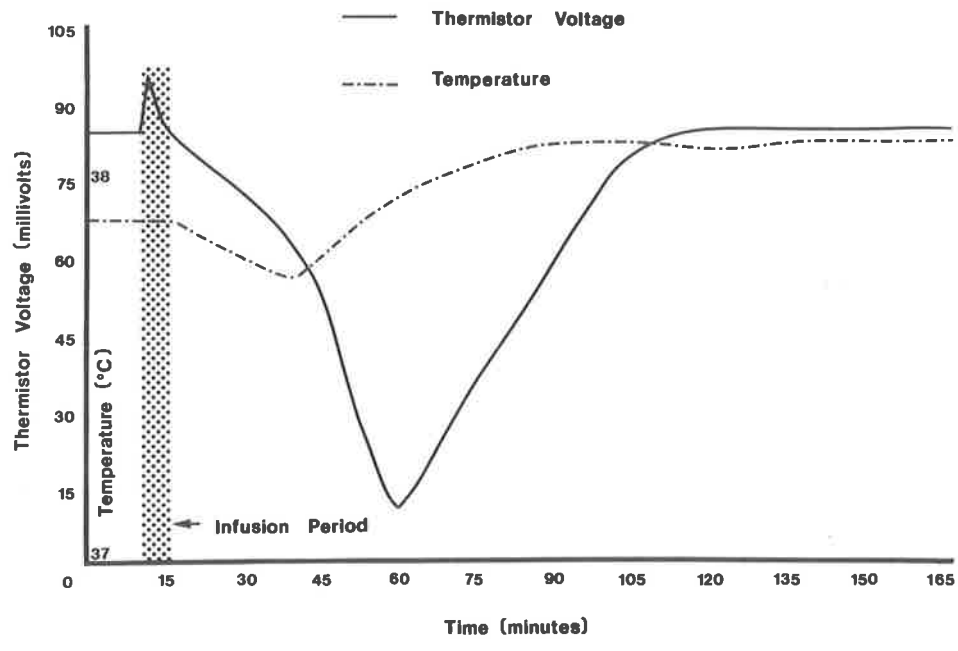


FIGURE 19.



values was calculated from Table 6 to represent an increase in thermistor bead temperature of 0.4°C The second infusion resulted in a further decrease in thermistor voltage values which represented an increase in thermistor bead temperature of 0.9°C. The third infusion resulted in a minimum milli-voltage value which corresponded to the maximum increase in the bead temperature of 1.3°C above the resting value. Subsequent infusions exhibited a gradual decline in response for the remainder of the experimental period (Figure 20).

2) BLOOD PRESSURE

The first of the multiple infusions of nicotine gave a similar blood pressure response to the single dose experiment. The second and subsequent infusions resulted in responses closely mimicking the single dose response but after the sixth infusion the resultant pressure changes were reduced in comparison with the early infusions. Blood pressure values remained elevated for the entire experimental period and stabilized at the value which was 14% above the pre-experimental value (Figure 20).

3) PERIPHERAL TEMPERATURE

Multiple infusions of nicotine caused an immediate steady decline in peripheral temperature to record a minimal value of 0.8°C lower than the pre-experimental value after 250 minutes (Figure 21).

FIGURE 20.

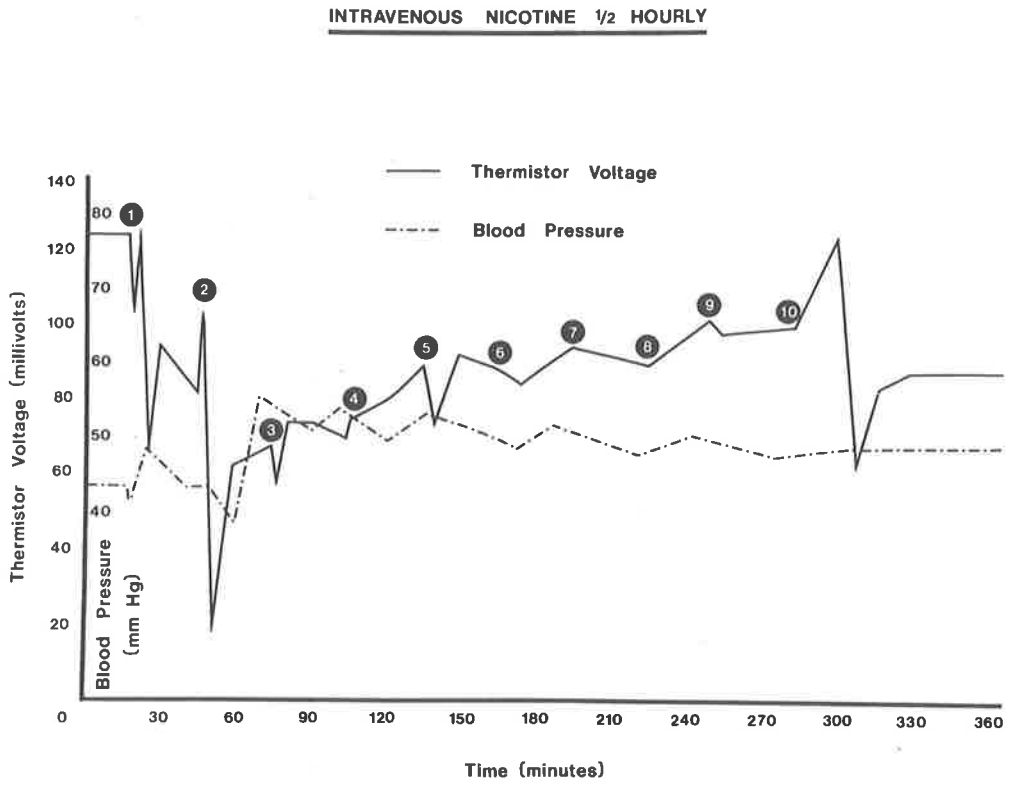
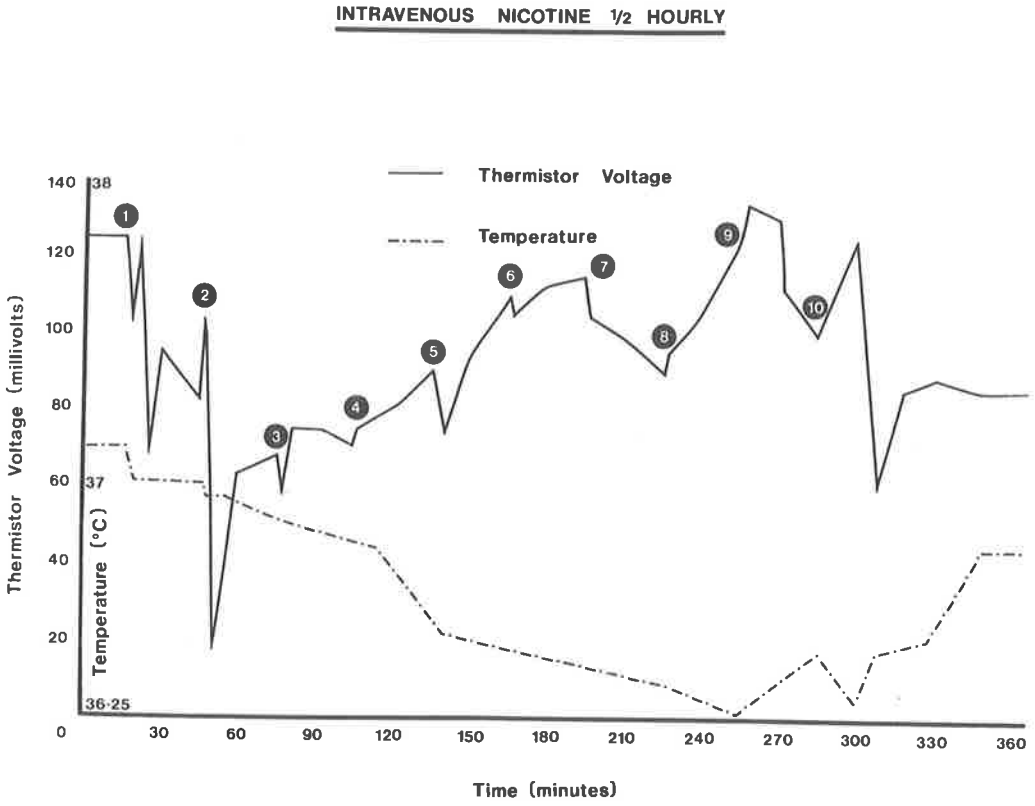


FIGURE 21.



C. INTRA-ARTERIAL ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

Intra-arterial nicotine infusion caused an immediate characteristic decrease of $0.2^{o}C$ in thermistor bead temperature (Table 7). The temperature of the bead then slowly increased during the following 60 minutes to record a maximum value of $0.5^{o}C$ above the resting value (Figure 22).

2) BLOOD PRESSURE

Intra-arterial infusion of nicotine caused an immediate fall in blood pressure from 55 mm Hg to 37 mm Hg within 2 minutes. From that point, the pressure began to rise to reach a peak value of 75 mm Hg at the cessation of infusion. Resting pressure value was restored approximately 30 minutes after infusion (Figure 22).

3) PERIPHERAL TEMPERATURE

The maximum temperature variation recorded during the experimental period was $0.4^{o}C$ below the resting value which occurred 60 minutes after nicotine infusion (Figure 23).

D. CONTINUOUS HALF HOURLY INTRA-ARTERIAL ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

The initial infusion resulted in a response which was virtually identical to the single dose experiment (Table 8).

FIGURE 22.

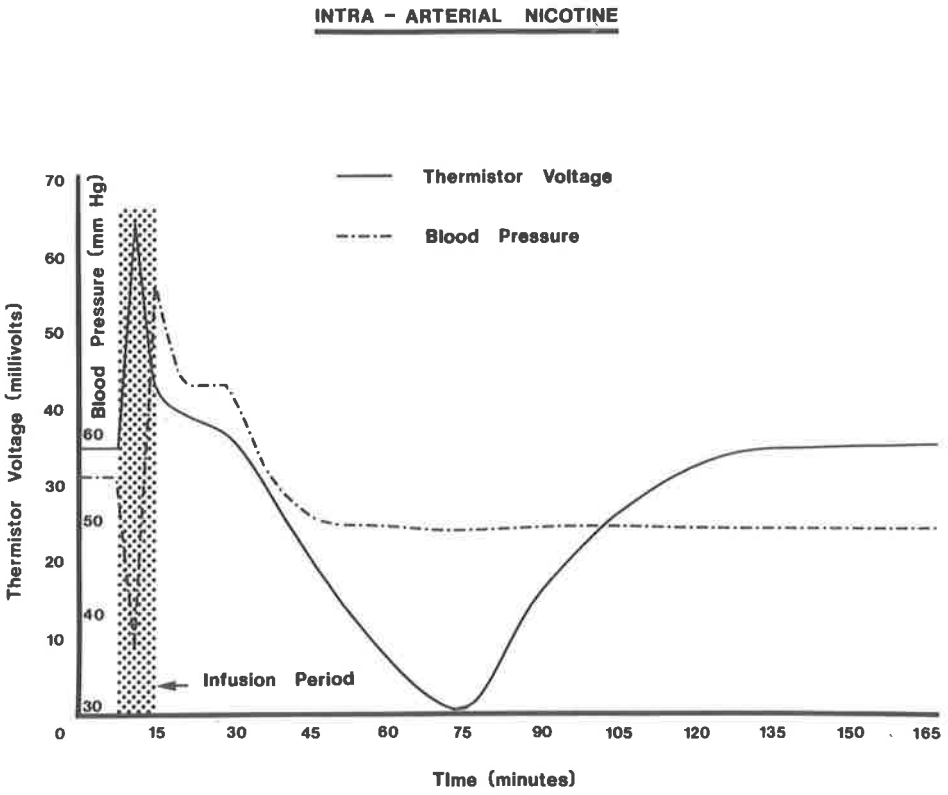
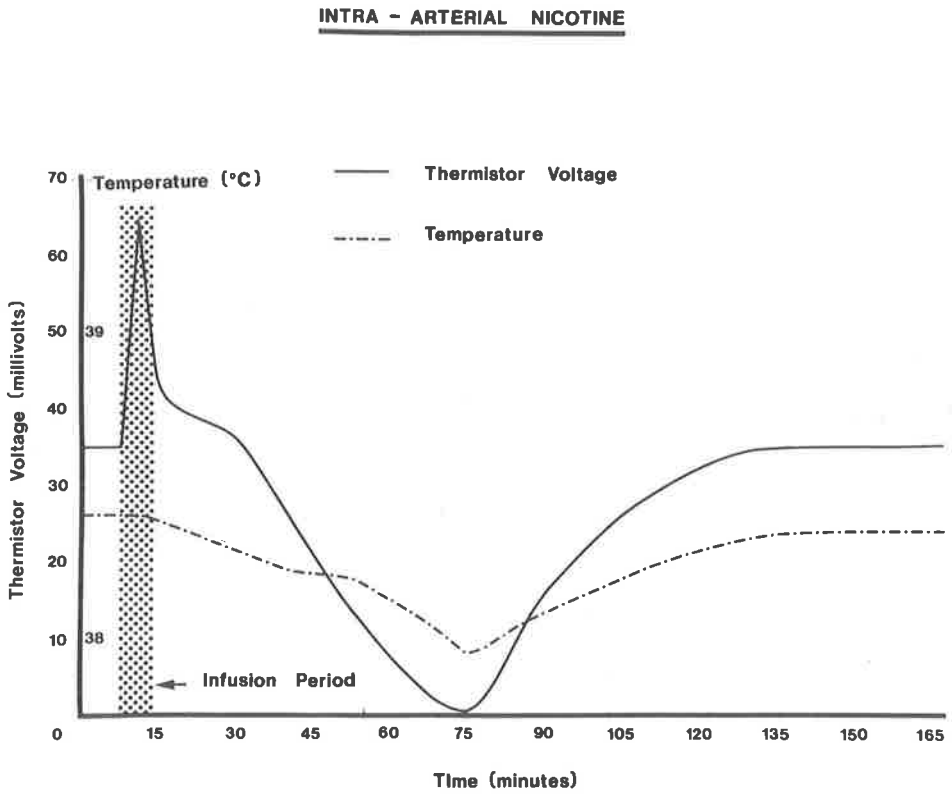


FIGURE 23.



The thermistor bead became progressively warmer during the experiment to reach a maximum value of 1.4°C above the resting level at the 200 minute mark (Figure 24).

2) BLOOD PRESSURE

The first of the half hourly nicotine infusions caused a response similar to the single dose experiment; blood pressure fell from 52 mm Hg to 40 mm Hg within 2 minutes of infusion before reaching a peak value of 79 mm Hg after 7½ minutes. Subsequent infusions followed the same basic pattern although blood pressure values diminished in response during the final three infusions (Figure 24).

3) PERIPHERAL TEMPERATURE

There was a progressive decline in peripheral temperature during the infusions with a minimum value recorded 200 minutes after the onset of the experiment (Figure 25).

E. SUMMARY

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

The effect of nicotine infusion upon millivoltage values was similar during both intravenous (IV) and intra-arterial (IA) administration. The minimum millivoltage recorded during IV presentation was reached earlier and demonstrated a quicker recovery to the pre-experimental value than during IA administration.

FIGURE 24.

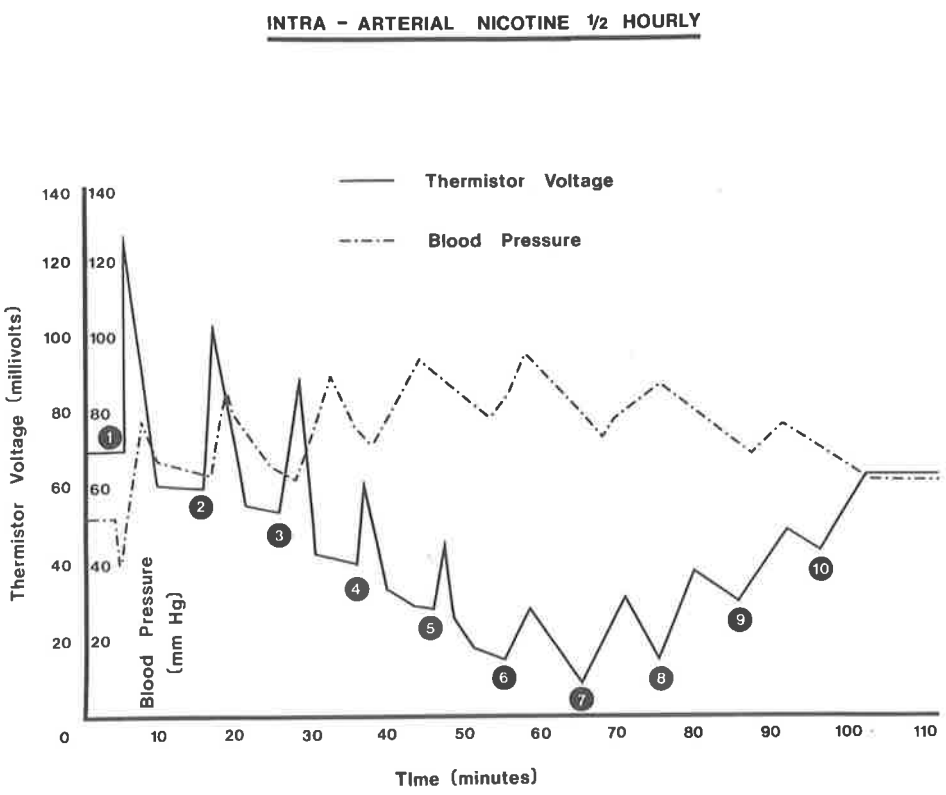
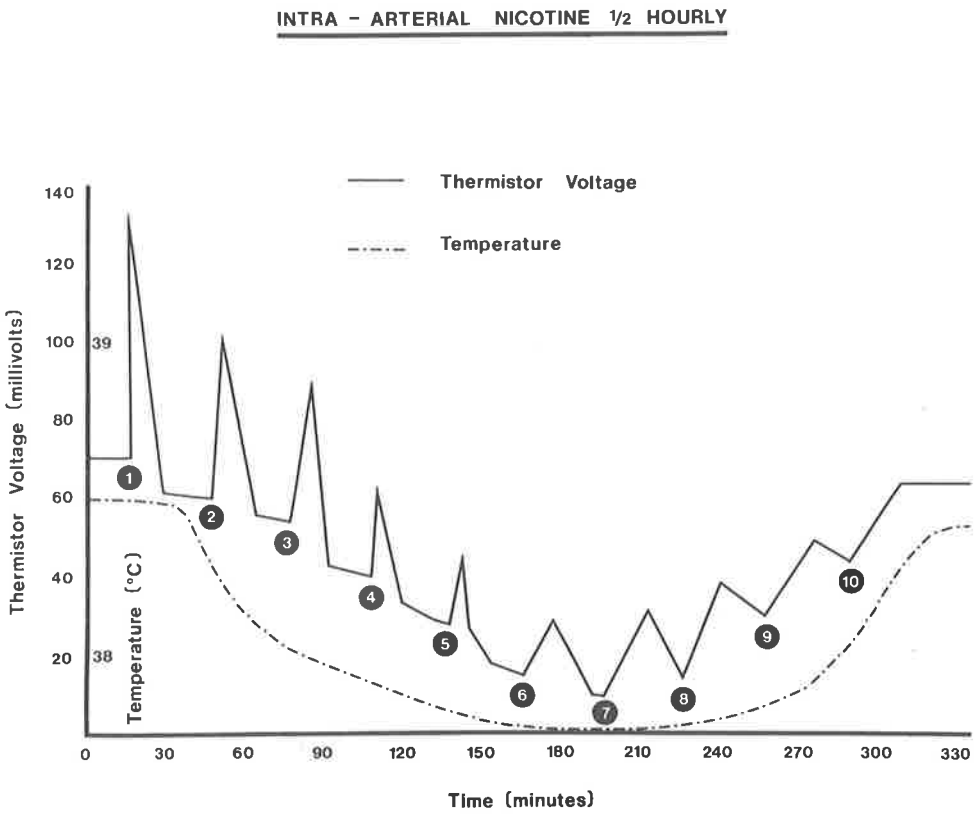


FIGURE 25.



Although both IV and IA routes exhibited different millivoltage responses to multiple infusions, the overall response to continuous half hourly nicotine infusions was one of vasoconstriction of gingival blood flow for a period of approximately 240 minutes. During both single and multiple infusions the temperature increase of the thermistor bead above the resting value was almost coincident.

2) BLOOD PRESSURE

Administration of nicotine by either IV or IA routes resulted in a rapid early fall in blood pressure with a slower rebound to an elevated value. The IA approach exhibited a quicker return to the resting pressure value.

Continuous half hourly nicotine infusions caused a substantial rise in blood pressure values throughout the entire experimental period.

3) PERIPHERAL TEMPERATURE

Peripheral temperature was closely allied to thermistor temperature during single dose IA nicotine infusions as evidenced by minimum peripheral temperature values recorded simultaneously with minimum millivoltage values.

Peripheral temperature values in response to IV infusion were allied with thermistor voltage but the major variations in temperature values occurred slightly earlier than fluctuations in bead temperature.

Continuous half hourly nicotine infusions caused a gradual decline in peripheral temperature to record a minimum value before equilibrium was finally restored.

F. DISCUSSION

The sharp initial decrease in blood pressure immediately following nicotine infusion may be attributed to a rapid vasodilation of blood vessels (Fewings, Rand, Scroop and Whelan, 1966) or the response of the central nervous system causing a decreased cardiac output. The rebound recovery in blood pressure after only a short period of time indicated that the effect was transient and probably mediated through the autonomic nervous control of tonus of the vessel walls.

It was assumed that the blood pressure change resulted from vasoconstriction of vessels, but it was not possible to determine from the experimental data whether the vasoconstrictive effect of nicotine on the circulation was centrally or locally mediated. The vasoconstrictive effect on the circulation was of longer duration than the initial vasodilatory response and persisted for 20-30 minutes after nicotine infusion ceased.

There is little information of the dilator action of nicotine on blood vessels. Kottegoda (1953) described vasodilation in the rabbit ear when nicotine was injected after treatment with tolazoline to block its constrictor effect but the cause of the dilation was not discussed.

Hilton (1954) reported dilation of blood vessels in the cat after intra-arterial doses of nicotine and attributed the response partly to an axon reflex in cholinergic vasodilator fibres and partly to a direct action of nicotine on the blood vessels.

Immediately following nicotine infusion there was an increase in millivoltage values indicating that the thermistor bead became cooler as a result of increased blood flow within the gingival vessels. The rise in millivoltage to a peak value after approximately 2 minutes of nicotine infusion corresponded closely to the minimum blood pressure recording. The rapid decline in blood pressure values was attributable to a rapid vasodilation of blood vessels following nicotine infusion (Fewings *et al.*, 1966) and it is reasonable to accept that the maximum millivoltage value of the thermistor assembly corresponded to maximal gingival blood flow. The vasoconstrictive changes within the gingival circulation recorded by the thermistor assembly closely correlated with the increase in blood pressure due to arteriolar constriction. Following infusion, the blood pressure remained at an elevated level for 25-35 minutes and during that time the temperature of the thermistor bead steadily increased due to a progressive reduction in gingival blood flow.

The use of the marginal ear vein for the IV approach may have had a delayed effect in achieving the maximal blood titre of nicotine. This delayed effect may account for the differences observed in thermistor voltage values compared with the IA approach into the carotid

vessel and the resultant direct path to the gingival microcirculation.

During nicotine infusions millivoltage and blood pressure values were in harmony but as the experiment progressed the millivoltage response began to lag behind the blood pressure response to such an extent that the minimum millivoltage value was recorded some 10-25 minutes after blood pressure values had returned to their pre-experimental value. From this information it would appear that the blood flow through the microcirculation of the gingiva has a more prolonged effect to blood borne chemicals than the central vessels of the experimental animal.

The increase in millivoltage values during the latter portion of the experimental period during continuous half hourly nicotine infusions may be explained by the hypothesis that with prolonged exposure to nicotine, two related mechanisms of physiological control of the vascular system are utilized. The first control mechanism is able to "absorb" the effects of nicotine but with increasing exposure to the drug the initial physiological response gradually deteriorates and becomes saturated (i.e. ever increasing vasoconstriction). When the first or short term control is extinguished then a second or long term control takes over in an effort to stabilize the vascular responses of the experimental animal. The postulated second or long term control of blood flow could either be a gradual relaxation of neural vasoconstrictor tone of the blood vessels or an increase in the discharge of vasodilator nerve endings.

Peripheral temperature appeared to mimic the responses of thermistor voltage during both IV and IA nicotine infusions. In comparison with IA administration where peripheral temperature and thermistor voltage closely coincided, peripheral temperature during IV administration responded more quickly than thermistor voltage. The minor features of less temperature drop and quicker recovery response upon IV administration can be explained; a) IV nicotine infusion produced a local vasodilatory response which allowed a greater blood flow and hence a cooler thermistor bead than that produced by IA administration; b) peripheral temperature should be more responsive than thermistor voltage because the sensor for temperature measurement was sited over the large femoral blood vessels which have an abundant blood supply in comparison with the terminal microvasculature of the gingiva.

A possible explanation for the observed decline in peripheral temperature during continuous half hourly nicotine infusions was that the dual homeostatic mechanism postulated earlier was either slower in action during IV nicotine exposure or was unable to effectively control the downslide in peripheral temperature values.

3. ADRENALINE

INTRODUCTION

In general the physiological response to adrenaline infusion resembles the effects of stimulation of adrenergic nerves. The most significant effect occurs in cardiac and smooth muscle resulting in a dramatic rise in blood pressure as a result of direct myocardial stimulation that increases the strength of ventricular contraction, increases heart rate and vasoconstriction. Arterioles are especially affected by constriction of the precapillary sphincter but capillaries and veins are also constricted by adrenaline. Arterio-venous (A-V) anastomoses are communications between smaller arteries or arterioles and the corresponding venous channels, through which blood may be shunted and capillary areas short circuited. The A-V anastomoses react in the same manner as arterioles to the administration of adrenaline (Zweifach, 1961).

RESULTS

A. INTRAVENOUS ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

Adrenaline infusion caused a small rise in millivoltage values equivalent to a 0.2^{o}C decrease in thermistor bead temperature before a minimum value was recorded approximately 10 minutes after cessation of infusion. The recorded minimum millivoltage value represented an increase in the bead

FIGURE 26.

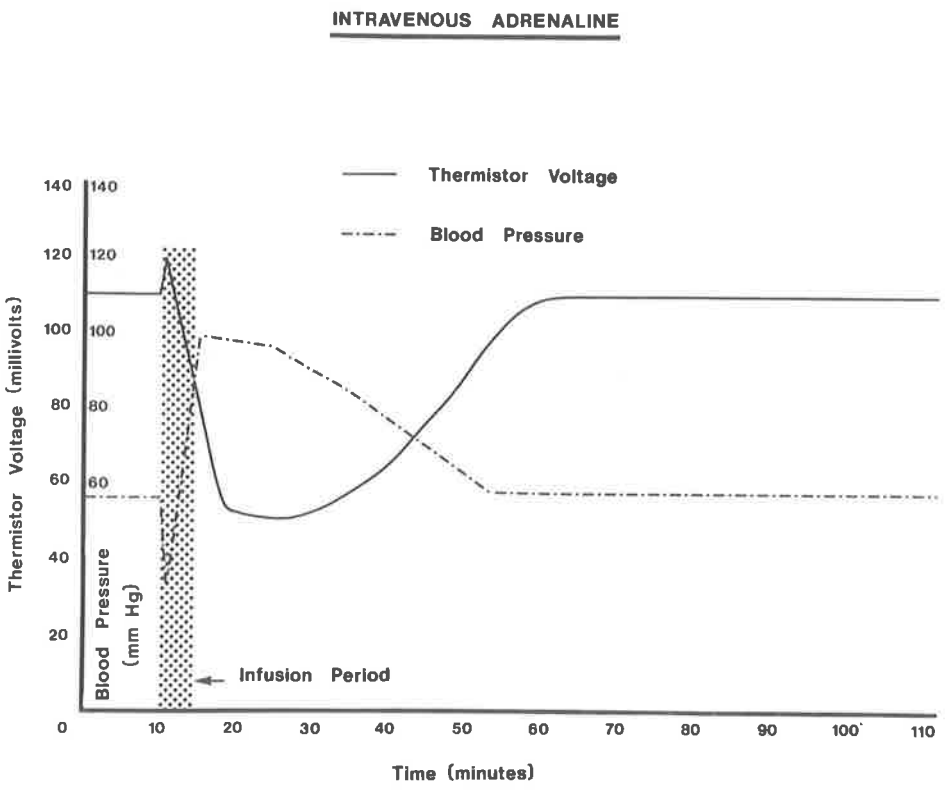
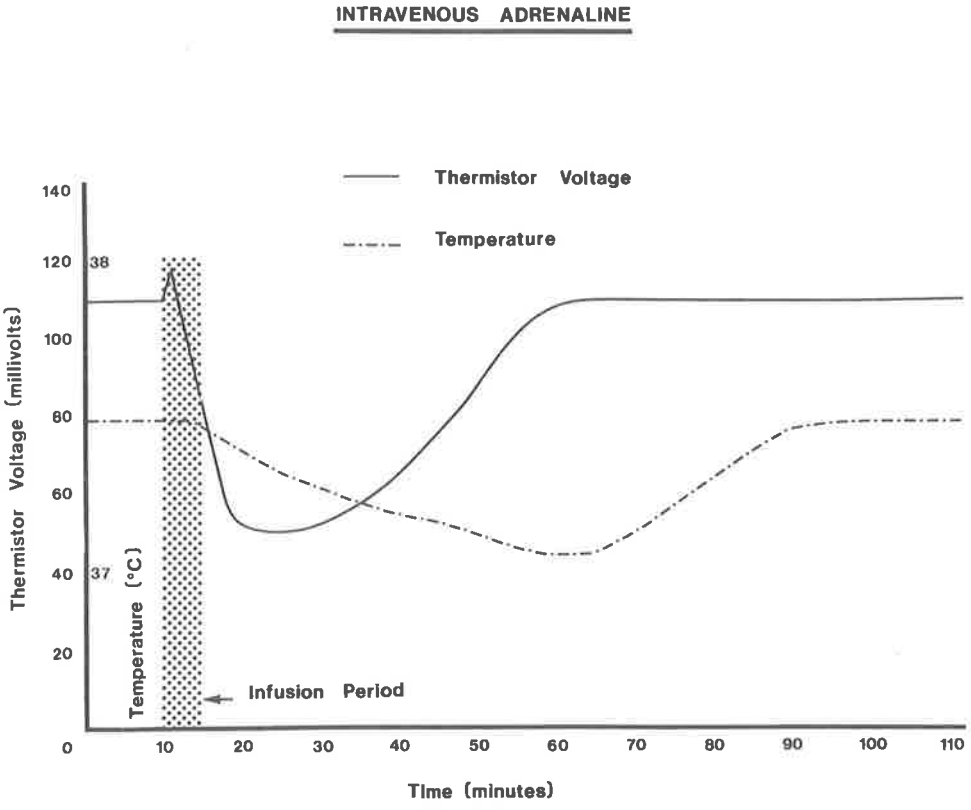


FIGURE 27.



temperature of 0.6°C (Table 9). The pre-experimental value was regained 35 minutes later (Figure 26).

2) BLOOD PRESSURE

A single infusion of adrenaline caused an immediate fall in blood pressure from 58 mm Hg to 35 mm Hg. After the infusion period, blood pressure peaked to a maximum value of 100 mm Hg before slowly decaying to a resting value approximately 40 minutes later (Figure 26).

3) PERIPHERAL TEMPERATURE

A decrease of 0.4°C in peripheral temperature was recorded approximately 50 minutes after adrenaline infusion. Pre-experimental temperature values were regained after a further 25 minutes (Figure 27).

B. CONTINUOUS HALF HOURLY INTRAVENOUS ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

Early adrenaline infusions caused a marked increase in thermistor bead temperature of 1.4°C above the resting value (Table 10). Later infusions did not cause the same degree of response (Figure 28).

2) BLOOD PRESSURE

Half hourly adrenaline infusions caused the characteristic rise in blood pressure values similar to the single dose experiment with the maximum response being registered in the fifth presentation.

FIGURE 28.

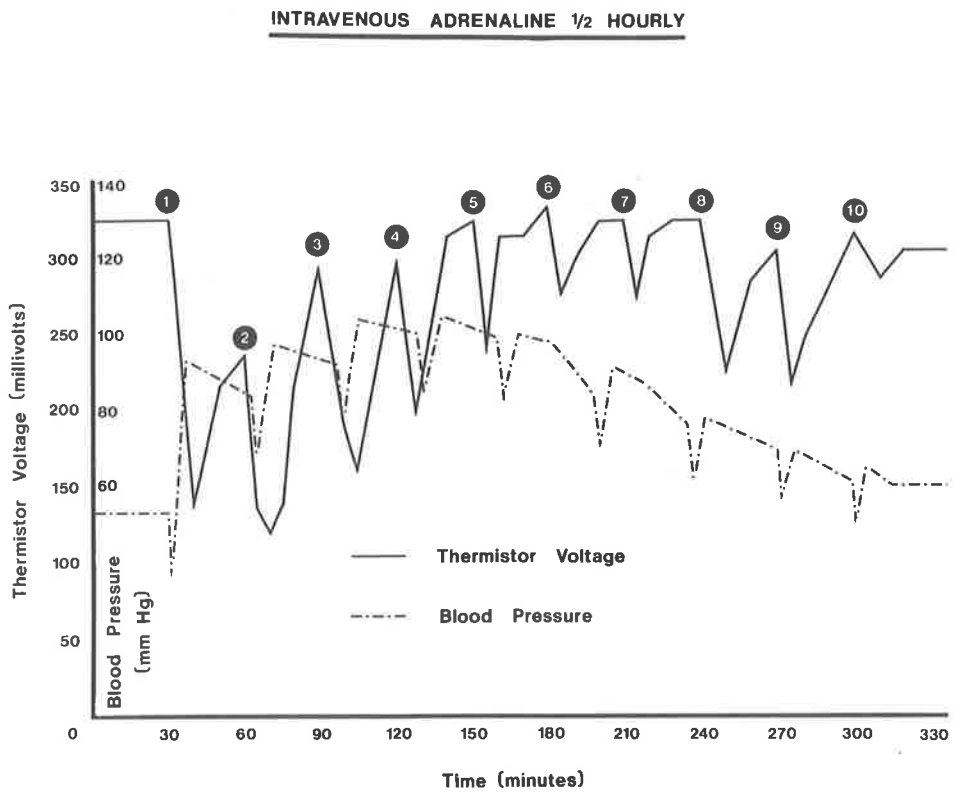
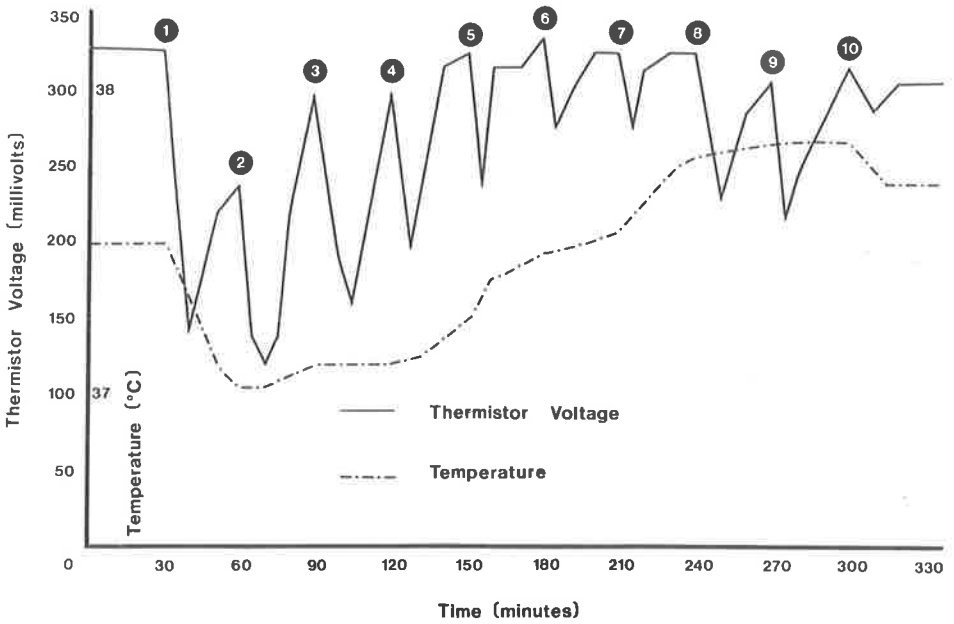


FIGURE 29.



Subsequent presentations exhibited a general decline in blood pressure values throughout the remainder of the experimental period (Figure 28).

3) PERIPHERAL TEMPERATURE

Peripheral temperature recorded a decline immediately following the initial adrenaline infusion. The minimum value recorded was $0.5^{o}C$ below the equilibrium value and occurred after 60 minutes (Figure 29).

C. INTRA-ARTERIAL ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

Adrenaline infusion caused a decrease of $0.1^{o}C$ in the bead temperature early in the experimental period (Table 11) but an increase in thermistor bead temperature of $0.7^{o}C$ above the resting value was recorded before the pre-experimental values were restored approximately 25 minutes later (Figure 30).

2) BLOOD PRESSURE

Intra-arterial adrenaline infusion caused an immediate fall in blood pressure from 55 mm Hg to 35 mm Hg. After 3 minutes blood pressure rebounded strongly to a peak value of 116 mm Hg before pre-experimental values were regained 20 minutes later (Figure 30).

3) PERIPHERAL TEMPERATURE

Adrenaline infusion caused a $0.6^{o}C$ decrease in

FIGURE 30.

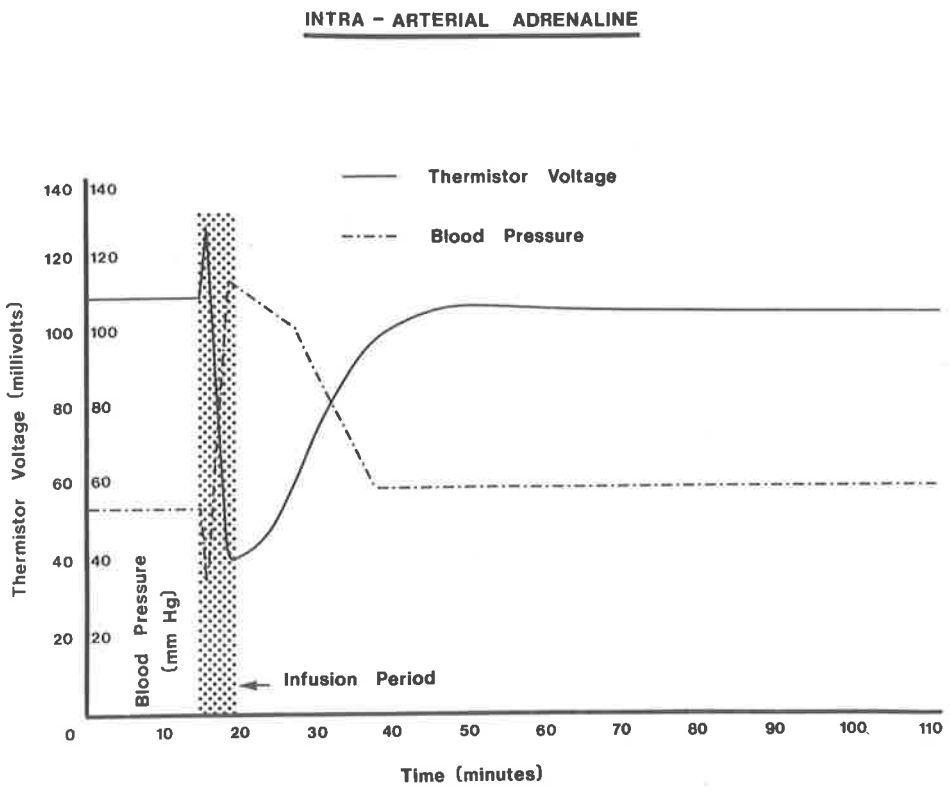
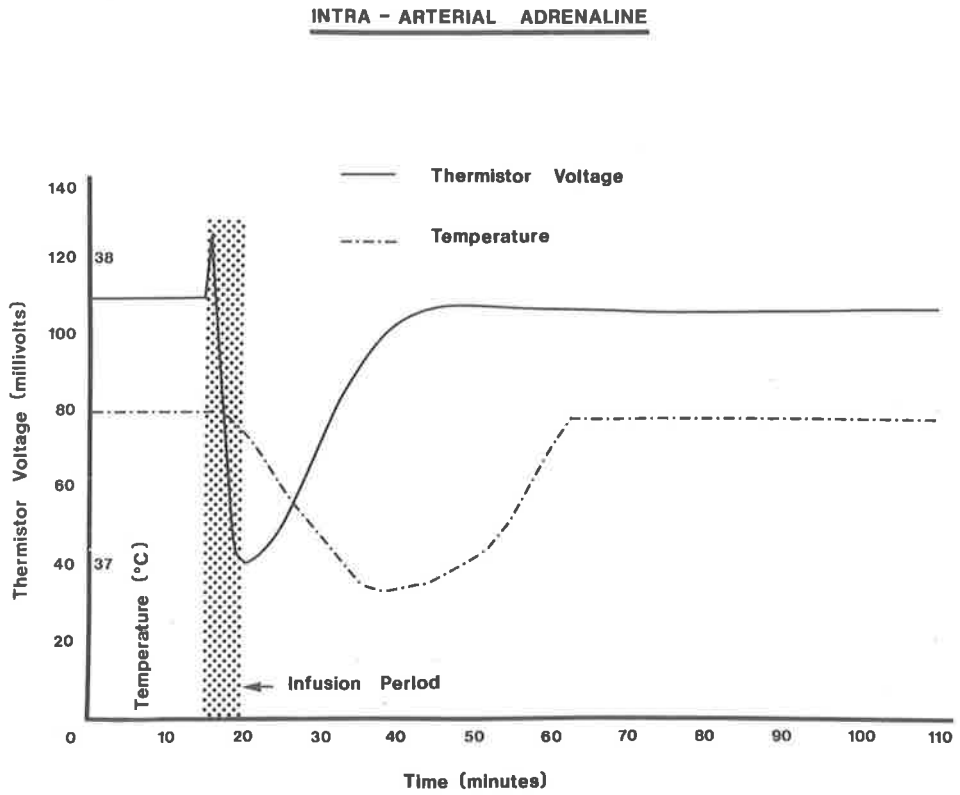


FIGURE 31.



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peripheral temperature which was registered approximately 20 minutes after infusion. Pre-experimental values were regained 20 minutes later (Figure 31).

D. CONTINUOUS HALF HOURLY INTRA-ARTERIAL ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

Each infusion caused a rise in bead temperature during and immediately following infusion as in the single dose experiment (Table 12). The maximum bead temperature rise of 1.6^{o}C occurred following the third infusion (Figure 32).

2) BLOOD PRESSURE

Each presentation of adrenaline at 30 minute intervals gave a blood pressure response similar to the single dose experiment (Figure 32).

3) PERIPHERAL TEMPERATURE

Multiple adrenaline infusions caused peripheral temperature to record a minimum value after 170 minutes which was 0.8^{o}C below the resting value (Figure 33).

E. SUMMARY

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

The effect on thermistor bead temperature following adrenaline infusion was very similar irrespective of the mode of presentation. During infusion,

FIGURE 32.

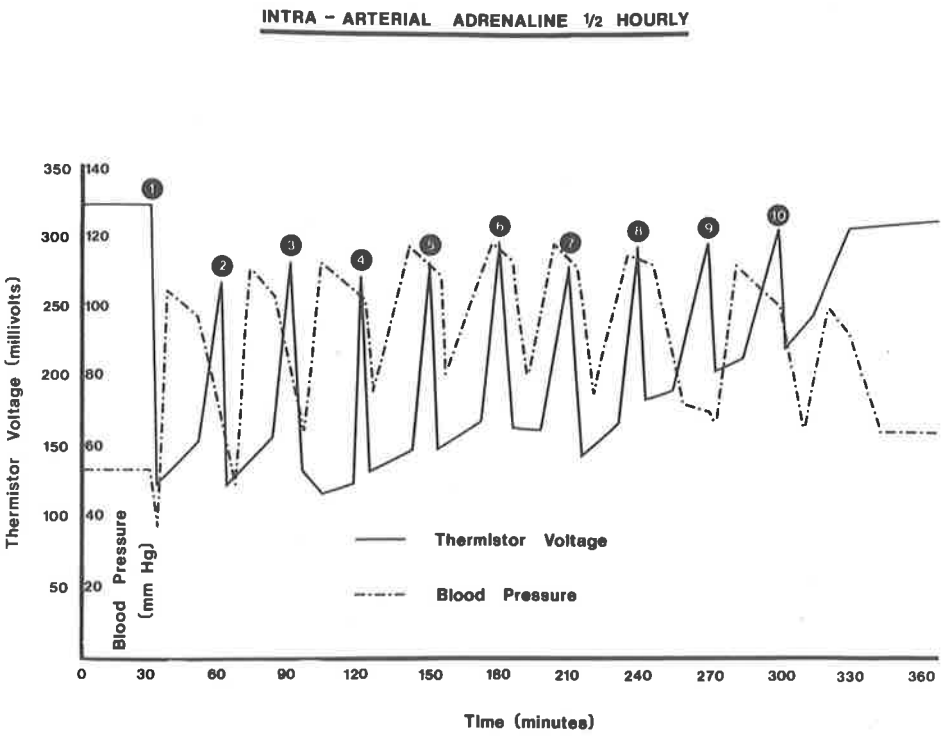
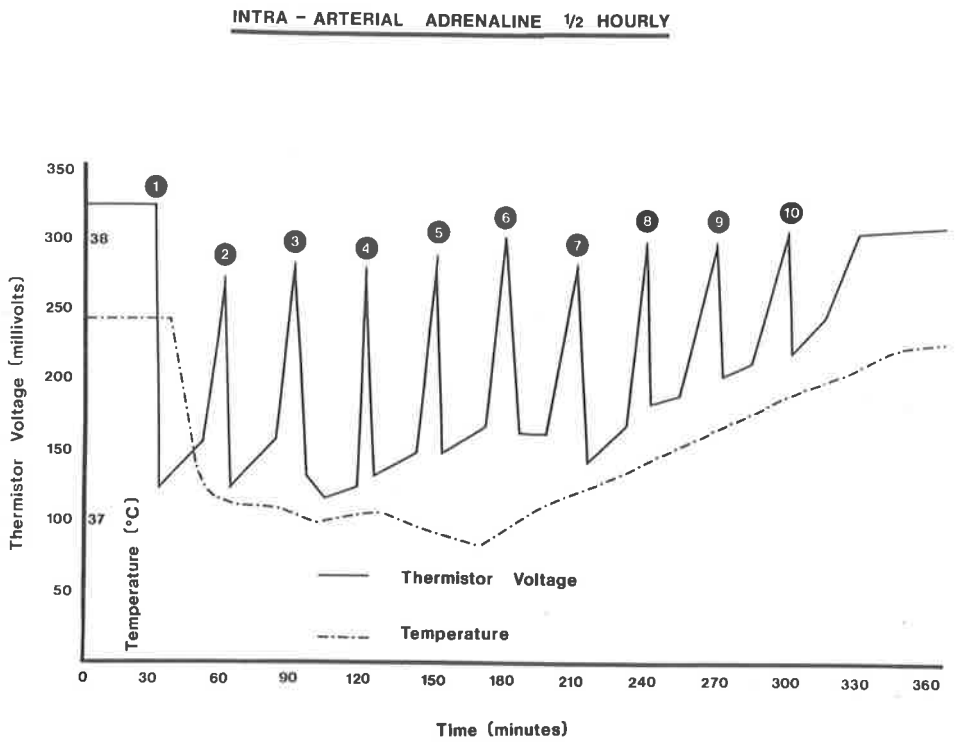


FIGURE 33.



thermistor temperature showed an initial transient decrease followed immediately by a rapid increase in temperature. The intra-arterial (IA) mode of presentation showed a greater degree of response and a quicker return to the pre-experimental value than the intravenous (IV) route.

Continuous half hourly adrenaline infusions caused bead temperature to remain elevated throughout the entire experimental period.

The overall effect of adrenaline infusion was a prolonged vasoconstriction with IA infusion causing the greatest increase in thermistor bead temperature.

2) BLOOD PRESSURE

Adrenaline infusion effected the same basic response during both IV and IA administration. The blood pressure response was characterized by a sudden fall in pressure which was immediately followed by a rapid increase before pre-experimental values were regained.

Continuous half hourly adrenaline infusions showed an overall increase in blood pressure values throughout the entire experimental period with a peak value being recorded during the earlier presentations.

3) PERIPHERAL TEMPERATURE

The same basic peripheral temperature response to adrenaline infusion was seen during both IV and IA administration but the rate of response was more

rapid during IA administration.

Continuous half hourly adrenaline infusion evoked a similar response to peripheral temperature values as the single dose experiment but the return to the pre-experimental value was delayed.

F. DISCUSSION

The rapid return to homeostatic blood pressure values following adrenaline infusion was probably due to the central control mechanism of the experimental animal. The mechanism has vascular and neural components that quickly respond to restore homeostasis and minimize the physiological changes subsequent to adrenaline infusion. Although early presentations of adrenaline infusions caused a peak blood pressure response, later presentations did not register the same effect. The postulated central control mechanism could explain the effects of the variation in blood pressure readings during continuous adrenaline infusion.

Early adrenaline infusions caused a maximal vasoconstrictive effect which was far too rapid in its action for the control mechanism to respond appropriately; only with continued drug infusions over a longer period of time did the control mechanism have the capacity or potential to effect a gradual decline in vasoconstriction as evidenced by the fall in blood pressure values after the fifth drug presentation.

The increase in thermistor bead temperature was a result of a decreased gingival blood flow. There was an

inverse correlation between the maximum blood pressure value and the marked decrease in gingival blood flow (Figures 17 and 21). Despite an increase in blood pressure, increasing the pressure gradient within the gingival circulation was the most powerful factor in causing a decreased blood flow (Poiseuille's Law) The increase in temperature of the thermistor bead was indicative of the marked vasoconstriction of the gingiva and it is possible that irreversible and irreparable damage could occur to the delicate tissues of the interdental gingivae if this drug was present in the circulation for prolonged periods.

The A-V anastomoses of the microcirculation have been shown to be very effective in controlling body temperature i.e. increased temperature induces vasodilation and decreased temperature, constriction. When open these channels offer very low vessel resistance and large volumes of blood flow through them for heat exchange. Rushmer (1960) concluded that the role of the sympathetic vasoconstrictor nerves differs greatly in various tissues but that sympathetic vasoconstrictors completely dominate the calibre of the A-V shunts in the skin and mucous membranes, which are of great importance in the control of heat exchange. A convincing study carried out by Giddon *et al.* (1963) showed that when blood volume pulses of the gingival papilla and pulses of the left finger pad were monitored at rest and during the cold pressor test, the average amplitudes of the volume pulse of both gingiva and finger were significantly reduced in response to the decreased temperature, which indicated vasoconstriction in both areas. The study by Giddon *et al.*

lends powerful support to the results obtained in the experiments which clearly demonstrated that a decrease in peripheral body temperature is indicative of vaso-constriction.

4. SALINE, NICOTINE AND ADRENALINE

INTRODUCTION

This experiment was designed to determine whether significant changes occurred within the parameters of thermistor bead temperature, blood pressure and peripheral temperature when the experimental animal was subjected to the combined effects of saline, nicotine and adrenaline.

RESULTS

A. INTRAVENOUS ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

The combined effects of saline, nicotine and adrenaline infusion caused a decrease of $0.1^{o}C$ in the temperature of the thermistor bead before recording an increase of $0.6^{o}C$ above the resting value (Table 13). Pre-experimental values were regained approximately 35 minutes later (Figure 34).

2) BLOOD PRESSURE

Within one minute of the combined drug infusion, blood pressure fell rapidly from the resting value of 54 mm Hg to 26 mm Hg before a peak value of 96 mm Hg was recorded 4 minutes after infusion ceased. Normal blood pressure was regained approximately 30 minutes later (Figure 34).

FIGURE 34.

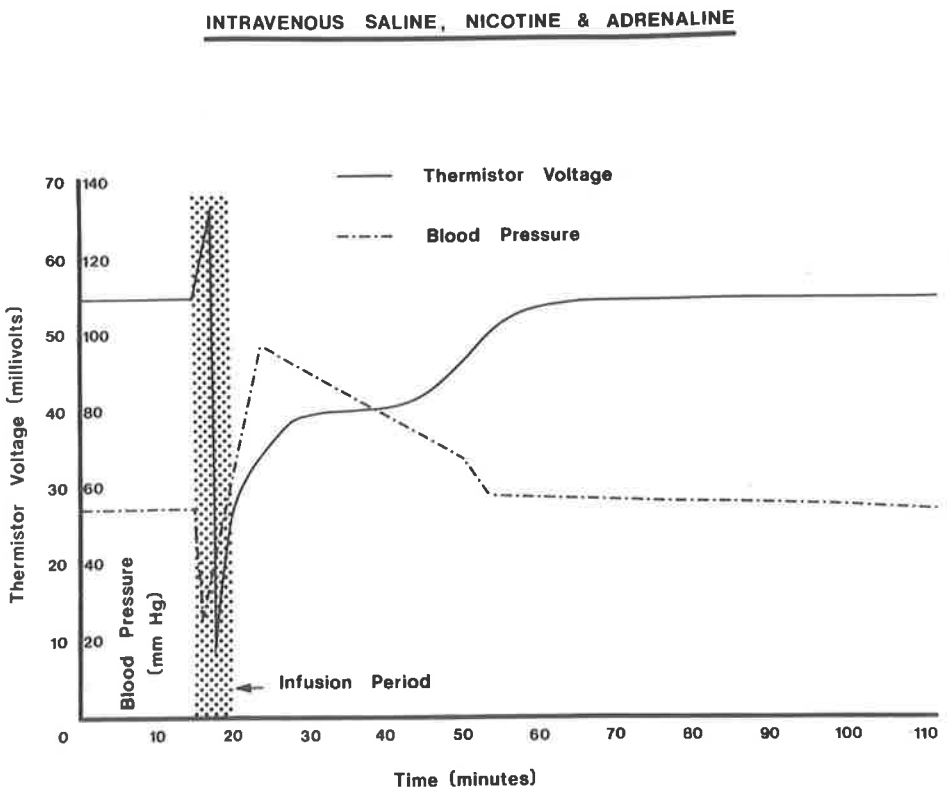
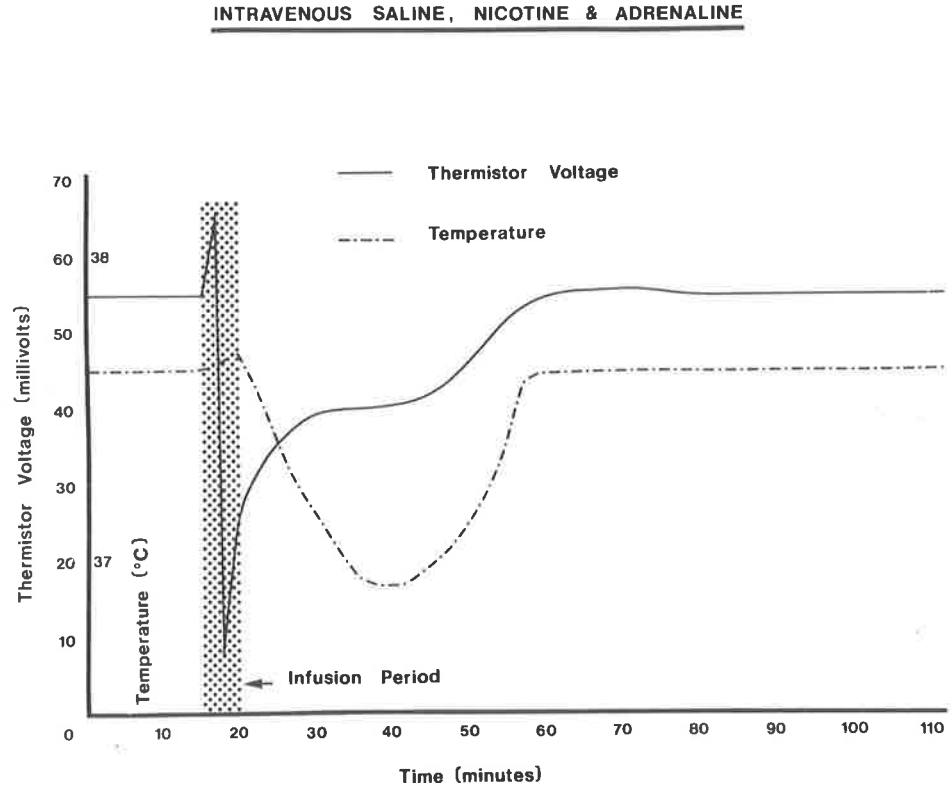


FIGURE 35.



3) PERIPHERAL TEMPERATURE

The major variation in peripheral temperature following combination drug infusion was a decrease of 0.6°C from the resting value which was recorded after 40 minutes (Figure 35).

B. CONTINUOUS HALF HOURLY INTRAVENOUS ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

During multiple drug infusion thermistor bead temperature recorded a maximum value of 1.5°C at the second presentation (Table 14). Subsequent presentations did not effect the same magnitude of response (Figure 36).

2) BLOOD PRESSURE

Throughout the entire experimental period multiple infusions of the drug combination demonstrated a blood pressure response similar to the single dose experiment (Figure 36).

3) PERIPHERAL TEMPERATURE

A decrease in peripheral temperature of 0.7°C from the resting value occurred at the second drug presentation and remained at this stable value for approximately 200 minutes before pre-experimental values were regained (Figure 37).

FIGURE 36.

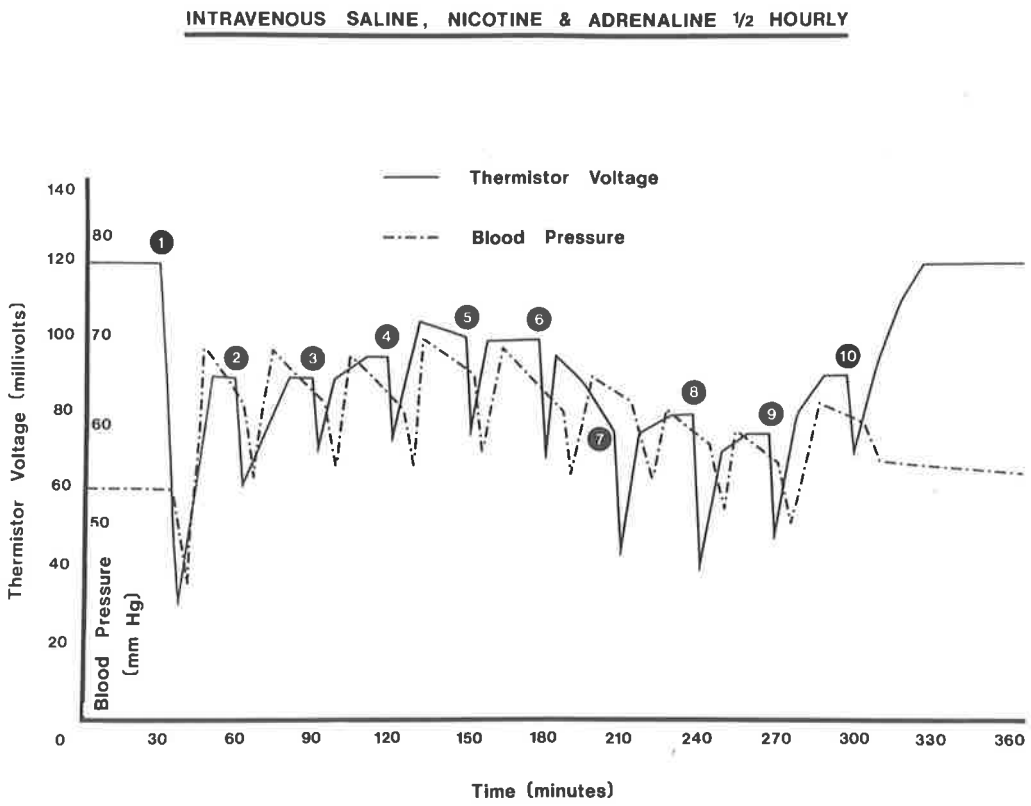
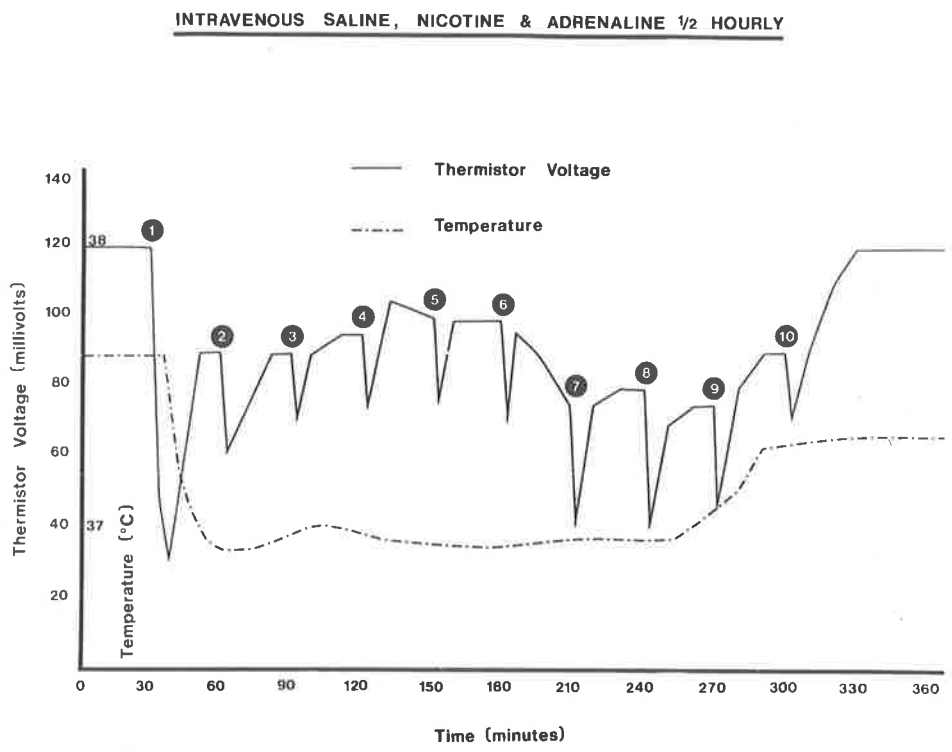


FIGURE 37.



C. INTRA-ARTERIAL ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

Intra-arterial infusion of the drug combination caused an immediate $0.2^{o}C$ fall in the temperature of the thermistor bead. An increase of $1.2^{o}C$ in the temperature of the thermistor bead was recorded 10 minutes after infusion (Table 15). Pre-experimental values were regained approximately 50 minutes later (Figure 38).

2) BLOOD PRESSURE

Within 2 minutes of drug infusion the blood pressure fell sharply from the resting value of 60 mm Hg to a minimum of 22 mm Hg before rebounding to a peak of 120 mm Hg. Pre-experimental values were regained after 60 minutes (Figure 38).

3) PERIPHERAL TEMPERATURE

The combined infusion of saline, nicotine and adrenaline caused peripheral temperature to fall by $0.8^{o}C$ from the resting value within 25 minutes (Figure 39).

D. CONTINUOUS HALF HOURLY INTRA-ARTERIAL ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

During infusion thermistor temperature recorded a $0.1^{o}C$ fall in temperature (Table 16). An increase in the bead temperature of $1.6^{o}C$ above the resting

FIGURE 38.

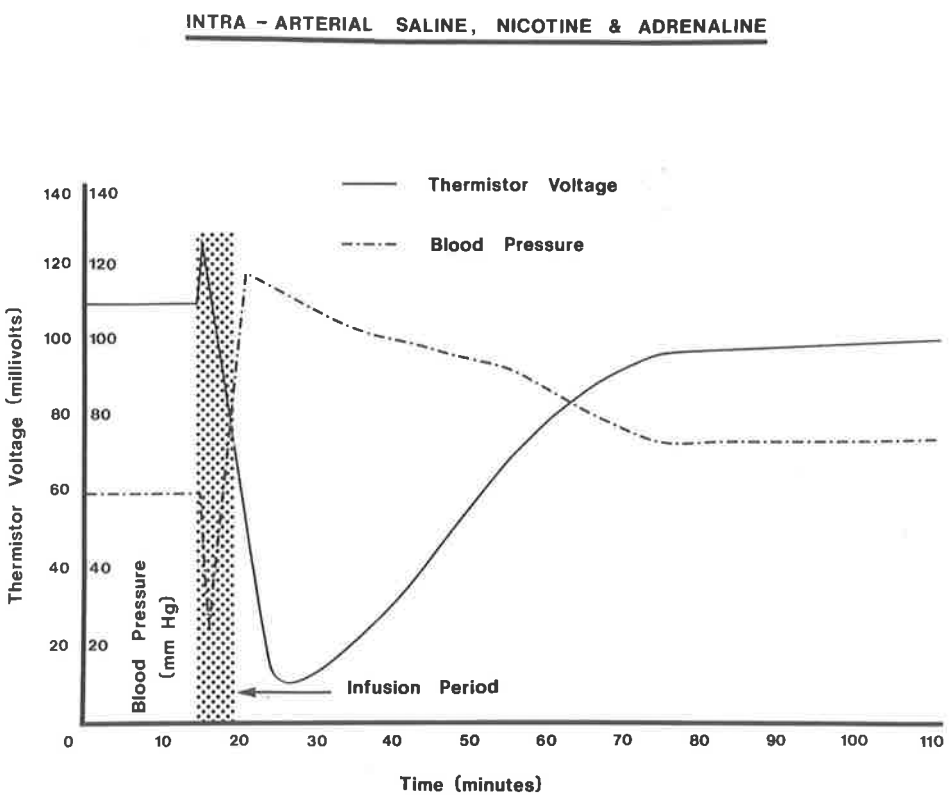
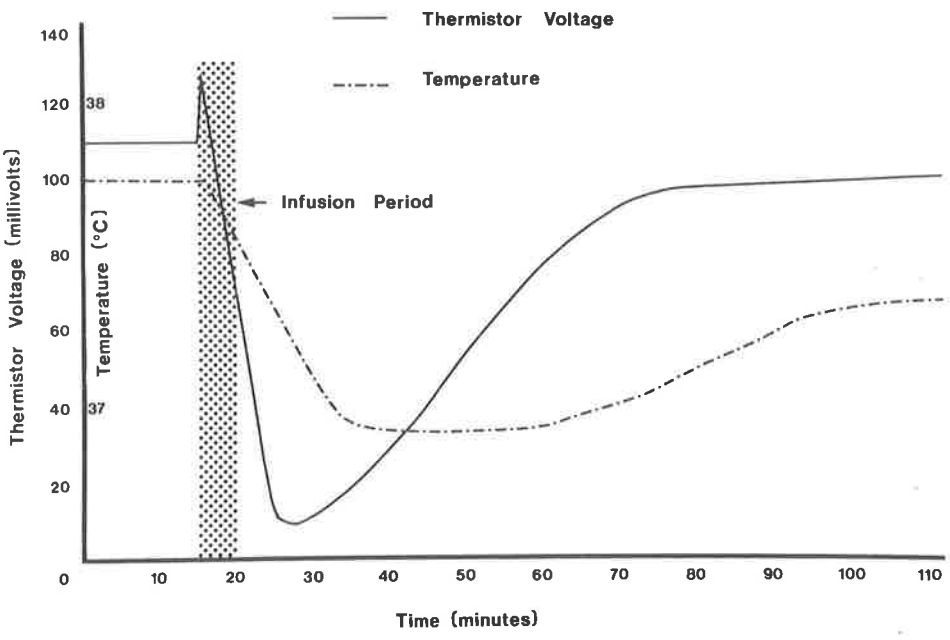


FIGURE 39.



value was the maximum recorded during the first infusion (Figure 40).

2) BLOOD PRESSURE

Initially, multiple infusion of the drug combination caused a transient hypotensive response which was immediately followed by a sharp increase to record the maximum blood pressure value. Subsequent infusions maintained the overall increase in blood pressure values but to a lesser extent than the earlier infusions (Figure 40).

3) PERIPHERAL TEMPERATURE

An overall drop of 1.0^{o}C in peripheral temperature was recorded over the entire experimental period following multiple infusions of saline, nicotine and adrenaline (Figure 41).

E. SUMMARY

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

Thermistor temperature values following infusion of the drug combination were similar for both intravenous (IV) and intra-arterial (IA) routes, although the IA route gave marginally higher values.

2) BLOOD PRESSURE

The blood pressure response to the drug combination was similar for both IV and IA administration. During infusion there was immediate fall in blood pressure but a peak of 120 mm Hg was recorded

FIGURE 40.

INTRA - ARTERIAL SALINE, NICOTINE & ADRENALINE ½ HOURLY

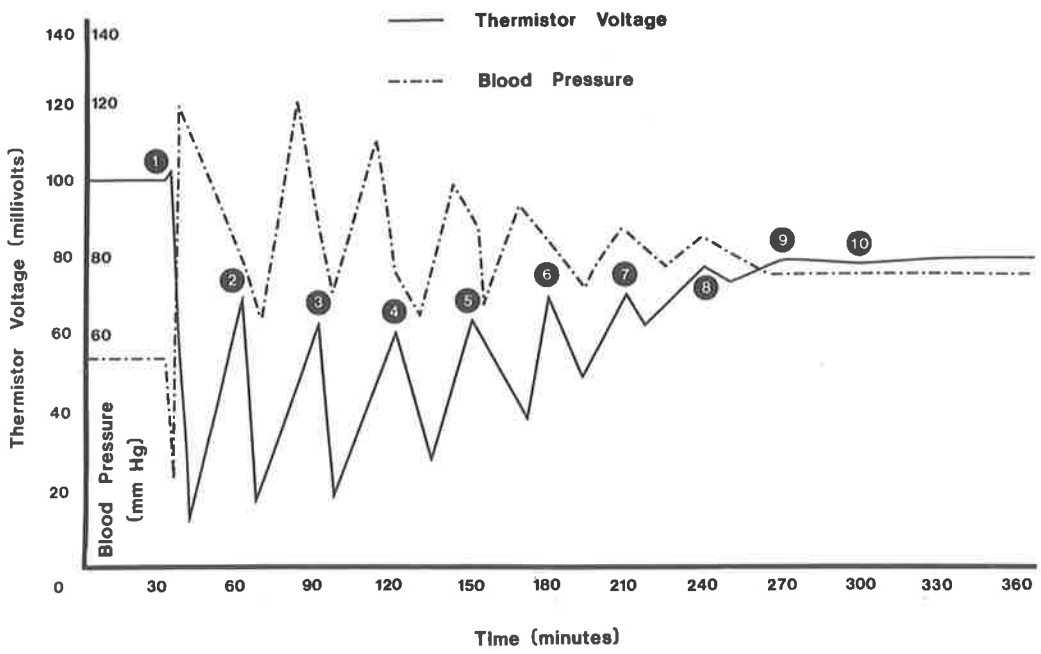
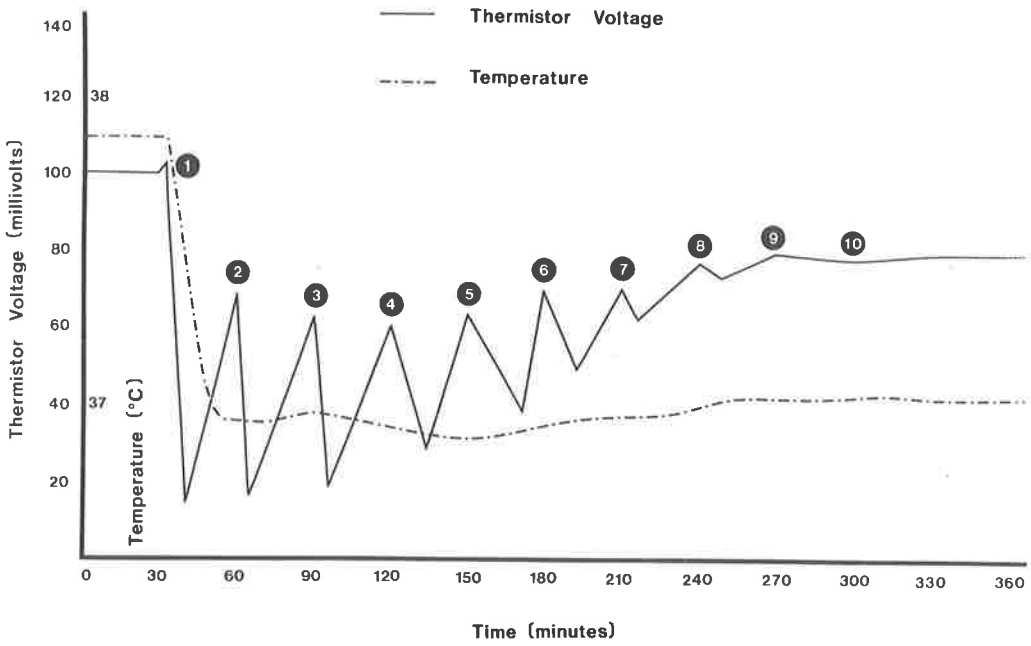


FIGURE 41.

INTRA - ARTERIAL SALINE, NICOTINE & ADRENALINE ½ HOURLY



before returning to the pre-experimental value.

Continuous half hourly infusions of the drug combination caused a general increase in blood pressure values throughout the entire experimental period during both IV and IA administration.

3) PERIPHERAL TEMPERATURE

Peripheral temperature demonstrated an immediate decline following drug infusion to record a minimum value before the resting value was recorded. Intra-arterial infusion produced the greatest temperature gradient.

Continuous half hourly drug infusions exhibited a decrease in temperature following the early presentations to record a minimum value which was maintained for the majority of the experimental period.

F. DISCUSSION

Although blood pressure values were recorded from the carotid artery of the central circulation and milli-voltage values were recorded from within the gingival crevice which constitutes part of the peripheral circulation, an inverse relationship between the two parameters was noted. The minimum millivoltage value of the microthermistor assembly closely corresponded to the minimum blood pressure value of the animal - both results are indicative of

vasoconstriction. Blood pressure remained elevated after cessation of half hourly infusions which proves that the prolonged effects of vasopressor drugs such as nicotine and adrenaline have the potential to cause marked irreversible changes within the circulation. The continued exposure to the drugs under test must have caused irreversible changes within the gingival micro-circulation because after cessation of infusion, thermistor voltage values remained depressed.

Peripheral temperature also did not return to the pre-experimental value following drug presentations which means that the combined pharmacological effects of adrenaline and nicotine are very powerful and effect the circulatory and neural systems so that they are unable to respond appropriately. The infusion of saline, nicotine and adrenaline as a 5 ml "slug" could be responsible for this phenomenon by acting on the heat regulating centres in the hypothalamus as well as the circulatory control mechanisms. The introduction of pharmacologically active drugs into the circulation over a short period of time could have depressed either the heat regulating centres or the circulatory control mechanisms.

5. SIX HOUR CAROTID CLAMP

INTRODUCTION

An attempt to clarify the effects of vaso-constriction upon the gingival circulation was carried out by total occlusion of the right carotid artery and observation of the resultant effects on thermistor voltage and peripheral temperature.

In vascular terms, occlusion of the carotid artery resulted in a total or absolute loss of blood flow and the results of this experiment were used as a comparison of the vascular reactivity of the gingival microcirculation to the chemicals used during infusion.

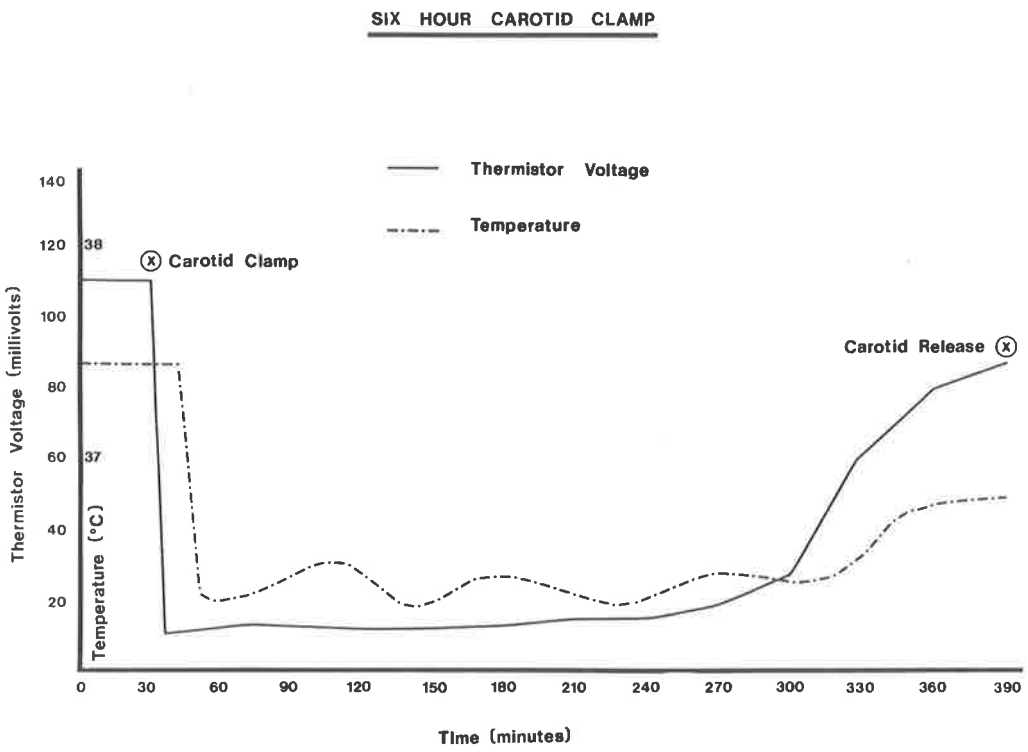
RESULTS

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

When the common carotid artery was clamped to completely obstruct blood flow, millivoltage values fell rapidly to a minimum value which was calculated to be equivalent to an increase of 1.7^{o}C in the temperature of the thermistor bead above the resting value (Table 17).

Following release of the carotid clamp after 6 hours, thermistor bead temperature fell slowly to a resting value (Figure 42).

FIGURE 42.


SIX HOUR CAROTID CLAMP
Thermistor Voltage
Temperature
Carotid Clamp
Carotid Release
Thermistor Voltage (millivolts)
Temperature (°C)
38
37
140
120
100
80
60
40
20
0
30
60
90
120
150
180
210
240
270
300
330
360
390
Time (minutes)

2) PERIPHERAL TEMPERATURE

Peripheral temperature recorded a minimum value of 1.2°C below the experimental level within 30 minutes of carotid clamping and this value was maintained for the entire experimental period. Following release of the carotid clamp, temperature values slowly increased but failed to return to the pre-experimental level.

A. SUMMARY

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

Occlusion of the carotid artery caused instantaneous and immediate increase in thermistor bead temperature to record a peak value which was maintained for almost 4 hours.

2) PERIPHERAL TEMPERATURE

Peripheral temperature demonstrated a marked decrease in temperature values within 30 minutes of the carotid artery occlusion.

B. DISCUSSION

Carotid occlusion showed conclusively that the technique of indirect measurement of gingival blood flow by means of a microthermistor assembly inserted into the gingival crevice of the experimental animal was a reliable technique which constituted the fundamental basis for the previously described experiments. When the carotid artery was totally occluded, minimum millivoltage values recorded

were equivalent to an increase of 1.70°C in the temperature of the thermistor bead. This information had important ramifications: if the temperature of the thermistor bead increased by a value of 1.70°C or more during experimentation then this would correspond to total occlusion of the gingival microcirculation. Total occlusion of the microcirculation within the gingiva could not be tolerated before irreversible damage occurred.

The gradual increase in thermistor voltage from the minimum value after 4 hours was possibly due to collateral circulation from across the midline of the experimental animal.

Temperature homeostasis is achieved by an intimate inter-relationship between neural and circulatory mechanisms. When the carotid artery was occluded, important information normally supplied from both the chemo- and pressure receptors was lacking as well as probable changes in the chemical composition of the blood and accumulation of metabolites. The lack of information from the circulation would tend to depress or paralyze the effectiveness of the central neural mechanisms which are responsible for temperature equilibrium.

6. PHYSIOLOGICAL TEMPERATURE VARIATION

INTRODUCTION

Before any comparisons were made regarding the possible effects of drug administration in the experimental animal, the physiological fluctuations in peripheral temperature were recorded over a six hour period under the same experimental conditions as drug infusion experiments.

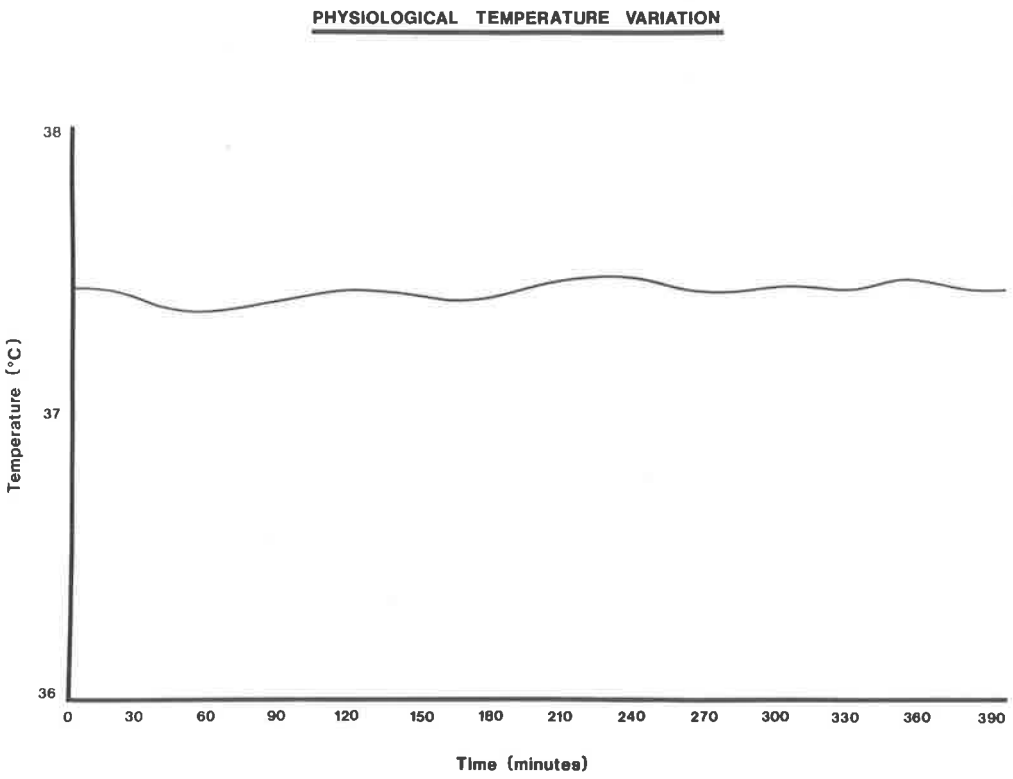
RESULTS

From Figure 43 it was seen that peripheral temperature exhibited a minor variation of $0.1^{o}C$ over the 360 minute experimental period.

A. DISCUSSION

This experiment served as a baseline indicator of temperature values when comparison was made of the effects of drug infusion upon peripheral temperature. Any changes greater than $0.1^{o}C$ in peripheral temperature could be attributed to the specific pharmacological properties of the drug under test.

FIGURE 43.


PHYSIOLOGICAL TEMPERATURE VARIATION
38
37
36
Temperature (°C)
0
30
60
90
120
150
180
210
240
270
300
330
360
390
Time (minutes)

7. MICROSPHERE INFUSION

INTRODUCTION

The technique employed to indirectly estimate gingival blood flow using a thermal diffusion method was supported by a histological investigation using black plastic microspheres. The infusion of microspheres also indirectly measures blood flow by measuring the radius of vessels. The use of this technique provided an additional dimension in which estimation could be made of the influence, if any, of the chemicals under evaluation, upon the patency of gingival blood vessels.

Using the assumption that flow is greater where the radius of the vessel is greater, then correlation of these two indirect methods provide strong evidence of the effect of nicotine and adrenaline on gingival blood flow and should enhance the findings of any one method observed alone.

A series of twenty experiments were undertaken in this study using plastic microspheres to substantiate histologically that the reduction in gingival blood flow was in fact due to constriction of the peripheral blood vessels supplying the gingival tissues.

The basal epithelial cell layer of the gingival crest and the most inferior point of the basal cell layer of the junctional epithelium were used as the two reference

points from which comparative measurements were made of microsphere impaction.

It was assumed that the microspheres measured in this study were 15 μ in diameter although it was possible that some smaller microspheres gained entry to the gingival capillaries before those of the predominant dimension. The consistency of the observations found in this experiment lends support to the validity of this assumption.

Mean numbers of microsphere impactions for each chemical under test were compared statistically. Testing was carried out for both raw data (untransforms) and for natural log transforms. Descriptive statistics (means and variances) for raw and transformed data are presented in Appendix III, Tables 1 and 2, while Table 3 gives the results of the analysis of variance for log scores.

The most appropriate graphical format for microsphere impaction was found by plotting cumulative frequency percent against distance (mm). The curves drawn from both reference points were essentially similar and are described together (see Figures 44 and 45).

FIGURE 44.

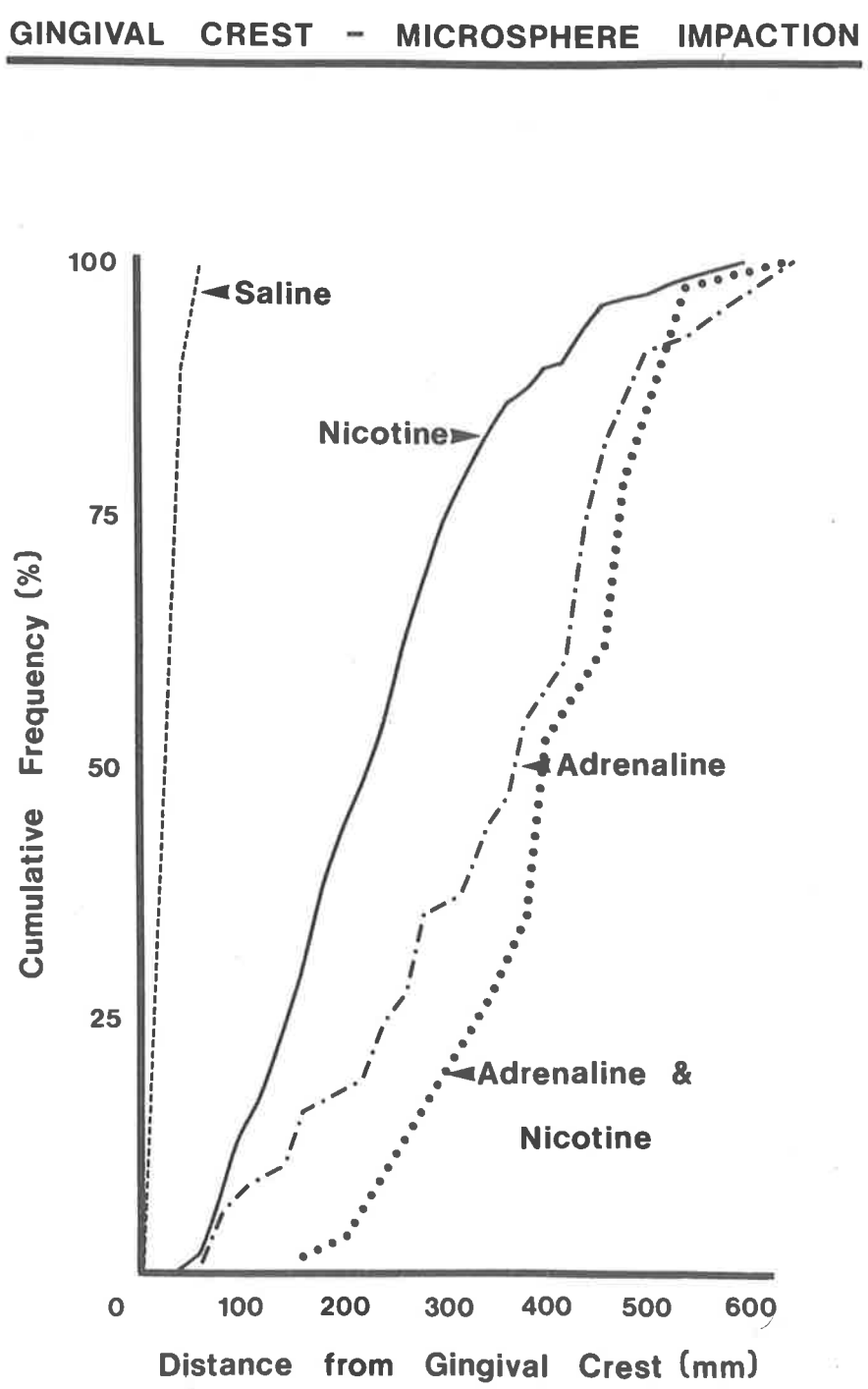

GINGIVAL CREST - MICROSPHERE IMPACTION
100
75
50
25
0
Cumulative Frequency (%)
Saline
Nicotine
Adrenaline
Adrenaline &
Nicotine
100
200
300
400
500
600
Distance from Gingival Crest (mm)

FIGURE 45.

EPITHELIAL ATTACHMENT - MICROSPHERE IMPACTION

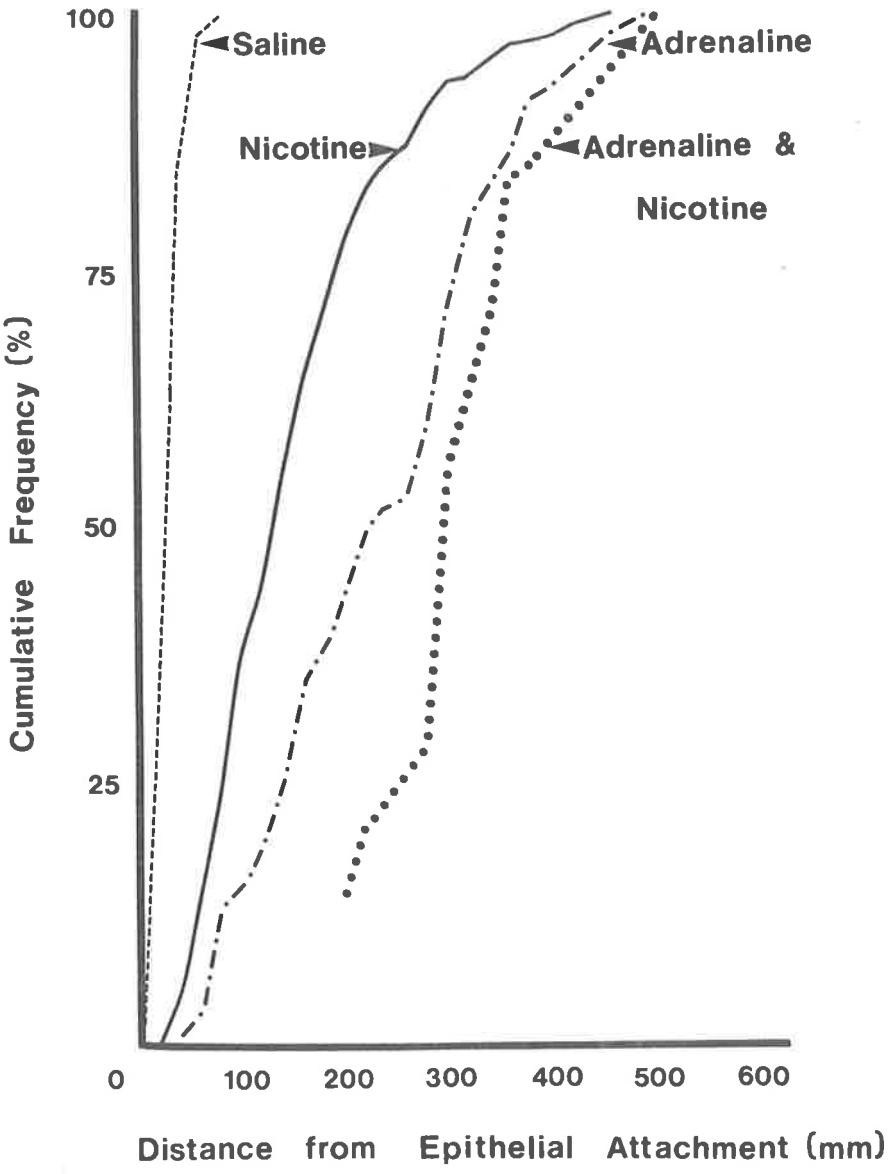


FIGURE 46. Microsphere impaction following saline infusion.

(Ehrlich's haemotoxylan and eosin. X 210).

B = Basement membrane
M = Microspheres

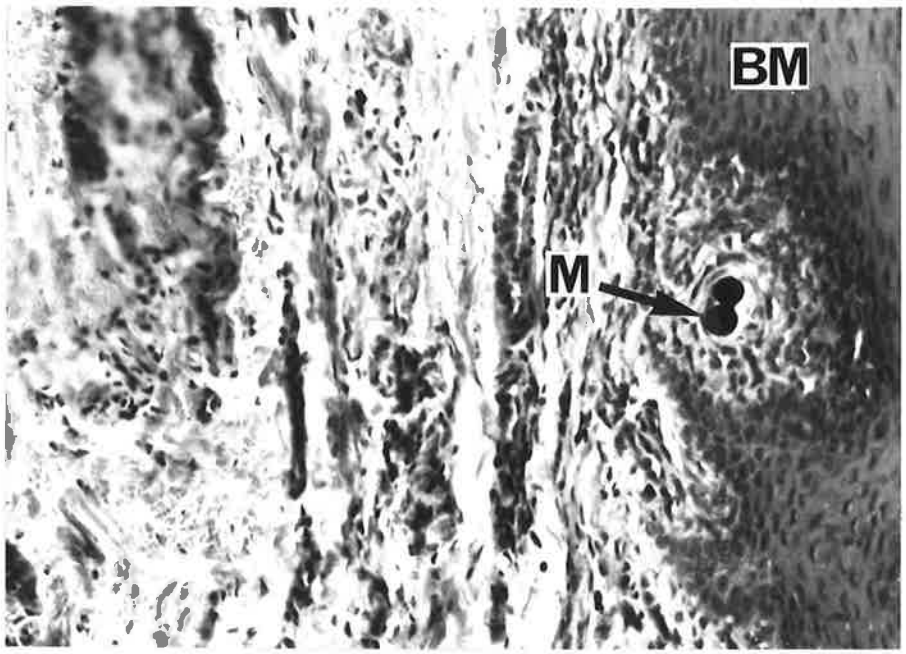


FIGURE 47. Microsphere impaction following nicotine infusion.

(Ehrlich's haemotoxylan and eosin. X 210)

B = Basement Membrane
M = Microspheres

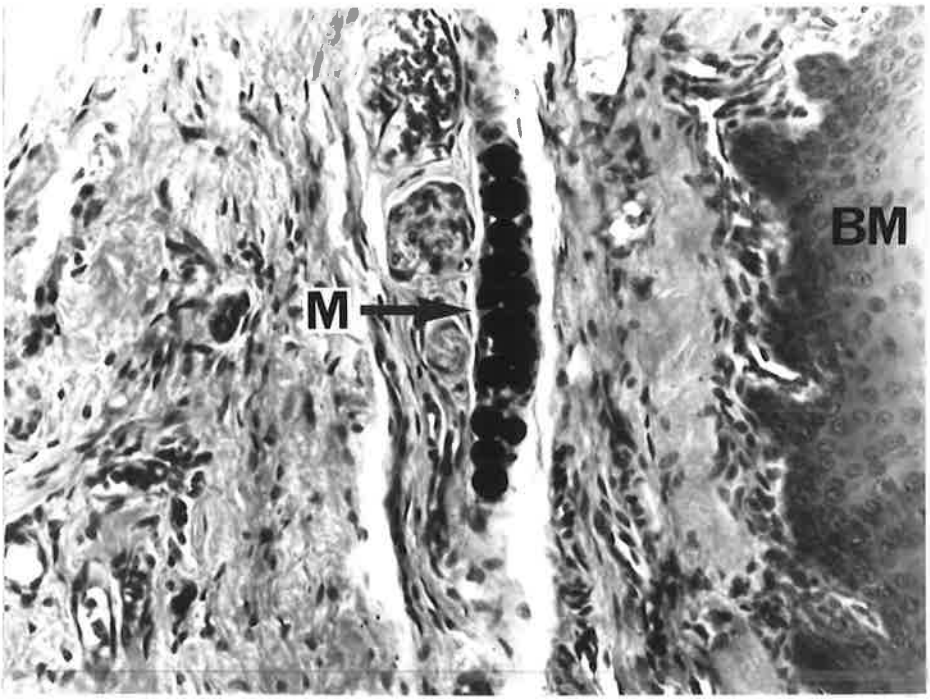


FIGURE 48. Microsphere impaction following adrenaline infusion. (Ehrlich's haemotoxylan and eosin. X 210).

B = Basement Membrane
M = Microspheres

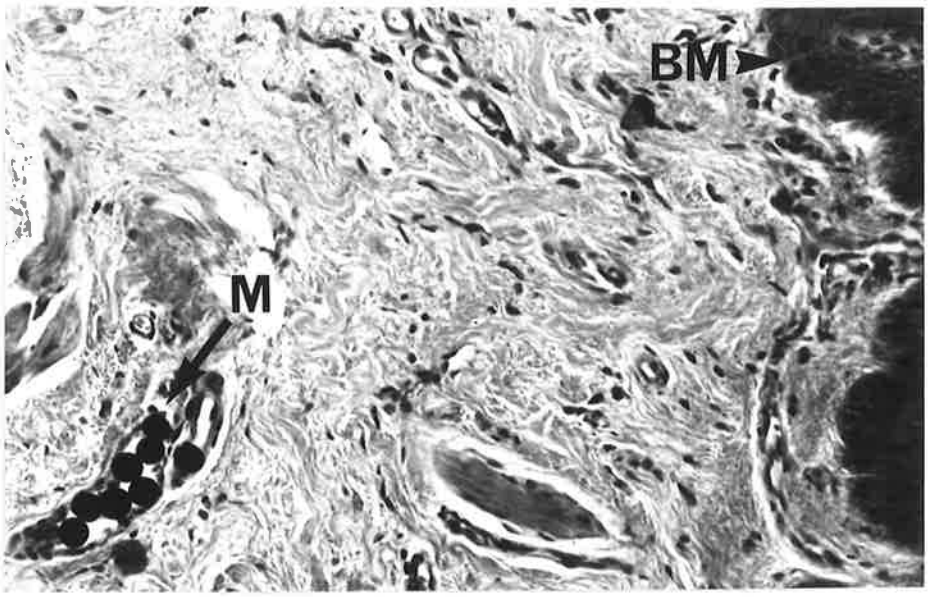


FIGURE 49. Microsphere impaction following saline, nicotine and adrenaline infusion. (Ehrlich's haemotoxylan and eosin. X 210).

B = Basement Membrane
M = Microspheres

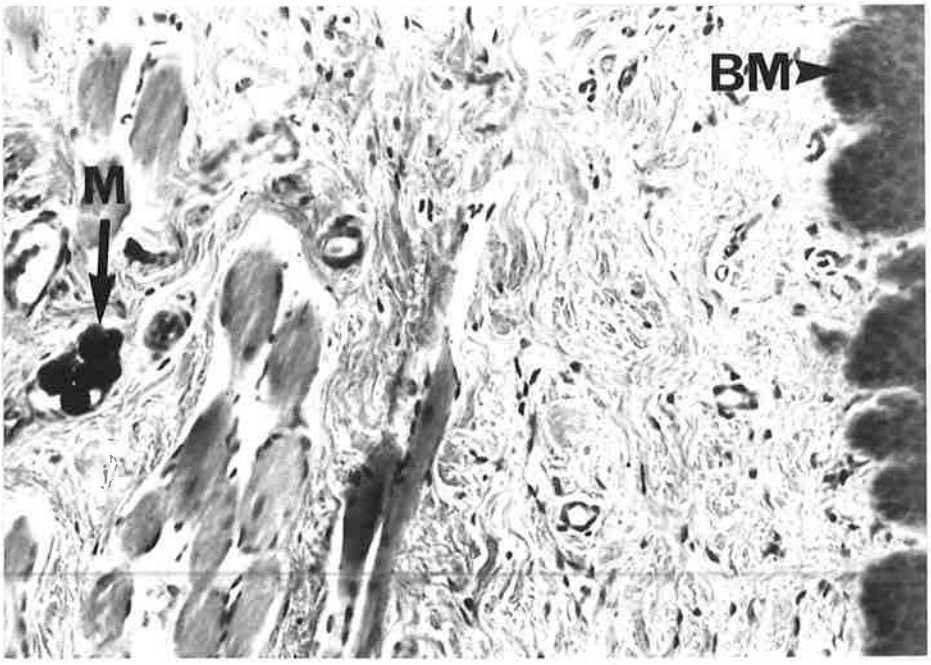


Table 4 shows the microsphere blockage levels of saline, nicotine and adrenaline as well as the comparative distance ratios of microsphere blockage for each drug from the reference points.

When saline was used as the vehicle for the microspheres, it was apparent that 100% of the microspheres were found within 0.06 mm of the gingival crest. Virtually identical results were obtained from the second reference point in the junctional epithelium.

When compared to the gingival crest, no significant difference in the diameter of capillaries adjacent to the junctional epithelium could be measured using saline borne microspheres. The spheres observed in relation to the crestal and junctional regions were found to be similar in distance from the basement membrane in the control experiments. Impaction had occurred close to the basement membrane in both locations where the diameter of the vessels would be expected to be less than that of the plastic spheres.

Infusion of the vehicle containing nicotine and the microspheres resulted in measurements indicating that 80% of spheres were lodged within 0.33 mm of the gingival crest; 100% of spheres were found within 0.6 mm of the crestal epithelium.

From the junctional epithelium reference point, 80% of the microspheres were found to be within 0.2 mm; 100% of microspheres were within 0.4 mm of the junctional epithelium. The distance doubled between the impaction of

80% and 100% of the lodged spheres from both reference points, although the spheres were closer in absolute values to the junctional epithelium.

Adrenaline had a greater effect upon the lumen of the capillaries than nicotine as shown in Table 4. Eighty percent of the microspheres were found with 0.45 mm (0.65 mm for 100% of spheres) compared with 0.33 mm using nicotine. The junctional epithelium reference point gave a value of 0.32 mm for 80% of microspheres (0.51 mm for 100% spheres) compared with 0.2 mm for nicotine and 0.04 mm for saline. Adrenaline increased the vasoconstriction in comparison to nicotine at both sites in absolute terms resulting in the most distant spheres being located at greater distances from the reference point when compared with nicotine.

A. SUMMARY

The use of black plastic microspheres as intra-vascular "markers" has shown that intra-arterial administration of saline had virtually no effect on the microvasculature of the gingiva. The microspheres were found trapped in capillaries close to the basement layer of the epithelium at both the gingival crest and the junctional epithelium. The average diameter of each microsphere was 15 μ and the entrapment of the spheres in these locations was assumed to be due to the spheres having an equal diameter to the vessels in those areas.

The intra-arterial infusion of nicotine significantly reduced the diameter of the gingival vessels,

the microspheres became trapped further away from the basal epithelial layer. There was a difference factor of 10 orders of magnitude between nicotine and saline measurements in the crestal region; six orders of magnitude of difference were found in the junctional region.

Intra-arterial infusion of adrenaline using the microspheres showed that the obstruction to the markers occurred in the vessels even further away from the epithelium than observed in the nicotine experiment. Using saline as the reference for the comparison of the effect of adrenaline on vessels, it was found that the difference of 11 and 8 orders of magnitude occurred in the crestal and junctional epithelium respectively.

B. DISCUSSION

The statistical analysis of saline, nicotine and adrenaline administration with microsphere infusion showed a high variance ratio between groups which was highly significant (see Tables 1, 2 and 3).

The statistical analysis can be interpreted to mean that the administration of saline, nicotine and adrenaline each had a variable effect on the micro-circulation of the gingiva. The impaction of the plastic microspheres at different distances from both the junctional epithelium and the gingival crest in response to the different chemicals used for infusion was indicative of their varying vasoconstrictive potential.

Adrenaline/nicotine infusion was not included in the analysis of variance because of the very small variance values for both reference points. A possible explanation for this phenomenon was that the combined or total vasoconstrictive action of these two chemicals was so great as to have caused microsphere impaction much farther back in the microcirculation compared with each individual chemical. In the histological evaluation of microsphere impaction it was noted that nicotine/adrenaline infusion caused impaction of microspheres in the dense connective tissues of the gingiva whereas all other impaction occurred relatively much closer to the reference points.

From Table 4 it can be seen that the vasoconstrictive properties of adrenaline were greater than nicotine by a factor range of 1.08 - 1.6 as evidenced by the different blockage distance of the microspheres from both the gingival crest and epithelial attachment. Although the vasoconstrictive properties of both adrenaline and nicotine were basically similar, each was powerfully potent with respect to saline by a factor of at least 10 at the gingival crest and a factor of at least 6.6 at the epithelial attachment.

The pharmacological action of nicotine evokes a response similar to that evoked by adrenaline administration, which is principally vasoconstriction of the peripheral vascular beds. Using Poiseuille's Law, where $\text{Flow} = \frac{\text{Pressure}}{\text{Resistance}}$, it can be appreciated that any peripheral vasoconstriction (nicotine or adrenaline administration) would markedly increase the resistance of the vascular bed and correspondingly reduce blood flow. As noted earlier,

the crucial parameter affecting blood flow is the radius of the blood vessels and this appears to be the major contributor to the vasoconstriction of the terminal vascular beds following either nicotine or adrenaline infusion. Any alteration in the lumen size of the arterioles will be reflected by a change to the 4th power in blood flow.

The observed constriction of the vessels was greater at the crest than adjacent to the junctional epithelium, when both adrenaline and nicotine were injected. A possible explanation could be found in the anatomical distribution of gingival vessels and the closer proximity of arterioles to the junctional epithelium than to the crest.

8. HUMAN STUDY

A total of ten experiments were undertaken using the crown form thermistor assemblies, electronic thermometer and acrylic stents. A typical result of these experiments is shown in Figure 50.

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

Variations in thermistor voltage during a typical experiment were as follows:

a) a baseline level of thermistor voltage was attained for a 20 minute period before smoking.

b) one cigarette was smoked in 7.5 minutes. During this time, thermistor voltage fell by 25 millivolts (8.8%).

c) in the post smoking period thermistor voltage continued to fall for a further 75 minutes before showing a rise in voltage values at the 105 minute mark.

Using the temperature v's resistance chart modified from manufacturer's data to include only the range of resistances observed in this study -

Thermistor temperature at R_{MAX} = 55.5^{o}C

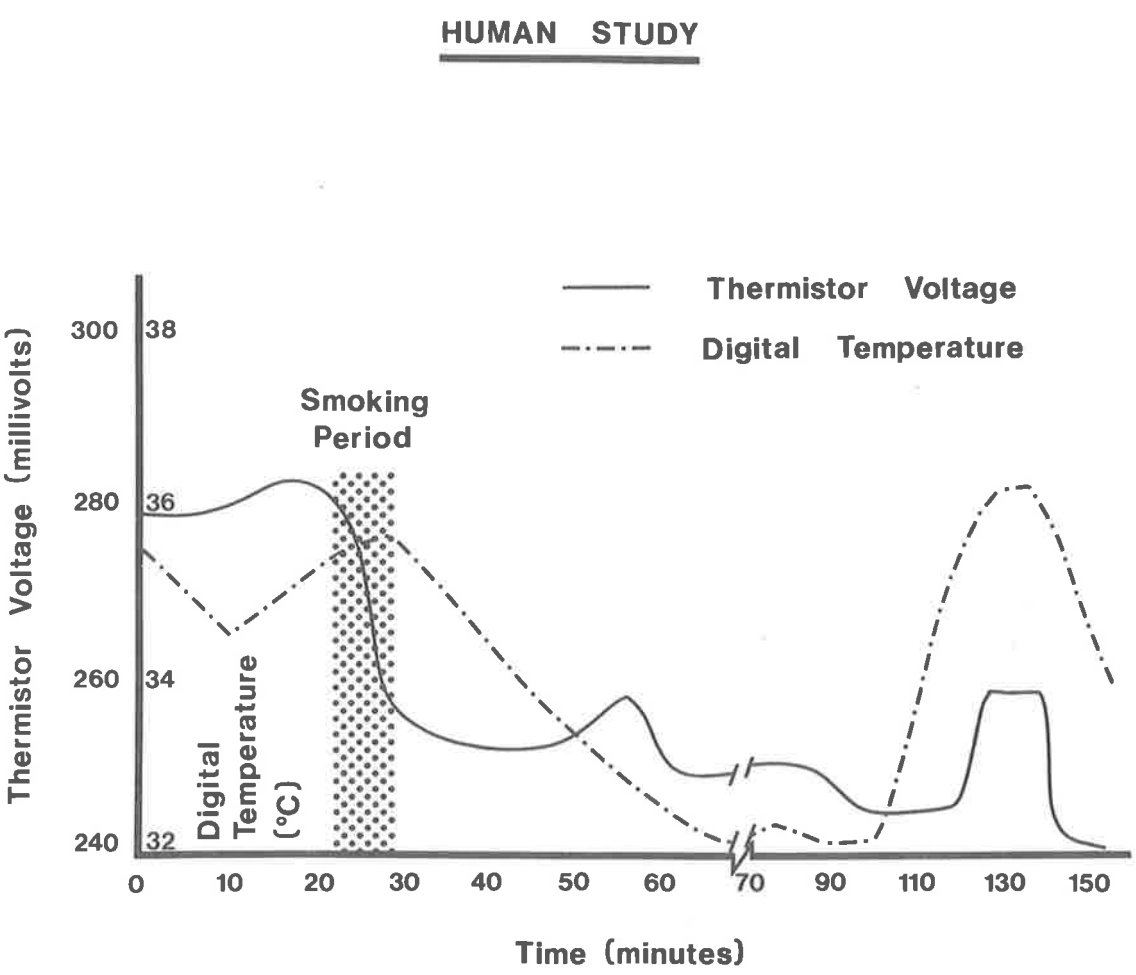
Thermistor temperature at R_{MIN} = 55.9^{o}C

These results indicate a rise of 0.4^{o}C in the temperature of the thermistor bead during smoking.

2) DIGITAL TEMPERATURE

Variations in the electronic thermometer's voltages, plotted as digital temperature were as

FIGURE 50.


HUMAN STUDY
Thermistor Voltage
Digital Temperature
Smoking Period
Thermistor Voltage (millivolts)
300
280
260
240
38
36
34
32
Digital Temperature (°C)
0
10
20
30
40
50
60
70
90
110
130
150
Time (minutes)

follows:

a) in the 22.5 minutes before smoking, digital temperature fell 1.0°C to 34.5°C after 10 minutes but rose to 35.5°C just prior to smoking.

b) during the smoking period digital temperature peaked at 35.7°C.

c) as soon as smoking was completed, digital temperature continued to fall over a 40 minute period before rising to 36.3°C after a further 65 minutes.

A. DISCUSSION

The significant fall in digital temperature following smoking was attributed to the effects of nicotine released by tobacco smoking. Over a period of 40 minutes after smoking, digital temperature decreased by 3.5°C indicating a progressive decrease in local blood supply due to peripheral vasoconstriction. Release of the vasoconstrictor action of nicotine occurred 70 minutes after smoking, demonstrating its prolonged vascular influence. Digital temperature peaked above the pre-smoking baseline level 105 minutes after smoking, suggesting a rebound vasodilation phenomenon - overcompensating for the prolonged peripheral vasoconstriction.

Maddock and Coller (1932), early researchers in this field, found a decrease in finger temperature of 5.9°C after three cigarettes had been smoked. At the termination

of their experiment 40 minutes later, digital temperature remained depressed. Roth, McDonald and Sheard (1944) reported an average decrease of 3.2°C in digital temperature after two cigarettes, while body core temperature remained constant. The quantitative changes of digital temperature as reported in this study are compatible with the findings of other workers in this field. The consistent findings of prolonged depression of digital temperature after smoking suggest the pharmacologically active agent(s) in tobacco smoke to be responsible, with only minimal influence from environmental changes.

It is concluded that the absorption of 1.24 - 1.52 mg of nicotine released by smoking one cigarette caused -

a) prolonged depression of digital temperature,

b) significant vasoconstriction of gingival blood vessels.

This experiment demonstrated that changes in thermistor voltage values were due to variations in gingival blood flow, seen concurrently as changes in another part of the peripheral circulation, as measured by digital temperature.

CHAPTER V

DISCUSSION

The hypothesis postulated by Kardachi and Clarke (1974) was that the known predisposing factors of A.N.U.G., chronic inflammation, (chronic marginal gingivitis) smoking and stress act together to powerfully influence the blood supply to the gingiva. They suggested that if the resultant ischaemia from this triad of factors were maintained, then a loss of vitality would occur in the epithelium of gingival papillary tips where there is no collateral gingival circulation. They argued that the known predisposing factors would cause ischaemia as a result of circulatory stasis, and be accompanied by poor nutrition of the dependent tissue. The established factors predisposing to coronary thrombosis are the same as those for A.N.U.G., if the stasis of chronic inflammation is equated to atheroma of the coronary artery.

The present investigations were devised to monitor gingival blood flow, blood pressure and peripheral body temperature in an animal model when circulatory nicotine and adrenaline were introduced into the circulation either singly or in combination.

Nicotine released into the circulation of the experimental animal has the potential to release adrenaline from the adrenal medulla and noradrenaline from the chromaffin cells, as well as from stores near to or in

vessel walls; both of these substances cause vasoconstriction (Courant, Paunio and Gibbons, 1965). Previous animal studies have shown a fall in gingival circulation which could be due to these vasoconstriction effects (Forsslund, 1959). The action of nicotine on the circulation is complex, acting on the vasomotor and vagus centres in the medulla, the sympathetic and para-sympathetic ganglia, the adrenal medulla and the chemoreceptors of the carotid sinus and aortic body. The rise in blood pressure is the combined result of stimulation of the medullary vasoconstrictor centres and the ganglia of the vasoconstrictor nerves, and the release of adrenaline and noradrenaline (Schmiterlow, 1948) from the adrenal gland and vessel walls. The released catecholamines quicken the pulse rate, increase the force of contraction of the myocardium and cause vasoconstriction in the peripheral vessels.

Hazard, Beauvallet and Larno (1957) and Malmejac, Neverre and Bianchi (1957) observed comparable increases in the release of adrenaline from the adrenal glands of dogs following the intravenous administration of nicotine. Adrenaline accounted for between 80-90 percent of catecholamines of the adrenal vein blood. These investigations have shown that intravenous nicotine increases the secretion of adrenaline by the adrenal glands. The data indicated that nicotine is one of the most effective compounds available to release adrenaline from the adrenal glands.

Investigations into the effect of nicotine on the peripheral circulation by Maddock and Coller (1933) and Moyer and Maddock (1940) showed that the vasoconstriction

produced by smoking cigarettes was analogous to that produced by intravenous injection of as much nicotine as was contained in the cigarette smoked. Weatherby, in 1942, found that vasoconstriction took place after smoking standard brands of cigarettes but that when such cigarettes were denicotinized and smoked, the vasoconstriction was abolished almost completely. These observations indicated that nicotine was the most important agent which contributed to the circulatory changes.

Schofield and Walker (1953) reported from perfusion experiments in an animal model that nicotine acted directly on the blood vessels, since the vasoconstrictor action of nicotine occurred after the removal of sympathetic chains and of the spinal nerves. Indirect measurements of constriction and dilation of the peripheral blood vessels has been obtained by using a plethysmograph. Most investigators found a decrease in blood flow and associated constriction of the peripheral blood vessels during nicotine administration.

Previous work has shown that the hypertensive episode following nicotine administration is due to the stimulation of the vasomotor centre, autonomic ganglia and the adrenal medulla. Experimentation by Watts (1956) showed definitely that stimulation of the adrenal medulla and the appearance of massive quantities of adrenaline in the peripheral circulation is one of the most important factors in producing the hypertensive episode. This study substantiated the finding of previous investigators who demonstrated a marked hypertensive episode in response to nicotine administration.

Rapaport, Frank and Massell (1950) showed that lumbar sympathectomy abolished the peripheral vasoconstriction in the lower extremities of 19 patients and concluded that the vasoconstriction was mediated by sympathetic vasomotor fibres. Other evidence shows that vasoconstrictor fibres are responsible for blood pressure homeostasis (vascular adjustments derived from the baro- and chemo- receptors) and for regulation of heat loss by the skin blood flow. There may be a generalized or a strictly segmented or regional engagement, depending upon the type of stimulus. Vasoconstrictor fibres, together with sympathetic vasodilator fibres to skeletal muscles, may affect specific vascular areas in a discharge pattern, causing marked redistribution of blood flow. The vasoconstrictor fibres are looked upon as the main neurogenic adjustors of the peripheral circulation showing prompt and regional adjustments to any change in environment, especially those changes that affect the hypothalamus and the vasoconstrictor centre in the medulla. Any arterial blood pressure deviation causes compensatory reflexes, through the baroreceptors, to re-establish a normal blood pressure. The efferent pathways to the heart for these baroreceptor vasomotor reflexes are the vagus and the sympathetic; in addition, the sympathetic vasoconstrictor fibres adjust the size of the "resistance" and "capacity" vessels in control of peripheral resistance to blood flow.

Further work has suggested that vasoconstriction might be a humoral effect and the studies of Burn and Grewal (1951) have shown that nicotine has an antidiuretic action, probably due to stimulation of the hypothalamus

with secretion from the posterior lobe of the pituitary, which in turn produces vasoconstriction.

The blood flow through a peripheral vascular bed is determined by a number of factors, the chief of which is the calibre of the terminal arterioles. This in turn is determined by the response of the smooth muscle of the vessels to stimuli which may be nervous, hormonal or chemical in nature and which act to adjust the peripheral circulation according to the local needs of the tissue itself or to the needs of the body as a whole.

The results of the present study showed that the characteristic rise in blood pressure during adrenaline infusion was of greater magnitude than the pressor response of nicotine administration. It is well established that adrenaline is one of the most potent vasopressor drugs known to effect a rapid increase in blood pressure. Adrenaline causes a raised blood pressure in three ways; a direct myocardial stimulation that increases the strength of ventricular contraction, an increased heart rate, and vasoconstriction, especially in the veins and precapillary resistance vessels of skin and mucosa. The chief vascular action of adrenaline is accentuated on the smaller arterioles, precapillary sphincters and sub-papillary venules of the microcirculation (Goodman and Gilman, 1970).

Adrenaline infusion also exhibited the same vaso-constrictive effect upon the microcirculation of the gingiva except that the decrease in blood flow was more profound although of shorter duration than nicotine. These results

indicate that adrenaline caused a rapid and marked vasoconstriction of the gingival circulation whereas nicotine caused less constriction for a longer period upon the terminal blood vessels. This effect of nicotine could result in damaging long-term effects to the gingival tissues explained later in the text. A study by Forsslund (1959) showed a reduced gingival circulation following systemic injection of adrenaline in humans and dogs by means of a stereoscopic microscope. Giddon *et al.* (1963) used a photo-electric method for continuously monitoring gingival vascular reactions and showed that both local and systemic administration of small amounts of adrenaline markedly reduced the vascular activity in the gingivae of dogs. Ito *et al.* (1973) measured gingival capillary flow rate by using a double thermocouple technique and showed that an intra-arterial injection of 1 ug adrenaline caused a rise in carotid arterial pressure and a substantial decrease in gingival blood flow. They also noted that the effects of adrenaline on the gingival circulation lasted longer than the effects upon the carotid arteries.

In the present study the greatest increase in blood pressure values was seen when saline, nicotine, and adrenaline was infused together into the experimental animal. The duration of the gingival vasoconstriction differed from the results obtained from the drugs when used alone.

Many of the accepted concepts regarding vascularization of the oral tissues have been obtained from anatomical and histological studies. Measurement of the actual blood flow into a tissue without disturbing the physiological

status of the tissue is a difficult problem. The microthermistor assembly which was inserted into the gingival crevice of the experimental animal in this study proved to be an excellent, reliable and sensitive indicator of gingival blood flow.

The second technique used to assess the effects of vasoconstriction on the gingival circulation following drug infusion also proved most satisfactory. Black plastic microspheres were infused after the test drug and the impaction sites of the spheres were compared with control sections. It was statistically significant that both nicotine and adrenaline infusion caused impaction of the microspheres at greater distances from the anatomical reference points than saline. The results obtained from the microsphere experiment confirmed the findings of the thermal diffusion method.

The skin plays an important role in dissipation of metabolic heat and regulation of blood volume, therefore circulatory responses and peripheral temperature changes are closely inter-related. Temperature changes can be effected by local mechanisms but the heat regulating centres in the hypothalamus ultimately are responsible for heat regulation. Blood flow in the skin may be diminished by increased activity of vasoconstrictor tone and by inhibition of vasodilator nerves. The skin is abundantly supplied with capillary loops that drain into a sub-capillary venous plexus which is capable of containing large volumes of blood. Arteriovenous anastomoses are present between smaller arteries and arterioles and the

corresponding venous channels, through which blood may be shunted and capillary areas short circuited. The coiled arterio-venous anastomotic vessels contain thick muscular walls abundantly supplied with nerve endings. The presence of a direct or indirect regulatory mechanism should be included in the shunt concept of arterio-venous anastomoses.

These communications have been studied mainly in animals, especially the rabbit. In the rabbit ear, arterio-venous anastomoses have been observed to undergo rhythmic contraction and dilation. The nature of their nervous supply in unknown, yet they react in the same manner, but at greater speed than arterioles, to the administration of adrenaline and noradrenaline. In the rabbit ear, arterio-venous anastomoses have been shown to be very effective in controlling body temperature. When open these channels offer low vessel resistance and large volumes of blood can flow through them for heat exchange. It has been demonstrated using microspheres that more than one third of the total blood flow through the rabbit ear may pass through such shunts (Meyer and Tschetter, 1966). Another important function of the arterio-venous anastomoses may be in regulation of vascular haemodynamics.

Nicotine infusion caused an immediate fall in peripheral temperature values and these changes closely correlated with the observations obtained from gingival blood flow. These findings support the study by Lampson (1935) who used a plethysomographic technique to show that blood volume reduction and falling skin temperatures were associated during smoking. The significance of these

results was further supported by the observations of Roth *et al.* (1944). They noted that intravenous injection of 1 to 2 milligrams of nicotine produced a significant drop in skin temperature which was strikingly similar to that observed during cigarette smoking. Maddock and Coller (1933) and Moyer and Maddock (1940) showed that the vasoconstriction of peripheral vessels after smoking cigarettes was analogous to that produced by intravenous injection of as much nicotine as was contained in the cigarette smoked. Furthermore, they noted that smoking cigarettes which did not contain nicotine produced no appreciable effects on the cutaneous temperature of the extremities. The potent vasoconstrictive and peripheral temperature effect of nicotine was also shown by Roth (1951). She found that a slight fall in cutaneous temperature of the extremities occurred when intravenous saline solution was given but when nicotine was added to the solution the decrease in peripheral cutaneous temperature was more rapid and of greater magnitude.

Roth also found a decrease of the cutaneous temperature of the extremities in subjects following consumption of two cigarettes. This decrease of cutaneous temperatures continued for up to one hour after smoking. Freund and Ward (1960) found that following cigarette smoking there was a significant reduction in digital skin temperature. The effects of nicotine and lowered peripheral temperature were shown to be additive (Wood, 1960).

In the present study adrenaline caused a greater reduction in peripheral temperature values than nicotine.

The drug combination of saline, nicotine and adrenaline caused the greatest decline in peripheral temperature values. This finding is similar to the results obtained from a combination effect of the drugs upon gingival blood flow and blood pressure. It is possible that the cumulative effect of the two vasopressor drugs could result in the establishment of a gingival ischaemia in chronic marginal gingivitis, ultimately resulting in papillary necrosis giving rise to the clinical lesion of A.N.U.G.

A feature of this study was the proven inter-relationship between gingival blood flow, blood pressure and peripheral temperature. The study showed that nicotine and adrenaline profoundly affected the peripheral circulation of the experimental animal. Thermistor bead temperature rose by 1.6^{o}C under the influence of the combined drugs compared to a rise of only 1.7^{o}C following total carotid occlusion.

The severity of reduction of gingival blood flow from the combined use of the drugs was therefore marginally less severe than the reduction consequent upon total carotid occlusion. The maximal reduction in blood flow occurred more quickly with total mechanical occlusion than occurred when drugs were used, but the combined effects of nicotine and adrenaline have the potential to profoundly restrict gingival blood flow.

Circulatory disturbances have been implicated as causal factors in periodontal disease. Provenza *et al.*

(1959) have speculated that complete or partial occlusion of blood vessels are related to the pathogenesis of periodontosis. In 1963 Lammie cited ischaemia as a primary cause of periodontal ligament breakdown leading to disease. Kennedy and Zander (1969) studied the effects of ischaemia within the gingival epithelium in a monkey model. They found that ischaemia of less than 10 hours duration did not induce epithelial lesions; focal necrosis occurred if ischaemia lasted between 10 and 14 hours, while ischaemia in excess of 24 hours affected necrosis in all layers of the epithelium. They concluded that the severity of epithelial pathology was directly related to the duration of ischaemia. These findings verify the dependency of the integrity of gingival epithelium upon the vascular supply to the underlying connective tissue. Forsslund (1953) in an animal study demonstrated a decrease in gingival circulation which was thought to be due to these vaso-constricting effects. Forsslund (1964) microscopically studied the sub-epithelial blood vessels and noted that they appeared to be carrying blood at a maximum rate. He postulated that the failure to find capillaries which are devoid of erythrocytes near the gingival surface may have indicated that this part of the gingival circulation was in service maximally at all times. If this hypothesis is valid, then reduced blood flow could alter the gingival physiology and increase the susceptibility of the tissue. The marked reduction of blood flow induced by a cool environment was accompanied by a further reduction in blood flow with the additional stimulus of smoking (Wood, 1960). It was suggested that if the circulation to the skin is greatly compromised, mild cold or smoking might be

detrimental. The present study indicates that smoking may be similarly implicated in A.N.U.G. Also the fact that a cool environment could jeopardize gingival blood flow, especially in people with a mouth breathing tendency, further augments the A.N.U.G. hypothesis of Kardachi and Clarke (1974) who suggest that A.N.U.G. is more prevalent during the winter months.

Emotional stress and the correlation with the endocrine and autonomic mechanisms of the body has been associated with A.N.U.G. in physchological studies using personality tests. Giddon, Zackin and Goldhaber (1964) have shown that in groups of college students there was a greater proportion of A.N.U.G. in students who withdrew from school than in those who remained. Moulton *et al.* (1952) found that each of the A.N.U.G. patients in their study experienced a period of stress before the onset of the disease. Although the relationship of emotional stress to A.N.U.G. is not totally conclusive, the emotional factor appears to be one of the most important aetiological factors.

Emotional stress stimulates the release of adrenaline from the adrenal gland and the release of noradrenaline from sympathetic nerve endings in the vascular bed: - adrenaline has a cutaneous vasopressor effect, noradrenaline is an overall vasoconstrictor. Manhold (1956) suggested that long-continued stress could cause a periodontal condition as a result of continued vessel constriction, causing a maintained lack of oxygen and nutrient materials within the periodontal tissues. He

considered that long periods of stress or extreme emotions could be a significant factor in pathological periodontal breakdown. His hypothesis was based on an experiment by Glickman, Turesky and Manhold (1950) who studied the oxygen consumption of healing gingival tissue of dogs. The study demonstrated the inter-relationship between cellular activity and the amount of oxygen consumed.

Moulton *et al.* (1952) pointed out that emotional stress can affect the gingiva directly or indirectly. The direct route involves overt habits partly or incompletely under voluntary control and may include such problems as poor oral hygiene, poor dietary habits and smoking (or increased smoking). The indirect route may alter the resistance of the periodontium to infection by acting with the autonomic nervous system and the endocrine system to affect such factors as the gingival circulation and circulating antibodies.

The results of this study using systemic administration of adrenaline to simulate the stress situation, and the results of the previously described studies of the psychosomatic effects upon the gingival circulation, lend further strong support to the proposed hypothesis for the aetiology of A.N.U.G. being related to the onset of tissue ischaemia from environmentally induced arteriolar spasm.

In other organs spastic constrictions of the arterioles may occur in the extremities with resulting cyanosis, pain and ultimately trophic lesions, ending in

gangrene and loss of tissue. These spastic vascular reactions may be initiated by cold temperature, local inflammatory changes or emotional instability. There is now good evidence to show that A.N.U.G. is such a lesion.

All experiments in this study were carried out in healthy gingival tissue unaffected by chronic inflammation. The results show that nicotine and adrenaline both separately and collectively markedly influence gingival blood flow by virtue of their vaso-constrictive action upon peripheral blood vessels. The vasopressor effect of nicotine upon the peripheral circulation was of longer duration but of lesser magnitude than adrenaline.

The results obtained from the series of experiments and further substantiated by histological evidence and a human study, confirmed that at least two of the suggested predisposing factors of Kardachi and Clarke's (1974) hypothesis for the aetiology of A.N.U.G. are valid. The predisposing factors of smoking (nicotine) and stress (adrenaline) had a powerful effect upon gingival blood flow with extreme and prolonged vaso-constriction of the gingival vessels being the predominant features.

CHAPTER VI

CONCLUSIONS

1. The results of the animal study showed that nicotine infusion in rabbits markedly reduced gingival blood flow for prolonged periods while the combination nicotine/adrenaline infusion caused a greater vasoconstriction than either drug used singly. In some instances vasoconstriction of the gingival vessels almost equalled total occlusion of the carotid artery.

2. The human study demonstrated the peripheral vasoconstrictor action of nicotine, absorbed during the smoking of one cigarette, on gingival blood flow and digital temperature.

3. The known predisposing factors of A.N.U.G. were postulated by Kardachi and Clarke (1974) to form a triad of factors that powerfully influence the tonus of blood vessels. Vasoconstriction was suggested to result in epithelial ischaemia in end-arterial organs. The evidence in this report strongly supports the hypothesis of Kardachi and Clarke as it relates to the peripheral vasoconstrictor effect of nicotine (smoking) and adrenaline (stress).

CHAPTER VII

APPENDICES

APPENDIX I. HISTOLOGIC PREPARATION

The lower jaw containing the central incisors was dissected free and fixed in 10 per cent neutral buffered formalin. The specimens were decalcified for three weeks in DECAL* and then divided into control and experimental blocks. All specimens were processed simultaneously for paraffin wax blocking by the double embedding method, serially sectioned bucco-lingually at 8 μm and stained with Ehrlich's Haemotoxylin and Eosin. A 10X magnification graticule (1 division = 0.01 mm) was used to sequentially analyze the location of impaction of the microspheres from the two reference points.

* OMEGA CHEMICAL CORP., COLD SPRING, NEW YORK.

APPENDIX II. GINGIVAL BLOOD FLOW

1. SALINE

A. INTRAVENOUS ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

A graph of thermistor temperature and thermistor resistance was reproduced from published data to enable thermistor voltage to be calculated in terms of thermistor bead temperature (Figure 4).

From Ohm's Law:- $E = IR$

where E = voltage in volts

I = current in amps

R = resistance in ohms,

resistance can be calculated because current and voltage are both known. In this series of experiments the maximum value for thermistor voltage was 70 millivolts and a constant current of 8 milli-amps was used to heat the thermistor bead.

Using Ohm's Law:- $R = \frac{.070}{.008}$

$= 8.8$ ohms

The balancing resistance applied to the circuit was 450 ohms, so the total resistance at maximum voltage was 458.8 ohms.

Using the temperature versus resistance chart from published data and modified to include only the resistance range observed in this study, it was shown that the resistance of the thermistor assembly at the maximum voltage value was

equivalent to a thermistor bead temperature of 66.2°C. Other values for the thermistor assembly are shown in Table 1.

TABLE 1. THERMISTOR ASSEMBLY VALUES

Millivolts	Resistance (OHMS)	Thermistor Temperature (°C)	Temperature Difference from Mean (°C)
57	457.1	66.5	+0.1
60	457.5	66.4	0
65	458.2	66.3	-0.1
70	458.8	66.2	-0.2

B. CONTINUOUS HALF HOURLY INTRAVENOUS ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

In this series of experiments the maximum value for thermistor voltage was 70 millivolts and a constant current of 8 milli-amps was used to heat the thermistor bead. The balancing resistance applied to the circuit was 450 ohms, so the total resistance at maximum voltage was 458.8 ohms. Other values for the thermistor assembly are shown in Table 2.

TABLE 2. THERMISTOR ASSEMBLY VALUES

Millivolts	Resistance (OHMS)	Thermistor Temperature (^{o}C)	Temperature Difference from Mean (^{o}C)
57	457.1	66.5	+0.1
60	457.5	66.4	0
65	458.2	66.3	-0.1
70	458.8	66.2	-0.2

C. INTRA-ARTERIAL ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

In this series of experiments the maximum value for thermistor voltage was 340 millivolts and a constant current of 10 milli-amps was used to heat the thermistor bead. The balancing resistance applied to the circuit was 428.5 ohms, so the total resistance at maximum voltage was 462.5 ohms. Other values for the thermistor assembly are shown in Table 3.

TABLE 3. THERMISTOR ASSEMBLY VALUES

Millivolts	Resistance (OHMS)	Thermistor Temperature (^{o}C)	Temperature Difference from Mean (^{o}C)
325	460.5	66.1	+0.1
330	461.0	66.0	0
335	462.0	65.9	-0.1
340	462.5	65.8	-0.2

D. CONTINUOUS HALF HOURLY INTRA-ARTERIAL ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

In this series of experiments the maximum value for thermistor voltage was 340 millivolts and a constant current of 10 milli-amps was used to heat the thermistor bead. The balancing resistance applied to the circuit was 428.5 ohms, so the total resistance at maximum voltage was 462.5 ohms. Other values for the thermistor assembly are shown in Table 4.

TABLE 4. THERMISTOR ASSEMBLY VALUES

Millivolts	Resistance (OHMS)	Thermistor Temperature (^{o}C)	Temperature Difference from Mean (^{o}C)
325	460.5	66.1	+0.1
330	461.0	66.0	0
335	462.0	65.9	-0.1
340	462.5	65.8	-0.2

2. NICOTINE

A. INTRAVENOUS ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

In this series of experiments the maximum value for thermistor voltage was 95 millivolts and a constant current of 8 milli-amps was used to heat the thermistor bead. The balancing resistance applied to the circuit was 453 ohms, so the total resistance at maximum voltage was 464.9 ohms. Other values for the thermistor assembly are shown in Table 5.

TABLE 5. THERMISTOR ASSEMBLY VALUES

Millivolts	Resistance (OHMS)	Thermistor Temperature (^{O}C)	Temperature Difference from Mean (^{O}C)
12	454.5	66.5	+0.6
15	454.9	66.5	+0.6
30	456.8	66.5	+0.6
45	458.6	66.2	+0.3
60	460.5	66.0	+0.1
75	462.4	66.0	+0.1
85	463.6	65.9	0
90	464.2	65.9	0
95	464.9	65.7	-0.2

B. CONTINUOUS HALF HOURLY INTRAVENOUS ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

In this series of experiments the maximum value for thermistor voltage was 135 millivolts and a constant current of 8 milli-amps was used to heat the thermistor bead. The balancing resistance applied to the circuit was 400 ohms, so the total resistance at maximum voltage was 416.9 ohms. Other values for the thermistor assembly are shown in Table 6.

TABLE 6. THERMISTOR ASSEMBLY VALUES

Millivolts	Resistance (OHMS)	Thermistor Temperature (^{o}C)	Temperature Difference from Mean (^{o}C)
16	402.0	71.0	+1.3
20	402.5	71.0	+1.3
40	405.0	70.9	+1.2
60	407.5	70.7	+1.0
80	410.0	70.5	+0.8
100	412.5	70.1	+0.4
120	415.0	70.0	+0.3
125	415.6	69.7	0
135	416.9	69.6	-0.1

C. INTRA ARTERIAL ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

In this series of experiments the maximum value for thermistor voltage was 65 millivolts and a constant current of 10 milli-amps was used to heat the thermistor bed. The balancing resistance applied to the circuit was 380 ohms, so the total resistance at maximum voltage was 386.5 ohms. Other values for the thermistor assembly are shown in Table 7.

TABLE 7. THERMISTOR ASSEMBLY VALUES

Millivolts	Resistance (OHMS)	Thermistor Temperature (^{o}C)	Temperature Difference from Mean (^{o}C)
2	380.2	73.4	+0.5
10	381.0	73.3	+0.4
20	382.0	73.2	+0.3
30	383.0	72.9	0
35	383.5	72.9	0
40	384.0	72.9	0
50	385.0	72.8	-0.1
60	386.0	72.8	-0.1
65	386.5	72.7	-0.2

D. CONTINUOUS HALF HOURLY INTRA-ARTERIAL ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

In this series of experiments the maximum value for thermistor voltage was 135 millivolts and a constant current of 9 milli-amps was used to heat the thermistor bead. The balancing resistance applied to the circuit was 465 ohms, so the total resistance at maximum voltage was 480 ohms. Other values for the thermistor assembly are shown in Table 8.

TABLE 8. THERMISTOR ASSEMBLY VALUES

Millivolts	Resistance (OHMS)	Thermistor Temperature (^{o}C)	Temperature Difference from Mean (^{o}C)
10	466.1	66.3	+1.4
20	467.2	66.1	+1.1
40	469.4	65.9	+0.9
60	471.6	65.7	+0.7
70	472.9	65.0	0
80	474.9	64.9	-0.1
100	476.1	64.9	-0.1
120	478.3	64.8	-0.2
135	480.0	64.8	-0.2

3. ADRENALINE

A. INTRAVENOUS ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

In this series of experiments the maximim value for thermistor voltage was 120 millivolts and a constant current of 8 milli-amps was used to heat the thermistor bead. The balancing resistance applied to the circuit was 445 ohms, so the total resistance at maximum voltage was 460.5 ohms. Other values for the thermistor assembly are shown in Table 9.

TABLE 9. THERMISTOR ASSEMBLY VALUES

Millivolts	Resistance (OHMS)	Thermistor Temperature (^{o}C)	Temperature Difference from Mean (^{o}C)
50	451.2	66.8	+0.6
60	452.5	66.7	+0.5
80	455.0	66.5	+0.3
100	457.5	66.4	+0.2
110	458.7	66.2	0
120	460.5	66.0	-0.2

B. CONTINUOUS HALF HOURLY INTRAVENOUS ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

In this series of experiments the maximum value for thermistor voltage was 325 millivolts and a constant current of 10 milli-amps was used to heat the thermistor bead. The balancing resistance applied to the circuit was 425 ohms, so the total resistance at maximum voltage was 457.5 ohms. Other values for the thermistor assembly are shown in Table 10.

TABLE 10. THERMISTOR ASSEMBLY VALUES

Millivolts	Resistance (OHMS)	Thermistor Temperature (^{o}C)	Temperature Difference from Mean (^{o}C)
120	437.0	67.8	+1.4
150	440.0	67.7	+1.3
200	445.0	67.3	+0.9
250	450.0	66.9	+0.5
300	455.0	66.5	+0.1
325	457.5	66.4	0

C. INTRA-ARTERIAL ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

In this series of experiments the maximum value for thermistor voltage was 130 millivolts and a constant current of 8 milli-amps was used to heat the thermistor bead. The balancing resistance applied to the circuit was 447 ohms, so the total resistance at maximum voltage was 463.2 ohms. Other values for the thermistor assembly are shown in Table 11.

TABLE 11. THERMISTOR ASSEMBLY VALUES

Millivolts	Resistance (OHMS)	Thermistor Temperature (^{o}C)	Temperature Difference from Mean (^{o}C)
40	452.0	66.7	+0.7
60	454.5	66.6	+0.6
80	457.0	66.5	+0.5
100	459.5	66.1	+0.1
110	460.7	66.0	0
120	462.0	66.0	0
130	463.2	65.9	-0.1

D. CONTINUOUS HALF HOURLY INTRA-ARTERIAL ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

In this series of experiments the maximum value for thermistor voltage was 325 millivolts and a constant current of 10 milli-amps was used to heat the thermistor bead. The balancing resistance applied to the circuit was 420 ohms, so the total resistance at maximum voltage was 452.5 ohms. Other values for the thermistor assembly are shown in Table 12.

TABLE 12. THERMISTOR ASSEMBLY VALUES

Millivolts	Resistance (OHMS)	Thermistor Temperature (^{o}C)	Temperature Difference from Mean (^{o}C)
115	431.5	68.3	+1.6
150	435.0	68.0	+1.3
200	440.0	67.7	+1.0
250	445.0	67.3	+0.6
300	450.0	66.9	+0.2
325	452.5	66.7	0

4. SALINE, NICOTINE AND ADRENALINE

A. INTRAVENOUS ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

In this series of experiments the maximum value for thermistor voltage was 66 millivolts and a constant current of 8 milli-amps was used to heat the thermistor bead. The balancing resistance applied to the circuit was 430 ohms, so the total resistance at maximum voltage was 438.3 ohms. Other values for the thermistor assembly are shown in Table 13.

TABLE 13. THERMISTOR ASSEMBLY VALUES

Millivolts	Resistance (OHMS)	Thermistor Temperature (^{o}C)	Temperature Difference from Mean (^{o}C)
8	431.0	68.5	+0.6
10	431.2	68.4	+0.5
20	432.2	68.3	+0.4
30	433.7	68.2	+0.3
40	435.0	68.0	+0.1
50	536.2	68.0	+0.1
55	436.9	67.9	0
60	437.5	67.8	-0.1
66	438.2	67.8	-0.1

B. CONTINUOUS HALF HOURLY INTRAVENOUS ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

In this series of experiments the maximum value for thermistor voltage was 120 millivolts and a constant current of 10 milli-amps was used to heat the thermistor bead. The balancing resistance applied to the circuit was 425 ohms, so the total resistance at maximum voltage was 437 ohms. Other values for the thermistor assembly are shown in Table 14.

TABLE 14. THERMISTOR ASSEMBLY VALUES

Millivolts	Resistance (OHMS)	Thermistor Temperature (^{o}C)	Temperature Difference from Mean (^{o}C)
30	428.0	69.3	+1.5
40	429.0	68.8	+1.0
60	431.0	68.4	+0.6
80	433.0	68.3	+0.5
100	435.0	68.0	+0.2
120	437.0	67.8	0

C. INTRA-ARTERIAL ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

In this series of experiments the maximum value for thermistor voltage was 130 millivolts and a constant current of 7 milli-amps was used to heat the thermistor bead. The balancing resistance applied to the circuit was 425 ohms, so the total resistance at maximum voltage was 443.5 ohms. Other values for the thermistor assembly are shown in Table 15.

TABLE 15. THERMISTOR ASSEMBLY VALUES

Millivolts	Resistance (OHMS)	Thermistor Temperature (^{o}C)	Temperature Difference from Mean (^{o}C)
10	426.4	68.9	+1.2
20	427.9	68.7	+1.0
40	430.7	68.4	+0.7
60	432.5	68.3	+0.6
80	436.1	68.0	+0.3
100	439.3	67.8	+0.1
110	440.7	67.7	0
120	441.1	67.6	-0.1
130	443.5	67.5	-0.2

D. CONTINUOUS HALF HOURLY INTRA-ARTERIAL ADMINISTRATION

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

In this series of experiments the maximum value for thermistor voltage was 102 millivolts and a constant current of 8 milli-amps was used to heat the thermistor bead. The balancing resistance applied to the circuit was 373 ohms, so the total resistance at maximum voltage was 387.7 ohms. Other values for the thermistor assembly are shown in Table 16.

TABLE 16. THERMISTOR ASSEMBLY VALUES

Millivolts	Resistance (OHMS)	Thermistor Temperature (^{o}C)	Temperature Difference from Mean (^{o}C)
15	374.9	74.1	+1.6
20	375.2	73.8	+1.3
40	378.0	73.5	+1.0
60	380.5	73.3	+0.8
80	383.0	72.9	+0.4
100	385.5	72.5	0
102	385.7	72.4	-0.1

5. SIX HOUR CAROTID CLAMP

1) THERMISTOR TEMPERATURE (MILLIVOLTAGE)

In this series of experiments the maximum value for thermistor voltage was 110 millivolts and a constant current of 7 milli-amps was used to heat the thermistor bead. The balancing resistance applied to the circuit was 400 ohms, so the total resistance at maximum voltage was 415.7 ohms. Other values for the thermistor assembly are shown in Table 17.

TABLE 17. THERMISTOR ASSEMBLY VALUES

Millivolts	Resistance (OHMS)	Thermistor Temperature (^{o}C)	Temperature Difference from Mean (^{o}C)
10	401.4	71.3	+1.7
20	402.9	71.0	+1.4
40	405.7	70.7	+1.1
60	407.5	70.3	+0.7
80	411.4	70.2	+0.6
100	414.3	69.8	+0.2
110	415.7	69.6	0

APPENDIX III. MICROSPHERE INFUSION

Table I shows the raw data:-

TABLE I. DISTANCE OF MICROSPHERE IMPACTIONS FROM REFERENCE POINTS

1). CREST

	No. Observations	Mean	Variance
Saline	67	26.791	147.35
Nicotine	198	238.535	14665.36
Adrenaline	87	347.701	21415.00
Adrenaline/ Nicotine	39	425.897	17403.78

2). JUNCTIONAL EPITHELIUM

	No. Observations	Mean	Variance
Saline	67	28.269	172.02
Nicotine	204	150.172	7929.40
Adrenaline	86	230.814	13622.27
Adrenaline/ Nicotine	42	266.071	3149.43

The raw data was then transformed into logarithmic values in order to validate the distribution scale of the results. See Table 2 for logged data.

TABLE 2. LOG OF DISTANCE OF MICROSPHERE IMPACTIONS FROM REFERENCE POINTS

1). CREST

	No Observations	Log of Mean	Log of Variance
Saline	67	3.162	.29
Nicotine	198	5.333	.32
Adrenaline	87	5.726	.32
Adrenaline/ Nicotine	39	5.998	.13

2). JUNCTIONAL EPITHELIUM

	No Observations	Log of Mean	Log of Variance
Saline	67	3.229	.25
Nicotine	204	4.832	.40
Adrenaline	86	5.283	.37
Adrenaline/ Nicotine	42	5.561	.05

An analysis of variance on the logged data was then carried out for saline, nicotine and adrenaline, It should be noted that adrenaline/nicotine combination was not included in table 3 because of their very small variance values for both crest and junctional epithelium references.

TABLE 3. ANALYSIS OF VARIANCE OF LOG. SCORES: SALINE, NICOTINE AND ADRENALINE

1) CREST

	Sum of Squares (SS)	Degrees of Freedom (DF)	Mean of Squares (MS)	Variance Ratio (VR)
Between Groups	294.05	2	147.03	470.20
Within Groups	109.13	349	.31	
Total	403.18	351		

2) JUNCTIONAL EPITHELIUM

	Sum of Squares (SS)	Degrees of Freedom (DF)	Mean of Squares (MS)	Variance Ratio (VR)
Between Groups	176.47	2	88.24	242.35
Within Groups	128.89	354	.36	
Total	305.36	356		

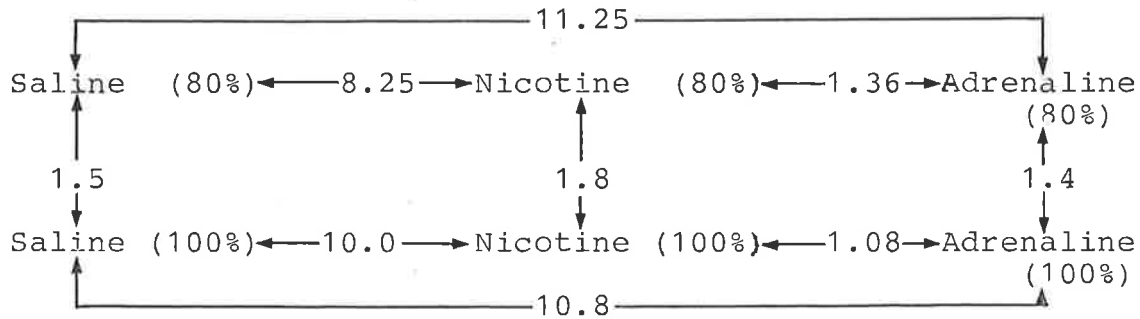
From Table 3 it can be seen that the variance ratio (VR) between the groups was highly significant for both reference points.

TABLE 4. MICROSPHERE BLOCKAGE LEVELS OF SALINE, NICOTINE AND ADRENALINE

1) CREST

		Saline	Nicotine	Adrenaline
Microsphere Blockage level in mm from crest	80%	.04	.33	.45
	100%	.06	.60	.65

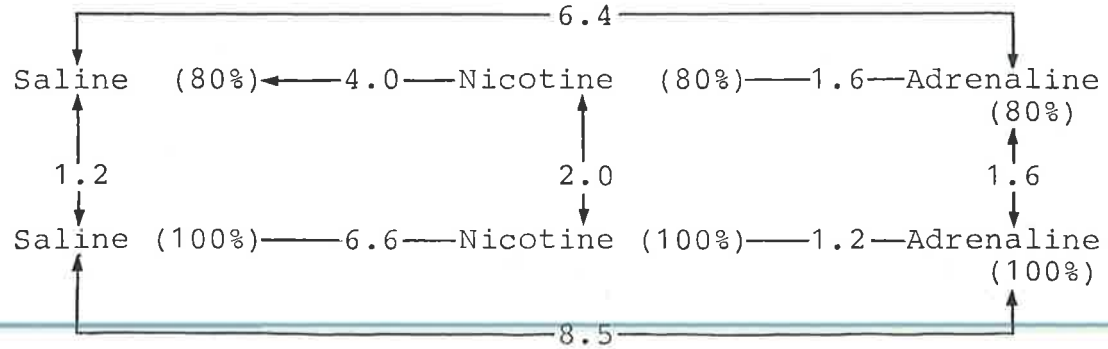
COMPARATIVE DISTANCE RATIOS OF MICROSPHERE BLOCKAGE LEVELS OF SALINE, NICOTINE AND ADRENALINE



2) JUNCTIONAL EPITHELIUM

		Saline	Nicotine	Adrenaline
Microsphere Blockage level in mm from junctional epithelium	80%	.05	.20	.32
	100%	.06	.40	.51

COMPARATIVE DISTANCE RATIOS OF MICROSPHERE BLOCKAGE LEVELS OF SALINE, NICOTINE AND ADRENALINE



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