



THE ROLE OF CERTAIN VASOACTIVE SUBSTANCES IN THE CONTROL
OF THE PERIPHERAL CIRCULATION IN MAN,

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DECLARATION AND ACKNOWLEDGEMENTS

I declare that this thesis is of my own composition and that it is a record of original work conducted during the years 1964, 1965, 1966 in the Department of Human Physiology and Pharmacology, University of Adelaide and 1967 in the Department of Physiology, University of Southern California School of Medicine, Los Angeles, California. The work described herein has not been submitted for any other degree, award or diploma.

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3.

S E C T I O N I.

GENERAL INTRODUCTION.

This thesis examines the role of several vasoactive substances, especially serotonin, in the control of the peripheral circulation in man and includes studies of the capacity and fluid exchange properties of the peripheral circulation. This division of the systemic circulation can be subdivided into three principal functional areas:

- (1) the resistance vessels, mainly the arterioles
- (2) the exchange vessels, exchange of solutes and water occurring across capillary walls, and,
- (3) the capacity vessels, the veins.

The peripheral circulation is involved in two sets of control mechanisms, one local and the other general. On the one hand it plays a part in the supply of nutrients and removal of waste products according to the local needs of each tissue, and on the other it is involved in generalized adjustments such as those of cardiac output, systemic blood pressure and body temperature. At both of these levels the necessary adjustments of the circulation through a given tissue are effected by the action on the smooth muscle of the resistance and capacity vessels of hormonal or chemical agents and by nervous influences, which act ultimately by way of chemical transmitters.

The effects of a number of these agents on the peripheral

circulation have been investigated intensively and the mechanisms of action of such vasoactive substances as adrenaline, noradrenaline, serotonin and angiotensin are becoming clarified. However, under physiological conditions the vessels will often be exposed to the effects of two or more of these agents acting simultaneously and little is known of the ways in which they may interact with one another. Work described in Section 2 of this thesis examines the actions of some of the naturally occurring vasoactive substances on the resistance vessels and relates ways in which their actions may be influenced by the effects of others and by the presence or absence of sympathetic innervation.

Emphasis throughout is placed on the circulation in the skin and muscle of the upper limb since the limb vascular beds are the most readily accessible to methods of measurement which do not themselves interfere greatly with the circulation. Most studies have involved indirect measurements of hand or forearm blood flow by the technique of venous occlusion plethysmography, using either water-displacement or electro-capacitance plethysmographs. In general, local effects of the various vasoactive substances have been studied by infusing them intra-arterially into the main artery supplying the vascular bed. Relatively high concentrations can thus be given locally but the substance is so diluted when it reaches the general circulation that it has no widespread effects. The circulation

through the corresponding vascular bed of the opposite limb can thus be used as a control. General effects of the vasoactive substances were assessed by intravenous administration. General effects, however, are often more difficult to interpret since the effects of the vasoactive substances are often complicated by reflex cardiovascular effects and respiratory and emotional effects on the subject. Thus a preference is shown for the intra-arterial studies since the effects of the vasoactive substances are more clearly apparent.

The studies of the peripheral vascular effects of serotonin, angiotensin and noradrenaline were followed by a study of the role of a number of vasoactive substances (especially acetylcholine) in the phenomenon of post-exercise hyperaemia of the resistance vessels of skeletal muscle (Section 3). The search for the elusive vasoactive substance or substances responsible for this well-recognized phenomenon has absorbed the interest of investigators for many years. In addition to the study in man, the study of post-exercise hyperaemia was extended to animal work (the sheep) since it seemed appropriate to extend the search for vasoactive substances to lymph, and this was not feasible in man. Finally, in the sections on the resistance vessels the effects of ethyl alcohol were studied as an example of a vasoactive substance which is not a naturally occurring one (Section 4).

In Sections 5 and 6 are described studies on venous tone and on capillary filtration which were undertaken at the University of Southern California, while all the previous investigations in Sections 2-4 were performed at the University of Adelaide. The study of venous distensibility in normotensive and hypertensive patients and the effects of antihypertensive treatment on the latter provided data on normal and abnormal responses of the capacity vessels. Capillary filtration rate and capillary filtration coefficient were measured plethysmographically and the effect of adrenaline iontophoresis (to the skin) on the rate of accumulation of tissue fluid in the forearm was assessed,

Throughout all these studies it was apparent that the general (central) and local control of the peripheral vessels was influenced in a highly specific manner by the interaction of various hormonal, chemical and nervous factors.

THE AUTONOMIC NERVOUS SYSTEM, ADRENALINE AND NORADRENALINE

Galen (circa 200 A.D.) was probably the first to distinguish the sympathetic trunks in the thorax. He regarded them as "costal branches of the sixth pair of cerebral nerves" (now known as the vagi). He also recognised sympathetic ganglia and is credited with originating the concept that "sympathy" or "consent" exists between all parts of the body and that the brain and nerves play an essential role in this relationship. At that time the "humoral theory" of "animal" and "vital spirits" was generally accepted and he and his followers believed that hollow nerves conveyed "humors" from one part of the body to another so that every part was influenced and was brought into sympathy. Galen's ideas on the autonomic nervous system as well as many other facets of medicine dominated medical thinking for some 1400 years. When Willis (1664) provided a reasonably accurate description of the sympathetic and vagus nerves and differentiated voluntary and involuntary movements he still adhered to the humoral theory in that he believed that the "intercostal" nerves, as he called the sympathetics, carried "animal spirits" to and from the cerebellum.

Winslow (1732) was probably first to use the term "sympathetic nerves" to indicate their importance in effecting sympathy between various organs. Whytt (1751) provided the basis of the modern concept of the reflex nature of sympathetic control in

some otherwise rather philosophical essays. He interpreted the contraction of the intestines and bladder which occurs with distension or irritation as a reflex response involving the sympathetic nerves and also described the responses of the pupils to light.

In 1858, Claude Bernard described the vasomotor functions of sympathetic nerves. He found that stimulation of the sympathetic fibres to the submandibular gland produced vasoconstriction whereas stimulation of the chorda tympani produced vasodilatation. He also punctured the floor of the fourth ventricle and provided the first evidence for the existence of higher centres controlling the activity of the autonomic nervous system. This marked the beginning of a new era for it saw a shift in emphasis from the purely descriptive approach to an experimental mode of study which has accounted for the relatively rapid progress in our understanding of the autonomic system over the past 100 years.

The structural and functional relationships of the sympathetic nerves and ganglia were demonstrated by Gaskell (1886) who differentiated the thoraco-lumbar from the cranio-sacral outflow. He noted that the vagus was inhibitory, whereas the sympathetic was excitatory. Gaskell's concepts were extended by Langley (1893) who is responsible for the definition of the autonomic nervous system as we know it. He introduced the terms "preganglionic" and "postganglionic" and in 1898 suggested the title "autonomic nervous system"

to classify the cranial, thoraco-lumbar and sacral outflows. At this time, largely due to the work of Gaskell and Langley, it was apparent that stimulation of craniosacral and thoraco-lumbar outflows produced opposite effects. The emergence of various vaso-active substances at about the turn of the century, especially adrenaline, aided elucidation of the functions of the autonomic nervous system. Oliver and Schafer (1894) discovered adrenaline: Oliver had administered an extract of calf adrenal gland (then known as the "supra-renal capsule") by mouth to one of his children and noted a rise in blood pressure and a decrease in the size of the radial artery. Langley (1901) established the sympathetic effects of adrenal gland extract. This finding along with evidence that pilocarpine has effects similar to stimulation of fibres in the cranio-sacral outflow tracts led Langley (1905) to introduce the term "parasympathetic" for effects analogous to cranio-sacral outflow effects and to reserve the term "sympathetic" for thoraco-lumbar outflow effects. At this time noradrenaline had also been identified, in fact Stolz had synthesised it in 1904 and found that it raised arterial blood pressure in animals in a manner similar to that of adrenaline, but was less toxic than the latter. But like so many naturally occurring substances a considerable period of time elapsed before its biological importance was revealed - not till the 1940's did noradrenaline assume full importance as a sympathetic neurotransmitter. At about 1900 adrenaline was regarded

as the sole adrenal medullary hormone. In 1905, Elliott foreshadowed the concept of autonomic nerve transmitter substances when he remarked on the similarity between the effects of adrenaline administration and sympathetic nerve activity in animals and suggested that adrenaline might act as a chemical transmitter of sympathetic nerve impulses as well as being the specific hormone of the adrenal medulla. But it was not until a further 16 years that the first direct evidence for release of a sympathetic transmitter substance was produced by Loewi (1921) who described "acceleranzstoff" released by sympathetic nerve stimulation of the frog's heart. Loewi later considered that this was probably adrenaline. Even before this, however, some doubt had been cast on the premise that this was in fact adrenaline; Barger and Dale (1910) pointed out that adrenaline exaggerated inhibitory compared with motor effects to a greater degree than did the sympathetic transmitter and, therefore, suggested that other members of the catechol group (e.g. amino- and ethylaminobases) may be involved. They also were responsible for introducing the term "sympathomimetic". This was the first intimation that noradrenaline might somehow be involved. Cannon and Rosenblueth (1933) postulated that "Sympathin I" and "Sympathin E" were formed at different end organs by combination of released adrenaline (which was generally accepted as the sympathetic transmitter) with substances in the effector cells. Bacq (1934), however, considered that noradrenaline was the transmitter and suggested that Sympathin I was

adrenaline (i.e. the inhibitory substance) and Sympathin E was noradrenaline (i.e. the excitatory substance). The concept of the sympathins is in disrepute now and serves only to confuse. The term "adrenergic" was introduced by Dale (1934a) to refer to the sympathetic part of the autonomic nervous system. In 1950, von Euler demonstrated the presence of 1-noradrenaline in the sympathetic chain in man, following the demonstration of noradrenaline in splenic nerves of cattle and various organs of other animals. Holton (1949) found both adrenaline and noradrenaline in extracts of three human pheochromocytomata of the adrenal medulla and subsequently their presence and release from normal adrenals in animals and man was confirmed. Both catecholamines are considered to be released by the adrenal medulla, the proportions varying with the type of stress (von Euler, 1956).

It is now clear that adrenergic sympathetic stimulation is more closely matched by noradrenaline than by adrenaline and therefore noradrenaline is regarded by most as the neurotransmitter in man. Recently some direct evidence for noradrenaline being the transmitter has been gained with a fluorescent histochemical method for identifying noradrenaline; Falck and Rorsman (1963) demonstrated the presence of a catecholamine, probably noradrenaline, in sympathetic nerve endings lying between the adventitia and media of vessels of human skin, and Waterson (1967) identified noradrenaline in nerve fibres in the same medio-adventitial region in arteries of human

dental pulp. Frewin and Whelan (1968) produced some indirect evidence supporting the above with the finding that tyramine, administered by intra-arterial infusion to the human forearm, released a substance with the physiological properties of noradrenaline. This latter substance was probably released from sympathetic nerve endings since the above authors have confirmed that the action of tyramine is dependent on the presence of the sympathetic nerves.

Some investigators, however, have challenged the hypothesis that noradrenaline is the neurotransmitter at sympathetic post-ganglionic "adrenergic" nerve endings. Burn and Rand (1965) have reviewed evidence which suggests that there is a cholinergic link in adrenergic transmission. For example, stimulation of the splenic nerves or sympathetic supply to the nictitating membrane of the cat leads to a release of acetylcholine which is considered to cause a secondary release of noradrenaline from sympathetic nerve endings or tissue stores. There is, however, no evidence to support this hypothesis in man: although there are well recognised cholinergic sympathetic dilator fibres to muscle and skin vessels in man vasoconstrictor fibres are considered to be purely adrenergic i.e. release noradrenaline (Whelan and Skinner, 1963).

Understanding the often very complex effects of sympathetic nerve stimulation and of administration of catecholamines has been aided substantially by the concept of the alpha (α) and beta (β)

adrenoreceptors (syn adrenergic receptors or adrenotropic receptors) proposed by Ahlquist (1948). In the blood vessels, the α adreno-receptor responds to adrenaline by inducing a contraction of its smooth muscle cell and therefore vasoconstriction occurs, conversely the β adrenoreceptor causes a relaxation of the vascular smooth muscle cell and hence vasodilatation results. The β receptors of the heart respond to adrenaline by increasing the rate (positive chronotropic effect) and strength (positive inotropic effect) of ventricular contraction. The development of pharmacological adreno-receptor blocking drugs has resulted in a much clearer understanding of the normal pharmacological and physiological effects of noradrenaline and adrenaline. Noradrenaline has predominantly α effects whereas adrenaline has both α and β effects. The synthetic catecholamine isoprenaline is a pure β adrenoreceptor stimulating substance.

von Euler (1965) has reviewed the physiological and pharmacological actions of noradrenaline which is

α - 3 - 4 - dihydroxy - phenyl - β - aminoethanol, molecular weight 169, and differs from adrenaline only by absence of one methyl group, hence its name nor-adrenaline. The 1- isomer is the physiologically active form. Overall it is a vasoconstrictor substance (resistance and capacity vessels) and details of its actions will be discussed in the appropriate sections of this thesis.

SEROTONIN (5-hydroxytryptamine, 5HT)

Serotonin is the name given by Rapport, Green and Page (1948) to a substance which they isolated from beef serum and which they found increased vascular tone. Soon after this Rapport (1949) then established that serotonin was 5-hydroxytryptamine creatinine sulphate, an indolealkylamine with the formula $C_{14}H_{23}N_5O_7S$ and a molecular weight of 405. Hamlin and Fischer (1951) were first to synthesise it and confirmed the structure as proposed by Rapport. The presence of a vasoconstrictor substance in serum was well-known for about forty years before Rapport et al (1948) purified it. Battelli (1905) described "vasoconstrictines des serums sanguins" and Verney and Starling (1922) had learnt that "vasotonins" were removed from defibrinated dog's blood if it was perfused through a heart-lung preparation. Gaddum, Hebb, Silver and Swan (1953) showed that serotonin activity was removed by perfusion of blood through the lungs (which are rich in monoamine oxidase, the principal enzyme responsible for destruction of serotonin).

Serotonin is one of the most ubiquitous substances, occurring widely among various tissues of animals and plants. Erspamer, in two comprehensive reviews (1954 and 1961) has collected data on the occurrence of 5-hydroxytryptamine which he demonstrated was identical with enteramine, the substance he had found in the

enterochromaffin cells (Erspamer and Asero, 1952). The principal natural site of occurrence of serotonin is in tissue containing cells belonging to the enterochromaffin system, cells of which are found in the gastro-intestinal mucosa of all mammals, birds, reptiles and amphibia and some fish. Erspamer regarded the enterochromaffin cells of the gut as a diffuse endocrine system. Dalglish, Toh and Work (1953) confirmed the presence of serotonin in the alimentary canal. Other cells of this enterochromaffin system are found in the posterior salivary glands of the octopus, in the cutaneous glands of amphibia and in the nematocysts (stinging organelles) of some coelenterates.

The venom of wasps, scorpions and hornets contains serotonin which presumably serves a protective function. The tissue mast cells of rats and mice (but not man) contain serotonin as well as histamine.

Blood is the second major site where serotonin is found. Erspamer (1961) gave the serotonin content of human whole blood as 0.06 - 0.4 $\mu\text{g/ml}$, virtually all of which is in the platelets which are believed to obtain their content of serotonin from the enterochromaffin cells during passage through capillaries of the alimentary system. Thrombocytes (platelets) have the capacity to absorb serotonin from the surrounding medium (Humphrey and Toh, 1954) and thus the serotonin is protected from monoamine oxidase to which it would otherwise inevitably be exposed. The spleen is rich in

serotonin derived from disintegrating platelets.

The central nervous system of all vertebrates contains serotonin, Twarog and Page (1953) being first to demonstrate serotonin in brain. The pineal gland of man and the apes is particularly rich in serotonin. Molluscan ganglia and hearts also contain appreciable amounts of serotonin (Welsh, 1954a).

Among plants, serotonin content is high in the stinging nettle, banana (especially the peel) avocado, plum, pineapple and tomato.

The biosynthesis and fate of serotonin have been reviewed by Lewis (1957). The amino acid tryptophan is oxidised by tryptophan oxidase to 5-hydroxytryptophan which is then converted to 5-hydroxytryptamine by 5-hydroxytryptophan decarboxylase. The principal pathway for the destruction of serotonin is by enzymatic inactivation: monoamine oxidase converts 5-hydroxytryptamine to 5-hydroxyindole acetic acid, a process of oxidative deamination. The liver and lung are particularly rich in monoamine oxidase. 5-hydroxyindole acetic acid (5HIAA) is excreted in the urine and its estimation plus measurement of whole blood serotonin give the best estimate of serotonin output (Warner, 1967). Figure 1-1 summarises this principal metabolic pathway of serotonin. In addition to oxidative deamination, some serotonin undergoes rupture of the indole ring,

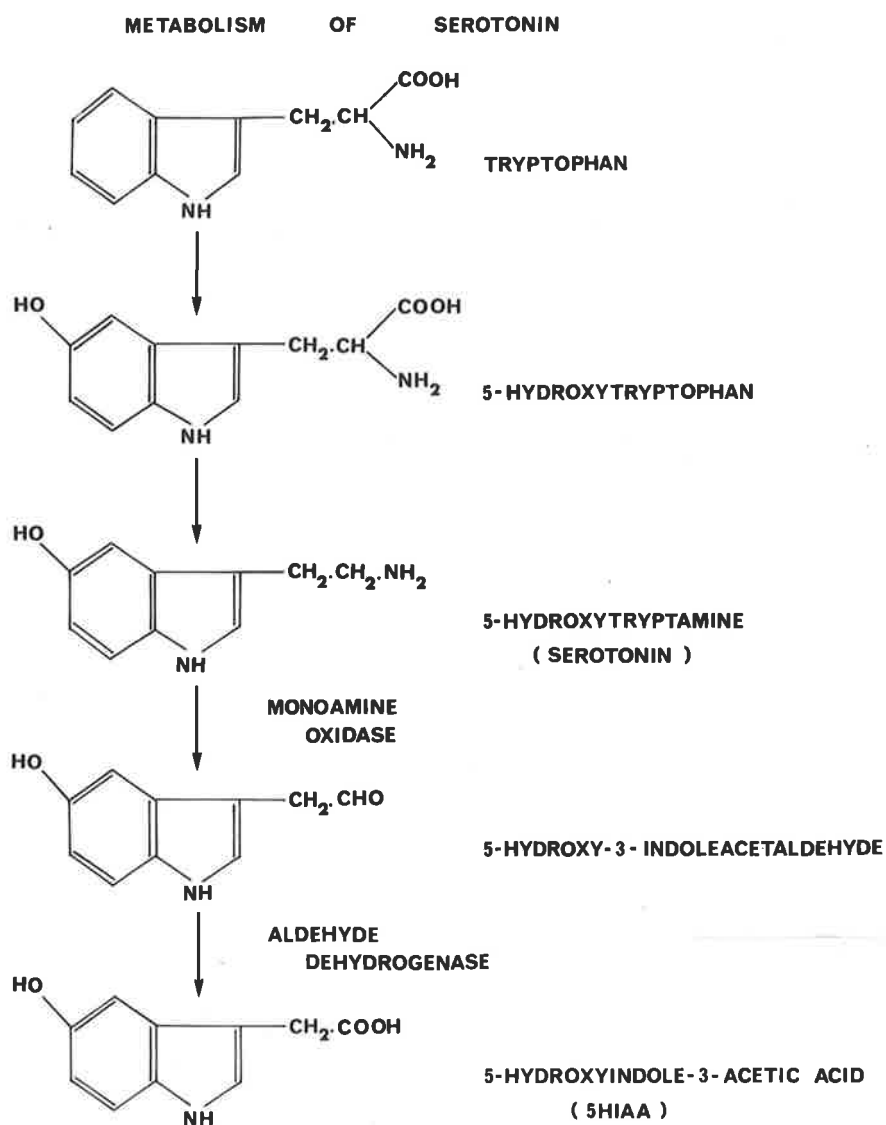


Fig 1-1. Principal metabolic pathway of serotonin.

some is excreted unchanged in the urine, some is oxidised to pigments or may be taken up by platelets (eventually however this platelet serotonin is converted to 5HIAA). Estimation of tissue or blood serotonin is performed most accurately by spectro-fluorometric assay. Using this chemical technique (versus biological) Udenfriend (1962) reported normal values in human blood as 0.1 - 0.3 $\mu\text{g}/\text{G}$.

Passing now to the actions of serotonin, in general it is a smooth muscle stimulant both in vivo and in vitro. The stomach, oestrus uterus and colon of the rat are particularly sensitive, so that serotonin may be assayed biologically on these preparations: the fundal strip of the rat stomach is the most sensitive (Vane, 1957). Serotonin contracts most animal vascular preparations: the perfused central artery of the rabbit ear, perfused hind limb preparations and carotid artery strips of the cat or dog exhibit vasoconstriction during serotonin administration (Ginzel and Kottegoda, 1953).

Reid (1952) was probably first to demonstrate a direct vascular action of serotonin in man when he observed that serotonin injected under the skin caused constriction of a nearby vein. This potent venoconstrictor action has been confirmed plethysmographically by Glover, Greenfield, Kidd and Whelan (1958) who gave intra-arterial serotonin and recorded a reduction in the capacity of the veins of both the hand and forearm, indicating an increased venous

tone. The direct constrictor action of serotonin on the resistance vessels of the forearm and hand was studied by Roddie, Shepherd and Whelan (1955). Serotonin administered by close intra-arterial infusion into the brachial artery led to a substantial fall in hand or forearm blood flow, the forearm vasoconstrictor response, however, was preceded by a transient but marked vasodilatation. Similar findings were reported by Bock, Dengler, Kuhn and Matthes (1957) who, using a Hensel needle, recorded an increase in blood flow through muscle of the arm or calf during intra-arterial infusions. If, however, higher concentrations of serotonin were administered a decrease in muscle blood flow supervened. Skin blood flow, as assessed by heat-flow calorimetry, invariably decreased. Intra-brachial infusion of serotonin to the dog forelimb resulted in a rise in brachial (muscle) venous outflow and a fall in cephalic (skin) venous outflow (Daugherty, Scott, Emerson and Haddy, 1968). Thus there is considerable evidence that serotonin tends to have a vasodilator action on muscle as well as a vasoconstrictor action on the skin, but the precise effects on skin and muscle vessels and the mechanism are obscure. The studies reported in this thesis attempt to elucidate the peripheral vascular effects of serotonin in man.

The effect of serotonin on systemic blood pressure in various animals was found to be particularly erratic and unpredictable by Page and McCubbin (1953), who also administered serotonin

intravenously in normal human subjects and found a variable blood pressure response. Hence they suggested that serotonin was not an overall vasoconstrictor but exhibited spasmogenic or spasmolytic effects on vascular smooth muscle depending on vasomotor tone. They introduced the term "amphibatic" to describe the variable systemic arterial blood pressure response to serotonin. Later, using the perfused dog hind limb and kidney preparations, McCubbin, Kaneko and Page (1962) suggested that adrenergic vasodilator receptors mediate the ability of serotonin to oppose neurogenic vasoconstriction. An increase in systolic and diastolic blood pressures following intravenous injections of serotonin in doses ranging from 0.5 - 5.0 mg was reported by Spies and Stone (1952). Bock et al (1957) found variable responses, but usually an initial decrease followed by an increase above baseline blood pressure resulted from intravenous injection of serotonin. At the same time muscle blood flow increased and skin blood flow decreased. Infusion of serotonin, 1, 2 and 3 mg/min intravenously for 10 min, raised systemic blood pressure in normal subjects (LeMessurier, Schwartz and Whelan, 1959) and was associated with tachycardia (probably due to a direct action of serotonin on the heart), respiratory stimulation and an increase in forearm blood flow. The forearm blood flow increased 3- to 4- fold initially and then stabilised at a level approximately 20-30% above resting level. This vasodilator action was probably direct since it persisted after blockade of the sympathetic nerve supply to the upper limb. Ersamer

(1961) summarised the blood pressure response most appropriately when he concluded that "5HT is neither a pure hypertensive nor a pure hypotensive agent, but, according to the dose, the route of administration, the anaesthetic used, the neurogenic vasoconstrictor tone, the general condition of the cardiovascular apparatus and above all the animal species, 5HT can elicit hypotensive, hypertensive or mixed responses."

The respiratory stimulating action of serotonin is mediated by the peripheral chemoreceptors: Skinner and Whelan (1962) showed that the respiratory stimulating effect of intra-carotid injections of small doses of serotonin in man was abolished by removal of the carotid body. This chemoreceptor stimulating action of serotonin had been demonstrated previously in animals by Douglas and Toh (1953) and Ginzel and Kottegoda (1954).

Several investigators have suggested that serotonin may release other vasoactive substances. Reid and Rand (1952) injected serotonin into the stump of the superior mesenteric artery of the eviscerated cat and obtained a discharge of adrenaline from the adrenal medulla. The possible histamine-releasing property of serotonin was indicated by Feldberg and Smith (1953) when they demonstrated that intra-arterial serotonin (2.5 mg) caused the appearance of histamine (assayed on the guinea pig ileum) in the venous effluent from a perfused skin flap of the dog or perfused skin or muscle of the cat. Whether these pharmacological effects of serotonin have

any physiological significance is obscure but there is some evidence that the high circulating levels of serotonin occurring in carcinoid disease may cause a secondary release of histamine (Pernow and Waldenstrom, 1957).

In 1954, Erspamer and Sala found that subcutaneous injections of serotonin had a potent antidiuretic action in the rat even in doses too small to affect systemic blood pressure. Vasoconstriction of the afferent arteries of the glomeruli with consequent diminution of renal blood flow and glomerular filtration rate was postulated as the mechanism and they suggested that serotonin may be a factor regulating renal haemodynamics and renal function. This antidiuretic effect was confirmed in all mammals (including man) studied, but only in the rat did the renal actions fulfill the main conditions necessary for it to be considered physiological (Erspamer, 1961). Thus a specific physiological renal action of serotonin lacks experimental evidence in man.

The presence of large amounts of serotonin in the alimentary tract led Bulbring and Lin (1958) to suggest that serotonin may be important in the intrinsic motility of the gut. They found that intraluminal serotonin facilitated peristalsis in the guinea pig ileum and rabbit jejunum, an effect which was inhibited by the serotonin antagonists lysergic acid diethylamide (LSD) and bromolysergic acid diethylamide (BOL). In addition, serotonin, a substance formed and stored locally in the intestinal mucosa, was

released in proportion to the increase in intraluminal pressure.

The above investigators postulated that it sensitises mucosal sensory pressure receptors and is thus a hormone with a physiological modulating function in the regulation of intestinal peristalsis.

Intravenous infusions of serotonin were associated with an increased activity in the small intestine but a decrease in activity was recorded in the large intestine in man by Murrell, Wangel and Deller (1966). Studies of the carcinoid syndrome certainly support the pharmacological effect of serotonin in increasing intestinal motility. On the basis of the actions of various blocking drugs on the serotonin-induced contractions of guinea-pig ileum, Gaddum and Picarelli (1957) postulated the existence of two kinds of tryptamine receptors. The first, the M receptors, were blocked by morphine, and also by atropine, cocaine and methadone, and were considered to be in nervous tissue, whereas the second kind, the D receptors, were blocked by dibenzylamine, and also by LSD and dihydro-ergotamine, and were thought to be in muscle.

Although serotonin has no effect on blood coagulation it may be involved in haemostasis by virtue of its vasoconstrictor action. Zucker and Borelli (1955) demonstrated serotonin in platelets and in retrospect Zucker considered that it was probably the "vasoconstrictor substance" which she had described in 1947 as being liberated from platelets aggregated at the site of vessel injury. This "vasoconstrictor substance" caused spasm of adjacent

uninjured vessels, even in denervated preparations and was therefore probably a hormone. Similarly it has been postulated that serotonin release may be responsible for some of the effects of pulmonary embolism, viz. pulmonary vasoconstriction, oedema and bronchoconstriction (Lewis, 1957).

The findings of large amounts of serotonin in the ganglia and hearts of some molluscs along with the observation that some molluscan hearts were very sensitive to serotonin led Welsh (1954b) to postulate that serotonin was a chemical transmitter in this situation. The increase in amplitude and frequency of the molluscan heart beat was so sensitive that it could be used as a biological assay for serotonin. The effect was also inhibited by LSD. This appears to be one of the few clearly defined physiological roles of serotonin, viz. chemical mediator of the excitatory innervation of some molluscan hearts. In mammalia serotonin has a cardiac stimulating action but there is no evidence to suggest it is a transmitter. There is however considerable evidence that serotonin is important in central nervous system functions. Even before serotonin had been isolated, Stoll (1947) had described the hallucinogenic effects of the serotonin antagonist LSD and subsequent investigators promptly suggested that serotonin was a neurotransmitter in the brain. Woolley and Shaw (1954) suggested that serotonin has a role in mental processes and that suppression

of its action results in mental disorder. Their suggestion was based mainly on two simple observations: firstly, that the brain contains serotonin, and secondly, that several drugs antagonistic to serotonin (especially LSD) lead to mental and nervous disturbance. Cerletti and Rothlin (1955) found that BOL, which has pharmacological actions on smooth muscle which are similar to those of LSD, induced no psychic disturbances, and therefore cast doubt on the assertion that inhibition of serotonin led to mental disturbance. Erspamer, in his review of 1961, supported this suggestion that the hallucinogenic effects of LSD were not simply due to serotonin antagonism. He considered that biogenic amines (serotonin, noradrenaline, dopamine) probably play an important part in central nervous system function but the details of their roles were very clouded. This interesting facet of serotonin physiology is still subject to considerable investigation. One area where vascular and nervous actions possibly overlap is in discussion of migrainous headaches. Sicuteri (1959) has implicated serotonin in the prodromal phase of migraine, the phase of vasoconstriction of extracranial and intracranial arteries. Kimball, Friedman and Vallejo (1960) and Lance, Curran and Anthony (1965) have suggested that serotonin depletion is an important factor in the prodromal phase of migraine. Support for this hypothesis was obtained by Hintenberger, Anthony and Vagholkar (1968) who demonstrated a fall in serotonin content of circulating platelets during

a migrainous attack. Wolff (1963) however, commented that there was no good evidence for involvement of serotonin and his group has implicated a polypeptide (neurokinin) as the cause of the vasodilatation, local oedema and pain of the fully-developed phase of migrainous headache.

Pickles (1956) suggested that serotonin caused a selective alteration in cell membrane permeability of the red blood cell and later confirmed this on frog skin. Serotonin decreased the potential difference across the cell membranes and therefore passive movement of ions increased. This membrane effect may explain the potentiation by serotonin of the vasoconstrictor actions of nor-adrenaline and serotonin on the central artery of the rabbit ear (de la Lande, Cannell and Waterson, 1966) and possibly serotonin's stimulating action on the superior cervical ganglion of the cat (Trendelenberg, 1956).

In addition to its vasoconstrictor actions serotonin increases capillary permeability, and also causes cutaneous pain if applied to a blister base. Subcutaneous or intradermal injections of serotonin produced oedema in rats (Rowley and Benditt, 1956; Parratt and West, 1957) and oedema of the hand occurred during intra-arterial administration of 16 $\mu\text{g}/\text{min}$ dose of serotonin in man (Roddie et al, 1955).

The possibility of a role for serotonin in antigen-antibody reactions has been mentioned by Page (1958). Serotonin also bestows some protection against X - radiation to rats and mice.

Clinically one major abnormality of serotonin physiology, the carcinoid syndrome, has provided considerable insight into the normal physiology of serotonin. In 1952, Biorck, Axen and Thorson described the association of a carcinoid tumour with tricuspid valve insufficiency and peculiar cyanosis in a 19 year old Swedish boy. Lembeck (1953) found overproduction of serotonin in carcinoid disease and so also did Pernow and Waldenstrom (1954). They reported elevated blood and urinary serotonin levels and considered that serotonin, released especially from liver metastases, accounted for the carcinoid syndrome, viz. association of a tumour of enterochromaffin tissue with facial flushing, diarrhoea and valvular lesions in the right side of the heart. Udenfriend (1962) reported elevated blood serotonin levels ranging from 0.5 - 3.0 $\mu\text{g/G}$ in patients with the carcinoid syndrome. Case reports of variants of the syndrome rapidly abounded in the literature but it was soon apparent that intravenous serotonin did not reproduce all the features including the flushing (Page, Corcoran, Udenfriend, Sjoerdsma and Weissbach, 1955). The flushing attacks could be induced by small intravenous doses of adrenaline or noradrenaline (Peart, Robertson and Andrews, 1959), and sometimes by alcohol

(Snow, Lennard-Jones, Curzon and Stacey, 1955). Plasma serotonin levels sometimes rose during adrenaline-provoked flushing episodes in 13 patients studied by Robertson, Peart and Andrews (1962) but they suggested that substances other than serotonin may be released from the tumour to produce the flush. This was in fact the case: Oates, Melmon, Sjoerdsma, Gillespie and Mason (1964) demonstrated that a kinin peptide was released into the hepatic venous blood from liver metastases during the flushes in 9 patients with the carcinoid syndrome. Furthermore, the enzyme kallikrein which catalyses the formation of kinins was found to be present in metastatic carcinoid tumour tissue. Injection of the nonapeptide bradykinin produced flushing episodes which resembled the spontaneous flushes in carcinoid patients. Oates, Pettinger and Doctor (1966) showed that the kinin produced by the patients was identical to the synthetic nonapeptide (bradykinin) which has the structure:

ARG - PRO - PRO - GLY - PHE - SER - PRO - PHE - ARG

and is released from α -2 globulin precursors (kininogens) by the action of the enzymes kallikrein or trypsin (Rocha e Silva, Beraldo and Rosenfeld, 1949). The kinins are powerful vasodilator substances and thus explain the facial flushing and also the hypotension and tachycardia which are sometimes a feature of the carcinoid syndrome. Thus both serotonin and bradykinin are involved in the carcinoid syndrome.

Increased output of serotonin is probably involved in several other clinical conditions; Warner (1967) described increased circulating levels of serotonin in intestinal malabsorption states (including the "dumping syndrome"), symptomatic hyperserotoninaemia, and in association with intravascular thrombosis and infarction. A role for serotonin in pre-eclamptic toxemia is also remotely possible.

Thus, in summary, serotonin is an ubiquitous substance which may be a factor influencing normal peristalsis, arteriolar tone, renal function, mental processes and haemostasis, but its normal physiological action in man is ill-defined. Section 2 (Parts A and B) of this thesis concentrates on the peripheral circulatory effects of serotonin and includes some observations on the carcinoid syndrome.

THE RENIN-ANGIOTENSIN SYSTEM

In 1836, Bright observed that there was a direct correlation between the severity of renal disease and the extent of hypertensive cardiac disease and on the basis of this he suggested that a humoral factor may be released from diseased kidneys and be involved in the pathogenesis of hypertension. Brown-Sequard (1893) proposed that the kidney produced an internal secretion which was of importance to the whole body, and in 1898 Tigerstedt and Bergman produced the first direct experimental evidence to support the former two investigators when they injected a saline extract of the cortex of fresh rabbit kidney into normal rabbits and observed a rise in systemic blood pressure. They named this pressor substance renin and demonstrated that its pressor action was still present after cardiac denervation, cervical cord transection or even destruction of the spinal cord. Tachyphylaxis consequent upon repeated injections was also demonstrated.

These findings laid the foundation of a hypothesis to explain the association of renal disease and hypertension through release of a renal pressor substance, but not till 1934 did Goldblatt, Lynch, Hanzal and Summerville develop a reliable experimental model in animals when they reported that sustained hypertension could be produced in dogs by partially obstructing the renal artery of one or both kidneys. This was subsequently confirmed by Goldblatt's

group and by many other investigators in a variety of animals.

From his experiments Goldblatt (1937) concluded that unilateral renal ischaemia produced hypertension through a humoral mechanism rather than by a neurogenic mechanism. In support of this

Fasciolo, Houssay and Taquini (1938) demonstrated increased vasoconstrictor activity in the renal venous blood of "ischaemic"

kidneys, Page and Helmer (1940) suggested that renin (from the juxta-glomerular cells) was the most probable humoral agent

involved in the pathogenesis of experimental renal hypertension

The observation that renin itself had no vasoconstrictor activity and required blood colloids for its activation led them and also an

independent South American group (Braun-Menendez, Fasciolo, Leloir and Munoz, 1939; 1940) to the realisation that renin was an enzyme

which formed the active vasoactive substance angiotonin (Page and

co-workers) or hypertensin (Braun-Menendez and co-workers) from a

plasma globulin fraction. The names angiotonin and hypertensin

were combined eventually and so this vasoactive substance is now

known as angiotensin (Braun-Menendez and Page, 1958).

Angiotensin is a polypeptide, existing in two forms: the decapeptide (Angiotensin I) and the octapeptide (Angiotensin II).

The latter is the physiologically active form. A converting

enzyme in the circulation converts angiotensin I to angiotensin II.

The amino acid sequence of angiotensin I was worked out in 1955 by

Skeggs, Marsh, Kahn and Shumway and the same group determined the amino acid sequence of angiotensin II in 1956 (Skeggs, Lentz, Kahn, Shumway and Woods).

Angiotensin II is:

H - ASP - ARG - VAL - TYR - ILEU - HIS - PRO - PHE - OH

and has a molecular weight of 877.

Page and Bumpus (1962) summarised structural aspects relating to the octapeptide and postulated that it requires the specific three dimensional configuration of an alpha helix to be functional. They stressed that it is the most powerful pressor substance known and contracts the smooth muscle of isolated blood vessels, uterine and intestinal tissue. Shortly after determination of amino acid composition and sequence the synthesis of angiotensin II was achieved by Bumpus, Schwartz and Page (1957) and by Rittel, Iselin, Kappeler, Rinniker and Schwyzer (1957).

The methods of purification and estimation of renin and angiotensin have been reviewed by Page and McCubbin (1968) and by Peart (1965). One of the most sensitive assay methods for plasma renin was described by Skinner, McCubbin and Page (1964) who incubated aliquots of blood containing renin with plasma substrate and assayed the angiotensin generated by matching the blood pressure responses of the anaesthetised, ganglion-blocked (with pentolinium) rat with known doses of angiotensin. Using this assay technique they

demonstrated that renal venous renin content was inversely proportional to mean renal perfusion pressure and, therefore, postulated that a "renal baroreceptor" mechanism controls renin output rather than ischaemia per se. However, Hodge, Lowe and Vane (1966) produced evidence that the renal sympathetic nerves are involved in regulation of renin release by the kidney when they showed that blocking the renal nerves abolished the changes in circulating angiotensin which normally occurred during alterations in blood volume of the dog. Angiotensin was estimated directly by a continuous blood-bathed organ assay technique - the rat colon being particularly sensitive to angiotensin. Using this same experimental model, Hodge, Ng and Vane (1967) investigated the destruction of angiotensin and refuted many of the earlier in vitro and in vivo animal studies which suggested that blood angiotensinases terminated the action of angiotensin when they demonstrated that removal by peripheral tissues (with the exception of the lungs) was the principal factor, so that angiotensin had a half-life of approximately one circulation time. Angiotensin incubated in arterial blood survived for an equivalent of 10-20 circulation times.

The availability of the pure synthetic substance which was biologically identical to the naturally occurring substance facilitated determination of its pharmacology - in essence a continuation of the earlier work with renin begun by Tigerstedt and Bergman (1898).

Abell and Page (1942, 1946) demonstrated that intravenous angiotensin (= angiotensin II) and renin constricted arterioles of the rabbit ear and elevated blood pressure. Hill and Andrus (1940) and Lorber (1942) found that angiotensin reduced coronary artery blood flow but increased the force of contraction without change in heart rate in the isolated perfused cat heart. Battro, Braun-Menendez, Lanari and Leloir (1940) and Battro, Gongalez-Segura and Lanari (1941) were among the earliest to investigate cardiovascular actions of angiotensin in man, demonstrating a pressor action (both systolic and diastolic pressures increased), a reduction in renal blood flow and a compensatory bradycardia mediated by the vagus. Similarly, Corcoran, Kohlstaedt and Page (1941) demonstrated a rise in blood pressure and a fall in renal blood flow. They attributed the latter effect to a constrictor action on the efferent arterioles of the glomeruli since they demonstrated an increase in filtration fraction i.e. evidence of increased intraglomerular pressure. They postulated a role for angiotensin in hypertension.

A more detailed study by Wilkins and Duncan (1941) showed an increase in mean arterial pressure and central venous pressure, and a decrease in limb blood flow, skin temperature, heart rate and cardiac output during intravenous angiotensin administration in normal man. The bradycardia was abolished by atropine and converted to a tachycardia. Similar changes in blood pressure and cardiac output were reported by Bradley and Parker (1941), Taylor and Page

(1943) and Middleton and Wiggers (1944). Thus there was general agreement that angiotensin was pressor in man and in fact was the most powerful pressor agent so far described. Gregory, Levine and Lindley (1944) showed that its pressor effect was not modified by spinal anaesthesia in man or animals, and dibenamine pretreatment in dogs did not modify its pressor action (Youmans and Rankin, 1947). Therefore, since it caused no increase in cardiac output, but usually a slight fall due to bradycardia, angiotensin's pressor action was considered to be a consequence of increased total peripheral resistance by a direct vasoconstrictor action on specific non-adrenergic receptors. The direct vasoconstrictor action observed when angiotensin was administered into the supplying artery of the forearm, calf or hand was described by Bock, Krecke, and Kuhn (1958), de Bono, Lee, Mottram, Pickering, Brown, Keen, Peart and Sanderson (1963), Daly and Duff (1960) and Wilkins and Duncan (1941). Although angiotensin is a potent constrictor of resistance vessels it is a rather weak constrictor of capacitance vessels (Folkow, Johansson and Mellander, 1961; de Pasquale and Burch, 1963).

There have been many studies which have attempted to relate the renin-angiotensin system to systemic hypertension. Prolonged infusions of angiotensin have been administered to various animals in an attempt to reproduce hypertension. Results suggest that with prolonged infusions the initial pressure rise is

due to a direct action of angiotensin on the blood vessels but that it is subsequently replaced by an indirect sympathetic mechanism which maintains the elevation of the systemic arterial blood pressure (Blacket, Depoorter, Pickering, Sellers and Wilson, 1950; Brown, Chapuis and Robertson, 1964; Dickinson and Lawrence, 1963; Hill and Pickering, 1939; and Yu and Dickinson, 1965). McCubbin, DeMoura, Page and Olmsted (1955) supported this when they found that a hypertensive effect of angiotensin, in doses which were initially subpressor, appeared after several days of continuous infusion in the dog. In renal hypertension in particular it seems that the initial mechanism is a humoral one (renin-angiotensin) and is unmodified by various forms of sympathectomy whereas in the chronic state a major part of the elevation in pressure appears to be through a neurogenic mechanism (McCubbin and Page, 1963; Pickering, 1945; Reed, Sapirstein, Southard and Ogden, 1944; Taggart and Drury, 1940; and Taquini, Blaquier and Bohr, 1961). Kaplan and Silah (1964) have shown that the blood pressure response to intravenous angiotensin is less marked in patients with renovascular hypertension than in those with hypertension of non-renal origin, and on this basis have proposed an angiotensin infusion test. The decreased reactivity in renovascular hypertension is attributed to elevated levels of angiotensin already circulating leading to a reduction in response to exogenous angiotensin (i.e. the phenomenon of tachyphylaxis). Similarly, Scroop and Whelan

(1968a) found a decreased sensitivity of the hand vessels to intra-arterial angiotensin in three patients suffering from renovascular hypertension (corroborated by elevated plasma renin levels) whereas the responses in 19 patients with essential (primary) hypertension and normal renin levels were within the range of normotensive subjects. Thus there is considerable evidence to support a role for the renin-angiotensin system in renovascular hypertension but little evidence to implicate it in other forms of hypertension.

Although most of the early work was concerned with purification, chemistry and with trying to correlate the cardiovascular actions of the renin-angiotensin system with the haemodynamic changes of essential and renal hypertension a second important role of the renin-angiotensin system became apparent, viz. its association with the secretion of the mineralocorticoid aldosterone from the adrenal cortex. Dunihue (1946) suggested that a close relationship existed between the juxta-glomerular apparatus of the kidney and the adrenal cortex when he observed increased granularity (i.e. renin content) of juxta-glomerular cells in the rabbit, dog and monkey following adrenalectomy. The body of evidence supporting association between the juxta-glomerular apparatus and the adrenal cortex grew and, in 1958, Gross suggested that the renin-angiotensin system controlled aldosterone secretion from the adrenal cortex and, therefore, was intimately connected with sodium balance and control of circulating blood volume.

This was confirmed in many animals and also in man by Laragh, Angers, Kelly and Lieberman (1960).

Although the early studies of angiotensin suggested it was mainly a directly-acting vasoconstrictor substance it soon became apparent that several indirect mechanisms may be involved, such as release of adrenal medullary hormones, central sympathetic stimulating action, peripheral sympathetic stimulation and interaction with other vasoactive substances. Williams and Grossman (1938) were first to suggest release of adrenal medullary hormones when they observed a biphasic pressor response to hog renin in rats: an initial adrenaline-like rise in blood pressure and a secondary renin-like prolonged pressor effect. Braun-Menendez et al (1940) showed that angiotensin released adrenaline from both the normal and denervated adrenal gland. Kaneko, McCubbin and Page (1961) demonstrated adrenal medullary discharge by angiotensin in the dog following sensitisation with a ganglion stimulating agent (DMPP). Angiotensin (1-2 ng threshold) and bradykinin (0.05-1.0 μ g threshold) were found to be potent releasers of adrenal catecholamines when injected into the central stump of the coeliac artery of an eviscerated cat (Feldberg and Lewis, 1964). These investigators also reported evidence that catecholamine release occurred with intravenous angiotensin (0.1 μ g) in the same preparation. Intravenous infusions or injections of angiotensin in the dog increased the level of circulating catechol-

amines (both noradrenaline and adrenaline) in blood taken from the inferior vena cava above the outflow from the adrenal veins at the peak of the pressor response (Peach, Cline and Watts, 1966). Catecholamines were assayed by fluorimetric analysis. White and Ross (1966) using an electromagnetic flow probe, measured blood flow changes in the femoral artery of the cat during intravenous angiotensin administration and found indirect evidence for adrenaline release since the muscle vasodilatation occurring during angiotensin infusion was abolished by adrenal blood vessel ligation. Staszewska-Barczak and Vane (1967) doubt the physiological importance of catecholamine release, however, since they found release in both cats and dogs with only very high intra-arterial or intravenous doses and even then the secretion was transitory. Similarly Vincent, Kashemsant, Cuddy, Fried, Smulyan and Eich (1965) found no change in urinary vanillylmandelic acid levels during pressor infusions of angiotensin in man.

Using synthetic angiotensin II, Bickerton and Buckley (1961) described a central sympathetic stimulating action in cross-perfused dogs. Angiotensin reaching only the cerebral circulation of one of the dogs caused a nervously mediated rise in systemic arterial blood pressure in the same dog. Laverty (1963) observed a nervously mediated vasoconstriction in the hind limb of the anaesthetised rat with angiotensin and concluded that it was due to central sympathetic stimulation. However, Laurence and Nagel

(1963) were unable to modify the pressor response to angiotensin in man with either bretylium or guanethidine.

Evidence for a peripheral sympathetic stimulating action was obtained by Benelli, Della Bella and Gandini (1964) who found potentiation of contractions of the guinea pig vas deferens during electrical stimulation of the hypogastric nerve and decrease of the pressor response in cats by tetramethylammonium during angiotensin administration. Liebau, Distler and Wolff (1966) described a tyramine-like (i.e. release of noradrenaline from sympathetic nerve endings) action of angiotensin and Zimmerman (1962) and Zimmerman and Gomez (1965) suggested that angiotensin enhances normal sympathetic nerve transmission by either facilitating release or inhibiting reuptake of the transmitter. Lewis and Reit (1965) proposed a ganglionic site of action, and Nishith, Davis and Youmans (1962) and Krasney, Paudler, Smith, Davis and Youmans (1965) observed that positive inotropic and chronotropic effects of angiotensin on the heart were mediated via the sympathetic nervous system. The direct action of angiotensin on the isolated heart of the cat, dog or frog has not revealed a positive chronotropic effect (Hill and Andrus, 1940; Lorber, 1942; and Koch-Weser, 1964). McGiff and Fasy (1964) abolished the renal vasoconstrictor action of intravenous angiotensin by guanethidine sympathetic blockade or renal denervation.

The first evidence for a possible interaction with other vasoactive substances was reported by Verney and Vogt (1938) who found an increase in the pressor response to intravenous tyramine in renal hypertensive dogs. Thus another mechanism which may be involved in the vasoconstrictor action of angiotensin is its interaction with and potentiation of the vasoconstrictor effects of other circulating substances (especially catecholamines). Braun-Menendez et al (1939 ; 1940) and Braun-Menendez, Fasciolo, Leloir, Munoz and Taquini (1946) reported a similar potentiation of the pressor action of angiotensin by tyramine and also by another sympathomimetic drug, ephedrine, in the dog. McCubbin and Page (1963) observed potentiation of endogenously released noradrenaline (released by tyramine) when in combination with angiotensin in the dog. However the responses to exogenous noradrenaline were unchanged. Similarly, Sakurai and Hashimoto (1965) demonstrated an enhanced vasoconstrictor response of the vessels of the perfused rabbit ear to noradrenaline and tyramine when administered in combination with subthreshold amounts of angiotensin. The pressor response to intravenous tyramine was also enhanced by subpressor amounts of angiotensin. Marked potentiation by serotonin of the constrictor response to noradrenaline and angiotensin in the isolated perfused central artery of the rabbit ear was observed by de la Lande, Cannell and Waterson (1966).

Thus there is considerable evidence for involvement of several indirect mechanisms in the overall vasoconstrictor action of angiotensin. Adrenal catecholamine release, central and peripheral sympathetic stimulation and interaction with other vasoactive substances may reinforce the more obvious direct vasoconstrictor effects of angiotensin. However the contribution of these indirect mechanisms to its total pressor effect is still subject to conjecture. Species differences in responses to angiotensin probably confuse the picture further. The studies outlined in this thesis (Section 2, Parts C and D) attempt to clarify the direct actions of angiotensin in man and also examine the relationship of angiotensin to the autonomic nervous system by comparing its actions with those of noradrenaline. The interactions between angiotensin, noradrenaline and serotonin on the peripheral vessels have also been examined.

OTHER VASOACTIVE SUBSTANCES - ACETYLCHOLINE, HISTAMINE AND BRADYKININ

Only a brief mention of these three vasodilator substances is being made in this general introduction. Their possible roles in the phenomenon of post-exercise hyperaemia will be examined more closely in Section 3.

The monoamine histamine, established as a normal constituent of blood by Barsoum and Gaddum (1935), has a depressor action on the systemic blood pressure of animals and man when administered intravenously (reviewed by Whelan, 1967). In man its administration systemically is associated with flushing, tachycardia and increased cardiac output. Infusion into the brachial artery causes a marked vasodilatation in the forearm and hand (Duff and Whelan, 1954). Histamine has been postulated to play a part in adrenaline vasodilatation, reactive and post-exercise hyperaemia and cold vasodilatation, but its role in physiological responses is still unsubstantiated. It may, however, be important in anaphylactic reactions and wound healing.

Acetylcholine was found to be liberated from parasympathetic nerves by Loewi in 1921 and is now generally accepted as the neurotransmitter released from parasympathetic nerve endings and from "cholinergic" sympathetic nerve endings to skeletal muscle (Blair, Glover, Greenfield and Roddie, 1959; Barcroft, Brod, Hejl,

Hersjarvi and Kitchin, 1960) during emotional stress, and from "cholinergic" sympathetic nerve endings supplying forearm skin (but not hand skin) during the reflex dilatation of body heating (Roddie, Shepherd and Whelan, 1957a). Ellis and Weiss (1932) observed tachycardia, slight hypotension, cutaneous flushing and salivation during intravenous administration of acetylcholine to man. Injection into the femoral artery induced vasodilatation and sweating. The vasodilator action is short-lived since acetylcholine is rapidly destroyed by circulating cholinesterase enzyme (Duff, Greenfield, Shepherd and Thompson, 1953). Acetylcholine's principal role in the control of the peripheral circulation is its role as a transmitter substance at cholinergic nerve endings but its possible role in the phenomenon of post-exercise hyperaemia of skeletal muscle is examined in Section 3.

The nonapeptide bradykinin, the most powerful vasodilator substance yet found in man, has been discussed briefly in relation to the carcinoid syndrome. The possible involvement of bradykinin in functional vasodilatation of exocrine glands, reactive and post-exercise hyperaemia and response to tissue injury has been summarised by Whelan (1967).

SUMMARY

1. Historical aspects of the autonomic nervous system, catecholamines, serotonin and angiotensin have been reviewed.
2. Noradrenaline, which is probably the sympathetic post-ganglionic transmitter at adrenergic nerve endings is a vasoconstrictor substance whose action on peripheral vessels is due mainly to stimulation of the α -adrenoreceptors.
3. Serotonin (5-hydroxytryptamine) is the hormone of the enterochromaffin system. Though widely distributed in animals and plant tissues its physiological importance is largely unknown. It may be a factor influencing peripheral vascular tone, peristalsis, central nervous system function, renal function and haemostasis.
4. Angiotensin, an octapeptide and the most powerful pressor substance known, is generated in plasma by an enzyme (renin) released from an "ischaemic" kidney. Angiotensin increases total peripheral resistance by both direct and sympathetically-mediated vasoconstrictor actions and is probably an important agent involved in the pathogenesis of renal hypertension. In addition it is the trophic hormone for aldosterone release and

46.

therefore influences sodium and water metabolism.

5. The peripheral circulatory effects and inter-relationships of these vasoactive substances are the principal investigations of this thesis.

47.

GENERAL METHODS.

VENOUS OCCLUSION PLETHYSMOGRAPHY

Measurement of blood flow in limb segments.

In most investigations performed for this thesis assessment of blood flow in a limb segment, hand or forearm, was made by the technique of venous occlusion plethysmography (plethysmos= increase, graphein= to write). The principle is a simple one in that a pneumatic congesting cuff, encircling the limb proximal to the segment being studied, is inflated to a pressure below diastolic blood pressure, usually 60-70 mm Hg, so that arterial blood flows into the segment and is pooled in the capacity vessels. The rate of increase in the volume of the part tends to be linear until the capacity vessels are distended and then some blood escapes back under the cuff. Greenfield and Patterson (1954) described three phases occurring during venous occlusion: phase 1, unimpeded arterial inflow where volume increase of the limb segment is equal to arterial inflow; phase 2, capacity vessels have filled sufficiently so that back pressure progressively impedes arterial inflow; and phase 3, in addition to the above, the pressure in the capacity vessels has risen sufficiently for blood to leak back under the congesting cuff. The latter investigators calculated that arterial inflow would be unaltered by venous back pressure until the increase in resting volume exceeded 2% - and this usually takes much longer than the 10-15 sec collecting time used in the present studies.

The increase in volume of the segment against time is recorded on a kymograph drum or electronic recorder and the arterial inflow in ml/100 ml/min can be indirectly deduced from the slope of the record. The validity of this method has been confirmed by Landowne and Katz (1942), Formel and Doyle (1957) and Greenfield (1960).

When forearm blood flow was measured plethysmographically a second pneumatic cuff was applied to the wrist immediately distal to the plethysmograph and inflated to above systolic blood pressure, usually to 200 mm Hg, to exclude the hand circulation, since Grant and Pearson (1938) had found that such an arterial occlusion cuff considerably reduced forearm blood flow in a warm subject. The inflation of the proximal venous occluding cuff (in the absence of the distal cuff) results in the accumulation of venous blood not only from the forearm but also from the hand, and therefore the values of forearm flow are a rather meaningless resultant of forearm and hand flow. To reduce artefacts the arterial occlusion cuff was applied for 60-90 sec before flow measurements were commenced as suggested by Kerslake (1949). Discomfort caused by the pressure in the wrist cuff and ischaemia limited each phase of an experiment to 20-25 min in the case of the forearm.

Inflation and deflation of the pneumatic cuffs was achieved by the apparatus shown in Fig 1-2. The high pressure arterial occlusion cuffs were inflated to 200 mm Hg by air obtained from a compressed air cylinder through a two stage reducing valve.

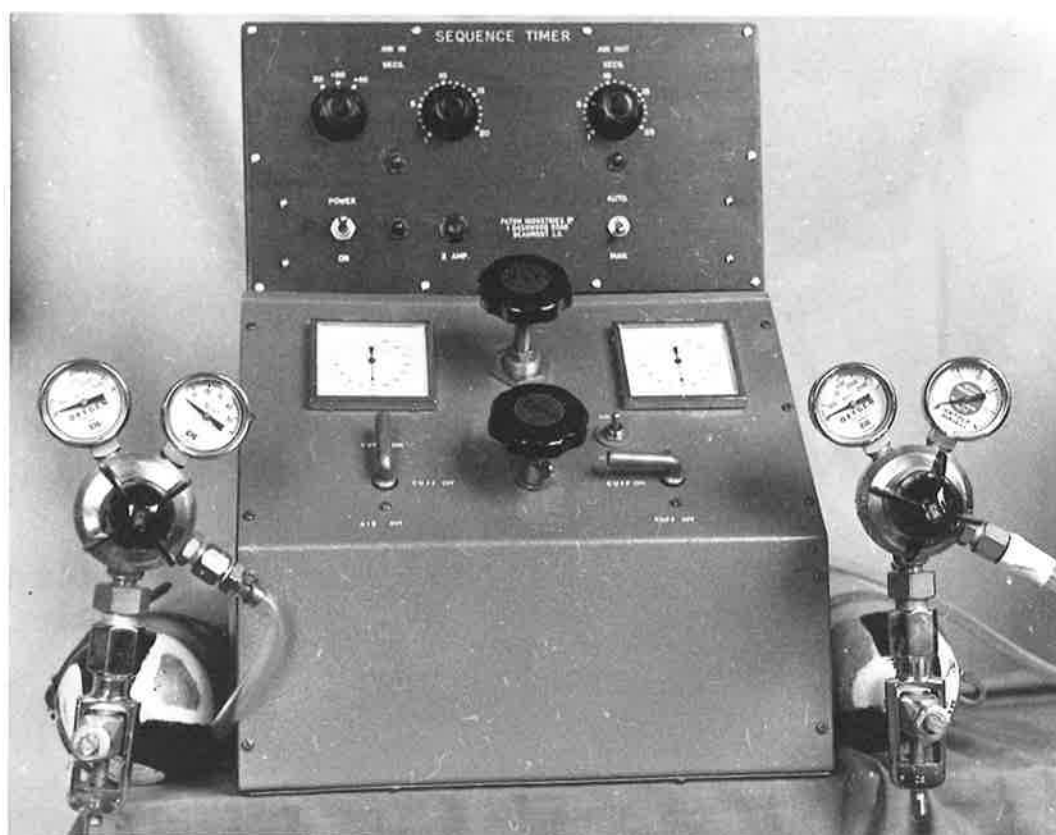


Fig 1-2. Apparatus used to inflate and deflate pneumatic cuffs during venous occlusion plethysmography.

A two-way gas stop-cock permitted the cuffs to be inflated and deflated. The venous occlusion cuffs were inflated and deflated 3-4 times each minute from a reservoir of air maintained at constant pressure (usually 60-70 mm Hg) by a compressed air cylinder through two reducing valves. A sequence timer (Paton Industries, Adelaide) operated solenoid inlet and outlet valves and could be set to function automatically with inflation periods ranging from 1 to 60 sec and deflation periods from 1 to 20 sec or manually so that inflation and deflation could be effected at will. For normal operation an "air in" period of 9-12 sec and an "air out" phase of 4-5 sec on automatic position were used. The apparatus is schematically represented in Fig 1-3. Care was taken to position the limb segment above heart level during flow measurements so that adequate venous drainage during the "air out" phase ensured that the capacity vessels were relatively empty for the subsequent flow estimation.

Conversion of the volume increment versus time recordings to blood flow in ml/100ml/min was achieved using the sliding caliper apparatus shown in Fig 1-4. The apparatus was placed on the record so that one of the horizontal lines overlay or was parallel to the base line (time trace). One of the parallel lines on the moveable perspex arm was then placed so that it overlay the recorded blood flow measurement and the height in cm read at the point of intersection of the lower border of the perspex arm and the inner border of the vertical centimetre rule. The distance

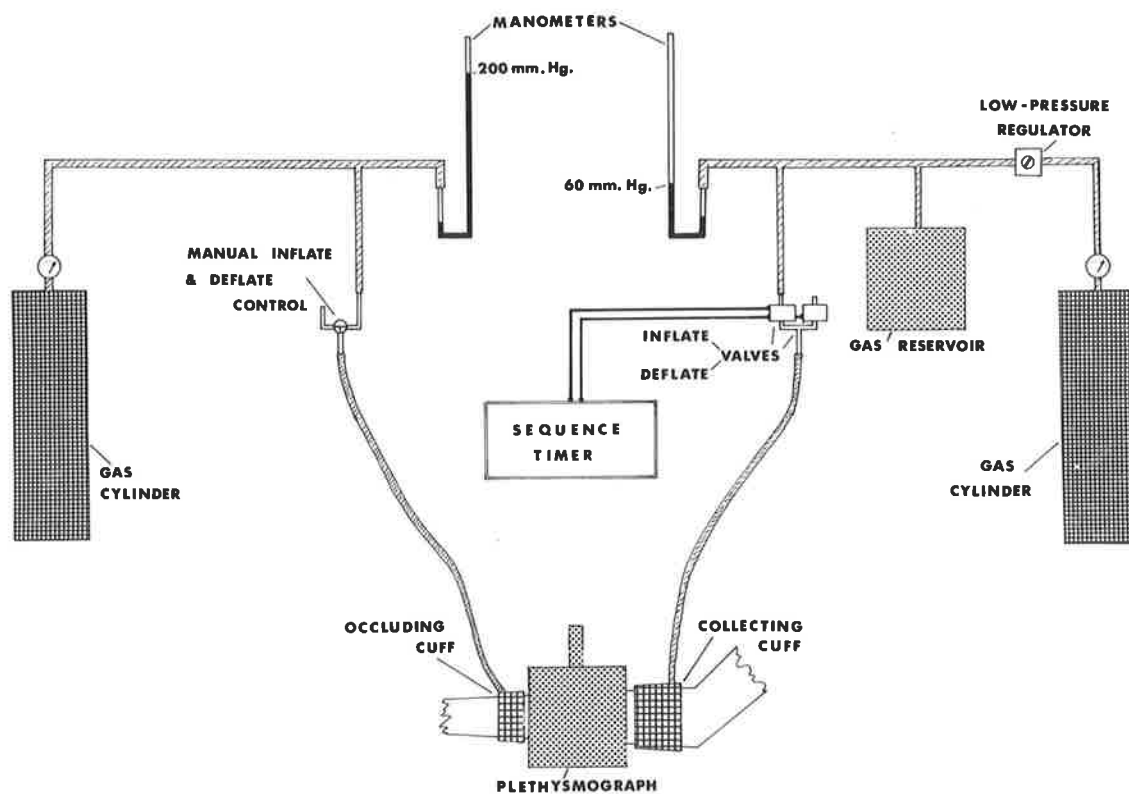


Fig 1-3. Schematic diagram of sequence timer and controls for pneumatic cuff inflation used during venous occlusion plethysmography.

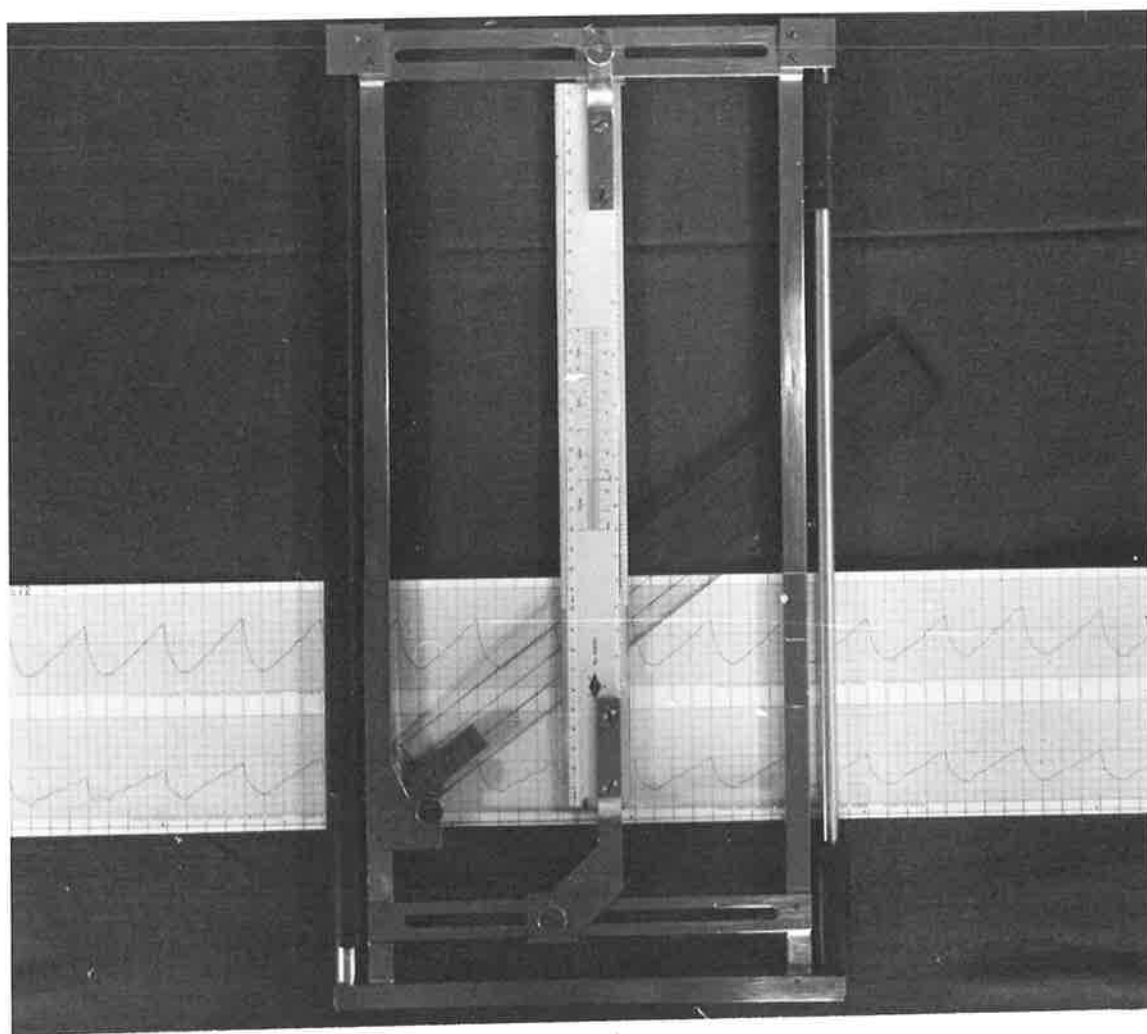


Fig 1-4. The sliding caliper used to quantitate the recorded blood flow measurements. The tracing was obtained from capacitance plethysmographs.

between the pivot of the perspex arm and the inner border of the centimetre rule (which is moveable) is variable (= time base: T_x) and must be calculated for each experiment. Each cm on the vertical scale is equivalent to 1 ml/100ml/min.

Verification and formula for calculating the time base (T_x): the blood flow (F) in ml/100ml/min is given by the equation

$$F = \frac{T_{60}}{T_x} \cdot \frac{H}{h} \cdot \frac{1}{V} \dots\dots\dots(1)$$

where T_{60} = distance in cm travelled by recording trace in 60 sec.

T_x = distance in cm travelled by recording trace in x sec

H = vertical movement of recorder writing point in cm
while recorder travels T_x cm

h = vertical movement of recorder writing point in cm
for a volume increment of 1.0 ml

$V = \frac{\text{Volume of limb segment}}{100}$

As T_{60} , h and V are constant in any one experiment a value of T_x can be chosen so that

$$T_x = \frac{T_{60}}{hV} \dots\dots\dots (2)$$

Substituting for T_x in equation (1)

$$F = \frac{T_{60} \cdot h \cdot V}{T_{60}} \cdot \frac{H}{h} \cdot \frac{1}{V}$$

therefore $F = H$

T_x in any experiment can, therefore, be calculated from equation (2) and when the pivot of the moveable arm and inner border of the rule are set this distance apart the blood flow can be assessed in ml/100ml/min.

The water-filled plethysmograph

Probably the first investigator to use the water-filled plethysmograph (= volume displacement plethysmograph) was Francis Glisson, in 1677. He measured volume changes during muscular contractions with the whole arm sealed in a water-filled glass cylinder with a narrow outlet at the distal end. By application of intermittent venous occlusion, as suggested by Brodie and Russell (1905), the rate of blood flow could be determined quantitatively. This modification was adapted for assessment of blood flow in the human arm by Hewlett and van Zwaluwenberg (1909) who measured total forearm flow with an air-filled glass plethysmograph and stated, "By means of this method it is possible to demonstrate in man the

tremendous acceleration of the blood stream following exercise, as well as that which occurs in overheating by the general hot tub." A plethysmograph of truncated cone shape which enclosed the wrist, forearm and elbow was used by Lewis and Grant (1925) to assess volume changes and blood flow during observations on reactive hyperaemia. The use of a rubber sleeve or glove to seal the plethysmograph (Krogh, Landis and Turner, 1932), the application of a high pressure wrist cuff during measurements of forearm blood flow (Grant and Pearson, 1938) and control of water temperature to 32°C and 34°C in the hand and forearm plethysmographs respectively (Barcroft and Edholm, 1946) added considerable refinement to the technique and permitted accurate measurement of flow. The method used in the present studies and the calculations outlined previously are basically the same as those described by Barcroft and Swan (1953). The plethysmograph used (Fig 1-5) is similar to the type described by Greenfield (1954): the hand or forearm, separated from the water by a glove or sleeve, is admitted to the inner of two separate chambers, and the temperature adjustments effected by adding water of appropriate temperature to the outer chamber. A 12-volt battery drives a small motor which stirs the water in both chambers to prevent temperature layering effects. Volume changes in the inner chamber are recorded via wide bore polythene tubing by means of a float recorder on a smoked kymograph drum (diagrammatically represented in Fig 1-6). This type of plethysmograph is also



Fig 1-5. The water-filled, temperature-controlled plethysmograph, and the rubber glove and sealing plate used for hand plethysmography.

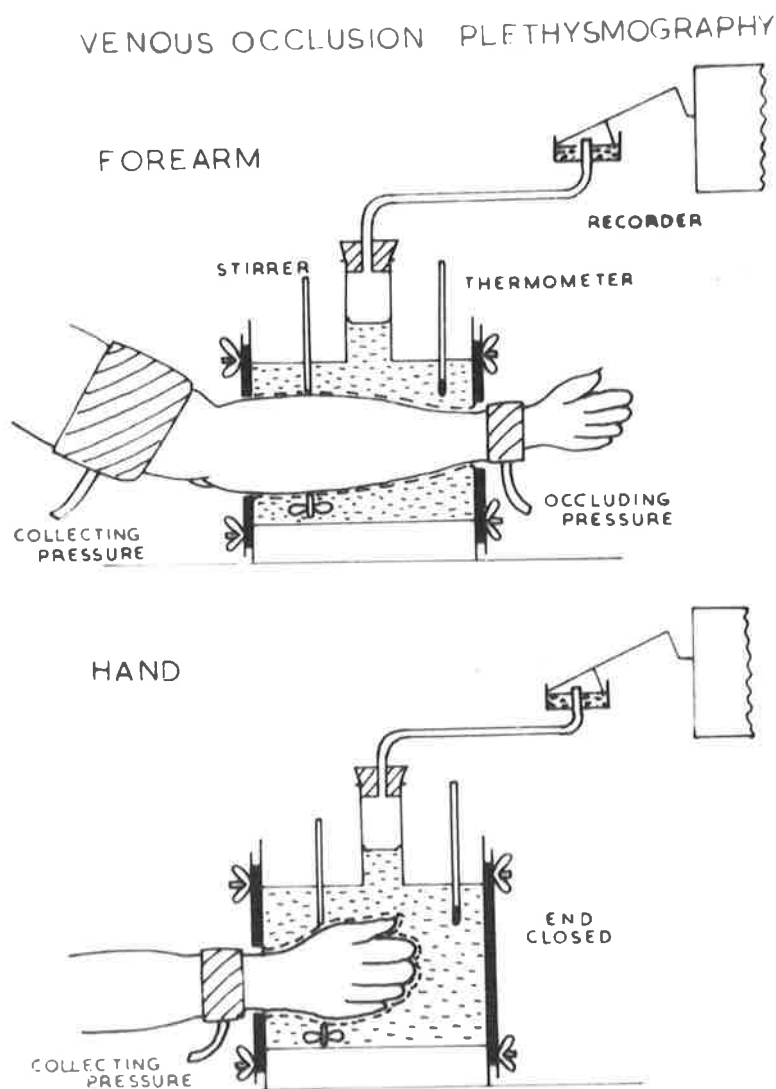


Fig 1-6. Diagrammatic representation of the water-filled plethysmograph adapted for measurement of blood flow in the forearm (upper frame) and the hand (lower frame).

suitable for measuring flow in the lower limb: an example of a "calf" plethysmograph is shown in Fig 1-7.

The capacitance plethysmograph

Although the water-filled plethysmograph is reliable, accurate, and permits temperature control it is nevertheless somewhat cumbersome, is not readily portable and since its application takes some considerable time it is not particularly suitable for clinical use or where rapid assessment of flow is required. Therefore several attempts have been made to produce plethysmographs which are simpler to use and secondly which give an electrical output. The mercury-in-rubber strain gauge plethysmograph, introduced by Whitney (1953), was one of the earlier electrical methods for assessing flow. In principal, increase in circumference of a limb segment increases electrical resistance in a thin column of mercury contained in a length of silicone-rubber tubing encircling the limb. However, the strain-gauge samples only a small fraction of the limb volume change and furthermore probably inhibits expansion of the underlying tissues by exerting counterpressure. Deficiencies in existing methods, therefore, prompted Figar (1959) to develop a plethysmograph based on the principle of an electrical capacitor i.e. changes in the capacity existing between two charged surfaces varies inversely with the distance between them. Figar's original frequency modulated

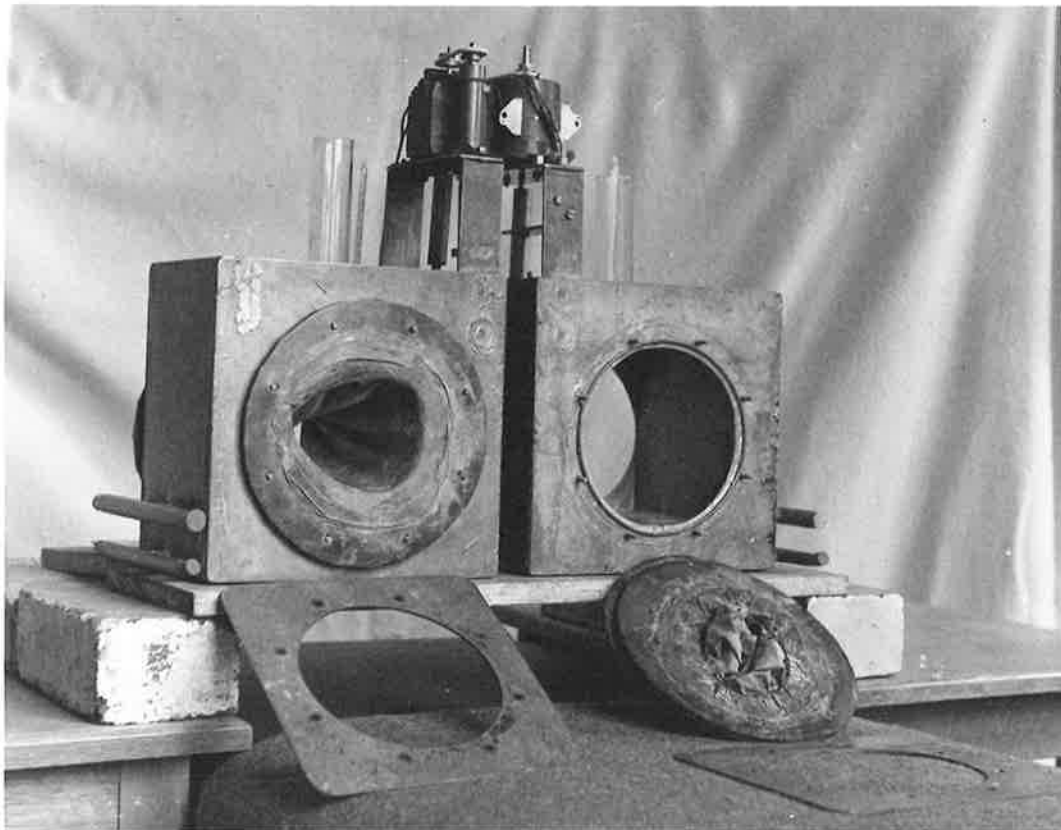


Fig 1-7. The water-filled plethysmograph for measurement of calf blood flow.

electro-capacitance plethysmograph was used to assess digital blood flow but was subsequently modified to measure forearm or calf blood flow (Hyman, Burnap and Figar, 1963). This Hyman capacitance plethysmograph, an early example of which is shown in Fig 1-8, consists of two screens: the inner "live" copper screen is woven or knitted from insulated 29-gauge stranded hook-up wire to exclude any possibility of electrical contact with either the skin beneath it or the outer shielding screen (bronze window screening) to which it is fixed. The two screens are formed into a light, flexible open sheet which can be wrapped loosely around a segment of forearm or calf to provide a conically shaped plethysmograph with a reasonably uniform relationship to the skin surface. There is slight overlap of the ends of the sheet when the plethysmograph is placed around the limb segment and secured with tape.

The insulated "live" inner screen functions as one plate of a capacitor (or condenser), while the outer shield together with the skin to which it is connected (via a skin electrode) forms the earthed (or grounded) plate. Venous occlusion and the resultant volume increase moves the skin surface toward the "live" screen thereby increasing electrical capacitance. The change in capacitance modifies the frequency in the oscillator circuit connected to the plethysmograph, this alters the output of a phased discriminator in direct proportion to change in volume. The volume/capaci-

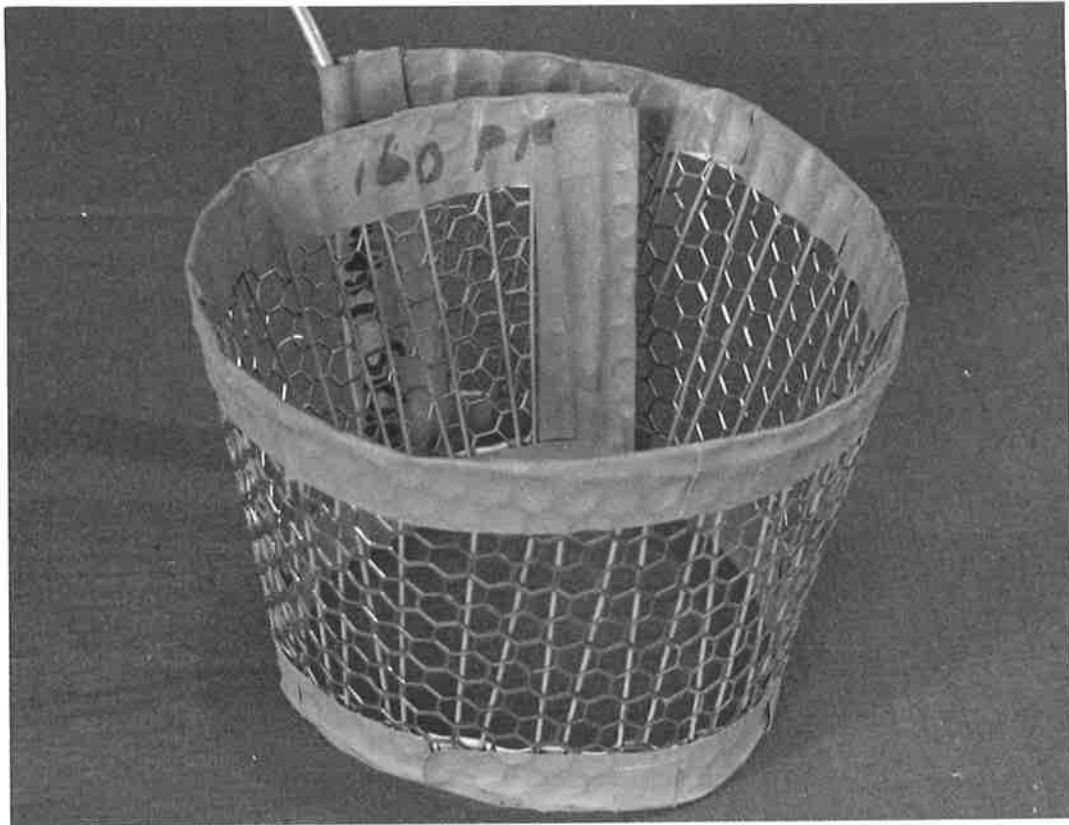


Fig 1-8. An early example of the Hyman capacitance plethysmograph.

tance relationship is sufficiently linear to permit satisfactory assessment of the volume changes involved in blood flow measurement by venous occlusion plethysmography.

Calibration was obtained initially by noting changes in output when measured volumes of saline were added to, or removed from, a small flat polythene bag which was applied closely to the skin beneath the capacitance screen. A simple electrical calibration system is now used and has been found to be more consistent and practical (Hyman and Wong, 1968). The capacitance plethysmograph measures the mean distance between the charged layers on the "live" screen and on the surface of the skin, so that displacement of a fixed area of the charge from the skin layer through a known distance toward the screen should serve to calibrate the system. Six strips, each 0.6 cm wide and 4.5 cm long, made up of 1 mm thick vinyl plastic separating two layers of copper foil (Fig 1-9) were interconnected and applied around the limb segment so that one set of copper strips was in contact with the skin. For each calibration, the upper set of copper strips was short-circuited by a miniature relay to the same potential as skin (earthed), and thus this effectively moved the charges from a corresponding area of skin through 1 mm to the upper level. Because the strips were placed at intervals around the segment and orientated parallel to the axis of the part some of the inherent asymmetries of the plethysmograph - skin spacing were averaged out. Direct calculation of

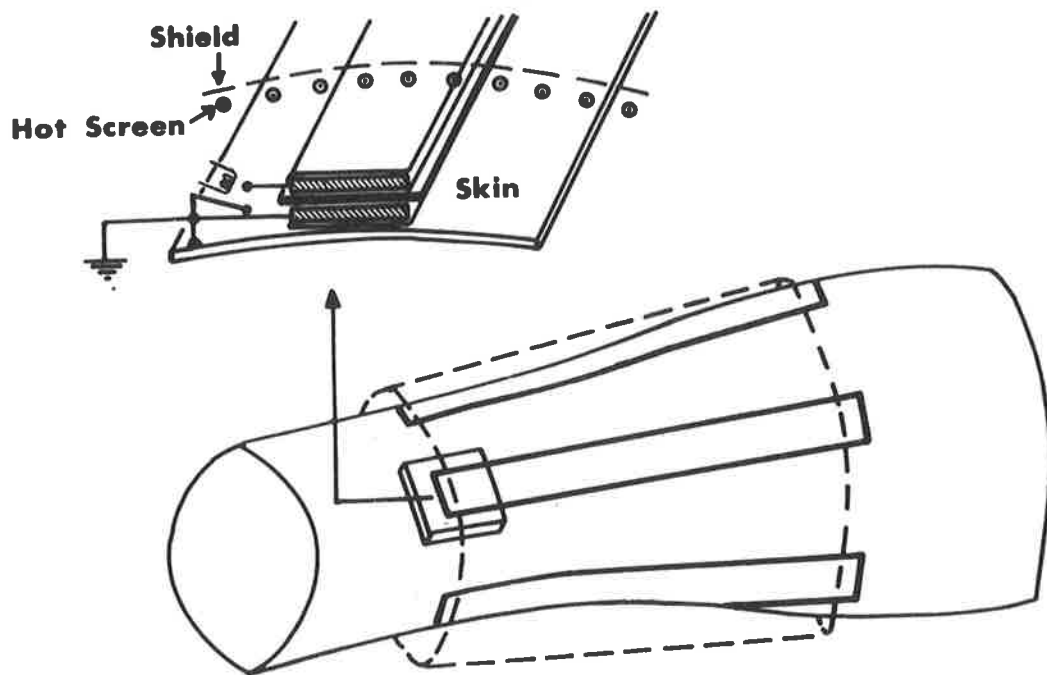


Fig 1-9. Charge displacement calibration system for Hyman plethysmograph. Copper strips (hatching) separated by vinyl (solid). Lower strips are connected to skin at all times; relay can be activated to connect upper strips to this potential.

the volume equivalent of the calibrator was not satisfactory so the volume equivalent was obtained by comparing the calibration height with values obtained from a simulated forearm, a saline-filled rubber truncated cone, into which known volumes of saline could be introduced. The simulated forearm was designed so that volume increases occurred only beneath the encircling plethysmograph. Consistent results were obtained and these also compared favourably with the saling bag calibrations.

The Hyman plethysmograph and electrical calibrator were used in the Venous Distensibility and Capillary Filtration studies described in Sections 5 and 6. Figure 1-10 shows the Hyman plethysmograph with a knitted "live" screen (which confers greater sensitivity) and the saline bag and electrical calibrators. Figure 1-11 shows the apparatus in use.

Although the Hyman-type capacitance plethysmograph is sensitive, versatile, easy to apply and has an electrical output it has two main disadvantages. Firstly, since the plethysmograph is flexible and hangs loosely on the limb segment being studied there are asymmetries inherent in the plethysmograph to arm relationship. Secondly, calibration by the distensible saline bag or electrical calibrator is not entirely satisfactory. There is a third, theoretical, disadvantage in that the volume/capacitance relationship is not completely linear. These considerations there-



Fig 1-10. The frequency-modulated, electro-capacitance plethysmograph of Hyman et al (1963), with saline bag and electrical calibrators.

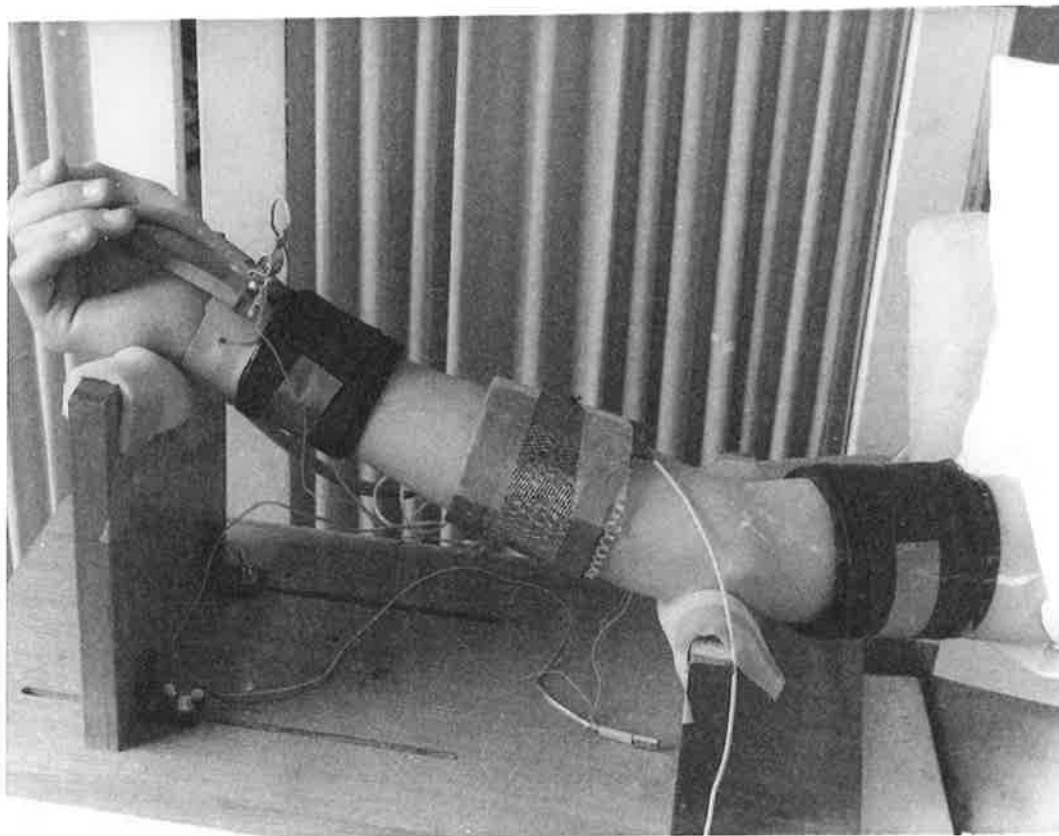


Fig 1-11. Arrangement for measurement of forearm blood flow with the Hyman plethysmograph.

fore led to the design of a forearm capacitance plethysmograph of fixed dimensions, with a built in electrical calibration system and with a linear relationship between volume and capacity changes (Willoughby, 1965; Fewings, 1965; and Fewings and Whelan, 1966). Professor Willoughby (Department of Electrical Engineering, University of Adelaide) deduced that a fixed frequency, constant current system with a mean spacing of 1.25 ± 0.25 cm between the surfaces of the forearm and plethysmograph would enable volume changes to be measured accurately (error $<1\%$). A constant current (100 mA) at a fixed frequency, 6.59 Mc/sec, is passed across the capacitor, the two "plates" of which are formed by the inner surface of the plethysmograph and the arm surface (which is earthed to the outer screen of the plethysmograph as is the case with the Hyman plethysmograph.) Following results obtained from field plotting the ends of the outer screen were shaped to confine the electrical field within the plethysmograph (Willoughby, 1965). Several pairs of forearm plethysmographs were constructed to give a selection of sizes so that the mean spacing of 1.25 ± 0.25 cm could be attained with each subject. This was achieved by making plaster casts of the forearms of a representative group of subjects and then the casts were built up to a thickness of 1.25 cm with wax. Contoured metal cones (the "active" plates) were built up on the wax bases by an electroforming technique. A shielding screen surrounded the "active" plate and was insulated from it by polythene rings. A probe unit, mounted on the plethysmograph, contained the calibration

unit and was connected with the control unit by a low capacity coaxial cable which conveyed the radiofrequency (R.F.) input to the probe unit and the output back to the control unit. Venous occlusion resulted in an increase in capacity and a decrease in output voltage. The voltage was rectified, amplified and fed into a rectilinear pen-writer system (Oscilloriter, Model 323B medium frequency recorder, Texas Instruments Inc.). Figures 1-12 to 1-15 depict this "Adelaide plethysmograph".

As the plethysmograph actually measures the volume of the space between the plethysmograph and the skin surfaces i.e. the volume between the "plates", the volume of the forearm segment can be calculated.

$$\text{i.e.} \quad V_a = V_p - V_t \quad \dots\dots\dots(1)$$

It was found that

$$V_a = V_p - \frac{A^2}{3.6\pi C_t} \quad \dots\dots\dots(2)$$

where V_a = volume of forearm segment in cm^3

V_p = volume of plethysmograph in cm^3

V_t = total volume of the space in cm^3

A = $\frac{1}{2}$ (surface area of forearm + surface area of plethysmograph) in cm^2

C_t = total plethysmograph to arm capacitance in pF.

THE CAPACITANCE PLETHYSMOGRAPH

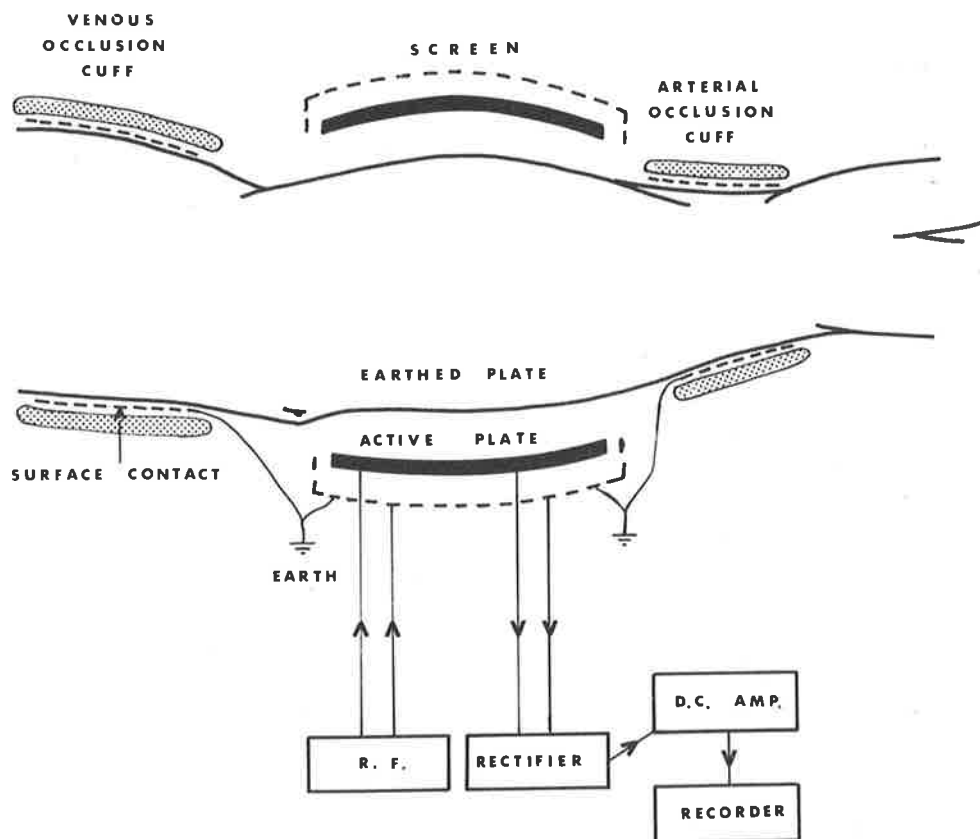


Fig 1-12. A diagrammatic longitudinal section of the Adelaide capacitance plethysmograph applied to the forearm. The forearm is centered within the plethysmograph and the limb is earthed by connecting skin electrodes to the outer screen of the plethysmograph. A simplified schematic electrical circuit is shown. Venous and arterial occlusion cuffs are shown in position.

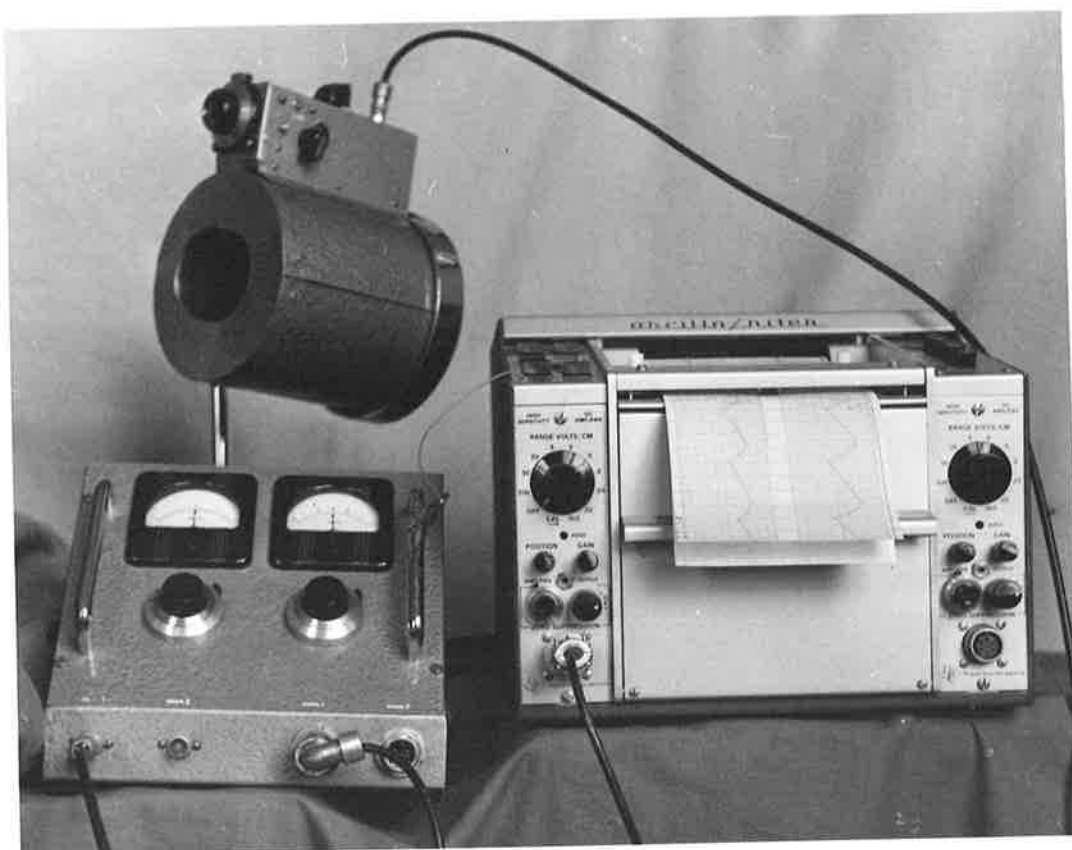


Fig 1-13. The control unit, Adelaide capacitance plethysmograph (with probe unit attached), coaxial cable, and recorder (Oscilloriter, Texas Instruments Inc.).

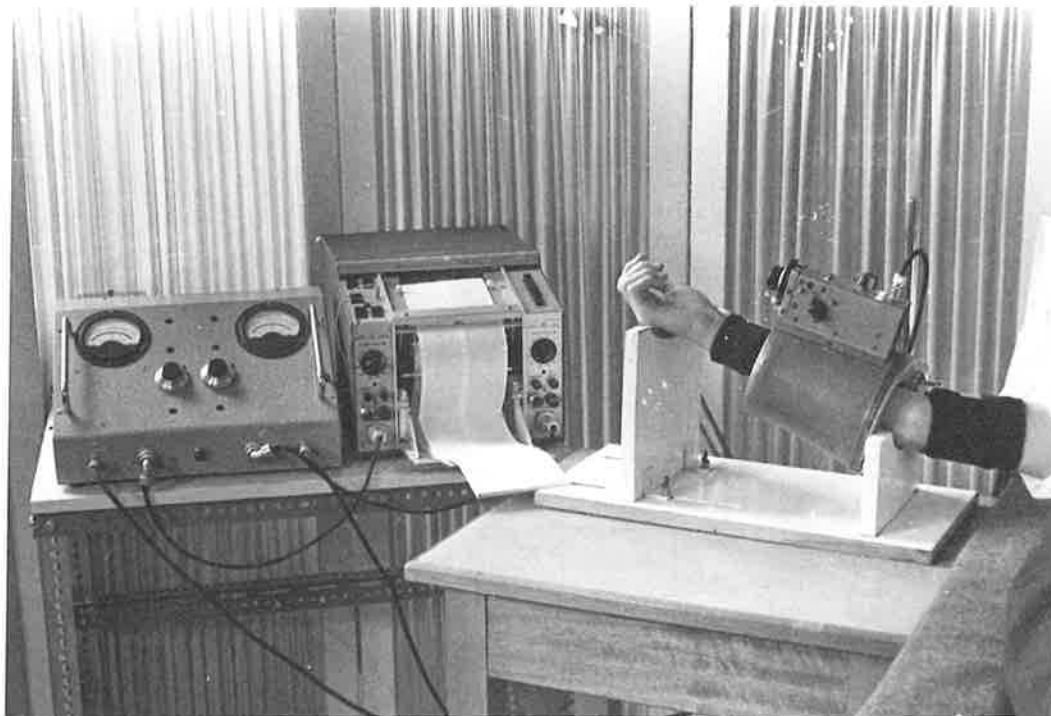


Fig 1-14. The Adelaide capacitance plethysmograph, control unit and recorder.

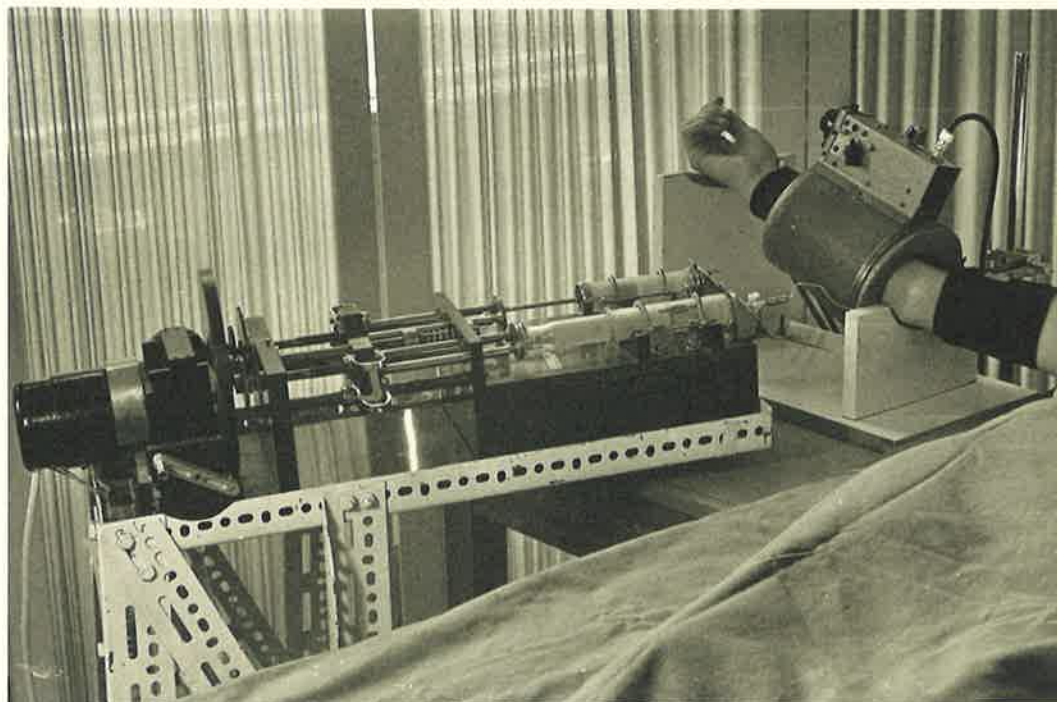


Fig 1-15. The arrangement for intra-arterial infusion, showing the infusion pump, 50 ml syringe and polythene connection, and the Adelaide capacitance plethysmograph with pneumatic cuffs applied to the upper limb.

Calibration was achieved by introducing an inductance (0.27 μH) in series with a capacitance equivalent to the plethysmograph-arm capacitance to give a voltage change of the same order as the changes produced by resting blood flow measurements (approximately 4 ml). The formula derived to calculate the volume change (ΔV) equivalent to that produced by introducing the calibration inductance was:

$$\Delta V = \frac{\omega^2 \cdot A_a^2 \cdot L_o}{36 \pi \times 10^{11}} \quad \text{cm}^3 \quad \dots\dots\dots (3)$$

where ω = frequency in Mc/sec

A_a = surface area of forearm segment (calculated by multiplying mean circumference of segment by effective length of plethysmograph.)

L_o = inductance (Henries)

Using an inductance of 0.27 μH and a frequency of 6.59 Mc/sec this equation (3) simplifies to:

$$\Delta V = 4.1 \times 10^{-5} \times A_a^2 \quad \text{cm}^3 \quad \dots\dots\dots (4).$$

For flow measurements the arm is centered within the plethysmograph and earthed to the outer shield via a shim brass electrode placed on the upper arm. Adjustable supports permit independent positioning of the limb and apparatus. Comparison

with the air-filled plethysmograph showed excellent agreement of values of resting and hyperaemic flows (Fewings and Whelan, 1966). Some differences between capacitance and water-filled plethysmograph results were noted and were attributed to the presence of hydrostatic pressure on the limb and abnormal local temperature effects consequent on confinement of the limb in a water-filled box in the latter. The measurement of flow by a device which is not in actual contact with the segment being studied is probably more "physiological" than the water-filled method and in addition has the advantage of portability, and electrical calibration and output, and has therefore been used in most of the forearm flow studies in this thesis.

Measurement of venous distensibility

Venous distensibility, the ability of the capacity vessels to contain blood at different distending pressures, was examined by two plethysmographic techniques.

(1) Changes in the reaction of the capacity vessels of the hand or forearm were studied using the method described by Glover, Greenfield, Kidd and Whelan (1958). The hand or forearm was enclosed in a water-filled plethysmograph and venous congestion produced by inflating a pneumatic cuff applied to the limb just proximal to the plethysmograph. Increases in the volume of the part displaced water from the plethysmograph into a burette cali-

brated in millilitres. The position of the burette was continuously adjusted to keep the meniscus at a level 10 cm above the centre of the limb segment, and the burette reading was noted at 30 sec intervals. Venous congesting pressures of 20, 40 and 60 mm Hg above the minimal pressure which just caused a measurable change in volume were applied and each was maintained until the rate of increase of volume of the part fell below 0.15 ml/100 ml of tissue/min, usually a period of 3-4 min. The pressure was then returned to atmospheric and the limb volume allowed to stabilize at the resting level before further measurements were made, usually 2-3 min. During measurements of forearm venous distensibility a wrist cuff was inflated to 200 mm Hg to exclude the hand circulation. Venous distensibility was expressed as ml/100 ml of tissue or percentage increase of forearm volume at each congesting pressure.

(2) The second method of assessing venous distensibility was developed by Professor Hyman and myself in California in 1967. For certain theoretical reasons, which are discussed in Section 5 in the Venous Distensibility in Essential Hypertension study, measurement of venous distensibility on the emptying phase following venous congestion, rather than on the filling phase as in the method previously described, seemed desirable. The Hyman capacitance plethysmograph was placed on the forearm (elevated above heart level to facilitate venous drainage) as for flow

measurement and the proximal congesting cuff inflated to 50 mm Hg and the increase in forearm volume was recorded continuously. When a "plateau" was reached (1.5-3 min) the congesting pressure was lowered in 5 mm Hg steps with sufficient time to establish a new "plateau" at each pressure. Each new pressure was momentarily undershot by 3-4 mm Hg to facilitate emptying of the veins. The percentage increase in forearm volume above the baseline of zero congesting pressure was plotted against each corresponding congesting cuff pressure and thus a Δ volume/ Δ pressure curve was obtained. Venous distensibility (VV_{30}) was expressed as the percentage increase in forearm volume at a cuff pressure 30 mm Hg above the minimal pressure which caused a measurable increase in forearm volume (usually 3-10 mm Hg and deduced by extrapolating the curve through zero volume change). The method allowed rapid assessment of the capacity of the vascular bed of the forearm and produced (in normal subjects) results very similar to those obtained by other investigators using different methods. The assessment of venous distensibility by this second method was equally suited to the Adelaide capacitance and also the conventional water-filled plethysmograph. For further discussion the reader is referred to Section 5.

Measurement of capillary filtration rate and capillary filtration coefficient

The plethysmographic technique was first used to assess capillary filtration rate in the human limb by Drury and Jones

(1927). Prolonged venous occlusion leads to a rapid increase in volume at first, and then a sustained slow increase in volume which might be attributed to interstitial fluid accumulation, active modification of vessel calibre or to a slow continuous increase in venous capacity, but the interstitial fluid accumulation probably accounts for over 90% of the sustained slow increase (Landis and Gibbon, 1933). Pappenheimer and Soto-Rivera (1948) recognised two phases during venous congestion: an initial rapid phase considered to be due to distension of capacity vessels and a slower but continuous phase of outward filtration. Krogh, Landis and Turner (1932), using a water-filled plethysmograph which was pressurised to 55 cm H₂O before and after each period of accumulation of interstitial fluid in an attempt to obtain a "reduced arm volume", performed some of the early investigations on the relationship of hydrostatic pressure and colloid osmotic pressure to the movement of fluid through the capillary wall. Subsequently, Landis and Gibbon (1933), Brown, Wise and Wheeler (1947) and Kitchin (1963) found that a 200 mm Hg counter-pressure was required to obtain a stable baseline level for "reduced arm volume" and hence assessment of the amount of tissue fluid which had accumulated per minute during prolonged venous occlusion.

Hyman and Wong (1968) described a simplified technique, using their capacitance plethysmograph, for measuring capillary filtration rate and capillary filtration coefficient: volume change

of a forearm segment was recorded continuously during 10-15 min of venous occlusion when a proximal pneumatic cuff was inflated to 40, 50 and 60 mm Hg in three consecutive recordings. The rate of volume increase determined from the late phase of the volume record (usually 5th-15th min) was considered a measure of capillary filtration. Capillary filtration rate, i.e. volume increment per 100 ml of forearm tissue per min, was plotted against venous congesting pressure, and capillary filtration coefficient i.e. volume increment per 100 ml of forearm tissue per min per mm Hg, was read as the slope of the straight line relating capillary filtration rate to applied venous pressure. The method was validated in terms of consistency of values obtained on the same individual at different times, the correspondence between values obtained on a group of normal individuals, and by the agreement between the values obtained and those reported in the literature. This latter method was used in the Capillary Filtration study reported in Section 6.

SUPERFICIAL AND DEEP VENOUS BLOOD OXYGEN SATURATION ESTIMATIONS

Although the plethysmograph gives a satisfactory measurement of total blood flow through a limb segment it gives little information about changes in flow in the different vascular beds within the segment. Since many vasoactive substances and reflex phenomena have qualitatively and quantitatively different effects on different vascular beds, skin and muscle in particular, a method of differentiating changes in blood flow through each of these vascular beds is desirable.

Several methods for "separating" skin and muscle blood flow have been described: iontophoresis of adrenaline to exclude skin blood flow (Cooper, Edholm and Mottram, 1955); clearance of radio-isotopes injected intradermally or intramuscularly (Winsor and Hyman, 1965); application of counter-pressure to minimise skin blood flow over a short segment of forearm (Hyman, Greenson, Clem and Winsor, 1964); and estimation of muscle blood flow with a Hensel needle and skin flow with heat-flow calorimetry (Bock, Dengler, Kuhn and Matthes, 1957). All of these methods provide useful information but the method preferred by myself and my colleagues is assessment of skin and muscle blood flow by the technique of superficial and deep venous blood oxygen saturation estimations.

Assessment of forearm muscle and skin blood flow by the technique of blood oxygen saturation measurements arose from

observations on muscle venous blood oxygen saturations made by Mottram (1954). His technique of retrograde catheterisation and blood sampling from a deep (muscle) vein in the antecubital fossa was extended by Roddie, Shepherd and Whelan, 1956; 1957b. Polythene catheters (Intracath No. 17, Bardic) were inserted centrifugally into a deep (muscle) vein and a superficial (skin) vein in the antecubital fossa and, following inflation of a wrist cuff to 200 mm Hg to exclude hand flow, serial sampling of venous effluent from skin and muscle vascular beds was commenced and then the effect of the particular procedure, e.g. infusion of serotonin into the brachial artery, was studied. Samples of blood (1.0 ml) were collected anaerobically into heparinised plastic syringes and haemolysed by a saponin in sodium carbonate solution. Estimation of the oxygen saturation of each sample was determined rapidly by estimating the optical density (actually the percentage transmission of light) with a Unicam SP. 1400 prism absorptiometer (= spectrophotometer) using a wavelength of 660 m μ and a self-flushing cuvette (Fig 1-16). Fully oxygenated and fully reduced standards were prepared from each subject's blood so that the percentage saturation of each test sample could be derived.

If it is assumed that metabolic activity and, therefore, oxygen requirement of a tissue remains constant during the test procedure then changes in the oxygen saturation of the returning venous blood will reflect changes in the blood flow through that

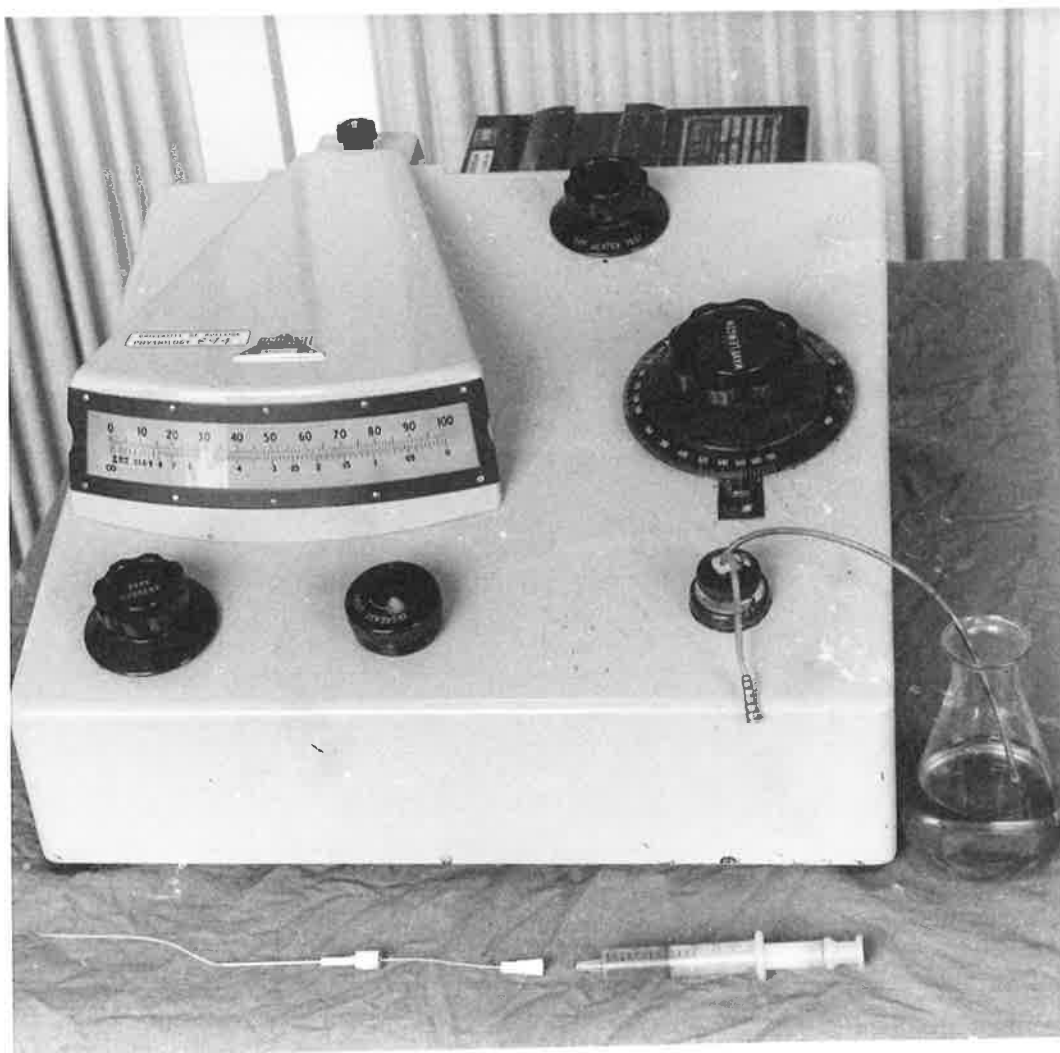


Fig 1-16. The Unicam prism absorptiometer used to estimate the oxygen saturation of samples of venous blood from skin or muscle.

tissue. Thus, a rise in oxygen saturation of samples from the deep catheter would indicate an increased muscle flow i.e. diminished arteriovenous difference in oxygen saturation due to diminished transit time; similarly, a decrease in saturation of samples obtained from the superficial catheter would indicate stasis in the skin vessels. In most subjects anatomically distinct superficial and deep venous systems are present. The method while not permitting quantitative estimation of skin and muscle flow, nevertheless allows valuable qualitative assessment of flow through these two principal vascular beds in the forearm.

INTRAVASCULAR INFUSIONS

In the GENERAL INTRODUCTION it was noted that the action of vasoactive substances on the peripheral vessels is influenced greatly by the route of administration. Intra-arterial infusions permit study of the direct or local actions of vasoactive substances and the effect on these local actions caused by administration of various receptor blocking drugs or blockade of the regional sympathetic nerve supply. With intravenous infusions the direct effects may be accompanied by general effects such as stimulation of medullary vasomotor centres, baroreceptor and chemoreceptor stimulation, and release of endocrine secretions so that a combined response which is a resultant of direct and indirect actions is observed. Study of the effects of a vasoactive substance by both intravenous and intra-arterial administration aids elucidation of its role in the control of the peripheral circulation.

Intra-arterial infusions

These were administered through a 22-gauge short bevel buttless needle (Willen Bros., London) inserted centripetally into the brachial artery at the elbow under local anaesthesia (lignocaine 2%). The needle (3.2 cm in length) was threaded up the lumen of the artery to prevent displacement during the experiment. A 30 cm length of polythene tubing (Sterivac No. 1, int. diam. 0.5 mm) connected the needle to a mechanically driven 50 ml glass syringe

which delivered solutions at a constant rate of 2.0 ml/min. Saline (sodium chloride 0.9% w/v or 9 G/L) was infused during control periods and was used as a vehicle for the drugs. The dead space in the polythene tubing was 0.24 ml and, therefore, at 2.0 ml/min caused a delay of 7 secs before a solution entered the brachial artery from the syringe.

By using this intra-arterial route of administration in studies of drug responses in the human hand or forearm it is possible to use small doses and to confine the effects to the infused limb because the drug is so diluted on reaching the general circulation that it has no systemic effects. This enables the opposite uninfused limb to act as a control during the infusions and thus allowance can be made for spontaneous variations in flow unrelated to the drug action. This is particularly important in regard to flow measurements in the hand where sympathetic vasomotor activity, and hence flow, is particularly variable. Observations were made on both limbs simultaneously, three or four records being taken each minute. Calculation of percentage change in blood flow during intra-arterial infusion of a vasoactive substance was made by obtaining the average of blood flow measurements taken during the 2 min immediately preceding the infusion and comparing it with the average flow during the last 2 min of the infusion. Where appropriate, correction for spontaneous changes in the blood flow was made by reference to the control side, since it can be assumed

that in the absence of the drug infusion the two sides would maintain the same flow relationship (Duff, 1952). Figure 1-15 shows the arrangement for intra-arterial infusion.

Intravenous Infusions

Intravenous infusions were administered through a polythene catheter (Intracath No. 17 or No. 19, Bardic) inserted centripetally into a vein in the antecubital fossa under local anaesthesia (Lignocaine 2%). When forearm blood flow was being measured care was taken to ensure that the catheter tip lay proximal to the pneumatic congesting cuff so that intermittent obstruction of the infused solution was avoided. The infusion apparatus and connecting tubing were identical to that used for intra-arterial infusions, and similarly saline or solution was delivered at 2.0 ml/min.

PRESSURE MEASUREMENTS

The intra-arterial measurement of blood pressure

Systemic blood pressure was measured directly from a catheter (occasionally from a needle) inserted into the brachial artery and connected by a 30 cm length of saline-filled polythene tubing (Portex 49A, int diam 0.86 mm) to a Statham P23DC or P23AC pressure transducer and the output was recorded on a Grass Polygraph, model 5D (Fig 1-17). Heart rate was read from the blood pressure tracing. The catheter (Intramedic, PE90, int diam 0.76 mm) was inserted into the brachial artery by a modified Seldinger technique (Seldinger, 1953). Firstly, a 19-gauge short bevel needle was inserted into the brachial artery; secondly, a "leader", a short measured length of nylon fishing line (Perlon, diam 0.5 mm), was inserted through the needle and threaded well into the artery; and thirdly the needle was removed. Finally the PE90 catheter was passed into the artery along the nylon "leader" which was then removed. In some of the earlier experiments blood pressure was recorded from a 21-gauge siliconised needle inserted into the brachial artery and connected by saline-filled polythene tubing to a differential electro-capacitance manometer (N.E.P., London) and the signal from this passed to a Bevelec carrier amplifier and recorded on an ultraviolet oscillograph (N.E.P. type 1050). Heparinised saline filled all polythene tubing used in pressure

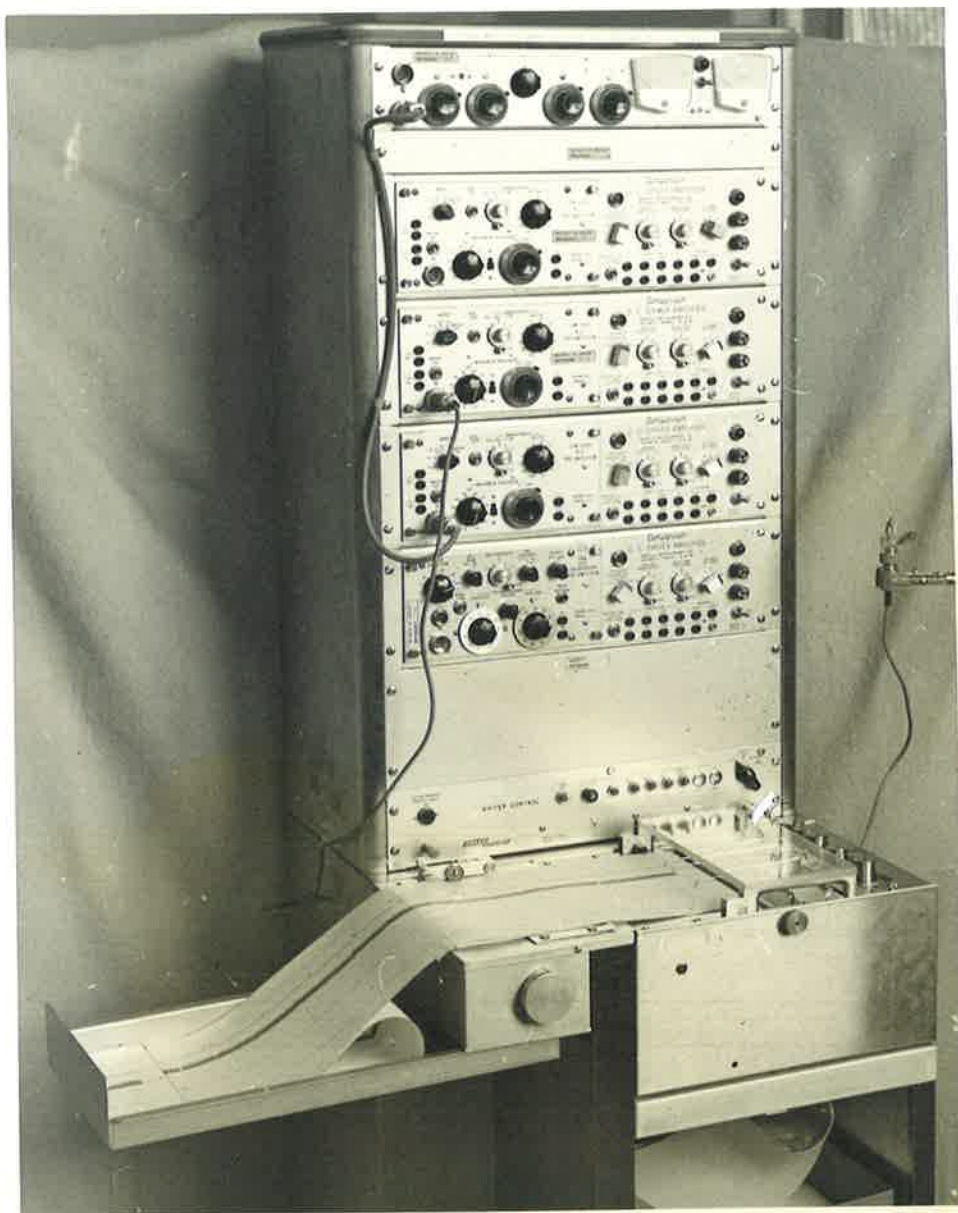


Fig 1-17. The Grass polygraph (Model 5D) and Statham pressure transducer (P23DC) used for intra-arterial measurement of blood pressure.

recordings and was also used for intermittent flushing of the needle or catheter. An electrical calibration system was built into each system but was checked with a mercury manometer at the end of each experiment.

Calculation of peripheral resistance

Peripheral resistance in the vascular bed of the hand or forearm was calculated from the ratio:

Mean blood pressure (mm Hg)

Blood flow (ml/100ml of hand or forearm tissue/min).

(in arbitrary peripheral resistance units).

Mean blood pressure = Diastolic pressure

+ 1/3 (Systolic pressure-diastolic pressure).

The measurement of central venous pressure

Central venous pressure was recorded from a polythene catheter (Intramedic PE90) inserted into an ante-cubital vein by the modified Seldinger technique described earlier. The catheter length required was determined in each subject prior to insertion to ensure that the tip lay in the great veins in the thorax. Recordings were made using the differential-electrocapacitance manometer and ultraviolet oscillograph (N.E.P. London) described earlier. The system was calibrated against a simple water manometer and the readings expressed in terms of cm of water.

STATISTICAL ANALYSES

Statistical analyses were performed following generally accepted procedures for biological research as summarised by Moroney (1960).

$$\text{Mean } (\bar{x}) = \frac{\sum x}{n}$$

where $\sum x$ = sum of individual values (x)

n = frequency or number of values.

$$\text{Standard deviation } (s) = \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}} = \sqrt{\frac{1}{n-1} \cdot \left(\sum x^2 - \frac{\sum x^2}{n} \right)}$$

$$\text{Standard error (S.E.)} = \frac{(s)}{\sqrt{n}} = \sqrt{\frac{1}{n(n-1)} \cdot \left(\sum x^2 - \frac{\sum x^2}{n} \right)}$$

"Student's" t distribution:

One set of observations: i.e. pair differences within individuals.

$$t = \frac{\bar{x} - 0}{\frac{s}{\sqrt{n}}}$$

where there are $n-1$ degrees of freedom.

Two pairs of observations: i.e. difference between means.

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} \quad \text{where there are } n_1 + n_2 - 2 \text{ degrees of freedom.}$$

Correlation coefficient (r) between two sets of values χ and y :

$$r = \frac{n \Sigma \chi y - (\Sigma \chi) (\Sigma y)}{\sqrt{(n \Sigma \chi^2 - \Sigma \chi^2) (n \Sigma y^2 - \Sigma y^2)}}$$

where n = number of pairs of observations.

χ and y = corresponding paired values of each set of observations.

Statistical tables of Fisher and Yates (1963) were consulted to assess the Probabilities of the results. A Probability limit of less than 0.05 was considered to denote a significant difference from a null hypothesis.

76.

SECTION 2

SEROTONIN, ANGIOTENSIN, NORADRENALINE
AND THEIR INTERACTIONS.

77.

PART A

THE ANTAGONISM BY METHYSERGIDE OF PERIPHERAL
VASCULAR EFFECTS OF SEROTONIN AND THE SPECI-
FICITY OF SEROTONIN RECEPTORS.

INTRODUCTION.

The vascular responses in the upper limbs of normal human subjects to intra-arterially administered serotonin (5-hydroxytryptamine) were described by Roddie, Shepherd and Whelan (1955) and by Bock, Dengler, Kuhn and Matthes (1957). Glover, Marshall and Whelan (1957) found the lysergic acid derivative 2-bromo- (+)- lysergic acid diethylamide bitartrate (BOL 148) to be a specific antagonist of the constrictor response of forearm and hand blood vessels to intra-arterial infusions of serotonin.

Methysergide (1-methyl-d-lysergic acid butanolamide bimalate), a more recent lysergic acid derivative, has been used therapeutically in the long term management of migrainous headaches in the pathogenesis of which serotonin may be involved (Ekblom, 1962; Freidman and Elkind, 1963; Curran and Lance, 1964; Graham, 1964). Its effectiveness in reducing diarrhoea in patients suffering from the carcinoid syndrome has been reported by Peart and Robertson (1961), Melmon, Sjoerdsma, Oates and Laster (1965) and Mengel (1965). The possible use of methysergide in the management of post-gastrectomy syndromes and post-vagotomy diarrhoea has been indicated by Peskin and Miller (1965).

The present study was undertaken to determine the effects of methysergide on the responses of limb blood vessels to infused serotonin and other vasoactive substances in normal subjects and

also to investigate the specificity of serotonin receptors in blood vessels. Since serotonin has been shown to have both constrictor and dilator effects on the vessels of the upper limb the use of a specific competitive antagonist of the lysergic acid group may be expected to aid clarification of the precise effects of serotonin on the vascular beds of the hand and forearm in man, for, as was found with the catecholamines, one of the most effective methods for studying the actions of a pharmacological agent is its administration before and after various blocking agents. This study, therefore, employs methysergide as a pharmacological tool in determining the precise effects of serotonin on skin and muscle vessels in the upper limb of man, and investigates the relationship of serotonin receptors to the α and β adrenoreceptors, acetylcholine and histamine receptors, and to the peripheral sympathetic innervation.

METHODS

The subjects were colleagues and volunteer medical and physiotherapy students (ages 18-28 yr) and two patients who had undergone cervical sympathectomy (ages 31 and 37 yr). They lay supine on a couch in a temperature-controlled laboratory (22-24°C) for at least 30-40 min before observations began, during which time the recording apparatus was applied and needles or catheters inserted. Blood flow through the hands was recorded with the water-filled, temperature-controlled plethysmographs (Greenfield, 1954), the temperature being maintained at 32°C. Forearm blood flow was measured with capacitance plethysmographs of the type described by Willoughby (1965) and Fewings and Whelan (1966).

In three subjects the effects of serotonin and methysergide on the vessels of the skin and muscle of the forearm were followed independently by determining the changes in percentage oxygen saturation of samples of blood taken from catheters inserted into a superficial and a deep forearm vein respectively.

Changes in the reaction of the capacity vessels of the hand and forearm were studied each in one subject using the method described by Glover, Greenfield, Kidd and Whelan (1958), where the volume increments of the limb segment at venous congestion pressures of 20, 40 and 60 mm Hg were determined with the water-filled plethysmographs on the filling phase of the venous congestion. A

wrist cuff was inflated to 200 mm Hg during measurements of forearm blood flow and venous capacity in order to exclude the hand circulation.

Intra-arterial infusions of drugs were administered through a 22-gauge needle inserted under local anaesthesia (Lignocaine 2%) into the brachial artery in the cubital fossa. The needle was connected by a 30 cm length of 0.5 mm bore polythene tubing to a mechanically-driven syringe. Saline (0.9% W/V) was infused continuously at a rate of 2 ml/min throughout the experiment and was used as a vehicle for the drugs. Calculation of the percentage change in blood flow during intra-arterial infusion of serotonin was made by obtaining the average of blood flow measurements taken during the 2 min immediately prior to the infusion and comparing it with the average flow during the last 2 min of the infusion. Where appropriate, correction for spontaneous changes in the blood flow was made by reference to the control side (Duff, 1952). The drugs used were acetylcholine chloride (Roche), adenosine triphosphate (Light), adrenaline hydrochloride (D.H.A.), angiotensin II (val⁵ - hypertensin II-asp- β -amide, Hypertensin CIBA), bradykinin (BRS 640, Sandoz), bretylium tosylate (Darenthin, B.W), histamine acid phosphate (B.D.H.), hyoscine hydrobromide (Farmer-Hill), mepyramine maleate (Anthisan, M.B.), methysergide (1-methyl-d-lysergic acid butanolamide bimaleate, UML 491, Deseril, Sandoz), noradrenaline bitartrate monohydrate (Levophed, Winthrop),

phenoxybenzamine hydrochloride (Dibenyline, S.K.F.), propranolol (Inderal, I.C.I.) and serotonin (5-hydroxytryptamine creatinine sulphate, SR134, Sandoz). Doses are expressed as weights of the salts. Ascorbic acid (1:50,000) was added to the adrenaline and noradrenaline solutions to prevent oxidation.

RESULTS.Forearm blood flow

In eight subjects infusions of serotonin of 2,4,8 $\mu\text{g}/\text{min}$ and in one subject 16 $\mu\text{g}/\text{min}$ for 5 min were given into one brachial artery and the blood flow measured on both sides.

Serotonin caused an initial transient dilatation followed by a sustained fall in flow, the latter fall being dose dependent. Red flushing of forearm skin was observed and prickling sensations were often experienced. These changes were similar to those described by Roddie et al (1955). The infused arm was then treated with methysergide (25 $\mu\text{g}/\text{min}$ for 10 min) and doses of serotonin were repeated. The initial dilatation was not consistently or greatly altered by methysergide administration but the subsequent vasoconstriction was abolished or converted to a slight dilatation as shown in Fig 2-1. Similar results were obtained in each of the eight subjects (Table 2-1).

The above dose of methysergide was effective for at least 20 min as repeated infusions of serotonin produced no vasoconstriction until 25 min after the end of the methysergide infusion. An additional infusion of methysergide (25 $\mu\text{g}/\text{min}$ for 5 min) was given when 20 min had elapsed. Methysergide itself produced no change in blood flow with the above dose.

METHYSERGIDE

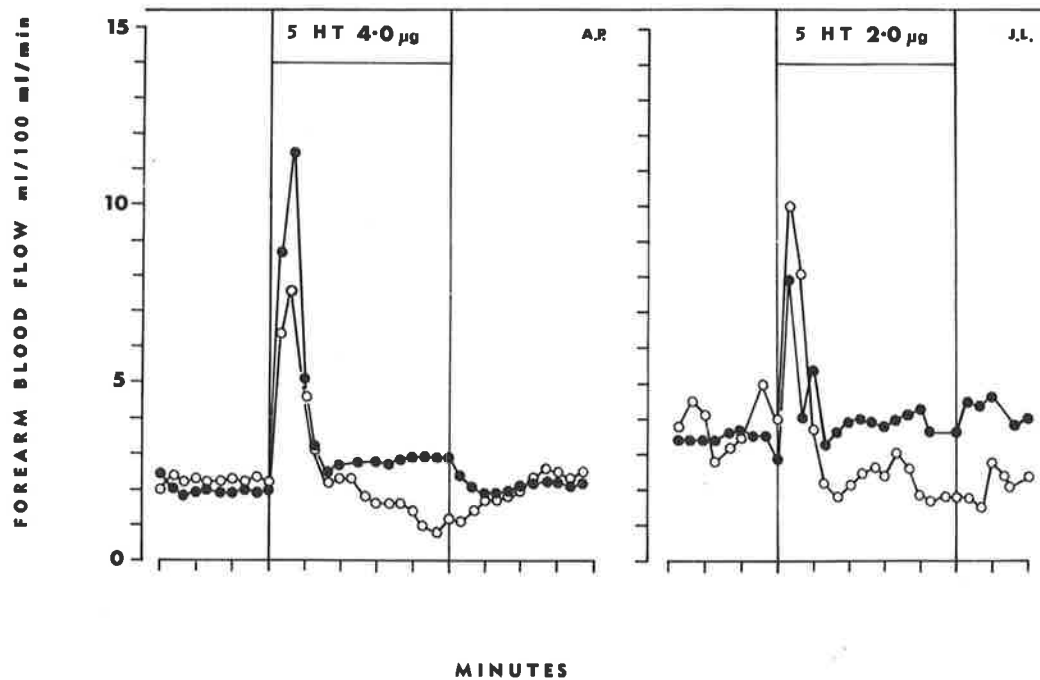


Fig 2-1. The effect of intra-arterial infusions of serotonin (5HT, 4 and 2 $\mu\text{g}/\text{min}$) on blood flow through the forearm before (○) and after (●) intra-arterial infusion of methysergide (25 $\mu\text{g}/\text{min}$ for 10 min) in two subjects. Blood flow in ml/100 ml/min.

In three subjects superficial and deep forearm venous blood oxygen saturation estimations were made during intra-arterial infusion of serotonin before and after treatment with methysergide. These indicated that the modification of the total forearm response to serotonin by methysergide (Fig 2-1) was due to an abolition of the vasoconstriction in skin and an enhanced vasodilator response of muscle vessels, as the oxygen saturation of superficial samples did not fall and that of the deep samples showed a more sustained elevation during the serotonin infusion subsequent to methysergide treatment. Figure 2-2 illustrates the results of one of these experiments.

Intravenous serotonin (1.0 mg/min for 8 min) was administered to two subjects via a polythene catheter (Intracath, Bardic) before and after intra-arterial infusion of methysergide (25 µg/min for 10 min) to one forearm. The normal vasodilator response of the forearm to serotonin infused intravenously (LeMessurier, Schwartz and Whelan, 1959) was enhanced in the forearm pretreated with methysergide in each subject.

Hand blood flow

The effect of methysergide on the response of the hand vessels to intra-arterial serotonin (8 or 16 µg/min for 5 min) was studied in six subjects (Table 2-1). The normal vasoconstrictor response (Roddie et al, 1955) was replaced by a significant dilata-

METHYSERGIDE

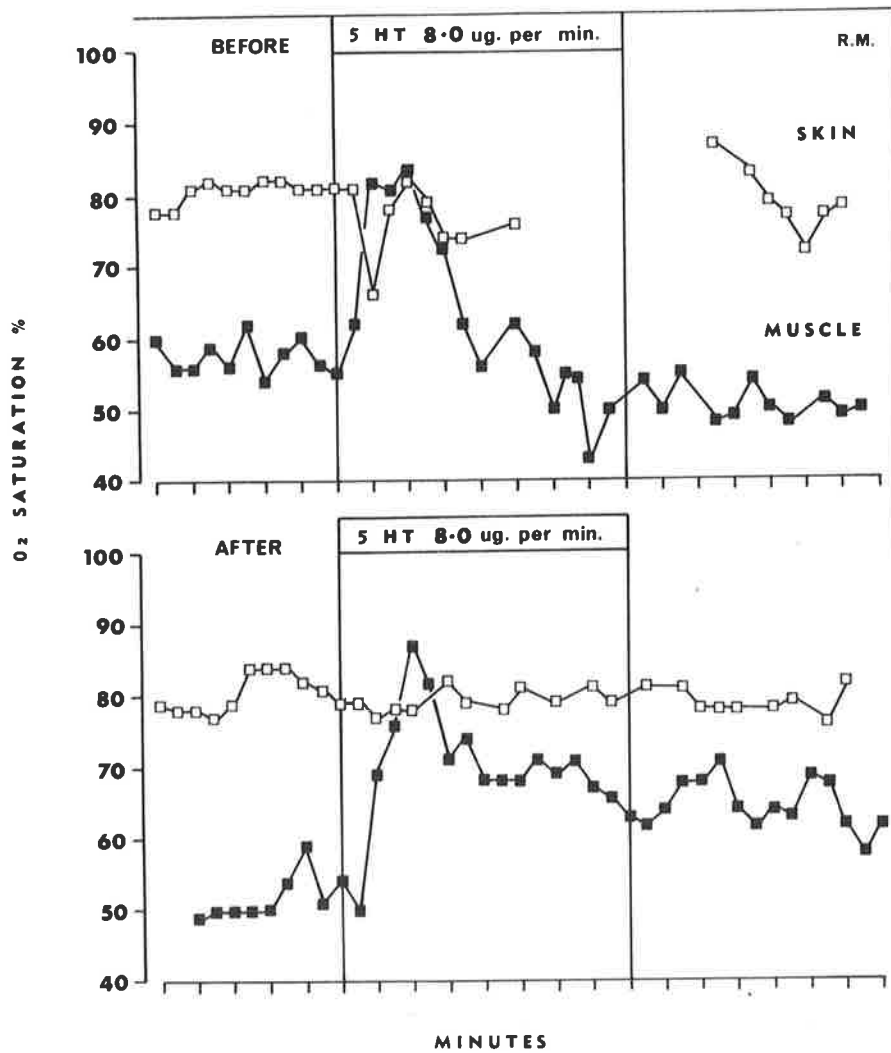


Fig 2-2. The effect of intra-arterial infusion of serotonin (5HT, 8 μ g/min for 8 min) on the % oxygen saturation of blood samples taken from veins which drained the skin ($\square$) and muscle ($\blacksquare$) of the same forearm before (upper frame) and after (lower frame) intra-arterial infusion of methysergide (25 μ g/min for 10 min).

tion in four subjects (as in O. C., Fig 2-3) and greatly reduced in two subjects (as in J.R., Fig 2-3).

Figure 2-4 summarises the results of 7 infusions on the above 6 subjects (hand blood flow) and 14 infusions on the 8 subjects in whom forearm flow was measured before and after methysergide 250 μ g. In all cases there was antagonism of the vasoconstrictor action of serotonin and in most cases a vasodilatation occurred, the dilatation being more marked with the smaller dose of serotonin.

Venous capacity

Administration of serotonin intra-arterially (4 μ g/min continuously) produced a reduction in venous capacity of the vessels of the hand (one subject) and forearm (one subject). This potent venoconstrictor action of serotonin was partially antagonised by methysergide 300 μ g and 500 μ g respectively (Fig 2-5).

Flush

Methysergide abolished the skin flushing which occurred during the control infusions of serotonin.

Specificity of the action of methysergide

The effect of methysergide on responses of upper limb vessels to other vasoactive substances administered intra-arterially

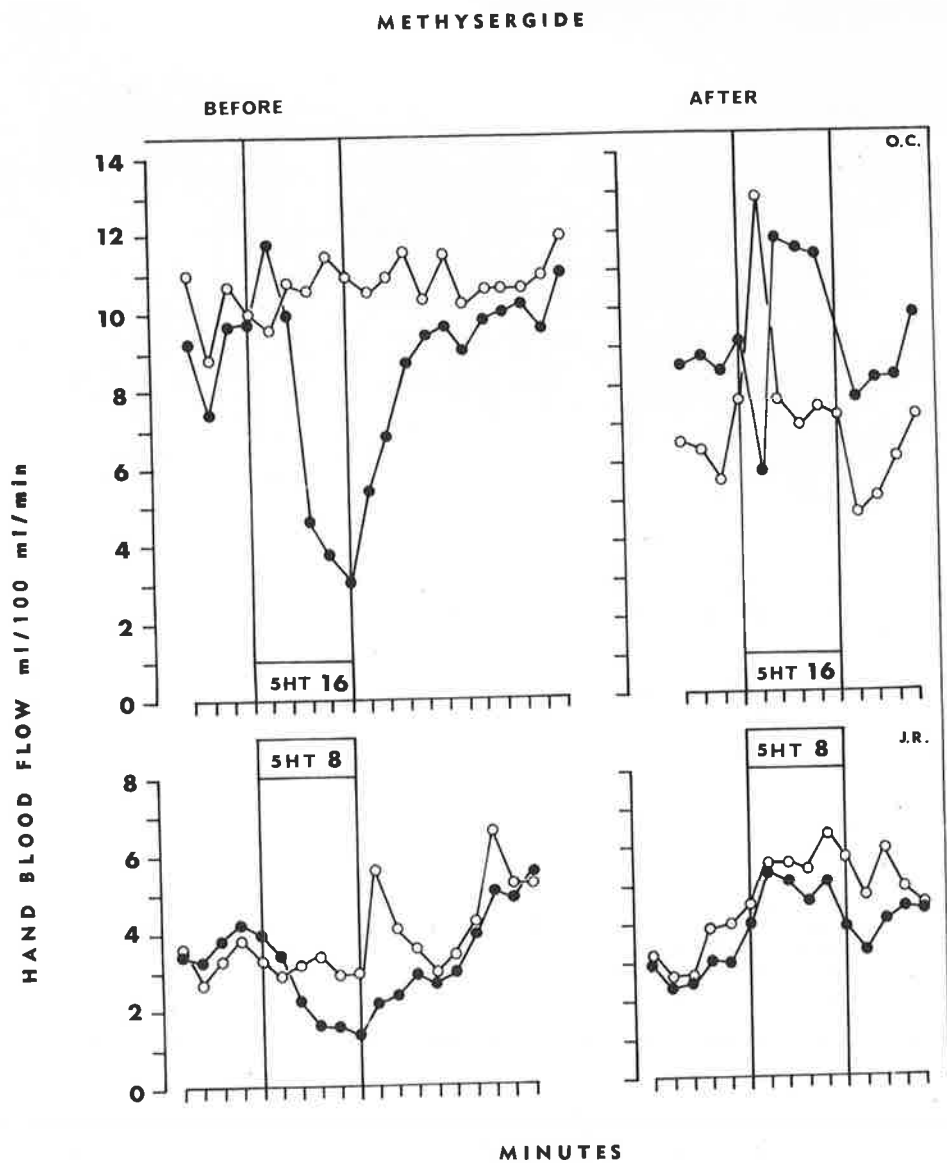
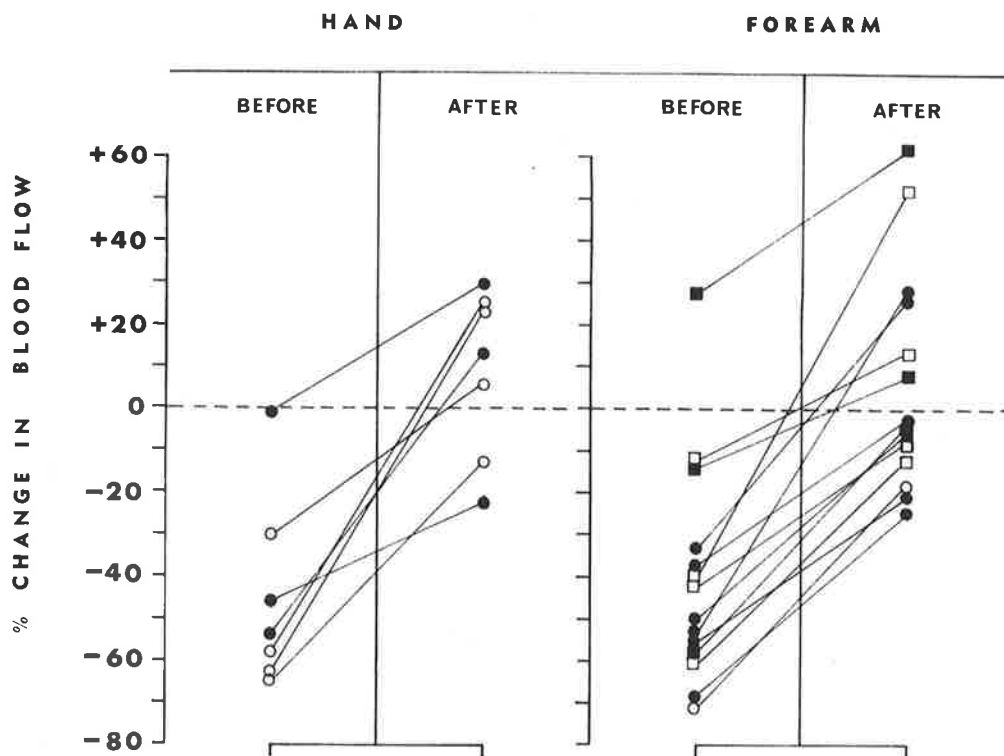


Fig 2-3. The effect of intra-arterial infusion of serotonin (5HT, 16 and 8 $\mu\text{g}/\text{min}$) on the blood flow through the hand (ml/100 ml/min) before (left hand frames) and after (right hand frames) intra-arterial infusion of methysergide (25 $\mu\text{g}/\text{min}$ for 10 min) in two subjects. ● , infused side; ○ , control side.

I. A. METHYSERGIDE



5-HYDROXYTRYPTAMINE

Fig 2-4. The pooled data from 14 normal subjects of the per cent changes in blood flow through the hand (left hand frame) and forearm (right hand frame) resulting from intra-arterial infusions of serotonin before and after intra-arterial infusion of methysergide (25 µg/min for 10 min). Dose of serotonin (µg/min): ■, 2; □, 4; ●, 8; ○, 16.

I. A. 5-HYDROXYTRYPTAMINE

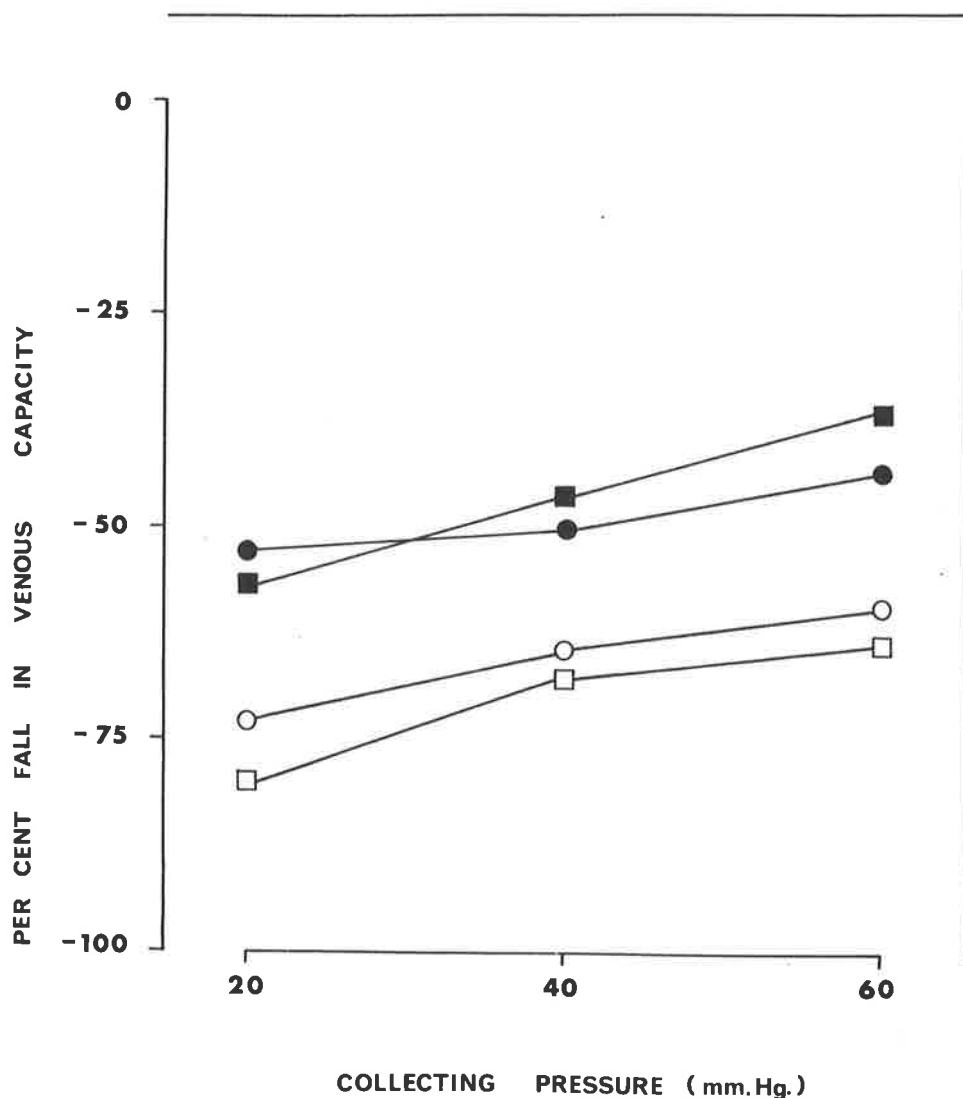


Fig 2-5. The effect of intra-arterially administered methysergide on the per cent fall in venous capacity of the vessels of the upper limb resulting from intra-arterial infusion of serotonin ($4 \mu\text{g}/\text{min}$ continuously) in two subjects. □, before; ■, after methysergide $300 \mu\text{g}$ (hand). ○, before; ● after methysergide $500 \mu\text{g}$ (forearm). Venous collecting pressures: 20, 40, 60 mm Hg.

was studied in many of the above subjects. The fall in hand blood flow which occurs with noradrenaline and adrenaline (0.2 $\mu\text{g}/\text{min}$ for 4 min) was studied before and after methysergide in two subjects. The vasoconstrictor action of the catecholamines was unaffected by methysergide whereas the vasoconstrictor effect of serotonin was converted to a dilatation. Figure 2-6 shows the result of one of these experiments.

The responses of the forearm vessels to adrenaline (0.1-0.4 $\mu\text{g}/\text{min}$ for 4 min; four subjects), histamine (0.5-5.0 $\mu\text{g}/\text{min}$ for 3 min; three subjects), acetylcholine (2.5 $\mu\text{g}/\text{min}$ for three min; two subjects), bradykinin (0.05 $\mu\text{g}/\text{min}$ for 1 min; one subject) and adenosine triphosphate (50 $\mu\text{g}/\text{min}$ for 2 min; one subject) were unchanged by administration of methysergide. Typical examples are shown in Fig 2-7. Similarly there was no change in the response of the forearm vessels to the vasoconstrictor drugs noradrenaline (0.05 $\mu\text{g}/\text{min}$ for 4 min; two subjects) and angiotensin (0.004 $\mu\text{g}/\text{min}$ for 4 min; one subject; Fig 2-8). These results indicate that methysergide is a specific antagonist of peripheral vasoconstrictor effects of serotonin.

Specificity of serotonin receptors

Phenoxybenzamine. The alpha-adrenoreceptor blocking drug phenoxybenzamine (250 $\mu\text{g}/\text{min}$ for 8 min) was administered to three subjects after control infusions of adrenaline and serotonin

METHYSERGIDE

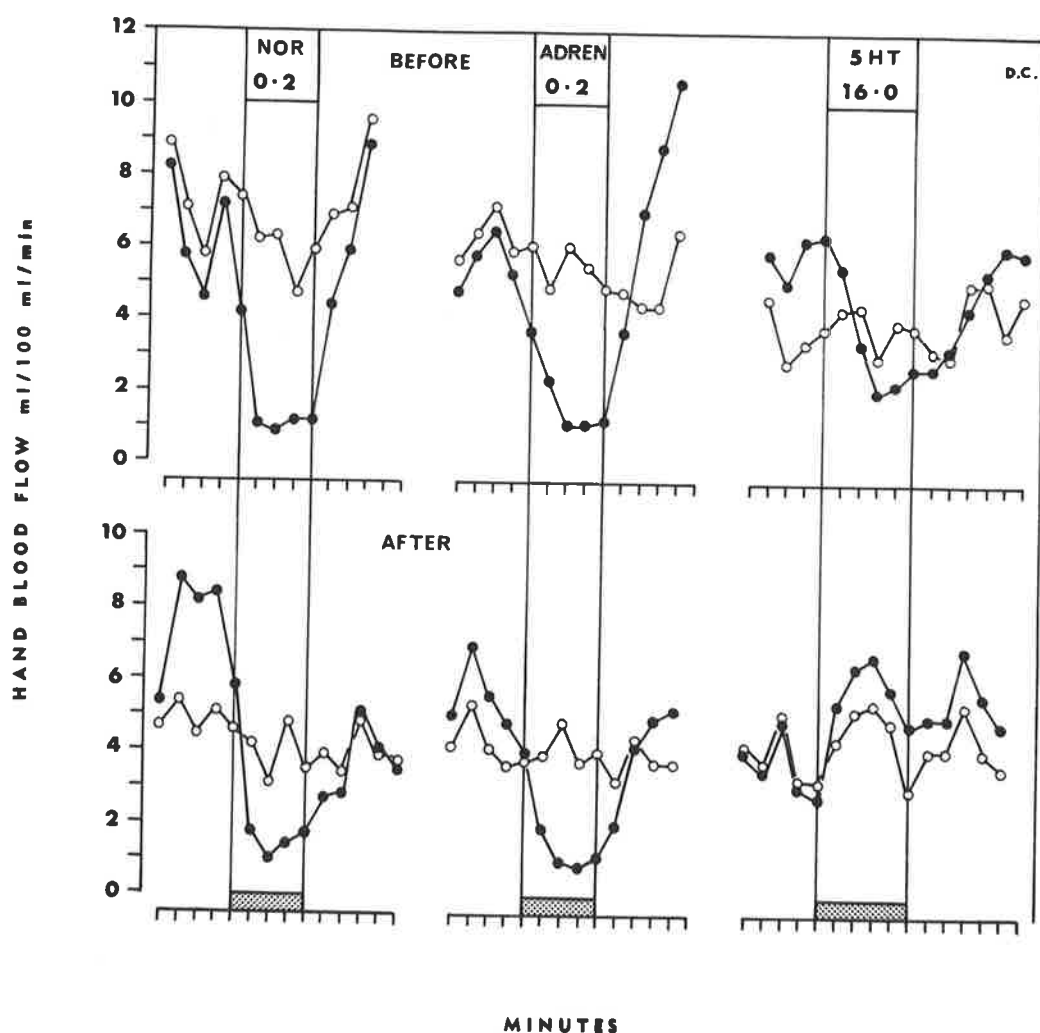


Fig 2-6. The effect on the blood flow through the hand (ml/100 ml/min) of intra-arterial infusions of noradrenaline and adrenaline (0.2 μ g/min for 4 min) and serotonin (5HT, 16 μ g/min for 5 min) before (upper frames) and after (lower frames) intra-arterial infusion of methysergide (25 μ g/min for 10 min). ● , infused side; ○ , control side.

I.A. METHYSERGIDE

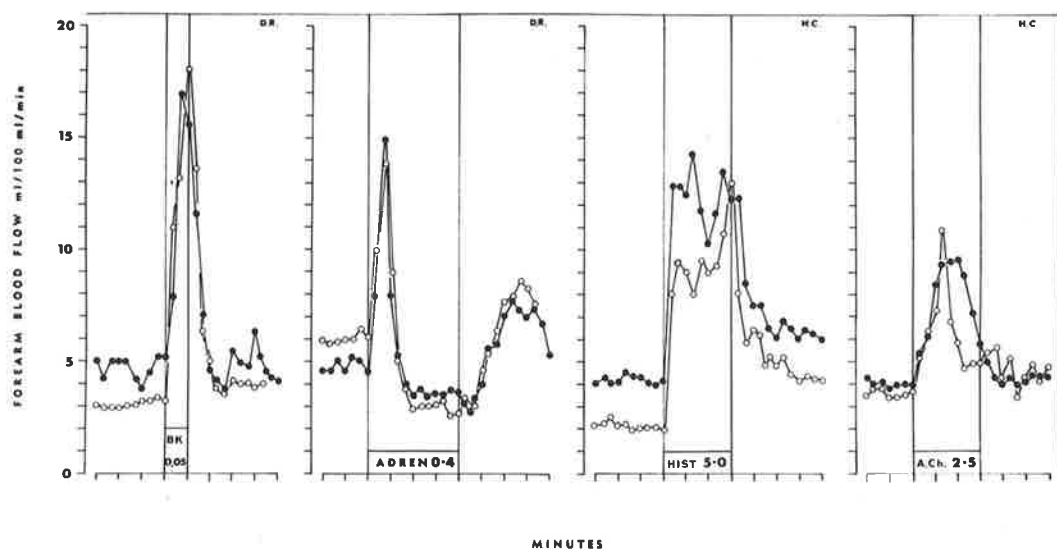


Fig 2-7. The effect of intra-arterial infusion of methysergide on the forearm blood flow responses (ml/100 ml/min) to intra-arterial infusions of bradykinin (BK 0.05 μ g/min), adrenaline (ADREN 0.4 μ g/min), histamine (HIST 5 μ g/min) and acetylcholine (ACh 2.5 μ g/min) in two subjects (D.R. and H.C.). ○ , before; ● , after methysergide (25 μ g/min for 10 min).

METHYSERGIDE

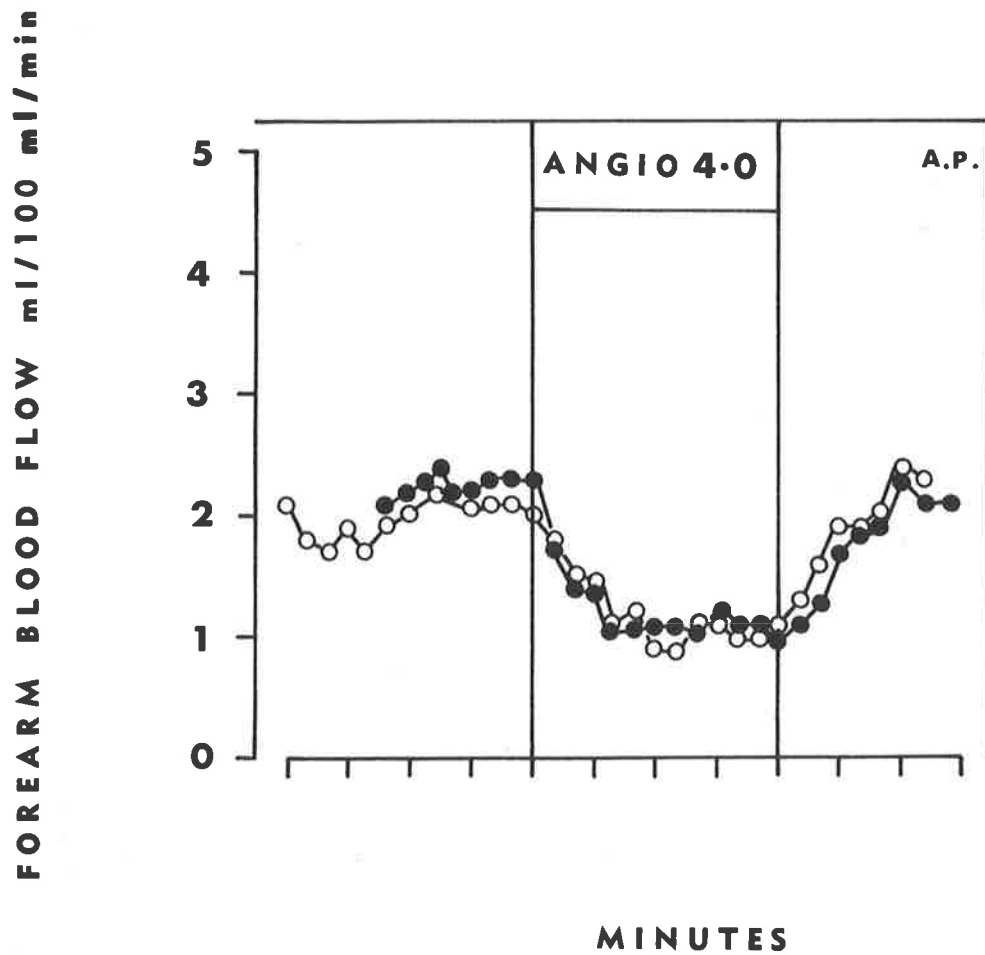


Fig 2-8. The effect of intra-arterial infusion of methysergide on the forearm blood flow response (ml/100 ml/min) to intra-arterial infusion of angiotensin (4 ng/min). $\bigcirc$, before; $\bullet$, after methysergide (25 μ g/min for 10 min).

to the hand (two subjects) and forearm (one subject). Figure 2-9 shows abolition of the adrenaline-induced vasoconstriction in one subject after treatment of the hand with phenoxybenzamine but persistence of the fall in hand blood flow caused by serotonin. This result was confirmed on the second subject where hand blood flow was measured. Similarly, phenoxybenzamine abolished the vasoconstrictor effect of adrenaline on the forearm, replacing it with a pronounced dilatation, whereas the pattern of response to serotonin was essentially unchanged although the resting blood flow increased due to release of vessel tone by the phenoxybenzamine (Fig 2-10).

Propranolol. Intra-arterial infusion of the beta adrenoreceptor blocking agent propranolol (0.1 mg/min for 5 min) was administered to three subjects after control responses to adrenaline and serotonin had been recorded. The serotonin responses were not significantly modified whereas adrenaline now produced vasoconstriction only, the dilator effect being completely abolished (Fig 2-11, upper frame).

Hyoscine. Intra-arterial infusion of the post-ganglionic acetylcholine receptor blocking agent hyoscine (0.1 mg/min for 4 min; two subjects) did not alter the response of the forearm vessels to serotonin (4 μ g/min) whereas the dilator effect of acetylcholine (5 μ g/min) was completely abolished. Figure 2-11 (lower frame) shows the result of one of these two experiments.

I.A. PHENOXYBENZAMINE 2.0 mgm

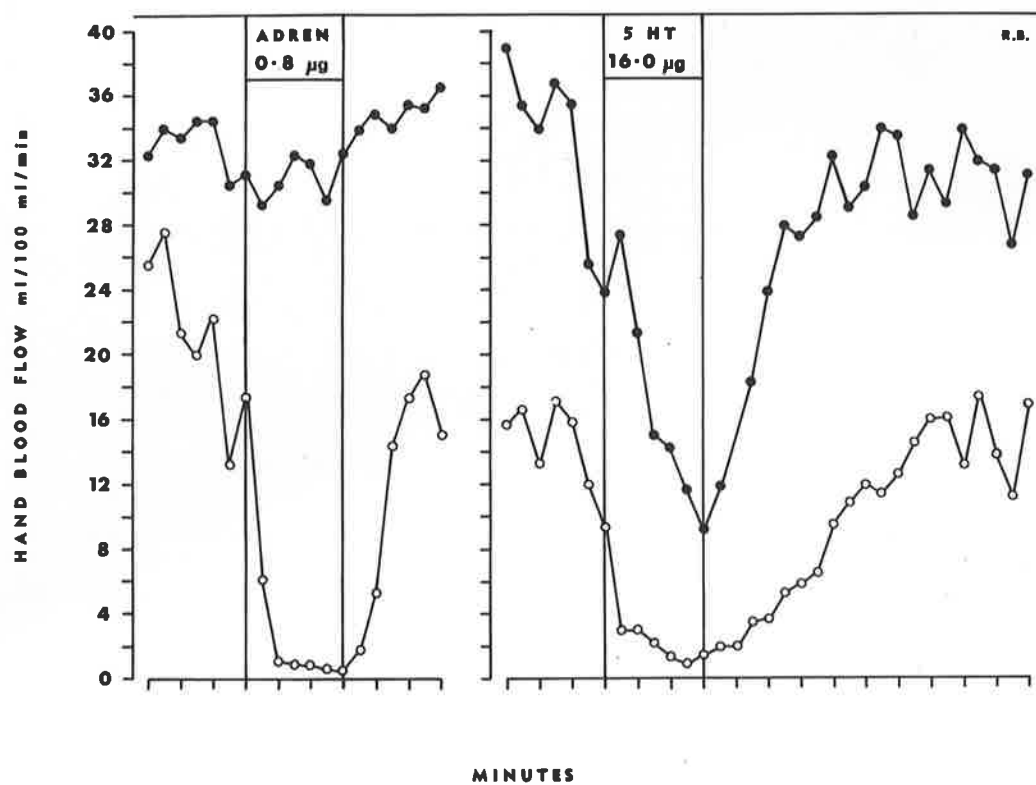


Fig 2-9. The effect of intra-arterial infusion of adrenaline (ADREN, 0.8 µg/min) and serotonin (5HT, 16.0 µg/min) on the blood flow through the hand before (○) and after (●) intra-arterial infusion of phenoxybenzamine (250 µg/min for 8 min). Blood flow in ml/100 ml/min.

I. A. PHENOXYBENZAMINE

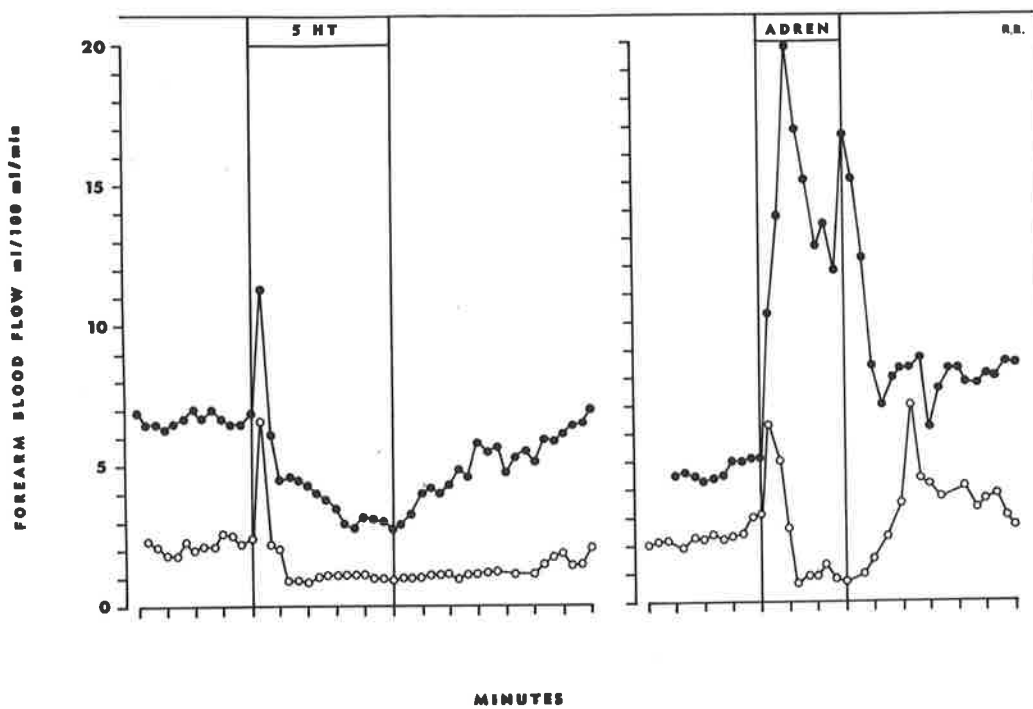


Fig 2-10. The effect of intra-arterial infusion of serotonin, 8 µg/min (5HT) and adrenaline, 0.4 µg/min (ADREN) on the blood flow through the forearm before (○) and after (●) intra-arterial infusion of phenoxybenzamine (250 µg/min for 8 min). Blood flow in ml/100 ml/min.

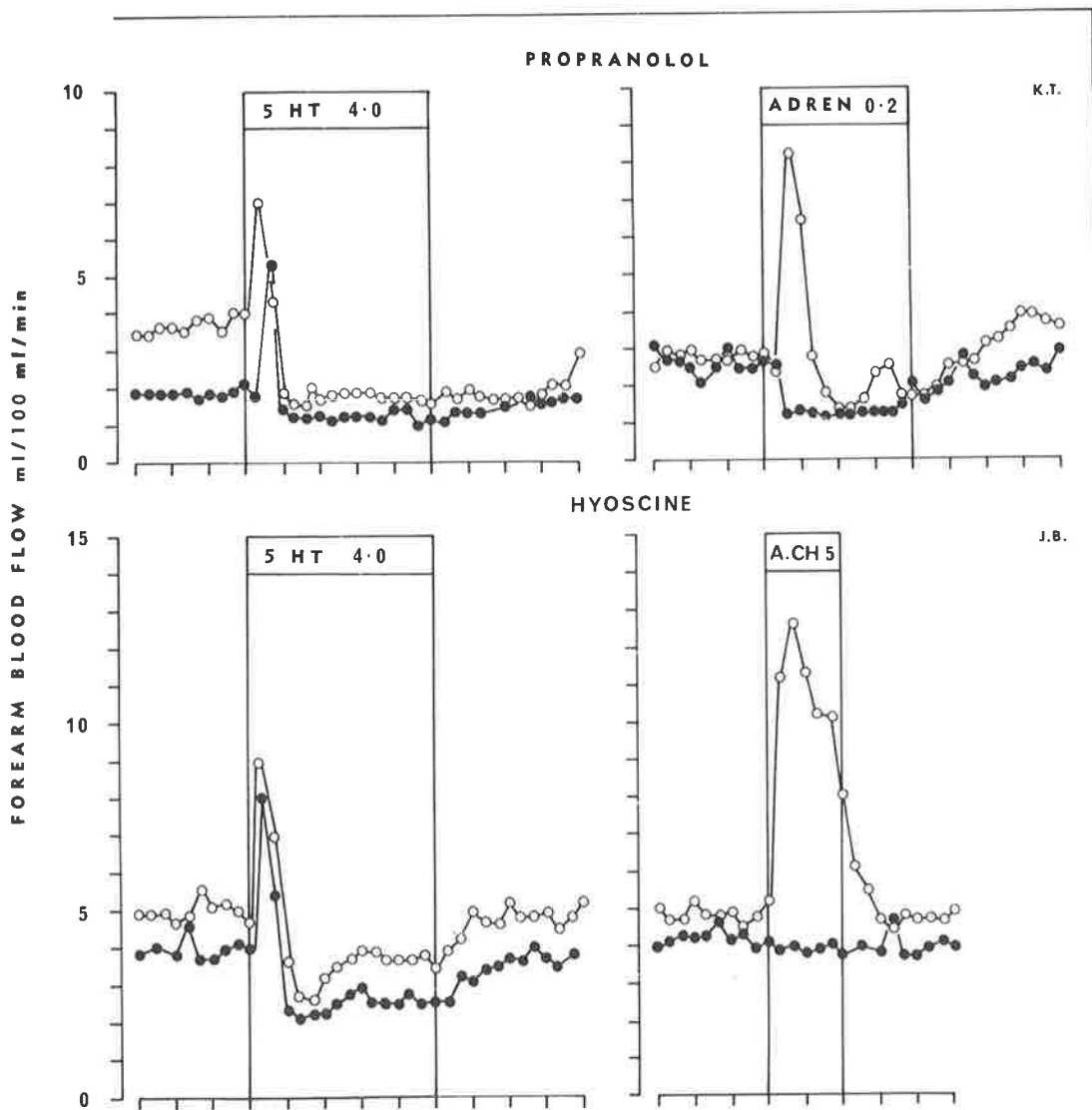


Fig 2-11. Upper frame (K.T.). The response of the forearm blood flow to intra-arterial infusion of serotonin (5HT, 4 $\mu\text{g/min}$) and adrenaline (ADREN, 0.2 $\mu\text{g/min}$) before ($\bigcirc$) and after ($\bullet$) intra-arterial infusion of propranolol (0.1 mg/min for 5 min). Lower frame (J.B.). The response of the forearm blood flow to intra-arterial infusion of serotonin (5HT, 4 $\mu\text{g/min}$) and acetylcholine (ACh, 5 $\mu\text{g/min}$) before ($\bigcirc$) and after ($\bullet$) intra-arterial infusion of hyoscine (0.1 mg/min for 4 min). Blood flow in ml/100 ml/min.

Mepyramine. Intra-arterial infusion of the specific antihistamine drug mepyramine (2.5 mg/min for 10 min) was administered to two subjects after control responses of the forearm vessels to serotonin (1 and 2 μ g/min for 4 min) and histamine (1 μ g/min for 3 min) had been recorded. The pattern of the response to serotonin was not altered in either subject by mepyramine whereas the dilator effect of histamine was almost completely abolished. Figure 2-12 illustrates the result of one of these experiments.

Serotonin and sympathetic innervation. The influence of sympathetic innervation on the vasoconstrictor action of serotonin on the hand was investigated in four experiments. Intra-arterial serotonin (2 and 4 μ g/min for 5 min) was infused before and after intra-arterial administration of the sympathetic nerve-blocking agent bretylium (4 mg/min for 5 min) in two subjects. The effect of serotonin was unchanged. The effectiveness of the bretylium-induced sympathetic nerve block was demonstrated by abolition of the sympathetic constrictor reflex response of hand vessels to the stimulus of ice applied to the neck (Cooper, Fewings, Hodge and Whelan, 1963). This independence of serotonin of the autonomic nerve supply was confirmed by the observation of normal responses of the hand vessels to intra-arterial serotonin in two patients who had undergone cervical sympathectomy many months previously and in whom all sympathetic nerve activity was absent as evidenced by the

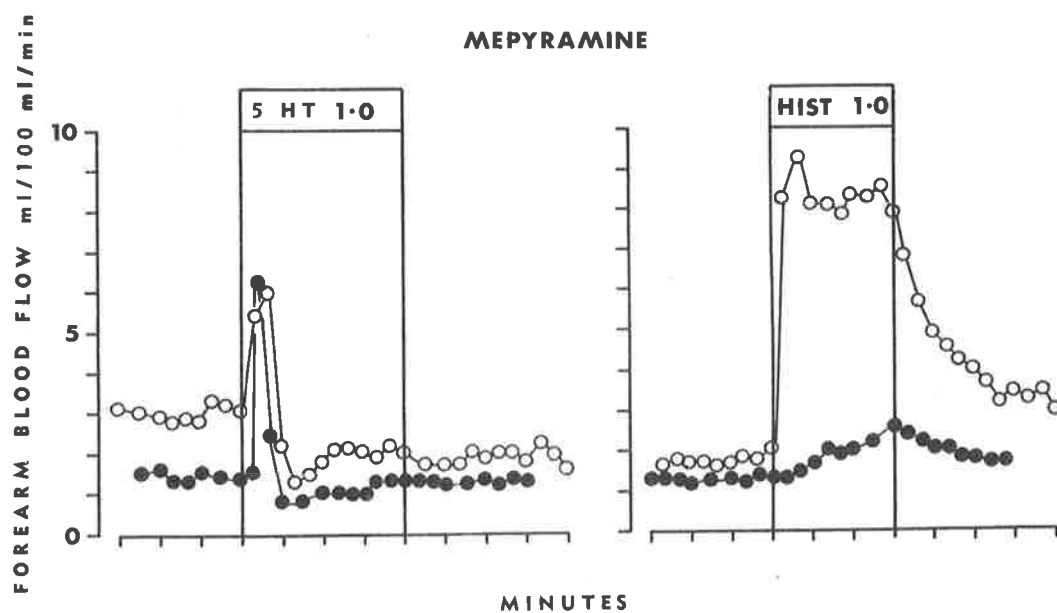


Fig 2-12. The response of the forearm blood flow to intra-arterial infusion of serotonin (5HT, 1.0 $\mu\text{g}/\text{min}$) and histamine (HIST, 1.0 $\mu\text{g}/\text{min}$) before (○) and after (●) intra-arterial administration of mepyramine 2.5 mg/min for 10 min.

lack of reflex sympathetic responses and loss of vasoconstriction to intra-arterial ephedrine (Parks, Skinner and Whelan, 1961).

Figure 2-13 illustrates the results of one experiment where bretylium was administered (left hand frame) and one in which serotonin was infused into a sympathectomised limb (right hand frame).

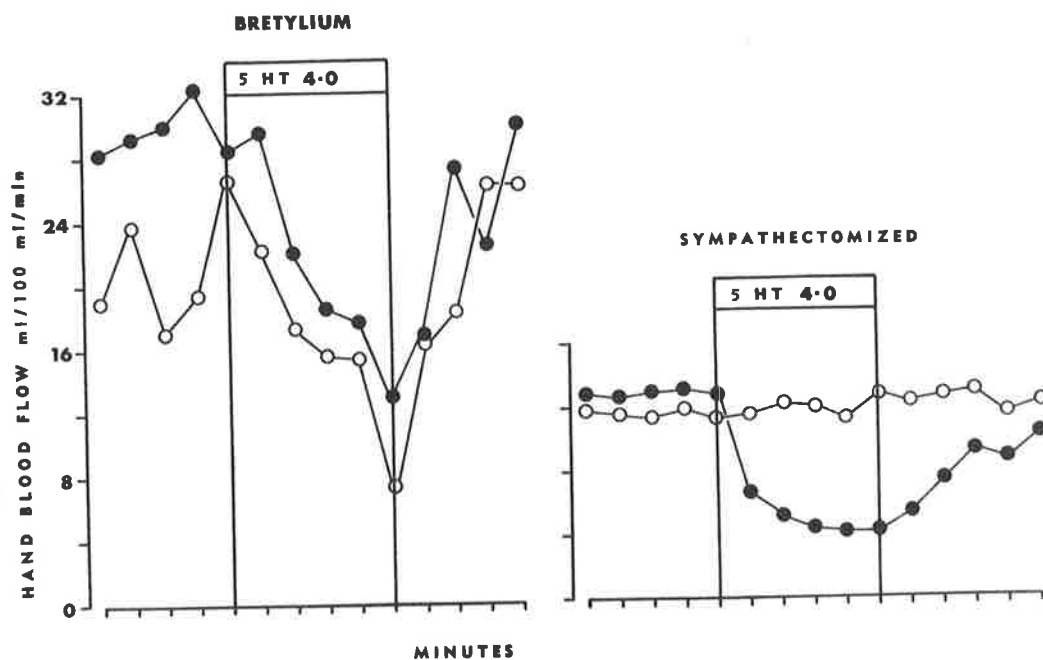


Fig 2-13. Left hand frame. The response of the hand blood flow to intra-arterial serotonin (5HT, 4.0 $\mu\text{g}/\text{min}$) before ($\bigcirc$) and after ($\bullet$) intra-arterial infusion of bretylium 4 mg/min for 5 min. Right hand frame. The effect of intra-arterial infusion of serotonin (5HT, 4.0 $\mu\text{g}/\text{min}$) on the blood flow through the hand (ml/100 ml/min) of a chronically sympathectomised limb ($\bullet$); $\bigcirc$, control side.

TABLE 2-1

The percentage changes in blood flow in the hands and forearms during intra-arterial infusion of serotonin before and after intra-arterial infusion of methysergide, 25 µg/min for 10 min. The minus and plus signs represent decreases and increases in flow respectively.

FOREARMS

Subject	Dose of intra-arterial serotonin							
	2 µg/min		4 µg/min		8 µg/min		16 µg/min	
	Before	After	Before	After	Before	After	Before	After
L.A.	-	-	-61.0	-12.0	-56.0	-20.0	-	-
S.S.	-	-	-	-	-50.0	- 6.3	-71.4	-18.0
V.H.	-13.6	- 4.0	- 4.6	-18.1	-37.5	- 4.5	-	-
D.R.	+28.2	+62.2	-	-	-53.7	+28.2	-	-
J.L.	-58.0	+18.3	-	-	-69.2	-25.0	-	-
B.T.	-	-	-	-	-33.3	+27.3	-	-
H.C.	-	-	-11.7	+13.3	-	-	-	-
A.P.	-	-	-40.9	+52.6	-	-	-	-
Average	-14.3	+25.5	-29.5	+ 8.9	-49.9	-0.08	-71.4	-18.0

HANDS

O.C.	-	-	-	-	- 1.1	+30.2	-58.2	+25.0
J.D.	-	-	-	-	-46.1	-23.2	-	-
M.L.	-	-	-	-	-	-	-30.0	+ 6.1
W.C.	-	-	-	-	-	-	-65.0	-12.3
J.R.	-	-	-	-	-54.2	+13.0	-	-
D.C.	-	-	-	-	-	-	-63.3	+25.0
Average	-	-	-	-	-37.1	+ 6.6	-54.1	+10.9

DISCUSSION.

The antagonism by methysergide of vasoconstrictor effects of serotonin on the vessels of the hand and forearm in man is of the same nature as the antagonism by BOL 148 described by Glover et al (1957). Lysergic acid and its derivatives, including BOL 148, have been shown to antagonise the effects of serotonin on isolated smooth muscle preparations (Gaddum and Hameed, 1954). Similarly, this more recent lysergic acid derivative, methysergide, has been found to be an effective antagonist of serotonin on animal preparations. Doepfner and Cerletti (1958) showed that methysergide has four to six times the potency of lysergic acid in antagonising serotonin-provoked oedema in the rat's paw.

Using the technique of superficial and deep venous blood oxygen saturation estimations to follow changes in skin and muscle blood flow, Whelan (1967) showed that intra-arterial infusion of serotonin to the forearm caused a fall in the oxygen saturation of blood draining from skin and a rise in that of blood draining from muscle. Assuming that the oxygen consumption of the tissues remains constant then a fall in oxygen saturation of the sampled blood denotes a decrease in blood flow, and a rise in saturation an increase. Thus the total forearm flow changes with infused serotonin were considered to be a resultant of vasoconstriction in skin and vasodilatation in muscle. A similar conclusion was reached

by Bock et al (1957) who measured the skin blood flow with a heat flow calorimeter and muscle blood flow with the Hensel needle. This observation that serotonin, although a constrictor of the hand vessels and those of the skin of the forearm, is a dilator of the vessels of the underlying muscle is in accord with the suggestion of Fox, Goldsmith, Kidd and Lewis (1961) and accounts for the fact that when the skin flow is high serotonin causes a fall in total forearm flow due to the predominant effect of the skin vessel constriction, whereas, when the skin flow is low an increase in forearm flow may be seen due to the muscle dilatation being more manifest (Barcroft and Whelan, 1962; personal communication; Fig 2-14). These opposite effects of serotonin on skin and muscle vessels resemble closely those of adrenaline. That there is a marked constrictor component to serotonin's action on muscle vessels is demonstrated by the fact that after antagonism of the constrictor effect by methysergide a greater and more sustained dilatation of muscle vessels occurred. It would appear that skin vessels have constrictor receptors to serotonin while the underlying muscle vessels have both constrictor and dilator receptors, the action on the latter normally predominating at low dosage levels of serotonin. At higher dosage levels the vasoconstrictor action on muscle assumes greater importance and hence, along with skin vasoconstriction, probably explains the dose-dependent fall in forearm blood flow observed with increasing doses of intra-arterial

I.A. 5-HYDROXYTRYPTAMINE

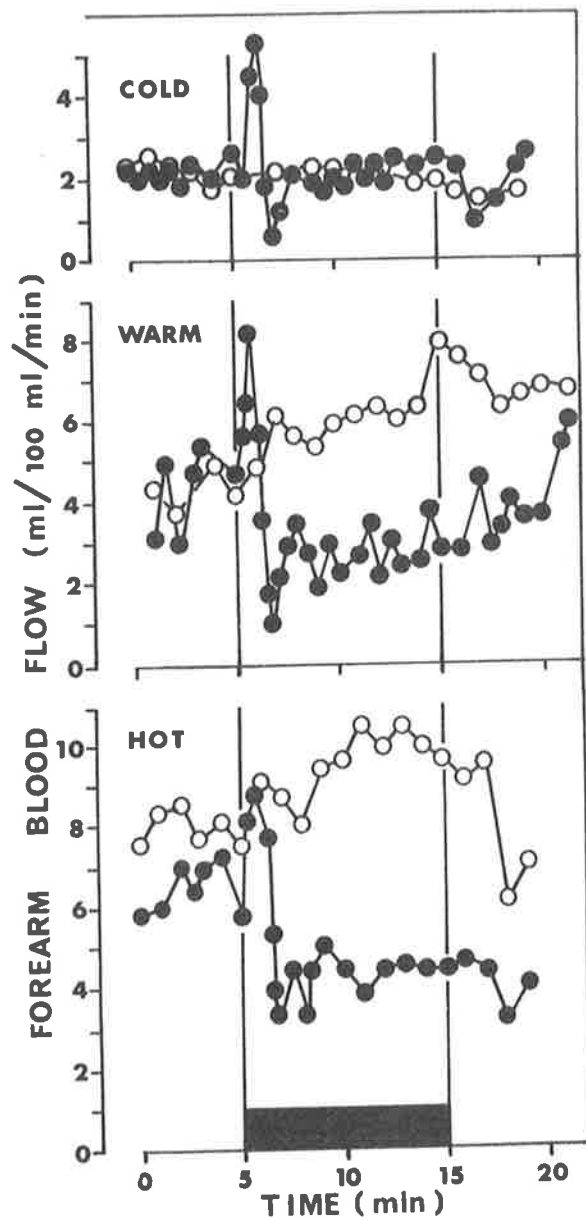


Fig 2-14. The response of the forearm blood flow to intra-arterial infusion of serotonin, 5 μ g/min for 10 min (black rectangle), at different levels of skin blood flow achieved by indirect body cooling or heating. Upper frame, cold; middle frame, warm; lower frame, hot; $\bullet$, infused side; $\circ$, control side. (Personal communication, Barcroft and Whelan, 1962).

serotonin.

As with BOL 148 the antagonism by methysergide is not a consequence of a non-specific inhibition of smooth muscle since the ability of the vessels to respond to other vasoactive drugs, both dilator and constrictor, was unaffected. Methysergide was therefore considered to be a specific and effective antagonist of peripheral vasoconstrictor effects of serotonin. Although it is difficult to extrapolate drug actions from one vascular bed to another this action of methysergide may contribute to its effectiveness in preventing migraine, the prodromal stage of which is associated with vasoconstriction of extra-cranial and intracranial arteries, an action ascribed in part to serotonin (Sicuteri, 1959). As methysergide itself has little effect on limb blood flow it is unlikely that its action in preventing migraine is due to direct antagonism of the phase of vasodilatation of cranial vessels, a conclusion which is supported by the fact that it has no therapeutic value during a fully-developed migrainous headache attack (Friedman and Elkind, 1963). This finding of absence of direct vasoconstrictor activity is supported by Wolff (1963) who has proposed that methysergide is effective in migraine prophylaxis because it potentiates endogenous noradrenaline, has an anti-inflammatory action, and dampens vasomotor reflexes and cranial vessel responses. However, Kimball, Friedman and Vallejo (1960)

and Lance, Curran and Anthony (1965) attributed methysergide's effectiveness in prevention of migraine to simulation of serotonin for they reported that depletion of serotonin is an important factor in the prodromal phase of migrainous headaches.

The effect of methysergide in opposing the potent veno-constrictor action of serotonin was observed in two experiments. This anti-venoconstrictor effect may explain the abolition of the skin flush which normally occurs during intra-arterial infusion of serotonin since Oyvin and Shegal (1965) attribute the serotonin flush to a hyperaemia of stasis, a consequence of constriction of large veins plus small vessel dilatation and oedema formation. Similarly, Haddy, Fleishman and Emanuel (1957) have found that intra-arterial serotonin increased large vein resistance and decreased small vein resistance in the dog's forelimb.

McCubbin, Kaneko and Page (1962) suggested that adrenergic dilator receptors mediate the ability of serotonin to oppose neuro-genic vasoconstriction in the perfused hind-limb of the anaesthetised dog. However, the β -adrenoreceptor blocking agent propranolol did not affect the pattern of response of the human forelimb to infused serotonin. Similarly, the α -adrenoreceptor blocking drug phenoxybenzamine did not block serotonin-induced vasoconstriction. Both blocking agents were effective antagonists of infusions of adrenaline in the same experiments. This lack of modification by phenoxybenzamine of the constrictor effect of serotonin on vascular smooth

muscle in the hand and forearm in man contrasts with the antagonistic effects of the α -adrenoreceptor blocking agents dibenzyline and dihydroergotamine on the spasmogenic action of serotonin on smooth muscle of the guinea pig ileum reported by Gaddum and Picarelli (1957). Lysergic acid and its derivatives, considered by the latter authors to block the same D receptors as dibenzyline and dihydroergotamine, however, are effective antagonists on both preparations. The difference in responses following administration of the α -adrenoreceptor blocking agents probably reflects species or dosage differences or differences in the properties of vascular and intestinal smooth muscle.

The dilator action of serotonin on the muscle vessels of the forearm was not blocked by methysergide nor was it modified by blockade of the β -adrenoreceptors. Therefore involvement of the acetylcholine or histamine receptors was considered. The acetylcholine receptor blocking agent hyoscine did not modify the effect of serotonin on the forearm indicating that its dilator action was independent of the acetylcholine vasodilator receptors, and similarly it was independent of histamine receptors, since the antihistamine mepyramine did not affect the vasodilator action of serotonin on the forearm, whereas the dilator effect of histamine was almost completely abolished. Thus there was no evidence in man to support either release of histamine from skin and muscle by serotonin (as reported in perfused skin and muscle of the cat and dog by

Feldberg and Smith, 1953) or direct action of serotonin on histamine receptors.

Acute blockade of the sympathetic nerve supply to the hand by bretylium and chronic blockade resulting from cervical sympathectomy with consequent degeneration of peripheral sympathetic nerves did not modify the effects of intra-arterially administered serotonin indicating that release of an autonomic transmitter substance or interaction with peripheral sympathetic nerves plays no part in the peripheral vascular actions of serotonin.

It is concluded that in the case of the vessels of the upper limb in man serotonin acts upon specific constrictor and dilator receptors which are independent of the α and β -adreno-receptors, of acetylcholine and histamine receptors and also of sympathetic innervation.

SUMMARY.

1. Serotonin infused into the brachial artery in man caused vasoconstriction in skin and vasodilatation in muscle, the latter masking a muscle vasoconstrictor action.
2. Intra-arterial methysergide specifically antagonised the vasoconstrictor effects of serotonin on skin and muscle of the upper limb in man.
3. Methysergide antagonised serotonin-induced venoconstriction and skin flush.
4. The peripheral effects of serotonin on the upper limb were independent of the adrenoreceptors, acetylcholine and histamine receptors and of sympathetic innervation.
5. It is concluded that specific serotonin receptors are present in vascular smooth muscle in the forearm and hand in man.

97.

PART B

SOME OBSERVATIONS ON THE ROLE OF SEROTONIN
AND BRADYKININ IN THE CARCINOID SYNDROME.

INTRODUCTION

As outlined in the GENERAL INTRODUCTION recent evidence has suggested that the carcinoid syndrome (the association of a tumour of enterochromaffin cells with diarrhoea, facial flushing and possibly endocardial lesions on the right side of the heart) is not simply due to overproduction of serotonin. The early work of Lembeck (1953) and Pernow and Waldenstrom (1954) has been amply confirmed, and serotonin overproduction undoubtedly occurs. However, many workers have remarked that the vascular response to serotonin does not explain the cutaneous vasodilatation in carcinoid attacks (Robertson, Peart and Andrews, 1962; Sjoerdsma and Melmon, 1964). Interest has recently been expressed in the plasma kinins as possible mediators of the flushing episodes. Bradykinin, a nonapeptide, which is the best known of these small peptides is released from α -2 globulin precursors (kininogens) by the action of various enzymes such as trypsin and a group of enzymes occurring widely in the body, collectively called kallikreins. Small amounts of bradykinin normally circulate in the blood (Zacest and Mashford, 1967) and are subject to attack by an enzyme in plasma called kininase, which destroys the biological activity of the peptide by removal of the C-terminal arginine residue.

Oates, Melmon, Sjoerdsma, Gillespie and Mason (1964) were able to detect increased amounts of kallikrein in liver metastases of nine patients with carcinoid lesions originating in the small

intestine. In addition, the hepatic venous blood contained large amounts of a kinin indistinguishable from bradykinin. Oates, Pettinger and Doctor (1966) extended these observations by identifying the kinin in hepatic venous blood as the nonapeptide, bradykinin. In the present study the systemic arterial blood levels of bradykinin were estimated in three patients with the carcinoid syndrome in the resting state and during attacks of flushing. Bradykinin and serotonin were administered intravenously to two of the patients in an attempt to mimic the features of the syndrome and the effect of methysergide on the flushing and the abdominal symptoms was investigated in these two patients also. The value of methysergide, a specific competitive antagonist of serotonin, in the management of intestinal symptoms (especially diarrhoea) in patients suffering from the carcinoid syndrome has been described by Peart and Robertson (1961), Melmon, Sjoerdsma, Oates and Laster (1965) and Mengel (1965).

Since prolonged exposure of tissues to a circulating vasoactive agent may be associated with the development of the phenomenon of tachyphylaxis, the sensitivity of the hand or forearm blood vessels to intra-arterially administered serotonin was studied in three patients.

Thus, the present study attempts to clarify the role of serotonin and bradykinin in the carcinoid syndrome.

CASE HISTORIES

Four patients were studied in whom carcinoid syndrome could be confidently diagnosed on the basis of the clinical history, excess urinary excretion of 5-hydroxy-indoleacetic acid (5HIAA) and ultimately by histopathology (biopsy or autopsy).

Case 1 (E.B.) - A female, aged 69 yr, who presented with diarrhoea (10-15 bowel actions daily), weight loss and facial flushing attacks which were induced by meals, bending or defaecation. Gross hepatomegaly was present. The urinary excretion of 5HIAA ranged from 165-687 mg in 24 hr (normal, 10 mg in 24 hr). The patient's condition deteriorated rapidly, and she died of hepatic failure 12 months after she was first diagnosed. Autopsy revealed an ileal carcinoid tumour with hepatic metastases.

Case 2 (L.C.) - A female, aged 71 yr, who in 1953 had undergone excision of an ileal tumour which was identified on histological examination as a carcinoid tumour. In 1962 she began to have diarrhoea and flushing attacks. In 1965 the symptoms had intensified (4-5 bowel actions daily), and gross hepatomegaly was present. Urinary excretion of 5HIAA ranged from 60-106 mg per 24 hr in 1965, during which time she remained ambulant. Her condition progressed downhill slowly and she died in 1968.

Case 3 (L.H.) - A male, aged 35 yr, who presented with weight loss, mental depression and continuous flushing, and was



found to have tricuspid stenosis and incompetence. Gross hepato-megaly was present. The urinary excretion of 5HIAA was 320 mg in 24 hr. His condition deteriorated rapidly, and autopsy revealed widespread metastases from a small bowel carcinoid tumour. During his time in hospital the patient had been continuously flushed, but had in addition noted a hot feeling in his face and trunk when his liver was compressed by twisting or bending movements, although there was no obvious change in his flushed appearance.

Case 4 (J.C.) - A male, aged 45 yr, who gave a history of recurrent diarrhoea and abdominal pain for 10 yr. Occasional facial flushing attacks had occurred during the past two years. A hard fixed mass in the lower right quadrant of the abdomen was found at laparotomy to be an inoperable ileal malignancy. Histopathology confirmed that it was a carcinoid tumour. Several hepatic metastases were found at the operation. Urinary 5HIAA was elevated to 35 mg in 24 hr. The patient's condition is gradually deteriorating with increase of diarrhoea and flushing symptoms and obvious enlargement of the inoperable primary tumour.

METHODS

Bradykinin levels in systemic blood were assayed by the method described by Zacest and Mashford (1967), where 30 ml samples of blood were withdrawn rapidly from the brachial artery into nylon syringes and the kininase activity of the sample immediately suppressed by transferring the blood into 96% ethanol at 0°C. Subsequent chemical extraction and assay on the oestrus rat uterus and duodenum permitted measurement of the kinin levels in all subjects studied; in normal subjects a mean resting level of 0.30 ng/ml (standard deviation, 0.27 ng/ml) was found, and recovery of the internal standard averaged 97%, with a range from 80% to 110%.

Blood pressure was recorded with a Statham pressure transducer (P23AC) and Grass Polygraph (Model 5D) from the arterial catheter used for sampling. Heart rate was counted from the blood pressure record. Intravenous and intra-arterial infusions were administered through a needle or catheter by a motor-driven syringe. Hand blood flow was measured with the water-filled plethysmograph and forearm flow with the Adelaide capacitance plethysmograph. Skin temperature on the forehead was registered by a copper-constantan thermocouple and Elektrolaboratoriet galvanometer and recorded at intervals of one-half or one min in Cases 1 and 2.

Advantage was made of the phenomenon observed by Peart,

Robertson and Andrews (1959) that facial flushing could be produced in patients with the carcinoid syndrome by intravenous doses of adrenaline or noradrenaline. Thus, flushing attacks were induced in three patients by intravenous administration of small doses of adrenaline. In the other patient (Case 3, who flushed continuously) an attempt was made to exacerbate the flushing by pressing on the liver.

In Case 1 (E.B.), an extract of tumour tissue (post-mortem) was assayed on the fundal strip of rat stomach (Vane, 1957) to determine serotonin content of the tumour.

The drugs used were adrenaline hydrochloride (D.H.A.), bradykinin (BRS640, Sandoz), methysergide (1-methyl-d-lysergic acid butanolamide bimaleate, UML491, Deseril, Sandoz) and serotonin (5-hydroxytryptamine creatinine sulphate, SR134, Sandoz).

RESULTS.Resting levels of bradykinin

In Case 2 (L.C.) the resting bradykinin level of 0.15 ng/ml in arterial blood was within the range for normal subjects (0.30 ± 0.27 ng/ml). In Case 3 (L.H.), who flushed continuously, the arterial level was 1.1 ng/ml, which was higher than has been found in any normal subject. However, the level of 10 ng/ml in Case 1 (E.B.) was by far the highest that has been found with this method, despite the absence of flushing at the time of sampling. Resting levels are summarised in Table 2-2. Estimation of bradykinin levels was not performed in Case 4 (J.C.).

Bradykinin levels during provoked flushing

Intravenous infusions of adrenaline were effective in provoking a flush in Case 1 (E.B.) at a dose rate of 0.5 μ g/min for 3 min, in Case 2 (L.C.) at a dose of 2 μ g/min for 4 min and in Case 4 (J.C.) at 4 μ g/min for 2 min. In each of the three patients an intense, red, hot, facial flush which extended down the neck appeared within two min of commencement of the infusion of these relatively low doses of adrenaline. Blood samples for bradykinin were taken at the height of the response. In each case concomitant with the flush there was a fall in blood pressure (systolic blood pressure decreased 40-50 mm Hg) and a tachycardia

developed. Forehead skin temperature rose $0.5-1.0^{\circ}\text{C}$ during flushing episodes in Cases 1 and 2, and forearm blood flow, measured in Cases 2 and 4, rose markedly. No abdominal symptoms occurred during any of the flushing episodes. Recovery from the cutaneous vasodilatation and general circulatory changes was complete within 5 min following cessation of the adrenaline infusion.

Figure 2-15 is a representative example of the response of systemic blood pressure and heart rate to the adrenaline-induced flushing. Adrenaline was not given in Case (L.H.) but blood samples for bradykinin estimation were taken while pressure was applied over the liver.

As summarised in Table 2-2 the arterial blood bradykinin levels rose during the provoked flushing. In Case 2 (L.C.) the bradykinin level doubled (0.15 to 0.3 ng/ml). In Case 3 (L.H.) a 20% increase in arterial bradykinin level (1.1 to 1.3 ng/ml) was associated with pressure over the liver, at which time the patient had complained of an increased sensation of heat in the face, and in Case 1 (E.B.) there was a 50% increase in arterial bradykinin (10.0 to 15.0 ng/ml) during the adrenaline-induced flush.

Effect of bradykinin, serotonin and methysergide

Infusion of synthetic bradykinin $16\text{ }\mu\text{g/min}$ for 5 min to Case 1 (E.B.) reproduced the subjective and objective features of the adrenaline-induced facial flushing: in particular, the flush,

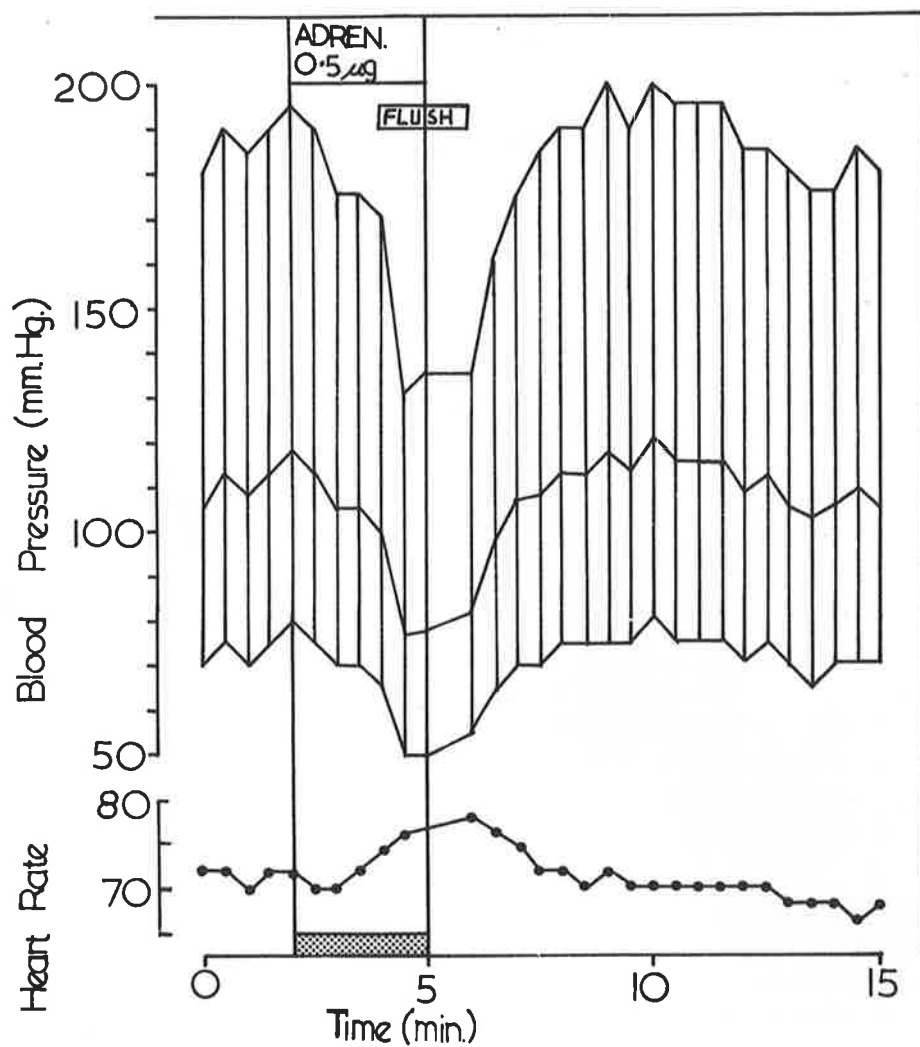


Fig 2-15. Case 1 (E.B.). Flush, fall in systemic arterial blood pressure and tachycardia associated with intravenous infusion of adrenaline, 0.5 µg/min.

the fall in systemic blood pressure (again of the order of 50 mm Hg systolic) and tachycardia (Fig 2-16). Similarly it was found that intravenous bradykinin (25 μ g/min for 5 min) mimicked the flush, tachycardia and transient hypotension observed during adrenaline infusion in Case 2 (L.C.). No gastro-intestinal symptoms occurred in either patient during administration of bradykinin.

The effect of intravenous administration of methysergide on adrenaline-induced flushing and the effects of intravenous serotonin were also examined in both Cases 1 and 2. Methysergide (100 μ g/min for 10 min intravenously failed to modify the adrenaline-provoked flush, depressor effect or tachycardia in either case.

Intravenous infusion of serotonin (2 mg/min for 5 min) caused quite a different response compared with the adrenaline or bradykinin response. In both of the above patients a rise in systolic pressure of approximately 20 mm Hg and a slight tachycardia (about 10 beats/min increase) was recorded during the serotonin infusions. No flushing or change in forehead skin temperature occurred but abdominal cramps prompted each patient to request termination of the infusion. Methysergide (1 mg) did not modify the cardiovascular effects of the intravenous serotonin infusions but completely abolished the abdominal cramps. Therefore, oral methysergide was administered therapeutically in an attempt to control the diarrhoea in Cases 1 and 2. Oral methysergide up to 18 mg daily over the period of hospitalisation produced a marked reduction in

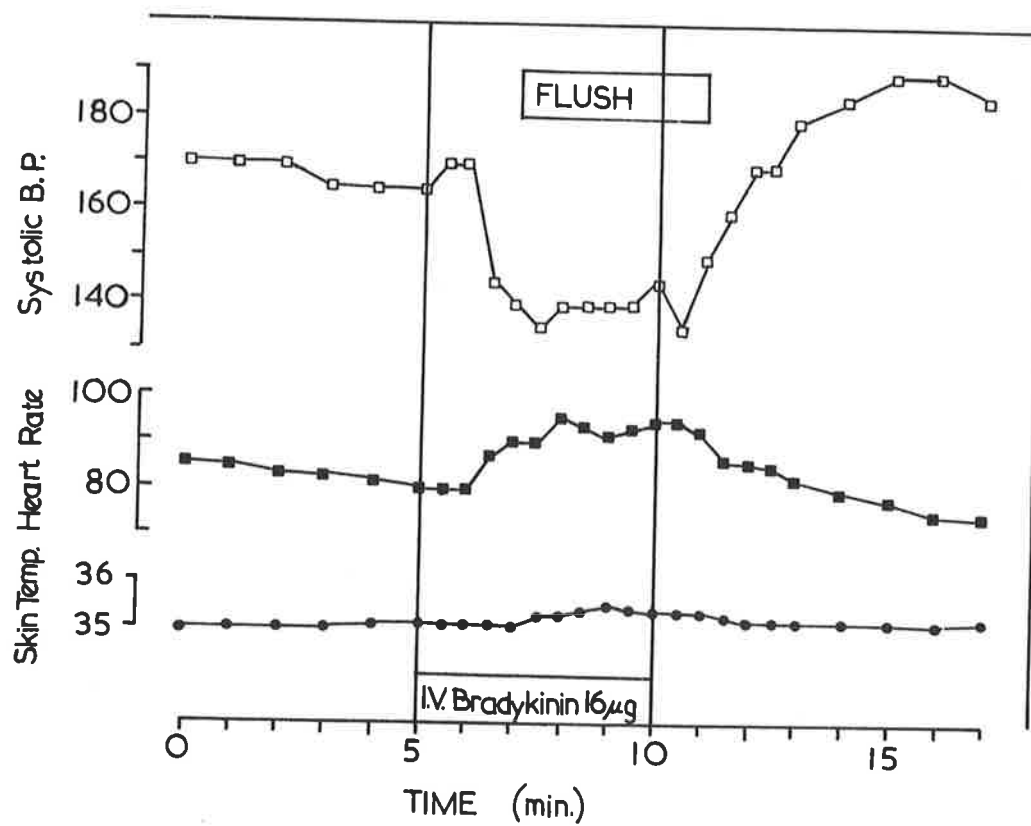


Fig 2-16. Case 1 (E.B.). Effect of intravenous bradykinin, 16 μ g/min, on systolic blood pressure (□), heart rate (■), and forehead skin temperature (●).

bowel frequency from 12-15 per day to 3-5 per day in Case 1 (E.B.). Concomitant with this, there occurred a parallel decrease in the frequency of flushing (Fig 2-17), which was probably due to removal of one of the principal stimuli, viz, defaecation with its associated increase in intra-abdominal pressure and hence pressure on the liver metastases. Diarrhoea recurred when methysergide therapy was temporarily interrupted. Oral methysergide also proved effective in controlling diarrhoea in Case 2 (L.C.). Methyl prednisolone, the efficacy of which has been reported by Ureles, Murray and Wolff (1962), was also administered to Case 1, but was found to be of no therapeutic value, and indeed increased the frequency of flushing attacks.

The suspected presence of large amounts of serotonin in the tumour tissue of Case 1 was confirmed by assay of tumour tissue extract on the fundal strip of rat stomach: the tumour contained serotonin 145 $\mu\text{g/g}$ wet tumour tissue, compared with the expected normal concentration of serotonin in ileal tissue of 1.2-4.6 $\mu\text{g/g}$ (Gowenlock and Platt, 1962).

Intra-arterial sensitivity studies

Continual exposure of vascular beds to increased circulating levels of a vasoactive substance may lead to development of a refractory state, the phenomenon of tachyphylaxis. For instance, in man, Scroop (1967) found decreased sensitivity of hand blood vessels

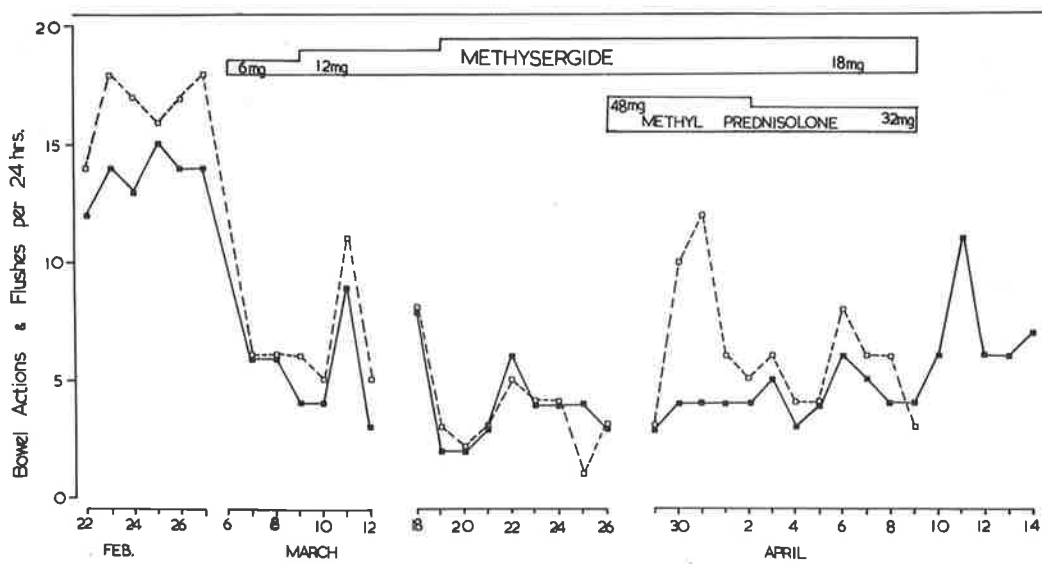


Fig 2-17. Case 1 (E.B.). Effect of oral methysergide and methyl prednisolone therapy (dosage: mg/24 hr) on daily frequency of spontaneous facial flushing attacks (□) and bowel actions (■).

to the vasoconstrictor action of intra-arterial noradrenaline in two patients with phaeochromocytomata, and similarly Scroop and Whelan (1968a) described gross reduction in sensitivity of hand vessels to the constrictor effect of angiotensin in three cases of renal hypertension. Since the levels of circulating serotonin are generally elevated in carcinoidosis, the sensitivity of hand or forearm vessels to intra-arterial serotonin was investigated to determine whether tachyphylaxis was a feature in this situation also. Intra-arterial infusions of serotonin in doses of 2, 4, 8 and 16 $\mu\text{g}/\text{min}$ for 5 min were administered to the hand vessels in Cases 1 and 2 and dose-response curves (percentage fall in blood flow/log dose) constructed. However the general instability of the hand blood flow measurements and unsatisfactory responses to the serotonin infusions prevented any conclusions being drawn regarding sensitivity when the responses were compared with dose-response curves obtained from 10 normal subjects (some from Section 2, Part A). Therefore the sensitivity of forearm blood vessels to intra-arterial serotonin was studied in the most recent case (J.C.). Doses of serotonin of 2, 4 and 8 $\mu\text{g}/\text{min}$ for 5 min failed to induce the usual fall in forearm flow (c.f. normal subjects, Table 2-1) and hence a reduction in sensitivity, i.e. tachyphylaxis, to serotonin is suggested by this one experiment.

PATIENT	ARTERIAL BLOOD BRADYKININ* LEVEL (ng./ml.)			URINARY 5 H.IAA EXCRETION (mg./24 hrs)	CLINICAL STATUS
	Resting	Flushing	Provocation		
L.C. ♀ 71 yrs.	0.15	0.31	I.V. ADREN.	60-106	Ambulant.
L.H. ♂ 35 yrs.	★ 1.1	1.3	PRESSURE on LIVER	320	Incapacitated; Right heart disease.
E.B. ♀ 69 yrs.	1.0	15	I.V. ADREN.	165-687	Rapid deterioration & death.

* NORMAL ARTERIAL BK LEVEL = 0.30 ng/ml. (S.D. 0.27 ng/ml)

★ PERMANENTLY FLUSHED

Table 2-2. A summary of arterial blood bradykinin levels (resting and during provoked flushing), 24 hr urinary 5-hydroxy-indoleacetic acid excretion, and clinical status in three patients with the carcinoid syndrome.

DISCUSSION.

These findings add to the accumulating evidence that the flushing attacks which characterise the carcinoid syndrome are associated with and presumably are due to elevated levels of a kinin peptide, probably bradykinin, in the blood. The blood levels of bradykinin reported here are lower than values which have been described by others, however the satisfactory recoveries of internal standards suggest that this method gives an accurate reflection of true levels. Recently others have reported changes in peripheral blood kinin levels during carcinoid flushes. Mason and Melmon (1966) found elevations of peripheral arterial blood concentrations of bradykinin (from 11 $\mu\text{g}/10\text{ ml}$ resting to 78 $\mu\text{g}/10\text{ ml}$ during adrenaline-induced flushing in 4 patients) and also kallikrein. As in the present study they found that the cardiovascular changes with intravenous bradykinin mimicked those of the flushing episodes. Zeitlin and Smith (1966) showed a rise in kinin levels in forearm venous blood during flushing in three or four carcinoid patients studied. The destruction of bradykinin by blood kininases is very rapid so that only approximately 10% of bradykinin infused into the brachial artery survives to appear in the venous effluent (Zacest and Mashford, 1967). Under these circumstances it is surprising that Zeitlin and Smith (1966) found that venous blood levels rose in the manner described.

An unexplained feature in Case 1 (E.B.), who had a blood bradykinin level higher than any other subject studied, was the absence of permanent vasodilatation. This implies a reduced sensitivity to the peptide. Case 2(L.C.) presents a problem in that she exhibited low-normal resting blood bradykinin levels, and, although this doubled during the adrenaline-induced flush, it was still well within the normal range. It seems, therefore, that there is considerable variation in the absolute sensitivity of vascular smooth muscle to the effects of bradykinin but that any substantial increase from the resting level may cause a response. The relationship between blood and tissue levels of bradykinin is also unknown and this may be an important factor.

The rise in arterial blood bradykinin levels caused by adrenaline infusions in the carcinoid syndrome is particularly striking in view of the fall reported in six normal subjects by Mashford and Zacest (1967) who attributed the decrease in bradykinin levels to activation of the bradykinin-destroying enzyme, kininase, by adrenaline. Hilton and Lewis (1955), from studies of the functional hyperaemia in the submandibular salivary gland, suggested that catecholamines release kallikrein from glandular tissue. Thus the mechanism of the flushing episodes provoked by adrenaline is probably entry of large amounts of kallikrein into the blood from the tumour tissue with consequent rise in blood bradykinin levels, but the mechanism is by no means clear. This augmented

release of kallikrein in the case of the carcinoid patients probably over-rides the tendency of bradykinin levels to fall under the kininase-activating action of adrenaline.

It is noteworthy that each of the patients in this study exhibited hepatomegaly from metastatic deposits of carcinoid tissue from mid-gut primary tumours. The features of the carcinoid syndrome are more common in association with hepatic infiltration when the hormones elaborated can enter the general circulation than when the carcinoid tissue drains into the portal system only since the liver is rich in monoamine oxidase which destroys serotonin (Sjoerdsma, 1959) and the increased circulation time probably permits breakdown of bradykinin in the blood in the latter case. The provocation of flushing by adrenaline administration, plus the refinement of bradykinin estimation would be a useful diagnostic test in assessing the presence and severity of carcinoid disease if bradykinin estimation was easier. The best routine screening test, however, is probably still the estimation of 24 hr urinary 5HIAA: as early as 1955, Sjoerdsma, Weissbach and Udenfriend found that elevated urinary 5HIAA excretion was a constant feature of this condition, and this was confirmed in the present series. Warner (1967) recommends estimation of blood levels of serotonin in addition to urinary 5HIAA in the assessment of serotonin overproduction.

The observation that increased intra-abdominal pressure,

associated with defaecation (Case 1) or pressing on the liver (Case 3), caused flushing and an elevation in blood bradykinin levels is in accord with the findings of Snow, Lennard-Jones, Curzon and Stacey (1955) who reported that squeezing, traction or massage of both primary and metastatic carcinoids would produce the syndrome.

While it is most likely that bradykinin is the principal factor responsible for the flushing attacks, the elevated bradykinin levels do not bear on the cause of the gastro-intestinal features of the syndrome. Bradykinin has variable effects on gut motility in animals (Winkler, Bauer and Gmeiner, 1965; Bauer, Gmeiner and Winkler, 1966) and its administration to two patients was not associated with abdominal discomfort. However, in these two patients (Cases 1 and 2) it was possible to reproduce the bowel symptoms by infusion of serotonin, without any pronounced vascular effect. Acute administration of methysergide abolished the bowel discomfort formerly complained of during serotonin administration, and chronic administration reduced the severity of the diarrhoea. The use of methysergide did not interfere in any way with the bradykinin effect or with the adrenaline-induced flushes. Thus these findings indicate that excess production of serotonin is responsible for the gastro-intestinal hurry of this syndrome. Peart (1966), one of many investigators who have reached a similar conclusion, noted that serotonin does not reproduce the warm red flush and also that the spontaneous flush of the syndrome is not

associated with elevated blood serotonin levels.

In addition to the above rather distinct actions of the two principal substances upon the cutaneous circulation and gastrointestinal motility it is possible that there is interaction between bradykinin and serotonin. Evidence for a synergism between these two vasoactive substances was reported by Weiner and Altura (1967) who found specific potentiation by topically-applied bradykinin of the venular constrictor action of serotonin on the mesocaecum of the rat.

Participation of other vasoactive agents is also likely. Pernow and Waldenstrom (1954) found evidence of histamine release in carcinoidosis and Sjoerdsma (1959) suggested local release of histamine by serotonin as a factor in the facial flushing. The occasional overproduction of the serotonin precursor 5-hydroxytryptophan, especially in tumours of fore-gut origin, has been documented by the latter author and by Sandler and Snow (1958). Involvement of substance P, a vasodilator substance originating from the gut, is remotely possible also (Robertson et al, 1962). Thus the variations in the clinical presentation of cases of carcinoid syndrome probably involve several vasoactive substances and complex interactions between them.

The intra-arterial sensitivity studies showed no evidence of tachyphylaxis to serotonin in the hand vessels but there was an

indication of the phenomenon in that the vasoconstrictor response of the forearm vessels to serotonin was absent in one subject. Examples of tachyphylaxis have been found in similar situations in which high circulating levels of vasoactive substances may exist - such as noradrenaline in phaeochromocytoma and angiotensin in some cases of renal hypertension (Scroop, 1967; Scroop and Whelan, 1968a). Tachyphylaxis is less likely to occur with serotonin, however, since almost all of the circulating serotonin is contained in the platelets and therefore the peripheral vessels are probably protected from exposure to high concentrations (Erspamer, 1954).

SUMMARY

1. Four cases are briefly discussed in which the carcinoid syndrome could be diagnosed with confidence.
2. Two patients showed elevated resting levels of arterial blood bradykinin and in three the level rose in association with provocation of flushing episodes by either intravenous infusion of adrenaline or by pressing on the liver.
3. Facial flushing was mimicked by bradykinin infusion and was unaffected by the serotonin antagonist methysergide.
4. Infusion of serotonin reproduced bowel symptoms, which could be abolished by methysergide administration.
5. Intra-arterial sensitivity studies suggested tachyphylaxis to serotonin in only one of three patients studied.
6. It was concluded that the flushing attacks which characterise the syndrome are associated with elevated circulating levels of bradykinin and that the gastrointestinal symptoms are related to serotonin overproduction. Bradykinin-serotonin synergism and participation of histamine, 5-hydroxytryptophan and substance P may be involved in the pathophysiology in some cases.

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PART C

A COMPARISON OF THE EFFECTS OF INTRA-ARTERIAL
AND INTRAVENOUS INFUSIONS OF ANGIOTENSIN AND
NORADRENALINE ON THE CIRCULATION IN MAN.

INTRODUCTION.

A number of studies which have compared the pressor actions of angiotensin and noradrenaline in man have shown that angiotensin is six to ten times more active on a weight basis or fifty to sixty times on a molar basis (Finnerty, Massaro, Chupkovich and Tuckman, 1961; McQueen and Morrison, 1961; de Bono, Lee, Mottram, Pickering, Brown, Keen, Peart and Sanderson, 1963). Both angiotensin and noradrenaline are potent vasoconstrictor agents and an intravenous infusion will elevate total peripheral resistance. It is to this property that they appear to owe their pressor actions rather than to any significant effect on cardiac output (Finnerty et al, 1961; Cumming, 1963). Although a number of vascular beds have been studied in man (de Bono et al, 1963; Wilkins and Duncan, 1941; Bock, Krecke and Kuhn, 1958; Daly and Duff, 1960) and in animals (Barer, 1961; Mandel and Sapirstein, 1962; Haddy, Molnar, Borden and Texter, 1962) the site of the major increase in peripheral resistance during angiotensin infusions remains uncertain.

The present study was undertaken to compare the actions of noradrenaline and angiotensin (val⁵-hypertensin II-asp- β -amide) on the peripheral circulation in man during both intravenous and intra-arterial administration. In addition, the pressor activity, heart rate changes and effects on central venous pressure were examined. The responses to noradrenaline have been used as a comparison since

the cardiovascular effects of this drug, in most cases, are due to direct receptor stimulation. This is almost certainly true of the hand vascular responses during both local and systemic infusions of noradrenaline and a comparison of the angiotensin responses during both methods of administration might expose the suggested indirect constrictor mechanisms of this agent which is the most powerful pressor substance known.

METHODS.

The subjects were volunteer medical students. The laboratory temperature was maintained at 23-24°C and the subjects rested on a couch for at least 40 min before observations began.

Intra-arterial infusions of drugs were administered from a motor-driven syringe delivering a volume of 2 ml/min through a 22-gauge short-bevel needle inserted into the brachial artery of one arm. Saline (0.9% W/V) was given throughout the control periods and was also used as a vehicle for the drugs. Blood flow in the hand or forearm was recorded three to five times every minute by venous occlusion plethysmography using water-filled plethysmographs. The flow in the opposite, uninfused, side was recorded as a control. The temperature of the water was 32°C and 34°C respectively for the hand and forearm.

Intravenous infusions were administered through a polythene catheter (Intracath, Bardic) inserted into an antecubital vein. Blood pressure was recorded from a needle or catheter in the brachial artery of one arm, using a capacitance electromanometer and ultra-violet oscillograph (N.E.P. type 1050) or a Statham pressure transducer (P23AC) and Grass Polygraph (Model 5D). Heart rate was read from the blood pressure tracing. In some experiments central venous pressure was also recorded with an electromanometer, a poly-

the catheter being inserted into an antecubital vein and passed centrally to the great veins in the thorax. During these latter experiments, assessment of changes in forearm muscle blood flow was made by the technique of venous blood oxygen saturation estimation.

The drugs used were noradrenaline bitartrate mono-hydrate (Levophed, Winthrop), and angiotensin II (val⁵-hypertensin II-aspartamide, Hypertensin CIBA). Doses of each drug are expressed as weights of the base. Ascorbic acid (1:50,000) was added to the noradrenaline solutions.

RESULTS.Intra-arterial infusions

Figure 2-18 (left hand frame) represents the responses of the hand vessels to the same three doses of angiotensin and noradrenaline (25, 50 and 100 ng/min for 4 min) in each of five experiments on five subjects. In most cases more than one infusion was given at each dose level (see legend, Fig 2-18). Similar responses were obtained in all subjects. Both angiotensin and noradrenaline reduced hand blood flow, the responses increasing with increasing doses, the effect of angiotensin being slower in onset and taking longer to wear off. To obtain the same degree of constriction a dose of angiotensin two to three times greater than that of noradrenaline was required.

Figure 2-18 (right hand frame) represents the responses of the forearm vessels to three doses of noradrenaline (16, 32 and 64 ng/min for 4 min) and of angiotensin (2, 4 and 8 ng/min for 4 min) in each of five experiments on five subjects. In all subjects each dose of noradrenaline and angiotensin was given twice. Both angiotensin and noradrenaline reduced forearm blood flow, the responses increasing with increasing doses, and being slower to develop with angiotensin and persisting for a longer period after

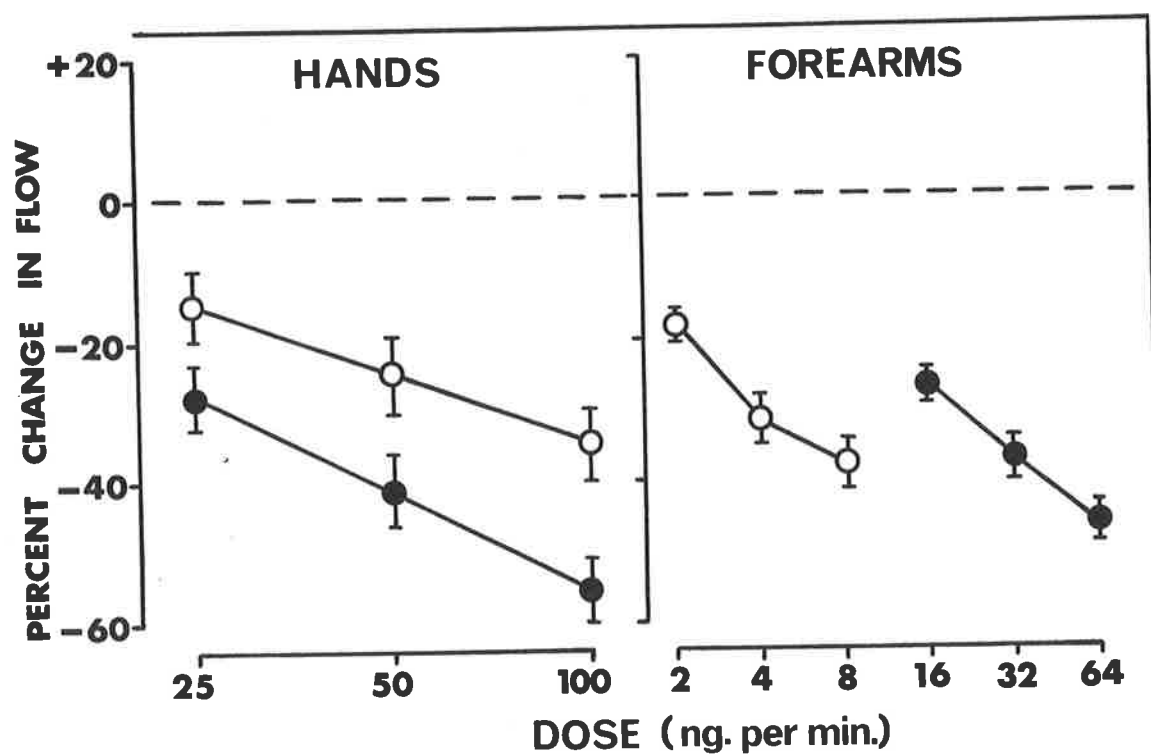


Fig 2-18. The mean percentage changes in hand blood flow (left hand frame) and forearm blood flow (right hand frame) during intra-arterial infusions of angiotensin (○) and noradrenaline (●) in the doses indicated. The hand flow values were obtained from five subjects, each point representing the mean of eight values in the case of angiotensin and nine values in the case of noradrenaline. The forearm flow values were obtained from five subjects, two infusions of each drug at each dose level being given in each subject. The vertical lines through the flow values represent one standard error on either side of the mean.

the infusion. To obtain the same response with both drugs approximately four times the dose of noradrenaline (by weight) was required.

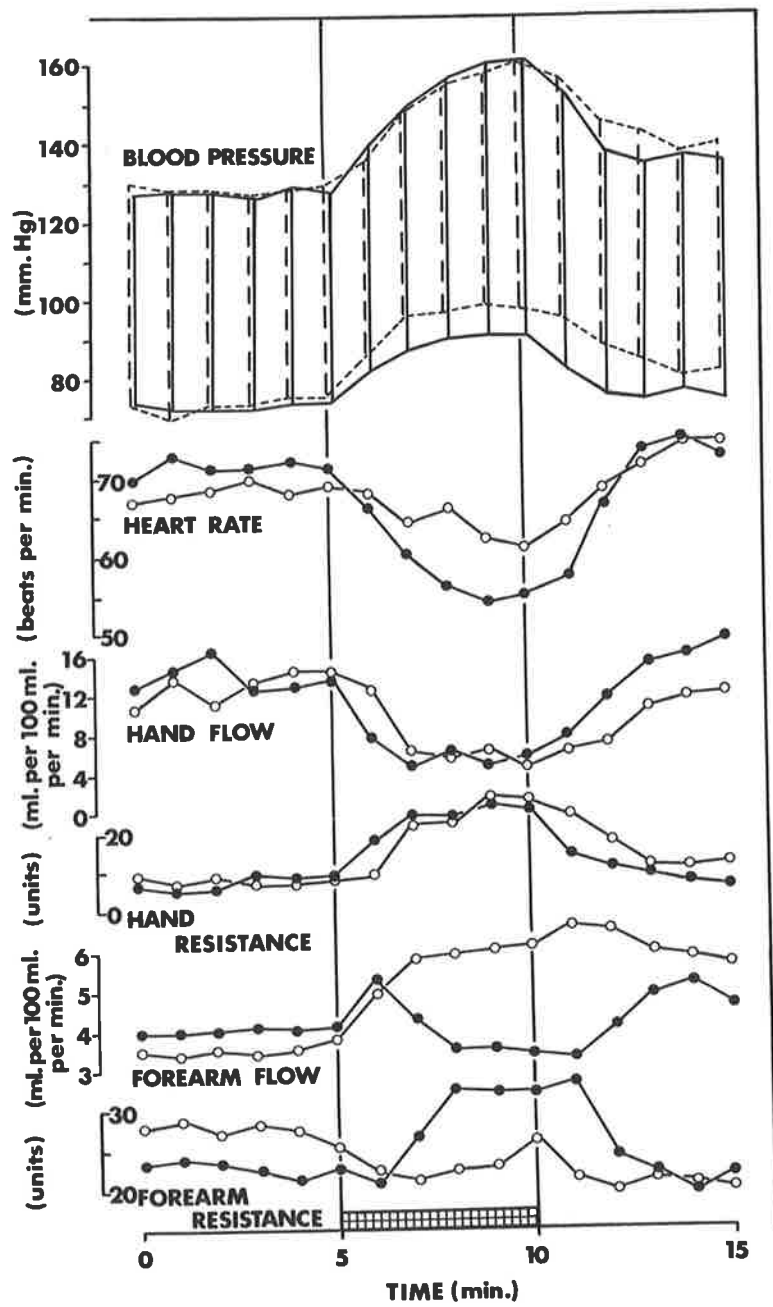
Intravenous infusions

Intravenous infusions of three doses of angiotensin (0.5, 1 and 2 $\mu\text{g}/\text{min}$ for 5 min) and three doses of noradrenaline (4, 8 and 16 $\mu\text{g}/\text{min}$ for 5 min) were given to each of seven subjects, and arterial blood pressure, heart rate and hand and forearm blood flow were recorded. Similar responses were obtained in all subjects. Approximately equal increases in systolic blood pressure were produced by 2 $\mu\text{g}/\text{min}$ angiotensin and 16 $\mu\text{g}/\text{min}$ noradrenaline, which were the largest doses given. The averaged data of the effects of these doses from the seven experiments are shown in Fig 2-19.

Blood pressure

While the rise in systolic pressure was approximately equal and followed the same time course with these doses of the two drugs, the diastolic pressure always rose more quickly and to a higher level with angiotensin and the rise in mean pressure was therefore correspondingly greater. After the noradrenaline infusion both the systolic and diastolic pressures returned to pre-infusion levels more rapidly than after angiotensin. After noradrenaline infusions the diastolic pressure fell to baseline within

Fig 2-19. The changes in heart rate, hand blood flow and resistance and forearm blood flow and resistance during intravenous infusions of 2.0 $\mu\text{g}/\text{min}$ angiotensin (○) and 16.0 $\mu\text{g}/\text{min}$ noradrenaline (●), being the averaged data of paired infusions from seven subjects. For each parameter the mean value for the appropriate minute has been calculated and plotted as an individual point. The blood pressure responses (top record) have been superimposed, that to noradrenaline being indicated by the solid lines and that to angiotensin by the dotted lines. The period of infusion (5 min in each case) is indicated by the hatched area.



3-4 min and the systolic within 6-7 min, whereas after angiotensin infusions both of the pressures were still elevated by 5-10 mm Hg at the end of 7 min, but baseline levels had usually been restored by 15 min after the end of the infusion.

Figure 2-20 shows the dose-response curve for the percentage increases in mean blood pressure with each drug, being the averaged responses from the seven subjects. On a weight basis angiotensin was approximately fifteen times more effective in elevating the mean blood pressure than was noradrenaline.

Heart rate

The heart rate was reduced during infusions of both drugs, but for similar increases in systolic (Fig 2-19) or mean pressure the effect was greater with noradrenaline. The relationship between rise in mean arterial pressure and the bradycardia for all doses of the two drugs is shown in Fig 2-21, which contains pooled data from seventeen subjects and includes data from experiments in which, for various reasons, the full set of six drug infusions was not given. In the case of noradrenaline there is a close relationship (correlation coefficient, $r = -0.57$, $n = 35$, $P < 0.001$) but no relationship was found with angiotensin where the bradycardia did not increase significantly with the increase in pressor effect ($r = +0.09$, $n = 39$, $P > 0.1$).

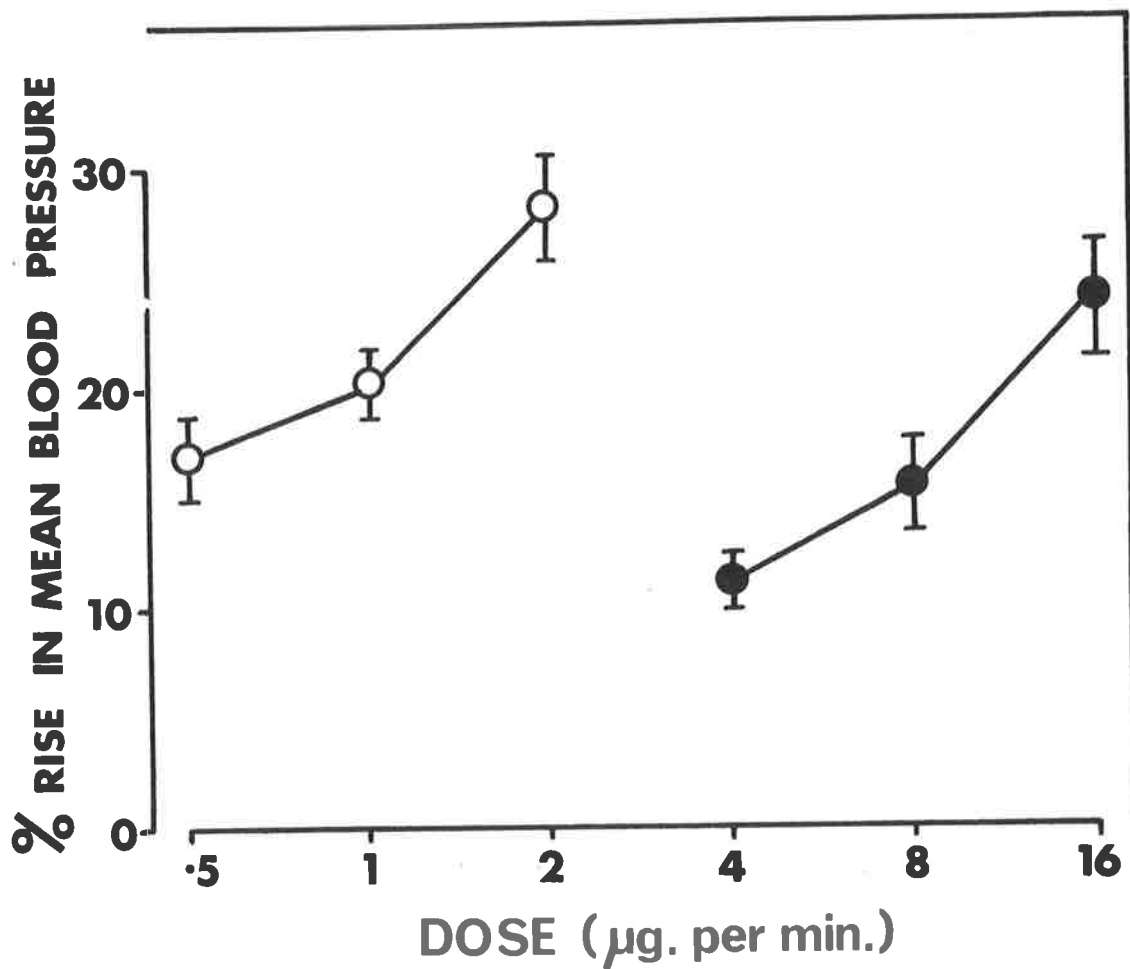
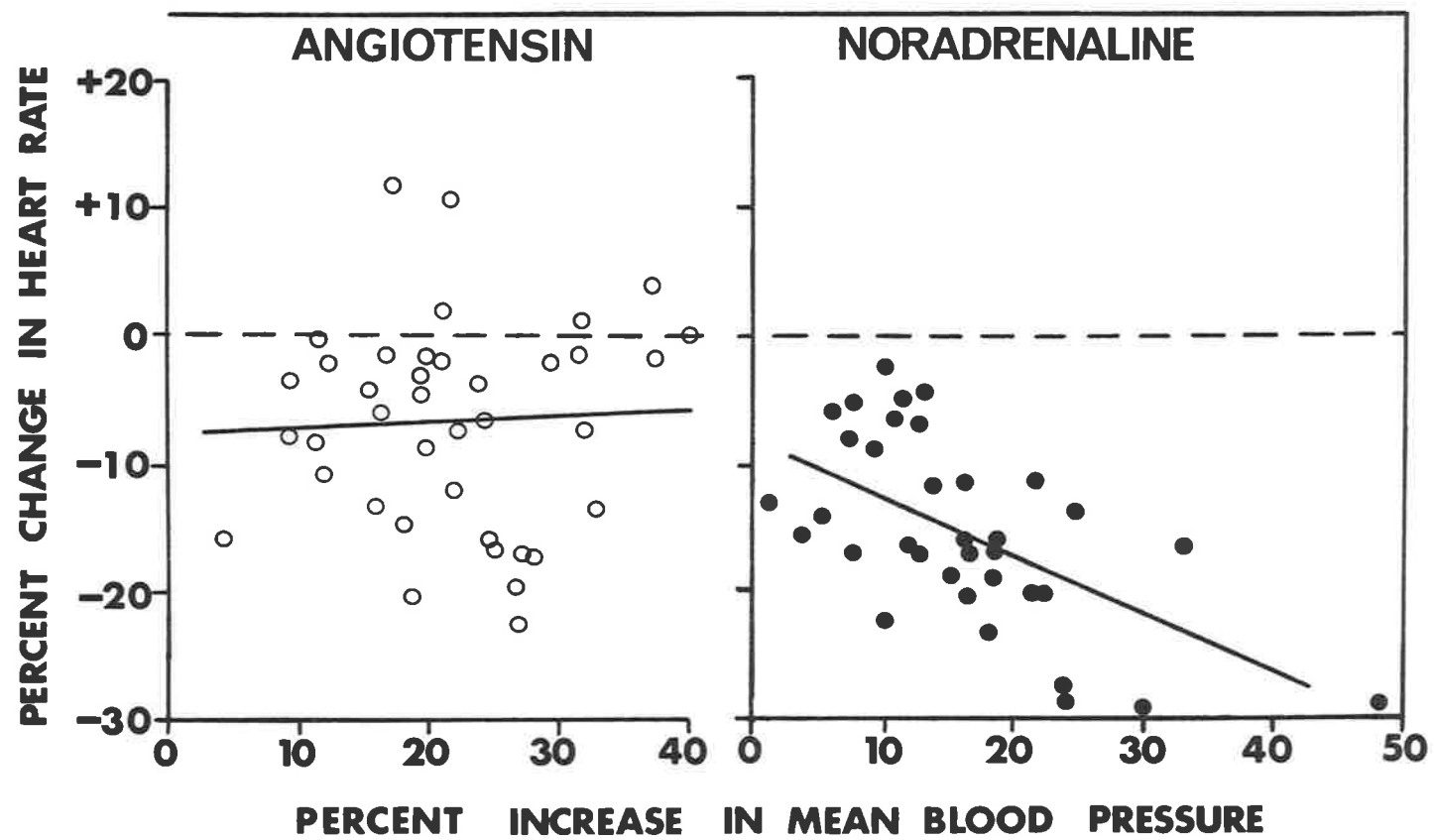


Fig 2-20. The percentage rise in mean blood pressure following intravenous infusions of three doses of angiotensin (○) and three doses of noradrenaline (●). Each point represents the mean of seven values from seven subjects and the vertical line through each point represents one standard error on either side of the mean.

Fig 2-21. The relationship between percentage change in heart rate and percentage increase in mean blood pressure in seventeen subjects during intravenous infusions of angiotensin (○) in doses ranging from 0.5 to 2.0 $\mu\text{g}/\text{min}$, and noradrenaline (●) in doses ranging from 2.5 to 16.0 $\mu\text{g}/\text{min}$. The regression lines for angiotensin ($y = 0.052 x - 7.621$) and noradrenaline $y = -0.449x - 8.02$.



Hand blood flow

The constrictor response of the hand vessels was approximately the same with doses of the two drugs which had similar effects on the systolic blood pressure although the fall in flow was a little slower to develop and wear off after angiotensin (Fig 2-19). A similar pattern was seen in calculated hand vascular resistance.

Figure 2-22 (left hand frames) illustrates the percentage changes in hand blood flow and vascular resistance with three doses of each drug given intravenously, each value being the average of the data obtained from seven subjects. To produce a given response with the two drugs eight to ten times the dose of noradrenaline (on a weight basis) was required. This ratio of potencies of the two drugs on the hand vessels with intravenous infusions contrasts strongly with the results of intra-arterial administration where angiotensin had only one third to one half the constrictor potency of noradrenaline.

Figure 2-23 (upper frames) illustrates the relationship between percentage change in hand vascular resistance and percentage increase in mean blood pressure for all doses of the two drugs and contains data from ten subjects, including data from the seven mentioned above and three others in whom for various reasons the full set of six infusions was not given. With both angiotensin and noradrenaline a positive correlation was found (angiotensin,

Fig 2-22. The mean percentage changes in hand blood flow and vascular resistance (left hand frames) and forearm blood flow and vascular resistance (right hand frames) during intravenous infusions of three doses of angiotensin (○) and three doses of noradrenaline (●) in seven subjects. The vertical lines through each point represent one standard error on either side of the mean.

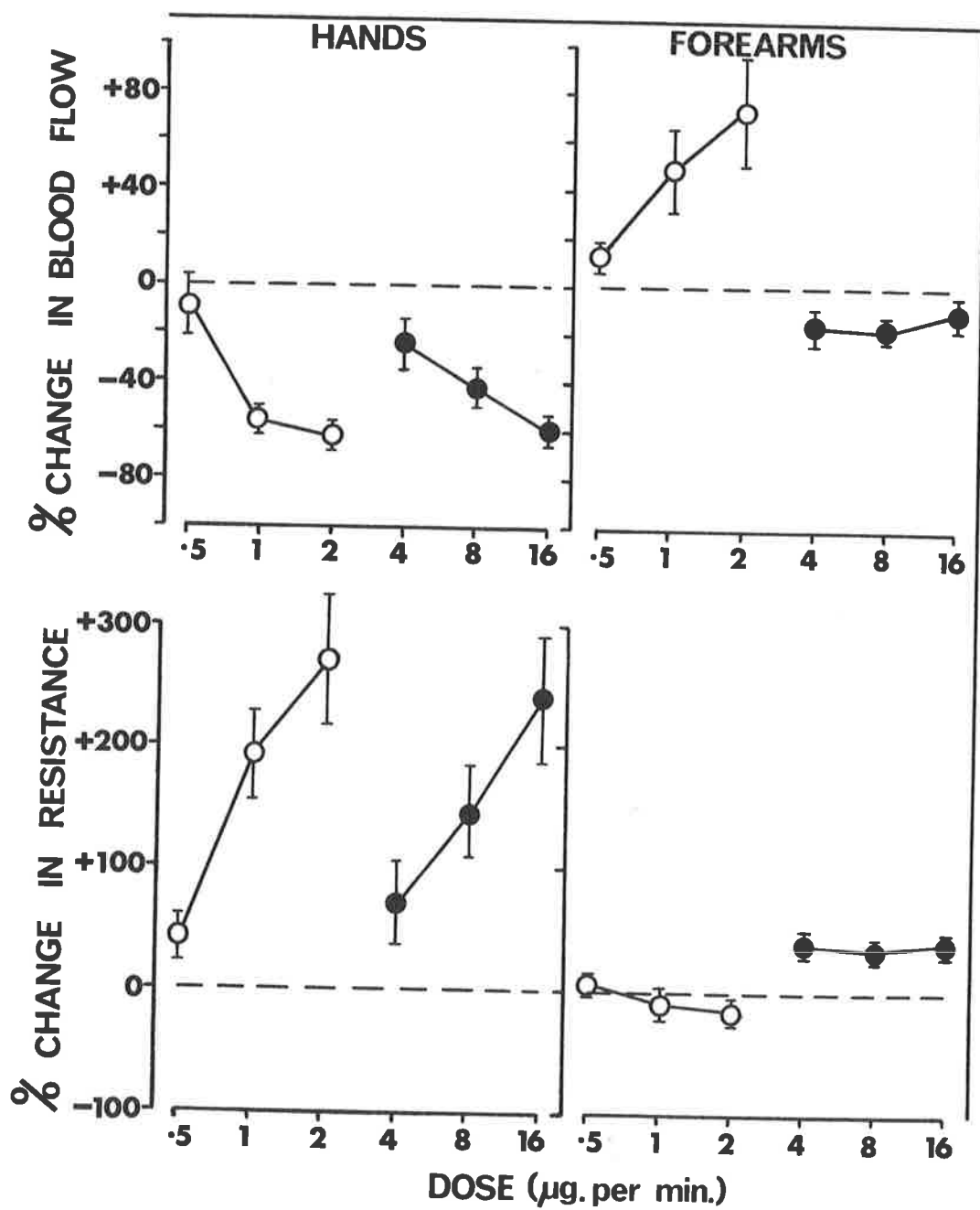
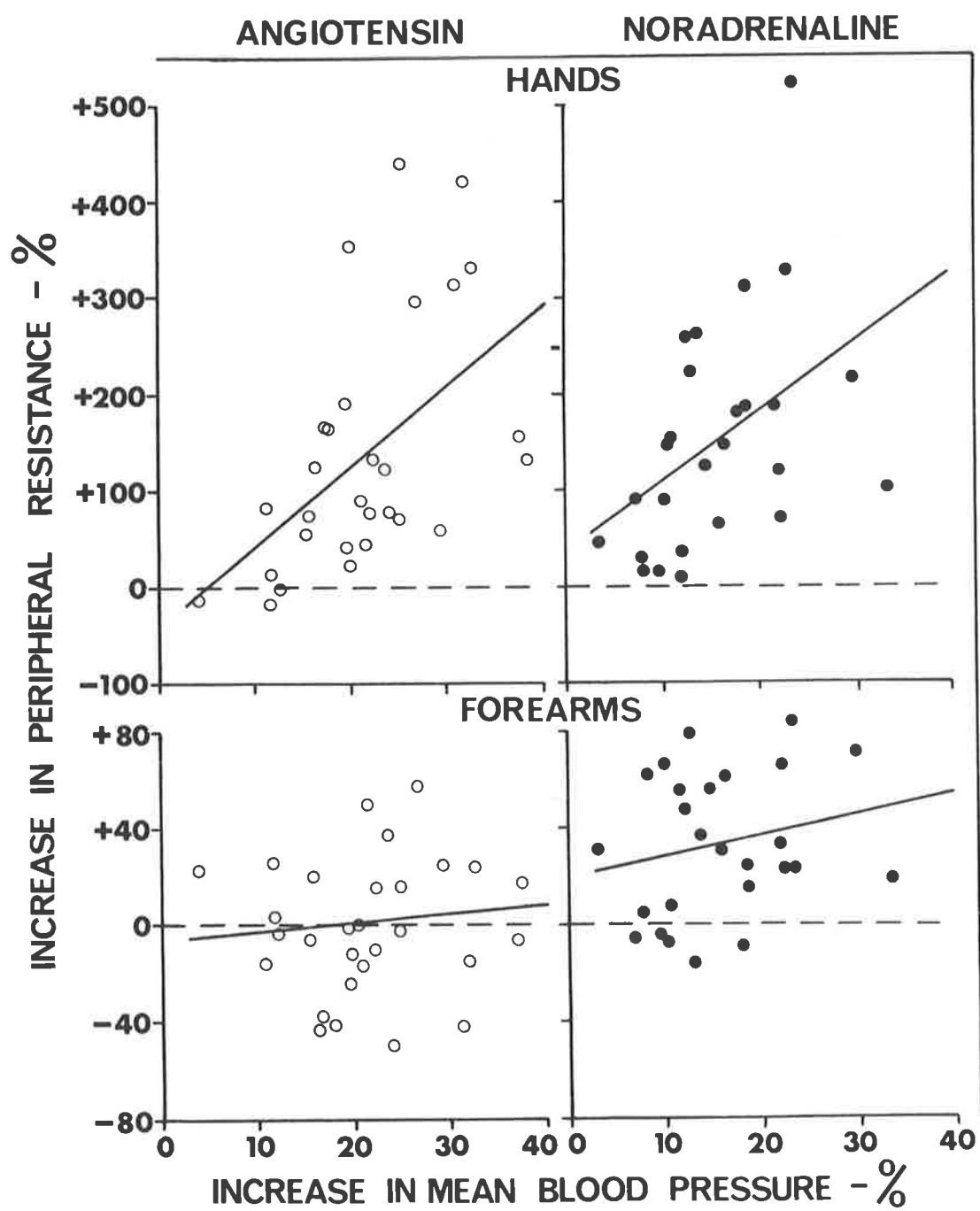


Fig 2-23. Relationship between percentage increase in mean blood pressure and percentage change in vascular resistance in the hand (upper frames) and the forearm (lower frames) during intravenous infusions in ten subjects of angiotensin (○) in doses ranging from 0.5 to 2.0 $\mu\text{g}/\text{min}$, and noradrenaline (●) in doses ranging from 4.0 to 16.0 $\mu\text{g}/\text{min}$. The regression lines for angiotensin (upper frame, $y = 8.6x - 45.01$; lower frame, $y = 0.28x - 8.04$) and noradrenaline (upper frame, $y = 7.37x + 35.25$; lower frame, $y = 0.805x + 19.34$) are shown.



$r = +0.53$, $n = 29$, $P < 0.01$; noradrenaline, $r = +0.45$, $n = 26$, $P < 0.05$) and there was no significant difference between their regression coefficients (t test, $0.8 > P > 0.7$).

Forearm blood flow

During noradrenaline infusions the forearm flow increased initially and then fell. After the infusion ceased the flow rose to exceed the control value before returning to the pre-infusion level (Fig 2-19). Forearm vascular resistance rose and had recovered within 3-5 min after the end of the infusion. With angiotensin the forearm blood flow consistently rose to about double the resting level and the effect lasted for up to 10 min after the infusion had ceased. Calculated forearm resistance fell slightly, but the change was not statistically significant.

Figure 2-22 (right hand frames) illustrates the percentage changes in forearm blood flow and vascular resistance with the three doses of the two drugs when given intravenously, each value being the average of the data obtained from seven subjects. During angiotensin infusions the forearm blood flow increased, the effect increasing with increasing doses. The calculated forearm resistance, however, did not exhibit any significant change. The increase in forearm blood flow was therefore probably the result of the increase in perfusion pressure, but an active dilator effect of angiotensin on muscle vessels, offset by skin vasoconstriction,

cannot be excluded. Noradrenaline caused a slight fall in forearm blood flow and approximately a 40% increase in vascular resistance, neither of which showed a relationship to dose. Noradrenaline intravenously often causes an increase in muscle blood flow which may offset the constriction in skin and result in little change in total forearm flow (Cooper, Fewings, Hodge, Scroop and Whelan, 1964) and since the muscle vasodilator action increases with increasing doses it could account for the constancy of the forearm flow and resistance changes at all dose levels. This more potent constrictor action of noradrenaline, compared with angiotensin, on the forearm vessels during intravenous administration contrasts with the results of intra-arterial administration where approximately four times the dose of noradrenaline (by weight) was required to obtain the same response with both drugs.

Figure 2-23 (lower frames) illustrates the relationship between percentage increase in forearm vascular resistance and percentage increase in mean blood pressure for all doses of the two drugs. No significant correlation was found with either angiotensin ($r = +0.07$, $n = 29$, $P > 0.1$) or noradrenaline ($r = +0.20$, $n = 26$, $P > 0.1$).

Central venous pressure

The central venous pressure and brachial arterial pressure were simultaneously recorded in six subjects and intra-

venous infusions of various doses of angiotensin and noradrenaline given. The central venous pressure rose with both drugs but this was much more marked with noradrenaline for a given rise in mean arterial pressure. In Fig 2-24 the increase in central venous pressure in cm of water is plotted against increase in mean arterial pressure for both drugs. A positive correlation was found for noradrenaline ($r = +0.74$, $n = 9$, $P < 0.05$) but no significant relationship appeared with angiotensin ($r = +0.32$, $n = 10$, $P > 0.1$) and there was a significant difference between their regression coefficients (t test, $P < 0.001$).

Forearm muscle venous blood oxygen saturation estimations

Samples of venous blood were taken for estimation of percentage oxygen saturation from a deep (muscle) catheter during intravenous angiotensin infusions in each of the above experiments where central venous pressure was monitored. In most cases an increase in the percentage oxygen saturation was recorded, indicating vasodilatation in the vascular bed of forearm muscle during infusion of angiotensin. Figure 2-25 illustrates the results of one of these experiments.

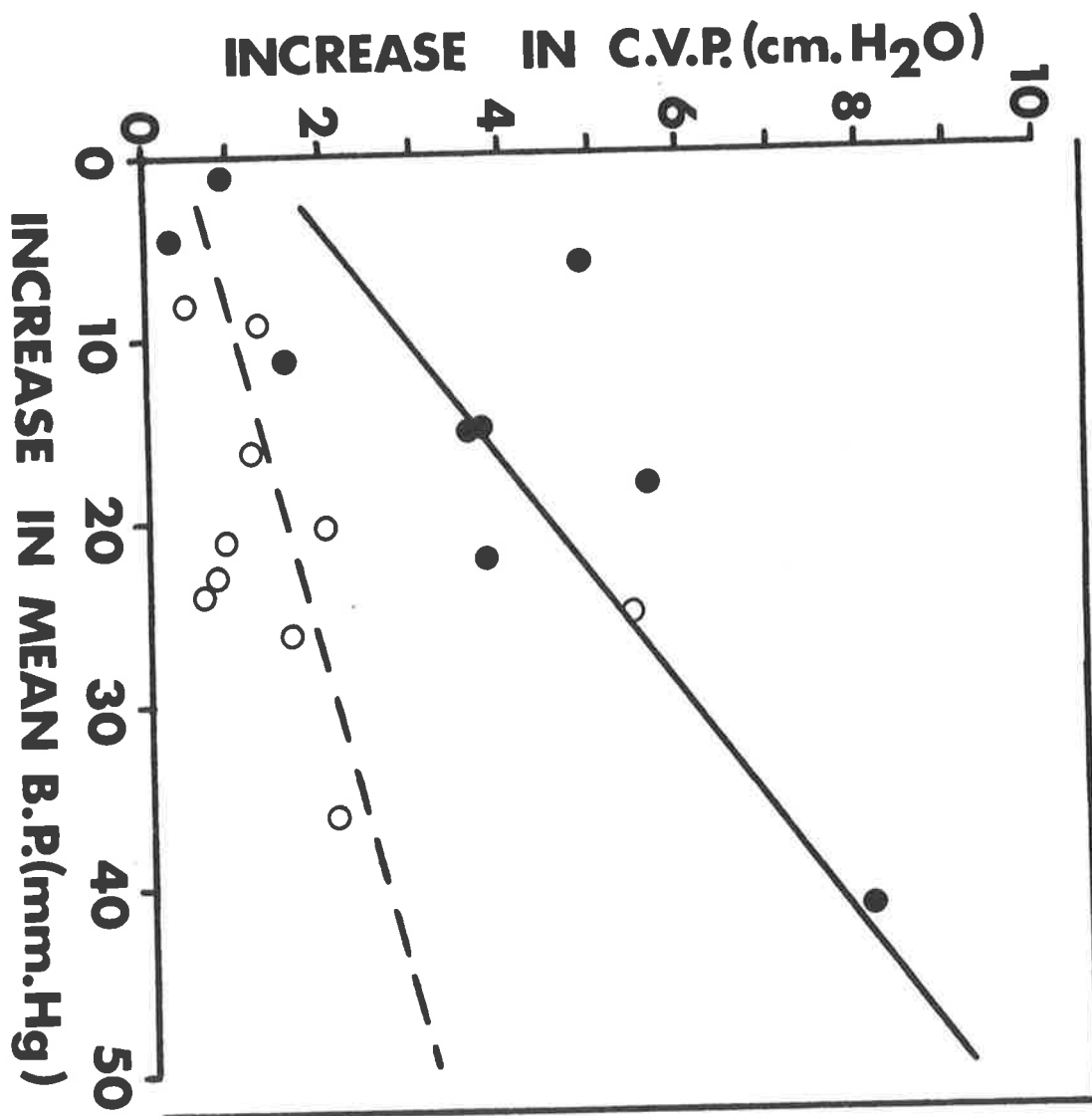


Fig 2-24. Relationship between the rise in central venous pressure and the rise in mean blood pressure in seven subjects during intravenous infusion of angiotensin (○) in doses ranging from 0.5 to 2.0 $\mu\text{g}/\text{min}$, and noradrenaline (●) in doses ranging from 2.5 to 10.0 $\mu\text{g}/\text{min}$. The regression lines for noradrenaline (solid line, $y = 0.126x + 1.53$) and angiotensin (dotted line, $y = 0.0372x + 0.78$) are shown.

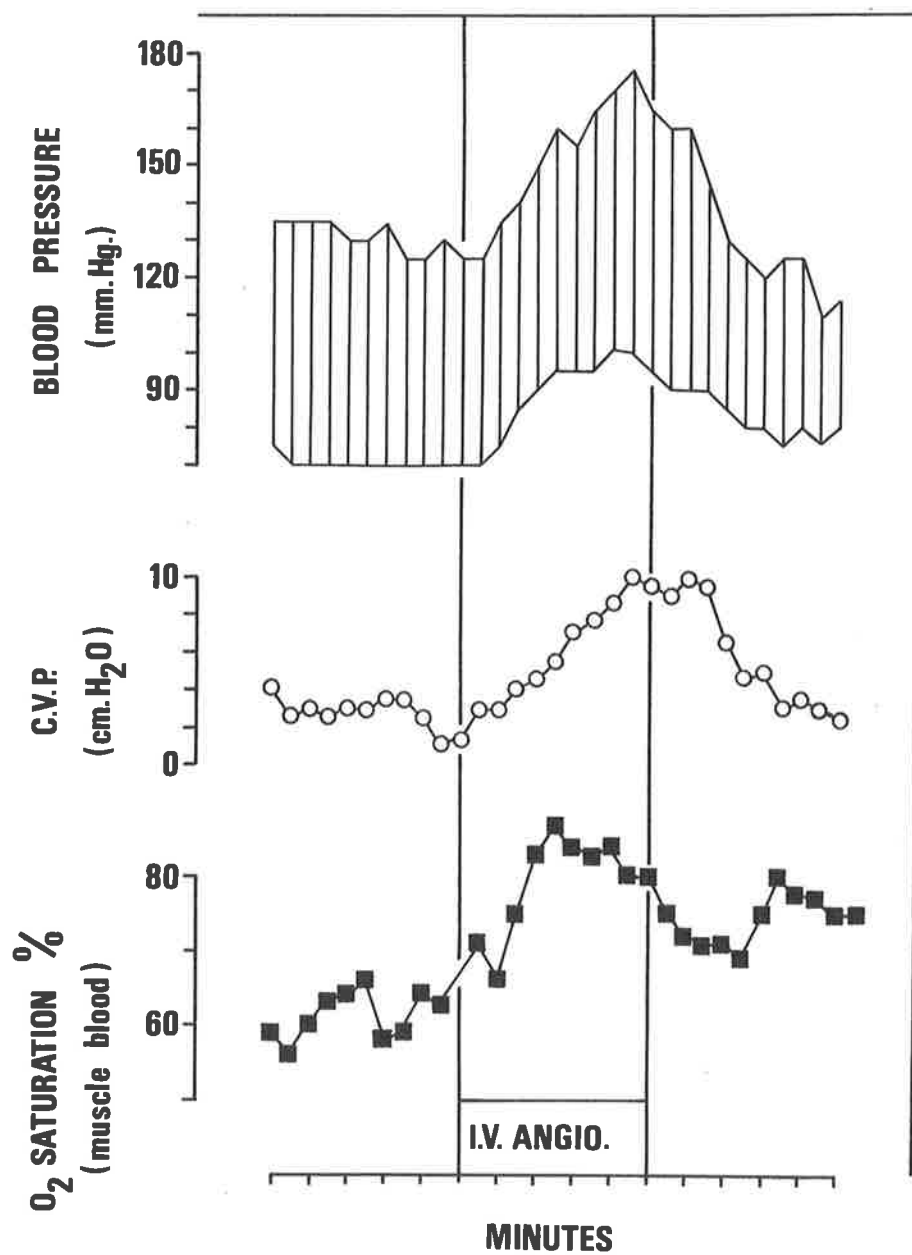


Fig 2-25. The changes in brachial arterial blood pressure, central venous pressure and forearm muscle blood oxygen saturation before, during and after intravenous infusion of angiotensin (2 μ g/min for 5 min).

DISCUSSION

The striking feature of the comparison of the effects of angiotensin and noradrenaline on the limb vessels is the difference in the ratio of relative activities of the two drugs with the intravenous and intra-arterial routes of administration.

Intra-arterial infusions

Investigation of the direct local actions of both vasoactive substances by intra-arterial infusion revealed that they reduced blood flow in both the forearm and hand, confirming previous findings that administration of angiotensin into the supplying artery results in a reduction in blood flow in various vascular beds in man (Wilkins and Duncan, 1941; Bock et al, 1958; Daly and Duff, 1960; de Bono et al, 1963). The constrictor response to angiotensin was somewhat slower in onset than that of a corresponding dose of noradrenaline and it took longer to wear off after cessation of the infusion. Comparison of dose response curves to the two constrictor agents showed that, on a weight basis, noradrenaline was two to three times more potent than angiotensin in reducing hand blood flow, but in the case of the forearm vessels angiotensin was four times more effective than noradrenaline (Fig 2-18). One possible explanation for this difference between forearm and hand responses to angiotensin might be a rapid uptake or inactivation in the blood stream reducing the effective dose of the drug by the time it travelled from the point

of the infusion at the elbow to the hand vessels, as occurs with bradykinin (Saameli and Eskes, 1962) and acetylcholine (Duff, Greenfield, Shepherd and Thompson, 1953). The degree of constriction produced with equal doses of noradrenaline, however, was the same for the two vascular beds, whereas with angiotensin similar constrictor responses in the forearm and hand required approximately twenty-five times the dose in the latter. Since the half-life of the two agents in the blood stream is similar, it seems likely that the forearm vessels have a greater sensitivity to angiotensin than do the hand vessels.

It has yet to be determined whether the fall in forearm flow produced by intra-arterial angiotensin is a consequence of constriction of skin or muscle vessels or of both. As the forearm is composed mainly of muscle it is often inferred that changes in total forearm blood flow measured plethysmographically are representative of changes in muscle flow alone. This is not necessarily so, since, as shown in Part A of this section, the total forearm vasoconstrictor response to intra-arterial serotonin is a resultant of vasoconstriction in skin and vasodilatation (plus some vasoconstriction) in muscle. However, angiotensin probably constricts both skin and muscle vessels in the forearm as Bock et al (1958) reported reduction in forearm muscle blood flow using the Hensel needle and also decrease in skin flow using heat flow calorimetry during intra-arterial administration.

Blockade of the alpha-adrenoreceptors of the hand vessels by phenoxybenzamine abolished the response to noradrenaline but left

that to angiotensin unaltered (Scroop and Whelan, 1966). Similarly sympathetic denervation had no appreciable effect on the responses of the hand vessels to intra-arterial angiotensin. These observations indicated that the direct action of angiotensin was not due to an effect on the alpha-adrenoreceptors either directly or by way of stimulation of the peripheral sympathetic nerve endings. This is in contrast to findings in animal preparations which indicate that angiotensin releases noradrenaline from the peripheral sympathetic nerve fibres (Liebau, Distler and Wolff, 1966; Kiran and Khairallah, 1968), but the concentrations used were very much higher than have been administered in human studies.

Intravenous infusions

Blood pressure, heart rate and central venous pressure

When the effect of doses of intravenous angiotensin and noradrenaline, which produced equal increases in systolic blood pressure were compared (Fig 2-19), it was found that the diastolic pressure always rose more quickly and to a higher level with angiotensin and the rise in mean pressure was therefore correspondingly greater. These observations are similar to those of other authors (Finnerty et al, 1961; de Bono et al, 1963). When the responses of mean systemic arterial blood pressure were related to dosage (Fig 2-20) it was found that angiotensin was approximately

fifteen times more potent than noradrenaline on a weight basis. If the comparison is made on a molar basis angiotensin is seen to be approximately eighty times more pressor than noradrenaline, but since the molecule-to-receptor relationship is unknown the weight basis for comparison is probably more appropriate. This potent pressor action suggests that indirect vasoconstrictor mechanisms may be involved in the systemic action of angiotensin.

The heart rate was reduced during infusions of both drugs, but for similar increases in systolic or mean pressure the effect was greater with noradrenaline. In the case of noradrenaline there was a correlation between the fall in heart rate and the rise in blood pressure, but no such relationship was found with angiotensin where bradycardia did not increase significantly with the increase in pressor effect.

The bradycardia seen with noradrenaline can be attributed to a reflex vagal effect (Goldenberg, Pines, Baldwin, Greene and Roh, 1948) initiated by the rise in arterial pressure. The bradycardia with angiotensin is also probably vagal in origin (Wilkins and Duncan, 1941) but the finding that it is less marked than with noradrenaline when given in approximately equipressor doses suggests that angiotensin may have a stimulating action on the heart which opposes the reflex slowing. Such a stimulating action is unlikely to be a direct effect of the drug since Hill and Andrus (1940), Lorber (1942) and Koch-Weser (1964) have not

observed a positive chronotropic effect of angiotensin on the heart of the kitten, frog, dog or cat. An indirect stimulating effect which increased with increasing doses could account for the observation that the bradycardia with angiotensin showed no correlation with the degree of hypertension. Support for this suggestion is indicated by the work of Nishith, Davis and Youmans (1962) and Krasney, Paudler, Smith, Davis and Youmans (1965) who observed that positive chronotropic and inotropic effects of angiotensin on the heart were mediated by the sympathetic efferent nerves. The lesser degree of bradycardia with angiotensin possibly accounts for the greater rise in diastolic pressure with this drug than with noradrenaline when the increase in systolic pressure is equal.

The increase in central venous pressure produced by both noradrenaline and angiotensin is probably due to the bradycardia, since it is reduced or abolished by atropine (Bock and Gross, 1961) and the greater rise in central venous pressure with noradrenaline may be related to the more marked bradycardia produced by this drug. It may also be relevant that angiotensin is not as marked a constrictor of capacitance vessels as is noradrenaline (Folkow, Johansson and Mellander, 1961; de Pasquale and Burch, 1963).

Hand blood flow

Intravenous infusion of a range of doses of angiotensin and noradrenaline showed that angiotensin was between eight and ten times

more potent as a constrictor of the hand vessels than was noradrenaline. This ratio of potencies was in marked contrast to that found during intra-arterial administration where angiotensin exhibited only one-half to one-third the constrictor potency of noradrenaline. The present experiments provide no explanation for this difference in potency with the two routes of administration, although a number of possibilities exist. Since the rates of inactivation of angiotensin and noradrenaline are very similar, the relative potencies of their direct actions should be unchanged by the route of administration. This suggests that the observed enhancement of the vasoconstrictor action of angiotensin on intravenous infusion is due to activation of some indirect mechanism, either adding to the direct constrictor action of angiotensin or opposing that of noradrenaline. There are a number of factors which might oppose the direct constrictor action of noradrenaline. With equipressor doses of the two drugs the eight-fold discrepancy remains so that the effect is unlikely to be a reflex from the high pressure baroreceptors. However, with such doses noradrenaline raised central venous pressure more than angiotensin and this could result in a reflex from low-pressure baroreceptors with release of sympathetic tone in the hand vessels as has been shown to occur in the forearm (Roddie, Shepherd and Whelan, 1957c). Noradrenaline has also been shown to have a central action in inhibiting sympathetic tone (Taylor and Page, 1951) and this may oppose the direct constrictor

action of the drug. Furthermore, Lever, Mowbray and Peart (1961) postulated the release of a vasodilator substance by intravenous noradrenaline, so that both a neural and a humoral mechanism could be activated on intravenous infusion and oppose the direct constrictor action of noradrenaline.

However, most data indicate that noradrenaline's action is predominantly vasoconstrictor through direct receptor stimulation and, therefore, the discrepancy between the constrictor potencies of the two drugs on the hand vessels when given by the two routes of administration is most probably due to activation of one or more indirect constrictor mechanisms by angiotensin (as outlined in General Introduction). Examination of the roles of central and peripheral sympathetic stimulation and of adrenal catecholamine release in the indirect constrictor action of angiotensin on the hand vessels followed logically from the present comparison study, and the results have been reported by Scroop and Whelan (1966) and Whelan, Scroop and Walsh (1968). The effect of intravenous infusion of angiotensin on the blood flow through both hands was determined before and after treatment of one hand with phenoxybenzamine infused into the brachial artery of that side. After phenoxybenzamine the constrictor response to angiotensin was abolished on the treated side while the control hand showed the expected fall in hand blood flow. Sympathetic reflex responses and the responses to noradrenaline were also abolished or very much reduced on the blocked side,

demonstrating the effectiveness of the blocking action of the phenoxybenzamine. These observations illustrated the adrenergic nature of the constrictor response to angiotensin, but did not distinguish between sympathetic nerve activity and the arrival of an adrenal medullary hormone, since phenoxybenzamine blocks both sympathetic nerve-induced activity and also the actions of circulating catecholamines on the hand vessels.

Treatment of the hand vessels with bretylium tosylate, however, indicated that sympathetic nerve activity was involved as the constrictor response of the hand vessels to intravenous angiotensin was very much reduced, but not completely abolished, after treatment with bretylium intra-arterially. These studies of the indirect effects of angiotensin were extended to examination of the responses of acutely nerve-blocked, chronically sympathectomised and traumatically denervated limbs and demonstrated that the response was mediated solely by the sympathetic nerves (Fig 2-26). In none of these did intravenous angiotensin have any constrictor effect on the hand vessels which were deprived of autonomic innervation, instead a slight increase in flow occurred which was probably a passive response to the rise in blood pressure since hand vascular resistance showed little change. Constrictor responses to nor-adrenaline were unimpaired. Similar findings were obtained from studies of a patient suffering from idiopathic autonomic degeneration in whom no sympathetic reflex activity could be demonstrated in the

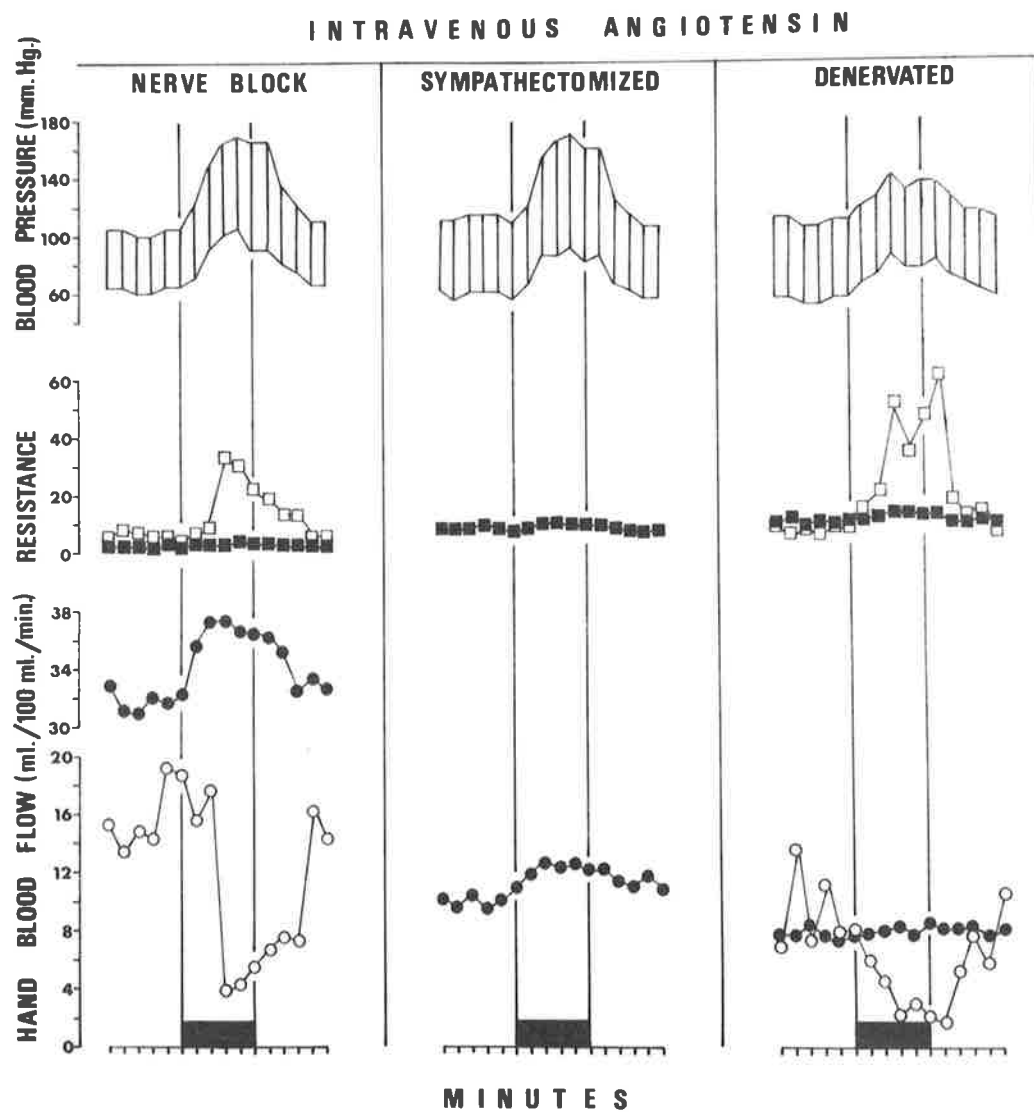


Fig 2-26. The response of the arterial blood pressure, hand vascular resistance and hand blood flow in control (○) and nerve-blocked, sympathectomized and denervated (●) hands of three subjects. Intravenous angiotensin infusions of 2, 0.5 and 1.5 $\mu\text{g}/\text{min}$, respectively, are indicated by the black rectangles.

limbs.

It can be concluded from the above observations that the increase in vascular resistance in the hands during intravenous administration of angiotensin can be attributed to a central action of angiotensin acting by way of the sympathetic nerves and that a direct local constrictor action does not play any part in the response of this vascular bed. This conclusion supports the central stimulating action of angiotensin on the vasomotor centres described in cross-perfused dogs by Bickerton and Buckley (1961) who found a central hypertensive effect of angiotensin due to sympathetic stimulation, and is also compatible with the observations of Johnsson, Henning, and Ablad (1965) who found that the constrictor effect of angiotensin on hand vessels was inhibited by phenoxybenzamine and guanethidine and concluded that the constriction was due, at least in part, to a central action eliciting increased sympathetic tone. Lavery (1963) also found evidence for a nervously mediated component to angiotensin's action on the vessels of the hind limb of the rat and concluded that it was due to central sympathetic stimulation.

There does not appear to be any evidence in man in support of the possibility that angiotensin might exert some of its constrictor effect by stimulating the release of transmitter from the peripheral nerve endings as has been suggested by Liebau,

Distler and Wolff (1966). If such an effect were present, a constriction of the hand vessels on intravenous administration would be expected in the acutely nerve-blocked limb in which the nerve endings were intact, but no such effect could be detected. Furthermore, the vasoconstrictor action of intra-arterial infusions was not diminished by sympathectomy indicating, as was the case with serotonin (Part A), independence of the sympathetic innervation.

It would appear also that the contribution to the cardiovascular action of angiotensin in man made by catecholamines released from the adrenal medulla is insignificant if, indeed, it occurs at all. A vasoconstrictor response of the sympathectomised, nerve-blocked and denervated hand vessels would have been expected during intravenous angiotensin infusion if catecholamine release had occurred. Similarly, Vincent, Kashemsant, Cuddy, Fried, Smulyan and Eich (1965) found no change in urinary vanillylmandelic acid levels during prolonged intravenous infusions of angiotensin in human subjects. This apparent absence of involvement of catecholamine release in the indirect action of angiotensin in man contrasts with the animal findings. Feldberg and Lewis (1964) have demonstrated release of adrenal medullary hormones by angiotensin in the cat, and Peach, Cline and Watts (1966) found a dose dependent increase in plasma catecholamine levels during intravenous administration of pressor doses of angiotensin in the dog.

Another possible indirect mechanism in the cardiovascular action of angiotensin is interaction with other vasoactive substances, and the interactions between angiotensin, noradrenaline and serotonin are discussed as a separate study in Part D of this Section.

Forearm blood vessels

A difference in intra-arterial and intravenous potencies was also observed in the case of the forearm vessels. Intra-arterially angiotensin had four times the constrictor action of noradrenaline whereas intravenously noradrenaline was more constrictor. Qualitatively also, intravenous angiotensin and noradrenaline had different effects on forearm blood flow. Noradrenaline caused an initial increase in flow, followed by a fall, and the forearm vascular resistance rose. During angiotensin infusions the forearm blood flow increased, the effect increasing with increasing doses, but the calculated forearm resistance did not exhibit any significant change. Increases in forearm blood flow have also been observed by Bradley and Parker (1941), Wilkins and Duncan (1941), de Bono et al (1963) and Bock et al (1958). The latter investigators, using calorimetric techniques, found that the blood flow in the muscle of the forearm increased during intravenous angiotensin administration, while that of the skin decreased. This observation has been confirmed in forearm muscle using changes in venous blood

oxygen saturation as an index of blood flow. The dilatation of muscle vessels may be a sympathetically mediated response to the rise in central venous pressure (Fig 2-25). Similar responses can be observed in association with alterations in posture when central venous pressure changes occur without any change in arterial pressure (Roddie et al, 1957 c). Wilkins and Duncan (1941) noted an increase, followed by a fall, in forearm and calf flow, the increase appearing before the expected arrival of angiotensin, and the appearance and amplitude of the increase were not related to the size of the dose or the degree of increase in arterial pressure. In a sympathectomised limb the dilatation was absent and only a constriction was seen. Similarly, the dilatation with intravenous angiotensin was absent in the forearm of a patient suffering from idiopathic autonomic degeneration and in whom no sympathetic reflexes could be induced in the upper limb (Whelan, 1967).

The above findings indicate that whereas in the case of the hand vessels all of the increase in resistance during intravenous angiotensin is mediated through the central action by way of the sympathetic nerves, in the case of the forearm the flow response is compounded of a direct constrictor action, probably confined to the vessels of the skin, and a reflex dilatation of muscle vessels, probably in response to low pressure baroreceptor stimulation. Since the component due to changes in skin flow will vary in magnitude with the state of tone of the skin vessels (as illustrated by

the response of forearm blood flow to intra-arterial serotonin in Fig 2-14), the variability in forearm responses and the apparent discrepancies in the reports of various authors are not surprising. Had there been some evidence of release of adrenaline from the adrenal medulla this could have offered an explanation for the hand and forearm findings, since adrenaline has constrictor effects on skin vessels and dilator effects on muscle vessels. However, no evidence for adrenaline release by angiotensin was found by Scroop and Whelan (1966).

Role of the various constrictor mechanisms in the pressor response to angiotensin

While the sympathetically-mediated constriction of hand vessels, which are mainly skin, may contribute to the pressor effect of angiotensin, such contribution of skin vessels to increase in total peripheral resistance is likely to be small. The forearm vascular bed does not contribute to the increased peripheral resistance during intravenous infusions of angiotensin since any constriction of skin vessels is offset by vasodilatation in the underlying muscle. Thus the major rise in total peripheral resistance with angiotensin must lie in vascular beds other than those of the limbs, either through a direct effect alone or combined with a sympathetically-mediated constriction. In a recent endeavour to determine the degree to which the central sympathetic stimulating action of angiotensin might contribute to its total pressor effect,

angiotensin was infused into normal subjects and the effect of intravenous phentolamine on the responses of systemic blood pressure and hand blood flow determined (Scroop and Whelan, 1968b). Phentolamine (a short-acting alpha-adrenoreceptor blocking agent) abolished the fall in hand flow which occurred in unblocked limbs but there was no significant modification of the rise in blood pressure in all six subjects studied. The above authors concluded, therefore, that the sympathetic stimulating action, which was responsible for virtually all of the vasoconstriction in the hand (and also the foot), did not make a significant contribution to the overall increase in peripheral resistance. This conclusion was further supported by the observation that intravenous angiotensin caused an enhanced pressor response in two patients suffering from advanced idiopathic autonomic degeneration. The failure of phentolamine to modify the pressor response to angiotensin also supports earlier findings that adrenal catecholamine release does not make a contribution to this pressor response to angiotensin in man. Similarly, Laurence and Nagle (1963) were unable to modify significantly the pressor response in man with either bretylium or guanethidine. This is contrary to most animal findings (outlined in General Introduction). However, it is possible that larger doses of angiotensin than those which can safely be given acutely in normal subjects, or a longer duration of exposure, might produce a significant degree of sympatho-adrenal stimulation.

Figure 2-27 is diagrammatic representation of the renin-angiotensin system with emphasis on the components of the vasoconstrictor action which have been deduced as a result of animal studies. Those mechanisms of action for which there is evidence in man are indicated by asterisks, viz, central sympathetic stimulation, direct action, potentiation of released noradrenaline (foreshadowing the results discussed in Part D of this Section), and aldosterone release (discussion of which is beyond the scope of the present study except for a mention of the importance of aldosterone in maintaining circulating blood volume by its sodium and water retaining action on the renal tubules).

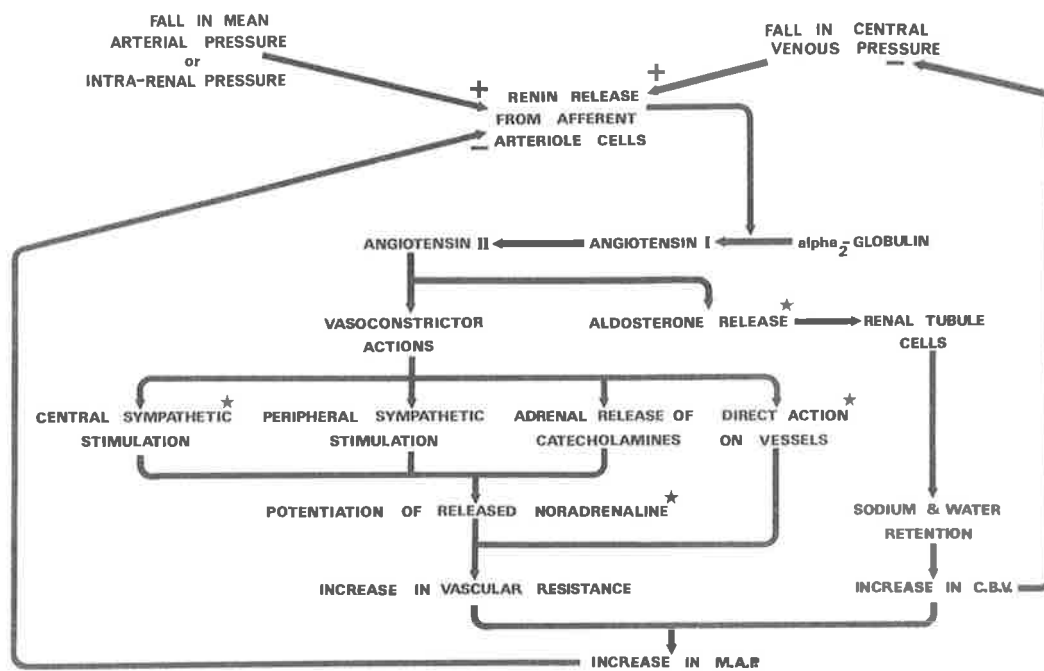


Fig 2-27. A diagrammatic representation of the renin-angiotensin system with emphasis on the components of the vasoconstrictor action. Those mechanisms of action for which there is evidence in man are indicated thus ★. C.B.V. = circulating blood volume; M.A.P. = mean arterial pressure.

SUMMARY

1. Intravenous and intra-arterial infusions of noradrenaline and angiotensin were given in normal human subjects and the changes in brachial arterial blood pressure, central venous pressure, heart rate and hand and forearm blood flow were recorded.
2. The ratio of activities of angiotensin and noradrenaline intravenously on hand vessels was 8-10:1. Intra-arterial infusions also constricted hand vessels but in this case noradrenaline was two to three times as effective as angiotensin.
3. Forearm blood flow was increased by intravenous angiotensin but vascular resistance did not change whereas noradrenaline decreased flow and increased vascular resistance. Intra-arterially, both drugs reduced forearm flow, angiotensin being four times as effective as noradrenaline.
4. Angiotensin was found to be fifteen times as effective in raising the mean arterial blood pressure as was noradrenaline when doses were expressed on a weight basis. The diastolic and mean pressures were elevated to a lesser degree by noradrenaline than by angiotensin with doses which had the same effect on systolic pressure.

5. The bradycardia which accompanied noradrenaline infusions showed a correlation with the rise in mean arterial pressure while that with angiotensin was less marked and did not correlate with the pressure rise.
6. Central venous pressure was increased with both drugs, the rise being more marked with noradrenaline for a given rise in mean arterial pressure. A positive correlation between central venous and arterial pressure rise was found with noradrenaline but not with angiotensin infusions.
7. The possibility of the involvement of central and peripheral vasomotor effects and release of adrenaline in the pressor action of angiotensin is discussed. It was found that angiotensin administered intravenously had a central stimulating action on the sympathetic vasomotor system and that, in the case of the hands, this was the sole cause of the vasoconstriction. There was no evidence of adrenal catecholamine release.
8. More recent studies, however, suggest that the central sympathetic stimulating action does not make a major contribution to the overall pressor effect of angiotensin in man.

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PART D

INTERACTION BETWEEN ANGIOTENSIN, NORADRENALINE
AND SEROTONIN ON THE PERIPHERAL BLOOD VESSELS
IN MAN.

INTRODUCTION.

The first report of an interaction between angiotensin and other vasoactive substances was that of Braun-Menendez, Fasciolo, Leloir and Munoz (1939; 1940) and Braun-Menendez, Fasciolo, Leloir, Munoz and Taquini (1946) who observed that both ephedrine and tyramine potentiated the pressor action of angiotensin in the dog. Prior to this Verney and Vogt (1938) had reported an increase in the pressor response to intravenous tyramine and occasionally that to adrenaline in renal hypertensive dogs. McCubbin and Page (1963) also observed an enhancement of the tyramine response in the presence of angiotensin but found that the response to exogenous noradrenaline was not similarly potentiated, and Khairallah, Page and Turker (1966) reported potentiation of vascular myotropic responses to angiotensin, serotonin and vasopressin by the sympathomimetic agents metanephrine, ephedrine and tyramine on the rabbit aortic strip. Zimmerman (1962) observed that the vasoconstrictor response to angiotensin administered intra-arterially to the perfused dog hind-limb was reduced after acute sympathectomy. Furthermore, the response of the dog cutaneous vessels and the guinea pig vas deferens to sympathetic nerve stimulation has been shown to be enhanced in the presence of angiotensin by Zimmerman and Gomez (1965) and Benelli, Della Bella and Gandini (1964) respectively. Sakurai and Hashimoto (1965) reported an enhanced response of the vessels of the perfused rabbit ear to noradrenaline and tyramine when administered in

combination with subthreshold amounts of angiotensin. The pressor response to tyramine in the intact rabbit was also enhanced by subpressor amounts of angiotensin.

Striking potentiation by serotonin of the constrictor response to angiotensin and noradrenaline in the isolated perfused central artery of the rabbit ear and human digital artery (obtained at post-mortem) was observed by de la Lande, Cannell and Waterson (1966). Hurwitz, Campbell, Gordon and Haddy (1961) were unable to demonstrate potentiation by serotonin of the constrictor responses to angiotensin and noradrenaline in the denervated dog forelimb as a whole, although the venous constrictor actions of both drugs were enhanced by serotonin.

These results of animal studies indicated that a similar mechanism might also exist in man. In order to examine this possibility the responses of the hand and forearm vessels have been observed during intra-arterial infusions of angiotensin, noradrenaline and serotonin in various combinations. In addition, the sensitivity of the forearm vessels to intra-arterial noradrenaline and angiotensin was examined before and during elevation of background serotonin by intravenous administration of the latter.

METHODS

The subjects were volunteer medical students who lay supine on a couch in a temperature-controlled laboratory for at least one hour before observations began, during which time infusion needles were inserted and recording apparatus applied.

The intra-arterial infusions were administered from a motor-driven syringe delivering a volume of 2 ml per min through a 22-gauge needle inserted centripetally into the brachial artery at the elbow under local anaesthesia (Lignocaine 2%). Saline (0.9% W/V) was given throughout the control periods and was also used as a vehicle for the drugs. Similarly, intravenous infusions were administered at 2 ml per min through a polythene catheter inserted into an antecubital vein.

Blood flow through the forearm and hand was measured by venous occlusion plethysmography using water-filled, temperature-controlled plethysmographs (Greenfield, 1954) with the water temperature maintained at 34°C and 32°C respectively. In some experiments forearm blood flow was measured with an electro-capacitance plethysmograph (Willoughby, 1965; Fewings and Whelan, 1966).

Intra-arterial/Intra-arterial interactions

In each subject an intra-arterial infusion of one of the three drugs was administered in a dose sufficient to cause an

obvious and reproducible effect on hand or forearm blood flow. Then a threshold dose (i.e., one which had little or no effect) of one of the other two drugs was determined. When these responses were established the threshold infusion was recommenced and after 4-5 min both drugs were given simultaneously from the same syringe, having been mixed in such concentrations that the dose rate of each was the same as that when administered singly.

Percentage changes in hand or forearm blood flow during infusions of each of the three drugs, whether alone or in combination with one of the other two, were determined from the averaged flow values for the two min prior to drug infusion and for the last two min of the infusion period, by which time the responses to the drugs had become stable. The doses used did not cause systemic effects and hence the blood flow in the opposite arm was regarded as a control. Allowance could then be made for spontaneous variations in flow unrelated to drug action by assuming that in the absence of the drug infusion the two sides would have maintained the same relationship to each other as in the pre-infusion period (Duff, 1952). Allowance was also made for any small effect of the threshold drug alone by appropriate correction of the theoretical flow value obtained as above.

Intra-arterial/Intravenous interactions

In those subjects in whom the background level of serotonin was elevated by intravenous administration, a dose response curve of the forearm vessels to three intra-arterial doses of noradrenaline or angiotensin was first obtained, then a threshold dose of serotonin was administered for 12-13 min: serotonin alone was infused for the first 8 min and then various intra-arterial doses of noradrenaline or angiotensin were infused for 4 and 5 min respectively while the serotonin was still being given. Systemic arterial blood pressure was measured directly from the brachial artery in several of these subjects and no change was observed during infusion of serotonin (0.5 mg/min intravenously). This dose, while not affecting blood pressure, invariably produced a transient increase in forearm blood flow during the first 1-2 min of the infusion, then blood flow settled down at a value very close to the pre-infusion level. Since the intravenous serotonin would be expected to affect both forearm vascular beds equally, the forearm not subjected to intra-arterial infusions could be used as a control and thus allowance could be made for any spontaneous changes in blood flow or changes due to the circulating serotonin.

The drugs used were angiotensin II (val⁵-hypertensinII-aspartic acid, Hypertensin CIBA), noradrenaline bitartrate monohydrate (Levophed, Winthrop) and serotonin (5-hydroxytryptamine creatinine sulphate, SR 134, Sandoz). Doses of noradrenaline are expressed as

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weights of the base and of angiotensin and serotonin as weights of their salts. Ascorbic acid (1:50,000) was added to the noradrenaline solutions.

RESULTSIntra-arterial/Intra-arterial interactionsHand vessels responses(a) Angiotensin-noradrenaline interaction

Figure 2-28 shows the hand vessel responses in one subject during intra-arterial infusion of noradrenaline alone (400 ng/min for 4 min) and then in combination with intra-arterial angiotensin (400 ng/min) during the last 4 min of an 8 min infusion of this drug. The first 4 min of this 8 min infusion period demonstrates that this dose of angiotensin alone in this particular subject had a negligible effect, but when the noradrenaline infusion, which by itself produced a 57% fall in hand blood flow, was repeated in combination with this threshold dose of angiotensin during the last 4 min the resultant fall in hand blood flow was 96%.

Similar results were obtained in three of four other subjects and the results from all five are summarised in Fig 2-29 (upper left-hand frame). In some subjects the combined infusions were repeated with almost identical results. When allowance was made for an occasional small constrictor effect of angiotensin by determining the effect of an 8 min infusion of this drug, it was found that angiotensin significantly enhanced the constrictor action of noradrenaline in the hand blood vessels ($0.02 < p < 0.05$).

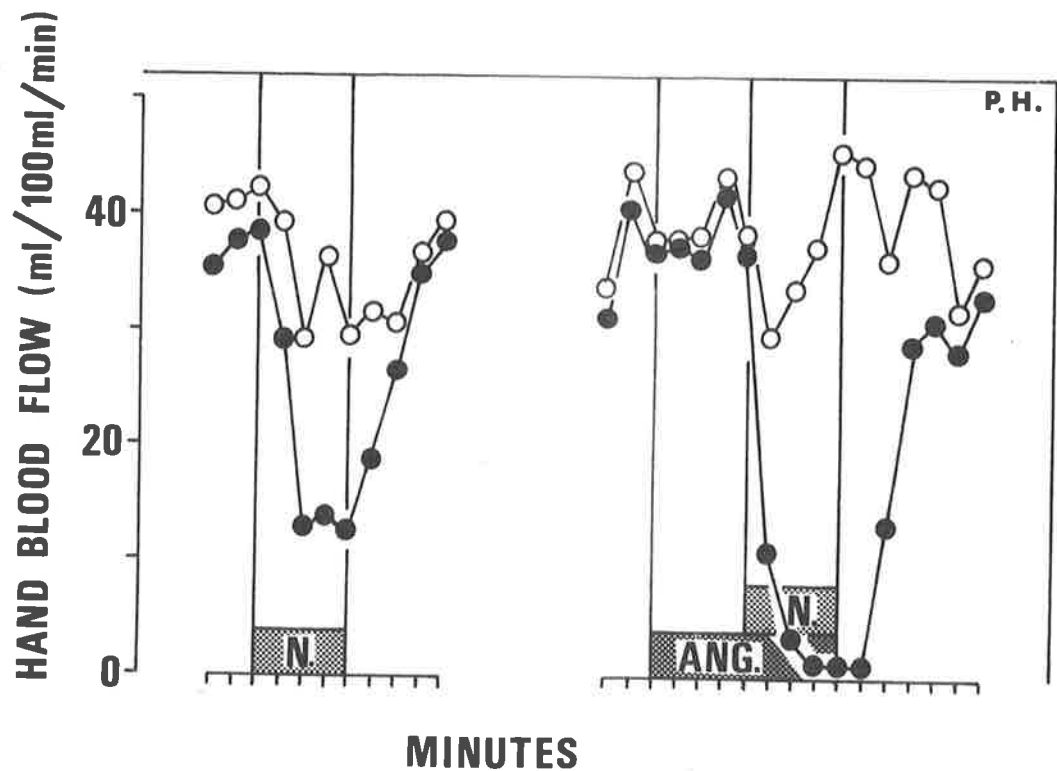
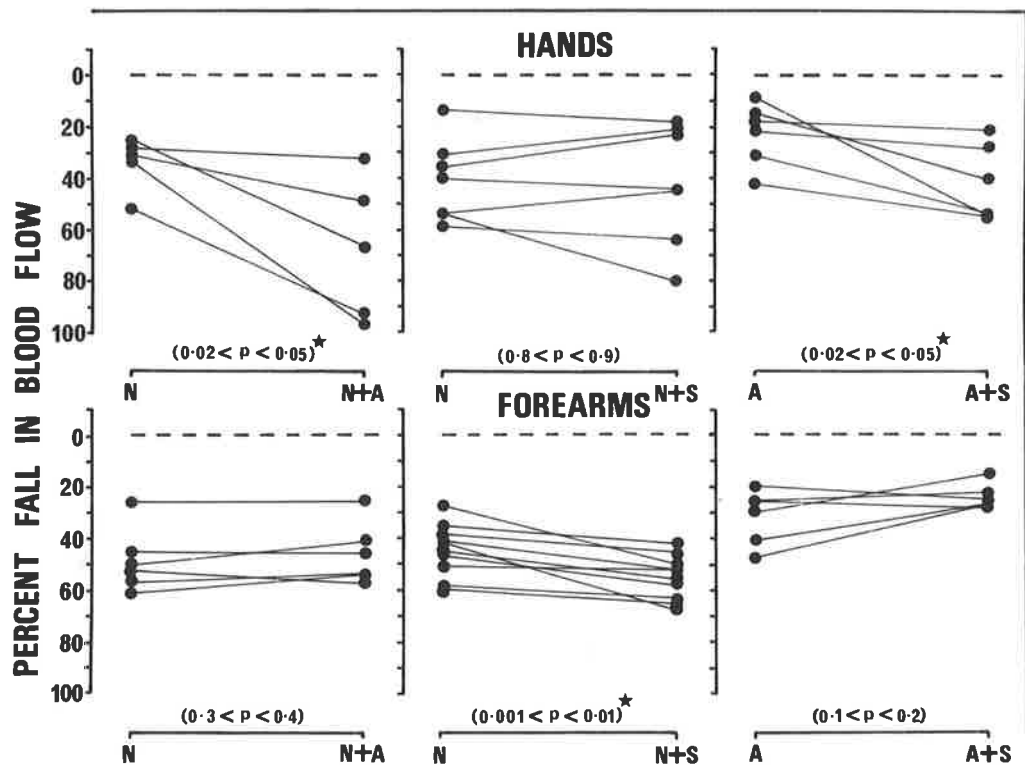


Fig 2-28. The effect of intra-arterial noradrenaline (400 ng/min for 4 min) on hand blood flow (● , infused hand; ○ , control hand) when given by itself (left of figure) and then during the last 4 min of an 8 min intra-arterial infusion of angiotensin (400 ng/min, right of figure).

Fig 2-29. The percentage falls in hand blood flow (upper three frames) and forearm blood flow (lower three frames) in response to intra-arterial infusions of noradrenaline alone (N) and then in combination with intra-arterial angiotensin (N+A, left-hand frames; 5 hand subjects and 6 forearm subjects) and serotonin (N+S, middle frames; 7 hand subjects and 10 forearm subjects), and to angiotensin alone (A) and then in combination with serotonin (A+S, right-hand frames; 6 hand subjects and 6 forearm subjects). Allowance has been made for the constrictor action of the "threshold" drug in each experiment. The results from the paired "t" tests are shown in brackets, those combinations resulting in significant potentiation being indicated thus ★ .



(b) Noradrenaline-serotonin interaction

Figure 2-29 (upper middle frame) shows the mean constrictor responses of the hand vessels of 7 subjects to noradrenaline alone and then the mean responses to the same dose when administered in combination with subthreshold doses of serotonin. As in the previous experiments each subject received several infusions of noradrenaline alone at the same dose level (usually 100 ng/min for 4 min) to obtain a mean dose-response value and then this dose was repeated during the last 4 min of an 8 min infusion of serotonin in a threshold dose (usually 250-500 ng/min for 8 min). Some of the combined infusions were repeated and a mean value obtained. In calculating the constrictor responses to noradrenaline in the presence of serotonin allowance was made for any small serotonin effect. The constrictor action of intra-arterial noradrenaline in the hand vessels was not significantly altered in the presence of serotonin ($0.8 < p < 0.9$) and in many cases the response was actually reduced.

(c) Angiotensin-serotonin interaction

Figure 2-30 shows the hand vessel responses in one subject during intra-arterial infusion of angiotensin alone (200 ng/min for 5 min) and then in combination with intra-arterial serotonin (500 ng/min) during the middle 5 min of a 15 min infusion period of this drug. In this subject this dose of serotonin alone had a

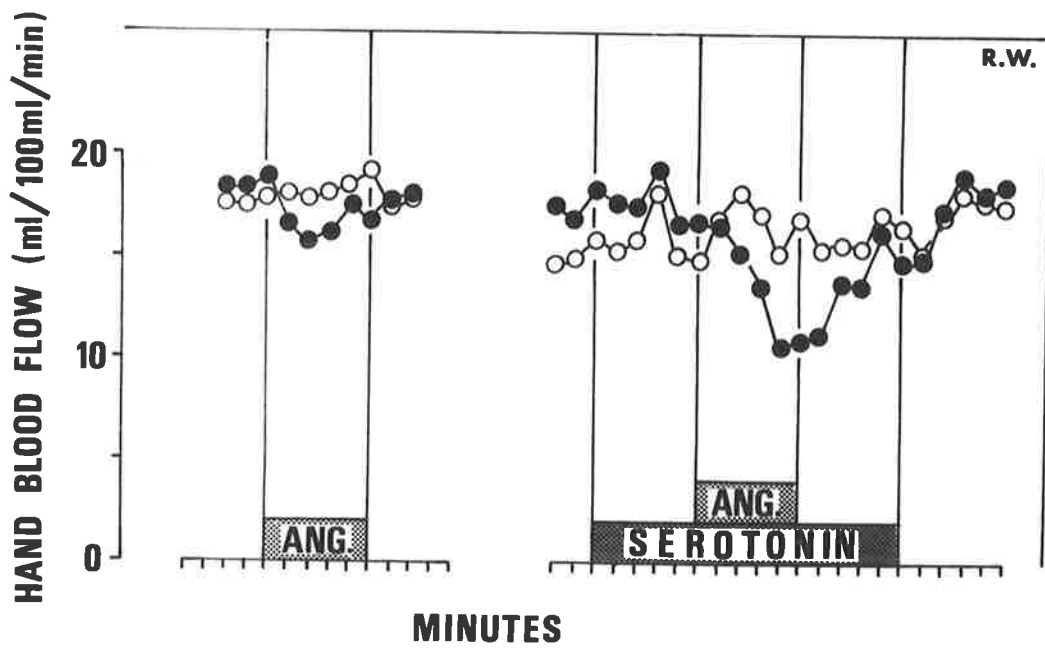


Fig 2-30. The effect of intra-arterial angiotenin (200 ng/min for 5 min) on hand blood flow (●, infused hand; ○, control hand) when given by itself (left of figure) and then during the middle 5 min of a 15 min intra-arterial infusion of serotonin (500 ng/min, right of figure).

negligible effect, but when the infusion of angiotensin, which alone produced a 14% fall in hand blood flow, was repeated against this background the enhancement of constrictor effect to produce a 38% fall in flow was greater than could be explained by summation alone. When the angiotensin infusion was terminated, the serotonin infusion continuing, the blood flow took much longer to return to pre-angiotensin infusion levels than it did following angiotensin alone.

A similar enhancement of the constrictor effect of intra-arterial angiotensin on the hand blood vessels in the presence of serotonin was seen in five other subjects and the enhancement was significant when allowance was made for any serotonin effect ($0.02 < p < 0.05$). The results from all six subjects are summarised in Fig 2-29 (upper right-hand frame).

Forearm vessel responses

(a) Angiotensin-noradrenaline interaction

Figure 2-29 (lower left-hand frame) shows the mean constrictor responses of the forearm vessels of six subjects to noradrenaline alone (in most experiments 25-50 ng/min for 4 min) and then the mean responses to the same dose when administered during the last 4 min of 8 min infusion periods of threshold doses of angiotensin (in most cases 0.25-0.5 ng/min for 8 min). To obtain mean responses most infusions were given more than once and in

calculating the constrictor responses to noradrenaline in the presence of angiotensin allowance was made for any small angiotensin effect. The constrictor action of intra-arterial noradrenaline on the forearm vessels was not significantly altered in the presence of angiotensin ($0.3 < P < 0.4$). This is contrary to the findings in the hand vessels with the same combination and experimental design.

(b) Noradrenaline-serotonin interaction

Figure 2-31 shows the forearm vascular responses in one subject during intra-arterial infusion of noradrenaline alone (50 ng/min for 4 min) and then together with a threshold dose of serotonin (500 ng/min) during the last 4 min of a 9 min infusion of this drug. Noradrenaline alone in this subject produced a 25% fall in forearm blood flow but when administered in combination with serotonin the resultant fall in forearm blood flow when corrected for the serotonin effect was 50%. Similar results were obtained in nine other subjects and the results from all ten have been summarised in Fig 2-29 (lower middle frame). In all subjects the constrictor responses of the forearm vessels to intra-arterial noradrenaline were significantly enhanced in the presence of serotonin ($0.001 < p < 0.01$).

(c) Angiotensin-serotonin interaction

Figure 2-29 (lower right-hand frame) illustrates

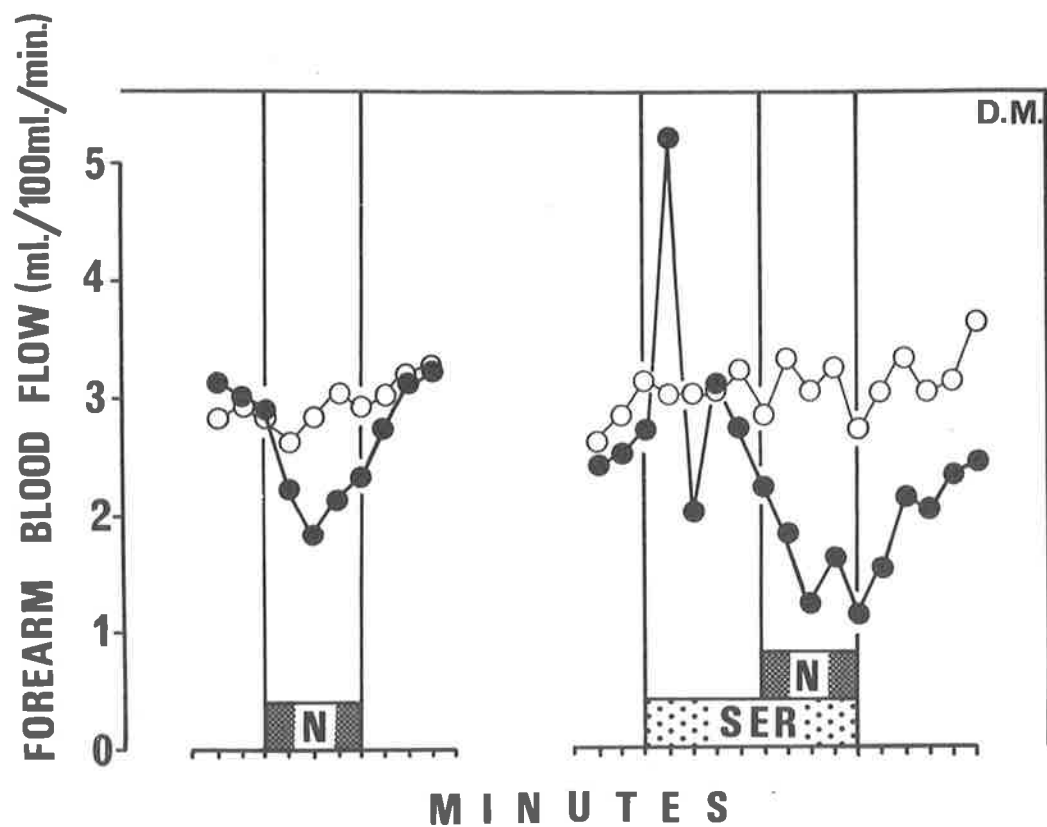


Fig 2-31. The effect of intra-arterial noradrenaline (50 ng/min for 4 min) on forearm blood flow (●, infused forearm; ○, control forearm) when given by itself (left of figure) and then during the last 4 min of a 9 min intra-arterial infusion of serotonin (500 ng/min, right of figure).

the percentage falls in forearm blood flow in each of six subjects in response to intra-arterial angiotensin alone (4, 8 or 16 ng/min for 5 min) and then in combination with a threshold intra-arterial infusion of serotonin (0.5-1 μ g/min). In three of the six subjects shown serotonin caused a marked reduction in the constrictor response to angiotensin whereas in the other three there was a small enhancement of the response. Collectively the constrictor action of angiotensin was not significantly altered in the presence of threshold amounts of serotonin ($0.1 < p < 0.2$). In contrast, significant potentiation was seen in the hand vessels. As in the other experiments the doses of serotonin used had very little effect on forearm blood flow but allowance was made for this in the calculations.

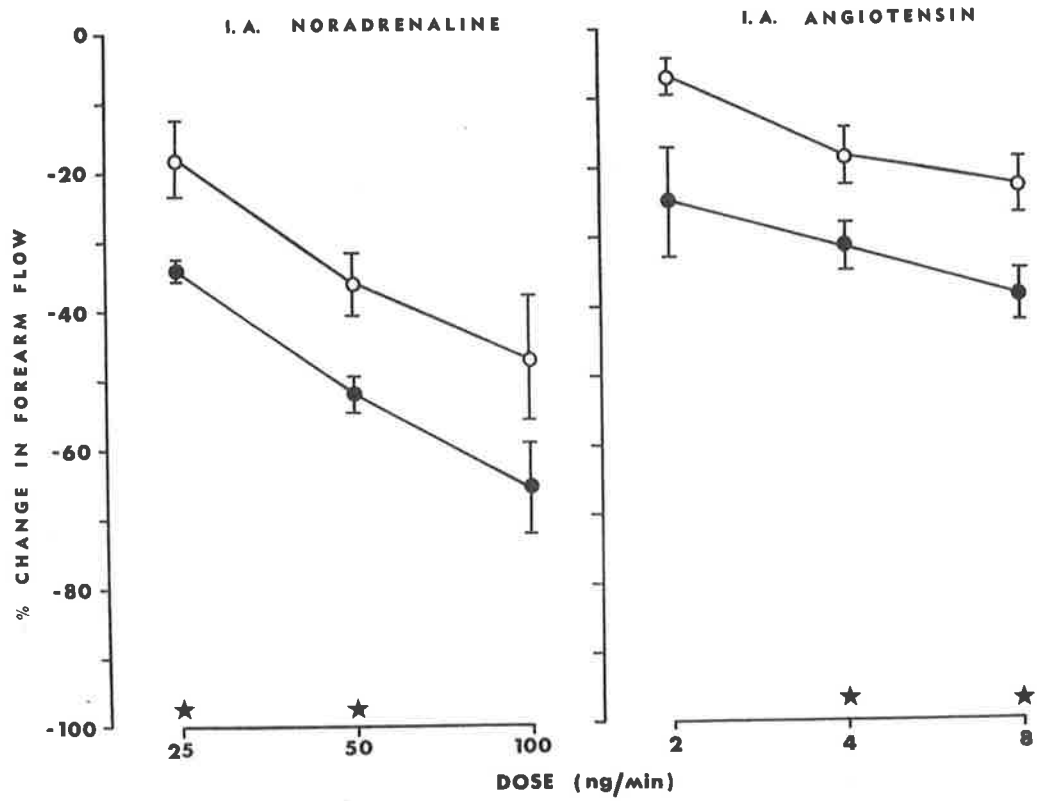
Intra-arterial/Intravenous interactions

(a) Noradrenaline-serotonin interaction

Figure 2-32 (left hand frame) illustrates the mean percentage falls in forearm blood flow (5 subjects) in response to intra-arterial noradrenaline alone (25, 50 and 100 ng/min for 4 min) and then in combination with threshold intravenous infusions of serotonin (0.5 mg/min). The difference between the mean percentage fall in flow before and during intravenous serotonin administration was significant with intra-arterial noradrenaline 25 and 50 ng/min but not with 100 ng/min. However, in each of the five subjects the

Fig 2-32. The mean percentage changes in forearm blood flow during intra-arterial infusions of noradrenaline (left-hand frame) and angiotensin (right-hand frame) before (○) and during (●) administration of threshold doses of serotonin intravenously (0.5 mg/min). The mean responses were obtained from 5 subjects in each group. The vertical lines through each value represent one standard error on either side of the mean. Those combinations resulting in significant potentiation are indicated thus ★ .

I.V. 5-HYDROXYTRYPTAMINE



constrictor responses of the forearm vessels to every dose of intra-arterial noradrenaline were significantly enhanced in the presence of serotonin ($P < 0.001$; paired data within individuals) as was the case with the intra-arterial versus intra-arterial interaction. The mean increase in vasoconstrictor potency was 16% during intravenous serotonin administration.

(b) Angiotensin-serotonin interaction

Figure 2-32 (right hand frame) shows the mean percentage decreases in forearm flow in 5 subjects during intra-arterial infusions of angiotensin (2, 4 and 8 ng/min for 5 min) alone and then when repeated during the last 5 min of 13 min threshold infusions of intravenous serotonin (0.5 mg/min). A significant increase in the vasoconstrictor effect of angiotensin was found during serotonin administration when the mean percentage decreases in flow at dosage rates of angiotensin 4 and 8 but not 2 ng/min were compared. On the basis of changes in individuals the constrictor potency of angiotensin increased 16% in the presence of intravenous serotonin: each dose of angiotensin in each subject showed potentiation of the vasoconstrictor effect on the forearm vessels ($P < 0.001$). This contrasts with the intra-arterial versus intra-arterial results where no significant interaction between angiotensin and serotonin was found in the case of the forearm vessels.

DISCUSSION

de la Lande, Frewin and Glover (personal communication) perfused human digital arteries, obtained immediately post mortem, with Krebs solution at a constant rate and followed the changes in vessel tone in response to added drugs by measuring the accompanying changes in perfusion pressure. When a single dose of angiotensin was added to the perfusing fluid it caused a constriction of the vessels. After this had worn off the vessel remained for some time more responsive than before to noradrenaline (Fig 2-33, upper frame). Likewise, when angiotensin was perfused through the vessel in a concentration which was below that required to produce constriction, responses to added noradrenaline were greater than those obtained with the same doses in the absence of angiotensin (Fig 2-33, lower frame).

Previously, de la Lande et al (1966) using the isolated, perfused central artery of the rabbit ear and the digital artery of man (obtained at post mortem) had shown potentiation of the constrictor responses to angiotensin and noradrenaline in the presence of sub-threshold concentrations of serotonin and concluded that serotonin was the sensitising agent in these experiments.

In the present study a complicated pattern of interactions between angiotensin, noradrenaline and serotonin was observed in the intra-arterial/intra-arterial studies and whether or not potentiation

Fig 2-33. Upper frame. The responses of the isolated perfused human digital artery to injections of noradrenaline (0, 25 ng) into the perfusion fluid before and after injection of angiotensin (50 ng).

Lower frame. Responses of the isolated perfused human digital artery to injections of noradrenaline (50 and 75 ng) before, during and after perfusion with angiotension (4 ng/min). The time scales of the abscissae are in minutes. (de la Lande, Frewin and Glover, personal communication).

occurred depended not only on the two drugs chosen but also on the vascular bed to which they were administered. Statistically significant potentiation was seen with angiotensin-noradrenaline and angiotensin-serotonin combinations in the hand but not in the forearm. Conversely, statistically significant potentiation was seen with combinations of noradrenaline and serotonin in the forearm but not in the hand. Furthermore, even in those combinations where collectively there was significant potentiation there were some experiments in which the combined response was very little different from the individual response. However, the interactions were more marked when forearm vessel responses to intra-arterial noradrenaline or angiotensin were studied against an elevated level of circulating serotonin achieved by continuous intravenous infusion of serotonin. The mean constrictor potency of both noradrenaline and angiotensin increased 16% against threshold intravenous serotonin within individuals, and in these experiments it was possible to show a marked shift of the dose-response curves to the left when the mean percentage falls in flow at each dose level were compared before and after serotonin infusions in the five subjects in each group. Figure 2-32 shows that to obtain the same vasoconstrictor response before and during intravenous serotonin administration only approximately half the dose of noradrenaline and only one quarter of the dose of angiotensin was required in the presence of serotonin. It is not apparent why the studies revealed a greater interaction when intravenous,

rather than intra-arterial, background levels of serotonin were raised. This difference is particularly striking in the case of the forearm responses to angiotensin. The explanation may involve differences in platelet bound versus free plasma serotonin levels or release of other vasoactive substances during systemic administration of serotonin.

The significance of these findings is obscure but certain speculations can be made. The intra-arterial/intravenous studies suggest that serotonin-noradrenaline and serotonin-angiotensin interactions could be important factors in the maintenance of normal peripheral vasoconstrictor tone. If this were so it would indicate a normal physiological function for the relatively high levels of circulating serotonin found in the blood. Hitherto, serotonin has generally been regarded as a rather passive circulating substance, both in the platelet bound and free forms. One would have expected, though that if this interaction phenomenon were of general importance consistent potentiation of vasoconstrictor responses would have been found with the intra-arterial/intra-arterial combinations. However our results suggest that while statistically significant degrees of potentiation can be demonstrated with some combinations of angiotensin, serotonin and noradrenaline the enhancement is usually small (especially in the case of the intra-arterial/intra-arterial studies) and is therefore of doubtful biological importance.

The degree of potentiation seen in isolated animal and human arterial preparations is often quite large, being of the order of a two to twenty-fold increase in constrictor effect (de la Lande et al, 1966). Such potentiation would be of considerable importance if it occurred in an intact vascular bed. In the case of isolated vessel studies the preparation has very little if any tone and is perfused at a low pressure with Krebs or other similar solution. It is therefore not exposed to the normal plasma levels of the hormones under study and may readily exhibit major degrees of potentiation when these are added. In the case of the vessels of the intact limb, however, maximal interaction may already be established between the normal circulating levels of angiotensin, noradrenaline and serotonin and little further potentiation could be achieved by increasing the levels in the perfusing blood. Thus the design of the present experiments may have precluded finding greater degrees of potentiation because the initial levels of circulating vasoactive substances were not known, and in any case these could not have been lowered to zero.

The mechanism of the potentiation is not clear from our experiments, nor were we able to decide which of the drugs was the more important sensitising factor. de la Lande et al (1966) suggest that the mechanism of sensitisation may be through changes in the resting membrane potential. For example, serotonin may shift the resting potential of the smooth muscle cell toward the

the critical firing level, thereby increasing excitability and enhancing the response to some additional constrictor agent. This suggestion is supported by the work of Pickles (1956) who postulated that serotonin caused a selective alteration in cell membrane permeability, thereby decreasing the potential difference across cell membranes and increasing passive movement of ions. Competitive interactions between the various drugs for receptor sites or catabolic enzymes are other possible mechanisms (Furchgott, 1954; Zeller, Barsky and Berman, 1955; Axelrod and Tomchick, 1960).

The type of interaction outlined in this discussion is also found with other substances. For example, Besse and Bass (1966) have described potentiation by hydrocortisone of responses to catecholamines in vascular smooth muscle, both in vitro and in vivo, suggesting that hydrocortisone modifies the interaction of the catecholamines with specific tissue receptors.

In conclusion, interactions between serotonin and other circulating vasoactive substances, such as noradrenaline and serotonin, may contribute to the maintenance of peripheral vascular tone in man, but the inconsistent, and relatively minor, degrees of potentiation compared with in vitro studies in animals make it unlikely that the interactions make a major contribution to the vasoconstrictor actions of these three hormones.

SUMMARY

1. With intra-arterial/intra-arterial studies the constrictor action of noradrenaline on the hand vessels was significantly enhanced in the presence of threshold amounts of angiotensin as was that of angiotensin in the presence of threshold amounts of serotonin. No such potentiation was seen with the same combinations in the forearm.
2. With intra-arterial/intra-arterial studies in the forearm significant potentiation was seen only with constrictor doses of noradrenaline in the presence of threshold amounts of serotonin. No potentiation was seen with this combination in the hand vessels.
3. The vasoconstrictor action of both noradrenaline and angiotensin on the forearm was enhanced significantly during intravenous infusion of threshold doses of serotonin.
4. These findings were discussed in relation to the more marked interactions described in in vitro situations. It was concluded that while it is possible that such interactions may contribute to maintenance of peripheral vascular tone, the inconsistency of some of the findings makes it unlikely that interactions between serotonin, noradrenaline and angiotensin make a major contribution to their vasoconstrictor actions.

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SECTION 3

THE ROLE OF VASOACTIVE SUBSTANCES IN THE HYPERAEMIA
WHICH FOLLOWS EXERCISE OF SKELETAL MUSCLE.

165.

PART A

THE ROLE OF ACETYLCHOLINE IN THE PHENOMENON
OF POST-EXERCISE HYPERAEMIA IN MAN.

INTRODUCTION

Gaskell (1877) was probably first to record the changes in the circulation through skeletal muscle during and after exercise. He assessed blood flow directly by measuring the rate of venous out-flow from the thigh muscles of the dog in association with tetanic contractions elicited by electrical stimulation of the motor nerve. In particular, he observed the large increase in flow which follows cessation of the stimulation - i.e. the phenomenon of post-exercise (or functional) hyperaemia.

Despite extensive investigation the cause of the increase in blood flow through skeletal muscle during and after its contraction remains unknown, although a wide range of causative factors have been examined.

As early as 1880, Gaskell had suggested that the hyperaemia was a local phenomenon due to chemical changes in active muscles rather than to impulses from vasodilator nerves or due to arrival of substances circulating generally. It appears unlikely that an intact sympathetic nerve supply to the muscles is necessary for the vascular response during contraction, since Grant (1938) and Barcroft (1964) studied sympathectomised limbs and did not observe any change in the post-exercise hyperaemia or in the patient's ability to exercise. Since Blair, Glover and Roddie (1961) found that vascular resistance of the nerve-blocked forearm was unchanged during vigorous leg

exercise it also seems unlikely that a muscle-vasodilating hormone is circulating. However, Fewings (1967) infused propranolol, a β adrenoreceptor blocking agent, intra-arterially in ten normal subjects and found a significant reduction in the degree and duration of the hyperaemia following 30 sec rhythmic exercise of the forearm muscles in six of these subjects. The latter author, therefore, suggested a contribution to the hyperaemia by circulating adrenaline released from the adrenals as a result of emotional stress involved in performing the exercise. The contribution of circulating substances is unlikely to be great, however, and probably can be excluded as one of the principal causes of the hyperaemia.

Since most evidence suggests that neither vasomotor nerve activity nor circulating vasodilator hormones account for the post-exercise hyperaemia it is probable that the phenomenon is due to release of one or more vasoactive substances in the muscle itself or at least to some physical or chemical change in the local environment. Much time and energy has been devoted to the search for the elusive local factor or factors responsible; involvement of changes in pO_2 , pCO_2 , pH, lactic acid and other Krebs intermediate metabolites, potassium ions, acetylcholine, histamine, bradykinin, adenine nucleotides and myogenic factors have all been suggested.

Chauveau and Kaufmann (1887) were probably first to demonstrate a fall in oxygen content of blood from exercising muscle and also among the first to demonstrate the hyperaemia of

intermittent exercise (in contrast to the post tetanic contraction hyperaemia). They measured blood flow by collecting the venous effluent from the upper lip of four horses at rest and while chewing, and observed a 5-10 fold increase in the amount of blood collected in each five min period during the chewing phase. The above authors also documented a rise in carbon dioxide and a fall in glucose content of the returning blood during mastication. Muscular contraction increases the local demand for oxygen and this demand increases with the severity of the muscular activity (Quensel and Kramer, 1939; Love, 1955; Barcroft, Greenwood and Whelan, 1963).

The effect on muscle blood flow of decreasing the oxygen tension of the perfusing blood has been examined by several workers. Crawford, Fairchild and Guyton (1959) reported that the blood flow through the hind limb of the anaesthetised dog doubled when the oxygen saturation of the arterial blood was reduced to 30% of normal, and similarly, Ross, Fairchild, Weldy and Guyton (1962) found a progressive increase in blood flow in the same preparation as the oxygen saturation was decreased from 100% to 0%. In the somewhat analogous phenomenon of reactive (post-ischaemic) hyperaemia, Fairchild, Ross and Guyton (1966) reported failure of recovery from the hyperaemia in the absence of oxygen - the hind limb of the dog, perfused at constant pressure with venous blood, showed no decrease from the high flow levels till reoxygenated blood was perfused. These observations suggest that anoxia may be an important local

factor in the regulation of the tone of the resistance vessels. On the other hand, Ross, Kaiser and Klocke (1964) found that the hyperaemic responses in rhythmically contracting dog gastrocnemius muscle were similar when either arterial or venous blood was perfused through the limb, and concluded that lowered pO_2 did not produce sufficient vasodilatation (either at rest or during exercise) to explain functional hyperaemia in skeletal muscle.

Dornhorst and Whelan (1953) examined the effects of reduced arterial oxygen supply on post-exercise hyperaemia in man and found only a slight increase in the levels of resting and hyperaemic flows in the calf during breathing of 8% oxygen, and the rate of recovery after exercise was the same as in the control state breathing room air. Love (1955) and Barcroft et al (1963) found that venous blood oxygen saturation estimations were elevated during the phase of post-exercise hyperaemia and thus it was unlikely that there was any tissue anoxia at this stage. Barcroft (1964) and Haddy (1966) suggested that while changes in oxygen tension may play a role in local regulation of resistance there is no general relationship between muscle blood flow and tissue oxygen tension.

Involvement of carbon dioxide in the hyperaemic response was suggested by Gaskell (1880) and an increase in carbon dioxide content in venous blood during muscular contraction was described by Chauveau and Kaufmann (1887). Stein and Weinstein (1942) and

Glover and Greenfield (1958) recorded increases in plethysmographically-measured blood flow when the calf and hand respectively were exposed to water saturated with carbon dioxide, indicating a direct vasodilator action of carbon dioxide on skin. Kontos and Patterson (1964) found a correlation between venous $p\text{CO}_2$ and blood flow during reactive hyperaemia in man and concluded that accumulation of carbon dioxide during ischaemia was an important factor in the production of reactive hyperaemia. However, when post-exercise hyperaemia was examined in this respect, Kontos, Richardson and Patterson (1966) concluded that local hypercapnia (and local hypoxia) could account for only part of the hyperaemia. Inhalation of gaseous mixtures with a high content of carbon dioxide causes a decrease in forearm blood flow which is probably in the muscle vascular bed (McArdle, Roddie, Shepherd and Whelan, 1957) but, as emphasised by Daugherty, Scott and Haddy (1967), both generalised hypercapnia and hypoxia, by their central actions, antagonise the local effects of hypercapnia and hypoxia on vascular smooth muscle; and therefore inhalation studies are difficult to interpret. Thus, local hypercapnia, while it appears to have a mild dilator action on some blood vessels, is of doubtful significance in explaining post-exercise hyperaemia.

The pH of venous effluent from the exercising gastronemius muscle of the dog decreased during activity (Gollwitzer-Meier, 1950) but there was no correlation between pH and flow changes. Similarly,

Barcroft, Greenwood and Rutt (1963) recorded a fall in pH in samples of venous blood draining from forearm muscles following sustained contractions, but again the pH and blood flow changes were not closely related. Richardson, Wasserman and Patterson (1961) found that either a rise or a fall in pH exerted a dilating action on vessels of a limb following sympathetic blockade by intra-arterial phenoxybenzamine, and therefore suggested that vasoconstrictor reflexes normally oppose the direct dilating action of altered blood pH. Changes in pH may, therefore, contribute to the total hyperaemic response.

Muscular exercise results in release of considerable amounts of lactic acid, but it can probably be excluded as a factor responsible for the functional hyperaemia because, firstly, patients suffering from myophosphorylase deficiency (McArdle, 1951), and who consequently are unable to convert glycogen to lactic acid, still exhibit a normal post-exercise hyperaemia, and secondly, infusion of a range of doses of lactic acid failed to induce an increase in forearm blood flow (de la Lande and Whelan, 1962) even when lactate levels of the venous effluent were markedly increased by the intra-arterial infusions. While there is little evidence to suggest involvement of lactic acid, Haddy (1966) notes that little attention has been paid to the Krebs intermediate metabolites as a whole and these may play a part in local regulation of vascular tone. Frohlich (1965) found a vasodilatation in the dog forelimb when the sodium salts of

acetate, citrate, α -ketoglutarate, succinate, fumarate, malate and oxalacetate were each infused separately.

Potassium is released from skeletal muscle during activity in animals and man (Dawes, 1941; Skinner, 1961) and has a dilator action on muscle vessels. Kjellmer (1965) found vasodilatation using the hind limb of the cat during intra-arterial infusions of potassium salts and postulated that potassium ions released from muscle cells during exercise account for up to 65% of the hyperaemic response. Administration of potassium chloride into the brachial artery had a vasodilator effect on the blood vessels of the forearm (Glover, Roddie and Shanks, 1962) but the dilator action was only a modest one even when the infusions resulted in a large increase in potassium concentration in the venous effluent. It is unlikely that potassium plays a major role in the production of post-exercise hyperaemia.

Various vasoactive hormones have been suggested as mediators of the functional vasodilator phenomenon. Acetylcholine, the transmitter at the motor end plate in animals (Dale, 1934b) and probably also in man, is a potent vasodilator substance and it may be involved in exercise hyperaemia. However, Hilton (1953) using curarised muscle was unable to produce vasodilatation during stimulation of the motor nerve, whereas tetanic contraction, elicited by direct electrical stimulation of the muscle led to the usual functional dilatation. This suggested that acetylcholine released

at the motor end plate during motor nerve activity was not directly involved in the production of post-exercise hyperaemia.

Increases in the histamine content of the venous blood from exercising muscle have been reported in the dog (Anrep and Barsoum, 1935), and man (Anrep, Barsoum, Salama and Souidan, 1944). These results, however, have not been confirmed in animals or man. For instance, the administration of antihistaminic drugs intra-arterially to the forearm in doses which effectively abolished the response of the forearm vessels to injected histamine did not significantly influence the course of the hyperaemia following 30 sec rhythmic exercise (Whelan, 1956). Similarly, a role for histamine in post-exercise hyperaemia was considered unlikely by Hilton (1953) on the basis of studies using antihistamine drugs.

The naturally occurring nonapeptide bradykinin is a potent vasodilator substance; Fox, Goldsmith, Kidd and Lewis (1961) reported that forearm blood flow increased during intra-arterial infusions of bradykinin. Hilton and Lewis (1955) have shown that bradykinin is probably responsible for the functional vasodilatation in the secreting submandibular salivary gland of the cat, and Fox and Hilton (1958) have observed that the bradykinin content of human skin increases during the hyperaemia of indirect body heating. It is possible, therefore, that bradykinin is also involved in the production of post-exercise hyperaemia. Allwood and Lewis (1964) and Mashford and Zacest (1965, personal communication) were, however,

unable to detect an increase in the bradykinin content of the venous effluent from human forearm muscle during exercise. More recently, Webster, Skinner and Powell (1967) were able to block the vasodilator effect of bradykinin on the isolated perfused gracilis muscle preparation of the dog with the kininase carboxypeptidase B, but found that the latter had no effect on the functional hyperaemia of muscle contraction. Thus bradykinin does not appear to be involved in post-exercise hyperaemia.

The adenine nucleotides, adenosine tri-, di- and monophosphate (ATP, ADP and AMP) are potent vasodilator substances (Frohlich, 1963; Berne, 1964) and Duff, Patterson and Shepherd (1954) have shown that ATP infused intra-arterially causes a marked increase in the blood flow through the forearm and hand. Since adenine nucleotides (particularly ATP) play an important role in muscle contraction it is possible that they may be released from the muscle cells and produce vasodilatation. Hilton (1962) considered that the nucleotides did not pass through the cell membranes of intact skeletal muscle cells and were, therefore, unlikely to be involved. However, Haddy (1966) suggests that various adenine nucleotides do escape into venous effluent and that with more refined assay techniques a better understanding of their role in regulation of vascular resistance may be achieved.

In addition to the above chemical and hormonal factors, participation of myogenic factors may be involved in the local

hyperaemic response to exercise. A drop in transmural pressure induced by reduction in perfusion pressure has been shown to cause vascular relaxation (Bayliss, 1902). Thus a myogenic relaxation of resistance vessels may reinforce the vasodilatation initiated by local vasoactive substances. Similarly, Hilton (1959) described dilatation of proximal arterial vessels during exercise of distal leg muscles and, on the basis of stripping and denervation procedures, concluded that this effect was non-nervous in origin and that the conducting elements lay in the media of the vessels. This myogenic effect is probably of considerable importance in reactive hyperaemia where arterial transmural pressures fall very low, but it appears unlikely that such physical factors are involved to any great extent in post-exercise hyperaemia.

Thus many factors have been investigated in an attempt to find the cause of this functional vasodilatation in skeletal muscle. It may well be that all of the above factors are involved to some extent and that the post-exercise hyperaemia is the resultant of interactions between many vasodilator mechanisms; and, indeed, recently Skinner and Powell (1967) have shown reinforcement of the vasodilator effects of low oxygen and high potassium content on perfused dog gracilis muscle when both the pO_2 and the potassium concentration were still within the normal physiological range. On the other hand, very little fall in resistance was observed in this preparation with either an equivalent degree of hypoxia or

elevated blood potassium alone. Further investigations into the interactions between the various factors will probably reveal the importance of the combined actions of a number of agents. One difficulty in postulating such interactions arises when one considers that the hyperaemic response normally follows a simple exponential pattern i.e. the blood flow rises rapidly to a peak when the muscular contraction ceases and then blood flow declines at a constant relatively simple exponential rate. If the hyperaemic response were due to local vasodilatation caused by accumulated products of metabolism acting on the resistance vessels, then a more complicated pattern of blood flow response would be expected since changes in the concentration of the dilator would affect subsequent removal of the dilator metabolites. This may be explained, however, by the concept of dual circulations - a nutritive circulation concerned with removal of and regulated by local metabolites, and a by-pass or non-nutritive circulation (Hyman and Lenthall, 1962; Hyman, Paldino and Zimmerman, 1963). Thus if a major fraction of the excess flow is through the non-nutritive circulation, provided the nutritive circulation is maximally dilated, then the vasodilator metabolites would be removed at a fixed rate, and thus the kinetics would conform to the simple exponential relationship observed.

Since most investigators have implicated vasoactive substances as causative agents in post-exercise hyperaemia, it was the purpose of the present study to examine the venous blood draining

from human skeletal muscle for the presence of active substances liberated as a result of skeletal muscle contraction, using the opposite forearm and the guinea pig ileum as the test organs. This subsequently led to the investigation of the role of acetylcholine in the mechanism of post-exercise hyperaemia in man and (in Part B) to the examination of sheep lymph for active substances.

METHODS

The subjects in all experiments were normal healthy males who lay supine on a couch in a temperature-controlled laboratory (22-25°C) for 30-60 min before observations began, during which time the apparatus was applied and catheters and needles inserted.

Blood flow was measured by the technique of venous occlusion plethysmography, using water-filled plethysmographs in the initial experiments and Adelaide capacitance plethysmographs in the latter experiments. Pneumatic arterial occlusion cuffs encircled the wrists and were inflated to 200 mm Hg 2 min before blood flow measurements were made or venous blood samples were taken.

Cross-transfusion experiments

Fig 3-1 shows diagrammatically the method used to examine the venous blood from one forearm by injecting the blood into the brachial artery of the opposite arm and measuring the blood flow through the forearm of this latter side.

Venous blood draining from forearm muscle was withdrawn through a nylon catheter introduced through a 17-gauge needle into a deep (muscle) vein located in the cubital fossa of one arm. The catheter was kept patent by the occasional injection of a small volume of saline (0.9% W/V) containing heparin 1 U/ml. A siliconised 22-gauge needle was introduced into the brachial artery in

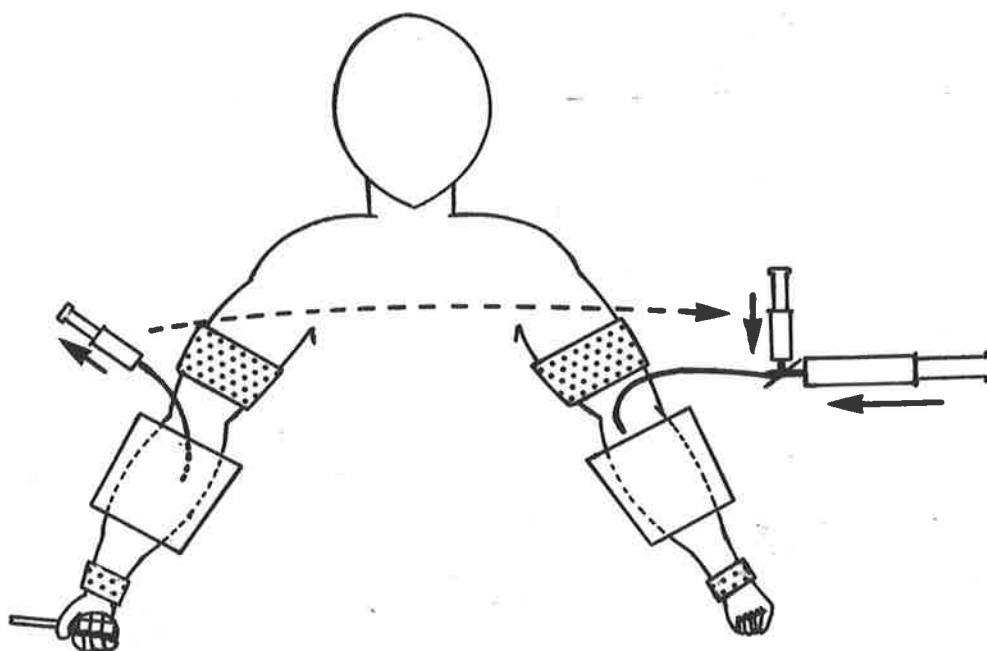


Fig 3-1. A schematic diagram showing the assay of venous blood, draining from forearm muscle by slow injection into the brachial artery of the opposite arm during venous occlusion plethysmography.

the cubital fossa of the opposite arm and connected by a short length of polythene tubing to a 3-way polythene stopcock attached to a siliconised glass syringe. A constant infusion apparatus delivered saline containing heparin 1 U/ml at a rate of 4 ml/min into the brachial artery. Oxygen saturation measurements made according to the method of Roddie, Shepherd and Whelan (1957b) on samples of venous blood ensured that the blood obtained was, in fact, draining from the forearm muscles.

The usual experimental procedure was to measure bilateral resting forearm blood flow for a few minutes. A 5ml sample of venous blood, collected in a nylon syringe, was then taken from the resting forearm of one side and 4 ml of this sample injected via the 3-way stopcock into the brachial artery of the opposite testing forearm over a period of 1 min, blood flow measurements being continued throughout this procedure. After a further 4 or 5 min of blood flow measurements, the forearm from which venous samples were obtained was exercised rhythmically by squeezing a rubber bulb and raising a column of water 75 cm in a glass tube of 0.7 cm internal diameter which was connected to the bulb by a length of rubber tubing. The standard exercise for this series of experiments was 30 contractions/min for 4 min. A venous sample was collected from the exercising forearm during a sustained contraction at the end of the 4 min exercise period. This sample was then injected into the brachial artery of the opposite arm in the same way as the control

sample. Bilateral blood flow measurements were made throughout the exercise period and for up to 10 min after completion of the exercise.

Superfused guinea pig ileum

Six week old guinea-pigs weighing 150-200 g were used in all experiments. The ileum was superfused according to the method described by Gaddum (1953) with Tyrode solution aerated with O_2 (95%) and CO_2 (5%) and warmed to $37^{\circ}C$. The solution contained NaCl 8 g; glucose 1 g; $NaHCO_3$ 1.1 g; KCl 0.2 g; NaH_2PO_4 0.05 g; $CaCl_2$ 0.2 g; H_2O 1.0 L. The superfusing Tyrode solution was interrupted for 15 sec every 2 min to allow superfusion with a test solution which was acetylcholine in Tyrode, Tyrode itself or whole blood. Venous blood samples were obtained from human subjects in the manner described above for the cross transfusion experiments. Forearm blood flow measurements were not made during this series of experiments.

Acetylcholine and post-exercise hyperaemia

For this study forearm blood flow was measured on both sides. A needle was inserted into the brachial artery for the infusion of drugs into the arm to be exercised. A dose response curve to acetylcholine was obtained and 2 or 3 standard exercises performed (18 rhythmic contractions in 30 sec). Hyoscine or atropine (0.1 mg/min) was then infused into the brachial artery

for 2 and 3 min respectively before exercise commenced, and maintained during the 30 sec of exercise and for 1.5 min after it ceased. The effectiveness of the atropine and hyoscine was then determined by repeating an infusion of acetylcholine. A second exercise was then performed. In the case of the experiments with atropine, the second exercise was accompanied by a further dose of the drug.

In another series of experiments neostigmine (40 μ g/min for 5 min) was infused into the brachial artery 10-15 min before the exercise was performed. The effectiveness of the neostigmine was monitored by measuring the potentiation of the response of the forearm flow to a dose of acetylcholine which was just sufficient to give a dilatation prior to the use of neostigmine. Acetylcholine was given before and after the exercise was performed. In some subjects a second exercise was performed after the infusion of neostigmine. In other subjects a further exercise was performed during an intra-arterial infusion of atropine or hyoscine. In all cases the effectiveness of the acetylcholine-blocking action of atropine or hyoscine was determined by intra-arterial infusions of acetylcholine.

The results of these experiments were analysed by calculating the differences in blood flow between the control post-exercise hyperaemia and the post-exercise hyperaemias obtained after treatment in each case. These differences were then pooled and the mean value for each quarter-min period of the post-exercise hyperaemia calculated.

The drugs used were acetylcholine chloride (Roche), atropine sulphate (D.H.A.), histamine acid phosphate (B.D.H.), hyoscine hydrobromide (Farmer-Hill), and neostigmine methylsulphate (Prostigmin, Roche), and the doses are expressed as weights of the salts.

RESULTS

Cross-transfusion experiments

Provided that the metal and glass components of the intra-arterial infusion system were kept to a minimum and that these were well siliconised, no vascular response in the test forearm was produced by intra-arterial injection of samples of blood taken from the muscle of the opposite forearm either during control resting periods or in the periods of exercise of the forearm muscles. This was the case in 12 experiments on 6 subjects, and Fig 3-2 shows the results on 1 of these. In 1 subject, where a non-siliconised system was used, a marked vasodilatation occurred in the test forearm, probably due to the formation or release of vasoactive substances in the blood by contact with metal and glass (Fig 3-3). This observation demonstrated that the vessels of the forearm were capable of responding to blood-formed dilator substances.

That the blood samples withdrawn from the catheter came from the forearm muscle was illustrated by the fact that the oxygen saturation of the samples showed the typical fall during, and subsequent rise after, the exercise period (Barcroft et al, 1963).

Superfused guinea-pig ileum

In all experiments the guinea-pig ileum used for assay responded to at least 1.0 pg/ml of acetylcholine or 10.0 pg/ml of

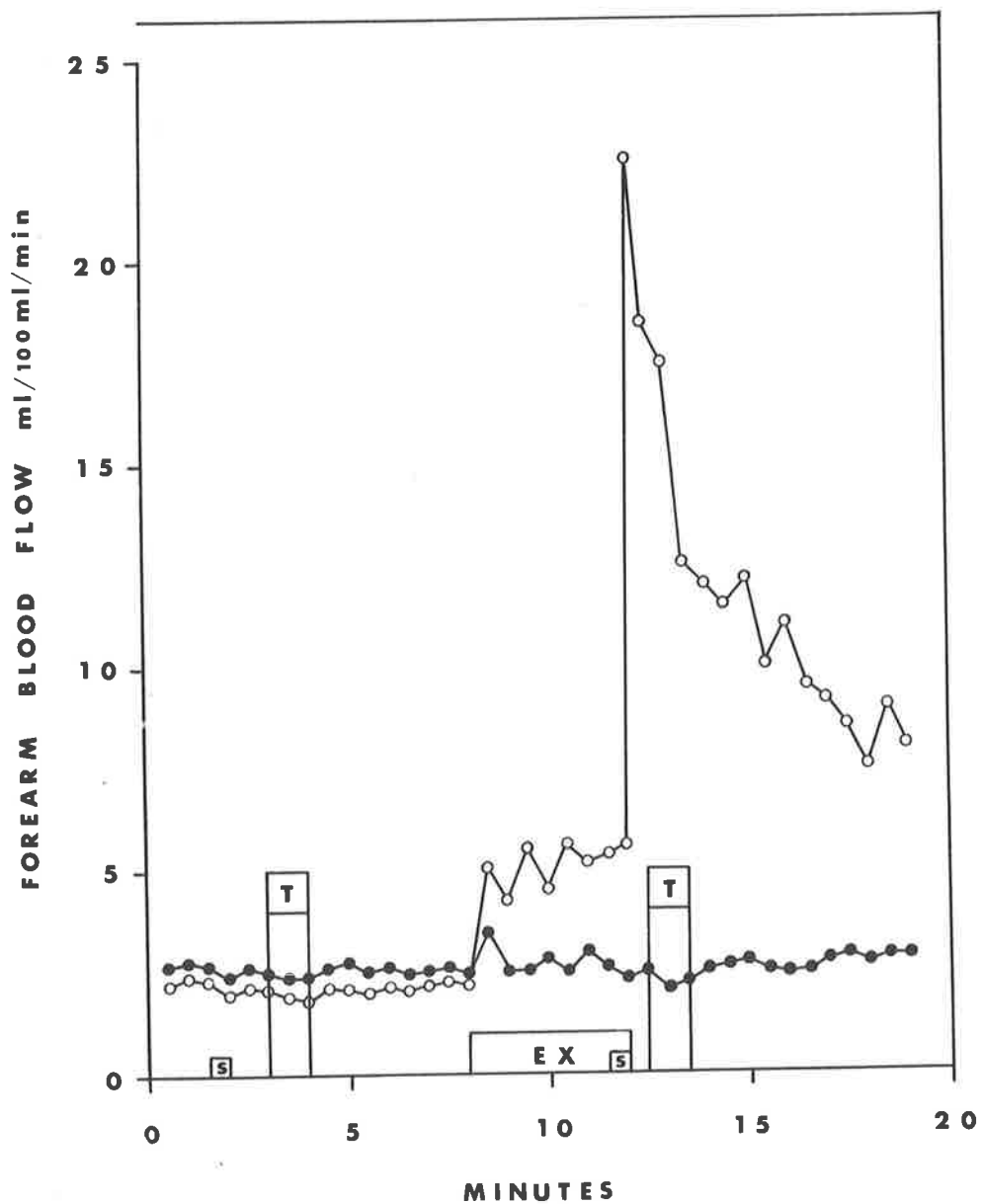


Fig 3-2. The effect on blood flow through the test forearm (●) of cross transfusion (T_1) of 4 ml venous blood collected from the opposite forearm (○) during rest (S_1) and of cross-transfusion (T_2) of blood collected at the end (S_2) of a 4 min period of rhythmic exercise (EX).

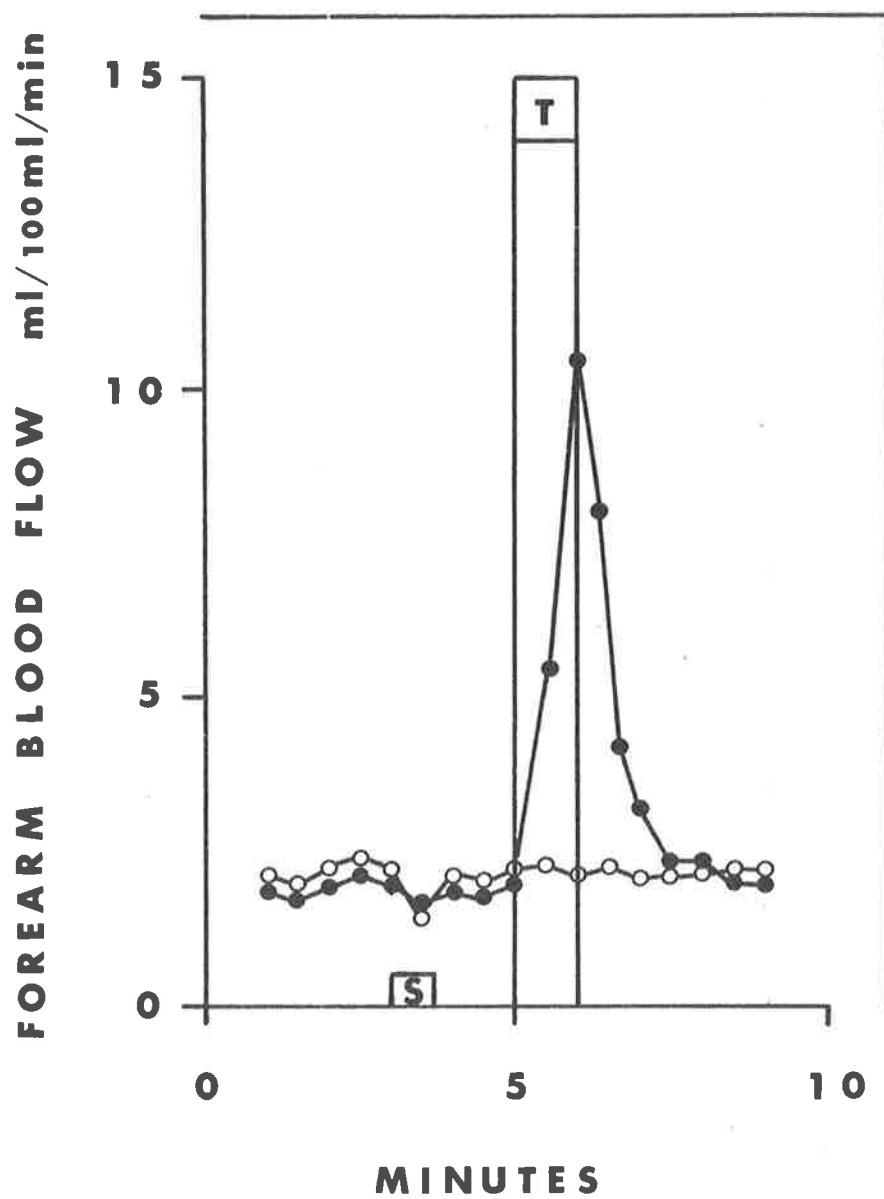


Fig 3-3. The effect on blood flow through the test forearm (●) of cross transfusion through a non-siliconised system (T) of 4 ml venous blood collected from the opposite forearm (○) during rest (S).

histamine. If the preparation was less sensitive than this it was discarded. Approximately 1 preparation in 3 exhibited this degree of sensitivity.

Fig 3-4 shows a portion of the kymograph tracing from one experiment. The preparation was extremely sensitive to acetylcholine, giving a greater response to 0.5 pg/ml acetylcholine in Tyrode than to Tyrode solution alone. The response of the preparation to venous blood obtained at the end of the exercise period differed from that to venous blood obtained from the resting forearm. A further venous sample taken from the resting forearm 30 min after exercise was without effect on the preparation, and a venous sample taken at the end of a second period of exercise again produced a response similar to that produced by the blood sample taken at the end of the first exercise period. Similar responses of the guinea-pig ileum to venous blood taken at the end of the exercise period were obtained in 4 other experiments, and smaller but definite responses in 3 experiments. In 3 further experiments there was no increase in the response.

The substance responsible for the altered response has not been definitely identified, but the rapid onset of contraction was similar to that obtained with acetylcholine. Histamine caused a much slower onset. In one experiment in which the response was greater to the blood sample taken during exercise than to the control sample the ileum was treated with atropine. This considerably

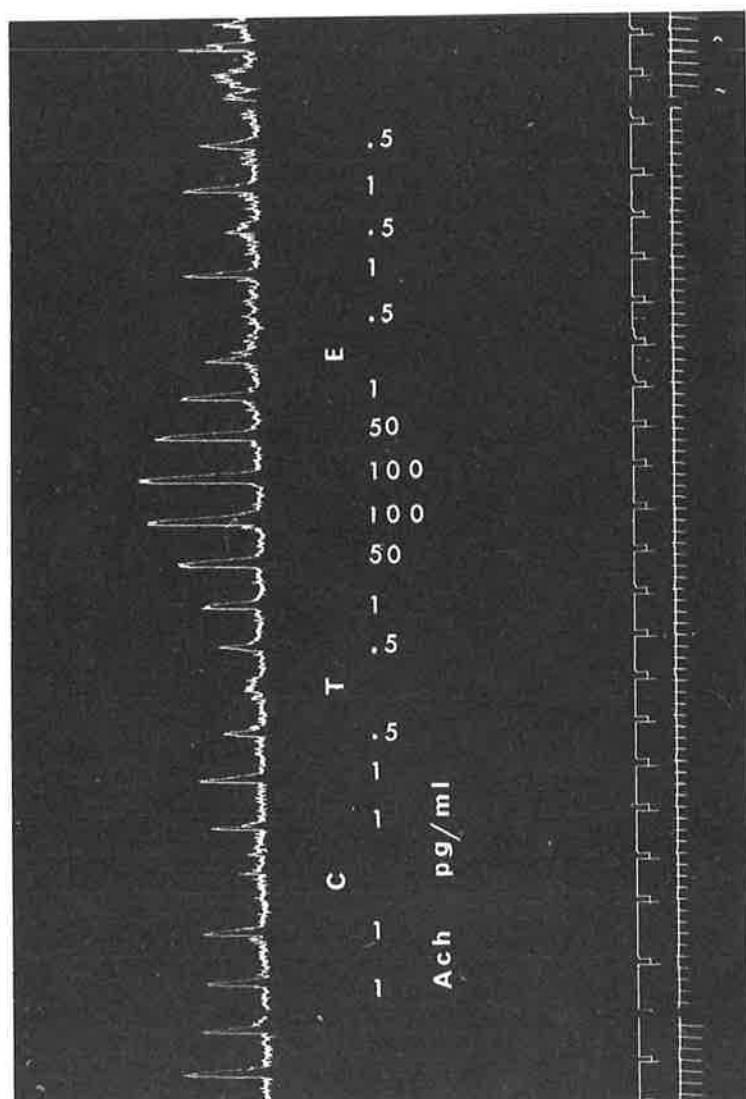


Fig 3-4. The response of the superfused guinea-pig ileum to Tyrode solution (T), venous blood collected at rest (C) and venous blood collected at the end of a 4 min period of rhythmic forearm exercise (E). The response of the preparation to different concentrations of acetylcholine (pg/ml) is also shown. Superfusion of test solutions was at 2 min intervals. Time tracing 30 sec intervals.

reduced the sensitivity of the preparation to acetylcholine, but not to histamine, and there was now no difference in the responses to the blood obtained during the control and exercise periods. This observation was not repeated in other experiments in which it was attempted, as the sensitivity of the preparations diminished rapidly after addition of blood and was unsatisfactory by the time atropine was added.

Acetylcholine and post-exercise hyperaemia

The effect of acetylcholine blocking and potentiating drugs was determined by recording post-exercise hyperaemia before and after their introduction intra-arterially into the forearm. No effect on the resting forearm blood flow was produced by the infusions of atropine, hyoscine or neostigmine in the doses used.

The result of a typical experiment showing the effect of an intra-arterial infusion of hyoscine on the post-exercise hyperaemia is shown in Fig 3-5. Although the vascular effect of acetylcholine was virtually abolished by hyoscine, there was no reduction in the post-exercise hyperaemia. A similar result was obtained in 3 other experiments on 2 subjects in which hyoscine was used and 8 experiments on 4 subjects in which atropine was given. Fig 3-6 shows the pooled differences between the control post-exercise hyperaemia and that following the use of hyoscine or atropine in each of the 12 experiments. There is no significant difference between

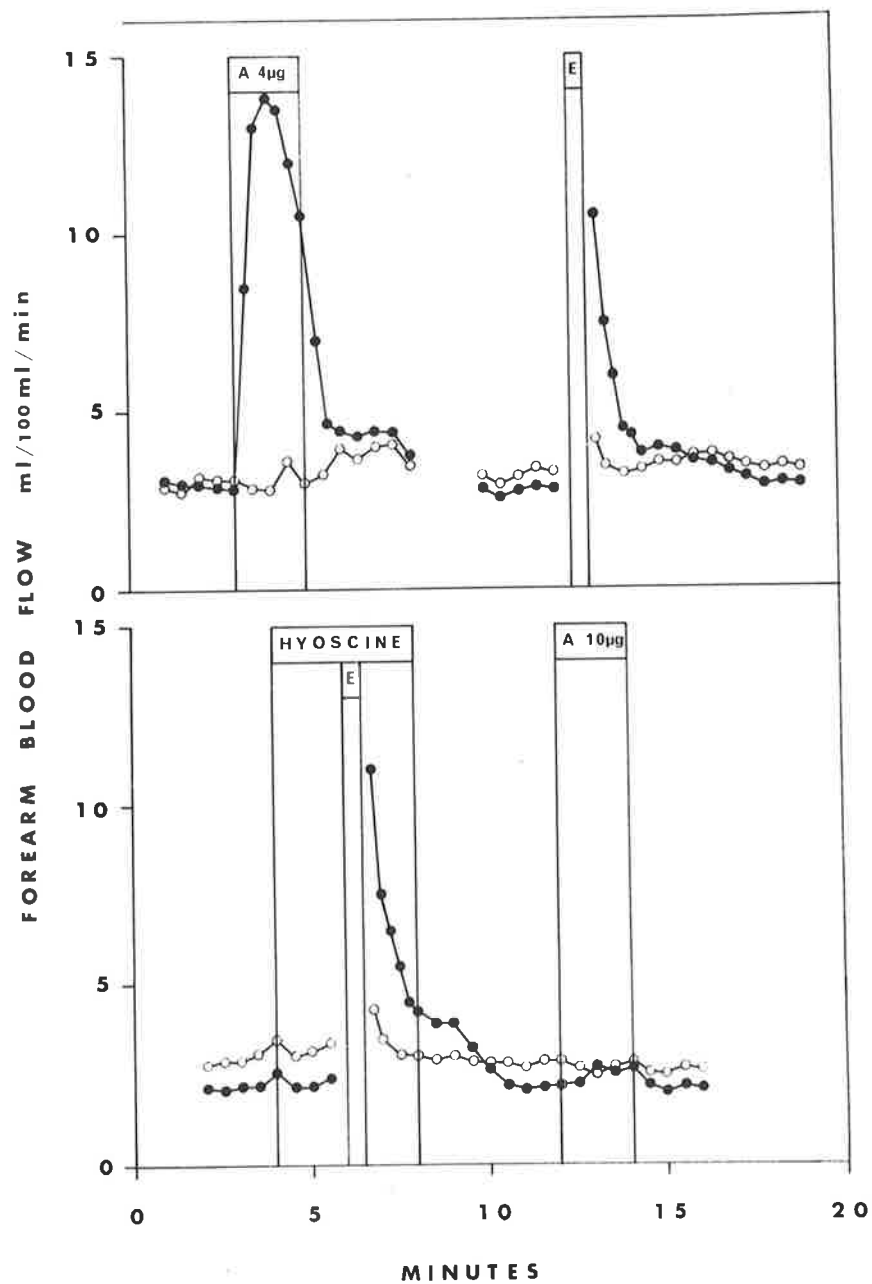


Fig 3-5. The changes in blood flow through the forearm caused by the intra-arterial infusion of acetylcholine (A, μ g/min) and a 30 sec period of rhythmic forearm exercise (E) before (upper frame) and after (lower frame) intra-arterial infusion of hyoscine (0.1 mg/min for 4 min).
 ● injected side; ○ control side.

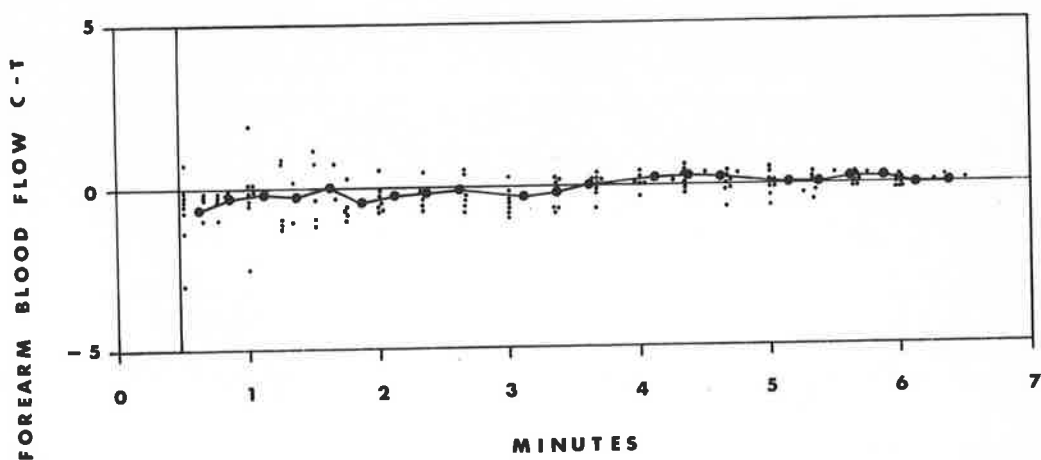


Fig 3-6. The pooled differences in the blood flow through the forearm following 30 sec rhythmic forearm exercise performed before (C) and after (T) intra-arterial infusion of atropine or hyoscine. The small dots represent the differences in each individual experiment; the large closed circles represent the mean value for each quarter-min period.

them.

The effect of an intra-arterial infusion of neostigmine on the post-exercise hyperaemia was examined in 12 experiments on 8 subjects. Fig 3-7 shows the results of 1 such experiment. The effectiveness of neostigmine in preventing the destruction of acetylcholine was shown by the increased response of the forearm vessels to acetylcholine. The post-exercise hyperaemia after the use of neostigmine was greater than that following the control exercise. Fig 3-8 shows the pooled differences between the control post-exercise hyperaemia and that following the use of neostigmine for all the experiments. There is a significant increase in the first minute of the post-exercise hyperaemia performed after the use of neostigmine. The hyperaemias are almost identical thereafter.

In 6 experiments on 6 subjects after neostigmine had been given, and shown to potentiate the post-exercise hyperaemia response, atropine (4 experiments) or hyoscine (2 experiments) was then administered and the exercise repeated. In all cases the post-exercise hyperaemia was reduced, though not completely restored to the pre-neostigmine level.

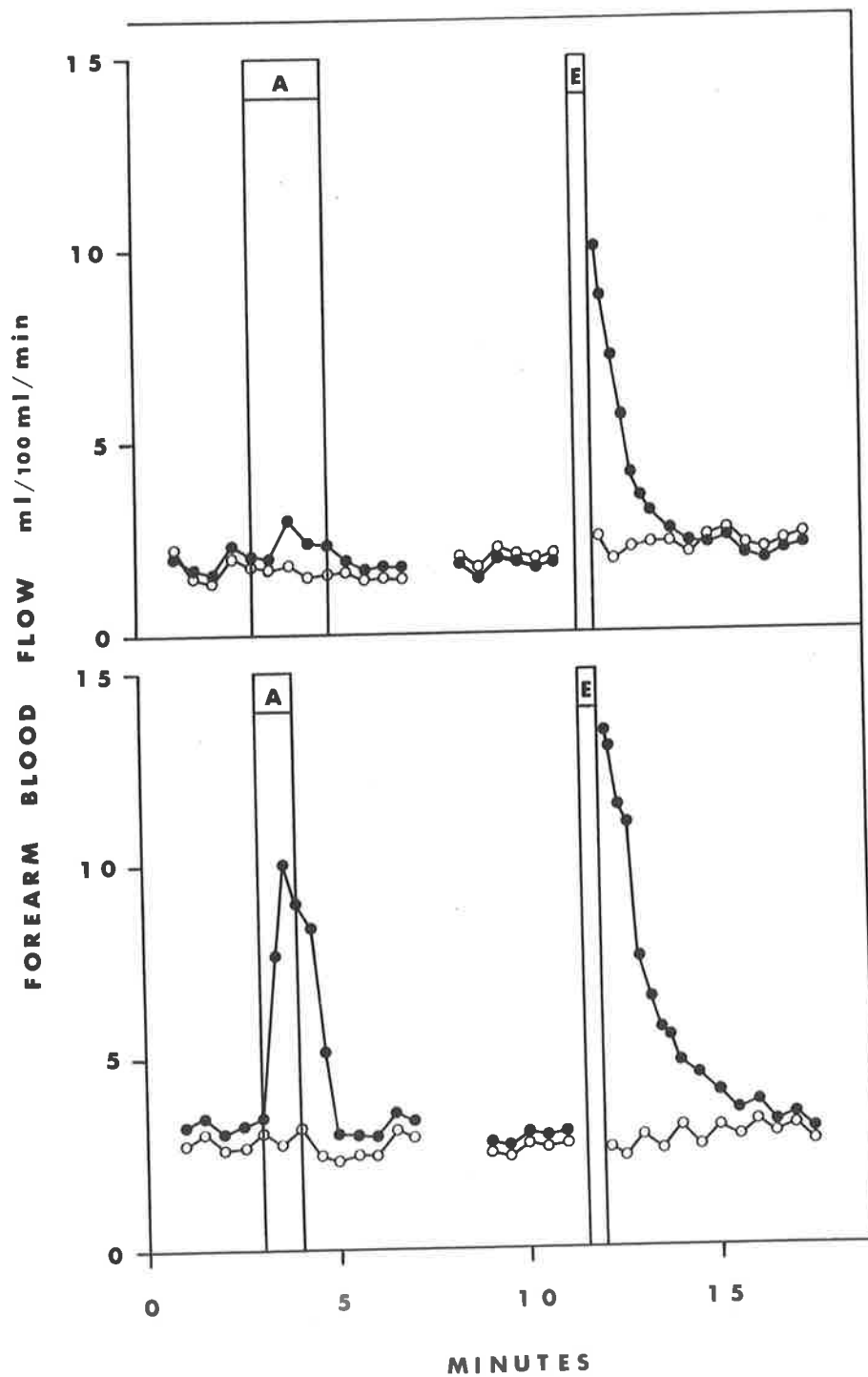


Fig 3-7. The changes in blood flow through the forearm caused by intra-arterial infusion of acetylcholine (A, 4 $\mu\text{g}/\text{min}$) and a 30 sec period of rhythmic forearm exercise (E) before (upper frame) and after (lower frame) intra-arterial infusion of neostigmine (40 $\mu\text{g}/\text{min}$ for 5 min).

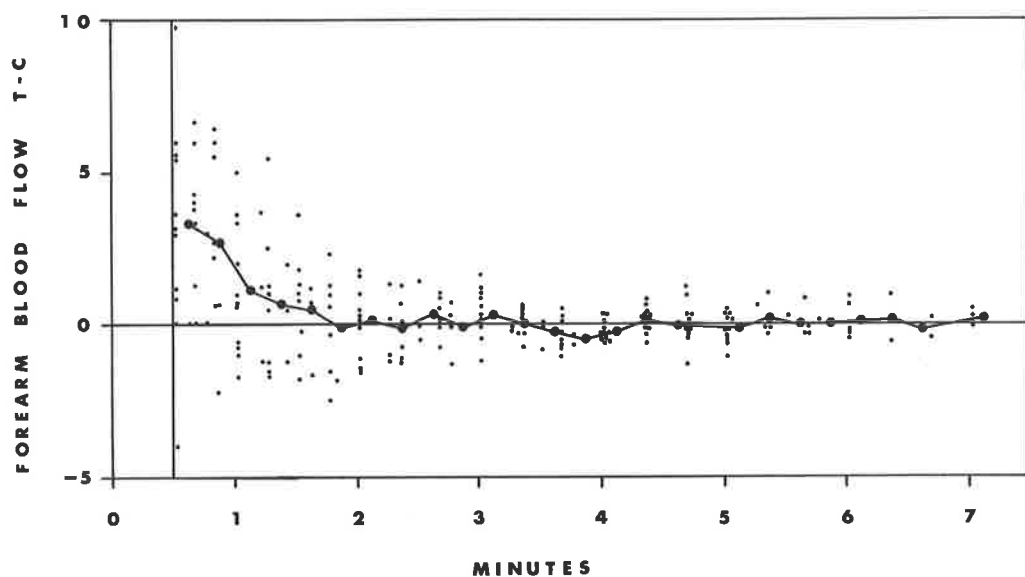


Fig 3-8. The pooled differences in the blood flow through the forearm following 30 sec rhythmic forearm exercise performed before (C) and after (T) intra-arterial infusion of neostigmine. The small dots represent the differences in each individual experiment, the larger closed circles represent the mean value for each quarter-min period.

DISCUSSION

Anrep and von Saalfeld (1935) studied the vasodilator properties of venous blood samples from the resting and stimulated gastronemius muscle of the anaesthetised dog. They were able to show a conspicuous hyperaemia when venous blood collected during activity of the muscle was reperfused through it. This was not observed on reperfusion of venous blood collected at rest. Anrep and Barsoum (1935) found an increased blood histamine content at the end of the period of stimulated contraction and suggested that histamine was the cause of the hyperaemia. Anrep et al (1944), using human subjects, observed an increase in histamine content of the venous blood draining from contracting skeletal muscle. The present findings do not support the above, for assay on the human forearm of venous blood from exercising muscle showed no evidence of vasoactivity, and the responses of the guinea pig ileum suggested acetylcholine, rather than histamine release. Similarly, Whelan (1956) was unable to show any reduction in post-exercise hyperaemia in the human forearm by the introduction of anti-histamine substances into the brachial artery.

In the present study the inability of the cross transfusion technique to demonstrate a vasodilator substance in blood draining from exercising forearm muscles could be due to a number of factors. The concentration of a postulated metabolite in the blood might be

so small that the human forearm is too insensitive an assay preparation to detect it; the substance might be rapidly destroyed on entering the blood stream or only inactive break-down products appear in the venous drainage; the substance might not enter the blood vessels but be destroyed in the tissue spaces or removed in the lymph.

It was because of the first of the above possibilities that samples were tested on the superfused guinea pig ileum, which was very sensitive to vasoactive substances such as acetylcholine and histamine. In the majority of such experiments exercise did appear to cause the presence in the venous effluent of a very small amount of an active substance. The response evoked more closely resembled that of acetylcholine than that of histamine and in one experiment the response was abolished by atropine. Convincing proof of the nature of the agent was not obtained, owing to the fact that the preparations usually deteriorated rapidly once blood had been in contact with them and only a few measurements could be made with each preparation.

However, the suggestion that a small amount of acetylcholine may be present in the muscle blood during exercise is supported by the observation that the post-exercise hyperaemia was regularly potentiated by the introduction of neostigmine into the arm. Neostigmine is known to have a direct stimulant action on skeletal muscle (Beckman, 1961) and most subjects were aware of some degree of spontaneous muscle twitching during and for a short time after the

infusion. As a result, some degree of muscle incoordination may have been present, and it is possible that more muscle fibres were undergoing activity during the exercise period after neostigmine than before and that this was responsible for the increased vascular response. Such an effect cannot, however, account for all of the potentiation since this was markedly diminished, although not completely abolished, by the subsequent introduction of atropine or hyoscine, suggesting that acetylcholine played at least some part in the potentiated response. The acetylcholine involved in the response to exercise after neostigmine probably originates from the active motor nerve endings. Acetylcholine has been shown to be the transmitter substance at the motor end-plates in animals (Dale, 1934b) and indirect evidence for acetylcholine being the transmitter substance in man has been advanced by Grob, Johns and Harvey (1956) who demonstrated motor activity in normal subjects and in patients with myasthenia gravis on intra-arterial injection of acetylcholine after treatment with anti-cholinesterases. The potent but transient vasodilator action of acetylcholine on limb vessels in man has been described by Duff, Greenfield, Shepherd and Thompson (1953). The fact that, in the absence of neostigmine, the post-exercise hyperaemia was not reduced by atropine or hyoscine indicates that acetylcholine does not play a significant role in the hyperaemia of exercise in normal circumstances.

SUMMARY

1. The role of various vasoactive agents in the phenomenon of post-exercise hyperaemia of skeletal muscle has been briefly reviewed.
2. A technique is described for the assay of vasoactive substances in venous blood draining from the forearm muscles of one arm by the injection of a sample of the venous blood into the brachial artery of the opposite arm and the measurement of blood flow through that forearm.
3. Using this technique, no vasoactive substance was detected in venous blood from exercising muscle.
4. An altered response of the superfused guinea-pig ileum to venous blood draining from human skeletal muscle at the end of a 4-min period of rhythmic exercise was observed in 8 of 11 experiments.
5. The precise identity of the substance responsible was not determined, but the nature of its effect on the guinea-pig ileum was more suggestive of acetylcholine than of histamine.
6. The role of acetylcholine in the post-exercise hyperaemia of the human forearm was examined by determining the effect of intra-arterial infusions of the acetylcholine-blocking agents atropine and hyoscine and the anti-

cholinesterase neostigmine.

7. Intra-arterial infusions of atropine and hyoscine did not alter the post-exercise hyperaemia and it is concluded that acetylcholine normally plays no part in the production of the hyperaemia.
8. Post-exercise hyperaemia was increased by the use of neostigmine, suggesting that, in this circumstance, acetylcholine liberated from the motor end-plates causes vasodilatation which was additive to the post-exercise hyperaemia.

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PART B

VASOACTIVE SUBSTANCES IN LYMPH DURING
EXERCISE IN SHEEP.

INTRODUCTION.

As outlined in the introduction of Section 3, Part A, the cause of the increase in blood flow through skeletal muscle during and immediately after contraction has continued to elude investigators since it was first observed, probably by Gaskell (1877). Since section of the autonomic nerve supply to the muscle group studied does not affect the hyperaemia (Grant, 1938; Barcroft, 1964), and since the hyperaemia can be localised to the muscle group under study, local factors are held to be responsible.

As it is possible that the "local metabolites" produced during exercise are removed from the tissue by either the blood or lymph and as minute concentrations of an active substance, probably acetylcholine, have been detected in the venous blood during forearm exercise (Part A of this Section), the present study was designed to examine the lymph collected from a conscious exercising animal for active substances. The sheep, a relatively large and placid animal, was chosen for these investigations. The lumbar lymphatics were cannulated since they drain the hind quarters which are composed mainly of muscle. Although there are no lymphatics lying amongst individual skeletal muscle fibres nevertheless the muscle has adequate lymphatic drainage due to the existence of rich plexuses of lymphatic vessels in the fibrous tissue septa and enveloping fascia

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of the muscle, and these might be expected to drain tissue fluid from the muscles (J.R. Casley-Smith, personal communication).

METHODS

The sheep used were 2 to 3 year old merino ewes. Anaesthesia was induced by intravenous injection of sodium pentobarbitone (veterinary Nembutal, Abbott, 600-900 mg) and maintained by halothane and oxygen via a No. 9 Magill's endotracheal tube in a closed circuit. The aseptic surgical technique used was similar to that described by Lascelles and Morris (1961). The lumbar region was closely shorn and the skin prepared with a 1 in 30 solution of cetrimide in 70% ethyl alcohol. The lumbar lymphatics were approached through a right transverse lumbar skin incision with muscle splitting. The inferior vena cava was located transperitoneally and two large lumbar lymphatics were normally found closely applied to it. The superficial surface of one of these lymphatics was denuded of adventitia and an aneurysm needle used to pass black silk ties (4/0) underneath it. A transverse slit was made with fine scissors and the lymphatic was cannulated in a retrograde direction using a heparin-filled polythene cannula (Boots, size 49 to 52) which was fixed in position by suturing it to the neighbouring supporting tissue. The free end was brought to the surface through a separate stab incision in the flank. All remaining large lymphatics were tied off. The wound was closed in layers with chromic cat-gut and linen thread. A small polythene strip, used for holding the polythene collection bottle, was sutured to the flank near the exit of the cannula (Fig 3-9). The sheep was then allowed to recover and experiments were

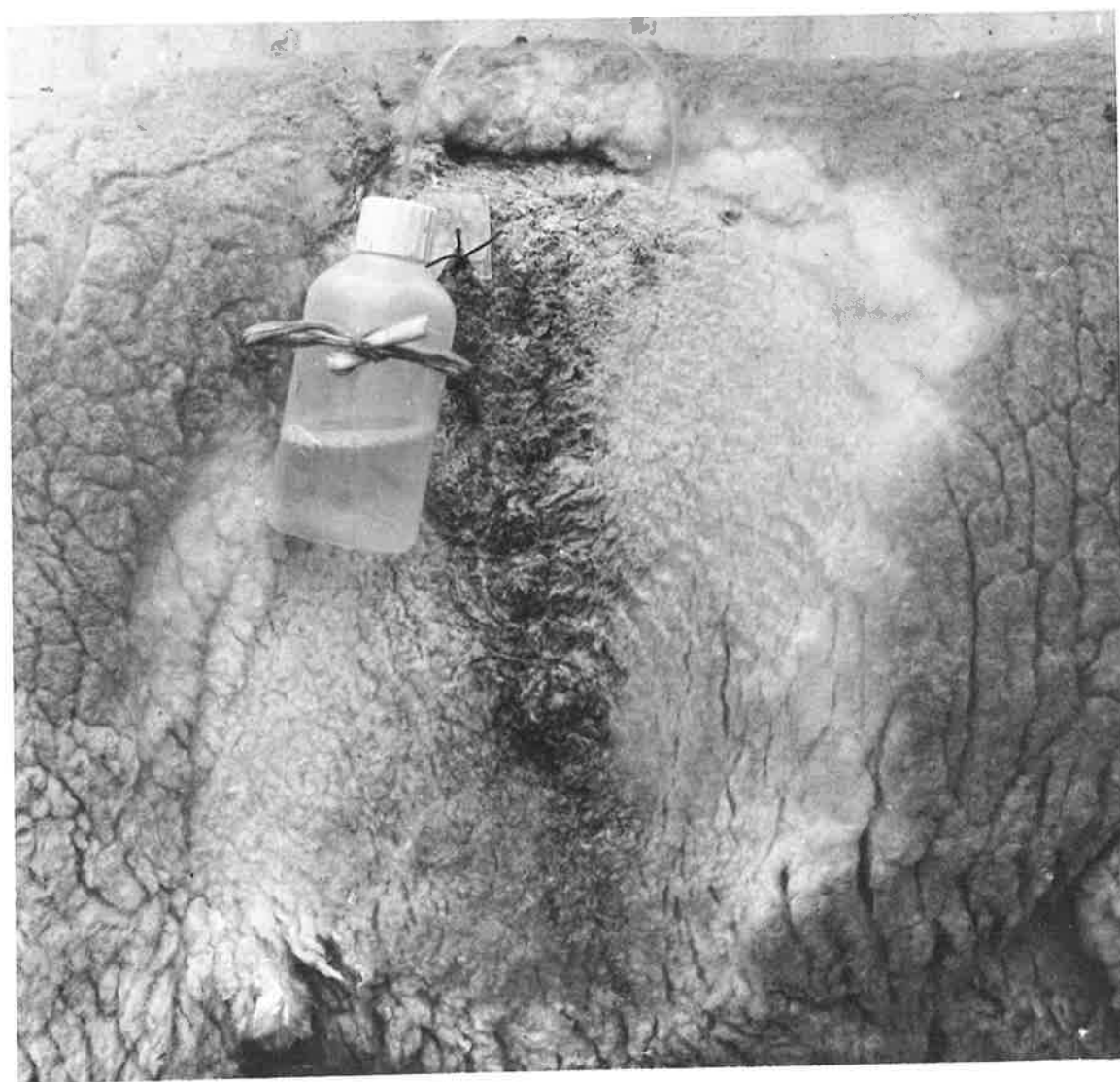


Fig 3-9. A close up view of the flank of one sheep showing the cannula, supporting polythene strip and polythene collection bottle containing lymph.

carried out 1-3 days after operation.

Collection of lymph

Lymph for volume measurement only was collected in a small polythene container. Lymph for assay was collected and frozen immediately by placing the collection bottle (heparinised) in a large insulated polythene container filled with a freezing mixture of dry-ice and ethyl alcohol (Fig 3-10). Lymph was collected with the sheep at rest (standing in a wire cage) and during exercise which was performed by vigorously walking the sheep for periods of up to 20 min. The lymph was subsequently deep frozen until assay could be performed and then small amounts were thawed immediately prior to use. All collection, storage and assay apparatus was siliconised to minimise kinin formation.

Assay

The lymph was assayed for acetylcholine and histamine by superfusion onto the guinea pig ileum. The superfusion technique (Gaddum, 1953) permits a pharmacological assay which is more sensitive than the organ bath technique. Six week old guinea pigs weighing 150-250 g were used. The ileum was superfused with Tyrode solution (NaCl 8.0 g, glucose 1.0 g, NaHCO_3 1.0 g, KCl 0.2 g, NaH_2PO_4 0.05 g, CaCl_2 0.2 g, MgCl_2 0.1 g, H_2O to 1.0 L), warmed to 37°C and aerated with O_2 95%, CO_2 5%. The superfusing solution was interrupted for

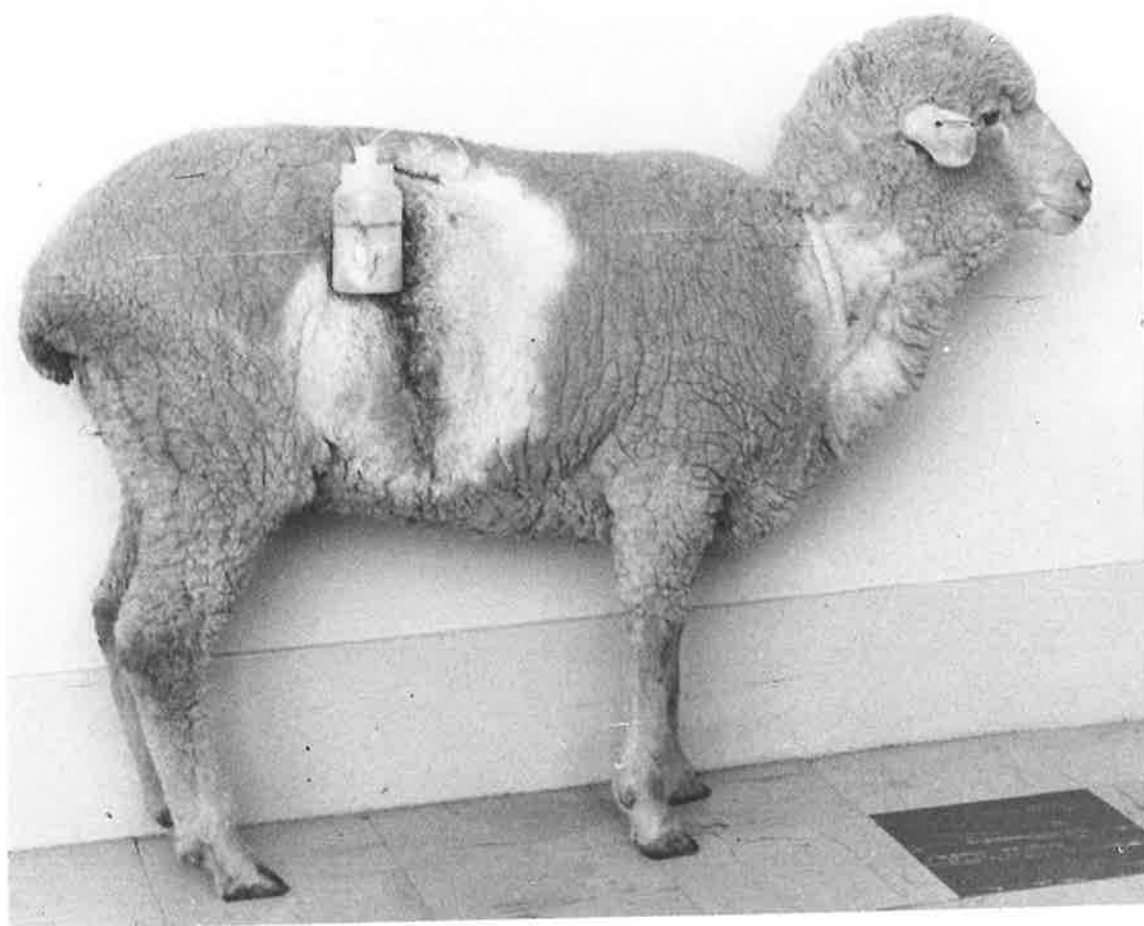


Fig 3-10. A merino sheep showing the position of the lymph collection bottle, which is surrounded by a larger polythene container filled with a freezing mixture of dry-ice and alcohol.

15 sec every 2 min to allow superfusion with 4 drops of a pre-warmed test solution which was Tyrode itself, acetylcholine or histamine in Tyrode, or lymph. At least 6-10 min were allowed between each application of lymph. Only those preparations which responded to 100 pg/ml acetylcholine or 1 ng/ml histamine were used for assay. Less sensitive preparations or those which exhibited spasmodic contractions were discarded.

Assay for bradykinin was carried out by superfusion of the oestrus rat uterus and duodenum. The rat received a subcutaneous injection of stilboestrol 0.1 mg 24 hr before the removal of the organs for assay. The lymph activity was assayed against bradykinin in de Jalon's solution (Gaddum, Peart and Vogt, 1949).

Lymph was examined for the presence of catecholamines by testing it on the perfused artery of the rabbit ear preparation (de la Lande and Rand, 1965) against a noradrenaline monitor.

The drugs used were acetylcholine chloride (Roche), atropine sulphate (D.H.A.), histamine acid phosphate (B.D.H.), mepyramine maleate (Anthisan, May and Baker) and noradrenaline bitartrate (Levophed, Winthrop). Doses are expressed as weights of the salts.

RESULTS

Lymph was obtained at rest and during exercise from two sheep. There was a large increase (approximately three-fold) in the flow of lymph during exercise and Fig 3-11 shows the effect of a 20 min period of exercise on the rate of lymph flow in sheep 1. A similar result was obtained with sheep 2.

The lymph collected from both sheep during exercise caused a greater contraction of the guinea pig ileum than lymph collected at rest in each of eight assays. In three experiments with lymph collected from sheep 1, atropine (10^{-7} g/ml) was superfused onto the ileum. On each occasion the activity of the exercise lymph was almost abolished (Fig 3-12). In one experiment mepyramine (10^{-7} g/ml) had no effect on the response. In two separate assays of the lymph collected from sheep 2, atropine (10^{-7} g/ml) reduced the response to the exercise lymph and the contraction was completely abolished by a combination of atropine and mepyramine (10^{-7} g/ml) as shown in Fig 3-13.

To obtain an estimate of cholinesterase activity of lymph a control sample was incubated with acetylcholine (20 ng/ml lymph) at 37°C . After 11 min incubation this test sample produced a contraction of the ileum similar to that produced by acetylcholine 1 ng/ml, indicating hydrolysis of the added acetylcholine. The inactivation of the acetylcholine was complete at 60 min whereas

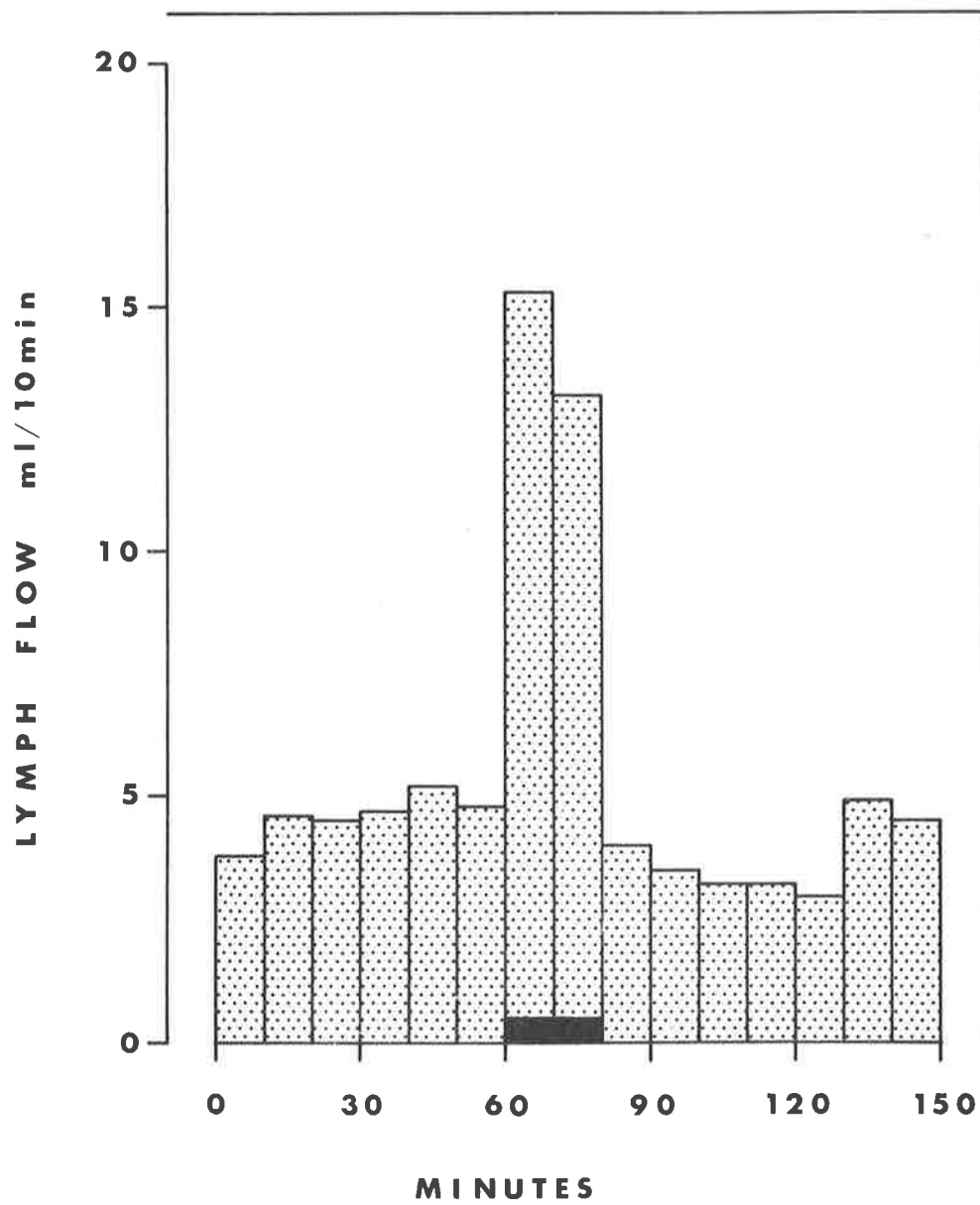


Fig 3-11. The effect of a twenty minute period of exercise (black rectangle) on lymph flow from the hind quarters of sheep 1.

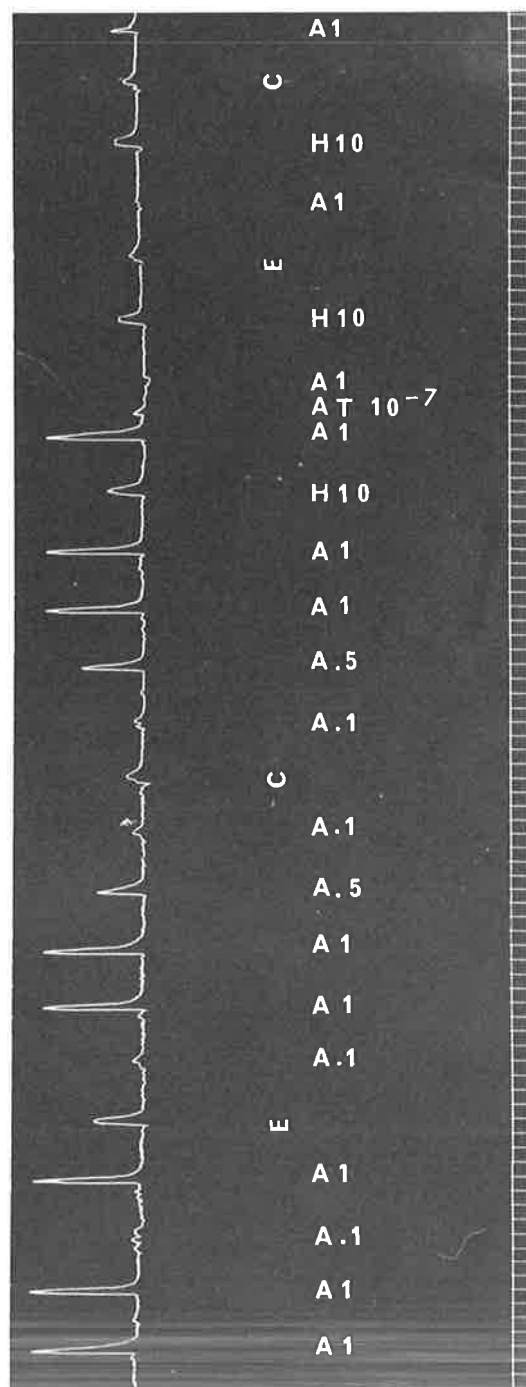


Fig 3-12. The effect of superfusion onto guinea pig ileum of lymph collected from sheep 1 during a period of rest (C) and exercise (E) before and after atropine (AT, 10^{-7} g/ml). The responses to acetylcholine (A, ng/ml) and histamine (H, ng/ml) are shown. Time tracing: 30 intervals.

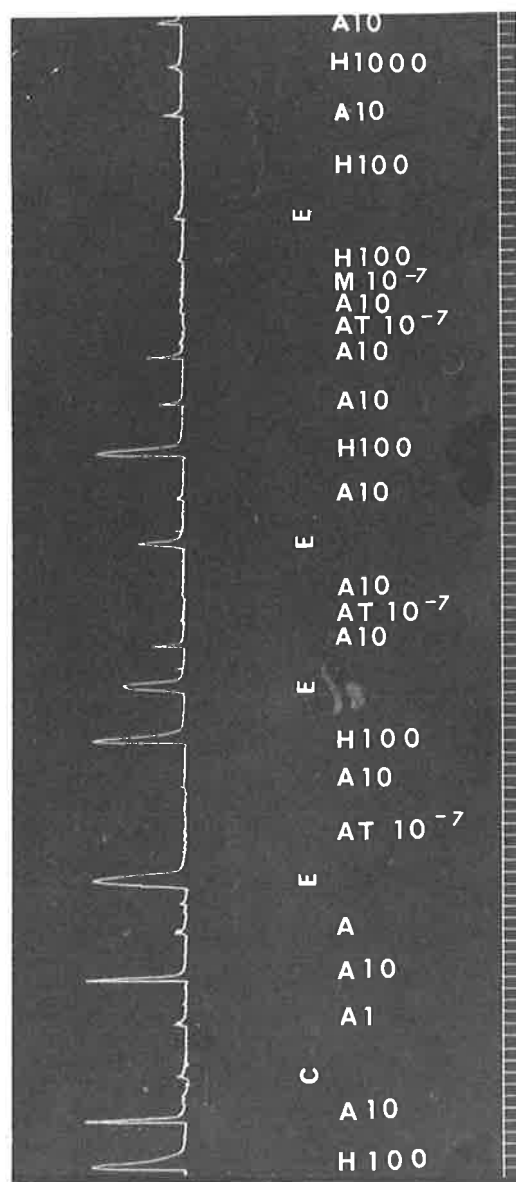


Fig 3-13. The effect of superfusion onto guinea pig ileum of lymph collected from sheep 2 during a period of rest (C) and exercise (E) before and after atropine (AT, 10^{-7} g/ml) and mepyramine (M, 10^{-7} g/ml). The responses to acetylcholine (A, ng/ml) and histamine (H, ng/ml) are also shown. Time tracing: 30 sec intervals.

the response to acetylcholine incubated in Tyrode solution for a similar period was unchanged.

Lymph collected from sheep 2 and stored for 10 days was assayed for kinin activity by superfusion onto the oestrus rat uterus and duodenum. There was no evidence of increased kinin activity in the lymph collected during exercise (Fig 3-14) although there was a considerable amount of kinin present (approximately 8 ng/ml) in both control and exercise samples.

Neither control nor exercise lymph from sheep 1 produced any change in the perfused artery of the rabbit ear preparation whereas the noradrenaline monitor (1 to 2 ng/ml) caused a significant increase in vessel resistance. Hence there was no evidence for the presence of noradrenaline in amounts of 1 to 2 ng/ml.

Potassium ion concentration in exercise and control lymph was estimated on many samples by flame photometry, e.g. in sheep 2: control lymph average = 4.2 mEq/L; exercise lymph, average = 4.1 mEq/L.

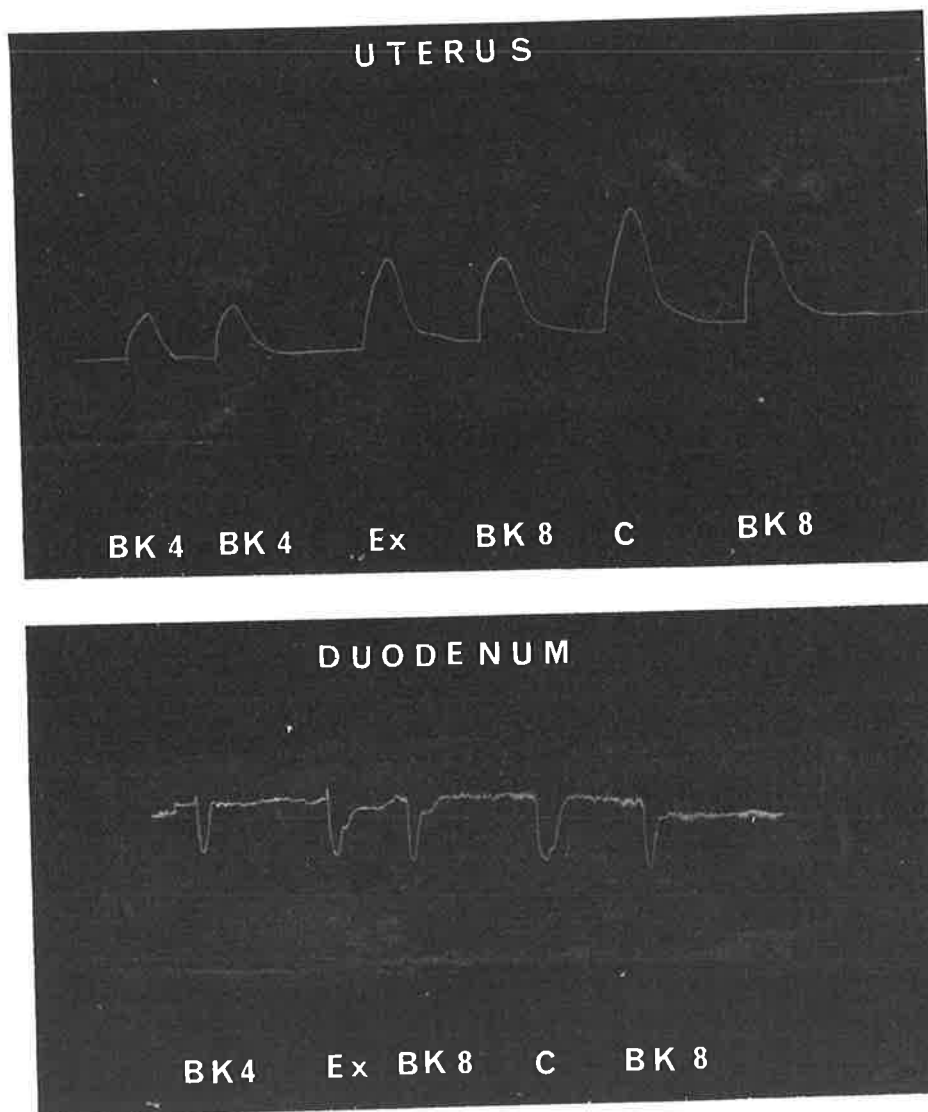


Fig 3-14. The effect of superfusion onto the oestrus rat uterus and duodenum of lymph collected from sheep 2 during a period of rest (C) and exercise (Ex). The response to bradykinin (BK, ng/ml) is also shown.

DISCUSSION

Exercise caused a marked increase in the flow of lymph from the exercising lower limbs of two sheep. Similar observations have been made in the anaesthetised cat (Jacobsson, and Kjellmer, 1964) and in the conscious sheep (Hall, Morris and Woolley, 1965).

Lymph for assay was frozen immediately upon collection to prevent destruction of labile substances and to minimise the formation of vasoactive peptides during storage. The detection of both acetylcholine and cholinesterase activity in lymph obtained during exercise suggests that lymph is involved in the removal and destruction of acetylcholine under normal conditions. The acetylcholine presumably comes from the neuromuscular junction and this finding is in agreement with the observations of Dale (1934b). Earlier studies (Part A) have shown that acetylcholine does not normally participate in the production of the hyperaemia which follows light exercise, but it may be present in the blood during more prolonged exercise. It is possible that prolonged neuromuscular activity overtaxes the local cholinesterase and some of the released acetylcholine is removed by the blood or the lymph.

The detection of histamine in the lymph of one sheep during exercise requires confirmation and further investigation to determine the implications of this finding. Anrep and Barsoum (1935), Anrep

and von Saalfeld (1935) and Anrep, Barsoum, Salama and Souidan (1944) have documented evidence that histamine is released during skeletal muscle contraction. The majority of evidence, however, is against histamine being a factor in the production of post-exercise hyperaemia. For instance, as discussed previously, the anti-histaminic substance mepyramine does not alter the hyperaemia in the limb of the cat (Hilton, 1953) or the human (Whelan, 1956).

Attempts have been made to reproduce the characteristics of exercise and post-exercise hyperaemia by intra-arterial infusion of vasoactive substances: Kjellmer and Odelram (1965) infused acetylcholine, histamine, adenosine triphosphate and bradykinin into the calf muscle of anaesthetised cats but were unable to match the vascular response to exercise.

Kjellmer (1965) has suggested that the potassium ion (K^+) may be responsible for up to 65% of the post-exercise hyperaemia occurring in the exercising hind limb of the cat. Glover, Roddie and Shanks (1962) administered a range of doses of potassium chloride intra-arterially in man and observed some increase in forearm blood flow. This dilatation was not marked when compared with the dilatation following muscular contractions which cause an increase in potassium ion concentration in venous effluent. No significant change occurred in the concentration of the potassium ion in the exercise, compared with control lymph, in this investigation.

It has been postulated that as bradykinin, a potent vaso-active nonapeptide, is involved in the production of the functional hyperaemia of salivary glands (Hilton and Lewis, 1955) and the skin of the human forearm during sweat gland activity (Fox and Hilton, 1958) it might also be involved in the hyperaemia of exercise (Hilton and Lewis, 1958). Since bradykinin is removed from the tissues in lymph (Lewis, 1960) it is probable that if it is involved in the production of the hyperaemia of exercise an increased amount would be detectable in the lymph from the exercising muscles. In the present study only one assay was performed for bradykinin and although the lymph exhibited kinin activity (probably generated during storage since deep freezing slows but does not completely halt the kinetics of kinin formation) there was no difference in the activity of the control and exercise samples.

The presence of increased amounts of catecholamines was excluded by perfusing lymph through the central artery of the rabbit ear nerve-blood vessel preparation. While catecholamines are unlikely to be involved in the vasodilatation mechanism of exercise hyperaemia they may be able to modify the hyperaemia by constriction of the resistance vessels as suggested by Fewings, Roberts, Stepanas and Whelan (1965) from experiments on body tilting in man.

It would appear, therefore, that during exercise the flow of lymph is increased, acetylcholine is present in increased amounts

and histamine level may be elevated but bradykinin and catecholamines are probably not present in increased amounts. However, the basic factors responsible for the hyperaemia are still unknown, but it is likely that the local interaction of many vasoactive substances is involved.

SUMMARY

1. Lymph was collected from the lumbar lymphatics of conscious sheep standing at rest and during walking exercise.
2. The rate of flow and the pharmacological activity of the lymph increased with exercise.
3. Acetylcholine was present in lymph in increased amounts during exercise in two sheep and histamine was demonstrated in one sheep. Bradykinin was not demonstrated in increased amounts.
4. Catecholamines were not demonstrated by a sensitive nerve-blood vessel preparation.
5. It was concluded that lymph may remove some of the acetylcholine liberated from motor nerve endings during skeletal muscle activity, but that acetylcholine is unlikely to play a major role in the phenomenon of post-exercise hyperaemia of skeletal muscle.

205.

SECTION 4

THE EFFECTS OF ETHYL ALCOHOL ON THE PERIPHERAL
VESSELS.

INTRODUCTION

Alcohol is generally considered to have a dilator action on blood vessels, but there is little documentation of its effect on the circulation either in animals or in man. Miles (1924) and Cook and Brown (1932) reported a rise in skin temperature of the fingers and toes of normal subjects after oral administration of whisky. This was confirmed by Grollman (1942) who gave both oral whisky and alcohol as a 40% aqueous solution, and by Montgomery (1942) who observed that oral whisky had little effect on pulse rate, blood pressure or cardiac output. Abramson, Zazeela and Schkloven (1941) reported that after administration of whisky an increase in blood flow was generally seen in the hand and little or no effect in the leg or forearm. Edwards, Jones, McConnell, Pemberton and Watson (1952) described the use of infusions of ethyl alcohol into the femoral artery in the treatment of occlusive vascular disease, and observed an increase in skin temperature of the toes in one of six patients so treated. Conrad and Green (1964) found an increase in digital blood flow after ingestion of 60 ml of 86 proof alcohol which was less marked in patients suffering from arterial disease than in normal subjects.

Horwitz, Montgomery, Longaker and Sayen (1949) postulated that, since blood pressure and cardiac output remained unchanged after oral whisky, the vasodilatation in skin must be accompanied by a vasoconstriction elsewhere, but no direct evidence of a con-

strictor action of alcohol on blood vessels in man was then available.

During a recent investigation by Douglas, Fewings, Casley-Smith and West (1966) of a chronic alcoholic patient who suffered recurrent episodes of rhabdomyolysis, it was observed that administration of alcohol into the brachial artery reduced the blood flow through the forearm.

This was probably the first demonstration of a vasoconstrictor action of alcohol in man, but Stephen Hales, Vicar of Teddington, had demonstrated the vasoconstrictor action of alcohol on the mesenteric vessels of a dog as early as 1733. Hales, who was also first to measure blood pressure (by cannulating the femoral artery of a mare with a length of copper pipe and observing the height to which the blood column rose in a vertical glass tube connected to the cannula), cannulated the aorta of a dog and measured the resistance to flow of liquids through the mesenteric vessels by counting the number of seconds taken for measured volumes to gravitate through the preparation. He noted that brandy took longer to pass through the mesenteric vessels than water and concluded, "Hence we see that brandy contracts the fine capillary arteries of the guts, and that water soon relaxes them again, by diluting and carrying off the spirituous part of brandy, which as it is well known, not only contracts the coats of the blood vessels, but also thickens the blood and humours, both which effects con-

tribute to the sudden heating of the blood, by much increasing thereby its friction in the contracted capillary vessels".

Thus, the above conflicting reports of vasodilator and vasoconstrictor effects of ethyl alcohol prompted the present investigation of the effects of alcohol on the blood vessels of the upper limbs in human subjects. As in previous studies (particularly the angiotensin studies) both local and systemic effects were investigated in an attempt to elucidate the mechanisms underlying the vasoactive properties of this substance, which, unlike the other substances studied previously in this thesis, is not a naturally-occurring vasoactive agent.

METHODS

The subjects were volunteer medical students. Two patients were also studied, one who had undergone cervical sympathectomy five years previously for mild Raynaud's disease and another who had suffered unilateral brachial plexus avulsion 12 months previously. In neither case did the vessels of the limbs studied show any sympathetic reflex responses nor were they constricted by ephedrine (Parks, Skinner and Whelan, 1961; Fewings, Rand, Scroop and Whelan, 1966) which demonstrated that the sympathetic nerves had completely degenerated.

The subjects lay supine on a couch in a temperature-controlled laboratory (24 to 26°C) for at least 30 min before observations began. Blood flow through the hands was recorded using the water-filled plethysmographs. Capacitance plethysmographs (Adelaide type) were used to measure forearm blood flow. A wrist cuff was inflated to 200 mm Hg during measurements of forearm flow. Intra-arterial infusions of alcohol or of other drugs were given through a 22-gauge needle inserted, under local anaesthesia (lignocaine 2%), into the brachial artery at the elbow. Saline (0.9% W/V) was infused continuously at a rate of 2 ml/min throughout the experiment; being interrupted when required for the infusion of ethyl alcohol (95%; 75 mg/ml) or phenoxybenzamine (Dibenyline S.K.F. 250 µg/min for 8 to 10 min) diluted in saline to the desired concen-

tration. Calculation of the percent fall in blood flow during intra-arterial infusion of alcohol was made by averaging the blood flow for 3 min immediately prior to the infusion and during the last 4.5 min of the infusion. Where appropriate, correction for the spontaneous changes in the blood flow was made by reference to the control side (Duff, 1952).

In five subjects alcohol was administered in the form of 100 ml brandy (30.9% W/V, diluted with ginger ale), which was taken over a period of 1 to 5 min. The blood flow through the forearm was recorded in one arm, and the blood flow through the hand in the other. In addition, arterial blood pressure was recorded from a 21-gauge needle inserted into the brachial artery of one arm and connected by means of 30 cm length of saline-filled polythene tubing to an electromanometer and ultraviolet light recorder (N.E.P., London). Samples of venous blood were withdrawn 15, 45, 75 and 105 min after alcohol administration, through a polythene catheter (Bardic, Intracath) inserted under local anaesthesia into a vein in the cubital fossa. The alcohol content of these samples was subsequently measured by the method of Kozelka and Hine (1958).

In two experiments the effects of alcohol (in one subject administered orally and in the other intra-arterially) on the vessels of the skin and muscle of the forearm were followed independently by determining the percentage oxygen saturation of

samples of blood taken from catheters inserted into a superficial and deep forearm vein respectively.

In two further subjects 40 ml ethyl alcohol (76% W/V) was administered orally diluted with 110 ml ginger ale and in a third the same amounts were given by stomach tube. In three subjects control runs were carried out in which ginger ale alone was administered. Sherry (300 ml; 14% W/V ethyl alcohol) was given to one subject and rum (100 ml; 30.9% W/V ethyl alcohol flavoured with cloves) to another. In the above group of subjects forearm and hand blood flows only were recorded.

RESULTSIntra-arterial infusions

Twenty infusions of alcohol were given into the brachial artery in four normal subjects and bilateral hand blood flow measured. The amounts given ranged from 7.5 to 600 mg/min for 5 min. Doses below 150 mg/min had little or no effect on blood flow, doses between 150 and 250 mg/min caused a small reduction in flow and doses above 300 mg/min caused an intense burning sensation in the forearm and hand and a fall in blood flow through both hands which was more marked on the infused side (Fig 4-1, upper frame and Table 4-1). An after-dilatation usually, but not always, followed cessation of the infusions.

Twenty-two infusions of alcohol were given into the brachial artery in seven normal subjects and the forearm blood flow measured on both sides. The amounts given ranged from 7.5 to 150 mg/min for 5 min. A constrictor effect was seen in every case, the forearm blood flow falling by 4 to 33% (Fig 4-1 lower frame and Table 4-2). An after-dilatation was often seen.

The constrictor action of intra-arterial alcohol on the forearm vessels was not abolished by previous infusion of phenoxybenzamine (250 µg/min for 8 or 10 min), which abolished or markedly reduced the response of the vessels to intra-arterial infusion of noradrenaline (Fig 4-2, Table 4-2). No reduction in the constrictor

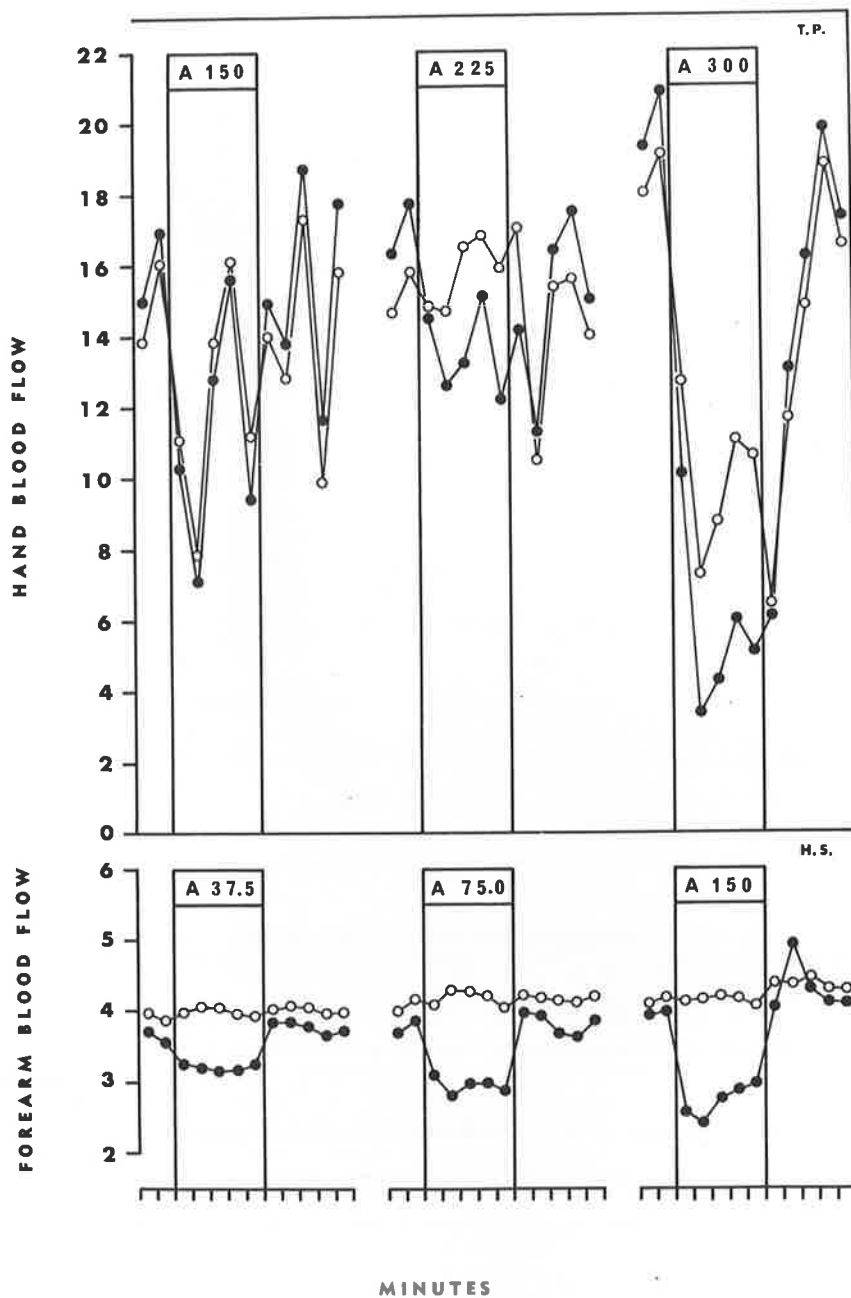


Fig 4-1. The effect of intra-arterial infusions of ethyl alcohol (A, mg/min) on blood flow through the hand (upper frame) and forearm (lower frame). Blood flow in ml/100 ml/min. ● , injected side; ○ , control side.

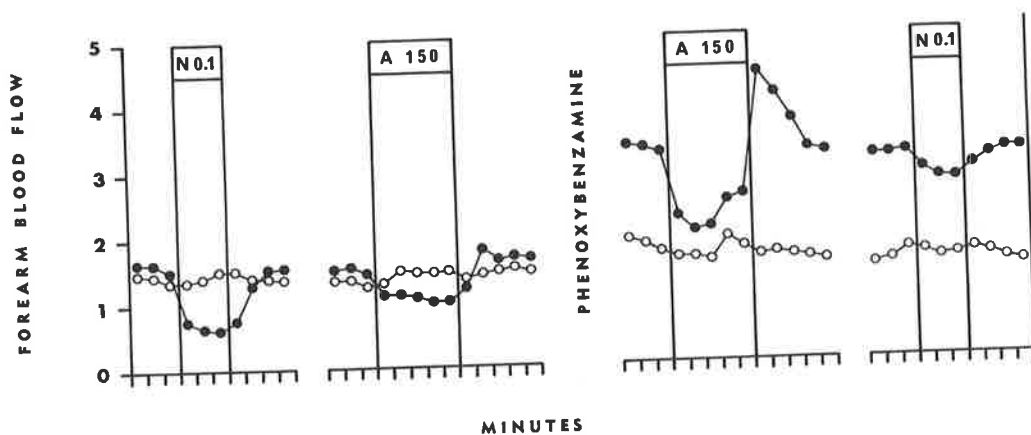


Fig 4-2. The effect of intra-arterial infusions of noradrenaline (N, $\mu\text{g}/\text{min}$) and ethyl alcohol (A, mg/min) on the blood flow through the forearm before and after intra-arterial infusion of phenoxybenzamine ($250\mu\text{g}/\text{min}$ for 8 min). Blood flow in $\text{ml}/100\text{ml}/\text{min}$.

● , injected side; ○ , control side.

response of the hands to alcohol was seen after phenoxybenzamine in one subject (A.R., Table 4-1). In two other subjects (D.H. and J.F., Table 4-1) the constrictor response of the hand vessels to alcohol expressed as a percentage fall in flow from resting level was reduced but not abolished by phenoxybenzamine. Since a reduction in flow in the control hand was usually seen with doses above 300 mg/min and can be attributed to a reflex response to the painful stimulus, it seems likely that the diminution in response seen after phenoxybenzamine in D.H. and J.F. represents the blockade of this component, the residual constriction being due to a direct action of the alcohol.

Further evidence for a direct constrictor action of ethyl alcohol on the limbs vessels was obtained from the patient who had undergone cervical sympathectomy (W.W.). Intra-arterial infusions of doses ranging from 75 to 300 mg/min resulted in falls in both hand and forearm blood flow (Fig 4-3, Tables 4-1 and 4-2).

In one subject the effect of intra-arterial administration of alcohol on the blood flow through the muscle and skin of the forearm was determined. Ethyl alcohol (150 mg/min for 5 min) was given intra-arterially and total forearm blood flow measured. Approximately 30 min later a similar infusion of alcohol was given and venous blood samples were taken from veins which drained the muscle and skin of the same forearm and the percentage oxygen saturation of each sample estimated. Blood flow measurements and the

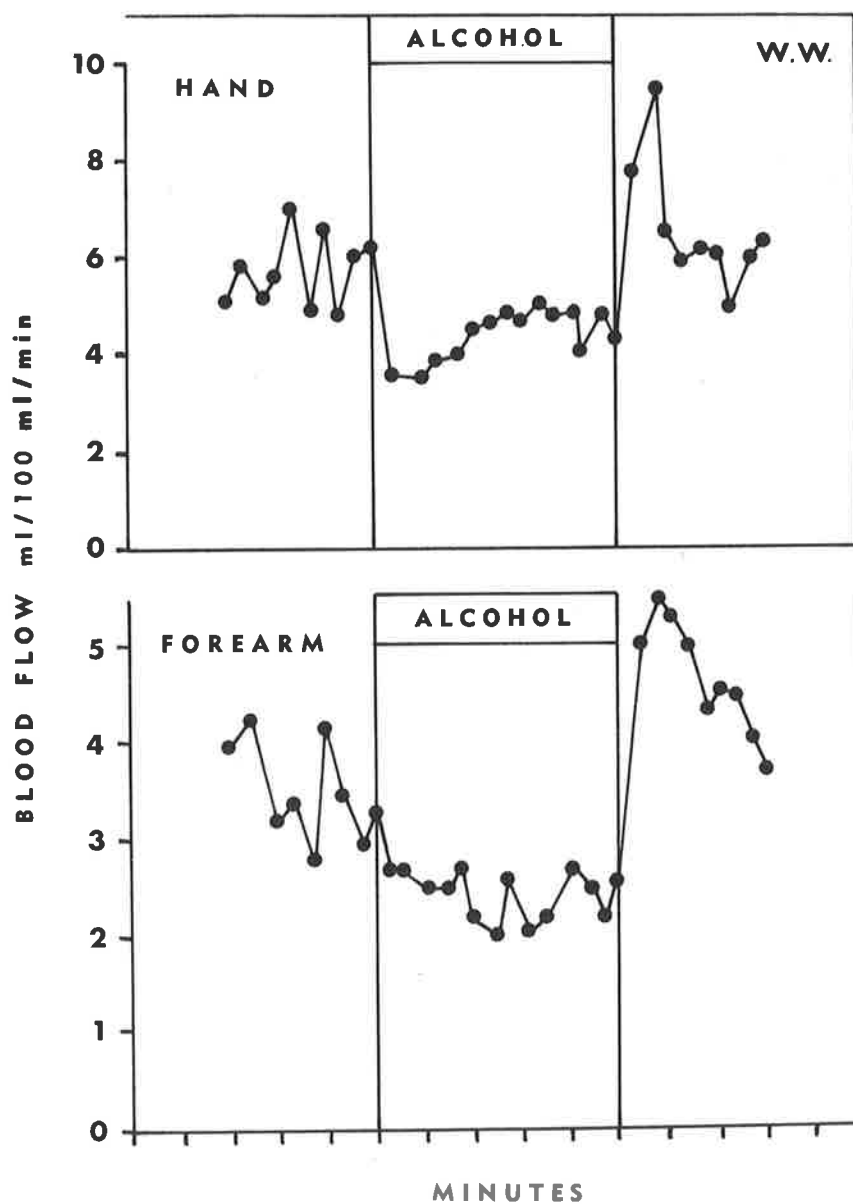


Fig 4-3. The effect of intra-arterial infusion of ethyl alcohol (225 mg/min) on the blood flow through the hand and forearm of a patient whose upper limb was sympathetically denervated. The flow is expressed in ml/100 ml/min.

collection of venous samples were not performed simultaneously as the collecting cuff pressure during blood flow measurements may divert skin venous blood into the muscle bed (Roddie, Shepherd and Whelan, 1956). Fig 4-4 shows the results of this experiment. There was a fall in total forearm blood flow and the changes in venous oxygen saturation indicated a fall in blood flow through muscle and skin. An after-dilatation was seen in the muscle after cessation of the infusion.

Oral administration

Five normal subjects whose body weights ranged from 65 to 80 kg consumed 100 ml brandy over a period of 1 to 5 min. The blood level rose rapidly to reach a peak of 35 to 60 mg/100 ml, and the increase was paralleled in every case by a rise in hand blood flow and a fall in hand vascular resistance. Forearm blood flow showed a variable response. In two subjects there was a slight increase in flow, in two there was a decrease and in one approximately a three-fold increase. Mean blood pressure fell by 6 mm Hg in one subject, rose by 4 to 8 mm Hg in three and was unchanged in one. Heart rate was increased by 5 and 9 beats/min in two subjects and was unchanged in three. The averaged data from these five experiments are illustrated in Fig 4-5.

The variability of the forearm blood flow changes can be

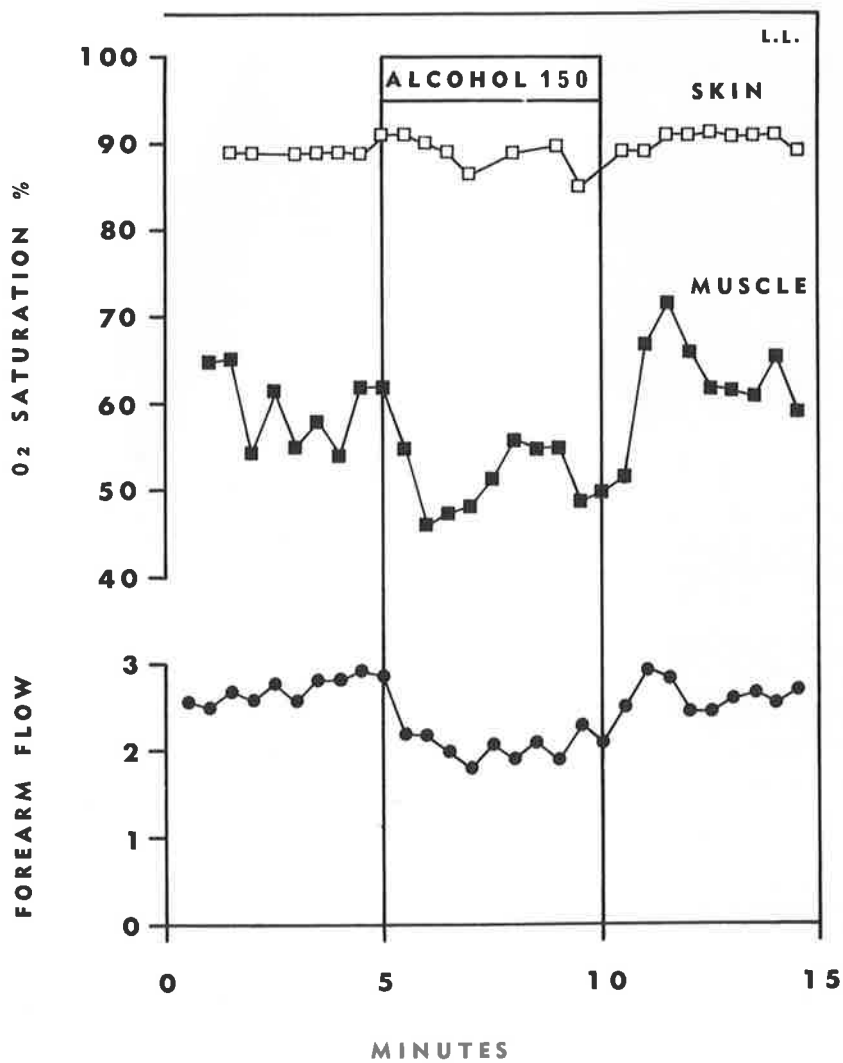
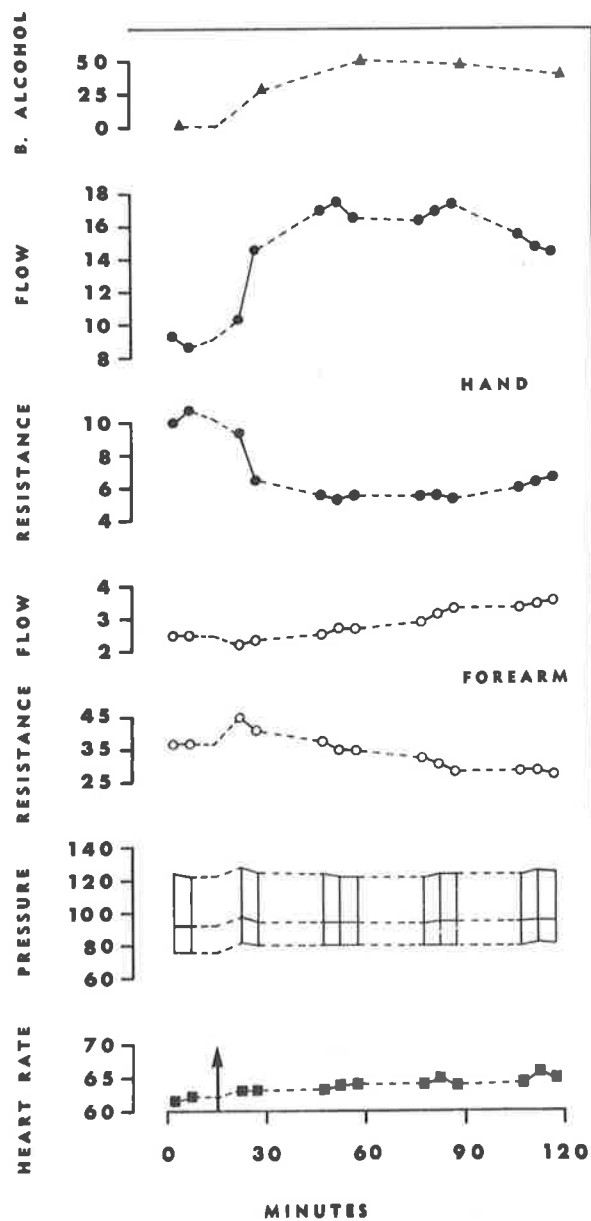


Fig 4-4. The effect of intra-arterial infusions of ethyl alcohol (150mg/min for 5 min) on the blood flow through the forearm (● , ml/100ml/min) and on the percent oxygen saturation of blood samples taken from veins which drained the skin (□) and muscle (■) of the same forearm.

Fig 4-5. The averaged data from 5 normal subjects (body weight 65-80 Kg) who consumed 100 ml brandy by mouth (arrow). Each point represents the mean value for a period of 5 min except for the blood alcohol estimations which were made on venous blood samples collected 15, 45, 75 and 105 min after alcohol.

- ▲ venous blood alcohol concentration in mg/100 ml.
- hand blood flow in ml/100 ml/min and hand resistance in arbitrary units.
- forearm blood flow in ml/100 ml/min and forearm resistance in arbitrary units.
- arterial blood pressure in mm Hg.
- heart rate in beats/min.



attributed to the fact that an increase in flow through the skin was accompanied by a fall in that through the underlying muscle (Fig 4-6).

Figure 4-7 shows the response to oral brandy of the vessels of the normal and denervated hand of the patient who had suffered brachial plexus avulsion. The normal hand showed a dilator response and the flow through the denervated hand was unchanged. A less marked but similar pattern of response was seen in the forearm during a separate experiment (Fig 4-8).

When brandy (75 ml) was taken orally by the patient with bilateral sympathectomy the blood alcohol rose to a level similar to that seen in the normal subjects, but the blood flow through the hand and forearm fell slightly during the first 45 min and then rose slowly over the subsequent 90 min to a little above the pre-alcohol value (Fig 4-9).

Ethyl alcohol (40 ml, 76% W/V) was given orally with 110 ml ginger ale to two subjects, sherry (300 ml, 14% W/V) was given to one subject and rum (100 ml, 30.9% W/V, flavoured with cloves) to one subject. In each case a marked increase in hand blood flow resulted with either no change or a slight rise in forearm flow. A further subject who was able to swallow only 20 ml ethyl alcohol with 55 ml ginger ale did not show any significant change in limb blood flow.

Ginger ale alone (110 ml) was taken by three subjects.

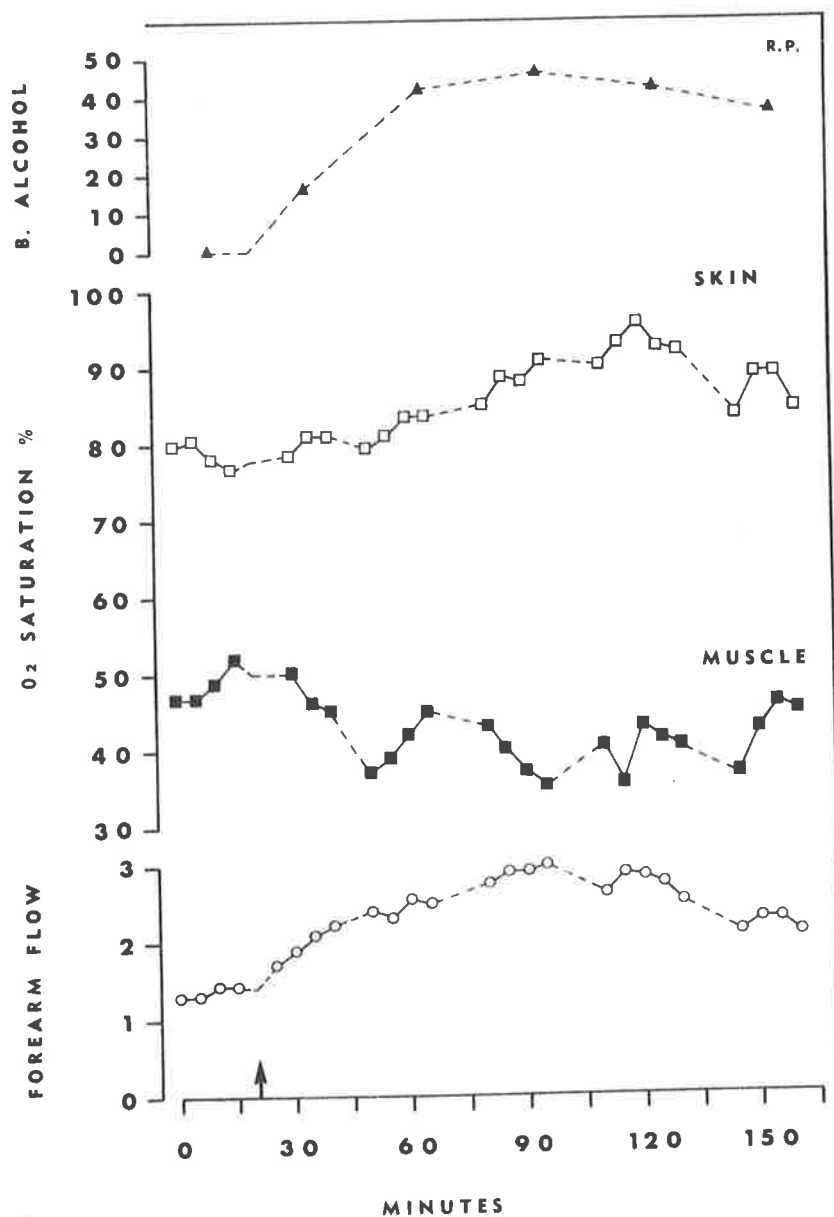
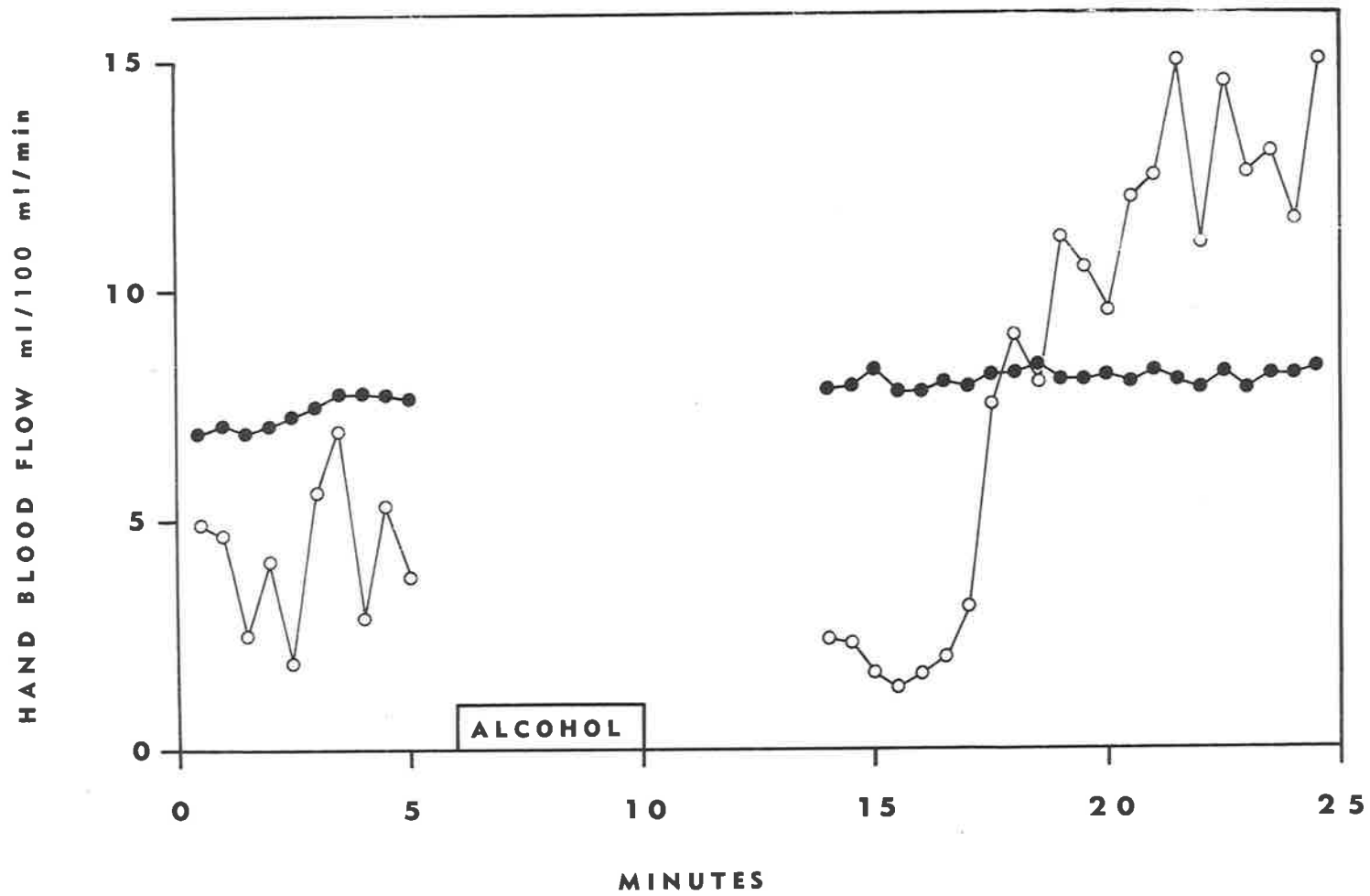


Fig 4-6. The effect of 100ml brandy by mouth (arrow) on blood flow through one forearm ($\bigcirc$, ml/100ml/min) and the percent oxygen saturation of blood samples taken from veins which drained the skin ($\square$) and muscle ($\blacksquare$) of the other forearm. $\blacktriangle$, venous blood alcohol concentrations in mg/100 ml.

Fig 4-7. The effect of 100 ml brandy by mouth on the blood flow through a totally denervated hand (●) and the opposite normal hand (○) of a patient who had suffered unilateral brachial plexus avulsion 12 months previously. The venous blood alcohol concentration 15 min after the consumption of alcohol was 40 mg/100 ml.



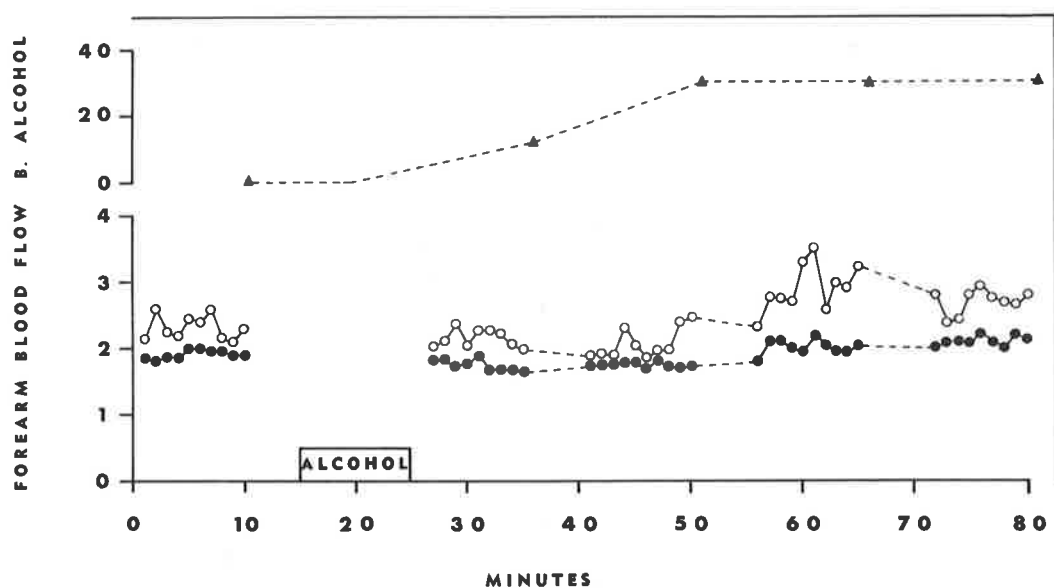


Fig 4-8. The effect of 75 ml brandy by mouth on the blood flow through the totally denervated forearm (●) and the opposite normal forearm (○) of the patient who had suffered brachial plexus avulsion. Blood flow in ml/100 ml/min.

▲, venous blood alcohol concentration mg/100 ml.

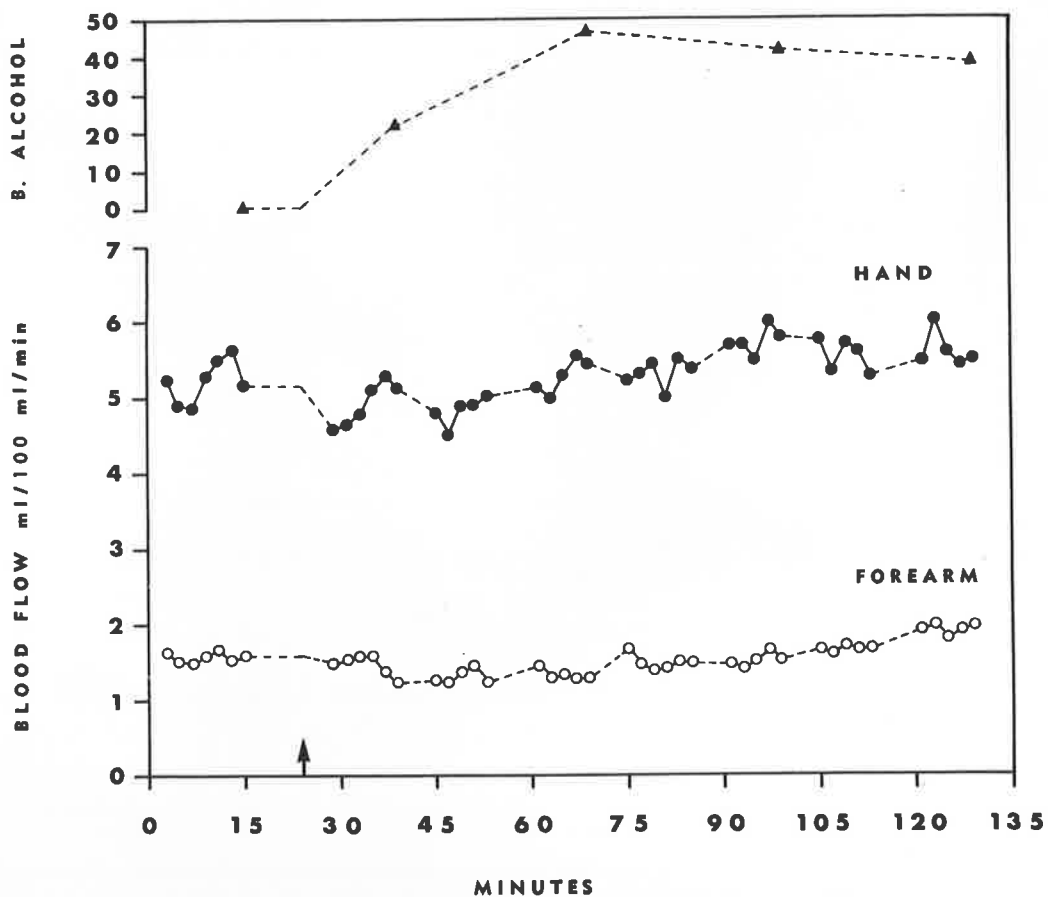


Fig 4-9. The effect of 75 ml brandy by mouth (arrow) on the blood flow through the hand (●) and forearm (○) of a patient who had undergone bilateral cervical sympathectomy 5 years previously. ▲, venous blood alcohol concentration in mg/100 ml.

There was no effect on hand or forearm blood flow. Two of the subjects also rinsed out their mouths over five min with ethyl alcohol in ginger ale but did not swallow any of the mixture. This procedure did not affect the circulation in the hand or forearm.

One subject given 110 ml ginger ale by stomach tube showed no change in hand or forearm circulation, but 40 ml ethyl alcohol in 110 ml water given in the same way produced a marked rise in hand blood flow and a slight increase in forearm flow.

TABLE 4-1

EFFECT OF INTRA-ARTERIAL INFUSION OF ALCOHOL ON HAND BLOOD FLOW

Subject	I.a. dose alcohol mg/min.	Hand blood flow ml/min.		Per cent fall in hand blood flow†
		before infusion	during infusion	
A.R.	75	75	96	-
	300	80	87	20
	450	60	12	65
	600	60	22	34
	375*	110	28	40
T.P.	75	37	32	7
	150	60	53	9
	225	70	60	26
	300	80	25	50
D.H.	7.5	38	37	-
	18.75	32	35	8
	37.5	40	34	4
	75	33	36	5
	150	37	26	19
	225	24	15	48
	225*	115	92	16
J.F.	75	45	51	-
	150	55	40	28
	225	50	24	36
	300	40	16	54
	375	60	18	62
	450	60	23	50
	375*	115	99	13
	450*	110	94	16
W.W. cervical sympathectomy	75	17	14	19
	150	20	18	14
	225	23	18	19
	300	21	16	26

* Denotes after phenoxybenzamine.

† These values have been corrected for spontaneous changes in blood flow by reference to the opposite control side (Duff, 1952).

TABLE 4-2

EFFECT OF INTRA-ARTERIAL INFUSION OF ALCOHOL ON FOREARM BLOOD FLOW

Subject	Forearm blood flow during infusion ml/min.	I.a. dose alcohol mg/min.	Calculated arterial alcohol concentration mg/100 ml	Per cent fall in forearm blood flow
J.S.	32	18.75	59	4
	26	37.5	144	12
	32	75.0	234	15
G.C.	18	37.5	208	9
	19	75.0	395	6
	16	150.0	937	20
H.S.	23	37.5	163	8
	18	75.0	417	19
	20	150.0	750	32
T.P.	19	75.0	395	12
	18	150.0	833	33
J.N.	21	7.5	34	7
	21	18.75	86	7
	20	37.5	180	16
	17	75.0	426	8
	16	150.0	892	25
R.M.	11	7.5	70	13
	8	18.75	234	13
	9	37.5	417	25
	9	75.0	833	25
	8	150.0	1,875	31
	17*	150.0	882	26
L.L.	16	150.0	93	29
W.W.	18	150.0	830	16
cervical sympathectomy	16	225.0	1,406	29

* Denotes after phenoxybenzamine.

DISCUSSION

The alcohol levels of the arterial blood arriving in the forearm during the intra-arterial infusions have been calculated to range from 34 mg/100 ml (which represents the level produced in the blood by ingestion of about 50 ml brandy) to 1,875 mg/100 ml (which, if it pertained for the whole circulation, would represent the equivalent of the ingestion of more than 3 litres of brandy and be more than sufficient to induce alcoholic coma). The constriction of skin and muscle vessels caused by these concentrations appeared to be a direct action of alcohol on the smooth muscle of the arteriolar walls. Sympathetic vasoconstrictor nerve stimulation or release of catecholamines from peripheral storage sites does not appear to be involved in the vasoconstrictor action since neither sympathectomy nor administration of phenoxybenzamine abolished the response. Although intra-arterial administration of alcohol has been used in the treatment of peripheral vascular disease (Edwards et al, 1952), the demonstration of a direct constrictor action on the vessels of normal limbs indicates that such administration is likely to be detrimental if alcohol has similar effects on both normal and diseased vessels.

The dilator effect on the limb vessels of moderate amounts of alcohol taken orally was found to be confined to the vessels of the skin. The response was sympathetically mediated since it was absent in the sympathectomised and denervated limbs, and was presum-

ably due to inhibition of sympathetic vasomotor tone due to an action on either the vasomotor centres or the sympathetic ganglia. The fall in muscle blood flow which was present at the time of the skin dilatation probably represented the direct local action of the alcohol on the muscle vessels, since a fall in forearm flow occurred in the sympathectomised limb. This suggested that the dilator action of alcohol was due to a selective action on the vasomotor centres controlling the skin vessels rather than to ganglionic blockade which might have been expected to result in increased muscle flow as well as skin vasodilatation. That the skin and muscle vessels of the forearm are independently innervated and that specific controlling centres are present for the two vascular beds has been demonstrated by Roddie, Shepherd and Whelan (1956, 1957c). They showed that general body heating results in dilatation of the vessels of the skin without any effect on the vessels of the underlying muscles, whereas increases in pressure on the low pressure side of the intra-thoracic vascular bed cause reflex dilatation of the muscle vessels in the limbs without any such change in the skin.

This central action of alcohol to produce vasodilatation appears to be the first demonstration of a drug with such a discriminating action on one part of the central controlling mechanism. Angiotensin given intravenously has been shown to cause a constriction of the vessels of the hand which is central in origin and is mediated by the sympathetic nerves (Scroop and Whelan, 1966). Whether the action

of angiotensin is due to an effect on vasomotor centres or on the ganglia is not yet certain, but the former site seems the more likely.

Pure ethyl alcohol was not given in most of the experiments involving oral administration because it was unpleasant to the taste and not all subjects were able to ingest the required amount. However, two subjects succeeded in taking 40 ml with 110 ml ginger ale and a third received ethyl alcohol by stomach tube. The responses of forearm and hand vessels were similar to those seen when brandy, sherry and rum were administered orally. Ginger ale alone, orally or by stomach tube, was without effect, as was rinsing the mouth with the ethyl alcohol-ginger ale mixture. It seems reasonable to conclude that the responses of the limb vessels to the ingestion of brandy, sherry and rum can be attributed to their content of ethyl alcohol rather than to any flavouring agents or other constituents or to emotional effects accompanying ingestion of fluid.

The fact that the blood flow through muscle is reduced after oral administration of alcohol indicates that caution should be exercised in advocating its use by mouth to improve the peripheral circulation in vascular disease. While the blood flow through the hands and digits may be enhanced, a concomitant decrease in muscle blood flow may have adverse effects in an already ischaemic muscle. This may also pertain to cardiac as well as skeletal muscle since Webb and Degerli (1965) found significant reduction in coronary artery

blood flow and Regan, Koroxenidis, Moschos, Oldewurtel, Lehan and Hellems (1966) described diminished left ventricular function during systemic administration of alcohol to anaesthetised dogs.

In conclusion, the present studies revealed that the direct, local actions of ethyl alcohol on the skin and muscle vessels of the upper limb in man are entirely constrictor, but an indirect, sympathetically-mediated vasodilator action on skin vessels is manifest when alcohol is administered systemically.

SUMMARY

1. Ethyl alcohol given into the brachial artery in normal subjects caused a reduction in the blood flow through the hand and forearm. Skin and muscle vessels were both constricted.
2. The constrictor action appeared to be a direct one on the vessels since it was present in sympathectomised limbs and in normal limbs treated by intra-arterial infusion of phenoxybenzamine.
3. Brandy, sherry, rum and ethyl alcohol given by mouth caused a vasodilatation in the hand. The skin vessels of the forearm were also dilated by oral alcohol. The dilatation was sympathetically mediated and was absent in sympathectomised and denervated limbs.
4. The blood flow through the forearm muscles was reduced after oral alcohol, probably due to a direct vasoconstrictor action.
5. As a result of these studies it is concluded that intra-arterial administration of alcohol is contra-indicated in the treatment of peripheral vascular disease, and while oral administration may cause dilatation of skin vessels

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the accompanying constriction of muscle vessels could prove to be a disadvantage where muscle ischaemia is present.

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SECTION 5

VENOUS DISTENSIBILITY IN ESSENTIAL HYPERTENSION

INTRODUCTION

The role of the arterioles (resistance vessels) in essential hypertension and their responses to various hypotensive agents have been subject to intense investigation (Pickering, 1955; Folkow, Grimby and Thulesius, 1958; and Freis, 1960). In contrast, the role of the veins (capacity vessels) in hypertension has received little attention. Since the veins have sympathetic innervation (Donegan, 1921), variations in venous tone may be expected, and since approximately two-thirds of the systemic blood is normally contained in these vessels (Green, 1944), these variations and the attendant changes in venous capacity may be expected to influence other vascular beds. Thus, Braunwald, Ross, Kahler, Gaffney, Goldblatt and Mason (1963) and Mason and Braunwald (1964), from studies of the dog and man, concluded that the responses of capacity vessels were important in the action of various anti-hypertensive drugs. Wood (1961), however, studied venous tone (measured as venous distensibility) of forearm vessels of hypertensive patients and control subjects and found no difference between the two groups. The present study was undertaken to examine venous distensibility and its response to increased sympathetic nerve activity (produced by tilting) in the forearms of normotensive and hypertensive subjects, and to evaluate the effect of anti-hypertensive drugs on these features in the latter.

METHODSSelection of subjects

Studies were carried out on 36 volunteers of whom 20 were healthy normotensives (16 female, 4 male; ages 19-59 years; 9 Negro, 7 Caucasian, 2 Oriental and 2 American Indian) and 16 were patients from the Hypertension Clinic of the Los Angeles County General Hospital (11 female, 5 male; ages 28-63 years; 15 Negro and 1 Caucasian) diagnosed as having essential hypertension. Neither the normal subjects nor the hypertensive patients had had previous experience of this or other experimental techniques, and the arm volumes and body builds of both groups were similar. The patients showed no evidence of malignant or accelerated hypertension or cardiac decompensation, and serum urea nitrogen levels were below 25 mg%. All patients selected had exhibited a diastolic blood pressure greater than 90 mm Hg and/or a mean blood pressure above 110 mm Hg on several occasions. Blood pressure was measured sphygmomanometrically on both normotensive and hypertensive subjects during each study. At least 3 determinations were made with the subject horizontal and then the subjects were tilted. The first blood pressure measurement was made at least 10 min after the subject was at rest. Mean blood pressure was calculated as diastolic plus one-third of the pulse pressure. After venous distensibility measurements had been made without treatment, thirteen of the hypotensives received specific anti-hypertensive medications. A

standard regime of 50 mg of hydrochlorothiazide and 15 mEq of potassium 3 times daily was given. In addition, 4 patients received guanethidine, 2 reserpine and 2 methyldopa. Doses are indicated in Table 5-3. Patients received medication for at least one month prior to subsequent venous distensibility measurements. The retest measurements on both normal and hypertensive subjects were made at monthly intervals.

Plethysmographic details

The capacity of the vascular bed of the forearm was measured with an electro-capacitance plethysmograph (Hyman, Burnap and Figar, 1963). The subject rested quietly on a tilt-table in a temperature-controlled laboratory (21-22°C) for at least 15 min while the apparatus was applied. An electrical calibrator with a volume equivalent of 1.46 ml, as described by Hyman and Wong (1968), was applied around the forearm segment beneath the plethysmograph. A pneumatic congesting cuff encircled the upper arm. An adjustable arm rest ensured that the forearm segment was above the right atrial level in both the horizontal and tilted positions.

With the subject lying horizontal, the congesting cuff was inflated to 50 mm Hg and the increase in forearm volume was recorded continuously. When a "plateau" was reached (1.5-3 min) the congesting pressure was lowered in 5 mm Hg steps with sufficient time to establish a new "plateau" at each pressure. Each new pressure was

momentarily undershot by 3-4 mm Hg to facilitate emptying of the veins (Fig 5-1). The baseline at zero congesting pressure was reached in 4-7 min. Following a short rest period the procedure was repeated. Then the subject was tilted 60° feet down and, after allowing 4 min for venous pressure to stabilise (Samueloff, Browse and Shepherd, 1966), two measurements of venous distensibility, separated by a 2-3 min rest period, were made. During the tilt the subject's weight was supported by a saddle to facilitate venous pooling in the inactive dependent lower limbs. The percentage increase in forearm volume above the baseline of zero congesting pressure was plotted against the corresponding congesting cuff pressures for each of the 4 determinations as in Fig 5-2.

Venous distensibility (VV_{30}) is expressed as the percentage increase in forearm volume at a cuff pressure 30 mm Hg above the minimal pressure which caused a measurable increase in forearm volume (usually 3-10 mm Hg and deduced by extrapolating the volume/pressure curve through zero volume change as in Fig 5-2). The average of the two VV_{30} values, horizontal and tilted, for each subject and the calculated absolute and percentage changes associated with tilting are reported in the tables. The same sphygmomanometer, plethysmograph and calibrator were used throughout the study to minimise errors which might arise from variations in characteristics of the apparatus.

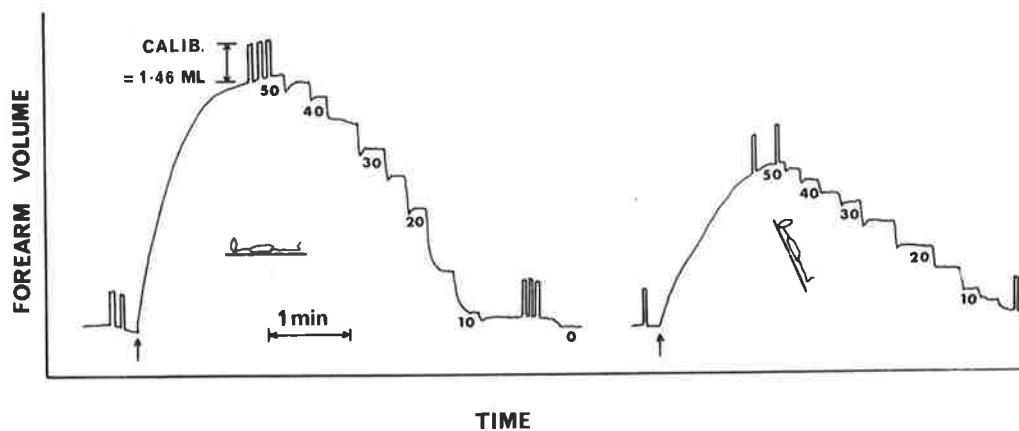


Fig 5-1. Tracing of venous distensibility records, horizontal and tilted, from subject R.A., obtained with electro-capacitance plethysmograph (Hyman, Burnap and Figar, 1963). CALIB.= electrical calibrations equivalent to 1.46 ml. Arrows indicate inflation of proximal venous congesting cuff to 50 mm Hg.

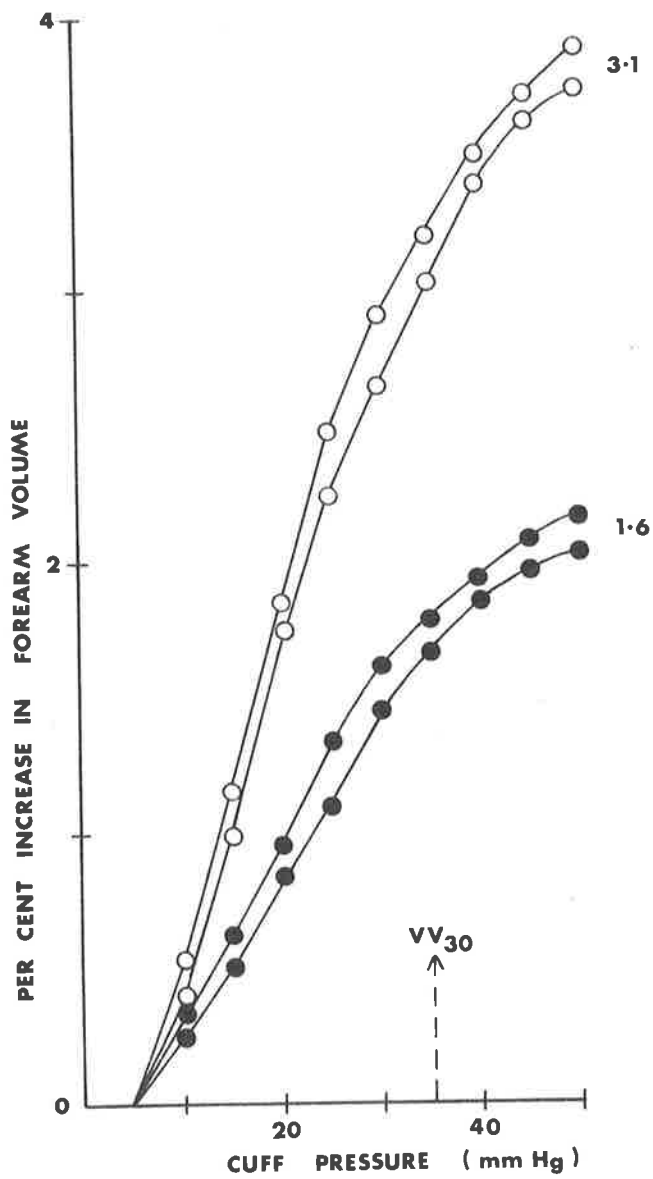


Fig 5-2. Venous volume/pressure curves from subject R.A. 0, pretilt; ●, tilted determinations. Average VV_{30} values for paired determinations, horizontal and tilted, are indicated,

Statistical analyses were performed using the Student "t" test on pair differences within individuals and differences between means. A probability limit of less than 0.05 was considered significant.

Validation of the method

The validity of the method used in the principal study above was examined by two additional sets of experiments using different plethysmographic techniques and different normal subjects. In the first, venous distensibility in 5 subjects was measured simultaneously by a water-filled plethysmograph (Greenfield, 1954) on one forearm and an electro-capacitance plethysmograph, of the type described by Willoughby (1965) and Fewings and Whelan (1966), on the other. VV_{30} , horizontal and tilted, was deduced from the volume/pressure relationship obtained on the emptying phase following venous congestion as described above. Venous pressure was recorded from a polythene catheter inserted centrifugally into an antecubital vein and connected to a Statham pressure transducer (P23BC) and Grass Polygraph (Model 5D). The catheter was placed so that the tip lay beneath the capacitance plethysmograph.

In the second set of experiments, changes in forearm volume at congesting cuff pressures 20, 40 and 60 mm Hg above the minimal pressure which caused a measurable change in volume were studied in 6 normal subjects, horizontal and tilted, by the method described by

Glover, Greenfield, Kidd and Whelan (1958). Increase in volume of the forearm displaced water from a water-filled plethysmograph into a burette calibrated in millilitres. The position of the burette was continuously adjusted to keep the meniscus at a level 10 cm above the centre of the limb segment, and the burette reading was noted at 30 sec intervals until a "plateau" was reached. The cuff pressure was then returned to atmospheric and the limb volume allowed to stabilise at the resting level before further measurements were made.

RESULTS

Venous distensibility in control subjects

A tracing of a record of a typical experiment on a normotensive control subject (R.A.) is shown in Fig 5-1. Application of the congesting pressure caused forearm volume to increase rapidly at first and then more slowly until a relative "plateau" was reached. The subsequent stepwise decrements in limb segment volume reflect corresponding decreases in congesting cuff pressure. Figure 5-2 presents the individual volume/pressure curves in the pre-tilt and tilted positions measured on subject R.A.. The volume change at each congesting pressure is less in the tilted than in the horizontal posture. This response pattern was obtained in 23 of the 28 experiments performed on the 20 control subjects and was statistically significant: in 4 experiments a slight rise in VV_{30} was recorded and in one there was no change (Table 5-1). The mean and standard error of the venous distensibility measurements (VV_{30}) was 3.15 ± 0.16 in the horizontal position and fell to 2.29 ± 0.16 with tilting - a fall of 27.3% ($P < 0.001$). On the basis of pair differences in individuals a fall of $25.7 \pm 4.7\%$ occurred ($P < 0.001$). Figure 5-3 (open bars) summarises the responses of the control group.

Venous distensibility in untreated hypertensive patients

Twenty-four venous distensibility experiments were performed

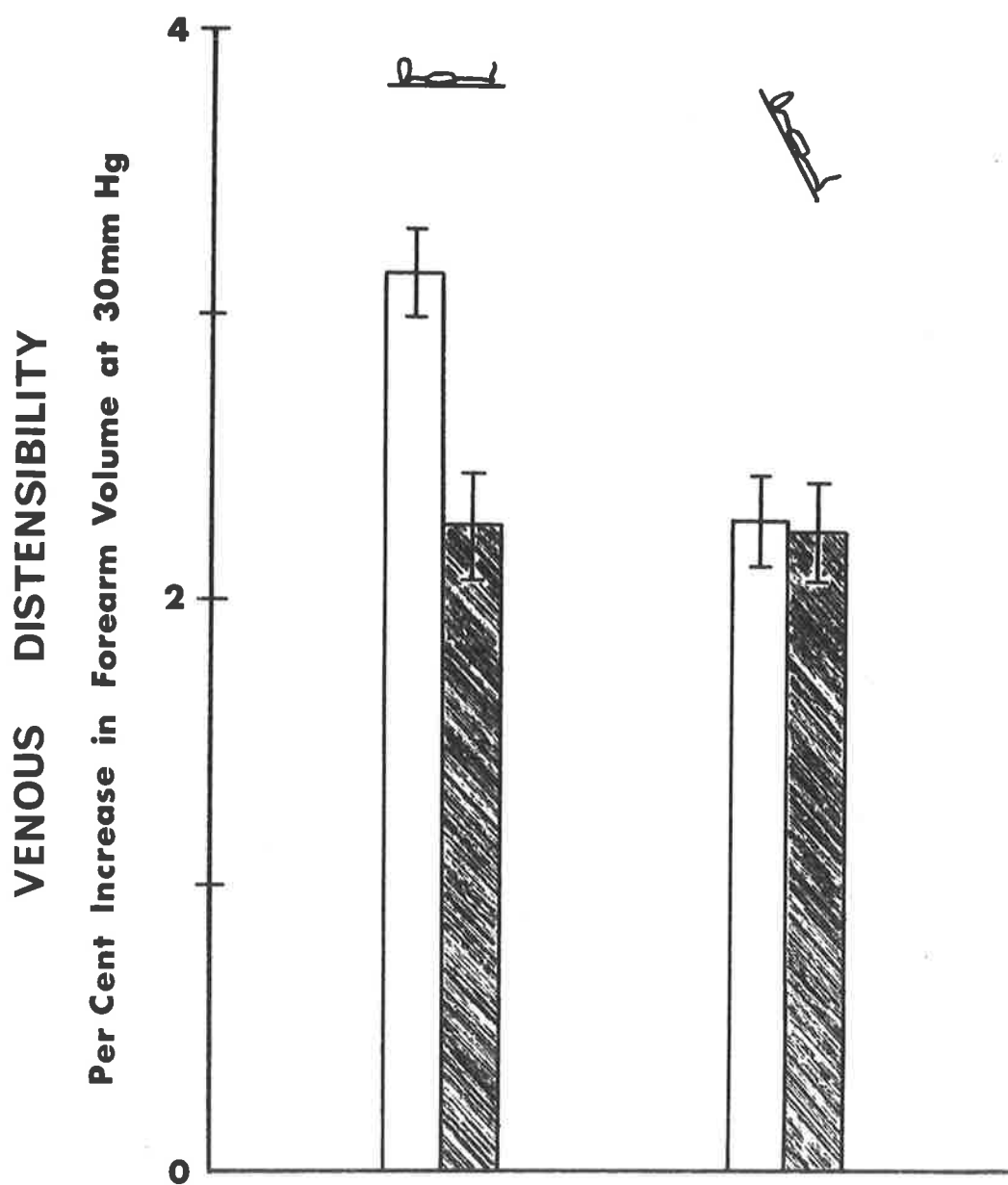


Fig 5-3. Effect of tilting on mean venous distensibility (VV_{30}) in 28 experiments on 20 normal subjects (open bars) and 24 experiments on 16 hypertensive patients (shaded bars). The vertical lines through each bar represent one standard error on either side of the mean.

Fig 5-4. Venous distensibility (VV_{30}) horizontal before (open bars) and during (shaded bars) treatment in each of 13 patients.

Guanethidine = guanethidine plus hydrochlorothiazide.

Reserp. = reserpine plus hydrochlorothiazide.

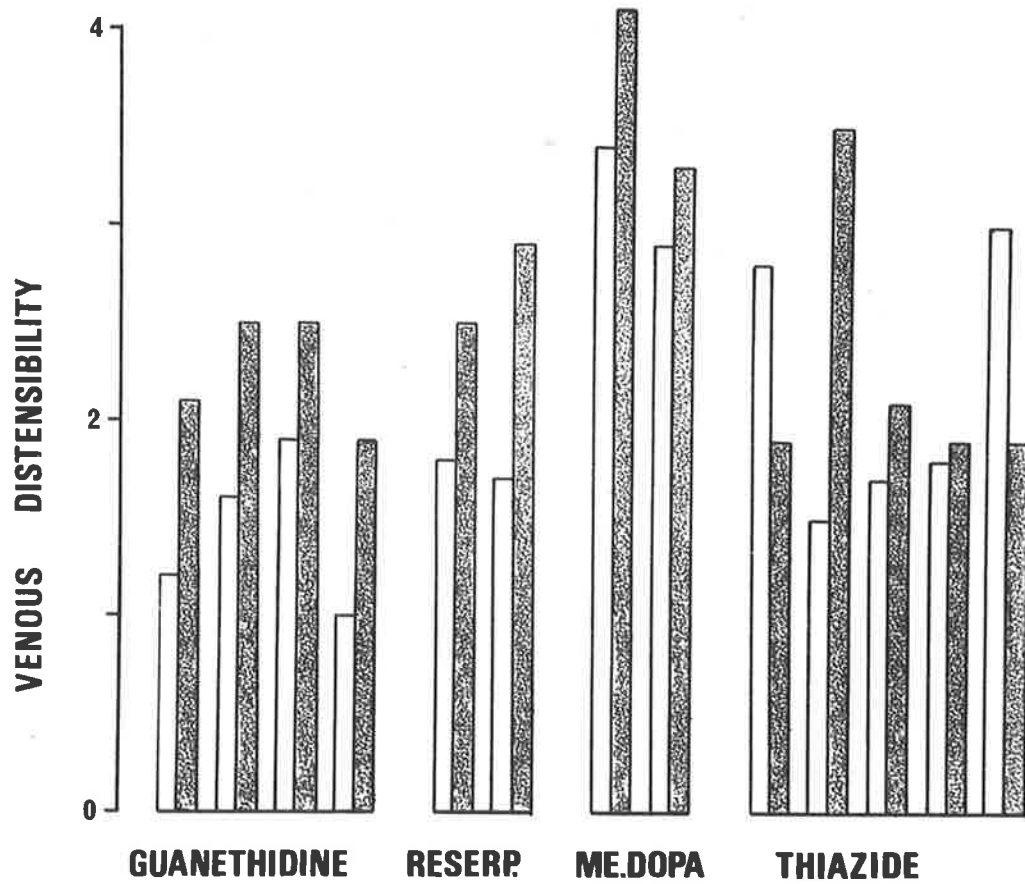
Me.Dopa = methyldopa plus hydrochlorothiazide.

Thiazide = hydrochlorothiazide alone.

on the 16 untreated hypertensive patients (Table 5-2). The mean of the VV_{30} measurements supine (2.27 ± 0.19) was significantly lower than that of the control group ($P < 0.001$), but no further decrease in VV_{30} occurred with tilting (2.25 ± 0.17). This mean VV_{30} tilted was also almost identical to the corresponding mean of the control group. Figure 5-3 (shaded bars) summarises the results of the untreated hypertensive group.

Effect of treatment on venous distensibility in hypertensive patients

After preliminary measurements without therapy 13 of the 16 hypertensive patients were placed on antihypertensive therapy. Venous distensibility (VV_{30}), both horizontal and tilted, was determined for each subject, and the absolute and percentage changes from the pretreated state for each individual and for the means of each group are summarised in Table 5-3. The mean venous distensibility in the horizontal position increased by 37.7% with antihypertensive therapy: the rise being more marked in those patients who received guanethidine or reserpine in addition to thiazide than in those treated with thiazide alone. In two of the five patients treated with thiazide alone venous distensibility fell; only a small rise (20.6 and 13.8%) occurred in the two patients treated with methyldopa plus thiazide. The mean VV_{30} values horizontal, before and during treatment, for each of the 13 patients are indicated in Fig 5-4. As illustrated in Fig 5-5, the mean VV_{30} horizontal for all of the



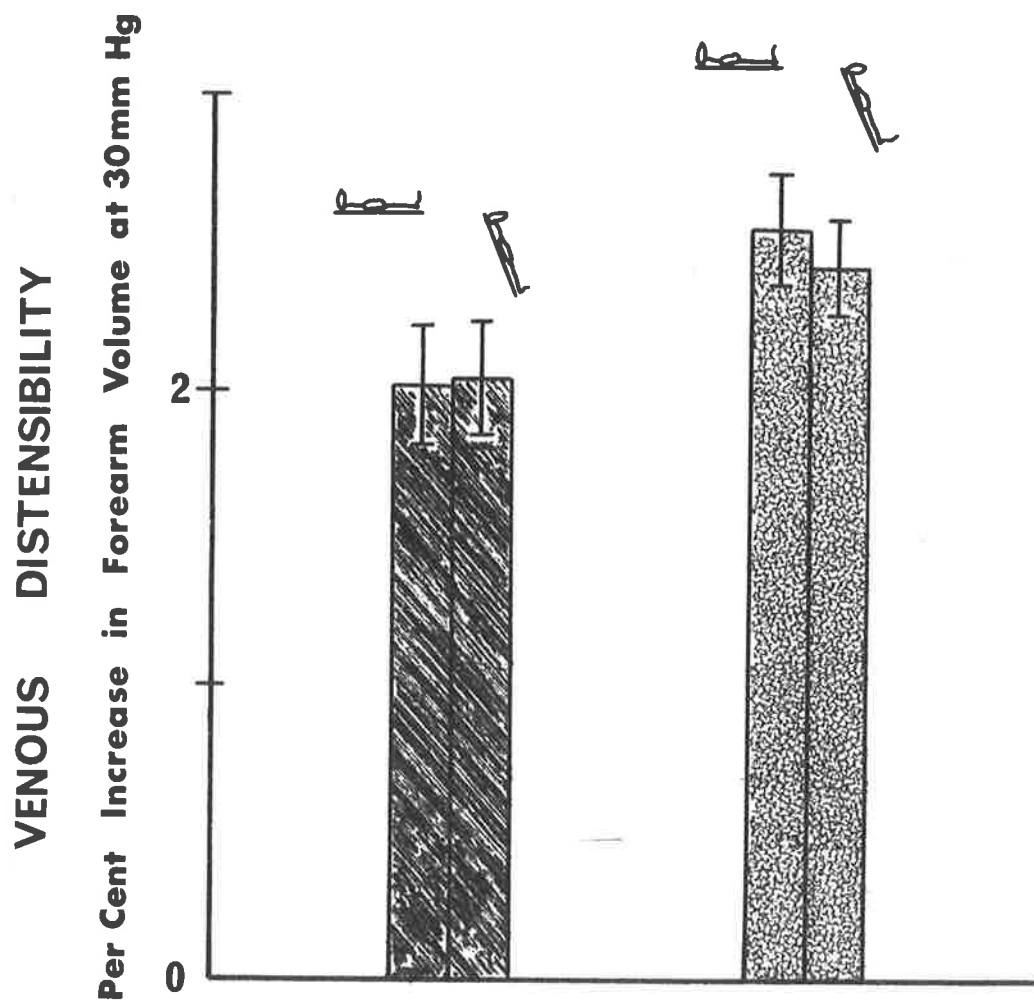


Fig 5-5. Effect of antihypertensive treatment on mean venous distensibility (VV_{30}) in 13 patients. Left hand shaded bars represent pretreatment values, horizontal and tilted, and right hand shaded bars represent the corresponding values after one or more months of treatment. The vertical lines through each bar indicate one standard error on either side of the mean,

patients on treatment rose to 2.55 from a pre-treatment mean of 2.02; this difference was not significant when the means were compared ($0.05 < P < 0.10$), but was significant on the basis of percentage change ($37.7 \pm 12.7\%$, $0.01 < P < 0.02$) or absolute change in individual patients ($+0.52 \pm 0.22$, $0.02 < P < 0.05$). Similarly, the mean VV_{30} tilted changed from 2.04 to 2.42. The lower value of the mean VV_{30} horizontal for the 13 patients (2.02) compared with that of all 16 hypertensives (2.27) is explained by exclusion of 3 subjects with high initial VV_{30} (I.F., W.S., and A.H.), who received no antihypertensive therapy.

Blood pressure observations

Tilting increased the average diastolic blood pressure of the normotensive subjects from 72 to 79 mm Hg, and the average mean blood pressure increased from 86 to 91 mm Hg. The same manoeuvre increased average diastolic blood pressure in 24 experiments on the 16 hypertensive patients, before treatment, from 104 to 110 mm Hg and the average mean blood pressure from 122 to 127 mm Hg. The arterial blood pressure values reported in Table 5-2 are the average of 3 determinations on each patient while resting in the horizontal position. The 13 patients who later received antihypertensive therapy had an average diastolic blood pressure of 103 horizontal, 109 tilted and an average mean pressure of 123 horizontal, 127 mm Hg tilted. During therapy their average diastolic blood pressure decreased to 96 horizontal, 99 tilted and the average mean pressure

to 116 horizontal and 115 mm Hg tilted, the fall in pressure being more marked in those patients receiving thiazide plus one of the more potent antihypertensive agents than with thiazide alone. These changes in blood pressure are essentially the same as those found by Maronde, Haywood and Barbour (1961). The increases of mean arterial pressure of approximately 5 mm Hg as a response to tilting in the normotensives and untreated hypertensives are also similar to those described by Frohlich, Tarazi, Ulrych, Dunstan and Page (1967) who utilised the tilting response to classify patients in regard to severity of the hypertension: they found that neurogenic activity was highest in mild early hypertension and as hypertension progressed other mechanisms became more important. The subjects in this group, therefore, fall into the relatively early hypertensive group which exhibits neurogenic activity on tilting. Symptoms attributable to postural hypotension were not noted in any of the subjects.

Verification of method

(a) Results obtained in 5 subjects using a water-filled plethysmograph and an electro-capacitance plethysmograph showed no significant difference from those obtained in the principal study using an identical protocol, although the mean VV_{30} values were somewhat higher than in the principal study. Venous distensibility horizontal averaged 3.85 (water-filled) and 4.00 (capacitance) and

decreased to 2.91 and 2.97, respectively, with tilting - a fall in VV_{30} of 24.4% and 25.8%, respectively (Fig 5-6).

The forearm venous pressure, recorded directly via a polythene catheter and pressure transducer, ranged between 1-3 mm Hg in the elevated forearm during rest periods (5 subjects). Increments or decrements in applied cuff pressure produced equal changes in forearm venous pressure provided a minimal starting pressure (3-8 mm Hg) was exceeded. The same relationship was found in the tilted position, care having been taken to re-position the pressure-sensitive diaphragm of the transducer at the same level as that of the catheter tip in the forearm vein. These observations were essentially the same as those of Wood (1965) who reported that pressure changes within limb veins equalled changes in cuff pressure once local venous pressure had been reached. Therefore, intravenous pressure measurements were omitted from the principal study and the volume/pressure curve was corrected for the minimal starting pressure by extrapolating the curve through zero volume change as in Fig 5-2.

(b) The results of 6 experiments in which venous distensibility was measured with the water-filled plethysmograph on the filling phase during prolonged venous congestion are summarised in Fig 5-7. Venous distensibility decreased by an average of 22, 11 and 19% during 60° feet down tilting when measurements were made at cuff pressures of 20, 40 and 60 mm Hg respectively. These results were

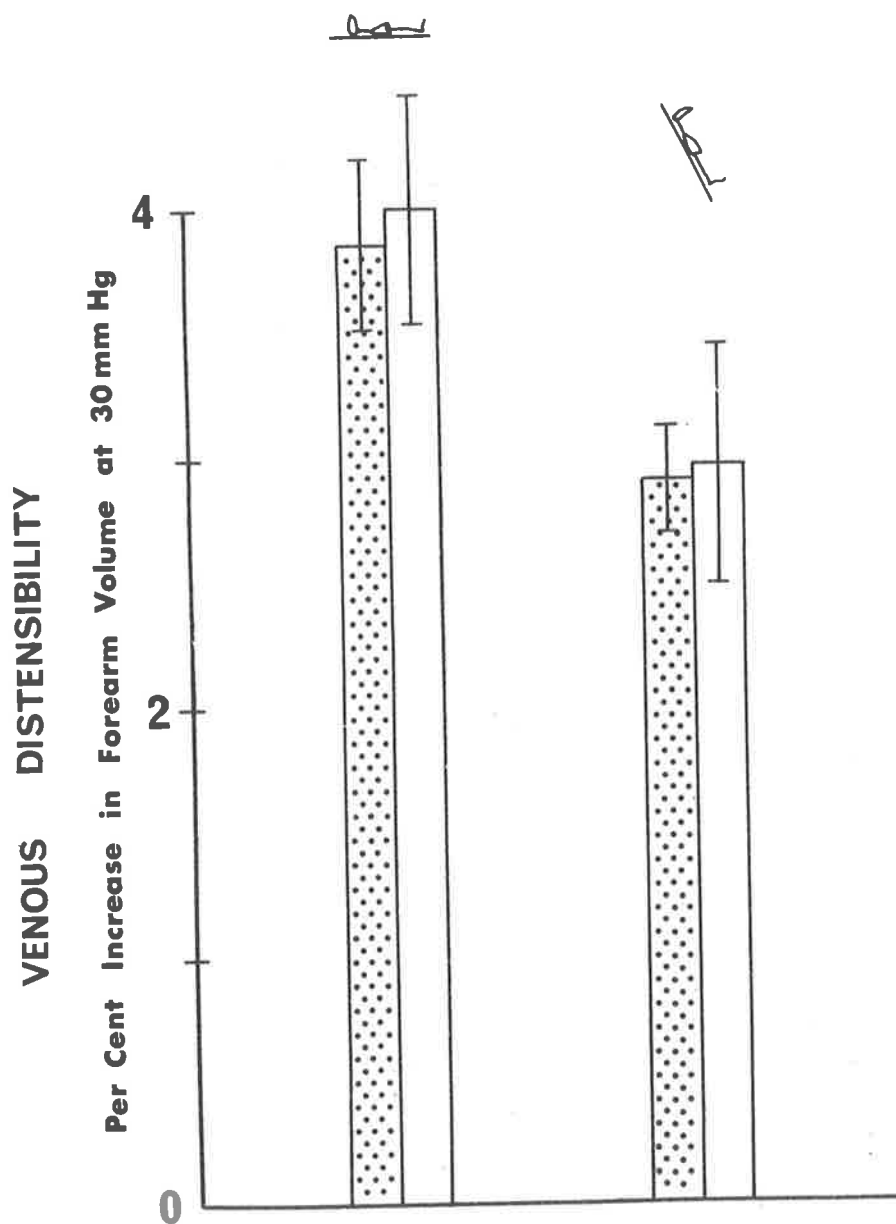


Fig 5-6. Comparison of venous distensibility (percentage increase in forearm volume at 30 mm Hg), horizontal and tilted, as determined on 5 normal subjects by water-filled (shaded bars) and Adelaide capacitance plethysmograph (open bars). The vertical lines through each bar represent one standard error on either side of the mean.

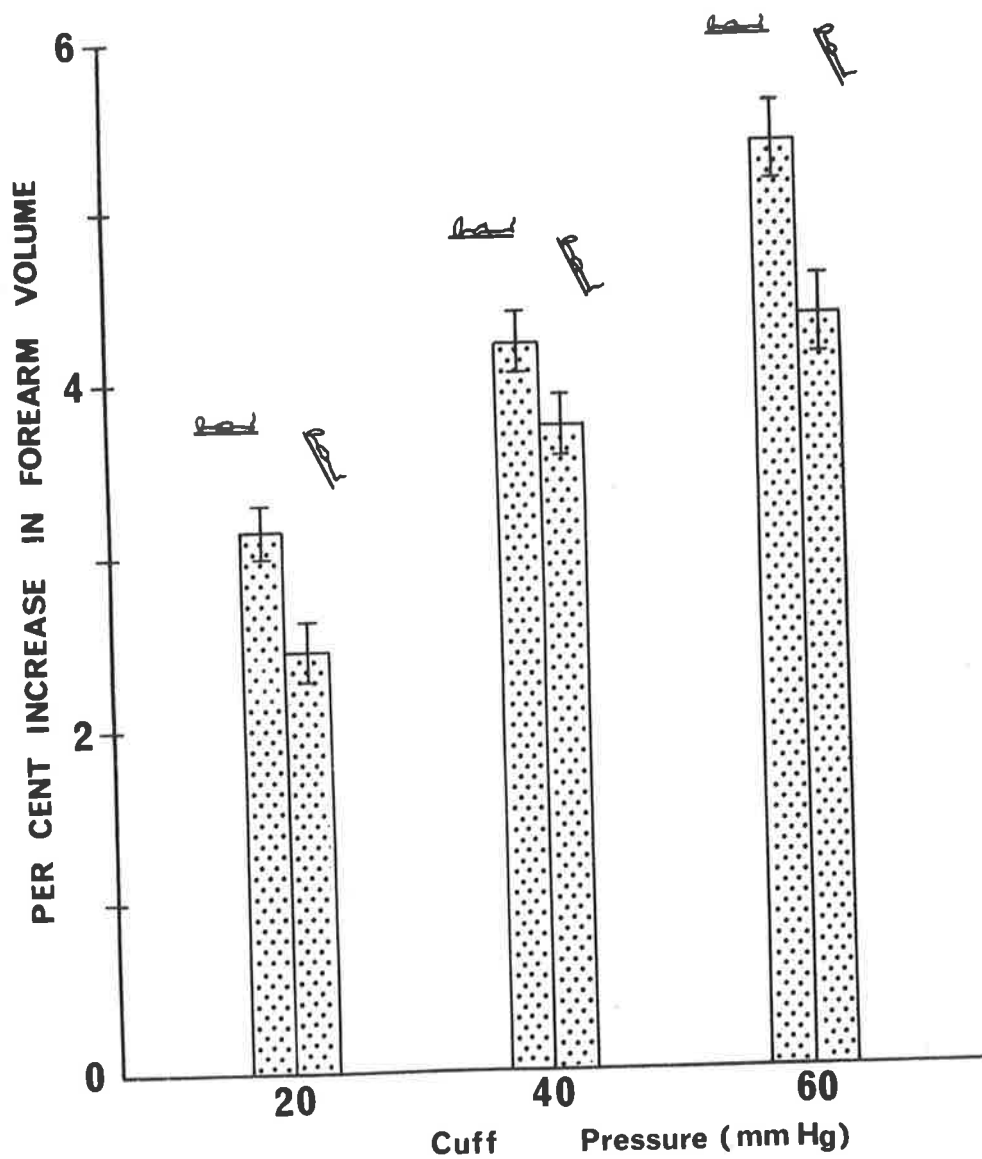


Fig 5-7. The percentage increase in forearm volume, horizontal and tilted, recorded at cuff pressures of 20, 40 and 60 mm Hg with a water-filled plethysmograph. Records were obtained during the filling phase of venous congestion and represent the mean responses in 6 normal subjects.

essentially similar to the emptying phase experiments.

Thus the forearm vascular capacity of normal subjects decreased by approximately 20-25% during 60⁰ feet down tilting irrespective of whether an electro-capacitance or water-filled plethysmograph was used or whether the forearm volume/pressure relationship was studied on the filling or emptying phase of venous congestion.

TABLE 5-1

VENOUS DISTENSIBILITY (VV_{30}) IN NORMOTENSIVE CONTROL SUBJECTS

<u>SUBJECT</u>	<u>AGE</u>	<u>SEX</u>	<u>RACE</u> ⁺	<u>PRE-TILT</u>	<u>TILT</u>	<u>CHANGE*</u>	<u>%CHANGE</u>
M.D.	33	F	A	3.9	2.9	-1.0	-25.6
V.P.	24	F	N	1.9	1.2	-0.7	-36.8
				3.2	2.8	-0.4	-12.5
L.I.	22	F	O	2.4	0.7	-1.7	-70.8
				4.6	4.1	-0.5	-10.9
K.S.	20	F	N	2.7	2.5	-0.2	-7.4
D.W.	25	F	C	4.2	1.1	-3.1	-73.8
				3.2	2.6	-0.6	-18.8
L.J.	19	F	A	2.5	1.2	-1.3	-52.0
D.H.	22	F	N	2.0	2.3	+0.3	+15.0
L.A.	45	F	N	4.2	1.8	-2.4	-57.1
G.J.	19	M	N	4.1	3.2	-0.9	-22.0
				3.0	3.3	+0.3	+10.0
M.B.	48	F	C	2.9	2.4	-0.5	-17.2
				2.4	1.4	-1.0	-41.7
				5.2	3.5	-1.7	-32.7
N.D.	42	F	C	3.9	1.9	-2.0	-51.3
				2.7	1.6	-1.1	-40.7
R.A.	36	F	C	3.1	1.6	-1.5	-48.4
				2.9	2.2	-0.7	-24.1
L.M.	42	F	N	3.7	2.3	-1.4	-37.8
F.F.	30	F	N	2.0	1.7	-0.3	-15.0
T.W.	36	F	N	2.4	2.0	-0.4	-16.7

TABLE 5-1 (cont.)

<u>SUBJECT</u>	<u>AGE</u>	<u>SEX</u>	<u>RACE</u> ⁺	<u>PRE-TILT</u>	<u>TILT</u>	<u>CHANGE*</u>	<u>%CHANGE</u>
G.D.	36	M	C	3.2	3.3	+0.1	+3.1
G.F.	36	M	N	2.1	2.7	+0.6	+28.6
W.J.	45	M	C	3.7	3.7	0	0
C.N.	59	F	O	3.5	1.7	-1.8	-51.4
B.A.	25	F	C	2.6	2.3	-0.3	-11.5
MEAN				3.15	2.29	-0.86	-25.7
STANDARD DEVIATION				0.84	0.84	0.85	24.8
STANDARD ERROR				0.16	0.16	0.16	4.7
				P < 0.001		P < 0.001	P < 0.001

* (-) and (+) signs indicate decrease and increase in VV_{30} respectively.

+ A = American Indian ; N = Negro; O = Oriental; C = Caucasian.

Each value of VV_{30} is the average of 2 determinations.

TABLE 5-2

VENOUS DISTENSIBILITY (VV_{30}) IN UNTREATED HYPERTENSIVE PATIENTS

PATIENT	AGE	SEX	RACE ⁺	PRE-TILT	TILT	CHANGE*	%CHANGE	B.P. MEAN	HORIZONTAL DIASTOLIC (mm Hg)
L.R.	56	F	N	1.0	1.0	0	0	158	128
B.M.	57	F	N	1.9	1.0	-0.9	-47.4	142	119
A.S.	28	F	N	1.7	1.9	+0.2	+11.8	137	117
K.M. ^C F.	45	M	N	4.4	3.5	-0.9	-20.5	135	119
				2.4	2.1	-0.3	-12.5	133	117
D.B.	39	F	N	1.6	2.1	+0.5	+31.3	131	117
A.K.	39	F	N	1.8	1.9	+0.1	+5.6	126	109
				1.8	2.0	+0.2	+11.1	118	99
V.P.	62	F	N	1.5	2.0	+0.5	+33.3	124	97
I.F.	52	F	N	2.4	2.6	+0.2	+8.3	120	105
W.S.	44	M	N	3.3	2.9	-0.4	-12.1	117	99
				3.9	3.0	-0.9	-23.1	111	98
				2.6	1.7	-0.9	-34.6	125	108
A.H.	30	F	N	2.2	4.0	+1.8	+81.8	117	104
M.C.	63	F	N	1.8	2.9	+1.1	+61.1	118	87
E.D.	54	F	N	2.6	3.2	+0.6	+23.7	115	98
				3.3	2.7	-0.6	-18.2	119	98
W.E.	48	M	N	4.0	3.3	-0.7	-17.5	113	96
				1.8	2.1	+0.3	+16.7	123	119
I.C.	56	F	N	1.2	1.2	0	0	111	97
				1.2	0.8	-0.4	-33.3	110	91
S.P.	60	M	C	2.8	2.3	-0.5	-17.9	112	90

TABLE 5-2 (cont.)

PATIENT	AGE	SEX	RACE ⁺	PRE-TILT	TILT	CHANGE*	%CHANGE	B.P. MEAN	HORIZONTAL DIASTOLIC (mm Hg)
E.D.	42	M	N	1.5	1.5	0	0	103	90
				1.8	2.4	+0.6	+33.3	104	91
MEAN				2.27	2.25	-0.02	+3.37	122	104
STANDARD DEVIATION				0.91	0.82	0.67	29.8		
STANDARD ERROR				0.19	0.17	0.14	6.1		
				0.90 < P < 0.95			0.5 < P < 0.6		

+ N = Negro; C = Caucasian

* (-) and (+) signs indicate decrease and increase in VV_{30} respectively.

Each value of VV_{30} is the average of 2 determinations.

TABLE 5-3

EFFECT OF TREATMENT ON VENOUS DISTENSIBILITY (VV_{30})

IN HYPERTENSIVE PATIENTS

PATIENT	<u>HORIZONTAL</u>		CHANGE*	%CHANGE	<u>TILTED</u>		THERAPY (mg)
	VV_{30} Before Therapy	VV_{30} During Therapy			VV_{30} Before Therapy	VV_{30} During Therapy	
I.C.	1.2	2.1	+0.9	+75.0	1.0	2.3	G 12.5
D.B.	1.6	2.5	+0.9	+56.3	2.1	2.3	G 25
B.M.	1.9	2.5	+0.6	+31.6	1.0	2.6	G 37.5
L.R.	1.0	1.9	+0.9	+90.0	1.0	2.0	G 125
A.K.	1.8	2.5	+0.7	+38.9	1.9	2.2	R 0.5
A.S.	1.7	2.9	+1.2	+70.6	1.9	2.5	R 0.5
K.M ^C F.	3.4	4.1	+0.7	+20.6	2.8	4.1	M 500
W.E.	2.9	3.3	+0.4	+13.8	2.7	2.5	M 750
S.P.	2.8	1.9	-0.9	-32.1	2.3	2.2	
V.P.	1.5	3.5	+2.0	+133.3	2.0	1.8	
E.D.	1.7	2.1	+0.4	+23.5	2.0	2.8	
M.C.	1.8	1.9	+0.1	+ 5.6	2.9	2.2	
E.D.	3.0	1.9	-1.1	-36.7	2.9	1.9	
MEAN	2.02	2.55	+0.52	+37.7	2.04	2.42	
STANDARD DEVIATION	0.72	0.68	0.78	45.8	0.67	0.56	
STANDARD ERROR	0.20	0.19	0.22	12.7	0.19	0.16	
	0.05 < P < 0.10		0.02 < P < 0.05		0.01 < P < 0.02		0.1 < P < 0.2

TABLE 5-3 (cont.)

* (-) and (+) signs refer to decrease and increase in VV_{30} respectively.

Each value of VV_{30} is an average of all estimations made on each subject under the conditions specified above. In every case at least two and in several as many as 4 or 6 estimations were made, e.g. $K.M^C F$: above values represent average of 4 measurements untreated and 4 treated.

THERAPY: each patient received hydrochlorothiazide 150 mg and 45 mEq potassium daily; in addition some patients received guanethidine (G) reserpine (R) or methyldopa (M): daily doses (mg) are indicated.

DISCUSSION

Methods

Since Bayliss and Starling (1894) recognised neurogenic venoconstriction investigators have studied the role of the venous (capacity) system in the control of the circulation. Many ingenious methods have been used to study peripheral venous function in man:

(1) Construction of Δ Volume/ Δ Pressure curves (Glover et al , 1958; Wood and Eckstein, 1958); (2) Venous pressure changes in "isolated arms" where circulation is arrested proximally (Wallace, 1956; Samueloff, Bevegard and Shepherd, 1966); (3) Changes in pressure in "isolated vein" segments (Duggan, Love and Lyons, 1953; Burch and Murtadha, 1956); (4) Volume changes at constant collecting cuff pressure (Bevegard and Shepherd, 1965; Ardill, Bhatnagar and Fentem, 1968). Each has its particular advantages and disadvantages, but the "isotonic" volume/pressure measurements possibly provide more useful information than the "isometric" methods (Wood, 1965; Gilbert and Stevens, 1966; Oberg, 1967) since they give a measure of the ability of the system to contain blood rather than an indication of ability of the venous walls to raise pressure in a closed system, as is the case with the "isometric" methods. Our modification of the former approach was designed to provide a rapid index of the capacity of the veins suitable for a survey.

Possible errors due to stress relaxation and yield phenomena (i.e. distension due to the visco-elastic and plastic properties of veins) plus accumulation of capillary filtration fluid have been pointed out by Alexander (1963). He also showed that the initial state of venoconstriction or venodilatation has appreciably greater influence on the distensibility curve during filling than during emptying. Similar conclusions were reached by Brown, Greenfield, Goei and Plassaras (1966), who assumed that non-uniform venous filling was due to differences in flow in different tissues and interruption of hydrostatic continuity by the valves, and therefore recommended measuring distensibility on the emptying phase of the curve. Accordingly, our technique recorded the volume-pressure relationship on the emptying phase, following a prolonged venous congestion at 50 mm Hg. Because trauma from venepuncture may change venous compliance (Stevens, 1966), and since preliminary experiments had indicated that changes in cuff pressure produced equal changes in venous pressure provided a minimal pressure was exceeded, intravascular pressure measurements were avoided.

Expressing venous distensibility in terms of change in total forearm vascular capacity introduces several unavoidable errors. Firstly, changes in the capacity of the pre-capillary vessels will influence forearm volume. Wood (1965) and Oberg (1967), however, calculated that gross changes in the capacity of the pre-capillary vessels were likely to affect total vascular volume of the forearm by

less than 5%. Secondly, since a pressure gradient exists along the veins, not all of the capacity vessels are exposed to the same changes in distending pressure. Thus, while the pressure in the large veins will approach applied pressure (attenuated only by transmission through limb tissues and resistance of veins to collapse), the pressure in the immediate post-capillary vessels may be 20-25 mm Hg initially and therefore net change in distending pressure may be slight. If, however, as data of Weideman (1963) suggest, most of the venous capacity is approximately equally divided between the large veins, small veins and venules, and only a relatively small volume is to be found in the post-capillary vessels and capillaries, a congesting pressure of 30-40 mm Hg should distend most of the capacity vessels. Since the $\Delta \text{volume} / \Delta \text{pressure}$ relationship is relatively linear over the range 0-30 mm Hg, choosing a single point (VV_{30}) to express the relationship provides an index of venous capacity in the physiological range of venous transmural pressures. The electro-capacitance plethysmograph used has the advantage, unlike the water-filled plethysmograph, that it imposes no counter-pressure nor abnormal temperature effects on the forearm segment. However, similar responses were obtained with the water-filled plethysmograph and the two types of electro-capacitance plethysmograph.

Normotensive subjects

Almost identical values for horizontal control subjects were reported by Newberry and Bryan (1967) who recorded a mean VV_{30} of

3.2% in 9 healthy males with the mercury-in-rubber strain gauge (present study: mean VV_{30} of 3.15%). Similar values were also obtained by the earlier investigators who used a water-filled plethysmograph: Wood and Eckstein (1958) reported an average VV_{30} of 3.0 ml/100 ml from 21 experiments on 10 subjects, and Glover et al (1958) found an average venous capacity of 1.9% at 20 mm Hg and 3.6% at 40 mm Hg in 12 experiments on 10 subjects. If the Δ volume/ Δ pressure relationship were linear over the range, this would give a VV_{30} of 2.75%. Similar calculations from our preliminary experiments, using this latter method (Fig 5-7), yield an average venous capacity of 3.1% at 20 mm Hg and 4.2% at 40 mm Hg and hence a VV_{30} of 3.65%. This high value may be due to the effect of high environmental temperature since these values were recorded during the Australian summer.

Tilting from horizontal to the 60° feet down position depressed the mean VV_{30} by 27.3% in the 20 normotensive subjects. This apparent venoconstriction may be a compensatory response to gravitational pooling of the blood in the dependent lower limbs, a diminished venous return and consequent stimulation of low and even high pressure baroreceptors. An analogous response of the capacity vessels to reduced circulating blood volume, by haemorrhage or venous pooling, has been studied by many investigators. Increased venous tone in various vascular beds due to stimulation of the

carotid sinus baroreceptors is well documented in man and animals (Fleisch, 1931; Gollwitzer-Meier and Schulte, 1931; Landis and Hortenstine, 1950; Alexander, 1954; Alexander, 1955; Page, Hickam, Sieker, McIntosh and Pryor, 1955; Salzman, 1957; Heymans and Neil, 1958; Bartelstone, 1960; Ross, Frahm and Braunwald, 1961a; Sharpey-Schafer, 1961; Braunwald et al, 1963; Folkow and Mellander, 1964; Gilbert and Stevens, 1966; and Mason, Kopin and Braunwald, 1966). Similarly, evidence for the participation of the low pressure baroreceptors in the control of venous tone has been presented by many investigators (Alexander, 1956; Henry, Gauer and Sieker, 1956; Ross, Frahm and Braunwald, 1961b; Neil, 1962 and Braunwald et al, 1963). The analogous mechanism which causes an increased sympathetic vasoconstrictor discharge to resistance vessels in states of reduced circulating blood volume has been described by Brigden, Howarth and Sharpey-Schafer (1950).

A few investigators, however, have reported studies which suggest that the capacity vessels have no role in adjustments to diminution of effective circulating blood volume, e.g. as with tilting or negative pressure to lower half of body. Gauer and Thron (1962) and Samueloff, Browse and Shepherd (1966) found only transient increases in the tone of hand and forearm veins on tilting, using the "occluded limb" technique, and Plassaras, Brown, Goei and Greenfield (1963) found no change in distensibility of forearm veins, with the water-filled plethysmograph, when the lower part of the body

was exposed to subatmospheric pressures. Decreases in carotid sinus pressure had little effect on the venous pressure in the occluded hind limb of the dog (Browse, Donald and Shepherd, 1966), and Bevegard and Shepherd (1966) found no evidence of venodilatation when the carotid sinus was stimulated in the opposite direction to the above (i.e. increased transmural pressure) by subatmospheric pressures applied around the neck. These apparent discrepancies are probably due to differences in the methods used. The "occluded limb" technique measures the ability of the venous wall to generate tension rather than the ability of the system to contain blood and also, and perhaps more important, prevents access of circulating substances. Similarly, lower body negative pressure and orthostasis are not strictly comparable; Gilbert and Stevens (1966) found increases in forearm venous tone to be greater with orthostasis compared with lower body negative pressure. Absence of venodilatation when the carotid sinus was stimulated by subatmospheric pressure around the neck is not surprising since there is minimal resting venous tone in a warm environment (Abboud, Schmid and Eckstein, 1968) and thus the veins may have already been maximally dilated.

Hypertensive patients

The reduced venous distensibility in hypertensive patients suggests a state of increased venous tone, i.e. resistance to distension by the applied transmural pressure. This decrease in venous

distensibility tended to be exaggerated in patients with higher mean and diastolic pressures. The failure to show a further reduction in venous distensibility on tilting implies a maximal venous tone in these patients when horizontal. Wood (1961) reported a similar failure of hypertensive subjects to respond to venous pooling under conditions which, in a group of 18 normal subjects, reduced the VV_{30} from 3.1 to 2.5 ml/100 ml. However, in contrast to our findings, Wood found no difference in resting VV_{30} in the two groups. This, too, may be due to differences in method since Wood's subjects were tilted 30° feet down throughout the experiment, and the horizontal position was simulated by inflation of pneumatic leggings to prevent venous pooling, whereas tilting was simulated by inflation of thigh congesting cuffs to achieve venous pooling. In support of our findings Caliva, Napodano, Stafford, Loftus and Lyons (1963) found a decreased distensibility of the digital veins in a large series of hypertensive patients.

Examination of Tables 5-1 and 5-2 reveals considerable variation in some of the test/retest data in both the normal subjects and the untreated hypertensive patients. The reason for the variation is not immediately apparent but since the retest measurements were made at monthly intervals then environmental, as well as emotional, factors may have influenced resting tone of the forearm veins. When, however, VV_{30} measurements were repeated on the same day close correspondence between the successive values was found.

There is also considerable overlap in some of the VV_{30} values found in individual normotensive and hypertensive subjects, but the overall results show that the hypertensives tend to distribute about a different VV_{30} level compared with normotensives. If the difference had been more clearly defined then venous distensibility measurements may have been useful as a diagnostic procedure.

The mean age of the two groups differed considerably (normal subjects, 33.2 yr; hypertensive patients, 48.4 yr) but this is unlikely to influence the overall findings since no significant correlation was found between age and VV_{30} ($r = -0.19$, 52 pairs of observations, $P > 0.1$).

That the reduced distensibility in hypertensive patients is not due to an irreversible structural change in the veins is suggested by the observation that the veins tended towards normal distensibility when effective antihypertensive therapy was administered. It seems more likely that the reduced venous distensibility in the hypertensive group is due to an increase in venous tone resulting from increased sympathetic venomotor activity rather than from an organic change. However, increased circulating levels of a venoconstrictor substance such as a catecholamine cannot be excluded nor can increased sensitivity to normal circulating levels of the latter: Doyle, Fraser and Marshall (1959) reported increased sensitivity of forearm arterioles to intra-arterially administered

noradrenaline in 25 hypertensive patients.

Certain suggestions can be made regarding the possible significance of the above findings. The reduced venous distensibility in essential hypertensives, if generalised, may result in an overall reduction in venous capacity and hence in a redistribution of blood volume to other vascular beds: a condition which could aggravate the hypertensive state. There is some indirect evidence for this hypothesis since the finding of exaggerated natriuresis accompanying fluid overloading and increased responsiveness to reduction in plasma volume and extracellular fluid in essential hypertensives suggests a reduced venous capacity (Freis, 1960). Most investigations have shown that circulating blood volume is slightly but significantly decreased in both males and females in essential hypertension (reviewed by Dickinson, 1965) and thus the reduced venous capacity may be related to a reduced blood volume - but which is of prime importance is not known. The increased venous tone in hypertension may provide support for the hypothesis of a neurogenic pathogenesis of essential hypertension since Dickinson (1965) suggested that venous as well as arteriolar constriction might be expected if neurogenic activity were high in early essential hypertension. Folkow and Rubinstein (1966) were able to induce chronic hypertension in rats by long-term intermittent hypothalamic stimulation, and Henry, Meehan and Stevens (1967) produced hypertension in mice by repeated noxious stimuli.

Extrapolating to man, essential hypertension may represent an increased autonomic response to repeated noxious stimuli, a response which is eventually perpetuated by associated arteriolar changes (Folkow et al, 1958). The decreased venous distensibility of the hypertensive patients may thus be a result of a general increase in sympathetic nerve activity, another part of the response being the increased arteriolar resistance.

The restoration of distensibility towards that of the control group during guanethidine therapy supports the suggestion of Richardson and Wyso (1960) that venous pooling is an important factor in the hypotensive action of guanethidine. The latter investigators found no significant change in calculated total peripheral resistance with tilting in 30 patients receiving doses of guanethidine sufficient to produce a substantial fall in systolic blood pressure, and concluded that guanethidine's hypotensive action was not primarily due to modification of arteriolar resistance but resulted from venous pooling. Similarly, Mason and Braunwald (1964) reported that guanethidine, reserpine and methyldopa block reflex venoconstriction, as well as arteriolar constriction. Thus sympathetic blockade is probably a major factor causing the relative increase in venous distensibility during antihypertensive therapy and also may explain the absence of significant venoconstriction on tilting the treated hypertensives. Hickler, Wells, Tyler and Hamlin (1959) established that release of noradrenaline is required

for the normal compensatory vasoconstriction (arteriolar and venous) that occurs when the upright stance is assumed. Therefore interference with or depletion of this sympathetic post-ganglionic neurotransmitter substance is probably the basis of the increased venous distensibility recorded during therapy. In addition, part of the increase in venous distensibility accompanying therapy may be secondary to a change in electrolyte composition of the vessel walls due to hydrochlorothiazide, i.e. sodium depletion leading to decreased contractility: hydrochlorothiazide has been found to augment the hypotensive effect of guanethidine by Maronde et al (1961).

Thus in normal subjects it has been found that upright tilting results in a significant reduction in the capacity of the vascular bed of the forearm, probably due to increased sympathetic nerve activity. Hypertensives, however, exhibit a reduced venous capacity at rest and show no further reduction with tilting. Since sympathetic blocking drugs, guanethidine in particular, tend to reverse this state of increased venous tone, it is suggested that the capacity vessels share in the generalised increase in sympathetic nerve activity which is a feature in essential hypertension.

Addendum

Venous distensibility was studied in 2 additional patients. The first, a Negro woman, aged 25 yr who was five months pregnant, showed an increased distensibility: the paired VV_{30} determinations supine were 5.85 and 5.65. The second was a Caucasian male aged 33 yr suffering from orthostatic hypotension. His VV_{30} values of 4.74 and 5.10 supine did not decrease when he was tilted.

These results obtained from the two additional patients are in agreement with findings of other investigators. The increased venous distensibility found in the pregnant patient is similar to that reported by McCausland, Hyman, Winsor and Trotter (1961) and Goodrich and Wood (1964), and may well be due to elevated oestrogen levels. Deficient or absent venomotor responses have been found in postural hypotensives by Page et al (1955) and in familial dysautonomia by Mason et al (1966). The elevated VV_{30} values and absence of response to tilting in the patient who exhibited autonomic degeneration with postural hypotension agree with these findings. Treatment with a fluid retaining steroid (9 α fluoro-hydrocortisone) returned his VV_{30} to within normal limits.

SUMMARY

1. Venous distensibility (expressed as VV_{30} = percentage increase in volume at 30 mm Hg) of forearm vessels has been studied in 20 normal subjects and in 16 untreated essential hypertensives by an electro-capacitance plethysmographic technique and the effects of tilting and treatment investigated.
2. A reduced venous distensibility, compared with the normotensive group, was recorded in the hypertensive patients. In addition, hypertensive patients showed no change in venous distensibility during 60° feet down tilting whereas a 27% decrease occurred in normal subjects.
3. Treatment of hypertensive patients with hydrochlorothiazide plus guanethidine, reserpine or methyldopa resulted in an increase in venous distensibility above the pre-treatment values.
4. It is concluded that there is an increased forearm venous tone in essential hypertension. The significance of these findings in relation to the patho-physiology of essential hypertension is discussed and it is concluded that the capacity vessels participate in the generalised increase in sympathetic nerve activity which is a feature of early essential hypertension.

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SECTION 6

C A P I L L A R Y F I L T R A T I O N S T U D Y :

THE EFFECT OF IONTOPHORESIS OF ADRENALINE ON THE

RATE OF ACCUMULATION OF TISSUE FLUID IN THE

FOREARM.

INTRODUCTION

The principal investigations of this thesis have examined the role of various vasoactive substances in the control of blood flow through the resistance vessels, and the preceding section shifted emphasis to the capacity properties of the circulation and examined the influence of the autonomic nervous system on the forearm veins in normal and hypertensive patients. This, the final study, briefly looks at the capillary circulation, in particular at an aspect of water exchange.

It is generally assumed that the loose areolar part of the human skin and subcutaneous tissues is the principal site for accumulation of tissue fluid. Impressions derived from the sign of pitting oedema and, historically, from the use of Southey's tubes inserted into the subcutaneous tissue to drain excess fluid in gross ankle oedema, are the principal bases for this assumption. Further evidence is derived from the observation that intravenously administered saline increased the water and sodium chloride content of subcutaneous tissues of the cat, with little or no change in muscle (Skelton, 1927), and the more recent measurements by an ultra-sound echo technique which demonstrated increased thickness of the soft tissue overlying the medial aspect of the tibia in oedema (Fischer and Schneider, 1966). In contrast, accumulation of fluid in muscle is probably restricted by the tight investing fascia, since small

amounts of filtrate will rapidly raise tissue pressure and limit further filtration of fluid (Kjellmer, 1964a). This study was undertaken to examine the hypothesis that the superficial tissues are the major site for accumulation of extracellular fluid in the extremities in man. Pharmacological "skinning" of the forearm by iontophoresis of adrenaline permitted measurement of the rate of fluid accumulation in the underlying muscle and, knowing the rate of fluid accumulation in the untreated forearm, the contribution by the skin could be calculated.

METHODS

The subjects were six healthy volunteers (ages 20-34 yr) who lay supine on a couch in a temperature-controlled laboratory (22-24°C) for 20-30 min before observations began.

Blood flow and capillary filtration rate were measured on both forearms with electro-capacitance plethysmographs (Hyman, Burnap and Figar, 1963). The adaptation of the electro-capacitance plethysmograph for measurement of capillary filtration rate and capillary filtration coefficient has been described in General Methods.

Blood flow through the superficial tissues in a 10 cm forearm segment was minimised by adrenaline iontophoresis (1:2000 adrenaline hydrochloride at 20 mA for 20 min) following the method described by Cooper, Edholm and Mottram (1955). The adrenaline iontophoresis was judged to be successful if persistent blanching was observed and if values of forearm blood flow were reduced by approximately one third compared with pre-iontophoresis values. A wrist cuff was inflated to 200 mm Hg during all measurements of forearm blood flow and values for forearm blood flow through the iontophoresed limb were corrected for spontaneous variations by reference to corresponding measurements on the untreated control limb (Duff, 1952).

Capillary filtration rate was measured by recording the

volume increase of the limb segments during 8-12 min of venous congestion when proximal pneumatic cuffs were inflated to 40, 50 and 60 mm Hg in three consecutive recordings. Electrical outputs were recorded simultaneously from the plethysmographs on both the iontophoresed and control arms and plotted on the same record by use of an alternating switching circuit. The volume change during venous congestion showed two phases similar to those discussed by Pappenheimer and Soto-Rivera (1948): an initial rapid phase considered to be due to distension of capacity vessels and a slower but continuous phase which tended to be constant from the 3rd-12th min, the phase of outward filtration. The rate determined from the late phase of the volume record, usually from 5th-10th or 12th min, was considered a measure of capillary filtration. Measurements of capillary filtration rate were completed within one hour of adrenaline iontophoresis. Capillary filtration rate i.e. the volume increment per 100 ml of forearm tissue per min was plotted against venous congesting pressure and capillary filtration coefficient i.e. volume increment per 100 ml of forearm tissue per mm Hg, was read as the slope of the straight line relating capillary filtration rate to applied venous pressure (mm Hg).

RESULTS

A typical result from one of the six experiments is shown in Fig 6-1. The upper slope of the tracing represents the volume increase of the untreated forearm, the lower slope of the tracing the volume increase of the iontophoresed forearm at a venous congesting pressure of 40 mm Hg (Subject B.C.). Electrical calibrations are included. The slope of the record from the untreated arm is steeper and hence the rate of volume increase is greater than that obtained from the iontophoresed forearm. A similar pattern of response was found at each of the venous congesting pressures in each subject, and the magnitude of the volume increase was linearly related to the venous congesting pressure. Spontaneous vasomotor changes were minimal and caused no difficulty in assessing the slope of the volume increment against time. Figure 6-2 shows capillary filtration rate plotted against venous congesting pressure for one subject (P.P.). The capillary filtration rates of the iontophoresed arm are greatly reduced compared with those of the untreated side. Similar findings were made in each subject and are summarised in Table 6-1. The straight lines relating capillary filtration rate and venous congesting pressure for treated and untreated limbs both crossed the pressure axis at 20.7 ± 7.6 mm Hg and their slopes provide a measure of the capillary filtration coefficients. This intercept of approximately 20-25 mm Hg on the pressure axis probably indicates that the applied venous congesting pressure, and hence the hydrostatic

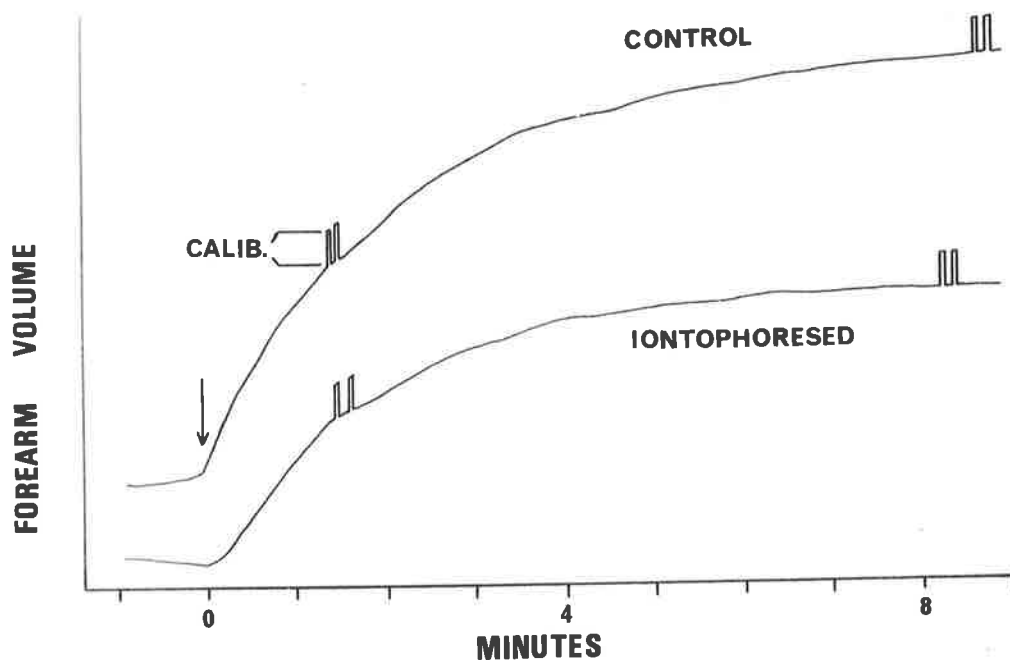


Fig 6-1. Tracing of a recording, using an alternating switching circuit, of volume in untreated (upper slope) and iontophoresed (lower slope) forearm during prolonged venous congestion at 40 mm Hg (subject B.C.). Calibrations (CALIB.) equal 1.46 ml.

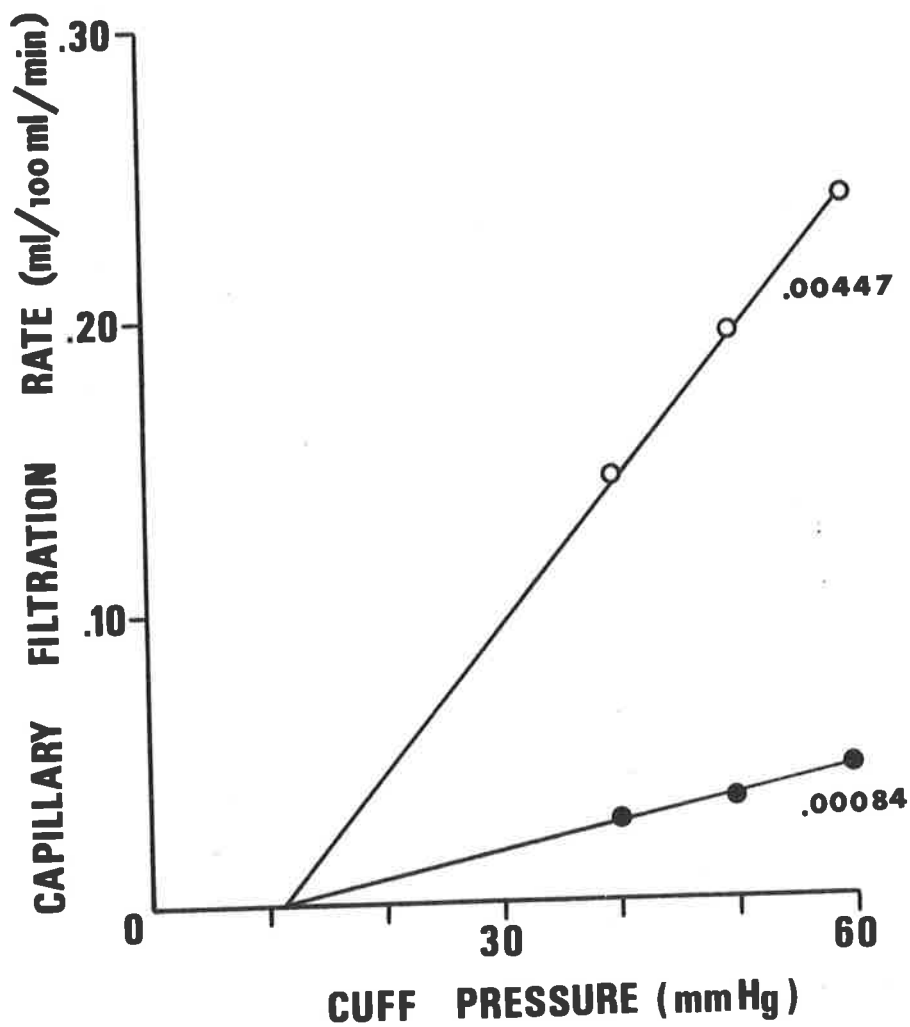


Fig 6-2. Relationship of capillary filtration rate to venous congesting pressure (cuff pressure) in untreated (○) and iontophoresed (●) forearm segments (subject P.P.). Respective capillary filtration coefficients (ml/100 ml/min/mm Hg) viz. 0.0047 and 0.00084 are indicated.

pressure in the post-capillary vessels, must exceed the colloid osmotic pressure for filtration into the tissues to occur. Figure 6-3 shows pooled data of the capillary filtration coefficients with the mean plus one standard deviation on either side of the mean. The mean capillary filtration coefficient fell from 0.00521 ml/100 ml/min/mm Hg in the untreated limbs to 0.00195 ml/100 ml/min/mm Hg in the limbs subjected to adrenaline iontophoresis - a fall of 62%.

The average forearm blood flow fell from 3.9 to 2.5 ml/100 ml/min (a 36% fall) and was still reduced by the same amount one hour after the completion of adrenaline iontophoresis. There was no correlation between blood flow and capillary filtration rate.

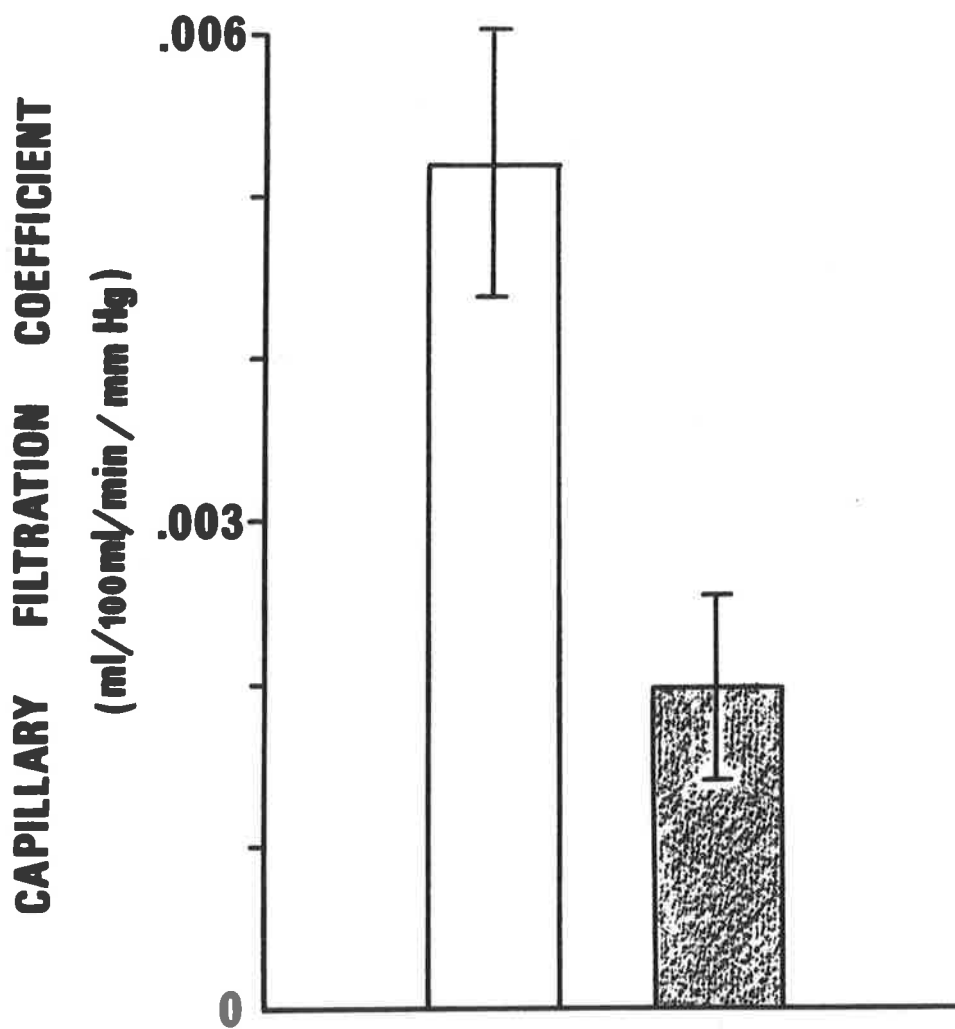


Fig 6-3. Mean $\pm$ standard deviation of capillary filtration coefficient on 6 subjects. Untreated (open bar) and iontophoresed forearm segments (hatched bar).

TABLE 6-1

CAPILLARY FILTRATION RATES (ml/100 ml/min) and
CAPILLARY FILTRATION COEFFICIENTS(C.F.C., units:
ml/100 ml/min/ mm Hg.)

<u>ADRENALINE IONTOPHORESIS</u>							<u>CONTROL</u>			
Venous Collecting Pressure (mm Hg)			40	50	60		40	50	60	
			C.F.C.				C.F.C.			
<u>Sub- ject</u>	<u>Age</u>	<u>Sex</u>								
P.P.	34	F	.0274	.0034	.0447	.00084	.149	.193	.239	.00447
D.L.	20	M	.0135	.1080	.0728	.00277	.089	.182	.126	.00440
W.M.	26	M	.0155	.0331	.0607	.00183	.059	.126	.171	.00593
C.D.	22	F	.0296	.0555	.0694	.00206	.118	.140	.197	.00457
M.J.	33	F	.0283	.0500	.1920	.00209	.094	.214	.282	.00663
B.C.	27	F.	.0437	.0601	.0897	.00210	.132	.162	.265	.00523
MEAN						.00195	.00521			
STANDARD DEVIATION						+.00057	+.00083			

P<< 0.001

TABLE 6-2

REPORTED VALUES FOR CAPILLARY FILTRATION COEFFICIENT

Refer- ence	Tissue Studied	Method of study	No. of Subjects	No. of Experi- ments	Period of con- gestion (min)	Capillary filtration coefficient (ml/100ml/ min/mm Hg)
Krogh, Landis & Turner (1932)	Human forearm	Pressure Plethys- mograph (55cm H ₂ O)	2	8	15-30	0.0031
				5		0.0048*
Landis & Gibbon (1933)	Human forearm	Pressure Plethys- mograph (200mmHg)	2	7	10	0.0045
				3	30	0.0038
Brown, Wise & Wheeler (1947)	Human forearm	Pressure Plethys- mograph (200mmHg)	4	4	10	0.0036
Kitchin (1963)	Human forearm	Pressure Plethys- mograph (200mmHg)	6	6	15	0.0036
Mellander & Oberg (1967)	Human Forearm	Water Plethys- mograph	12	28	5-6	0.0061
Hyman & Wong (1968)	Human forearm	Electro- capaci- tance plethys- mograph	11	11	10-15	0.0058
	Human calf		28	28	10-15	0.0048
Present study	Human forearm	Electro- capaci- tance plethys- mograph	6	6	8-12	0.0052

* on basis of unit change in colloid osmotic pressure.

DISCUSSION

It has been estimated that the skin and subcutaneous tissues comprise approximately 13-16% of the total forearm volume (Abramson and Ferris, 1940; Cooper et al , 1955). Under conditions of controlled oedema formation in this series of experiments the capillary filtration coefficient was diminished by 62% when a relatively small fraction of the total forearm volume was rendered ischaemic by the iontophoresis of adrenaline. It is unlikely that flow through the superficial vessels was completely stopped by the adrenaline ion transfer so that in the untreated arm at least 62% of the tissue fluid accumulated in a fraction of the skin and subcutaneous tissues. Studies of the clearance from superficial depot sites of I^{131} iodoantipyrene indicated penetration of the adrenaline to approximately 4 mm beneath the skin surface since clearance of the radio-isotope fell markedly after iontophoresis, indicating vasoconstriction and consequent interference with exchange function (La Joie, personal communication).

Previously, Hyman and Wong (1968) had suggested that the superficial tissues were the major site for the accumulation of extracellular fluid on the basis of some of their findings. A venous congesting pressure of 50 mm Hg applied proximal to the test segment of the limb with a counter-pressure of 15 mm Hg in a 10 cm wide cuff around the segment gave a capillary filtration rate corresponding to

that expected at a venous congesting pressure of 35 mm Hg. A counter-pressure of 5-20 mm Hg applied to a 7.5 cm segment of forearm has been shown to affect only the superficial tissues (Hyman, Greeson, Clem and Winsor, 1964). These considerations led to the suggestion that effective transmural pressure in the superficial tissues, 35 mm Hg, rather than that in the deeper tissues, 50 mm Hg, accounted for the overall capillary filtration rate for the whole segment.

It was assumed that the venous pressure applied proximal to the plethysmograph is reflected directly as an unattenuated increase in pressure in the filtration area. Kitchin (1955) and Kjellmer (1964b) have taken an arbitrary value of 80% of the applied venous pressure to calculate capillary filtration rate based on the early suggestion of Bayliss and Starling (1894) that the ratio of pre-capillary to post-capillary resistance determines the fraction of the increment in venous pressure which reaches the capillary level. This value makes some questionable assumptions and the situation would be expected to change with e.g. exercise, drugs, or diversion phenomena. Either argument recognises that, under steady conditions, the pressure in the capillaries must exceed the pressure in the veins and therefore the pressure in the venous occlusion cuff.

The mean of the capillary filtration coefficients of the control forearm segments, viz. 0.0052 ml/100 ml/min/ mm Hg, agrees

with the values Hyman and Wong (1968) reported for the mean of estimations on forearm segments of 11 subjects (0.0058) and for the mean of similar determinations made on calf segments of 28 subjects (0.0048). Similarly, Landis and Gibbon (1933) using the pressure plethysmograph, found a mean capillary filtration coefficient of 0.0045 and Mellander and Oberg (1967) a value of 0.0061 (assuming 80% transmission of increment in venous pressure to capillaries) with the water-filled plethysmograph using a technique similar to that described in this series. Somewhat lower values have been reported by Krogh, Landis and Turner (1932), Brown, Wise and Wheeler (1947) and Kitchin (1963). Table 6-2 summarises this data on reported capillary filtration coefficients. The differences are probably due to environmental factors or differences in the methods used.

It is concluded that the skin and subcutaneous tissues, i.e. the tissues whose vessels are accessible to vasoconstriction by adrenaline administered by ion transfer, are the major site for accumulation of extracellular fluid in the forearm in man.

SUMMARY

1. Capillary filtration rate and capillary filtration coefficient have been measured by an electro-capacitance plethysmographic technique in the forearm of man in control limbs and in limbs subjected to iontophoresis of adrenaline to minimise blood flow through superficial tissues.
2. A significant reduction (62%) in the rate of tissue fluid accumulation occurred in the iontophoresed limbs. This finding is discussed in relation to the fraction of total forearm volume occupied by skin and subcutaneous tissues.
3. It is concluded that the superficial tissues are the major site for accumulation of tissue fluid in the forearm in man.

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GENERAL SUMMARY

A survey of historical aspects of the autonomic nervous system, catecholamines, serotonin and angiotensin was presented in the General Introduction (Section 1). A review of previous observations on the peripheral vascular effects of the naturally occurring hormones serotonin and angiotensin in man revealed that understanding of their roles in the control of the circulation through the resistance vessels and the mechanisms of their actions were clouded and incomplete. Thus the principal investigations of this thesis (Section 2) have examined the role of serotonin and angiotensin in the control of the peripheral circulation.

Plethysmographic techniques have constituted the principal method of study in all sections of the thesis, investigations being confined to the upper limbs in man. The conventional water-filled plethysmograph was used to assess hand blood flow, but more recent electro-capacitance plethysmographic techniques were generally preferred when forearm blood flow (and also venous distensibility and capillary filtration rate) were measured. Local effects of the various vasoactive agents were assessed by intra-arterial infusion into the brachial artery and general effects by intravenous administration.

In the first part of Section 2, the effects of serotonin on the limb vessels were studied by direct (intra-arterial) infusion. Use of the lysergic acid derivative methysergide, which was found to

be a specific antagonist of vasoconstrictor effects of serotonin on the vessels of the forearm and hand, revealed that the effects of serotonin were a resultant of vasoconstriction in skin and vasodilatation in muscle, but that there was also a vasoconstrictor effect on muscle vessels which became more manifest as the dose of serotonin was increased. The peripheral effects of serotonin were independent of the adrenoreceptors, acetylcholine and histamine receptors and of sympathetic innervation, since the pattern of response was not modified by phenoxybenzamine, propranolol, hyoscine, mepyramine or by sympathetic blockade. It was concluded that serotonin exerts its peripheral vasoconstrictor effects by acting on specific serotonin receptors in vascular smooth muscle.

The serotonin study was extended to an examination of its role, and also the role of bradykinin, in the carcinoid syndrome. Systemic administration of serotonin reproduced gastrointestinal symptoms and these were abolished by methysergide. On the other hand, the facial flushes, the second major feature of the syndrome, were associated with elevated levels of arterial blood bradykinin and the facial flushing was mimicked by intravenous infusions of bradykinin. These findings are in agreement with the suggestion of other investigators that the flushing attacks are due to elevated circulating levels of bradykinin and that the gastrointestinal symptoms are due to serotonin overproduction.

Next, a comparison was made of the cardiovascular effects of angiotensin and noradrenaline. It was found that the local action of angiotensin on the hand vessels was only one third to one half as potent as that of noradrenaline; whereas, on intravenous infusion angiotensin's constrictor potency was greatly enhanced and it became eight to ten times more active than noradrenaline. Angiotensin was fifteen times as effective as noradrenaline in raising the mean arterial blood pressure. Thus it seemed likely that one or more indirect vasoconstrictor mechanisms were involved in the systemic action of angiotensin. Angiotensin was subsequently shown to have a central stimulating action on the sympathetic vasomotor system in man, and, in the case of the hands, this was the sole cause of the vasoconstriction. However, the direct action of angiotensin, which is presumably on specific receptors, makes the most important contribution to its overall vasoconstrictor action. In contrast to findings in animal studies, there was no evidence of increase in the circulating levels of catecholamines or of a peripheral sympathetic stimulating action in man.

Finally, in Section 2, interactions between various combinations of angiotensin, serotonin and noradrenaline were investigated, since animal studies have suggested that the vasoconstrictor effects of some circulating vasoactive substances may be enhanced

by interaction with other circulating agents. When combinations of angiotensin and noradrenaline, and of angiotensin and serotonin, were administered to the hand vessels by intra-arterial infusion the constrictor response was greater than could be accounted for by summation of effects alone. No such potentiation was found with the same combinations in the forearm where only the noradrenaline-serotonin combinations showed potentiation when both drugs were infused intra-arterially. When the background level of serotonin was raised by intravenous administration, however, the vasoconstrictor effects of both noradrenaline and angiotensin on the forearm were significantly enhanced. These findings suggest that such interactions may play a role in increasing the vasoconstrictor effects of these substances, but the degree of potentiation was often small and thus such interactions are unlikely to make a major contribution to the overall vasoconstrictor effects.

In Section 3, the role of various vasoactive substances suggested as playing a part in the phenomenon of post-exercise (or functional) hyperaemia of skeletal muscle was reviewed briefly. This prompted the examination of venous effluent from exercising forearm muscles for the presence of active substances since most investigators have suggested that local chemical factors are probably involved in the phenomenon, and it is possible that they may be removed in the blood. Assay of venous blood on the opposite forearm showed no vasoactivity, but assay on the superfused

guinea pig ileum revealed the presence of an active substance, probably acetylcholine, in the venous effluent from exercising muscle. This led to examination of the role of acetylcholine in the hyperaemic response by determining the effects of intra-arterial infusions of acetylcholine-blocking and potentiating drugs on post-exercise hyperaemia. Neither atropine nor hyoscine altered the response but neostigmine potentiated the hyperaemia suggesting that acetylcholine, liberated from the motor nerve endings, caused a vasodilatation which was additive to the post-exercise hyperaemia. The study was extended to examination of sheep lymph (collected from lumbar lymphatics) for active substances, and assay of exercise lymph on the guinea pig ileum showed increased amounts of acetylcholine in two sheep and of histamine in one sheep. Thus both the venous blood and the lymph may remove some of the acetylcholine liberated during motor nerve activity, but it was concluded that acetylcholine is unlikely to play an important role in the phenomenon of post-exercise hyperaemia of skeletal muscle.

In Section 4, the final section concerned with the resistance vessels, the effect on the peripheral vessels of local and systemic administration of ethyl alcohol was investigated. The direct (intra-arterial) actions of alcohol on the skin and muscle vessels of the upper limb in man were found to be entirely constrictor. On the other hand, systemic (oral) administration revealed an indirect, sympathetically-mediated vasodilator action

on skin vessels. The skin vasodilatation was absent in sympathectomised and denervated limbs. This effect of alcohol of inducing a central sympathetically-mediated vasodilator effect on skin is analogous to the stimulating effect of angiotensin on the vasomotor centres which in this latter case induced vasoconstriction in hand skin. These two examples illustrate particularly well how the direct, local actions of a vasoactive substance may be modified when the substance is administered systemically so that autonomic nervous system influences are superimposed on the direct effects. This comparison of the indirect effects of alcohol and angiotensin also illustrates the considerable refinement of the responses of the vasomotor centres in that specific dilator and constrictor effects respectively on skin blood vessels have resulted from administration of these two substances.

In Section 5, emphasis shifted to the part that the capacity vessels play in the circulation. Forearm venous distensibility was measured in normotensive subjects and in patients with essential hypertension in both the horizontal and 60⁰ feet-down positions to determine the capacity of the venous vessels and their response to increased sympathetic nerve activity in the two groups. The hypertensive patients exhibited a reduced venous distensibility compared with the normal subjects, and, furthermore, showed a minimal change on being tilted, whereas distensibility decreased 27% in the

normal subjects. Treatment of the hypertensives with various antihypertensive drugs increased venous distensibility above pre-treatment values. These findings suggest that the capacity vessels share in the generalised increase in sympathetic nerve activity which is a feature of essential hypertension.

The effect of iontophoresis of adrenaline on the rate of accumulation on tissue fluid in the forearm was studied in Section 6 (capillary filtration study) to examine the proposition that the loose areolar part of the human skin and subcutaneous tissues is the principal site for accumulation of tissue fluid. Controlled oedema formation was achieved by prolonged venous congestion at various applied pressures, and pharmacological "skinning" of the forearm by adrenaline ion transfer showed that the skin was in fact the major site for accumulation of tissue fluid.

The most striking feature to emerge from the studies outlined in this thesis was the great variation in the responses of the peripheral circulation to the various vasoactive substances, for, in the final analysis, the smooth muscle cells of the vessels have only one response mechanism i.e. variation in tone by contraction or relaxation. The complex, but usually very specific local actions of the vasoactive substances on their specific receptors are subject to autonomic nervous system influences when administered systemically. The angiotensin and alcohol studies in particular

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demonstrated that the response of the peripheral vessels varied markedly with local and systemic administration. It is concluded that the central and local control of the peripheral vessels is influenced in a highly specific manner by different vasoactive substances and by different stimuli.

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