VASOACTIVE HORMONES IN STRESS.

A THESIS

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RODNEY CHARLES HALL, M.Sc., M.B.,Ch.B.

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PREFACE

"Science moves but slowly slowly, creeping on from point to point."
"Sonnet to Science", Edgar Allan Poe.

Extensive studies have been carried out on a wide range of species to examine the possible mechanisms for maintaining circulatory homeostasis in response to stresses such as haemorrhage, hypoxia and endotoxins. These studies have examined neural and humoral responses to stress and in particular the sympatho-adrenal and renin-angiotensin systems. However, few studies have measured both catecholamines and angiotensin levels simultaneously.

The aim of the present study was to examine the inter-relationship of the renin-angiotensin and sympatho-adrenal systems in similar stress situations, both within and between species, by concurrent estimations of both angiotensin and catecholamines. The stresses applied were endotoxin shock and haemorrhage to cats and dogs, hypoxia to dogs, and tilting to man. In cats and dogs, changes in arterial levels of angiotensin and catecholamine levels were determined simultaneously using a superfusion technique of Vane so that the timing of the changes in the circulating levels of each hormone could be estimated. Further preliminary studies on cats, dogs and sheep examined the possible mechanism of action of endotoxin and the role of aspirin in inhibiting the cardiovascular effects of endotoxin.

DECLARATION AND ACKNOWLEDGEMENTS

I declare that this thesis is of my own composition and that it is a record of original work conducted in the Department of Human Physiology and Pharmacology, at the University of Adelaide, during the years 1968 to 1972.

The experiments described herein have not been submitted for any other degree or diploma.

I am indebted to Dr R. L. Hodge for his supervision, stimulation and patient understanding throughout the period of this study. I also wish to thank Dr E. R. Lumbers for her interest and many hours of provocative discussion. I am also grateful to Mr R. Irvine, Miss C. Karausch and Miss E. Pavy for technical assistance. I also wish to thank those colleagues who volunteered for those experiments described.

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CONTENTS

HISTORICAL INTRODUCTION

(A) GENERAL

The experimental observations on the nature of the circulation made by William Harvey (1628) established one way in which the various systems of the body were closely interrelated and could interact. Claude Bernard (1878) introduced the concept of a "milieu intérieur" and so extended the concept of circulation within blood vessels to include a free exchange of fluids throughout the body. He went further and said (1878): "All the vital mechanisms, however varied they may be, have only one object, that of preserving the condition of life in the internal environment." This statement remains true nearly 100 years later and much investigation has been directed towards determining the mechanisms responsible for maintaining this "milieu intérieur".

Following a series of experiments using testicular extracts, Brown-Séquard (1889) proposed that internal secretions from specific organs may be released into the circulation to have generalised effects. The observations of Brown-Sequard set the scene for further fundamental experiments. Oliver and Schäfer (1894, 1895) clearly demonstrated the effects of extracts of the adrenal gland on the cardiovascular system of the cat. Four years later, in Sweden, Tigerstedt and Bergman (1898) published their observations in which extracts of rabbit kidney injected into the circulation of a normal rabbit caused a prolonged rise in arterial pressure. They called this pressor extract renin.

In 1905 Starling coined the term hormone to describe chemical messengers which are released into the circulation from specific glands in small quantities to have profound effects on the "milieu" of other systems and organs. Cannon introduced and developed the concept of homeostasis to describe those interactions of the circulation, the nervous system, hormones, and diet which maintain a constant "milieu intérieur" (Cannon, 1926a, 1926b; 1929).

(B) RENIN-ANGIOTENSIN SYSTEM

Renin.

The work of Tigerstedt and Bergman (1898), showing that kidney extracts could elevate arterial pressure, followed Bright's description of the combination of hypertension, cardiac hypertrophy and renal disease in man (1836). Much of the subsequent experimental work was devoted to ascertaining the role of the kidney in the aetiology of hypertension rather than to determining the physiological role of renin in cardiovascular homeostasis. The classic experiments of Goldblatt, in which partial occlusion of the remaining renal artery in a uninephrectomised animal was associated with sustained and severe hypertension, heightened the search for a pathological role of renin (Goldblatt, Lynch, Hanzal & Summerville, 1934). Similar results were obtained in the monkey (Goldblatt, 1937). Goldblatt's observations were confirmed by others in dogs (Collins, 1936; Fasciolo, Houssay & Taquini, 1938; Verney & Vogt, 1938), and in rabbits (Pickering & Prinzmetal, 1938a).

The classic experiments of Tigerstedt and Bergman (1898) showed that renin was thermo-labile, non-dialysable, and soluble in water but insoluble in alcohol. Furthermore, renin was present only in the cortex of the kidney. The phenomenon of tachyphylaxis to renin was also demonstrated. These workers concluded that, since renin was present in normal kidneys, it was probably released into the blood flowing through the kidney. In 70 years of further research the findings and conclusions of Tigerstedt and Bergman on the physico-chemical properties of renin still stand.

The establishment of a simple reliable method for producing experimental hypertension led to a period of intensive study on the properties of renin. Two groups of workers established simultaneously that renin was an enzyme with a substrate referred to as renin activator (Page & Helmer, 1940) and as hypertensinogen (Braun-Menéndez, Fasciolo, Leloir & Muñoz, 1940). The pressor end-product of the renin-substrate reaction was named angiotonin (Page & Helmer,

1940) and hypertensin (Muñoz, Braun-Menéndez, Fasciolo and Leloir, 1939).

The present nomenclature, angiotensin, was agreed on in 1958 (Braun-Menéndez & Page, 1958).

Renin is produced in the juxtaglomerular apparatus of the kidney, but the precise site of synthesis and storage is not established beyond doubt. Using histological techniques, Goormagtigh (1945) suggested that renin may be produced in the juxtaglomerular cells and that the granules were the storage reserves of renin. Using micro-dissection techniques, Cook & Pickering (1962) showed that the granulation associated with renin was limited to the vascular pole of the juxtaglomerular apparatus. Similar results were obtained using fluoroscein-labelled antirenin antibodies (Hartroft, 1963). Evidence in favour of the macula densa as the site of renin storage was provided by Bing & Kazimierczak (1962). Whether nerves play any direct role in the release of renin is not yet clear, but a nerve supply to the juxtaglomerular apparatus has been demonstrated in cats, rats, monkeys and mice with some species differences in the density of innervation (Harman & Davies, 1948; Barajas, 1964).

Renin substrate.

Page, McSwain, Knapp & Andrus (1941) and Leloir, Muñoz, Taquini, Braun-Menéndez & Fasciolo (1942) showed that renin substrate was formed in the liver. Evidence that substrate was an α_2 globulin was provided by Plentl, Page & Davis (1943) and by Cohn, Oncley, Strong, Hughes & Armstrong (1944). Finally, in a detailed study of the chemical nature of substrate, the tetra-deca-peptide structure was established and synthesis was achieved (Skeggs, Lentz, Kahn & Shumway, 1958).

Angiotensin.

The existence of two forms of angiotensin, I and II, was first demonstrated by Skeggs and co-workers, who showed that incubation of hog renin with chloride-free plasma produced angiotensin I and that incubation of angiotensin I with plasma containing chloride ions produced angiotensin II (Skeggs, Marsh, Kahn & Shumway, 1954a;

Skeggs, Kahn & Shumway, 1956a). Angiotensin I was purified and a non-apeptide sequence suggested (Skeggs, Marsh, Kahn & Shumway, 1954b). The amino-acid sequence of angiotensin I was later determined and it was shown to be a decapeptide (Lentz, Skeggs, Woods, Kahn & Shumway, 1956). These workers also purified angiotensin II, established that it was an octapeptide and determined the amino-acid sequence (Lentz *et al.*, 1956; Skeggs, Kahn & Shumway, 1956b; Skeggs, Lentz, Kahn, Shumway & Woods, 1956). These observations were subsequently confirmed by others (Peart, 1956; Elliott & Peart, 1957; Bumpus, Schwarz & Page, 1957).

Synthesis of isoleucyl^5 angiotensin II was carried out by Bumpus, Schwarz & Page in 1957. Synthesis of the β asp-Val^5 angiotensin II amide, now commonly used in experiments, was achieved by Schwyzer, Iselin, Kappeler, Riniker, Rittel & Zuber in 1958.

Converting enzyme.

It was shown that the conversion of angiotensin I to angiotensin II was due to a chloride-dependent enzyme in plasma, named converting enzyme (Skeggs *et al.*, 1954a). The physiological importance of converting enzyme was established by perfusing isolated rat kidneys and showing that angiotensin II was a potent vasoconstrictor, while angiotensin I had little pressor activity (Skeggs *et al.*, 1956a). It was concluded that the conversion of angiotensin I to II occurs rapidly in plasma, thus accounting for the pressor activity of angiotensin II. However, recently it has been shown that rapid *in vivo* conversion of angiotensin I to II occurs in the lungs which also contain converting enzyme (Ng & Vane, 1967; Biron & Huggins, 1968; Oparil, Tregear, Koerner, Barnes & Haber, 1971).

Angiotensinases.

Several enzymes capable of inactivating angiotensin II, including trypsin, chymotrypsin, leucine amino-peptidase and carboxypeptidase, are present in plasma (Elliott & Peart, 1957; Regoli, Riniker & Brunner, 1963). The presence of angiotensinase activity has been established in red blood cells (Khairallah, Bumpus, Page & Smeby, 1963) and in a variety of other tissues (Regoli *et al.*, 1963).

However, despite the numerous angiotensinases present in blood, recent studies have shown that tissue angiotensinases remove approximately 50-75% of angiotensin I and II in one passage through a systemic vascular bed, while the half-life of angiotensin II in blood is approximately 120 sec (Hodge, Ng & Vane, 1967; Ng & Vane, 1968; Biron, Meyer & Panisett, 1968).

Assay of components of renin-angiotensin system.

The various components of the renin-angiotensin system are shown in Fig. 1. The assay of each component of the renin-angiotensin system has been fraught with problems related partly to the poor reliability and reproducibility of bioassay techniques and partly to the fact that a method for purification of renin is not yet available. Bioassay techniques have until recently provided the only means of determining circulating levels of each component. Early workers assayed renin by injecting plasma into intact rabbits (Pickering & Prinzmetal, 1938b) or dogs nephrectomised to increase their sensitivity (Wakerlin & Chobot, 1939a). Such techniques are relatively non-specific and there is considerable variation in the response of different animals. Other techniques depend on the fact that renin is an enzyme so that the principles of enzyme kinetics can be used. Thus the amount of the end product, angiotensin I, formed will depend on the concentration of substrate and enzyme. If the substrate concentration is constant or higher than the rate limiting concentration, then the amount of angiotensin generated will depend on the amount of renin present. In such kinetic studies, angiotensinase activity must be inhibited by adding chelating agents, or by low pH treatment, in order to prevent destruction of the end-product.

The earlier techniques of assay have been discussed by Braun-Menéndez, Fasciolo, Leloir, Muñoz & Taquini (1946). More recently, other techniques have been described which use the pressor response of the ganglion-blocked rat as a means of determining the amount of angiotensin formed (Peart, 1955; Boucher, Veyrat, de Champlain & Genest, 1964; Skinner, 1967). The disadvantage of these methods of renin assay is that the true nature of renin has not been established.

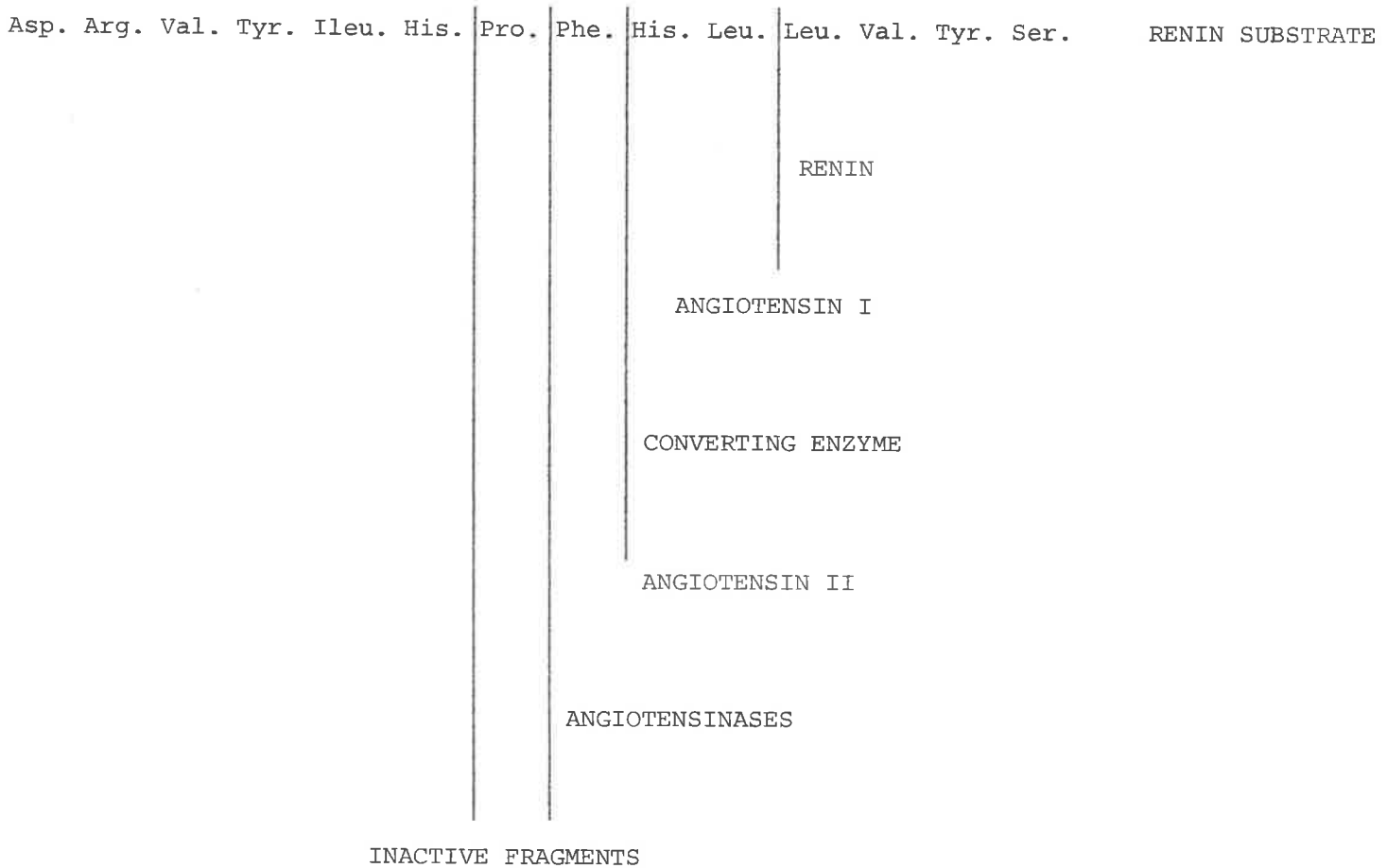


Fig. 1. Amino-acid sequence of the components of the renin-angiotensin system.

Thus the recent suggestion that renin may exist in both active and inactive forms (Lumbers, 1971) makes difficult the interpretation of results of assay methods which use pH treatment of renin below 4.0 to destroy substrate (Skinner, 1967). Furthermore, the renin-renin-substrate reaction produces angiotensin I which gives a pressor response in the rat only after conversion in the lungs to angiotensin II, a factor which contributes to variability of up to 20% (Lumbers, 1969). More recently, a radioimmunoassay of angiotensin I has been developed for estimation of plasma renin activity (Boyd, Adamson, Fitz & Peart, 1969). This technique has distinct advantages over bioassay, since the end-product of the renin-renin-substrate reaction, angiotensin I, is measured directly.

The levels of angiotensin II in plasma are normally too small to be determined by direct bioassay techniques. Techniques for extraction of angiotensin from blood followed by a concentration step and subsequent bioassay on rats have been developed (Skeggs, Kahn & Shumway, 1952; Scornik & Paladini, 1964). Regoli & Vane (1964) described a sensitive method for continuously assaying angiotensin in arterial blood. This technique uses the rat colon superfused with blood as an assay tissue. In blood, a contraction of the rat colon is almost specific for angiotensin. The technique offers the advantage of requiring only small amounts of blood, as well as giving a continuous assessment of circulating levels of angiotensin; however, it can only determine changes in concentration from an unknown basal level.

Radioimmunoassay of angiotensin II was developed simultaneously by two groups (Catt, Cain & Coghlan, 1967; Boyd, Landon & Peart, 1967). This technique offers advantages over any previous techniques, since relatively small amounts of blood are required and the assay is relatively specific for the physiologically active end-product. However, hexapeptide and heptapeptide fragments of angiotensin II may also be immuno-reactive (Cain, Catt & Coghlan, 1969).

Action of angiotensin.

Renin itself has no pressor activity (Friedman, Abramson & Marx,

1938; Braun-Menéndez *et al.*, 1940; Kohlstaedt, Page & Helmer, 1940), and thus the pressor response to renin is dependent on the generation of angiotensin I and on its conversion to angiotensin II. Angiotensin, weight for weight, is the most potent vasoconstrictor agent known. Its constrictor action on isolated vessels (Friedman *et al.*, 1938; Kohlstaedt *et al.*, 1940; Braun-Menéndez *et al.*, 1940) and its pressor action in the ganglion-blocked rat (Peart, 1955) established beyond doubt that a major part of its pressor activity may be due to its direct action on blood vessels. The role of the central nervous system in the pressor response to angiotensin in intact animals is not, as yet, clearly resolved (Ferrario, Guildenburg & McCubbin, 1972). Tigerstedt & Bergman (1898) claimed that the central nervous system had no part to play in the pressor response. These workers thought that much of the action may be related to the peripheral nerves. Sympathectomy and section of anterior spinal nerves did not affect the development of renal hypertension in dogs (Page, 1935; Goldblatt & Wartman, 1937; Goldblatt, Gross & Hanzal, 1937; Freeman & Page, 1940). However, Page (1939; 1943) suggested that lesions in the region of the medulla or mid-brain of dogs were associated with a decrease in the pressor response to renin. von Euler & Sjostrand (1941) showed that lesions in the mid-brain or medulla of cats and rabbits were associated with a decrease in the pressor response to renin. Experiments with pithed animals (Collins & Hamilton, 1940; Dock, 1940) also suggest that part of the pressor response to renin is indirect. More recently, a central site of action of angiotensin has been demonstrated in dogs (Bickerton & Buckley, 1961; Scroop & Lowe, 1968), in cats and dogs (Severs, Daniels & Buckley, 1967), cats (Davis, Dutta, Booker & Pradhan, 1969), rabbits (Yu & Dickinson, 1965), and in man (Scroop & Whelan, 1966). Some evidence suggests that part of the central action of angiotensin in dogs is mediated via the area postrema where angiotensin causes an inhibition of vagal tone on the heart and a subsequent tachycardia (Scroop & Lowe, 1969). Thus there is good evidence that the pressor response to angiotensin may be a combination of a direct action on

blood vessels as well as an indirect effect mediated via the mid-brain. Furthermore, Feldberg & Lewis (1964) showed that angiotensin in large doses could act directly on the adrenal medulla to cause a release of adrenaline, which may also contribute to the pressor response of angiotensin or renin.

Apart from its effects on the cardiovascular system, angiotensin affects salt and water balance by stimulating the release of aldosterone from the adrenal cortex of several species including man, sheep and dogs (Gross, Brunner & Ziegler, 1965), and of anti-diuretic hormone from the posterior pituitary of dogs (Mouw, Bonjour, Malvin & Vander, 1971). The release of aldosterone stimulates the reabsorption of sodium from the distal tubule of the kidney and, as a consequence, the reabsorption of water (Turner & Bagnara, 1971). Specially significant is the fact that these effects of angiotensin on both the adrenal cortex and the posterior pituitary occur with sub-pressor or minimally pressor doses of angiotensin.

Role of the renal sympathetic nerves.

The renal arteries have a rich plexus of sympathetic nerves which pass to the afferent and efferent arterioles. Some fibres also pass to the juxtaglomerular cells (Harman & Davies, 1948; Barajas, 1964). However, the precise role of the sympathetic nerves in controlling renin release is not certain. It has been clearly established that the renal nerves are not necessary for the development of hypertension of the Goldblatt type (Page, 1935; Collins, 1936; Fasciolo, Houssay & Taquini, 1938; Kohlstaedt & Page, 1940; Verney & Vogt, 1938). However, chronic stimulation of the renal nerves causes a rise in arterial pressure in dogs (Kottke, Kubiceck & Visscher, 1945). Furthermore, the release of renin or the rise in angiotensin levels during bilateral carotid occlusion can be inhibited by local anaesthetics injected around the renal plexus (Bunag, Page & McCubbin, 1966; Hodge, Lowe & Vane, 1966a). Experiments on conscious dogs (Brubacher & Vander, 1968) showed that renal denervation delayed the rise in renin levels following commencement of a low salt diet, but did not affect the final level reached.

These observations suggest that the physiological role of the renal nerves may be one of modulation rather than to cause release by direct stimulation. However, evidence to the contrary has been provided by recent experiments, using a non-filtering kidney, in which papaverine inhibited the release of renin by adrenaline but had no effect on the release of renin by noradrenaline or renal nerve stimulation (Johnson, Davis & Witty, 1971).

Homeostasis and the renin-angiotensin system.

The demonstration that the kidney produced renin which could cause a rise in arterial pressure implied not only that renin might be an aetiological factor in hypertension, but also that it might play an important role in the maintenance of a normal arterial pressure.

Wakerlin & Chobot (1939b) found no conclusive evidence for a homeostatic role of renin in dogs following haemorrhage or splanchnic nerve stimulation, but the renin content of the kidneys was estimated. The more critical study would have been the estimation of changes in renal vein renin levels. A renin-like substance was demonstrated in the blood of dogs haemorrhaged 24 hours previously (Sapirstein, Ogden & Southard, 1941). However, the assay method used was the guinea pig ileum, and so these workers were probably estimating several substances, including angiotensin. In a more detailed examination of a homeostatic role of renin, Hamilton & Collins (1942) observed a rise in the pressor activity of arterial and renal vein blood, after removal of 35-40 ml/kg blood, as assayed by injection of blood samples into control dogs. The pressor activity of renal vein blood was usually very much greater than that of arterial blood and was neither affected by adrenalectomy, nor, in one instance, by renal denervation. Furthermore, adrenalectomised, nephrectomised dogs showed no increase in pressor activity in arterial blood, and dogs with the renal circulation excluded had an impaired ability to maintain arterial pressure following haemorrhage. Other workers have shown a rise of renin levels in dogs, rats and man following haemorrhage (Huidobro & Braun-Menéndez, 1942; Dexter, Frank, Haynes & Altschule, 1943; Field & Laverty, 1958; Brown, Davies, Lever,

Robertson & Verniory, 1966). Simultaneous estimations of renin and angiotensin following haemorrhage in the dog demonstrated a parallel rise in both (Brown, Hodge, Lever, Lowe, Robertson & Vane, 1967). Thus it is well established that both renin and angiotensin levels rise in response to a stress such as haemorrhage.

Hypoxia is another stress which has been studied. Huidobro & Braun-Menéndez (1942) could not demonstrate a rise in renin levels during hypoxia. However, Gould & Goodman (1970) observed a three-fold increase in plasma renin levels and a doubling of renal renin content in rats subjected to hypoxia for up to 15 days.

White, Gold & Vaughn (1966) provided indirect evidence of a homeostatic role of the renin-angiotensin system in dogs in endotoxin shock. It was shown that nephrectomy was associated with a more rapid decline in arterial pressure after administration of endotoxin. Furthermore, this fall in arterial pressure in nephrectomised animals was prevented by an infusion of angiotensin. Further indirect evidence of a homeostatic role of the renin-angiotensin system in endotoxin shock was the finding that administration of angiotensin to dogs (Spink & Vick, 1962) and to monkeys (Spink, Vick, Melby & Finstad, 1963) was associated with an improved survival rate.

(C) THE SYMPATHO-ADRENAL SYSTEM

The demonstration by Oliver & Schäfer (1894; 1895) that an intravenous injection of dog suprarenal extract into cats caused a marked rise in arterial pressure heralded a period of intensive research into the role of the sympatho-adrenal system in response to a variety of stresses. The observations of Oliver & Schäfer were repeated in sheep by Abel & Crawford (1897) and in cats by Langley (1901). Elliott (1904) showed that the action of adrenaline was not by excitation of autonomic ganglia and was, therefore, directly on blood vessels. Further work established that the splanchnic nerves were important in causing a release of catecholamines from the adrenal medulla (Elliott, 1912). Adrenaline was the first hormone

to be purified (Abel & Crawford, 1897) and it was assumed to be the transmitter substance at all sympathetic nerve endings. The role of noradrenaline in neurotransmission was not established until the work of von Euler (1946). However, because of certain differences in the responses of isolated tissues and of the cardiovascular system to these two catecholamines, it appeared that the transmitter substance at sympathetic nerve endings was more likely to be noradrenaline than adrenaline (Barger & Dale, 1910). Cannon named the transmitter substance Sympathin, and later proposed the names Sympathin E for the excitatory effect of sympathetic stimulation and Sympathin I for the inhibitory effects (Cannon & Rosenblueth, 1933). A detailed study of the physiological, pharmacological and physico-chemical properties of sympathin as compared to adrenaline brought Bacq and his co-workers close to the identification of noradrenaline (Bacq, 1934; Bacq & Fredericq, 1935; 1940). Finally, in 1946 von Euler and his group isolated noradrenaline from the sympathetic nerve endings of cattle and horses and so established the nature of sympathin. Furthermore, it was established that adrenaline accounted for less than 2% of the total catecholamines in sympathetic nerve terminals (von Euler, 1948).

Nature of adrenal medullary secretion.

Early workers assumed that the adrenal medulla contained only adrenaline. However, in 1932 it was suggested that a second catecholamine was present (Annau, Huszak, Svirbely & Szent-Gryorgi, 1932). The name noradrenaline was proposed. Since techniques for differentiating between noradrenaline and adrenaline have become available, it has been established that the adrenal medulla contains a mixture of both adrenaline and noradrenaline, the proportions of which vary between species and within species, depending on age (von Euler, 1956). The ratio between the two catecholamines released varies with differing physiological and pathological states, both between and within species (Malmejac, 1964). The precise physiological significance of a change in the ratio is not clear but may relate to the greater potency of adrenaline than noradrenaline in inducing metabolic

changes such as hyperglycaemia and the mobilisation of fat. The observation that in a single passage through the lungs 20% of the circulating noradrenaline is extracted but no extraction of adrenaline occurs (Vane, 1968; 1969) suggests that adrenaline may be more important than noradrenaline as a circulating hormone.

Measurement of catecholamines.

Early biological and chemical techniques for the estimation of catecholamines have been discussed by von Euler (1956). Bioassay remained the only technique for assaying small amounts of catecholamines until the advent of more reliable fluorimetric or other techniques. Numerous bioassay systems have been used, including blood pressure of vagotomised cats, the cat pupil, cat nictitating membrane, rabbit duodenum, rat uterus, chick rectum and rat stomach strip. By using a continuous superfusion of the rat stomach strip with blood, Vane (1968; 1969) has determined changes in the circulating levels of catecholamines. The addition to the superfusion circuit of the chick rectum, which is selective for adrenaline, enables simultaneous differential assays of adrenaline and noradrenaline to be performed. As with the assay of angiotensin, the superfusion technique offers a means of continuous assay of catecholamines but only changes from an unknown base line are recorded.

Chemical methods for estimating catecholamines depend on the fact that fluorescence of the catecholamine molecule or of its derivatives can be detected. Earlier methods were not sufficiently sensitive, nor were the fluorescent products stable enough, to differentiate between noradrenaline and adrenaline, but later techniques with successive modifications have been developed (Lund, 1950; Weil-Malherbe & Bone, 1952; von Euler & Floding, 1955). The most recent modifications enable a differential assay of the resting levels of catecholamines in man to be determined (Fiorica, 1965; Renzini, Brunori & Valori, 1970). However, fluorimetric techniques still suffer from the disadvantage of requiring extraction and concentration steps before estimations can be carried out, and the sampling volume (20 ml) is prohibitive for repetitive estimations in

small animals such as cats. An enzyme technique employing tritiated noradrenaline offers the highest hopes of a micro-technique without loss of specificity (Engelman & Portnoy, 1970). In this technique specificity is achieved by using the conversion of adrenaline and noradrenaline to their respective metanephrines by catechol-O-methyl transferase. Sensitivity is achieved by using tritiated noradrenaline and specificity by using an enzyme.

Action of catecholamines.

The most important actions of catecholamines are their effects on the cardiovascular system and on metabolism. The proof that noradrenaline was the neurotransmitter at sympathetic nerve endings on blood vessels (von Euler, 1946; 1948) emphasised the significance of the pressor role of catecholamines, previously demonstrated for the adrenal medulla (Oliver & Schäfer, 1894; Abel & Crawford, 1897; Langley, 1901; Elliott, 1912). The physiological role of noradrenaline appears to be limited to the cardiovascular system, while adrenaline has actions which prepare the animal for fight or flight (Cannon, 1940). Thus the cardiovascular effects of adrenaline are an increased cardiac output and muscle blood flow, but a fall in skin and mesenteric blood flow, while the metabolic effects are to increase the blood sugar level by glycogenolysis and to mobilise fats (Turner & Bagnara, 1971). The possibility of different end organ receptors for adrenaline was suggested by the observation of Dale (1906), who showed that ergot converted the pressor response to adrenaline in the cat to a depressor response.

In 1948 Ahlquist proposed a two-receptor theory to explain the actions of adrenaline and noradrenaline. The excitatory effects of adrenaline and noradrenaline on blood vessels are mediated by α receptors, while the effects on the heart, gut, uterus, some blood vessels and bronchial muscles are related to β receptors.

Sympatho-adrenal system and homeostasis.

The role of the adrenal medulla has been studied in a wide variety of stresses, including haemorrhage, hypoxia, asphyxia, endotoxin shock, hypoglycaemia and emotional stimulation. It has

been assumed that, together with the sympathetic nerves, the adrenal medulla plays an important part in cardiovascular homeostasis.

The early work of Cannon, Elliott and Bedford, using cats and dogs, established that in almost any severe stress to an animal, large amounts of adrenaline are released (Cannon & Hoskins, 1911; Cannon & de la Paz, 1911; Elliott, 1912; Bedford, 1917).

Subsequent investigations using more refined techniques for estimating catecholamines have confirmed that haemorrhage in dogs is invariably associated with a rise in total catecholamine levels, but the ratio of adrenaline to noradrenaline is variable (Fowler, Shabetai & Holmes, 1961; Walker, Zileli, Reuter, Shoemaker, Friend & Moore, 1959; Walton, Richardson, Walton & Thompson, 1959; Greever & Watts, 1959; Glaviano, Bass & Nykiel, 1960; Darby & Watts, 1964; Feuerstein & Gutman, 1971). Little information on the precise stimulus to medullary secretion can be gained from these studies since haemorrhage was severe and rapid in all studies, except one, in which it was observed that catecholamine levels did not rise until arterial hypotension occurred (Watts, 1956). Severe hypoxia in dogs (Fowler *et al.*, 1961; Harrison & Seaton, 1965), rabbits (Iriki, Pleschka, Walther & Simon, 1971), and moderate hypoxia in man (Becker, 1968) is associated with a rise in catecholamines and little change in the adrenaline-noradrenaline ratio.

The administration of lethal doses of endotoxin is associated with severe haemodynamic changes and a rise in catecholamines in the cat (Hokfelt, Bygdeman & Sekkenes, 1962), and dog (Egdahl, 1959; Rosenburg, Liltehei, Longerbeam & Zimmerman, 1961; Nykiel & Glaviano, 1961; Spink, Reddin, Zak, Peterson, Starzecki & Seljeskog, 1966). Hypoglycaemia is a potent stimulus for medullary secretion in the dog (de Schaepdryver, 1959) and the cat (Feuerstein & Gutman, 1971). Furthermore, hypoglycaemia is the most potent stimulus for increasing the adrenaline-noradrenaline ratio. The mechanism whereby this ratio is changed is not clear. However, since stimulation of discrete areas of the hypothalamus of the cat can cause an increase in the adrenaline-noradrenaline ratio, it has been suggested that

hypothalamic control of adreno-medullary secretions may be important (Folkow & von Euler, 1954).

The precise stimulus to secretion of the adrenal medulla in the various stresses is not clear, but experimental evidence supports the original observation of Elliott (1912) on the importance of the splanchnic nerves. In haemorrhage, the onset of arterial hypotension and subsequent stimulation of arterial baroreceptors appears to be important in stimulating the release of catecholamines (Watts, 1956). Also, bilateral carotid occlusion is associated with a rise in catecholamine secretion in the cat (Kaindl & von Euler, 1951) and dog (de Schaepdryver, 1959). The release of catecholamines during hypoxia is markedly reduced if the adrenal medulla is denervated (Kellaway, 1919; Houssay & Molinelli, 1926), or the carotid and aortic chemoreceptors are denervated (Gellhorn & Lambert, 1939). Furthermore, studies in foetal sheep and foals show that the response of the adrenal medulla to hypoxia is not fully developed until innervation is complete (Comline & Silver, 1961; 1971). Using superfusion to assay catecholamines, it has been shown that the stress of myocardial infarction is associated with an immediate release of catecholamines (Staszewska-Barcak & Ceremuzynski, 1968).

There is thus strong evidence in support of the adrenal medulla playing some part in the response to severe stress situations in all species studied. However, whether secretion from the adrenal medulla is continuous or reserved for fight and flight reaction (Cannon, 1940) is not yet clear, since the procedures required to obtain samples for catecholamine estimation cannot be taken in a truly basal state.

(D) SUMMARY

Investigations on a wide range of species have established the nature of the components of the renin-angiotensin system and several physiological actions of angiotensin have been demonstrated. Both the renin-angiotensin and sympatho-adrenal systems are stimulated in a variety of stress situations, including haemorrhage, hypoxia and

endotoxin shock. The sympathetic innervation of the kidney may provide a link between the two systems, but since renal denervation does not abolish the release of renin, the two systems can function independently. Since circulating levels of renin, angiotensin and catecholamines rise in stress situations, one might expect these hormones to be playing a homeostatic role. However, in spite of the numerous investigations, the two systems have not yet been investigated simultaneously.# The precise role and interactions of these hormones in stress in different species is not clear. While it is tempting to postulate that their actions may be limited to their effects on the cardiovascular system, the metabolic effects of angiotensin on salt and water balance, and of adrenaline on carbohydrate and fat metabolism may be the more important physiological role.

As part of a study on angiotensin II like activity in blood of new-born rabbits, a qualitative simultaneous assay of catecholamines has been carried out (Broughton Pipkin, Mott & Roberton, 1971).

SECTION 1A
GENERAL METHODS

1. *ANIMALS*

(a) *Cats and dogs.* No selection was made with regard to age, sex, weight or breed. Animals were not starved and had free access to water prior to surgery.

(b) *Sheep.* Adult Merino or Corriedale wethers were used. Sheep were starved for 12-24 hours prior to surgery but had free access to water.

(c) *Rats.* Adult albino or hooded wistar rats were used for assay tissues. Male albino rats (150-200 g) were used for determination of amounts of angiotensin generated.

(d) *Human studies.* Subjects were healthy volunteers of either sex. Prior to consenting to be a subject, the aim of the study and all experimental procedures were explained, and each subject was fully aware of the risks of all procedures.

2. *EQUIPMENT*

(a) *Recorders.* A Beckman 6 channel Type R dynograph recorder was used for the majority of experiments. However, in some experiments it was replaced by a two channel Rika-Denki recorder, together with a Brodie-Starling Kymograph and smoked drum.

Arterial pressure was recorded using an S.E. Laboratories transducer (Model SEM 4-82) or P23 AC Statham transducer; central venous pressure was recorded using a P23 BC or P23 Bb Statham transducer.

Responses of the assay tissues were measured using Harvard strain gauges or auxotonic levers (Paton, 1957) and a smoked drum.

(b) *Infusion pumps.* Drugs were infused with a Sage pump (Model 521, Orion Research Instruments), a Harvard 2 channel pump (Model 600-910/20) or a roller pump driven by a variable speed motor (Servo-Mex Controls Ltd). The tubing used in the roller pump was translucent vinyl tubing (Portex 2E Int. Diameter 1.0 mm) and was calibrated each time it was changed. This pump was used for intravenous infusions.

Siliconised glass syringes (20 ml or 50 ml) were used with the Sage and Harvard pumps. Each syringe was calibrated prior to use. **The Harvard and Sage pumps could operate against an opposing pressure of 200 mm Hg.**

(c) *Superfusion pump.* A roller pump made of perspex with 6 stainless steel rollers was used. The motor was designed to enable the pump to give a constant flow of blood despite changes in arterial pressure on the input to the pump, or changes in resistance in the tubing leading from the pump.

Vinyl tubing (Portex NT 6) was used in the roller pump and was changed after each experiment.

(d) *Cannulae and tubing.* Polyethylene cannulae (Portex or Dural Plastics Pty Australia) were used for all experiments.

The cannula selected depended on the size of the vessel; where a cannula of internal diameter 2.8 mm, external diameter 4.1 mm could not be used, the maximum size possible was selected.

After each experiment, all cannulae were soaked overnight in a nontoxic, pyrogen free, deproteinising solution (Diversey, Diversey, A/asia). All cannulae were replaced at intervals. Silicone-rubber tubing was used to join cannulae together.

(e) *Respirator.* A positive pressure ventilator (C. F. Palmer) was used when required.

(f) *Drugs.*

(i) *Diluents.* Saline was prepared daily as a 0.15 M solution from a stock 3 M solution.

Ascorbic saline was prepared by adding 100 mgm ascorbic acid per litre 0.15 M saline and adjusting the pH to 5.6.

(ii) *Anaesthetics.* Alpha-chloralose (Koch Light; B.D.H.; Merck Darmstadt) was prepared as a 1% solution.

Halothane (Fluothane, I.C.I.) or anaesthetic ether (B.D.H.) were administered by a face mask or anaesthetic box.

Pentobarbitone sodium (Nembutal, Abbott Laboratories) was used to anaesthetise rats.

Thiopentone (Intraval Sodium, May & Baker) was prepared as a 1% solution.

(iii) *Vaso-active drugs.* Adrenaline bitartrate (Sigma) was prepared in ascorbic saline.

β asparaginyl val^5 angiotensin amide (Hypertensin, Ciba Ltd) was

prepared as a stock solution of 10 µg/ml at -20°C. For use, appropriate dilutions were made using 0.15 M saline. On no occasion was the stock solution refrozen. Dilutions were kept on ice and were never stored overnight.

Aspartyl isoleucyl^5 angiotensin I (Bioschwarz Research) was prepared as a stock solution of 1 µg/ml and stored at -20°C.

5-Hydroxytryptamine creatinine sulphate (Sigma) was prepared in 0.15 M saline.

Pentolinium Tartrate (Ansolysen, May & Baker) was stored as a stock solution of 5 mgm/ml.

Prostaglandin. $PGF_{2\alpha}$ (Upjohn Chemical Co., Kalamazoo) was stored as a stock solution of 5 mgm/ml at -20°C and diluted as required.

(iv) *Miscellaneous*. Aspirin (Ajax Chemicals, Australia) was prepared as a 3% solution at 37°C or a 1% solution at room temperature.

2 Brom lysergic acid diethylamide (BOL 148, Sandoz) was stored as a 1 mgm/ml solution at -20°C.

Endotoxin. E. coli endotoxin (Twyford Laboratories, Twyford, England) was stored as a freeze dried powder. The same batch of endotoxin was used for each group of experiments. Endotoxin was prepared as a 1 mgm/ml solution.

Heparin (Boots Ltd, Commonwealth Serum Laboratories or Weddell Pharmaceuticals) was stored as a stock solution of 1000, 5000 or 25,000 i.u./ml at 10°C.

Indocyanine Green (Cardio-Green, Hynson, Westcott & Dunning) was prepared as a 5 mgm or 2.5 mgm per ml solution in the solvent provided.

Propranolol (Inderal, I.C.I.) was prepared as a 1 mgm/ml solution in Krebs solution.

3. *SURGERY*

(a) *Dogs*. Dogs were anaesthetised with i.v. thiopentone, approximately 25 mgm/kg. Anaesthesia was maintained with i.v. chloralose (100 mgm/kg). If necessary, anaesthesia was supplemented with one-third the original dose of chloralose. Through a mid-line incision an external jugular vein was exposed cannulated and the catheter advanced until it was in the vicinity of the right atrium.

This catheter was used for recording central venous pressure.

A carotid artery was cannulated for recording arterial pressure.

Blood for the superfusion circuit was taken from a femoral artery and returned via a femoral vein.

A second vein, either femoral or external jugular, was cannulated for infusion of drugs.

(b) *Cats*. Anaesthesia was induced with halothane in an enclosed box and maintained with ether on a face mask until a femoral vein had been cannulated through which chloralose (80 mgm/kg) was administered. The cannula was then used for infusions of drugs.

An external jugular vein was cannulated, as in the dog, to record central venous pressure.

A femoral artery was cannulated to record arterial pressure.

Blood for the superfusion circuit was taken from a carotid artery and returned via an external jugular vein.

(c) *Sheep*. Anaesthesia was induced with intravenous thiopentone (25 mgm/kg) and maintained with chloralose 50 mgm/kg.

Cannulations were the same as for the dog.

Tracheostomy was performed on all animals. Dogs were respired by positive pressure ventilation; cats and sheep were respired only if the chloralose or endotoxin depressed respiration.

After completion of surgery all animals received heparin intravenously (1000 i.u./kg).

(d) *Rats*. Rats were used for bioassay of angiotensin in the determination of plasma renin activity, plasma renin concentration or renin-substrate. Rats were anaesthetised with intraperitoneal Nembutal (60 mgm/kg); anaesthesia was maintained with further doses of Nembutal either intravenous or intramuscular. Through a mid-line incision in the neck a tracheostomy was performed, an external jugular vein was cannulated for injection of drugs, and a carotid artery was cannulated for recording arterial pressure. Pentolinium tartrate (25 mgm/kg) was administered subcutaneously. After completion of surgery, heparin (1000 i.u./kg) was administered intravenously.

SECTION 1B
ASSAY TECHNIQUES

1. *ANGIOTENSIN AND CATECHOLAMINES*

Changes in angiotensin and catecholamines in arterial blood were determined using the superfusion technique of Vane first described for measuring angiotensin (Regoli & Vane, 1964) and later developed for other hormones by selecting appropriate tissues (Vane, 1969).

This technique has several advantages over other techniques. Thus a continuous estimation of changes in the circulating levels of one or more hormones can be performed enabling a direct determination of the timing of the release of each hormone, and the inter-relationship between hormones to be carried out. Furthermore, apart from the blood removed initially to prime the superfusion circuit, no further blood need be lost, thus enabling detailed studies to be carried out on small animals such as cats.

Superfusion circuit. A diagrammatic representation of the superfusion circuit is shown in Fig. 1.1. The priming volume was 8-10 ml for cats and 15-20 ml for dogs. Blood was taken from a carotid or femoral artery and pumped at a constant rate by a roller pump at between 10 and 15 ml/min. A Y connector on the input side of the pump enabled either blood or Krebs solution to be pumped.

Blood was pumped from the roller pump through a length of polyethylene tubing (Portex PP 160) which was heated to 38^{o}C by a countercurrent flow of water from a Braun Thermomix pump. After the water jacket, blood passed from one tissue to the next (Fig. 1.2) before collecting in a reservoir and returning to the animal by gravity.

The assay tissues were mounted in 50 ml polypropylene centrifuge tubes. A ¼" diameter hole was drilled in the base of the tube and a polyethylene connector cemented into the hole so that blood could be directed from one tissue to the next.

The inside of the polypropylene tubes was coated with a defoaming

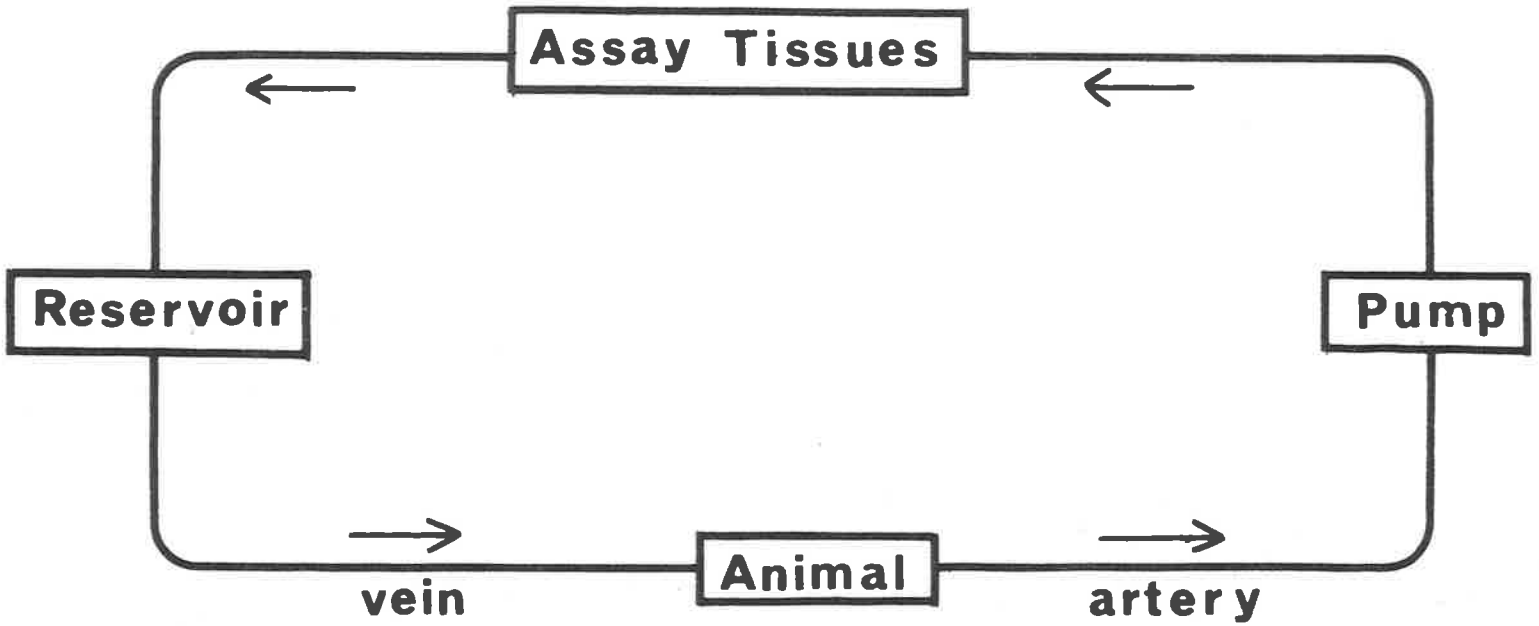


Fig. 1.1

Diagrammatic representation of the extra-corporeal circuit for superfusion.

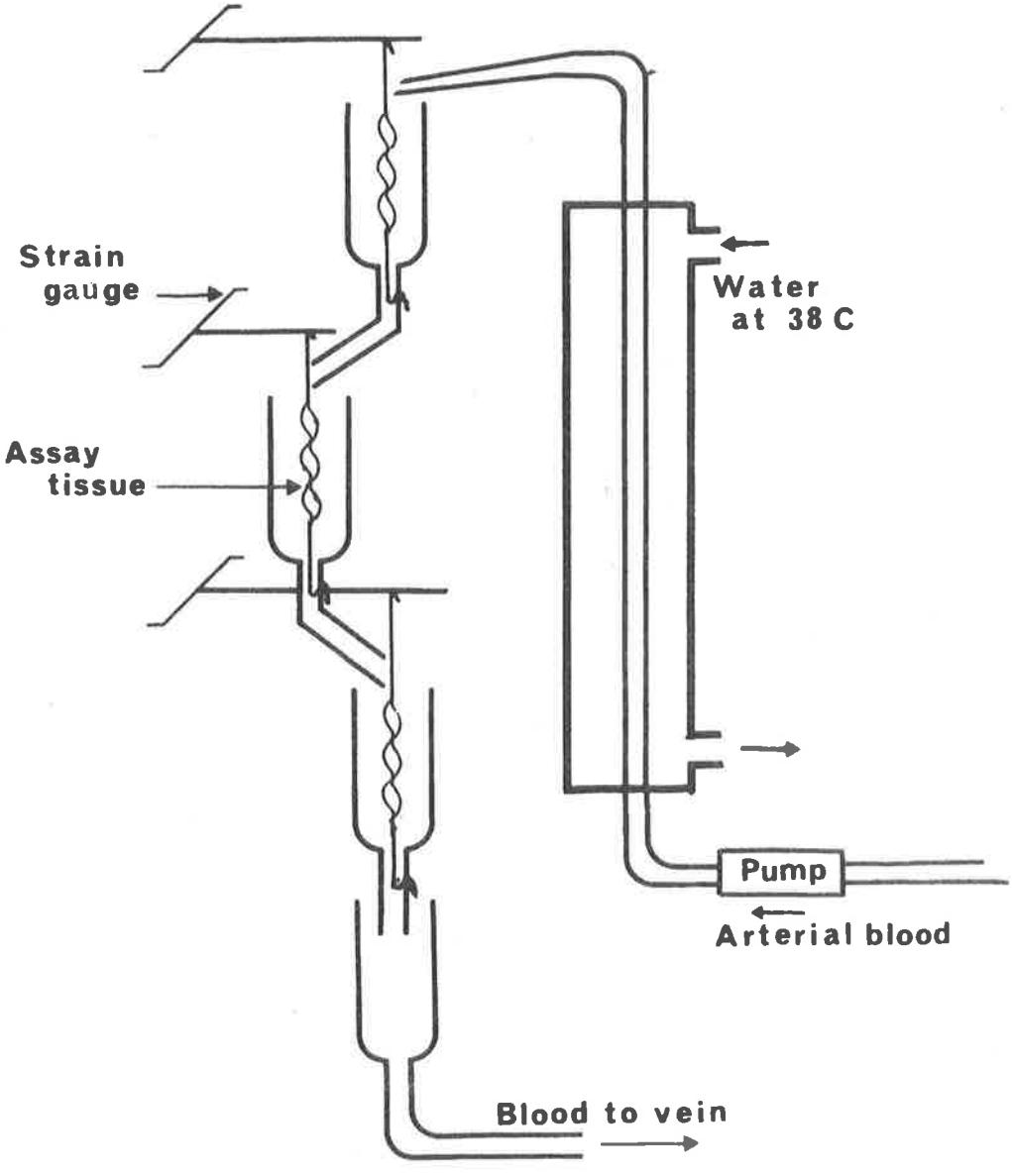


Fig. 1.2

Arrangement of polypropylene pots, assay tissues and strain gauges. The temperature of the blood superfusing the first tissue was 38°C. A 1°C fall occurred over three assay tissues.

preparation (Anti-Foam A, Ajax Chemicals) to prevent platelet aggregation.

Assay tissues. The tissues appropriate for the vasoactive agents being studied are shown in Table 1.1. The tissues used for catecholamines and angiotensin were the rat stomach strip and the rat colon, respectively. A third tissue was either a second rat stomach strip or rat colon.

Rats were killed by stunning and the appropriate tissues immediately removed and placed in Krebs solution (Appendix 1.1).

Rat stomach strip. The fundus was removed from the stomach by transection at the level of the oesophagus. Any muscle coat remaining on the fundus was removed. The fundus was then converted into a strip of tissue by making cuts with scissors as shown in Fig. 1.3. Care was taken during preparation of the tissue to keep all surfaces moist with Krebs solution. A length of mersil suture thread (3/0) was then tied to the end of the tissue.

Rat colon. The portion of the rat colon used was the ascending colon. The colon was sectioned at the junction of the terminal ileum and the first flexure (Fig. 1.4). The mesentery was cut close to its colonic attachment, care being taken not to perforate the colon. Having removed the colon, the lumen was then washed several times with Krebs solution. A length of mersil suture thread (3/0) was tied to the proximal end of the colon so that the lumen of the colon was occluded (Fig. 1.4).

While Krebs solution was pumped through the superfusion circuit, the tissues were mounted in the polypropylene tubes, one ligature passing through the base of the polypropylene tube, where it was held by silicone rubber tubing, and the other being attached to the Harvard strain gauge.

Once mounted, the tissues were superfused with Krebs solution for periods of up to 15 minutes to ensure removal of any remaining particulate matter. Tissues were then superfused with blood.

The Beckman recorder, together with pressure transducers, Harvard strain gauges and polypropylene pots are shown ready for use

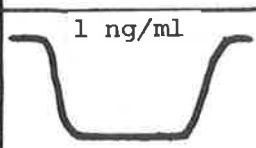
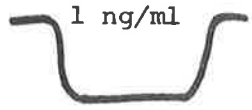
HORMONE	RAT COLON	RAT STOMACH STRIP	CHICK RECTUM
ANGIOTENSIN	0.1 ng/ml 		
ADRENALINE		1 ng/ml 	1 ng/ml 
NORADRENALINE		1 ng/ml 	
5-HT			
$PGF_{2}\alpha$			

Table 1.1

The relative responses of assay tissues superfused with blood to which each hormone has been added in concentrations not exceeding 5 ng/ml. Contraction is indicated by a rise in the base line, relaxation by a fall. Values refer to the minimal acceptable sensitivities of each tissue. Tissues less sensitive than these values were rejected.

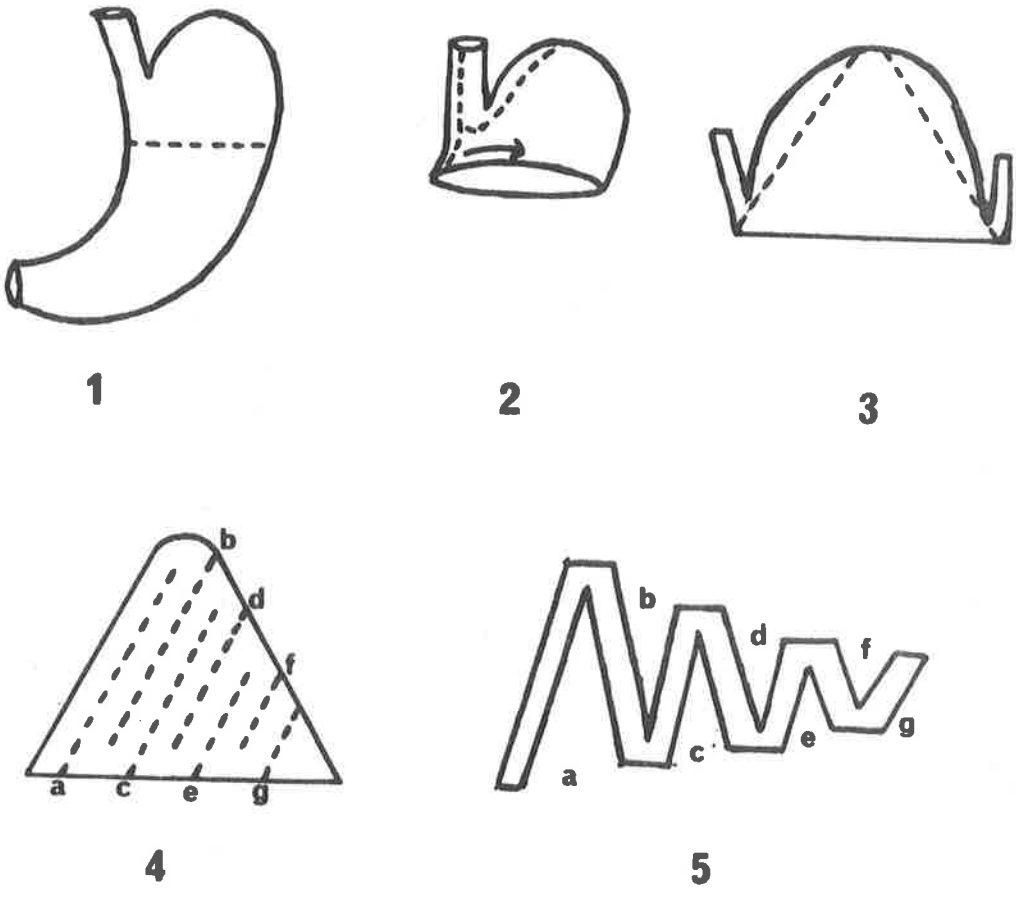


Fig. 1.3

Preparation of the rat stomach strip.

1. Stomach is transected at the level of the oesophagus.
2. A second cut is made along the lesser curve to the top of the fundus.
3. The stomach is opened and any remaining muscle removed.
4. Cuts a-g are made in the fundus to give a strip of tissue (5).

Modified from Vane's technique to achieve more stable assay preparation.

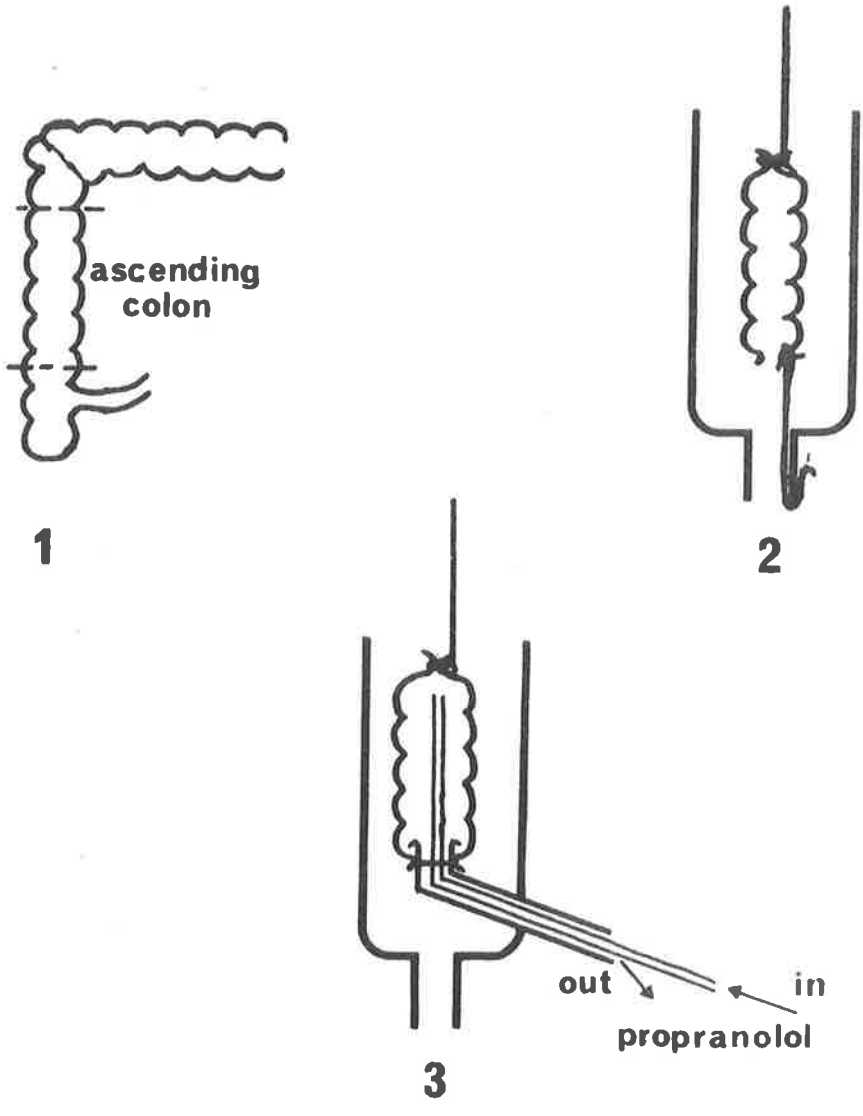


Fig. 1.4

Preparation of the rat colon.

1. The ascending colon is removed.
2. The proximal end is tied occluding the lumen and a ligature passed through the wall of the distal end.
3. A rat colon mounted to enable infusion of propranolol.

in Fig. 1.5.

When superfusion with blood commenced, all the tissues contracted to a variable degree. Approximately 20 to 30 mins was allowed for the tissues to achieve steady base lines.

A variable degree of spontaneous activity was recorded from the rat colon superfused with blood. If this activity was such as to interfere with the assay of angiotensin, a condenser (1 Farad capacity) was switched into the recording circuit. Most spontaneous activity was then removed from the recording without prejudicing the assay of angiotensin. When steady base lines were obtained, the tissues were calibrated.

Calibration of the tissues. Different doses of adrenaline or angiotensin were infused intravenously or directly into the superfusion circuit. The responses of the tissues to angiotensin and adrenaline infusions are shown in Fig. 1.6. Changes in catecholamine or angiotensin levels induced by an experimental procedure were assayed whenever possible by bracketing the response with infusions of adrenaline or angiotensin, except in experiments with endotoxin where the assay preparations usually did not recover.

The rat colon responds to angiotensin by contracting. However, catecholamines inhibit the tone of the rat colon. Thus a rise in catecholamine levels may diminish the response of this tissue to angiotensin. The effect of catecholamines was inhibited by infusing the β blocking agent propranolol into the lumen of the rat colon (Fig. 1.4). Propranolol (1 mgm/ml) was prepared in Krebs solution and infused at 0.25 ml/min. Since the colon was not perforated during preparation, propranolol could not reach the general circulation.

Since propranolol is a competitive antagonist of adrenaline, the rat colon will relax if catecholamines rise to a level sufficient to overcome the β blockade and any rise in angiotensin will thus be underestimated. The level of catecholamines which would overcome the competitive block of propranolol was determined during calibration of the tissues with intravenous adrenaline.

While the rat stomach strip is preferentially sensitive to catecholamines, to which it responds by relaxing, it does respond to

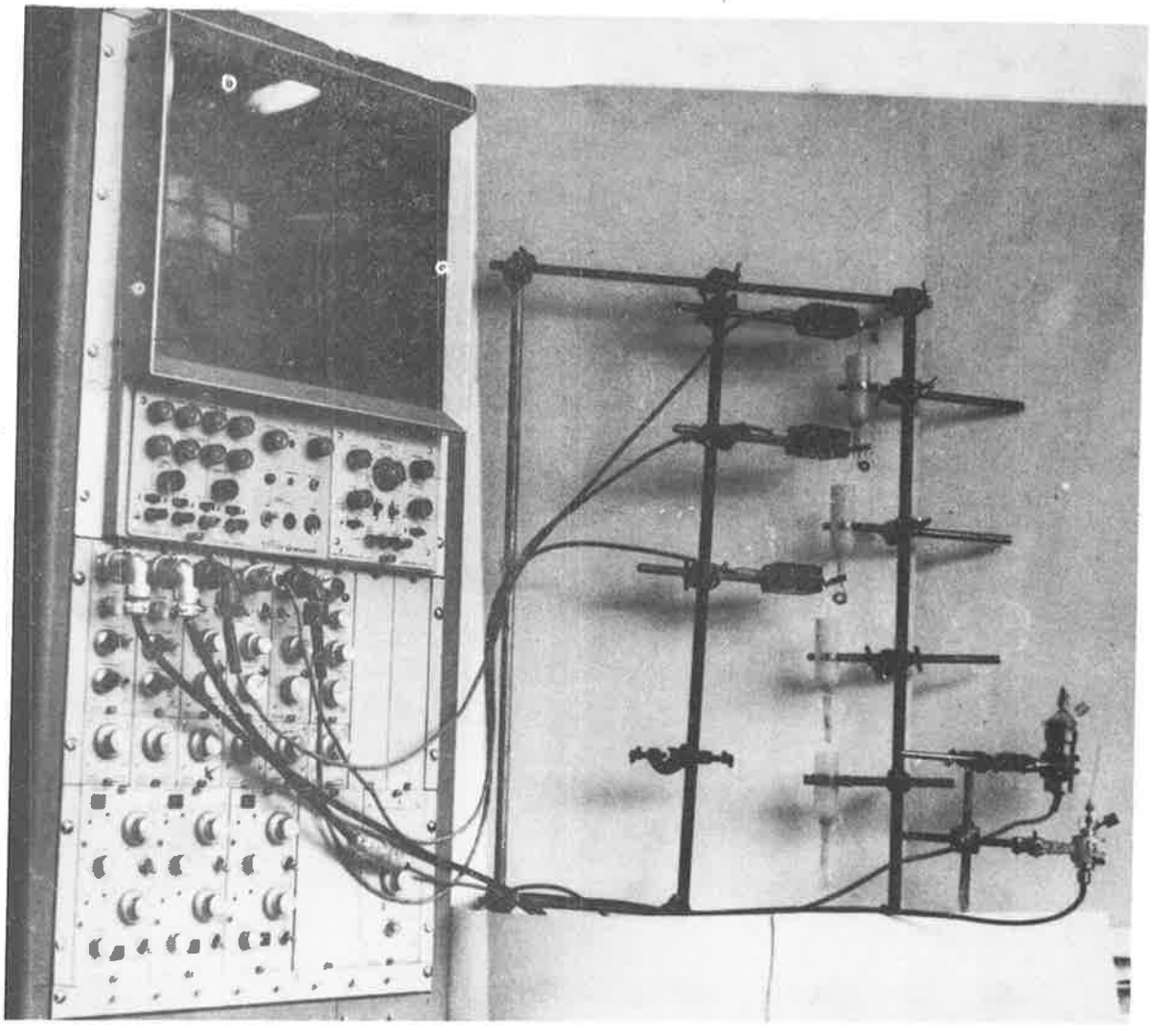


Fig. 1.5

The Beckman recorder, strain gauges and pots for holding the assay tissues.

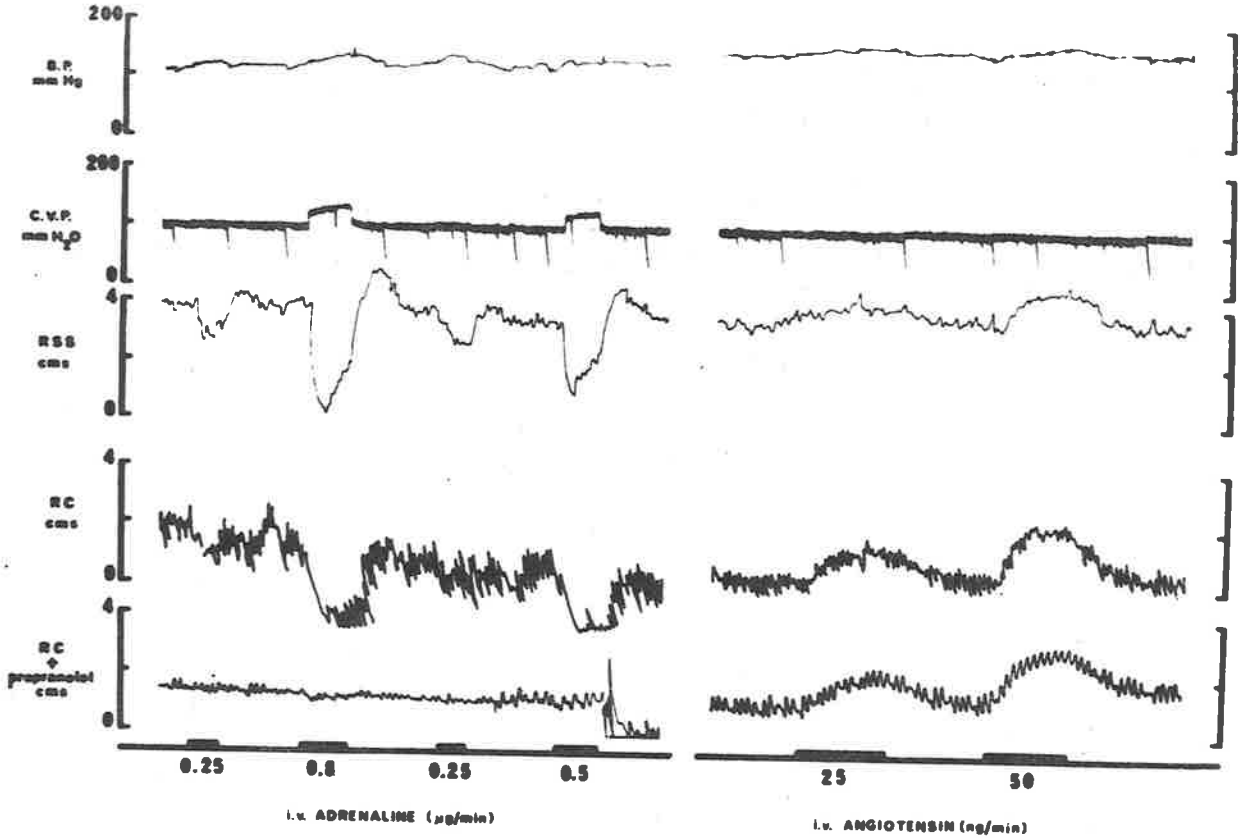


Fig. 1.6

Cat: A tracing showing the response of arterial pressure (B.P.), central venous pressure (C.V.P.), a rat stomach strip (R.S.S.), a rat colon (R.C.), and a rat colon + propranolol to infusions of adrenaline and angiotensin. The unblocked rat colon is inhibited by adrenaline, while the base line of the blocked colon is unaffected.

angiotensin by contraction. The degree to which various isolated strip preparations respond to angiotensin varies from tissue to tissue and can only be ascertained during the calibration of the tissues with intravenous doses of angiotensin. However, if interference between angiotensin and catecholamines should occur, any change in the circulating levels of catecholamines will be underestimated since angiotensin causes a contraction of the rat stomach strip.

Changes in the base line of the tissues were expressed as the response to be expected from an equivalent intravenous dose of angiotensin or adrenaline. Since the rat stomach strip was used, total catecholamines were estimated in terms of their adrenaline equivalent; in some experiments a qualitative assay of noradrenaline was carried out by including a chick rectum as an assay tissue.

Changes in sensitivity of the tissues to adrenaline or angiotensin were monitored by infusing low concentrations of the appropriate hormone into the superfusion circuit immediately before the pump.

Since there was a large range in body weight both within and between species, the humoral response to a particular stress was standardised by expressing the amount of angiotensin generated or catecholamines released in ng/kg/min.

Nictitating membrane. It has been shown that a fall in the oxygen tension of the superfusate causes a relaxation of the rat stomach strip, thus mimicking the response to catecholamines (Smith & Vane, 1966). Endotoxin may cause a fall in PO_2 in cats (Gilbert, 1960). Therefore, in some experiments in which endotoxin was given to cats, changes in catecholamine levels were assayed by the response of these cats' nictitating membranes as well as on the superfused rat stomach strip.

The cat's head was held firmly in position with clamps passing into each external auditory meatus and across the upper jaw. The postganglionic fibres supplying the nictitating mebrane whose response was to be recorded were identified by means of supra-maximal electrical stimulation and then severed. A ligature was tied to the medial border of the membrane and attached to a Grass instrument FTO3 strain

gauge. The tension applied to the membrane was 5 grams. The responses of the nictitating membrane to different intravenous infusions of adrenaline were then obtained at the same time as the stomach strip.

2. *PLASMA RENIN*

The generation of angiotensin I depends on the action of renin on substrate. Since the reaction is enzymatic, the rate of generation will be dependent on renin and substrate concentrations. Skinner (1967) has developed a technique to determine separately the overall activity of the renin angiotensin system in plasma, plasma renin activity, the renin concentration, plasma renin concentration and the substrate concentration. The technique depends on the fact that dialysis to pH 4.5 has no effect on renin or renin substrate but destroys angiotensinases, while dialysis to pH 3.3 destroys renin substrate but the enzyme renin remains active.

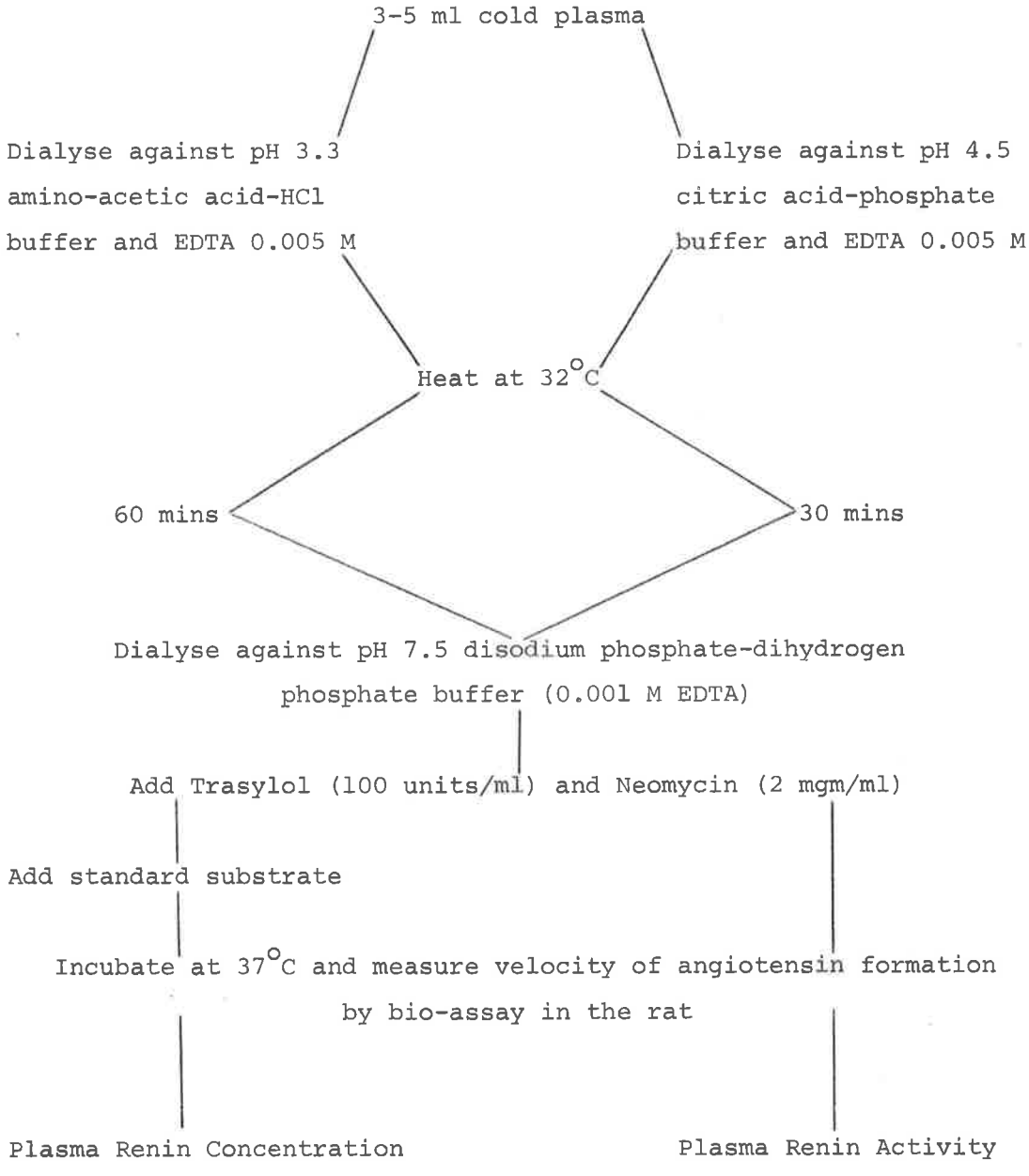
Plasma renin activity and concentration. A flow sheet showing the treatment of each sample is shown in Fig. 1.7. Details of the buffers used are given in the Appendix (1.2). Venous blood samples (10 ml) were taken into iced tubes containing 100 i.u. of heparin, centrifuged at 4000 g for 20 minutes, and the plasma decanted and stored at -20°C until dialysis.

Dialysis. Approximately 2 ml aliquots of plasma were weighed and dialysed in 8/32 Visking cellophane casing against pH 4.5 buffer or pH 3.3 buffer for 24 hrs at 4°C. Dialysis bags were tied under pressure in order to ensure reduction of the volume during dialysis. After dialysis at pH 4.5, samples were incubated at 32°C for 30 minutes to destroy angiotensinases; pH 3.3 samples were incubated at 32°C for 60 minutes to destroy both substrate and angiotensinases. Samples were then transferred to pH 7.5 buffer for 24 hrs.

Following dialysis, samples were removed from pH 7.5 buffer and the initial weight restored by adding a bacteriostatic agent (2 mgm/ml neomycin sulphate, Ethnor, Australia), a kallikrein inhibitor (100 units/ml Trasylol, F.B.A. Pharmaceuticals, Australia) and pH 7.5 buffer. Samples were then stored at -20°C until incubation

FIGURE 1.7

Flow sequence for handling plasma for estimation of plasma renin concentration (PRC) and plasma renin activity (PRA).



and assay.

Incubation and assay. For determination of plasma renin activity samples were incubated at 37°C without further treatment.

For determination of plasma renin concentration an excess of sheep substrate was added to the samples prior to incubation at 37°C.

Incubation times were selected in order to give at least three points on a linear velocity plot. The angiotensin generated at each time was determined by bracket assay in the ganglion blocked rat (Peart, 1955; Skinner, 1967), using aspartyl-isoleucyl^{5} angiotensin I as standard.

The rate of generation of angiotensin in ng/ml/hr is an expression of the activity of plasma renin and substrate or of the plasma renin concentration.

Substrate. Substrate concentration was estimated by the addition of an excess of human renin to samples dialysed to pH 4.5. Incubation was then carried out as for plasma renin activity.

SECTION 2

CONTROL EXPERIMENTS

Experiments were divided into four groups. In each group the validity of the superfusion technique in determining changes in levels of angiotensin or adrenaline was determined.

1. *Assay of angiotensin* (4 dogs). During an i.v. infusion of angiotensin II cardiac output was estimated using indocyanine green. Blood was sampled from the carotid artery and the concentration of dye determined with a Waters cuvette and densitometer. Cardiac output was calculated with an Olivetti 101 programma computer (Hall & Tyler, 1971).

The expected rise in circulating angiotensin during an infusion of the drug was calculated from the formula:

$$\text{Expected concentration (ng/ml)} = \frac{\text{Amount of angiotensin infused (ng/min)}}{\text{Cardiac output ml/min}}$$

The change in circulating levels of hormones was also assayed by bracketing responses of the rat colon to i.v. infusions of angiotensin with infusions into the superfusion circuit. The concentration of hormone during the latter infusions is given by the formula:

$$\text{Concentration (ng/ml)} = \frac{\text{Amount of angiotensin infused (ng/min)}}{\text{Flow rate in superfusion circuit (ml/min)}}$$

A total of 12 different doses of i.v. angiotensin was administered to 4 dogs. In Fig. 2.1 the rise in circulating levels of angiotensin calculated from the cardiac output is plotted against the rise determined by bracket assay. Details of data are shown in the Appendix (2.1). There was a significant correlation between the two concentrations ($r = 0.9742$ $P<0.001$). The positive intercept of the ordinate was not significantly greater than 0.

2. *Assay of catecholamines* (3 dogs). Adrenaline was infused intravenously and cardiac output estimated as in group 1. Changes in adrenaline levels were assayed using the rat stomach strip. Rises in circulating levels of adrenaline calculated from the cardiac output and from bracket assay were determined as for angiotensin.

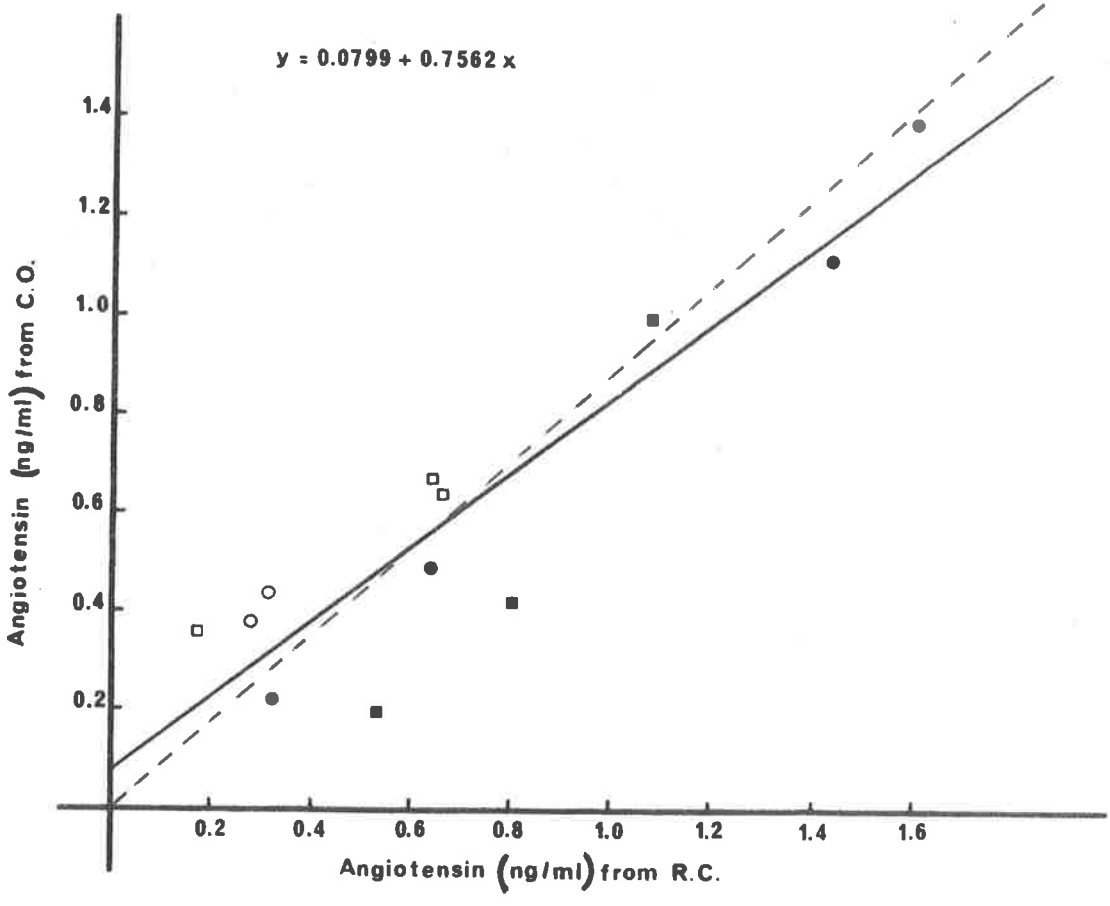


Fig. 2.1

Correlation between increases in blood concentration of angiotensin (ng/ml) as calculated from the cardiac output (C.O.) and as determined by bioassay using the rat colon (R.C.) (Appendix 2.1) Different symbols are different dogs.

A total of 13 doses of i.v. adrenaline was administered to 3 dogs.

In Fig. 2.2 the rise in circulating levels of adrenaline calculated from the cardiac output is plotted against the rise determined by assay. Details of the data are shown in Appendix 2.2. There was a significant correlation between the two concentrations ($r = 0.983$; $P<0.001$).

3. *Effect of a prolonged infusion of angiotensin* (3 dogs). Responses of the rat stomach strip and rat colon were obtained to i.v. adrenaline and angiotensin. A 60 minute i.v. infusion of angiotensin was then commenced. Responses were again obtained to i.v. adrenaline and angiotensin during the infusion of angiotensin.

The responses of the rat stomach strip to i.v. adrenaline were unchanged by the rise in circulating levels of angiotensin.

The responses of the rat colon to i.v. angiotensin were diminished but were of the same order as those to be expected if the same total dose of angiotensin had been given as a single dose prior to infusion.

At the end of the prolonged infusion, the tissue base lines returned to pre-infusion levels.

Apart from the expected rise during angiotensin or adrenaline infusions, arterial and central venous pressures remained unchanged throughout the experimental period of 2-3 hours.

4. *Effect of a prolonged infusion of adrenaline*. Responses of the rat stomach strip and a β-blocked colon to i.v. adrenaline and angiotensin were obtained. A 60 minute i.v. infusion of adrenaline was then commenced. Intravenous infusions of adrenaline and angiotensin were repeated. The responses of the blocked rat colon to angiotensin during the infusion of adrenaline were similar to those preceding the adrenaline infusion.

The responses of the rat stomach strip obtained during the i.v. infusion of adrenaline were the same as those to be expected if the same total dose of adrenaline was administered as a single infusion.

At the end of the prolonged infusion of adrenaline, the tissue

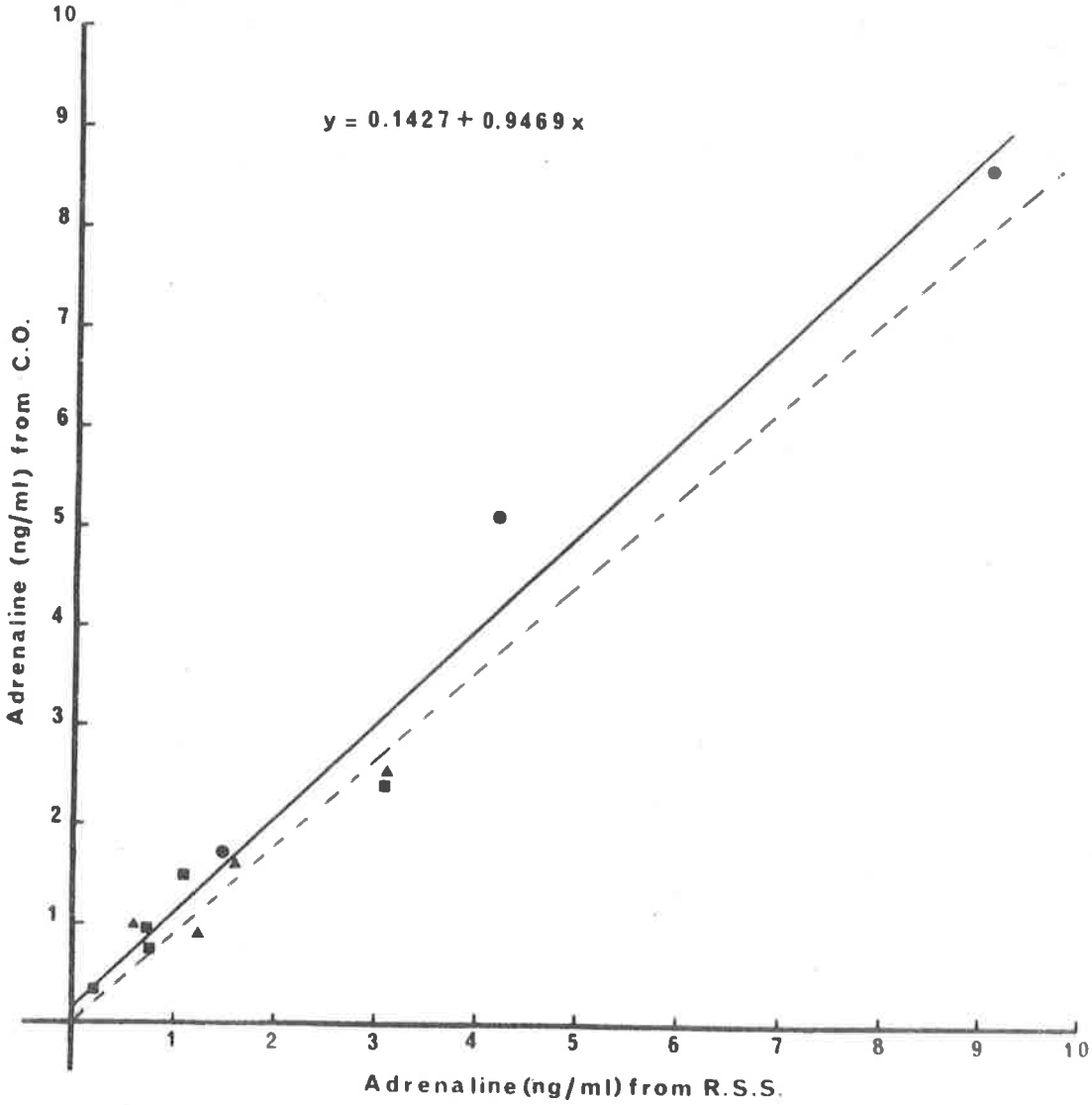


Fig. 2.2

Correlation between increases in blood concentration of adrenaline (ng/ml) as calculated from the cardiac output (C.O.) and as determined by bioassay using the rat stomach strip (R.S.S.) (Appendix 2.2) Different symbols are different dogs.

base lines returned to pre-infusion levels.

Except for the expected responses to the adrenaline and angiotensin infusions, arterial and central venous pressures remained unchanged throughout the experimental period of 2-3 hours.

DISCUSSION

The results obtained in groups 1 and 2 show that the assay technique is capable of recording changes in circulating levels of catecholamines and angiotensin within the range expected to be measured during the experimental procedures used in the studies described in this thesis. Furthermore, the assay technique is at least as accurate as the dye dilution technique used for estimating cardiac output. The accuracy of the latter is 3-5% (Guyton, 1963); the bioassay technique thus compares very favourably with other techniques for estimating catecholamines or angiotensin (Fiorica, 1965; Renzini *et al.*, 1970; Catt *et al.*, 1967; Boyd *et al.*, 1971).

In the studies to be described, rises in both angiotensin and catecholamines were observed. The results obtained in groups 3 and 4 show that a rise in one hormone has no effect on the response of the appropriate assay tissue to the other hormone. Furthermore, these experiments show that the use of an extracorporeal circuit does not cause a significant change in either arterial or central venous pressure for a period of up to 3 hours, which was the average duration of experimental observation.

SECTION 3
CHANGES IN CATECHOLAMINE LEVELS IN THE CAT AND DOG DURING HAEMORRHAGE

Several studies have been carried out to determine separately the effects of haemorrhage on the circulating levels of catecholamines, renin or angiotensin. The role of the sympathetic nervous system in haemorrhage has been reviewed (Chien, 1967). It is well established in the dog that haemorrhage severe enough to cause systemic hypotension is associated with a rise in catecholamine levels (Watts & Bragg, 1957; Walton *et al.*, 1959; Walker *et al.*, 1959; Fiorica, Iampietro, Burr & Moses, 1969). In these studies haemorrhage was sufficient to cause a fall in blood pressure to 40-50 mm Hg. However, moderate falls in blood pressure were also associated with a rise in catecholamine levels (Fowler *et al.*, 1961). In a study of the effects of slow haemorrhage in the dog, a gradual rise in catecholamine levels was also observed, which commenced after the onset of hypotension (Watts, 1956).

The response of the renin angiotensin system of the dog to haemorrhage has been studied by several workers. Severe haemorrhage is consistently associated with a rise in angiotensin levels (Scornik & Paladini, 1964) and renin levels (Brown *et al.*, 1966). Haemorrhage insufficient to cause a fall in blood pressure is associated with a rise in angiotensin levels (Hodge, Lowe & Vane, 1966b; Regoli & Vane, 1966). A concurrent estimation of renin and angiotensin levels showed that a rise in both occurred during haemorrhage in the absence of any fall in systemic blood pressure (Brown *et al.*, 1967).

The response of the cat to haemorrhage has been studied less extensively. A rise in catecholamines in adrenal vein blood was observed following severe hypotension (Hökfelt *et al.*, 1962). These workers also observed a rise in catecholamine levels during slow, intermittent haemorrhage before blood pressure had fallen significantly. They suggested that these results may provide evidence that

adreno-medullary stimulation may occur in the cat before the onset of hypotension.

Previous studies have not correlated the response of the renin angiotensin and sympatho-adrenal systems to haemorrhage and, furthermore, haemorrhage has been too severe in most studies to establish the nature of the stimulus to each system. The present study was carried out to determine simultaneously the changes in circulating levels of angiotensin and catecholamines during controlled moderate haemorrhage, and also to compare two species, the cat and dog, since there is some evidence that the sympatho-adrenal system may be more responsive in the cat than the dog during stress (Bacq & Brouha, 1934; Cannon, 1940).

EXPERIMENTAL GROUPS

1. *The effect of the rate of haemorrhage* (7 cats, 5 dogs). Each animal was bled at two rates: a mean of 0.5 ml/kg/min (slow haemorrhage) and 3.0 ml/kg/min (fast haemorrhage). The mean volume of blood removed was 8.6 ml/kg. After each haemorrhage the blood was retransfused. The interval between successive haemorrhages was 20-40 mins and the order in which haemorrhages were carried out varied. Blood which had been removed was kept in siliconised containers and warmed to 38^{o}C prior to retransfusion.

In dogs, both slow and fast haemorrhage was carried out from an artery. In cats, fast haemorrhage was carried out from an artery and slow haemorrhage from a vein.

2. *The effect of the volume of haemorrhage* (5 dogs). Five additional dogs were subjected to continuous haemorrhage at a mean rate of 1.0 ml/kg/min until 25 ml/kg had been removed.

RESULTS

1. *Rate of haemorrhage*. The results obtained from a single experiment on a cat are shown in Fig. 3.1. Both slow and fast haemorrhage were associated with a fall in the base line of the rat stomach strip and of the unblocked rat colon, indicating a rise in the circulating levels of catecholamines, and a contraction of the blocked rat colon, indicating a generation of angiotensin. In this

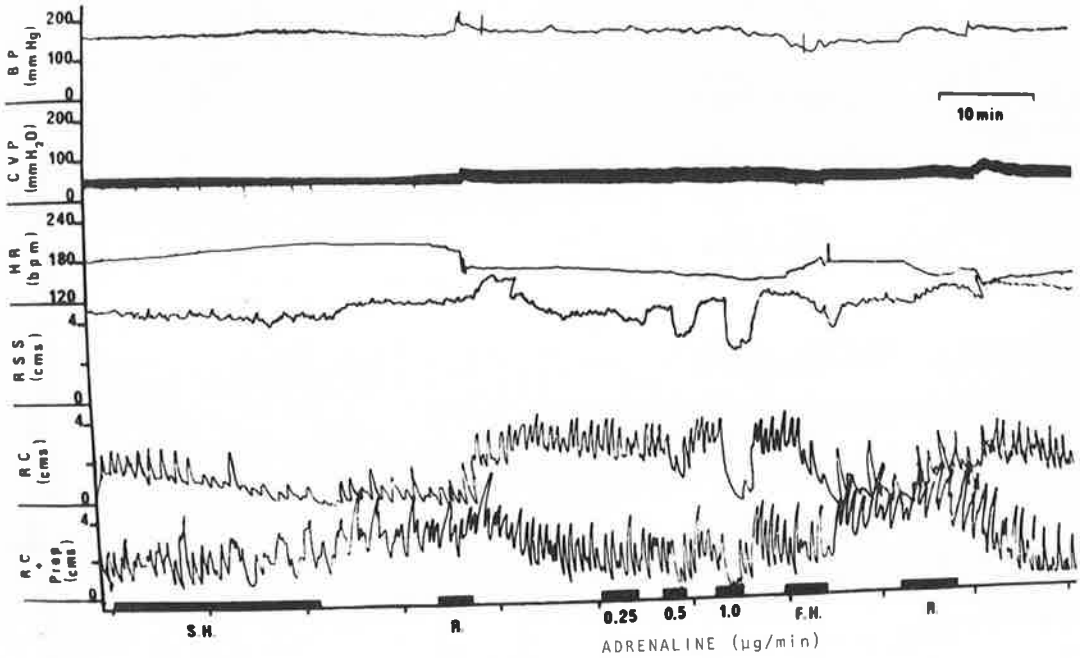


Fig. 3.1

Cat 3.1 kg. Female. Shows responses during slow haemorrhage (S.H.) and fast haemorrhage (F.H.). Note fall in base line of both the rat stomach strip and the rat colon without propranolol indicating a rise in circulating catecholamines during both slow and fast haemorrhage. A rise in circulating angiotensin levels is indicated by the rise in the base line of the propranolol-treated rat colon. Tissues return to control base lines after retransfusion (R).

experiment, the dose of adrenaline required to overcome the competitive β-blockade of the rat colon by propranolol was an intravenous infusion of adrenaline at a rate of 0.5-1.0 μg/min. This was in excess of the levels of catecholamines estimated during haemorrhage and, therefore, catecholamines would not be expected to interfere with the assay of angiotensin. A similar degree of β-blockade of the rat colon was established in all other experiments.

In all experiments, the return of the shed blood was associated with a return of the tissues to the prehaemorrhage baselines, indicating a return of circulating hormone levels to control values. However, the time course of the return of the tissues to the control levels varied for the rat stomach strip and the rat colon, the former occurring within 5 minutes, while the latter took 15-20 minutes. This is presumably a reflection of the half lives of catecholamines and renin in the circulation.

The changes in blood pressure, central venous pressure, angiotensin and catecholamine levels in cats and dogs are shown in Fig. 3.2. The mean changes ± 1 S.E. are plotted for both slow and fast rates of haemorrhage. Details of the data are shown in the appendix (Appendix 3.1 and 3.2). The statistical treatment of the results, showing changes within species and between species, is tabulated in Table 3.1.

Effect of haemorrhage on cats.

(a) Slow haemorrhage. During slow haemorrhage there was no significant change in arterial pressure. The change in central venous pressure was variable. Thus, there was a rise in one cat, no change in two, and a fall in four. The mean change was a fall of 5 mm H_2O: this was not significant. However, haemorrhage was associated with a significant elevation in both catecholamine and angiotensin levels. The mean rise in secretion rate of catecholamines was equivalent to 81 ng/kg/min ($P<.01$). The mean rise in angiotensin levels was equivalent to 17.6 ng/kg/min ($P<0.001$).

(b) Fast haemorrhage. During fast haemorrhage there was a significant fall in arterial pressure ($P<0.01$) but no significant

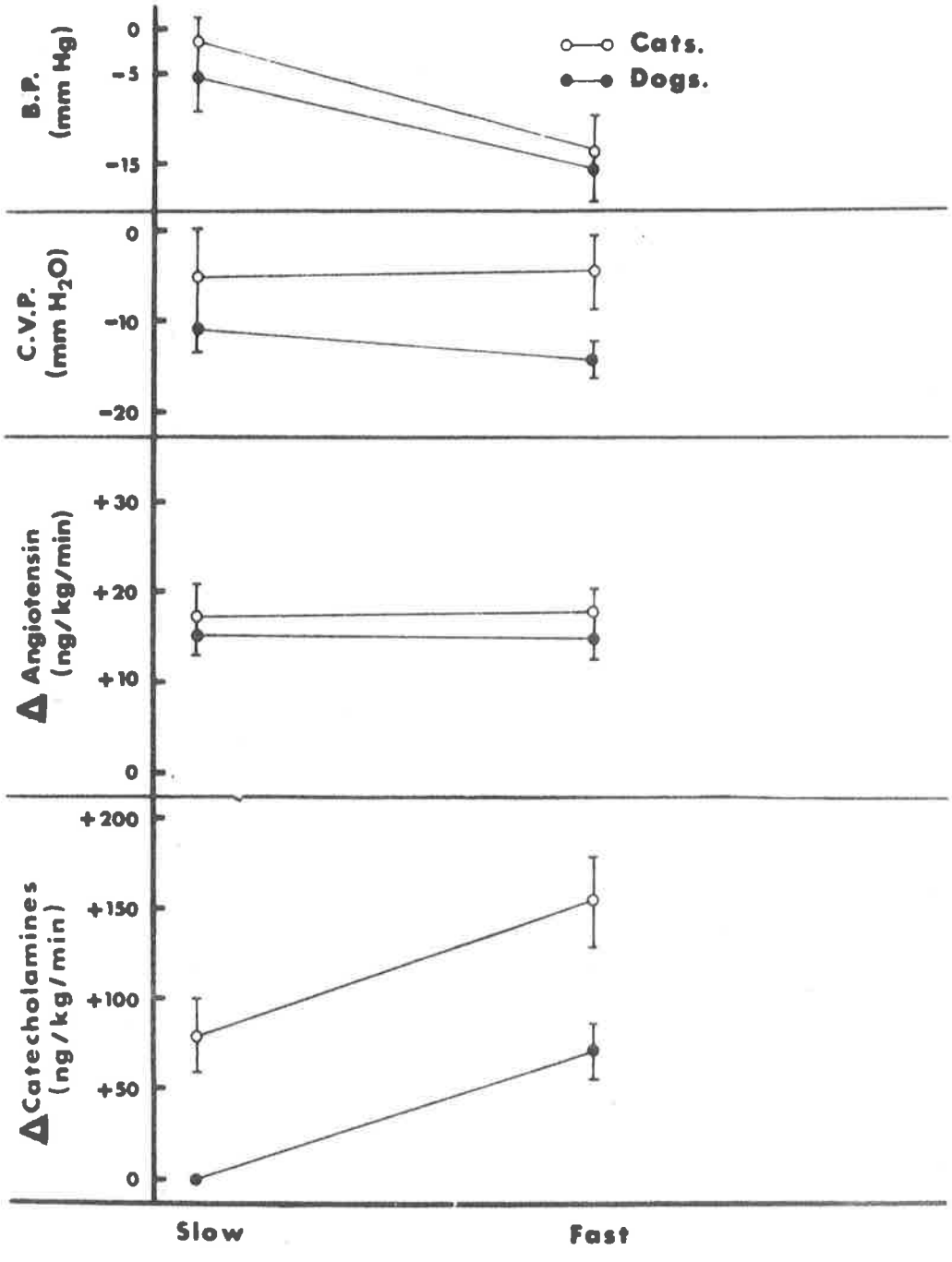


Fig. 3.2

Effect of rate of haemorrhage in cats and dogs. Changes from control values in blood pressure (B.P., mm Hg), central venous pressure (C.V.P., mm H_2O), angiotensin and catecholamine levels in cats (o) and dogs (●) during slow haemorrhage (Slow) and fast haemorrhage (Fast). Mean changes ± 1 S.E. are plotted. Significance data are shown in Table 3.1. (Appendix 3.1, 3.2)

TABLE 3.1

Statistical significance of data from which Fig. 3.1 was drawn (paired "t" test within species, unpaired "t" test between species).

	Slow Haemorrhage				*Fast Haemorrhage*			
	B.P.	C.V.P.	A-T	C-A	B.P.	C.V.P.	A-T	C-A
1	N.S.	N.S.	<0.001*	<0.01*	<0.01*	N.S.	<0.001*	<0.001*
2	N.S.	<0.01*	<0.001*	-	<0.01*	<0.001*	<0.001*	<0.01*
3	N.S.	N.S.	N.S.	<0.01*	N.S.	N.S.	N.S.	<0.05*

Shows significant P values for Student "t" test on changes from control values in blood pressure (B.P.), central venous pressure (C.V.P.), angiotensin (A-T) and catecholamine (C-A) levels during slow and fast haemorrhage.

1 Comparing changes in cats with control values.

2 Comparing changes in dogs with control values.

3 Comparing cats and dogs for each parameter.

N.S. No significant difference (P>0.05).

* Significant difference from control or between species.

fall in central venous pressure. The levels of angiotensin generated during fast haemorrhage were the same as those during slow haemorrhage. There was, however, a mean rise in catecholamine levels equivalent to 156 ng/kg/min above control levels. This was significantly greater than those observed during slow haemorrhage ($P<0.05$). The failure of central venous pressure to fall during fast haemorrhage may be due to the associated relatively high circulating levels of catecholamines. If this dose of adrenaline is infused into an intact cat, there is invariably a rise in central venous pressure of the order of 5-10 mm H_2O.

Effect of haemorrhage in dogs.

(a) Slow haemorrhage. Slow haemorrhage was associated with a small but significant fall in central venous pressure ($P<0.01$) but no significant change in arterial pressure. There was a significant rise in the circulating levels of angiotensin ($P<0.001$), equivalent to an intravenous infusion of 15.5 ng/kg/min. A rise in catecholamine levels was not observed in any dogs.

(b) Fast haemorrhage. Fast haemorrhage caused a significant fall in both central venous pressure ($P<0.001$) and arterial pressure ($P<0.01$). The fall in central venous pressure was not greater than that observed during slow haemorrhage. There was also no significant difference between the amount of angiotensin generated during fast haemorrhage as compared with slow haemorrhage. However, there was now a significant elevation of catecholamine levels equivalent to 73 ng/kg/min ($P<0.01$).

Comparison of cats and dogs. There was no significant difference between species in the change in arterial pressure during slow or fast haemorrhage. There was a more marked fall in central venous pressure in dogs, as compared with cats, at both rates of haemorrhage. This was, however, not significant (slow haemorrhage $P>0.4$; fast haemorrhage $P>0.05$). There was no significant difference between the angiotensin levels generated in either species at the slow or fast rate of haemorrhage. However, there was a significant difference between species with regard to catecholamine secretion. Slow

haemorrhage was not associated with a rise in catecholamine levels in the dog, while there was a significant rise in cats. Furthermore, this species difference was maintained at fast rates of haemorrhage, since catecholamine secretion in dogs was equivalent to 73 ng/kg/min, while in cats it was 156 ng/kg/min.

2. *Volume of haemorrhage.* Five dogs in this group were subjected to a slow continuous haemorrhage. The mean changes (± 1 S.E.) in arterial pressure, central venous pressure, angiotensin and catecholamine levels are shown in Fig. 3.3. Details of the data are tabulated in the appendix (Appendix 3.3).

The first parameter to change was central venous pressure which had fallen significantly after the removal of 5 ml blood per kg. This was associated with a rise in the circulating levels of angiotensin which was significant ($P<0.05$) after 10 ml/kg had been removed. There was at this time no fall in arterial pressure. Further angiotensin was generated as haemorrhage progressed until a mean generation rate of 18 ng/kg/min was reached. At this stage, 15 ml blood per kg had been removed and the fall in arterial pressure was 9 mm Hg from a mean control level of 135 mm Hg (± 4.2 S.E.). With continuing haemorrhage to 25 ml/kg arterial pressure fell to 80 mm Hg (± 12 S.E.), but there was no further significant rise in angiotensin levels.

There was no rise in the circulating levels of catecholamines until arterial pressure had fallen to 105 mm Hg (± 10.7 S.E.). As arterial pressure continued to fall, there was a rise in catecholamine levels which reached a secretion rate equivalent to 84 ng/kg/min when 25 ml/kg of blood had been removed and arterial pressure was 80 mm Hg. Adrenaline administered as a calibrating infusion to dogs prior to haemorrhage in a dose of approximately 84 ng/kg/min was associated with a pressor response ranging from 8 to 15 mm Hg and an invariable rise in central venous pressure of the order of 5-10 mm H_2O.

DISCUSSION

The results obtained by the simultaneous estimation of circulating levels of catecholamines and angiotensin confirm the results

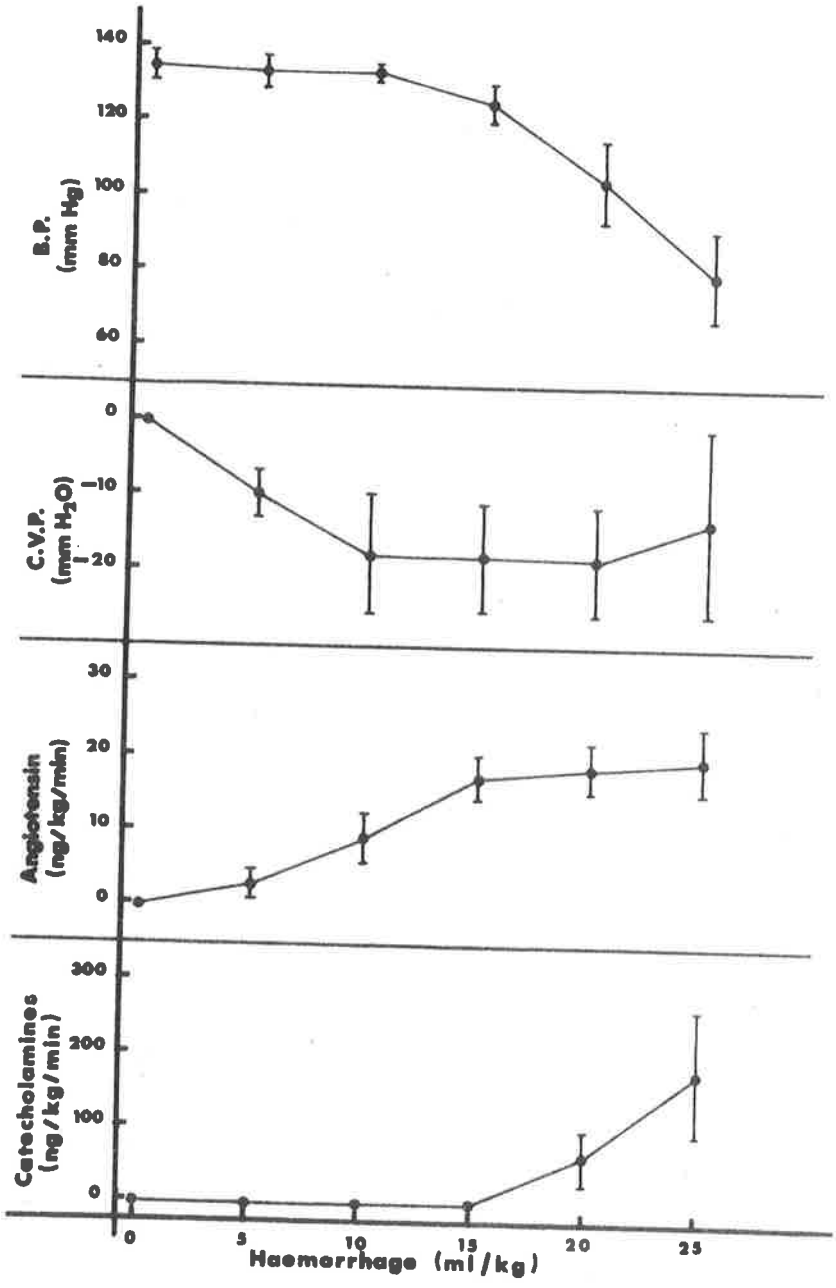


Fig. 3.3

Effect of slow progressive haemorrhage in dogs. On abscissa is plotted haemorrhage in ml/kg body weight. On ordinate is shown B.P. (mm Hg) and changes in central venous pressure (C.V.P., mm H_2O), angiotensin and catecholamine levels. The means ± 1 S.E. are plotted. (Appendix 3.3).

of previous workers who considered each hormone separately. Since a continuous estimation was carried out, it is possible not only to relate the renin-angiotensin and sympatho-adrenal systems to the severity of haemorrhage, but also to establish the timing of changes in the circulating levels of each hormone.

These results show that the relative importance of the renin-angiotensin and sympatho-adrenal systems in response to a given stress cannot be assessed if the stress imposed is severe, since a fall in arterial pressure to 40-50 mm Hg will activate both systems simultaneously.

The results show that there is no species difference in the response of the renin-angiotensin system and, furthermore, the maximum generation rate of angiotensin of approximately 17 ng/kg/min precedes any significant fall in arterial pressure.

The observations in the dog provide strong evidence that a fall in central venous pressure is the primary stimulus to renin release, the afferent pathway presumably being via the vagus nerve (Hodge, Lowe, Ng & Vane, 1969) and the efferent via the sympathetic nerves (Hodge *et al.*, 1966a).

In contrast to the changes in angiotensin levels, there is a marked species difference in the release of catecholamines following an equivalent stress, providing support for the early observations of Bacq & Brouha (1934) and Cannon (1940). Thus, in the dog, no increase in catecholamine levels was detected until there was a fall in arterial pressure, while in the cat there was a consistent rise in catecholamines during slow haemorrhage although there was no significant fall in arterial pressure. These observations in the cat confirm those of Hökfelt *et al.* (1962), who suggested that a fall in blood volume may be an important stimulus to catecholamine release. However, since central venous pressure showed no significant fall in the present study, the precise stimulus for an increased activity of the sympatho-adrenal system remains obscure.

These experiments cannot provide a definite answer as to the possible afferent pathways or receptors involved in detecting the

haemodynamic changes during haemorrhage. However, in the dog there was no detectable elevation of catecholamines until there was a fall in arterial pressure, suggesting that at least in this species baroreceptor control of the adrenal medulla is important. Thus, in group 1, a constant volume of blood was removed, but at two rates. The faster rate was associated with both a significant fall in arterial pressure and a significant rise in catecholamines. In contrast, during the slower rate of haemorrhage, while there was a significant fall in central venous pressure, there was no significant fall in arterial pressure and no change in catecholamine levels was observed.

Haemorrhage is associated with hyperventilation, an elevated arterial PO_2 and a fall in arterial PCO_2 in the cat (Daly, Lambertsen & Schweitzer, 1954) and dog (Bjurstedt & Coleridge, 1962). Stimulation of the chemoreceptors and subsequent stimulation of the adrenal medulla is thus unlikely. However, the precise stimulus to hyperventilation which occurs following haemorrhage is unknown and may also stimulate the sympatho-adrenal system in both the cat and dog.

In both species, the greater cardiovascular stress of fast haemorrhage was not associated with a greater generation of angiotensin. Furthermore, during progressive slow haemorrhage in the dog, an apparent peak generation rate of angiotensin of 15-20 ng/kg/min was observed after 15 ml/kg of blood had been removed. This suggests that the generation of angiotensin may have been approaching maximal physiological rates. If this amount of angiotensin II is administered as an intravenous infusion to a non-haemorrhaged dog, it has a minimal pressor effect. This, then, raises the question of the role of angiotensin in circulatory homeostasis and it appears that its direct effects on blood vessels are of minor importance. However, the action of low doses of angiotensin on the area postrema and the associated circulatory effects appear to be important in maintaining arterial pressure during haemorrhage in the greyhound (Katic, Joy, Lavery, Lowe & Scroop, 1971).

A further possibility is a selective action of angiotensin

on a specific vascular bed such as the kidney. In this respect, it is interesting to note that in the few studies in which low doses of angiotensin have been used, the renal response is an anti-natriuresis and a fall in glomerular filtration rate (Gross *et al.*, 1965; Malvin & Vander, 1967; Schmid, 1968). The stimulation of aldosterone release and antidiuretic hormone by subpressor doses of angiotensin (Bonjour & Malvin, 1970) is further evidence that the homeostatic mechanism of the renin-angiotensin system is related to salt and water balance, and hence extracellular fluid volume and blood volume, rather than being directly concerned with arterial pressure.

SECTION 4

THE EFFECT OF HYPOXIA ON CATECHOLAMINE LEVELS IN THE DOG

The responses of the cardiovascular and the sympathetic nervous systems to hypoxia have been reviewed by Korner (1959) and Gowdey (1966). Several studies in a variety of species have confirmed the original observations of Cannon & Hoskins in the cat (1911) that asphyxia is associated with elevated catecholamine levels (Anrep, 1912; Kellaway, 1919; Houssay & Molinelli, 1926). However, asphyxia not only causes hypoxia but also hypercarbia. Kellaway (1919) recognised this and demonstrated a rise in catecholamine levels in conscious cats during hypoxia alone. The techniques used in these early experiments could not quantitate the catecholamines released.

More recent studies have determined the amount of catecholamines released in hypoxia and haemorrhage using the rabbit aortic strip (Fowler *et al.*, 1961) or fluorimetric techniques (Harrison & Seaton, 1965). However, the degree of hypoxia in these studies was severe (PO_2 30-50 mm Hg). The present study was carried out to determine the effect of varying degrees of hypoxia on the circulating levels of catecholamines. Since catecholamines were assayed by superfusion, the timing of their release could also be studied.

METHODS

Experiments were carried out on 20 dogs, weighing from 11-36 kg. Surgery was as described in the Methods section. Hypoxia was induced by adding nitrogen to the inlet side of the Palmer respirator. Eight dogs were also haemorrhaged to a mean arterial pressure of 60 mm Hg and the change in catecholamine levels were assayed. Haemorrhages were carried out after these animals had been subjected to a wide range of hypoxia.

Catecholamine levels were assayed by superfusing either two rat stomach strips and a rat colon, or a rat stomach strip, a chick rectum and a rat colon. The use of a rat stomach strip and a chick rectum enables a differential assay of noradrenaline and adrenaline to be carried out. However, in this study, changes in catecholamine

levels were expressed as total catecholamines equivalent to a known infusion of adrenaline: only a qualitative differential assay was carried out.

Moderate falls in the PO_2 of the blood superfusing the rat stomach strip cause this tissue to relax (Smith & Vane, 1966), thus mimicking the response to a rise in circulating catecholamine levels. Therefore, the superfusion circuit was modified by inserting a bubble oxygenator after the roller pump and before the assay tissues (Fig. 4.1). Air was bubbled through the oxygenator; PO_2 levels were measured simultaneously in arterial blood before the roller pump (dog P_aO_2) and before the tissues (tissue P_aO_2).

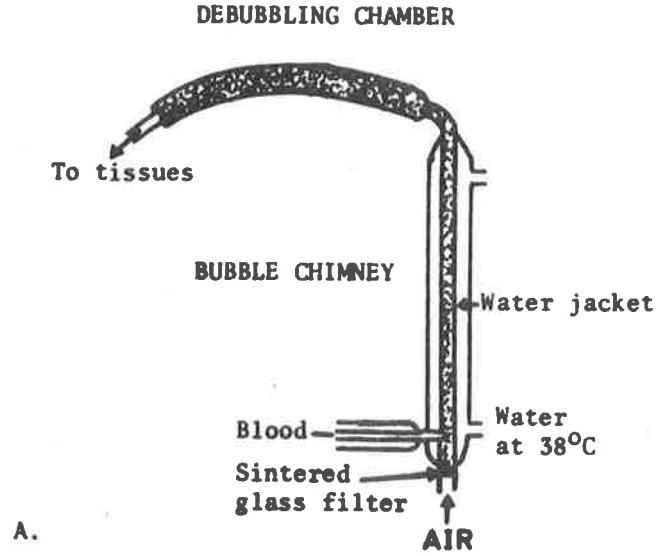
Arterial samples were taken for measurement of PO_2, PCO_2 and pH using a Radiometer Blood Micro system (BMS 3) and Acid Base Analyser (PHM 72). In some dogs continuous oxygen tension measurements were made using a Titron oxygen macro-electrode.

RESULTS

The results obtained in a single experiment are shown in Fig. 4.2, which shows the responses obtained to intravenous adrenaline (2.5 µg/min) and a period of nitrogen hypoxia.

During the infusion of adrenaline, there was a moderate rise in both arterial and central venous pressure. Both the rat stomach strip and chick rectum subsequently relaxed and the spontaneous activity of the unblocked rat colon was inhibited. The P_aO_2 was unchanged at 120 mm Hg. In this experiment the addition of nitrogen to the inspired air reduced arterial PO_2 from 120 mm Hg to approximately 40 mm Hg. During hypoxia there was a rise in arterial pressure but no change in central venous pressure. The rat stomach strip responded by a relaxation approximately equivalent to the infusion of adrenaline. However, in this experiment the chick rectum relaxed considerably less than during the infusion of adrenaline, indicating that both adrenaline and noradrenaline were released. Further support for this is provided by the fact that there was no inhibition of the spontaneous action of the rat colon; in fact, the rise in base line indicates a simultaneous generation of angiotensin.

The results for blood gas analysis in all animals are shown in



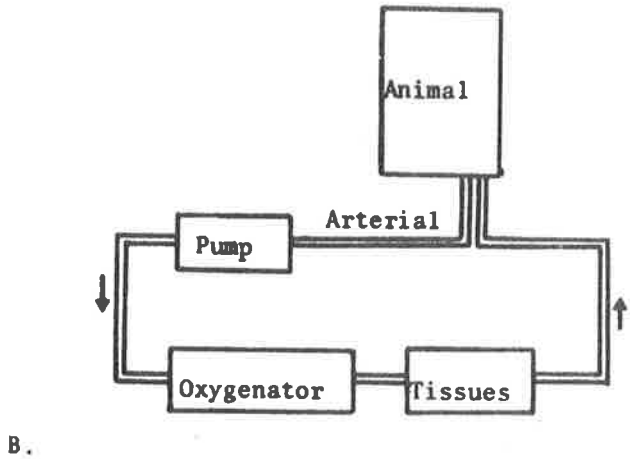


Fig. 4.1

A. Bubble oxygenator.
B. Modification of superfusion circuit.

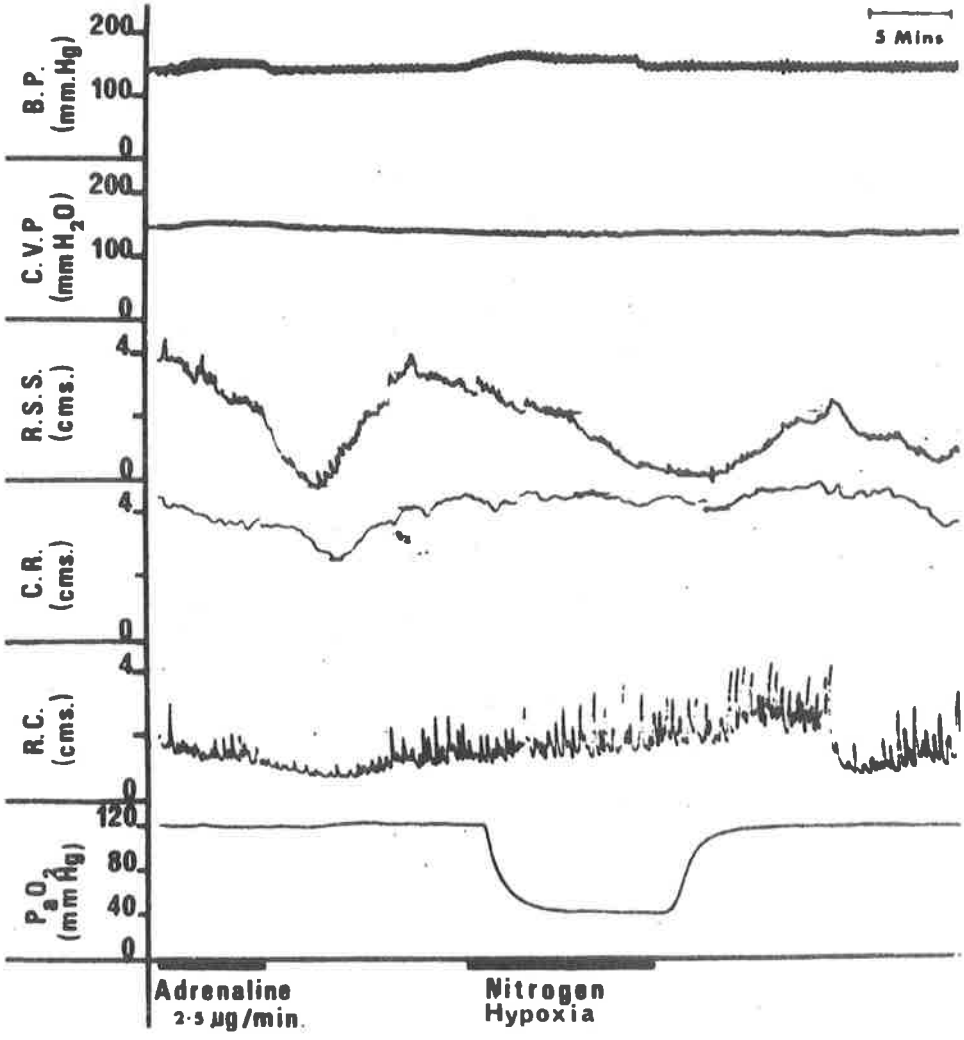


Fig. 4.2

Female Dog, 16 kg. From top to bottom, mean arterial pressure (mm Hg), central venous pressure (mm H_2O), rat stomach strip (R.S.S.), chick rectum (C.R.), rat colon (R.C.) and arterial PO_2. The responses to an infusion of 2.5 µg/min adrenaline and a period of hypoxia (P_aO_2 = 40 mm Hg) are shown.

Table 4.1. The mean arterial oxygen tension was 109 mm Hg (S.D. 16). Control arterial pH and PCO_2 values were 7.36 and 20.9 mm Hg, respectively. During periods of hypoxia there was no significant change in pH or PCO_2. Thus the responses recorded were to hypoxia alone.

There was no change in pH or PCO_2 of the arterial blood superfusing the tissues in hypoxic periods as compared to control periods. The mean oxygen tension during control periods was 160 mm Hg (S.D. 14); during hypoxia the mean oxygen tension was 156 mm Hg (S.D. 16). While this was a significant reduction ($P<0.05$), a mean fall in PO_2 of 4 mm Hg has no effect on the rat stomach strip (Smith & Vane, 1966). Changes in the base line of the rat stomach strip were thus due to changes in circulating catecholamine levels.

The results obtained from all experiments are shown in Fig. 4.3, which shows the rise in mean arterial pressure and catecholamine levels for a range of P_aO_2 values 30 mm Hg - 80 mm Hg in 10 mm Hg increments. Details of the data are shown in Appendix 4.1. A fall in arterial PO_2 was invariably associated with a rise in mean arterial pressure. This rise was significant for PO_2 of less than 70 mm Hg, and, furthermore, this rise in arterial pressure during P_aO_2 30-40 mm Hg was significantly greater than the rise which occurred in the range 40-70 mm Hg.

The changes in catecholamine levels were variable both between animals and within the same animal. However, there was a progressive rise in mean catecholamine levels for arterial oxygen tensions 80 mm Hg - 50 mm Hg. This rise was a significant elevation above controls for P_aO_2 below 70 mm Hg. However, despite progressively lower arterial oxygen tensions, there was no further significant elevation in catecholamines.

For comparative purposes, the change in catecholamine levels in 8 dogs bled to 60 mm Hg is also shown in Fig. 4.3. Details of the data are shown in Appendix 4.1. In all animals the rise in catecholamine levels was considerably greater than the rise occurring during oxygen tensions of 30-50 mm Hg in the same animals.

TABLE 4.1

Mean pH, PCO_2 and PO_2 during control periods and periods of hypoxia in blood from the dog and blood after the bubble oxygenator. The means ± 1 S.D. are shown. Significance levels shown are as determined by paired "t" test.

	DOG			TISSUE		
	pH	PCO_2	PO_2	pH	PCO_2	PO_2
Control	7.36±0.06	20.9±6.0	109±16	7.47±0.11	<8	160±14
Hypoxia	7.37±0.07	21.6±5.5	-	7.46±0.12	<8	156±16
Significance	N.S.	N.S.	-	N.S.	-	P<0.05

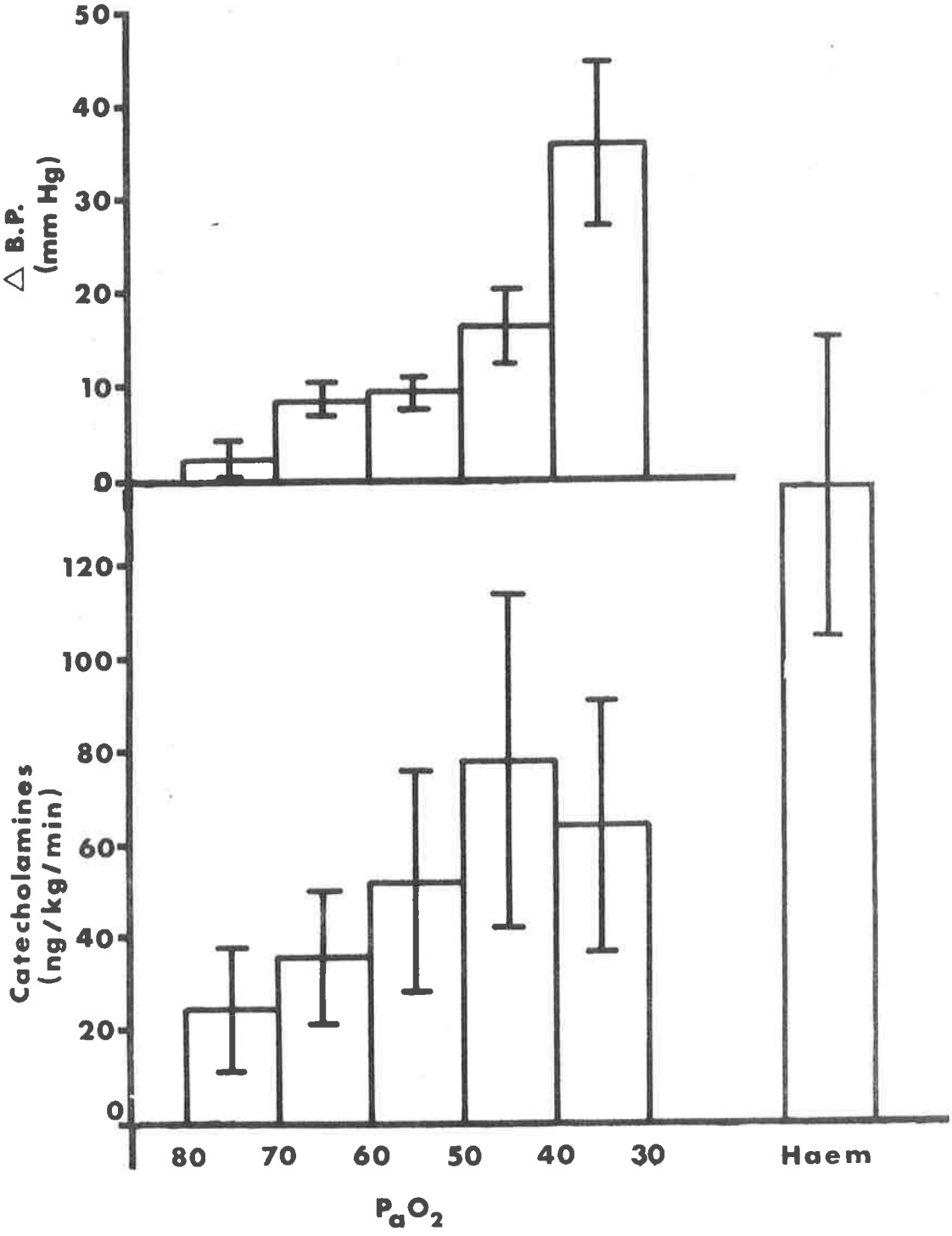


Fig. 4.3

The changes in arterial pressure and catecholamine levels for progressively severe periods of hypoxia (80-30 mm Hg). For direct comparison the response to haemorrhage to a B.P. of 60 mm Hg (Haem) is also shown. (Appendix 4.1)

DISCUSSION

Despite marked falls in arterial PO_2 in these experiments, there was no significant change in either PCO_2 or pH. The responses observed were therefore to pure hypoxia. Furthermore, the mean change in oxygen tension in the blood superfusing the assay tissues (4 mm Hg) was insufficient to cause a relaxation of the rat stomach strip. Thus a relaxation observed during hypoxia was due to an increase in circulating catecholamine levels and not due to hypoxia *per se*.

These results show that while severe hypoxia is invariably associated with a marked rise in arterial pressure, the change in circulating catecholamine levels is more variable. Furthermore, the change in catecholamines is moderate when compared to the change associated with haemorrhage. These findings are similar to those reported previously (Fowler *et al.*, 1961) in which catecholamines were measured during hypoxia and moderate and severe haemorrhage. However, in these studies animals were subjected to a single hypoxic stimulus and, since intermittent sampling was used, the timing of the rise in circulating levels of catecholamines could not be established. The results in the present study show that the release invariably occurred within one minute of rendering the animal hypoxic and, furthermore, the change in catecholamines was sustained for the period of hypoxia.

The assays as performed cannot differentiate quantitatively between adrenaline and noradrenaline. However, the responses of the chick rectum indicate that noradrenaline as well as adrenaline levels were elevated during hypoxia. Basal catecholamine secretion in adrenal vein blood of the dog contains approximately 10% noradrenaline, and this percentage is unchanged, or decreased, during hypoxia (Malmejac, 1958). Since the technique used in the present study measures changes from a control level, these results suggest a greater proportion of noradrenaline, rather than adrenaline, may be released during hypoxia. The marked rise in arterial pressure during hypoxia is secondary to increased sympathetic nerve activity (Korner, 1959; Iriki *et al.*, 1971). Noradrenaline is the transmitter substance at sympathetic nerve terminals, and the increase in noradrenaline in

arterial blood may be due in part to noradrenaline from vascular sympathetic nerve endings diffusing through the vessel wall and therefore reaching the general circulation.

The precise stimulus to catecholamine secretions is unknown, but probably involves chemoreceptors in the aortic and carotid bodies, since a fall in P_aO_2 of blood perfusing the isolated carotid body of the dog is associated with a tachycardia despite cardiac denervation (Daly & Scott, 1962). In the conscious rabbit, chemoreceptors accounted for an increase in arterial pressure, heart rate and cardiac output during hypoxia (Korner, 1965). The importance of the splanchnic nerves have been demonstrated by experiments showing increased sympathetic activity in visceral nerves during hypoxia (Iriki *et al.*, 1971) and the failure of severe hypoxia to cause catecholamine release in spinal dogs (Cantu, Nahas & Manger, 1966). Furthermore, hypoxia is a weak stimulus to catecholamine release in the isolated adrenal gland (Bulbring, Burn & de Elio, 1948). In the present study a significant elevation in catecholamines occurred with a reduction of P_aO_2 to the range 60-70 mm Hg. However, despite a further rise in mean catecholamine levels during a reduction in P_aO_2 to 40-50 mm Hg, there was no further significant rise in catecholamine levels. Furthermore, the mean rise in catecholamines for the lowest range of P_aO_2 studied (30-40 mm Hg) was less than for the P_aO_2 range 40-50 mm Hg. Since haemorrhage at the end of some experiments was consistently associated with a rise in catecholamine levels greater than those observed during the most severe periods of hypoxia, exhaustion of the catecholamine content of the adrenal medulla is not a possible explanation for the fall in response to severe hypoxia. A possible explanation for this fall in catecholamine levels is the fact that the rise in arterial pressure during P_aO_2 30-40 mm Hg was significantly higher than for any previous level of hypoxia. Bilateral carotid occlusion is associated with increased catecholamine levels in cats (Kaindl & von Euler, 1951) and dogs (de Schaepdryver, 1959). Thus it is possible that a marked rise in arterial pressure is associated with a specific inhibition of the adrenal medulla despite marked sympathetic activity elsewhere.

SECTION 5

CHANGES IN CIRCULATING LEVELS OF ANGIOTENSIN AND CATECHOLAMINES IN ENDOTOXIN SHOCK IN CATS AND DOGS

In a previous section of this thesis the rise in circulating levels of angiotensin in dogs during haemorrhage was related to a fall in central venous pressure rather than a fall in arterial pressure. Intravenous administration of endotoxin causes rapid and severe haemodynamic changes in both the cat and dog (Gilbert, 1960). Following administration of endotoxin in both species there is a variable brief period of systemic hypotension, succeeded by a temporary recovery and a later progressive decline in blood pressure. However, the cause of the initial hypotensive phase in the two species is different. In the cat, marked pulmonary vasoconstriction leads to pulmonary hypertension together with a rise in central venous pressure and a fall in cardiac output (Kuida, Gilbert, Hinshaw, Brunson & Visscher, 1961; Greenway, Lautt & Stark, 1969). In the dog, there is a marked rise in portal venous pressure associated with a fall in central venous pressure (Maclean & Weil, 1956; Weil *et al.*, 1956; Kuida *et al.*, 1961). Because of the species differences in the haemodynamic response to endotoxin and, in particular, the opposite effects on central venous pressure, it was decided to investigate the changes in both catecholamine and angiotensin levels following endotoxin. In order to compare the responsiveness of the renin-angiotensin system in cats and dogs, graded aortic occlusion was carried out and the angiotensin generated was bracket-assayed.

EXPERIMENTAL GROUPS

1. Endotoxin. The vascular response to endotoxin was determined in 12 cats and 10 dogs. Changes in catecholamine levels were determined in 12 cats and 8 dogs; changes in angiotensin levels were determined in 9 cats and 9 dogs.

After calibration of the tissues all animals received 2 mg/kg E. coli endotoxin as a 5-15 minute i.v. infusion. Observations were then continued for up to 90 minutes. All experimental animals

except one cat survived the experimental period.

2. *Reduced renal perfusion pressure* (4 dogs, 6 cats). In this group the generation of angiotensin in response to graded reduction in renal perfusion pressure was studied. The total experimental period was of the same order as in group 1.

The aorta was exposed through a mid-line incision and a loose ligature was passed around the aorta above the renal arteries. Arterial pressure was recorded from a carotid and a femoral artery in dogs. In cats, the pressure above the occlusion was recorded either from a carotid artery, or via a catheter threaded into the thoracic aorta via a femoral artery. Pressure below the occlusion was recorded from a femoral artery. Graded reduction in renal artery perfusion pressure was produced by tightening the aortic ligature. Changes in angiotensin levels were bracket-assayed with i.v. angiotensin II for each reduction in pressure.

3. *Direct effect of endotoxin on assay tissues* (3 dogs). Endotoxin was infused into the extra-corporeal circuit in amounts calculated to be of the same order as those expected to be circulating in animals in group 1. The contact time of the endotoxin with blood was equivalent to the circulation time from the femoral artery to the assay tissues in group 1.

RESULTS

1. *Endotoxin.* The results obtained from a single cat are shown in Fig. 5.1. After endotoxin, there was a rise in central venous pressure associated, in this animal, with little change in blood pressure. There was a relaxation of both rat stomach strip preparations, indicating a rise in catecholamine levels. There was a later contraction of the rat colon, indicating a rise in angiotensin levels.

The changes in mean arterial pressure (± 1 S.E. of the mean) and central venous pressure (± 1 S.E. of the mean) in cats and dogs are shown in Fig. 5.2. Detailed data is given in Appendix 5.1.-5.4 While the pattern of the blood pressure change is similar in the two species, the effect of the endotoxin on central venous pressure is

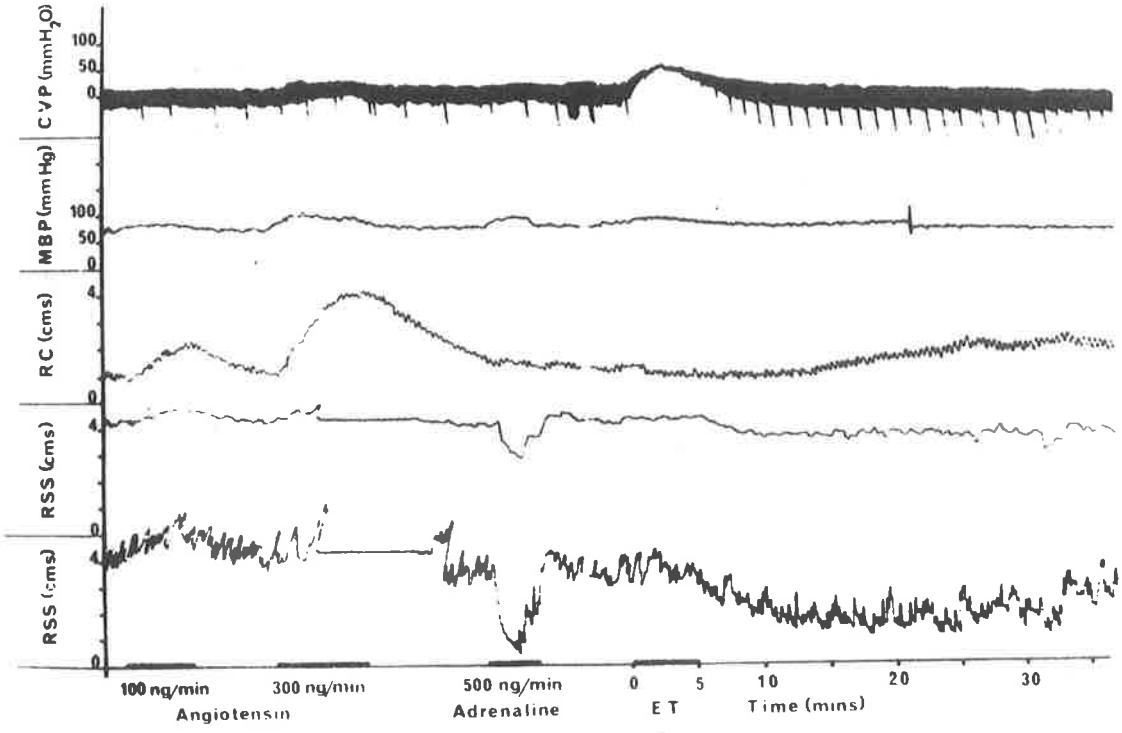


Fig. 5.1

Cat 2.2 kg. Female. From top to bottom, central venous pressure (mm H_2O), mean arterial pressure (mm Hg), a rat colon (R.C.) and two rat stomach strips (R.S.S.). The response to i.v. infusions of angiotensin (100 ng/min and 300 ng/min) and adrenaline 500 ng/min are shown. At time O endotoxin was administered as a 5 min i.v. infusion. There followed a relaxation of both rat stomach strips and a later contraction of the rat colon. (Response of R.S.S. to 300 ng/min angiotensin not recorded due to contraction of R.S.S.)

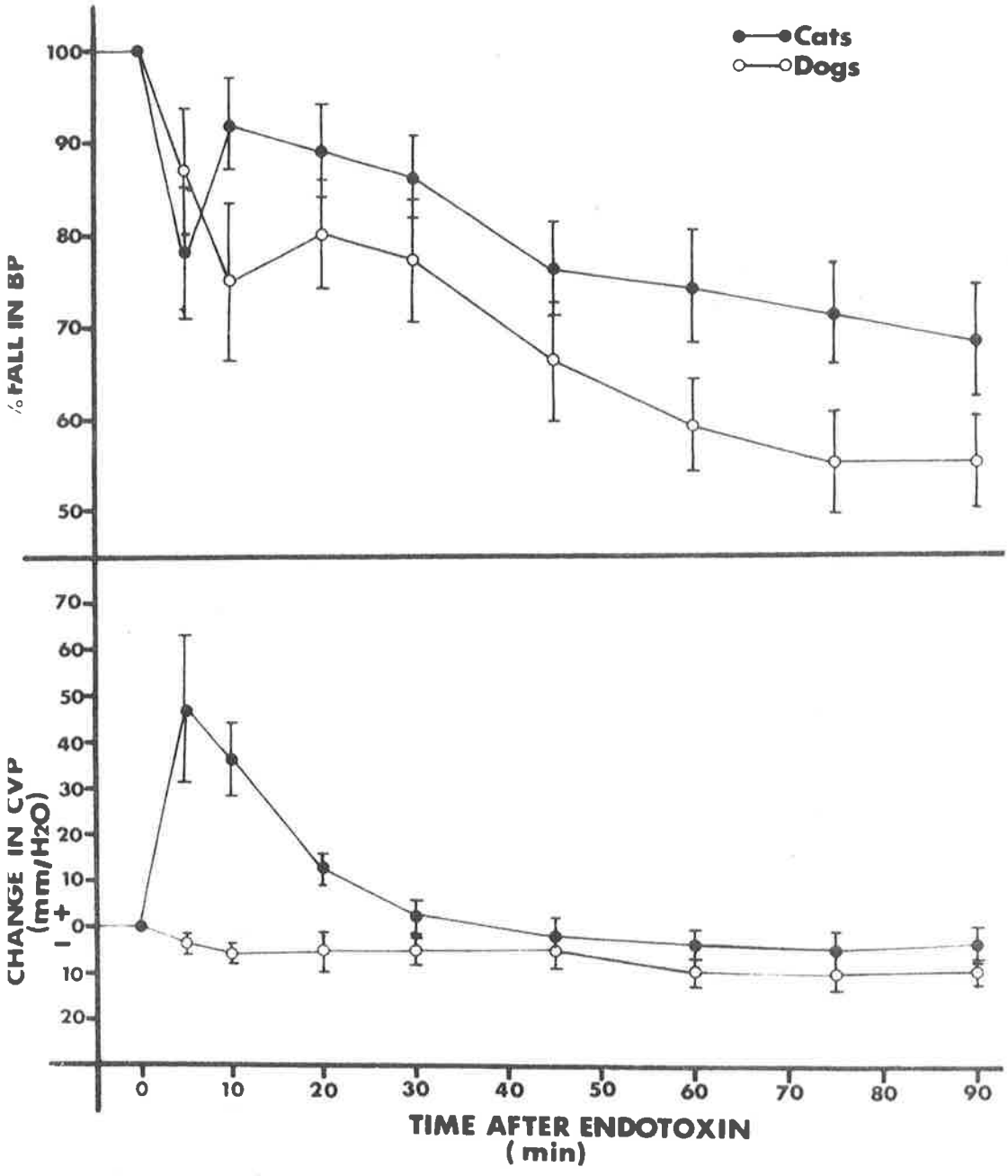


Fig. 5.2

Effect of endotoxin on blood pressure (% control value) and central venous pressure (mean change) in cats and dogs. The means ± 1 S.E. are plotted. (Appendix 5.1-5.4)

different for 30-40 min following administration of endotoxin. Thus a rise in central venous pressure occurred in all cats, reaching a peak 5 min after the endotoxin, whilst in dogs there was a progressive fall in central venous pressure. After 30-40 min the central venous pressure had fallen in all cats and it now followed a pattern similar to that seen in dogs.

The changes in circulating levels of catecholamines are compared in Tables 5.1a and b; changes in circulating levels of angiotensin are compared in Tables 5.2a and b.

The mean changes in catecholamine and angiotensin levels in cats and dogs are shown in Fig. 5.3. The standard errors and the significance level of the difference between the means at each time are tabulated in Table 5.3. In some animals the responses were greater than the maximal tissue response obtained during calibration. Hence, the value taken as the mean (Fig. 5.3) is an underestimate of the true change. Since the pre-endotoxin hormone levels cannot be determined by the superfusion technique, the results shown refer to changes from the control values.

Catecholamine levels in the cat. In nine out of twelve cats, there was an initial rise in catecholamine levels, followed by a fall and a subsequent secondary rise. The timing of the initial rise was variable, but in eight out of twelve cats had commenced within 5 min. Estimation of catecholamine output, using the nictitating membrane, gave similar results to those obtained using the rat stomach strip, showing that if hypoxia occurred after giving the endotoxin (Gilbert, 1960), it could not account for the relaxation of the rat stomach strip.

Catecholamine levels in the dog. In two out of eight dogs, there was a rise in catecholamine levels within 5 min. The large output in one of these (23) was associated with marked hypotension. During the succeeding 5 min there was a rise in a further two dogs. There was a variable rise in catecholamine levels occurring within 45-60 min in the remaining dogs.

Angiotensin levels in the cat. Circulating levels of

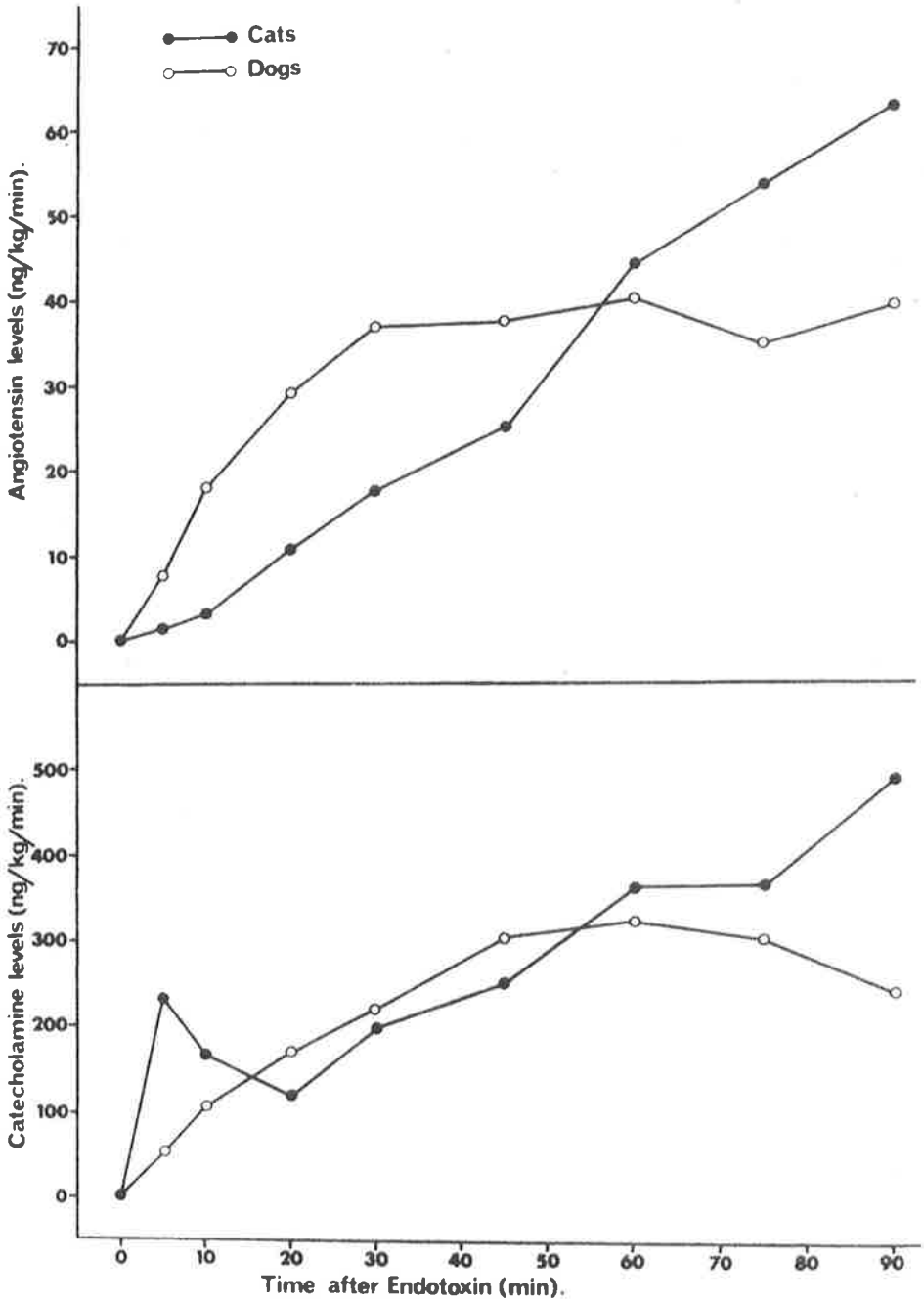


Fig. 5.3

Effect of endotoxin on circulating levels of angiotensin and catecholamines in cats (●) and dogs (o). The mean values shown in Tables 5.1a and b, and 5.2a and b are plotted. Statistical treatment of the data is shown in Table 5.3.

TABLE 5.1A

Increases in catecholamine levels in cats expressed as ng/kg/min following endotoxin.

	Minutes after Endotoxin							
Cat	5	10	20	30	45	60	75	90
1	0	130	0-100	130	130	130	200	230
2	0	220	280	280	280	280	310	310
4	720	0	320	320	400	480	800	1200
6	0	91	136	*	*	*	*	*
12	300	200	200	200	200	400	400	400
13	500	200	200	200	250	200	250	-
14	35	185	20	56	56	65	83	93
16	0	0	0	200	430	1000	430	*
17	110	280	0	0	90	185	295	555
18	370	130	0	*	*	*	*	-
19	>450	310	220	190	*	*	-	-
20	320	240	290	420	420	520	520	645
Mean	234	166	143	200	251	362	365	490

TABLE 5.1B

Increases in catecholamine levels in dogs expressed as ng/kg/min.

	Minutes after Endotoxin							
Dog	5	10	20	30	45	60	75	90
2	0	0	0	0	0	0	0	150
3	30	37	0	0	0	0	0	0
7	0	0-80	250	500	>800	400	250	150
10	0	0	0	0	170	400	>700	>700
15	0	0	340	460	460	570	500	-
18	0	320	320	570	570	640	570	570
20	0	0	0	0	130	260	110	110
23	>450	>450	>450	*	*	*	*	*
Mean	60	106	170	219	304	324	304	280

* Angiotensin inhibiting the response of the rat stomach strip.

TABLE 5.2A

Increases in angiotensin levels in cats expressed as ng/kg/min following endotoxin.

	Minutes after Endotoxin							
Cat	5	10	20	30	45	60	75	90
2	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0
6	0	0	34	57	57	91	91	125
12	0	0	0	0	25	45	75	100
14	0	0	0	3	4	5	6	8
16	0	0	0	16	16	130	>130	>130
17	0	0	0	20	55	*	*	*
18	0	0	0	0-10	37	56	56	67
20	13	29	>65	>65	31	>65	>65	>65
Mean	1.4	3.2	11.0	17.9	25.0	49.0	52.9	61.9

TABLE 5.2B

Increases in angiotensin levels in dogs expressed as ng/kg/min following endotoxin.

	Minutes after Endotoxin							
Dog	5	10	20	30	45	60	75	90
2	0	25	56	80	80	80	80	80
3	30	37	62	75	75	78	-	63
6	30	30	30	30	30	30	30	30
7	0	20	20	*	*	*	*	*
10	10	10	10	10	10	17	17	17
15	0	0	9	9	9	9	9	9
18	0	36	57	>70	>>70	>>70	>>70	>>70
20	0	0	11	13	13	20	20	20
23	0	6	7	7	12	15	20	23
Mean	7.8	18.2	29.1	36.8	37.4	38.9	35.4	39.0

* Catecholamines inhibiting the response of the rat colon.

TABLE 5.3

Comparison of changes in catecholamine and angiotensin levels in cats and dogs expressed as ng/kg/min following endotoxin. The mean levels ± 1 S.E. of the mean and the P value for Student "t" test for the significance between species at each time are tabulated.

		Minutes after Endotoxin							
		5	10	20	30	45	60	75	90
Cats	Catecholamine levels	234	166	143	200	251	362	365	490
	± 1 S.E.	71	29	36	39	48	95	69	138
Dogs	Catecholamine levels	60	106	170	219	304	324	304	280
	± 1 S.E.	56	62	67	104	117	96	108	116
	P	0.1-0.05	0.4-0.3	0.8-0.7	0.9-0.8	0.7-0.6	0.8-0.7	0.7-0.6	0.3-0.2
Cats	Angiotensin levels	1.4	3.2	11.0	17.9	25	49	52.9	61.9
	± 1 S.E.	1.4	3.2	7.7	8.5	7.3	16.6	16.8	19.2
Dogs	Angiotensin levels	7.8	18.2	29.1	36.8	37.4	39.9	35.1	39.0
	± 1 S.E.	4.3	4.9	7.7	11.5	11.3	10.8	10.6	9.7
	P	0.2-0.1	0.05-0.02	0.2-0.1	0.3-0.2	0.4-0.3	0.7-0.6	0.5-0.4	0.4-0.3

angiotensin rose in only one cat within 10 min. Within 20 min after the endotoxin a second cat showed a rise in angiotensin levels. There was then a progressive rise in all but two of the nine cats.

Angiotensin levels in the dog. In all dogs, there was a progressive rise in angiotensin levels. The generation of angiotensin commenced within 5 min in three dogs, and during the following 5 min was observed in all but two dogs. Within 20 min of giving the endotoxin all dogs showed a rise in circulating levels of angiotensin.

Comparison of humoral responses in cats and dogs.

The changes in catecholamine and angiotensin levels are compared in Table 5.3. Mean catecholamine levels were higher in the cat than the dog up to 10 min after giving the endotoxin. This difference was barely significant 5 min after giving endotoxin ($P<0.1$). At all other times, there was no significant difference between catecholamine levels in the two species.

Mean angiotensin levels in the dog were higher until 60 min after giving the endotoxin. This difference was significant 10 min after endotoxin ($P<0.05$).

2. *Reduced renal perfusion pressure.* The effect of partial occlusion of the aorta above the renal arteries on circulating levels of angiotensin is shown in Fig. 5.4.

In all animals, partial aortic occlusion was associated with increased levels of angiotensin. In some animals in which the response to graded constriction of the aorta was studied, the amount of angiotensin generated increased with increasing reductions in renal artery pressure. No species difference was apparent.

3. *Direct effect of endotoxin on assay tissues.* The circuit time from animal to assay tissues was 0.75 min for dogs and 0.5 min for cats in experiments in group 1. Endotoxin was infused into the extra-corporeal circuit to allow incubation in blood for up to 1 min. In none of the experiments was there a change in the base line of the rat colon. In two experiments, there was a minimal contraction of the rat stomach strip: a relaxation was never observed.

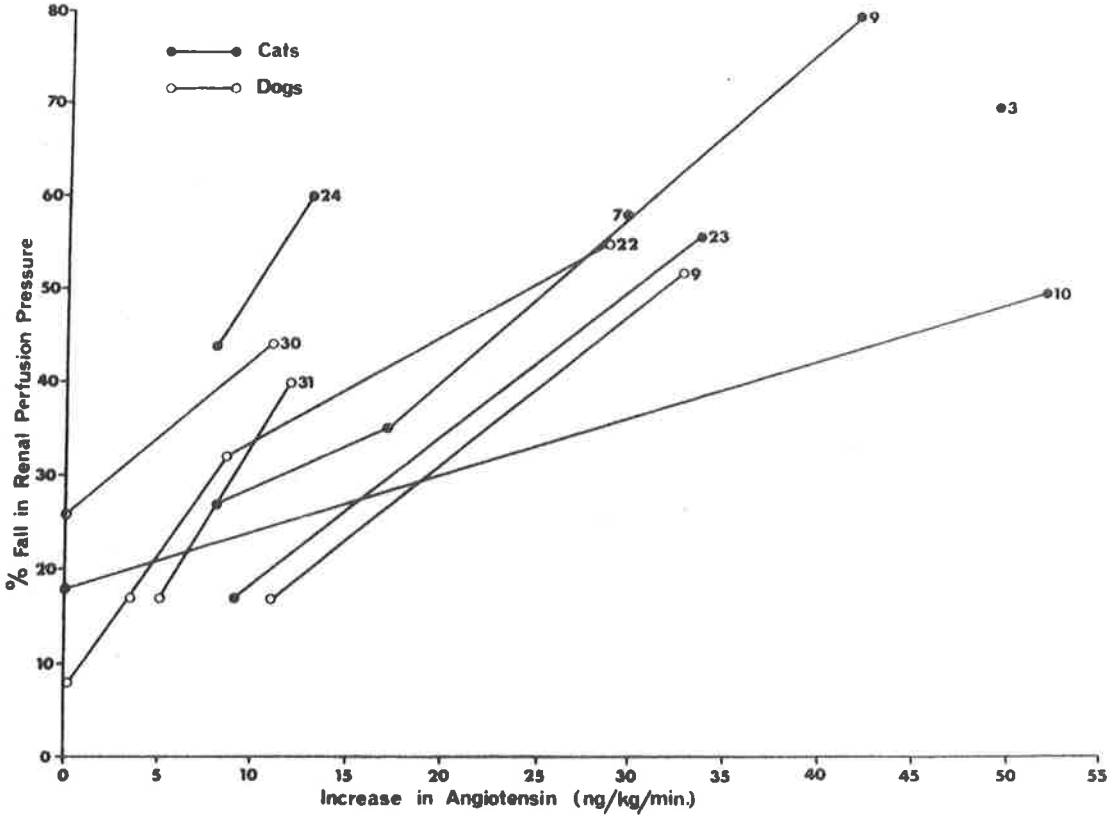


Fig. 5.4

Effect of partial aortic occlusion on angiotensin levels in cats (●) and dogs (o). Cats 3 and 7 represent responses to single occlusions. All other animals represent responses to two or more occlusions in the same animal.

DISCUSSION

The cardiovascular effects of endotoxin administered to cats and dogs were similar to those previously reported (Maclean & Weil, 1956; Weil *et al.*, 1956; Kuida, Hinshaw, Gilbert & Visscher, 1958; Kuida *et al.*, 1961; Greenway *et al.*, 1969). Thus in both cats and dogs there was a variable acute fall in systemic arterial pressure, followed by a rise, before the onset of steadily progressive hypotension. Central venous pressure showed the expected rise in cats and fall in dogs (Gilbert, 1960).

Despite similar changes in systemic arterial pressure, the timing of the changes in circulating levels of catecholamines and angiotensin were strikingly different in the two species. In the cat there was a rise in catecholamines within 5 minutes and an increase in angiotensin levels 20-30 minutes after endotoxin. In contrast, in the dog there was a rise in angiotensin levels within 5 minutes, while there was a delayed rise in catecholamine levels. However, the final levels of both hormones were of the same order in both cats and dogs. An explanation for these differences is not immediately apparent.

The control experiments show that incubation of endotoxin for up to one minute with blood did not cause the release of any vasoactive agents from the blood into the extra-corporeal circuit to which these assay tissues responded. It is probable, therefore, that the responses of the assay tissues were due to increases in angiotensin or catecholamine levels. These elevations could be due to a decreased rate of destruction of angiotensin and catecholamines, or to an increased rate of production of these hormones. Since the physiological response to stress is increased production rather than decreased breakdown (Brown *et al.*, 1967), the latter is the more likely explanation.

The rise in the circulating levels of the hormones studied is almost certainly caused by several factors. Despite the early release of angiotensin in the dog, there is a progressive decline in systemic blood pressure, which may be the stimulus for the delayed release of catecholamines. A delayed release of catecholamines in

the dog following intravenous endotoxin has been reported (Heiffer, Mundy & Grim, 1958; Spink *et al.*, 1966; Vick, 1965). However, other workers described an immediate rise in catecholamines (Rosenburg *et al.*, 1961). These differences in results appear to be due to the dose, potency and the rate of infusion of endotoxin (Spink *et al.*, 1966). The only cardiovascular parameter consistently associated with a release of catecholamines was hypotension. The results in this section, together with those in section three of this thesis (Hall & Hodge, 1971a), support the concept of systemic hypotension being one of the main stimuli of the adrenal medulla in the dog.

All cats showed a marked and rapid rise in catecholamine levels, confirming the observations of Hökfelt *et al.* (1962). The early release of catecholamines may be due to the more important role the sympatho-adrenal system plays in stress in this species as compared to the dog (Hall & Hodge, 1971a). A second factor may be the progressive hypoxia which occurs in this species following the administration of endotoxin (Gilbert, 1960). Such a degree of hypoxia does not occur in the dog (Stein & Thomas, 1967). A third factor may be the acute strain imposed on the right ventricle due to the onset of pulmonary hypertension following endotoxin. It has been shown that myocardial infarction of the left ventricle in dogs is associated with the immediate release of adrenaline (Staszewska-Barczak & Ceremuzynski, 1968).

The early rise in circulating levels of angiotensin in dogs as compared to cats is evidence that changes in central venous pressure are important in the control of renin release. These findings are similar to the observations of White *et al.* (1966), who observed a rise in plasma renin activity 10-20 minutes after administration of endotoxin to dogs. Central venous pressure was not measured but is likely to have fallen.

The exact stimulus for renin release in the dog is not clear, but renin release may be a normal response to maintain circulatory homeostasis as suggested by Regoli & Vane (1966). The importance of the afferent vagal fibres as a monitor of central venous pressure and

the renal sympathetic nerves as the efferent pathway to renin release has been shown (Hodge *et al.*, 1966a, 1966b). The fall in central venous pressure observed following endotoxin would thus lead to a rise in renin levels and, secondarily, angiotensin.

Another possible explanation for renin release is a direct effect of endotoxin on the renal vasculature. This has been suggested by a rapid fall in renal blood flow in the dog following endotoxins (Hinshaw, Bradley & Carlson, 1959; Hinshaw, Spink, Vick, Mallett & Finstad, 1961; White *et al.*, 1966). This fall in renal blood flow is presumably due to constriction of renal afferent arterioles which could then be followed by a release of renin (Vander, 1967). While the renal sympathetic nerves may play a part in renal vasoconstriction, evidence for a direct effect of endotoxin on renal blood vessels is provided by the observations that a fall in renal blood flow occurs in isolated heart-lung-kidney preparations after endotoxin (Hinshaw *et al.*, 1959).

If the amounts of angiotensin generated twenty minutes after endotoxin administration to dogs is compared to the amounts generated during graded aortic occlusion, then endotoxin administration was associated with a fall in renal perfusion pressure equivalent to 30-40%. Since systemic arterial pressure at this time had fallen approximately 20%, this implies that intrarenal perfusion pressure may have fallen to a greater extent than that indicated by the systemic pressure. This suggests either a direct action of endotoxin on renal blood vessels, or a selectively greater increase in renal sympathetic nerve activity secondary to the fall in central venous pressure.

The difference between cats and dogs in the timing of the generation of angiotensin raises several possibilities. Endotoxin may not have a direct action on the renal vasculature of the cat as suggested in the dog. However, renal function in the cat following endotoxin has not been studied.

The results obtained with graded aortic occlusion and those obtained during haemorrhage show that there is no species difference between cats and dogs in the response of the renin-angiotensin system

(Hall & Hodge, 1971a). The most likely explanation is that the acute haemodynamic changes, and in particular the rise in central venous pressure, induced by endotoxin may, in fact, inhibit renin release in the cat. After administration of endotoxin, central venous pressure invariably rises, reaching a peak at 5 minutes and returning to control values 20-30 minutes after endotoxin, before finally dropping below control values. The rise in angiotensin levels occurred at a time when central venous pressure was either approaching, or had fallen below, control values. Since aspirin abolishes the acute rise in central venous pressure following administration of endotoxin to cats (Hall, Hodge, Irvine & Middleton, 1971; Greenway and Murthy, 1971), the effect of aspirin on the timing of the release of angiotensin was studied.

SECTION 5A

THE EFFECT OF ASPIRIN ON THE HUMORAL RESPONSE TO ENDOTOXIN

It has previously been shown that aspirin can abolish the acute rise in portal venous pressure in dogs following the administration of endotoxin (Northover & Subramanian, 1962; Hinshaw, Solomon, Erdos, Reins & Gunter, 1967). Aspirin also abolishes the acute vascular response to endotoxin in cats (Hall *et al.*, 1971; Greenway & Murthy, 1971).

In the previous section (5) it was shown that the generation of angiotensin was significantly delayed in cats as compared to dogs following endotoxin despite a similar response in both species to haemorrhage (Hall & Hodge, 1971a; 1971b). The present study was carried out to determine if the timing of the change in circulating angiotensin in cats is altered when the acute rise in central venous pressure is abolished by aspirin.

METHODS

The study was carried out on 6 cats. Aspirin was administered as a loading dose (50 mgm/kg) followed by a continuous infusion (50 mgm/kg/hr). Endotoxin (2 mgm/kg) was administered approximately one hour after the commencement of the aspirin infusion. Arterial and central venous pressures and changes in circulating levels of angiotensin and catecholamines were recorded as in Section 5. Observations were continued for 60 minutes after administration of endotoxin.

RESULTS

The mean changes in arterial pressure, central venous pressure, angiotensin and catecholamine levels following endotoxin are shown in Fig. 5a.1. Standard errors are plotted for changes in arterial pressure and central venous pressure. The changes in angiotensin and catecholamines were variable, and the actual values, from which the data for Fig. 5a.1 were obtained, are shown in Table 5a.1. In 2 cats, only a qualitative assay of catecholamines was possible. However, since the main interest of the present experiments was the change in angiotensin levels, further experiments were not carried out to

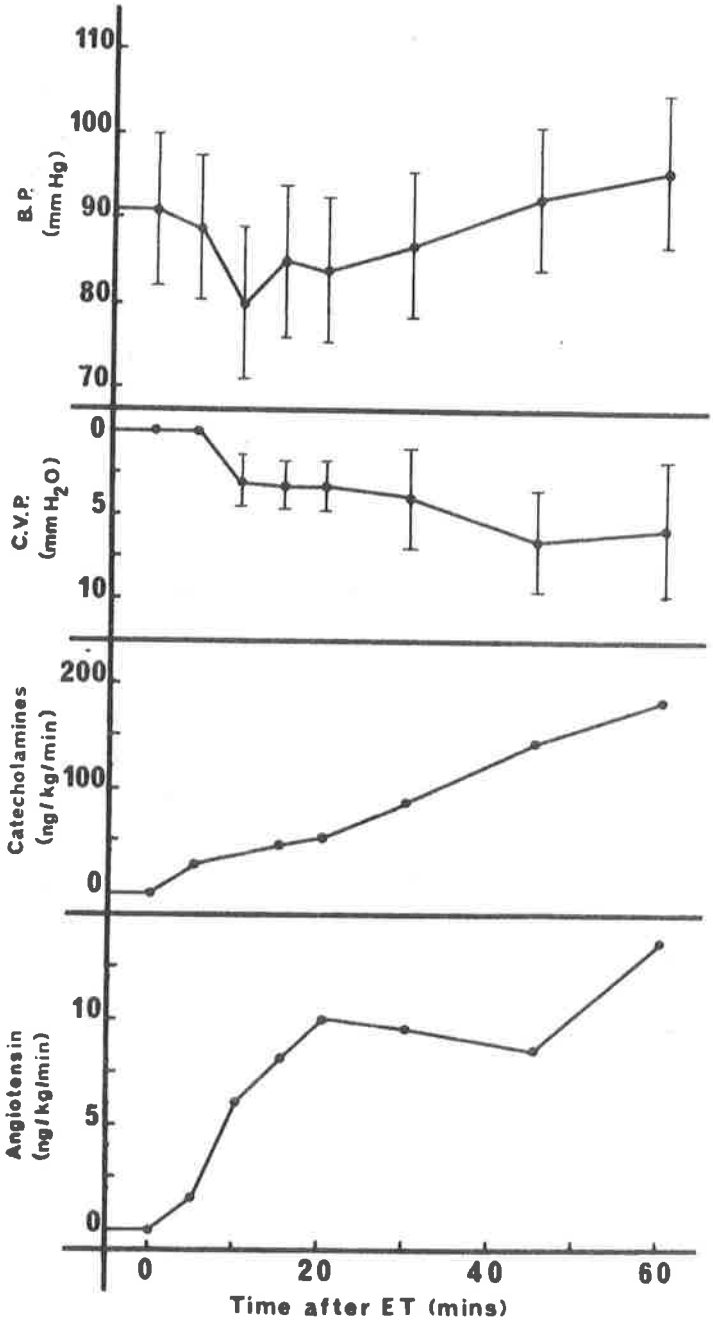


Fig. 5A.1

Changes in mean arterial pressure (mm Hg), central venous pressure (mm H_2O), angiotensin and catecholamine levels (ng/kg/min) after endotoxin in aspirin-treated cats. (Appendix 5A.1, Table 5A.1)

TABLE 5A.1

Changes in circulating catecholamine (C-A) and angiotensin (A-T) levels (ng/kg/min) following i.v. endotoxin to aspirin-treated cats.
(*control data Table 5.1A and 5.2A.*)

	Minutes after Endotoxin							
Cat	0	5	10	15	20	30	45	60
C-A								
37	0	0	0	0	0	0	++	++
38	0	0	0	116	A-T			
39	0	0	+	++	++	++	++	++
40	0	87	0	0	87	109	126	139
41	0	0	0	0	0	0	0	0
42	0	0	0	0	21.7	21.7	91.3	134.8
Mean	0	14.5	0	23.2	27.2	43.6	72.4	91.3
± S.E.	-	14.5	-	23.2	20.6	33.3	37.6	45.6
A-T								
37	0	0	0	0	0	0	44	87
38	0	6.4	18.4	25.2	>30	>30	>30	>30
39	0	4.0	8.0	12.0	13.6	8.0	0	0
40	0	0	0	0	5.2	7.4	0	7.4
41	0	0	0	0	0	0	6.9	24.2
42	0	0	10.4	12.17	11.7	13.0	10.4	11.7
Mean	0	1.73	6.1	8.2	10.1	9.7	8.6	13.7
± S.E.	-	1.1	3.1	4.2	4.6	4.5	4.6	4.6

quantitate the changes in catecholamine levels.

For 10-15 min following endotoxin, changes in arterial pressure were similar to those observed in non-aspirin-treated animals. Thereafter arterial pressure was better maintained. Aspirin abolished the acute rise in central venous pressure, and a progressive fall in central venous pressure was now observed. However, this fall was not significant up to 60 min after endotoxin.

The abolition of any significant changes in arterial and central venous pressures following endotoxin was associated with more variable and less marked rises in both angiotensin and catecholamine levels than those observed in non-aspirin-treated cats. However, in contrast to the results in the previous section, the rise in angiotensin levels preceded, or occurred simultaneously with, the rise in catecholamines in all but one cat (Table 5a.1). Thus there was a rise in angiotensin levels in 2 out of 6 cats in 5 minutes and a further 2 cats in 20 minutes. This rise was significant 60 minutes after endotoxin ($P<0.05$), when a rise in angiotensin levels had occurred in all cats.

DISCUSSION

These results show that although the humoral response is partially inhibited, the timing of changes in angiotensin and catecholamine levels in aspirin-treated cats are similar to those observed in dogs following endotoxin. The most likely explanation of this observation is the abolition by aspirin of the acute rise in central venous pressure which normally occurs following endotoxin.

Reflex release of renin, angiotensin and catecholamines occurs in cats and dogs during bilateral carotid occlusion (Kaindl & von Euler, 1951; de Schaepdryver, 1959; Bunag *et al.*, 1966; Hodge *et al.*, 1966a). Thus one might expect simultaneous elevations in other stress procedures.

A qualitative comparison of the response of the cat to endotoxin, aspirin + endotoxin, and haemorrhage is shown in Fig. 5a.2.

In cats subjected to haemorrhage there was a moderate fall in arterial pressure and central venous pressure. These changes were associated with simultaneous increases in circulating levels of

	E T	ASP+ET	HAEM
B.P.	− −	− −	− −
C.V.P.	++++	−	−
A-T	0	++	++
C-A	++++	++	+++

Fig. 5A.2

Qualitative comparison of the changes in blood pressure (B.P.), central venous pressure (C.V.P.), angiotensin (A-T) and catecholamine (C-A) levels in cats following endotoxin (ET) given to aspirin-treated cats (ASP + ET) and during haemorrhage (Haem).

angiotensin and catecholamines.

In cats receiving endotoxin there was a fall in arterial pressure similar to that occurring in haemorrhaged animals. In contrast to haemorrhaged cats, endotoxin administration was associated with a marked increase in central venous pressure. These cardiovascular changes were associated with greater rises in circulating catecholamines than those observed during haemorrhage, but in these animals the rise in catecholamines was not associated with a rise in angiotensin levels.

Endotoxin administration to cats treated with aspirin caused falls in both arterial and central venous pressure - changes similar to those observed in haemorrhaged cats. In these animals there was a simultaneous rise in angiotensin and catecholamine levels qualitatively similar to changes observed in haemorrhaged cats.

These results provide strong evidence for specific reflex control of renin release dependent on changes in central venous pressure, so that, despite stimulation of the sympatho-adrenal system generally, rises in central venous pressure cause a specific inhibition of renal sympathetic nerve activity.

Other workers have also provided evidence for the existence of a selective vagal-renal sympathetic nerve reflex path (Chalmers, Korner & White, 1967; Oberg & White, 1970a; 1970b; Karim, Kidd, Malpus & Penna, 1970; Pelletier, Edis & Shepherd, 1971; Brennan, Malvin, Jochim & Roberts, 1971). However, further experiments using direct recording of activity in the vagus, renal sympathetic nerves and other splanchnic nerves following endotoxin administration to control and aspirin-treated cats are required to clarify the precise afferent and efferent nervous pathways.

SECTION 6

THE EFFECT OF ASPIRIN ON THE RESPONSE TO ENDOTOXIN

In all species the acute response to endotoxin depends on the presence of platelets and one or more constituents of plasma (Kuida *et al.*, 1958; Vick, 1960; des Prez, Horowitz & Hook, 1961; Hinshaw, Emerson, Iampietro & Brake, 1962). After the administration of endotoxin to dogs and rabbits there is a rapid transient fall in platelet count associated with a rise in circulating 5-Hydroxytryptamine (5-HT) levels (Davis, Meeker & McQuarrie, 1960; Davis, Meeker & Bailey, 1961). Endotoxin also causes the release of 5-HT from platelets *in vitro* (des Prez *et al.*, 1961). Further indirect evidence that 5-HT contributes to the pathogenesis of endotoxin shock has been provided by studies which have shown a protective effect of 5-HT antagonists (Gilbert, 1959; Kalas & Jacobson, 1964).

In the dog, aspirin abolishes the delayed fall in arterial pressure which occurs after endotoxin (Northover & Subramanian, 1962). Other workers have confirmed this finding and also shown that aspirin abolishes the acute rise in portal venous pressure following endotoxin (Hinshaw *et al.*, 1967). It was concluded that the lethal effects of endotoxin are secondary to the haemodynamic changes in the portal circulation. However, no experimental evidence was provided as to the possible mechanism of action of aspirin. Other anti-inflammatory agents, including indomethacin, aminopyrine, flufenamic acid and phenylbutazone, modify but do not abolish the acute vascular response to endotoxin in the dog (Erdos, Hinshaw & Gill, 1967; Culp, Erdos, Hinshaw & Holmes, 1971). Recently, it has been shown that while aspirin abolishes the acute effects of endotoxin on the pulmonary vascular bed of the cat, the delayed fall in arterial pressure still occurs (Greenway & Murthy, 1971).

The effect of aspirin on platelet function has been reviewed (Collier, 1969; Mustard & Packham, 1970). Aspirin reduces platelet adhesiveness (Weiss, Aledort & Kochwa, 1968; O'Brien, 1968) and alters the release of vasoactive substances following aggregation

(Evans, Packham, Nishizawa, Mustard & Murphy, 1968; Weiss *et al.*, 1968; Smith & Willis, 1971).

The dog is unusual in that the acute effect of endotoxin is on the portal vascular bed, so, in addition to this species, the effects of aspirin were examined in the cat and sheep, in which the pulmonary vascular bed is the site of the acute effects of endotoxin. Since endotoxin causes the release of 5-HT from platelets, both *in vitro* and *in vivo*, the effect of aspirin on the release of 5-HT in blood by endotoxin was also studied.

METHODS

Experiments were carried out on cats, dogs and sheep of either sex. Arterial and central venous pressures were recorded as described in Section 1A. Platelets were counted on an automatic Coulter counter, or manually in the case of sheep platelets, since they do not centrifuge adequately for processing by the automatic technique.

EXPERIMENTAL GROUPS

1. Effect of aspirin on the vascular and platelet response to endotoxin.

(a) Cats.

(i) Vascular responses. Four animals were given a loading dose of aspirin of 50 mg/kg intravenously followed by an infusion of 50 mg/kg/hr. One hour after the commencement of the aspirin administration all animals received *E. coli* endotoxin (2 mg/kg) intravenously.

(ii) Platelet counts. Estimations were performed on 4 cats which had endotoxin alone and on the 4 cats above which had aspirin and endotoxin. Three arterial blood samples were taken during the hour before endotoxin was given and others were taken 5, 10, 15, and 60 min after its administration.

(b) Dogs.

(i) Vascular responses. Aspirin was administered as a loading dose of 50 mg/kg followed by an infusion of 50 mg/kg/hr to 4 dogs. *E. coli* endotoxin (2 mg/kg) was administered i.v. one hour after the aspirin infusion was commenced.

(ii) Platelet counts. Estimations were carried out on the four

aspirin-treated dogs above and on 7 dogs given endotoxin alone. Three samples were taken prior to the endotoxin and other samples were taken 5, 15, 30 and 60 minutes after endotoxin.

Control data for cats and dogs.

The vascular responses of cats and dogs to endotoxin without aspirin have already been studied (Hall & Hodge, 1971b).

(c) Sheep (6 aspirin-treated, 6 control).

(i) Vascular responses. A loading dose of aspirin (5 mgm/kg) was followed by an infusion of 5 mgm/kg/hr. One hour after the commencement of the aspirin infusion, *E. coli* endotoxin (0.5 mgm/kg) was administered intravenously. Sheep are more sensitive to endotoxin, so this dose was chosen to allow the animals to survive for times comparable to those in dogs and cats. Similarly, the dose of aspirin could also be reduced. Control animals received no aspirin, but were given the same dose of endotoxin one hour after the commencement of the experiment.

(ii) Platelet counts. Three arterial blood samples were taken for platelet counts prior to endotoxin. In aspirin-treated animals further samples were taken at 5, 10 and 15 minutes after endotoxin, while in control animals they were taken at 5, 10, 30 and 60 minutes after endotoxin.

2. Effect of aspirin on the vascular response to 5-HT (5 sheep).

The effects of intravenous infusions of 5-HT (0.15 µg/min, 0.3 µg/min and 0.6 µg/min) on arterial and central venous pressure were obtained before and after the administration of aspirin. At least two responses were obtained for each dose of 5-HT in the control period. When control responses had been obtained, aspirin was given in a loading dose of 5 mgm/kg followed by an infusion of 5 mgm/kg/hr. The infusions of 5-HT were then repeated during the aspirin infusion.

3. Effect of aspirin on the half-life of 5-HT in blood and on the release of a 5-HT-like substance by endotoxin.

In this group there were 21 dogs: 11 control and 10 aspirin-treated. As in other groups, aspirin was administered as a loading dose of 50 mgm/kg followed by an infusion of 50 mgm/kg/hr.

In 5 control animals and 5 aspirin-treated animals the half-life of 5-HT in blood was determined. The technique used was similar to that described by Thomas & Vane (1967) using the rat stomach strip to determine changes in concentration of 5-HT. The assay tissues were superfused with heparinized arterial blood from a femoral artery using a roller pump delivering blood at a constant rate for each dog (usually about 15 ml/min). A length of silicone-rubber tubing, whose priming volume was 50 ml, was included in the extra-corporeal circuit prior to the roller pump and immersed in a water bath at 38°C. Thus, for flow rates of 15 ml/min, blood was incubated for 3-3½ min after leaving the dog and before superfusing the assay tissues. A dose of 5-HT was infused at the beginning of the incubation circuit and a response of the assay tissues obtained. This contraction was then bracketed by those produced by 5-HT infused into the blood immediately before it superfused the assay tissues. The amount of 5-HT remaining after passing through the incubation circuit was calculated from these bracket assays and the half-life was determined from a semilog plot of the residual 5-HT concentration against time of incubation.

The rate of generation of a 5-HT-like substance in blood, with and without aspirin, in the presence of endotoxin was determined by infusing endotoxin at the beginning of the incubation circuit. The rate of infusion of endotoxin was adjusted to produce concentrations in the blood of the incubation circuit of 20 µg/ml (6 dogs) and 67 µg/ml (5 dogs) in control animals, and 65 µg/ml (5 dogs) and 200 µg/ml (5 dogs) in aspirin-treated animals. The latter two doses were selected as the best to determine whether inhibition of the release of 5-HT was competitive or non-competitive. Since the incubation time varied from 2.5 to 3.8 min, the amount of 5-HT generated in ng/ml, as determined by bracket assay, was standardised by dividing the concentration by the incubation time to give a final generation rate in ng/ml/min. For the range of incubation times and endotoxin doses used control experiments have shown that the release of 5-HT is linear.

Salicylate levels.

Blood samples were taken from 5 dogs in group 3 one hour after

the beginning of aspirin administration for estimation of salicylate levels (Trinder, 1954).

Plasma assays.

Assays on plasma were carried out on samples taken from 8 dogs: 4 control and 4 aspirin-treated.

When endotoxin was incubated with blood for approximately 3 min, there was a consistent contraction of the rat stomach strip. This activity was assayed against 5-HT infused into the blood just before it superfused the assay tissues. During the response of the assay tissues to infusions of endotoxin, blood samples were taken into ice-cold tubes and centrifuged at 4000 g, and platelet-free plasma was decanted and stored at -20°C until assay.

Vasoactive substances in the plasma were assayed in terms of 5-HT using a rat stomach strip superfused with Krebs solution before and after the addition of BOL 148 (1 µg/ml). As controls, the responses to prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) were also obtained before and after the addition of BOL 148. $PGF_{2\alpha}$ was selected because it is the only prostaglandin which, when given intravenously, causes a rise in central venous pressure similar to that observed following endotoxin.

RESULTS

1. Effect of aspirin on the vascular and platelet response to endotoxin.

(i) Vascular response.

(a) Cats. Fig. 6.1 shows the effect of aspirin on the change in arterial and central venous pressure after endotoxin. Detailed data are given in Appendix 6.1. The acute rise in central venous pressure after endotoxin (Hall & Hodge, 1971b) was abolished by aspirin. However, thirty minutes after endotoxin there was no difference between the two groups. There was no significant difference at any time between arterial pressures of the two groups.

(b) Dogs. Fig. 6.2 shows the results obtained in dogs. Detailed data are given in Appendix 6.2. Changes in arterial pressure up to 15-20 min after endotoxin were similar to those observed in dogs given endotoxin alone (Hall & Hodge, 1971b). However, in aspirin-treated

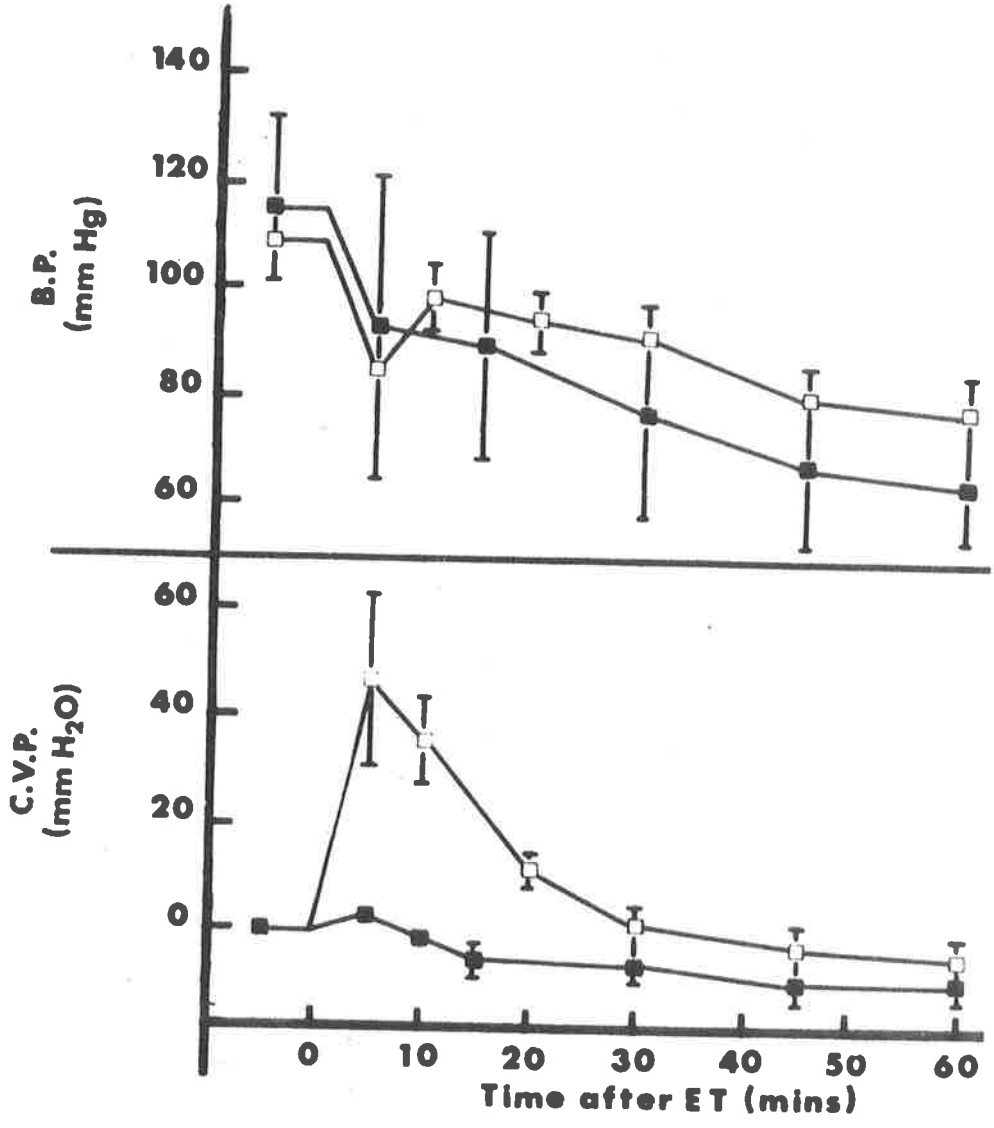


Fig. 6.1

Cats. The effect of endotoxin (2 mgm/kg) on blood pressure and central venous pressure (mm H_2O) in control cats (□) and aspirin-treated animals (■). The means ± 1 S.E. are plotted. The data for control cats has been replotted from experiments in Section 5 and is included for purposes of comparison. (Appendix 6.1)

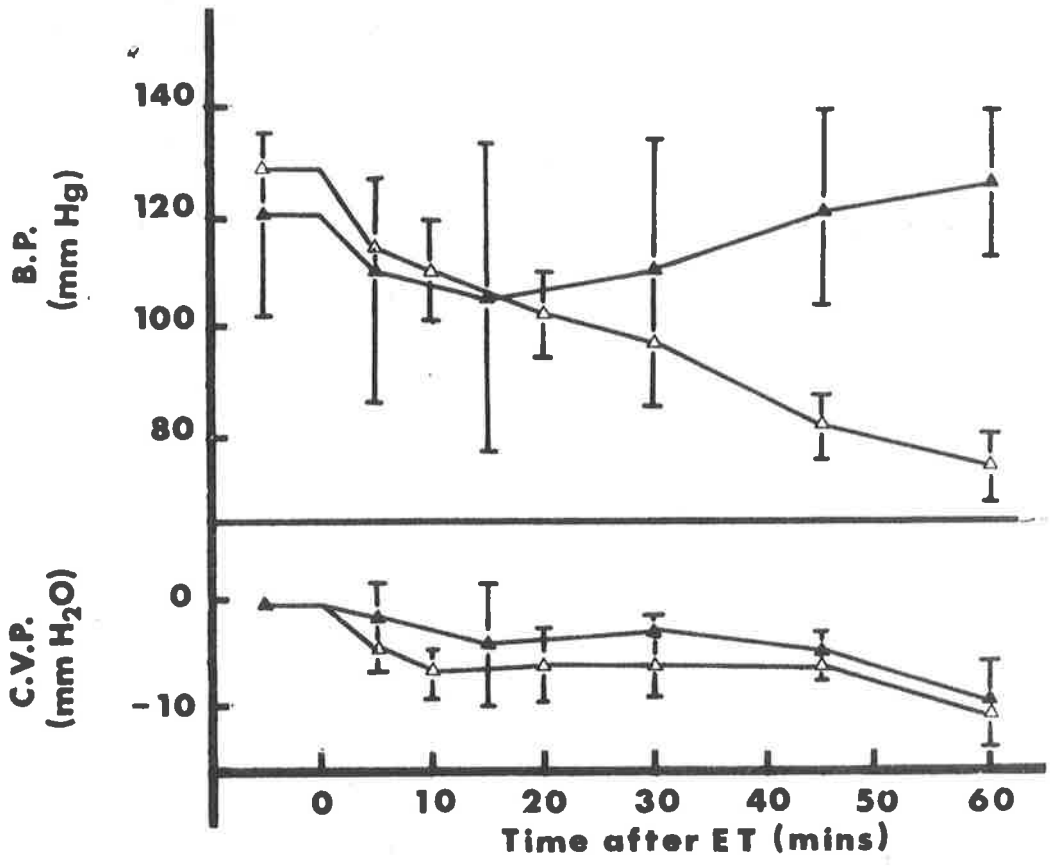


Fig. 6.2

Dogs. The effect of endotoxin (2 mgm/kg) on blood pressure (mm Hg) and central venous pressure (mm H_2O) in control dogs (Δ) and aspirin-treated dogs (▲). The means ± 1 S.E. are plotted. The data for control dogs has been replotted from a previous Section (5) and is included for purposes of comparison. (Appendix 6.2)

animals, arterial pressure then began to rise, and was significantly higher than in non-aspirin-treated animals 45 min ($P<0.02$) and 60 min after endotoxin ($P<0.002$). There was no significant difference between the two groups in the change in central venous pressure following endotoxin.

(c) Sheep. Fig. 6.3 shows the results obtained in sheep. Detailed data are given in Appendix 6.3a and 6.3b. As with cats, the transient increase in central venous pressure was abolished by aspirin, but there was no significant difference between groups 15 and 30 min after endotoxin. Forty-five minutes after endotoxin and thereafter central venous pressure in the aspirin-treated sheep was lower than in control animals, but this difference was not significant. The immediate fall and subsequent rise in arterial pressure in control animals following endotoxin was abolished by aspirin. However, as in cats, the later fall still occurred.

(ii) Platelet response.

Fig. 6.4 shows the changes in platelet count following endotoxin expressed as a percentage of pre-endotoxin values in control and aspirin-treated cats, dogs and sheep. Detailed data are given in Appendix 6.4.

(a) Cats. In control cats, platelets fell to 36% of pre-endotoxin levels 10 min after endotoxin, rising to control values 60 min after endotoxin. In aspirin-treated cats, the platelet count was 22% of control values 5 min after endotoxin. When the fall in platelet count of the two groups was compared, it was significantly lower ($P<0.02$) in aspirin-treated cats 5 min after endotoxin, but there was no significant difference between groups thereafter.

(b) Dogs. In control dogs, the platelet count fell to 22% of pre-endotoxin values 10 min after endotoxin, rising to 56% of control values 60 min after endotoxin. In aspirin-treated animals, the platelet count fell to 44% of control values. There was then a rise to 94% of control values 60 min after endotoxin. However, there was no significant difference between the two groups at any time.

(c) Sheep. In control sheep, there was no significant change in

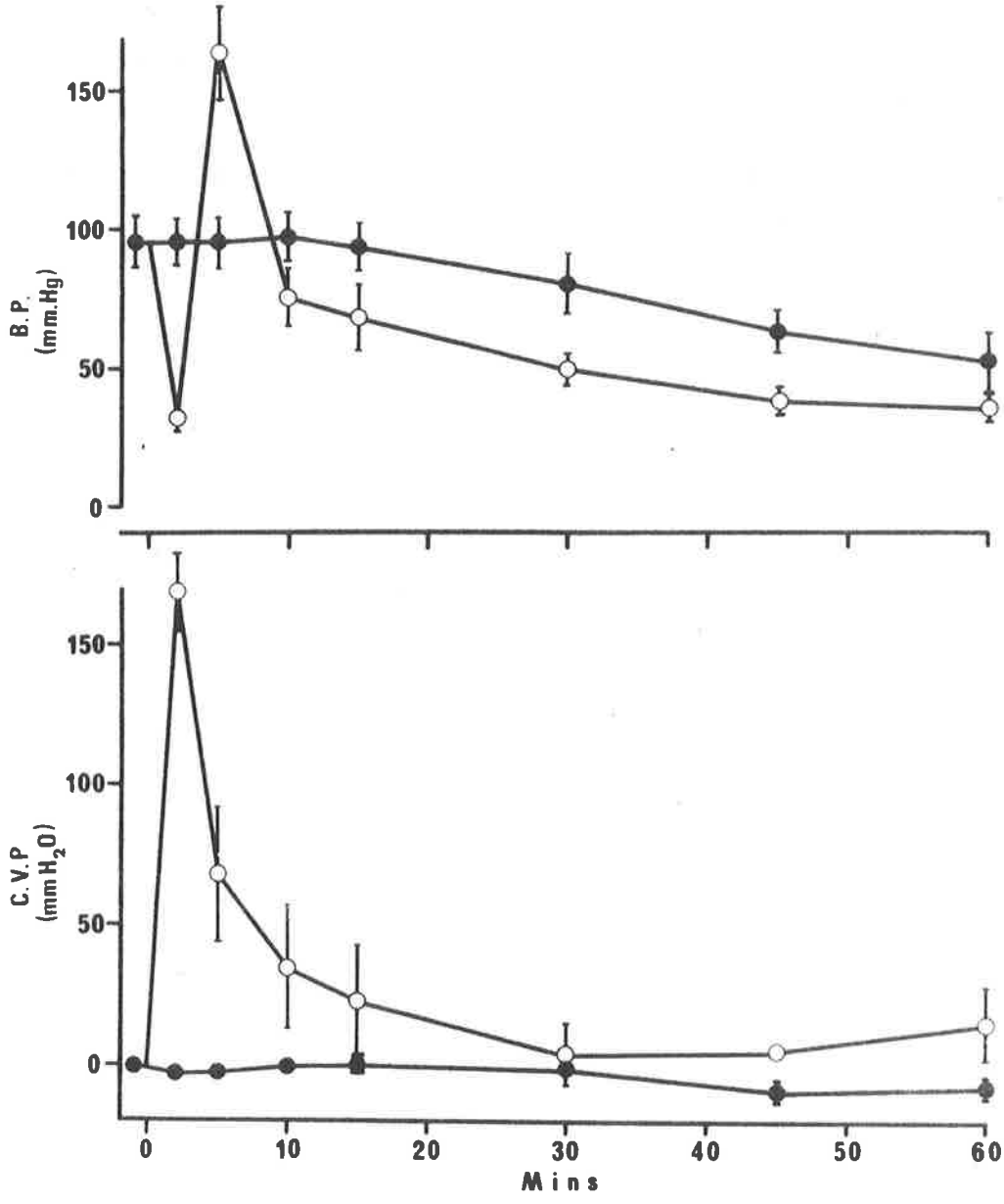


Fig. 6.3

Sheep. The effect of endotoxin (0.5 mgm/kg) on blood pressure (mm Hg) and central venous pressure (mm H_2O) in control sheep (O—O) and aspirin-treated sheep (●—●). The means ± 1 S.E. are plotted. (Appendix 6.3a, 6.3b)

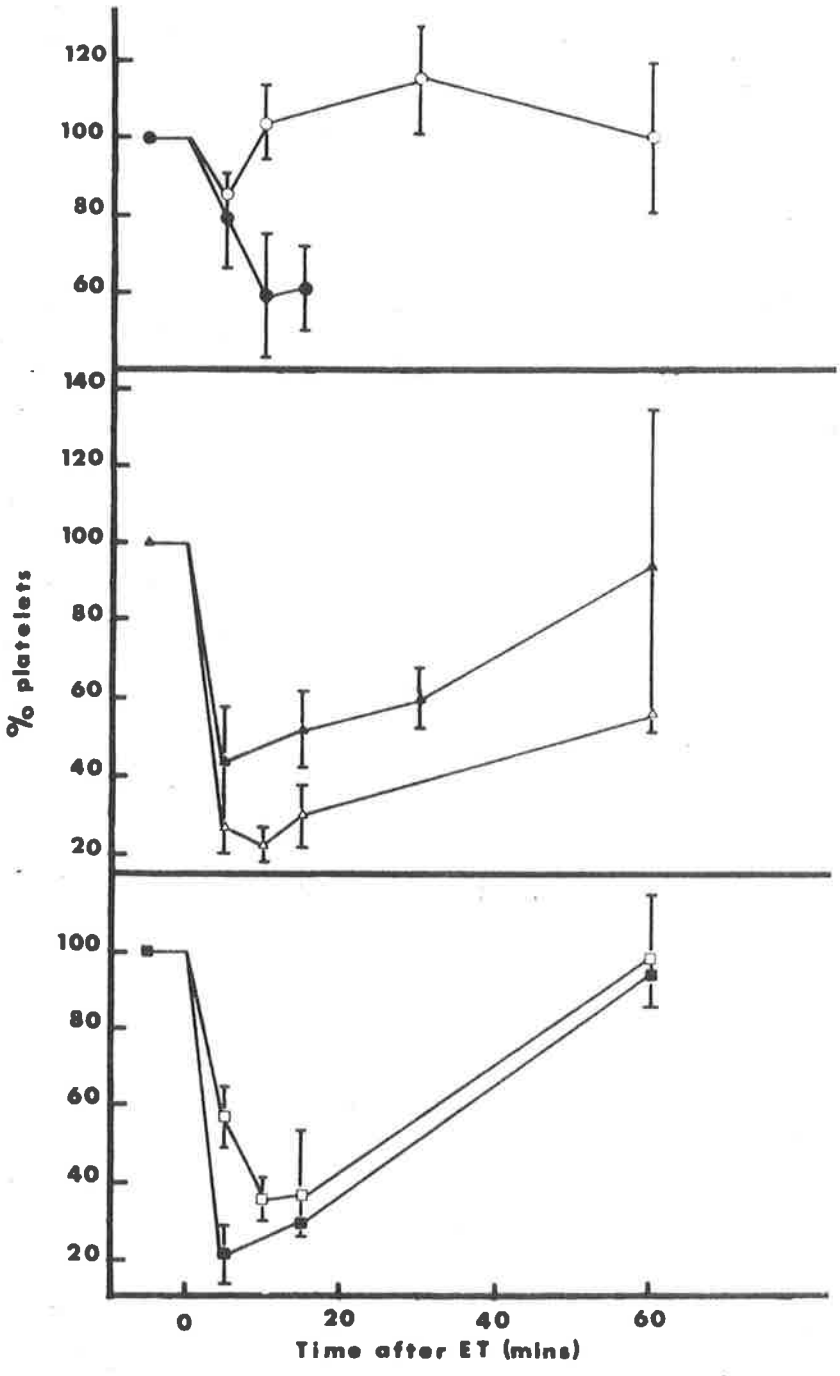


Fig. 6.4

Effect of endotoxin on platelet count in control and aspirin-treated sheep (top panel), dogs (centre panel) and cats (bottom panel). Control animals: open symbols; aspirin-treated animals: closed symbols. (Appendix 6.4)

platelet count up to 60 min after endotoxin. In aspirin-treated animals, platelets fell to 59% of pre-endotoxin levels 10 min after endotoxin. This difference between groups, however, did not reach significance at the 5% level ($P>0.05$).

2. *Effect of aspirin on the vascular response to 5-HT.*

Fig. 6.5 shows that aspirin had no effect on the mean changes in arterial pressure in sheep during infusion of 5-HT. Aspirin did not affect the rise in central venous pressure with the lower doses of 5-HT (0.15 µg/min and 0.3 µg/min), but caused a significantly greater rise in central venous pressure with the highest dose ($P<0.05$). Detailed data are given in Appendix 6.5.

3. *Effect of aspirin on the release of a 5-HT-like substance by endotoxin and on the half-life of 5-HT in blood.*

Fig. 6.6 shows the amounts of a 5-HT-like substance released from blood in the incubation circuit into which endotoxin was infused to give mean concentrations of 20 and 67 µg/ml endotoxin in control animals, and 65 and 200 µg/ml endotoxin in aspirin-treated animals. Detailed data are given in Appendix 6.6. The release of this substance in both control and aspirin-treated animals was dose-dependent, but aspirin inhibited the effect of endotoxin in a competitive manner. The maximum dose ratio was 3.85 and the minimum 3.16, giving a geometric mean of 3.49.

Table 6.1 shows the effects of aspirin on the half-life of 5-HT in blood in the incubation circuit and the salicylate levels. The mean half-life in control animals was 1.72 ± 0.12 min. The mean half-life in the presence of aspirin was 3.1 ± 0.46 min, which was a significant increase ($P<0.02$). The mean salicylate levels were 21.2 ± 1.9 mgm/100 ml plasma.

Plasma assays.

Results from a single assay are shown in Fig. 6.7. Plasma samples taken during an infusion of endotoxin into blood gave a contraction of the assay tissues equivalent to a mean concentration of 238 ng/ml 5-HT. BOL 148 invariably abolished more than 90% of this plasma activity. Although the responses of some, but not all, assay

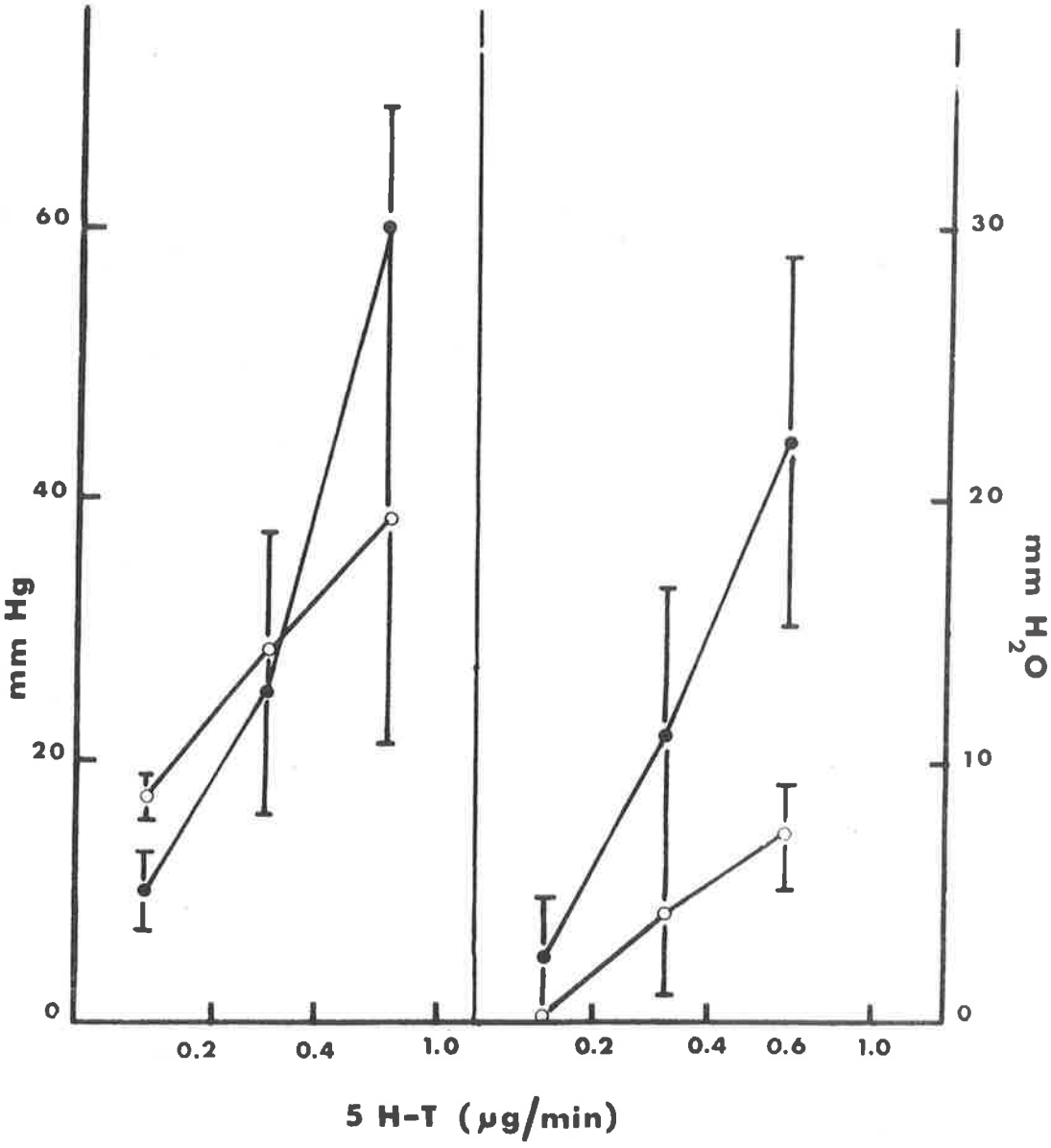


Fig. 6.5

Sheep. Effect of aspirin on the response to i.v. infusions of 5-HT. Left hand panel: responses of arterial pressure (mm Hg) before (O–O) and after (●–●) aspirin. Right hand panel: responses of central venous pressure (mm H_2O) before (O–O) and after (●–●) aspirin. (Appendix 6.5)

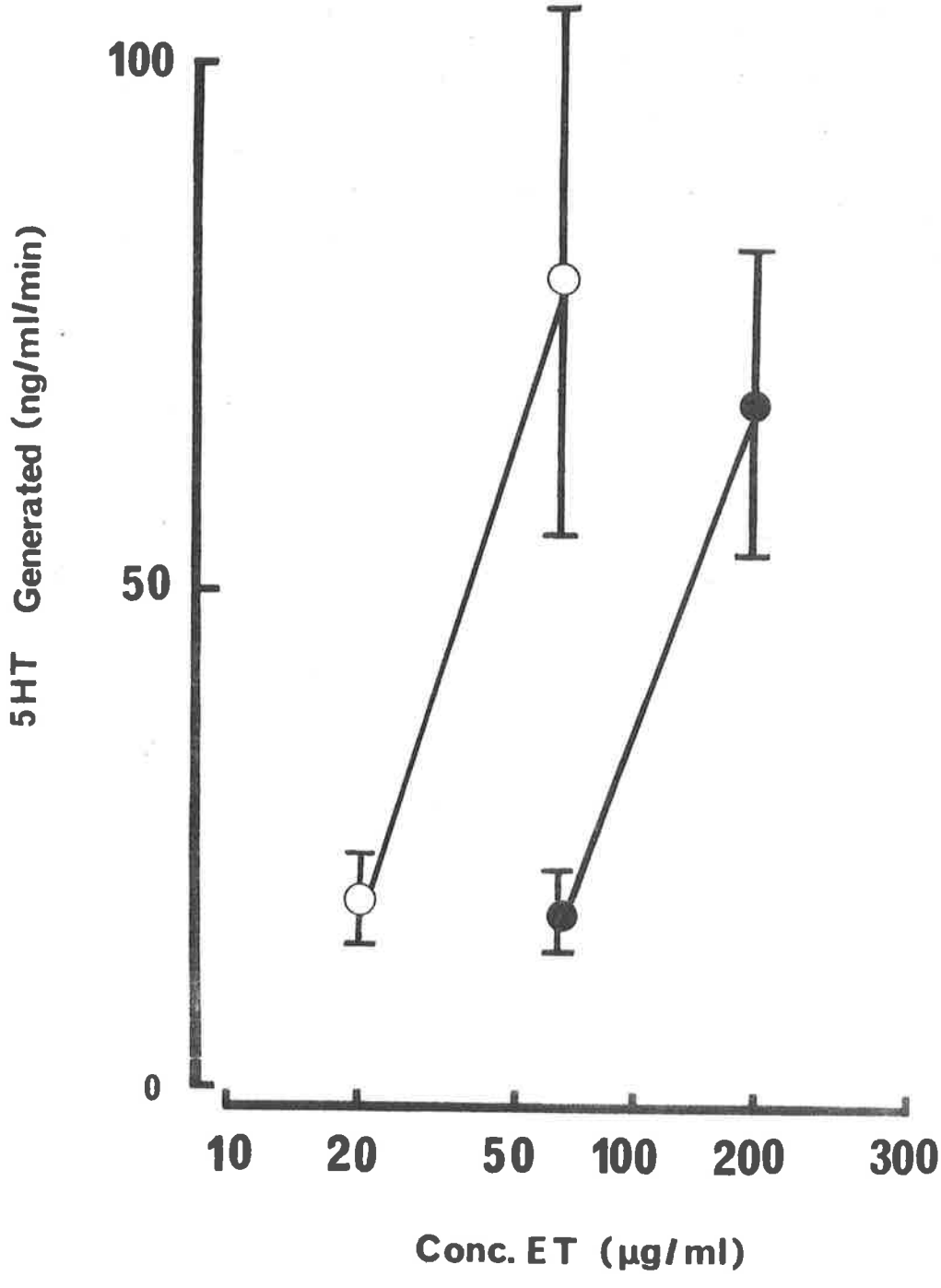


Fig. 6.6

Dogs. The generation of 5-HT (ng/ml/min) during infusions of endotoxin into the incubation circuit in control animals (○—○) and aspirin-treated animals (●—●). (Appendix 6.6)

TABLE 6.1

Salicylate levels and the effect of aspirin on the half-life of 5-HT in dogs' blood.

CONTROL		ASPIRIN-TREATED		
Initial Conc. 5-HT (ng/ml)	Half-life (mins)	Initial Conc. 5-HT (ng/ml)	Half-life (mins)	Salicylate level (mgm/100 ml)
417	1.6	333	2.6	22
200	1.5	333	2.1	16
312	2.2	214	3.1	28
214	1.7	357	4.8	25
277	1.6	333	2.9	18
		-	-	18
Mean	1.72		3.1	21.2
± S.E.	0.12		0.46	1.9

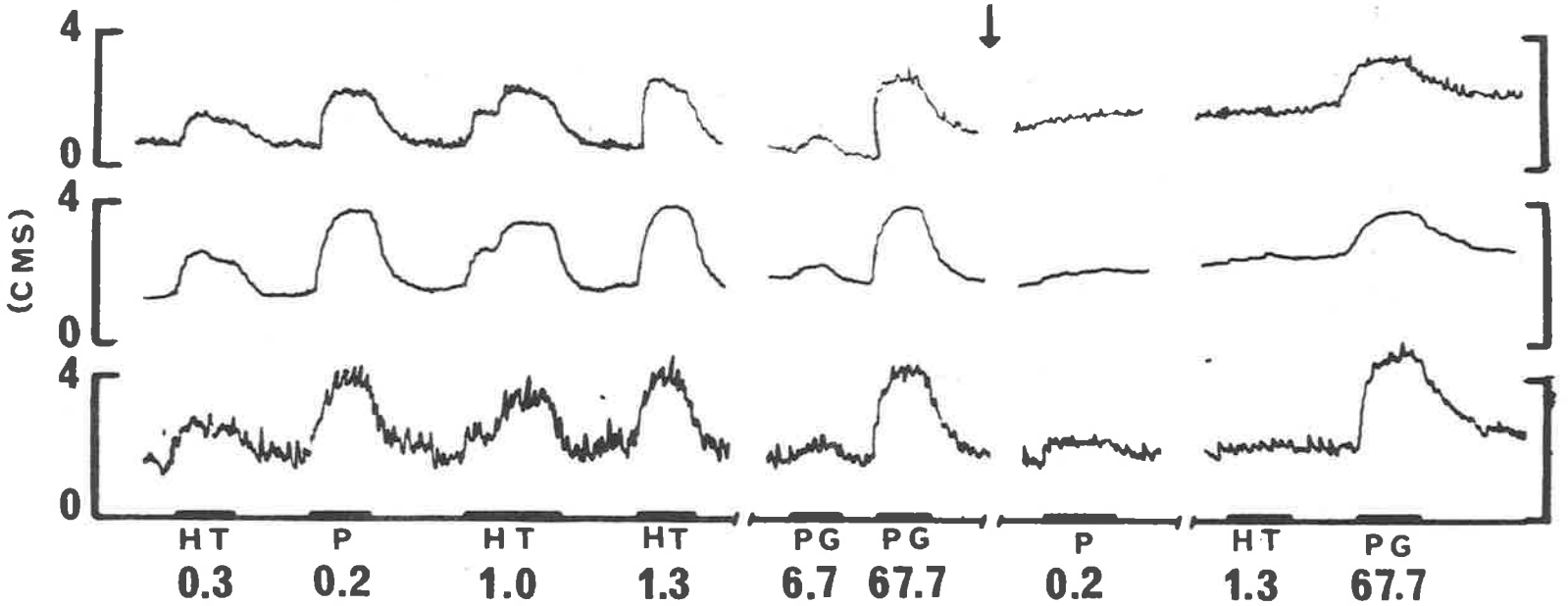


Fig. 6.7.

The responses of 3 rat stomach strips to infusions of 5-HT, $PGF_{2\alpha}$, and plasma from blood sampled at the end of the incubation circuit during an infusion of endotoxin into the circuit. The numbers refer to the concentration of 5-HT (HT) and $PGF_{2\alpha}$ (PG) in ng/ml of the superfusate and to the rate of infusion of plasma (P) in ml/min into the superfusate. From the time marked by the arrow the superfusate contained BOL 148 (1 µg/ml).

tissues to $PGF_{2\alpha}$ were depressed by the dose of BOL 148 used, this was considerably less than the reduction in the response to plasma, suggesting that a considerable portion of the activity in plasma was due to 5-HT. The residual contraction to plasma after BOL 148 indicates that other active substances are present or are released by endotoxin (Fig. 6.7).

DISCUSSION

These results show that aspirin abolishes the acute vascular response to endotoxin in cats and sheep. Aspirin also abolishes the acute rise in portal venous pressure following endotoxin in the dog (Hinshaw *et al.*, 1967). In the present study, dogs differed markedly from cats and sheep in relation to the delayed response to endotoxin. Thus, aspirin did not affect the delayed fall in arterial pressure in cats and sheep following endotoxin administration, but it inhibited the delayed fall in arterial pressure in the dog, which confirms previous observations (Hinshaw *et al.*, 1967; Greenway & Murthy, 1971). In view of the marked species difference observed in the present section, the effects of aspirin will be discussed in relation to the acute and delayed effects of endotoxin.

The pulmonary vascular bed is involved in the acute response to endotoxin in cats and sheep and the portal vascular bed is involved in the dog (Gilbert, 1960; Halmagyi, Starzecki & Horner, 1963). Despite this difference in the site of action of endotoxin, aspirin is an effective inhibitor of the acute response to endotoxin in all three species, suggesting that there may be a similar mechanism of action. 5-HT is released by endotoxin *in vivo* (Davis *et al.*, 1960; Davis *et al.*, 1961) and *in vitro* (des Prez *et al.*, 1961). The vascular response to 5-HT is complex but includes a rise in pulmonary artery pressure in dogs, cats and sheep (Aviado, 1965). Sodium salicylate inhibits the local effects of 5-HT on forearm vessels in man (Glover, Marshall & Whelan, 1957). However, in the present study, aspirin did not affect the change in central venous pressure or arterial pressure of sheep caused by 5-HT infusion. In fact, the highest dose of 5-HT had a significantly greater effect on central venous pressure in the

presence of aspirin.

Endotoxin induces an acute fall in platelet count in rabbits and dogs (Davis *et al.*, 1960; Davis *et al.*, 1961; Stein & Thomas, 1967). *In vitro* studies have shown that the addition of endotoxin to platelet-rich rabbit plasma is associated with both platelet aggregation and the release of 5-HT (des Prez *et al.*, 1961). Aspirin combines irreversibly with the platelet membrane, alters platelet adhesiveness and inhibits platelet aggregation (O'Brien, 1968; Weiss *et al.*, 1968; Evans *et al.*, 1968). The release of 5-HT by small doses of thrombin and collagen is inhibited by aspirin (Evans *et al.*, 1968), but the response to large doses of thrombin is unaffected, suggesting a competitive inhibition. Aspirin could therefore affect the response to endotoxin either by altering platelet aggregation, or by inhibiting the release of the vasoactive agents, or both. However, aspirin had no effect on the fall in platelet count following endotoxin in dogs, and, if anything, there was a greater fall in cats and sheep. Thus aggregation was not inhibited. However, aspirin did inhibit the release by endotoxin of a 5-HT-like substance into blood. This inhibition was competitive and, in this respect, was similar to the effects of aspirin on the release reaction initiated by thrombin (Evans *et al.*, 1968; Smith & Willis, 1971). The increase in half-life of 5-HT in blood from 1.74 to 3.1 minutes in the presence of aspirin suggests that aspirin not only affects the release of 5-HT but also its uptake. Since the active removal of 5-HT from blood is by platelets (Born & Gillson, 1959; Pletscher, 1968), aspirin is presumably acting on these elements.

It is possible that aspirin may also affect the release by endotoxin of other vasoactive agents such as prostaglandins and histamine. Release of prostaglandins from the spleen during nerve stimulation is inhibited by both aspirin and indomethacin (Ferreira, Moncada & Vane, 1971). Aspirin also inhibits the release of prostaglandings from platelets *in vitro* (Smith & Willis, 1971). The results of the plasma assays show that a vasoactive agent other than 5-HT may be released by the action of endotoxin on the constituents of

blood. The precise nature and amount of this substance requires further investigation.

A second aspect of our results is the marked species difference in the effect of aspirin on the delayed fall in arterial pressure following endotoxin. Hinshaw *et al.* (1967) concluded that the delayed fall in arterial pressure following endotoxin in dogs was secondary to the acute rise in portal venous pressure, since aspirin abolished both effects of endotoxin in this species. These results show that this explanation cannot be so in cats and sheep, since the abolition of the acute vascular response did not alter the delayed vascular effects. The explanation for the persistence of these delayed vascular effects in cats and sheep is not clear, but may be related to a failure of the lungs to remove vasoactive substances (Vane, 1969). However, in Section 5A, aspirin abolished the delayed effects of endotoxin on arterial pressure of cats. Animals in this section were not identical to those in the present section, since an extra-corporeal circuit for superfusion was included and this may have modified the delayed effects by increasing the catabolism of vasoactive agents normally removed by the lungs. Further studies on the pulmonary extraction of vasoactive agents following endotoxin in all three species are necessary.

The levels of salicylate in man at which toxic effects become apparent is 25-30 mgm/100 ml plasma (Smith, 1959). The dose of aspirin used in this study to abolish the delayed fall in arterial pressure in dogs gave a mean plasma level of 21.9 mgm %. In sheep, the levels were not measured, but would have been considerably lower than this, since the dose of aspirin used was one-tenth that in dogs. These results suggest that the effect of high circulating levels of aspirin in the therapy of endotoxin shock in man should be investigated.

SECTION 7

THE EFFECT OF ORAL CONTRACEPTIVE THERAPY ON THE RESPONSE TO TILTING

An association between oral contraceptive therapy and the onset of hypertension is well described (Laragh, Sealey, Ledingham & Newton, 1967; Woods, 1967; Harris, 1969; Macintosh, 1968; Weir, Tree & Fraser, 1969; Clezy, Foy, Hodge & Lumbers, 1972). Furthermore, cessation of oral contraceptive therapy may be associated with a remission of hypertension (Laragh *et al.*, 1967; Harris, 1969; Macintosh, 1968; Clezy *et al.*, 1972). The cause of hypertension in these subjects is unknown, but oral contraceptive therapy is associated with reversible elevations in renin substrate and plasma renin activity (Skinner, Lumbers & Symonds, 1969; Laragh *et al.*, 1967), and as a consequence angiotensin II (Cain, Walters & Catt, 1971) and aldosterone levels are also raised (Layne, Meyer, Vaishwanar & Pincus, 1962).

Assumption of the upright posture is a repeated stress on the cardiovascular system compensated for by both the sympatho-adrenal (Vendsalu, 1960; Molzahn, Dissman, Halim, Lohmann & Oelkers, 1972) and renin-angiotensin systems (Catt, Cain, Coghlan, Zimmet, Cran & Best, 1970; Molzahn *et al.*, 1972).

The present preliminary study was designed to examine the effect of oral contraceptive therapy on the cardiovascular response to the stress of tilting.

METHODS

Subjects (4 males, 3 females).

Seven apparently normal subjects, aged 27-39 years, were subjected to 85^{o} passive feet down tilting before and 10-12 days after the commencement of oral contraceptive therapy (Anacyclin: 5 subjects; Eugynon: 1 subject; Ovulen: 1 subject; see Appendix 7.6 for chemical composition of agents).

All subjects rested recumbent for one to two hours prior to the onset of postural stress. During this time a Bardic intracath (17G) or a Bardic angiocath (16G) was inserted into an antecubital vein

for collection of venous blood samples. A catheter (No. 1 Portex Nylon tubing) was threaded into the superior vena cava through a Bardic 17G needle inserted into a second antecubital vein. This catheter was used for injection of indocyanine green (Cardio-Green, Hynson, Westcott & Dunning) for estimation of cardiac output. Heart rate was calculated from electrocardiograms obtained at 10-minute intervals preceding, during, and after tilting.

The subjects were tilted feet down to an angle of 85^{o} to the horizontal for periods of up to 30 minutes. The period of tilting was reduced only when subjects fainted. Muscular activity in the lower limbs was **minimised** in all the subjects by supporting them on a saddle.

In four subjects cardiac outputs were estimated at 10-minute intervals using indocyanine green (5 mgm/2 ml). The change in arterial concentration of dye was measured by a Waters Densitometer (XP-302) and a dichromatic ear piece and recorded on a Texas Instruments servo-recorder. This technique has previously been shown to provide an adequate estimate of relative changes in cardiac output (Gabe, Tuckman & Shillingford, 1962). Areas under the dye-dilution curves were calculated using an Olivetti Programma 101 computer (Hall & Tyler, 1971).

Venous blood samples (10 ml) were collected for estimation of plasma renin activity, plasma renin concentration and renin substrate levels at the following times: immediately before tilting, three samples to provide control values; at 10-minute intervals following tilting of the subjects; and then for three 10-minute intervals following resumption of the horizontal position.

Plasma renin activity, plasma renin concentration and renin substrate levels were estimated as previously described in Section 1B.

RESULTS

1. Cardiovascular response.

Before oral contraceptive therapy three subjects fainted 10-15 minutes after tilting and a fourth subject fainted 30 minutes after tilting. However, during oral contraceptive therapy none of the

subjects fainted.

(a) Heart rate. The changes in heart rate in response to tilting before and during oral contraceptive therapy are shown in Fig. 7.1. Detailed data are shown in Appendix 7.1. Mean control heart rate before tilting was 65.7 b.p.m. (± 1.15 S.E.). Heart rate increased on tilting; this increase became significant 30 minutes after tilting. Ten minutes after resuming the horizontal position, heart rate had returned to control values and thereafter there was no further significant change.

During oral contraceptive therapy, mean control heart rate was 59 b.p.m. (±1.15 S.E.), which was significantly lower than control heart rates before oral contraceptive therapy (paired "t" test: $P<0.05$). Furthermore, there was a significant increase in heart rate 10 minutes after tilting ($P<0.01$). This increase in heart rate was maintained until return to the horizontal when heart rates returned to pre-tilting values within 10 minutes.

(b) Cardiac output. The per cent. changes in cardiac output before and during oral contraceptive therapy are shown in Fig. 7.2. Detailed data are shown in Appendix 7.2. Before oral contraceptive therapy 2 out of 4 subjects, in whom cardiac output was estimated, fainted 10-15 minutes after tilting. Mean cardiac output had fallen to 82.5% of control values within 10 minutes of tilting. This fall was significant ($P<0.01$). In the two subjects who did not faint, cardiac output continued to fall. After return to the horizontal, cardiac output returned to 87.8% of pre-tilt values within 10 minutes, a value not significantly different from control values.

During oral contraceptive therapy none of the subjects fainted during tilting. Since the cardiac outputs were related to areas under the curve rather than absolute values, no comment can be made on the effect of oral contraceptive therapy on resting cardiac output. However, 10 minutes after tilting, cardiac output had fallen to a mean of 85.0% of control levels ($P<0.1$). In marked contrast to the changes prior to oral contraceptive therapy, cardiac output rose during the succeeding 20 minute tilt period to 88.0% and 89.5% of

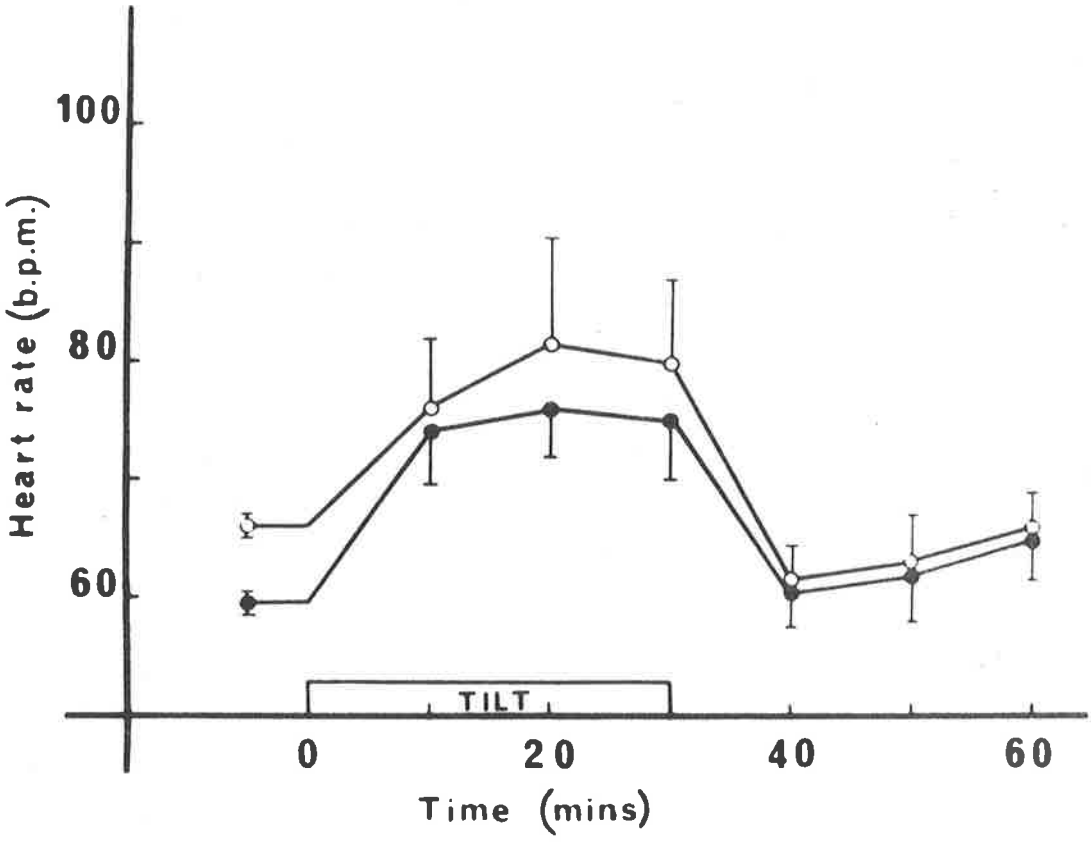


Fig. 7.1

Changes in heart rate (mean ± 1 S.E.) in response to tilting before (O) and during (●) oral contraceptive therapy. (Appendix 7.1)

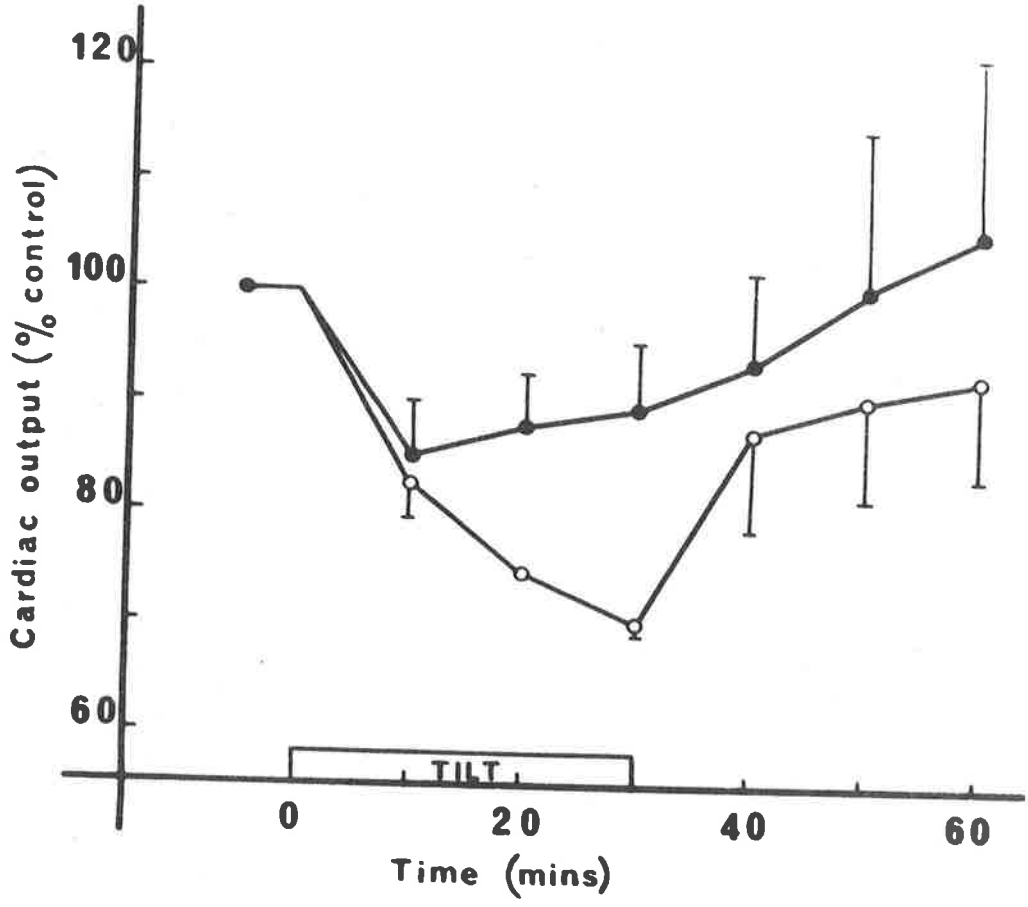


Fig. 7.2

Per cent. change in cardiac output (mean ± 1 S.E.) in response to tilting before (○) and during (●) oral contraceptive therapy. (Appendix 7.2)

control values, neither of which were significantly below control values. After returning to the horizontal, there was a further rise in cardiac output which was not significant.

2. Response of the renin-angiotensin system.

(a) Substrate. Mean changes in renin substrate in five out of seven subjects following commencement of oral contraceptive therapy are shown in Fig. 7.3. Detailed data are shown in Appendix 7.3. In all subjects there was a rise in renin substrate which was significant (Wilcoxon Ranking test: $P<0.001$). Changes were not significant, as tested by a paired "t" test, as one subject showed a marked elevation in renin substrate. There was no change in renin substrate levels during tilting either before or after commencement of oral contraceptive therapy.

(b) Plasma renin activity. The mean changes in plasma renin activity in six out of seven subjects are shown in Fig. 7.4 and the per cent. change in Table 7.1. Detailed data are shown in Appendix 7.4. One subject had an unexplained marked elevation of plasma renin activity prior to tilting both before and during oral contraceptive therapy. The results for this subject have been excluded from the values used for statistical analysis on the basis that they were an outlying observation, as tested by Dixon's gap test or the ratio (Range/ Standard Deviation) (Bliss, 1967).

Before oral contraceptive therapy, there was a rise in plasma renin activity in all subjects within 10 minutes of tilting. In those who did not faint there was a further rise 20 and 30 minutes after tilting. This rise was significant 30 and 40 minutes after tilting ($P<0.02$). Following a return to the horizontal position, there was a slow variable fall in all subjects.

After commencement of oral contraceptive therapy, control plasma renin activity was significantly elevated ($P<0.02$). Despite higher control levels, all subjects showed a rise in plasma renin activity during tilting, and this elevation was significant 30 minutes after tilting ($P<0.05$). The per cent. change in plasma renin activity was similar to that observed prior to treatment (Table 7.1.). Return

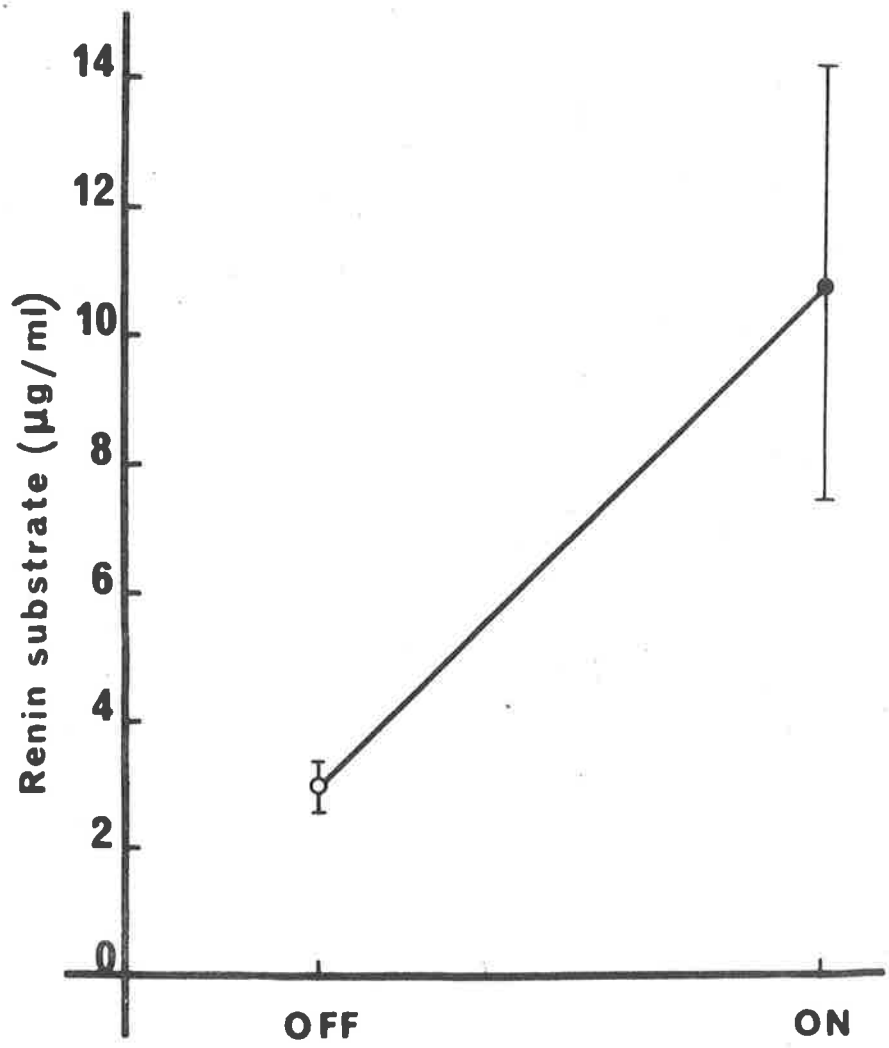


Fig. 7.3

Changes in renin substrate levels (mean ± 1 S.E.) before (○) and during (●) oral contraceptive therapy. (Appendix 7.3)

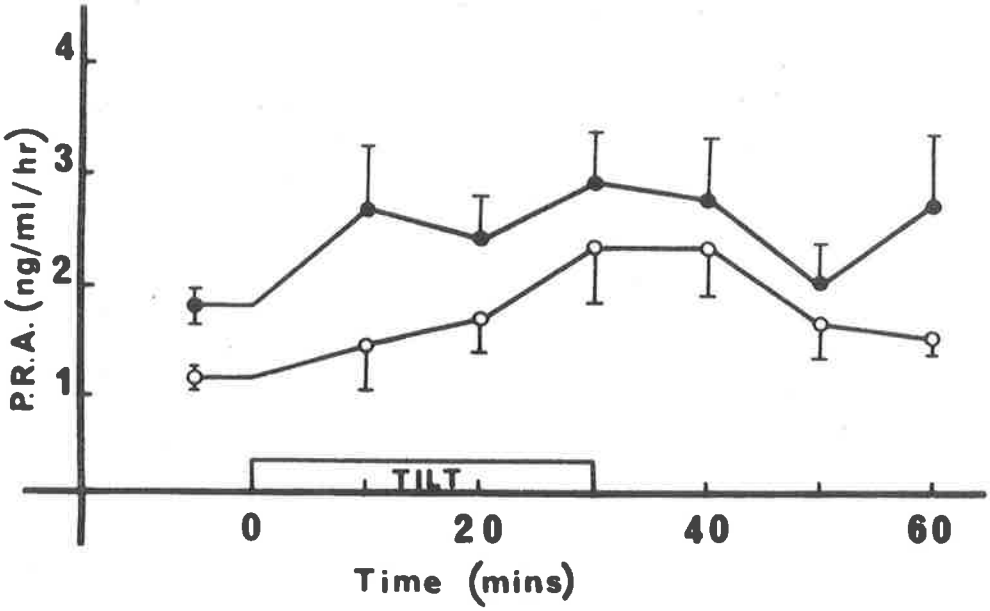


Fig. 7.4

Changes in P.R.A. (mean ± 1 S.E.) in response to tilting before (O) and during (●) oral contraceptive therapy. (Appendix 7.4)

TABLE 7.1

Percentage change in plasma renin activity and plasma renin concentration. Pre-tilting values ≡ 100%.

		TILT			POST-TILT		
P.R.A.	Off Pill	131.6 ±24.2	147.9 ±14.1	209.9 ±24.4	176.4 ±28.0	152.3 ±20.7	145.6 ±14.1
	On Pill	155.1 ±34.8	146.4 ±27.2	175.9 ±28.0	164.3 ±24.6	123.3 ±11.5	155.3 ±20.4
P.R.C.	Off Pill	108.0 ±11.2	110.2 ±17.8	120.1 ±14.6	98.7 ± 5.2	103.8 ± 5.7	97.0 ± 7.5
	On Pill	100.2 ± 5.0	71.7 ±10.6	176.5 ±24.1	107.3 ±13.5	82.4 ±14.3	78.1 ± 5.7

to the horizontal position was again associated with a gradual return to control plasma renin activity in all subjects.

(c) Plasma renin concentration. Changes in plasma renin concentration in seven subjects during tilting, before and after commencement of oral contraceptive therapy, are shown in Fig. 7.5. Detailed data are shown in Appendix 7.5. Per cent. changes are shown in Table 7.1. Before commencement of oral contraceptive therapy, tilting was associated with a variable rise in plasma renin concentration which was not significant. Resumption of the horizontal position was associated with a fall in plasma renin concentration.

Administration of oral contraceptives was associated with a rise in plasma renin concentration in four subjects, no change in one, and a fall in two. Overall, there was a slight rise which was not significant. Tilting was associated with a rise in plasma renin concentration in five out of seven subjects, the rise being significant 30 minutes after tilting ($P<0.05$). The mean per cent. rise in plasma renin concentration 30 minutes after tilting (176.5) was not significantly different from the per cent. rise in plasma renin activity (175.9). Return to the horizontal position was associated with a variable slow fall in plasma renin concentration.

DISCUSSION

These results clearly show that the administration of oral contraceptives is associated with a marked increase in the ability of the cardiovascular system to compensate for the relatively severe stress of passive feet down tilting. Thus during oral contraceptive therapy no subject fainted during tilting, while prior to oral contraceptive therapy three out of seven subjects fainted within 15 minutes, and a fourth fainted at the end of the tilt period.

The mechanism of this increased compensation by the cardiovascular system probably depends on several factors. Before tilting, subjects receiving oral contraceptive therapy had significantly lower heart rates than those recorded prior to oral contraceptive therapy, but during tilting similar heart rates were recorded. Thus oral contraceptive therapy was associated with an increased cardiac

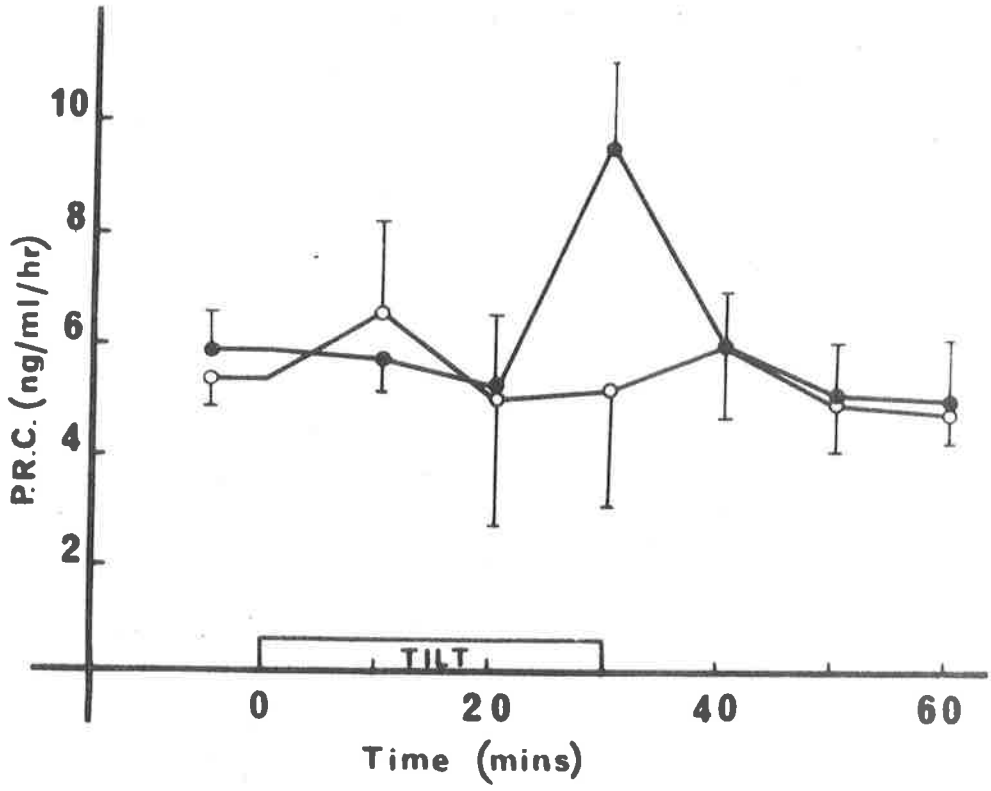


Fig. 7.5

Changes in P.R.C. (mean ± 1 S.E.) in response to tilting before (O) and during (●) oral contraceptive therapy. (Appendix 7.5)

response to postural stress. Further evidence for such a response is provided by the changes in cardiac output. Thus, prior to oral contraceptive therapy, tilting caused a progressive fall in cardiac output, while during oral contraceptive therapy, after initially falling to similar values, there was a progressive rise in cardiac output during the tilt period. It is possible that there was also a greater increase in peripheral resistance, thus maintaining arterial pressure high enough for adequate cerebral perfusion.

The increased cardiac response to tilting may be related to the changes in the renin-angiotensin system caused by oral contraceptive therapy. Oral contraceptive therapy was associated with a significant rise in both renin substrate and plasma renin activity, confirming the observations of others (Laragh *et al.*, 1967; Skinner *et al.*, 1969; Walters & Lim, 1970). These changes in renin activity are associated with increased angiotensin II levels (Cain *et al.*, 1971). Despite significantly higher control plasma renin activities during oral contraceptive therapy, tilting was associated with a further significant increase. Furthermore, tilting caused a significant increase in plasma renin concentration not observed prior to oral contraceptive therapy.

An increase in angiotensin II levels is associated with immediate and delayed effects. The latter are related to the effect of angiotensin on salt and water balance due to an increase in aldosterone levels (Layne *et al.*, 1962; Laragh *et al.*, 1967). Thus oral contraceptive therapy is associated with a significant increase in cardiac output, blood volume, and mean arterial pressure (Walters & Lim, 1970), all of which may be explained by an increase in aldosterone levels. While such changes may account for the increased ability of the cardiovascular system to compensate for postural stresses, the effects of an acute rise of angiotensin levels on the cardiovascular system may also play a part. The pressor response to intravenous infusions of angiotensin in man is due in part to a central stimulation of peripheral sympathetic nerves (Scroop & Whelan, 1966). In greyhounds, angiotensin has an indirect effect, important

in circulatory homeostasis during haemorrhage (Katic *et al.*, 1971). This response may be due to an inhibition of vagal tone on the heart, thus causing a relative increase in sympathetic activity (Scroop & Lowe, 1969).

A further possibility is that the administration of oral contraceptives is associated with an increased sensitivity of the cardiovascular system to either angiotensin or noradrenaline, or both.

Thus, the presence of angiotensin in the perfusate of isolated vessels is associated with an increased response to noradrenaline and, furthermore, angiotensin infusions *in vivo* inhibit the uptake of noradrenaline (Khairallah, Davila, Papanicolaou, Glende & Meyer, 1971).

In the present study there was a significant increase in plasma renin concentration in response to tilting during oral contraceptive therapy not observed prior to therapy. An explanation for this difference is not immediately apparent. However, the technique used in the present study to determine plasma renin concentration reduces plasma pH to 3.3 before dialysis to pH 7.5. It has been shown that exposure of renin in amniotic fluid to pH below 4.5 is associated with a marked increase in enzyme activity (Lumbers, 1971).

Thus renin at physiological pH exists in two forms, one only activated by low pH treatment. The physiological significance of the "inactive" renin present in plasma is unknown, but, since oral contraceptive therapy was associated with a rise in plasma renin concentration during tilting, it is probable that there is an increased amount of "inactive" renin released during postural stress.

Finally, these results offer an explanation for the onset of hypertension in some individuals during oral contraceptive therapy. Such individuals may have heightened responses to the daily repeated stress which upright posture imposes, or they may be unable to produce inactive renin. In such individuals the inactive-active renin ratio would approach one, and consequently rises in angiotensin II may be greater.

Simultaneous estimations of catecholamines, angiotensin and active-inactive renin during tilting in normotensive and hypertensive

subjects are necessary in order to establish the significance of such a mechanism in the aetiology of oral contraceptive-induced hypertension.

REFERENCES

Abel, J. J. & Crawford, A. C. (1897). On the blood-pressure-raising constituent of the suprarenal capsule. John Hopkins Medical Bulletin, 8, 151.

Ahlquist R. P. (1948). A study of the adrenotropic receptors. Am. J. Physiol., 153, 586.

Annau, E., Huszak, S. T., Svirbely, J. L. & Szent-Gyorgi, A. (1932). The function of the adrenal medulla. J. Physiol., 76, 181.

Anrep, G. von (1912). On the part played by the suprarenals in the normal vascular reactions of the body. J. Physiol., 45, 307.

Aviado, D. M. (1965). The Lung Circulation. Pergamon Press Ltd, p. 275-294.

Bacq, Z. M. (1934). La pharmacologie du système nerveux autonome, et particulièrement du sympathique d' après la theorie neuro-humorale. Annales de physiologie et de physicochemie biologique, 10, 467.

Bacq, Z. M. & Brouha, L. (1934). Sympathectomie totale, en une seule opération, chez le chat et le chien (avec présentation d'animaux). Compte rendus de societé de biologie, 117, 69.

Bacq, Z. M. & Fredericq, H. (1935). Recherches sur la physiologie et la pharmacologie du système nerveux autonome. XIV. Modifications apportées par deux dérivés de l'amino methyl benzodioxane (883 F & 933 F) aux effets de l'adrenaline et de l'excitation sympathique sur la membrane nictitante du chat. Arch. Internationale de Physiologie, 40, 454.

Bacq, Z. M. & Fredericq, H. (1940). Recherches sur la physiologie et la pharmacologie du système nerveux autonome. XI. Essai d'identification du médiateur chimique libéré dans la membrane nictitante du chat par l'excitation sympathique. Arch. Internationale de Physiologie, 40, 297.

Barajas, L. (1964). The innervation of the juxta-glomerular apparatus. An electron microscope study of the innervation of glomerular arterioles. Lab. Invest., 13, 916.

Barger, G. & Dale, H. H. (1910). Chemical structure and sympathomimetic action of amines. J. Physiol., 41, 19.

Becker, E. J. (1968). Sympatho-adrenal response to hypoxia. Pflügers Arch., 304, 1.

Bedford, E. A. (1917). The epinephrine content of the blood in conditions of low blood pressure and shock. Am. J. Physiol., 43, 235.

Bernard, C. (1878). Lecons sur les phénomeñes de la vie communs aux animaux et aux végétaux. Paris. J. B. Baillière et Fils.

Bickerton, R. K. & Buckley, J. P. (1961). Evidence for a central mechanism in angiotensin induced hypertension. Proc. Soc. Exp. Biol. Med., 106, 834.

Bing, J. & Kazimierczak, J. (1960). Renin content of different parts of the periglomerular circumference. Acta Path. Microbiol. Scan., 50, 1.

Biron, P. & Huggins, C. G. (1968). Pulmonary activation of synthetic angiotensin I. Life Science, 7, 965.

Biron, P., Meyer, P. & Panisset, J. C. (1968). Removal of angiotensins from the systemic circulation. Canad. J. Physiol. Pharmac., 46, 175.

Bjurstedt, H. & Coleridge, J. C. G. (1962). Observations on haemorespiratory effects of acute haemorrhage. In: Shock, Pathogenesis & Therapy. Ed. K. D. Bock, p. 200-204.

Bliss, C. I. (1967). Statistics in Biology, Vol. 1. McGraw-Hill, New York, p. 153-155.

Bonjour, J. P. & Malvin, R. L. (1970). Stimulation of ADH release by the renin angiotensin system. Am. J. Physiol., 218, 1555.

Born, G. V. R. & Gillson, R. E. (1959). Studies on the uptake of 5-Hydroxytryptamine by platelets. J. Physiol. (Lond.), 146, 472.

Boucher, R., Veyrat, R., Champlain, J. de & Genest, J. (1964). New procedures for measurement of human plasma angiotensin and renin activity levels. Canad. Med. Ass. J., 90, 194.

Boyd, G. W., Adamson, A. R., Fitz, A. H. & Peart, W. S. (1969). Radioimmunoassay determination of plasma-renin activity. Lancet, 1, 213.

Boyd, G. W., Landon, J. & Peart, W. S. (1967). Radioimmunoassay for determining plasma levels of angiotensin II in man. Lancet, 2, 1002.

Braun-Menéndez, E., Fasciolo, J. C., Leloir, L. F. & Muñoz, J. M. (1940). The substance causing renal hypertension. J. Physiol., 98, 283.

Braun-Menéndez, E., Fasciolo, J. C., Leloir, L. F., Muñoz, J. M. & Taquini, A. C. (1946). Renal Hypertension. Translated by L. Dexter, Springfield, Illinois, C. C. Thomas, p. 108-113.

Braun-Menéndez, E. & Page, I. H. (1958). Suggested revision of nomenclature. Angiotensin. Science, 127, 242.

Brennan, L. A., Malvin, R. L., Jochim, K. E. & Roberts, D. E. (1971). Influence of right and left atrial receptors on plasma concentrations of ADH and renin. Am. J. Physiol., 221, 273.

Bright, R. (1836). Tabular view of the morbid appearances in 100 [illegible] 30.

Broughton Pipkin, F., Mott, J. C. & Roberton, N. R. C. (1971). Angiotensin II-like activity in circulating arterial blood in immature and adult rabbits. J. Physiol., 218, 385.

tson, hanges in posture. Clin. Sci., 30, 279.

Brown, J. J., Davies, D. L., Lever, A. F., Robertson, J. I. S. & Verniory, A. (1966). The effect of acute haemorrhage in the dog and man on plasma renin concentration. J. Physiol., 182, 649.

Brown, J. J., Hodge, R. L., Lever, A. F., Lowe, R. D., Robertson, J. I. S. & Vane, J. R. (1967). Concurrent measurement of renin and angiotensin in the circulation of the dog. Nature (Lond.),

Brown-Séquard, C.E. (1889). Lancet, 2, 105.

Brubacher, E. S. & Vander, A. J. (1968). Sodium deprivation and renin secretion in unanaesthetised dogs. Am. J. Physiol., 214, 15.

Bulbring, E., Burn, J. H. & Elio, F. J., de (1948). The secretion of adrenaline from the perfused adrenal gland. J. Physiol., 107, 222.

Bumpus, F. M., Schwarz, H. & Page, I. H. (1957). Synthesis and pharmacology of the octapeptide angiotonin. Science, 125, 886.

Bunag, R. D., Page, I. H. & McCubbin, J. W. (1966). Neural stimulation of release of renin. Circulat. Res., 19, 851.

Cain, M. D., Catt, K. J. & Cochlan, J. P. (1969). Chromatography and radioimmunoassay of angiotensin II and metabolites in blood. Biochimica et Biophysica Acta., 194, 322.

Cain, M. D., Walters, W. A. & Catt, K. J. (1971). Effects of oral contraceptive therapy on the renin-angiotensin system. J. Clin Endocrin. Metab., 33, 671.

Cannon, W. B. (1926a). Physiological regulation of normal states: some tentative postulates concerning biological homeostatics. À Charles Richet; ses amis, ses collègues, ses élèves, 22 Mai 1926. Auguste Pettit, Paris Les Éditions Médicales.

Cannon, W. B. (1926b). Some general features of endocrine influence on metabolism. Am. J. Med. Sci., 171, 1.

Cannon, W. B. (1929). Organisations for physiological homeostasis. Phys. Rev., 9, 399.

Cannon, W. B. (1940). The adrenal medulla. Bulletin of New York Acad. Med., 16, 3.

Cannon, W. B. & Hoskins, R. G. (1911). The effects of asphyxia, hyperpnoea and sensory stimulation on adrenal secretion. Am. J. Physiol., 29, 274.

Cannon, W. B. & Paz, D., de la (1911). Emotional stimulation of adrenal secretion. Am. J. Physiol., 28, 64.

Cannon, W. B. & Rosenblueth, A. (1933). Sympathin E and sympathin I. Am. J. Physiol., 104, 557.

Cantu, J. R., Nahas, G. G. & Manger, W. M. (1966). Effect of hypercapnic acidosis and of hypoxia on adrenal catecholamine output of the spinal dog. Proc. Soc. Exp. Biol. Med., 38, 500.

Catt, K. J., Cain, M. D. & Coghlan, J. P. (1967). Measurement of angiotensin II in blood. Lancet, 2, 1005.

Catt, K. J., Cain, M. D., Coghlan, J. P., Zimmet, P. Z., Cran, E. & Best, J. B. (1970). Metabolism and blood levels of angiotensin II in normal subjects, renal disease and essential hypertension. Circulat. Res., 26-27, Suppl. II, 177.

Chalmers, J. P., Korner, P. I. & White, S. W. (1967). Effects of haemorrhage on the distribution of the peripheral blood flow in the rabbit. J. Physiol., 192, 561.

Chien, S. (1967). Role of the sympathetic nervous system in haemorrhage. Phys. Rev., 47, 214.

Clezy, T. M., Foy, B. N., Hodge, R. L. & Lumbers, E. R. (1972). Oral contraceptives and hypertension: an epidemiological survey. Br. Heart J. (in press).

Cohn, E. J., Oncley, F. L., Strong, L. E., Hughes, W. L. & Armstrong, S. H. (1944). Chemical, clinical and immunological studies on the products of human plasma fractionation. J. Clin. Invest., 23, 417.

Collier, H. O. J. (1969). A pharmacological analysis of aspirin. Advances in Pharmacology and Chemotherapy 7, 333.

Collins, D. A. (1936). Hypertension from constriction of the arteries of denervated kidneys. Am. J. Physiol., 116, 616.

Collins, D. A. & Hamilton, A. S. (1940). Pressor responses following short complete renal ischaemia: characteristics, mechanism, specificity for kidney. Am. J. Physiol., 130, 784.

Comline, R. S. & Silver, M. (1961). The release of adrenaline and noradrenaline from the adrenal glands of the foetal sheep. J. Physiol., 156, 424.

Comline, R. S. & Silver, M. (1971). Catecholamine secretion by the adrenal medulla of the foetal and new born foal. J. Physiol., 216, 659.

Cook, W. F. & Pickering, G. W. (1962). The location of renin in the kidney. Biochem. Pharmac., 9, 165.

Culp, J. R., Erdos, E. G., Hinshaw, L. B. & Holmes, D. D. (1971). Effects of anti-inflammatory drugs in shock caused by injection of living *E. coli* cells. Proc. Soc. Exp. Biol. Med., 137, 219.

Dale, H. H. (1906). On some physiological actions of ergot. J. Physiol., 34, 163.

Daly, M. de Burgh, Lambertsen, C. J. & Schweitzer, A. (1954). Observations on the volume of blood flow and oxygen utilisation of the carotid body in the cat. J. Physiol., 125, 67.

Daly, M. de Burgh & Scott, M. J. (1962). An analysis of the primary cardiovascular reflex effects of stimulation of the carotid body chemoreceptors of the dog. J. Physiol., 162, 555.

Darby, T. D. & Watts, D. T. (1964). Acidosis and blood epinephrine levels in haemorrhagic hypotension. Am. J. Physiol., 260, 1281.

Davis, D. A., Dutta, S. N., Booker, W. M. & Pradhan, S. W. (1969). Central effects of angiotensin II in cats. Pharmacologist, 11, 271.

Davis, R. B., Meeker, W. R. & Bailey, W. L. (1961). Serotonin release by bacterial endotoxin. Proc. Soc. Exp. Biol. Med., 108, 774.

Davis, R. B., Meeker, W. R. & McQuarrie, D. G. (1960). Immediate effects of intravenous endotoxin on serotonin concentrations and blood platelets. Circulat. Res., 8, 234.

Dexter, L., Frank, H. A., Haynes, F. W. & Altschule, M. D. (1943). Traumatic shock. VI. The effect of haemorrhagic shock on the concentration of renin and hypertensinogen in the plasma in unanaesthetised dogs. J. Clin. Invest., 22, 847.

Dock, W. (1940). Vasoconstriction in renal hypertension abolished by pithing. Am. J. Physiol., 130, 1.

Egdahl, R. H. (1959). The effect of bacterial endotoxin on adrenal medullary function. Clin. Res., 7, 158.

Elliott, D. F. & Peart, W. S. (1957). The amino acid sequence in a hypertensin. Biochem. J., 65, 246.

Elliott, T. R. (1904). On the action of adrenalin. J. Physiol., 31, Proc. XX.

Elliott, T. R. (1912). The control of the suprarenal glands by splanchnic nerves. J. Physiol., 44, 374.

Engelman, K. & Portnoy, B. (1970). A sensitive double-isotope derivative assay for norepinephrine and epinephrine: normal resting human plasma levels. Circulat. Res., 26, 53.

Erdos, E. G., Hinshaw, L. B. & Gill, C. C. (1967). Effect of indomethacin in endotoxin shock in the dog. Proc. Soc. Exp. Biol. Med., 125, 916.

Euler, U. S., von (1946). A specific sympathomimetic ergone in adrenergic nerve fibres (sympathin) and its relation to adrenaline and noradrenaline. Acta Physiol. Scand., 12, 73.

Euler, U. S., von (1948). Identification of the sympathomimetic ergone in adrenergic nerves of cattle (sympathin N) with laevo-noradrenaline. Acta Physiol. Scand., 16, 63.

Euler, U. S., von (1956). Noradrenaline. C. C. Thomas, Springfield, Illinois, U.S.A.

Euler, U. S., von & Floding, I. (1955). A fluorimetric micromethod for differential estimation of adrenaline and noradrenaline. Acta Physiol. Scand., 33, Suppl. 118, 45.

Euler, U. S., von & Sjostrand, T. (1941). Factors influencing renin pressor action. Acta Physiol. Scand., 2, 264.

Evans, G., Packham, M. A., Nishizawa, E. E., Mustard, J. F. & Murphy, E. A. (1968). The effect of acetylsalicylic acid on platelet function. J. Exp. Med., 128, 877.

Fasciolo, J. C., Houssay, B. A. & Taquini, A. C. (1938). The blood pressure raising secretion of the ischaemic kidney. J. Physiol., 94, 281.

Feldberg, W. & Lewis, G. P. (1964). The action of peptides on the adrenal medulla. Release of adrenaline by bradykinin and angiotensin. J. Physiol., 171, 98.

Ferrario, C. M., Gildenburg, P. L. & McCubbin, J. W. (1972). Cardiovascular effects of angiotensin mediated by the central nervous system. Circulat. Res., 30, 257.

Ferreira, S. H., Moncada, S. & Vane, J. R. (1971). Indomethacin and aspirin abolish prostaglandin release from the spleen. Nature (New Biol.), 231, 237.

Feuerstein, G. & Gutman, Y. (1971). Preferential secretion of adrenaline or noradrenaline by the cat adrenal *in vivo* in response to different stimuli. Br. J. Pharmac., 43, 764.

Field, L. W. & Laverty, R. (1958). Nervous and humoral responses to acute blood loss in the rat. J. Physiol., 143, 213.

Fiorica, V. (1965). An improved semi-automated procedure for fluorimetric determination of plasma catecholamines. Clin. Chim. Acta., 12, 191.

Fiorica, V., Iampietro, P. F., Burr, M. J. & Moses, R. (1969). Influence of blood pH on adrenomedullary response to haemorrhage. Am. J. Physiol., 217, 1211.

Folkow, B. & Euler, U. S., von (1954). Selective activation of noradrenaline and adrenaline producing cells in the cat's adrenal gland by hypothalamic stimulation. Circulat. Res., 2, 191.

Fowler, N. O., Shabetai, R. & Holmes, J. L. (1961). Adrenal medullary secretion during hypoxia, bleeding and rapid intravenous infusion. Circulat. Res., 9, 427.

Freeman, N. E. & Page, I. H. (1940). Hypertension produced by constriction of the renal artery in sympathectomised dogs. Am. Heart J., 14, 405.

Friedman, B., Abramson, D. I. & Marx, W. (1938). Pressor substance in the cortex of the kidney. Am. J. Physiol., 124, 285.

Gabe, I. T., Tuckman, J. & Shillingford, J. P. (1962). Determination of relative changes in cardiac output from non-calibrated earpiece dye-dilution curves. Circulat. Res., 11, 405.

Gellhorn, E. & Lambert, E. H. (1939). The vasomotor system in anoxia and asphyxia. Illinois Monograph. Med. Sci., 2, No. 3.

Gilbert, R. P. (1959). Effect of antihistaminic and antiserotonin drugs on vascular responses to *E. coli* endotoxin in the cat. Proc. Soc. Exp. Biol. Med., 100, 346.

Gilbert, R. P. (1960). Haemodynamic effects of endotoxin. Phys. Rev., 40, 245.

Glaviano, V. V., Bass, N. & Nykiel, F. (1960). Adrenal medullary secretion of epinephrine and norepinephrine in the dogs subjected to haemorrhagic hypotension. Circulat. Res., 8, 564.

Glover, W. E., Marshall, R. J. & Whelan, R. F. (1957). The antagonism of the vascular effects of 5-Hydroxytryptamine by BOL 148 and sodium salicylate in the human subject. Br. J. Pharmac. Chemother., 12, 498.

Goldblatt, H. (1937). The production of persistent hypertension in monkeys (Macaque) by renal ischaemia. J. Exp. Med., 65, 671.

Goldblatt, H., Gross, J. & Hanzal, R. F. (1937). The effect of resection of splanchnic nerves on experimental renal hypertension. J. Exp. Med., 65, 233.

Goldblatt, H., Lynch, J., Hanzal, R. F. & Summerville, W. W. (1934). Studies on experimental hypertension 1. The production of persistent elevation of systolic blood pressure by means of renal ischaemia. J. Exp. Med., 59, 347.

Goldblatt, H. & Wartman, W. B. (1937). The effect of section of anterior spinal nerve roots on experimental hypertension due to renal ischaemia. J. Exp. Med., 66, 527.

Goormagtigh, N. (1945). La fonction endocrine des arterioles rénales. Rev. Belge. Sci. Méd., 16, 65.

Gould, A. B. & Goodman, S. A. (1970). The effect of hypoxia on the renin-angiotensinogen systems. Lab. Invest., 22, 443.

Gowdey, C. W. (1966). The autonomic nervous system in hypoxia. Proc. Int. Symp. cardiovasc. respir. Effects of Hypoxia. Kingston, Ont., 1965, p. 232-247 (Karger, Basel), N.Y. 1966.

Greenway, C. V., Lautt, W. W. & Stark, R. D. (1969). Separation of the acute and delayed haemodynamic responses to endotoxin in the cat. Am. J. Physiol., 217, 518.

Greenway, C. V. & Murthy, V. S. (1971). Mesenteric vasoconstriction after endotoxin administration in cats pretreated with aspirin. Br. J. Pharmac., 43, 259.

Greever, C. J. & Watts, D. T. (1959). Epinephrine levels in the peripheral blood during irreversible haemorrhagic shock in dogs. Circulat. Res., 7, 192.

Gross, F., Brunner, H. & Ziegler, M. (1965). Renin angiotensin system, aldosterone and sodium balance. Rec. Prog. in Horm. Res., 21, 119.

Guyton, A. C. (1963). Circulatory Physiology: Cardiac Output and its Regulation. W. B. Saunders, Philadelphia, p. 40.

Hall, R. C. & Hodge, R. L. (1971a). Changes in catecholamine and angiotensin levels in the cat and dog during haemorrhage. Am. J. Physiol., 221, 1305.

Hall, R. C. & Hodge, R. L. (1971b). Vasoactive hormones in endotoxin shock: a comparative study in cats and dogs. J. Physiol., 213, 69.

Hall, R. C., Hodge, R. L., Irvine, R. J. & Middleton, J. M. (1971). The effect of acetylsalicylic acid on the response to endotoxin. Proc. Aust. Physiol. Pharmac. Soc., 2, 45.

Hall, R. C. & Tyler, M. J. (1971). A computer program to estimate cardiac output directly from dye-dilution curves. J. Appl. Physiol., 31, 145.

Halmagyi, D. F. J., Starzecki, B. & Horner, G. J. (1963). Mechanism and pharmacology of endotoxin shock in sheep. J. Appl. Physiol., 18, 544.

Hamilton, A. S. & Collins, D. A. (1942). The homeostatic role of a renal humoral mechanism in haemorrhage and shock. Am. J. Physiol., 136, 275.

Harman, P. J. & Davies, H. (1948). Intrinsic nerves in the mammalian kidney. J. Comp. Neurol., 89, 225.

Harris, P. W. R. (1969). Malignant hypertension associated with oral contraceptives. Lancet, 2, 466.

Harrison, T. S. & Seaton, J. (1965). The relative effects of hypoxia and hypercarbia on adrenal medullary secretion in anaesthetised dogs. J. Surg. Res., 5, 560.

Hartroft, P. M. (1963). Juxtaglomerular cells. Circulat. Res., 12, 525.

Harvey, W. (1628). Excertitatio anatomica de motu cordis et sanguinis in animalibus. Francofurti. Translated: K. J. Franklin. Blackwell Scientific Publications, Oxford, 1957.

Heiffer, M. H., Mundy, R. L. & Grim, L. J. E. (1958). Effect of bacterial liopolysaccharides on plasma catecholamine levels. Fed. Proc., 17, 68.

Hinshaw, L. B., Bradley, G. M. & Carlson, C. H. (1959). Effect of endotoxin on renal function in the dog. Am. J. Physiol., 196, 1127.

Hinshaw, L. B., Emerson, T. E., Iampietro, P. F. & Brake, C. M. (1962). A comparative study of the haemodynamic actions of histamine and endotoxin. Am. J. Physiol., 203, 600.

Hinshaw, L. B., Solomon, L. A., Erdos, E. G., Reins, D. N. & Gunter, B. J. (1967). Effects of acetylsalicylic acid on the canine response to endotoxin. J. Pharmac. Exp. Therap., 157, 665.

Hinshaw, L. B., Spink, W. W., Vick, J. A., Mallet, E. & Finstad, J. (1961). Effect of endotoxin on kidney function and renal hemodynamics in the dog. Am. J. Physiol., 201, 144.

Hodge, R. L., Lowe, R. D., Ng, K. K. F. & Vane, J. R. (1969). Role of the vagus nerve in the control of the concentration of angiotensin II in the circulation. Nature, 221, 177.

Hodge, R. L., Lowe, R. D. & Vane, J. R. (1966a). Increased angiotensin formation in response to carotid occlusion in the dog. Nature (Lond.), 211, 491.

Hodge, R. L., Lowe, R. D. & Vane, J. R. (1966b). The effects of alteration of blood-volume on the concentration of circulating angiotensin in the anaesthetized dog. J. Physiol. (Lond.), 185, 613.

Hodge, R. L., Ng, K. K. F. & Vane, J. R. (1967). Disappearance of angiotensin from the circulation of the dog. Nature, 215, 138.

Hökfelt, B., Bygdeman, S. & Sekkenes, J. (1962). The participation of the adrenal glands in endotoxin shock. In "Shock, Pathogenesis and Therapy" International Symposium. Ed. K. D. Bock, p. 151-161.

Houssay, B. A. & Molinelli, E. A. (1926). Adrenal secretion produced by asphyxia. Am. J. Physiol., 76, 538.

Huidobro, F. & Braun-Menéndez, E. (1942). The secretion of renin by the intact kidney. Am. J. Physiol., 137, 47.

Iriki, M., Pleschka, K., Walther, O. E. & Simon, E. (1971). Hypoxia and hypercapnia in asphyctic differentiation of regional sympathetic activity in the anaesthetised rabbit. Pflügers Arch., 328, 91.

Johnson, J. A., Davis, J. O. & Witty, R. T. (1971). Effects of catecholamines and renal nerve stimulation on renin release in the nonfiltering kidney. Circulat. Res., 29, 646.

Kaindl, F. & Euler, U. S., von (1951). Liberation of noradrenaline and adrenaline from the suprarenals of the cat during carotid occlusion. Am. J. Physiol., 123, 284.

Kalas, J. P. & Jacobson, E. D. (1964). The effects of vasoactive antagonists in endotoxin shock. Am. Heart J., 67, 764.

Karim, F., Kidd, C., Malpus, C. M. & Penna, P. E. (1970). The effects of stimulation of the left atrial receptors on sympathetic efferent nerve fibres. J. Physiol., 40P.

Katic, F., Joy, M. D., Lavery, H., Lowe, R. D. & Scroop, G. C. (1971). Role of central effects of angiotensin in response to haemorrhage in the dog. Lancet, 2, 1354.

Kellaway, C. H. (1919). The hyperglycaemia of asphyxia and the part played therein by the suprarenals. J. Physiol., 53, 211.

Khairallah, P. A., Bumpus, F. M., Page, I. H. & Smeby, R. R. (1963). Angiotensinase with a high degree of specificity in plasma and red cells. Science, 140, 672.

Khairallah, P. A., Davila, D., Papanicolaou, N., Glende, N. M. & Meyer, P. (1971). Effects of angiotensin infusion on catecholamine uptake and reactivity in blood vessels. Circulat. Res., 28, Suppl. 2, 96.

Kohlstaedt, K. G. & Page, I. H. (1940). Production of renin by

Kohlstaedt, K. G., Page, I. H. & Helmer, O. M. (1940). The activation of renin by blood. Am. Heart J., 19, 92.

Korner, P. I. (1959). Circulatory adaptations in hypoxia. Physiol. Rev., 39, 687.

Korner, P. I. (1965). The role of the arterial chemoreceptors and baroreceptors in the circulatory response to hypoxia of the rabbit. J. Physiol., 180, 279.

Kottke, F. J., Kubiceck, W. G. & Visscher, M. B. (1945). The production of arterial hypertension by chronic renal artery-nerve stimulation. Am. J. Physiol., 145, 38.

Kuida, H., Gilbert, R. P., Hinshaw, L. B., Brunson, J. G. & Visscher, M. B. (1961). Species differences in effect of gram-negative endotoxin on circulation. Am. J. Physiol., 200, 1197.

Kuida, H., Hinshaw, L. B., Gilbert, R. P. & Visscher, M. B. (1958). Effect of gram-negative endotoxin on pulmonary circulation. Am. J. Physiol., 192, 335.

Langley, J. N. (1901). Observations on the physiological action of extracts of the suprarenal bodies. J. Physiol., 27, 237.

Laragh, J. H., Sealey, J. E., Ledingham, J. G. G. & Newton, M. A. (1967). Oral contraceptives, renin, aldosterone and high blood pressure. J. Am. Med. Ass., 201, 918.

Layne, D. S., Meyer, C. J., Vaishwanar, P. S. & Pincus, G. (1962). The secretion and metabolism of cortisol and aldosterone in normal and steroid-treated women. J. Clin. Endocr., 22, 107.

Leloir, L. F., Muñoz, J. M., Taquini, A. C., Braun-Menéndez, E. & Fasciolo, J. C. (1942). La formacion de hypertensinogeno. Rev. Argent. de Cardiol., 9, 269.

Lentz, K. E., Skeggs, L. T., Woods, K. R., Kahn, J. R. & Shumway, N. P. (1956). The amino-acid composition of hypertensin II and its biochemical relationship to hypertensin I. J. Exp. Med., 104, 183.

Lumbers, E. R. (1969). The quantitation and significance of renin in biological fluids. M.D. Thesis, University of Adelaide.

Lumbers, E. R. (1971). Activation of renin in human amniotic fluid by low pH. Enzymologia, 40, 329.

Lund, A. (1950). Simultaneous fluorimetric determinations of adrenaline and noradrenaline in blood. Acta. Pharmac. Toxicol., 6, 137.

MacIntosh, A. M. (1968). Oral contraceptives and hypertension; a clinical observation. Med. J. Aust., 2, 1010.

MacLean, L. D. & Weil, M. H. (1956). Hypotension (shock) in dogs produced by *E. coli* endotoxin. Circulat. Res., 4, 546.

Malmejac, J. (1958). Sur la nature de la secretion médullo-surrénale. Discussion sur des théories dualiste et uniciste. Bull. Acad. Roy. Méd. Belg., 23, 50.

Malmejac, J. (1964). Activity of the adrenal medulla and its regulation. Physiol. Rev., 44, 186.

Malvin, R. L. & Vander, A. J. (1967). Effects of angiotensin infusion on renal function in the unanaesthetised rat. Am. J. Physiol., 213, 1205.

Molzahn, M., Dissman, T. H., Halim, S., Lohmann, F. W. & Oelkers, S. W. (1972). Orthostatic changes of haemodynamics, renal function, plasma catecholamines and plasma renin concentration in normal and hypertensive man. Clin. Sci., 42, 209.

Mouw, D., Bonjour, J., Malvin, R. L. & Vander, A. (1971). Central action of angiotensin in stimulating ADH release. Am. J. Physiol., 220, 239.

Muñoz, J. M., Braun-Menéndez, E., Fasciolo, J. C. & Leloir, L. F. (1939). Hypertensin: The substance causing renal hypertension. Nature, 144, 980.

Mustard, J. F. & Packham, M. A. (1970). Factors influencing platelet function: adhesion, release and aggregation. Pharm. Rev., 22, 97.

Ng, K. K. F. & Vane, J. R. (1967). Conversion of angiotensin I to angiotensin II. Nature, 216, 762.

Ng, K. K. F. & Vane, J. R. (1968). Fate of angiotensin I in the circulation. Nature, 218, 144.

Northover, B. J. & Subramanian, G. J. (1962). Analgesic-antipyretic drugs as antagonists of endotoxin shock in dogs. J. Path. Bact., 83, 463.

Nykiel, F. & Glaviano, V. V. (1961). Adrenal catecholamines in *E. coli* endotoxin shock. J. Appl. Physiol., 16, 348.

Öberg, B. & White, S. (1970a). Circulatory effects of interruption and stimulation of cardiac vagal afferents. Acta. Physiol. Scand., 80, 383.

Öberg, B. & White, S. (1970b). The role of vagal cardiac nerves and arterial baroreceptors in the circulatory adjustments to haemorrhage in the cat. Acta. Physiol. Scand., 80, 395.

O'Brien, J. R. (1968). Effects of salicylates on human platelets. Lancet, 1, 779.

Oliver, G. & Schäfer, E. A. (1894). On the physiological action of extract of the suprarenal capsules. J. Physiol. (Lond.), 16, 1.

Oliver, G. & Schäfer, E. A. (1895). The physiological effects of extract of the suprarenal capsules. J. Physiol. (Lond.), 18, 230.

Oparil, S., Tregear, G. W., Koerner, T., Barnes, B. A. & Haber, E. (1971). Mechanism of pulmonary conversion of angiotensin I to angiotensin II in the dog. Circulat. Res., 29, 682.

Page, I. H. (1935). The relationship of the extrinsic renal nerves to the origin of experimental hypertension. Am. J. Physiol., 112, 166.

Page, I. H. (1939). On the nature of the pressor action of renin. J. Exp. Med., 70, 521.

Page, I. H. (1943). Hypotension and loss of pressor response to angiotonin as the result of trauma to the central nervous system and severe haemorrhage. J. Exp. Med., 78, 41.

Page, I. H. & Helmer, O. M. (1940). A crystalline pressor substance (angiotonin) resulting from the reaction between renin and renin activator. J. Exp. Med., 71, 29.

Page, I. H., McSwain, B., Knapp, G. M. & Andrus, W. D. (1941). The origin of renin activator. Am. J. Physiol., 134, 214.

Paton, W. D. M. (1957). A pendulum auxotonic lever. J. Physiol., 137, 35P.

Peart, W. S. (1955). A new method of large scale preparation of hypertensin, with a note on its assay. Biochem. J., 59, 300.

Peart, W. S. (1956). The isolation of a hypertensin. Biochem. J., 62, 520.

Pelletier, C. L., Edis, A. J. & Shepherd, J. T. (1971). Circulatory reflex from vagal afferents, in response to haemorrhage in the dog. Circulat. Res., 29, 626.

Pickering, G. & Prinzmetal, M. (1938a). Experimental hypertension of renal origin in the rabbit. Clin. Sci., 3, 357.

Pickering, G. & Prinzmetal, M. (1938b). Some observations on renin, a pressor substance contained in normal kidney together with a method for its biological assay. Clin. Sci., 3, 211.

Plentl, A. A., Page, I. H. & Davis, W. W. (1943). The nature of renin activator. J. Biol. Chem., 147, 143.

Pletscher, A. (1968). Metabolism, transfer and storage of 5-Hydroxy-tryptamine in blood platelets. Br. J. Pharmac. Chemother., 32, 1.

Prez, R. M., des, Horowitz, H. I. & Hook, E. W. (1961). Effects of bacterial endotoxin on rabbit platelets. I. Platelet aggregation and release of platelet factors *in vitro*. J. Exp. Med., 114, 857.

Regoli, D., Riniker, B. & Brunner, H. (1963). The enzymatic degradation of various angiotensin II derivatives by serum, plasma or kidney homogenate. Biochem. Pharmac., 12, 637.

Regoli, D. & Vane, J. R. (1964). A sensitive method for the assay of angiotensin. Br. J. Pharmac., 23, 351.

Regoli, D. & Vane, J. R. (1966). The continuous estimation of angiotensin formed in the circulation of the dog. J. Physiol., 183, 513.

Renzini, V., Brunori, C. A. & Valori, C. (1970). A sensitive and specific fluorimetric method for the determination of noradrenaline and adrenaline in human plasma. Clinica. Chim. Acta., 30, 587.

Rosenberg, J. C., Lillehei, R. C., Longerbeam, J. & Zimmerman, B. (1961). Studies on haemorrhagic and endotoxin shock in relation to vasomotor changes and endogenous circulating epinephrine, norepinephrine and serotonin. Ann. Surg., 154, 611.

Sapirstein, L. A., Ogden, E. & Southard, F. D. (1941). Renin-like substance in blood after haemorrhage. Proc. Soc. Exp. Biol. Med., 48, 505.

Schaepdryver, A. F., de (1959). Physio-pharmacological effect on supra-renal secretion of adrenaline and noradrenaline in dogs. Arch. Int. Pharmacodyn., 71, 222.

Schmid, H. E. (1968). Action of angiotensin in normal, adrenalectomised and renal hypertensive trained dogs. Nephron, 5, 265.

Schwyzer, R., Iselin, B., Kappeler, H., Riniker, B., Rittel, W. & Zuber, H. (1958). Synthese, hochwirksamer octapeptide der vermutlichen amino sauresequenz des noch unbekannten Hypertensin II aus Rinderseum (val^5 Hypertensin II und val^5 Hypertensin II asp-beta-amid). Helv. Chim. Acta., 41, 1287.

Scornik, O. A. & Paladini, A. C. (1964). Angiotensin blood levels in haemorrhagic hypotension and other related conditions. Am. J. Physiol., 206, 553.

Scroop, G. C. & Lowe, R. D. (1968). Central pressor effect of angiotensin mediated by the parasympathetic nervous system. Nature (Lond.), 220, 1331.

Scroop, G. C. & Lowe, R. D. (1969). Efferent pathways of the cardiovascular response to vertebral artery infusions of angiotensin in the dog. Clin. Sci., 37, 605.

Scroop, G. C. & Whelan, R. F. (1966). A central vasomotor action of angiotensin in man. Clin. Sci., 30, 79.

Severs, W. B., Daniels, A. E. & Buckley, J. P. (1967). On the central hypertensive effect of angiotensin II. Int. J. Neuropharmac., 6, 199.

Skeggs, L. T., Kahn, J. R. & Shumway, N. P. (1952). The isolation and assay of hypertensin from blood. J. Exp. Med., 95, 241.

Skeggs, L. T., Kahn, J. R. & Shumway, N. P. (1956a). The preparation and function of the hypertensin converting enzyme. J. Exp. Med., 103, 295.

Skeggs, L. T., Kahn, J. R. & Shumway, N. P. (1956b). The purification of hypertensin II. J. Exp. Med., 103, 300.

Skeggs, L. T., Lentz, K. E., Kahn, J. R. & Shumway, N. P. (1958). The synthesis of a tetradecapeptide renin substrate. J. Exp. Med., 108, 283.

Skeggs, L. T., Lentz, K. E., Kahn, J. R., Shumway, N. P. & Woods, K. R. (1956). The amino-acid sequence of hypertensin II. J. Exp. Med., 104, 193.

Skeggs, L. T., Marsh, W. H., Kahn, J. R. & Shumway, N. P. (1954a). The existence of two forms of hypertensin. J. Exp. Med., 99, 275.

Skeggs, L. T., Marsh, W. H., Kahn, J. R. & Shumway, N. P. (1954b). The purification of hypertensin I. J. Exp. Med., 100, 363.

Skinner, S. L. (1967). Improved assay methods for renin "concentration" and "activity" in plasma. Circulat. Res., 20, 391.

Skinner, S. L., Lumbers, E. R. & Symonds, E. M. (1969). Alteration by oral contraceptives of normal menstrual changes in plasma renin activity, concentration and substrate. Clin. Sci., 36, 67.

Smith, D. J. & Vane, J. R. (1966). Effects of oxygen tension on vascular and other smooth muscle. J. Physiol., 186, 284.

Smith, J. B. & Willis, A. C. (1971). Aspirin selectively inhibits prostaglandin production in human platelets. Nature (New Biol.), 231, 235.

Smith, M. J. H. (1959). Salicylates and metabolism. J. Pharm. Pharmac., 11, 705.

Spink, W. W., Reddin, J., Zak, S. J., Peterson, M., Starzecki, B. & Seljeskog, E. (1966). Correlation of plasma catecholamine levels with haemodynamic changes in canine endotoxin shock. J. Clin. Invest., 45, 78.

Spink, W. W. & Vick, J. (1962). Canine endotoxin shock: reversal with aldosterone and angiotensin II (hypertensin). Proc. Soc. Exp. Biol. Med., 109, 521.

Spink, W. W., Vick, J. A., Melby, J. C. & Finstad, J. (1963). Influence of aldosterone and angiotensin II on endotoxin shock in the primate. Proc. Soc. Exp. Biol. Med., 112, 795.

Starling, E. H. (1905). The Croonian lectures on the chemical correlation of the functions of the body. Lancet, 2, 339.

Staszewska-Barczak, J. & Ceremuzynski, L. (1968). The continuous estimation of catecholamine release in the early stages of myocardial infarction in the dog. Clin. Sci., 34, 531.

Stein, M. & Thomas, D. P. (1967). Role of platelets in the acute pulmonary responses to endotoxin. J. Appl. Physiol., 23, 47.

Thomas, D. P. & Vane, J. R. (1967). 5-Hydroxytryptamine in the circulation of the dog. Nature (Lond.), 216, 335.

Tigerstedt, R. & Bergman, P. G. (1898). Niere und Kreislauf. Skand. Arch. Physiol., 8, 223.

Trinder, P. (1954). Rapid determination of salicylate in biological fluids. Biochem. J., 57, 301.

Turner, C. D. & Bagnara, J. T. (1971). General Endocrinology. 5th Ed. W. B. Saunders, Philadelphia, London, Toronto, p. 330.

Vander, A. J. (1967). Control of renin release. Physiol. Rev., 47, 359.

Vane, J. R. (1968). The release and assay of hormones in the circulation. The Scientific Basis of Medicine. Annual Reviews, 336.

Vane, J. R. (1969). The release and fate of vaso-active hormones in the circulation. Br. J. Pharmac., 35, 209.

Vendsalu, A. (1960). Studies on adrenaline and noradrenaline in plasma. Acta. Physiol. Scand., 49, Suppl. 173, 70.

Verney, E. B. & Vogt, M. (1938). An experimental investigation into hypertension of renal origin, with some observations on convulsive uraemia. Quart. J. Exp. Physiol., 28, 253.

Vick, J. A. (1960). Studies on the trigger mechanism involved in the vascular alterations induced by endotoxin. J. Lab. Clin. Med., 56, 953.

Vick, J. A. (1965). Bioassay of the prominent humoral agents involved in endotoxin shock. Am. J. Physiol., 209, 75.

Wakerlin, G. E. & Chobot, G. R. (1939a). A method for assay of renin. Am. J. Physiol., 126, 646.

Wakerlin, G. E. & Chobot, G. R. (1939b). Does renin play a role in the maintenance of normal blood pressure? Proc. Soc. Exp. Biol. Med., 40, 331.

Walker, W. F., Zileli, M. S., Reuter, F. W., Shoemaker, W. C., Friend, D. & Moore, F. D. (1959). Adrenal medullary secretion in haemorrhagic shock. Am. J. Physiol., 197, 773.

Walters, W. A. W. & Lim, Y. L. (1970). Haemodynamic changes in women taking oral contraceptives. J. Obstet. Gynaec. Br. Commonwealth, 77, 1007.

Walton, R. P., Richardson, J. A., Walton, R. P. Jr. & Thompson, W. L. (1959). Sympathetic influences during haemorrhagic hypotension. Am. J. Physiol., 197, 223.

Watts, D. T. (1956). Arterial blood epinephrine levels during haemorrhagic hypotension in dogs. Am. J. Physiol., 184, 271.

Watts, D. T. & Bragg, A. D. (1957). Blood epinephrine levels and automatic reinfusion of blood during haemorrhagic shock in dogs. Proc. Soc. Exp. Biol. Med., 96, 609.

Weil, M. H., MacLean, L. D., Visscher, M. B. & Spink, W. W. (1956). Studies on the circulatory changes in the dog produced by endotoxin from gram-negative micro-organisms. J. Clin. Invest., 35, 1191.

Weil-Malherbe, H. & Bone, A. D. (1952). The chemical estimation of adrenaline-like substances in blood. Biochem. J., 51, 311.

Weir, R. J., Tree, M. & Fraser, R. (1969). Effect of oral contraceptives on blood pressure and on plasma renin, renin substrate and corticosteroids. J. Clin. Path., 23, Suppl. 3, 49.

Weiss, H. J., Aledort, L. M. & Kochwa, S. (1968). The effect of salicylate on the haemostatic properties of platelets in man. J. Clin. Invest., 47, 2169.

White, F. N., Gold, E. M. & Vaughn, D. L. (1966). Renin-aldosterone system in endotoxin shock in the dog. Am. J. Physiol., 212, 1195.

Woods, J. W. (1967). Oral contraceptives and hypertension. Lancet, 2, 653.

Yu, R. & Dickinson, C. J. (1965). Neurogenic effects of angiotensin. Lancet, 2, 1276.

STATISTICAL METHODS

1. *Student unpaired "t" test.*

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{s^2}{n_1} + \frac{s^2}{n_2}}}$$

$\bar{x}$ = mean

s = standard deviation in total sample

n = number in each sample

2. *Student paired "t" test.*

$$t = \frac{\frac{\Sigma x_1 - \Sigma x_2}{n}}{\frac{s}{\sqrt{n}}}$$

where x_1 = pre-treatment observation

x_2 = post-treatment observation

s = standard deviation

n = number in sample

3. *Wilcoxon Ranking Test.*

(1) Values in the two groups are ranked in descending order 1 to N where N is the number in the two groups.

(2) The rank number for values in each group are totalled giving two totals T_1 and T_2.

(3) The smaller total is examined in the appropriate tables.

4. *Correlation coefficient.*

$$r = \frac{\Sigma\{(x-\bar{x})(y-\bar{y})\}}{\sqrt{\Sigma(x-\bar{x})^2 \Sigma(y-\bar{y})^2}}$$

r = correlation coefficient
x = abscissa
y = ordinate

5. *Linear regression.*

$y = a + bx$

b = slope

$$= \frac{\Sigma(x-\bar{x})(y-\bar{y})}{\Sigma(x-\bar{x})^2}$$

$$= \frac{\Sigma xy}{\Sigma x^2}$$

6. *Dixon Gap Test for outlying observations.*

$$R_1 = \frac{x_2 - x_1}{x_n - x_1} \qquad (n = 3 \text{ to } 8)$$

$$R_2 = \frac{x_3 - x_1}{x_{n-2} - x_1} \qquad (n = 8 \text{ to } 13)$$

$$R_3 = \frac{x_3 - x_1}{x_{n-1} - x_1} \qquad (n = 13 \text{ to } 30)$$

x_1 = suspected outlying value.

$x_n - x_1$ = range of values including suspected value.

7. *Pearson & Stephens' ratio (R) for outlying observations.*

$$R = \frac{x_n - x_1}{s}$$

$x_n - x_1$ = range including suspected value.

s = standard deviation of the total sample.

APPENDIX 1.1

KREBS SOLUTION

NaCl	0.1179 M
KCl	0.0047 M
$NaHCO_3$	0.025 M
KH_2PO_4	0.0011 M
Glucose	0.0055 M
$CaCl_2$	0.0025 M
$MgCl_2$	0.001 M

Gassed with 5% CO_2, 95% O_2.

APPENDIX 1.2

Buffers used to dialyse samples for estimation of plasma renin activity and concentration.

1. pH 3.3

Amino-acetic acid	0.05 M
HCl	0.01 M
EDTA	0.0051 M
NaCl	0.0949 M

2. pH 4.5

Citric acid	0.0028 M
$Na_2HPO_4.12H_2O$	0.045 M
EDTA	0.0051 M
NaCl	0.0821 M

3. pH 7.5

$NaH_2PO_4.12H_2O$	0.0122 M
$Na_2HPO_4.12H_2O$	0.0867 M
EDTA	0.001 M
NaCl	0.075 M

APPENDIX 2.1

Correlation between angiotensin concentration as calculated from cardiac output and as determined by bioassay.

	Concentration of Angiotensin (ng/ml)	
Dog	Expected from cardiac output	Determined by bioassay
1	0.44	0.31
	0.38	0.28
2	0.22	0.32
	0.49	0.64
	1.12	1.43
	1.4	1.6
3	1.0	1.07
	0.43	0.8
	0.2	0.53
4	0.64	0.66
	0.36	0.17
	0.67	0.64

APPENDIX 2.2

Correlation between adrenaline concentration as calculated from cardiac output and as determined by bioassay.

	Concentration of Adrenaline (ng/ml)	
Dog	Expected from cardiac output	Determined by bioassay
1	4.0	4.0
	1.7	1.5
	8.6	9.0
	5.1	4.2
2	2.4	3.1
	1.5	1.11
	0.74	0.74
	0.38	0.21
	0.95	0.72
3	0.92	1.25
	1.0	0.63
	2.5	3.1
	1.59	1.62

APPENDIX 3.1

DOGS

Effect of slow (S) and fast (F) haemorrhage on blood pressure (B.P.), central venous pressure (C.V.P.), angiotensin levels (A-T) and catecholamine levels (C-A).

	Vol ml/kg	Rate ml/kg/min		Δ B.P mm Hg		Δ C.V.P mm H_2O		Δ A-T ng/kg/min		Δ C-A ng/kg/min	
Dog		S	F	S	F	S	F	S	F	S	F
13	7.5	0.7	2.6	0	-15	-5	-10	-	-	0	70
14	17.0	0.7	6.7	5	-15	-20	-20	17	15	0	30
17	9.0	0.9	4.3	-20	-25	-12	-15	10	10	0	60
19	4.5	0.9	4.5	-5	0	-5	-10	14	>14	0	100
38	8.5	0.35	8.5	-5	-20	-10	-15	21	21	0	104
Mean	9.3	0.7	5.3	-5	15	-10.4	-14	15.5	15.0	0	72.8
± S.E.		0.1	1.0	4.2	4.2	2.8	1.9	2.3	2.3		13.6

APPENDIX 3.2

CATS

Effect of slow (S) and fast (F) haemorrhage on blood pressure (B.P.), central venous pressure (C.V.P.), angiotensin levels (A-T) and catecholamine levels (C-A).

	Vol ml/kg	Rate ml/kg/min		Δ B.P mm Hg		Δ C.V.P mm H_2O		Δ A-T ng/kg/min		Δ C-A ng/kg/min	
Cat		S	F	S	F	S	F	S	F	S	F
15	12.4	0.8	6.2	-5	-25	15	10	12	12	160	190
21	7.3	0.3	1.7	+10	-20	-30	-20	16	25	110	250
22	6.0	0.6	3.5	0	0	0	-5	20	25	25	150
26	6.5	0.8	3.2	+5	-20	-5	-10	13	13	++	++
27	9.5	0.6	4.3	0	0	-5	-5	36	24	25	167
28	6.9	0.3	2.8	-5	-10	0	-10	11	14	56	69
29	6.5	0.5	3.0	-15	-20	-10	+10	15	15	110	110
Mean	8.0	0.56	3.5	-1.4	-13.6	-5	-4.3	17.6	18.3	81	156
± S.E.		0.1	0.5	3.0	3.9	5.1	4.1	3.3	2.3	22	26

APPENDIX 3.3

DOGS

Effect of slow continuous haemorrhage on blood pressure (B.P.), central venous pressure (C.V.P.), angiotensin levels (A-T) and catecholamine levels (C-A).

Dog	0	5	10	15	20	25	ml/kg
14	125	125	130	125	100	-	B.P. (mm Hg)
34	135	130	130	125	100	70	
35	130	130	130	130	125	115	
36	150	150	140	110	70	60	
38	135	135	140	140	130	75	
Mean	135	134	134	126	105	80	
± S.E.	4.2	4.3	2.5	4.9	10.7	12.1	
14	0	-5	-10	-15	-10	-	Δ C.V.P. mm H_2O
34	0	-22	-48	-44	-36	-22	
35	0	-10	-15	-15	-30	-40	
36	0	-5	-5	0	5	20	
38	0	-5	-10	-15	-20	-10	
Mean	-	-9.4	-17.6	-17.8	-18.2	-13	
± S.E.	-	3.3	7.8	7.2	7.3	12.6	
14	0	0	7	17	17	-	Δ A-T ng/kg/min
34	0	0	0	7	7	7	
35	0	0	6.5	16.8	25	27.5	
36	0	10	20	25	25	25.0	
38	0	5.2	13.3	21	21	21.0	
Mean	-	3.0	9.4	17.4	19	20.1	
± S.E.	-	2.0	3.4	3.0	3.3	4.6	
14	0	0	0	0	160	-	Δ C-A ng/kg/min
34	0	0	0	0	170	385	
35	0	0	0	0	0	0	
36	0	0	0	0	0	240	
38	0	0	0	0	0	100	
Mean	-	-	-	-	66	181	
± S.E.	-	-	-	-	40	84	

APPENDIX 4.1

Changes in blood pressure and circulating catecholamines during hypoxia and haemorrhage to 60 mm Hg.

BLOOD PRESSURE (mm Hg)

	Arterial PO_2				
Dog	30-40	40-50	50-60	60-70	70-80
1				12	5
2		15	10	15	
3	22	3	2		
4	40		12		
5			5	12	7
6			4	3	
7				5	7
8	20		15		
9		10	13		
10	14				
11		13			-5
12	96	45			5
13			13		10
14	20	15			
15				3	
16	20	7			
17	60	15		2	
18		24			
19	30			15	
20					0
Mean	35.7	16.3	9.2	8.4	1.9
± S.E.	8.8	4.1	1.8	2.0	2.1

CATECHOLAMINES (ng/kg/min) (Adrenaline Equivalent)

	Arterial PO_2					
Dog	30-40	40-50	50-60	60-70	70-80	Haemorrhage
1				74	110	>120
2		154	115	0		
3	100	50	0		0	50
4	175		20			
5			87	72	36	
6			14	0		>31
7				20	13	
8	0		0			45
9		77	0			
10	5.3					
11		0			0	
12	0	0			0	63
13			370		38	
14	70	330				
15				105		
16	207	0				
17	0	90		0		70
18		0				
19	18			18		100
20					0	>69
Mean	63.9	77.9	52.0	36.1	24.6	68.5
± S.E.	26.9	36.2	24.0	14.6	13.5	10.3

APPENDIX 5.1

DOGS

Effect of endotoxin on blood pressure expressed as a percentage of control.

	Control B.P. (mm Hg)	Minutes after Endotoxin							
Dog	0	5	10	20	30	45	60	75	90
2	120	92	100	75	71	75	62	54	54
3	130	100	100	100	96	77	65	62	58
4	110	95	91	82	86	91	91	91	86
6	125	96	96	96	100	80	60	48	48
7	145	76	69	55	41	38	66	72	72
10	130	100	100	96	88	58	35	27	27
15	155	100	77	45	39	32	52	55	58
18	90	72	89	83	78	67	50	39	39
20	160	103	100	94	88	56	44	47	50
23	110	35	59	77	86	86	68	55	59
Mean	128	87	75	80	77	66	59	55	55
± S.E.	6.8	6.7	8.6	5.8	6.7	6.3	4.9	5.6	5.2

APPENDIX 5.2

DOGS

Effect of endotoxin on central venous pressure.

	Minutes after Endotoxin							
Dog	5	10	20	30	45	60	75	90
2				Not Recorded				
3	0	-5	-10	-10	-10	-15	-15	-15
4	-15	-15	-10	-10	-10	-5	-5	-5
6	-5	-10	-10	-7	-5	-7	-10	-7
7	0	0	+10	+10	0	0	0	0
10	0	-5	-10	-15	-15	-15	-15	-15
15	-10	-5	+5	0	-25	-25	-25	-20
18	+5	-2	-5	-2	-12	-12	-10	-10
20	-5	-15	-25	-20	-15	-15	-20	-20
23	-5	0	+5	+5	+5	+5	+5	+5
Mean	-4	-6	-5.6	-5.4	-5.6	-9.9	-10.6	-9.7
± S.E.	2.0	2.0	3.6	3.2	2.3	3.0	3.2	2.9

APPENDIX 5.3

CATS

Effect of endotoxin on blood pressure expressed as a percentage of control.

		Minutes after Endotoxin							
Cat	Control B.P mm Hg	5	10	20	30	45	60	75	90
1	85	88	71	82	71	59	47	47	41
2	100	100	105	105	105	100	100	100	105
4	100	110	85	100	100	90	90	75	65
6	85	106	100	106	94	82	82	65	59
12	75	53	113	87	87	80	80	80	87
13	110	36	82	95	95	100	105	100	55
14	100	60	125	105	100	90	90	90	85
16	115	100	100	95	100	74	74	74	74
17	135	56	81	81	78	74	70	70	59
18	135	74	81	81	78	67	63	59	70
19	125	60	68	52	52	40	36	40	-
20	145	93	90	74	70	59	48	52	45
Mean	109	78	92	89	86	76	74	71	68
± S.E.	6.5	7.1	5.0	4.6	4.7	5.2	6.3	5.7	5.8

APPENDIX 5.4

CATS

Effect of endotoxin on central venous pressure.

	Minutes after Endotoxin							
Cat	5	10	20	30	45	60	75	90
1	0	+18	+5	+5	+10	+10	+10	+10
2	-5	+60	+25	0	-5	-5	-5	-5
4	-	-	-	-	-	-	-	-
6	+30	+10	0	-5	-10	-15	-15	-10
12	-	-	-	-	-	-	-	-
13	+85	+50	+20	+10	0	0	0	+5
14	+145	+65	+20	+15	+10	+5	0	0
16	+15	+25	-5	-20	-25	-25	-30	-35
17	+45	+20	+10	+5	+5	+5	0	+5
18	+85	+65	+20	0	-10	-10	-5	-5
19	-	-	-	-	-	-	-	-
20	+25	+10	+15	+10	+5	0	0	0
Mean	47.2	35.9	12.2	2.2	-2.2	-3.9	-5.0	-3.9
± S.E.	16.3	7.9	3.4	3.4	3.8	3.7	3.8	4.4

APPENDIX 5A.1

Effect of endotoxin on blood pressure (B.P.) and central venous pressure (C.V.P.) of aspirin-treated cats.

	Minutes after Endotoxin							
Cat	0	5	10	15	20	30	45	60
B.P.								
37	100	100	95	90	85	75	75	80
38	55	55	50	45	50	55	65	75
39	100	100	75	80	85	90	105	100
40	105	105	95	105	115	115	125	135
41	115	105	105	105	95	100	95	85
42	70	70	60	85	75	85	90	100
Mean	90.83	89.17	80.0	85.0	84.17	86.67	92.5	95.83
± S.E.	9.43	8.7	8.94	9.04	8.80	8.43	8.73	8.89
C.V.P.								
37	0	0	0	0	0	0	-5	-5
38	0	0	-10	-10	-10	-15	-15	-10
39	0	0	0	0	0	+5	+5	+10
40	0	0	-2.5	-5	-5	-10	-15	-20
41	0	0	0	0	0	0	-5	-5
42	0	0	-5	-5	-5	-5	-5	-5
Mean	0	0	-2.92	-3.33	-3.33	-4.17	-6.67	-5.83
± S.E.	-	-	1.64	1.67	1.66	3.01	3.07	3.96

APPENDIX 6.1

Effect of endotoxin on blood pressure (B.P.) and central venous pressure (C.V.P.) of aspirin-treated cats.

	Minutes after Endotoxin						
Cat	0	5	10	15	30	45	60
B.P.							
1	170	155	150	140	135	110	95
2	100	50	60	75	60	55	65
3	60	40	40	40	40	45	40
4	130	125	115	105	75	60	60
Mean	115	93	91	90	78	68	65
± S.E.	23	28	25	21	20	15	11
C.V.P.							
1	0	+5	-5	-10	-10	-10	-15
2	0	0	-	-10	-10	-15	-10
3	0	+5	0	0	0	0	0
4	0	0	0	0	-5	-10	-10
Mean	0	2.5	-1.25	-5	-6.25	-8.75	-8.75
± S.E.	-	1.4	1.25	2.9	2.4	3.14	3.14

APPENDIX 6.2

Effect of endotoxin on blood pressure (B.P.) and central venous pressure (C.V.P.) of aspirin-treated dogs.

	Minutes after Endotoxin					
Dog	0	5	15	30	45	60
B.P.						
1	155	160	160	160	160	155
2	145	138	138	140	135	130
3	95	55	35	60	80	90
4	100	85	85	80	110	130
Mean	120	110	105	110	121	126
± S.E.	18	24	28	24	17	13
C.V.P.						
1	0	+5	0	-5	-10	-20
2	0	0	0	0	-2.5	-5
3	0	0	5	0	-5	-10
4	0	-10	-20	-5	0	0
Mean	0	-1.25	-3.75	-2.5	-4.4	-8.8
± S.E.	-	3.14	5.5	1.4	2.1	4.3

APPENDIX 6.3A

CONTROL SHEEP

Effect of endotoxin on blood pressure (B.P.) and central venous pressure (C.V.P.).

	Minutes after Endotoxin							
Sheep	0	2	5	10	15	30	45	60
B.P.								
1	105	30	175	70	45	35	20	20
2	75	20	185	50	45	45	35	25
3	115	20	150	-	120	70	55	45
4	70	35	95	70	50	35	30	30
5	115	35	220	115	85	60	50	50
6	100	50	150	70	60	50	40	40
Mean	97	32	163	75	68	49	38	35
± S.E.	8.0	5	17.0	10.7	12	6	5	4.8
C.V.P.								
1	0	175	25	15	-15	-25	+5	+35
2	0	200	75	100	75	25	10	20
3	0	195	75	-	-	-	-	-
4	0	125	15	5	0	0	-	-
5	0	150	150	20	30	15	0	-10
6	0	-	-	-	-	-	-	-
Mean	0	169	68	35	22.5	3.8	5.0	15
± S.E.		14	24	22	19.8	10.9	2.9	13

APPENDIX 6.3B

ASPIRIN-TREATED SHEEP

Effect of endotoxin on blood pressure (B.P.) and central venous pressure (C.V.P.).

	Minutes after Endotoxin							
Sheep	0	2	5	10	15	30	45	60
B.P.								
7	110	110	110	110	100	95	55	45
8	105	105	105	105	105			
9	75	75	75	75	70	65	55	55
10	125	125	125	125	120	110	105	90
11	72	72	72	70	65	50	40	30
12	85	80	85	95	95	80	60	35
Mean	95	95	95	97	93	80	63	51
± S.E.	9.0	9.0	9.0	9.0	9.0	11.0	11.0	11.0
C.V.P.								
7	0	0	0	0	-5	-15	-20	-20
8	0	0	0	0	0	-	-	-
9	0	0	0	0	+5	+15	-	-
10	0	0	0	0	0	0	-5	0
11	0	-15	-10	-5	-5	-5	-5	-5
12	0	0	0	+5	+5	0	-5	-5
Mean	0	-2.5	-1.7	0	0	-1.0	-9.0	-7.5
± S.E.	-	2.5	1.7	1.3	1.8	4.8	3.8	4.3

APPENDIX 6.4

CATS, DOGS, SHEEP

Platelet count in control (C) and aspirin-treated (A) animals following endotoxin, expressed as a percentage of pre-endotoxin counts.

	Minutes after Endotoxin							
	5		10		15		60	
Species	C	A	C	A	C	A	C	A
Cats	44	4	46	-	46	31	142	101
	49	21	25	-	7	26	68	69
	55	22	47	-	80	38	107	99
	79	40	25	-	16	25	74	106
Mean	57	22	36	-	37	30	98	94
± S.E.	8	7.4	6	-	17	3	17	8
Dogs	46	51	-	-	29	67	65	173
	28	78	10	-	11	71	57	-
	43	32	17	-	28	32	-	34
	6	14	14	-	17	36	60	76
	28	-	28	-	73	-	-	-
	11	-	35	-	25	-	53	-
	-	-	25	-	25	-	43	-
Mean	27	44	22	-	30	52	56	94
± S.E.	7	14	4	-	8	10	4	41
Sheep	101	88	117	-	142	77	140	-
	85	73	85	93	80	78	80	-
	84	74	-	80	107	82	80	-
	71	122	109	38	130	36	-	-
	-	40	-	27	-	33	-	-
Mean	85	79	104	59.5	115	61.2	100	-
± S.E.	6	13	10	16	14	11	20	-

APPENDIX 6.5

SHEEP

Effect of aspirin on the response of blood pressure and central venous pressure to intravenous infusions of 5-HT. (C = control; A = during aspirin)

	Dose of 5-HT (µg/min)						
	0.15		0.3		0.6		
Sheep	C	A	C	A	C	A	Δ
1	20	10	20	30	-		
2	-	-	25	15	50	80	B.P.
3	15	5	30	15	60	55	mm Hg
4	-	-	5	5	5	45	
5	15	15	60	60	-	-	
Mean	17	10	28	25	38	60	
± S.E.	1.7	2.9	9.0	9.6	16.9	10.4	
1	0	0	0	0	-		
2	-	-	5	5	5	15	C.V.P.
3	0	5	10	15	10	35	mm H_2O
4	-	-	0	25	5	15	
Mean	0	2.5	4	11	7	22	
± S.E.	0	2.5	2.4	5.5	1.7	6.7	

APPENDIX 6.6

DOGS

The generation rate in ng/ml/min of a 5-HT-like substance in dogs' blood following incubation with endotoxin in the presence and absence of aspirin. Each generation rate is determined in a different animal.

	CONTROL		ASPIRIN-TREATED	
	Concentration Endotoxin (µg/ml)		Concentration Endotoxin (µg/ml)	
	21.5	65	67	200
	15	120	24.8	35
	26.3	161	16.0	52
	12.9	62	<11.0	79.6
	6.4	40	10.9	119
	12.7	21.8	55	55.4
	16.7	-	-	-
Mean	15.0	81	13.64	68.2
± S.E.	2.7	26.0	3.3	14.6

APPENDIX 7.1

Effect of tilting on heart rate before and during oral contraceptive (O.C.) therapy.

BEFORE O.C. THERAPY

Subject	Pre-Tilt			Tilt			Post-Tilt		
1	-	66	68	90	106				
2	-	74	76	48	Fainted	-	-	-	-
3	66	66	66	90	Fainted	-	65	60	60
4	70	65	65	95	Fainted	-	55	65	70
5	72	72	66	72	84	78	55	54	66
6	60	60	60	70	70	75	60	60	60
7	60	58	62	66	66	66	60	58	60
Mean	65.7			76	81.5	79.8	61.5	62.8	66.0
± S.E.	1.15			6.3	9.0	7.2	2.9	3.7	3.3
	DURING O.C. THERAPY								
1	64	60	60	84	84	84	56	68	68
2	68	67	66	88	86	94	66	66	68
3	65	65	60	80	85	85	75	75	75
4	60	60	60	72	72	72	56	60	66
5	50	50	50	56	56	60	50	50	50
6	60	60	60	78	78	72	60	54	60
7	60	50	60	60	70	60	60	-	-
Mean	59.4			74	75.9	75.3	60.4	62.2	64.5
± S.E.	1.2			4.6	4.1	4.9	3.1	3.8	3.5

APPENDIX 7.2

Effect of tilting on the cardiac output before and during oral contraceptive (O.C.) therapy. Changes are expressed as a percentage of pre-tilt values.

BEFORE O.C. THERAPY

Subject	Pre-Tilt	Tilt			Post-Tilt		
3	100	77	Fainted	-	106	93	89
4	100	88	Fainted	-	96	113	118
5	100	87	75	71	63	69	76
6	100	78	74	69	86	87	86
Mean		82.5	74.5	70.0	87.8	90.5	92.3
± S.E.		2.9	0.5	1.0	9.2	9.1	9.0
	DURING O.C. THERAPY						
3	100	76	88	84	83	-	-
4	100	97	95	93	111	126	123
5	100	77	75	77	77	75	74
6	100	90	94	104	104	100	122
Mean		85	88	89.5	93.8	100.3	106.3
± S.E.		5.1	4.6	5.8	8.2	14.7	16.2

APPENDIX 7.3

Effect of oral contraceptive therapy on renin substrate concentration.

	Renin Substrate (µg/ml)	
Subject	Before	During
1	3.3	24.3
2	3.00	9.0
5	4.00	8.3
6	2.70	5.5
7	1.80	7.2
Mean	2.96	10.86
± S.E.	.36	3.41

APPENDIX 7.4

Effect of tilting on plasma renin activity before and during oral contraceptive (O.C.) therapy. Data from subject 4 are excluded in calculating the means.

BEFORE O.C. THERAPY

Subject	Pre-Tilt			Tilt			Post-Tilt		
1	0.96	0.84	0.60	0.66	1.02	1.44	1.3	0.9	1.6
2	0.8	0.86	0.8	0.9	Fainted	-	1.15	1.02	1.08
3	1.5	-	1.0	2.8	Fainted	-	3.2	2.5	1.5
4	5.2	8.6	9.6	10.8	Fainted	-	11.4	7.4	9.9
5	1.2	-	-	1.4	2.1	3.1	3.2	1.1	1.6
6	1.5	1.3	1.05	1.6	1.45	2.45	1.65	2.15	-
7	1.36	1.8	1.68	-	-	-	3.5	2.3	1.9
Mean	1.15			1.47	1.52	2.33	2.33	1.66	1.54
± S.E.	0.09			0.37	0.31	0.48	0.44	0.30	0.13
	DURING O.C. THERAPY								
1	1.26	1.86	1.26	1.56	2.4	3.2	1.8	1.6	1.7
2	0.6	0.5	1.0	1.8	1.8	1.8	1.8	1.1	1.1
3	1.6	2.3	1.7	1.9	1.1	3.2	2.4	2.1	2.8
4	9.0	7.8	7.2	3.0	-	4.8	8.4	9.6	8.0
5	2.4	2.3	2.3	2.8	3.2	4.9	5.3	3.7	5.8
6	1.6	1.7	2.5	5.2	3.0	2.4	2.6	2.0	2.6
7	2.4	3.1	2.2	1.9	3.07	2.1	3.0	1.9	2.4
Mean	1.81			2.52	2.43	2.93	2.82	2.07	2.73
± S.E.	0.16			0.56	0.34	0.46	0.53	0.36	0.67

APPENDIX 7.5

Effect of tilting on plasma renin concentration before and during oral contraceptive (O.C.) therapy.

BEFORE O.C. THERAPY

Subject	Pre-Tilt			Tilt			Post-Tilt		
1	7.0	6.8	7.0	9.2	9.7	9.4	8.1	9.0	5.0
2	6.3	5.0	6.8	4.5	Fainted	-	5.4	6.3	6.5
3	5.0	7.8	-	6.3	Fainted	-	6.3	6.3	5.7
4	5.4	9.6	8.6	13.0	Fainted	-	11.0	5.6	5.4
5	3.3	3.3	3.3	3.3	2.6	3.0	2.9	3.3	3.8
6	3.0	-	2.4	3.6	3.0	3.6	-	2.4	-
7	3.0	3.0	-	-	-	-	3.0	3.0	3.0
Mean	5.37			6.65	5.1	5.33	6.12	5.12	4.9
± S.E.	0.52			1.52	2.30	2.05	1.27	0.89	0.53
	DURING O.C. THERAPY								
1	10.8	9.5	9.1	11.7	5.9	15.5	9.7	3.6	8.3
2	-	7.7	7.4	6.8	7.4	12.4	6.8	7.9	4.5
3	3.3	2.4	6.0	3.6	3.8	9.6	4.5	3.5	2.9
4	9.0	9.0	8.1	6.0	10.2	8.1	9.0	9.0	10.5
5	2.6	3.3	3.2	2.7	1.5	6.3	5.1	3.5	3.0
6	6.1	2.4	9.6	6.6	3.2	6.3	5.4	3.9	4.2
7	3.0	3.0	3.0	3.0	-	-	2.4	-	2.4
Mean	5.93			5.77	5.33	9.70	6.13	5.23	5.11
± S.E.	0.67			1.18	1.29	1.5	0.97	1.03	1.17

APPENDIX 7.6

Anacyclin	Lynoestranol	2.5 mgm
	Mestranol	0.075 mgm
Eugynon	Norgestrel	0.5 mgm
	Ethinyloestradiol	0.05 mgm
Ovulen	Ethynodioldiacetate	0.5 mgm
	Ethinyloestradiol	0.05 mgm