# STUDIES ON EXPERIMENTAL HYPERTENSION IN THE RAT AND CAT

A thesis presented by Roger Lewis Whiting for the degree of DOCTOR OF PHILOSOPHY in the UNIVERSITY OF ASTON IN BIRMINGHAM.



Department of Pharmacy,
The University of Aston in Birmingham,
Gosta Green,
Birmingham B4 7ET

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## ABSTRACT

A study has been made of mechanisms involved in the production and maintenance of experimentally induced hypertension in the rat and cat.

An indirect tail cuff method for determination of rat blood pressures was developed and provided accurate values for the systolic blood pressure. An improved method for measuring the blood pressure of conscious, unrestrained cats was also developed.

Hypertension was produced consistently in the rat and cat by using either a DOCA-NaCl regimen or the method of Grollman. The pathologies of these hypertensions were found to be similar in both species.

No clear explanation for the rapid production of DOCA-NaCl hypertension in rats, which occurred in these laboratories, was found although the strain, age and housing of the rats were considered to be possibly implicated.

The involvement of the sympathetic nervous system in the production of DOCA-NaCl hypertension in rats was revealed in a study involving the inhibition of peripheral sympathetic nerves with guanethidine. Whilst a potent adrenergic neuronal blockade was present, the rise in blood pressure produced by a DOCA-NaCl regimen, was prevented. In a similar series of experiments a lack of dependency on the sympathetic nervous system for the production of one-kidney renal hypertension was shown.

Cardiovascular reactivity in both DOCA-NaCl hypertensive rats and renal hypertensive cats revealed a lack of true hypersensitivity of vascular smooth muscle to pressor agents. The involvement of

the renin-angiotensin system in the production of renal hypertension and the sympathetic nervous system in the maintenance of both DOCA-NaCl and renal hypertensions was also revealed.

The antihypertensive effect of both guanethidine and α-methyldopa in DOCA-NaCl hypertensive rats and renal hypertensive cats also provided evidence for the importance of the sympathetic nervous system. The mechanism of action of α-methyldopa was shown to be due to a central action of its metabolite, α-methylnoradrenaline.

CONTENTS

# HISTORICAL INTRODUCTION

## Blood Circulation and Measurement of Arterial Blood Pressure

Castiglioni (1958) quotes that in the 'Nei Ching' of 2600 B.C. the following passages appear: "The heart regulates all the blood in the body, the blood current flows continuously in a circle and never stops." However, the concept of circulation of the blood by the heart was not generally accepted until the classical work of Sir William Harvey. Harvey published his book "De Motu Cordis" in 1628 and in it established what is still the current concept of the circulatory system and hence paved the way for the development of successful treatments of cardiovascular disorders.

The measurement of arterial blood pressure was first undertaken by the Reverend Stephen Hales in 1733. He determined the height to which blood rose in a glass tube inserted into major arteries of various animals, including the horse.

This method was subsequently improved, first by Poiseuille (1828) who used a 'U' shaped mercury manometer and then by Ludwig (1847) who added a float to one limb of the manometer enabling a continuous record of blood pressure to be obtained using a kymograph. Since these methods involved the introduction of a tube into the lumen of an artery they were unsuitable for routine clinical use. The first instrument to overcome this problem was the sphygmomanometer of Von Basch (1881). This manometer was improved by several workers and the first clinically acceptable sphygmomanometer was that designed by Riva-Rocci (1896) which measured systolic blood pressure by occluding the brachial artery with an inflatable cuff. Von Recklinghausen (1901) showed that Riva-Rocci's instrument gave a falsely high estimate of systolic pressure and this was confirmed by Martin and remedied in his mercurial sphygmomanometer of 1905. In the same year Korotkoff reported on his auscultatory method of determining systolic and diastolic blood pressures and this method together with Martin's sphygmomanometer is now the

standard method of monitoring arterial blood pressure in all parts of the world. With the widespread use of this method has come the recognition of the greater stability of the diastolic over the systolic pressure and therefore of its greater diagnostic value.

The Recognition of Hypertension as a Disease Entity

Schroeder (1953) reported that Choun-You-J, a Chinese physician of 200 B.C. wrote "when the pulse upon depressing is very firm and upon superficial palpitation tight, then the disease has its seat in the kidney". However, it was not until 1827 that Bright associated the clinical findings of albuminuria, hardness and fulness of the pulse, oedema and hypertrophy of the left ventricle with the pathological finding of sclerosing, contracted kidneys. In 1836 Bright suggested that the quality of blood was changed to cause an increase in the resistance of flow through what he called "the minute and capillary circulation" thereby originating the concept of arterial hypertension with the kidneys as the cause. Glomerulonephritis is still often referred to as Bright's disease in recognition of his pioneering work in this field.

Gull & Sutton (1872) ascribed chronic Bright's disease to primary generalized 'arterio-capillary fibrosis' which they believed produced left ventricular hypertrophy and contracted kidneys. Mahomed (1874) recognised the condition later called essential hypertension which he termed the "pre-albuminuric stage of Bright's disease" and proposed that hypertension can cause renal changes. In 1893 Huchard in Paris and Allbutt in London recognized the frequency of hypertension and that it could occur in the absence of observable morphological changes in the kidneys and arteries. Two years later Allbutt (1895) stated that renal disease was not a necessary prelude to hypertension and that persistent hypertension may cause, but is not due to arteriosclerosis.

At the beginning of the twentieth century there were three major schools of thought regarding the pathogenesis of essential or primary hypertension. The Bright school maintained that the disease had its seat in the kidney whilst followers of Gull & Sutton considered it more likely to be due to widespread vascular disease. The followers of Huchard and Allbutt favoured generalized vasoconstriction unrelated to renal disease as the most likely cause. This latter view has received much support and essential or primary hypertension is still defined as elevated blood pressure of unknown cause.

Much experimental and clinical research on hypertension continued throughout the first quarter of the twentieth century (see review by Braun-Menendez et al., 1946) but fundamental advances in basic knowledge of the disease were much hampered by the inability to produce persistent hypertension in experimental animals.

Definition of Hypertension

Arterial hypertension is merely a physical sign indicating the presence of an underlying disease and the hypertension is classified according to the underlying disease (e.g. renal hypertension, neurogenic hypertension, contraceptive pill hypertension etc.)

Arterial hypertension in man may be defined as 'a condition in which the peripheral resistance to the flow of circulating blood is chronically increased to such a point that the diastolic blood pressure is 90 mm Hg or more as determined by the indirect method, using the disappearance of sound as a criterion'. When this is so the systolic blood pressure is usually 140 mm Hg or more (Schroeder, 1953).

Hypertensive vascular disease is defined as 'a disease characterized by pathological changes in the arteriolar walls which can be seen

microscopically (Schroeder, 1953). Hypertension is an intermediate cause of hypertensive vascular disease in the sense that whatever cause or causes lead to hypertension it is the hypertension as such which leads on to the hypertensive vascular disease (Smirk, 1967).

Hypertensive vascular disease is classified according to the level of blood pressure and associated arteriolar disease (see review by Schroeder, 1953).

Similar definitions apply to hypertension in experimental animals. However, since the basal blood pressures vary from species to species and with the method of blood pressure determination, no simple definition of the level of blood pressure which represents a hypertensive state can be given for all experimental animals. Normally a blood pressure level which is greater than three standard deviations of the normal blood pressure mean is generally taken to represent a hypertensive level in experimental animals (see review by Pickering, 1955).

Certain types of clinical hypertension have experimental analogues (e.g. Cushings syndrome in man and the administration of cortisone to animals). However, although the spontaneously hypertensive rat represents an experimental model which in many ways resembles essential hypertension in man (Okamoto, 1969) no experimentally induced hypertension exactly parallels human essential hypertension.

## Production of Experimental Hypertension

The various methods for the production of persistent experimental hypertension in animals can be classified into five main categories, depending upon the factor responsible.

### (a) Neurogenic Hypertension

The first persistent hypertension in experimental animals was

produced by Koch & Mies (1929) who sectioned the carotid sinus and aortic depressor nerves in rabbits. The hypertension produced has been termed "neurogenic" since it is due to sympathetic overactivity and is characterised by tachycardia and increased cardiac output, a state of affairs prevailing in the human hypertension of phaeochromocytoma but not in essential hypertension.

(b) Hypertension of Renal Origin

(i) "The Goldblatt method"

The greatest stimulus to research in experimental hypertension came with the report of Goldblatt, Lynch, Hanzal & Summerville (1934) that persistent hypertension could be produced in dogs by partial occlusion of a renal artery with an adjustable silver clamp. Goldblatt et al. (1934) found that the blood pressure began to rise between 24 and 48 hours after renal artery occlusion and reached a maximal steady level 2 to 10 days later. These workers reported that in order to obtain permanent hypertension in the dog both renal arteries must be constricted or alternatively one renal artery constricted and the contralateral kidney removed. However, Lupu et al. (1972) demonstrated that progressive reduction of renal blood flow over a period of several hours in the day resulted in chronic hypertension in the presence of the opposite untouched kidney.

The hypertension produced by the method of Goldblatt et al. (1934) resembled essential hypertension and in dogs with severe renal artery constriction a condition resembling human malignant hypertension occurred. The 'Goldblatt' method of producing hypertension in dogs was soon shown to be applicable to other species, for instance monkeys (Goldblatt, 1937), rabbits (Pickering & Prinzmetal, 1938), rats (Wilson & Byrom, 1939,

1941), goats and sheep (Goldblatt, Kahn & Lewis, 1943).

In rats it has been shown (Wilson & Byrom, 1939, 1941) that hypertension can be produced by constricting one renal artery and either leaving the opposite kidney intact (two - kidney Goldblatt hypertension) or performing contralateral nephrectomy (one-kidney Goldblatt hypertension).

The mechanism of the hypertension produced by the method of Goldblatt et al. (1934) was thought to be due to the arteriolar vasoconstriction caused by angiotensin II formed as a result of the release of renin from the ischaemic kidney(s) (see review by Braun-Menendez et al., 1946). It is now generally considered that although the renin-angiotensin system is almost certainly involved in the pathogenesis of experimental renal hypertension it is not the sole factor implicated in the development or maintenance of the hypertension (see reviews by Smirk, 1967; De Champlain, 1972 and Page, 1974).

Following the work of Goldblatt et al. (1934) minor modifications of their method were reported which also produced permanent experimental hypertension in laboratory species. Thus, partial occlusion of the aorta above, but not below, the level of the origin of the renal arteries resulted in hypertension in dogs (Goldblatt & Kahn, 1938; Goldblatt, Kahn & Hanzal, 1939) and in rats (Rytand, 1938). Loomis (1946) obtained hypertension in the rat by ligation of major branches of the renal arteries with or without concomitant unilateral nephrectomy. Selye & Stone (1946) produced hypertension in rats by partially occluding the aorta between the kidneys and many years later Rojo-Ortega & Genest (1968) showed that complete ligation of the aorta between the origins of the renal arteries produced hypertension in rats weighing more than 300 grams.

(ii) Methods involving the production of perinephritis

Page (1939) reported that permanent hypertension could be produced in dogs, cats and rabbits by enveloping the kidneys in transparent cellulose or silk. Within 3 to 5 days of the kidney wrapping a tissue reaction occurs leading to the formation in 2 to 3 weeks of a fibrocollaginous shell. This compresses the renal parenchyma and hypertension is usually established after about 5 weeks. The 'Page' method was shown to be applicable to the rat using either transparent cellulose (Friedman, Jarman & Klemperer, 1941), silk (Kempf & Page, 1942), rubber (Loring & Goracz, 1954) or latex (Abrams & Sobin, 1947). Greenwood, Nassim & Taylor (1939) obtained hypertension in dogs by enclosing the kidney in collodion and this was later reported for the rat (Hermann, Decherd & Erhard, 1941). Grollman (1944) produced hypertension in rats, rabbits and dogs by tying a cotton thread (in rats) or linen tape over the pole and body of the kidney and drawing it taut. Recently, Rosas et al. (1975) obtained hypertension in rats by ligating both poles of one kidney and performing contralateral nephrectomy one week later.

The mechanism by which perinephritis causes hypertension appears to depend on the constrictive action of the fibroblastic envelope formed which leads to an involvement of the renin-angiotensin system (see review by Braun-Menendez et al., 1946; Brace et al., 1974). The hypertension produced is very similar to that produced by interference with renal blood flow (Page, 1939; Rosas et al., 1975). The main advantages of this method over that of Goldblatt et al. (1934) are simplicity, especially when using small species such as the rat, and reproducibility.

(iii) Macromolecular hypertension

Hall & Hall (1961, 1962) demonstrated that daily subcutaneous injections of methylcellulose or polyvinyl alcohol combined with the substitution of 1% sodium chloride solution for normal drinking water produced severe hypertension of renal origin. Rats treated in this way developed a pathological picture resembling that of human lipid nephritis and some other forms of glomerulonephritis.

(iv) Experimental pyelonephritis

Heptinstall (1962) induced unilateral experimental pyelonephritis by the intravenous injection of Bacterium coli together with temporary occlusion of the ureter. The contralateral kidney was removed and moderate hypertension was produced. The hypertension was apparently caused by infective scarring or reduction in renal tissue mass although no relationship was established between the extent of the renal damage and the blood pressure elevation.

(v) Heterologous and homologous renin administration

Masson, Kashii, Matsunaga & Page (1964, 1966) reported that 3 daily subcutaneous injections of either homologous or heterologous renin extract into uninephrectomized rats produced hypertensive cardiovascular disease. The hypertension produced was similar to that resulting from renal artery clamping in as much that nephrosclerosis, renin depletion and stimulation of the zona glomerulosa of the remaining kidney occurred.

(vi) Renoprival hypertension

Jeffers, Lindauer, Twaddle & Wolferth (1940) showed that removal of 3 kidneys from a pair of rats joined in parabiosis resulted in hypertension only in the bilaterally nephrectomized member of the pair.

This observation was extended by Grollman & Rule (1943) who found that removal of both kidneys from one member of a parabiotic pair was sufficient to induce hypertension in the nephrectomized animal with virtually no effect on the blood pressure of its intact partner. Braun-Menendez & Von Euler (1947) were unable to repeat this result but obtained a reliable degree of hypertension in a bilaterally nephrectomized rat which was one of a paraboitic group of 3. They observed little or no rise in the blood pressure of the other pair of rats with normal kidney function. The precise cause of renoprival hypertension is not clear but may be due to the lack of some antihypertensive function of normal kidneys possibly acting through a specific hormonal agent (Grollman & Rule, 1943); release of pressor substances from the adrenal cortex (Ledingham, 1951) or increase in blood volume and extracellular fluid volume (Braun-Menendez & Von Euler, 1947).

(c) Endocrine Induced Hypertension

(i) Acute desoxycorticosterone acetate (DOCA) and sodium chloride

Selye, Hall & Rowley (1943) showed that twice daily subcutaneous injections of large doses of DOCA combined with substitution of 1% sodium chloride solution for drinking water resulted in the production of hypertension in rats. The hypertension was associated with malignant nephrosclerosis, lesions of the arteriolar walls in the pancreas and adrenal capsule, polydipsia, marked diuresis, increased excretion of sodium and chloride, proteinuria, cardiac hypertrophy and adrenal atrophy. Selye & Pentz (1943) showed that uninephrectomy, like sodium chloride, sensitised rats to the nephrosclerotic and hypertensive properties of DOCA.

The precise aetiology of acute DOCA-NaCl hypertension remains unclear (see review by De Champlain, 1972). The primary cause has variously been suggested as the kidney (Masson, Corcoran & Page, 1949);

the adrenal cortex (Selye, 1948); the sympathetic nervous system (see review by De Champlain, 1972) and disturbed electrolyte function (Tobian, 1960; and review by De Champlain, 1972).

(ii) Post DOCA-NaCl hypertension (Metacorticoid hypertension)

Friedman, Friedman & Nakashima (1951) demonstrated that in uni-nephrectomized rats treated by DOCA implantation and replacement of drinking water with 1% sodium chloride solution the resulting hypertension was sustained in about one third of the animals after removal of the DOCA implants. Green, Saunders, Wahlgren & Craig, (1952) recorded the effectiveness of DOCA implants plus sodium chloride in producing hypertension in the rat. The maximal level to which the systolic blood pressure was elevated in their experiments was 220 mm Hg which was reached after about 6 weeks and remained constant for a further 7 weeks and then slowly subsided to 200 mm Hg by the 16th week and thereafter remained constant. The activity of the DOCA implant was demonstrated to last for about 3 months after which the hypertensive disease was completely self-sustaining.

Metacorticoid hypertension is characterised by renal hypertrophy, progressive cardiac enlargement and generalized arteriolar thickening predominantly involving the muscular coat. There is an exaggerated urinary excretion of salt and water with the urinary sodium-to-potassium ratio elevated (see review by Sturtevant, 1958). The renin content of the kidney is low (Tobian, 1960). Green, Saunders, Wahlgren & Craig, (1952) demonstrated that metacorticoid hypertension is not mediated directly via the adrenals, the thyroid or parathyroid glands or the gonads. The hypertension is unaffected by drastic reduction of dietary sodium or potassium, and water and sodium intake are within normal limits. These workers suggested that the hypertension appears to involve the sympathetic nervous system as a final pathway with the pituitary

acting as an intermediate agency. Green, Craig, Saunders & Sturtevant (1952) demonstrated that total nephrectomy produced a fall in blood pressure in metacorticoid hypertensive rats. Thus the kidneys are apparently necessary for the maintenance of metacorticoid hypertension although the nature of their participation is as yet unclear and the pathogensis of the hypertension has not been fully elucidated.

(iii) Aldosterone-NaCl hypertension

Kumar, Hall, Nakashima & Gornall (1957) demonstrated that small daily or twice daily doses of aldosterone resulted in hypertension in rats. The hypertension was independent of salt intake and was unaffected by bilateral adrenalectomy. These results were confirmed by Gornall et al., (1957) and Masson, Mikasa & Yasuda, (1962) but could not be repeated by Gaunt, Ulsamer & Chart (1957). Gross, Loustalot & Meier (1957) found that in rats sensitised by unilateral nephrectomy and substitution of 1% sodium chloride solution for drinking water hypertension could be produced by the daily subcutaneous injection of large, but not small, doses of aldosterone, and this has been confirmed by Hall & Hall (1967). Lesions of the cardiovascular system and other organs were present but were much less severe than in metacorticoid hypertensive rats. Hypertension has also been produced in 2 strains of rats by daily subcutaneous injections of angiotensin I or II with combined salt loading (Muirhead, Leach & Armstrong, 1973) and these workers have suggested that the hypertension is mediated by a mineralocorticoid under the influence of angiotensin.

(iv) Cortisone acetate hypertension

Knowlton et al. (1952) demonstrated that bilaterally adrenalectomized rats maintained on a sodium restricted diet and given daily

subcutaneous injections of cortisone acetate rapidly developed hypertension. The severity of the hypertension was much less in normal rats or in those on a high sodium containing diet. The mechanism of the production of the hypertension is obscure although no lesions were found in the kidneys and the serum electrolyte pattern was normal. Serum cholesterol levels, however, were markedly elevated.

(v) Hydrocortisone hypertension

Friedman, Friedman & Nakashima (1952) showed that in rats daily subcutaneous injections of hydrocortisone acetate produced a mild hypertension. This hypertension was subsequently shown to be unaffected by uninephrectomy, substitution of 1% sodium chloride solution for drinking water or a combination of both. Bilateral nephrectomy was also without effect and young rats were shown to be more susceptible to the hypertension than old animals (Friedman, Friedman & Nakashima, 1953). The hypertension was associated with a moderate increase in serum potassium levels, cardiac and renal hypertrophy, adrenal atrophy and absence of renal lesions. Friedman et al. (1953) concluded that the hypertension could be due to a decreased ability of the adrenal glands to inhibit certain pressor agents.

(vi) Adrenal regeneration hypertension

Skelton (1955) reported that in rats sensitised by unilateral nephrectomy and substitution of sodium chloride solution for drinking water, unilateral adrenalectomy with contralateral adrenal enucleation produced severe hypertension. The development of the hypertension was associated with hypertrophy and lesions of the kidney, heart, adrenal gland and brain. In a later paper (Skelton, 1956) he demonstrated that for the development of this type of hypertension there must be actively

regenerating adrenal cortical tissue, a reduction in renal mass and availability of excess sodium chloride. This hypertension is pathologically similar to DOCA-NaCl hypertension.

Skelton (1953, 1969) has also described the production of hypertension in rats by the administration of methylandrostenediol, methyltestosterone, testosterone and metyrapone. He considered the aetiology of these hypertensions to be due to an inadequacy of adrenal cortical 11-β-hydroxylation leading to a dysfunctional state characterised by overproduction of DOCA. Hall, Ayachi & Hall (1974) obtained hypertension in rats, sensitised by unilateral nephrectomy and replacement of drinking water with 1% sodium chloride solution, by compression of the adrenal glands and concluded that the hypertension was the same as that produced by Skelton (1955) but was experimentally easier to produce.

(vii) Salt-induced hypertension

Selye & Stone (1943) showed that chicks given saline instead of drinking water developed nephrosclerosis. This was confirmed by Lenel, Katz and Rodbard (1948) who showed that this treatment also resulted in hypertension which persisted only whilst the sodium chloride was administered. Renal hypertrophy and lesions were observed but arteriolar lesions were absent. Sapirstein, Brandt & Drury (1950) elicited rapid hypertension in rats by replacing drinking water with 2% sodium chloride solution. The hypertension was associated with renal and cardiac hypertrophy but kidney lesions were absent. Meneely, Tucker, Darby & Auerbach (1953) confirmed this observation and further showed that the severity of the hypertension was increased as the concentration of sodium chloride in the diet was increased. Pathological lesions in blood vessels and kidneys appeared when the dietary concentration of sodium chloride was 7%

or more. Meneely & Ball (1958) showed that a concomitant increase in potassium intake reduced the hypertensive action of high sodium diets. Dahl (1961) produced hypertension in rats by chronic feeding of high doses of sodium salts and demonstrated that in the majority of cases the hypertension was self-sustaining.

Dahl, Heine & Tassinari (1962) showed that by selective inbreeding, a strain of rats susceptible to salt hypertension was developed. In the same way, by inbreeding insensitive rats a strain which was resistant to salt hypertension was produced. Rapp & Dahl (1972) reported that the adrenal gland of the insensitive strain had high 11-β -hydroxylase activity whilst those of the sensitive strain possessed high 18-hydroxylase activity leading to the production of 18-hydroxy-desoxycorticosterone. These workers also demonstrated that the inheritance of 18-and 11-β -hydroxylase activities in the adrenals of rats genetically susceptible or resistant to salt hypertension followed Mendelian inheritance.

(d) Dietary Hypertension

(i) Hypertension produced by dietary deficiency

Hartroft & Best (1949) produced hypertension in rats by subjecting them to a short period of dietary choline deficiency during early life. Grollman & White (1958) found that immature rats and dogs maintained on a choline or potassium deficient diet eventually developed hypertension, the onset and severity of which depended on the length of the dietary deficient period. The dietary deficiency caused kidney lesions resembling those found in human essential hypertension suggesting that this condition, in certain cases, may be due to early dietary deficiency.

(e) Hereditary Hypertensions

(i) Genetically inherited hypertensions

Alexander, Hinshaw & Drury (1954, 1956) demonstrated that by selective inbreeding of rabbits with a spontaneously elevated blood pressure then the majority of the offspring were also hypertensive. Similarly, Smirk & Hall (1958) demonstrated the inheritance of hypertension in the rat by intensive inbreeding and selective cross-breeding. They found the incidence of hypertension to be about 40%. These hypertensive rats have been repeatedly cross-bred and have been termed the New Zealand Strain Rats with Genetic Hypertension. Phelan (1968) has described the subsequent improvement of this strain to produce a level of spontaneous hypertension in the offspring of 90%.
Okamoto & Aoki (1963) described the production of a spontaneously hypertensive rat colony by selective inbreeding in which the incidence of spontaneous hypertension was 100% and of a severe nature. This colony of rats was termed the Japanese Strain of Spontaneously Hypertensive Rats, and has been suggested to be the best animal model for the study of essential hypertension in man (Okamoto, Aoki, Nosaka & Fukushima, 1964). Recently Okamoto, Yamori & Nagaoka (1974) have established a stroke-prone spontaneously hypertensive rat with the occurrence of severe cerebrovascular lesions.

Only the above workers have succeeded in isolating a strain of animals susceptible to the occurrence of spontaneous hypertension. However, other workers have recorded spontaneous hypertension in other species, for instance, Rosenfeld, Thomas & Drury (1954) in rabbits; Katz, Skom & Wakerlin (1957) in dogs and by teratogenic induction of hypertension in rats by Grollman & Grollman (1962).

(ii) Genetically susceptible hypertensions

Several workers have obtained rats which are genetically more susceptible to the induction of renal (Iwai, Dahl & Knudsen, 1973), salt (Dahl, Heine & Tassinari, 1962) and DOCA-NaCl (Ben-Ishay et al., 1972) hypertensions. Rats which are predisposed to the induction of experimental renal hypertension have been reported to possess lowered plasma and kidney renin activities (Iwai, Dahl & Knudsen, 1973) and this has also been suggested to be true for salt and DOCA-NaCl hypertensions (Gordon, 1966, 1973).

Aetiology of Hypertension

(a) Renin-angiotensin system

(i) Components of the system

The kidney has been suspected of involvement in the aetiology of hypertension since the time of Bright and the discovery by Tigerstedt and Bergman (1898) that saline extracts of kidney tissue contained a pressor substance, which they termed renin, further implicated the kidney in the development of hypertension. However, little further interest was taken in this observation until the production of permanent experimental hypertension by Goldblatt et al. (1934).

Braun-Menendez et al. (1940) and Page & Helmer (1940) simultaneously demonstrated that renin was a proteolytic enzyme that acts on a substrate in the plasma, later shown to be an $\alpha$-2-globulin (Green & Bumpus, 1954), to produce an active pressor agent termed, by agreement, angiotensin.

Renin is believed to be elaborated and stored in the granulated juxtaglomerular cells located in the wall of the afferent arteriole of the kidney (e.g. Goormaghtigh, 1939; Hartroft, Sutherland & Hartroft, 1964; Ajzen, Simmons & Woods, 1965).

The control of renin release from the juxtaglomerular cells is complex and not fully elucidated though available evidence provides support for four mechanisms which could alone or in conjunction control this release (see reviews by Vander, 1967; Davis 1971, 1973 and Assaykeen, 1972).

(i) Intrarenal Baroreceptor Theory: The renal afferent arterioles and juxtaglomerular cells respond to changes in stretch which could be secondary to changes in vascular volume and pressure. This idea was first proposed by Tobian, Tomboulian & Janecek (1959) and later supported by Skinner, McCubbin & Page (1964) and Eide, Loyning & Kiil (1973).

(ii) Macula Densa Theory: Renin release is mediated by either a decrease (Vander & Miller, 1964; Freeman et al., 1974) or increase (Thurau et al., 1967; Cooke et al., 1970) in sodium transport by the macula densa cells.

(iii) Renal Sympathetic Nerve Control: Much evidence has accumulated indicating a control of renin release by the renal sympathetic nerves (e.g. Vander, 1967; Mogil et al., 1969; Ganong, 1972a) an effect which is mediated via the β-adrenergic receptors in the kidney (Ganong, 1972b; Reid, Schrier & Earley, 1972).

(iv) Hormonal Control: Various humoral agents, sodium and potassium ions, angiotensin, catecholamines and prostaglandins have been reported as affecting renin release (Wathen et al., 1965; Veyrat et al., 1967; Michelakis, Caudle & Liddle, 1969; Itskovitz & McGill, 1974; Ayers et al., 1974).

Several groups of workers (Skeggs et al., 1955, 1956; Elliot & Peart, 1956, 1957) demonstrated that angiotensin was a polypeptide occurring in two forms:- a decapeptide, angiotensin I, which is

transformed to the physiologically more active octapeptide, angiotensin II, by a plasma enzyme termed "converting enzyme" (Lentz et al., 1956 and review by Erdos, 1975). Most of this conversion appears to occur in transit through the lungs rather than in the circulation itself (Ng & Vane, 1967, 1968 a & b; Oparil & Haber, 1972).

The octapeptide, angiotensin II, may still be regarded as the main effector of the renin - angiotensin system. Angiotensin II has been ascribed several physiological and pharmacological properties:-

(a) Effects on the cardiovascular system: Angiotensin II remains the most powerful pressor agent known. It has a direct action on blood vessel walls causing vasoconstriction, and most of the pressor effect observed in man and animals is due to this action. It acts principally on arterioles with very little effect on venules or veins (see reviews by Bumpus, Schwarz and Page, 1957, 1958; Page & Bumpus, 1961; Peart, 1965, 1975; and Page & McCubbin, 1968). Angiotensin II also stimulates cardiac muscle causing increased inotropic and chronotropic effects, (e.g. Hill & Andrus, 1941; Koch-Weser, 1964; Bonnardeaux & Regoli, 1974). However, this action on the heart is probably unimportant in vivo (Peart, 1975).

(b) Effects on nerves: Angiotensin II acts directly and indirectly on the brain to increase sympathetic nerve activity (see review by Ferrario, Gildenberg & McCubbin, 1972). The angiotensin II sensitive site of the brain has been shown to be the area prostrema of the medulla oblongata (Ueda, Katayama & Kato, 1972). Angiotensin II facilitates ganglionic transmission (Lewis & Reit, 1965; Farr & Grupp, 1967; Aiken & Reit, 1968), interacts with the peripheral sympathetic nervous system (e.g. McCubbin & Page, 1963; Zimmerman, 1967, 1973; Day & Owen, 1969) and stimulates the release of catecholamines from

the adrenal medulla (Braun-Menendez et al., 1940; Feldberg & Lewis, 1964; Aiken & Reit, 1968).

(c) Effects on the kidneys: Angiotensin II in small amounts in man and conscious animals produces a decrease in sodium excretion (Bock & Krecke, 1958; De Bono et al., 1963; Page & McCubbin, 1968; Granger et al., 1972) whilst in high doses and anaesthetised animals an increase in sodium excretion occurs (Vander, 1963; Leyssac, 1964). The possibility exists that angiotensin II has a direct action on tubular sodium reabsorption (see reviews by Peart, 1969, 1975).

(d) Effects on the adrenal glands: The close relationship of the kidney, the adrenal glands and aldosterone production described by Gross (1960) and later Davis (1962, 1963) led to the hypothesis that angiotensin II stimulated the zona glomerulosa of the adrenal cortex in man to release aldosterone. Further evidence accumulated suggesting that angiotensin II was a major physiological regulator of aldosterone secretion in many species except the rat (e.g. Genest, 1961; Davis, Ayers & Carpenter, 1961; Ganong & Mulrow, 1961; Miller et al., 1968). The specificity of the aldosterone stimulating effect of angiotensin II has been questioned. For example, it has been shown (Lowenstein et al., 1972) that in rabbits specific antibodies to angiotensin II fail to prevent aldosterone responses to renin release. Recent reports have shown that the naturally occurring heptapeptide fragment of angiotensin II, (des-aspartic$^{1}$)-angiotensin II, is a potent stimulator of aldosterone biosynthesis in rats, sheep and dogs, both in vitro (see review by Goodfriend and Peach, 1975) and in vivo (Blair-West et al., 1971; Campbell, Brooks & Pettinger, 1974; Spielman et al., 1974). It has been suggested that (des-aspartic$^{1}$)-angiotensin II may be the mediator of the renin-angiotensin system at the level of the

adrenal receptor, (see review by Peart, 1975). This hypothesis is supported by the fact that both the heptapeptide and octapeptide are more effectively inhibited in the adrenal by specific antagonists of (des-aspartic$^{1}$)-angiotensin II (see review by Goodfriend & Peach, 1975).

Angiotensin II is believed to be metabolised, mainly in the tissues, by peptidases termed "angiotensinases" (Khairallah et al., 1963; Pickens et al., 1965).

(ii) Role of the renin-angiotensin system in the production of hypertension

The renin-angiotensin system has been reported to be involved in the production and maintenance of various forms of experimental hypertension (see reviews by Page & McCubbin, 1968; Ledingham, 1971; Davis et al., 1974; Page, 1974 and Peart, 1975).

However, determinations of plasma levels of renin and angiotensin II have produced conflicting results. During the acute stages of experimental one and two-kidney renal hypertensions in rats, dogs and rabbits, there is strong evidence to suggest an involvement of the renin-angiotensin system (e.g. Page, 1940; Dell'Oro & Braun-Menendez, 1942; Govaerts & Verniony, 1952; Page, Kaneko & McCubbin, 1966; Koletsky et al., 1966; Lupu, Sambhi & Maxwell, 1967; Sen, Smeby & Bumpus, 1972; Gulati et al., 1975; Hutchinson et al., 1975) whilst the weight of evidence suggests no increase in circulating levels of renin or angiotensin II during the chronic stage of experimental one-kidney renal hypertension in rats, dogs and rabbits (e.g. Peart, Robertson & Grahame-Smith, 1961; Ebrihara & Grollman, 1968; Koletsky, Shook & Rivera-Valez, 1970; Baer, Knowlton & Laragh, 1972; Hutchinson et al., 1975). Recent evidence has indicated a positive correlation between blood pressure and plasma renin and angiotensin II

levels in the chronic stage of two-kidney renal hypertension in the rat (e.g. Leenen, De Jong & Dewied, 1973; Oates, Stokes & Storey, 1975; Hutchinson et al., 1975).

Decreased levels of circulating renin have been reported in other non-renal forms of experimental hypertension in rats, for example DOCA-NaCl hypertension (Gross, Loustalot & Sulser, 1956; Gross & Sulser, 1956; Goodwin, Knowlton & Laragh, 1969; De Champlain, 1972) and adrenal regeneration hypertension (Masuyama et al., 1969; Hasatani, Morimoto & Takeda, 1975).

In all the above forms of hypertension there have been reports which are contrary to those stated here. Until more accurate analytical methods become available for the determination of plasma renin and angiotensin II levels, no definite conclusions can be made.

Immunological studies employing either injection or endogenous production of renin antibodies have provided strong evidence that the renin-angiotensin system is directly responsible for the rise in blood pressure in experimental one and two-kidney renal hypertensions in rats, dogs and rabbits (e.g. Wakerlin & Johnson, 1941; Wakerlin, 1958; Frank, 1963; Hill, Chester & Wisenbaugh, 1970; Romero et al., 1973) but due to lack of antibody specificity a certain amount of doubt has been cast on these studies (see reviews by Page & McCubbin, 1968 and Peart, 1975).

The recent development of specific antibodies against angiotensin II has overcome this problem of specificity but the results have still been contradictory. Several reports (e.g. Christlieb, Biber & Hickler, 1969; Carretero et al., 1971) have stated that both one and two-kidney renal hypertensions do not develop in rats and rabbits

immunized against angiotensin II whilst other workers (e.g. Eide & Aars, 1969, 1970; Johnston et al., 1970; Eide, 1972) have reported the opposite. Walker, Ruiz-Maza & Horvath (1972) have suggested that the level of free (unbound) angiotensin II is sufficient for angiotensin II to participate in physiological processes despite high titers of antibodies for the octapeptide.

The formation of angiotensin I by renin can be inhibited by a naturally occurring phospholipid termed renin preinhibitor (Sen, Smeby & Bumpus, 1967). Injection of renin preinhibitor reduces the blood pressure in both short-term and long-term (40 weeks duration) one and two-kidney renal hypertensive rats (Sen, Smeby & Bumpus, 1968, 1969). However, the blood pressure never returns to normotensive levels, and plasma renin activity is not completely inhibited (see review by Davis et al., 1974).

Converting enzyme, which is responsible for the conversion of angiotensin I to angiotensin II, is inhibited by SQ 20881, a nonapeptide originally isolated from Bothrops jararuca venom (Ferreira et al., 1970). Romero, Mak & Hoobler (1974) have shown that SQ 20881 reduces the blood pressure, although not to normal levels, of rabbits with acute one and two-kidney renal hypertensions but has no effect on chronic one-kidney hypertension. These workers suggested that in the acute renal hypertensive model the hypertension is dependent on the renin-angiotensin system but not in the chronic one-kidney hypertensive animal.

The recent use of angiotensin II analogues, particularly 1-sarcosine-8-alanine angiotensin II (P 113), which act as specific competitive antagonists of angiotensin II has also provided evidence of the role of the renin-angiotensin system in experimental renal hypertensions. It has been stated that the renin-angiotensin system is

involved in the pathogenesis of acute one-kidney hypertension in rats and dogs, two-kidney hypertension in rats and chronic two-kidney hypertension in rats. A lack of involvement of the renin-angiotensin system in chronic one-kidney hypertension in rats, dogs and rabbits and chronic two-kidney hypertension in rabbits has been reported (see reviews by Davis et al., 1974 and Peart, 1975; Johnson et al., 1975; MacDonald, Boyd & Peart, 1975).

If renin and angiotensin II are involved in the production of hypertension then injections or infusions of these substances should be capable of producing hypertension in normotensive animals. As described in the previous section (p. 14) injections of renin are capable of producing hypertension in the rat (Masson et al., 1964, 1966; Masson, 1966). Similarly prolonged infusions of renin are reported to produce a sustained rise in the blood pressure of rabbits (Blacket et al., 1950). However, due to the lack of high purity renin and the formation of antirenin when using heterologus renin, the effect of prolonged infusions of angiotensin II has more often been employed to investigate the role of the renin-angiotensin system in the pathogenesis of hypertension.

Brown, Chapius & Robertson (1963, 1964) have succeeded in maintaining hypertension in conscious rabbits for as long as 3 months by continuous intravenous infusions of pressor dose levels of angiotensin II. Blood pressure fell rapidly to normal, or below normal, when the infusion was stopped. However, in conscious dogs, infusions of high doses of angiotensin II produce a large rise in blood pressure followed by a return to the pre-infusion levels despite continued infusions (Bock & Gross, 1961; Page & Olmsted, 1961). Inability to maintain an elevated blood pressure during infusions of high doses of angiotensin II

is probably due to the development of tachyphylaxis (Day, McCubbin & Page, 1965).

Subpressor infusions of angiotensin II are capable of producing a progressive rise in blood pressure in rabbits and dogs which is maintained for the duration of the infusions (e.g. Dickinson & Lawrence, 1963; Yu & Dickinson, 1965; McCubbin et al., 1965; Dickinson & Yu, 1967). The rise in blood pressure appears to be dependent, to a large degree, upon the action of angiotensin on the sympathetic nervous system (McCubbin & Page, 1963; McCubbin et al., 1965; Dickinson & Yu, 1967) since, for example, prior administration of bethanidine prevents the rise in blood pressure (Dickinson & Yu, 1967). The involvement of the autonomic nervous system gradually increases, as assessed by serial injections of ganglion-blocking drugs (Yu & Dickinson, 1965). Subpressor daily doses of angiotensin I or II combined with salt intake are also capable of producing hypertension in the rat (Muirhead, Leach & Armstrong, 1973).

The precise role of the renin-angiotensin system in the pathogenesis of hypertension is still not clear but the published evidence strongly suggests its involvement in acute one and two-kidney renal hypertensions.

(b) Role of the sympathetic nervous system in the aetiology of hypertension

There is a considerable body of evidence implicating the sympathetic nervous system in the production and maintenance of hypertension (see reviews by Page & McCubbin, 1968; De Champlain, 1972 and Schmid & Abboud, 1974).

Inconsistent results, as to the role of the sympathetic nervous system in hypertension, have been obtained using surgical sympathectomy and this is probably due to the difficulty in obtaining a total sympathectomy (see review by Braun-Menendez et al., 1946). However, total

destruction of the spinal cord in experimental renal hypertensive or normotensive dogs, rabbits or rats lowers the blood pressure to the same level (Dock, 1940; Dock, Shindler & Moy, 1942; Taquini, Blaquier & Bohr, 1961; Taquini, 1963) thus suggesting,in renal hypertensive animals,the nervous system is almost wholly responsible for the elevated blood pressure (see review by Page & McCubbin, 1968).

Immunosympathectomy has recently been made possible by the isolation of antiserum to nerve growth factor (Levi-Montalcini, 1964; Levi-Montalcini & Angletti, 1968). Whilst being ineffective in delaying the onset of renal hypertension in rats, immunosympathectomy has been reported to prevent the maintenance of the hypertension (Dorr & Brody, 1964; Ayitey-Smith & Varma, 1970). Immunosympathectomy in the rat has also been reported to prevent, in a large part, the development of DOCA-NaCl hypertension (Ayitey-Smith & Varma, 1970), triiodothyronine accelerated salt hypertension (Willard & Fuller, 1969) and genetic hypertension (Okamoto et al., 1967; Clark, 1969, 1971; Clark & Simpson, 1970). This method of study however, suffers from the fact that the immunosympathectomy, although irreversible, is subtotal (Zaimis, 1965; Zaimis, Berk & Callingham, 1965; Iversen, Glowinski & Axelrod, 1966; Levi-Montalcini & Angeletti, 1966, 1968).

Degeneration of the sympathetic post-ganglionic nerve terminals by 6-hydroxydopamine (see reviews by Thoenen, 1972 and Kostrzewa & Jacobowitz, 1974) has been employed to determine the role of the sympathetic nervous system in various forms of experimental hypertension. Grewal & Kaul (1971) demonstrated that in weanling rats systemically administered 6-hydroxydopamine prevented the development of renal hypertension whilst Grewal & Kaul (1971) and Finch & Leach (1970) showed that in adult rats systemically administered 6-hydroxydopamine did not affect the development or maintenance of renal hypertension.

Mueller & Thoenen (1970) reported that in adult rats systemically administered 6-hydroxydopamine delayed the onset of DOCA-NaCl hypertension but failed to prevent its development whilst Finch & Leach (1970) and Clarke, Smookler & Barry (1970) described a lack of effect on both the onset and maintenance of DOCA-NaCl hypertension. De Champlain & Ameringen (1972) demonstrated that the combined effect of chemical sympathectomy with 6-hydroxydopamine and bilateral adrenalectomy caused the blood pressures of both DOCA-NaCl hypertensive and normotensive rats to fall to the same level whilst individually each procedure had little effect on blood pressure. Systemic administration of 6-hydroxydopamine has been reported to prevent the production of genetic hypertension in rats (Clark, 1969; Finch, Haeusler & Thoenen, 1973; Vapaatalo et al., 1974; Clark & Phelan, 1975). Centrally administered 6-hydroxydopamine has been reported to prevent the production of DOCA-NaCl hypertension in rats (Finch, Haeusler & Thoenen, 1972; Dargie, Dollery & Lewis, 1975), renal hypertension in rabbits (Lewis, Reid, Chalmers & Dollery, 1973; Chalmers, Reid & Wing, 1974) and genetic hypertension in rats (Haeusler, Finch & Thoenen, 1972; Erinoff, Heller & Oparil, 1975). Centrally administered 6-hydroxydopamine has also been reported not to affect established genetic hypertension in rats (Haeusler, Gerold & Thoenen, 1972) but caused the blood pressure to fall in established renal hypertension in rabbits (Chalmers, Reid & Wing, 1974). An 'irreversible' sympathectomy can only be obtained if 6-hydroxydopamine is administered to newborn animals and the number of adrenergic neurones affected, especially centrally, is greater than that obtained with the 'reversible' sympathectomy which is obtained when 6-hydroxydopamine is administered to adult animals (see reviews by Thoenen, 1972 and Kostrzewa & Jacobowitz, 1974). When chemical

sympathectomy with 6-hydroxydopamine has been performed in young animals the importance of the sympathetic nervous system in the development and maintenance of all types of experimental hypertension has been demonstrated.

Chemical sympathectomy has also been shown to be produced by the chronic administration of large doses of guanethidine to adult rats (Jensen-Holm & Juul, 1970; Burnstock et al., 1971; Heath et al., 1972; Juul, 1973). In newborn rats guanethidine is more toxic to the sympathetic adrenergic neurones than in adult animals (Eranko & Eranko, 1971 a & b; Angeletti & Levi-Montalcini, 1972; Angeletti et al., 1972; Liuzzi et al., 1974). Douglas et al. (1975 a & b) have reported that chemical sympathectomy with guanethidine has no effect on the production of either one or two-kidney renal hypertension in rats. Chemical sympathectomy with guanethidine will, however, only be effective peripherally since it does not cross the blood-brain barrier (see review by Mull & Maxwell, 1967).

Hexamethonium has been found to lower the blood pressure of both hypertensive and normotensive animals and man to similar levels (Doyle & Smirk, 1954; Smirk & Hall, 1958; Smirk et al., 1958; Laverty & Smirk, 1961; Phelan, Eryetishir & Smirk, 1962; Phelan, 1966; Smirk, 1970; Louis, Doyle, Anavekar & Chua, 1973). This indicates that the elevated component of blood pressure in hypertension is maintained by the sympathetic nervous system and the action of sympatholytic drugs depends upon a reduction of this component for their useful clinical actions (Smirk, 1970).

The effectiveness of antihypertensive compounds which act by impairment of the peripheral sympathetic nervous system such as $\alpha$-blockers, adrenergic neurone blockers, transmitter depletors,

ganglion blockers, enzyme inhibitors of noradrenaline synthesis and drugs which lead to the release of false transmitters strongly indicate the involvement of a neurogenic component in the maintenance of both experimental and clinical hypertensions (see reviews by Smirk, 1967 and Frohlich, 1974).

Cardiovascular reactivity provides an approximate index of the sympathetic discharge to the heart and blood vessels (Smirk, 1970). Since cardiovascular hyper-reactivity is normally observed in hypertensives when compared to normotensives a sympathetic component in the development and maintenance of hypertension is indicated (see later section p. 38).

Hyper-reactivity of the sympathetic nervous system may be due to a dysfunction of the baroreceptors. If the blood pressure of an animal is raised and maintained high baroreceptor activity initially reflects the high blood pressure but after one to two days starts to decline, (see review by Aars, 1975). In animals with established renal or genetic hypertension baroreceptor activity at all pressures is lower than normal (e.g. McCubbin, Green & Page, 1956; McCubbin, 1958; Kreiger & Marseillan, 1966; Kezdi, 1967; Aars, 1968 a & b; Kreiger, 1970; Nosaka & Wang, 1972; Angell-James, 1973). For example, the aortic baroreceptors have a higher threshold and a lower sensitivity to pressure in renal hypertensive rabbits than normotensive rabbits (Aars, 1968b; Angell-James, 1973). These changes in baroreceptor activity have also been reported in hypertensive patients (e.g. Bristow et al., 1969; Wallin, Delius & Hagbarth, 1973). It has been suggested that these changes in baroreceptor activity are secondary to the reduction of arterial distensibility which occurs in hypertensive patients and animals (see reviews by Aars, 1975 and Korner, 1975).

It has been shown that in the hypertensive rabbit, aortic distensibility is reduced as is baroreceptor activity (Aars, 1968a; Angell-James, 1973) and simultaneous recordings of aortic and carotid sinus diameters and baroreceptor activity have suggested that receptor activity changes in parallel with the distensibility (Aars, 1969; Koushanpour & Kenfield, 1974). Thus the baroreceptor may be normal, the altered threshold and sensitivity being due to changes in other elements of the arterial wall caused by the hypertension (Aars, 1975).

Increased turnover of noradrenaline would indicate hyper-reactivity of the sympathetic nervous system. However, measurements of noradrenaline turnover in peripheral tissues of both human and experimental hypertensives have produced conflicting results (see reviews by De Champlain, 1972 and Schmid & Abboud, 1974). Recently measurements of noradrenaline turnovers in the brain of renal and genetic hypertensive animals have also produced conflicting results (see reviews by Schmid & Abboud, 1974; Haeusler, 1975 and Chalmers, 1975). In DOCA-NaCl hypertensive rats, Nakamura, Gerold & Thoenen (1971) and De Champlain & Ameringen (1973) have reported a decreased turnover of noradrenaline in the medulla oblongata without a change in other regions of the brain. In the same rats these workers found an increase in the turnover of noradrenaline in the heart. It has been suggested that in rats with DOCA-NaCl hypertension the reduced activity in noradrenergic neurones in the medulla oblongata is responsible for the increased activity of peripheral sympathetic nerves and hence the hypertension (see reviews by Chalmers 1975 and Haeusler, 1975).

Increases in plasma levels and urinary excretion of catecholamines and their metabolites may be considered to provide indirect evidence of sympathetic overactivity. However, the published results have been contradictary (see reviews by De Champlain 1972 and Schmid & Abboud, 1974).

Iriuchijima, Mizogami & Sokabe (1975) have measured the sympathetic 'tone' of the splanchnic nerve during the development of DOCA-NaCl and one and two-kidney renal hypertensions in rats. They reported that nervous activity, two weeks after initiation of the hypertension, was markedly increased in DOCA-NaCl hypertension, reduced in two-kidney renal hypertension and unchanged in one-kidney renal hypertension.

Although the role of the sympathetic nervous system in the pathogenesis of hypertension has not been absolutely clarified the published literature suggests a role in the development and maintenance of DOCA-NaCl hypertension and an involvement in the chronic stage of experimental renal and human hypertensions.

(c) Cardiovascular Reactivity

(i) Proposed role in the aetiology of hypertension

Cardiovascular reactivity has been defined as "the changing ability of blood vessels to respond to the same stimulus, whether nervous or humoral in origin" (Page & McCubbin, 1963). Though the pathogenesis of hypertension remains confused, it is generally agreed that the basic defect is an excessive resistance to blood flow due to a diminished calibre of arterial vessels, especially of the systemic arterioles (see reviews by Pickering, 1955; Page & McCubbin, 1968 and Page, 1974).

The exact mechanism causing the narrowing of small arteries and arterioles is not understood but two hypotheses have been proposed (Gordon & Nogueira, 1962; Holloway & Bohr, 1973):-

The mechanical hypothesis: Structural changes in the walls of small vessels may lead to narrowing of the lumen and increase in the wall-lumen ratio (see reviews by Giese, 1966 and Page, 1974). However, many of the changes such as vascular smooth muscle and fibrinoid necrosis in arteries from hypertensive animals and man appear to be secondary, and are either adaptive, or a pathological consequence of the

raised arterial pressure itself (Folkow, Grimby & Thulesius, 1958; Hinke, 1965; Hollander et al., 1968; Folkow, 1971, 1975; Wolinsky 1972; Folkow et al., 1973). If this hypothesis is true then vascular hyper-reactivity may be explained by an "apparent" rather than a "true" supersensitivity to pressor substances. The degree of smooth muscle cell shortening to pressor substances is normal but owing to the increased wall-lumen ratio the increase in peripheral resistance is greater than in normal vessels (Collis & Alps, 1975). In perfused arterial tissues this is evidenced by a steeper dose-response curve with an elevated maximum response (Folkow et al., 1970).

The contractile hypothesis: Increased tone in the vascular resistance beds will also lead to a narrowing of these vessels. This may result from increased nervous or chemical stimulation or hyper-reactivity of the resistance vessels to vasoconstrictor stimuli (see review by De Champlain, 1972). Neither increased nervous activity nor increased circulating levels of physiologically active pressor substances in human and experimental hypertensions have been conclusively demonstrated (see reviews by Braun-Menendez et al., 1946; Pickering, 1955, 1968; Page & McCubbin, 1968 and Page, 1974). Thus the idea that vascular smooth muscle of hypertensives might react excessively to normal nervous and humoral influences, particularly noradrenaline, has become increasingly popular (see review by De Champlain, 1972). If this hypothesis is true then vascular hyper-reactivity may be explained by a "true" supersensitivity (increased responsiveness) to pressor substances. Thus the same submaximal dose of agonist evokes a greater than normal degree of shortening in the vascular smooth muscle cell (Collis & Alps, 1975). In isolated arterial tissues, "true" super-sensitivity is evidenced by a displacement of the dose-response curve to

the left of the control with a lower vasoconstrictor threshold (Haeusler & Finch, 1972).

(ii) Evidence for cardiovascular hyper-reactivity as a factor in the aetiology of hypertension.

The large number of reports examining the role of cardiovascular reactivity in the aetiology of hypertension have produced confusing and conflicting results. This may be partly due to the various experimental conditions employed:-

(a) various types of human and experimental hypertension

(b) various types of preparation (e.g. whole animal, isolated organ, vascular bed)

(c) test material used (e.g. noradrenaline, angiotensin, 5HT, tyramine, oxygen, psychic stimuli)

(d) method of determination (e.g. single dose, dose-response curves. pressure-flow rates, intravenous or intra-arterial infusion)

(e) interpretation of results (e.g. mechanical, contractile, reflex).

Studies in hypertensive patients have suggested that there is increased cardiovascular reactivity in essential hypertension but not in various types of human renal hypertension (Mendlowitz, 1967). However, there has been disagreement about whether the cardiovascular hyper-reactivity resulted from increased responsiveness of vascular smooth muscle or from a different level of tone, or different wall-to-lumen ratio of the vessels (Sivertsson, 1970; Folkow et al., 1973; Mendlowitz, 1973; Horowitz et al., 1974; Folkow, 1975). This difficulty in interpreting results obtained from blood pressure measurements in the whole body led to the use of evaluating cardiovascular reactivity in local vascular beds or isolated arterial strips. This method has

produced a great deal of evidence for the existence of cardiovascular hyper-reactivity in essential hypertension (see review by Smirk, 1967).

Cardiovascular reactivity has also been examined in more direct observations on peripheral blood vessels. Griseman (1952, 1954, 1956) demonstrated increased digital capillary narrowing when noradrenaline was infused into hypertensive subjects compared with normotensives. Lee & Holze (1951) reported increased responsiveness of conjunctival vessels to directly applied adrenaline in essential hypertensives.

Mendlowitz (1967) has stated that cardiovascular reactivity studies are most productive if the methods employ either intra-arterial or intravenous infusions (preferably the former to minimise general baroreceptor and other responses affecting the general circulation) after inhibition of sympathetic nerve discharge (employing either ganglionic blockade or heat or both) and interpreting changes in flow and pressure in terms of vasoconstriction rather than resistance. Under these conditions essential hypertensive subjects have been demonstrated to possess cardiovascular hyper-reactivity to noradrenaline and angiotensin (Mendlowitz et al., 1965). However, under similar conditions a lack of cardiovascular hyper-reactivity to noradrenaline and angiotensin has been reported in human renal hypertension (Mendlowitz & Naftchi, 1958; Mendlowitz et al., 1961, 1962, 1965).

The greater antihypertensive action of many compounds in hypertensive man and animals compared with that in similar normotensive species has been claimed to demonstrate cardiovascular hyper-reactivity in hypertension (Stanton & White, 1964; Willard & Beckhelm, 1968; Nagaoka, Kikuchi & Aramaki, 1969; Frohlich, 1974).

The profusion of evidence relating to cardiovascular hyper-reactivity in experimental hypertension is even more conflicting and

confusing than that relating to human hypertension.

The effect on the blood pressure of renal hypertensive animals to an immense variety of pressor stimuli has produced contradictory results (see reviews by Page & McCubbin, 1968 and De Champlain, 1972).

Masson, Page & Corcoran (1950) reported no increase in blood pressure responses in DOCA-NaCl hypertensive rats to injections of adrenaline, renin and angiotensin compared with normotensive rats. Other workers (e.g. Sturtevant, 1953, 1956; Carlini, Sampaio & Paiva, 1959; Holloway, Sitrin & Bohr, 1972; Armstrong, 1972) have reported cardiovascular hyper-reactivity to a variety of pressor substances in DOCA-NaCl hypertensive rats.

A lack of cardiovascular hyper-reactivity to catecholamines and acetylcholine has been reported in adrenal regeneration hypertension in rats (Gardener & Honore, 1964). Cardiovascular hyper-reactivity to adrenaline, 5HT and methylisothiourea, but not to noradrenaline and angiotensin, has been shown in genetically hypertensive rats (see review by Laverty, McGregor & McQueen, 1967). Exaggerated cardiovascular responses to 'psychological stress' have been observed in spontaneously hypertensive rats (Hallback & Folkow, 1974; Hallback, 1975).

Studies in the whole animal are subject to the difficulties stated previously (p.38) and the use of perfused vascular beds and isolated tissues free from reflex controls have been employed.

In isolated vascular beds and tissues from renal hypertensive animals cardiovascular hyper-reactivity to a variety of pressor agents (usually angiotensin, noradrenaline and 5HT) has been reported (see reviews by Smirk, 1967; Page & McCubbin, 1968 and De Champlain, 1972). However, Redleaf & Tobian (1958) and Mallov (1959) using aortic strips from renal hypertensive rabbits and rats, respectively, failed to demonstrate any cardiovascular hyper-reactivity to adrenaline and

noradrenaline.

Collis & Alps (1975) reported an increased responsiveness (true increase in sensitivity) of the rat mesenteric vasculature to noradrenaline and angiotensin, but not potassium chloride, during the development of renal hypertension. However, these workers found that in the later stages of renal hypertension there was cardiovascular hyper-reactivity (apparent increase in sensitivity) of the mesenteric vasculature to all three compounds.

An increased responsiveness to noradrenaline has been observed in the saphenous and cephalic veins from renal hypertensive rabbits (Bevan et al., 1974) and the portal vein of spontaneously hypertensive rats (Greenberg & Bohr, 1975).

Demura et al. (1965) and Baum & Shropshire (1967) have reported cardiovascular hyper-reactivity of the isolated perfused rat hindquarters from DOCA-NaCl hypertensives to noradrenaline and angiotensin but not sympathetic stimulation. Similarly, Armstrong (1972) reported cardiovascular hyper-reactivity to noradrenaline and 5HT in this preparation. However, Oono (1966) reported no increase in cardiovascular reactivity to noradrenaline and angiotensin.

Hinke (1965) demonstrated an increased responsiveness of isolated perfused ventral caudal arteries of DOCA-NaCl hypertensive rats to noradrenaline, 5HT and rat serum.

Beilin et al. (1970) using the isolated perfused rat tail observed cardiovascular hyper-reactivity to noradrenaline but not 5HT in DOCA-NaCl hypertensive rats, whilst Beilin & Ziakus (1972) employing the same preparation reported cardiovascular hyper-reactivity to both noradrenaline and 5HT in metacorticoid hypertensive rats. Finch (1971, 1975) reported cardiovascular hyper-reactivity to noradrenaline

in perfused mesenteric arteries from DOCA-NaCl hypertensive rats. Contrary to these results Redleaf & Tobian (1958) and Mallov (1959) obtained smaller responses to adrenaline and noradrenaline from isolated aortic strips of DOCA-NaCl hypertensive rats than normotensive rats. Bohr & Sitrin (1970) and Hansen, Abrams & Bohr (1974) have reported increased responsiveness of the femoral artery from DOCA-NaCl hypertensive rats to potassium chloride, adrenaline and noradrenaline.

Laverty (1961) and Armstrong (1972) employing the isolated hind-limb preparation and McGregor & Smirk (1968, 1970) using the isolated mesenteric artery preparation have reported cardiovascular hyper-reactivity to 5HT, noradrenaline and angiotensin in genetically hypertensive rats. A lack of cardiovascular hyper-reactivity of aortic strips from spontaneously hypertensive rats to noradrenaline, angiotensin, potassium, barium and oubain has been shown (Spector et al., 1969; Clineschmidt et al., 1970; Shibata, Kurahashi & Mori, 1971). Lais & Brody (1975) found an increased responsiveness of the isolated hindquarters from spontaneously hypertensive rats to noradrenaline. Fujiwara, Kuchii & Shibata (1972) reported a decreased inotropic response to isoprenaline, adrenaline and noradrenaline on isolated atria from spontaneously hypertensive rats but an increased negative inotropic response to potassium ions.

Thus the majority of reports in the published literature suggest that there is cardiovascular hyper-reactivity in both essential hypertension in man and various forms of experimental hypertension in animals. However, whether the cardiovascular hyper-reactivity is due to an actual increase in the sensitivity of vascular smooth muscle cells or simply the result of adaptive structural changes in the blood vessels due to the elevated blood pressure has not been established.

Antihypertensive Agents

Evidence that an elevated blood pressure is harmful and that the lowering of blood pressure in hypertensive patients is beneficial has come from many sources (see reviews by Frohlich, 1974 and McKenny, 1974). No consistent abnormality in the sympathetic nervous system or of the biosynthesis, metabolism or concentrations of circulating catecholamines has been demonstrated (see reviews by Peart, 1966; Page & McCubbin, 1968; De Champlain, 1972 and Frohlich, 1974). However, pharmacological sympathectomy and antihypertensive agents affecting the sympathetic nervous system, by alteration in cardiac output or peripheral resistance, do permit effective control of blood pressure (see reviews by Hunninghake, 1969 and Frohlich, 1974). Of the antihypertensive agents acting by modification of sympathetic function, guanethidine and α-methyldopa are the compounds, at the time of writing, most used in general practice.

Guanethidine has been used extensively in the treatment of moderately severe hypertension (see reviews by Frohlich, 1974 and McKenny, 1974). However, Stanton & Cooper (1966, 1967) have found it ineffective in metacorticoid or adrenal regeneration hypertensions. The mode of action of guanethidine is complex and not fully elucidated (see reviews by Boura & Green, 1964; Green & Boura, 1964; Mull & Maxwell, 1967 and Frohlich, 1974).

Guanethidine is believed to act by preventing the release of adrenergic transmitter from the nerve endings (see reviews by Boura & Green, 1964; Mull & Maxwell, 1967 and Frohlich, 1974). Guanethidine does not penetrate the blood-brain barrier and is devoid of any central actions (Baum, Shropshire & Varner, 1972). The basis for its penetration of, and accumulation in, sympathetic nerve endings is the existence of a special amine concentrating mechanism probably located

at the level of the cell membrane and involved in the uptake of noradrenaline (see reviews by Malmfors, 1965; Carlsson, 1966; Mull & Maxwell, 1967 and Mitchell & Oates, 1970).

Guanethidine depletes catecholamine stores (see reviews by Boura & Green, 1965; Zaimis, 1964 and Frohlich, 1974) but this action is not considered important in its adrenergic neuronal blocking action since the degree of blockade does not parallel the loss of noradrenaline stores (Zaimis, 1964). Guanethidine also produces complete block of adrenergic transmission before it produces a measurable loss of noradrenaline stores, and the blockade disappears before the amine content is noticeably repleted (Cass & Spriggs, 1961; Sanan & Vogt, 1962). However, Burnstock et al. (1971) and Johnson, Cantor & Douglas (1975) have reported that prolonged treatment with high doses of guanethidine results in destruction of peripheral sympathetic nerves in adult and newborn rats.

Green (1960) and Bein (1960) demonstrated that guanethidine possessed powerful local anaesthetic effects and Boura & Green (1965) suggested that this was the basis of its action in blocking sympathetic nerves despite the demonstration that adrenergic neurone blockers do not suppress action potentials in postganglionic sympathetic nerves (Exley, 1957, 1960). Boura & Green (1965) proposed that guanethidine stabilised the intraneuronal storage granules by an action analogous to its depression of nerve conduction and this produced adrenergic neuronal blockade.

The possibility of a direct influence of guanethidine on vascular tone has been suggested by Abboud & Eckstein (1962) who reported that guanethidine produced vasodilation by stimulation of β-adrenergic

receptors or other dilator receptors which are blocked by dichloro-isoprenaline.

α-methyldopa has achieved widespread popularity for the treatment of moderately severe and severe hypertension (see reviews by Frohlich, 1974 and McKenny, 1974). The mode of action of α-methyldopa has been the subject of many investigations since the demonstration by Oates, Gillespie, Undenfriend & Sjoerdsma (1960) that it lowered the blood pressure of hypertensive patients (see reviews by Muscholl, 1966, 1972; Stone & Porter, 1966; Sourkes & Rodriguez, 1967 and Henning, 1969a).

Sourkes (1954) reported that α-methyldopa was a potent in vitro inhibitor of dopa decarboxylase. Dopa decarboxylase inhibition in vivo was demonstrated by Dengler & Reichel (1957) and Westermann, Balzer & Knell (1958).

Tissue levels of noradrenaline, dopamine and 5HT were reported to be lowered by α-methyldopa (Smith, 1960; Oates et al., 1960; Hess, Connamacher, Ozaki & Undenfriend, 1961; Kuntzman et al., 1961). It is now largely agreed that α-methyldopa lowers catecholamine levels chiefly by a mole for mole displacement of noradrenaline from its intraneuronal storage sites by the amine metabolites of α-methyldopa (Carlsson & Lindqvist, 1962; Maitre & Staehelin, 1963; Muscholl & Maitre, 1963; Anden, 1964; Shore, Busfield & Alpers, 1964).

During treatment with α-methyldopa the depleted amines are replaced by the metabolites of α-methyldopa (i.e. α-methyldopamine and α-methylnoradrenaline) and both have been identified in the brains and peripheral tissues of animals given α-methyldopa (Carlsson & Lindqvist, 1962; Muscholl & Maitre, 1963; Porter, Totaro & Burcin, 1965; Torchiana, Porter, Watson & Stone, 1965). Evidence has accumulated that α-methylnoradrenaline is stored in the same way

as normal transmitters (see review by Henning, 1969a). Muscholl & Maitre (1963) demonstrated that α-methylnoradrenaline formed from α-methyldopa was released during stimulation of the sympathetic nerves in isolated rabbit hearts.

Carlsson & Lindqvist (1962) suggested that the amines formed on metabolism of α-methyldopa may take over the function of dopamine and noradrenaline in the brain and later Day & Rand (1963) extended this hypothesis to the peripheral nerves. Day & Rand (1964) found α-methylnoradrenaline to have less vasoconstrictor activity in various animal preparations than noradrenaline and suggested that the substitution of α-methylnoradrenaline for noradrenaline would diminish the effectiveness of sympathetic impulses to the blood vessels, thus leading to a reduction in blood pressure, (i.e. the 'false transmitter' hypothesis). Many investigators attempted to establish the relative pressor activities of α-methylnoradrenaline and noradrenaline and the results have been conflicting (see review by Henning, 1969a).

Farmer (1965) confirmed the report of Day & Rand (1964) concerning the sympathetic nerve blocking action of α-methyldopa on the cat's nictitating membrane. However, Farmer (1965) hypothesised that from his results the antihypertensive action of α-methyldopa may be mediated via an antisympathetic effect of α-methyldopamine.

A central action of α-methyldopa was suggested when α-methyldopa was injected into the cerebral ventricles (Jaju, Tangri & Bhargava, 1966), into the vertebral arteries (Henning & Van Zwieten, 1967, 1968) and perfused into the vascularly isolated in situ brain (Ingenito, Barrett & Procita, 1970) of anaesthetised cats and falls in blood pressure reported which were equivalent to those observed with much larger doses given systemically.

By the use of differential (i.e. central and peripheral) dopa decarboxylase inhibitors Henning (1968, 1969b) obtained further results in rats and cats indicating a central mode of action for α-methyldopa mediated via its amine metabolites.

Henning & Rubenson (1971) showed that a dopamine-β-hydroxylase inhibitor prevented the fall in blood pressure to α-methyldopa in rats and suggested that α-methylnoradrenaline formation was necessary for the antihypertensive effect of α-methyldopa. Further evidence for a central mode of action of α-methyldopa mediated by α-methylnoradrenaline was provided by Day, Roach & Whiting (1972, 1973) and has been confirmed by Finch & Haeusler (1972), Torchiana et al., (1973) Cohen et al., (1974), Nijkamp, Ezer & De Jong, (1975) and Waldmeier, Hedwall & Maitre (1975).

In contrast to the mass of evidence suggesting a central mode of action of α-methyldopa other workers have cited evidence which they believe is contrary to this view (Mohammed et al., 1968; Ayitey-Smith & Varma, 1970; Tauberger & Kuhn, 1971; Lokhandwala, Buckley & Jandhyala, 1975; Altura, 1975).

α-methylnoradrenaline has been shown to produce a greater fall in blood pressure when given centrally to the anaesthetised cat, than either α-methyldopa or α-methyldopamine (Heise & Kroneberg, 1972). The antihypertensive action of α-methylnoradrenaline derived from α-methyldopa is probably due to an action on α-receptors in the brain (see review by Van Zwieten, 1973) since it is prevented by prior administration of α-blockers (Heise & Kroneberg, 1972; Nijkamp & De Jong, 1975). The area of the nucleus tractus solitarii has been proposed by Nijkamp & De Jong (1975) to be the part of the brain involved in the antihypertensive action of α-methylnoradrenaline

derived from α-methyldopa. The effect of α-methylnoradrenaline on α-receptors in the brain causes a decrease in impulse flow from the central nervous system (Tauberger & Kuhn, 1971; Baum, Shropshire & Varner, 1972) resulting in a reduction of the release of noradrenaline from peripheral sympathetic nerves (Anden & Henning, 1974).

## Objectives of the Research Reported in this Thesis

The first necessity of the research programme was to develop a method for the accurate measurement of blood pressure in the rat over a period of months. Similarly, an examination of the existing methods for the production of hypertension, whether acute or chronic, in the rat had to be undertaken in order to obtain a method which resulted in a good level of hypertension in a high percentage of operated animals.

After the development of methods for the production and measurement of hypertension in the rat it was decided, due to the contradictory nature of the many reports involving cardiovascular reactivity studies in experimental hypertension, to attempt to clarify the role of this phenomenon during the development and maintenance of experimental hypertension. This was initially to involve determination of whether or not cardiovascular hyper-reactivity occurred during the production and maintenance of acute and chronic hypertensions in the rat. It was also envisaged that if hypertension could be produced in the cat, cardiovascular reactivity studies would be undertaken during the development and maintenance of the hypertension. An attempt to determine the mechanism of cardiovascular hyper-reactivity, if present in the rat or cat during the development or maintenance phases of hypertension, would also be undertaken. This was thought to probably involve investigation of the role of either the renin-angiotensin

system or the sympathetic nervous system in the production and maintenance of hypertension.

The role of the sympathetic nervous system during the development and maintenance of experimental hypertension has also produced many conflicting reports. It was decided to attempt to investigate the role of the sympathetic nervous system in the production and maintenance of experimental hypertension using the adrenergic neurone blocker, guanethidine, since this compound appears to produce a more complete inhibition of the sympathetic nervous system than sympathectomy with 6-hydroxydopamine or anti-nerve growth factor.

The role of the sympathetic nervous system and the actual mechanism involved in the antihypertensive action of α-methyldopa has also produced many conflicting reports and an attempt to determine its mode of action was undertaken. The use of differential, central or peripheral, dopa decarboxylase and dopamine-β-hydroxylase inhibitors was thought to be useful in determining whether the mechanism of action of α-methyldopa was central or peripheral in origin and due to α-methyldopa or one of its metabolites.

# EXPERIMENTAL METHODS

All experiments, in this section, involving the use of rats, unless otherwise indicated, refer to male Wistar rats (Rattus norvegicus) supplied by Fisons Ltd., Hill Crest Farm, Loughborough, Leicestershire; maintained on a conventional 41B cube diet (supplied by Pilsbury's Ltd.) and water ad libitum.

After surgical procedures had been performed, animals were housed individually to prevent wounds being opened or cannulae dislodged by interference from other inmates.

Production of Hypertension in Rats

Rats with systolic blood pressures greater than 170 mm Hg as measured by the indirect tail cuff method were considered hypertensive.

(a) Renal hypertension induced by perinephritis

(i) Hypertension was induced by a modification of the method of Grollman (1944).

Rats weighing 50 - 60 g were anaesthetised with 3.5% halothane (Fluothane, I.C.I. Ltd.) in a mixture of 20% oxygen and 80% nitrous oxide. The anaesthesia was maintained by halothane 1.5% in the same mixture of oxygen and nitrous oxide.

The skin above the left kidney was shaved and then cleaned using a 1% solution of chlorhexidine (Hibitane, I.C.I. Ltd.) in 70% industrial methylated spirit. The kidney was exposed by a lumbar incision and the adrenal gland separated from its attachment to the kidney. The kidney was exteriorized through the incision and the renal capsule removed by gentle traction of the finger. A 'figure-of-eight' ligature was tied around the poles and body of the exposed kidney using non capillary braided silk suture (Abrasilk, Armour Pharmaceutical Co. Ltd.). The ligature was pulled taut, care being exercised to avoid occluding the renal blood vessels and ureter emerging from the hilus, until the

normal elipsoidal shape of the kidney was deformed.

The kidney was replaced into the abdominal cavity and a small quantity of sulphathiazole dusting powder (Thiazamide, May & Baker Ltd.) applied to the open wound to prevent infection. The abdominal wall was stitched using cotton thread and the skin brought together by the use of 'Michelle' clips.

The rat was allowed to recover and seven days later it was anaesthetised as before (p.50), the skin shaved and cleaned and a lumbar incision made in the area of the right kidney. The adrenal gland was separated from its attachment to the kidney and the kidney exteriorized through the lumbar incision. The renal blood vessels and ureter were clamped close to the hilus using Spencer-Well's artery forceps and ligatures placed both sides of the forceps. The kidney was removed by severing the renal blood vessels and ureter between the ligatures with a scalpel. Sulphathiazole dusting powder was applied to the open wound, the abdominal wall stitched and the skin brought together with 'Michelle' clips.

(ii) Hypertension was induced by a modification of the method of Page (1939).

Rats weighing 50 - 60 g were anaesthetised as before (p.50) and the left kidney exteriorized from the abdominal cavity in the same manner as described previously (p.50). A piece of transparent cellulose (Cellophane, W. H. Smith & Son Ltd.) was wrapped around the kidney and heat sealed, using a pair of preheated artery forceps, until the kidney was tightly and completely (except for the renal blood vessels and ureter originating at the hilus) enclosed. The wrapped kidney was lightly tied with cotton thread to ensure that the transparent cellulose remained correctly positioned and was then replaced into the abdominal cavity, sulphathiazole dusting powder applied and the open wound closed as described in the previous section (p.51).

Contralateral nephrectomy was performed at the same time using the procedure described before (p.51).

(b) Renal hypertension induced by partial interruption of renal blood flow

Hypertension was induced by a modification of the method of Loomis (1946).

Rats weighing 50 - 60 g were anaesthetised as before (p.50) and the left kidney exteriorized from the abdominal cavity as previously described. As the renal artery approaches the kidney it divides into two branches and one of these branches was ligated with cotton thread, the kidney returned to the abdominal cavity, sulphathiazole dusting powder applied and the abdominal wall stitched as before (p.51). Finally the skin was brought together using 'Michelle' clips.

The procedure was repeated with the right kidney.

(c) DOCA-NaCl hypertension

Hypertension was induced by a modification of the method of Selye. Hall & Rowley (1943).

Male Wistar rats weighing 50 - 60 g were anaesthetised as before (p.50) and right nephrectomy performed as previously described (p.51).

A small incision was made at the back of the neck and the skin separated from the underlying connective tissue using Spencer-Well's artery forceps. A 25 mg implant of DOCA (Organon Laboratories Ltd.) was inserted through the incision to a position level with the top of the right front leg and sulphathiazole dusting powder applied. The incision was stitched using cotton thread and the animal allowed to recover.

The rats were given 1% sodium chloride solution to drink instead

of drinking water for 14 days following the operation after which they were returned to normal tap water.

Direct Blood Pressure Measurement in Conscious, Unrestrained Rats

The method employed was based on that of Popovic & Popovic (1960) and involved cannulation of the aorta via the carotid artery in order to obtain direct blood pressure measurements.

Preparation of cannulae: The arterial and venous cannulae were both made from polyethylene tubing size PP 25 (Portex Plastics). Pieces of tubing about 25 cm in length were cut and a small enlargement (or 'bulb') was produced, by employing gentle heat, about 9 cm from one end. The cannulae were cut individually for each rat so that the length from the tip to the 'bulb' was such that the cannula lay in the aorta or vena cava when the 'bulb' was only just inside the cut artery or vein. The cannulae were placed in a 1% solution of chlorhexidine in 70% industrial methylated spirit for 10 minutes in order to sterilise them, they were then washed with sterile water and finally filled with heparinised saline solution (25 i.u. per ml) and the distal end heat-sealed.

Cannulation of Aorta and Vena Cava: Male Wistar rats were anaesthetised as previously described (p.50). A mid-line ventral incision was made in the neck and the left common carotid artery located and carefully dissected free from it's attachment to the vagus and superior cervical nerves. The arterial cannula was attached to a blood pressure transducer (Devices/C.E.C. type 4-327-L221) and the transducer to a Devices M.2. electronic recorder in order that the blood pressure could be continuously monitored. The carotid artery was ligated centrally, a 'bulldog clip' applied distally to the ligature and a small incision made, into which the cannula was inserted and loosely tied into the artery. The

'bulldog clip' was removed and the cannula slowly fed down the carotid artery into the aorta. If the cannula became 'kinked' the recording was lost whilst if it entered the heart the pulse pressure recorded was that of the left ventricle (i.e. diastolic pressure approached zero). In both these instances the cannula was withdrawn and the process repeated until the cannula lay in the aorta and a normal blood pressure trace was observed. When the tip of the cannula was thought to lie in the aorta the small 'bulb' was situated just inside the carotid artery and by placing ties both sides of the 'bulb' the cannula was securely held in position.

The right jugular vein was also occasionally cannulated to allow the intravenous injection of drugs. The jugular vein was dissected out and tied centrally, a small incision made and the venous cannula slowly fed down the vein until it was thought to lie in the vena cava when the small 'bulb' was then situated just past the incision. The cannula was secured by ties either side of the 'bulb'.

The cannulae were separately clamped and then threaded through the eye of a large, curved needle which was passed, under the skin, around the neck and brought out at the back of the head between the ears, thereby exteriorizing the cannulae. The arterial cannula was reconnected to the blood pressure transducer so that blood pressure was continuously monitored. Both cannulae were attached to muscle close to their emergence from the blood vessels, by small ligatures which ensured a smooth curvature of the cannulae and prevented 'kinking' as well as anchoring the cannulae firmly in position. Sulphathiazole dusting powder was applied to the open wound and the skin stitched together using silk. The venous and arterial cannulae were clamped, a small pin inserted into the end of each and the clamps removed.

The rat was allowed to recover and the cannulae flushed through daily and before each experiment, with 1 ml of heparinised saline (25 i.u. per ml). Some difficulties were encountered with this method due to the formation of blood clots in the cannulae, and rats occasionally chewing through their cannulae.

Recording of Blood Pressure: In order to measure the blood pressure directly the conscious rats were placed into individual compartments of a stainless-steel box which allowed adequate movement of the animals. The arterial cannula was clamped and a heparinised saline filled polyethylene tubing, size PP 60 (Portex Plastics) pushed over the cannula and the clamp removed. The PP 60 tubing was attached to a blood pressure transducer which was itself connected to an M.4. Devices electronic recorder to obtain a write-out of blood pressure. In certain instances heart rate was also monitored using a Neilson instantaneous ratemeter (Devices Instruments Ltd.) triggered by the pulsatile signal representing the blood pressure pulse.

The venous cannula was clamped and heparinised saline filled polyethylene tubing, size PP 25, (Portex Plastics) was attached to the cannula by means of a 'broken off' hypodermic needle. The volume of the tubing was 0.15 ml which, by using dose volumes of 0.1 ml and less, allowed the complete retention of the drug in the tubing until it was flushed in with 0.2 ml normal saline, hence the administration of the drug could be delayed if necessary.

Indirect Blood Pressure Measurement in Conscious Restrained Rats

Due to the lengthy operative procedure and difficulties encountered with the direct method of recording blood pressure in rats it was considered necessary to obtain a more rapid system involving conscious, unoperated animals. The method chosen was a modification of that of

Friedman & Freed (1949).

A cabinet, specially designed to accommodate six rats, provided an environment of 33.5°C, a temperature which was necessary for an arterial pulse to be detected in the tail. The arterial pulse was detected by a strain gauge, positioned distally to an occluding cuff, on the rat's tail. The cuff was slowly inflated to a pressure greater than arterial blood pressure at which point the pulse disappeared, the pressure was then allowed to slowly fall until the pulse reappeared, the average of these two positions being taken as the systolic blood pressure. A photograph of the complete apparatus employed in this method is shown in Figure 1.

The wooden cabinet, with dimensions of 88 x 62 x 37 cm was fitted with ventilation holes and polymethyl methacrylate (Perspex, I.C.I. Ltd.) doors. A thermostat (Associated Electrical Industries Ltd., adjustable bimetal thermostat type TS3) situated centrally on one side of the cabinet controlled the three 100 watt electric light bulbs which acted as the heat source and were positioned inside the cabinet near the base. To prevent urine and faeces falling onto the lights and also to restrict the degree of disturbance caused to the rats by the continual on - off switching of the lights a metal screen was placed over the whole of the heat source. Three expanded metal shelves were positioned across the full width of the cabinet about 20 cm apart and a fan on the top shelf provided circulation of the air. The thermostat was adjusted and the fan positioned in order that the temperature at all positions where the rats were situated was 33.5°C $\pm$ 0.5°C.

The rats were held in restraining cages as illustrated in Figure 2. The metal restraining cages were produced in four sizes (University of Reading, Instruments Department) which were capable of taking rats

ranging from 100 g to 450 g whilst rats weighing less than 100 g were placed in glass restraining cages (University of Aston in Birmingham, Physics Department).

The occluding cuff (Scientific and Research Instruments Ltd.), which was used to apply pressure to the rat's tail, consisted of a polymethyl methacrylate cylinder and end clamps holding a latex rubber tube in such a manner that the amount of cuff in contact with the rat's tail was 18 mm in length. This length enables the most accurate systolic blood pressure readings to be obtained (Maistrello & Matscher, 1969). Each cuff was checked to establish that a pressure of not more than 5 mm Hg (1mm Hg = 133 Pa) completely occluded the lumen of the cuff otherwise a falsely high systolic blood pressure reading is obtained (Kersten, Brosene, Ablondi & Subbarow, 1947). The occluding cuffs were connected by means of polyvinylchloride tubing, size NT/4, SH80 (Portex Plastics), to a simple luer fitting which enabled any of the six cuffs to be easily connected to the air line system. A cylinder of compressed air was used as the pressure source to inflate the occluding cuff, the cylinder tap being used to regulate the rate of inflation. The rate of deflation was adjusted by means of a screw clip compressing a rubber tube which formed a side arm of the air line system. The pressure within the system was measured using a blood pressure transducer (Devices / C.E.C. type 4-327-L221) which was connected to a DC 2 preamplifier within a Devices M.4. electronic recorder. The voltage output proportional to pressure was either monitored on a 2 channel oscilloscope (Cossor Instruments Ltd. Oscillograph Model 1049 Mk. III A) or a permanent record obtained on heat sensitive paper.

The arterial pulse was detected using a silicon semi-conductor strain gauge (Ether type 3A - 1A - 350 P) mounted in a polymethyl methacrylate clip.

The pulse detectors were manufactured in 2 sizes by Scientific and Research Instruments Ltd. and an example is shown in Figure 3. The strain gauge formed one arm of a Wheatstone bridge circuit and a 2 pole 6 position switch enabled any of the 6 strain gauges to be selected (see Figure 4). The bridge voltage supply was derived from a 4.5 V battery. The output from the bridge was fed to a Devices AC 1 pre-amplifier within the M.4. recorder and as with the pressure the output was monitored on the oscilloscope or recorded on heat sensitive paper. A block diagram of the complete system is illustrated in Figure 5.

Due to the low level of signal derived from the Wheatstone bridge circuit (less than 1 millivolt) and the high incidence of mains cables, for example to the fan and lights, it was found necessary to use screened wire throughout.

The heating source and fan were switched on and the cabinet left to attain a temperature of 33.5°C. Each of 6 rats was placed into a restraining cage of the correct size and held firmly in position by means of an adjustable metal end plate in the case of the metal cages and by adhesive tape in the case of the glass cages. In both cases the rats were fitted as tightly as possible into the cages, but also ensuring that they were comfortable, with the tail emerging from the rear of the cage. The rats were placed 3 to a shelf on the lower 2 shelves and left for 30 minutes. After this time an occluding cuff and strain gauge were fitted as near the base of the tail as possible, since this provides the most accurate readings of blood pressure (Sobin, 1946; Fregly, 1963; Maistrello & Matscher, 1969).

A rat held in a restraining cage with the strain gauge and occluding cuff in position is illustrated in Figure 6.

After a suitable warming-up period the pressure transducer and DC 2 preamplifier were balanced and calibrated and the oscilloscope was adjusted so that 1 mm on the screen graticule corresponded to 1 mm Hg blood pressure. The calibrated offset control was used so that the systolic blood pressure level lay within the central portion of the screen.

The battery was connected to the Wheatstone bridge circuit and a strain gauge and its corresponding occluding cuff selected. The gain controls on the Devices AC1 preamplifier and the oscilloscope were adjusted so that the amplitude of the displayed pulse was between 1 and 2 cm. Electrical interference (e.g. 50 Hz from the mains) was reduced by adjusting the filter control on the AC 1.

When a suitable pulse was obtained the occluding cuff was slowly inflated and the point at which the pulse disappeared noted from the oscilloscope. The air supply was turned off and the pressure fell, due to the slow leak, and the point of reappearance of the pulse noted. The procedure was repeated twice and the average of these 6 readings was taken as the systolic blood pressure of the rat. Similarly the systolic blood pressure was determined in the other 5 rats, no readings were taken when the rat's tail was moving since the movement of the animal created artifacts. An attempt to read the diastolic blood pressure was initially undertaken but this was found to be impractical due to the inability of obtaining a precise end-point.

When the blood pressure was to be determined at various times throughout the day the rats were taken out of the restraining cages after each reading and not returned until 30 minutes before the next reading.

## The Pithed Rat Preparation

Male Wistar rats were anaesthetised as previously described (p.50). A small incision was made in the skin of the neck and both carotid arteries located, the superior cervical and vagus nerves were carefully dissected away from the carotid arteries and both carotid arteries were ligated to prevent excessive bleeding from the head after pithing. The trachea was cannulated and anaesthesia maintained by using a cork plug in the delivery tube from the Boyle's apparatus, the cork contained a small bore tube which led the anaesthetic mixture from the anaesthetic apparatus to the tracheal cannula. The miniature 'Ideal' respiration pump (C. F. Palmer Ltd.) was started, the stroke volume adjusted to deliver a volume equal to 1 ml per 100 g body weight of the rat plus an amount equivalent to the 'dead space' of the apparatus; the stroke rate was set at 36 per minute. The rat was secured by its tail, using string, to the operating table and pithed by the method of Shipley & Tilden (1947) using a copper pithing rod prepared as described by Gillespie & Muir (1967). The rat was immediately connected to the respiration pump and then left for 15 - 30 minutes.

The left carotid artery was cannulated using saline filled polyethylene tubing PP 25 (Portex Plastics). The cannula was connected to a blood pressure transducer (Devices / C.E.C. type 4-327-L221) which was itself connected to a DC 2 contained within a Devices M.4. electronic recorder. The left jugular vein was cannulated using the same tubing as used for the artery and a small amount (50 i.u.) of heparin flushed into the animal to prevent blood coagulation. Heart rate was monitored using a Neilson instantaneous ratemeter connected to the Devices M.4. electronic recorder and triggered from the pulsatile signal representing the blood

pressure pulse.

All drugs were dissolved in normal saline and injected in volumes not exceeding 0.2 ml and flushed in with a further 0.1 ml normal saline.

Sympathetic stimulation: Stimulation of the complete sympathetic outflow from the spinal cord was performed as described by Gillespie & Muir (1967). The rats were anaesthetised with an intraperitoneal injection of pentobarbitone sodium (Nembutal, Abbott Laboratories Ltd.) 60 mg/kg, the animal pithed and prepared for intravenous administration of drugs and blood pressure recording as previously described (p.60). 50 i.u. of heparin (Evans Medical Ltd.), 3 mg/kg d-tubocurarine (Duncan, Flockhart & Co. Ltd.) and 1 mg/kg atropine (B.D.H.) were administered intravenously to each rat.

One lead from a constant voltage square wave stimulator (Scientific and Research Instruments Ltd.) was connected to the pithing rod which acted as one electrode whilst the other lead was connected to a piece of copper wire, introduced under the skin in the region of the left leg, which acted as the indifferent electrode.

A supramaximal voltage (80 V) and a pulse width of 1 msec. at various frequencies were used to stimulate the sympathetic outflow from the spinal cord for periods of 20 seconds. Heart rate was also recorded as previously described (p.60).

Intra-Cerebral Ventricular Injections into Conscious Rats

The method was based on that of Hayden, Johnson & Maickel (1966).

Preparation of Cannula: A sheet of polymethyl methacrylate, 6.4 mm thick was marked into blocks 6 x 7 mm using a metal scribe. A hole, 0.6 mm in diameter, was drilled through the centre of each block and the top of this hole expanded to 3.2 mm in diameter to a depth of 3 mm whilst the bottom part of the hole was expanded to 1 mm in diameter for a depth of 2 mm.

20 gauge stainless steel tube was cut to a length of 6 mm and placed in the 0.6 mm hole, this was sealed into position using epoxy cement (Araldite, Ciba-Geigy Ltd.) leaving a 4 mm length exposed. The epoxy resin was heated so that a firm seal was obtained and after it had cooled and set the polymethyl methacrylate sheet was cut into the individual cannula guides, the edges rounded off and excess epoxy resin removed using a small file. The cavity at the top of the guide was filled with a cold setting silicone rubber solution (Silescol SR 300, Esco Rubber Co.). After the rubber solution had set a stilette, 20 gauge stainless steel wire bent at 90° 4 mm from one end, was placed in the lumen of the cannula guide so that its tip coincided with that of the cannula guide. An illustration of a cannula and stilette is shown in Figure 7.

Implantation of Cannula: Male Wistar rats were anaesthetised as previously described (p.50) and placed on a wooden platform between two raised portions each of which contained one horizontally moving brass ear bar. The animal's head was held rigid in the horizontal plane by securing the brass bars into the external auditory meatus of each ear. The anaesthetic tube was held in a clamp so that anaesthesia could be maintained with the rat's head in its elevated position. A midsagittal incision was made just caudal to the eyes and rostral to the ears and the underlying tissues scraped to one side using a scalpel. The skull was cleaned with a 1% solution of chlorhexidine in 70% industrial methylated spirits to produce a dry, clean surface for drilling of the skull and application of dental acrylic cement. Using dividers, a point 2.4 mm lateral and 0.9 mm caudal to the bregma, was located and a hole drilled through the skull with a number 2 round dental burr. Three further holes were drilled around this point using the same burr and small stainless steel self tapping screws (1.4 mm in

diameter) fitted. The cannula guide was positioned on the skull so that the needle from the base fitted vertically into the centrally drilled hole and its sides were against the three screws. Dental acrylic cement (Sevriton Simplified, Amalgamated Dental Trade Distributors Ltd.) was built up around the screws and the sides of the cannula guide and allowed to harden. The open wound was stitched together with cotton thread and the animal treated with a subcutaneous injection of 125,000 i.u. benzylpenicillin (Crystapen, Glaxo Laboratories Ltd.). The animals were allowed to recover for four days before they were used for experiments.

All drugs were made up in saline and given as slow injections in a maximum volume of 40 µ litres. The injections were made from a microsyringe fitted with a 26 gauge (number 20 hypodermic) needle cut to a length of 11.3 mm and given a flat bevel (45°). Thus even though the tip of the cannula guide lay just outside the ventricles, when an injection was administered using the above needle its tip entered the lateral ventricle.

The Rat Isolated Aortic Strip

The method employed was a modification of that of Furchgott & Bhadrakom (1953).

Pairs of male Wistar rats, consisting of one normotensive control and one hypertensive animal, were killed by a blow on the head and exsanguinated. The thoracic cavity was opened and the descending thoracic aorta quickly dissected out and placed in Krebs-bicarbonate solution (1953) containing 0.01 M glucose. The cylindrical strips of aorta were then dissected free of extraneous fat and connective tissue. Helical strips were cut from the thoracic aorta, keeping the tissue moist at all times with the modified Kreb's solution, and gently trimmed free of surrounding connective tissue. The 2 strips were cut, on filter paper

soaked in the Kreb's solution, so that they were of similar length and width. The lowermost part of the aortic strip was attached by cotton thread to a tissue holder and placed in an organ bath of 30 ml working volume, containing the modified Kreb's solution maintained at 37°C and bubbled with 95% oxygen and 5% carbon dioxide. The top of the aortic strip was attached, by cotton thread, to an isotonic lever adjusted to give a 12.5 times magnification and counter weighted to exert 1 g tension on the aortic strips. Contractions of the aortic strips were recorded on a smoked drum revolving on a kymograph. A small vibrating motor was attached to the brass lever support so as to produce vibrations of small amplitude which minimised the lag in response due to the small amount of friction between the levers and the kymograph paper.

The organ bath was refilled from a reservoir containing the modified Kreb's solution which was also bubbled with 95% oxygen and 5% carbon dioxide. The solution from the reservoir passed to the organ bath via glass wool filters which served to remove any particles in the solution.

The aortic strips were allowed to relax for 2 hours after which time the tension on them was readjusted to 1 g. All drugs were dissolved in the modified Kreb's solution and added to the bath in volumes not exceeding 0.4 ml. Added drugs were maintained in contact with the tissues for 6 minutes, the bath was then rinsed twice with fresh Kreb's solution. Two further washings at 5 minute intervals preceeded the addition of a different concentration of the compound.

Production of Hypertension in Cats

Cats with diastolic blood pressures greater than 95 mm Hg as measured by the direct method were considered hypertensive.

(a) Renal Hypertension Induced by Perinephritis.

The method employed was a modification of that of Grollman (1944). Female cats, weighing 2 to 3 kg were anaesthetised with 3.5% halothane in a mixture of 20% oxygen and 80% nitrous oxide and anaesthesia was maintained with 1.5% halothane in the same mixture of oxygen and nitrous oxide.

The left lumbar region was shaved and cleaned with a 1% solution of chlorhexidine in industrial methylated spirits. An incision was made in the skin and the 3 underlying layers of muscle were separately and carefully teased apart. The kidney was exteriorized and cleaned of adhering fatty tissue. Cotton tape was sterilised by immersion, for 20 minutes, in the 1% solution of chlorhexidine in 70% industrial methylated spirits. After rinsing in sterile water this tape was passed over the pole and body of the kidney and drawn taut until the normal ellipsoidal shape of the kidney was deformed, care being taken to avoid the ureter and renal blood vessels emerging from the hilus. The kidney was returned to the abdominal cavity and the muscle layers brought together using braided silk sutures, previously soaked in the sterilising solution of chlorhexidine. The skin was sewn together using sterile silk sutures and the cat allowed to recover.

Seven days later, the right lumbar region was prepared and the kidney exteriorized and cleaned of fatty tissue as described above. The ureter and blood vessels arising from the hilus were clamped using a pair of Spencer Well's artery forceps and cotton ligatures placed on both sides of the forceps. The clamp was removed, the ureter and blood vessels between the ligatures severed and the kidney removed. The incision was sutured as described above and the cat allowed to recover.

A subcutaneous injection of 250,000 i.u. benzylpenicillin was administered after each experimental procedure. Similar doses of

benzylpenicillin were administered daily for the next 3 days.

(b) DOCA-NaCl hypertension:

The method used was based on that of Selye, Hall & Rowley (1943).

Female cats, weighing 2 to 3 kg were anaesthetised as previously described (p.65). The right lumbar region and the back of the neck were shaved and cleaned using a 1% solution of chlorhexidine in 70% industrial methylated spirits.

A mid-line incision was made at the back of the neck and 500 mg desoxycorticosterone acetate, consisting of 10 x 50 mg pellets (Organon Laboratories) were placed subcutaneously, using Spencer-Well's artery forceps, into positions between the incision and the right and left forelegs. The incision was closed using sterile silk sutures. Right nephrectomy was performed as previously described (p.65). Benzylpenicillin was administered as described earlier (p.65).

The cats were given a 1% solution of sodium chloride to drink instead of milk for 5 weeks after which they were returned to milk; normal cat food was supplied ad libitum.

Blood Pressure Measurement in Anaesthetised Cats

Cats were anaesthetised as previously described (p.65) and an incision made in the right leg. The femoral vein was located, dissected free from surrounding tissue and cannulated with polyethylene tubing size PP 90 (Portex Plastics) filled with heparinised saline. Chloralose (B.D.H.) 80 mg/kg (as a 1% solution in saline) was slowly injected to maintain anaesthesia, whilst simultaneously gradually lowering the halothane content of the anaesthetic mixture until half of the chloralose had been administered when the gaseous anaesthetic was removed. Occasionally

the left femoral vein was also cannulated to allow continuous intravenous infusions to be made from an automatic injection apparatus (C.F. Palmer Ltd.). A mid-line ventral incision was made in the neck, the trachea located and a metal cannula inserted to facilitate respiration. The right common carotid artery was dissected free from its attachment to the vagus and superior cervical nerves and cannulated with heparinised saline filled polyethylene tubing size PP 90 (Portex Plastics). The cannula was connected to a blood pressure transducer (Devices /C.E.C. type 4-327-L221) which was itself connected to a DC 2 contained within a Devices M.4. electronic recorder. Heart rate was also recorded using a Neilson instantaneous ratemeter connected to the M.4. and triggered from the pulsatile signal representing the blood pressure.

All drugs were dissolved in saline and injected via the femoral vein in volumes not exceeding 1 ml and flushed in with 2 ml of normal saline.

## Blood Pressure Measurement in Conscious, Unrestrained Cats

### Development of the Arterial Blood Pressure Recording System

Thuranszky (1966) first described a method for the continuous measurement of arterial blood pressure in conscious, unrestrained cats. The method consisted of insertion of a plastic cannula into the aorta via the common carotid artery which after plugging was exteriorized, led around the neck like a collar and held in position with adhesive tape. The danger of the catheter being pulled out and the difficulty of making a satisfactory connection to the recording device inherent in Thuranszky's method were overcome by Hall, Gomersall & Heneage (1967) who used a one-way valve made from polymethyl methacrylate and screwed into the skull.

This method whilst being an improvement on that of Thuranszky was still subject to certain problems and indicated that further improvements were necessary. Day & Owen (1970) screwed the arterial valve to a rectangular polymethyl methacrylate base which was sutured, via small holes in the corners, beneath the skin at the back of the neck. As well as appearing more acceptable to the cats than the system of Hall et al. (1967) it also allowed side-to-side movement of the valve which reduced the risk of it becoming dislodged from the catheter, another problem encountered using the method of Hall et al. (1967).

Day & Whiting (1972 a & b) abolished the sloughing of the skin at the base of the valve, the major problem encountered with the method of Hall et al. (1967), by constructing the whole valve and base from a single piece of polytetrafluoroethylene (PTFE) and increasing the height of the valve so that flat grooves could be incorporated on either side of the valve body so that it could be firmly held with a small spanner or forceps whilst making or breaking the valve connection, thus reducing the strain put upon the surrounding skin areas. For the same reason the base was made circular, with a bevelled edge, in design rather than rectangular as with the case of Day & Owen, (1970) thus doing away with any sharp corners.

The tendency for the valve to leak after prolonged use was abolished by increasing the length of the stainless steel spring and by replacing the steel ball and rubber washer used by Hall et al. (1967) with a nylon ball and neoprene washer.

The valve outlet was slightly bevelled to facilitate an easier connection to the arterial cannula than that in the method of Day & Owen (1970) whilst still preserving a very tight fit.

The valve device described together with the dust cap and connector are illustrated in Figure 8.

Implantation of the arterial valve and venous catheter: Cats were anaesthetised as previously described (p.65) and the front and back of the neck were shaved and cleaned with a 1% solution of chlorhexidine in 70% industrial methylated spirits. A mid-line ventral incision was made from the jaw to the chest whilst a smaller incision was made dorsally in the nape of the neck. A thick walled nylon tube size PP 400 (Portex Plastics) was passed, just underneath the skin, from one incision to the other and the arterial and venous cannulae, filled with heparinised saline and heat sealed, were passed through the nylon tube from the back of the neck. Removal of the nylon tube left the arterial and venous cannulae passing from the back of the neck and emerging at the front.

The right common carotid artery was located and carefully dissected from its attachment to the vagus and superior cervical nerves, the artery ligated cranially and a 'bulldog clip' applied distally to the ligature. The arterial cannula, polyvinylchloride tubing size SH 90 (Portex Plastics), was measured so that when a mark made on the cannula lay just at the point of insertion into the artery its tip should lie in the aorta. The cannula was attached to a blood pressure transducer (Devices /C.E.C. type 4-327-L221) which was itself connected to a Devices M.4. electronic recorder so that the passage of the cannula down the carotid artery into the aorta could be continuously monitored. A small incision was made in the carotid artery, the arterial cannula inserted, lightly tied and the 'bulldog clip' removed. The cannula was fed slowly down the carotid until the mark on the cannula appeared at the point of incision when the cannula tip

should lay in the aorta. Occasionally the cannula either passed into the heart and the blood pressure pulse became very large due to the fact that ventricular pressure was being recorded or it became 'kinked' when no arterial pulse was observed. In both cases the cannula was withdrawn and again passed down the carotid until it lay in the correct position in the aorta. When the cannula was considered to lie in the aorta it was tied into the carotid with at least four cotton ligatures previously soaked in the sterilising solution of chlorhexidine in industrial methylated spirits.

A branch of the right jugular vein was located and carefully dissected free from surrounding tissues. The venous cannula, polyethylene tubing size PP 30 (Portex Plastics), was slowly fed down the branch of the jugular vein until it lay in the superior vena cava, the necessary length having been previously measured and the cannula marked. The cannula was tied into the branch of the jugular vein with at least four cotton ligatures, previously soaked in the sterilising solution. It was considered that by cannulating a branch of the jugular vein rather than the jugular vein itself less interference of the normal blood flow would result.

Both cannulae were attached to muscle by means of single cotton ligatures, previously sterilised, which served to maintain a smooth curve on the cannula and prevent 'kinking' as well as firmly anchoring the cannula. Sulphathiazole dusting powder was applied to the open wound and then the wound was stitched using sterile silk sutures.

The venous cannula was clamped with rubber covered artery forceps, the cannula cut so that about a 150 mm length was left exteriorized at the back of the neck. The end of the cannula was closed with a

small pin and finally the forceps were removed.

Sulphathiazole dusting powder was applied to the open wound at the back of the neck. The arterial cannula was clamped, using rubber covered artery forceps, close to where it emerged from the incision and attached to the outlet tube on the valve device. The forceps were removed, saline flushed into the animal and the valve checked for leaks. Sterile ligatures were attached individually to the four holes in the base of the valve device which was then sutured beneath the skin at the back of the neck in such a manner that the open wound was also sutured. A dust cap was screwed into position on top of the valve device.

Benzylpenicillin (250,000 i.u.) was administered subcutaneously and the dose repeated each day for a further three days. Chlorpromazine (Largactil, May & Baker Ltd.) 1 mg/kg was administered intramuscularly after the gaseous anaesthetic had been removed. This served to prolong the recovery of the cat from the anaesthetic and abolished the excitation stage observed in the recovery of some cats to which chlorpromazine had not been administered. The chlorpromazine also served to sedate the animal for some hours after the operation which appeared to lessen any scratching of the skin around the operated areas. For the first four days after the operation and before each experiment the cannulae were flushed with heparinised saline (5 ml of 25 i.u. per ml) after which time the cannulae were flushed every other day with normal saline.

Experimental Procedure: After a four day recovery period the cats were allowed to become familiar with the cage into which they were placed for the duration of an experiment and trained to accept intravenous injections without moving. After about a week of training it was found that cats would either sit still or sleep for about three

hours whilst intravenous injections of compounds were administered.

The cat was placed into an expanded metal cage and the dust cap removed from the valve device. The connector, attached by a length of heparinised saline filled polyvinylchloride tubing size SH120 (Portex Plastics) to a blood pressure transducer (Devices /C.E.C. type 4-327-L221), was screwed onto the valve device. The blood pressure transducer was itself connected to a DC 2 preamplifier contained within a Devices M.4. electronic recorder to obtain a permanent record of the blood pressure. Heart rate was recorded using a Neilson instantaneous ratemeter connected to the M.4. and triggered from the pulsatile signal representing the blood pressure. The pin was removed from the venous cannula and connection made to a length of polyethylene tubing size PP 30 (Portex Plastics) by a blunted hypodermic needle. All drugs were dissolved in normal saline and injected in volumes not greater than 0.4 ml. The total volume of the tubing was 0.6 ml which enabled the complete injection to be retained in the tubing so that on the occasions when the cat was disturbed, flushing in could be delayed until the cat was again quiet. All drug solutions were flushed in with 0.8 ml normal saline. A cat set up for recording of blood pressure and intravenous administration of drugs is illustrated in Figure 9.

## DRUGS USED

All drugs were dissolved in normal saline except where otherwise stated. The doses of guanethidine, McN-A-343, noradrenaline and tyramine quoted in this thesis are expressed as free base.

| | |
|---|---|
| α-methyldopa | - Merck, Sharp & Dohme (Aldomet) solution for injection. |
| Angiotensinamide | - Ciba (Hypertensin) |
| Ascorbic acid | - B.D.H. |
| Atropine sulphate | - B.D.H. |
| Benzylpenicillin | - Glaxo (Crystapen G) |
| Chloralose | - B.D.H. |
| Chlorpromazine hydrochloride | - May & Baker (Largactil) solution for injection. |
| Desoxycorticosterone acetate | - Organon (DOCA) 25 mg pellets. |
| Guanethidine monosulphate | - Ciba (Ismelin) |
| Halothane | - I.C.I. (Fluothane) Inhalation with $N_2O$ and $O_2$ |
| Heparin | - Evans Medical solution for injection. |
| McN-A-343 | - McNeil |
| Noradrenaline bitartrate | - B.D.H. |
| Noradrenaline hydrochloride | - Sigma |
| Pentobarbitone sodium | - Abbott (Nembutal) solution for injection. |
| 1-Phenyl-3-(2-thiazolyl)-2-thiourea | - Upjohn (U-14,624) |
| Seryl-2,3,4-trihydroxybenzyl hydrochloride | - Hoffman-La Roche (Ro 4-4602) |
| Sodium diethyldithiocarbamate | - B.D.H. (DDC) |

| | |
|---|---|
| Sulphathiazole | - May & Baker (Thiazamide) dusting powder. |
| Tetraethylthiuram disulphide | - B.D.H. (Disulfiram) suspended in 0.9% sodium chloride solution with compound powder of tragacanth. |
| d-Tubocurarine | - Duncan, Flockhart & Co. Ltd. solution for injection. |
| Tyramine hydrochloride | - Sigma |

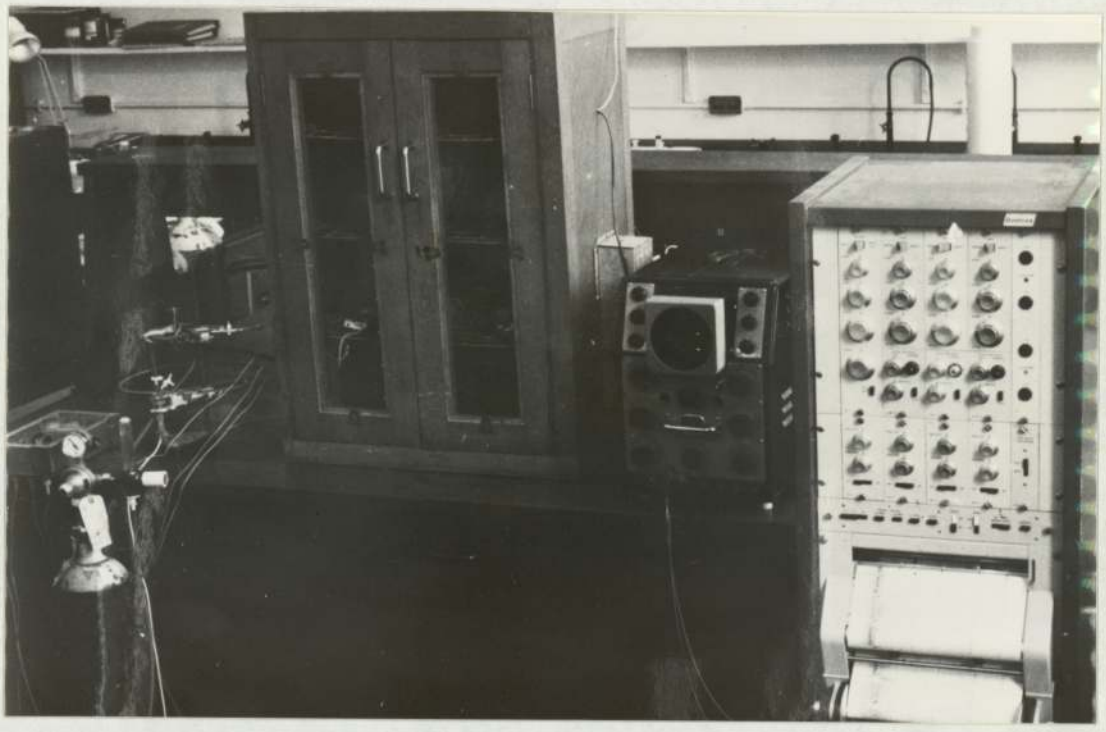

FIGURE 1: INDIRECT DETERMINATION OF THE SYSTOLIC BLOOD PRESSURE IN THE RAT.

The apparatus used for determining the systolic blood pressures of rats by a tail cuff method is shown above.

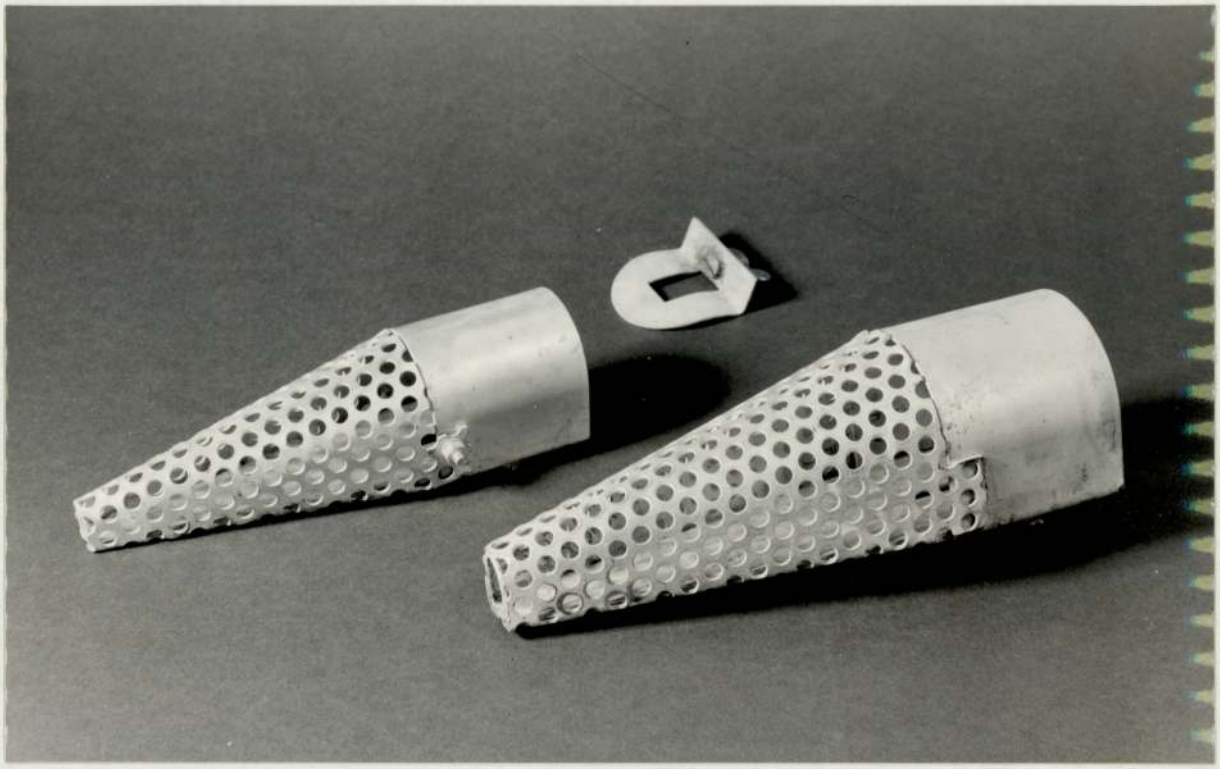

FIGURE 2: RAT RESTRAINING CAGES

After a rat was inserted into one of the cages it was placed in the heating cabinet to attain a temperature which produced adequate vasodilatation of the tail.

FIGURE 3: PULSE DETECTOR

The pulse from the tail artery was detected by a silicon semi-conductor strain gauge mounted in the polymethyl methacrylate clip.

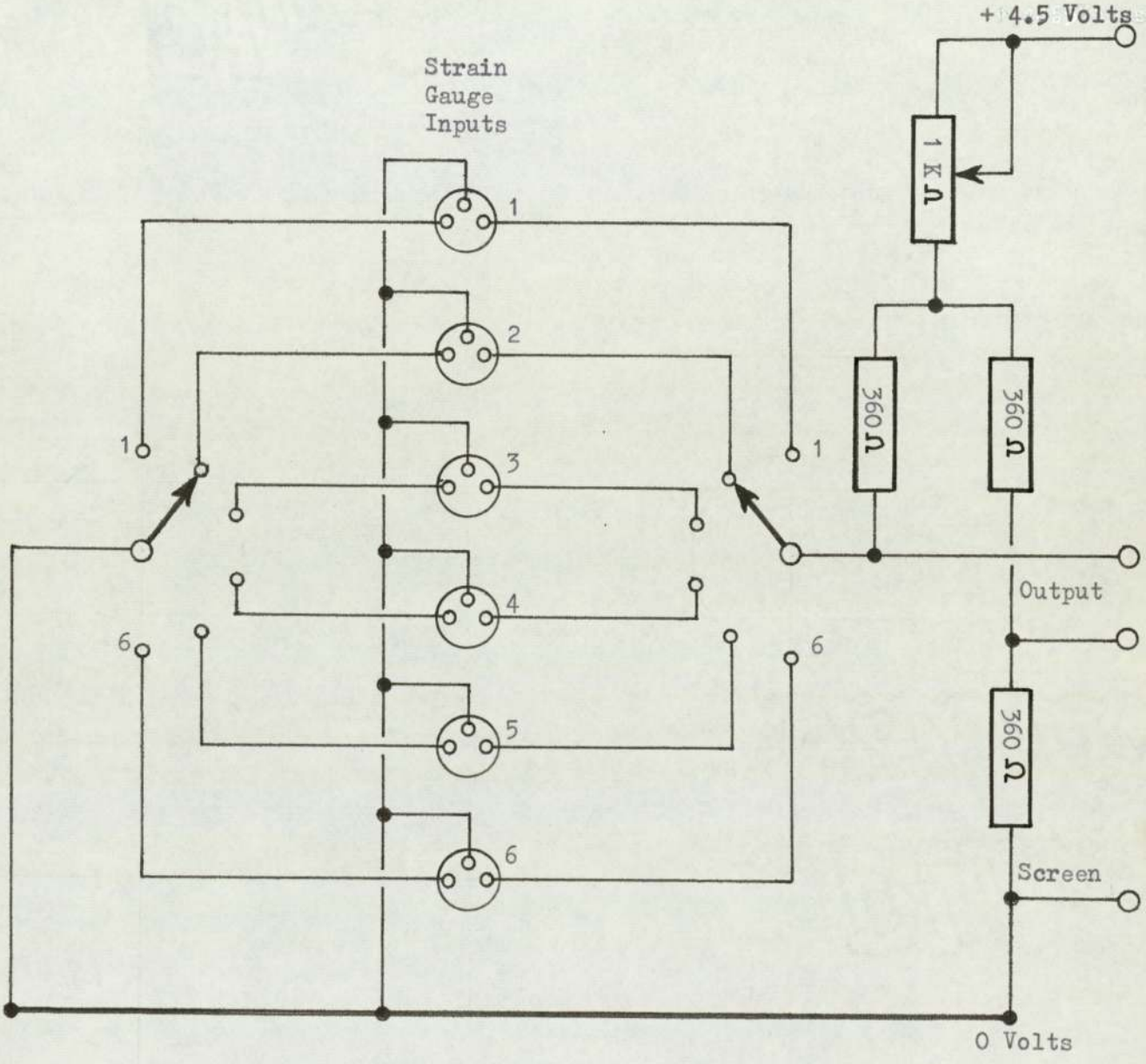


FIGURE 4: WHEATSTONE BRIDGE CIRCUIT AS USED WITH PULSE DETECTORS.

A switch enables one of 6 strain gauges to be connected into the bridge circuit. The output of the bridge is taken to the input of a medium gain A.C. amplifier e.g. E.C.G.

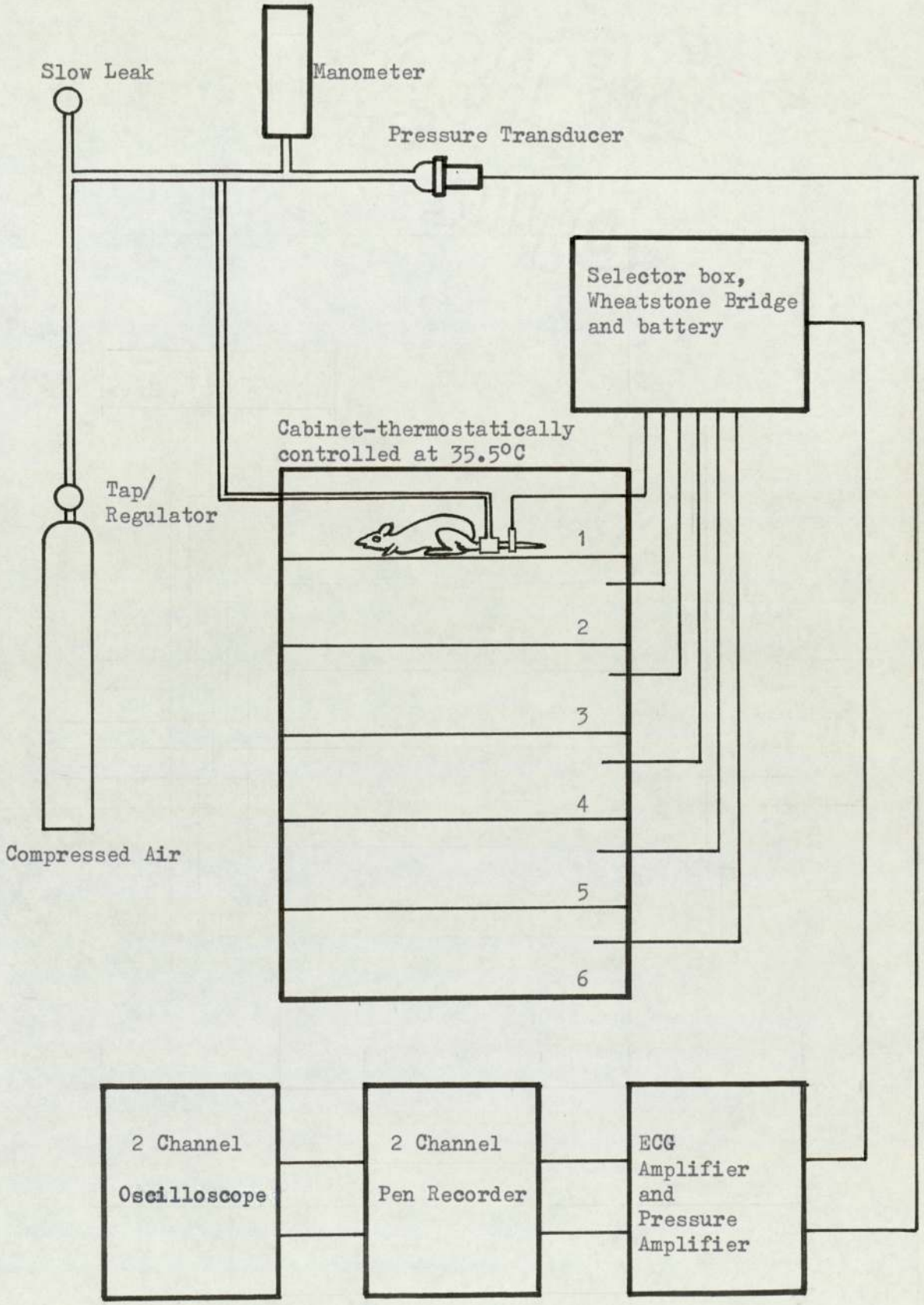


FIGURE 5: BLOCK DIAGRAM OF THE APPARATUS USED FOR MEASURING THE SYSTOLIC BLOOD PRESSURE OF A RAT BY THE TAIL CUFF METHOD.

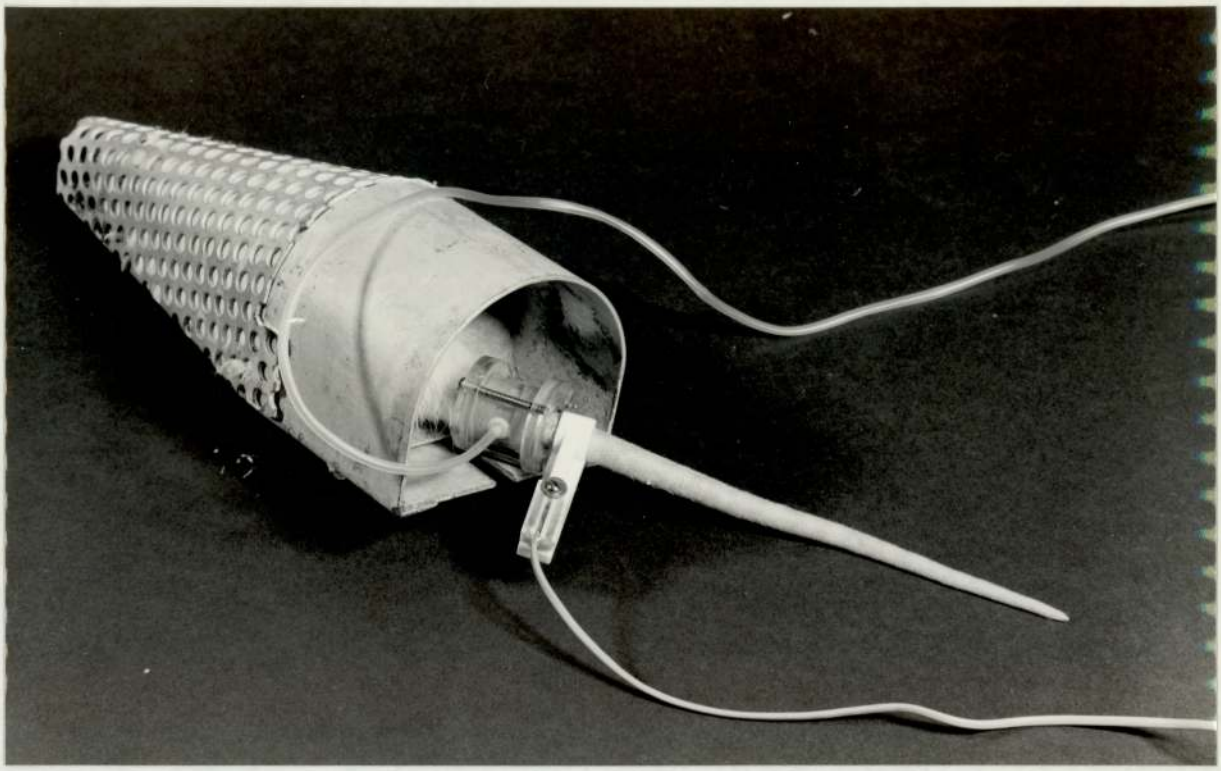

FIGURE 6: THE OCCLUDING CUFF AND PULSE DETECTOR ON THE TAIL OF A RAT.

This figure shows the positioning of the above equipment on the rat's tail when used to measure the systolic blood pressure.

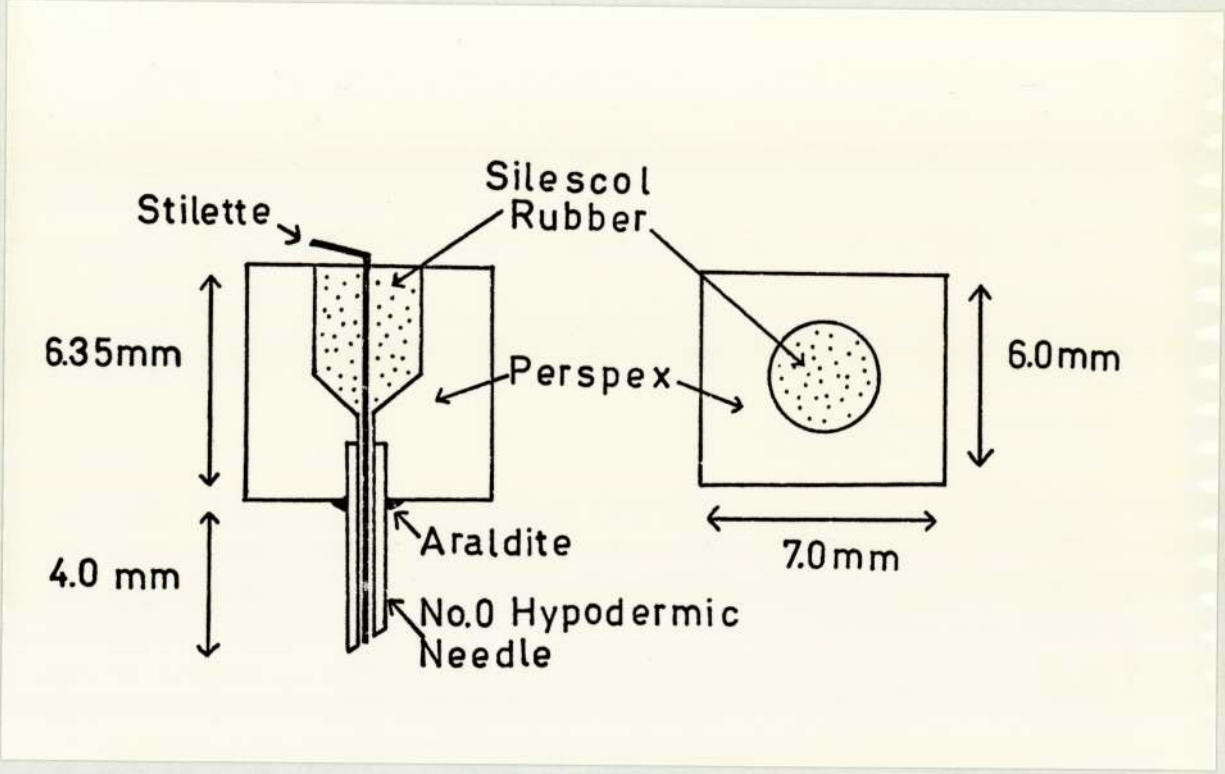


FIGURE 7: VERTICAL CROSS SECTION AND PLAN VIEWS OF THE CANNULA GUIDE USED FOR INJECTIONS INTO THE LATERAL VENTRICLES OF RATS.

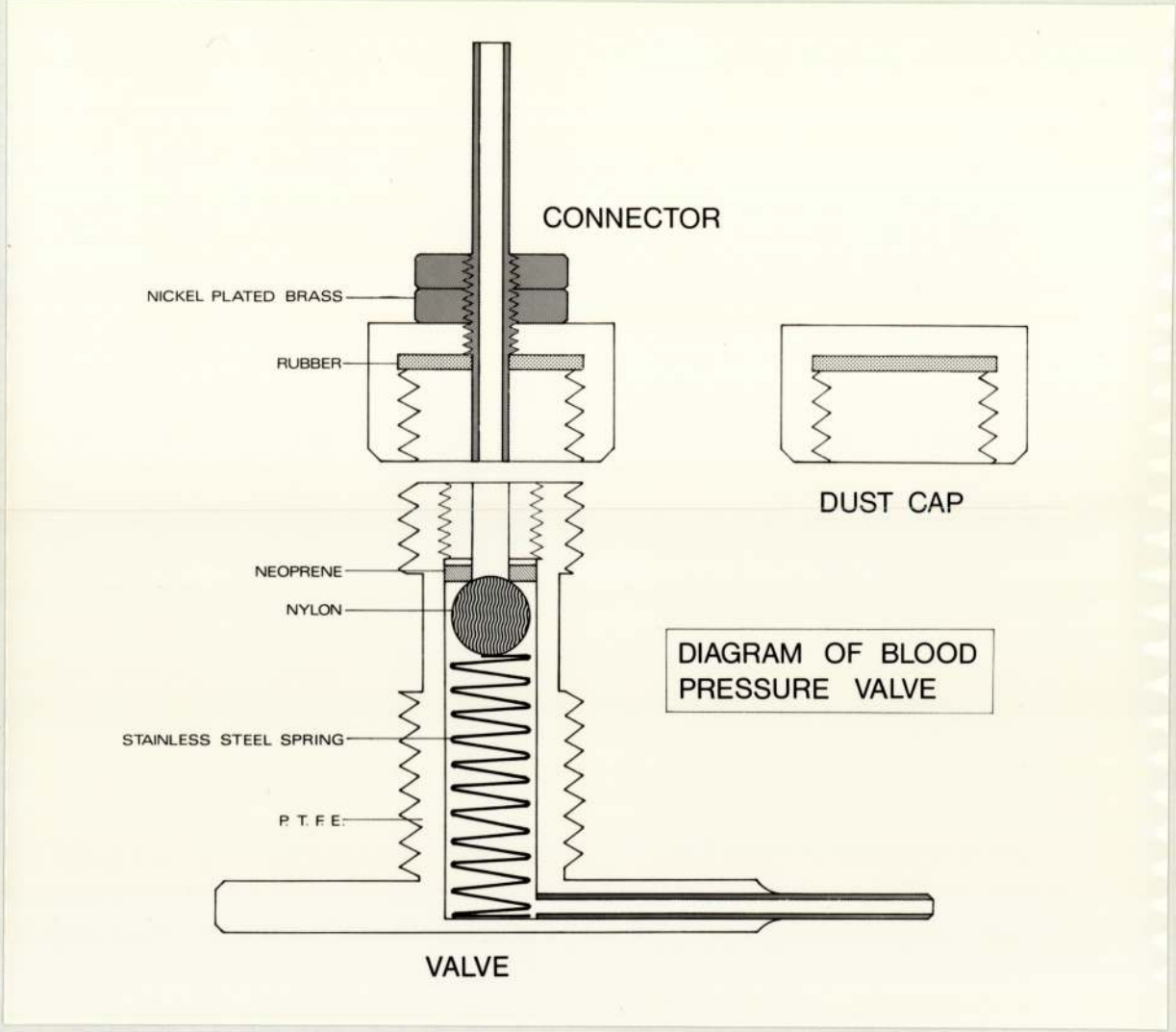


FIGURE 8: A CROSS SECTION DIAGRAM OF THE VALVE, CONNECTOR AND DUST CAP USED IN THE RECORDING OF BLOOD PRESSURE IN THE CONSCIOUS CAT.

FIGURE 9: BLOOD PRESSURE MEASUREMENT IN THE CONSCIOUS CAT

This photograph was taken during a typical experiment in which the aortic blood pressure of a conscious cat was continuously monitored.

# EXPERIMENTAL RESULTS

## SECTION 1: ARTERIAL HYPERTENSION IN THE RAT

### CHAPTER 1

### A Comparison of Direct and Indirect Systolic Blood Pressure Measurements in the Rat.

Initially an attempt was made to measure blood pressure chronically in the rat using cannulation of the aorta via a carotid artery. Although this method was found to be successful in the short term many problems, such as the formation of clots in the cannula, arose during the long term (i.e. greater than 4 weeks). Thus, although direct methods of measuring blood pressure offer greater accuracy (see review by Geddes, 1970), it was decided to construct a system capable of determining the blood pressures of rats by an indirect method.

Several workers (Fregly, 1963; Maistrello & Matscher, 1969; Pfeffer, Pfeffer & Frohlich, 1971) have stated that it is most important for each laboratory to validate its indirect pressure measurements for the circumstances under which the method is employed. Hence, after construction of the system enabling indirect systolic blood pressures of rats to be determined (see p.55) it was considered necessary to investigate the accuracy of the values obtained and also to determine the effect, on the rat's systolic blood pressure, of warming and restraining the animals. This was achieved by comparing the systolic blood pressures obtained indirectly from the caudal artery with those obtained directly from the aorta.

#### Results

A typical trace obtained during the determination of the indirect systolic blood pressure of a rat is illustrated in Fig. 10. The value for the systolic blood pressure obtained during inflation of the cuff was frequently higher than that obtained during deflation. However,

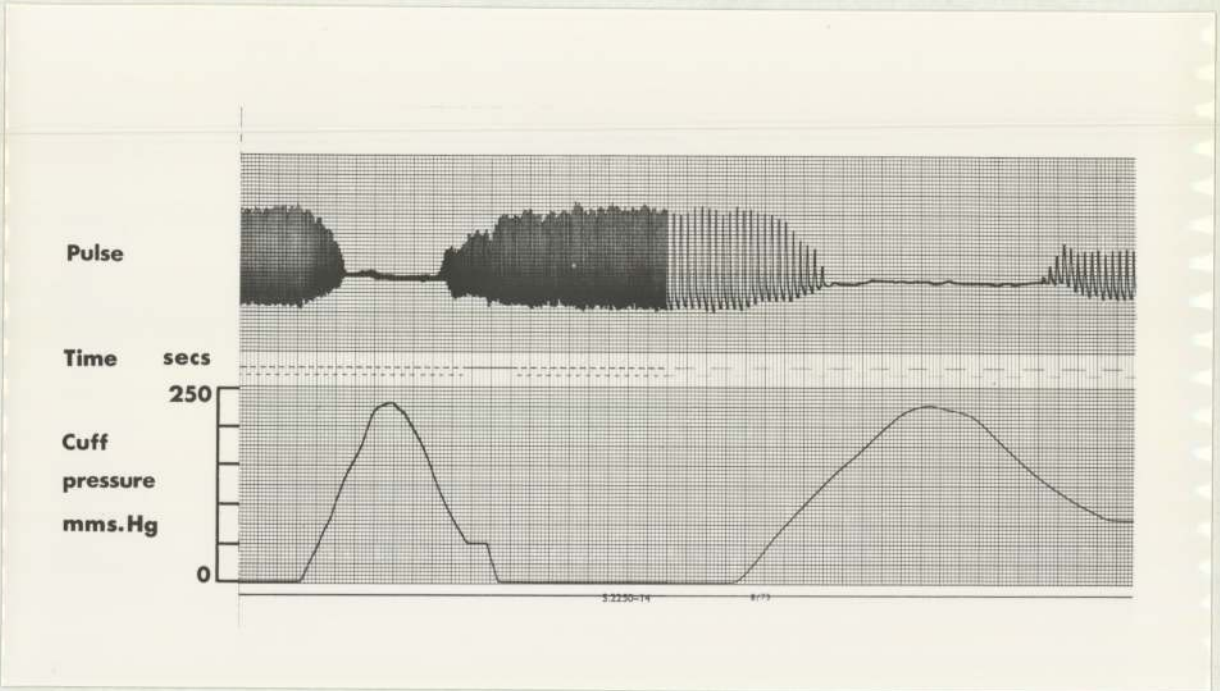


FIGURE 10: A TYPICAL TRACE OBTAINED DURING THE INDIRECT DETERMINATION OF THE SYSTOLIC BLOOD PRESSURE OF A RAT.

The upper part of the trace shows the pulse obtained from the caudal artery of the rat. The lower section represents the pressure in the occluding cuff. The pressures at which the pulse disappears and reappears are averaged and taken as the systolic blood pressure.

if the values obtained during inflation alone or deflation alone were considered for a single rat then a good consistency within a 5 to 10 mm Hg range was obtained. Repeated daily determinations of the systolic blood pressure of a single normotensive animal rarely varied more than 5 - 10 mm Hg. Similarly repeated determinations of the systolic blood pressure of a single animal throughout a working day rarely varied more than 5 - 10 mm Hg (see Fig. 58).

Simultaneous measurement of the indirect restrained systolic blood pressure and direct restrained blood pressure of a rat is illustrated in Fig. 11. Inflation and deflation of the occluding cuff appeared to have no effect on the resting blood pressure of the rat as seen from the direct recording of blood pressure.

A direct comparison of systolic blood pressures obtained from normotensive rats and from rats with varying degrees of DOCA-NaCl hypertension by simultaneous indirect and direct methods is illustrated in Fig. 12. Each point is the mean of 3 successive readings taken during deflation of the cuff, no individual reading differed from the mean by more than 10 mm Hg and the majority by less than 5mm Hg. The relationship between the indirect tail and simultaneous aortic systolic blood pressures was highly significant ($p < 0.001$, $r = 0.976$) with a regression coefficient of near unity (0.935). The means of the 48 direct aortic systolic pressure measurements was 179 mm Hg $\pm$ S.E.11.8; that of the simultaneously recorded, indirect systolic pressures was 167 mm Hg $\pm$ S.E.11.3. The equation of indirect (Y) to direct (X) was $Y = 0.935\ x - 1.044$.

The relationship of the indirect restrained systolic blood pressures to the systolic blood pressures of the rat in a near normal state, that is the measurements obtained from unrestrained rats at room temperature

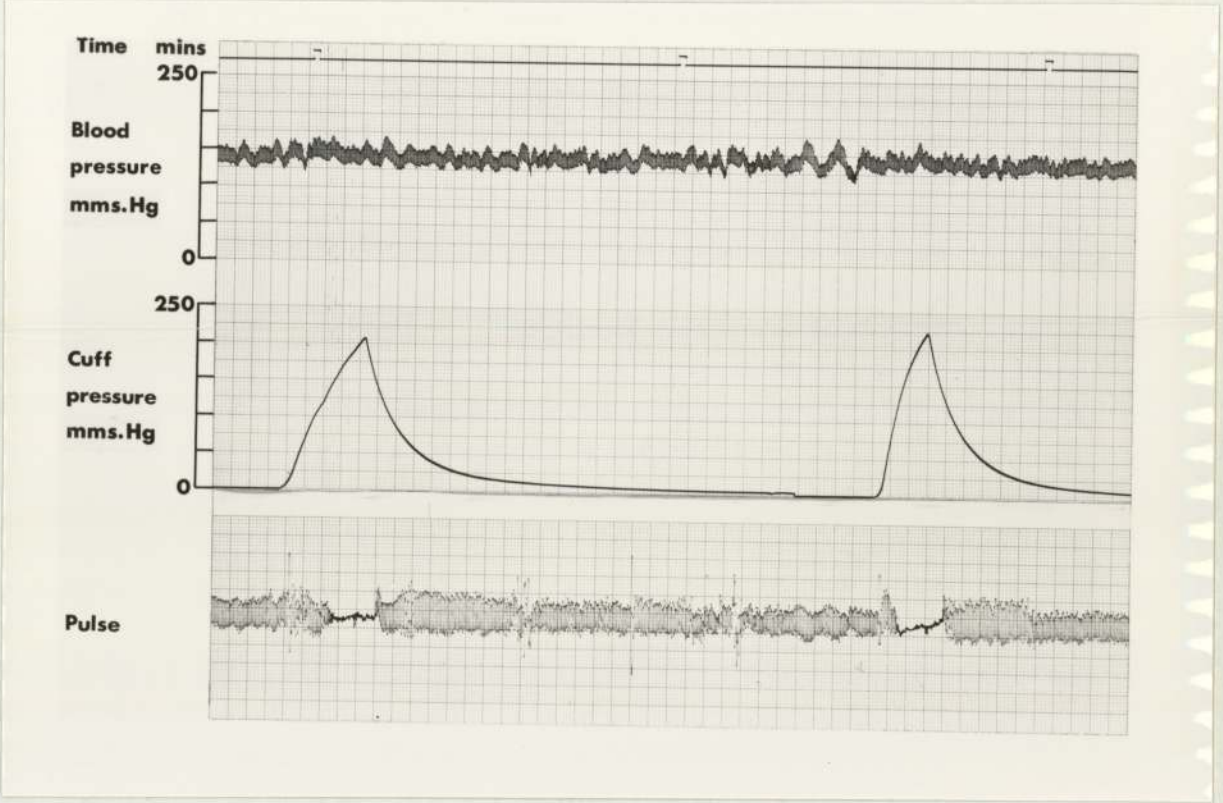


FIGURE 11: A TRACE SHOWING THE SIMULTANEOUS MEASUREMENT OF RAT BLOOD PRESSURE OBTAINED INDIRECTLY BY A TAIL CUFF METHOD AND DIRECTLY FROM AN AORTIC CANNULA.

The upper part of the trace shows the recording of blood pressure obtained directly from the aorta. The mid-portion of the trace represents the pressure in the occluding cuff and the lower part of the trace shows the pulse obtained indirectly from the caudal artery. The measurements of the systolic blood pressure obtained by each method are very similar.

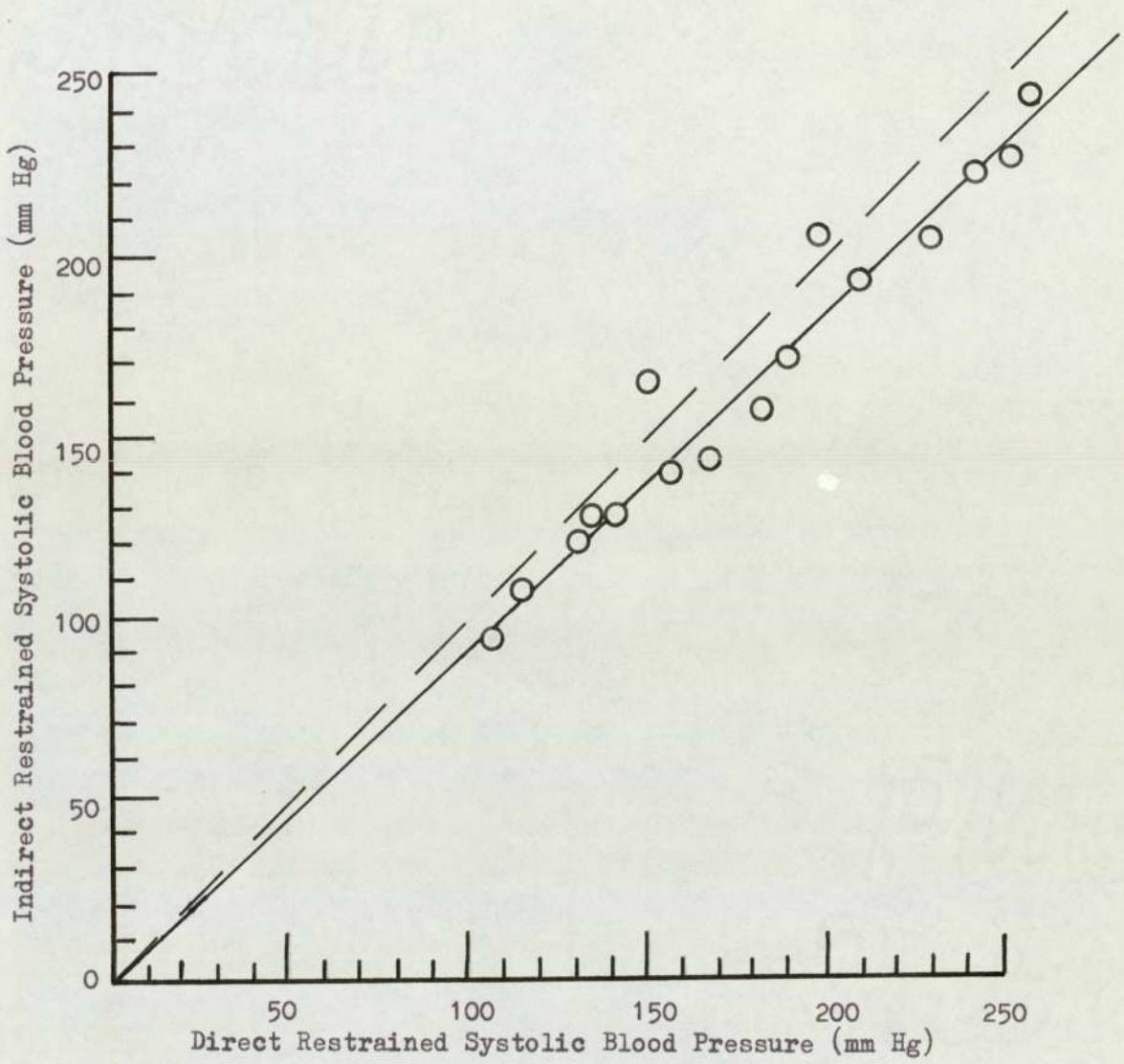


FIGURE 12: THE RELATIONSHIP BETWEEN SYSTOLIC BLOOD PRESSURE MEASUREMENTS OF RESTRAINED RATS OBTAINED BY DIRECT AND INDIRECT METHODS

Each circle represents the simultaneous measurement by an indirect and direct method of the systolic blood pressure of a single unanaesthetised rat. The line of perfect agreement is shown by the dotted line. The equation of indirect (Y) to direct (X) was $Y = 0.935 x - 1.044$ with a correlation coefficient $r = 0.976$.

by the direct method is illustrated in Fig. 13. The readings were not obtained simultaneously but a mean of 3 successive readings during deflation was obtained by the indirect tail method followed by 3 successive readings of the direct unrestrained rat in a normal environment at 3 arbitrary times to give a mean value. In both instances no individual reading differed from the mean by more than 10 mm Hg and the majority of readings differed by less than 5 mm Hg. The relationship between the indirect restrained systolic blood pressures and direct aortic unrestrained systolic blood pressures was highly significant ($p < 0.001$, $r = 0.989$) with a regression coefficient of near unity (1.298). The mean of the 51 direct aortic unrestrained systolic blood pressure measurements was 151 $\pm$ S.E.8.6 mm Hg, that of the indirect systolic blood pressures was 167 mm Hg $\pm$ S.E.11.3. The equation of indirect restrained (Y) to direct (X) was $Y = 1.298 x - 28.946$.

The effect of restraining and warming the rats on their systolic blood pressures is illustrated in Fig. 14. The reading of direct restrained systolic blood pressure was determined before that of the unrestrained systolic blood pressure and each point on the graph represents the mean of 3 successive readings as previously described (p.86). The relationship between the systolic blood pressures of restrained and unrestrained rats was highly significant ($p < 0.001$, $r = 0.980$) with a regression coefficient of 1.341. The mean of the 48 direct aortic systolic blood pressures of restrained rats was 179 mm Hg $\pm$ S.E.11.8; that of the unrestrained rats was 150 mm Hg $\pm$ S.E.8.6. The equation of direct restrained (Y) to direct unrestrained (X) was $Y = 1.341 x - 22.877$.

The effect of a 10 mg/kg intraperitoneal injection of guanethidine on the systolic blood pressures of restrained DOCA-NaCl hypertensive rats as recorded by both direct and indirect methods, is illustrated

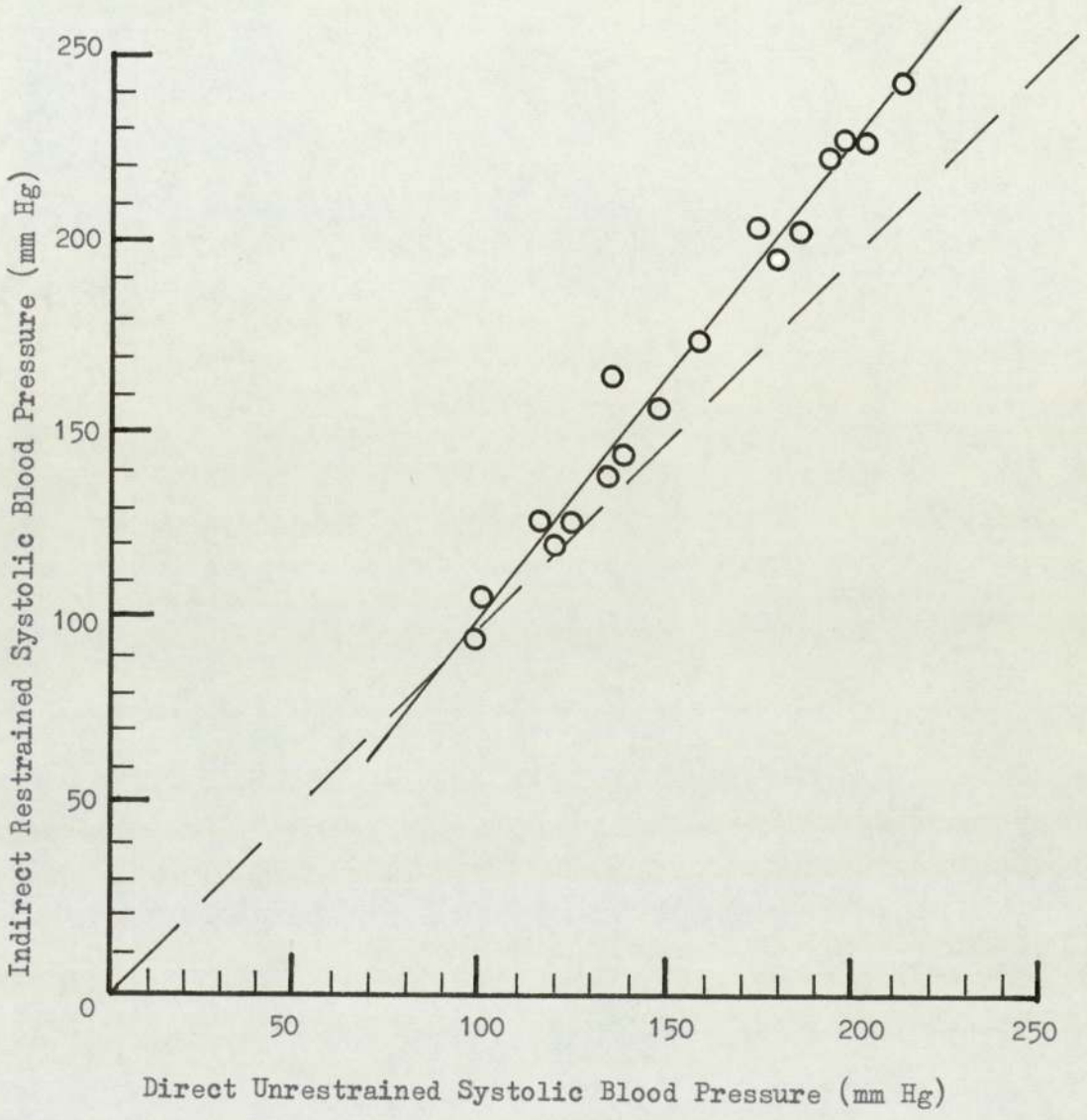


FIGURE 13: THE RELATIONSHIP BETWEEN DIRECT UNRESTRAINED AND INDIRECT RESTRAINED SYSTOLIC BLOOD PRESSURE MEASUREMENTS IN RATS.

Each circle represents the systolic blood pressure of a single unanaesthetised rat measured by the indirect method followed by the direct method. The line of perfect agreement is shown by the dotted line. The equation of indirect restrained (Y) to direct unrestrained (X) was $Y = 1.298 x - 28.946$ with a correlation coefficient $r = 0.989$.

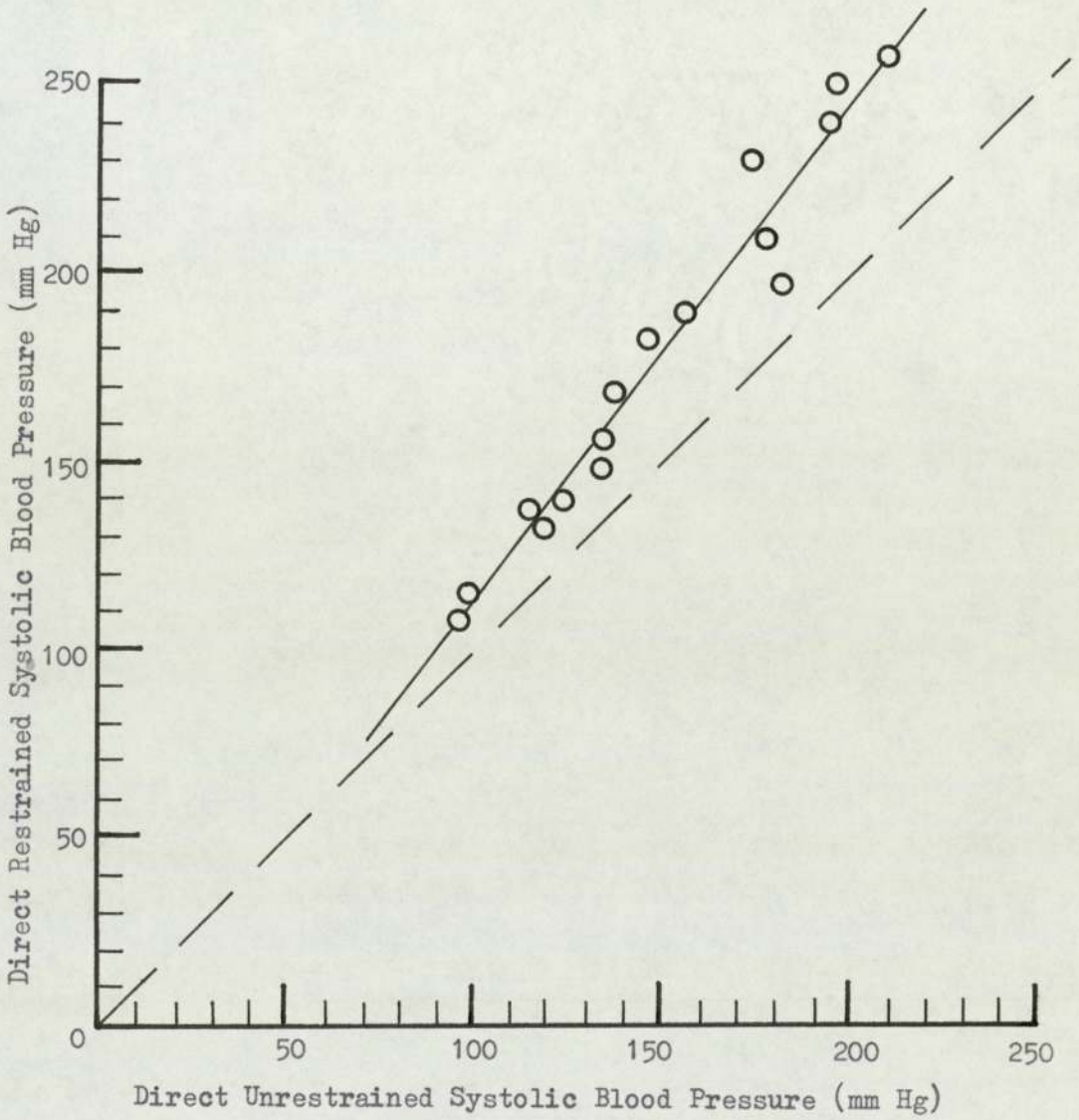


FIGURE 14: THE RELATIONSHIP BETWEEN DIRECT UNRESTRAINED AND DIRECT RESTRAINED SYSTOLIC BLOOD PRESSURE MEASUREMENTS IN RATS.

Each circle represents the systolic blood pressure of a single unanaesthetised rat measured directly in the restrained condition followed by a measurement taken in the unrestrained state. The line of perfect agreement is shown by the dotted line. The equation of direct restrained (Y) to direct unrestrained (X) was $Y = 1.341 x - 22.877$ with a correlation coefficient $r = 0.980$.

in Fig. 15. The direct aortic systolic blood pressure readings although higher than the indirect caudal systolic blood pressure readings throughout the duration of the experiment were at no time statistically significantly different (using the Students 't' test) from each other.

Discussion

The accuracy of any individual system for the measurement of blood pressure depends upon several variable factors. Certain of these parameters have been assessed and were adhered to in this study:-

(a) A pressure gradient exists along the rat's tail (Sobin, 1946; Fregly, 1963) and thus to obtain a systolic blood pressure reading employing the tail cuff method as closely related to that of aortic systolic pressure as possible the occluding cuff must be placed at the root of the tail.

(b) Although warming of the rat or it's tail has been shown to increase both blood pressure and heart rate (Proskauer, Neumann & Graef, 1945) it is essential not only to produce adequate vasodilatation of the caudal artery, and thus increase the signal level, but also to obtain an accurate value for the systolic blood pressure (Sobin, 1946; Fregly, 1963). The method of warming has been achieved in a variety of ways (see review by Geddes, 1970) and although local warming of the rat's tail has been claimed to provide more accurate values than warming the whole rat (Sobin, 1946) this has been disputed (Alexander, 1957) and no method of warming appears to afford any substantial increase in accuracy over any other. The temperature employed to warm the rat or it's tail has also varied. Maximum vasodilatation of the tail is obtained at 40 - 42°C (Sobin, 1946) but these temperatures have been reported to markedly increase the blood pressure and heart rate (Proskauer et al., 1945) whilst adequate

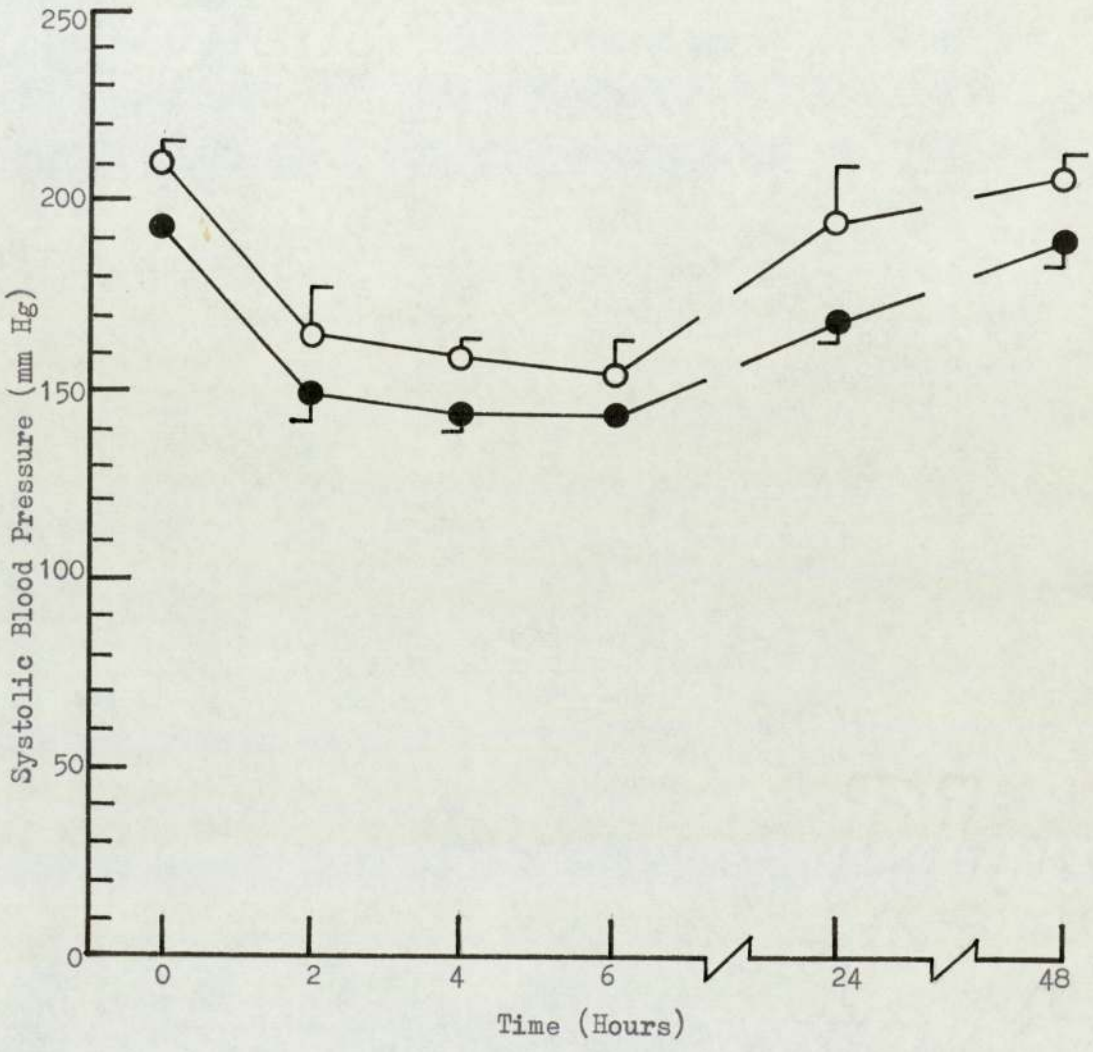


FIGURE 15: COMPARISON OF THE DIRECT AND INDIRECT METHODS OF MEASURING SYSTOLIC BLOOD PRESSURE AS ILLUSTRATED BY THE EFFECT OF AN INTRAPERITONEAL INJECTION OF 10 mg/kg GUANETHIDINE INTO RESTRAINED DOCA-NaCl HYPERTENSIVE RATS.

The line shown with open circles (O—O) represents the mean systolic blood pressure of six rats measured by direct cannulation. The line shown with solid circles (●—●) represents the mean systolic blood pressure of the same rats measured indirectly from the tail. Standard errors of the mean at each point are shown by the vertical bars.

vasodilatation of the tail without any appreciable rise in blood pressure and heart rate has been reported by Fregly (1963) in the temperature range 36.5 - 39°C. The temperature used in this study was 33.5 ± 0.5°C and although this does not produce a maximal signal level the end point was clearly defined and from the work of Proskauer et al. (1945) it can be presumed to produce little effect upon the blood pressure and heart rate.

(c) Although the foot, since it offers the advantage of being less vasoactive than the tail, has been used to determine the systolic blood pressure of rats (Griffith, 1934; Kersten et al., 1947; Olmsted, Corcoran & Page, 1951) most indirect methods employ the tail as the measuring site (e.g. Bonsmann, 1934; Kempf & Page, 1942; Friedman & Freed, 1949, 1952; Alexander, 1957; Maistrello & Matscher, 1969; Pfeffer, Pfeffer & Frohlich, 1971; Bunag, Page & McCubbin, 1971; Bunag, 1973) due to its greater convenience and the tail was used in the present study.

(d) To decrease the level of noise and hence increase the ratio of signal level to noise, so important in obtaining a clear end point, anaesthesia has been employed in some tail cuff methods (Byrom & Wilson, 1938; Gallagher & Grimwood, 1953; Dahl, 1960; Dowd & Jones, 1968; Maistrello & Matscher, 1969; Bunag, Page & McCubbin, 1971). However, anaesthesia has been criticised since it produces changes in the blood pressure (Ben-Ziv, Weinman & Sulman, 1964) and is less physiological than employing unanaesthetised rats (Pfeffer et al., 1971). In the present system unanaesthetised rats were used, the rats were also frequently handled and accustomed to the restraining system since this enables more reliable results to be obtained (Rowberg, Franklin & Van Citters, 1969; Pfeffer, Pfeffer & Frohlich, 1971).

(e) The reference artery, used to obtain the direct readings of systolic

blood pressure to compare with the indirect readings obtained from the caudal artery has usually been either the carotid or aorta (e.g. Alexander, 1957; Rowberg et al., 1969; Frangipane & Aporti, 1969; Pfeffer, Pfeffer & Frohlich, 1971; Bunag, 1973), though Geddes (1970) has suggested that the most desirable reference artery is the femoral since it arises from the aorta close to the caudal artery and the femoral has in fact been employed as the reference artery (Sobin, 1946; Fregly, 1963). However, since one is seeking to establish a relationship between systemic arterial pressure and the indirect readings obtained from the caudal artery it appeared advisable to use the aorta as the reference artery. The aorta also possesses the advantage that little disturbance to normal blood flow occurs when cannulated via a carotid artery and probably provides a more accurate value of blood pressure than that obtained from the femoral (Bunag, 1973).

(f) All indirect methods of measuring systolic blood pressure in the rat involve some form of restraining the animals. This may be performed by employing anaesthesia (e.g. Byrom & Wilson, 1938; Dowd & Jones, 1968; Maistrello & Matscher, 1969; Bunag, McCubbin & Page, 1971), or curare (Friedman & Freed, 1949), or in a restraining device (e.g. Sobin, 1946; Kersten et al., 1947; Fregly, 1963; Frangipane & Aporti, 1969; Bunag, McCubbin & Page, 1971; Pfeffer, Pfeffer & Frohlich, 1971; Bunag, 1973). The effect of this restraint on systolic blood pressure has not been previously reported and although in this study the effect of a combination of restraining and warming the animal on systolic blood pressure has been examined (Fig. 14) the effect of warming, as stated previously, can be considered to be minimal. The increase in systolic blood pressure due to restraining the animal can be seen to be directly proportional to the resting

level of systolic blood pressure indicating perhaps that rats with high blood pressure are more susceptible to stress or possess a more labile blood pressure. The presence of a significant difference between the two groups of systolic blood pressure measurements demonstrates that restraining rats will produce falsely elevated systolic blood pressure measurements when measured either directly or indirectly if the values obtained by the latter method are equivalent to direct readings. Since the rats used in this study had been frequently handled and accustomed to the restraining cages the lack of familiarity of the rats with the method cannot explain the rise in systolic blood pressure and would appear to be due to stress arising out of the confinement.

(g) All indirect methods of measuring rat systolic blood pressure employ an arterial occluding device, which is always an annular cuff in the case of the tail, and a means of detecting the pulse as the pressure in the occluding device falls below systolic and diastolic pressure and it is these parameters, (the width of the occluding cuff and the pulse detector), which are essential to obtain accurate systolic blood pressure readings, that are the subject of many reports and conflicting opinions.

(i) The accuracy of the systolic blood pressure measurement is partly determined by the length of the caudal artery which is occluded and this is described by the width of the occluding cuff (see review by Geddes, 1970). Although several studies have been undertaken to determine the width of cuff which provides the most accurate readings of systolic blood pressure (Maistrello & Matscher, 1969; review by Geddes, 1970; Bunag, 1973) no absolute value for this parameter has so far been obtained. The values for occluding cuff width have varied from 1 cm up to 7 cm though cuff widths as

narrow as 1 cm up to 1.4 cm will produce abnormally high systolic blood pressure values in small rats since although the diameter of the cuff remains constant the diameter of the tail increases with weight and hence the pressure of the cuff is exerted on a shorter tail segment in small rats, and a greater fraction of it is needed to overcome the resistance of the sleeve with the result of poorer compression of the artery. Occluding cuffs with widths from 1.8 to 2.8 cm are less susceptible to being influenced by variations in the length of the tail artery being compressed (Maistrello & Matscher, 1969). Occluding cuffs of large widths will produce abnormally low systolic blood pressure values due to the pressure gradient in existence along the rat's tail.

The detailed experiments of Shuler, Kupperman & Hamilton (1944), Maistrello & Matscher (1969) and Bunag (1973) proposed cuff widths of 1.6 cm, 1.8 cm and 1.5 cm respectively as providing the most accurate indirect systolic blood pressure readings from the caudal artery when employing the carotid artery as the reference artery. Sobin (1946) and Fregly (1963) used the femoral artery as the reference artery (as suggested by Geddes, 1970) and proposed cuff widths of 1 cm and 3.75 cm respectively as producing the most accurate indirect systolic blood pressure measurements from the caudal artery. However, Sobin (1946) only employed two cuff widths one of 1 cm and another of 4 cm and found the former the most satisfactory whilst Fregly (1963) obtained indirect systolic blood pressure measurements higher than the direct readings from the femoral artery which is contrary to the expected findings for such a large cuff width. Geddes (1970) stated that the cuff width necessary for the best agreement between direct (femoral artery or aorta) systolic blood pressure and indirect (caudal) systolic blood pressure lies between

1 cm and 3.75 cm. The cuff used in the present study was 1.8 cm which appeared initially to represent the ideal cuff width and since the comparison between the values obtained simultaneously for the indirect systolic blood pressures and the direct systolic blood pressures were so closely related ($r = 0.976$, regression coefficient 0.9354) it appears to indicate that such a cuff width is adequate to obtain accurate indirect systolic blood pressure measurements.

(ii) The systolic end point has been detected by various methods (see introductions by Frangipane & Aporti, 1969; Pfeffer, Pfeffer & Frohlich, 1971 and review by Geddes, 1970) and none has been conclusively demonstrated to produce any greater accuracy than any other. However, the microphonic method of Friedman & Freed (1949, 1952) is based upon the auscultatory method of Korotkoff (1905) which is the most consistently reproducible method (Geddes, 1970). In the present system the point at which blood is just forced into the caudal artery past the occluding cuff is detected by a strain gauge rather than a microphone as in the case of Friedman & Freed (1949, 1952) though these workers suggested a strain gauge as an alternative sensory device. Hence, the sensory system for the detection of systolic end point used in this case would appear to be as satisfactory as the microphonic manometer of Friedman & Freed (1949, 1952) and an improvement on the water plethysmograph of Byrom & Wilson (1938), its later electrical modifications (e.g. Skeggs & Leonards, 1946; Chittum, Hill & Grimson, 1953; Frangipane & Aporti, 1969) and optical methods (e.g. Diaz & Levy, 1939; Duncan, Hyman & Chambers, 1943; Alexander, 1957). The use of a write out also aids accuracy since the readings can be taken or verified at leisure.

The sensory device gave an easily identifiable systolic end point as can be seen from Figs.10 and 11. The three stages of arterial inflow designated by Pfeffer, Pfeffer & Frohlich (1971) can be clearly observed:

Stage I, when the occluding cuff pressure is above systolic pressure and the tracing from the pulse transducer is steady and noise free. Stage II, when the cuff pressure equals systolic pressure and low amplitude pulsations are observed and, Stage III, when the cuff pressure no longer impedes arterial flow and maximum amplitude pulsations occur.

The detection of the low amplitude pulsations was obtained with regularity and ease with the present system and thus allowed precise measurements of the systolic blood pressure to be made but the introduction of the maximum amplitude pulsations of Stage III, representing diastolic blood pressure was not easily detected and the system was considered unacceptable to obtain diastolic blood pressure readings with any degree of accuracy.

From the results and Fig. 12, it can be seen that the system used for obtaining indirect systolic blood pressure measurements in the present study was both accurate and reliable. The close relationship between the regression line for the simultaneously obtained readings of direct and indirect systolic blood pressures and the line of perfect agreement demonstrate the validity of the present tail-cuff system for the determination of the central (aortic) systolic blood pressure of rats.

Validity of other tail cuff methods has been repeatedly confirmed (see introduction by Bunag, Page & McCubbin, 1971) using anaesthetised

animals. However, only three studies have been reported using unanaesthetised animals; that of Bunag, McCubbin & Page (1971) which demonstrated that the tail cuff method for the indirect measurement of systolic blood pressure was only valid in anaesthetised rats and not in conscious animals and those of Pfeffer, Pfeffer & Frohlich (1971) and Bunag (1973) which demonstrated the validity of the tail cuff method in conscious animals. The cuff used by Bunag, McCubbin & Page (1971) was 2.5 cm in width and would be expected to produce abnormally low systolic blood pressure values due to the pressure gradient in existence along the rat's tail; this did in fact occur and the use of a smaller cuff, 1.25 cm in width, produced values closer to the directly measured aortic systolic blood pressure. Pfeffer, Pfeffer & Frohlich (1971) and Bunag (1973) obtained very close relationships between indirect and direct systolic blood pressure measurements using a 1.5 cm cuff which is much closer to the cuff size considered most desirable (see earlier) than those of Bunag, McCubbin & Page (1971). Pfeffer, Pfeffer & Frohlich (1971) considered that the primary explanation for acceptance of the validity of a tail-cuff technique was the type of sensory device employed but this cannot explain the difference in the findings of Pfeffer, Pfeffer & Frohlich (1971) and Bunag, McCubbin & Page (1971) since both used a modification of the method of Friedman & Freed (1949, 1952), using a pulse transducer (strain gauge), of exactly the same type, to detect the arterial pulsation, the same system as used in the present study. Pfeffer, Pfeffer & Frohlich (1971) considered that Bunag, McCubbin & Page (1971) failed to obtain any significant correlation between indirect and aortic systolic blood pressure measurements because of diminished stage III and hence stage II pulsations and this may be correct since even with the small cuff, which, as stated earlier (p. 97) should theoretically produce abnormally high indirect systolic blood

pressure readings Bunag, McCubbin & Page (1971) still obtained lower indirect systolic blood pressure readings than direct carotid artery systolic blood pressure measurements. Bunag (1973) considered his earlier study to be inaccurate due mainly to the width of the occluding cuff which he employed.

The accuracy of the present system would appear to be due to the easily identifiable systolic end point combined with an occluding cuff of size 1.8 cm though it would seem essential that each laboratory must validate each parameter in its indirect pressure measurements for the circumstances under which the method is used, as stated previously by several workers (Fregly, 1963; Maistrello & Matscher, 1969; Pfeffer, Pfeffer & Frohlich 1971).

Fig. 13 reveals that at low systolic blood pressure levels the agreement between the indirect restrained and direct unrestrained values is very good but this agreement gradually disappears at high systolic blood pressure levels. Thus the systolic blood pressure of restrained normotensive rats will bear a very close agreement with the systolic blood pressure of the rat in its near natural state, i.e. direct unrestrained readings at normal temperatures. The close agreement is due to the fact that systolic blood pressures measured indirectly in restrained rats are lower than the direct readings in similarly restrained rats (see Fig. 12), due to either the occluding cuff being slightly too large or the presence of inertia (time from blood just passing through the occluding cuff to trace being obtained on the recording system) in the recording system, or a combination of both, whilst the effect of restraining and warming caused a slight elevation of systolic blood pressure when compared with unrestrained normotensive rats maintained at room temperature (see Fig. 14) and

hence resulting in the blood pressures of restrained rats almost equalling those of unrestrained rats at normotensive levels. The lack of correlation observed at higher levels of systolic blood pressure is due to the effect of restraining and heating the animals, the former being the most important (see p.95).

Fig. 15 demonstrates that the tail cuff system used in the present study is capable of following changes in systolic blood pressure accurately.

Summary

1. Due to the problems associated with the measurement of blood pressure chronically in rats by direct arterial cannulation an indirect tail cuff method was developed.

2. The indirect tail cuff method was found to produce an easily identifiable end-point for the systolic blood pressure but not for the diastolic blood pressure.

3. The indirect tail cuff method was found to provide accurate values for the systolic blood pressure, over a wide range, when compared with systolic blood pressures obtained simultaneously by direct arterial cannulation.

4. At low levels of systolic blood pressure, but not at high levels, there was a very close agreement between the values obtained in restrained, heated rats by the indirect tail cuff method and those obtained in rats in a near normal state (i.e. unrestrained at room temperature) by direct arterial cannulation. The reasons for this are discussed.

5. Restraining and warming rats causes the systolic blood pressure to increase above that of rats in a near normal state in a manner directly proportional to the resting blood pressure level. The reasons for this are discussed.

6. The indirect tail cuff method is capable of following drug induced changes of the systolic blood pressure.

## CHAPTER 2

### The Development of Hypertension in the Rat

In order to investigate mechanisms involved in the production of hypertension in rats it was thought necessary to find a method capable of producing a consistent, stable elevation of blood pressure. Initially an attempt was made to produce hypertension acutely by ligation of the ureters or partial occlusion of the aorta, above the level of the renal arteries, in anaesthetised rats. Both methods produced rises in both systolic and diastolic blood pressures but it was found that a stable blood pressure could not be consistently obtained. The attempt to produce acute hypertension in anaesthetised rats was abandoned and methods capable of producing chronic hypertension in conscious animals examined.

There are many procedures for the production of chronic arterial hypertension in rats (see pages 10 to 22). It was decided to investigate the value of 3 methods involving interference with renal function and one method involving administration of DOCA, for producing hypertension in rats. Measurements of blood pressure and body weight were determined at intervals over a 2 month study period. After this time all animals were post-mortemed and the effect of hypertension on various organs was examined macroscopically and microscopically. The pilot study involved direct measurement of blood pressure and the subsequent, more extensive study employed the indirect tail cuff method for blood pressure measurement.

### Results

The pilot study employing implantation of a 25 mg DOCA pellet with unilateral nephrectomy and replacement of drinking water by 1% sodium chloride solution for 4 weeks, in a group of 10 rats, resulted in an

excessively large number of mortalities and a very high elevation of the systolic blood pressures (greater than 200 mm Hg) after 2 to 3 weeks.

Due to these findings 1% sodium chloride solution was substituted for drinking water for a period of 14 days only,in the subsequent investigation. The effects of this procedure on the systolic blood pressures of a group of 20 rats are illustrated in Fig. 16. A rapid rise in blood pressure began 6 days after the operation to induce hypertension which continued for a further 26 days to reach a maximum level of 200 mm Hg and thereafter remained at this level. All the surviving operated rats became hypertensive and 50% had systolic blood pressures greater than 200 mm Hg during the 2 month period of study. However, the mortality rate was very high (50% of the operated rats were dead after one month).

In the pilot study, where a 'figure-of-eight' ligature was made on the left kidney with simultaneous contralateral nephrectomy, a large number of deaths and severe elevation of blood pressure after 3 to 4 weeks occurred. In the subsequent study, involving the production of hypertension by the method of Grollman (1944), right nephrectomy was performed 7 days after the application of the 'figure-of-eight' ligature to the left kidney. The effect, on systolic blood pressure, of this procedure is illustrated in Fig. 17. The blood pressure began to increase 11 days after the introduction of the 'figure-of-eight' ligature and continued to gradually rise for a further 31 days to reach a maximum systolic blood pressure for the group of 195 mm Hg. The blood pressure of the group subsequently fell slightly but remained well above hypertensive levels. The percentage of rats which became hypertensive during the 2 month period of study was 75%, and 40% of these had blood pressures greater than 200 mm Hg.

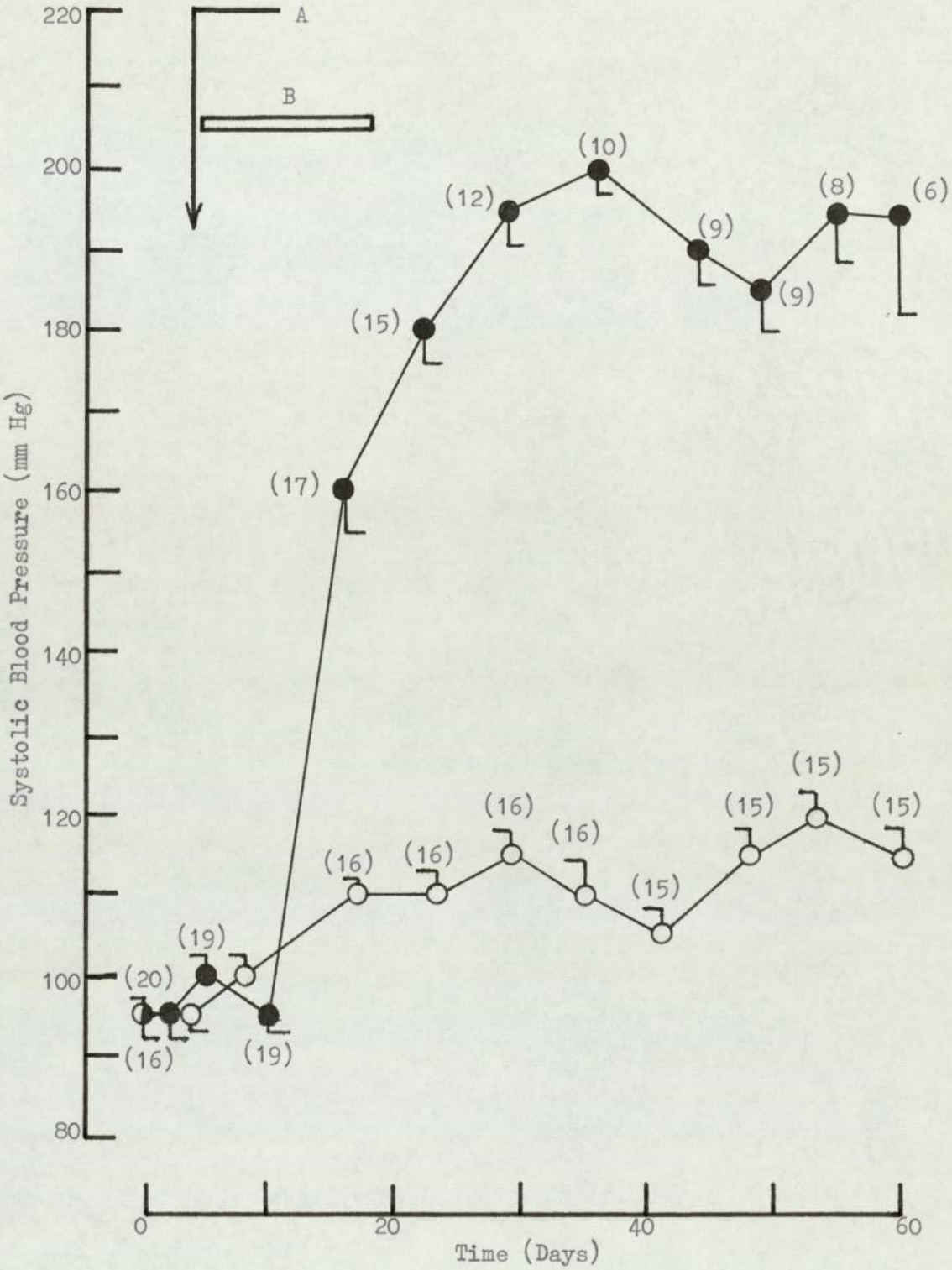


FIGURE 16: THE EFFECT OF A DOCA-NaCl REGIMEN ON THE SYSTOLIC BLOOD PRESSURES OF RATS.

The solid circles (●—●) represent the mean systolic blood pressure of rats in which a 25 mg DOCA pellet was implanted on day 4 (A) together with right nephrectomy. These rats were given 1% sodium chloride solution to drink for 14 days as indicated by the bar (B). The open circles (○—○) represent the mean systolic blood pressure of control rats. Each point represents the average value for the number of rats indicated in parenthesis. Standard errors of the mean are indicated for each point.

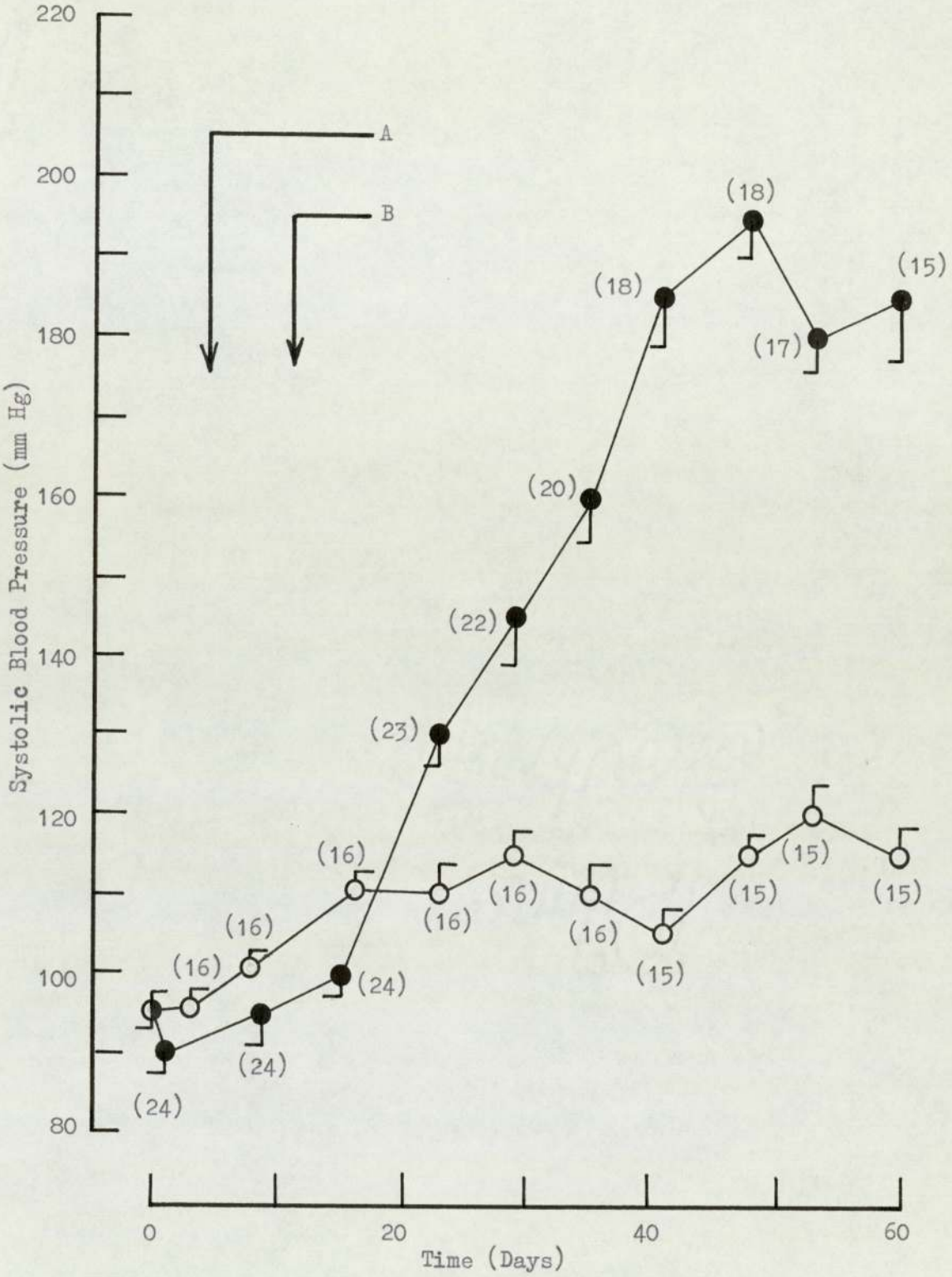


FIGURE 17: THE EFFECT OF THE METHOD OF 'GROLLMAN' ON THE SYSTOLIC BLOOD PRESSURES OF RATS.

The solid circles (●—●) represent the mean systolic blood pressure of rats in which a 'figure-of-eight' ligature was applied to the left kidney on day 4 (A) and contralateral nephrectomy performed 7 days later (B). The open circles (○—○) represent the mean systolic blood pressure of control rats. Each point represents the average value from the number of rats indicated in parenthesis. Standard errors of the mean are indicated for each point.

The mortality rate over the 2 month period of study was quite high at 40%.

In the pilot study, where the left kidney was wrapped in transparent cellulose, with simultaneous contralateral nephrectomy, only a small percentage of the operated rats developed hypertension. In the subsequent study the effect of the method of Page (1939) on systolic blood pressure showed that although the blood pressure was consistently elevated above the control group and 17% of the animals developed a blood pressure greater than 170 mm Hg the group as a whole did not develop hypertension. The mortality rate was quite low (25%) over the 2 month study period.

The pilot study where a branch of the renal artery to both kidneys was ligated revealed that no rat developed a systolic blood pressure greater than 170 mm Hg. In the rats in which an attempt to produce hypertension was made, by the method of Loomis (1946), the blood pressure began to rise one day after the operation and reached a maximum eight days later after which the blood pressure fell to a level not significantly different ($P > 0.05$) from that of the control group for the remaining period of study. The mortality rate was 30% and no animal developed a sustained blood pressure above 170 mm Hg.

The body weights of the four groups of operated rats compared with the control groups of unoperated normotensive rats are shown in Figs. 18 and 19. A significant difference ($P < 0.05$) in body weight was found between all the hypertensive groups and the controls immediately after the operation and this was sustained in two of the groups throughout the 2 month study period. However, the significant difference in body weight between the 'Loomis' and 'Page' operated rats and the unoperated control rats disappeared 4 and 31 days respectively after the operation to induce hypertension.

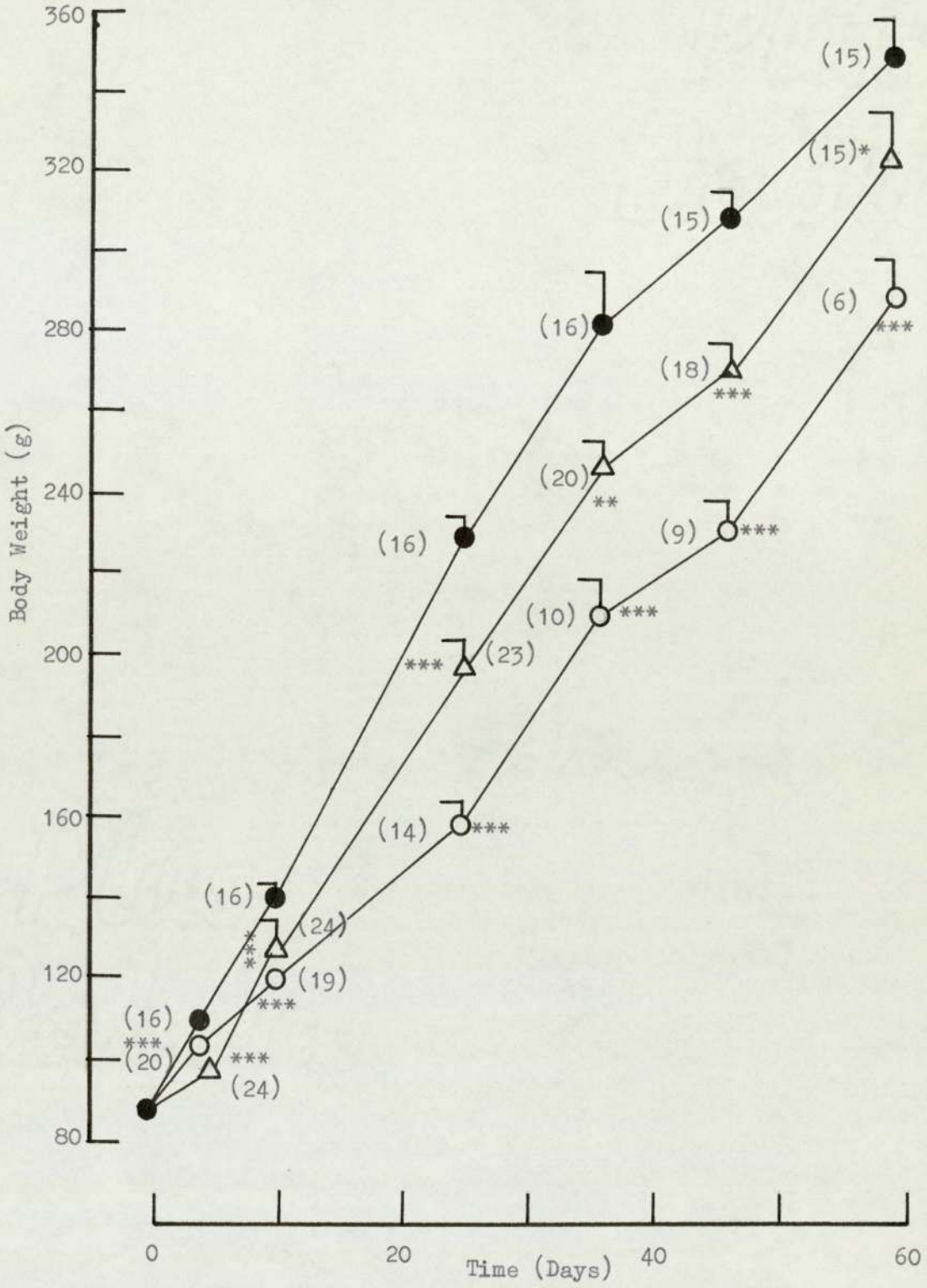


FIGURE 18: THE INCREASE IN BODY WEIGHT WITH TIME OF RATS IN WHICH HYPERTENSION WAS PRODUCED BY (1) A DOCA-NaCl REGIMEN AND (2) THE METHOD OF 'GROLLMAN' COMPARED WITH AN UNOPERATED CONTROL GROUP OF RATS.

The solid circles (●—●) represent the body weights of unoperated control rats. The open triangles (△—△) represent the body weights of the 'Grollman' rats and the open circles (○—○) the body weights of the rats which received a DOCA-NaCl regimen. Each point represents the average value for the number of rats indicated in parenthesis. Standard errors of the mean are indicated for each point. The difference in the body weights of the operated groups from the control group was evaluated using Student's 't' test and the level of significance is shown by the asterisks (*$P < 0.05$; **$P < 0.02$; ***$P < 0.001$).

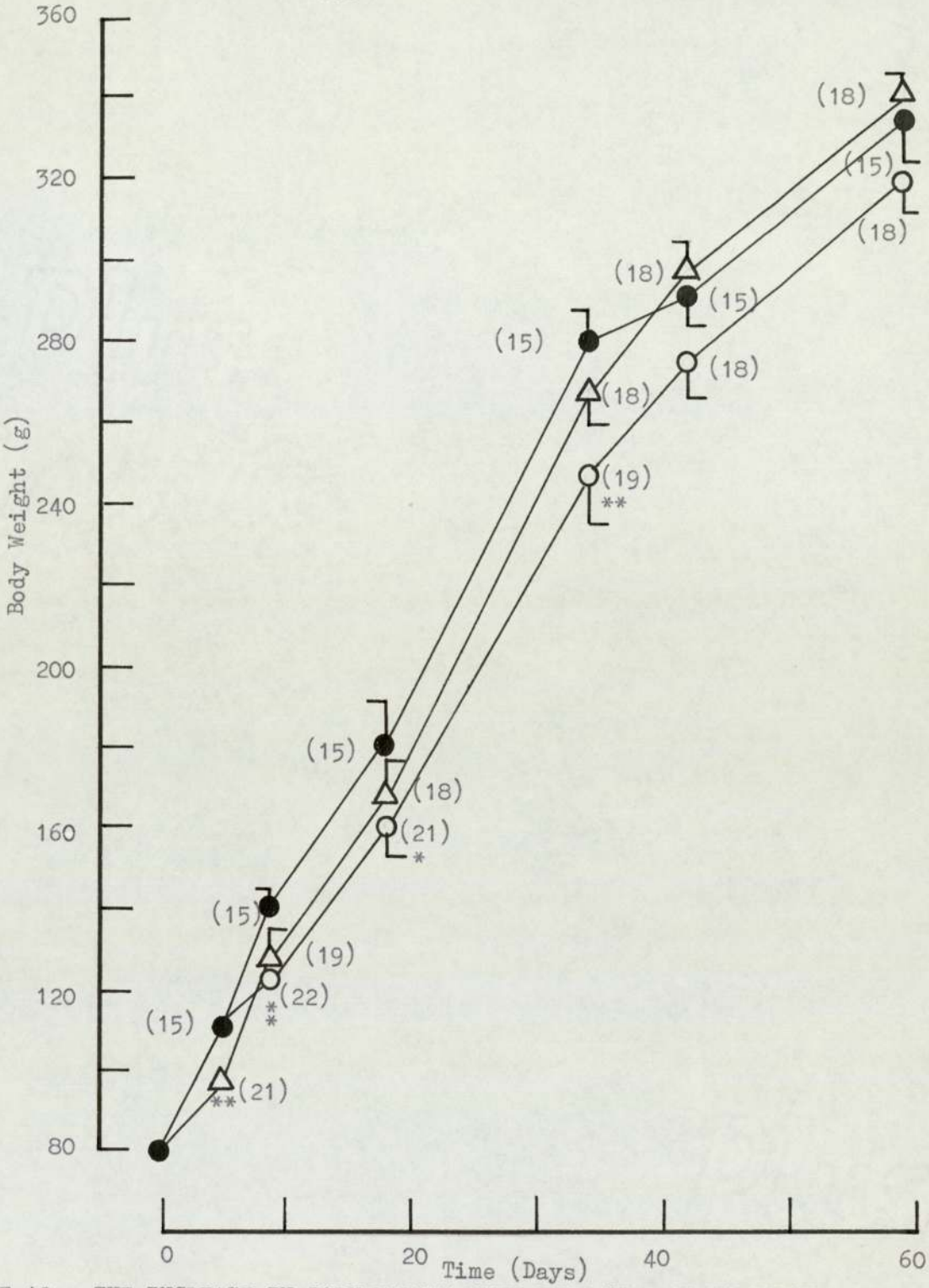


FIGURE 19: THE INCREASE IN BODY WEIGHT WITH TIME OF RATS IN WHICH HYPERTENSION WAS PRODUCED BY (1) THE METHOD OF 'LOOMIS' AND (2) THE METHOD OF 'PAGE' COMPARED WITH AN UNOPERATED CONTROL GROUP OF RATS.

The solid circles (●—●) represent the body weights of unoperated control rats. The open triangles (△—△) represent the body weights of the 'Loomis' rats and the open circles (○—○) the body weights of the 'Page' rats. Each point represents the average value for the number of rats indicated in parenthesis. Standard errors of the mean are indicated for each point. The difference in the body weights of the operated groups from the control group was evaluated using Student's 't' test and the level of significance is shown by the asterisks (*$P < 0.05$; **$P < 0.01$).

The weight of the single kidney of the DOCA-NaCl, 'Page' and 'Grollman' rat groups did not differ significantly from the combined weight of both kidneys in the unoperated controls. The weight of both kidneys in the 'Loomis' group of rats did not differ significantly from that of the unoperated control group. However, when the kidney weight of the DOCA-NaCl rats was expressed as a percentage of body weight a significant increase above that of the unoperated controls ($P < 0.001$) was observed. No significant increase was observed in the three other operated groups and there was a lack of correlation between the systolic blood pressure and the kidney weight expressed as a percentage of body weight when all the results from the four operated and two control groups were combined.

The heart weights of the DOCA-NaCl and 'Grollman' groups of rats were significantly greater than those of the unoperated control group ($P < 0.001$) when expressed as a percentage of body weight whilst the heart weights of the 'Loomis' and 'Page' groups of rats showed no significant difference from the unoperated controls. There was a highly significant correlation ($P < 0.001$) between the heart weight expressed as a percentage of body weight and the systolic blood pressure of the DOCA-NaCl, 'Grollman' and control rats ($r = 0.934$). The equation of systolic blood pressure (Y) to heart weight (X) was $Y = 1038.43x - 220.13$ (see Fig. 20).

The weights of the adrenal glands of the four operated groups showed no significant difference from the control group when expressed as absolute values. However, the 'Grollman' rats had a significantly greater adrenal gland weight ($P < 0.05$) when expressed as a percentage of body weight. The adrenal gland weight of DOCA-NaCl rats when

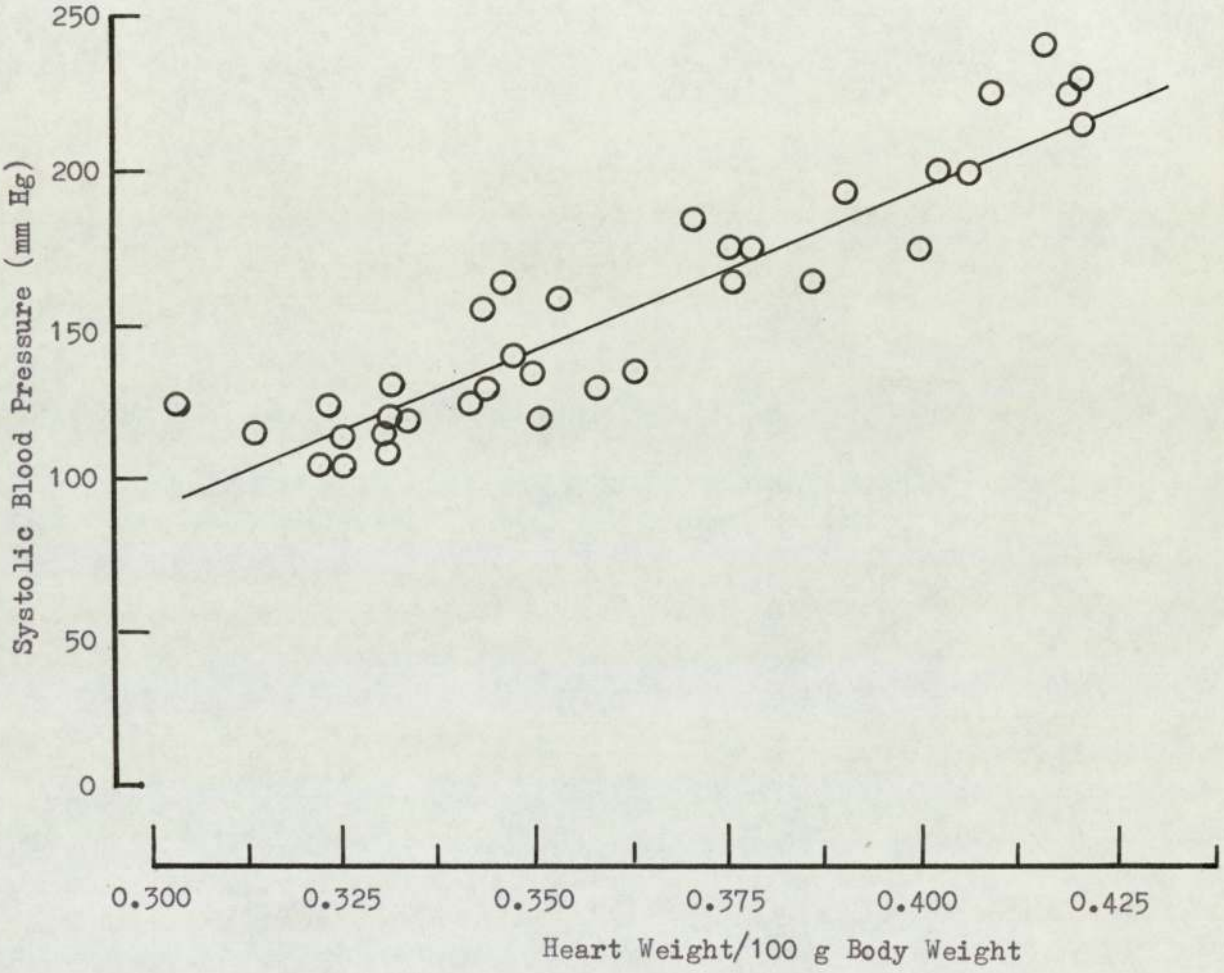


FIGURE 20: THE RELATIONSHIP BETWEEN SYSTOLIC BLOOD PRESSURE AND HEART WEIGHT/BODY WEIGHT RATIO IN RATS.

Each circle represents the heart weight/body weight ratio for the systolic blood pressure of a rat from either the control normotensive group, DOCA-NaCl group or 'Grollman' group. The equation of systolic blood pressure (Y) to heart weight/body weight ratio (X) was $Y = 1038.43x - 220.13$ with a correlation coefficient of $r = 0.934$.

expressed as a percentage of body weight, was increased but the difference was not statistically significant.

The general appearance of the 'Loomis' and 'Page' rats did not differ markedly from that of the unoperated controls except for the occasional appearance of blood in the urine of the 'Page' operated rats. The DOCA-NaCl and 'Grollman' rats displayed piloerection and were cold, the severity of these effects being greatest with the former group of animals. In general the DOCA-NaCl rats were emaciated, took up a 'hunched' posture and in extreme cases almost totally lacked their righting reflex. On handling these animals they were not only cold to the touch but became very 'jumpy' and appeared to be in pain. Blood was observed in the urine of both the DOCA-NaCl and 'Grollman' groups of rats.

The appearance at post-mortem of the DOCA-NaCl rats was of grossly enlarged hearts with engorged jugular veins. The kidneys were greatly hypertrophied and of a tougher texture than the kidneys from the control group of rats.

The occurrence of hypertrophied hearts and kidneys was also observed in the 'Grollman' group of rats with some engorgement of the jugular veins. The kidneys of the 'Grollman' rats were enveloped in a mass of connective tissue completely obscuring the 'figure-of-eight' ligature, although the indentation caused by the ligature could still be seen (see Fig. 21).

The rats of the 'Page' operated group occasionally displayed enlarged hearts, corresponding with elevated systolic blood pressures, and in all cases hypertrophied kidneys. However, the presence of a fibro-collaginous capsule around the kidney was observed in only a

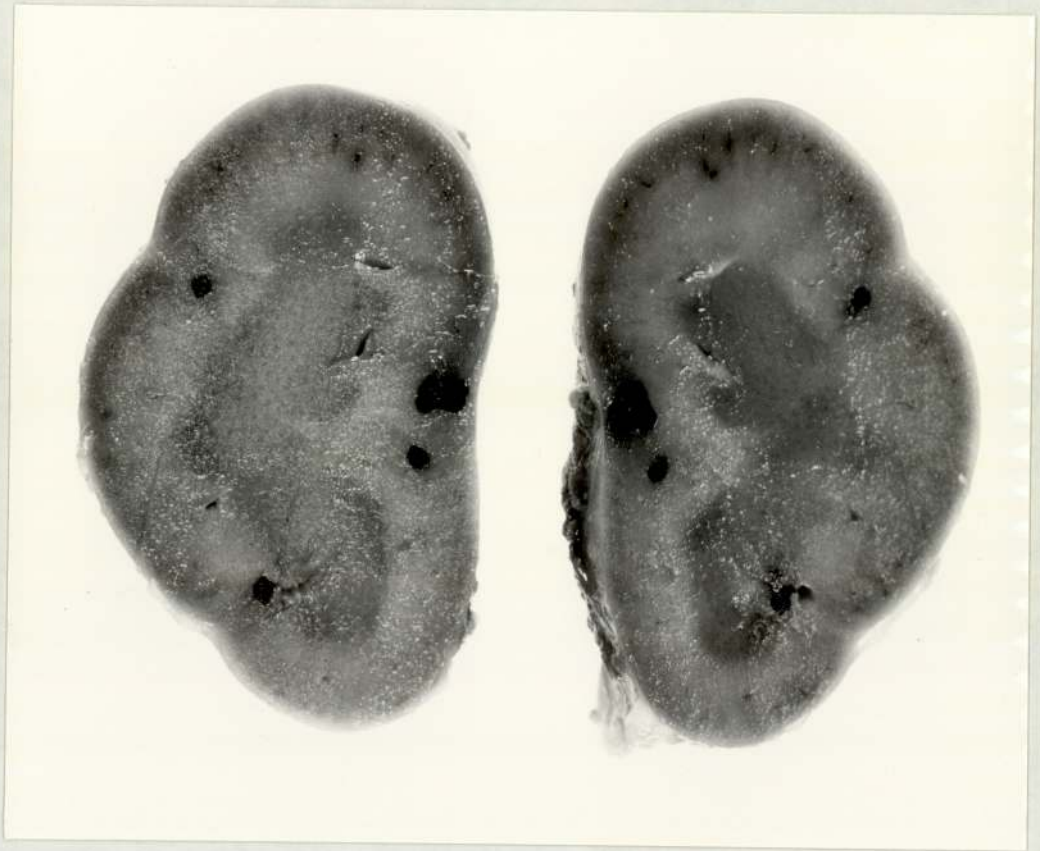

FIGURE 21: LONGITUDINAL SECTION OF THE LEFT KIDNEY FROM A RENAL HYPERTENSIVE RAT.

The cotton of the 'figure-of-eight' ligature can be seen to be completely surrounded by connective tissue although the indentations in the kidney surface caused by the ligature are still visible. (Magnification x 3).

few instances, in most cases the cellulose was only adhering to the kidney with the cotton visibly intact. The presence of a fibro-collaginous capsule around the kidney coincided in most cases, but not all, with an elevated systolic blood pressure.

The appearance of the 'Loomis' group of rats was in most instances the same as that of the control group of rats although in some cases connective tissue was observed around the ligatures on the branches of the renal artery. No obvious anastamoses of arterial renal vessels were observed in the animals studied.

The hearts of the three hypertensive groups in which hypertrophy occurred displayed similar characteristics upon histological examination. The hypertrophy of the heart occurred almost totally in the left ventricle and areas of muscle fibre degeneration were also observed in the left ventricle but in general there were no histopathological differences between the hypertrophied hearts of the hypertensive animals and the control, unoperated rats.

The kidneys of the three hypertensive groups displayed enlargement of both the cortex and medulla but mainly of the cortex. The cortex was also shown to contain distended tubules containing casts in some cases and fibrous tissue although there appeared to be no change in the glomeruli.

Fig. 22 shows the papillae from the kidney of a control unoperated rat and a hypertensive DOCA-NaCl rat. The presence of fibrous tissue at the base of the papillae of the kidney from the hypertensive rat can be seen as can the presence of proteinaceous casts in distended tubules. Although the upper part of the papillae is not so far affected degenerative changes would no doubt ensue due to the blockage of the tubules by the casts. Similar effects were observed in the two renal hypertensive groups in which hypertension had been induced

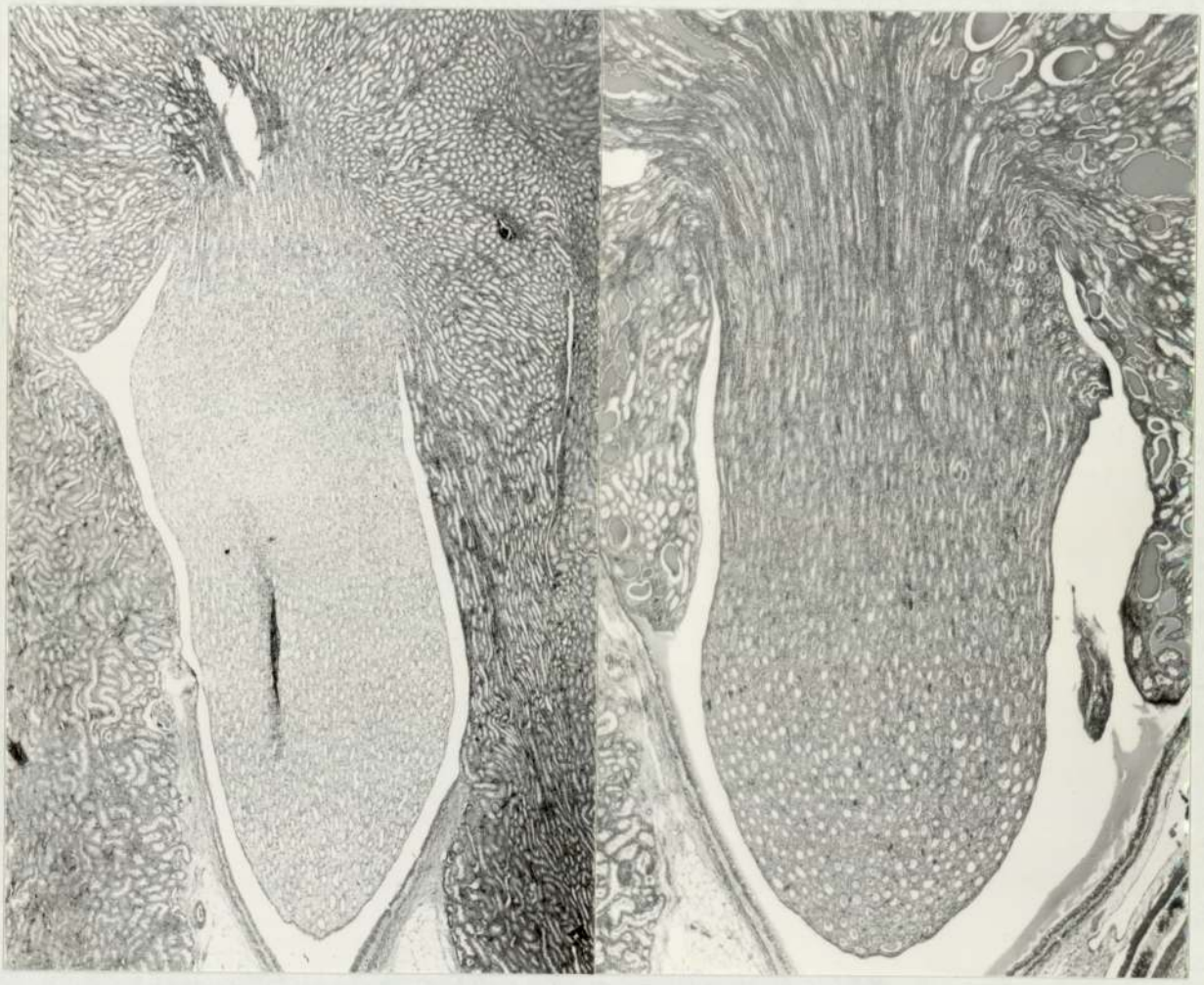

FIGURE 22: KIDNEY PAPILLAE FROM A DOCA-NaCl HYPERTENSIVE RAT AND A CONTROL NORMOTENSIVE RAT.

The presence of proteinaceous casts in distended renal tubules and fibrous tissue at the cortico-medullary junction of the papilla in the kidney from the DOCA-NaCl hypertensive rat is shown on the right. A normal kidney papilla is shown on the left. (Sections were stained with haemotoxylin and eosin. Magnification x 25).

although the presence of the proteinaceous casts was most marked in the DOCA-NaCl rats. The casts in the dilated tubules of a DOCA-NaCl hypertensive rat are shown in detail in Fig. 23.

The adrenal glands of hypertensive rats from the two renal hypertensive groups and the DOCA-NaCl group of rats occasionally showed hypertrophy of the cortex but not the medulla. There was also a lack of clarity of the division between the cortex and medulla as is illustrated in Fig. 24.

The aorta and mesenteric arteries of the operated groups showed no obvious changes from those of the control unoperated group.

Discussion

The development of DOCA-NaCl hypertension within 14 - 21 days was something of a surprise when considering the published literature on this type of hypertension (see Chapter 3). No reasons for this rapid production of hypertension were immediately obvious (see Chapter 3 for detailed examination).

The development of hypertension in the 'Grollman' operated rats was as expected (e.g. Mallov, 1959; Baum & Shropshire, 1967) and the necessity for performing contralateral nephrectomy some time after the application of the 'figure-of-eight' ligature was demonstrated (e.g. Grollman, 1944; Baum, 1968; Baum & Shropshire, 1969; Baum, Shropshire & Varner, 1972). Although Nickerson & Nomaguchi (1948) obtained only a 30% incidence of hypertension in rats this was probably due to the fact that contralateral nephrectomy was performed 4 to 6 weeks after the application of the 'figure-of-eight' ligature.

The failure to obtain a high incidence of hypertension in the 'Page' operated rats was not altogether unexpected since even though several workers have obtained hypertension employing transparent

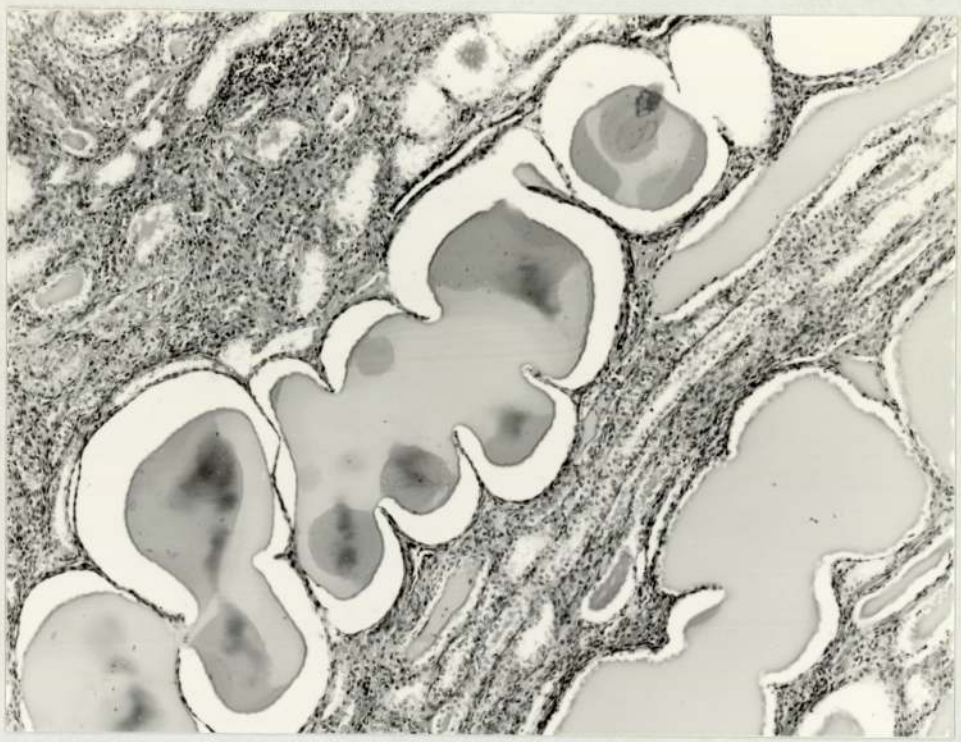

FIGURE 23: PROTEINACEOUS CASTS IN THE RENAL TUBULES FROM A DOCA-NaCl HYPERTENSIVE RAT.

(Section was stained with haemotoxylin and eosin. Mangification x 100).

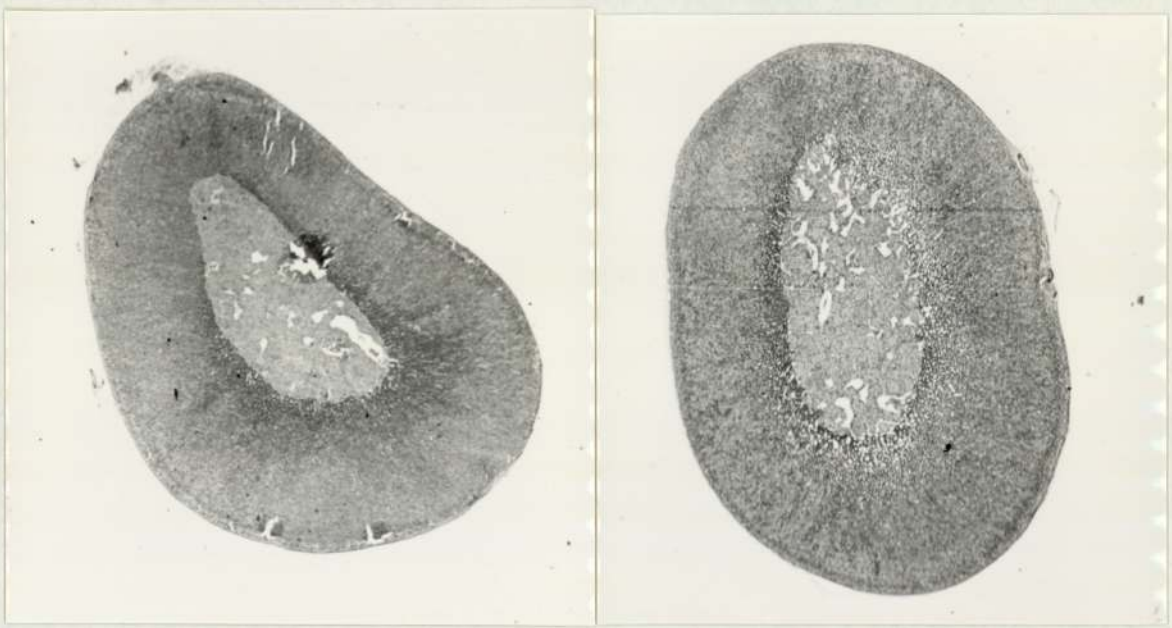

FIGURE 24: TRANSVERSE SECTIONS OF THE ADRENAL GLANDS FROM A DOCA-NaCl HYPERTENSIVE RAT AND A CONTROL NORMOTENSIVE RAT.

Slight hypertrophy of the adrenal cortex and early degenerative changes in the region of the cortico-medullary junction in the adrenal gland from the DOCA-NaCl hypertensive rat is shown on the right. A normal adrenal gland is shown on the left. (Sections were stained with haemotoxylin and eosin. Magnification x 20).

cellulose perinephritis (e.g. Friedman, Jarman & Klemperer, 1941; Schroeder, 1942; Gomez-Salazar, 1942; Chessar, Ferrario & McCubbin, 1972) the failure of transparent cellulose wrapping to produce hypertension in rats has been reported (Herman, Decherd & Erhard, 1941; Remington et al., 1944; Abrams & Sobin, 1947) and most recent reports on the production of hypertension by perinephritis employ latex encapsulation of both kidneys (e.g. Abrams & Sobin, 1947; Fregly, 1968; Fregly & Anton, 1969; Field, Janis & Triggle, 1972, 1973). The inability of the method of Page (1939) to produce a hypertensive state in a large percentage of rats in this study may possibly be associated with the strain of rat employed since in male Charles Rivers Sprague-Dawley rats a 70% success rate was obtained (Poyser, personal communication). Another possible reason for the lack of success of this method is the particular type of transparent cellulose used since a fibro-collaginous capsule developed in only a small percentage of the operated animals.

The method of 'Loomis' for the production of hypertension was rather disappointing with no rat in either study attaining a sustained hypertension. Koletsky (1955) found that ligation of major branches of both renal arteries resulted in an initial rapid rise of systolic blood pressure up to 7 days but not in a very high percentage of animals. Loomis (1946) also showed that ligation of a major branch of both renal arteries resulted in a rapid rise in systolic blood pressure which reached a maximum at one week and was followed by a fall to normotensive levels at 2 weeks as was observed in the present study. Loomis (1946), however, reported that the blood pressure once again began to rise and attained hypertensive levels at 5 weeks. It is

possible that a longer period of study may have revealed a secondary sustained rise of the blood pressure in the present study but the failure of many early workers to obtain a sustained hypertension and only a transitory rise (see review by Braun-Menendez et al., 1946) suggests that this may not be the case.

The fact that the number of mortalities is dependent upon the severity and period of hypertension has been frequently reported (see reviews by Hollander, 1973 and McKenny, 1974) and the present study is in agreement with these findings.

The body weights of hypertensive rats have been reported to be less than those of control normotensive animals during DOCA-NaCl (e.g. Masson, Corcoran & Page, 1949; Ueda, Nishimura & Yasuda, 1967; Beilin & Ziakus, 1972; Hansen, Abrams & Bohr, 1974) and renal hypertension, (e.g. Baum & Shropshire, 1967, 1969; Field, Janis & Triggle, 1973; Wolinsky, 1973) although no difference in body weight gain has been reported for DOCA-NaCl rats (e.g. Deming et al., 1958; Baum & Shropshire, 1967; De Champlain, Mueller & Axelrod, 1969; English et al., 1973; Holloway & Bohr, 1973) and renal hypertension (e.g. Adel et al., 1965; Phelan & Wong, 1968; Wolinsky, 1972; Holloway & Bohr, 1973; Lundgren, 1974). Although the growth rate is dependent upon the species as well as the severity of the hypertension (Molteni & Brownie, 1972) the duration of the hypertension appears to be the most important factor. In the short term, up to 14 weeks after the operation to induce hypertension, the growth rate of the hypertensive rats is slower than that of controls (e.g. Ueda et al., 1967; Baum & Shropshire, 1967, 1969; Beilin & Ziakus, 1972; Wolinsky, 1973; Hansen, Abrams & Bohr, 1974) for both DOCA-NaCl and renal hypertensive rats whilst the growth rate in the long term, over 15 weeks

after the operation to induce hypertension, the body weights of both DOCA-NaCl and renal hypertensive rats are not significantly different from those of control animals (e.g. Phelan & Wong, 1968; De Champlain, Mueller & Axelrod, 1969; Wolinsky, 1972; Holloway & Bohr, 1973). The significant difference in the rate of body weight gain between the DOCA-NaCl and 'Grollman' hypertensive rats and the lack of a significant difference in the other two renal operated groups from the normotensive control groups observed in the present study concurs with the results of the above authors.

The data on heart weight obtained in this study is in agreement with the published literature on the subject in that hypertrophy was observed in both renal (e.g. see review by Braun-Menendez et al., 1946; Phelan & Wong, 1968; Fregly & Anton, 1969; Field, Janis & Triggle, 1972, 1973; Wolinsky, 1973; Lundgren, 1974) and DOCA-NaCl (e.g. De Champlain, Krakoff & Axelrod, 1968; Hall & Hall, 1969; Beilin, Wade, Honour & Cole, 1970; Beilin & Ziakus, 1972) hypertensive animals. There was also a highly significant correlation between heart weight as a percentage of body weight and systolic blood pressure (Chanutin & Ferris, 1932; Chanutin & Barksdale, 1933; Benitz, Moraski & Cummings, 1961; Fregly, 1962) a fact which has led occasionally to the use of heart weight as a measure of the degree of elevation of blood pressure (Selye & Stone, 1946; Masson et al., 1949). The hypertrophy of the heart is due almost totally to the left ventricle which hypertrophies as a result of the increased work performed in overcoming the raised peripheral resistance (e.g. see reviews by Braun-Menendez et al., 1946; De Champlain, 1972 and Cohn, 1974).

The kidney hypertrophy observed in the present study is in agreement with the many reports of kidney enlargement following the

production of both DOCA-NaCl (e.g. Masson, Corcoran & Page, 1949; Ueda, Nishimura & Yasuda, 1967; Hall & Hall, 1969; English, et al., 1973) and renal (e.g. Wilson & Byrom, 1941; Ostrovsky, Papsin & Gornall, 1968; Field, Janis & Triggle, 1972, 1973) hypertensions. However, the exact cause of the hypertrophy has been the subject of much discussion. It is known that reduction of functioning renal mass, by for example unilateral nephrectomy, results in compensatory hypertrophy (e.g. Brunner, Desaulles, Regoli & Gross, 1962; Mason & Ewald, 1965; Royce, 1967). Royce (1967) has presented evidence for a rise in plasma concentrations of ribonuclease and other low molecular weight proteins following unilateral nephrectomy in rats and has suggested that an increased uptake of these proteins by the remaining kidney may be the stimulus for initiating compensatory hypertrophy. Ostrovsky, Papsin & Gornall (1968) have also suggested that the proteinuria of renal hypertension (see review by Braun-Menendez et al., 1946) may result in increased amounts of certain proteins in the glomular filtrate which may also stimulate renal hypertrophy even when both kidneys are present and these workers have suggested that hypertension may be a more powerful determinant of renal hypertrophy than the renoprival effect in both DOCA-NaCl and renal hypertensions.

However, Braun-Menendez (1952) and Goldstein (1960) do not consider hypertension as a mechanism of compensatory renal hypertrophy and the results of the present study appear to support this hypothesis as there was no significant difference between the kidney weight of the renal hypertensive rats, when expressed as absolute values or as a percentage of the body weight, irrespective of blood pressure. Also there was no significant correlation between kidney weight, expressed as a percentage of body weight, and blood pressure which is in agreement

with the work of Benitz, Moraski & Cummings (1961) but not that of Friedman & Friedman (1949), Grollman & Halpert (1949) and Fregly (1962) who observed direct relationships between kidney weight, expressed as a percentage of body weight, and systolic blood pressure. The DOCA-NaCl group of rats did exhibit a statistically significant increase in kidney weight, expressed as a percentage of body weight, when compared with either the normotensive controls or renal hypertensive rats indicating that a factor other than hypertension, possibly the much smaller body weight of the animals in this group, was responsible for the significantly greater relative weight of the kidneys in these rats.

The fact that unilateral nephrectomy is a powerful determinant of renal hypertrophy is also indicated by the fact that in the two renal hypertensive groups where this procedure was performed there was no significant difference between the kidney weights of normotensive and hypertensive members of the group although the weight of the single kidneys in these groups were highly significantly greater than those of the normotensive control and of the 'Loomis' groups of rats.

The absence of any significant increase in adrenal gland weight of DOCA-NaCl hypertensive rats is in agreement with the published literature. Decreased (e.g. Masson, Hazard, Corcoran & Page, 1950; Deane & Masson, 1951; Friedman et al., 1971; Friedman, Honore & Friedman, 1972) or unchanged (e.g. Masson, Corcoran & Page, 1949; Hall & Hall, 1969; De Champlain, Mueller & Axelrod, 1969 and review by De Champlain, 1972) adrenal gland weights have been frequently reported. There have been suggestions that the adrenal medulla is involved in the production of DOCA-NaCl hypertension (see review by De Champlain,

1972) and that the adrenal cortex is decreased in size (Friedman, Honore & Friedman, 1972) thus resulting in no overall change in the size or weight of the adrenal gland. However, the present study indicated that there was a possible increase in the adrenal cortex with no change in the adrenal medulla which is in general agreement with the bulk of the literature relating to adrenal gland weight in DOCA-NaCl hypertension, (see p. 124).

Increased adrenal gland weights in renal hypertensive rats has been observed many times (e.g. Rather, 1951; Deane & Masson, 1951; Fregly, 1962; Ostrovsky, Papsin & Gornall, 1968; Field, Janis & Triggle, 1972, 1973) and this is due mainly to an increase in the cortex particularly the zona glomerulosa (e.g. see reviews by Page & McCubbin, 1965, 1968) although an increase in the adrenal medulla has also been observed (Giampalmo, 1947; Rather, 1951; Liebefott, 1953). The increased size of the adrenal cortex in the one group of rats in which renal manipulation was consistently successful in producing hypertension in this study (i.e. the method of Grollman) is in agreement with the above reports and the lack of increase in the size of the adrenal medulla is also in agreement with most of the published literature.

The poor physical appearance of the rats receiving a DOCA-NaCl regimen in the present study, plus the other symptoms, such as polydipsia and polyuria, have been reported many times (e.g. Ueda, Nishimura & Yasuda, 1967; Panasevich, Belair, Trivedi & Yelnosky, 1969; English et al., 1973). The engorged jugular veins observed in the hypertensive rats, particularly the DOCA-NaCl group, represent a diminished ability of the heart to overcome the increased peripheral

resistance resulting in venous filling, a situation which has been frequently reported (e.g. see reviews by Braun-Menendez et al., 1946 and Cohn, 1974). The poor condition of the hypertensive rats appeared to be directly related to the elevation of the blood pressure but this was not true in the case of the occurrence and severity of lesions in the various organs examined.

Many authors have found no correlation between the degree of hypertension and the severity of arterial or organ lesions (see review by Giese, 1966) and several workers have suggested that the severity of the lesions is dependent upon the rate of rise of blood pressure (see review by Giese, 1966 and Lundgren, 1974). This present study is in general agreement with these findings in that the most severe cardiovascular and organ lesions occurred in the DOCA-NaCl rats, the blood pressures of which rose more rapidly than those of the 'Grollman' or 'Page' groups of rats.

The presence of only small degenerative changes in the hypertrophied left ventricles of hypertensive rats is not in general agreement with most reports since most workers have observed the occurrence of severe necrosis of muscle fibres of the left ventricle and the myocardium (see reviews by Giese, 1966 and Cohn, 1974). Similarly, the slight degenerative changes observed in the adrenal glands of hypertensive rats in this study are much less severe than most reports have observed (Deane & Masson, 1951 and review by Giese, 1966). The lack of degenerative changes in the hearts and adrenal glands of both renal and steroid hypertensive rats may be associated with the fact that the animals post-mortemed tended to be those in which the rate of blood pressure elevation was slowest. This was due to the

fact that rats in which the blood pressure rose rapidly were those which died or were in a poor physical condition. It is also possible that these changes were present in the hypertensive animals but were not observed since the histo-pathological examination involved only a small number of tissues.

The degenerative changes observed in the kidneys of hypertensive rats in this study are in general agreement with the published data of others but were not of such a severe nature (see reviews by Page & McCubbin, 1968 and Tobian, 1974). Reports of renal changes in DOCA-NaCl hypertension are very similar to those observed in the present study (e.g. Skelton, 1955; Heptinstall & Hill, 1967; Ljundqvist, 1969; Still & Dennison, 1969) except for the presence of proteinaceous casts.

The most surprising result of this study was the lack of obvious cardiovascular lesions or increase in wall-to-lumen ratio in the arteries of the heart or kidney or the mesenteric artery which have been reported in all types of hypertension (e.g. see reviews by Giese, 1966; Page & McCubbin, 1968; Hollander, 1973 and Page, 1974). It is very difficult to explain these findings although it is possible that the low biological sampling and the particular rats surviving at 2 months (that is, low rate of blood pressure elevation) may be relevant. However, Hansen, Abrams & Bohr (1974) failed to observe hypertrophy of femoral arteries from DOCA-NaCl rats and suggested that the short duration of treatment (5 weeks) was responsible. This may also explain the results observed in the present study.

Summary

1. Hypertension was produced in a high percentage of rats by either the method of Grollman or a DOCA-NaCl regimen. In the latter method the rapid production of a hypertensive state after only 14 days

substitution treatment with 1% sodium chloride solution was not in general agreement with the published literature.

2. Hypertension was only produced in a small percentage of rats using the method of Page and in none at all using the method of Loomis.

3. A significantly slower increase in body weight gain was observed in hypertensive rats when compared with normotensive operated or unoperated animals

4. Hypertrophy of the hearts of both steroid and renal hypertensive rats was observed and this hypertrophy was closely correlated with the systolic blood pressure.

5. Compensatory renal hypertrophy occurred in all groups in which unilateral nephrectomy was performed. The renal hypertrophy was not correlated with the systolic blood pressure.

6. Adrenal gland hypertrophy was only apparent in the 'Grollman' group of rats and the hypertrophy appeared to be due to an increase in the size of the adrenal cortex.

7. The observed arterial and organ lesions were not as severe as frequently reported in the literature.

## CHAPTER 3

### Rapid Development of DOCA-NaCl Hypertension in the Rat

The normal procedure for the production of DOCA-NaCl hypertension in rats involves either the injection or implantation of DOCA combined with sensitization of the animal to the action of DOCA by unilateral nephrectomy and/or replacement of drinking water by saline. However, quantitatively and sequentially the methods employed vary widely (e.g. Friedman, Friedman & Nakashima, 1951; Green, Saunders, Wahlgren & Craig, 1952; Sturtevant, 1956; Moore & Biliczki, 1968; Holloway & Bohr, 1973). Usually the reported methods for the production of DOCA-NaCl hypertension in rats have employed more severe experimental conditions than those used initially in these laboratories (see p.15). For example, the quantity of DOCA implanted or injected was normally greater than 25 mg and the period of sodium chloride substitution was usually longer than 4 weeks (e.g. Selye, Hall & Rowley, 1943; Mallov, 1959; Ostrovsky, Papsin & Gornall, 1968; Beilin & Ziakus, 1972; English et al., 1973; Hansen, Abrams & Bohr, 1974; Myers, Reid & Lewis, 1974). Despite these severe conditions the production of a hypertensive state normally required 5 to 8 weeks (e.g. Selye, Hall & Rowley, 1943; Deane & Masson, 1951; Friedman, Nakashima & Friedman, 1967; English et al, 1973; Myers, Reid & Lewis, 1975). Thus, the production of severe hypertension in a period of 14 - 21 days due to a 25 mg DOCA implant combined with unilateral nephrectomy and 1% sodium chloride substitution for 14 days as described in the previous chapter (see p.105) revealed a more rapid and severe production of DOCA-NaCl hypertension than had previously been reported at that time.

A study was undertaken in an attempt to establish the possible causes for this rapid development of DOCA-NaCl hypertension in rats.

Results

The two previous studies (see previous chapter p.104) involving the production of DOCA-NaCl hypertension employed sodium chloride made up in distilled water. A further batch of rats in which DOCA-NaCl hypertension was induced using sodium chloride solution made up in tap water showed no difference in the rate or severity of the subsequent hypertension from the previously reported studies.

Fig. 25 illustrates the development of DOCA-NaCl hypertension in two strains of Wistar rats. The rate of production of a hypertensive state was not significantly different in either group. Although the level of the systolic blood pressure in the 'Fisons' DOCA-NaCl rats was consistently higher than that of the 'Sc.P.F.' DOCA-NaCl rats no significant difference ($P > 0.05$) was observed. Throughout the period of study there was no significant difference between the two groups of unoperated normotensive control rats. A highly significant difference ($P < 0.001$) between the systolic blood pressures of the hypertensive and normotensive groups existed from day 19 of the study until its completion.

Fig. 26 illustrates the body weight gain of the four groups involved in the study. A significant difference in body weight was present in the two groups of DOCA-NaCl rats and their respective normotensive controls from immediately after the operation to induce hypertension until the completion of the study. In only one instance was there a significant difference in the body weights of the two hypertensive groups and only in two cases was there a significant difference between the normotensive control groups.

Fig. 27 illustrates the development of DOCA-NaCl hypertension in 'Fisons' rats of two different age groups. The 'young' rats had

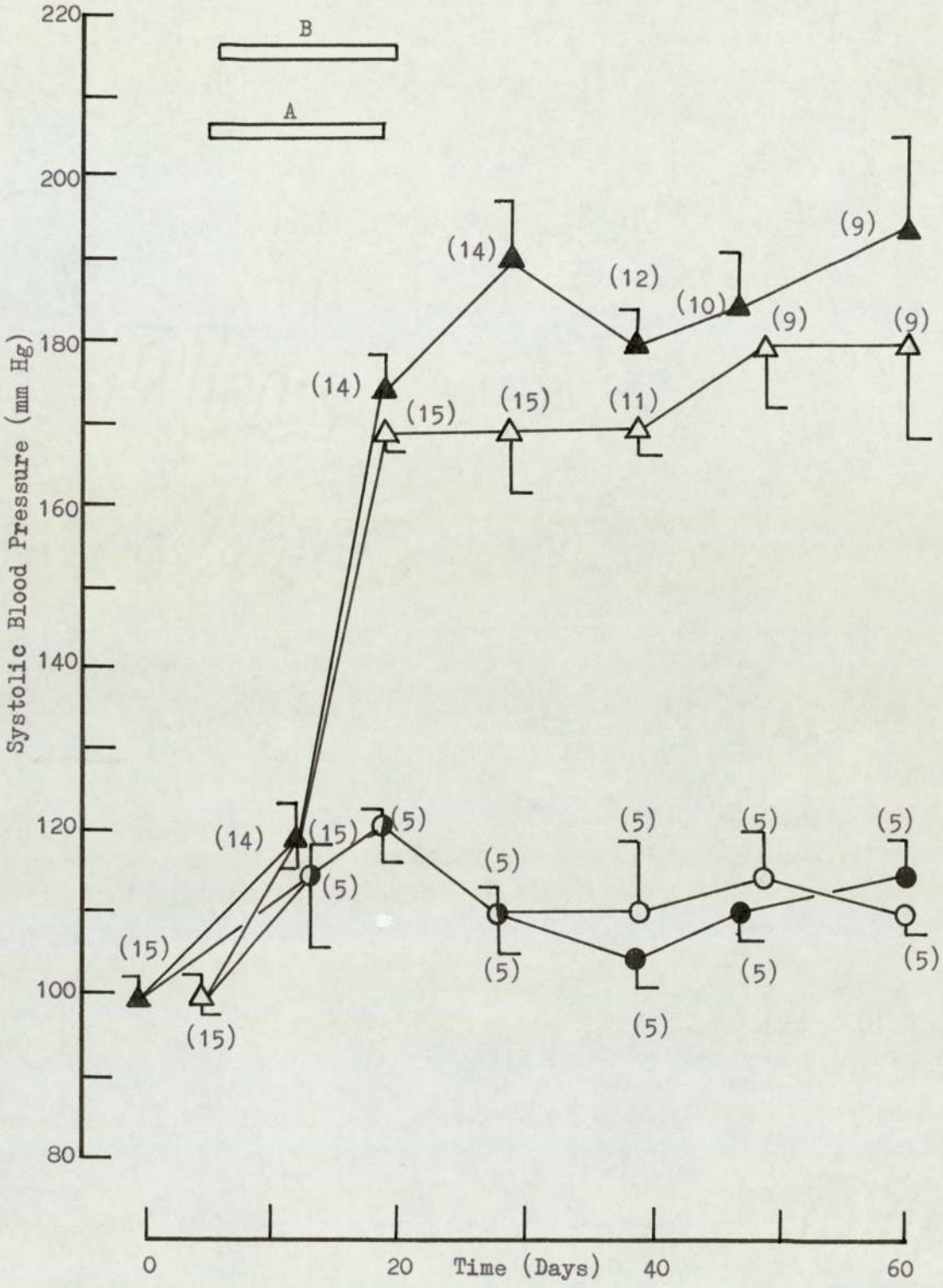


FIGURE 25: THE EFFECT OF A DOCA-NaCl REGIMEN ON THE SYSTOLIC BLOOD PRESSURE OF TWO STRAINS OF WISTAR RATS.

The solid triangles (▲—▲) shows the effect on the systolic blood pressure of a 25 mg implant of DOCA and right nephrectomy performed on day 5 in 'Fisons' Wistar rats. The line with open triangles (△—△) shows the effect on the systolic blood pressure of Wistar rats obtained from Scientific Products Farms after the same procedure was performed on day 6. Both groups of rats received 1% sodium chloride solution for 14 days as indicated by the bars (A=Fisons Wistar & B=Sc.P.F. Wistar). The line with closed circles (●—●) shows the systolic blood pressure of unoperated control 'Fisons' Wistar rats and the open circles (○—○) the systolic blood pressure of the control 'Sc.P.F.' Wistar rats. Each point is the mean of the number of animals indicated in parenthesis and the standard errors are shown by the vertical bars.

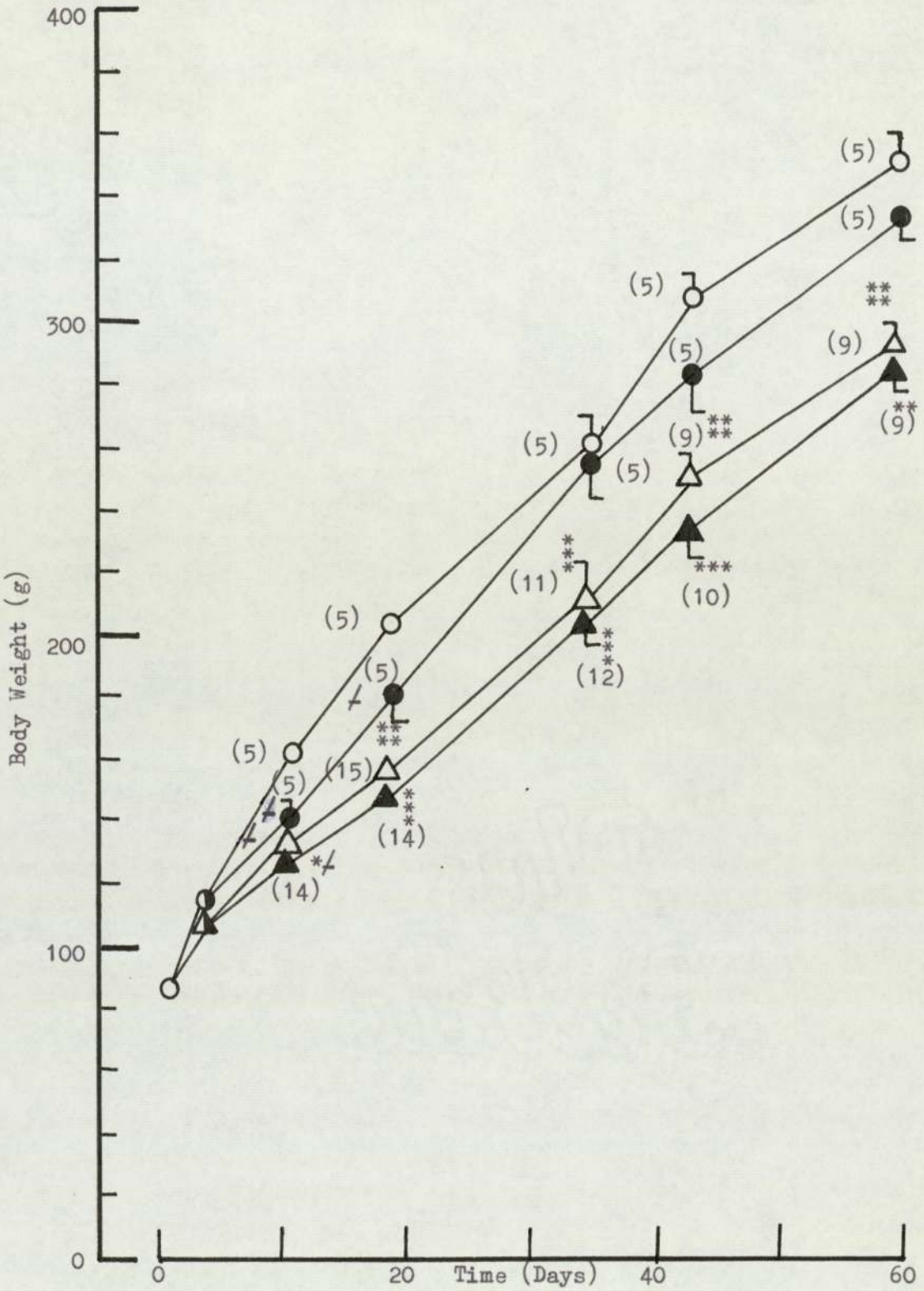


FIGURE 26: THE INCREASE IN BODY WEIGHT WITH TIME OF TWO DIFFERENT STRAINS OF RATS IN WHICH HYPERTENSION WAS INDUCED BY A DOCA-NaCl REGIMEN.

The circles represent the body weights of unoperated control rats (○—○ = Sc.P.F. ●—● = Fisons). The triangles represent the body weights of rats subjected to a DOCA-NaCl regimen (△—△ = Sc.P.F. ▲—▲ = Fisons). Each point represents the average value for the number of rats indicated in parenthesis. Standard errors of the mean are indicated for each point. The differences in the body weights of the operated groups from their respective control groups was evaluated using the Student's 't' test and the level of significance is shown by the asterisks (*P < 0.05; **P < 0.02; ***P < 0.01; ****P < 0.001). The differences in the body weights of the hypertensive groups or control groups were similarly evaluated and the level of significance is shown by / (/P < 0.05; //P < 0.02).

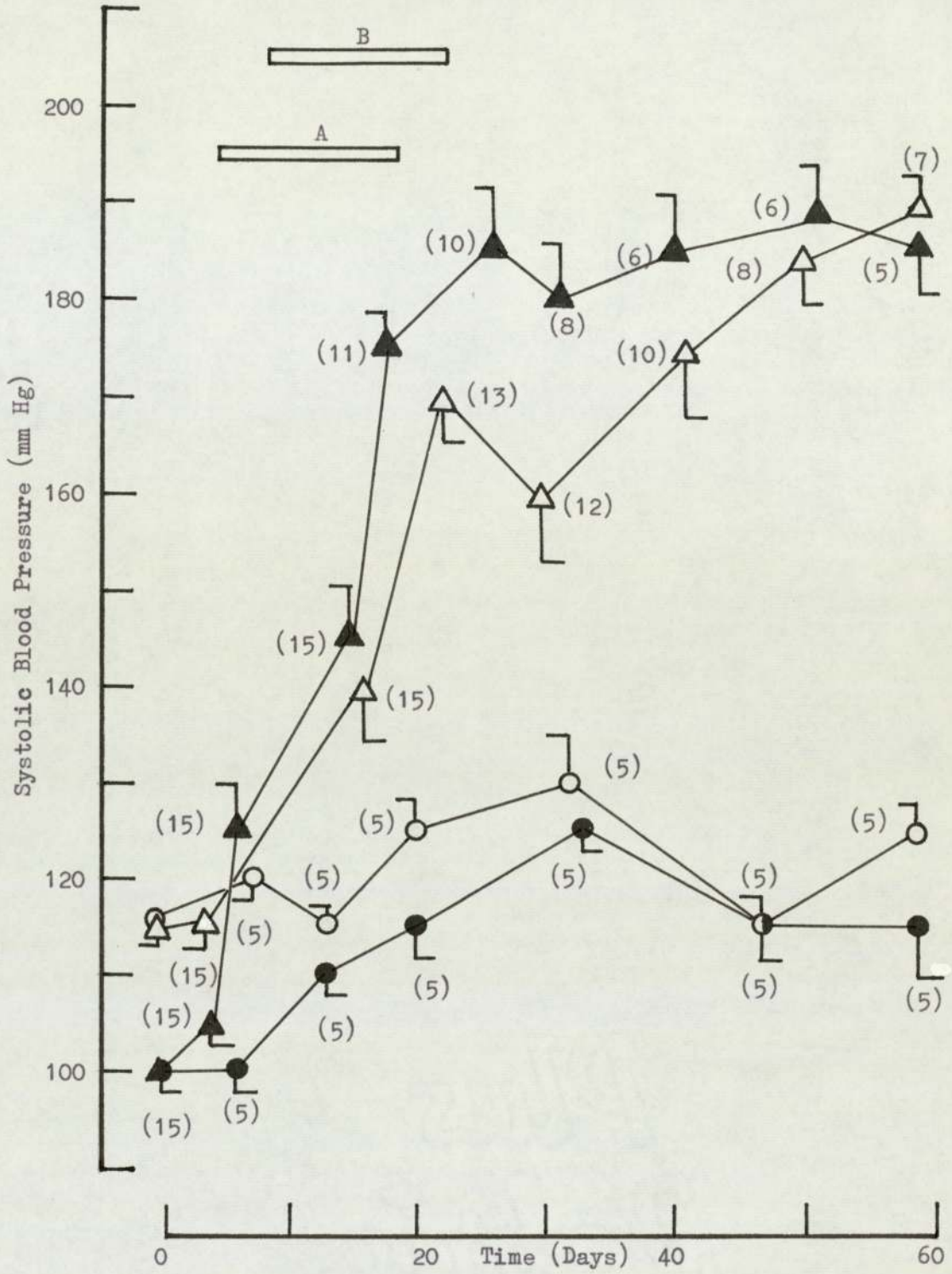


FIGURE 27: THE EFFECT OF AGE ON THE DEVELOPMENT OF HYPERTENSION INDUCED BY A DOCA-NaCl REGIMEN.

The solid triangles (▲—▲) show the systolic blood pressures of a group of rats which were 4 weeks old when they were subjected to the implantation of a DOCA pellet, 25mg, together with right nephrectomy. The open triangles (△—△) indicate the blood pressures of a group of rats which were 9 weeks old when subjected to the same procedure. Both groups of rats received 1% sodium chloride solution for 14 days as indicated by the bars (A=4 week old rats, B=9 week old rats). The circles indicate groups of identical rats which were not subjected to operative procedures (●—● 4 weeks old, ○—○ 9 weeks old). Each point represents the mean systolic blood pressure of the number of rats indicated in parenthesis. Differences between the operated groups of rats were evaluated using the Student's 't' test and no significant differences were observed throughout the study ($P > 0.05$).

body weights of 60 - 70 g and were about 5 weeks old whilst the 'old' rats had body weights of approximately 200 g and were about 9 weeks old.

Taking into account the four days difference in the date of the operation to induce hypertension there was no difference in the rate of production of a hypertensive state and although the systolic blood pressure of the 'young' group of DOCA-NaCl rats was consistently greater than that of the 'old' group of DOCA-NaCl rats until the last day of the study no significant difference was observed between the systolic blood pressures of the two groups except on the first day of the study ($P < 0.001$). No difference in the systolic blood pressures of the two normotensive control groups from day 13 of the study was observed. A highly significant difference in the systolic blood pressures of the 'young' DOCA-NaCl group of rats and the 'old' DOCA-NaCl group of rats and their respective normotensive control groups existed from day 6 and day 16 of the study respectively and remained so until the completion of the study.

Fig. 28 illustrates the changes in body weight of the four groups of rats at various times throughout the study. A highly significant difference in the body weights between the two groups of DOCA-NaCl rats existed throughout the study and also between the two groups of normotensive control rats. A significant difference between the two groups of DOCA-NaCl rats and their respective normotensive control groups was observed from after the time of the operation to induce hypertension until the completion of the study.

Fig. 29 illustrates the development of DOCA-NaCl hypertension in two groups of rats of different strain and age. The 'young' group of rats consisted of 'Fisons' Wistars of 70 - 80 g body weight whilst the 'old' group of rats were 'Sc.P.F.' Wistars of 200 g body weight.

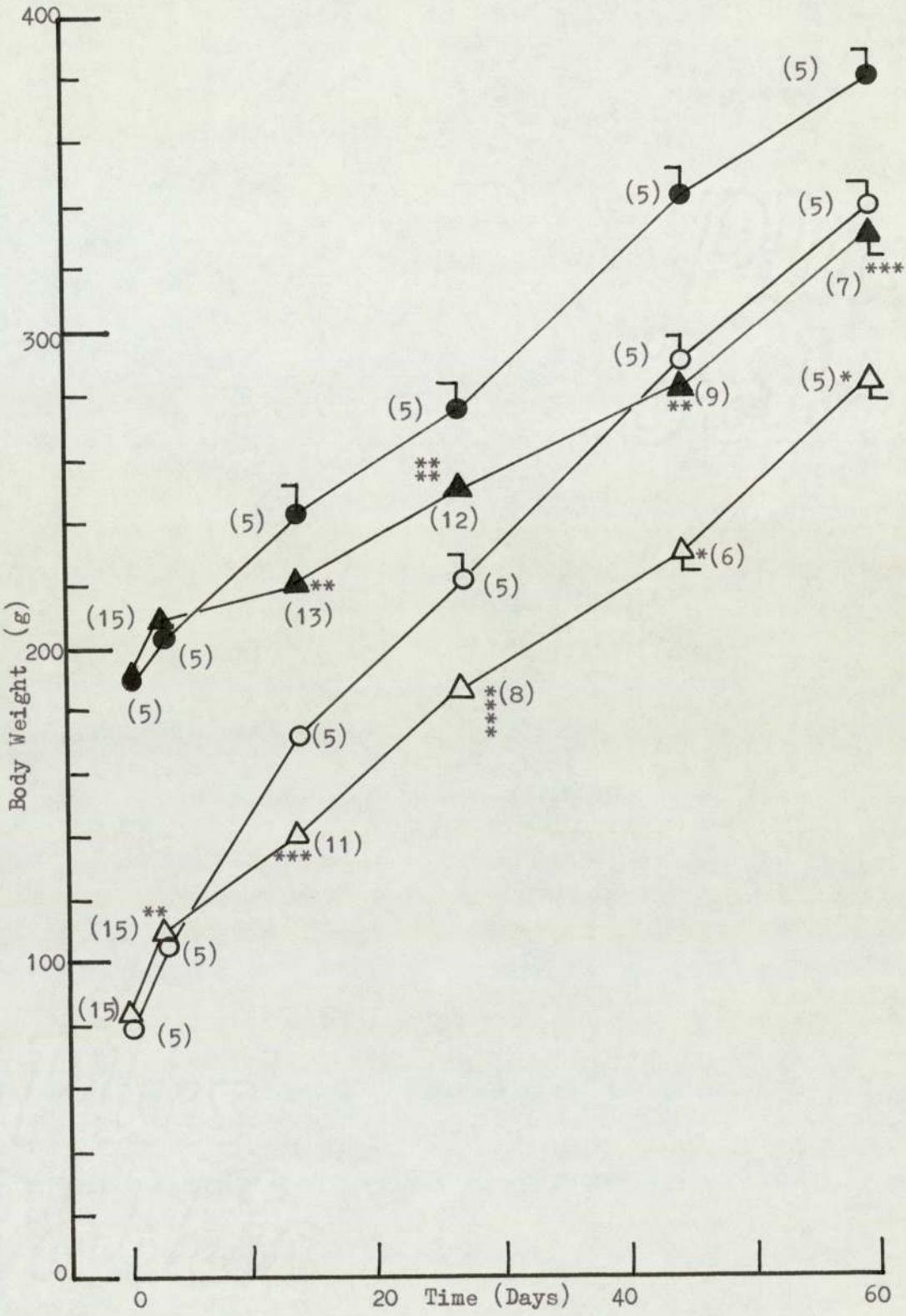


FIGURE 28: THE INCREASE IN BODY WEIGHT WITH TIME OF TWO GROUPS OF RATS OF DIFFERENT AGES IN WHICH HYPERTENSION WAS INDUCED BY A DOCA-NaCl REGIMEN.

The circles represent the body weights of unoperated control rats (●—● 9 weeks old; ○—○ 4 weeks old). The triangles represent the body weights of the rats subjected to a DOCA-NaCl regimen (▲—▲ 9 weeks old; △—△ 4 weeks old). Each point represents the average value for the number of rats indicated in parenthesis. Standard errors of the mean are indicated for each point. The differences in the body weights of the operated groups from their respective control groups was evaluated using the Student's 't' test and the level of significance is shown by the asterisks (*$P < 0.05$; **$P < 0.02$; ***$P < 0.01$; ****$P < 0.001$).

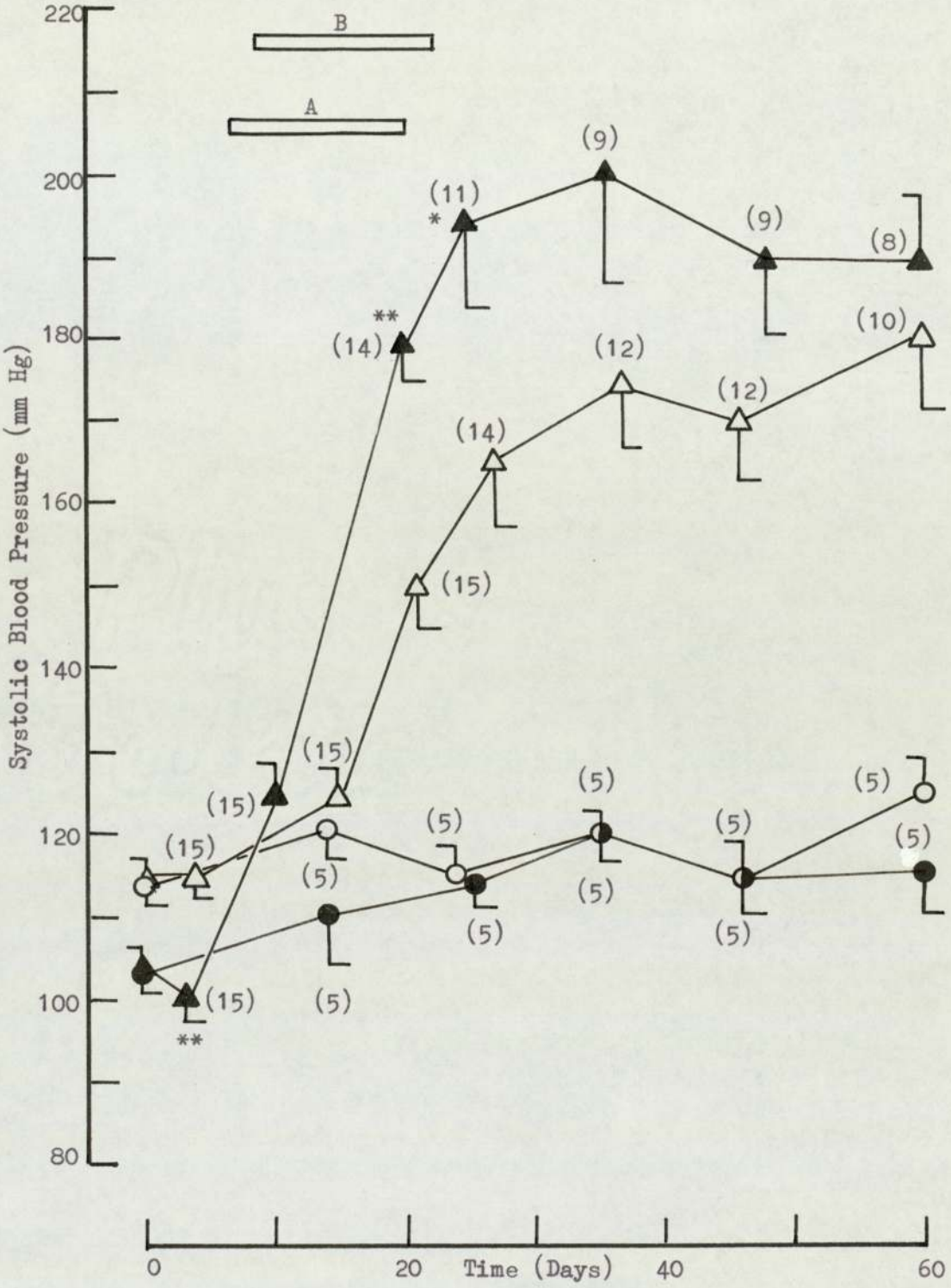


FIGURE 29: THE EFFECT OF A DOCA-NaCl REGIMEN ON THE SYSTOLIC BLOOD PRESSURES OF RATS OF DIFFERENT AGE AND STRAIN.

A DOCA pellet, 25mg, was implanted and right nephrectomy performed in Fisons rats which were 4 weeks old (▲—▲) and in Sc.P.F. rats which were 9 weeks old (△—△) and the effect on systolic blood pressure observed. Both groups of rats were given 1% sodium chloride solution to drink for 14 days as indicated by the bars (A=Fisons rats; B=Sc.P.F. rats). The circular symbols represent untreated but otherwise identical rats (●—● Fisons rats; ○—○ Sc.P.F. rats). Each point represents the mean systolic blood pressure of the number of rats indicated in parenthesis. Differences between the operated groups of rats were evaluated using the Student's 't' test and the level of significance indicated by asterisks (*$P < 0.02$; **$P < 0.001$).

Taking into account the two days difference in time of the operation to induce hypertension the 'young Fisons' group of rats attained a measured hypertensive level of systolic blood pressure fifteen days more quickly than the 'old Sc.P.F.' group of rats.

Before the operation to induce hypertension was performed the systolic blood pressure of the 'old Sc.P.F.' group of rats was highly significantly greater ($P < 0.001$) than that of the 'young Fisons' group of rats. However, the systolic blood pressure of the 'young Fisons' group of DOCA-NaCl rats was highly significantly greater ($P < 0.001$) than that of the 'old Sc.P.F.' group of DOCA-NaCl rats on day 20 - 22 of the study and significantly greater ($P < 0.02$) on day 25 - 27 of the study. No significant difference in the systolic blood pressure of these two groups was observed after this time throughout the remainder of the period of study.

A highly significant difference ($P < 0.001$) in the systolic blood pressures of the two groups of DOCA-NaCl rats and their respective normotensive control groups existed fourteen days after the operation to induce hypertension until the completion of the study.

Fig. 30 illustrates the body weights of the four groups of rats at various stages of the study. A significant difference in body weight in both groups of DOCA-NaCl rats from their respective normotensive control groups was evident from after the operation to induce hypertension until the completion of the study, except in the case of the 'old Sc.P.F.' DOCA-NaCl group of rats on day 10 of the study.

The body weights of the normotensive control groups were highly significantly different ($P < 0.001$) throughout the whole period of study as were the body weights of the DOCA-NaCl groups.

In all animals receiving a DOCA-NaCl regimen a marked polydipsia

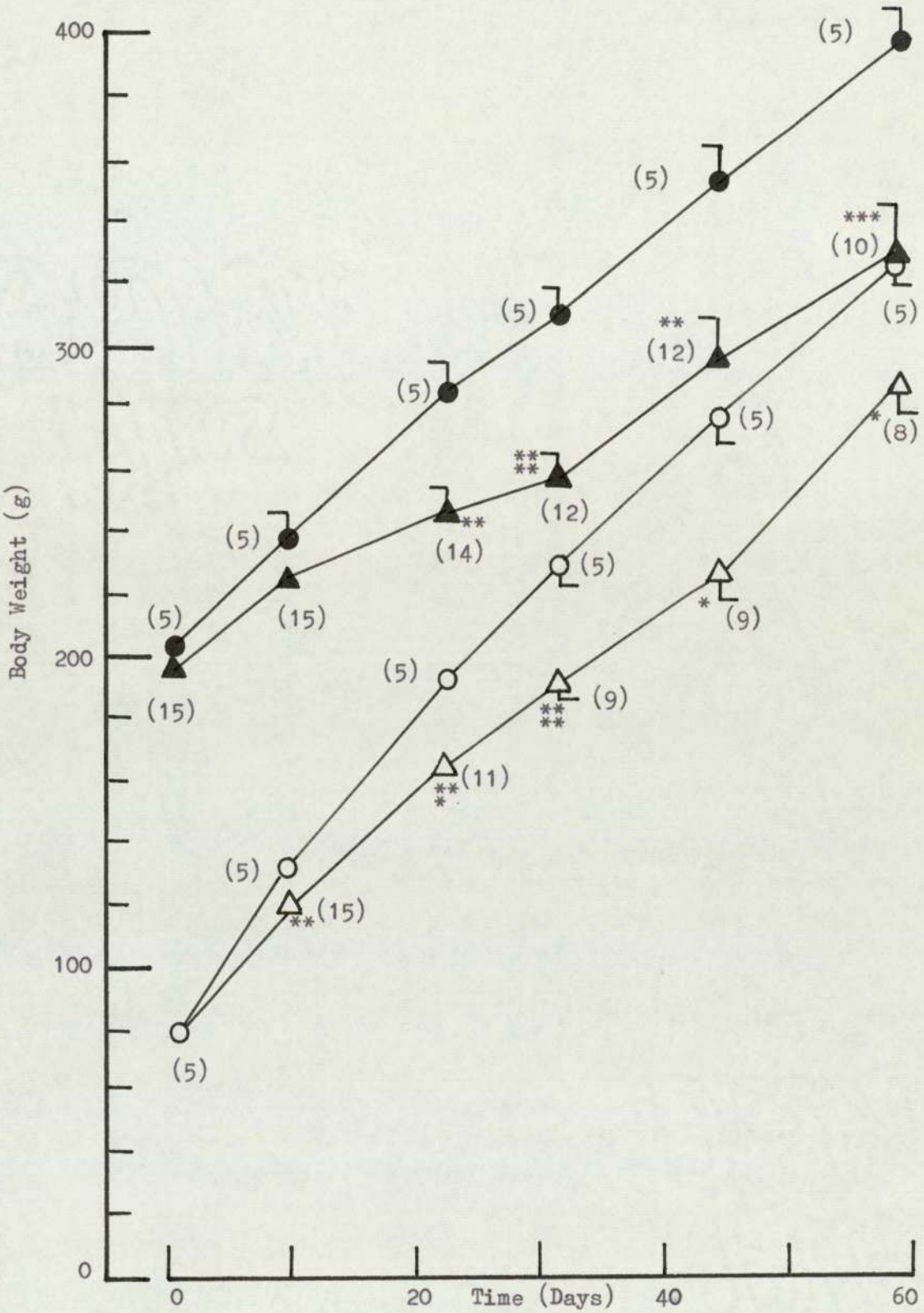


FIGURE 30: THE INCREASE IN BODY WEIGHT WITH TIME OF RATS OF DIFFERENT AGE AND STRAIN IN WHICH HYPERTENSION WAS INDUCED BY A DOCA-NaCl REGIMEN.

The circles represent the body weight of unoperated control rats (●—● = 9 week Sc.P.F. ○—○ = 4 week old Fisons). The triangles represent the body weights of rats which were subjected to a DOCA-NaCl regimen (▲—▲ = 9 week Sc.P.F. △—△ = 4 week old Fisons). Each point represents the average value for the number of rats indicated in parenthesis. The differences in the body weights of the operated groups from their respective control groups was evaluated using the Student's 't' test and the level of significance is shown by the asterisks. (*P < 0.05; **P < 0.02; ***P < 0.01; ****P < 0.001).

and polyuria was observed although measurement of sodium chloride intake was not undertaken.

Discussion

The occurrence of certain strains of rats which possess a high susceptibility to the development of a hypertensive state has been reported (Smirk & Hall, 1958; Okamoto & Aoki, 1963). Dahl, Heine & Tassinari (1962) have evolved two strains of rats which differed markedly in their susceptibility to salt-induced hypertension and were designated as 'sensitive' (S) and 'resistant' (R) depending upon their blood pressure response to dietary salt. The 'S' strain was also found to be very susceptible to DOCA-NaCl hypertension whilst the 'R' strain was resistant to the DOCA-NaCl regimen (Dahl, Heine & Tassinari, 1963). Recently Ben-Ishay, Saliternik & Welner (1972) have developed a colony of rats highly susceptible to the induction of DOCA-NaCl hypertension termed the 'H' strain and also an 'N' strain which remain normotensive despite the DOCA-NaCl regimen. Both groups of workers have suggested that the rapid development of a severe hypertension or lack of production of a hypertensive state may be due to genetic factors, possibly a difference in adrenal biosynthetic pathways (Rapp & Dahl, 1971 a & b; Dahl et al., 1972). It was considered that the 'Fisons' rats (Rattus norvegicus, Wistar strain F) might be similarly highly susceptible to the induction of DOCA-NaCl hypertension due to genetic factors. It is known (King, personal communication) that the Fisons rats, bred since 1922, have a syndrome of renal nephritis which was considered a possible cause for the susceptibility of these rats to the induction of DOCA-NaCl hypertension. However, the study comparing the production of hypertension in the above rats with another strain of Wistar rats supplied by

Scientific Products Farm (Rattus norvegicus, Wistar strain ASH/W) showed no significant difference in either the rate or severity of the induced hypertension. This suggests that a genetic factor was not responsible for the rapid production of hypertension in this study. Since, although only one other strain of rat was employed as a comparison, it would appear unlikely that both strains would have similar very high susceptibilities to DOCA-NaCl hypertension when other groups of workers (Dahl et al., 1962, 1963; Ben-Ishay et al., 1972) found it necessary to selectively inbreed their rats on a basis of susceptibility to salt or DOCA-NaCl hypertension for several years.

Young rats have been reported to be more susceptible to the development of a hypertensive state by experimental methods than more mature rats (Dahl et al., 1963). Many studies (e.g. Masson, Page & Corcoran, 1950; Deane & Masson, 1951; Ueda, Nishimura & Yasuda, 1967; Still & Dennison, 1969; Ayitey-Smith & Varma, 1970; Beilin et al., 1970; Beilin & Ziakus, 1972; Holloway & Bohr, 1973; Hansen, Abrams & Bohr, 1974) have employed more mature rats (150 - 225 g) than employed in the present study and it was considered that this might be an important factor in the rapid development of DOCA-NaCl hypertension in the just weaned rats. However, no significant difference was observed in either the rate of production or the severity of the DOCA-NaCl hypertension in 'Fisons' rats of two different age groups. Ben-Ishay et al., (1972) found that two month old rats (about 200 g body weight) developed a severe hypertension very rapidly whilst several workers employing just weaned rats did not obtain such a rapid production of hypertension (e.g. Friedman, Friedman & Nakashima, 1951; Green, Saunders, Wahlgren & Craig, 1952; Mallov, 1959; Hinke, 1965; Stanton & Cooper, 1966; English et al., 1973). These results and those of the present study

tend to suggest that the age of the rat within two weeks to two months has little effect on the rate of production or severity of hypertension.

It was found that 'mature Sc.P.F.' rats when compared with 'young Fisons' rats did have a significantly slower rate of development of DOCA-NaCl hypertension and the severity of this hypertension was significantly less until almost the end of the study. This was expected since although in the two previous experiments in this study no significant differences in the rate of production or severity of the hypertensions were observed the 'young Fisons' group of rats consistently had a higher systolic blood pressure immediately after the operation to induce hypertension. Thus, although 'mature Sc.P.F.' rats develop hypertension more slowly than 'young Fisons' rats they nevertheless do develop hypertension in four weeks from the time of the operation to induce hypertension whilst receiving 1% sodium chloride solution for only fourteen days. Thus, the cause of the rapid development of DOCA-NaCl hypertension in just weaned 'Fisons' rats is not totally due to either the age or the strain of these rats although these factors may be contributing factors. Recently, Molteni & Brownie (1972) have examined the susceptibility of several commonly used stocks of rats to salt-induced hypertension and observed that hypertension occurred frequently in the Holtzman Albino rats and not in the other groups of rats examined. These workers were not able to explain this susceptibility of the Holtzman Albino rats to salt-induced hypertension on a genetic basis. It is possible that the 'Fisons' and 'Sc.P.F.' Wistar rats might be highly susceptible to the induction of DOCA-NaCl hypertension in a similar manner although from the experiments performed no clear explanation for this susceptibility can be forwarded although certain possibilities exist. Hall, Ayachi & Hall (1972 a) found that

the Long-Evans strain of Sprague-Dawley rats was resistant to DOCA-NaCl hypertension and this was found to be due to the lack of development of an excessive thirst for sodium chloride (Hall, Ayachi & Hall, 1972 b).

Experiments performed recently at Beechams Research Laboratories (see Section 3, Chapter 2) have also shown that Wistar rats supplied by Animal Supplies Limited (ASL) do not develop DOCA-NaCl hypertension as rapidly as the strains involved in this study. Although exact measurements of sodium chloride intake were not performed in these rats, polydipsia and polyuria were obviously present. Thus, in contrast to the work of Hall, Ayachi & Hall (1972 b) it would appear improbable that the slow development of DOCA-NaCl in 'ASL' Wistar rats is due to a decreased sodium chloride intake.

The rapid production of DOCA-NaCl hypertension in just weaned 'Fisons' rats was not due to slight modifications in the technique for the induction of hypertension such as whether tap water or distilled water was used as the solvent for the sodium chloride or due to any differences in the batches of DOCA used since these did not affect either the rate of production or severity of the hypertension. The two variable factors which were not altered was the position of the DOCA implant and nephrectomy which was always performed on the right kidney. However, it is highly doubtful that either of these factors could be responsible for the rapid elevation of systolic blood pressure. Male rats were employed in this study and although Ben-Ishay et al. (1972) have stated that male rats are much more susceptible than female rats to DOCA-NaCl hypertension Selye & Pentz (1943) have stated the opposite view and since many studies involving DOCA-NaCl hypertension have employed both male (e.g. Mallov, 1959; Beilin & Ziakas, 1972; Holloway & Bohr, 1973) and female (e.g. Masson, Corcoran

& Page, 1949; Stanton & Cooper, 1966; Ben-Ishay & Welner, 1969) rats without any obvious variation in the development of DOCA-NaCl hypertension it would appear to be an insignificant factor in the rapid production of hypertension observed in this study.

Although it has been stated that the amount of saline consumed by rats on DOCA-NaCl therapy is not relative to the development of hypertension (Panasevich et al., 1969) it was noted in these studies, as stated in the preceeding chapter, that marked polydipsia was present in the 'young Fisons' rats. This polydipsia though greater than that observed in the 'Sc.P.F.' rats was still present in the latter group of animals and since saline sensitizes rats to the action of DOCA (Selye et al., 1943) it remains possible that the polydipsia observed in these two groups of rats may play a significant role in the rapid elevation of systolic blood pressure. This is confirmed by the work of Hall, Ayachi & Hall (1972 a & b) who found that DOCA-NaCl hypertension did not develop in the Long-Evans strain of Sprague-Dawley rats because their salt appetite did not increase after DOCA treatment, but when sucrose was added to sodium chloride drinking water polydipsia developed followed by hypertension. The later work involving ASL Wistar rats does not seem to support this hypothesis although for definite conclusions to be made it would be necessary to measure sodium chloride intake and correlate this with the resulting blood pressure.

One other factor that may be important in the rapid rise of the systolic blood pressure was that the animals were housed singly after the operation to induce DOCA-NaCl hypertension. Although the normotensive control rats were similarly housed and no elevation of systolic blood pressure was observed it is well known that changing a rat's

environmental situation in this way will produce animals which react in an exaggerated manner to stress (Hallback, 1975). Environmental stress has been shown to produce hypertension itself (e.g. Hudak & Buckley, 1961; Buckley et al., 1964; Rosecrans, Watzman & Buckley, 1966; Smookler & Buckley, 1969) and it remains a possibility that housing animals singly may be an important factor in the rapid development of DOCA-NaCl hypertension. The ASL Wistar rats used in the later study were housed together in groups of four. Thus, this idea of social isolation aggravating the hypertension due to a DOCA-NaCl regimen may be important. However, further studies would be necessary to clarify the importance of social isolation on the development of experimentally induced hypertensions.

One interesting point in this study, as stated in the previous chapter, was that the greater the rate of increase in the systolic blood pressure of a group of rats the slower the rate of body weight gain in that group, which was observed in both the 'Sc.P.F.' and 'Fisons' stocks of rats (e.g. Beilin & Ziakus, 1972; Hansen, Abrams & Bohr, 1974).

Although no single obvious reason for the rapid development of DOCA-NaCl hypertension can be forwarded from this study it would appear that a combination of the immature nature of the rats and the particular strain involved are important. Also the housing of rats singly and the observed marked intake of sodium chloride solution may be additional factors which play a role in the rapid production of DOCA-NaCl hypertension.

Summary

1. The possible cause or causes of the rapid development of DOCA-NaCl hypertension in 'Fisons' Wistar rats was investigated.

2. Minor alterations in the experimental procedure (i.e. different batches of DOCA implants or the sodium chloride vehicle) did not influence the rate of production of hypertension.

3. No significant difference in the production of hypertension in another strain of Wistar rats supplied by Scientific Products Farm was observed.

4. No significant difference in the production of hypertension in 'Fisons' rats of different ages was observed.

5. There was a significantly slower production of hypertension in 'old Sc.P.F.' rats than 'young Fisons' rats. However, the 'old Sc.P.F.' rats still developed hypertension after only 14 days sodium chloride substitution treatment.

6. Although no clear conclusion for the rapid production of DOCA-NaCl hypertension in 'Fisons' Wistar rats was found, possible explanations are discussed.

## SECTION 2: CARDIOVASCULAR REACTIVITY IN THE RAT

### CHAPTER 1

### Cardiovascular Reactivity in DOCA-NaCl Rats

Cardiovascular reactivity has been defined as 'the changing ability of blood vessels to respond to the same stimulus whether nervous or humoral in origin' (Page & McCubbin, 1963). Increased nervous activity or circulating amounts of physiologically active pressor substances have not been conclusively demonstrated in hypertension (see reviews by Braun-Menendez et al., 1946; Pickering, 1955, 1968; Page & McCubbin, 1968 and Page, 1974) and it is possible that cardiovascular hyper-reactivity to normal nervous and humoral influences, particularly noradrenaline, could be responsible for the increased blood pressure and peripheral resistance observed in both human and experimental hypertensions (see review by De Champlain, 1972).

There is a vast amount of conflicting literature on the subject of cardiovascular reactivity in both human and experimental hypertensions (see Introduction p. 38). A number of reports in which cardiovascular reactivity has been studied in clinical hypertension employing the whole cardiovascular system suggested that there was increased systemic cardiovascular reactivity in essential hypertension but not in various types of human renal hypertensions (Mendlowitz, 1967). However, these studies failed to consider the central regulatory mechanisms which maintain a constant blood pressure in the face of various pressor stimuli in hypertension as well as normotension (Doyle, Fraser & Marshall, 1959). The

increased pressor responses observed in hypertensive patients when compared to normotensive subjects was interpreted either as an actual increase in the responsiveness of vascular smooth muscle or simply a mechanical effect due to an increased wall-to-lumen ratio of the peripheral arterioles (Sivertsson, 1970; Folkow et al., 1973; Mendlowitz, 1973; Horowitz et al., 1974; Hamilton, 1975). Also complicating factors such as previous or current drug treatment of the hypertensive patients, the precise nature of their hypertension and the influence of secondary diseases on the responses observed has made interpretation of the results obtained in hypertensive subjects very difficult (Scroop & Whelan, 1968). Due to these difficulties cardiovascular reactivity in clinical hypertension has recently been studied in local vascular beds or isolated arterial strips and these results have suggested that there is an increased responsiveness of vascular smooth muscle from essential hypertensive patients (e.g. Conway, 1963; Dern & Leaverton, 1966; Ettinger, Siebel & Reicker, 1970). However, there is still the possibility that drug treatments and diseases other than hypertension may affect the responses of vascular smooth muscle and there is also the difficulty of obtaining comparable hypertensive and normotensive pairs of tissues. Experimental animals offer more accurate models in which to examine cardiovascular reactivity in hypertension.

Cardiovascular reactivity studies involving the response to various pressor substances in hypertensive and normotensive groups of animals in various types of experimental hypertension have produced conflicting results although cardiovascular hyper-reactivity has usually been reported (e.g. Schwartz et al., 1967; McGregor & Smirk, 1968, 1970; Baum & Shropshire, 1969; Armstrong, 1972).

However, studies in the whole animal are subject to the difficulties stated previously and thus the use of isolated vascular beds and arterial tissues free from reflex control have been employed. In tissues from renal, DOCA-NaCl and spontaneously hypertensive rats, increased responses to pressor substances have usually been observed although there have been reports to the contrary (see review by De Champlain, 1972).

However, when isolated tissues are removed from the animal or vascular beds are infused with physiological solutions ionic changes occur in the tissue cells and these changes are known to seriously affect responses of vascular smooth muscle to pressor substances (Holloway et al., 1972).

It was considered that the pithed rat would be ideally suited to cardiovascular reactivity studies since nervous reflexes are not present and the vascular smooth muscle is maintained in its normal physiological environment. Also it was considered useful for differentiating between the 'mechanical' and 'contractile' theories of increased peripheral vascular resistance.

Results

There was a highly significant difference ($P < 0.001$) between the systolic blood pressures of the DOCA-NaCl hypertensive rats (mean systolic blood pressure $\pm$ S.E. 200 $\pm$ 6.7 mm Hg; n = 30) and the control normotensive rats (mean systolic blood pressure $\pm$ S.E. 117 $\pm$ 1.7 mm Hg; n = 30). However, after pithing there was no significant difference between the resting blood pressures of the DOCA-NaCl and control groups (mean systolic blood pressures $\pm$ S.E. of 77 $\pm$ 6.6 mm Hg and 69 $\pm$ 2.2 mm Hg and mean diastolic blood pressures $\pm$ S.E. of 55 $\pm$ 3.9 mm Hg and 52 $\pm$ 2.2 mm Hg respectively).

The effect of electrical stimulation of the complete sympathetic outflow from the spinal cord on the systolic blood pressures of pithed DOCA-NaCl hypertensive and control normotensive rats is shown in Fig. 31. Although the pressor responses to all frequencies of electrical stimulation were smaller in the DOCA-NaCl rats than in the control rats, there was no statistically significant difference between the two groups.

Fig. 32 shows the effect of increasing doses of tyramine on the systolic blood pressure of DOCA-NaCl hypertensive and control normotensive pithed rats. The blood pressure response to low doses of tyramine was significantly greater ($P < 0.01$ to $P < 0.001$) in the DOCA-NaCl group of rats. At higher dose levels than 50 µg/kg there was no statistically significant difference in the blood pressure responses of the two groups. However, despite the 2 - 3 fold increase in response of DOCA-NaCl rats to tyramine there was no decrease in the threshold dose.

Angiotensin caused significantly greater ($P < 0.05$ to $P < 0.001$) blood pressure responses in DOCA-NaCl rats at all dose levels, although there was no increase in the threshold sensitivity to angiotensin (Fig. 33).

Similarly DOCA-NaCl hypertensive pithed rats showed a two fold increase in response to low doses of noradrenaline but no increase in the threshold sensitivity was observed. Dose levels of noradrenaline greater than 100 ng/kg produced no significant difference in the systolic blood pressure responses of the two groups (Fig. 34).

McN-A-343 is a substance which acts selectively to stimulate sympathetic ganglia and provides a measure of sympathetic activity (e.g. Roszkowski, 1961; Levy & Ahlquist, 1962; Jones, 1963; Smith, 1966).

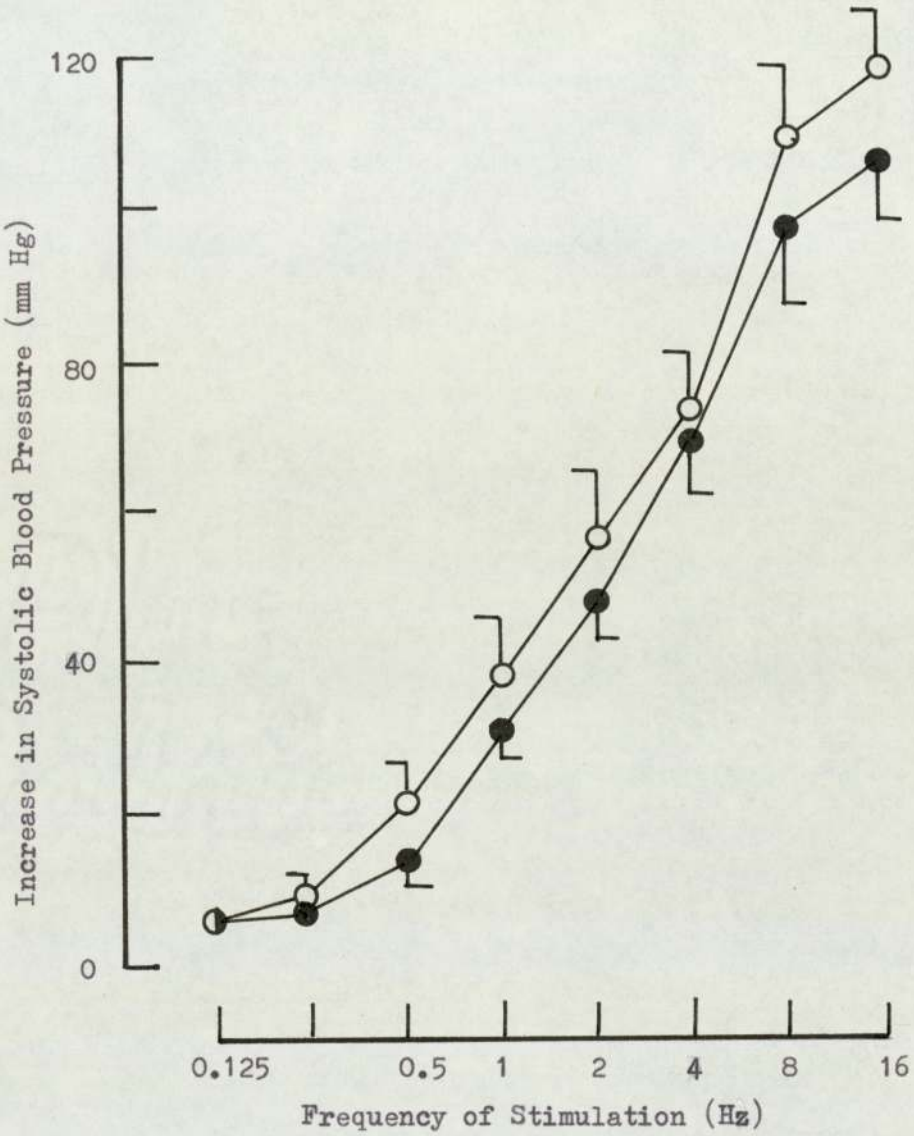


FIGURE 31: THE EFFECT OF ELECTRICAL STIMULATION OF THE COMPLETE SYMPATHETIC OUTFLOW FROM THE SPINAL CORD ON THE SYSTOLIC BLOOD PRESSURES OF PITHED RATS.

The solid circles (●—●) show the effect of increasing frequencies of stimulation on the systolic blood pressure of pithed hypertensive (DOCA-NaCl) rats. The open circles (○—○) show the effect of the same procedure on the systolic blood pressure of pithed normotensive rats. Each point represents the mean response of six rats and standard errors are shown by the vertical bars. Differences between the responses were evaluated using the Student's 't' test and no significant difference was observed at any frequency of stimulation.

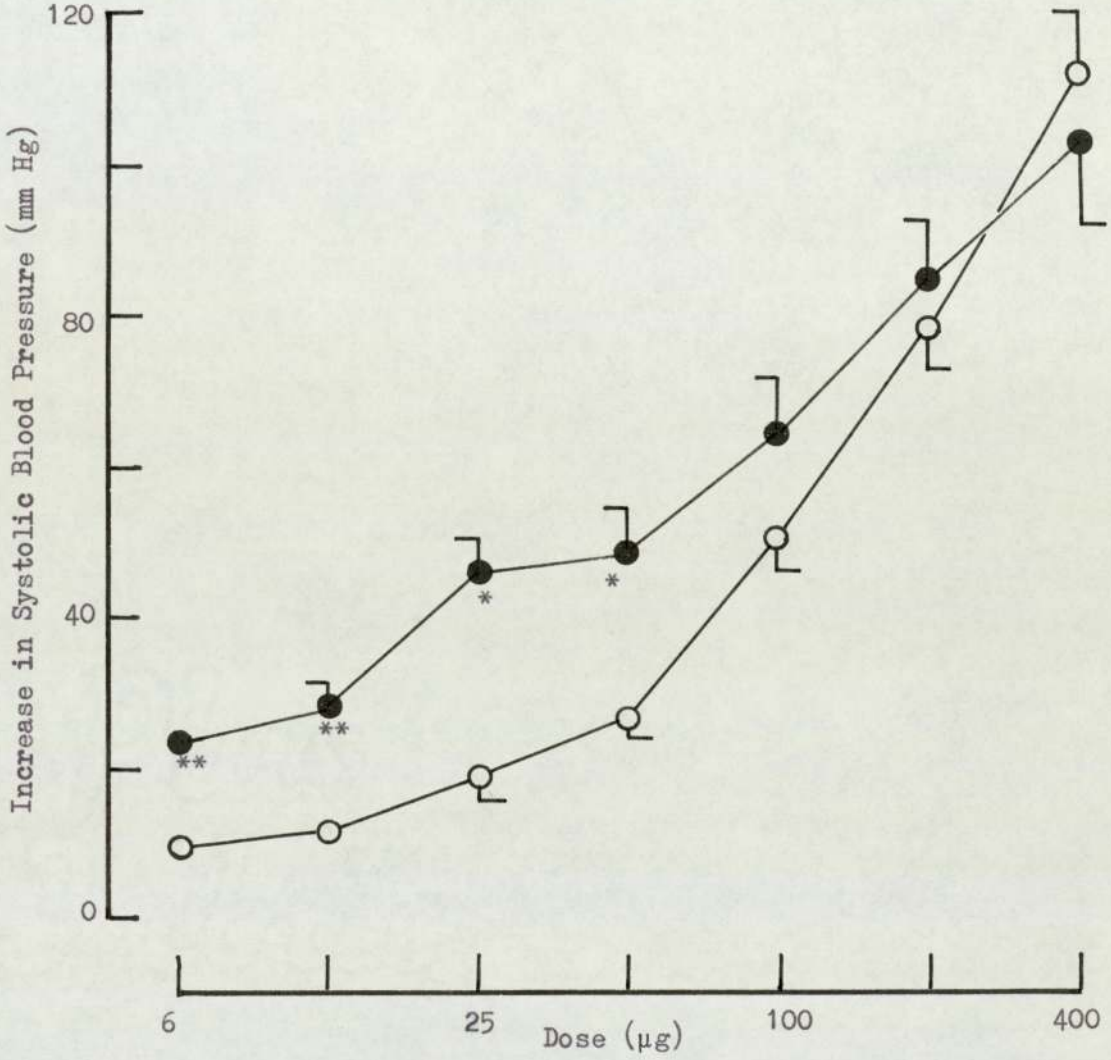


FIGURE 32: THE EFFECT OF TYRAMINE ON THE SYSTOLIC BLOOD PRESSURES OF PITHED RATS.

The closed circles (●—●) show the effect of increasing doses of tyramine on the systolic blood pressures of pithed hypertensive (DOCA-NaCl) rats. The open circles (○—○) show the effect of the same doses of tyramine on systolic blood pressure of pithed normotensive rats. Each point represents the mean response of a group of 6 rats. Standard errors are shown by the vertical bars. The difference in the responses of the hypertensive group compared to the normotensive group was evaluated using the Student's 't' test and the degree of significance is shown by the asterisks (*$P < 0.01$; **$P < 0.001$).

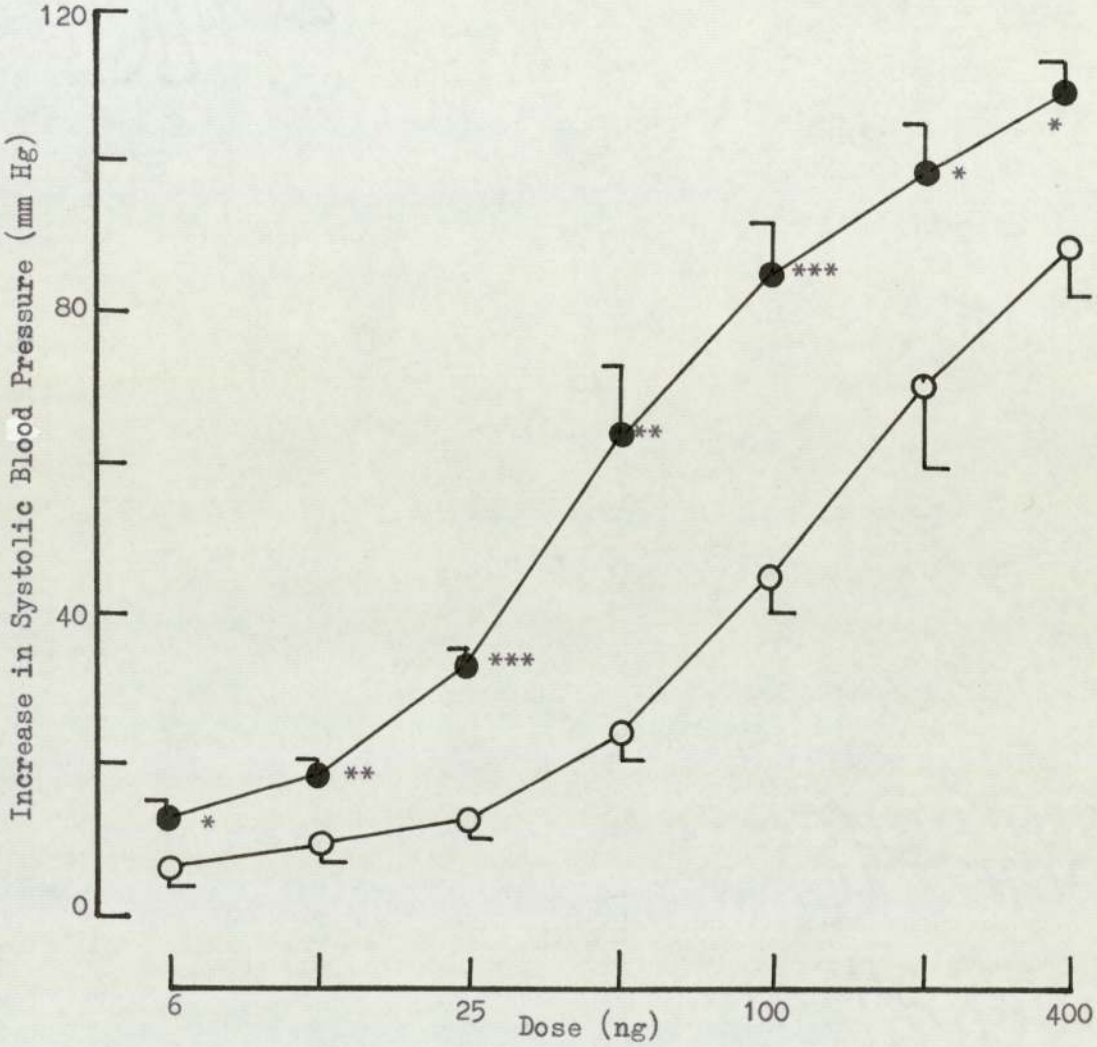


FIGURE 33: THE EFFECT OF ANGIOTENSIN ON THE SYSTOLIC BLOOD PRESSURES OF PITHED RATS.

The closed circles (●—●) show the effect of increasing doses of angiotensin on the systolic blood pressures of pithed hypertensive (DOCA-NaCl) rats. The open circles (○—○) show the effect of the same doses of angiotensin on the systolic blood pressures of pithed normotensive rats. Each point represents the mean response of a group of six rats. Standard errors are shown by the vertical bars. The difference in the responses to angiotensin of the hypertensive group compared to the normotensive group was evaluated using the Student's 't' test and the degree of significance is shown by the asterisks (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

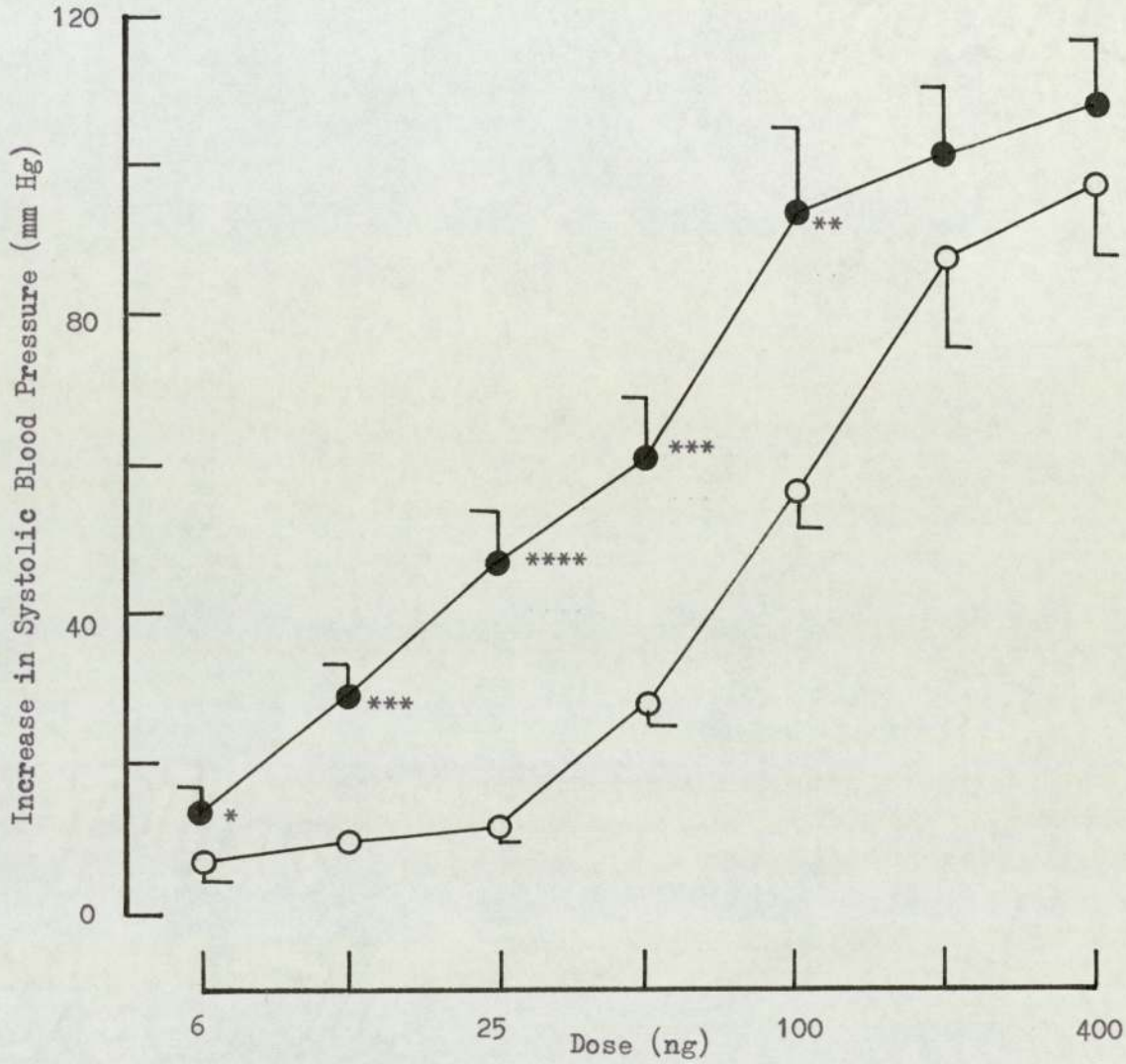


FIGURE 34: THE EFFECT OF NORADRENALINE ON THE SYSTOLIC BLOOD PRESSURES OF PITHED RATS.

The closed circles (●—●) show the effect of increasing doses of noradrenaline on the systolic blood pressures of pithed hypertensive (DOCA-NaCl) rats. The open circles (○—○) show the effects of the same doses of noradrenaline on the systolic blood pressures of pithed normotensive rats. Each point represents the mean response of a group of six rats. Standard errors are shown by the vertical bars. The difference in the responses of the hypertensive group compared to the normotensive group was evaluated using the Student's 't' test and the degree of significance is shown by the asterisks (*$P < 0.05$; **$P < 0.02$; ***$P < 0.01$; ****$P < 0.001$).

The usual response observed after an intravenous injection of McN-A-343 is a small transient fall in blood pressure (due to stimulation of peripheral muscarinic sites) followed by a prolonged large rise in blood pressure (due to stimulation of sympathetic ganglia) (e.g. Levy & Ahlquist, 1962). The usual fall in blood pressure observed after administration of McN-A-343 did not occur in either group of pithed rats. The systolic blood pressure response to McN-A-343 was consistently larger in the control normotensive group than in the DOCA-NaCl hypertensive group although this did not attain a statistically significant difference at any dose level (Fig. 35). There was no difference in the threshold sensitivity to McN-A-343 in either group.

It was thought possible that the increased responses to exogenous noradrenaline, tyramine and angiotensin might represent a true increase in sensitivity of vascular smooth muscle to noradrenaline. This was investigated by comparing the responses to noradrenaline of isolated aortic strips from DOCA-NaCl hypertensive and control normotensive rats (Fig. 36). The responses of the aortic strips from the DOCA-NaCl hypertensive group of rats to noradrenaline were consistently larger than those of the control normotensive group although this did not attain a statistically significant difference at any dose level.

Discussion

The resting blood pressures of the two groups of rats after pithing were not significantly different which strongly suggests that the elevated blood pressure of the DOCA-NaCl hypertensive group of rats was maintained by the sympathetic nervous system and not by a change in vascular smooth muscle resulting in an increased wall-to-lumen ratio. However, it was interesting to note that although the

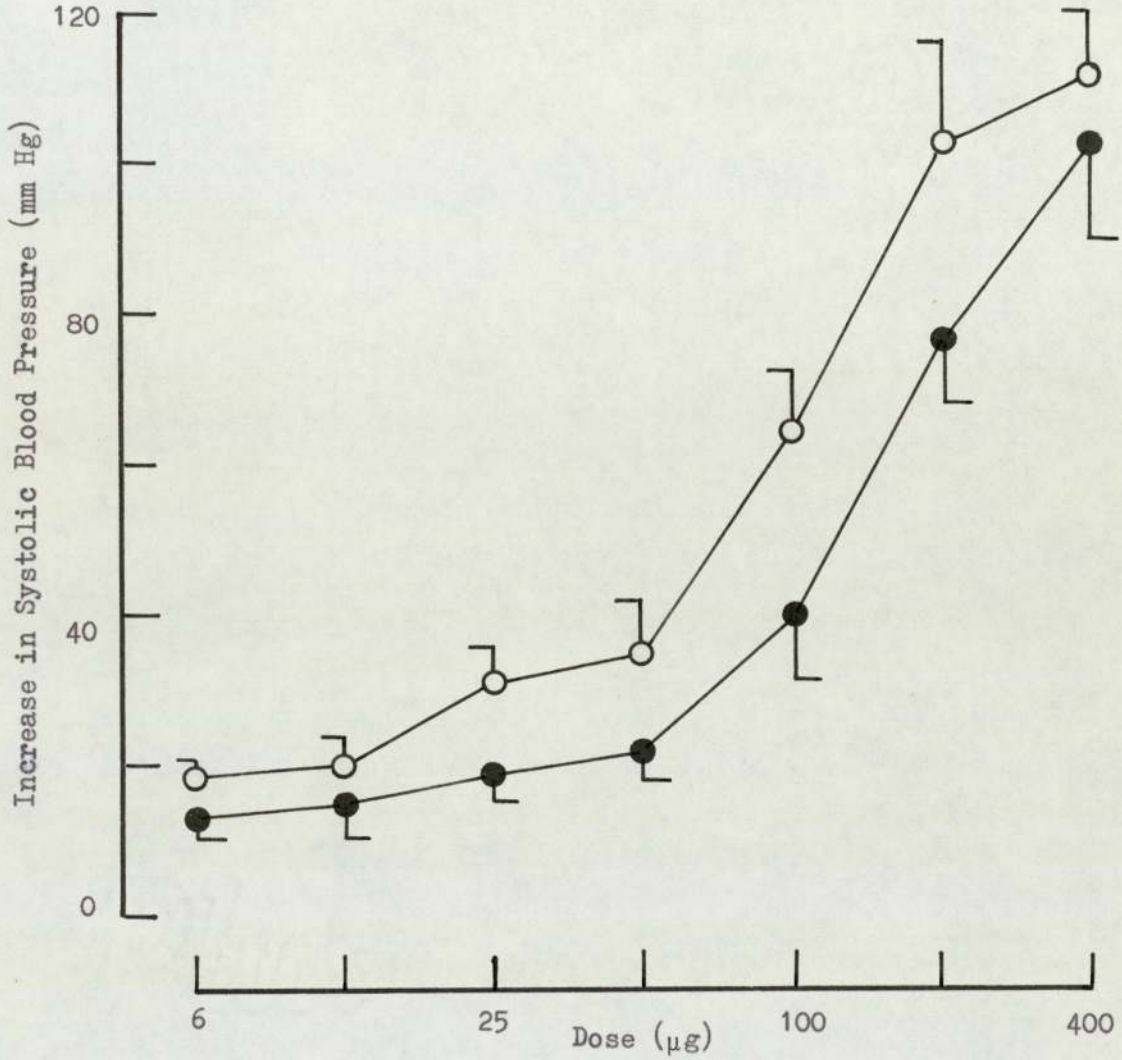


FIGURE 35: THE EFFECT OF McN-A-343 ON THE SYSTOLIC BLOOD PRESSURES OF PITHED RATS.

The closed circles (●—●) show the effect of increasing doses of McN-A-343 on the systolic blood pressures of pithed hypertensive (DOCA-NaCl) rats. The open circles (○—○) show the effect of the same doses of McN-A-343 on the systolic blood pressures of pithed normotensive rats. Each point represents the mean response of a group of six rats. Standard errors are shown by the vertical bars. The difference in the responses of the two groups was evaluated using the Student's 't' test and no significant difference ($P > 0.05$) was observed at any dose level.

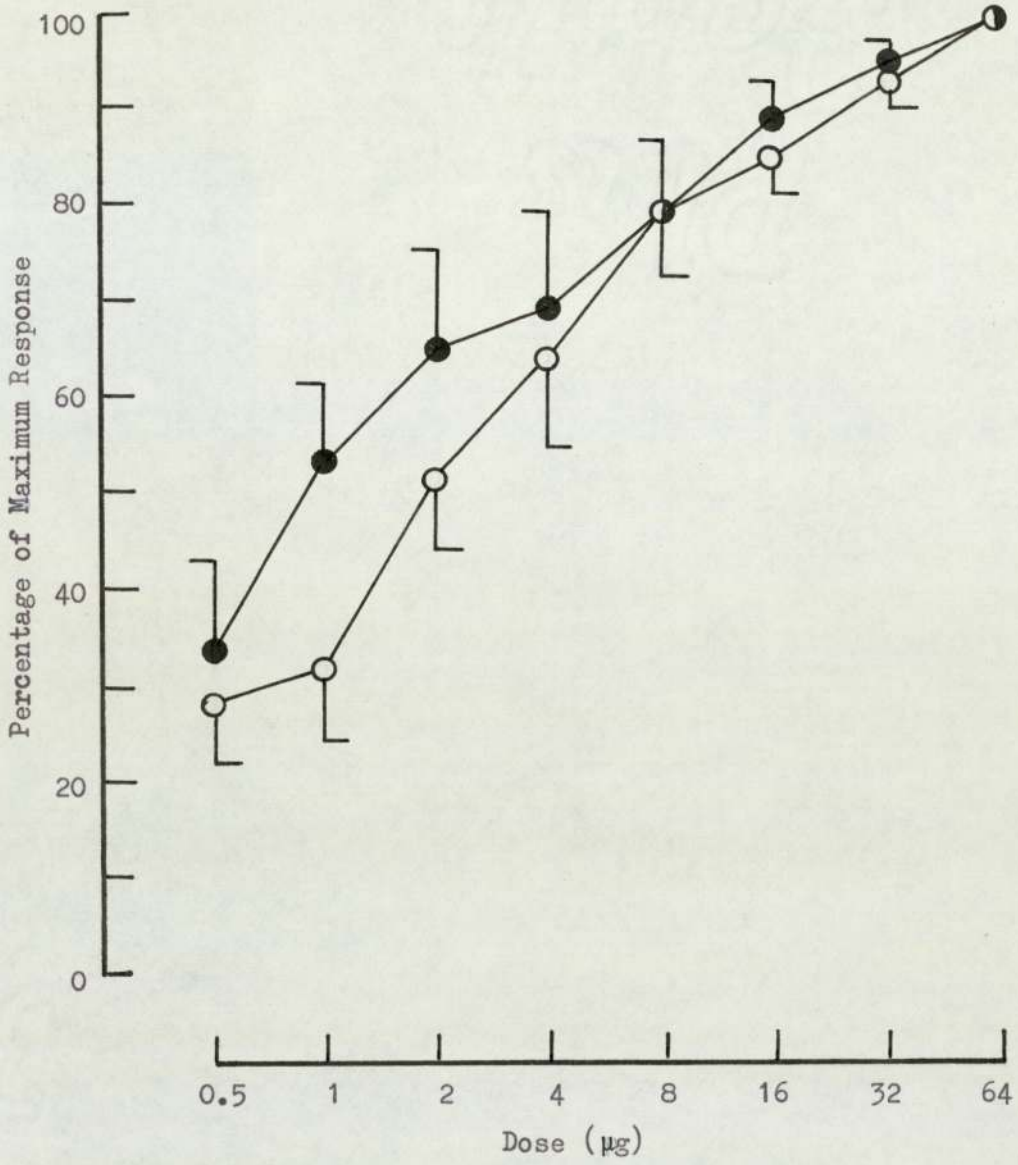


FIGURE 36: THE EFFECT OF NORADRENALINE ON AORTIC STRIPS FROM HYPERTENSIVE (DOCA-NaCl) AND NORMOTENSIVE RATS.

The solid circles (●—●) show the effect of increasing doses of noradrenaline on the response of aortic strips from hypertensive (DOCA-NaCl) rats. The open circles (○—○) show the effect of noradrenaline on the aortic strips from normotensive control animals. Each point represents the mean of six experiments and the standard errors are shown by the vertical bars. Differences between the two groups were evaluated using the Student's 't' test and there was no significant difference ($P > 0.05$) observed at any dose level.

blood pressures of the pithed DOCA-NaCl rats were not significantly greater than those of the control normotensive group they were invariably higher suggesting that some of the increase in peripheral resistance in DOCA-NaCl hypertension may be due to an increased wall-to-lumen ratio. These results are in agreement with those of Taquini (1963) and Finch (1971).

The contrast in the responses to endogenous noradrenaline, released on stimulation of the sympathetic outflow from the spinal cord, and exogenous noradrenaline was rather surprising. The increased responses in DOCA-NaCl rats to exogenous noradrenaline indicated that the vascular smooth muscle of the hypertensive rats possessed greater reactivity than that of the normotensive group. These results are in agreement with the subsequent reports of Finch (1971) and Armstrong (1972) who also observed a 1.5 to 3 fold steepening of the dose-response curve to exogenous noradrenaline, with no decrease in the threshold dose, in the pithed rat. The decreased blood pressure responses of DOCA-NaCl rats to endogenous noradrenaline when compared to control rats, is in contradiction to the report of Finch (1971). Similarly, the decreased cardiovascular reactivity of DOCA-NaCl rats to McN-A-343 observed in the present study was in contrast to the increased responses to 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP) which Finch (1971) reported in pithed DOCA-NaCl rats. However, the increased responsiveness of DOCA-NaCl rats to angiotensin and low dose levels of tyramine observed in the present study is in agreement with the report of Finch (1971).

These results suggest that smaller quantities of noradrenaline are released from the postganglionic sympathetic nerve endings of the DOCA-NaCl rats, during electrical or ganglionic stimulation, than from

the control normotensive rats. De Champlain and co-workers (see review by De Champlain, 1972) have observed a decreased ability of the sympathetic nervous system to store noradrenaline in DOCA-NaCl hypertensive rats and this may be reflected in the present study by the decreased blood pressure responses to endogenous noradrenaline, released by electrical and ganglionic stimulation, observed in the present study. The above workers have also reported an increased turnover of noradrenaline in DOCA-NaCl hypertension and increased extraneuronal levels of noradrenaline (see review by De Champlain, 1972). This excess extraneuronal noradrenaline may be responsible for the cardiovascular hyper-reactivity to tyramine and angiotensin, observed in the present study, since both tyramine and angiotensin responses are enhanced in the presence of increased amounts of circulating noradrenaline (e.g. Schmitt & Schmitt, 1967 a & b; Day & Owen, 1969). However, the response to exogenous noradrenaline is normally decreased or unchanged in the presence of excess circulating amounts of noradrenaline (e.g. Day & Owen, 1968; Page & McCubbin, 1968).

It was considered that the increased response to exogenous noradrenaline in pithed DOCA-NaCl rats might represent a true hypersensitivity of vascular smooth muscle to noradrenaline. However, the results obtained from isolated aortic strips of DOCA-NaCl and control rats showed no increased sensitivity of vascular smooth muscle to noradrenaline. This result was in agreement with those of Redleaf & Tobian (1958) and Mallov (1959) who observed smaller responses to noradrenaline in aortic strips from DOCA-NaCl hypertensive rats than in those from control normotensive animals. This does not preclude an actual increased sensitivity of vascular smooth muscle to noradren-

aline since the aorta plays little part in the maintenance of peripheral resistance and the smaller arterioles which maintain the elevated blood pressure in hypertension may be hyper-reactive to noradrenaline (Horowitz et al., 1974). Hinke (1965), Bohr & Sitrin (1970) and Hansen, Abrams & Bohr (1974) have indeed reported increased vascular responsiveness to noradrenaline when using smaller arteries than the aorta from DOCA-NaCl rats.

The cardiovascular hyper-reactivity to exogenous noradrenaline, tyramine and angiotensin observed in the present study may reflect a non-specific increase due to ionic changes in plasma and vascular smooth muscle of DOCA-NaCl hypertensive rats. DOCA and salt treatment has been reported to increase plasma sodium concentration (e.g. Heistad, Abboud & Eckstein, 1967; Nicholas, 1971) and potassium ion concentration of vascular smooth muscle (Tobian & Redleaf, 1957; Redleaf & Tobian, 1958). These changes may influence the membrane potential and contribute to hyper-reactivity of vascular smooth muscle (Jones, 1973). The ionic changes in plasma and vascular smooth muscle brought about by DOCA and salt treatment, resulting in a non-specific hyper-reactivity of vascular smooth muscle to pressor substances, would appear to be a possible explanation of the results observed in this study.

Thus, these results suggest that the sympathetic nervous system is important in maintaining the increased peripheral resistance observed in DOCA-NaCl induced hypertension and that the increased cardiovascular reactivity of smooth muscle in DOCA-NaCl hypertensive rats may be a consequence and not a cause of the hypertensive state.

Summary

1. Cardiovascular reactivity has been studied in pithed DOCA-NaCl hypertensive and control normotensive rats.

2. After pithing, the blood pressures of both the DOCA-NaCl hypertensive rats and the control normotensive rats fell to similar levels. This indicated that the elevated blood pressure in DOCA-NaCl hypertension is due to the sympathetic nervous system and not changed smooth muscle vasculature resulting in an increased wall-to-lumen ratio.

3. Reduced blood pressure responses were observed in pithed DOCA-NaCl rats, when compared with pithed control rats, to endogenous noradrenaline released by sympathetic stimulation and McN-A-343 with no change in threshold sensitivity.

4. Increased blood pressure responses were observed in pithed DOCA-NaCl rats, when compared with pithed control rats, to exogenous noradrenaline, tyramine and angiotensin with no change in threshold sensitivity.

5. Larger responses, to noradrenaline, were obtained in isolated aortic strips from DOCA-NaCl hypertensive rats than from control normotensive rats. However, these were not significant and threshold sensitivity was unchanged.

6. These results are discussed and appear to indicate that cardiovascular hyper-reactivity is a consequence of the hypertension and not the cause of the elevated blood pressure in DOCA-NaCl hypertensive rats.

## SECTION 3: EFFECTS OF ANTIHYPERTENSIVE AGENTS IN HYPERTENSION

### CHAPTER 1

### Effect of Postganglionic Adrenergic Neuronal Blockade on the Production of Experimental Hypertension in the Rat - Initial Studies.

There is a considerable body of evidence implicating the sympathetic nervous system in the production and maintenance of hypertension (see reviews by Page & McCubbin, 1968; De Champlain, 1972 and Schmid & Abboud, 1974).

However, studies involving surgical sympathectomy, immunosympathectomy or chemical sympathectomy have produced conflicting results as to the role of the sympathetic nervous system in the development and maintenance of experimentally induced hypertensions (see pp. 30 to 33). The conflicting nature of the results obtained has been suggested to be due to the varying degrees of sympathectomy obtained (see reviews by De Champlain, 1972 and Thoenen, 1972).

Guanethidine blocks sympathetic postganglionic transmission (see review by Boura & Green, 1965), is not subject to the rapid development of tolerance, (see review by Pardo, Vargas & Vidrio, 1965) and chronic administration depletes nerve endings of blood vessels of noradrenaline (see reviews by Boura & Green, 1965; Mull & Maxwell, 1967 and Frohlich, 1974). Thus, it was considered possible to investigate the role of the sympathetic nervous system in the production of DOCA-NaCl and renal hypertensions, by inhibiting the system with daily injections of guanethidine. By variation of the duration of guanethidine treatment it was hoped to determine at what stage, if any, the sympathetic nervous system becomes essential for the production and/or maintenance of hypertension.

Results

To test the effectiveness of the adrenergic neuronal blockade produced by guanethidine, rats were pithed 24 hours after receiving a 10 mg/kg i.p. dose and the sympathetic nervous system stimulated by the method of Gillespie & Muir (1967). Fig. 37 shows the 'frequency-response' curve for control and guanethidine pretreated rats. The 'frequency-response' curve of the control rats showed increasing responses up to 8 Hz and at this frequency cardiac arrythmias occurred which prevented higher frequencies being employed. The 'frequency-response' curve of the guanethidine pretreated rats was displaced to the right and the maximum response occurred at 64 Hz. The maximum response was considerably smaller in the guanethidine pretreated rats than in the control rats and the deleterious effects on the heart observed at stimulus frequencies of 4 and 8 Hz in the control rats were not evident in the guanethidine pretreated rats until 64 Hz.

The effect of various durations of treatment with guanethidine, which were begun just before the operations to induce hypertension were performed, on the production of DOCA-NaCl hypertension are shown in Figs. 38, 39, 40 and 41.

The normotensive control group showed a small gradual increase in systolic blood pressure over the 60 days study period. The four DOCA-NaCl control groups showed large, similar increases in systolic blood pressure attaining hypertensive levels within three weeks. This was followed by a continued gradual rise which stabilised at levels of 200 mm Hg or more.

After seven days treatment with guanethidine there was a highly

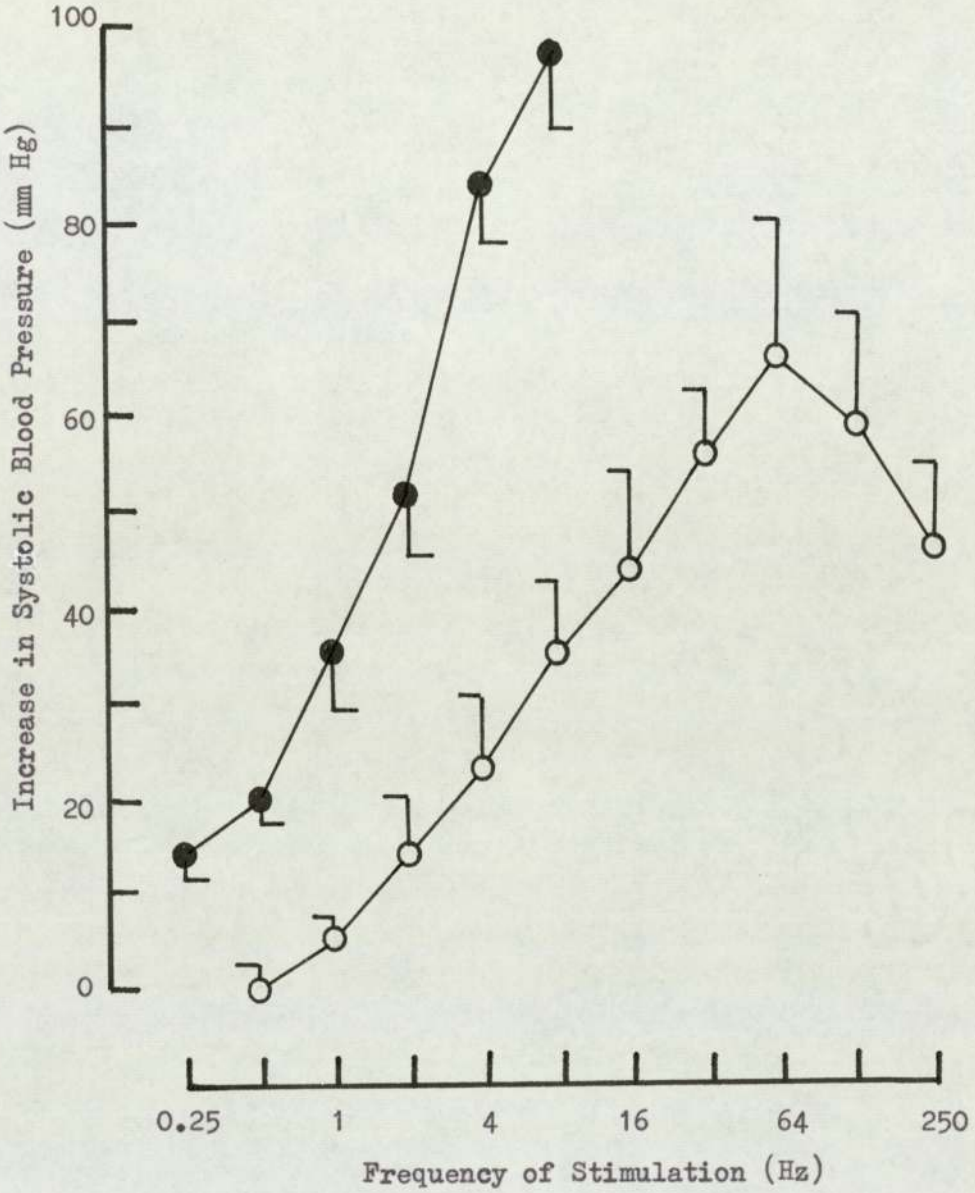


FIGURE 37: THE EFFECT OF GUANETHIDINE ON THE BLOOD PRESSURE RESPONSES TO ELECTRICAL STIMULATION OF THE SYMPATHETIC NERVOUS SYSTEM OF THE PITHED RAT.

The solid circles (●—●) represent the increase in systolic blood pressure caused by stimulation of the complete outflow of the sympathetic nervous system from the spinal cord of pithed rats using various frequencies of stimulation. The open circles (○—○) are pressor responses from pithed rats treated 24 hours previously, with 10 mg/kg i.p. guanethidine. Each point is the mean response from six animals and standard errors are indicated by vertical bars.

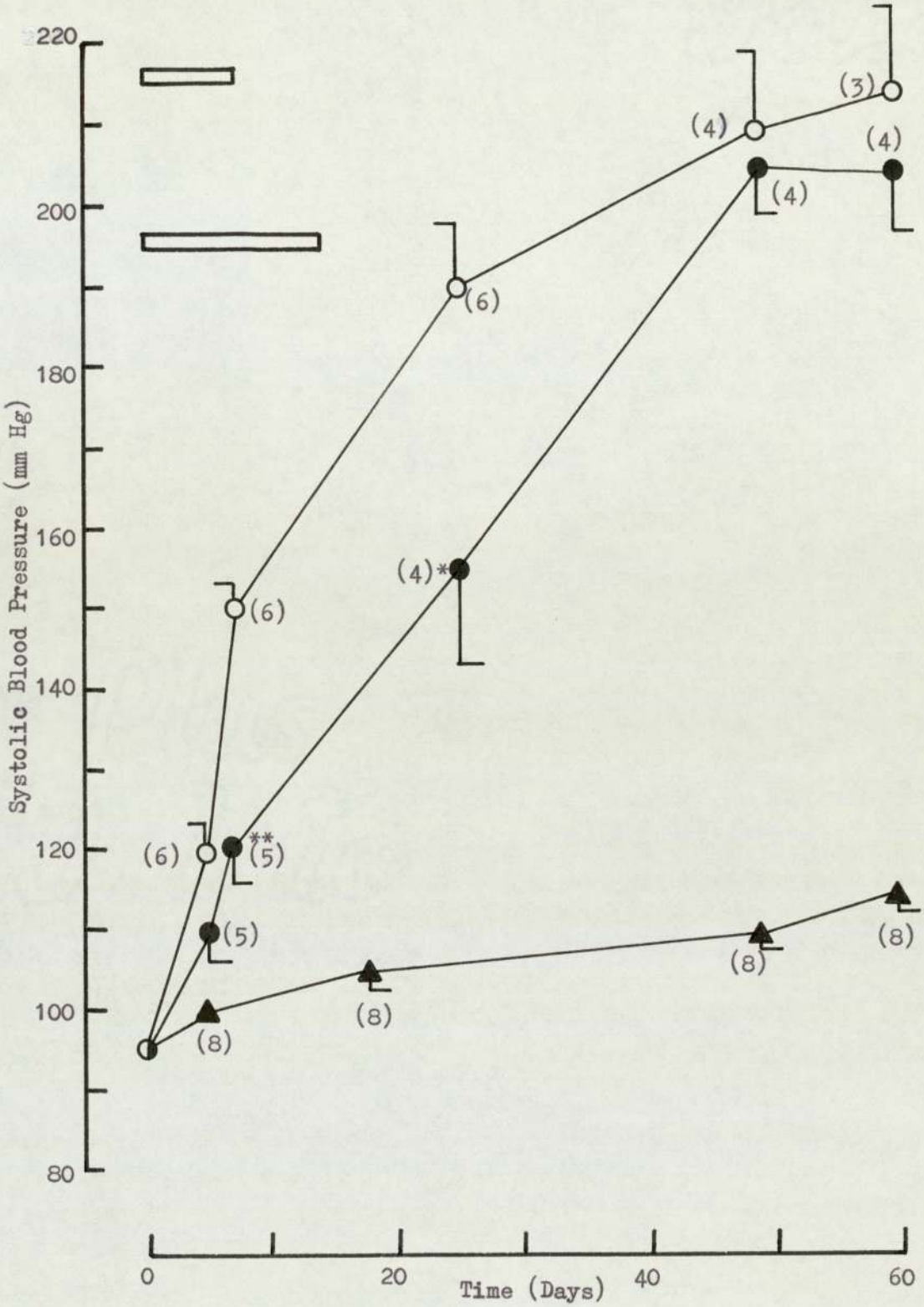


FIGURE 38: THE EFFECT OF GUANETHIDINE GIVEN DAILY FOR 7 DAYS UPON THE DEVELOPMENT OF HYPERTENSION PRODUCED BY A DOCA-NaCl REGIMEN.

The solid circles (●—●) represent the systolic blood pressures of a group of rats which were treated with guanethidine 10 mg/kg i.p. daily for 7 days, indicated by the upper bar, following the induction of hypertension by a DOCA-NaCl regimen (lower bar indicates the duration of replacement of drinking water with 1% sodium chloride solution). The open circles (○—○) show the blood pressures of a similar group of rats which did not receive guanethidine. Solid triangles (▲—▲) represent the blood pressures of normotensive control rats receiving no treatment. Each point represents the mean systolic blood pressure of the number of rats indicated in parenthesis. Standard errors are shown by vertical bars. The difference between the treated groups was evaluated using Student's 't' test and the degree of significance is shown by asterisks (*$P < 0.05$; **$P < 0.001$).

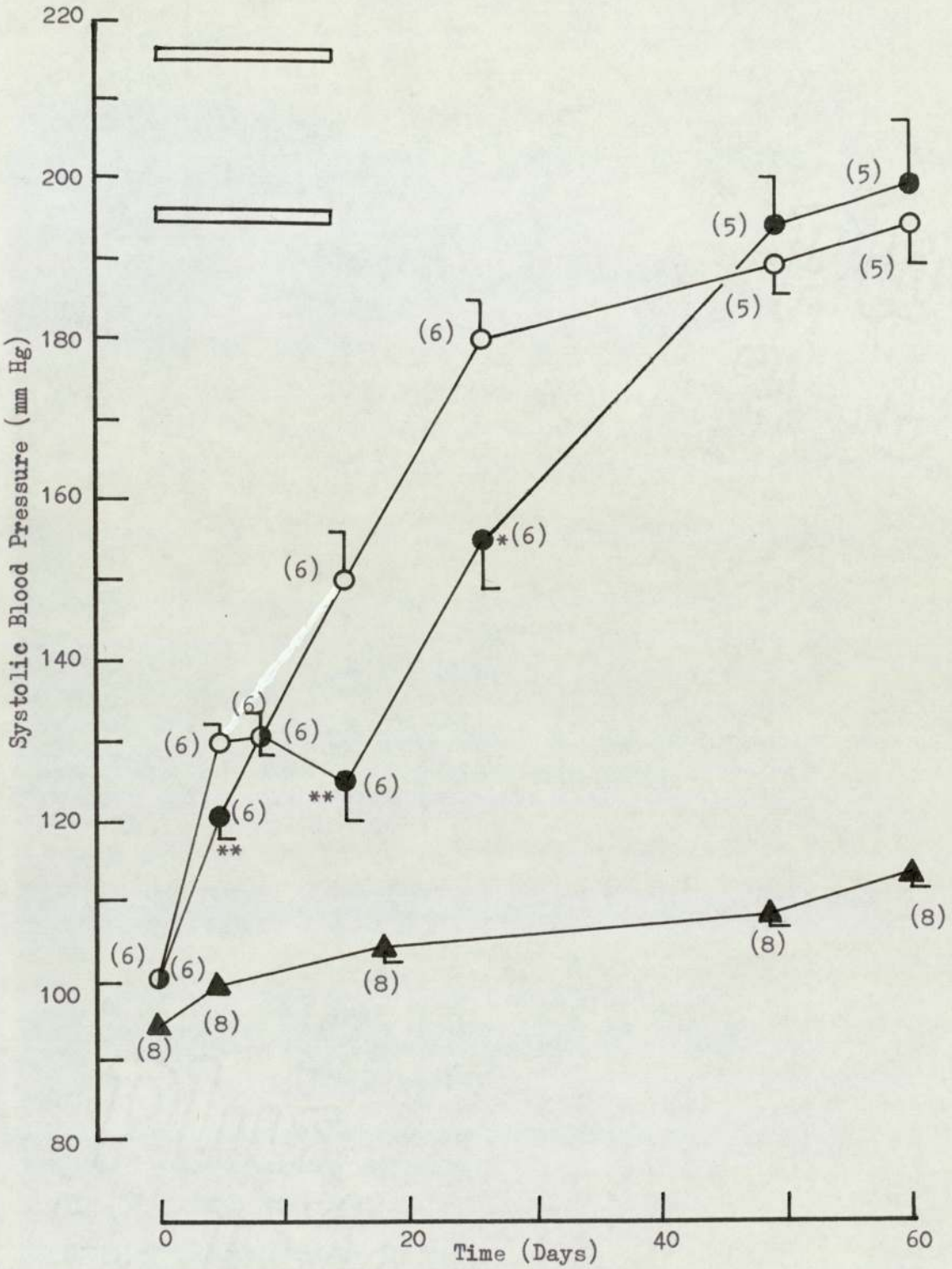


FIGURE 39: THE EFFECT OF GUANETHIDINE GIVEN DAILY FOR 14 DAYS UPON THE DEVELOPMENT OF HYPERTENSION PRODUCED BY A DOCA-NaCl REGIMEN.

The solid circles (●—●) represent the systolic blood pressures of a group of rats which were treated with guanethidine 10 mg/kg i.p. daily for 14 days, indicated by the upper bar, following the induction of hypertension by a DOCA-NaCl regimen (lower bar indicates the duration of replacement of drinking water with 1% sodium chloride solution). The open circles (○—○) show the blood pressures of a similar group of rats which did not receive guanethidine. Solid triangles (▲—▲) represent the blood pressures of normotensive control rats receiving no treatment. Each point represents the mean systolic blood pressure of the number of rats indicated in parenthesis. Standard errors are shown by vertical bars. The difference between the treated groups was evaluated using Student's 't' test and the degree of significance is shown by asterisks (*$P < 0.02$; **$P < 0.01$).

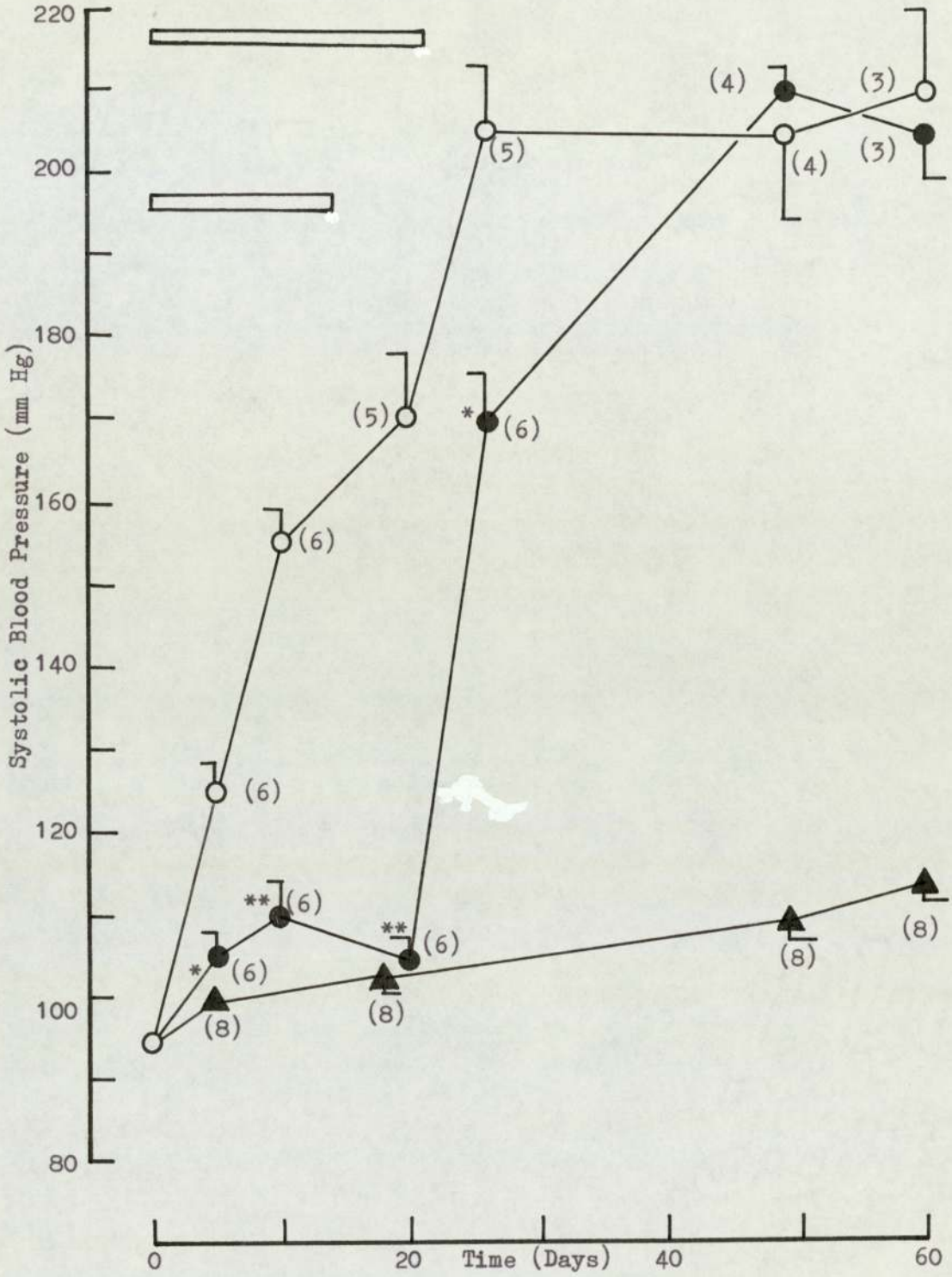


FIGURE 40: THE EFFECT OF GUANETHIDINE GIVEN DAILY FOR 21 DAYS UPON THE DEVELOPMENT OF HYPERTENSION PRODUCED BY A DOCA-NaCl REGIMEN.

The solid circles (●—●) represent the systolic blood pressures of a group of rats which were treated with guanethidine 10 mg/kg i.p. daily for 21 days, indicated by the upper bar, following the induction of hypertension by a DOCA-NaCl regimen (lower bar indicates the duration of replacement of drinking water with 1% sodium chloride solution). The open circles (○—○) show the blood pressures of a similar group of rats which did not receive guanethidine. Solid triangles (▲—▲) represent the blood pressures of normotensive control rats receiving no treatment. Each point represents the mean systolic blood pressure of the number of rats indicated in parenthesis. Standard errors are shown by vertical bars. The difference between the treated groups was evaluated using Student's 't' test and the degree of significance is shown by asterisks (*$P < 0.01$; **$P < 0.001$).

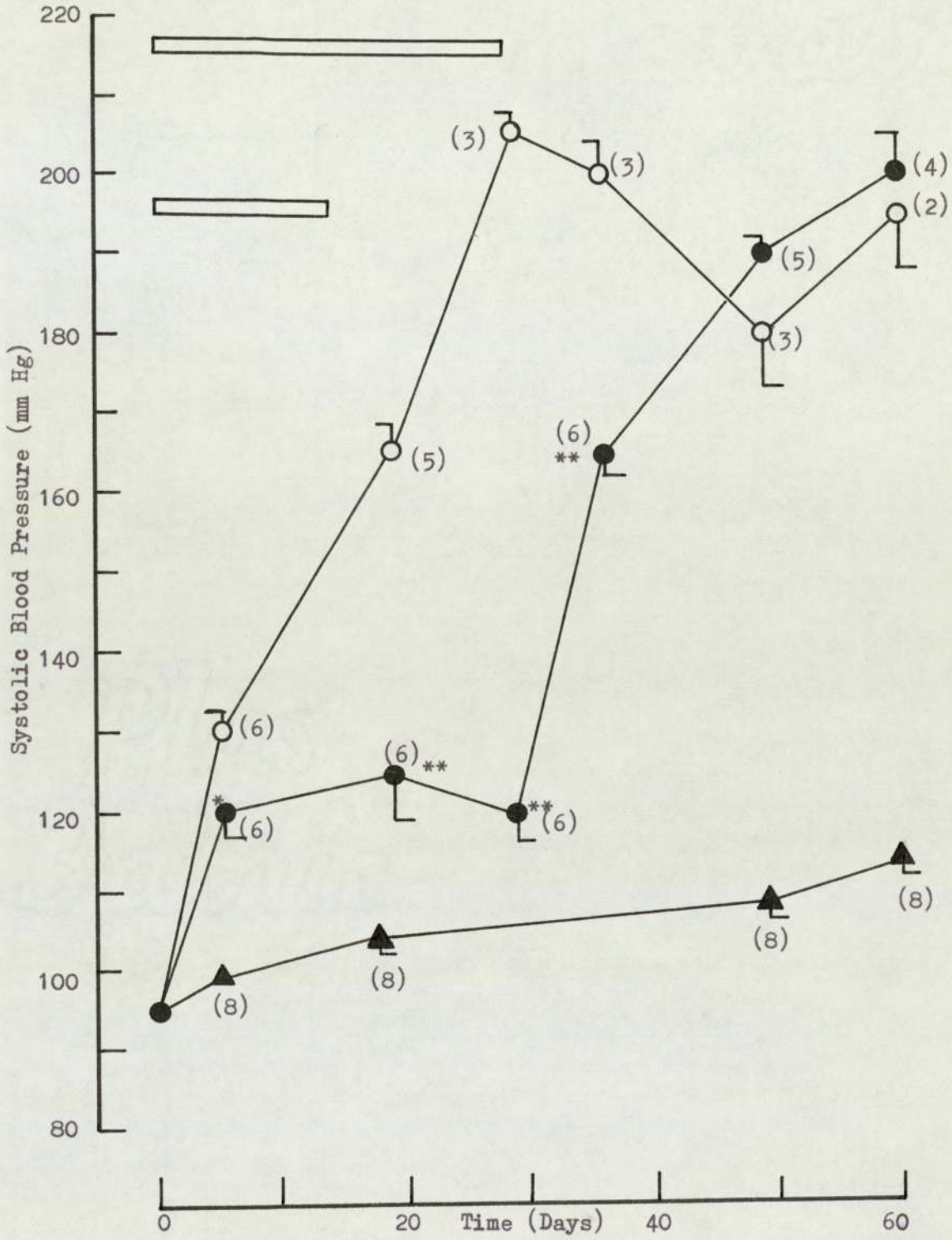


FIGURE 41: THE EFFECT OF GUANETHIDINE GIVEN DAILY FOR 28 DAYS UPON THE DEVELOPMENT OF HYPERTENSION PRODUCED BY A DOCA-NaCl REGIMEN.

The solid circles (●—●) represent the systolic blood pressures of a group of rats which were treated with guanethidine 10 mg/kg i.p. daily for 28 days indicated by the upper bar, following the induction of hypertension by a DOCA-NaCl regimen (lower bar indicates the duration of replacement of drinking water with 1% sodium chloride solution). The open circles (○—○) show the blood pressures of a similar group of rats which did not receive guanethidine. Solid triangles (▲—▲) represent the blood pressures of normotensive control rats receiving no treatment. Each point represents the mean systolic blood pressure of the number of rats indicated in parenthesis. Standard errors are shown by vertical bars. The difference between the treated groups was evaluated using Student's 't' test and the degree of significance is shown by asterisks (*$P < 0.05$; **$P < 0.001$).

significant ($P < 0.001$) decrease in the systolic blood pressure when compared with the control DOCA-NaCl group. After withdrawal of guanethidine treatment similar increases of the systolic blood pressure occurred in both operated groups which attained an observed non-significant difference forty-one days later.

Similar effects were observed in the group which received fourteen days guanethidine treatment. On day 15 the control DOCA-NaCl group had a significantly higher ($P < 0.01$) systolic blood pressure than that of the guanethidine treated group. This was then followed by similar increases in the systolic blood pressures of both groups which attained an observed non-significant difference thirty-four days later.

The group treated for twenty-one days with guanethidine showed a highly significant ($P < 0.01$ to $P < 0.001$) difference from the control DOCA-NaCl group but not from the control normotensive group, whilst treatment was in progress. After treatment with guanethidine was discontinued the systolic blood pressure increased and attained an observed non-significant difference with the control DOCA-NaCl group twenty-eight days later.

Throughout the twenty-eight day period of guanethidine treatment the systolic blood pressure of the treated group was significantly ($P < 0.05$ to $P < 0.001$) lower than that of the DOCA-NaCl control group. After dosing was discontinued there was an increase in systolic blood pressure which attained an observed non-significant difference from the control DOCA-NaCl group twenty days later.

The complete experiment was repeated and in this instance the systolic blood pressures were determined by an operator who had no knowledge of which group was being measured. The results of this 'blind' study were very similar to those observed in the first study

(for example, see Fig. 42). Statistically significant ($P < 0.01$ to $P < 0.001$) lower systolic blood pressures were recorded in all four guanethidine treated groups during the dosing period when compared with the control DOCA-NaCl group. In each case the systolic blood pressures of the guanethidine treated groups increased to attain observed non-significant differences from the control DOCA-NaCl group between 4 to 21 days after treatment was discontinued.

In both studies the mortality rates of the 7, 14 and 21 day guanethidine treated groups were similar to the control DOCA-NaCl group but the mortality rate was decreased in the 28 day guanethidine treated group. The general condition of the control DOCA-NaCl and guanethidine treated groups was similar and as described earlier (see p. 113), except for the 28 day dosed group, the animals of which were in a condition more similar to that of the control group.

The body weights of the guanethidine treated and control DOCA-NaCl groups in both studies were significantly lower ($P < 0.05$ to $P < 0.001$) than the control normotensive group throughout the sixty day period of study but at no time were they significantly different from each other.

A similar study involving the effect of postganglionic adrenergic neuronal blockade on the production of one-kidney renal hypertension in rats was performed. The injections were started just before the 'figure-of-eight' ligature was applied to the left kidney and continued for periods of 15, 30, 45 and 60 days. The results of this study are shown in Figs. 43, 44, 45 and 46.

The control normotensive group showed a slow, gradual increase in systolic blood pressure throughout the sixty day study. The four

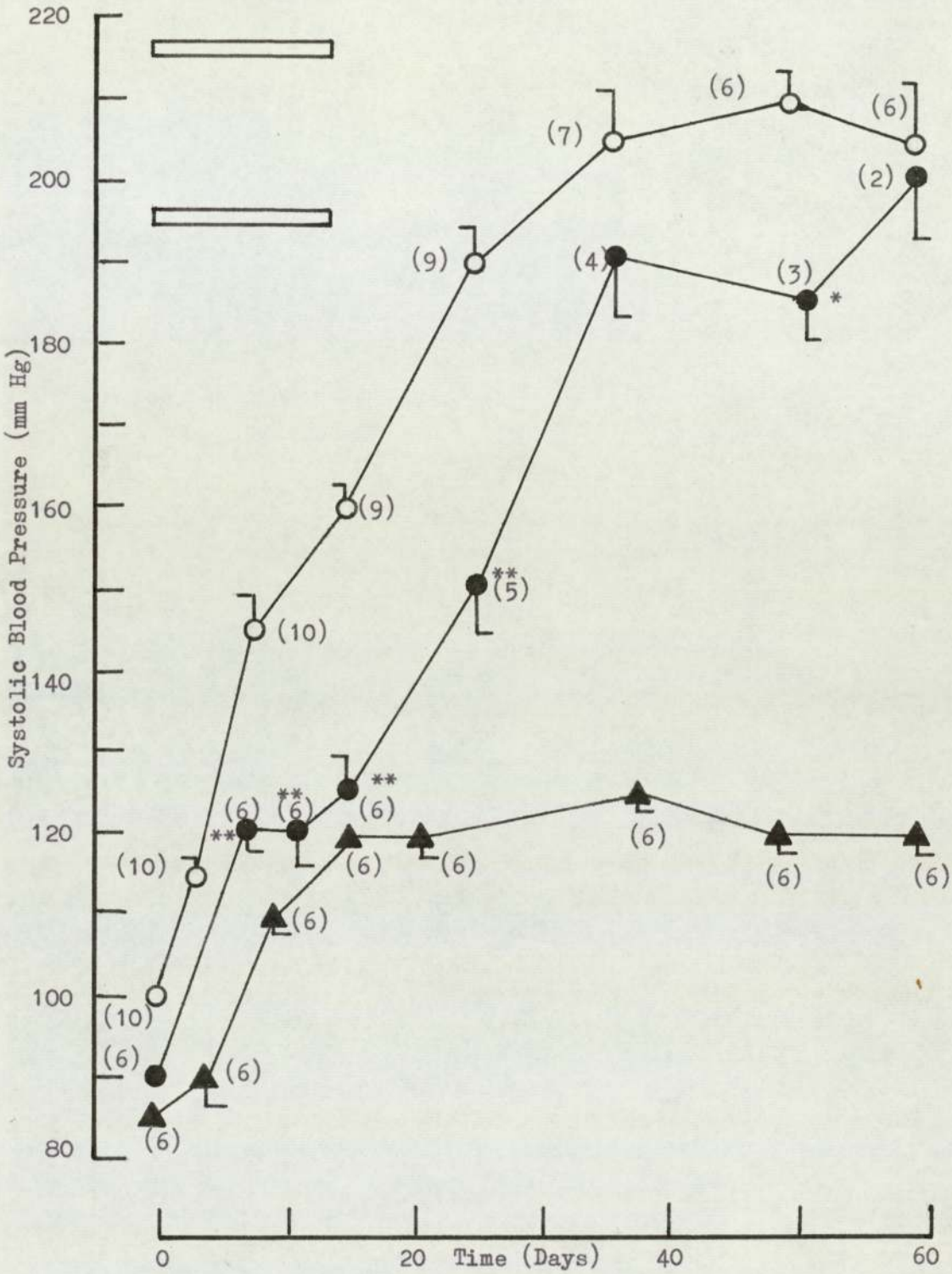


FIGURE 42: THE EFFECT OF GUANETHIDINE GIVEN DAILY FOR 14 DAYS UPON THE DEVELOPMENT OF HYPERTENSION PRODUCED BY A DOCA-NaCl REGIMEN (BLIND STUDY)

The solid circles (●—●) represent the systolic blood pressures of a group of rats which were treated with guanethidine 10 mg/kg i.p. daily for 14 days, indicated by the upper bar, following the induction of hypertension by a DOCA-NaCl regimen (lower bar indicates the duration of replacement of drinking water with 1% sodium chloride solution). The open circles (○—○) show the blood pressures of a similar group of rats which did not receive guanethidine. Solid triangles (▲—▲) represent the blood pressures of normotensive control rats receiving no treatment. Each point represents the mean systolic blood pressure of the number of rats indicated in parenthesis. Standard errors are shown by vertical bars. The difference between the treated groups was evaluated using Student's 't' test and the degree of significance is shown by asterisks (*$P < 0.01$; **$P < 0.001$).

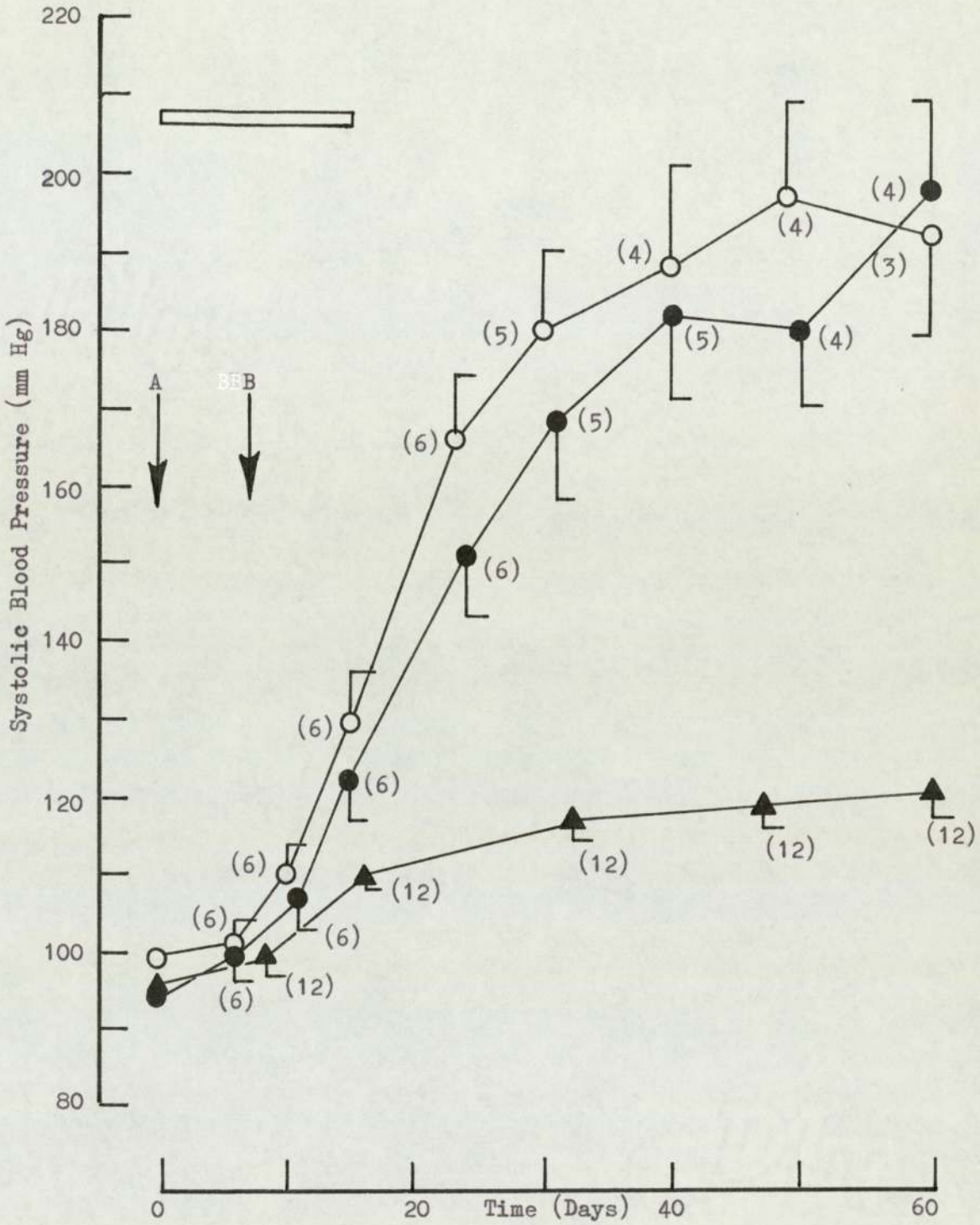


FIGURE 43: THE EFFECT OF GUANETHIDINE GIVEN DAILY FOR 15 DAYS UPON THE DEVELOPMENT OF HYPERTENSION PRODUCED BY THE METHOD OF 'GROLLMAN'.

The solid circles (●—●) represent the systolic blood pressures of a group of rats which were treated with guanethidine 10 mg/kg i.p. daily for 15 days, indicated by the bar, following the induction of hypertension by the application of a 'figure-of-eight' ligature on the left kidney (A) and right nephrectomy 7 days later (B). The open circles (○—○) show the blood pressures of a similar group of rats which did not receive guanethidine. Solid triangles (▲—▲) represent the blood pressure of normotensive control rats receiving no treatment. Each point represents the mean systolic blood pressure of the number of rats indicated in parenthesis. Standard errors are shown by vertical bars. The difference between the treated groups was evaluated using Student's 't' test and no significant difference was observed.

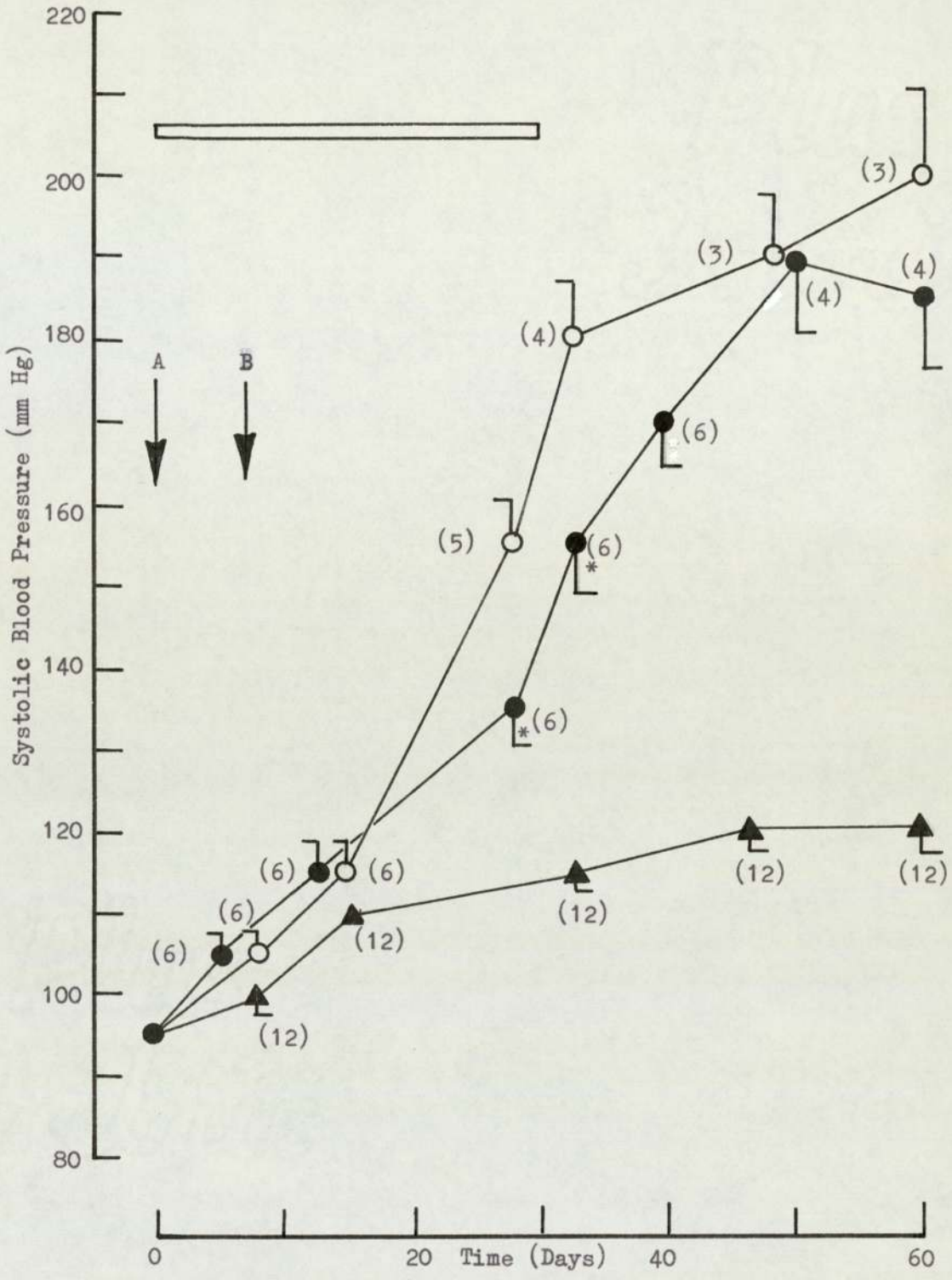


FIGURE 44: THE EFFECT OF GUANETHIDINE GIVEN DAILY FOR 30 DAYS UPON THE DEVELOPMENT OF HYPERTENSION PRODUCED BY THE METHOD OF 'GROLLMAN'.

The solid circles (●—●) represent the systolic blood pressures of a group of rats which were treated with guanethidine 10 mg/kg i.p. daily for 30 days, indicated by the bar, following the induction of hypertension by the application of a 'figure-of-eight' ligature on the left kidney (A) and right nephrectomy 7 days later (B). The open circles (○—○) show the blood pressures of a similar group of rats which did not receive guanethidine. Solid triangles (▲—▲) represent the blood pressure of normotensive control rats receiving no treatment. Each point represents the mean systolic blood pressure of the number of rats indicated in parenthesis. Standard errors are shown by vertical bars. The difference between the treated groups was evaluated using Student's 't' test and the degree of significance is shown by asterisks, ($*P < 0.05$).

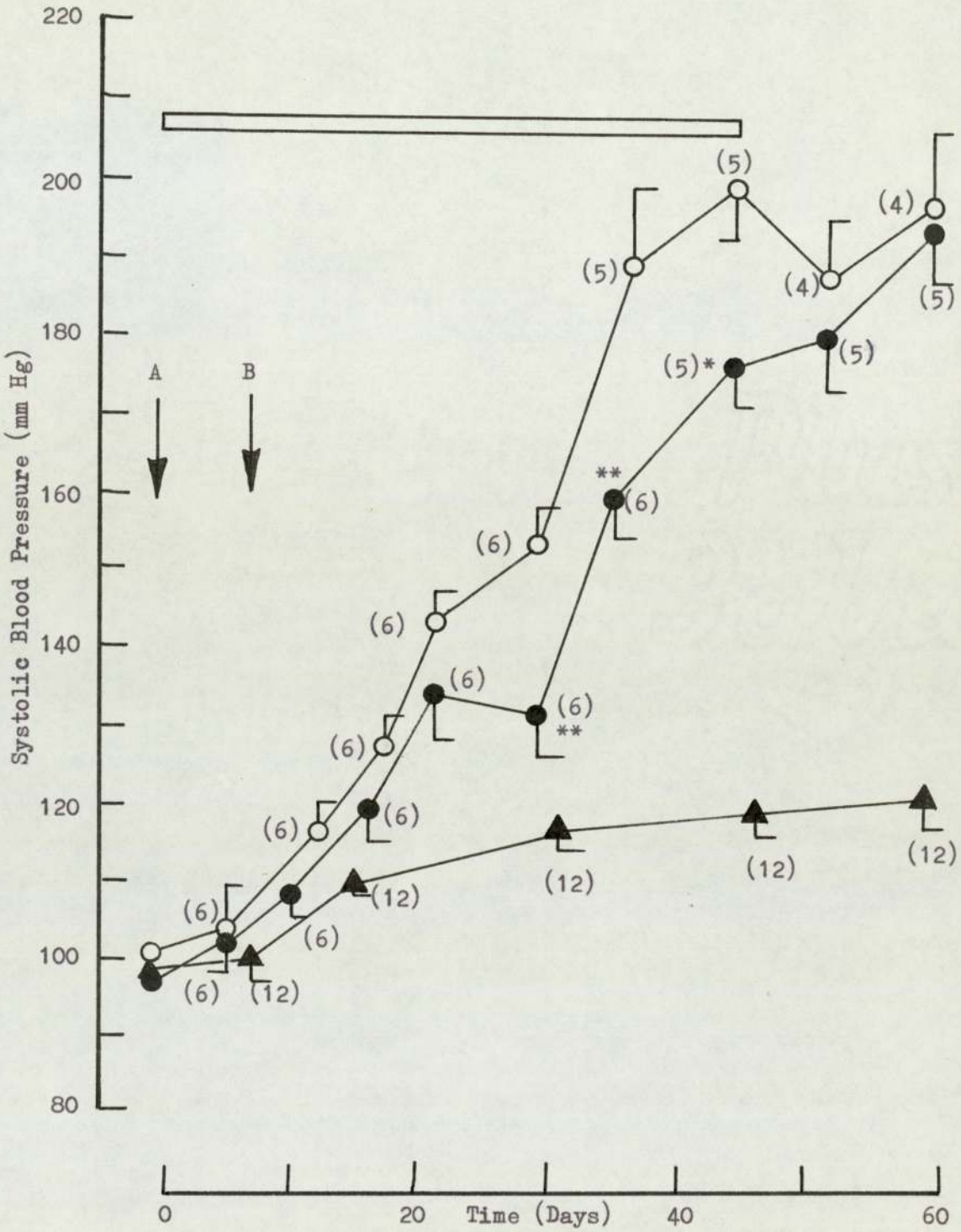


FIGURE 45: THE EFFECT OF GUANETHIDINE GIVEN DAILY FOR 45 DAYS UPON THE DEVELOPMENT OF HYPERTENSION PRODUCED BY THE METHOD OF 'GROLLMAN'.

The solid circles (●—●) represent the systolic blood pressures of a group of rats which were treated with guanethidine 10 mg/kg i.p. daily for 45 days, indicated by the bar, following the induction of hypertension by the application of a 'figure-of-eight' ligature on the left kidney (A) and right nephrectomy 7 days later (B). The open circles (○—○) show the blood pressures of a similar group of rats which did not receive guanethidine. Solid triangles (▲—▲) represent the blood pressure of normotensive control rats receiving no treatment. Each point represents the mean systolic blood pressure of the number of rats indicated in parenthesis. Standard errors are shown by vertical bars. The difference between the treated groups was evaluated using Student's 't' test and the degree of significance is shown by asterisks, (*$P < 0.05$; **$P < 0.02$).

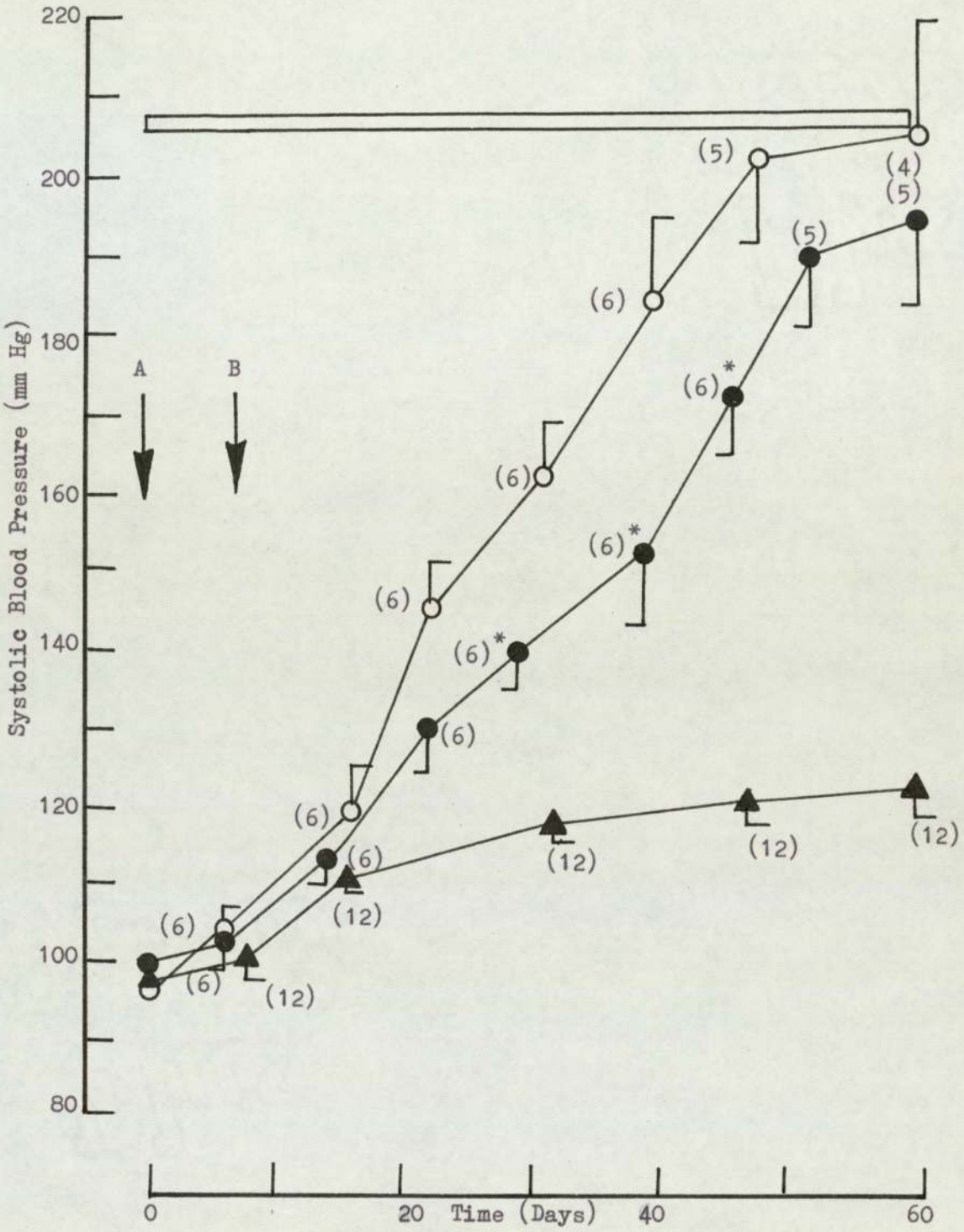


FIGURE 46: THE EFFECT OF GUANETHIDINE GIVEN DAILY FOR 60 DAYS UPON THE DEVELOPMENT OF HYPERTENSION PRODUCED BY THE METHOD OF 'GROLLMAN'.

The solid circles (●—●) represent the systolic blood pressures of a group of rats which were treated with guanethidine 10 mg/kg i.p. daily for 60 days, indicated by the bar, following the induction of hypertension by the application of a 'figure-of-eight' ligature on the left kidney (A) and right nephrectomy 7 days later (B). The open circles (○—○) show the blood pressures of a similar group of rats which did not receive guanethidine. Solid triangles (▲—▲) represent the blood pressure of normotensive control rats receiving no treatment. Each point represents the mean systolic blood pressure of the number of rats indicated in parenthesis. Standard errors are shown by vertical bars. The difference between the treated groups was evaluated using Student's 't' test and the degree of significance is shown by asterisks, ($*P < 0.05$).

control renal operated groups showed large, similar increases in systolic blood pressure attaining hypertensive levels within five weeks. This was followed by further gradual increases in blood pressure before stabilising at levels of 180 mm Hg or more.

Fifteen days treatment with guanethidine had no effect on either the development or maintenance of the hypertension produced by the method of Grollman. However, the guanethidine treated group had a lower systolic blood pressure than that of the control renal operated group throughout the study.

The mean systolic blood pressure of the 30 day guanethidine treated group increased in a similar manner to that of the control renal operated group, although it was significantly lower ($P < 0.05$) near the end of the dosing period. After guanethidine treatment was discontinued the systolic blood pressure continued to rise to attain an observed non-significant difference from the control renal operated group 18 days later, which was maintained for the remainder of the study.

The systolic blood pressure of the 45 day treated group was significantly lower ($P < 0.02$ to $P < 0.05$) than that of the control renal operated group from day 30 of the study until treatment was discontinued. The systolic blood pressures of the two operated groups attained an observed non-significant difference from each other 7 days later and this was maintained until the end of the study.

Similar effects were observed in the 60 day guanethidine treated group. The systolic blood pressure of the treated group was significantly lower ($P < 0.05$) from day 29 to 48 than that of the control renal operated group. However, although guanethidine treatment

continued up to 60 days there was no significant difference between the operated groups at this time.

The complete study was repeated and in this case the operator who determined the systolic blood pressures was unaware of which group was being studied. The results again showed that guanethidine treatment did not prevent the production of one-kidney renal hypertension, although the systolic blood pressures of the guanethidine treated group were consistently, though they rarely achieved a statistically significant difference, lower than the control renal operated group (for example, see Fig. 47)

In both series of experiments the mortality rates of both the guanethidine treated and control renal operated groups were similar throughout the sixty days study and similar to that previously described (see p.108). The body weights of the operated groups were smaller than those of the control group throughout the study but a statistically significant difference was only observed for a short duration after the operative procedures.

Discussion

The potent adrenergic neuronal blocking action of guanethidine was confirmed in this study. Twenty-four hours after a single i.p. injection of 10 mg/kg guanethidine, the sympathetic nervous system was severely depressed, the 'frequency-response' curve being moved far to the right and the maximum response greatly decreased. The potent adrenergic neuronal block was also observed on the sympathetic cardiac nerves, increases in heart rate were observed in controls at 0.25 Hz but after guanethidine, increases in heart rate did not occur until 4 Hz and the maximum effect was severely reduced

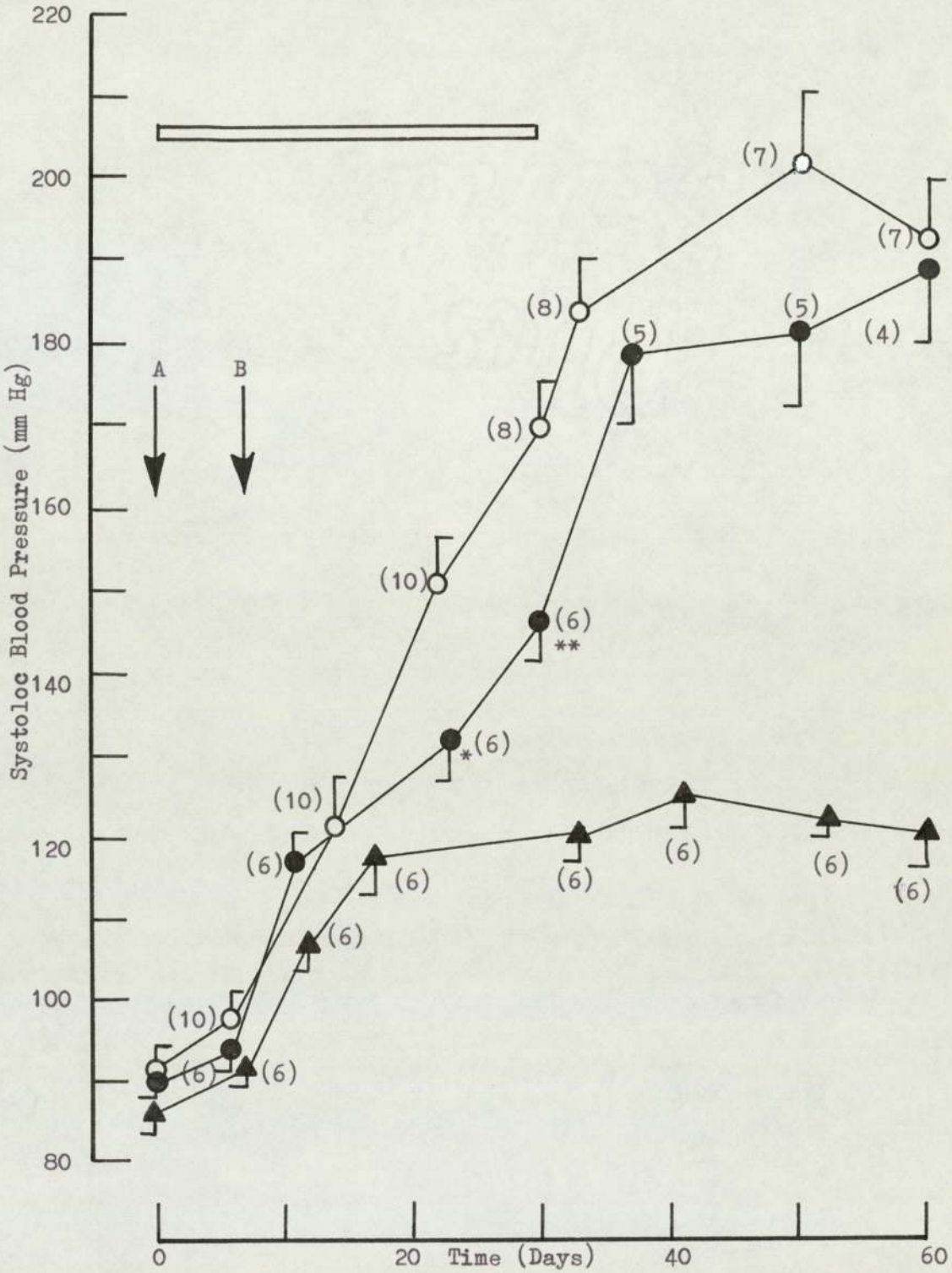


FIGURE 47: THE EFFECT OF GUANETHIDINE GIVEN DAILY FOR 30 DAYS UPON THE DEVELOPMENT OF HYPERTENSION PRODUCED BY THE METHOD OF 'GROLLMAN'. (BLIND STUDY)

The solid circles (●—●) represent the systolic blood pressures of a group of rats which were treated with guanethidine 10 mg/kg i.p. daily for 30 days, indicated by the bar, following the induction of hypertension by the application of a 'figure-of-eight' ligature on the left kidney (A) and right nephrectomy 7 days later (B). The open circles (○—○) show the blood pressures of a similar group of rats which did not receive guanethidine. Solid triangles (▲—▲) represent the blood pressure of normotensive control rats receiving no treatment. Each point represents the mean systolic blood pressure of the number of rats indicated in parenthesis. Standard errors are shown by vertical bars. The difference between the treated groups was evaluated using Student's 't' test and the degree of significance is shown by asterisks, ( $*P < 0.05$; $**P < 0.02$)

when compared to the increase in control rats. This corresponds closely with the work of Armstrong & Boura (1973) who reported the same shift of the 'frequency-response' curve after stimulation of the sympathetic cardiac nerves immediately after an i.v. dose of 2 mg/kg guanethidine. Thus, the adrenergic neuronal blockade produced by a single i.p. dose of guanethidine is potent, of long duration and ideally suited to the present study.

The results of these studies strongly suggest that the sympathetic nervous system is essential for the production of DOCA-NaCl hypertension in rats.

The systolic blood pressures of the guanethidine treated rats did increase significantly ($P < 0.001$) above those of the control group during the first 14 days. Although there was no statistically significant difference between the systolic blood pressures of the guanethidine treated rats and their respective unoperated control groups after 14 days and up to 28 days the systolic blood pressures of the former were higher. This increase is probably due to the release of catecholamines from the adrenal medulla which is not blocked by guanethidine (see review by Boura & Green, 1965). De Champlain (1972) reported similar results, after chemical sympathectomy with 6-hydroxydopamine in DOCA-NaCl rats and also suggested that this was due to increased release of catecholamines from the adrenal medulla.

After discontinuation of guanethidine treatment the systolic blood pressures of each of the four groups rose in an almost parallel manner to that of their respective operated control groups. This indicates that the sympathetic nervous system is essential for the production of DOCA-NaCl hypertension and once the peripheral sympathetic nervous

system is again functional blood pressure elevation due to the DOCA-NaCl regimen occurs.

The results of this study lend support to the hypothesis of De Champlain and co-workers (see review by De Champlain, 1972) that the rise in blood pressure to DOCA-NaCl treatment is due to a dysfunctional state of the peripheral sympathetic nervous system, characterised by an increased turnover rate of noradrenaline, mediated via decreased sympathetic activity in the medulla oblongata. These workers also reported that the increased turnover rate of noradrenaline could be normalised by the administration of ganglion blockers and result in the blood pressure returning to normotensive levels. Guanethidine would presumably produce a similar normalisation of the increased turnover rate or prevent its occurrence and thus prevent the rise in blood pressure to DOCA-NaCl treatment. Once the adrenergic neuronal blocking action of guanethidine has waned, the noradrenaline turnover rate of peripheral sympathetic nerves will increase and result in the usual blood pressure elevation as observed in the present study.

The decrease in body weight gain in the DOCA-NaCl control groups has been reported and discussed earlier in this thesis (see p.121). It might have been expected that the guanethidine treated groups would have shown body weight gains more similar to the control normotensive group, rather than decreased body weight gains similar to those of the control DOCA-NaCl group which was in fact observed. This effect may well have been due to the stress involved in administering the daily i.p. injections of guanethidine which were not controlled by saline injections in the two control groups.

The results of this present study indicate that the sympathetic nervous system is not necessary for the induction of one-kidney renal hypertension in rats.

The systolic blood pressures of the 15 day guanethidine treated animals at no time showed significant differences from those of the control renal operated groups. This is in accord with the hypothesis that the renin-angiotensin system and not the peripheral sympathetic nervous system is responsible for the production of one-kidney renal hypertension in rats. However, in the 30, 45 and 60 day guanethidine treated animals the systolic blood pressures did show occasional significant decreases from the control renal operated groups and generally the systolic blood pressures of the guanethidine treated rats were lower than those of the control renal operated groups. This is probably due to the fact that with resetting of the baroreceptors at a higher level (see reviews by Aars, 1975 and Korner, 1975) the sympathetic nervous system becomes involved in the maintenance of renal hypertension (see reviews by Page & McCubbin, 1968; De Champlain, 1972 and Schmid & Abboud, 1974) and becomes susceptible to guanethidine. However, the blood pressure continued to rise and did not fall to control normotensive levels thus indicating other systems must be maintaining the elevated blood pressure. These may involve continued influence of the renin-angiotensin system (see reviews by De Champlain, 1972 and Tobian, 1974), release of catecholamines from the adrenal medulla which is not affected by guanethidine (see review by Boura & Green, 1965) or the induced supersensitivity of smooth muscle to catecholamines by guanethidine (Boura & Green, 1962) and angiotensin (e.g. Zimmerman, 1967; Lowe & Scroop, 1970). Also it has been shown that in established renal hypertension, if the sympathetic nervous system is blocked or destroyed the blood pressure falls but within a few days arterial pressure returns to its previous level by largely, non-neurogenic mechanisms (see review by Page & McCubbin, 1968).

The systolic blood pressures of the 60 day guanethidine treated groups did not differ significantly from the control renal hypertensive group although it would be expected that at this stage when the blood pressure had stabilised at a high level guanethidine would produce a fall in blood pressure. Since it is known that guanethidine produces a fall in the blood pressure of one-kidney renal hypertensive rats to normotensive levels (see review by Boura & Green, 1965) this lack of effect in the present study may be due to the development of tolerance to guanethidine although this possibility was not examined.

Although it may be suggested from these studies that the sympathetic nervous system is involved in the production of DOCA-NaCl hypertension in rats but is not essential for the production of one-kidney renal hypertension in rats the experimental procedures employed were subject to several criticisms. It was considered that the length of time between the systolic blood pressure determinations of each group were excessive and possibly important changes in blood pressure may have been overlooked. Also the increase in systolic blood pressure in DOCA-NaCl rats after discontinuation of guanethidine treatment, may have been due to an 'overshoot' phenomenon of the drug and in order to examine whether or not this was true a control normotensive group receiving guanethidine treatment was essential. It was also probable that the lack of a hypotensive effect of guanethidine when one-kidney renal hypertension was established may have been due to the development of tolerance. It was considered that this possibility could be examined by assessing the degree of adrenergic neuronal blockade in pithed guanethidine treated rats at the completion of each treatment period. The apparent lack of a protective action of guanethidine to the hypertensive

disease processes, resulting from either a DOCA-NaCl regimen or 'figure-of-eight' perinephritis was not investigated in this study. More information could have been obtained by examining various organs from rats of each group both macroscopically and microscopically, at the end of the study. Finally, the control groups were not subject to laparotomy or daily i.p. injections of 0.9% saline which would have provided information as to whether or not the stress, possibly produced by the operation to induce hypertension and by the daily administration of guanethidine, was responsible for any of the observed results, particularly the decreased body weight gains observed in the guanethidine treated groups. Thus it was decided to repeat these studies in the light of these criticisms.

Summary

1. The role of the sympathetic nervous system in the production and maintenance of DOCA-NaCl and one-kidney renal hypertensions in rats has been studied by blocking the peripheral sympathetic nervous system, for various periods of time, with guanethidine.

2. The adrenergic neuronal blocking action of a 10 mg/kg i.p. dose of guanethidine was shown to be both potent and of long duration.

3. The blood pressures of DOCA-NaCl rats treated with guanethidine for 7, 14, 21 and 28 days remained at normotensive levels whilst guanethidine was administered. Discontinuation of guanethidine treatment resulted in a rise of blood pressure of these rats, in a manner parallel to that of control DOCA-NaCl rats, to hypertensive levels.

4. The production of one-kidney renal hypertension in rats was only slightly affected by 15, 30, 45 and 60 days guanethidine treatment when compared with control renal operated animals.

5. The experiments were repeated in a 'blind' study and the results

obtained confirmed those of the initial studies.

6. It was concluded from these studies that the sympathetic nervous system was involved in the production of DOCA-NaCl hypertension in rats but was not essential for the production of one-kidney renal hypertension in the same species. However, the experimental procedures employed in these experiments were criticised and it was decided to repeat the study taking these criticisms into consideration.

## CHAPTER 2

## Effect of Postganglionic Adrenergic Neuronal Blockade on the Production of Experimental Hypertension in the Rat - Further Studies.

Although the previous study, examining the role of the sympathetic nervous system in the production of both DOCA-NaCl and one-kidney renal hypertensions, produced some idea of the aetiologies of these hypertensions, the experimental procedures employed were subject to several criticisms (see pp. 181 to 182). Thus it was decided to repeat the study, in a more detailed manner, taking the objections raised into consideration.

Unfortunately it was found impossible at the time of this study to obtain Wistar rats from the same suppliers (Fisons Limited) as those used in the previous study. The rats used in this study were of the same strain but obtained from a different supplier (Animal Supplies Limited) and it was thought unlikely that these rats would respond differently to the drugs and/or experimental procedures to be used.

### Results

The production of a hypertensive state in ASL rats, using a DOCA-NaCl regimen, was found to be slower than that when using Wistar rats from the previous supplier and it was found necessary to maintain the animals on 1% sodium chloride solution for 4 weeks to produce a satisfactory degree of hypertension. Guanethidine was therefore administered for periods of 15, 30, 45 and 60 days rather than 7, 14, 21 and 28 days as used in the previous study. The effects of these periods of guanethidine treatment on the production of DOCA-NaCl hypertension in rats are shown in Figs. 48,

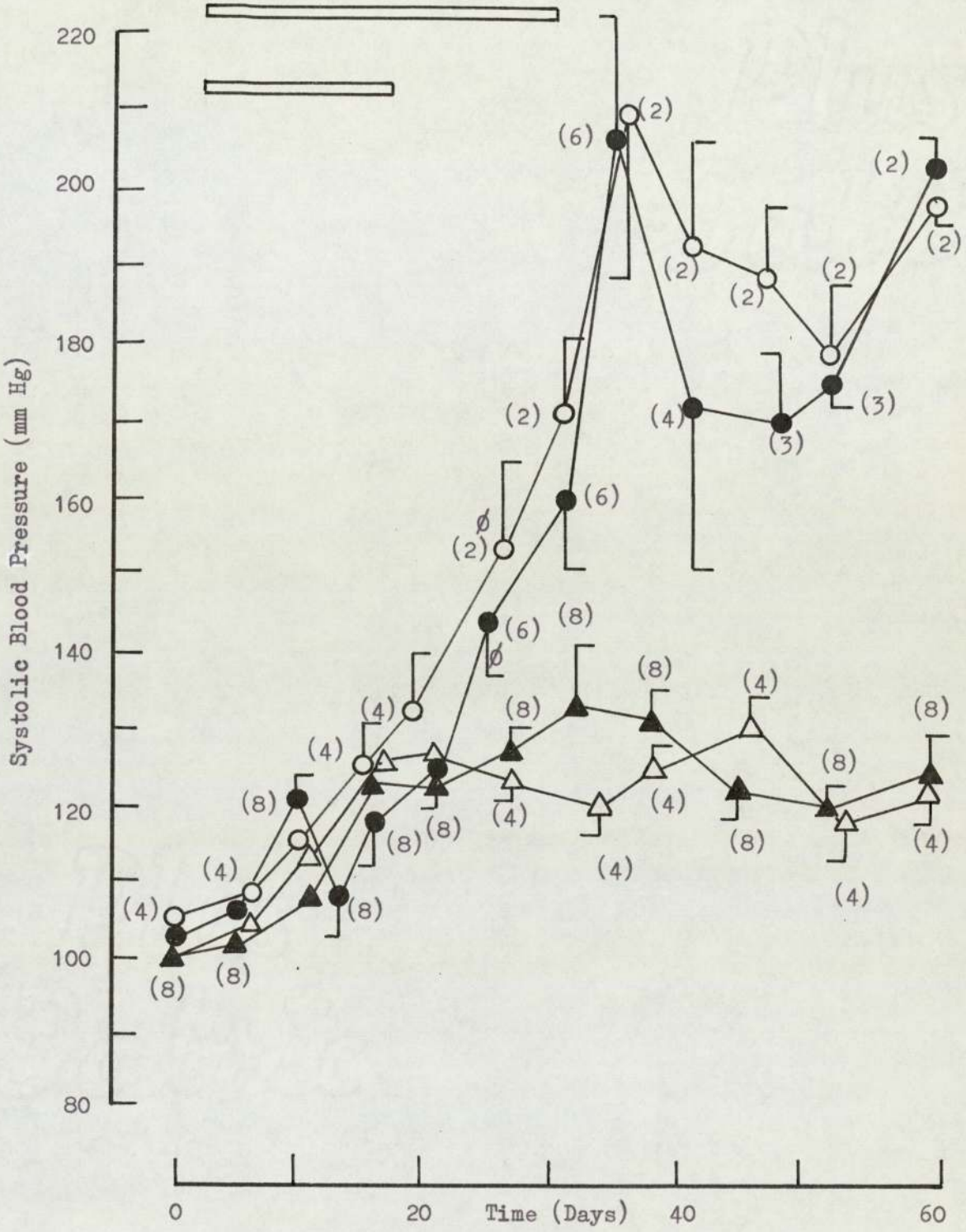


FIGURE 48: THE EFFECT OF GUANETHIDINE GIVEN DAILY FOR 15 DAYS UPON THE DEVELOPMENT OF HYPERTENSION PRODUCED BY A DOCA-NaCl REGIMEN.

Systolic blood pressures of 'sham-operated' control normotensive rats (△—△), 'sham-operated' guanethidine treated normotensive rats (▲—▲), control DOCA-NaCl rats (○—○) and guanethidine treated DOCA-NaCl rats (●—●) are shown above. The upper bar shows the duration of replacement of drinking water with 1% NaCl solution. The lower bar represents the duration of guanethidine treatment. Each point represents the mean of the number of animals, shown in parenthesis where possible. Standard errors are shown by vertical bars. Significant differences between the DOCA-NaCl groups were evaluated using the Student's 't' test and no significant difference was observed. The point at which the blood pressures of DOCA-NaCl groups became significantly different from their control normotensive groups is shown by Ø.

49, 50 and 51.

The systolic blood pressures of the control DOCA-NaCl groups of rats gradually increased from the time of the operation to attain hypertensive levels 22 - 33 days later. The blood pressure continued to rise after this time and usually stabilised at levels of 200 mm Hg or more. The systolic blood pressures of the control normotensive groups of rats increased gradually before stabilising at levels of 125 - 130 mm Hg.

The systolic blood pressures of the normotensive groups of rats receiving guanethidine were very similar to those of the control normotensive groups of animals receiving i.p. injections of 0.9% saline and a statistically significant difference in the blood pressures of these two groups was only observed in one instance. However, the blood pressure of the 30, 45 and 60 day guanethidine treated normotensive groups of rats rose abruptly between day 26 and 35 of the study.

Fifteen days treatment with guanethidine had no statistically significant effect on either the production or maintenance of DOCA-NaCl hypertension when compared with the control DOCA-NaCl group.

The systolic blood pressures of the 30 day guanethidine treated DOCA-NaCl group were significantly ($P < 0.5$ to $P < 0.001$) lower than those of the control DOCA-NaCl group from day 21 to day 35 of the study. The systolic blood pressure of the guanethidine treated group increased from day 31 of the study to attain an observed non-significant difference from the control DOCA-NaCl group 10 days later and this was maintained throughout the rest of the study.

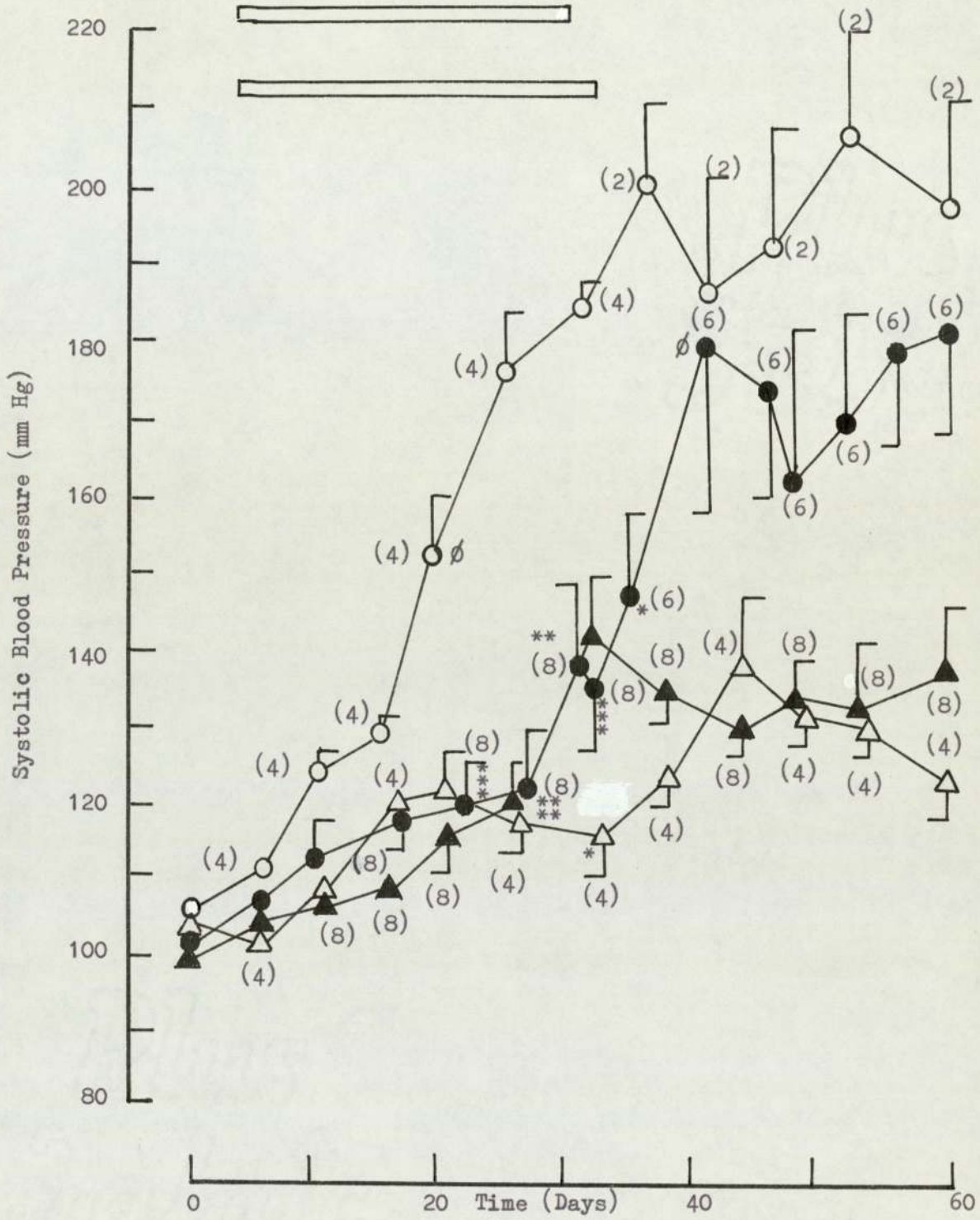


FIGURE 49: THE EFFECT OF GUANETHIDINE GIVEN DAILY FOR 30 DAYS UPON THE DEVELOPMENT OF HYPERTENSION PRODUCED BY A DOCA-NaCl REGIMEN.

Systolic blood pressures of 'sham-operated' control normotensive rats (△—△), 'sham-operated' guanethidine treated normotensive rats (▲—▲), control DOCA-NaCl rats (○—○) and guanethidine treated DOCA-NaCl rats (●—●) are shown above. The upper bar shows the duration of replacement of drinking water with 1% NaCl solution. The lower bar represents the duration of guanethidine treatment. Each point represents the mean of the number of animals, shown in parenthesis where possible. Standard errors are shown by vertical bars. Significant differences between the DOCA-NaCl groups were evaluated using the Student's 't' test as were those between the normotensive groups and the level of significance is shown by asterisks (*P < 0.05; **P < 0.02; ***P < 0.01; ***P < 0.001). The point at which the blood pressures of DOCA-NaCl groups became significantly different from their control normotensive groups is shown by ø.

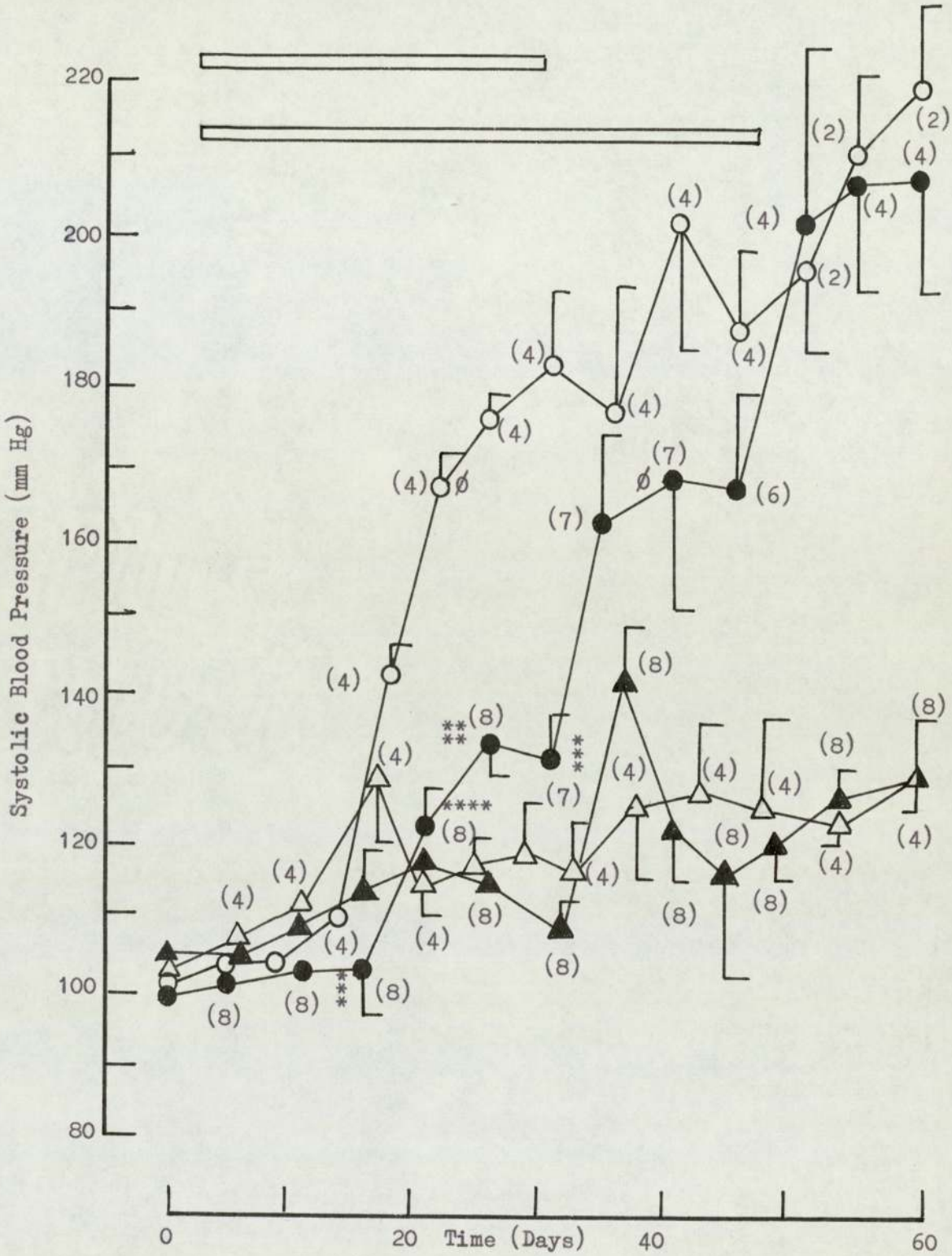


FIGURE 50: THE EFFECT OF GUANETHIDINE GIVEN DAILY FOR 45 DAYS UPON THE DEVELOPMENT OF HYPERTENSION PRODUCED BY A DOCA-NaCl REGIMEN.

Systolic blood pressures of 'sham-operated' control normotensive rats (△—△), 'sham-operated' guanethidine treated normotensive rats (▲—▲), control DOCA-NaCl rats (○—○) and guanethidine treated DOCA-NaCl rats (●—●) are shown above. The upper bar shows the duration of replacement of drinking water with 1% NaCl solution. The lower bar represents the duration of guanethidine treatment. Each point represents the mean of the number of animals, shown in parenthesis where possible. Standard errors are shown by vertical bars. Significant differences between the DOCA-NaCl groups were evaluated using the Student's 't' test and the level of significance is shown by asterisks ($^{**}P < 0.01$; $^{***}P < 0.001$). The point at which the blood pressures of DOCA-NaCl groups became significantly different from their control normotensive groups is shown by ø.

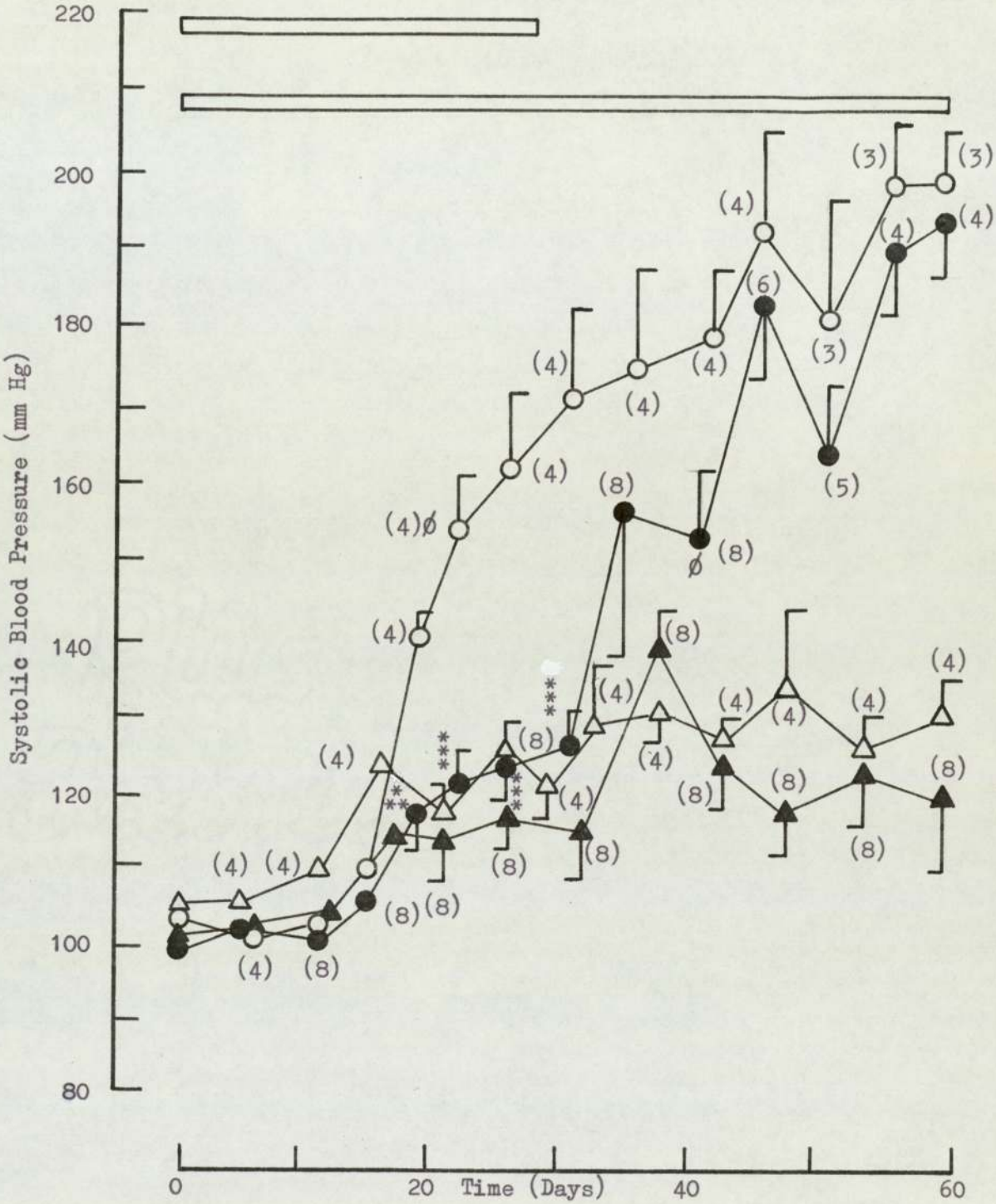


FIGURE 51: THE EFFECT OF GUANETHIDINE GIVEN DAILY FOR 60 DAYS UPON THE DEVELOPMENT OF HYPERTENSION PRODUCED BY A DOCA-NaCl REGIMEN.

Systolic blood pressures of 'sham-operated' control normotensive rats (△—△), 'sham-operated' guanethidine treated normotensive rats (▲—▲), control DOCA-NaCl rats (○—○) and guanethidine treated DOCA-NaCl rats (●—●) are shown above. The upper bar shows the duration of replacement of drinking water with 1% NaCl solution. The lower bar represents the duration of guanethidine treatment. Each point represents the mean of the number of animals, shown in parenthesis where possible. Standard errors are shown by vertical bars. Significant differences between the DOCA-NaCl groups were evaluated using the Student's 't' test and the level of significance is shown by asterisks (**P < 0.01). The point at which the blood pressures of DOCA-NaCl groups became significantly different from their control normotensive groups is shown by ø.

Similarly the systolic blood pressures of the 45 and 60 day guanethidine treated DOCA-NaCl groups were significantly ($P < 0.01$ to $P < 0.001$) lower than those of their respective control groups until day 31 of the study. The systolic blood pressures of the guanethidine treated DOCA-NaCl groups attained observed non-significant differences from their respective control groups 4 days later which were maintained for the remaining period of study.

Due to the slower production of DOCA-NaCl hypertension in the Wistar rats from ASL it was decided that a hypertensive state would be more readily obtained in ASL rats by performing unilateral nephrectomy at the same time as applying the 'figure-of-eight' ligature to the left kidney rather than 7 days later as was the case in the initial study. The effect of 15, 30, 45 and 60 days period of guanethidine treatment on the production of one-kidney renal hypertension in rats is shown in Figs. 52, 53, 54 and 55.

The systolic blood pressures of the control renal operated groups of rats gradually increased from the time of the operation to induce hypertension until 21 to 32 days later when hypertensive levels were obtained. The systolic blood pressures continued to increase after this time and stabilised at levels of 185 mm Hg or above. The systolic blood pressures of the control normotensive groups of rats increased gradually before stabilising at levels of 130 - 135 mm Hg.

The systolic blood pressures of the normotensive groups of rats receiving guanethidine were very similar to those of the control normotensive animals receiving i.p. injections of 0.9% saline and a statistically significant difference in the blood pressure of these two groups was only observed in four instances.

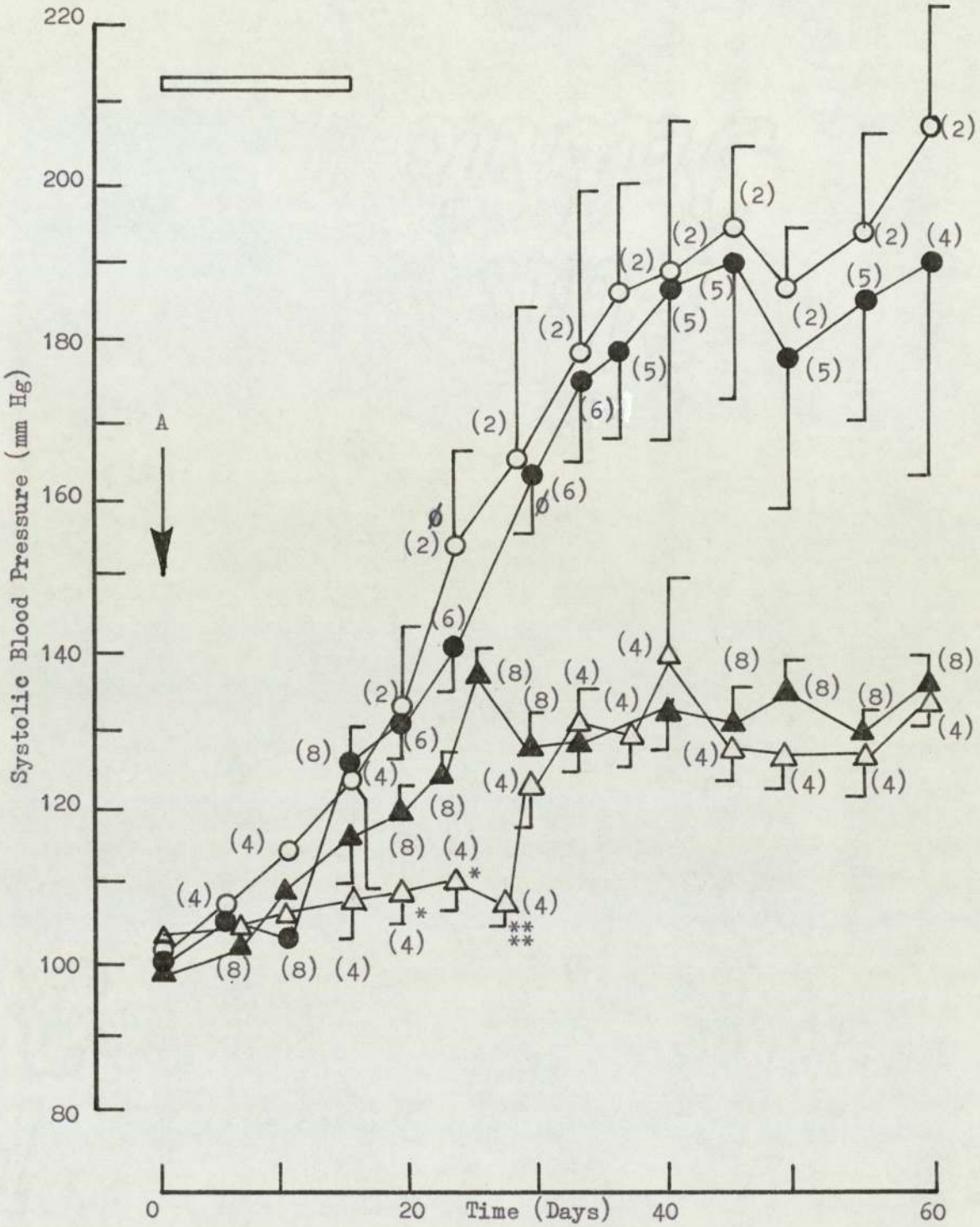


FIGURE 52: THE EFFECT OF GUANETHIDINE GIVEN DAILY FOR 15 DAYS UPON THE DEVELOPMENT OF HYPERTENSION PRODUCED BY THE METHOD OF 'GROLLMAN'.

Systolic blood pressures of 'sham-operated' control normotensive rats (△—△), 'sham-operated' guanethidine treated normotensive rats (▲—▲), control 'Grollman' rats (○—○) and guanethidine treated 'Grollman' rats (●—●) are shown above. At (A) a 'figure-of-eight' ligature was applied to the left kidney and contralateral nephrectomy performed. The bar represents the duration of guanethidine treatment. Each point represents the mean of the number of animals, shown in parenthesis where possible. Standard errors are shown by vertical bars. Significant differences between the 'Grollman' groups were evaluated using the Student's 't' test as were those between the normotensive groups and the level of significance is shown by asterisks (*P < 0.05; **P < 0.001). The point at which the blood pressures of 'Grollman' groups became significantly different from their control normotensive groups is shown by Ø.

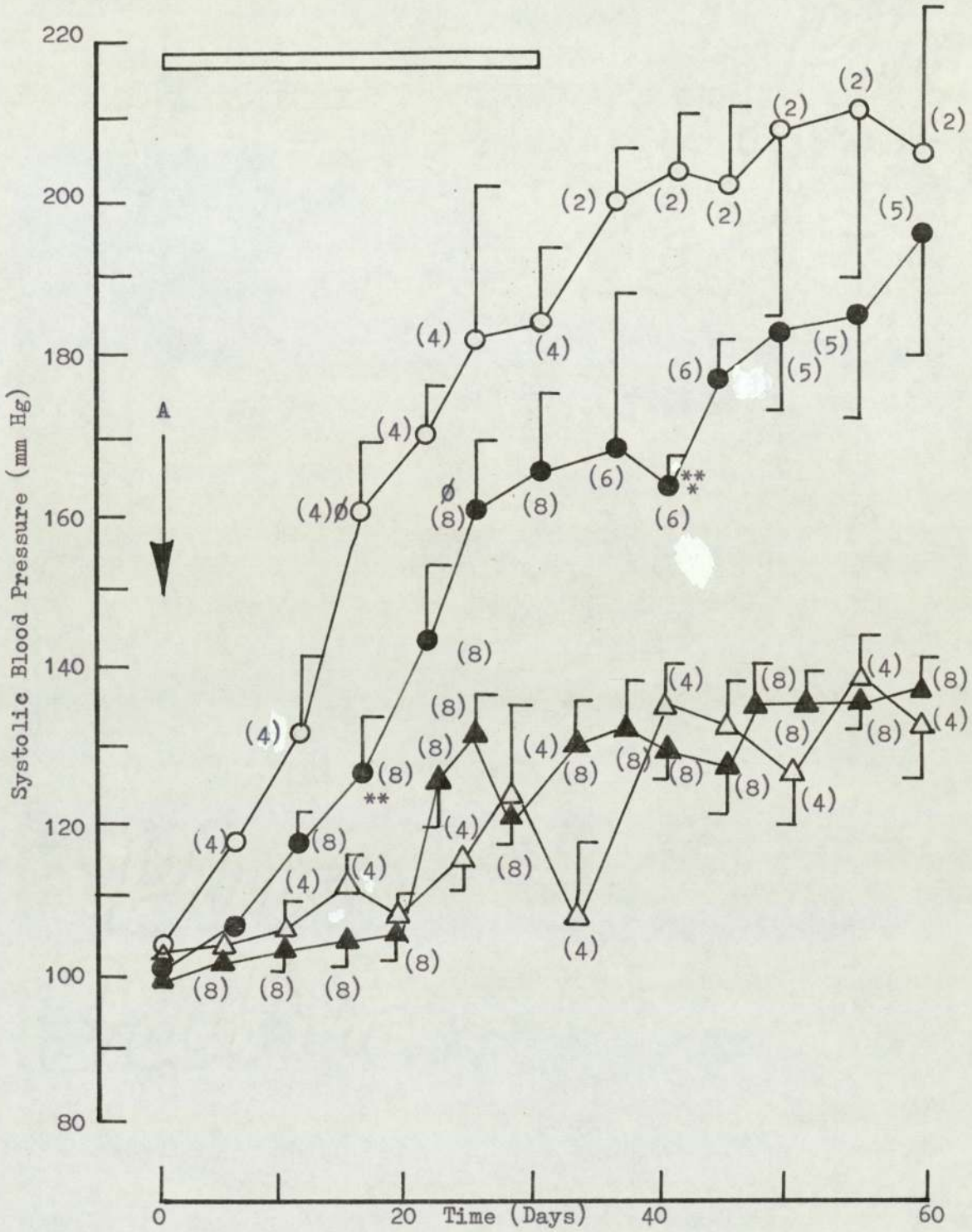


FIGURE 53: THE EFFECT OF GUANETHIDINE GIVEN DAILY FOR 30 DAYS UPON THE DEVELOPMENT OF HYPERTENSION PRODUCED BY THE METHOD OF 'GROLLMAN'.

Systolic blood pressures of 'sham-operated' control normotensive rats (△—△), 'sham-operated' guanethidine treated normotensive rats (▲—▲), control 'Grollman' rats (○—○) and guanethidine treated 'Grollman' rats (●—●) are shown above. At (A) a 'figure-of-eight' ligature was applied to the left kidney and contralateral nephrectomy performed. The bar represents the duration of guanethidine treatment. Each point represents the mean of the number of animals, shown in parenthesis where possible. Standard errors are shown by vertical bars. Significant differences between the 'Grollman' groups were evaluated using the Student's 't' test and the level of significance is shown by asterisks (**P < 0.02; $\overset{**}{*}$P < 0.01). The point at which the blood pressures of 'Grollman' groups became significantly different from their control normotensive groups is shown by ø.

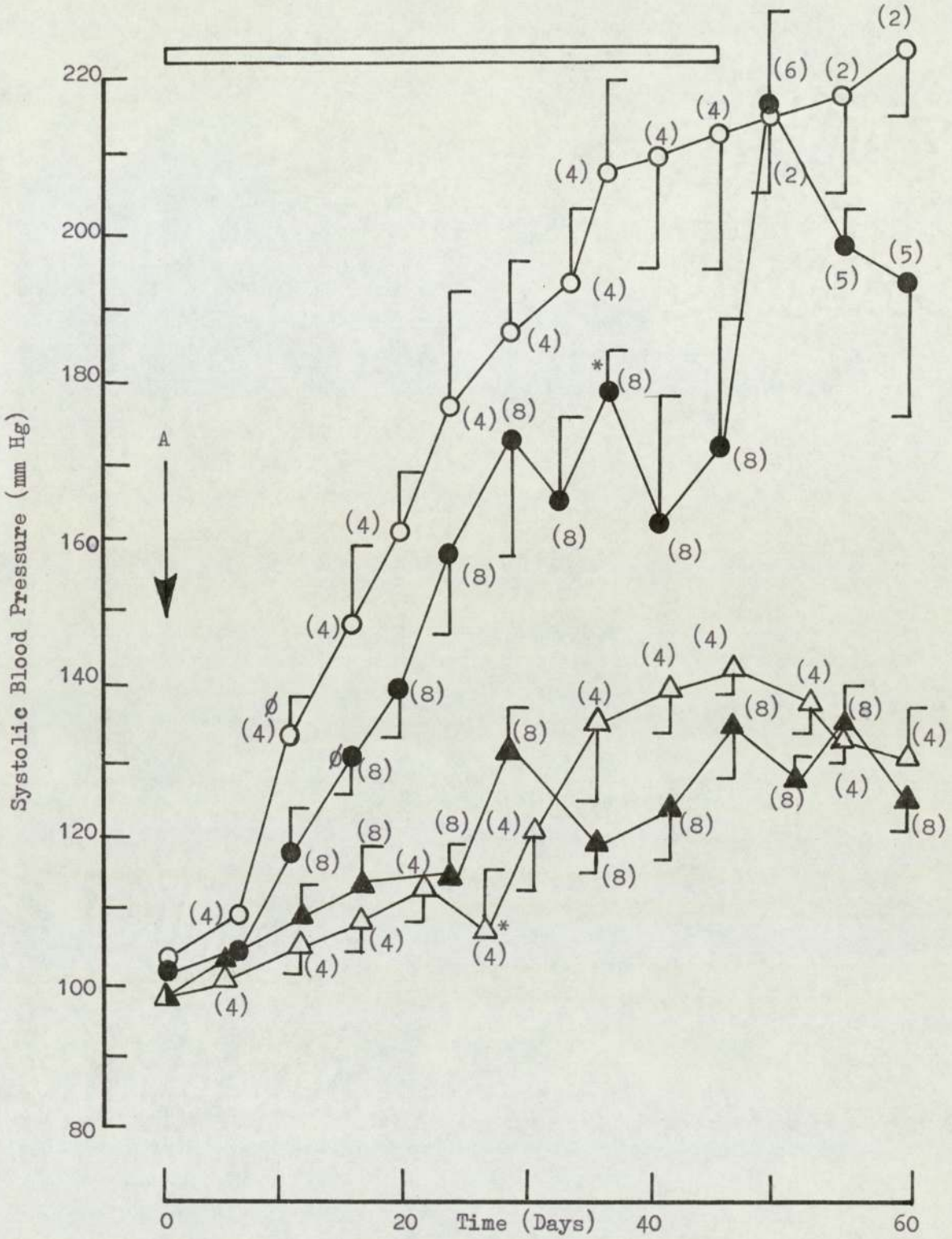


FIGURE 54: THE EFFECT OF GUANETHIDINE GIVEN DAILY FOR 45 DAYS UPON THE DEVELOPMENT OF HYPERTENSION PRODUCED BY THE METHOD OF 'GROLLMAN'.

Systolic blood pressures of 'sham-operated' control normotensive rats (△—△), 'sham-operated' guanethidine treated normotensive rats (▲—▲), control 'Grollman' rats (○—○) and guanethidine treated 'Grollman' rats (●—●) are shown above. At (A) a 'figure-of-eight' ligature was applied to the left kidney and contralateral nephrectomy performed. The bar represents the duration of guanethidine treatment. Each point represents the mean of the number of animals, shown in parenthesis where possible. Standard errors are shown by vertical bars. Significant differences between the 'Grollman' groups were evaluated using the Student's 't' test as were those between the normotensive groups and the level of significance is shown by asterisks (*$P < 0.05$). The point at which the blood pressures of 'Grollman' groups became significantly different from their control normotensive groups is shown by ø.

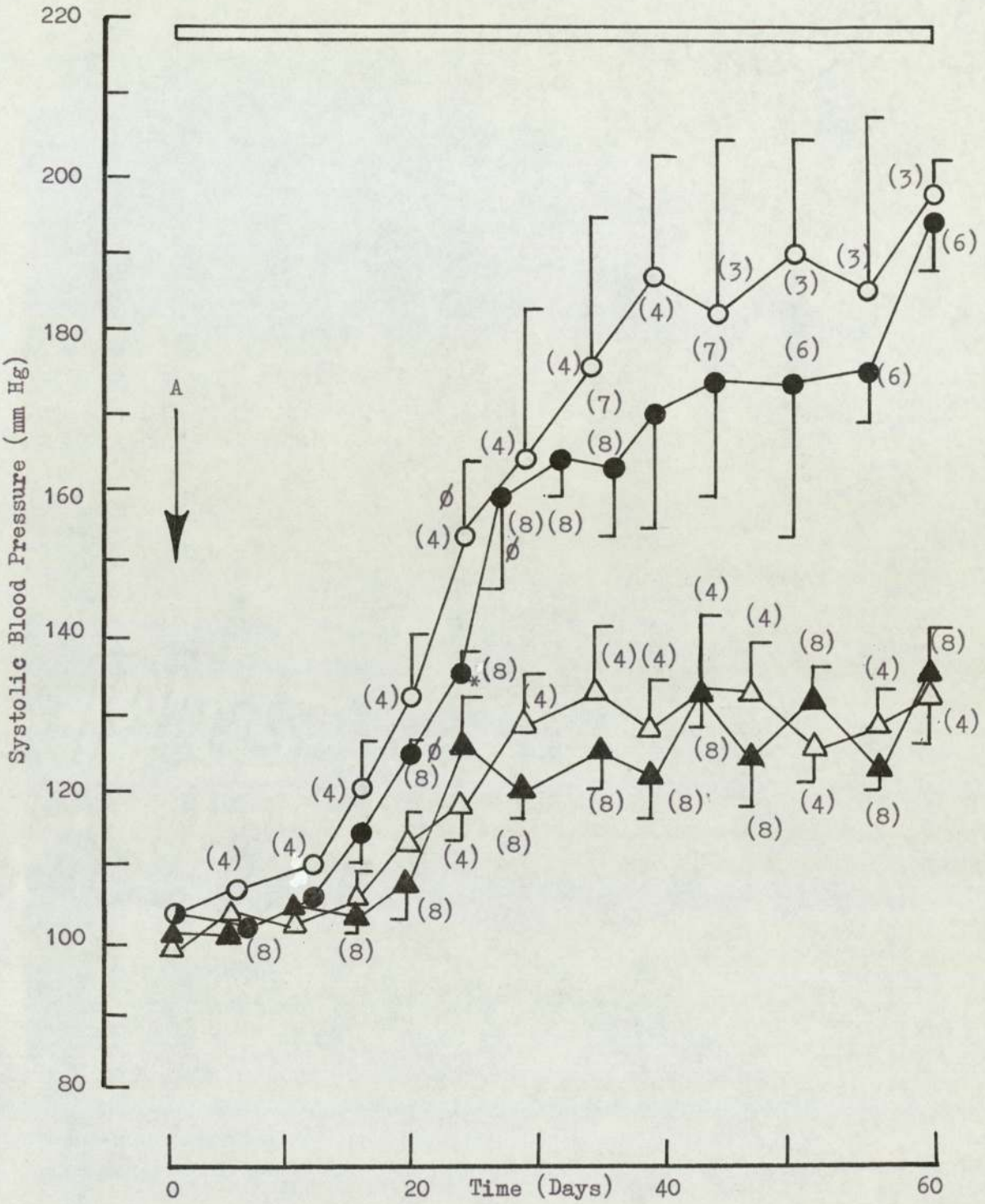


FIGURE 55: THE EFFECT OF GUANETHIDINE GIVEN DAILY FOR 60 DAYS UPON THE DEVELOPMENT OF HYPERTENSION PRODUCED BY THE METHOD OF 'GROLLMAN'.

Systolic blood pressures of 'sham-operated' control normotensive rats (△—△), 'sham-operated' guanethidine treated normotensive rats (▲—▲), control 'Grollman' rats (○—○) and guanethidine treated 'Grollman' rats (●—●) are shown above. At (A) a 'figure-of-eight' ligature was applied to the left kidney and contralateral nephrectomy performed. The bar represents the duration of guanethidine treatment. Each point represents the mean of the number of animals, shown in parenthesis where possible. Standard errors are shown by vertical bars. Significant differences between the 'Grollman' groups were evaluated using the Student's 't' test and the level of significance is shown by asterisks (*P < 0.05). The point at which the blood pressures of 'Grollman' groups became significantly different from their control normotensive groups is shown by ø.

Fifteen days treatment with guanethidine had no statistically significant effect on either the production or maintenance of one-kidney renal hypertension when compared with the control renal operated group.

The systolic blood pressures of the 30 day guanethidine treated renal operated group of rats were significantly ($P < 0.02$ to $P < 0.01$) lower than those of the control renal operated animals on only two separate occasions throughout the 60 days study period. However, the blood pressures of the guanethidine treated group of rats were always lower than those of the control renal operated group of rats.

Similarly the systolic blood pressures of the 45 and 60 day guanethidine treated renal operated groups of rats, although consistently lower than those of their respective control renal operated groups, were significantly ($P < 0.05$) lower on only one occasion in each case.

In these further studies, involving the production of either DOCA-NaCl or renal hypertensions, no statistically significant difference was observed in the heart rates of any of the four groups for each period of treatment of guanethidine. However, in the early periods of guanethidine treatment the heart rates of both hypertensive and normotensive guanethidine treated groups of rats were lower than those of their respective control groups.

In both the DOCA-NaCl and renal hypertensive studies the body weights of the four groups of rats at each of the four periods of guanethidine treatment were in each case in the order:- control normotensive > guanethidine treated normotensive > guanethidine treated hypertensive > control hypertensive from day 12 (in the case of the DOCA-NaCl experiments) and day 15 (in the case of the renal experiments). However, due to the wide variation in the body weights of the control and guanethidine treated hypertensive groups of rats

no statistically significant difference was observed between any of the four groups of rats during each of the four periods of guanethidine treatment.

In the DOCA-NaCl study the general condition of the animals from each of the four groups at each period of guanethidine treatment were similar although some of the rats from the control and guanethidine treated DOCA-NaCl groups of rats exhibited the typical appearance of DOCA-NaCl hypertensive rats described earlier (see p.113). The duration of guanethidine treatment did not appear to effect the general condition of the rats and the control DOCA-NaCl rats were in general in a condition more similar to that of control normotensive animals than either Fisons or Sc.P.F. DOCA-NaCl hypertensive rats (see p.140). Similarly the mortalities observed in this study were lower than those observed in the initial study. Similar results were observed in the study involving renal hypertension although the general condition of the rats and the mortalities which occurred in these further studies were similar to those observed in the initial experiments.

The weights of the various organs, expressed as a percentage of body weight, taken on post-mortem of the rats at the completion of the DOCA-NaCl study did show some significant differences. Both heart and kidney weights of the control and guanethidine treated DOCA-NaCl groups were significantly increased ($P < 0.001$) over their respective normotensive groups. There was no significant difference between the weights of the liver and adrenal glands between any of the groups of rats. There was no significant difference in the weights of any of the organs from the control or guanethidine treated DOCA-NaCl

groups of rats. Similarly there was no significant difference in the organ weights of the normotensive groups of rats. In the study involving renal hypertension similar results (that is, increased heart and kidney weights in the renal operated animals) were obtained although the lack of increased adrenal gland weight was surprising.

Histological examination of the hearts, kidneys, livers and adrenal glands from one rat of each of the 16 groups of animals involved in these further studies was performed. No apparent effects were observed in any of the tissues from rats of the normotensive groups. The histological appearance of the tissues from the control and guanethidine treated hypertensive rats were similar and as described earlier (see pp. 115 to 117).

The effect of stimulation of the sympathetic outflow from the spinal cord of two guanethidine treated DOCA-NaCl and renal operated rats and two of their respective control hypertensive rats at the completion of each period of guanethidine treatment was examined. In both the control DOCA-NaCl and control renal hypertensive rats the blood pressure responses increased with increasing stimulation frequencies from 1.25 to 16 Hz. The occurrence of cardiac arrythmias at 16 Hz prevented any higher stimulation frequencies being used. In both the guanethidine treated DOCA-NaCl and guanethidine treated renal operated rats the 'frequency-response' curve was moved to the right with maximum increases in blood pressure occurring at 64 Hz. The maximum blood pressure responses of the control hypertensive and guanethidine treated DOCA-NaCl and renal operated rats after each period of guanethidine treatment are shown in Fig. 56. It can be seen that an almost complete block of the peripheral sympathetic

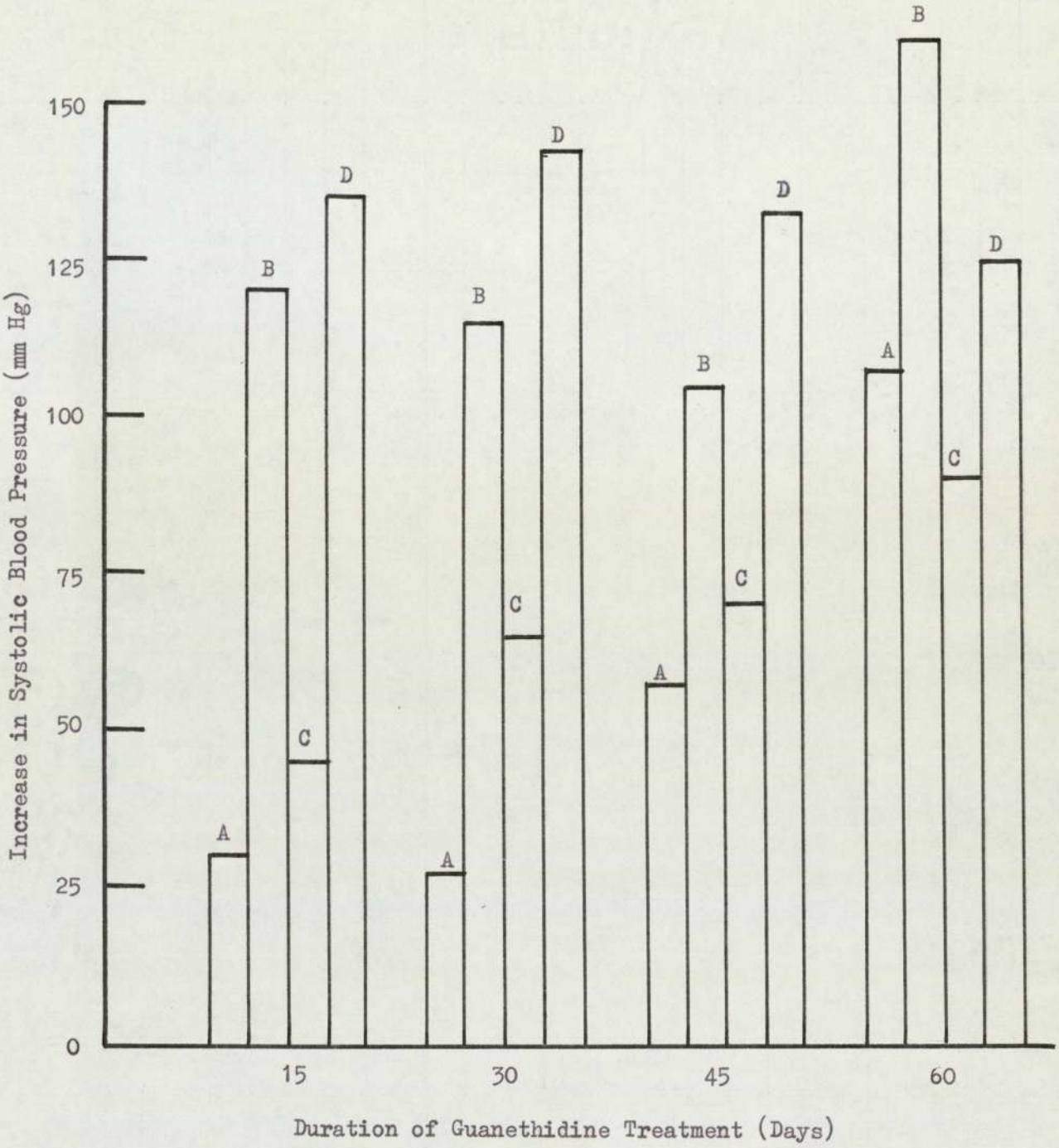


FIGURE 56: THE EFFECT OF VARIOUS PERIODS OF GUANETHIDINE TREATMENT ON THE MAXIMUM RESPONSE TO STIMULATION OF THE SYMPATHETIC OUTFLOW FROM THE SPINAL CORD OF DOCA-NaCl HYPERTENSIVE AND ONE-KIDNEY RENAL HYPERTENSIVE RATS.

Each histogram represents the mean maximum response of two rats to various frequencies of stimulation (groups B & D 16 Hz; groups A & C 64 Hz) of guanethidine treated DOCA-NaCl rats (A), control DOCA-NaCl rats (B), guanethidine treated one-kidney renal operated rats (C) and control one-kidney renal operated rats (D).

nervous system was present in the guanethidine treated DOCA-NaCl rats at 15 and 30 days. However, the adrenergic neuronal blockade was progressively reduced, but was still present, in the 45 and 60 days guanethidine treated DOCA-NaCl rats. An almost complete block of the peripheral sympathetic nervous system was present in the guanethidine treated renal operated rats at 15 days. The adrenergic neuronal blockade was progressively reduced, but was still present, in the 30, 45 and 60 days guanethidine treated renal operated rats.

The effect on blood pressure of the pithed rats to a 50 ng/kg i.v. dose of noradrenaline is shown in Fig. 57.

It can be seen that in both the guanethidine treated DOCA-NaCl rats and guanethidine treated renal operated rats, after each period of guanethidine treatment, the pressor response to noradrenaline was greater than that obtained in their respective control rats. The response to noradrenaline in the guanethidine treated rats was increased in duration as well as magnitude when compared with the control hypertensive rats.

Discussion

The results of this study support those of the initial study concerning the role of the sympathetic nervous system in the production of both DOCA-NaCl and one-kidney renal hypertensions in rats.

The slower development of DOCA-NaCl hypertension in rats obtained from ASL compared with that produced in rats obtained from Fisons Limited made comparisons of the initial and these further studies more difficult. This slow development of hypertension was responsible for the lack of any significant difference between the control DOCA-NaCl group of rats and 15 day guanethidine treated DOCA-NaCl group

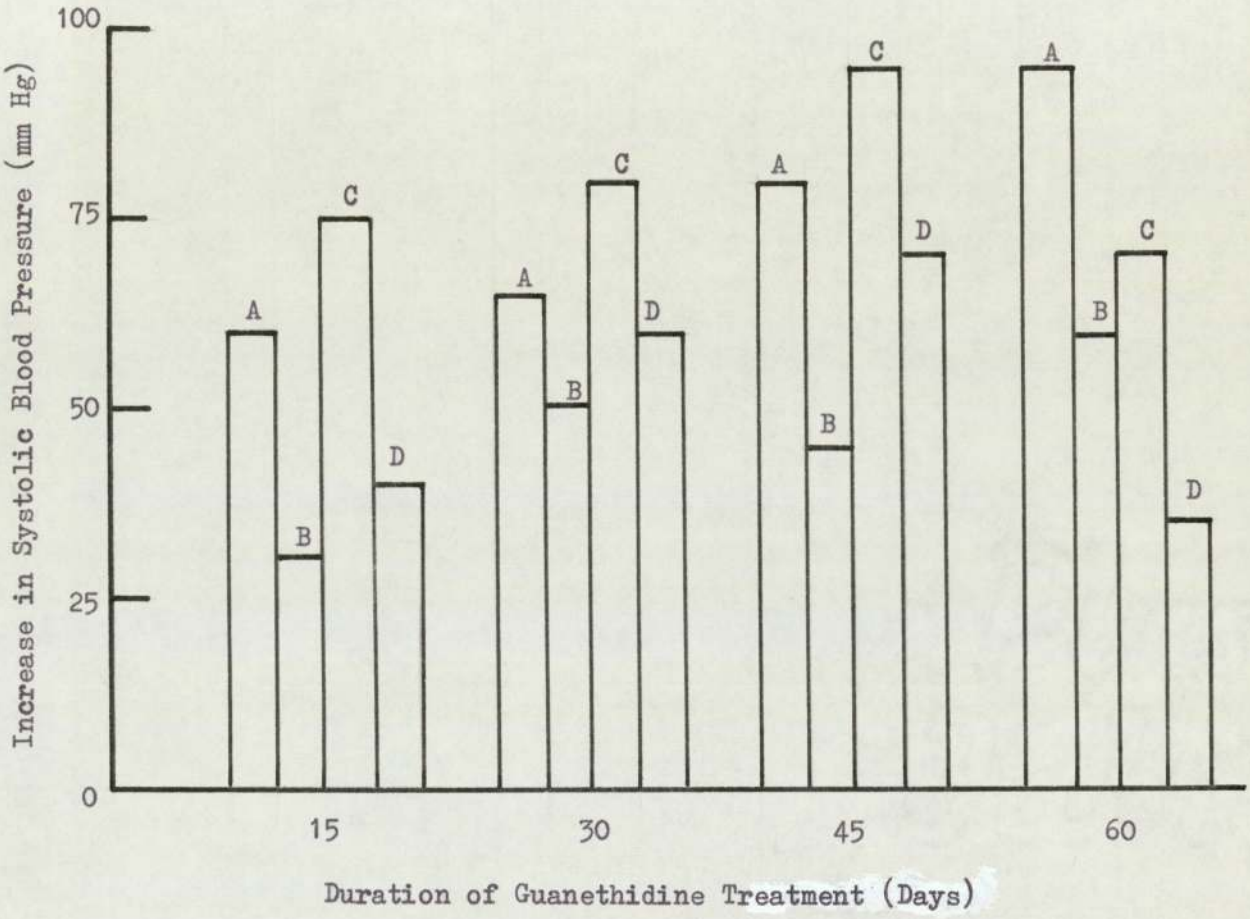


FIGURE 57: THE EFFECT OF VARIOUS PERIODS OF GUANETHIDINE TREATMENT ON THE RESPONSE TO NORADRENALINE IN DOCA-NaCl HYPERTENSIVE AND ONE-KIDNEY RENAL HYPERTENSIVE RATS.

Each histogram represents the mean response of two rats to a 50 ng/kg i.v. dose of noradrenaline of guanethidine treated DOCA-NaCl rats (A), control DOCA-NaCl rats (B), guanethidine treated one-kidney renal operated rats (C) and control one-kidney renal operated rats (D).

since at 15 days the blood pressures of the control DOCA-NaCl group were not significantly different from the 'sham-operated' normotensive groups of rats.

The results of the 30 day guanethidine experiment fully supported the findings of the initial study in that the blood pressures of the guanethidine treated group remained normotensive during guanethidine administration, whilst the blood pressure of the control DOCA-NaCl group had attained hypertensive levels. As in the initial study once guanethidine treatment was discontinued the blood pressure of the guanethidine treated DOCA-NaCl group rose in a manner parallel to that of the control DOCA-NaCl group. The results of the 45 and 60 day guanethidine experiments were complicated by the development of tolerance to guanethidine as shown by the results obtained in pithed rats to sympathetic stimulation. However, whilst there was evidence of a potent adrenergic neuronal blockade (that is, at least up to 30 days of guanethidine treatment) the blood pressures of the guanethidine treated groups of rats were significantly lower than those of their respective control DOCA-NaCl groups. The rises in blood pressure of the 45 and 60 day guanethidine treated DOCA-NaCl groups to hypertensive levels whilst guanethidine administration was still in progress, may have been due, wholly or partly, to the development of tolerance to the adrenergic neuronal blocking action of guanethidine. However, it is also possible that the development, by smooth muscle, of supersensitivity to catecholamines caused by chronic administration of guanethidine (Boura & Green, 1962) may also be responsible for the rise in blood pressure. This is indicated by the fact that although the adrenergic neuronal blockade was decreased in the 45 and 60 day guanethidine treated DOCA-NaCl

pithed rats the potentiation of the noradrenaline response was not decreased.

The rise in blood pressure after discontinuation of guanethidine treatment in DOCA-NaCl rats in the initial studies was considered to be possibly due to an 'overshoot' phenomenon of the drug. The results of this study demonstrate that this was not the case since the blood pressures of the 'sham-operated' guanethidine treated groups did not increase to hypertensive levels after discontinuation of guanethidine treatment. Although the blood pressures did increase, on about day 30 of the study in the 30, 45 and 60 day control guanethidine treated groups it was a relatively small increase and in the renal hypertensive study the blood pressures of similar control guanethidine treated groups did not increase in the same manner. Thus the results of this study indicate that the rise in blood pressure after discontinuation of guanethidine administration in the treated DOCA-NaCl groups in the initial study and in the 30 day group in this study is due to the removal of adrenergic neuronal blockade and not an 'overshoot' phenomenon.

The results of both studies indicate that the sympathetic nervous system is essential for the production of DOCA-NaCl hypertension in rats. This is in agreement with most of the published literature on this subject (see pp. 30 to 36). The involvement of the sympathetic nervous system in DOCA-NaCl hypertension may be explained by the hypothesis of De Champlain and co-workers (see review by De Champlain, 1972) and the results of these studies lend support to this hypothesis (see previous chapter for more detailed discussion).

The results obtained in this study are also in agreement with those of the initial study in that they show that the sympathetic nervous system is not essential for the production of one-kidney renal

hypertension in rats. The blood pressures of the guanethidine treated one-kidney renal operated rats in the 30, 45 and 60 day studies demonstrate that the initial rise in blood pressure is due to factors other than the sympathetic nervous system, probably the renin-angiotensin system (see previous chapter for detailed discussion). Although after this time the blood pressure rises at a slower rate than that of the control renal operated groups, probably due to the involvement of the sympathetic nervous system via resetting of the baroreceptors, the blood pressure still increases, indicating the involvement of other factors (for detailed discussion see previous chapter). The suggestion, stated in the previous chapter that the lack of effect of guanethidine in lowering the blood pressure of established renal hypertensive rats was due to the development of tolerance to guanethidine and/or the development, by smooth muscle, of supersensitivity to noradrenaline has been substantiated by the results involving sympathetic stimulation and noradrenaline administration in pithed renal hypertensive rats. Although investigation of the role of the sympathetic nervous system in the production of one-kidney renal hypertension has produced conflicting results (see pp. 30 to 36) Johnson et al. (1975 a & b) have reported that chemical sympathectomy with guanethidine did not affect either the production or maintenance of one-kidney renal hypertension in rats which is in agreement with the results of the studies presented here.

The detailed investigation of the pathologies of the animals, used in this study, revealed that the hypertensive disease in both guanethidine treated and control hypertensive rats was similar and no protection was afforded by guanethidine administration. This was not surprising since pathological examinations were performed at

the completion of the studies when the blood pressures of these groups of rats were similar. It was not possible to perform post-mortems in these groups of rats immediately after discontinuation of guanethidine treatment, which may have provided more useful information, since the number of animals remaining was too small.

The results regarding body weight are similar to those reported in the initial studies except that the body weights of the guanethidine treated DOCA-NaCl and control DOCA-NaCl groups of rats were not significantly lower than those of the control normotensive groups as observed in the initial studies. This may have been due to the fact that performing laparotomy and control daily injections of 0.9% saline in the normotensive groups may have produced stress which was considered an explanation for the differences in body weight observed in the initial studies. However, it is possible that the slower rate of increase in the blood pressure of the control DOCA-NaCl groups of rats may have been responsible for the lack of a significant difference between the hypertensive groups. The 'sham-operation' appeared to have little effect since the blood pressures and body weights of these normotensive groups in this study were similar to those observed in the initial studies.

Summary

1. Further studies, investigating the role of the sympathetic nervous system in the production and maintenance of DOCA-NaCl and renal hypertensions in rats have produced results similar to those of the initial studies.

2. Whilst a potent adrenergic neuronal blockade was present, the blood pressures of DOCA-NaCl guanethidine treated rats remained at normotensive

levels, although at this time the blood pressures of control DOCA-NaCl rats had attained hypertensive levels. Discontinuation of guanethidine treatment or the development of tolerance to guanethidine resulted in a rise in blood pressure of the treated DOCA-NaCl rats to hypertensive levels.

3. Guanethidine treated one-kidney renal hypertensive rats developed a hypertensive state in a similar manner to control renal hypertensive rats.

4. The development of tolerance to the adrenergic neuronal blocking action of guanethidine after 30 days and the continued supersensitivity of smooth muscle to noradrenaline was shown in guanethidine treated DOCA-NaCl and renal hypertensive pithed rats.

5. Results in guanethidine treated normotensive rats indicated that the rise in blood pressure following discontinuation of guanethidine treatment was not due to an 'overshoot' phenomenon.

6. Post-mortems revealed that guanethidine treatment did not protect rats to hypertensive vascular disease resulting from a DOCA-NaCl regimen or the method of Grollman and reasons for this are discussed.

7. 'Sham-operations' and/or i.p. injections of 0.9% saline in control hypertensive and normotensive groups of rats did not appear to produce effects which conflicted with those observed in the initial studies.

8. The results of these further experiments substantiate those of the initial studies in that it can be concluded that the sympathetic nervous system is essential for the production of DOCA-NaCl hypertension in rats but is not necessary for the production of one-kidney renal hypertension.

## CHAPTER 3

### Mechanism of Action of α-Methyldopa in the Rat

Since the introduction of α-methyldopa into clinical practice (Oates et al., 1960) the mechanism of action of the drug in lowering blood pressure has been the subject of much investigation and some controversy (see reviews by Muscholl, 1966, 1972; Stone & Porter, 1966; Sourkes & Rodriguez, 1967 and Henning, 1969 a).

Sourkes (1954) originally reported that α-methyldopa was an inhibitor of decarboxylation and tissue levels of dopamine, noradrenaline and 5HT were found to be decreased after administration of the drug (e.g. Hess, Ozaki & Undenfriend, 1960; Smith, 1960; Hess, Connamacher, Ozaki & Undenfriend, 1961; Porter, Totaro & Leiby, 1961). The depletion of dopamine and noradrenaline was found to occur mainly through a stoichiometric exchange of these amines with the metabolites of α-methyldopa, that is, α-methyldopamine and α-methylnoradrenaline respectively (Carlsson & Lindqvist, 1962; Carlsson, 1964).

Carlsson & Lindqvist (1962) suggested that the amines formed on metabolism of α-methyldopa may take over the function of dopamine and noradrenaline in the brain and later Day & Rand (1963) extended this hypothesis to the peripheral nerves also. It was shown that in these nerves, α-methylnoradrenaline formed from α-methyldopa was released after electrical stimulation (Muscholl & Maitre, 1963) and a number of investigators have stated that the activity of α-methylnoradrenaline on the adrenergic receptors was less than that of noradrenaline (e.g. Mueller & Horwitz, 1962; Day & Rand, 1963, 1964; Brunner, Hedwell, Maitre & Meier, 1966, 1967; Boakes, Candy & Wolstencroft, 1968, 1973). This release of a substitute or 'false' transmitter was proposed by Day & Rand (1963) to explain the hypotensive action of α-methyldopa.

However, doubt has been thrown on the 'false transmitter' hypothesis since the time relation between the noradrenaline depletion and the decrease in blood pressure was poor (Torchiana, Porter, Watson & Stone, 1965; Henning, 1967, 1969 a) and this discrepancy implied that the 'false transmitter' concept in its simplest outline was not sufficient to explain the antihypertensive action of α-methyldopa.

Farmer (1965) confirmed the report of Day & Rand (1964) concerning the sympathetic nerve blocking action of α-methyldopa on the cat's nictitating membrane and suggested from his data that the antihypertensive effect of α-methyldopa may be mediated via an antisympathetic effect of α-methyldopamine.

A central action of α-methyldopa was suggested when α-methyldopa was infused into the cerebral ventricles of dogs (Jaju, Tangri & Bhargava, 1966), into the vertebral arteries of cats (Henning & Van Zweiten, 1967, 1968) and perfused into the vascularly isolated 'in situ' cat brain (Ingenito, Barrett & Procita, 1970) and falls in blood pressure obtained which were equivalent to those obtained with much larger doses given systemically.

In renal hypertensive rats, the antihypertensive effect of α-methyldopa was shown by Henning (1968, 1969 b) to be related to central decarboxylation of this amino acid. The peripheral inhibitor of decarboxylation, carbidopa, did not alter the response to α-methyldopa while inhibition of peripheral and central decarboxylation by Ro 4-4602 abolished the response. Henning & Rubenson (1971) have reported that in normotensive rats, the dopamine-β-hydroxylase inhibitor FLA 63 abolished the fall in blood pressure caused by α-methyldopa, thus suggesting that α-methylnoradrenaline formation is necessary for the antihypertensive effect of α-methyldopa. Heise

& Kroneberg (1972) demonstrated that the central effects of α-methyldopa and its metabolites could be inhibited by α-blocking drugs, suggesting that the antihypertensive effect of α-methyldopa is produced, at least in part, by stimulation of central α-receptors.

In contrast to the mass of evidence suggesting a central mode of action of α-methyldopa other workers have cited evidence which they believe is contrary to this view (Mohammed et al., 1968; Ayitey-Smith & Varma, 1970). However, Tauberger & Kuhn (1971) demonstrated that α-methyldopa reduced sympathetic nerve firing and this was confirmed by Baum, Shropshire & Varner (1972).

The present study was designed to examine the action of α-methyldopa administered either systemically or centrally in conscious hypertensive rats in which either central or peripheral dopa decarboxylase was inhibited. In addition, the effects of central and peripheral inhibition of dopamine-β-hydroxylase on the antihypertensive effect of α-methyldopa was studied in order to test the hypothesis of Farmer (1965).

Results

The effect of 200 mg/kg i.p. dose of α-methyldopa on the systolic blood pressure of a group of six DOCA-NaCl hypertensive rats is shown in Fig. 58. The blood pressure began to fall immediately after the injection, and reached a maximum fall in systolic blood pressure of 57 ± 2 mm Hg at 4.5 hours post-dose, with a return to pre-dose levels at 24 hours. The vehicle in which the commercial 'Aldomet' injection of α-methyldopa is formulated contains a number of constituents and was tested for possible effects on blood pressure. When administered i.p. to hypertensive rats in volumes similar to those used when administering α-methyldopa it had little effect on the blood pressure

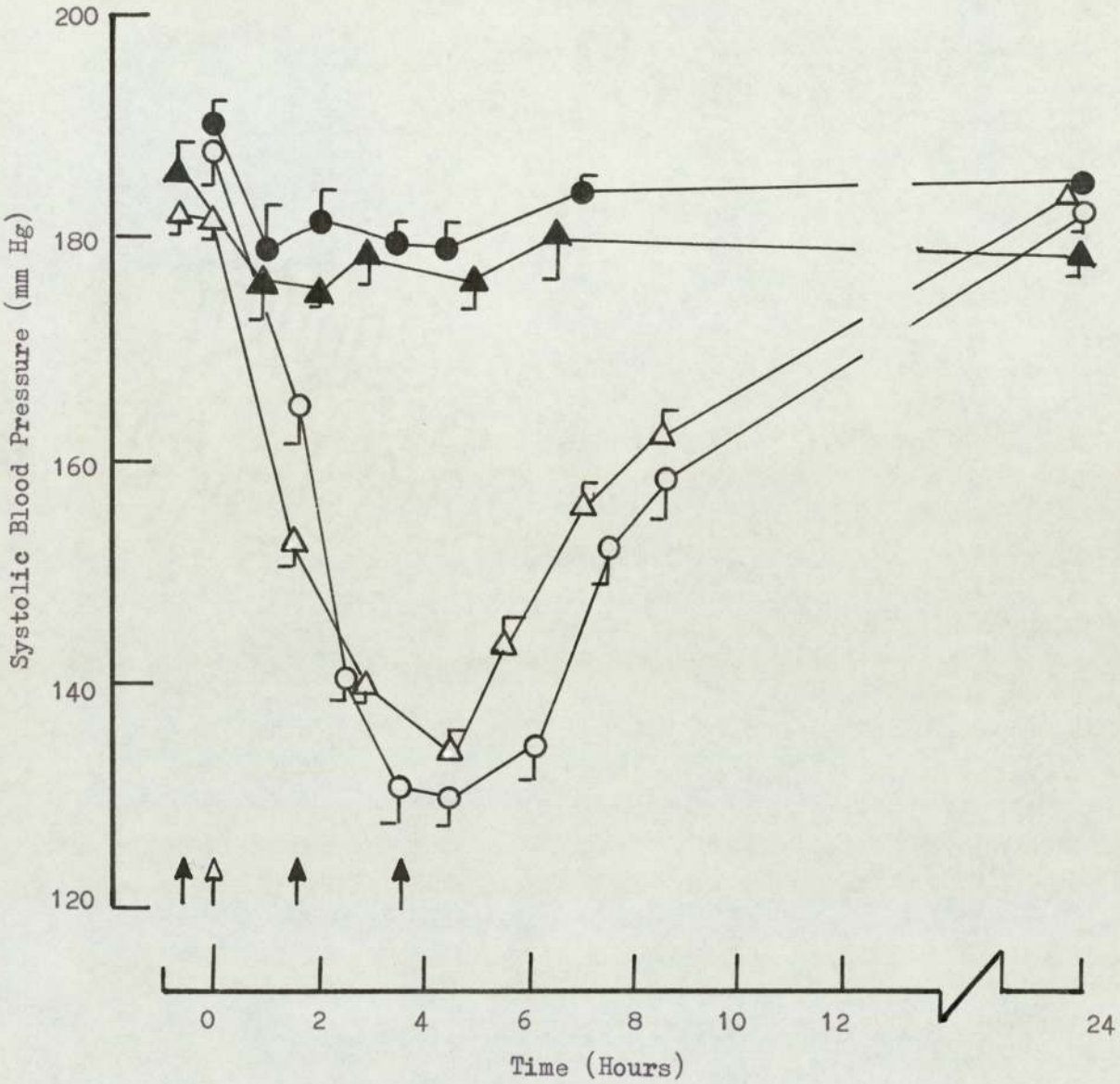


FIGURE 58: THE EFFECT OF PERIPHERAL DOPA DECARBOXYLASE INHIBITION WITH Ro 4-4602 ON THE ANTIHYPERTENSIVE ACTION OF α-METHYLDOPA.

The effects of 'Aldomet' vehicle (●—●), Ro 4-4602 + 'Aldomet' vehicle (▲—▲), α-methyldopa (○—○) and Ro 4-4602 + α-methyldopa (△—△) on the systolic blood pressures of DOCA-NaCl hypertensive rats are shown above. α-Methyldopa was administered intraperitoneally (i.p.) at a dose level of 200 mg/kg at time zero (△). Ro 4-4602 was administered in 3 separate doses (▲) of 50 mg/kg i.p. (total dose 150 mg/kg). Each point represents the mean of six rats and standard errors are shown by the vertical bars.

(Fig. 58). In a further group of six hypertensive rats the blood pressure was unaffected by 3 i.p. doses, each of 50 mg/kg of Ro 4-4602 given at two hourly intervals.

This dose regimen of Ro 4-4602 (total dose 150 mg/kg) which was shown by Kuruma et al. (1972) to inhibit peripheral but not central dopa decarboxylase was repeated in a group of hypertensive rats also given α-methyldopa, 200 mg/kg i.p., 0.5 hours after the first Ro 4-4602 dose. In this experiment α-methyldopa produced its usual fall in blood pressure.

Fig. 59 illustrates a similar group of experiments in which Ro 4-4602 was administered in a similar pattern at a dose of 200 mg/kg per injection (total dose 600 mg/kg). This treatment, which is known to inhibit central as well as peripheral dopa decarboxylase (Kuruma et al., 1972), itself produced a significant antihypertensive effect (peak effect at 6.75 hours was a mean fall in systolic blood pressure of 36 ± 1 mm Hg). When α-methyldopa, 200 mg/kg was administered during treatment with this regimen of Ro 4-4602 its antihypertensive effect was markedly reduced and was in fact less than that produced by this dose level of Ro 4-4602 alone. Thus, a mutual antagonism exists between the antihypertensive effects of α-methyldopa and Ro 4-4602.

Disulfiram, 100 mg/kg i.p., given alone or in combination with the 'Aldomet' injection vehicle caused a moderate fall in blood pressure (peak effect at 5 hours was a mean fall in systolic blood pressure from 191 ± 2 to 159 ± 2 mm Hg). In another group of rats disulfiram, 100 mg/kg i.p., was given 2 hours before α-methyldopa, 200 mg/kg i.p.; the antihypertensive effect of α-methyldopa was abolished (Fig.60).

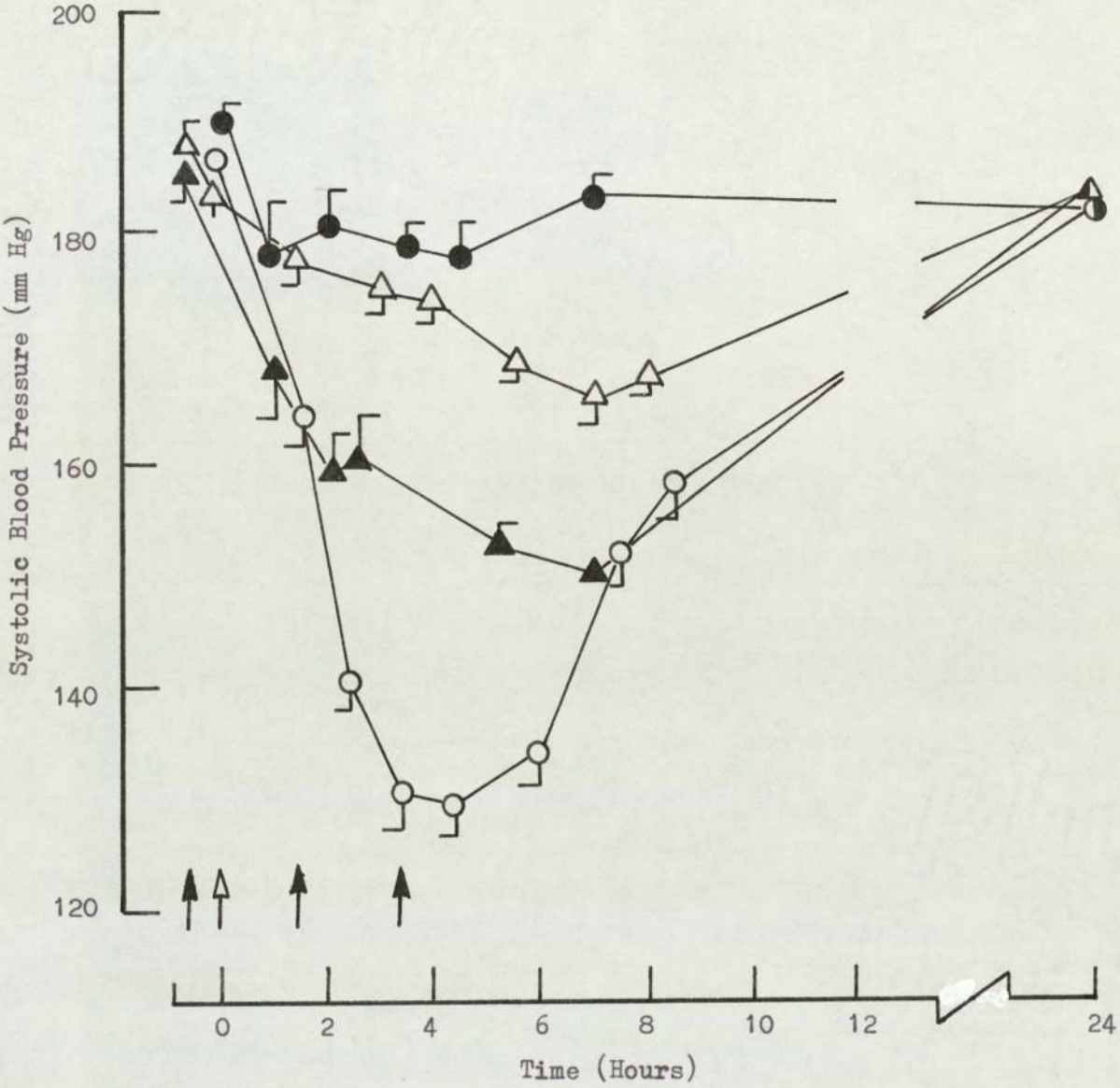


FIGURE 59: THE EFFECT OF SIMULTANEOUS PERIPHERAL AND CENTRAL DOPA DECARBOXYLASE INHIBITION WITH Ro 4-4602 ON THE ANTIHYPERTENSIVE ACTION OF α-METHYLDOPA.

The effects of 'Aldomet' vehicle (●—●), Ro 4-4602 + 'Aldomet' vehicle (▲—▲), α-methyldopa (○—○) and Ro 4-4602 + α-methyldopa (△—△) on the systolic blood pressures of DOCA-NaCl hypertensive rats are shown above. α-Methyldopa at a dose level of 200 mg/kg i.p. was administered at time zero (↑). Ro 4-4602 was administered in 3 separate doses (↑) of 200 mg/kg i.p. (total dose 600 mg/kg). Each point represents the mean of six rats and standard errors are shown by the vertical bars.

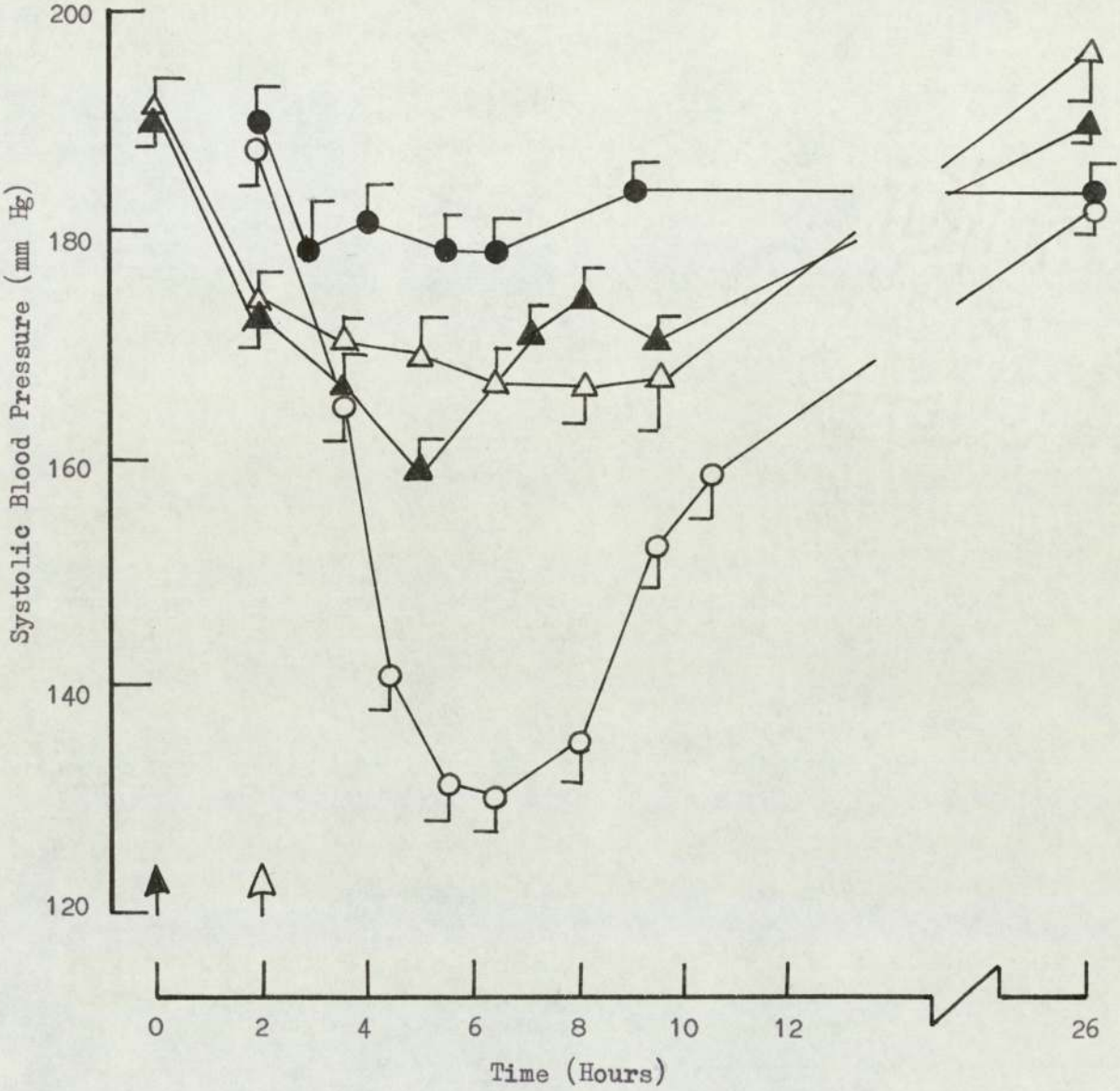


FIGURE 60: THE EFFECT OF SIMULTANEOUS PERIPHERAL AND CENTRAL DOPAMINE-β-HYDROXYLASE INHIBITION WITH DISULFIRAM ON THE ANTIHYPERTENSIVE ACTION OF α-METHYLDOPA.

The effects of 'Aldomet' vehicle (●—●), disulfiram + 'Aldomet' vehicle (▲—▲), α-methyldopa (○—○) and disulfiram + α-methyldopa (△—△) on the systolic blood pressure of DOCA-NaCl hypertensive rats are shown above. α-Methyldopa, 200 mg/kg i.p., was administered at 2 hours (⇧). Disulfiram was administered at a dose level of 100 mg/kg i.p. 2 hours before α-methyldopa (⇧). Each point represents the mean of six rats and standard errors are shown by the vertical bars.

Diethyldithiocarbamate (DDC), 100 mg/kg i.p. was substituted for disulfiram in another series of experiments and was shown itself to cause a similar fall in blood pressure as disulfiram but with a more rapid onset and peak effect (Fig.61). DDC treatment produced an identical abolition of the antihypertensive response to α-methyldopa as did disulfiram. It is interesting to note that α-methyldopa did not antagonise the fall in pressure produced by disulfiram and DDC as it did the fall caused by Ro 4-4602 (Fig.59).

Johnson et al. (1970) reported that U-14,624 was a more powerful inhibitor of central than of peripheral dopamine-β-hydroxylase. U-14,624, 200 mg/kg i.p., produced a slight fall in pressure which had returned to pre-treatment levels after 12 hours at which time α-methyldopa, 200 mg/kg i.p., was administered. Pre-treatment with U-14,624 effectively abolished the antihypertensive effect of α-methyldopa (Fig.62).

α-Methyldopa, 2 mg/kg, administered by icv injection into hypertensive rats caused a fall in blood pressure of rapid onset (peak effect at 1.5 hours was a fall in group mean systolic pressure from 190 ± 3 to 148 ± 2 mm Hg). A 4 mg/kg dose of α-methyldopa produced a somewhat larger response (peak effect at 1.5 hours was a fall in group mean systolic pressure from 191 ± 2 to 138 ± 3 mm Hg). The effect of both these doses of α-methyldopa given centrally was relatively transient compared with the effect of the 200 mg/kg dose given systemically, the blood pressure having returned to pre-treatment levels by 5 hours (Fig.63). The 'Aldomet' injection vehicle produced no significant effect on blood pressure when injected in volumes similar to those injected using the 2 mg/kg and 4 mg/kg doses of α-methyldopa (10 µl and 20 µl respectively).

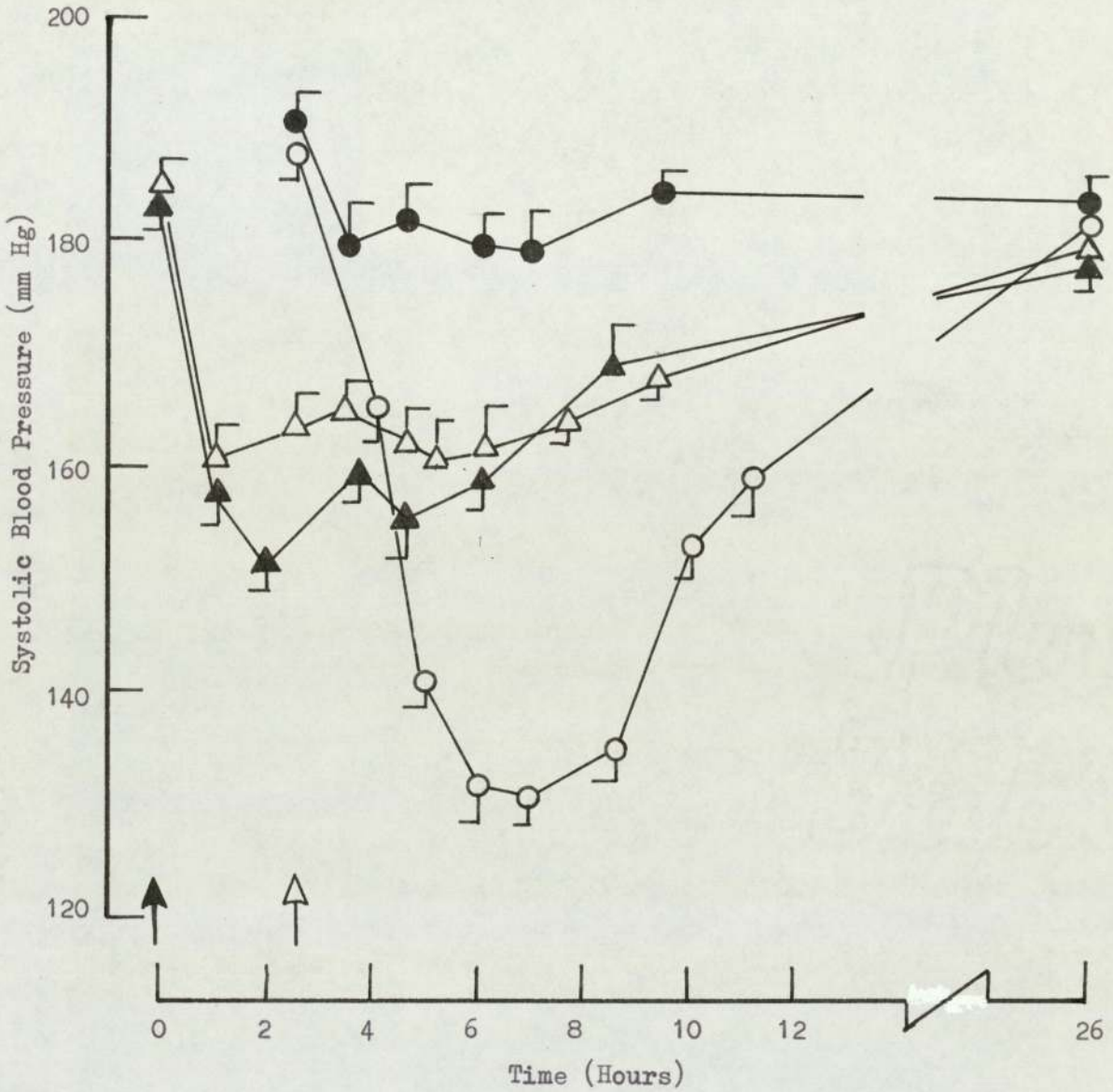


FIGURE 61: THE EFFECT OF SIMULTANEOUS PERIPHERAL AND CENTRAL DOPAMINE-β-HYDROXYLASE INHIBITION WITH SODIUM DIETHYLDITHIOCARBAMATE (DDC) ON THE ANTIHYPERTENSIVE ACTION OF α-METHYLDOPA.

The effects of 'Aldomet' vehicle (●—●), DDC + 'Aldomet' vehicle (▲—▲), α-methyldopa (○—○) and DDC + α-methyldopa (△—△) on the systolic blood pressures of DOCA-NaCl hypertensive rats are shown above. DDC was administered (▲) at a dose level of 100 mg/kg i.p. 2.5 hours before dosing rats with 200 mg/kg i.p. α-methyldopa (△). Each point represents the mean of six rats and standard errors are shown by the vertical bars.

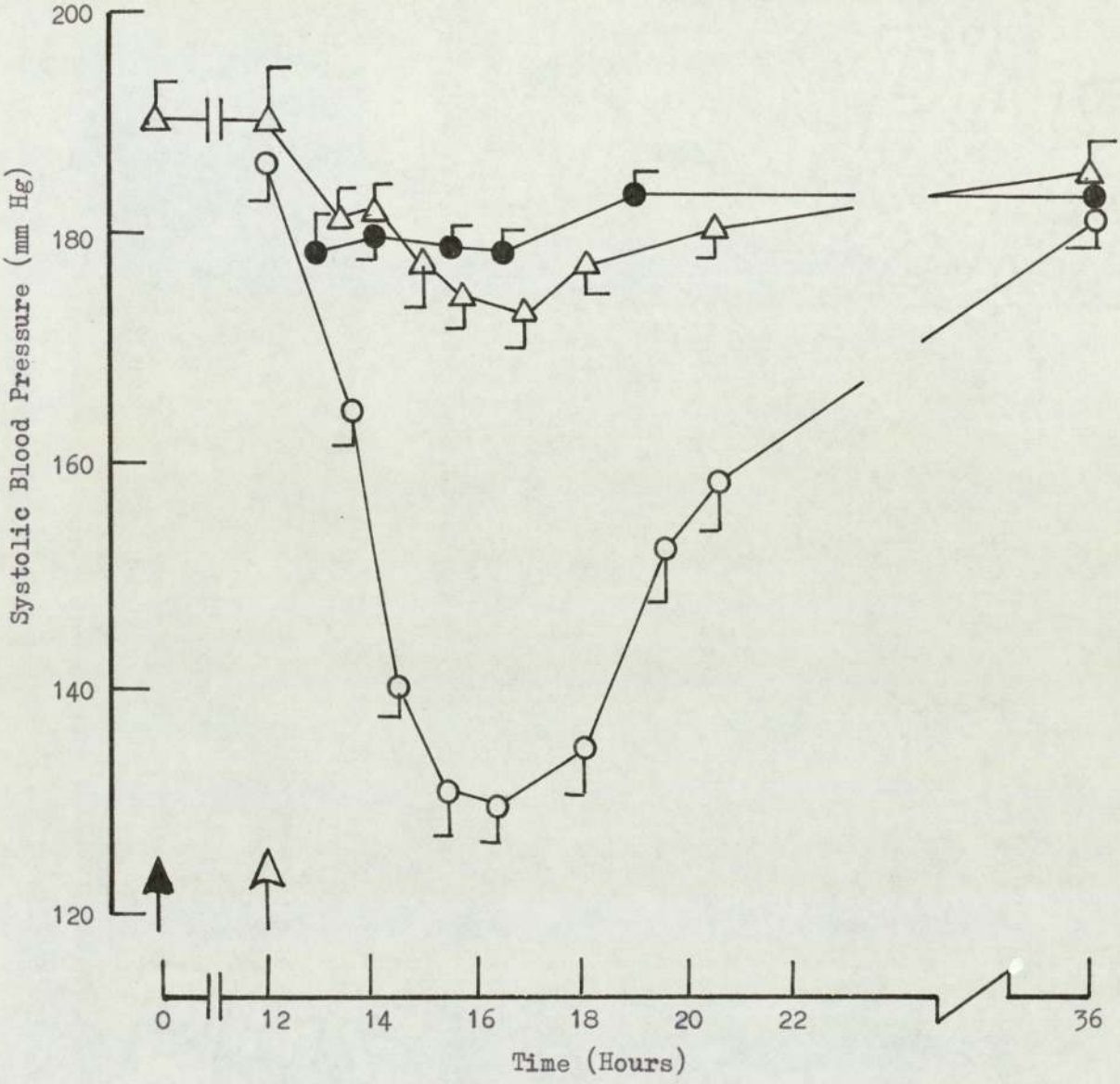


FIGURE 62: THE EFFECT OF CENTRAL DOPAMINE-β-HYDROXYLASE INHIBITION WITH U-14,624 ON THE ANTIHYPERTENSIVE ACTION OF α-METHYLDOPA.

The effects of 'Aldomet' vehicle (●—●), α-methyldopa (○—○) and U-14,624 + α-methyldopa (△—△) on the systolic blood pressures of DOCA-NaCl hypertensive rats are shown above. U-14,624 was administered at a dose level of 200 mg/kg i.p. (▲) 12 hours before dosing the rats with α-methyldopa 200 mg/kg i.p. (△). Each point represents the mean of six rats and standard errors are shown by the vertical bars.

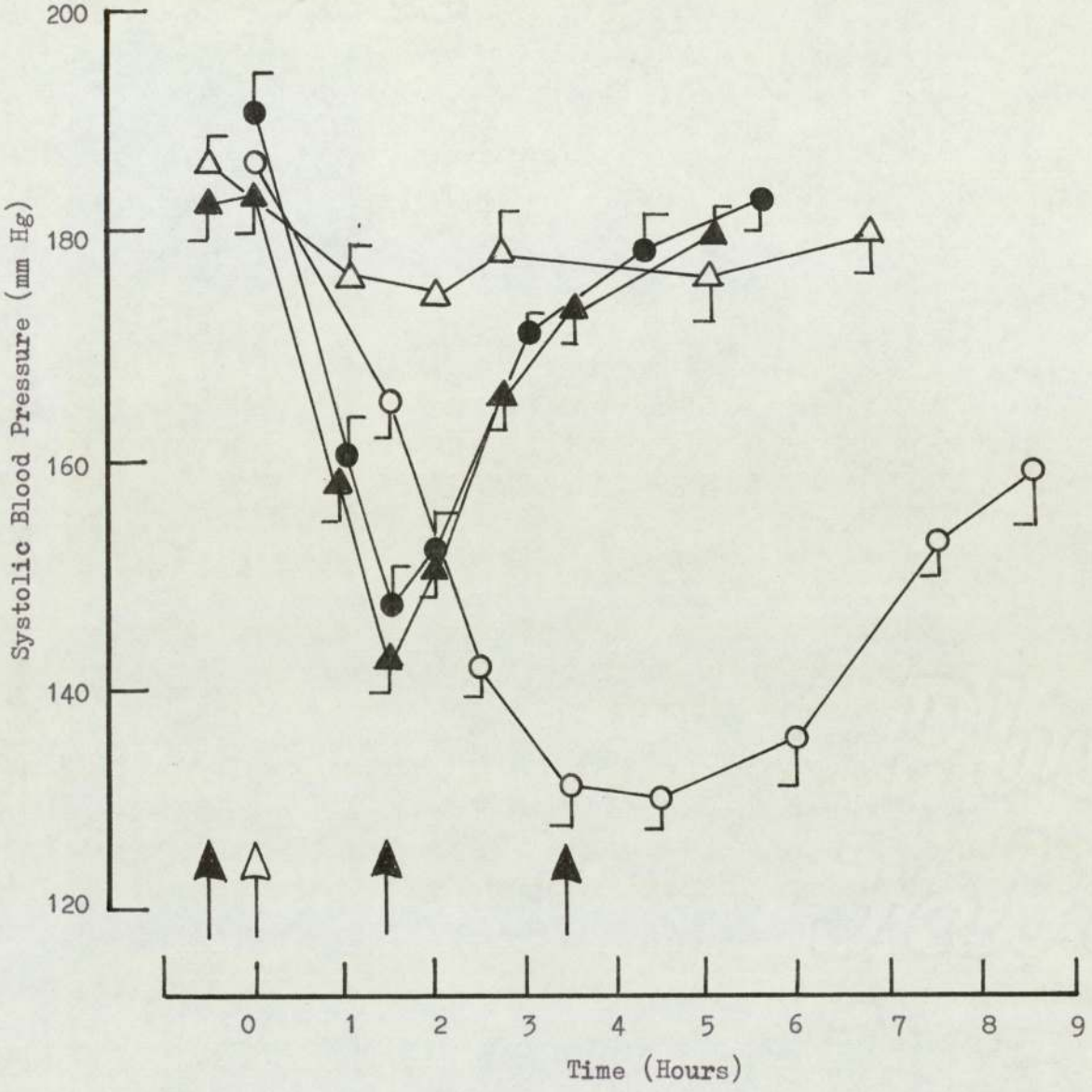


FIGURE 63: THE EFFECT OF PERIPHERAL DOPA DECARBOXYLASE INHIBITION WITH Ro 4-4602 ON THE ANTIHYPERTENSIVE ACTION OF CENTRALLY ADMINISTERED α-METHYLDOPA.

The effects of α-methyldopa (●—●), Ro 4-4602 + 'Aldomet' vehicle (△—△) and Ro 4-4602 + α-methyldopa (▲—▲) on the systolic blood pressures of DOCA-NaCl hypertensive rats are shown above. α-Methyldopa was administered by intracerebroventricular injection (icv) at time zero (↑) in a dose of 2 mg/kg. Ro 4-4602 was injected in 3 doses (↑) of 50 mg/kg i.p. (total dose 150 mg/kg). The effect of a 200 mg/kg i.p. dose of α-methyldopa (○—○) on the systolic blood pressures of DOCA-NaCl rats is also shown for comparison purposes. Each point represents the mean of six rats and standard errors are shown by the vertical bars.

In hypertensive rats, in which peripheral dopa decarboxylase was inhibited by the low dose regimen of Ro 4-4602, 3 x 50 mg/kg doses, i.p., the antihypertensive effect of α-methyldopa, 2 mg/kg given centrally, was unaffected (Fig. 63).

In another series of experiments, in which central as well as peripheral dopa decarboxylase was inhibited by the high dose regimen of Ro 4-4602, 3 x 200 mg/kg doses, i.p., the antihypertensive effect of α-methyldopa was abolished. It should be noticed that the antihypertensive effect produced by this high dose regimen of Ro 4-4602 was also abolished by the centrally administered α-methyldopa (Fig. 64). This strongly suggests a central site for the blood pressure lowering effect of Ro 4-4602.

Ro 4-4602 was administered icv in three doses each of 0.2 mg/kg given at 2 hourly intervals. This treatment did not produce any significant effect on blood pressure. However, in a parallel experiment α-methyldopa (200 mg/kg) was administered i.p. 30 minutes after the first dose of Ro 4-4602 and its antihypertensive effect was completely abolished (Fig. 65).

Discussion

The large fall in systolic blood pressure of hypertensive rats to peripherally administered α-methyldopa with a maximal effect at 4.5 hours and recovery to pre-dose levels at 24 hours is in agreement with previous reports (see review by Henning, 1969 a). The lack of effect on the systolic blood pressure of the vehicle in which commercial 'Aldomet' is formulated, administered either systemically or centrally, has not been previously reported and was rather surprising as it is acidic and composed of a number of constituents. However, the lack of effect of the vehicle demonstrated that falls in blood

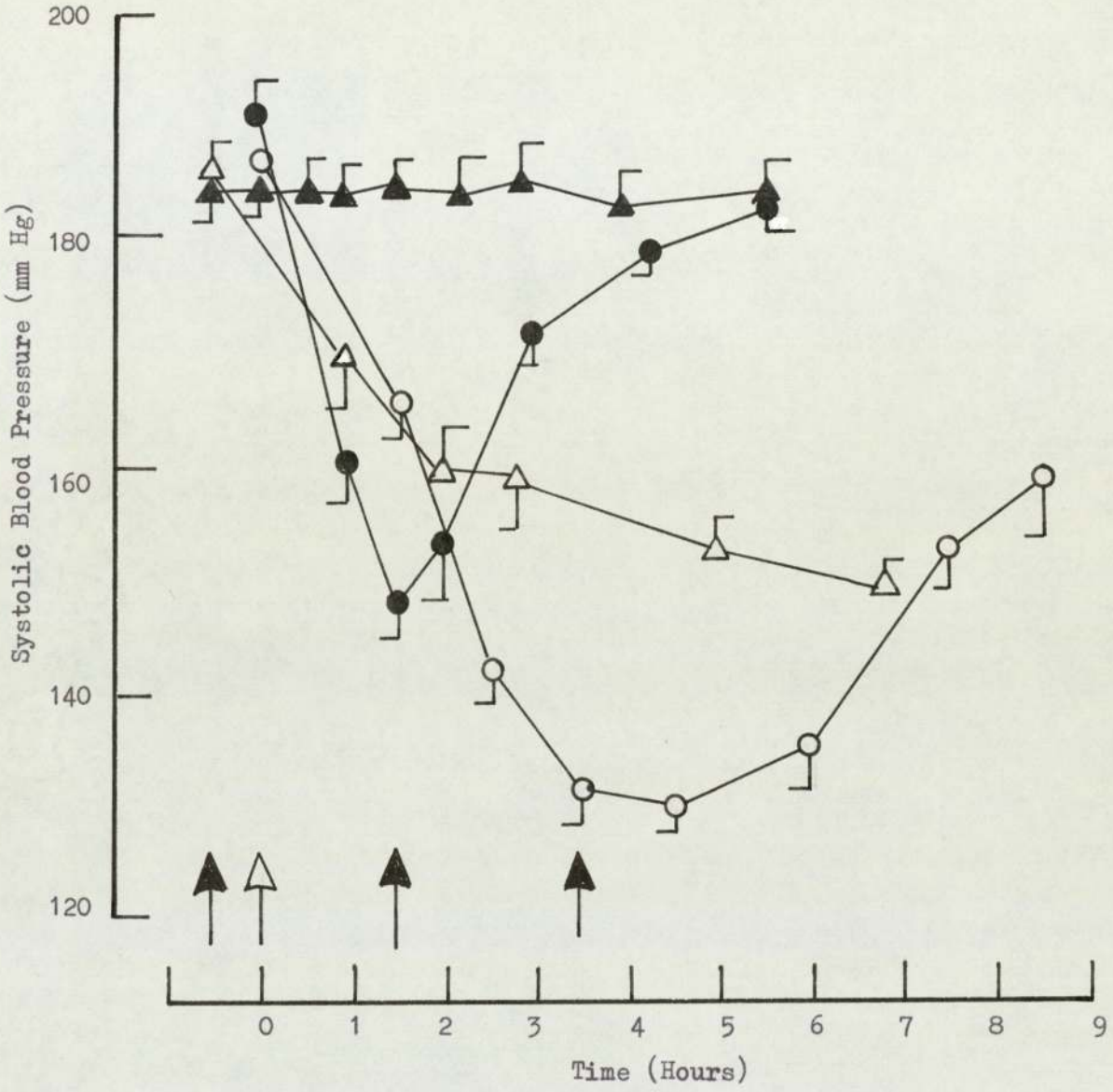


FIGURE 64: THE EFFECT OF SIMULTANEOUS PERIPHERAL AND CENTRAL DOPA DECARBOXYLASE INHIBITION WITH Ro 4-4602 ON THE ANTI-HYPERTENSIVE ACTION OF CENTRALLY ADMINISTERED α-METHYLDOPA.

The effects of α-methyldopa (●—●), Ro 4-4602 + 'Aldomet' vehicle (△—△) and Ro 4-4602 + α-methyldopa (▲—▲) on the systolic blood pressures of DOCA-NaCl hypertensive rats are shown above. α-Methyldopa at a dose level of 2 mg/kg icv was administered at time zero (⇧). Ro 4-4602 was injected in 3 doses (⇧) of 200 mg/kg i.p. (total dose 600 mg/kg). The effect of a 200 mg/kg i.p. dose of α-methyldopa (○—○) on the systolic blood pressures of DOCA-NaCl rats is also shown for comparison purposes. Each point represents the mean of six rats and standard errors are shown by the vertical bars.

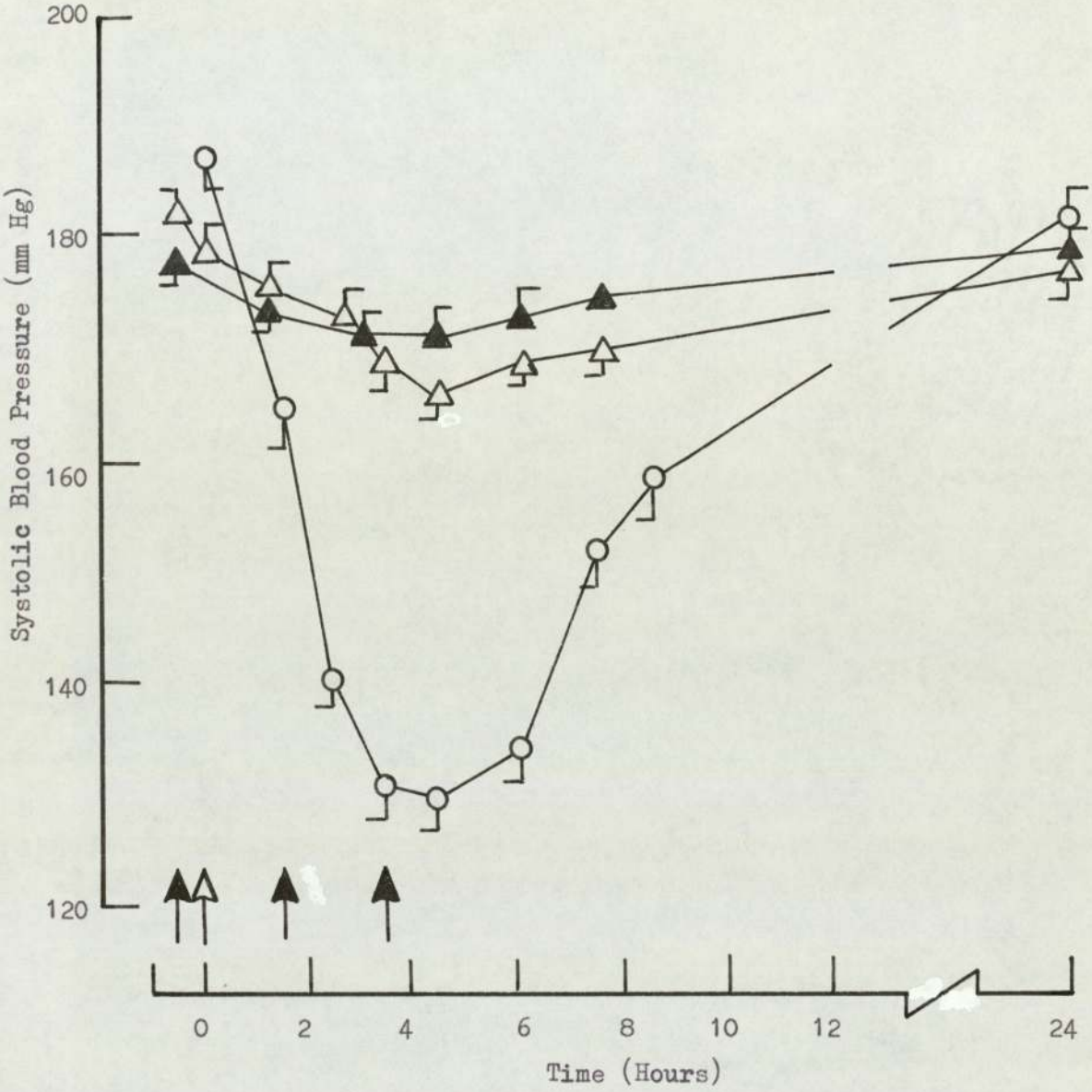


FIGURE 65: EFFECT OF CENTRAL DOPA DECARBOXYLASE INHIBITION PRODUCED BY CENTRALLY ADMINISTERED Ro 4-4602 ON THE ANTIHYPERTENSIVE ACTION OF α-METHYLDOPA.

The effects of α-methyldopa (○—○), Ro 4-4602 + 'Aldomet' vehicle (▲—▲) and Ro 4-4602 + α-methyldopa (△—△) on the systolic blood pressures of DOCA-NaCl hypertensive rats are shown above. Ro 4-4602 was injected centrally in 3 doses (↑) of 0.2 mg/kg. α-Methyldopa was administered at a dose level of 200 mg/kg i.p. at time zero (↑). Each point represents the mean of six rats and standard errors are shown by the vertical bars.

pressure following administration of commercial 'Aldomet' were due purely to the effects of α-methyldopa.

Administration of Ro 4-4602 at dose levels which inhibit predominantly peripheral dopa decarboxylase (Kuruma et al., 1972) did not affect the antihypertensive action of α-methyldopa. However, at higher dose levels which are known to inhibit both central and peripheral dopa decarboxylase (Kuruma et al., 1972) Ro 4-4602 severely inhibited the antihypertensive effect of α-methyldopa. At the higher dose level Ro 4-4602 produced a fall in blood pressure itself which was reduced when given in combination with α-methyldopa and possibly represents a competitive antagonism at some site in the central nervous system. The results obtained in these experiments are in agreement with those of Henning (1969 b) Cohen et al. (1974) and Nijkamp, Ezer & De Jong (1975) and strongly suggest that central decarboxylation of α-methyldopa must occur for it to reduce blood pressure.

Disulfiram and its reduction product DDC are known to inhibit both central and peripheral dopamine-β-hydroxylase (Musacchio et al., 1966) and in this study both produced falls in blood pressure of similar magnitude but with a much quicker onset for DDC. However, despite these falls the antihypertensive effect of systemically administered α-methyldopa was severely reduced. Similarly U-14,624 which has been shown to be a relatively specific inhibitor of central dopamine-β-hydroxylase (Johnson et al., 1970) almost totally abolished the antihypertensive effect of systemically administered α-methyldopa at a time when its own blood pressure lowering effect had subsided. These results strongly suggest that the formation of α-methylnoradrenaline in the central nervous system, is essential for the antihypertensive effect of α-methyldopa. This is in agreement with

the report of Henning & Rubenson (1971) who abolished the fall in blood pressure to α-methyldopa in normotensive rats by prior administration of FLA 63, a potent dopamine-β-hydroxylase inhibitor (Svensson & Waldeck, 1969). This work has been confirmed by Cohen et al. (1974) in renal hypertensive rats. These results disagree with the concept of Farmer (1965) that α-methyldopamine may be the active compound causing sympathetic impairment. Also treatment with disulfiram, DDC and FLA 63 all cause tissue levels of dopamine to increase in normal animals and therefore, if the concept of Farmer (1965) was correct, then this treatment would be expected to increase the antihypertensive effect of α-methyldopa. Finally, Torchiana et al. (1973) have shown that a block of central dopaminergic receptors in the anaesthetised cat does not effect the response to centrally administered α-methyldopa.

Jaju et al. (1966) reported that small doses of α-methyldopa administered centrally to anaesthetised dogs, produced a short lasting fall in blood pressure. Heise & Kroneberg (1972) and Torchiana et al. (1973) have obtained falls in blood pressure in the anaesthetised cat after perfusion of the cerebral ventricles, with doses which are not effective when administered systemically. Similarly, Henning & Van Zwieten (1967, 1968) obtained falls of blood pressure in anaesthetised cats after infusions into the vetebral artery of α-methyldopa which had no effect when administered systemically. The results of the present study are in agreement with these reports in that α-methyldopa injected into the lateral ventricles of conscious hypertensive rats produced a marked fall in systolic blood pressure in a dose approximately 100 x less than that used systemically and provides further evidence for a central mode of action of

α -methyldopa. The time course of the blood pressure fall to centrally administered α-methyldopa, in this study, was of a quicker onset and shorter duration than that reported by the above workers. This is probably due to species difference, although the use of anaesthesia and normotensive animals, by the above workers, may also be responsible. The onset and duration of the response to centrally administered α -methyldopa were both much shorter than the responses to systemic administration. The differences in responses may be due to the different availability of α -methyldopa at central sites. Hence, α -methyldopa administered systemically must be absorbed into the blood stream and cross the blood-brain barrier to its site of metabolism, thus increasing the time of onset of its action. On the other hand, the large systemic dose of α -methyldopa used, would act as a depot for the slow release of the substance and would explain the longer duration of action.

The fact that the antihypertensive effect of α -methyldopa, administered either centrally or systemically is mediated through a similar mechanism, is strongly suggested by the observation that Ro 4-4602 given systemically in doses known to inhibit central dopa decarboxylase, prevented the antihypertensive effect of centrally administered α-methyldopa. Similarly, small doses of Ro 4-4602 administered centrally abolished the antihypertensive effect of a large systemic dose of α-methyldopa. These results once again suggest that the antihypertensive action of α-methyldopa is mediated in the central nervous system via the amine metabolites of α -methyldopa, as proposed by Henning & Rubenson (1971).

More recently Finch & Haeusler (1972) have shown that, in rats, the antihypertensive effect of α -methyldopa is dependent upon

central noradrenergic neurones, since their destruction by 6-hydroxydopamine prevented the response to α-methyldopa but not to the α-adrenoceptor agonist clonidine, thus demonstrating the necessity of uptake of α-methyldopa into central adrenergic neurones and conversion to α-methylnoradrenaline. α-Methylnoradrenaline has been shown to produce a greater fall in blood pressure when given centrally to the anaesthetised cat, than either α-methyldopa or α-methyldopamine (Heise & Kroneberg, 1972). The action of α-methylnoradrenaline derived from α-methyldopa probably acts on α-receptors in the brain (see review by Van Zwieten, 1973) since it is prevented by prior administration of α-blockers (Heise & Kroneberg, 1972). Nijkamp & De Jong (1975) have suggested that the central site of action of α-methylnoradrenaline derived from α-methyldopa is α-receptors in the area of the nucleus tractus solitarii of the medulla oblongata. The effect of α-methylnoradrenaline on α-receptors in the brain causes a decreased impulse flow from the central nervous system (Tauberger & Kuhn, 1971; Baum, Shropshire & Varner, 1972) resulting in an inhibition of release of noradrenaline from the sympathetic nerves (Anden & Henning, 1974).

These results and those of the present study are consistent with the hypothesis of Henning & Rubenson (1971) in that the antihypertensive effect of α-methyldopa is mediated via α-methylnoradrenaline produced centrally and with that of Day & Rand (1963) that α-methylnoradrenaline may replace noradrenaline as the transmitter substance in noradrenergic neurones.

Summary

1. The mechanism of antihypertensive action of α-methyldopa was investigated in DOCA-NaCl hypertensive rats.

2. α-Methyldopa administered systemically to hypertensive rats produced a marked antihypertensive effect which was unaffected by peripheral dopa decarboxylase inhibition with Ro 4-4602 but was abolished by simultaneous central and peripheral inhibition.

3. Inhibition of central and peripheral dopamine-β-hydroxylase with either disulfiram or sodium diethyldithiocarbamate (DDC) produced an abolition of the fall in blood pressure to i.p. administered α-methyldopa. Central inhibition of dopamine-β-hydroxylase using U-14,624 produced the same effect.

4. When administered by i.c.v. injection α-methyldopa produced a marked antihypertensive effect at a dose level only 1/100 of that used systemically.

5. The fall in blood pressure to centrally administered α-methyldopa was abolished by Ro 4-4602 in doses inhibiting central dopa decarboxylase. Small doses of Ro 4-4602 given centrally antagonised the antihypertensive effect of a large dose of α-methyldopa given systemically.

6. It was concluded that α-methyldopa lowers blood pressure by a central mechanism involving the production of α-methylnoradrenaline which may act as a false neurohumoral transmitter substance.

## SECTION 4: ARTERIAL HYPERTENSION IN THE CAT

### CHAPTER 1

### Production of Hypertension in the Cat

The conscious cat in the normotensive state has been shown to be eminently suitable to study the effects of drugs on the cardiovascular system (Owen, 1969; Day & Owen, 1970). This work provided the impetus to examine the possibility of obtaining a stable hypertension in cats so that the effects of some antihypertensive agents could be examined in such cats whilst in the conscious state.

Although no detailed information on the production of hypertension in cats has been reported several workers (Wilson, 1965; Zanchetti, Guazzi & Baccelli, 1966; Cohn & Notargiacomo, 1969; Guazzi, 1969; Guazzi, Ellsworth & Fries, 1971) have obtained hypertension in cats using the method of Goldblatt et al., (1934). Similarly when Page (1939) reported his method for producing hypertension in rats and dogs by wrapping the kidneys in cellophane, he mentioned that it was also successful in the cat.

Hypertension has also been obtained by stimulation of the hypothalamus (Kell, Langford, Hoff & Henningar, 1960; Gerola & Grossi, 1965) or of a part of the cerebral cortex (Morin, Corriol, Naquet, Ricci & Berard-Badier, 1954) but the rise in blood pressure in both instances was temporary. Similarly a temporary hypertension was obtained in cats by Johansson et al. (1970) and Haggendal & Johansson (1972) by the rapid intravenous injection of metaraminol.

However, none of these reports provided information on the development of hypertension in the cat or its pathology. Also the effects of drugs on hypertension in cats was not investigated. Recently

Poyser, Shorter & Whiting (1974) have reported on the production of hypertension in cats by three methods and the effects of some antihypertensive agents on the resulting hypertensive state. This work was a development of that initially undertaken in these laboratories.

Results

Figs.66,67 & 68 illustrate the development of hypertension in three cats induced by the 'figure-of-eight' ligature method of Grollman (1944). In all three cases the application of the 'figure-of-eight' ligature to the kidney caused no increase in blood pressure. However, after contralateral nephrectomy a rise in blood pressure occurred within seven days. The rise in one case was very rapid, followed by a large fall in blood pressure and death. In the other two cats a fairly gradual rise in blood pressure was observed and there was no overall change in heart rate. However, in the cat whose blood pressure rose very rapidly there was also an increase in heart rate.

Figs. 69 & 70 illustrate the effect of a DOCA-NaCl regimen on the blood pressure and heart rate of two cats. A gradual rise in blood pressure was obtained in both cases with a gradual fall in heart rate and a fall in blood pressure in one case after replacement of the sodium chloride solution with the normal drinking fluid, milk.

Although cats are averse to drinking water (Carver & Waterhouse, 1962) in this study it was found that although this was true initially, once the cat had begun to drink sodium chloride solution polydipsia developed.

The general condition of cats on DOCA-NaCl therapy deteriorated; they lost weight, became listless and the normal glossy sheen of their coats disappeared.

The general condition of the cats in which hypertension had been

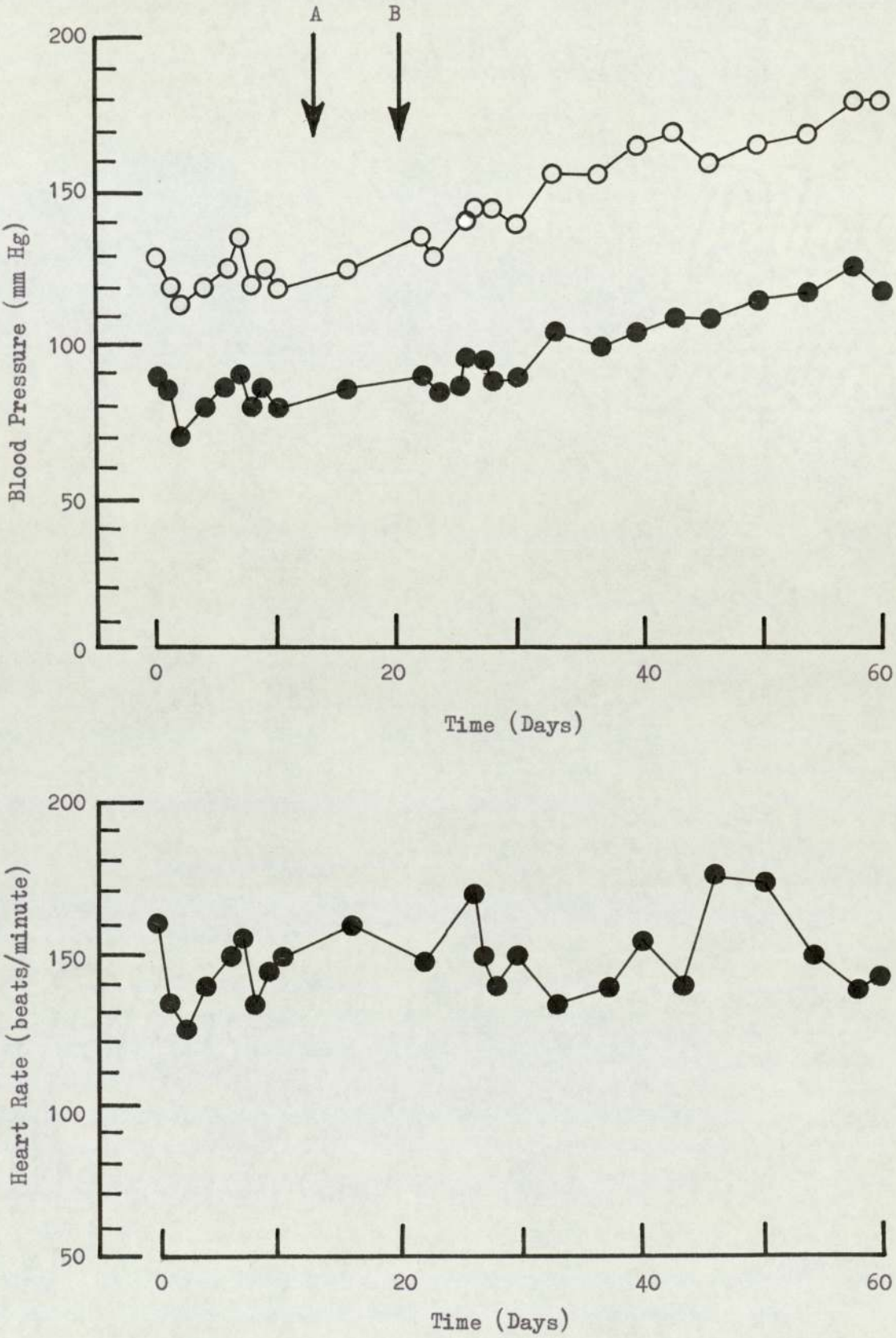


FIGURE 66: THE EFFECT OF PERINEPHRITIS INDUCED BY A 'FIGURE-OF-EIGHT' LIGATURE ON THE BLOOD PRESSURE AND HEART RATE OF CAT A3.

The upper graph shows the systolic (O—O) and diastolic (●—●) blood pressures and the lower graph the heart rate (●—●). After consistent values for the blood pressure had been obtained for several days a 'figure-of-eight' ligature was made on the left kidney with cotton tape (A). Seven days later the right kidney was removed (B).

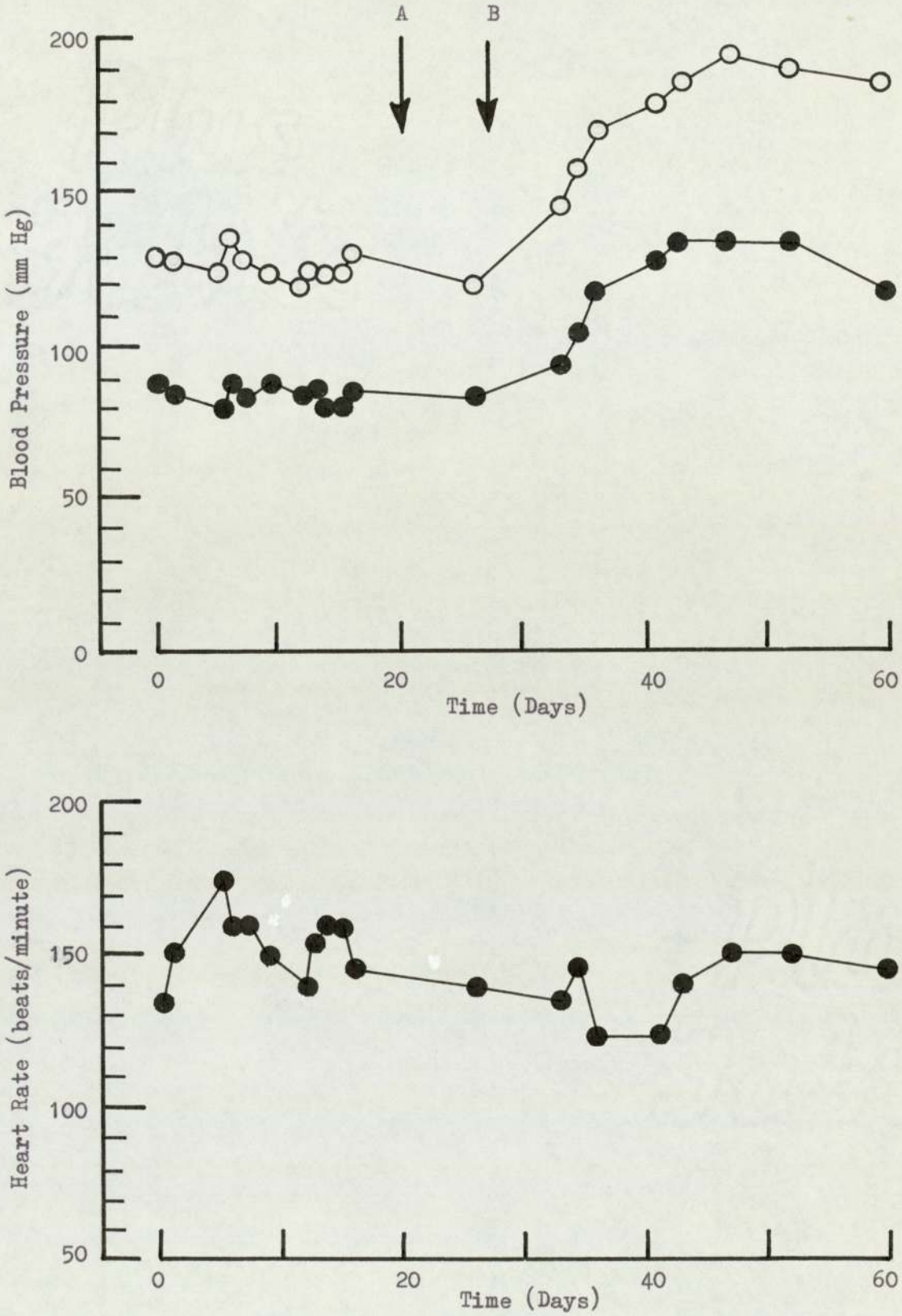


FIGURE 67: THE EFFECT OF PERINEPHRITIS INDUCED BY A 'FIGURE-OF-EIGHT' LIGATURE ON THE BLOOD PRESSURE AND HEART RATE OF CAT A4.

The upper graph shows the systolic (O—O) and diastolic (●—●) blood pressures and the lower graph the heart rate (●—●). After consistent values for the blood pressure had been obtained for several days a 'figure-of-eight' ligature was made on the left kidney with cotton tape (A). Seven days later the right kidney was removed (B).

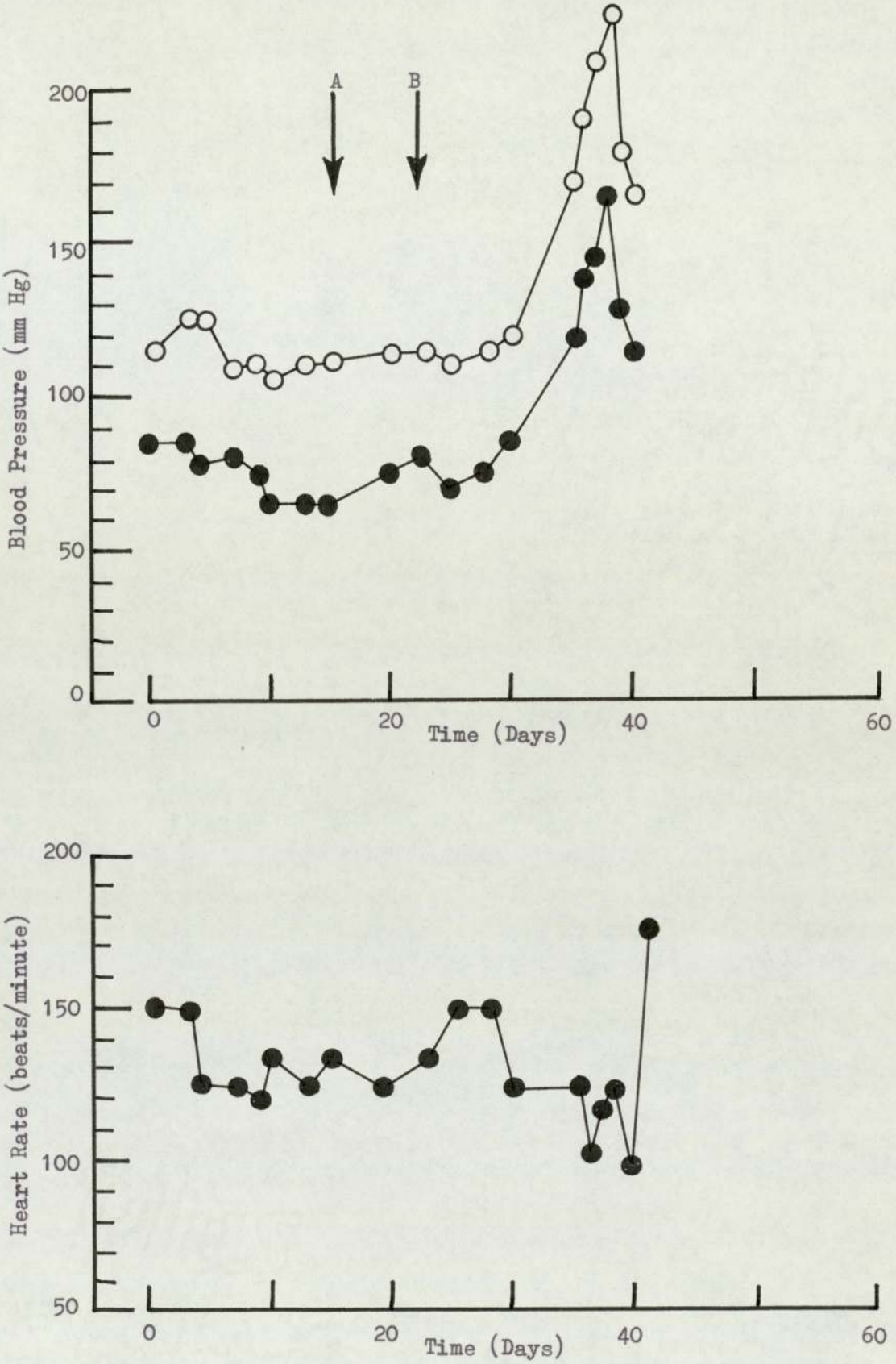


FIGURE 68: THE EFFECT OF PERINEPHRITIS INDUCED BY A 'FIGURE-OF-EIGHT' LIGATURE ON THE BLOOD PRESSURE AND HEART RATE OF CAT A5.

The upper graph shows the systolic (○—○) and diastolic (●—●) blood pressures and the lower graph the heart rate (●—●). After consistent values for the blood pressure had been obtained for several days a 'figure-of-eight' ligature was made on the left kidney with cotton tape (A). Seven days later the right kidney was removed (B).

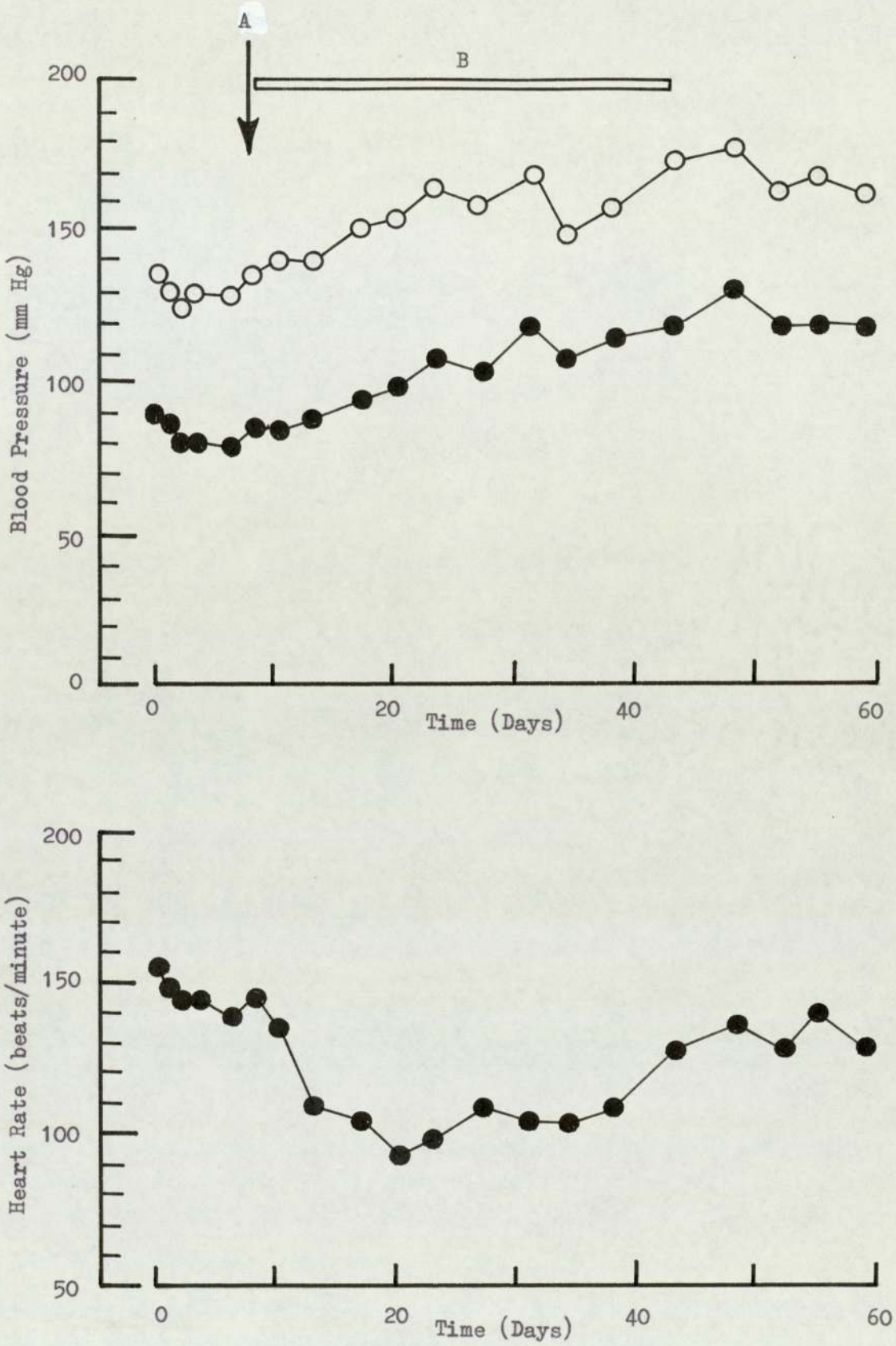


FIGURE 69: THE EFFECT OF A DOCA-NaCl REGIMEN ON THE BLOOD PRESSURE AND HEART RATE OF CAT A1.

The upper graph shows the systolic (O—O) and diastolic (●—●) blood pressures and the lower graph the heart rate (●—●). After consistent values for the blood pressure had been obtained for several days 500 mg desoxycorticosterone acetate (as 10 x 50 mg pellets) was implanted subcutaneously. At the same time the right kidney was removed (A). The normal drinking fluid, milk, was replaced by a 1% solution of sodium chloride for 5 weeks, as shown by the bar (B).

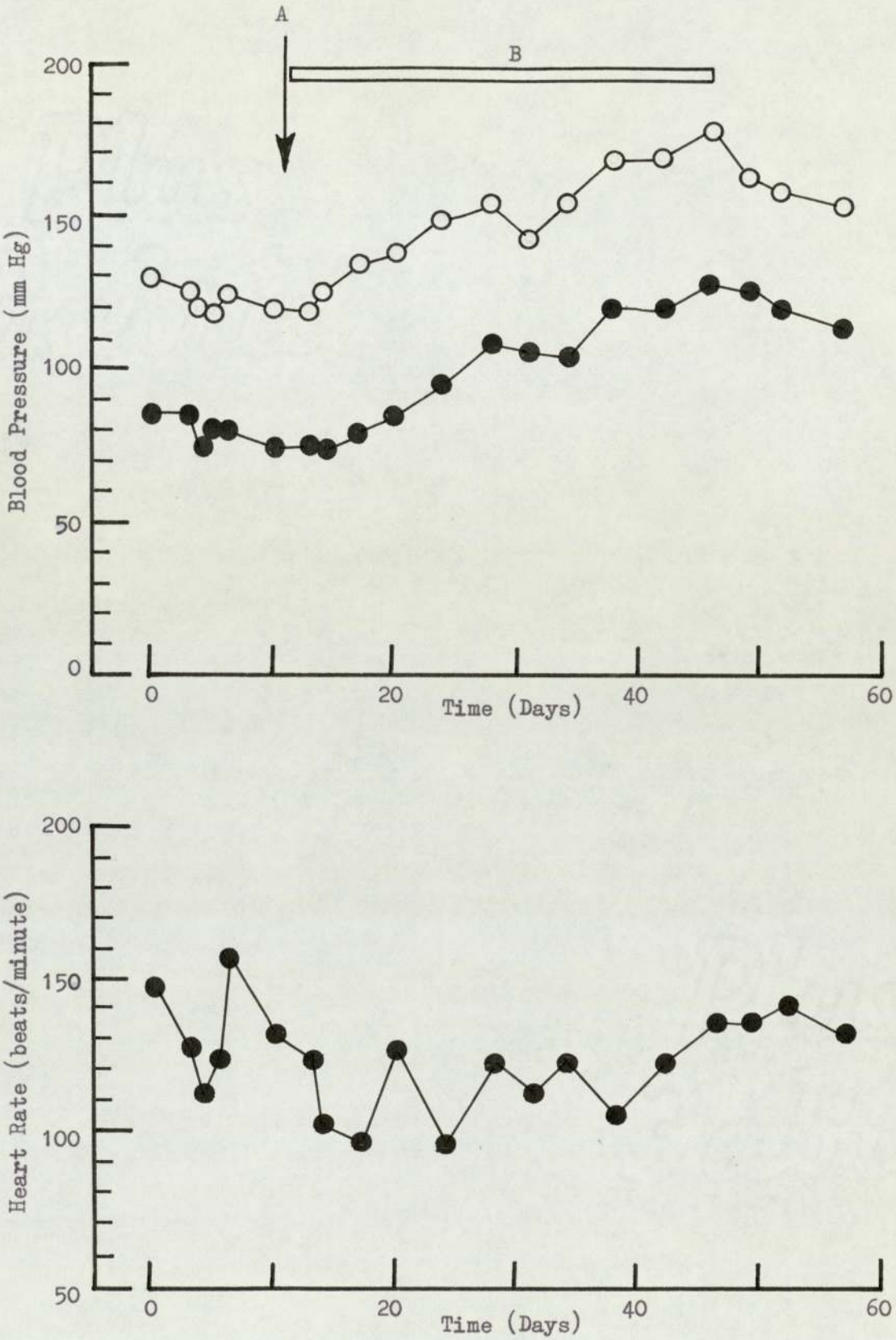


FIGURE 70: THE EFFECT OF A DOCA-NaCl REGIMEN ON THE BLOOD PRESSURE AND HEART RATE OF CAT A2.

The upper graph shows the systolic (O—O) and diastolic (●—●) blood pressures and the lower graph the heart rate (●—●). After consistent values for the blood pressure had been obtained for several days 500 mg desoxycorticosterone acetate (as 10 x 50 mg pellets) was implanted subcutaneously. At the same time the right kidney was removed (A). The normal drinking fluid, milk, was replaced by a 1% solution of sodium chloride for 5 weeks, as shown by the bar (B).

induced by a 'figure-of-eight' ligature was apparently normal, except for a loss in body weight and lethargy. The cat whose blood pressure rose very quickly lost weight dramatically and immediately prior to death became very listless and finally almost lost its righting reflex.

On post-mortem examination the kidneys and hearts were obviously hypertrophied in both DOCA-NaCl and renal hypertensive cats. Similarly, the aortas and adrenal glands of the renal hypertensive cats were seen to be hypertrophied but no obvious hypertrophy of these organs was observed in DOCA-NaCl hypertensive cats. The hypertrophy of the heart, kidney, adrenal gland and aorta of a renal hypertensive cat compared with a normotensive control cat of the same sex, weight and age is shown in Figs. 71, 72, 73 & 74.

Post-mortem examination also revealed adhesions of the kidney to the body wall, peritoneum, vessels supplying the peritoneum and the pancreas in both renal and DOCA-NaCl hypertensive cats. In one cat (A.5) there was fatty infiltration of the spleen and adhesions of the liver to the body wall.

The kidneys of the renal hypertensive cats were covered in connective tissue especially around the cotton tape forming the 'figure-of-eight' ligature. Macroscopic examination of the transverse sectioned kidneys of renal hypertensive cats showed an uneven appearance of the cortico-medullary junction. Microscopic examination revealed dilation of the blood vessels in the cortex and their walls were thickened. Many glomeruli showed mild distension of Bowman's capsule with evidence of tubular necrosis and degeneration in both the cortex and medulla. The adrenal glands and heart of the renal hypertensive cats showed no recognisable lesions although hypertrophy of the left ventricle of the latter and the cortex of the former

FIGURE 71: LONGITUDINAL SECTIONS OF THE HEARTS FROM A RENAL HYPERTENSIVE CAT AND A CONTROL NORMOTENSIVE CAT.

Hypertrophy of the left ventricle of the heart from a renal hypertensive cat can be seen on the right. The heart from a control normotensive cat is shown on the left. (Sections were stained with haemotoxylin and eosin. Magnification x 3).

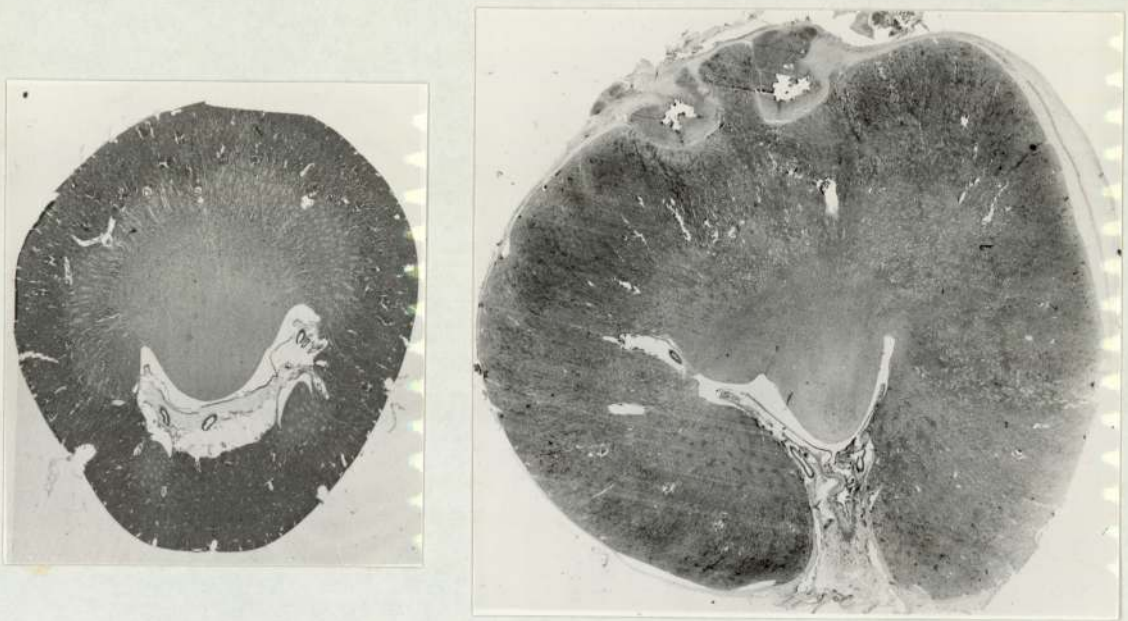

FIGURE 72: TRANSVERSE SECTIONS OF THE KIDNEYS FROM A RENAL HYPERTENSIVE CAT AND A CONTROL NORMOTENSIVE CAT.

The hypertrophy of the cortex of the kidney from a renal hypertensive cat (shown on the right) is seen when compared with a normal kidney shown on the left. (Sections were stained with haemotoxylin and eosin. Magnification x 3).

FIGURE 73: LONGITUDINAL SECTIONS OF THE ADRENAL GLANDS FROM A RENAL HYPERTENSIVE CAT AND A CONTROL NORMOTENSIVE CAT.

The hypertrophy of the adrenal cortex of the adrenal gland from a renal hypertensive cat (shown on the right) is seen when compared with a normal adrenal gland shown on the left (Sections were stained with haemotoxylin and eosin. Magnification x 10).

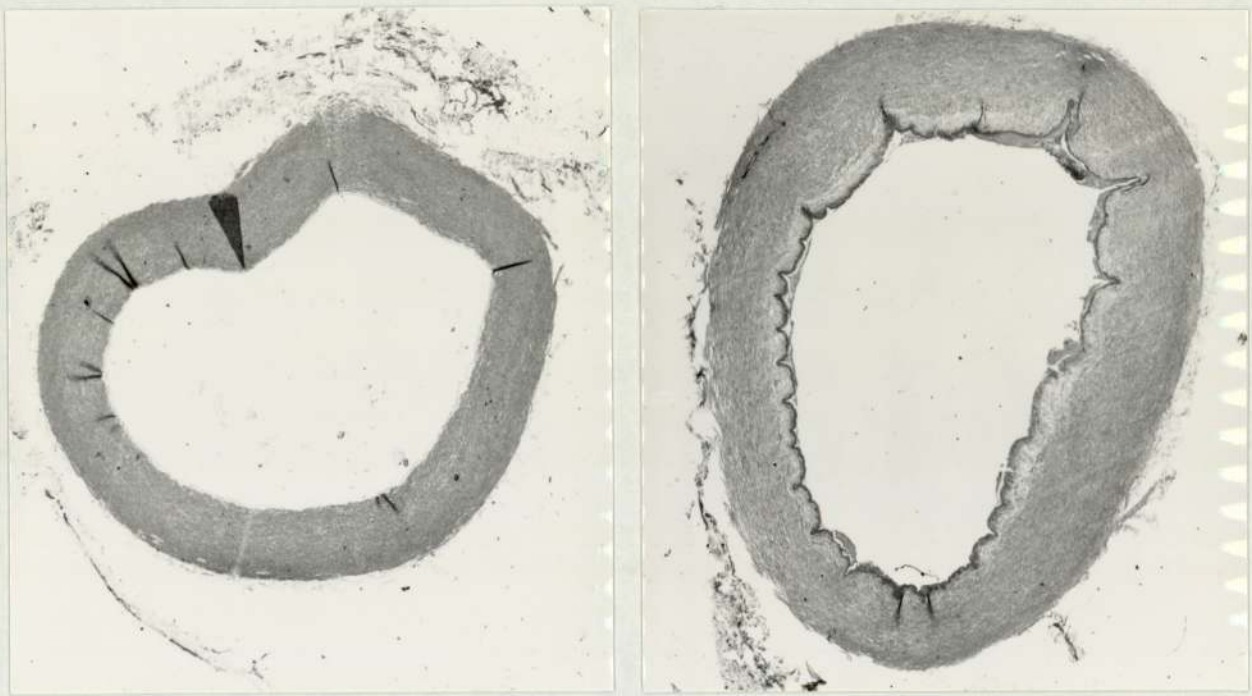

FIGURE 74: TRANSVERSE SECTIONS OF THE AORTAS FROM A RENAL HYPERTENSIVE CAT AND A CONTROL NORMOTENSIVE CAT.

The hypertrophy of the aorta from a renal hypertensive cat (shown on the right) is seen when compared with a normal aorta shown on the left. (Sections were stained with haemotoxylin and eosin. Magnification x 25).

were present. The aorta displayed signs of necrosis in the intima and hypertrophy of the elastic tissue in the media.

The hearts of the DOCA-NaCl cats showed left ventricular hypertrophy, with no obvious lesions present. The kidneys of the DOCA-NaCl cats showed dilatation of the tubules in both the cortex and medulla with thickening of the walls of blood vessels in the cortex although the overall picture was far less severe than that observed in the renal hypertensive cats. There was no obvious change from a control in either the adrenal glands or aorta of DOCA-NaCl hypertensive cats.

The effect of an oral dose of 30 mg/kg guanethidine on the blood pressure of a renal hypertensive cat is shown in Fig. 75. After dosing, a large rise in blood pressure associated with an increase in heart rate occurred. This was followed by a gradual fall in blood pressure to reach a level of 50 mm Hg below the pre-dose value at 9 hours post-dose. The systolic blood pressure had attained pre-dose levels at 24 hours although the diastolic pressure was still returning to pre-dose levels and did not attain them until 48 hours post-dose. The nictitating membrane was still relaxed at 24 hours and did not recover completely until 72 hours post-dose. The heart rate fell slightly after the initial rise to reach a level of 20 beats/minute below the pre-dose value at 5 hours and remained depressed during the remaining period of study. The heart rate had returned to pre-dose levels at 24 hours post-dose.

Fig. 76 shows the effect of a 100 mg/kg oral dose of α-methyldopa on the blood pressure of a renal hypertensive cat. The blood pressure gradually fell after dosing to reach a level of 35 mm Hg below the pre-dose value at 4 hours post-dose. The blood pressure stabilised

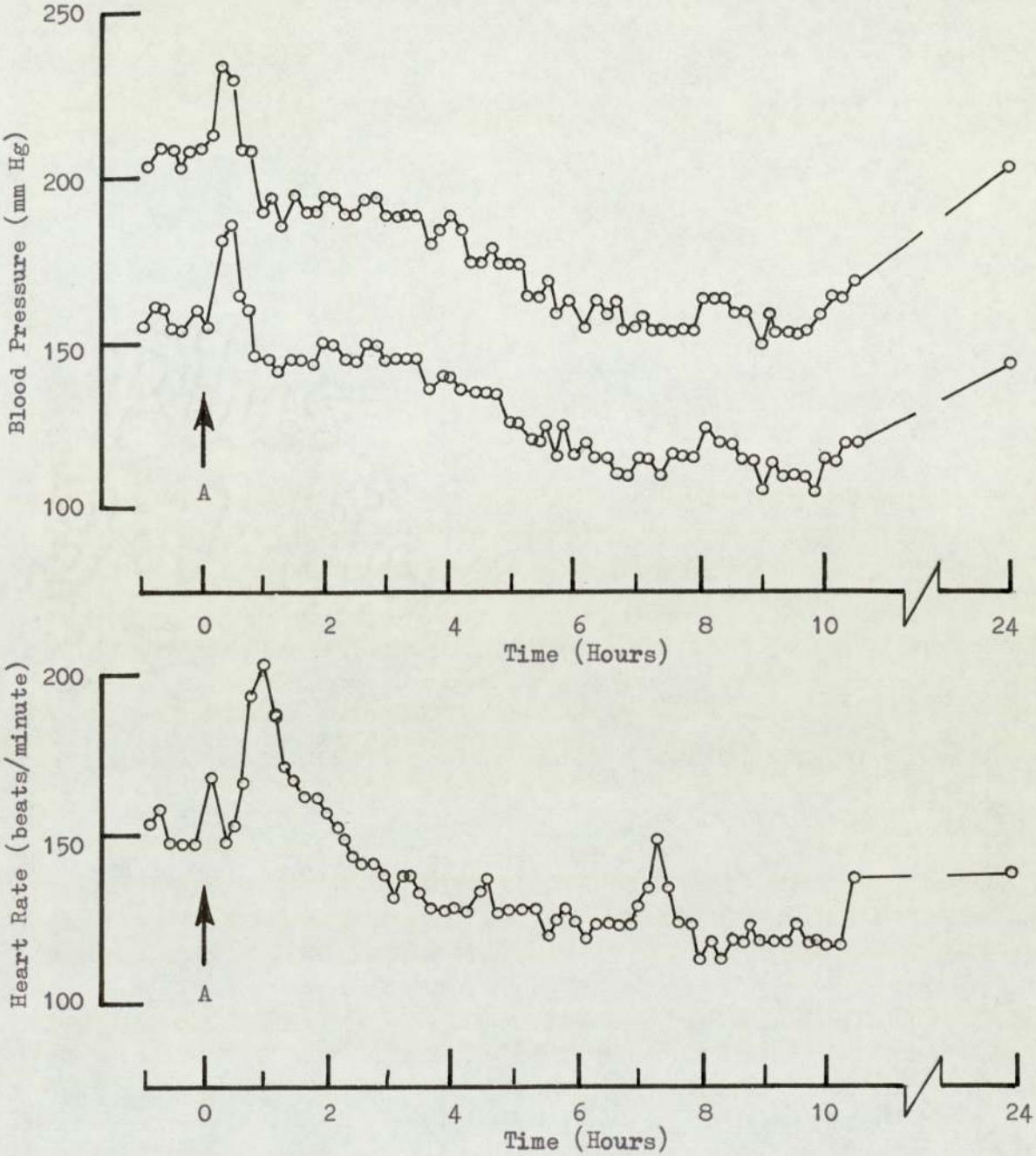


FIGURE 75: THE EFFECT OF AN ORAL DOSE OF 30 mg/kg GUANETHIDINE ON THE BLOOD PRESSURE AND HEART RATE OF A RENAL HYPERTENSIVE CAT.

The upper graph shows the systolic and diastolic blood pressures and the lower graph the heart rate. After obtaining consistent values for the blood pressure for at least 30 minutes the cat was dosed orally with 30 mg/kg guanethidine (A).

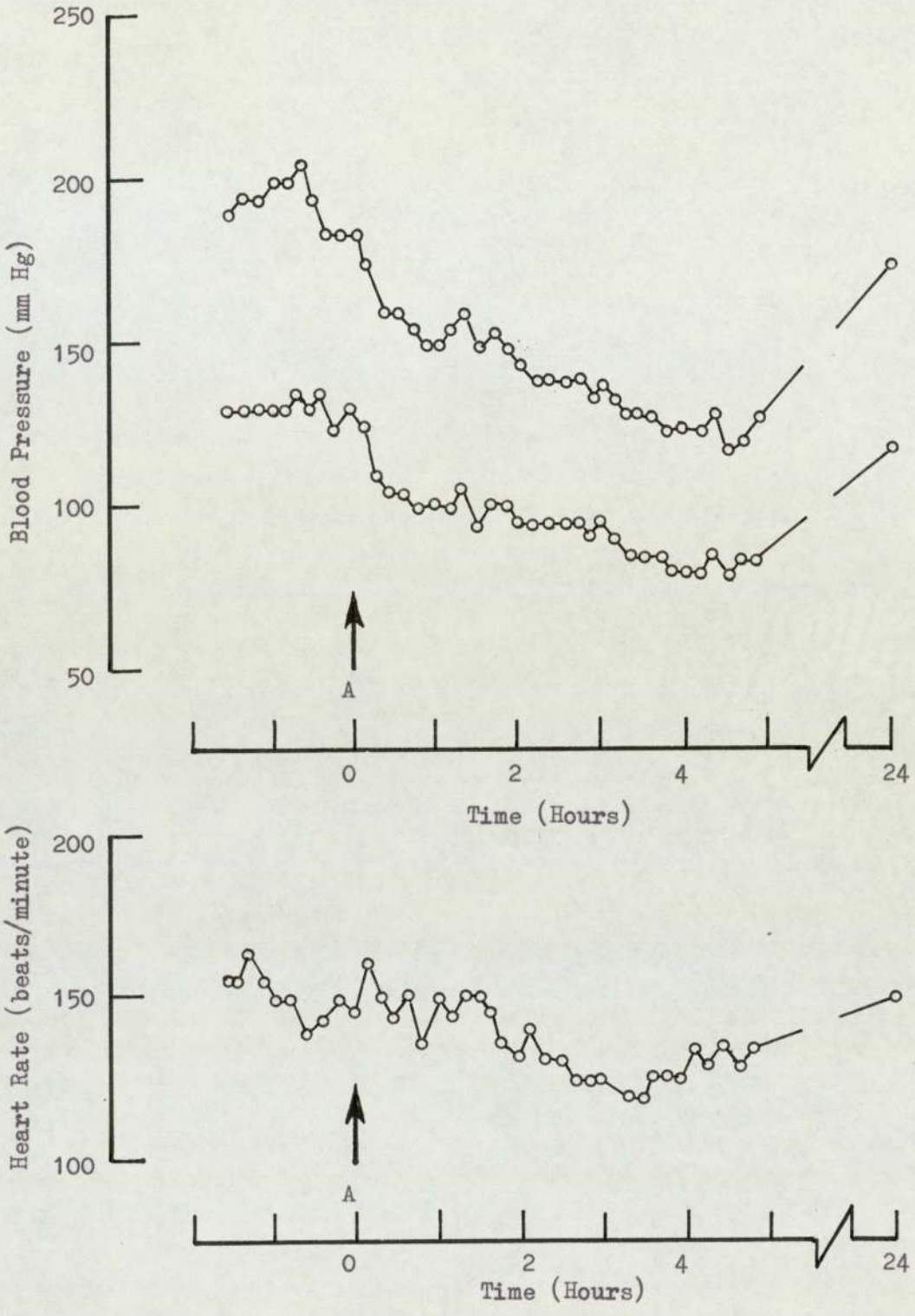


FIGURE 76: THE EFFECT OF AN ORAL DOSE OF 100 mg/kg α-METHYLDOPA ON THE BLOOD PRESSURE AND HEART RATE OF A RENAL HYPERTENSIVE CAT.

The upper graph shows the systolic and diastolic blood pressures and the lower graph the heart rate. After obtaining consistent values for the blood pressure for at least 30 minutes the cat was dosed orally with 100 mg/kg α-methyldopa (A).

at this level up to 5 hours post-dose but had attained pre-dose levels at 24 hours post-dose. The heart rate tended to follow the blood pressure.

Discussion

This study shows that renal hypertension can be produced in the cat by one of the classical methods (that is, the method of Grollman, 1944). Similarly, hypertension can be obtained in the cat by a DOCA-NaCl regimen although the general ill health of the animals tends to preclude its use as a routine method for the production of a hypertensive colony. It is possible that a lower dosage of steroid or a shorter duration of salt substitution for milk may produce hypertension without the ill effects observed. From the results of the present study the 'figure-of-eight' method of Grollman was far superior to that employing a DOCA-NaCl regimen for the production of a sustained high blood pressure. Similar results were obtained by Poyser, Shorter & Whiting (1974) who showed that cellophane perinephritis also produced hypertension in cats as initially reported by Page (1939). In these later studies the authors obtained a stable hypertension in cats after the same DOCA-NaCl regimen employed in the present study without any obvious ill effects by using large muscular cats (body weights greater than 3.5 kg).

The importance of these studies is that the conscious cat in the normotensive state has been shown to be ideally suited to the study of drugs on the cardiovascular system. The fact that the cat is in the conscious state removes any artefacts due to anaesthesia and the lack of restraint obviously produces blood pressure responses more akin to normal. Day & Owen (1970) showed that the responses to a pressor agent were consistent not only during several hours of study

but also from day to day and this reproducibility of the blood pressure and response to drugs enables a cat to serve as its own control. The fact that the conscious cat can serve as its own control allows regular determinations of the blood pressure responses to various agents to be made before, during the development of, and when the blood pressure has stabilised at hypertensive levels, which may provide useful information on the aetiology of experimental hypertension. Also the conscious unrestrained cat in the hypertensive state may be ideally suited to the study of potential antihypertensive agents administered chronically.

The fact that single doses of antihypertensive agents produce a fall in both systolic and diastolic blood pressures in the conscious hypertensive cat is very encouraging since although the hypertensive rat also responds to single doses of antihypertensive agents the direct measurement of blood pressure in the rat is difficult (Geddes, 1970). The dog is well suited to direct measurement of blood pressure but its nature is more playful than that of the cat, the blood pressure varies with the respiration and very few antihypertensive agents lower the blood pressure of hypertensive dogs acutely and centrally acting antihypertensive agents (e.g. clonidine, propranolol) do not lower the blood pressure of conscious hypertensive dogs even by chronic administration (Shorter, personal communication). The falls in blood pressure obtained in conscious hypertensive cats by both guanethidine and α-methyldopa after single oral doses of these antihypertensive agents closely resemble those obtained in conscious rats at similar dose levels (see review by Boura & Green, 1965; Day, Roach & Whiting, 1972, 1973). The rise in blood pressure with an associated rise in heart rate produced by guanethidine is well

documented as being due to the release of noradrenaline from intraneuronal stores of the sympathetic nervous system (Abbs, 1966 and review by Frohlich, 1974). The following fall in blood pressure is due to adrenergic neuronal blockade (see reviews by Boura & Green, 1965 and Frohlich, 1974) and the observed relaxation of the nictitating membrane is another useful indicator of drug action seen in this preparation.

α-Methyldopa produced a maximum fall in blood pressure 4 hours post-dose in the conscious hypertensive cat which agrees with results obtained in the conscious rat (e.g. Day, Roach & Whiting, 1972, 1973). The fall in blood pressure is believed to be due to a central action of α-methylnoradrenaline in the central nervous system (Day, Roach & Whiting, 1972, 1973).

Hence, this study has shown that a stable elevation of the normal blood pressure of cats can be obtained and that this elevated blood pressure can be reduced towards normal levels with single doses of two antihypertensive agents. This work has been confirmed and expanded by Poyser, Shorter & Whiting (1974).

The pathology of the hypertension in cats is very similar to that observed in rats. The hypertrophy of the heart, kidney, adrenal glands and aorta of the renal hypertensive cats are the typical effects observed in other renal hypertensive animals (e.g. Ostrovsky, Papsin & Gornall, 1968) demonstrating the similar course of the disease as in other species. Similarly, the hypertrophy of the heart and kidney and lack of hypertrophy of the adrenals in DOCA-NaCl hypertensive cats has been reported many times in the literature on the pathology of DOCA-NaCl hypertension in rats (e.g. Friedman, Honore & Friedman, 1972). The lack of hypertrophy of the aorta in

DOCA-NaCl cats is perhaps not too surprising since the aorta is not intimately involved in the maintenance of peripheral resistance and microscopic examination of a smaller artery or arteriole may reveal the presence of hypertrophy as reported in the case of DOCA-NaCl rats (e.g. see review by Plummer, 1967).

The presence of severe lesions in the kidney, slight lesions in the aorta and none in the heart and adrenal glands of renal hypertensive cats (except for the generalised hypertrophy of the former and the cortex of the latter) is also in agreement with the literature concerning renal hypertension in other species (e.g. see review by Giese, 1966). The absence of lesions in the organs of the DOCA-NaCl hypertensive cats is in agreement with much of the literature on steroid hypertension in other species although there is some disagreement on the pathology of DOCA-NaCl hypertension in rats (see Chapter 2, Section 1).

Thus this present study has shown that hypertension can be produced by two methods, that is a DOCA-NaCl regimen and Grollman's method of 'figure-of-eight' perinephritis, the pathology of the resulting hypertension is similar to that in other species in which hypertension has been produced and that the blood pressure of these hypertensive cats can be lowered by a single dose of either of two widely used antihypertensive agents.

Summary

1. Hypertension has been produced in the cat by two methods, that is, by a DOCA-NaCl regimen and by the 'figure-of-eight' perinephritic method of Grollman. Although both procedures resulted in the production of a hypertensive state the DOCA-NaCl regimen caused general ill-health of the cats and was not considered useful as a routine method for the production of a hypertensive colony.

2. The pathologies of these two types of hypertension in the cat closely resemble the pathologies of the same hypertensions in the rat.

3. Single doses of two widely used antihypertensive agents produce falls in both the systolic and diastolic blood pressures of conscious, unrestrained renal hypertensive cats.

4. The possible importance and uses of conscious hypertensive cats in studies on hypertension are discussed.

## CHAPTER 2

### Cardiovascular Reactivity in Renal Hypertensive Cats

Due to the consistent day to day reproducibility of blood pressure responses to various pressor agents in the conscious, unrestrained cat (Day & Owen, 1970) this preparation can act as its own control. Thus, cardiovascular reactivity to certain pressor agents can be studied before, during the development of and when the hypertension is established in a single cat.

Cardiovascular reactivity has usually been studied during established hypertension invariably by comparing the responses obtained to pressor substances in a group of anaesthetised control animals to a similar group of hypertensive animals (see Chapter 1, Section 2). Cardiovascular reactivity has never been examined in the hypertensive cat and similarly it has not been examined in a single animal during the development of hypertension. Thus the conscious, unrestrained cat offered a unique opportunity to examine cardiovascular reactivity during the various stages of hypertension.

### Results

Fig. 77 shows the changes in response to noradrenaline, tyramine, angiotensin and McN-A-343 before and during the development of hypertension induced by the 'figure-of-eight' method of Grollman (1944) in cat A4. Fig. 78 shows the changes in response to the same pressor agents before and during the development of hypertension, induced in the same manner as above, in cat A3.

In both cats the responses to the four pressor substances were very consistent during a days period of study (never greater than 10 mm Hg and usually less than 5 mm Hg) and fairly consistent from day to day (never greater than 20 mm Hg and normally about 5 - 10 mm Hg (see

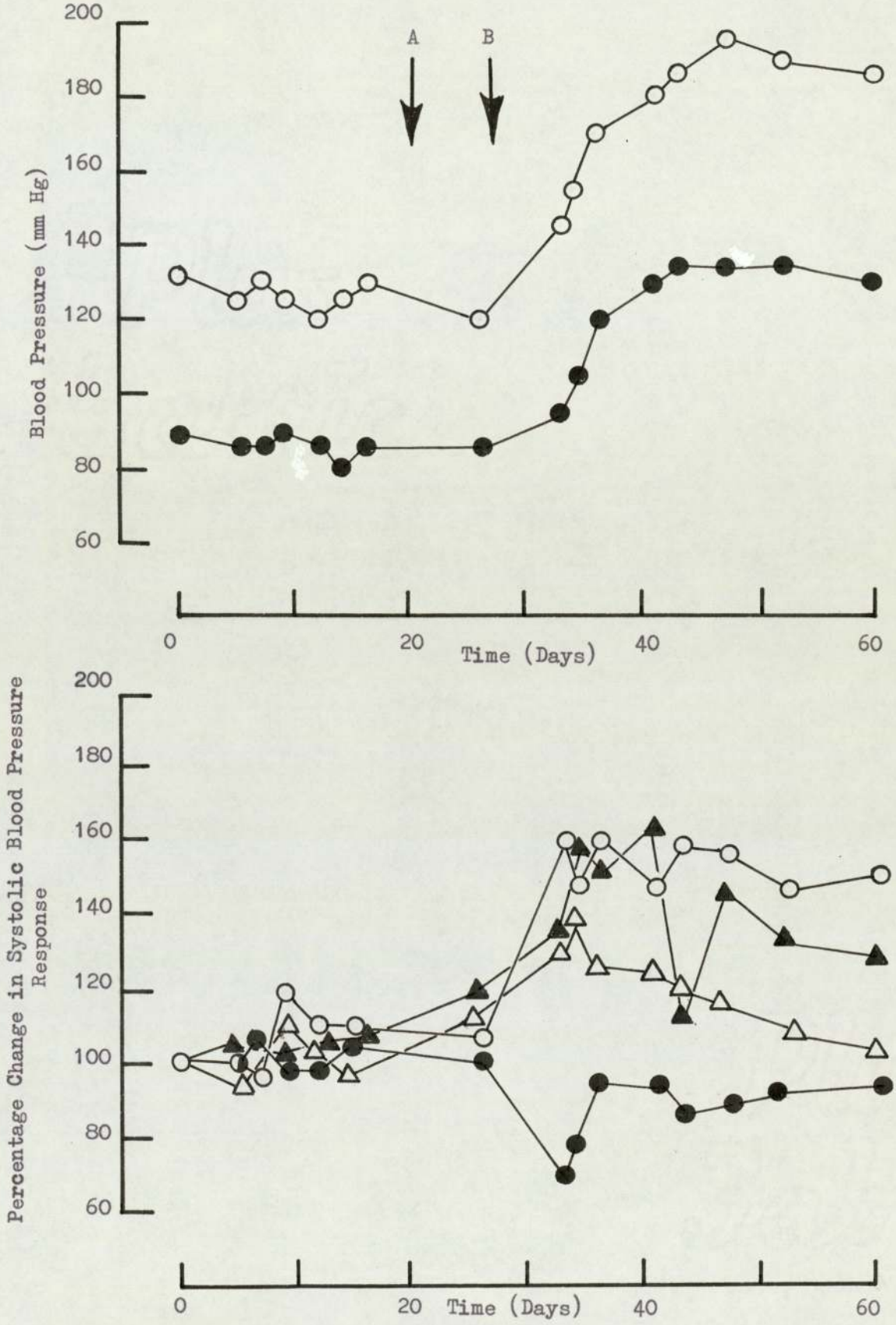


FIGURE 77: THE CHANGES IN RESPONSES TO VARIOUS PRESSOR AGENTS DURING THE DEVELOPMENT OF RENAL HYPERTENSION IN CAT A4.

The upper graph shows the systolic (○—○) and diastolic (●—●) blood pressures. The lower graph shows the blood pressure responses to angiotensin (●—●); McN-A-343 (○—○); noradrenaline (△—△) and tyramine (▲—▲) before and during the development of hypertension induced by making a 'figure-of-eight' ligature on the left kidney (A) and performing right nephrectomy 7 days later (B).

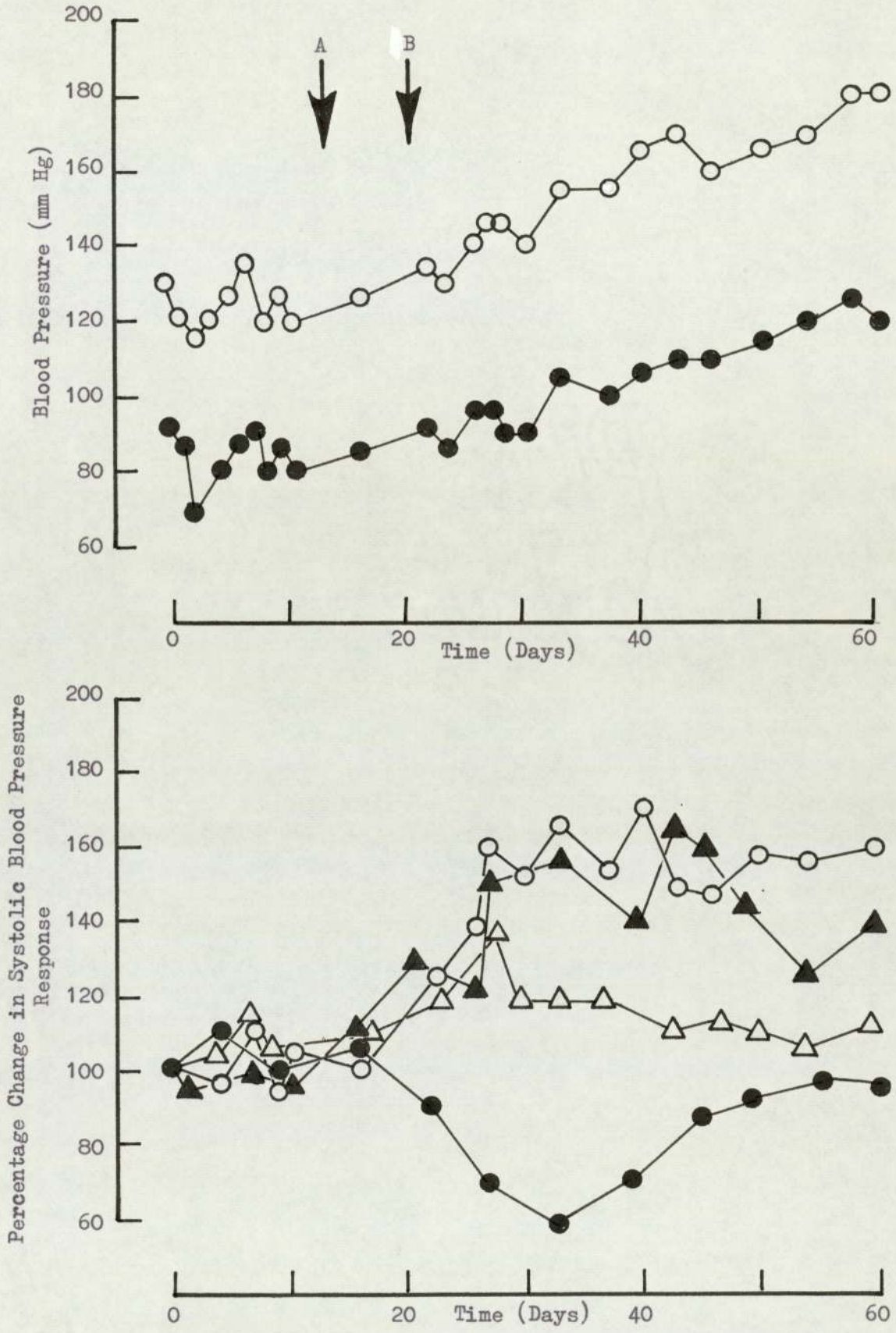


FIGURE 78: THE CHANGES IN RESPONSES TO VARIOUS PRESSOR AGENTS DURING THE DEVELOPMENT OF RENAL HYPERTENSION IN CAT A3.

The upper graph shows the systolic (○—○) and diastolic (●—●) blood pressures. The lower graph shows the blood pressure responses to angiotensin (●—●); McN-A-343 (○—○); noradrenaline (△—△) and tyramine (▲—▲) before and during the development of hypertension induced by making a 'figure-of-eight' ligature on the left kidney (A) and performing right nephrectomy 7 days later (B).

Figs. 79 & 80).

Similarly the doses of the pressor agents required to produce increases of systolic blood pressure of about 90 mm Hg were similar in both cats (noradrenaline 500 ng/kg and 400 ng/kg; angiotensin 25 ng/kg in both cases; tyramine 100 µg/kg and 75 µg/kg; McN-A-343 100 µg/kg and 75 µg/kg).

Also the changes in responses to the pressor substances during the development of hypertension were similar in both cats.

The response to angiotensin decreased rapidly from the time of unilateral nephrectomy whilst not changing in the seven previous days which followed application of the 'figure-of-eight' ligature to the left kidney. The angiotensin response decreased to its lowest level 6 - 10 days (cats A4 and A3 respectively) after unilateral nephrectomy and then quickly returned to control values in cat A4 and gradually in cat A3. Similarly the response to noradrenaline changed very little after the application of the 'figure-of-eight' ligature but increased rapidly after unilateral nephrectomy to reach a maximum seven days later and then gradually decreased to control levels.

The response to McN-A-343 also increased rapidly only after unilateral nephrectomy to reach a maximum 9 - 20 days later (cats A4 and A3 respectively) after which the response remained elevated throughout the remaining period of study.

The response to tyramine increased slightly after the application of the 'figure-of-eight' ligature to the left kidney and then quite markedly after unilateral nephrectomy to reach a maximum 14 - 23 days later (cats A4 and A3 respectively) after which there was a gradual fall in the response towards control values but remained well above these values until the end of the study.

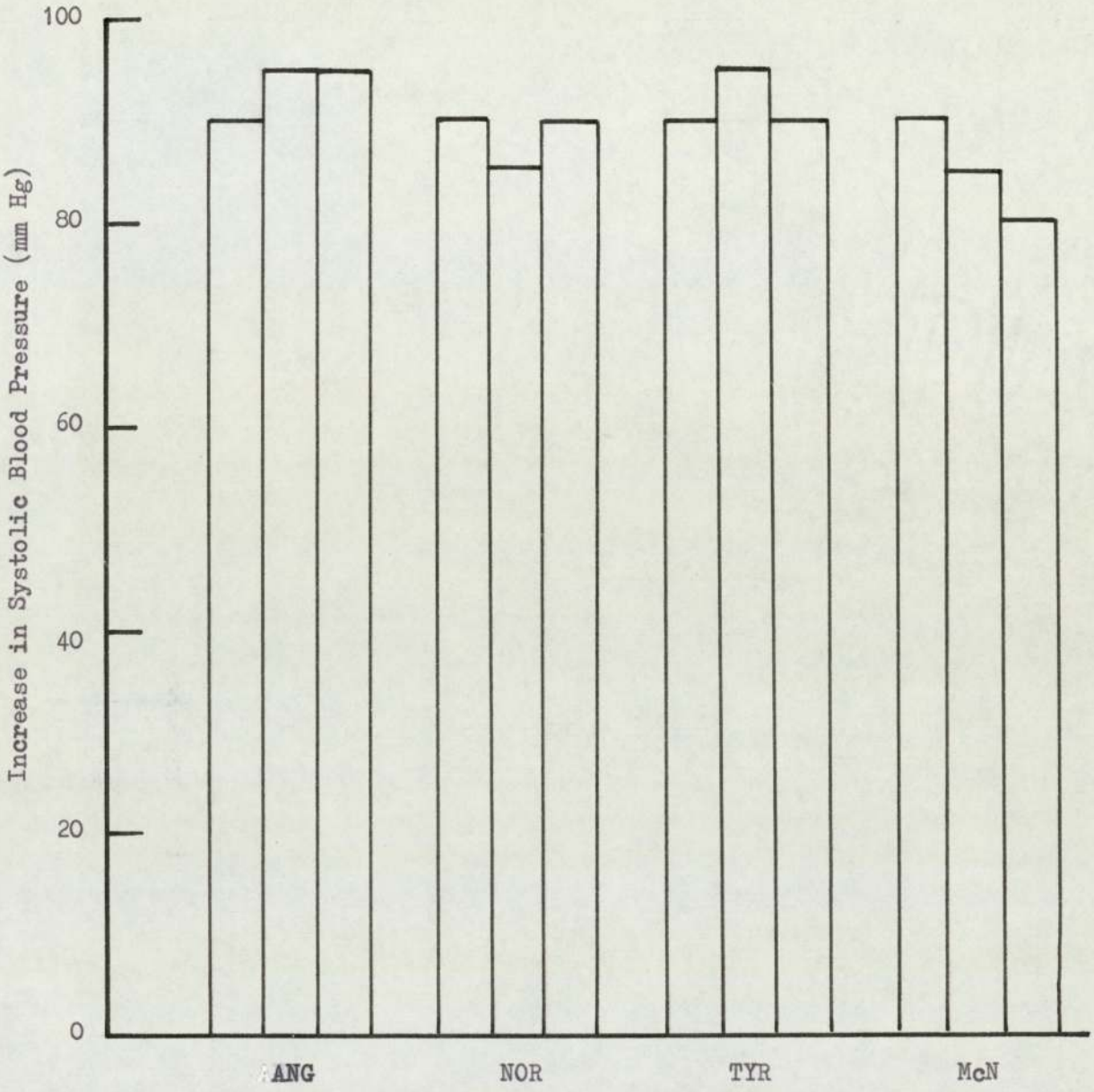


FIGURE 79: THE REPRODUCIBILITY OF CONSECUTIVE RESPONSES TO PRESSOR AGENTS IN CONSCIOUS CATS.

Each histogram represents the response to each of three consecutive i.v. doses of angiotensin (ANG) 25 ng/kg, noradrenaline (NOR) 500 ng/kg, tyramine (TYR) 100 µg/kg and McN-A-343 (McN) 100 µg/kg in cat A4.

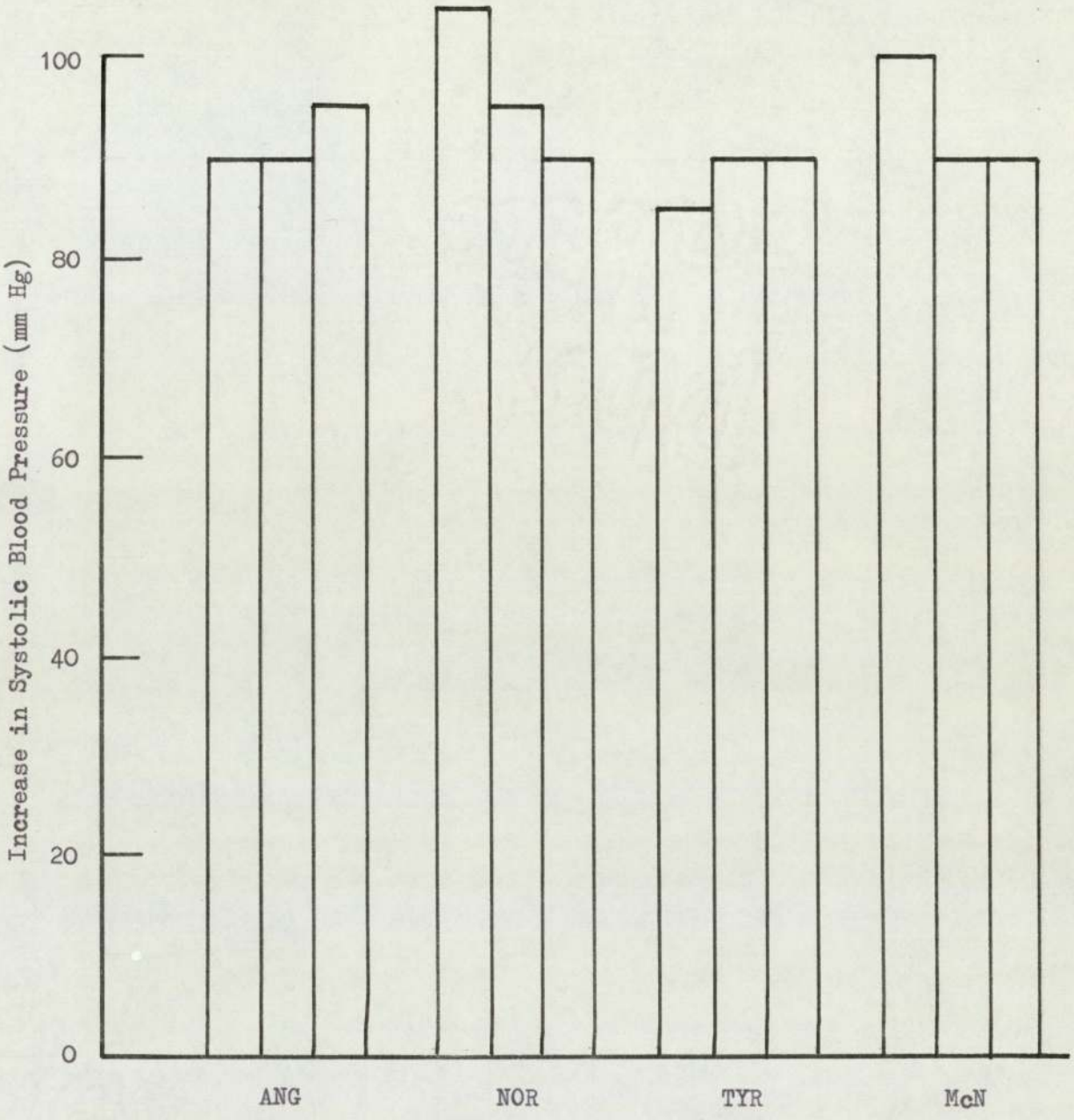


FIGURE 80: THE REPRODUCIBILITY OF RESPONSES TO PRESSOR AGENTS FROM DAY TO DAY IN CONSCIOUS CATS.

Each histogram represents the mean of three consecutive responses on each of three consecutive days. The histograms represent mean responses to i.v. doses of angiotensin (ANG) 25 ng/kg, noradrenaline (NOR) 500 ng/kg, tyramine (TYR) 100 µg/kg and McN-A-343 (McN) 100 µg/kg in cat A4.

The slower increase in the blood pressure of cat A3 after the operation to induce hypertension was not associated with a slower increase or decrease in the responses to the pressor agents than those observed in cat A4.

The reflex falls in heart rate to the four pressor substances increased or decreased from control levels dependent upon the change in the blood pressure response.

The effect of subpressor infusions of angiotensin (5 ng/kg/min. and 10 ng/kg/min.) on the responses to the four pressor substances was examined in chloralosed anaesthetised cats 30 - 45 minutes after the start of the infusion (see Fig. 81). The responses to noradrenaline were increased almost 100% and those to tyramine and McN-A-343 by 40 - 45% whilst the responses to angiotensin were decreased by 10 - 15% from control values.

Pressor infusions of angiotensin (25 ng/kg/min. and 100 ng/kg/min.) produced similar increases in the responses to noradrenaline, tyramine and McN-A-343 to those observed at the lower infusion doses. The response to angiotensin decreased as the infusion rate increased (20 - 25% at 25 ng/kg/min. and 40 - 45% at 100 ng/kg/min.).

Discussion

The consistency of the responses to the pressor substances during a single day's period of study, from day to day and from cat A3 to cat A4 are in agreement with the results of Day & Owen (1970) although slightly greater variations from day to day were observed in the noradrenaline and McN-A-343 responses than those workers reported. These results illustrate that a single cat can act as its own control and is well suited to cardiovascular reactivity studies in hypertension.

The results show a clear increase in cardiovascular reactivity to

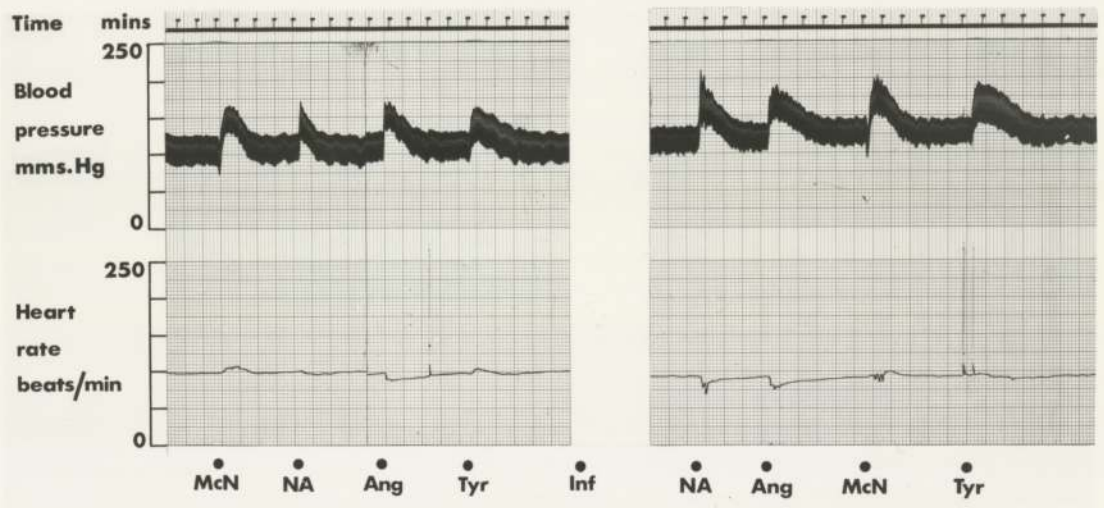


FIGURE 81: THE EFFECT OF AN INFUSION OF ANGIOTENSIN ON THE BLOOD PRESSURE RESPONSES TO FOUR STANDARD PRESSOR AGENTS.

The changes in the blood pressure of an anaesthetised cat caused by McN-A-343, 12 µg/kg i.v., (McN), noradrenaline, 200 ng/kg i.v. (NA), angiotensin, 50 ng/kg i.v., (Ang) and tyramine, 50 µg/kg i.v., (Tyr) are shown above. These standards were given before and approximately 30 minutes after the start of an infusion (indicated by Inf) of angiotensin 5 ng/kg/min.

McN-A-343 and tyramine, to a lesser extent to noradrenaline and a decrease in cardiovascular reactivity to angiotensin during the development of hypertension, the increase or decrease in cardiovascular reactivity closely paralleling the rise in blood pressure in all cases except noradrenaline. However, with the stabilisation of the blood pressure at a high level cardiovascular hyper-reactivity occurred only in the cases of McN-A-343 and tyramine, the responses to noradrenaline and angiotensin having returned to control levels.

There is much evidence that the renin-angiotensin system is involved in the rise in blood pressure during the acute states of renal hypertension (e.g. see reviews by Page & McCubbin, 1968; Ledingham, 1971; Tobian, 1974; Page, 1974 and Peart, 1975) and the effect of a pressor infusion of angiotensin in the anaesthetised cat produced changes in the responses to the pressor agents similar to those observed during the initial rise in blood pressure in the conscious cats. It would thus appear very probable that the changes in cardiovascular reactivity and the concurrent rapid rise in blood pressure observed in the conscious cat are due to the presence of increased amounts of circulating renin-angiotensin.

Infusions of both pressor and subpressor doses of angiotensin produce an elevation of the blood pressure, the former a transitory rise probably due to the development of tachyphylaxis and the latter a sustained hypertension (see review by Page & McCubbin, 1968).

Infusions of angiotensin in various species produce a dose dependent decrease in the response to injections of angiotensin (e.g. see review by Page & McCubbin, 1968; Aoki & Masson, 1969; Deheneffe & Bernard, 1974), an increase in the response to tyramine and other procedures which release noradrenaline from sympathetic neurones

(thus McN-A-343) (e.g. McCubbin & Page, 1963; Page & McCubbin, 1968; Day & Owen, 1969; Basso, 1975) and either an increase (Schmitt & Schmitt, 1967 a & b; Basso, 1975) or no change (e.g. McCubbin & Page, 1963; Page, Kaneko & McCubbin, 1966; Louis & Doyle, 1966; Day & Owen, 1969) in the response to exogenous noradrenaline. Hence, the changes in cardiovascular reactivity to the pressor substances used in this study would appear to be probably due to increased circulating amounts of renin-angiotensin which initiate the rise in blood pressure.

When the blood pressure had stabilised at its higher level the responses to angiotensin and noradrenaline had returned to control levels indicating that circulating levels of renin-angiotensin had returned to normal values. There is a great deal of evidence suggesting that during the chronic phase of one-kidney renal hypertension renin-angiotensin levels are not raised (e.g. see reviews by Page & McCubbin, 1968; Ledingham, 1971 and Davis, Freeman, Johnson & Spielman, 1974) and other mechanisms, as yet not fully identified, are responsible for the maintenance of the increased peripheral resistance. There is evidence, however, which strongly implicates the sympathetic nervous system as the mechanism which maintains the elevated pressure (see reviews by Page & McCubbin, 1968; De Champlain, 1972 and Schmid & Abboud, 1974) in conjunction with a resetting of the baroreceptors at a higher level (e.g. Kezdi, 1967; Aars, 1968 a & b; Bristow, Honour, Pickering, Sleight & Smyth, 1969; Wallin, Delius & Hagbarth, 1973). Although the baroreceptors are reset at a higher level very soon after the initiation of hypertension (see review by Korner, 1975) baroreflex modulation of the sympathetic outflow is still present (see reviews by Aars, 1975 and Korner, 1975) and thus the increased cardiovascular reactivity to pressor substances cannot be explained

on this basis and this is illustrated in the present study in that the response to noradrenaline returned to normal levels. The continued hyper-reactivity of vascular smooth muscle to tyramine and McN-A-343 during the chronic phase of hypertension would appear to reflect the presence of a further component, probably the sympathetic nervous system, which maintains the elevated blood pressure.

McN-A-343 is a substance which acts selectively at sympathetic ganglia and provides an index of sympathetic integrity (e.g. Roszkowski, 1961; Levy & Ahlquist, 1962; Jones, 1963; Smith, 1966). Hence, the response to McN-A-343 is severely reduced after reserpine pretreatment (Day & Owen, 1970) and the increased response observed in the present study may be due to increased intraneuronal noradrenaline. Similarly, infusions of noradrenaline are known to increase the blood pressure responses to tyramine (Burn & Rand, 1960; Day & Owen, 1969) and cause no change or a decrease in the response to exogenous noradrenaline (Page & McCubbin, 1968). Although these reports would support the changes observed in the present study angiotensin responses are also increased during noradrenaline infusions or procedures which release noradrenaline (Haas & Goldblatt, 1959; Schmitt & Schmitt, 1967 a & b; Pals & Fulton, 1968; Day & Owen, 1969). The lack of potentiation of the angiotensin response during the chronic stage of renal hypertension in the cat may reflect an opposition of effects of increased sympathetic activity and the presence of slightly elevated circulating levels of renin-angiotensin.

Although changes in the vasculature occur in renal hypertensive animals soon after the induction of hypertension creating an increased wall-to-lumen ratio (e.g. see review by Giese, 1966; Folkow et al.,

1973; Hallback, Weiss & Folkow, 1974) and this has been suggested as causing increased cardiovascular reactivity to pressor substances (see review by Folkow et al., 1972) this cannot be the case in the present study since a non specific increase in responses to pressor agents would have been observed.

It would appear from the results of this study that one-kidney renal hypertension is instigated in the cat by an involvement of the renin-angiotensin system. This is followed by a fall in renin-angiotensin levels and an increase in sympathetic nerve activity with a resetting of the baroreceptors to a higher level. Although changes in the vasculature of the cat have occurred at this time (see Section 4, Chapter 1) these changes do not cause increased responses to pressor substances as initially suggested by Conway (1958) and this is in agreement with the reports of other workers (e.g. Gordon & Noguiera, 1962; McGregor & Smirk, 1970; Lais & Brody, 1975) who state that there is an inherent increase in the sensitivity of vascular smooth muscle. However, the lack of cardiovascular hyper-reactivity in the cat to noradrenaline and angiotensin during the initial phase of established hypertension indicates that cardiovascular hyper-reactivity to these agents, often reported during hypertension in other species and man (e.g. see reviews by Doyle, 1968 and De Champlain, 1972) may be due to species difference or cardiovascular changes which occur during the chronic phase of the disease. Many workers have also failed to demonstrate cardiovascular hyper-reactivity to these pressor substances during the established phase of hypertension (e.g. Spector, Fleish, Maling & Brodie, 1969; McGregor & Smirk, 1970; Clineschmidt, Geller, Govier & Sjoerdsma, 1970; and review by De Champlain, 1972) as observed in this present study.

These results, although from a small number of animals, suggest that the increase in cardiovascular reactivity to various pressor agents in the conscious hypertensive cat is a secondary effect and not a primary increase in sensitivity of arteriolar smooth muscle.

Summary

1. Cardiovascular reactivity has been studied during the production and maintenance of one-kidney renal hypertension in conscious, unrestrained cats.

2. Consistent blood pressure responses, to the pressor agents used, were obtained during a day study and from day to day which enabled each cat to serve as its own control.

3. Increased pressor responses to tyramine, McN-A-343 and noradrenaline, and decreased pressor responses to angiotensin, were observed during the acute stage of the hypertension. Similar responses to the pressor agents were obtained in anaesthetised cats during infusions of angiotensin. It is suggested that the changes in the pressor responses observed during the acute stage of hypertension may be due to increased circulating levels of angiotensin.

4. Increased pressor responses to tyramine and McN-A-343 but not to angiotensin and noradrenaline were observed during the chronic stage of the hypertension. It is suggested that these changes may be due to increased activity of the sympathetic nervous system.

5. It is suggested that the observed cardiovascular hyper-reactivity is a secondary effect and not a primary increase in sensitivity of vascular smooth muscle or a changed vasculature resulting in an increased wall-to-lumen ratio.

# GENERAL DISCUSSION

The initial problem of the work described in this thesis was to produce a consistent hypertension, either acute or chronic, in rats. This also involved a further problem, how to measure the blood pressure of rats simply and accurately. The initial approach was to produce acute hypertension in the rat and subsequently measure the blood pressure directly in the anaesthetised animal. However, although acute hypertension was found to be easily producible it was very difficult to control. This led to the idea of producing chronic hypertension in the rat and measuring the blood pressure directly by cannulation of the aorta via the carotid artery. This method of blood pressure measurement in the rat was found to be adequate in the short term, however, it possessed many disadvantages for long term studies. Thus, it was decided to develop a system for the measurement of the systolic blood pressures of rats by an indirect tail cuff method. After construction of such a system it was found that a clear 'end point' for the systolic blood pressure was obtained and consistent values were obtained in a rat throughout a day.

The accuracy of the systolic blood pressure readings obtained by the tail cuff method were evaluated by comparison with values of the systolic blood pressure obtained directly by cannulation of the aorta via the carotid artery and a very close agreement was observed. These results were in agreement with those of Pfeffer, Pfeffer & Frohlich (1971) and Bunag (1973) who also found that a tail cuff method used for the determination of systolic blood pressures in unanaesthetised rats produced values very similar to those obtained directly by arterial cannulation. The tail cuff method was also found to accurately follow drug induced changes of the systolic blood pressure in unanaesthetised rats.

With the development of a system which produced accurate and reproducible values for the systolic blood pressure, when measured indirectly, a study was again undertaken to determine a suitable method for producing hypertension in the rat. It was found that hypertension could be produced consistently by employing either the method of 'Grollman' (1944) or a DOCA-NaCl regimen but not by the methods of 'Page'(1939) or 'Loomis'(1946).

The pathologies of the DOCA-NaCl and renal induced hypertensions were found to agree in general with those reported in the literature (see Section 1, Chapter 2). However, the lack of cardiovascular lesions and hypertrophy of arteries observed in this study are not in accord with the wealth of evidence on this subject (see review by Giese, 1966). Recently, however, Hansen, Abrams & Bohr (1974) also failed to observe hypertrophy of femoral arteries from DOCA-NaCl hypertensive rats and suggested that this was due to the short duration of treatment (5 weeks) which they employed. This explanation may well be applicable to the results obtained in the present study. In addition, the fact that the tissues examined were from rats which in most cases developed hypertension rather slowly, may also have been a factor, since the severity of vascular lesions is apparently directly related to the rate of rise of the blood pressure (see review by Giese, 1966).

The one suprising fact to emerge from this study, on the production of hypertension in rats, was the rapidity with which a DOCA-NaCl regimen produced very high levels of systolic blood pressure in rats. Since this had not been reported in the literature at that time it was decided to investigate the cause of this rapid production of hypertension by a DOCA-NaCl regimen. However, no clearly defined explanation could be found, although 'just weaned' rats supplied by Fisons Ltd.

did develop hypertension significantly more quickly than rats of 9 weeks of age supplied by Scientific Products Farm and a combination of the two factors of age and strain would appear to be involved in this rapid elevation of blood pressure caused by a DOCA-NaCl regimen. Recently Molteni & Brownie (1972) and Hall, Ayachi & Hall (1972 a & b) reported that commonly used stocks of rats vary widely in their susceptibility to the induction of hypertension by either a DOCA-NaCl regimen or the administration of salt and it is possible that the Wistar rats bred by Fisons Ltd. and Scientific Products Farm are both sensitive to the hypertensive effect of a DOCA-NaCl regimen. This hypothesis is enhanced by the fact that Wistar rats supplied by ASL required administration of 1% saline for periods in excess of 14 days for the production of a hypertensive state. This sensitivity of Wistar rats supplied by Fisons Ltd. and Scientific Products Farm to the hypertensive action of a DOCA-NaCl regimen may be associated with the marked polydipsia observed in both stocks of rats. Although Panasevich et al. (1969) found no relationship between the rise in blood pressure produced by a DOCA-NaCl regimen and the sodium chloride intake in rats, Hall, Ayachi & Hall (1972 a & b) have reported that in the Long Evans strain of Sprague-Dawley rats the failure of DOCA to induce polydipsia resulted in no elevation of the blood pressure and reversal of this effect by the addition of sucrose to the sodium chloride solution resulted in a subsequent rapid rise of blood pressure. Isolation of the rats may have also been important since it is known that this produces animals which react in an exaggerated manner to stress (Hallback, 1975). A DOCA-NaCl regimen has been reported to produce a stress-like syndrome (Selye, 1948). Thus the housing of the Sc.P.F. and Fisons Wistar rats singly and the ASL Wistar

rats in groups of four may have played a part in the different rates of production of hypertension in these rats. Although no clear explanation for the rapid production of DOCA-NaCl hypertension in the Fisons Wistar rats could be made from the studies presented in this thesis, factors such as strain, age and isolation may separately, or in combination, be important.

The development and maintenance of experimentally induced hypertension has been ascribed to various causes (see reviews by Page & McCubbin, 1968; De Champlain, 1972; Page, 1974 and Peart, 1975) and it was decided to investigate the role of the sympathetic nervous system in the production and maintenance of both DOCA-NaCl and one-kidney renal hypertensions. After an initial study revealed that an effective blockade of the peripheral postganglionic sympathetic neurones could be obtained by a single i.p. dose of 10 mg/kg guanethidine the effect of daily injections of guanethidine at this dose level, before and subsequent to, the operation to produce hypertension by the above methods was examined. The results, obtained in two different strains of Wistar rats, strongly suggested that the sympathetic nervous system was involved in the production of DOCA-NaCl hypertension but is not essential for the production of one-kidney renal hypertension in rats. These results are in agreement with those of De Champlain and co-workers (see review by De Champlain, 1972) but other reports have produced conflicting results, with regard to the involvement of the sympathetic nervous system in experimentally induced hypertension (see pp. 30 to 33). These other workers employed immunosympathectomy or chemical sympathectomy and the conflicting nature of the results has been suggested to be due to the varying

degrees of sympathectomy obtained (see reviews by De Champlain, 1972 and Thoenen, 1972).

The sympathetic nervous system was shown, in rats, not to be essential for the production of one-kidney renal hypertension and this lack of involvement of the sympathetic nervous system in one-kidney renal hypertension was supported by the work concerning cardiovascular reactivity during the development of one-kidney renal hypertension in cats. The observed changes in the response to pressor agents during the development of renal hypertension in conscious cats was closely paralleled by the responses obtained in the anaesthetised cat during infusions of angiotensin suggesting that the renin-angiotensin system plays the major role in the production of one-kidney renal hypertension. The change in responses to the pressor agents during the sustained phase of hypertension in conscious cats suggested a decreased role of the renin-angiotensin system and an increased one for the sympathetic nervous system, probably via upward resetting of the baroreceptors. These results are generally in agreement with the published literature on this subject in rats (see reviews by Page & McCubbin, 1968 and De Champlain, 1972). The lack of effect of guanethidine in lowering the blood pressure of renal hypertensive rats during the sustained phase of the disease has been discussed at length earlier in this thesis (see Section 3, Chapters 1 and 2) and is probably due to the development of tolerance to the hypotensive action of guanethidine and development of supersensitivity of vascular smooth muscle to noradrenaline. It has been shown (see review by Page & McCubbin, 1968) however, that even though the sympathetic nervous system is involved in the maintenance of renal hypertension other non-neuronal mechanisms can maintain the elevated blood pressure

after removal of sympathetic nerve function.

The involvement of the sympathetic nervous system in the development of DOCA-NaCl induced hypertension in rats has been suggested by De Champlain and co-workers (see review by De Champlain, 1972) to be due to a dysfunction of the sympathetic nervous system with increased turnover of noradrenaline in peripheral postganglionic neurones. This dysfunction has been shown by De Champlain (see review by De Champlain, 1972) to precede the rise in blood pressure after DOCA-NaCl treatment and can be reversed by ganglion blockers with a subsequent return of the blood pressure to normotensive levels. Guanethidine may well act in a similar manner to prevent the centrally produced increase in the peripheral sympathetic nervous activity and this would provide a possible answer for the lack of development of DOCA-NaCl induced hypertension in rats receiving guanethidine treatment.

Similarly the decreased responses to electrical stimulation of the sympathetic outflow from the spinal cord and McN-A-343 in the DOCA-NaCl hypertensive pithed rat may be due to this dysfunction of the sympathetic nervous system since it has been stated by De Champlain (see review by De Champlain, 1972) to result in decreased intraneuronal stores of noradrenaline in peripheral sympathetic nerves. The general increase in responses of pithed hypertensive rats to the other pressor agents used in this study suggested that a non-specific increase in vascular smooth muscle reactivity had occurred. This non-specific increase in vascular smooth muscle reactivity has been suggested to be due to altered permeability of vascular smooth muscle due to changes in plasma and tissue ion concentrations (Jones, 1973). It was considered unlikely that this non-specific increase in vascular

reactivity of smooth muscle could be explained by a simple mechanical change, that is, increased wall-to-lumen ratio, since no such hypertrophy of arteries had been observed in DOCA-NaCl induced hypertensive rats and the resting blood pressures of the DOCA-NaCl hypertensive and control normotensive rats did not show a statistically significant difference after pithing. This suggested that the increased peripheral resistance in DOCA-NaCl induced hypertension in the rat was due to increased sympathetic nerve activity and not simply a mechanical change in the walls of vascular smooth muscle. The results of the present studies showing increased cardiovascular reactivity to various pressor agents during the sustained phase of hypertension in rats and cats suggest that it is a consequence of, and not the cause of, the hypertension.

Further evidence for the involvement of the sympathetic nervous system in the maintenance of DOCA-NaCl induced hypertension in the rat was provided by the observed hypotensive effects of both guanethidine and α-methyldopa in hypertensive rats after single i.p. doses of these compounds.

The action of α-methyldopa was studied in detail using selective inhibition of peripheral and central dopa decarboxylase and dopamine-β-hydroxylase activities before peripheral or central administration of α-methyldopa. The results obtained strongly suggest a central mode of action of α-methyldopa mediated via α-methylnoradrenaline as proposed by Henning & Rubenson (1971). The results obtained in this study have subsequently been confirmed by Anden & Henning (1974).

The production of hypertension in the cat by employing either a DOCA-NaCl regimen or the method of 'Grollman', was highly encouraging due to the fact that Day & Owen (1970) had shown that the conscious, unrestrained cat was ideally suited to the study of drugs on the

cardiovascular system. The hypertension induced by a DOCA-NaCl regimen was associated with general ill health of the animal as previously observed in the rat. The pathologies of the hypertensions induced in the cat by either a DOCA-NaCl regimen or the method of 'Grollman' were similar to those observed in rats in which hypertension had been produced by the same procedures.

The falls in blood pressure of one-kidney renal hypertensive conscious cats to single oral doses of guanethidine and α-methyldopa indicates that not only is the sympathetic nervous system important in the maintenance of renal hypertension in the cat but that this species may be very useful in the study of antihypertensive agents. Similarly, the results of the present study involving cardiovascular reactivity during the production and maintenance of hypertension in the conscious cat, suggest that this model may provide many opportunities to examine the aetiology of experimentally induced hypertension so that artefacts produced by anaesthesia and restraining the animal do not occur.

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# REFERENCES

AARS, H. (1968 a)
Aortic baroreceptor activity in normal and hypertensive rabbits. Acta physiol. scand., 72, 298 - 309.

AARS, H. (1968 b)
Static load-length characteristics of aortic strips from hypertensive rabbits. Acta physiol. scand., 73, 101 - 110.

AARS, H. (1969)
Relationship between aortic diameter and aortic baroreceptor activity in normal and hypertensive rabbits. Acta physiol. scand., 75, 406 - 414.

AARS, H. (1975)
The baroreflex in arterial hypertension. Scand. J. clin. Lab. Invest., 35, 97 - 102.

ABBOUD, F. M. & ECKSTEIN, J. W. (1962)
Observations on the mechanism of the local vasodilator effect of guanethidine seen in man and in dog. Circulation, 24, 873.

ABBS, E.T. (1966)
The release of catecholamines by choline 2, 6-xylyl ether, bretylium and guanethidine. Br. J. Pharmac. Chemother., 26, 162 - 171.

ABRAMS, M. & SOBIN, S. (1947)
Latex rubber capsule for producing hypertension in rats by perinephritis. Proc. Soc. exp. Biol. Med., 64, 412 - 416.

ADEL, H. N., DEMING, Q.B., DALY, M.M., RAEFF, V. M. & BRUN, L. M. (1965)
The effect of experimental hypertension on cholesterol synthesis in the rat. J. Lab. clin. Med., 66, 571 - 581.

AIKEN, J. W. & REIT, E. (1968)
Stimulation of the cat stellate ganglion by angiotensin. J. Pharmac. exp. Ther., 159, 107 - 114.

AJZEN, H., SIMMONS, J. L. & WOODS, J. W. (1965)
Renal vein renin and juxtaglomerular activity in sodium-depleted subjects. Circulation Res., 17, 130 - 134.

ALEXANDER, C. S. (1957)
A new simple method for indirect determination of blood pressure in the rat. Proc. Soc. exp. Biol. Med., 94, 368 - 372.

ALEXANDER, N., HINSHAW, L. B. & DRURY, D. R. (1954)
Development of a strain of spontaneously hypertensive rabbits. Proc. Soc. exp. Biol. Med., 86, 855 - 858.

ALEXANDER, N., HINSHAW, L. B. & DRURY, D. R. (1956)
Further observations on development of a colony of spontaneously hypertensive rabbits. Proc. Soc. exp. Biol. Med., 92, 249 - 253.

ALLBUTT, T. C. (1893)
On Visceral Neuroses. Gulstonian Lectures. J. and A. Churchill, London.

ALLBUTT, T. C. (1895)
Senile plethora or high arterial pressure in elderly persons. Trans. Hunterian Soc., 77, 38 - 57.

ALTURA, B. M. (1975)
Pharmacological effects of alpha-methyldopa, alpha-methylnorepinephrine, and octopamine on rat arteriolar, arterial, and terminal vascular smooth muscle. Circulation Res., 36 - 37, suppl. I., I - 233 - I - 240.

ANDEN, N. K. (1964)
On the mechanism of noradrenaline depletion by alpha-methyl-metatyrosine and metaraminol. Acta pharmac. tox., 21, 260 - 271.

ANDEN, N. K. & HENNING, M. (1974)
Urinary excretion of noradrenaline after treatment with α-methyldopa: inhibition by a central nervous mechanism. Acta physiol. scand., 90, 69 - 72.

ANGELL-JAMES, J. E. (1973)
Characteristics of single aortic and right subclavian baroreceptor fiber activity in rabbits with chronic renal hypertension. Circulation Res., 32, 149 - 161.

ANGLETTI, P. U. & LEVI-MONTALCINI, R. (1972)
Growth inhibition of sympathetic cells by some adrenergic blocking agents. Proc. natn. Acad. Sci., U.S.A., 69, 86 - 88.

ANGLETTI, P. U., LEVI-MONTALCINI, R. & CARAMIA, F. (1972)
Structural and ultrastructural changes in developing sympathetic ganglia induced by guanethidine. Brain Res., 43, 515 - 525.

AOKI, K. & MASSON, G. M. C. (1969)
Pressor responsiveness to renin and angiotensin in renal hypertensive rats. Nephron, 6, 484 - 497.

ARMSTRONG, J. M. (1972)
Vascular reactivity to noradrenaline and 5-hydroxytryptamine in hypertensive rats. Br. J. Pharmac., 45, 183P - 184P.

ARMSTRONG, J. M. & BOURA, A. L. A. (1973)
Effects of clonidine and guanethidine on peripheral sympathetic nerve function in the pithed rat. Br. J. Pharmac., 47, 850 - 852.

ASSAYKEEN, T. A. (1972)
Control of Renin Secretion. Plenum Press, New York and London.

AYERS, C. R., DARRACOTT VAUGHAN, E. Jr., YANCEY, M. R. BING, K. T., JOHNSON, C. C. & MORTON, C. (1974)
Effect of 1-sarcosine-8-alanine angiotensin II and converting enzyme inhibitor on renin release in dog acute renovascular hypertension. Circulation Res., 34 - 35, suppl. I., I - 27 - I - 43.

AYITEY-SMITH, E. & VARMA, D. R. (1970)
An assessment of the role of the sympathetic nervous system in experimental hypertension using normal and immunosympathectomized rats. Br. J. Pharmac., 40, 175 - 185.

BAER, L., KNOWLTON, A. & LARAGH, J. H. (1972)
Role of sodium balance and the pituitary-adrenal axis in the hypertension of spontaneously hypertensive rats. In Spontaneous Hypertension. ed. by K. Okamoto, pp. 203 - 209. Igaku Shoin Ltd., Tokyo.

BASSO, N. (1975)
Effect of subpressor infusions of angiotensin on cardiovascular reactivity in the conscious rat. Archs int. Pharmacodyn. Ther., 215, 266 - 275.

BAUM, T. (1968)
Sympathetic responses in cardiac tissue in experimental hypertension. J. Pharmac. exp. Ther., 165, 176 - 180.

BAUM, T. & SHROPSHIRE, A. T. (1967)
Vasoconstriction induced by sympathetic stimulation during development of hypertension. Am. J. Physiol., 212, 1020 - 1024.

BAUM, T. & SHROPSHIRE, A. T. (1969)
Stimulation of central cardiovascular centres in hypertension. Archs int. Pharmacodyn. Ther., 181, 405 - 413.

BAUM, T., SHROPSHIRE, A. T. & VARNER, L. L. (1972)
Contribution of the central nervous system to the action of several antihypertensive agents (methyldopa, hydrallazine and guanethidine). J. Pharmac. exp. Ther., 182, 135 - 144.

BEILIN, L. J., WADE, D. N., HONOUR, A. J. & COLE, T. J. (1970)
Vascular hyper-reactivity with sodium loading and with desoxycorticosterone induced hypertension in the rat. Clin. Sci., 39, 793 - 810.

BEILIN, L. J. & ZIAKUS, G. (1972)
Vascular reactivity in post-deoxycorticosterone hypertension in rats and its relation to 'irreversible' hypertension in man. Clin. Sci., 42, 579 - 590.

BEIN, H. J. (1960)
Some pharmacological properties of guanethidine. In Adrenergic Mechanisms. ed. by J. R. Vane, G. E. W. Wolstenholme and M. O'Connor, pp.162 - 170. Churchill, London.

BEN-ISHAY, D., SALITERNIK, R. & WELNER, A. (1972).
Seperation of two strains of rats with inbred dissimilar sensitivity to Doca-salt hypertension. Experientia, 28, 1321 - 1322.

BEN-ISHAY, D. & WELNER, A. (1969)
Sensitivity to experimental hypertension and aggressive reaction in rats. Proc. Soc. exp. Biol. Med., 132, 1170 - 1173.

BENITZ, K. F., MORASKI, R. M. & CUMMINGS, J. R. (1961)
Relation of heart weight, ventricular ratio, and kidney weight to body weight and arterial blood pressure in normal and hypertensive rats. Lab. Invest., 10, 934 - 946.

BEN-ZIV, G., WEINMAN, J. & SULMAN, F. G. (1964)
A photoplethysmographic method for measurement of systolic blood pressure in the rat. Archs int. Pharmacodyn. Ther., 149, 527 - 536.

BEVAN, J. A., BEVAN, R. D., PEGRAM, B. L., PURDY, R. E. & SU, C. (1974)
Increased responsiveness of veins to adrenergic stimulation in experimental hypertension. Blood Vessels., 11, 241 - 244.

BLACKET, R. B., DEPOORTER, A., PICKERING, G. W., SELLARS, A. L. & WILSON, G. M. (1950)
Hypertension produced in the rabbit by long continued infusions of renin. Clin. Sci., 9, 223 - 245.

BLAIR-WEST, J. R., COGHLAN, J. P., DENTON, D. A., FUNDER, J. W., SCOGGINS, B. A. & WRIGHT, R. D. (1971)
Effect of the heptapeptide (2 - 8) and hexapeptide (3 - 8) fragments of angiotensin II on aldosterone. J. clin. Endocr. Metab., 32, 575 - 578.

BOAKES, R. J., CANDY, J. M. & WOLSTENCORFT, J. H. (1968)
Agonistic and antagonistic effects of alpha-methylnoradrenaline at central receptors. Brain Res., 11, 450 - 452.

BOAKES, R. J., CANDY, J. M. & WOLSTENCORFT, J. H. (1973)
Antagonistic actions of α-methylnoradrenaline derived from α-methyldopa. J. Pharm. Pharmac., 25, 491 - 492.

BOCK, K. D. & GROSS, F. (1961)
Renin and angiotensin tachphylaxis. Circulation Res., 9, 1044 - 1050.

BOCK, K. D. & KRECKE, H. J. (1958)
Die wirkung von synthetischem hypertensin II auf die P.A.H. und insulin - clearance, die renare hamodynamik und die diurese biem menschen. Klin. Wschr., 36, 69 - 74.

BOHR, D. F. & SITRIN, M. D. (1970)
Regulation of vascular smooth muscle contraction: changes in experimental hypertension. Circulation Res., 26 - 27, suppl. II., II - 83 - II - 90.

BONNARDEAUX, J. L. & REGOLI, D. (1974)
Action of angiotensin and analogues on the heart. Can. J. Physiol. Pharmac., 52, 50 - 60.

BONSMANN, M. R. (1934)
Blutdruckversuche in der maus und ratte mittels photozelle. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak., 175, 460 - 467.

BOURA, A. L. A. & GREEN, A. F. (1962)
Comparison of bretylium and guanethidine: tolerance, and effects on adrenergic nerve function and responses to sympathetic amines. Br. J. Pharmac. Chemother., 19, 13 - 41.

BOURA, A. L. A. & GREEN, A. F. (1964)
Antihypertensive agents. In Evaluation of Drug Activities: Pharmacometrics. Volume 1. ed. by D. R. Laurence and A. L. Bacharach, pp. 431 - 456. Academic Press, London and New York.

BOURA, A. L. A. & GREEN, A. F. (1965)
Adrenergic neurone blocking agents. A. Rev. Pharmac., 5, 183 - 212.

BRACE, R. A., JACKSON, T. E., FERGUSON, J. D., NORMAN, R. A. Jr. & GUYTON, A. C. (1974)
Pressure generated by scar tissue contraction: perinephritis hypertension. IRCS (Med. Sci.)., 2, 1683.

BRAUN-MENENDEZ, E. (1952)
Hypertension and relation between kidney weight and body weight. Stanford med. Bull., 10, 65 - 75.

BRAUN-MENENDEZ, E., FASCIOLO, J. C., LELOIR, L. F. & MUNOZ, J. M. (1940)
The substance causing renal hypertension. J. Physiol., Lond., 98, 283 - 298.

BRAUN-MENENDEZ, E., FASCIOLO, J. C., LELOIR, L. F., MUNOZ, J. M. & TAQUINI, A. C. (1946)
Renal Hypertension. Charles C. Thomas, Springfield, Illinois.

BRAUN-MENENDEZ, E. & VON EULER, U. S. (1947)
Hypertension after bilateral nephrectomy in the rat. Nature, Lond., 160, 905.

BRIGHT, R. (1827)
Reports of Medical Cases, Volume 1. Longman, London.

BRIGHT, R. (1836)
Cases and observations illustrative of renal disease accompanied with the secretion of albuminous urine. Guy's Hosp. Rep., 1, 338 - 400.

BRISTOW, J. D., HONOUR, A. J., PICKERING, G. W., SLEIGHT, P. & SMYTH, H. S. (1969)
Diminished baroreflex sensitivity in high blood pressure. Circulation, 39, 48 - 54.

BROWN, J. J., CHAPIUS, G. & ROBERTSON, J. I. S. (1963)
The effect of long-continued intravenous infusions of angiotensin in the rabbit. Lancet, 1, 1356 - 1357.

BROWN, J. J., CHAPIUS, G. & ROBERTSON, J. I. S. (1964)
The effect of prolonged intravenous infusion of angiotensin in the rabbit. Clin. Sci., 26, 165 - 175.

BRUNNER, H., DESAULLES, P. A., REGOLI, D. & GROSS, F. (1962)
Renin content and excretory function of the kidney in rats with experimental hypertension. Am. J. Physiol., 202, 795 - 799.

BRUNNER, H., HEDWELL, P. R., MAITRE, L. & MEIER, M. (1966)
Antihypertensive wirkung von α-methylierten catecholaminanalogen an ratten. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak., 255, 5 - 6.

BRUNNER, H., HEDWELL, P. R., MAITRE, L. & MEIER, M. (1967)
Antihypertensive effects of alpha-methylated catecholamine analogues in the rat. Br. J. Pharmac. Chemother., 30, 108 - 122.

BUCKLEY, J. P., KATO, M. S., KINNARD, W. J., ACETO, M. D. G. & ESTEVEZ, J. M. (1964)
Effects of reserpine and chlorpromazine on rats subjected to experimental stress. Psychopharmacologia, 6, 87 - 95.

BUMPUS, F. M., SCHWARZ, H. & PAGE, I. H. (1957)
Synthesis and pharmacology of the octapeptide angiotonin. Science, 125, 886 - 887.

BUMPUS, F. M., SCHWARZ, H. & PAGE, I. H. (1958)
Synthesis and properties of angiotonin. Circulation, 17, 664 - 667.

BUNAG, R. D. (1973)
Validation in awake rats of a tail-cuff method for measuring systolic pressure. J. appl. Physiol., 34, 279 - 282.

BUNAG, R. D., PAGE, I. H. & McCUBBIN, J. W. (1971)
Lack of correlation between direct and indirect measurements of arterial pressure in unanaesthetized rats. Cardiovascular Res., 5, 24 - 31.

BURN, J. H. & RAND, M. J. (1960)
The relation of circulating noradrenaline to the effect of sympathetic stimulation. J. Physiol., Lond., 150, 295 - 305.

BURNSTOCK, G., EVANS, B., GANNON, B. J., HEATH, J. W. & JAMES, V. (1971)
A new method of destroying adrenergic nerves in adult animals using guanethidine. Br. J. Pharmac., 43, 295 - 301.

BYROM, F. B. & WILSON, C. (1938)
A plethysmographic method for measuring systolic blood pressure in the intact rat. J. Physiol., Lond., 93, 301 - 304.

CAMPBELL, W. B., BROOKS, S. N. & PETTINGER, W. A. (1974)
Angiotensin - II - and angiotensin - III - induced aldosterone release in vivo in the rat. Science, 184, 994 - 996.

CARLINI, E. A., SAMPAIO, A. H. & PAIVA, A. C. M. (1959)
Vascular reactivity of rats with desoxycorticosterone and metacorticoid hypertension. Acta physiol. latinoam., 9, 138 - 142.

CARLSSON, A. (1964)
Functional significance of drug-induced changes in brain monamine levels. Prog. Brain Res., 8, 9 - 27.

CARLSSON, A. (1966)
Pharmacology of the sympathetic nervous system. In Antihypertensive Therapy. Principles and Practice. An International Symposium. ed. by F. Gross, pp. 5 - 14. Springer-Verlag, Berlin.

CARLSSON, A. & LINDQVIST, M. (1962)
In vivo decarboxylation of α-methyldopa and α-methylmetatyrosine. Acta physiol. scand., 54, 87 - 94.

CARRETERO, O. A., KUK, P., PIWONSKA, S., HOULE, J. A. & MARIN-GREZ, M. (1971)
Role of the renin-angiotensin system in the pathogenesis of severe hypertension in rats. Circulation Res., 29, 654 - 663.

CARVER, D. S. & WATERHOUSE, H. N. (1962).
The variation in the water consumption of cats. Proc. Anim. Care Panel, 12, 267 - 270.

CASS, R. & SPRIGGS, T. L. B. (1961)
Tissue amine levels and sympathetic blockade after guanethidine and bretylium. Br. J. Pharmac. Chemother., 17, 442 - 450.

CASTIGLIONI, A. (1958)
Far eastern medicine: systems of scholastic medicine. In A History of Medicine. Second Edition. p. 102. Knopf, New York.

CHALMERS, J. P. (1975)
Brain amines and models of experimental hypertension. Circulation Res., 36, 469 - 480.

CHALMERS, J. P., REID, J. L. & WING, L. M. H. (1974)
Intracisternal 6-hydroxydopamine (6-OHDA) and 5, 6-dihydroxytryptamine (5, 6-DHT) in experimental hypertension. Biochem. Pharmac., 23, suppl. 2, 727 - 729.

CHANUTIN, A. & BARKSDALE, E. E. (1933)
Experimental renal insufficiency produced by partial nephrectomy: relationship of left ventricular hypertrophy, width of cardiac muscle fiber, and hypertension in rat. Archs. intern. Med., 52, 739 - 751.

CHANUTIN, A. & FERRIS, E. B. Jr. (1932)
Experimental renal insufficiency produced by partial nephrectomy. Archs. intern. Med., 49, 767 - 787.

CHESSAR, J. R., FERRARIO, C. M. & McCUBBIN, J. W. (1972)
Acceleration of renal hypertension accompanied by increase in plasma volume as a result of prior thoracic sympathectomy. Can. J. Physiol. Pharmac., 50, 1108 - 1111.

CHITTUM, J. R., HILL, H. C. & GRIMSON, K. S. (1953)
An accoustical indicator for the systolic end point in rat blood pressure. Proc. Soc. exp. Biol. Med., 66, 486 - 488.

CHRISTLIEB, A. R., BIBER, T. U. L. & HICKLER, R. B. (1969)
Studies on the role of angiotensin in experimental renovascular hypertension: an immunological approach. J. Clin. Invest., 48, 1506 - 1518.

CLARK, D. W. J. (1969)
Effects of immunosympathectomy on the blood pressure of genetically hypertensive rats. Proc. Univ. Otago Med. Sch., 47, 42 - 44.

CLARK, D. W. J. (1971)
Effects of immunosympathectomy on development of high blood pressure in genetically hypertensive rats. Circulation Res., 28, 330 - 336.

CLARK, D. W. J. & PHELAN, E. L. (1975)
Blood pressure and hindlimb perfusion pressure following chronic sympathectomy of genetically hypertensive and normotensive rats. Clin. exp. Pharmac. Physiol., Suppl. 2., 153 - 157.

CLARK, D. W. J. & SIMPSON, F. O. (1970)
Effect of immunosympathectomy in preventing hypertension in rats from a genetically hypertensive colony. Australas Ann. Med., 19, 190.

CLARKE, D. E., SMOOKLER, H. H. & BARRY, H. (1970)
Sympathetic nerve function and Doca-NaCl induced hypertension. Life Sci., 9, 1097 - 1108.

CLINESCHIDT, B. V., GELLER, R. G., GOVIER, W. C. & SJOERDSMA, A. (1970)
Reactivity to norepinephrine and nature of the alpha adrenergic receptor in vascular smooth muscle of a genetically hypertensive rat. Europ. J. Pharmac., 10, 45 - 50.

COHEN, Y., WEPIERRE, J., JACQUOT, C. & RAPIN, J. (1974)
Relation entre l'activite antihypertensive de l'a methyldopa et l'apparition de ses metabolites dan le coeur et le cerveau des rats hypertendus. Archs int. Pharmacodyn. Ther., 207, 348 - 360.

COHN, J. N. (1974)
Hypertension - 1974. Archs. intern. Med., 133, 911 - 913.

COHN, J. N. & NOTARGIACOMO, A. V. (1969)
Clinical application of a simple, specific bioassay technique for measuring renin activity. Am. J. med. Sci., 257, 344 - 351.

COLLIS, M. G. & ALPS, B. J. (1975)
Vascular reactivity to noradrenaline, potassium chloride, and angiotensin II in the rat perfused mesenteric vasculature preparation, during the development of renal hypertension. Circulation Res., 9, 118 - 126.

CONWAY, J. (1958)
Vascular reactivity in experimental hypertension measured after hexamethonium. Circulation, 17, 807 - 810.

CONWAY, J. (1963)
Vascular abnormality in hypertension: study of blood flow in the forearm. Circulation, 27, 520 - 529.

COOKE, R. C., BROWN, R. C., ZACHERLE, B. J. & WALKER, W. G. (1970)
The effect of altered sodium concentration in the distal nephron segments on renin release. J. Clin. Invest., 49, 1630 - 1638.

DAHL, L. K. (1960)
Effects of chronic excess salt feeding. J. exp. Med., 112, 635 - 651.

DAHL, L. K. (1961)
Effects of chronic excess salt feeding. Induction of self-sustaining hypertension in rats. J. exp. Med., 114, 231 - 236.

DAHL, L. K., HEINE, M. & TASSINARI, L. (1962)
Role of genetic factors in susceptibility to experimental hypertension due to chronic excess salt ingestion. Nature, Lond., 194, 480 - 482.

DAHL, L. K., HEINE, M. & TASSINARI, L. (1963)
Effects of chronic excess salt ingestion. Role of genetic factors in both DOCA - salt and renal hypertension. J. exp. Med., 118, 605 - 617.

DAHL, L. K., KNUDSON, K. D., IWAI, J., RAPP, J. P. & JAFFE, D. (1972)
Some genetically determined differences between hypertension - prone and hypertension - resistant rats. In Hypertension '72. ed. by J. Genest and E. Koiw, pp. 337 - 349. Springer-Verlag, Berlin.

DARGIE, H. J., DOLLERY, C. T. & LEWIS, P. J. (1975)
Prevention of DOCA saline hypertension by central 6-hydroxydopamine; role of saline intake. Br. J. Pharmac., 53, 455P.

DAVIS, J. O. (1962)
The control of aldosterone secretion. Physiologist, Wash., 5, 65 - 86.

DAVIS, J. O. (1963)
The role of the adrenal cortex and the kidney in the pathogenesis of cardiac edema. Yale J. Biol. Med., 35, 402 - 428.

DAVIS, J. O. (1971)
What signals the kidney to release renin? Circulation Res., 28, 301 - 306.

DAVIS, J. O. (1973)
Control of renin release. Am. J. Med., 55, 333 - 350.

DAVIS, J. O., AYERS, C. R. & CARPENTER, C. C. J. (1961)
Renal origin of aldosterone stimulating hormone in dogs with thoracic caval constriction in sodium depleted dogs. J. Clin. Invest., 40, 1466 - 1474.

DAVIS, J. O., FREEMAN, R. H., JOHNSON, J. A. & SPIELMAN, W. S. (1974)
Agents which block the action of the renin-angiotensin system. Circulation Res., 34, 279 - 285.

DAY, M. D., McCUBBIN, J. W. & PAGE, I. H. (1965)
Limited hypertensive effects of infusion of angiotensin. Am. J. Physiol., 209, 264 - 268.

DAY, M. D. & OWEN, D. A. A. (1968)
The interaction between angiotensin and sympathetic vasoconstriction in the isolated artery of the rabbit ear. Br. J. Pharmac., 34, 499 - 507.

DAY, M. D. & OWEN, D. A. A. (1969)
Potentiation by angiotensin of pressor responses to endogenously released noradrenaline in the pithed rat. Archs int. Pharmacodyn. Ther., 179, 469 - 479.

DAY, M. D. & OWEN, D. A. A. (1970)
The effect of reserpine on the pressor responses to angiotensin in the conscious cat. Br. J. Pharmac., 39, 414 - 427.

DAY, M. D. & RAND, M. J. (1963)
A hypothesis for the mode of action of α-methyldopa in relieving hypertension. J. Pharm. Pharmac., 15, 221 - 224.

DAY, M. D. & RAND, M. J. (1964)
Some observations on the pharmacology of α-methyldopa. Br. J. Pharmac. Chemother., 22, 72 - 86.

DAY, M. D., ROACH, A. G. & WHITING, R. L. (1972)
The mode of action of α-methyldopa. Br. J. Pharmac., 45, 168P - 169P.

DAY, M. D., ROACH, A. G. & WHITING, R. L. (1973)
The mechanism of the antihypertensive action of α-methyldopa in hypertensive rats. Europ. J. Pharmac., 21, 271 - 280.

DAY, M. D. & WHITING, R. L. (1972 a)
An improved technique for the continuous measurement of arterial blood pressure in the conscious unrestrained cat. Br. J. Pharmac., 45, 182P.

DAY, M. D. & WHITING, R. L. (1972 b)
An improved valve device for the continuous measurement of arterial blood pressure in the conscious unrestrained cat. J. Pharm. Pharmac., 24, 263 - 264.

DEANE, H. W. & MASSON, G. M. C. (1951)
Adrenal cortical changes in rats with various types of experimental hypertension. J. clin. Endocr. Metab., 11, 193 - 208.

DE BONO, E., LEE, G. de J., MOTTRAM, F. R., PICKERING, G. W., BROWN, J. J., KEEN, H., PEART, W. S. & SANDERSON, P. H. (1963)
The action of angiotensin in man. Clin. Sci., 25, 123 - 157.

DE CHAMPLAIN, J. (1972)
Hypertension and the sympathetic nervous system. In Perspectives in Neuropharmacology. ed. by S. H. Snyder, pp.215 - 265. Oxford University Press.

DE CHAMPLAIN, J. & AMERINGEN, M. R. V. (1972)
Regulation of blood pressure by sympathetic nerve fibers and adrenal medulla in normotensive and hypertensive rats. Circulation Res., 31, 617 - 628.

DE CHAMPLAIN, J. & AMERINGEN, M. R. V. (1973)
Role of sympathetic fibres and of adrenal medulla in the maintenance of cardiovascular homeostasis in normotensive and hypertensive rats. In Frontiers in Catecholamine Research. ed. by E. Usdin and S. Snyder, pp. 859 - 864. Pergamon Press, Oxford.

DE CHAMPLAIN, J., KRAKOFF, L. R. & AXELROD, J. (1968)
Relationship between sodium intake and norepinephrine storage during the development of experimental hypertension. Circulation Res., 23, 479 - 491.

DE CHAMPLAIN, J., MUELLER, R. A. & AXELROD, J. (1969)
Turnover and synthesis of norepinephrine in experimental hypertension in rats. Circulation Res., 25, 285 - 291.

DEHENEFFE, J. & BERNARD, A. (1974)
The pressor response to intravenously infused angiotensin II: correlation with plasma renin activity. Clin. Sci. Mol. Med., 46, 149 - 161.

DELL'ORO, R. & BRAUN-MENENDEZ, E. (1942)
Dosaje de renina en la sangre de perros hipertensos por isquemia renal. Revta Soc. argent. Biol., 18, 65 - 70.

DEMING, Q. B., MOSBACH, E. H., BEVANS, M. D., DALY, M. M., ABELL, L. L., MARTIN, E., BRUN, L. M., HALPERN, E. & KAPLAN, R. (1958)
Blood pressure, cholesterol content of serum and tissues and atherogenesis in the rat. J. exp. Med., 107, 581 - 598.

DEMURA, H., FUKUCHI, S., TAKAHASHI, H. & GOTO, K. (1965)
The vascular reactivity to vasoactive substances and the electrolyte contents in arterial walls. Tohoku J. exp. Med., 86, 366 - 379.

DENGLER, H. J. & REICHEL, G. (1957)
Influence of a decarboxylase inhibitor on the blood pressure effects of 3-(3,4-dihydroxyphenyl) alanine (Dopa) and threo - (3,4-dihydroxyphenyl) serine (Dops). Naunyn-Schmiedebergs Arch. exp. Path. Pharmak., 232, 324 - 326.

DERN, P. L. & LEAVERTON, P. (1966)
The effect of angiotensin II on the "clearance" of radioiodine from the skin in hypertension. J. Lab. clin. Med., 67, 265 - 272.

DIAZ, J. T. & LEVY, S. E. (1939)
An indirect method for determinations of blood pressure of rats. Proc.Soc. exp. Biol. Med., 40, 402 - 407.

DICKINSON, C. J. & LAWRENCE, J. R. (1963)
A slowly developing pressor response to small concentrations of angiotensin. Lancet, 1, 1354 - 1356.

DICKINSON, C. J. & YU, R. (1967)
Mechanisms involved in the progressive pressor response to very small amounts of angiotensin in conscious rabbits. Circulation Res., 20 - 21, suppl. II., II - 157 - II - 165.

DOCK, W. (1940)
The role of the central nervous system in renal hypertension. J. Clin. Invest., 19, 769 - 770.

DOCK, W., SHINDLER, F. & MOY, B. (1942)
The vasomotor center essential in maintaining renal hypertension. Am. Heart J., 23, 513 - 521.

DORR, L. B. & BRODY, M. J. (1964)
The sympathetic nervous system and the development of experimental renal hypertension. Clin. Res., 12, 362 - 369.

DOUGLAS, J. R. Jr., HEIST, J. F., JOHNSON, E. M. Jr., MARSHALL, G. R. & NEEDLEMAN, P. (1975 a)
The development and maintenance of renal hypertension in normal and sympathectomized rats. Pharmacologist, 16, 296.

DOUGLAS, J. R. Jr., JOHNSON, E. M. Jr., MARSHALL G. R., HEIST, J., HARTMAN, B. K. & NEEDLEMAN, P. (1975 b)
Development and maintenance of renal hypertension in normal and guanethidine sympathectomized rats. Circulation Res., 36 - 37, suppl. I., I - 171 - I - 178.

DOWD, D. A. & JONES, D. R. (1968)
A method for recording baby rat systolic blood pressure. J. appl. Physiol., 25, 772 - 774.

DOYLE, A. E. (1968)
Vascular reactivity in human hypertension. N. Z. med. J., 67, 295 - 303.

DOYLE, A. E., FRASER, J. R. E. & MARSHALL, R. J. (1959)
Reactivity of forearm vessels to vasoconstrictor substances in hypertensive and normotensive subjects. Clin. Sci., 18, 441 - 454.

DOYLE, A. E. & SMIRK, F. H. (1954)
The neurogenic component in hypertension. Circulation, 12, 543 - 552.

DUNCAN, G. W., HYMAN, C. & CHAMBERS, E. L. (1943)
Determination of blood pressure in rats by direct observation of blood vessels. J. Lab. clin. Med., 28, 886 - 889.

EBRIHARA, A. & GROLLMAN, A. (1968)
Pressor activity of renal venous effluent following constriction of renal artery in dogs. Am. J. Physiol., 214, 1 - 5.

EIDE, I. (1972)
Renovascular hypertension in rats immunized with angiotensin II. Circulation Res., 30, 149 - 157.

EIDE, I. & AARS, H. (1969)
Renal hypertension in rabbits immunized with angiotensin. Nature, Lond., 222, 571.

EIDE, I. & AARS, H. (1970)
Renal hypertension in rabbits immunized with angiotensin - II. Scand. J. clin. Lab. Invest., 25, 119 - 127.

EIDE, I., LOYNING, E. & KIIL, F. (1973)
Evidence for hemodynamic autoregulation of renin release. Circulation Res., 32, 237 - 245.

ELLIOT, D. F. & PEART, W. S. (1956)
Amino acid sequence in a hypertensin. Nature, Lond., 177, 527 - 528.

ELLIOT, D. F. & PEART, W. S. (1957)
The amino acid sequence in a hypertensin. Biochem. J., 65, 246 - 254.

ENGLISH, P. D., MORGAN, B., POYSER, R. H. & WHITING, R. L. (1973)
Changes in serum and tissue lipid levels induced by metacorticoid hypertension in rats. Br. J. Pharmac., 49, 147P.

ERANKO, L. & ERANKO, O (1971 a)
Effect of guanethidine on nerve cells and small intensely fluorescent cells in sympathetic ganglia of newborn and adult rats. Acta pharmac. tox., 30, 403 - 416.

ERANKO, O. & ERANKO, L. (1971 b)
Histochemical evidence of chemical sympathectomy by guanethidine in newborn rats. Histochem. J., 3, 451 - 456.

ERDOS, E. G. (1975)
Angiotensin I converting enzyme. Circulation Res., 36, 247 - 255.

ERINOFF, L., HELLER, A. & OPARIL, S. (1975)
Central catecholamine depletion and the prevention of hypertension. Fedn. Proc. Fedn. Am. Socs. exp. Biol., 34, 817.

ETTINGER, U., SIEBEL, K. & REICKER, G. (1970)
Reactivity of isolated small arteries for norepinephrine in essential hypertension. Int. Z. Klin. Pharmakol. Ther. Toxik., 4, 121 - 122.

EXLEY, K. A. (1957)
The blocking action of choline 2:6-xylyl ether bromide on adrenergic nerves. Br. J. Pharmac. Chemother., 12, 297 - 305.

EXLEY, K. A. (1960)
The persistence of adrenergic nerve conduction after TM10 or bretylium in the cat. In Adrenergic Mechanisms ed. by J. R. Vane, G. E. W. Wolstenholme and M. O'Connor, pp. 158 - 161. Churchill, London.

FARMER, J. (1965)
Impairment of sympathetic nerve responses by dopa, dopamine and their α-methylated analogues. J. Pharm. Pharmac., 17, 640 - 646.

FARR, W. & GRUPP, G. (1967)
Sympathetically mediated effects of angiotensin on the dog heart in situ. J. Pharmac. exp. Ther., 156, 528 - 537.

FELDBERG, W. & LEWIS, G. P. (1964)
Further studies on the effects of peptides on the suprarenal medulla of cats. J. Physiol., Lond., 178, 239 - 251.

FERRARIO, C. M., GILDENBERG, P. L. & McCUBBIN, J. W. (1972)
Cardiovascular effects of angiotensin mediated by the central nervous system. Circulation Res., 30, 257 - 261.

FERREIRA, S. H., GREENE, L. J., ALABASTER, V. A., BAKHLE, Y. S. & VANE, J. R. (1970)
Activity of various fractions of bradykinin-potentiating-factor against angiotensin I-converting enzyme. Nature, Lond., 225, 379 - 380.

FIELD, F. P., JANIS, R. A. & TRIGGLE, D. J. (1972)
Aortic reactivity of rats with genetic and experimental renal hypertension. Can. J. Physiol. Pharmac., 50, 1072 - 1079.

FIELD, F. P., JANIS, R. A. & TRIGGLE, D. J. (1973)
Relationship between aortic reactivity and blood pressure of renal hypertensive, hyperthyroid and hypothyroid rats. Can. J. Physiol. Pharmac., 51, 344 - 353.

FINCH, L. (1971)
Cardiovascular reactivity in the experimental hypertensive rat. Br. J. Pharmac., 42, 56 - 65.

FINCH, L. (1975)
An increased reactivity in hypertensive rats unaffected by prolonged antihypertensive therapy. Br. J. Pharmac., 54, 437 - 443.

FINCH, L. & HAEUSLER, G. (1972)
Further evidence for a central hypotensive action of α-methyldopa. Br. J. Pharmac., 45, 167P - 168P.

FINCH, L., HAEUSLER, G. & THOENEN, H. (1972)
Failure to induce experimental hypertension in rats after intraventricular injections of 6-hydroxydopamine. Br. J. Pharmac., 44, 356P - 357P.

FINCH, L., HAEUSLER, G. & THOENEN, H. (1973)
A comparison of the effects of chemical sympathectomy by 6-hydroxydopamine in newborn and adult rats. Br. J. Pharmac., 39, 249 - 260.

FINCH, L. & LEACH, G. D. H. (1970)
The contribution of the sympathetic nervous system to the development and maintenance of experimental hypertension in the rat. Br. J. Pharmac., 39, 317 - 324.

FOLKOW, B. (1971)
The haemodynamic consequences of adaptive structural changes of the resistance vessels in hypertension. Clin. Sci., 41, 1 - 12.

FOLKOW, B. (1975)
Relationship between physical vascular properties and smooth muscle function: its importance for vascular control and reactivity. Clin. exp. Pharmac. Physiol., Suppl. 2, 55 - 61.

FOLKOW, B., GRIMBY, G. & THULESIUS, O. (1958)
Adaptive structural changes of the vascular walls. Acta physiol. scand., 44, 255 - 272.

FOLKOW, B., HALLBACK, M., LUNDGREN, Y., SIVERTSSON, R. & WEISS, L. (1973)
Importance of adaptive changes in vascular design for establishment of primary hypertension, studied in man and spontaneously hypertensive rats. Circulation Res., 32 - 33, suppl. I., I - 2 - I - 16.

FOLKOW, B., HALLBACK, M., LUNDGREN, Y. & WEISS, L. (1970)
Background of increased flow resistance and vascular reactivity in spontaneously hypertensive rats. Acta physiol. scand., 80, 93 - 106.

FOLKOW, B., HALLBACK, M., LUNDGREN, Y & WEISS, L. (1972)
The effect of "immunosympathectomy" on blood pressure and vascular "reactivity" in normal and spontaneously hypertensive rats. Acta physiol. scand., 84, 512 - 523.

FRANGIPANE, G. & APORTI, F. (1969)
Improved indirect method for the measurement of systolic blood pressure in the rat. J. Lab. clin. Med., 73, 872 - 876.

FRANK, M. H. (1963)
Renin in experimental renal hypertension in monkeys. Circulation Res., 12, 241 - 255.

FREEMAN, R. H., DAVIS, J. O., GOTSHALL, R. W., JOHNSON, J. A. & SPIELMAN, W. S. (1974)
The signal perceived by the macula densa during changes in renin release. Circulation Res., 35, 307 - 315.

FREGLY, M. J. (1962)
Relationship between blood pressure and organ weight in the rat. Am. J. Physiol., 202, 967 - 970.

FREGLY, M. J. (1963)
Factors affecting indirect determination of systolic blood pressure of rats. J. Lab. clin. Med., 62, 223 - 230.

FREGLY, M. J. (1968)
Effect of o,p'-DDD and metyrapone (SU-4885) on development of renal hypertension in rats. Toxic. appl. Pharmac., 12, 548 - 559.

FREGLY, M. J. & ANTON, A. H. (1969)
Aminotriazole, thyroid function, and catecholamines in nephrogenic hypertension in rats. Can. J. Physiol. Pharmac., 47, 407 - 414.

FRIEDMAN, B., JARMAN, J. & KLEMPERER, P. (1941)
Sustained hypertension following experimental unilateral renal injuries. Effects of nephrectomy. Am. J. med. Sci., 202, 20 - 29.

FRIEDMAN, M. & FREED, S. C. (1949)
Microphonic manometer for indirect determination of systolic blood pressure in the rat. Proc. Soc. exp. Biol. Med., 70, 670 - 672.

FRIEDMAN, M. & FREED, S. C. (1952)
Microphonic manometer for indirect determination of systolic blood pressure. In Methods in Medical Research. ed. by A. C. Corcoran, Vol. 5, p. 251. Year Book Medical Publishers Inc., Chicago.

FRIEDMAN, S. M. & FRIEDMAN, C. L. (1949)
Observations on the role of the rat kidney in hypertension caused by desoxycorticosterone acetate. J. exp. Med., 89, 631 - 641.

FRIEDMAN, S. M., FRIEDMAN, C. L. & NAKASHIMA, M. (1951)
Sustained hypertension following the administration of desoxycorticosterone acetate. J. exp. Med., 93, 361 - 371.

FRIEDMAN, S. M., FRIEDMAN, C. L. & NAKASHIMA, M. (1952)
Hypertensive effect of compound F acetate (17-OH-corticosterone-21-acetate) in rat. Endocrinology, 51, 401 - 405.

FRIEDMAN, S. M., FRIEDMAN, C. L. & NAKASHIMA, M. (1953)
Further observations on the hypertensive properties of compound F acetate in the rat. Endocrinology, 53, 633 - 639.

FRIEDMAN, S. M., FRIEDMAN, C. L. & NAKASHIMA, M. (1971)
Adrenal cortico-medullary transplant with antihypertensive activity in the rat. Nature, New Biol., 233, 23 - 25.

FRIEDMAN, S. M., HONORE, R. L. H. & FRIEDMAN, C. L. (1972)
The cortico-medullary zone of the adrenal and the hypertensive process. In Hypertension '72. ed. by J. Genest and E. Koiw, pp. 375 - 383. Springer-Verlag, Berlin.

FRIEDMAN. S. M., NAKASHIMA, M. & FRIEDMAN, C. L. (1967)
Neurohypophysial function in desoxycorticosterone acetate-hypertension in the rat. Endocrinology, 81, 1231 - 1240.

FROHLICH, E. D. (1974)
Inhibition of adrenergic function in the treatment of hypertension. Archs. intern. Med., 133, 1033 - 1048.

FUJIWARA, M., KUCHII, M. & SHIBATA, S. (1972)
Differences of cardiac reactivity between spontaneously hypertensive and normotensive rats. Europ. J. Pharmac., 19, 1 - 11.

FURCHGOTT, R. F. & BHADRAKOM, S. (1953)
Reactions of strips of rabbit aorta to epinephrine, isopropylarterenol, sodium nitrite and other drugs. J. Pharmac. exp. Ther., 108, 129 - 143.

GALLAGHER, D. J. A. & GRIMWOOD, L. H. (1953)
A simple electrical method for measuring systolic blood pressure in the intact rat. J. Physiol., Lond., 121, 163 - 166.

GANONG, W. F. (1972 a)
Effects of sympathetic activity and ACTH on renin and aldosterone secretion. In Hypertension '72. ed. by J. Genest and E. Koiw, pp. 4 - 13. Springer-Verlag, Berlin.

GANONG, W. F. (1972 b)
Sympathetic effects of renin secretion: mechanisms and physiological role. In Control of Renin Secretion. ed. by T. A. Assaykeen, pp. 17 - 32. Plenum Press, New York and London.

GANONG, W. F. & MULROW, P. J. (1961)
Evidence of secretion of an aldosterone-stimulating hormone by the kidney. Nature, Lond., 190, 1115 - 1116.

GARDNER, D. L. & HONORE, L. H. (1964)
Vascular response to catecholamines and to acetylcholine in adrenal-regeneration hypertension. Archs int. Pharmacodyn. Ther., 150, 504 - 515.

GAUNT, R., ULSAMER, G. J. & CHART, J. J. (1957)
Aldosterone and hypertension. Archs int. Pharmacodyn. Ther., 110, 114 - 118.

GEDDES, L. A. (1970)
The Direct and Indirect Measurement of Blood Pressure. Year Book Medical Publishers Inc., Chicago.

GENEST, J. (1961)
Angiotensin, aldosterone and human arterial hypertension. Can. med. Ass. J., 84, 403 - 419.

GEROLA, A. & GROSSI, A. (1965)
L'ipertensione ventricolare destra da stimolazione ipotalamica nel gatto curarizzato. III. Registrazione contemporanea della pressione atriale sinistra. Boll. Soc. ital. Biol. sper., 41, 348 - 352.

GIAMPALMO, A. (1947)
Sullo stato delle ghiandole soprarenali in coincidenza di alcune condizioni pathologiche e della varie eta. Pathologica, 39, 36 - 49.

GIESE, J. (1966)
The Pathogenesis of Hypertensive Vascular Disease. P. J. Schmidt, Copenhagen, Denmark.

GILLESPIE, J. S. & MUIR, T. C. (1967)
A method of stimulating the complete sympathetic outflow from the spinal cord to blood vessels in the pithed rat. Br. J. Pharmac. Chemother., 30, 78 - 87.

GOLDBLATT, H. (1937)
Studies on experimental hypertension: III. The production of persistent hypertension in monkeys (macaque) by renal ischemia. J. exp. Med., 65, 671 - 675.

GOLDBLATT, H. & KAHN, J. R. (1938)
Experimental hypertension. Constriction of the aorta at various levels. J. Am. med. Ass., 110, 686.

GOLDBLATT, H., KAHN. J. R. & HANZAL, R. F. (1939)
Studies on experimental hypertension: IX. The effect on blood pressure of constriction of the abdominal aorta above and below the site of origin of both main renal arteries. J. exp. Med., 69, 649 - 674.

GOLDBLATT, H., KAHN, J. R. & LEWIS, H. A. (1943)
Studies on experimental hypertension: XIX. The production of persistent hypertension in sheep and goats. J. exp. Med., 77, 297 - 307.

GOLDBLATT, H., LYNCH, J., HANZAL, R. F. & SUMMERVILLE, W. W. (1934)
Studies on experimental hypertension: I. Production of persistent elevation of systolic blood pressure by means of renal ischemia. J. exp. Med., 59, 347 - 379.

GOLDSTEIN, D. J. (1960)
The mechanism of compensatory renal hypertrophy. Leech, Johannesb., 30, 115 - 121.

GOMEZ-SALAZAR, J. (1942)
Hipertension arterial experimental en la rata. Tesis Doct. med., Univ. Catolica, Chile.

GOODFRIEND, T. L. & PEACH, M. J. (1975)
Angiotensin III: (des-aspartic acid[1])-angiotensin II. Evidence and speculation for its role as an important agonist in the renin-angiotensin system. Circulation Res., 36 - 37, suppl. I., I - 38 - I - 48.

GOODWIN, F. J., KNOWLTON, A. I. & LARAGH, J. H. (1969)
Absence of renin suppression by deoxycorticosterone acetate in rats. Am. J. Physiol., 216, 1476 - 1480.

GOORMAGHTIGH, N. (1939)
Existence of an endocrine gland in the media of the renal arterioles. Proc. Soc. exp. Biol. Med., 42, 688 - 689.

GORDON, D. B. (1966)
Renin and hypertension. Lancet, 2, 320 - 323.

GORDON, D. B. (1973)
Low renin in experimental hypertension. Circulation Res., 33, 757.

GORDON, D. B. & NOGUEIRA, A. (1962)
Increased vascular reactivity in experimental hypertension. Circulation Res., 10, 269 - 273.

GOVAERTS, P. & VERNIONY, A. (1952)
The vasoconstrictor properties of the vena cava blood of dogs with acute and renal hypertension. Acta. med. scand., 266, 419 - 428.

GRANGER, P., ROJO-ORTEGA, J. M., GRUNER, A., DAHLHEIM, H., THURAU, K., BOUCHER, R. & GENEST, J. (1972)
On the intrarenal role of the renin angiotensin system. In Control of Renin Secretion. ed. by T. A. Assaykeen, pp. 131 - 144. Plenum Press, New York and London.

GREEN, A. A. & BUMPUS, F. M. (1954)
The purification of hog renin substrate. J. biol. Chem., 210, 281 - 286.

GREEN, A. F. (1960)
The effects of bretylium and allied agents on adrenergic neurones. In Adrenergic Mechanisms. ed. by J. R. Vane, G. E. W. Wolstenholme and M. O'Connor, pp. 148 - 157. Churchill, London.

GREEN, A. F. & BOURA, A. L. A. (1964)
Depressants of peripheral sympathetic nerve function. In Evaluation of Drug Activities: Pharmacometrics. Volume 1. ed. by D. R. Laurence and A. L. Bacharach, pp. 369 - 430. Academic Press, London and New York.

GREEN, D. M., CRAIG, R. L., SAUNDERS, F. J. & STURTEVANT, F. M. (1952).
Mechanisms of desoxycorticosterone action. VIII. Effects of nephrectomy. Am. J. Physiol., 170, 477 - 485.

GREEN, D. M., SAUNDERS, F. J. WAHLGREN, N. & CRAIG, R. L. (1952).
Self-sustaining, post-DCA hypertensive cardiovascular disease. Am. J. Physiol., 170, 94 - 106.

GREENBERG, S. & BOHR, D. F. (1975)
Venous smooth muscle in hypertension: enhanced contractility of portal veins from spontaneously hypertensive rats. Circulation Res., 36 - 37, suppl. I, I - 208 - I - 215.

GREENWOOD, W. F., NASSIM, R. & TAYLOR, N. B. (1939)
The production of hypertension by the prevention of kidney hypertrophy. Can. med. Ass. J., 41, 443 - 445.

GREWAL, R. S. & KAUL, C. L. (1971)
Importance of the sympathetic nervous system in the development of renal hypertension in the rat. Br. J. Pharmac., 42, 497 - 504.

GRIFFITH, J. Q. (1934)
Indirect method for determining blood pressure of rats. Proc. Soc. exp. Biol. Med., 40, 402 - 407.

GRISEMAN, S. E. (1952)
The reactivity of the capillary bed of the nailfold to circulating epinephrine and nor-epinephrine in patients with normal blood pressure and with essential hypertension. J. Clin. Invest., 31, 782 - 788.

GRISEMAN, S. E. (1954)
Reaction of the capillary bed of the nailfold to the continuous intravenous infusion of levo-norepinephrine in patients with normal blood pressure and with essential hypertension. J. Clin. Invest., 33, 975 - 983.

GRISEMAN, S. E. (1956)
The relation of angiotensin and l-norepinephrine to essential hypertension as determined by the reaction of the nailfold capillary bed. J. exp. Med., 103, 477 - 486.

GROLLMAN, A. (1944)
A simplified procedure for inducing chronic renal hypertension in the mammal. Proc. Soc. exp. Biol. Med., 57, 102 - 104.

GROLLMAN, A. & GROLLMAN E. F. (1962)
The teratogenic induction of hypertension. J. Clin. Invest., 4, 710 - 714.

GROLLMAN, A. & HALPERT, B. (1949)
Renal lesions in chronic hypertension induced by unilateral nephrectomy in the rat. Proc. Soc. exp. Biol. Med., 71, 394 - 399.

GROLLMAN, A. & RULE, C. (1943)
Experimentally induced hypertension in parabiotic rats. Am. J. Physiol., 138, 587 - 592.

GROLLMAN, A. & WHITE, F. N. (1958)
Induction of renal hypertension in rats and dogs by potassium and chloride deficiency. Am. J. Physiol., 193, 144 - 146.

GROSS, F. (1960)
Adrenocortical function and renal pressor mechanisms in experimental hypertension. In Essential Hypertension. An International Symposium. ed. by K. D. Bock and P. T. Cottier, pp. 92 - 111. Springer-Verlag, Berlin.

GROSS, F., LOUSTALOT, P. & MEIER, R. (1957)
Production of experimental hypertension by aldosterone. Acta endocr., Copenh., 26, 417 - 423.

GROSS, F., LOUSTALOT, P. & SULSER, F. (1956)
Die bedeutung von kochsalz fur den cortexan-hochdruck der ratte und den nieren an pressorischen substanzen. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak., 229, 381 - 388.

GROSS, F. & SULSER, F. (1956)
Pressorische substanzen in den nieren experimentell hypertonischer ratten. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak., 229, 374 - 380.

GUAZZI, M. (1969)
Variazioni nel tempo della risposta pressoria alla noradrenalina dopo stenosi delle arterie renali in gatti con sistema simpatico integro e in gatti totalmente simpaticectomozzati. Boll. Soc. ital. Biol. sper., 45, 1207 - 1211.

GUAZZI, M., ELLSWORTH, O. T. & FRIES, E. D. (1971)
Influence of the adrenergic system in renovascular hypertension. Cardiovascular Res., 5, 71 - 80.

GULATI, O. P., CARRETERO, O. A., OZA, N. B., FERNANDEZ, L. A. & SCHORK, A. (1975)
Role of renin in the pathogenesis of renal hypertension. Circulation Res., 36 - 37, suppl. I, I - 187 - I - 193.

GULL, W. W. & SUTTON, H. G. (1872)
On the pathology of the morbid state commonly called chronic Bright's disease with contracted kidney. Med. chir. Trans., 55, 273 - 326.

HAAS, E. & GOLDBLATT, H. (1959)
Effects of an anhihypertensive drug, pentolinium. Am. J. Physiol., 196, 763 - 768.

HAEUSLER, G. (1975)
Cardiovascular regulation by central adrenergic mechanisms and its alteration by hypotensive drugs. Circulation Res., 36 - 37, suppl. I., I - 223 - I - 232.

HAEUSLER, G. & FINCH, L. (1972)
Vascular reactivity to 5-hydroxytryptamine and hypertension in the rat. Naunyn-Schmiedebergs Arch. Pharmac., 272, 101 - 116.

HAEUSLER, G., FINCH, L. & THOENEN, H. (1972)
Central adrenergic neurones and the initiation and development of experimental hypertension. Experientia, 28, 1200 - 1203.

HAEUSLER, G., GEROLD, M. & THOENEN, H. (1972)
Cardiovascular effects of 6-hydroxydopamine injected into the lateral brain ventricle of the rat. Naunyn-Schmiedebergs Arch. Pharmac., 274, 211 - 228.

HAGGENDAL, E. & JOHANSSON, B. (1972)
On the pathophysiology of the increased cerebrovascular permeability in acute arterial hypertension in cats. Acta. Neurol. scandinav., 48, 265 - 270.

HALES, S. (1733)
Statical Essays: Containing Haemastaticks. Volume II. W. Innys and R. Manby, London.

HALL, C. E., AYACHI, S. & HALL, O. (1972 a)
Salt appetite and hypertensive response to salt and to deoxycorticosterone in Sprague-Dawley and Long-Evans rats. Tex. Rep. Biol. Med., 30, 143 - 153.

HALL, C. E., AYACHI, S. & HALL, O. (1972 b)
Salt hypertension produced by sucrose facilitation of saline consumption in Long-Evans rats. Tex. Rep. Biol. Med., 30, 155 - 162.

HALL, C. E., AYACHI, S & HALL, O. (1974)
Hypertensive vascular disease produced in rats by compression of the adrenal glands and its relationship to adrenal-regeneration hypertension. Endocrinology, 94, 355 - 362.

HALL, C. E. & HALL. O. (1961)
Experimental hypertension elicited by injection of methyl cellulose. Experientia, 17, 544 - 545.

HALL, C. E. & HALL, O. (1962)
Macromolecular hypertension: Hypertensive cardiovascular disease from subcutaneously administered polyvinyl alcohol. Experientia, 18, 38 - 40.

HALL, C. E. & HALL, O. (1967)
The comparative h ypertensive activities of the acetates of D-aldosterone and deoxycorticosterone. Acta. Endocr., Copenh., 54, 399 - 410.

HALL, C. E. & HALL, O. (1969)
Interaction between desoxycorticosterone treatment, fluid intake, sodium consumption, blood pressure, and organ changes in rats drinking water, saline or sucrose solution. Can. J. Physiol. Pharmac., 47, 81 - 86.

HALL, G. H., GOMERSALL, J. C. R. & HENEAGE, E. (1967)
A simple device for recording blood pressure or for intravenous injection of drugs in the conscious unrestrained cat. Physiol. Behav., 3, 205 - 206.

HALLBACK, M. (1975)
Consequence of social isolation on blood pressure, cardiovascular reactivity and design in spontaneously hypertensive rats. Acta physiol. scand., 93, 455 - 465.

HALLBACK, M. & FOLKOW, B. (1974)
Cardiovascular responses to acute mental "stress" in spontaneously hypertensive rats. Acta physiol. scand., 90, 684 - 698.

HAMILTON, T. C. (1975)
Influence of anti-hypertensive drug treatment on vascular reactivity in spontaneously hypertensive rats. Br. J. Pharmac., 54, 429 - 436.

HANSEN, T. R., ABRAMS, G. D. & BOHR, D. F. (1974)
Role of pressure in structural and functional changes in arteries of hypertensive rats. Circulation Res., 34 - 35, suppl. I., I - 101 - I - 107.

HARTROFT, P. M., SUTHERLAND, L. E. & HARTROFT, W. S. (1964)
Juxtaglomerular cells as the source of renin. Further studies with the fluorescent antibody technique and the effect of passive transfer of antirenin. Can. med. Ass. J., 90, 163 - 166.

HARTFOFT, S. W. & BEST, C. H. (1949)
Hypertension of renal origin in rats following less than one week of choline deficiency in early life. Br. med. J., 1, 423 - 426.

HARVEY, W. (1628)
Exercitatio Anatomica de Motu Cordis et Sanguinis in Animalibus. William Fitzery, Frankfurt.

HASATANI, K., MORIMOTO, S. & TAKEDA, R. (1975)
Suppressed plasma renin activity in adrenal regeneration hypertension. Endocrinology, 96, 1300 - 1303.

HAYDEN, J. F., JOHNSON, L. R. & MAICKEL, R. P. (1966)
Construction and implantation of a permanent cannula for making injections into the lateral ventricle of the rat brain. Life Sci., 5, 1509 - 1515.

HEATH, J. W., EVANS, B. K., GANNON, B. J., BURNSTOCK, G. & JAMES, V. B. (1972)
Degeneration of adrenergic neurons following guanethidine treatment: an ultrastructural study. Virchows Arch. Zellpath., 11, 182 - 197.

HEISE, A. & KRONEBERG, G. (1972)
α -Sympathetic receptor stimulation in the brain and hypotensive activity of α-methyldopa. Europ. J. Pharmac., 17, 315 - 317.

HEISTAD, D. D., ABBOUD, F. M. & ECKSTEIN, J. W. (1967)
Serum sodium concentration and vascular responsiveness in man. Clin. Res., 15, 407.

HENNING, M. (1967)
Blood pressure and noradrenaline levels after treatment with α-methyldopa, α-methyldopamine and α-methyl-m-tyrosine. J. Pharm. Pharmac., 19, 775 - 779.

HENNING, M. (1968)
Effect of different dopa decarboxylase inhibitors on the hypertensive response to alpha-methyldopa in rats. Br. J. Pharmac., 34, 233P - 234P.

HENNING, M. (1969 a)
Studies on the mode of action of α-methyldopa. Acta physiol. scand., 75, suppl. 322, 1 - 37.

HENNING, M. (1969 b)
Interaction of dopa decarboxylase inhibitors with the effect of α-methyldopa on the blood pressure and tissue monoamines in rats. Acta pharmac. tox., 27, 135 - 148.

HENNING, M. & RUBENSON, A. (1971)
Evidence that the hypotensive action of methyldopa is mediated by central actions of methylnoradrenaline. J. Pharm. Pharmac., 23, 407 - 411.

HENNING, M. & VAN ZWIETEN, P. A. (1967)
Central hypotensive effect of α-methyldopa. J. Pharm. Pharmac., 19, 403 - 405.

HENNING, M. & VAN ZWIETEN, P. A. (1968)
Central hypotensive effect of α-methyldopa. J. Pharm. Pharmac., 20, 409 - 417.

HEPTINSTALL, R. H. (1962)
Experimental pyelonephritis. The effect of chronic infection on the blood pressure in the rat. Br. J. exp. Path., 43, 333 - 339.

HEPTINSTALL, R. H. & HILL, G. S. (1967)
Experimental pyelonephritis and hypertension. A study on the immunity of pyelonephritic glomeruli from hypertensive change. Lab. Invest., 16, 96 - 111.

HERMANN, G., DECHERD, G. & ERHARD, P. (1941)
Production of cardiac hypertrophy in rats. Proc. Soc. exp. Biol. Med., 47, 464 - 465.

HESS, S. M., CONNAMACHER, R. H., OZAKI, M. & UNDENFRIEND, S. (1961)
Effect of α-methyl and α-methyl-meta-tyrosine on the metabolism of norepinephrine and serotonin in vivo. J. Pharmac. exp. Ther., 134, 129 - 138.

HESS, S. M., OZAKI, M. & UNDENFRIEND, S. (1960)
The effects of α-methyl dopa and α-methyl meta tyrosine on the metabolism of serotonin and norepinephrine. Pharmacologist, 2, 81.

HILL, R. W., CHESTER, J. E. & WISENBAUGH, P. E. (1970)
The effect of intravenous antirenin injection on chronic experimental and acute renin-induced hypertension in dogs. Lab. Invest., 22, 404 - 410.

HILL, W. H. P. & ANDRUS, E. C. (1941)
The cardiac factor in the "pressor" effects of renin and angiotonin. J. exp. Med., 72, 91 - 103.

HINKE, J. A. M. (1965)
In vitro demonstration of vascular hyper-responsiveness in experimental hypertension. Circulation Res., 17, 359 - 371.

HOLLANDER, W. (1973)
Hypertension, antihypertensive drugs and atherosclerosis. Circulation, 48, 1112 - 1127.

HOLLANDER, W., KRAMSCH, D. M., FARMELANT, M. & MADOFF, I. M. (1968)
Arterial wall metabolism in experimental hypertension of coarctation of the aorta of short duration. J. Clin. Invest., 47, 1221 - 1229.

HOLLOWAY, E. T. & BOHR, D. F. (1973)
Reactivity of vascular smooth muscle in hypertensive rats. Circulation Res., 33, 678 - 685.

HOLLOWAY, E. T., SITRIN, M. D. & BOHR, D. F. (1972)
Calcium dependence of vascular smooth muscle from normotensive and hypertensive rats. In Hypertension '72. ed. by J. Genest and E. Koiw, pp. 400 - 408. Springer-Verlag, Berlin.

HOROWITZ, D., CLINESCHMIDT, B. V., VAN BUREN, J. M. & OMMAYA, A. K. (1974)
Temporal arteries from hypertensive and normotensive man. Reactivity to norepinephrine and characteristics of alpha-adrenergic receptors. Circulation Res., 34 - 35, suppl. I., I - 109 - I - 115.

HUCHARD, H. (1893)
Maladies du Coeur et des Vaisseaux. Second Edition. O'Dion, Paris.

HUDAK, W. J. & BUCKLEY, J. P. (1961)
Production of hypertensive rats by experimental stress. J. Pharm. Sci., 50, 263 - 264.

HUNNINGHAKE, D. B. (1969)
Drug therapy of essential hypertension. Postgrad. Med., 46, 113 - 117.

HUTCHINSON, J. S., MATTHEWS, P. G., DAX, E. & JOHNSTON, C. I. (1975)
Plasma renin and angiotensin levels in experimental hypertension in the rat. Clin. exp. Pharmac. Physiol., Suppl. 2, 83 - 88.

INGENITO, A. J., BARRETT, J. P. & PROCITA, L. (1970)
A centrally mediated peripheral hypotensive effect of α-methyldopa. J. Pharmac. exp. Ther., 175, 593 - 599.

IRIUCHIJIMA, J., MIZOGAMI, S. & SOKABE, H. (1975)
Sympathetic nervous activity in renal and DOC hypertensive rats. Jap. Heart J., 16, 36 - 43.

ITSKOVITZ, H. D. & McGILL, J. C. (1974)
Hormonal regulation of the renal circulation. Circulation Res., 34 - 35, suppl. I., I - 65 - I - 73.

IVERSEN, L. L., GLOWINSKI, J. & AXELROD. J. (1966)
The physiologic disposition and metabolism of norepinephrine in immunosympathectomized animals. J. Pharmac. exp. Ther., 151, 273 - 284.

IWAI, J., DAHL, L. K. & KNUDSEN, K. D. (1973)
Genetic influence on the renin-angiotensin system, low renin activities in hypertension-prone rats. Circulation Res., 32, 673 - 684.

JAJU, B. P., TANGRI, K. K. & BHARGAVA, K. P. (1966)
Central vasomotor effects of α-methyldopa. Can. J. Physiol. Pharmac., 44, 687 - 690.

JEFFERS, W. A., LINDAUER, M. A., TWADDLE, P. H. & WOLFERTH, C. C. (1940)
Experimental hypertension in nephrectomized parabiotic rats. Am. J. med. Sci., 199, 815 - 818.

JENSEN-HOLM, J. & JUUL, P. (1970)
Ultrastructure of rat sympathetic ganglia following guanethidine. Acta pharmac. tox., 28, suppl. I, 56.

JOHANSSON, B., LI, C., OLSSON, Y. & KLATZO, I. (1970)
The effect of acute arterial hypertension on the blood-brain barrier to protein tracers. Acta Neuropath., 16, 117 - 124.

JOHNSON, E. M. Jr., CANTOR, E. & DOUGLAS, J. R. Jr. (1975)
Biochemical and functional evaluation of the sympathectomy produced by the administration of guanethidine to newborn rats. J. Pharmac. exp. Ther., 193, 503 - 512.

JOHNSON, G. A., BOUKMA, S. J. & KIM, E. G. (1970)
In vivo inhibition of dopamine β-hydroxylase by 1-phenyl-3-(2-thiazolyl) -2-thiourea (U-14,624). J. Pharmac. exp. Ther., 171, 80 - 87.

JOHNSON, J. A., DAVIS, J. O., SPIELMAN, W. S. & FREEMAN, R. H. (1974)
The role of the renin-angiotensin system in experimental renal hypertension in dogs. Proc. Soc. exp. Biol. Med., 147, 387 - 391.

JOHNSTON, C. I., HUTCHINSON, J. S. & MENDELSOHN, F. A. (1970)
Biological significance of renin angiotensin immunization. Circulation Res., 26 - 27, suppl. II, II - 215 - II - 222.

JONES, A. (1963)
Ganglionic actions of muscarinic substances. J. Pharmac. exp. Ther., 141, 195 - 205.

JONES, A. W. (1973)
Altered ion transport in vascular smooth muscle from spontaneously hypertensive rats, influences of aldosterone, norepinephrine, and angiotensin. Circulation Res., 33, 563 - 572.

JUUL, P. (1973)
Effects of various antihypertensive guanidine derivatives on the adult rat superior cervical ganglion: histology, ultrastructure, and cholinesterase histochemistry. Acta pharmac. tox., 32, 500 - 512.

KATZ, J. I., SKOM. J. H. & WAKERLIN, G. E. (1957)
Pathogenesis of spontaneous and pyelonephritic hypertension in the dog. Circulation Res., 5, 137 - 143.

KELL, J. F. Jr., LANGFORD, H. G., HOFF, E. C. & HENNIGAR, G. R. (1960)
Differential production of vascular changes and temporarily sustained hypertension in cats and dogs. Surg. Forum., 11, 386 - 387.

KEMPF, G. F. & PAGE, I. H. (1942)
Production of experimental hypertension and the indirect determination of systolic blood pressure in rats. J. Lab. clin. Med., 27, 1192 - 1196.

KERSTEN, H., BROSENE, W. G., ABLONDI, F. & SUBBAROW, Y. (1947)
A new method for the indirect measurement of blood pressure in the rat. J. Lab. clin. Med., 32, 1090 - 1098.

KEZDI, P. (1967)
Resetting of the carotid sinus in experimental renal hypertension. In Baroreceptors and Hypertension. ed. by P. Kezdi, pp. 301 - 306. Pergamon Press, Oxford.

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 - 674.

KNOWLTON. A. I., LOEB, E. N., STOERK, H. C., WHITE, J. P. & HEFFERNAN, J. F. (1952)
Induction of arterial hypertension in normal and adrenalectomized rats given cortisone acetate. J. exp. Med., 96, 187 - 205.

KOCH, E. & MIES, H. (1929)
Cronischer arterieller hochdruck durch experimentelle dauerausschaltung des blutdruckzugler. Krankheitsforschung, 7, 241 - 256.

KOCH-WESER, J. (1964)
Myocardial actions of angiotensin Circulation Res., 14, 337 - 344.

KOLETSKY, S. (1955)
Necrotizing vascular disease in rat. Archs Path., 59, 312 - 320.

KOLETSKY, S., JACKSON, E. B., HESS, B. M., RIVERA-VELEZ, J. M. & PRITCHARD, W. H. (1966)
Role of pressor substance in unilateral renal hypertension. Proc. Soc. exp. Biol. Med., 122, 941 - 945.

KOLETSKY, S., SHOOK, P. & RIVERA-VELEZ, J. M. (1970)
Lack of increased renin-angiotensin activity in rats with spontaneous hypertension. Proc. Soc. exp. Biol. Med., 134, 1187 - 1192.

KORNER, P. I. (1975)
Central and peripheral 'resetting' of the baroreceptor system. Clin. exp. Pharmac. Physiol., Suppl. 2., 171 - 178.

KOROTKOFF, N. S. (1905)
On the subject of methods of measuring blood pressure. Izv. voenno-med. Akad., 11, 365 - 367.

KOSTRZEWA, R. M. & JACOBOWITZ, D. M. (1974)
Pharmacological actions of 6-hydroxydopamine. Pharmac. Rev., 26, 199 - 288.

KOUSHANPOUR, E. & KENFIELD, K. J. (1974)
Locus of resetting of carotid sinus baroreceptors in chronic renal hypertensive dogs. Fedn. Proc. Fedn. Am. Socs. exp. Biol., 33, 360.

KREIGER, E. M. (1970)
Time course of baroreceptor resetting in acute hypertension. Am. J. Physiol., 218, 486 - 490.

KREIGER, E. M. & MARSEILLAN, R. F. (1966)
Neural control in experimental hypertension: The role of baroreceptor and splanchnic fibers. Acta physiol. latinoam., 16, 343 - 352.

KUMAR, D., HALL, A. E. D., NAKASHIMA, R. & GORNALL, A. G. (1957)
Studies on aldosterone: II. Hypertension as a cumulative effect of aldosterone administration. Can. J. Biochem. Physiol., 35, 113 - 118.

KUNTZMAN, R., SHORE, P. A., BOGDANSKI, D. & BRODIE, B. B. (1961)
Microanalytical procedures for fluorometric assay of brain DOPA-5HT decarboxylase, norepinephrine and serotonin and a detailed mapping of decarboxylase activity in brain. J. Neurochem., 6, 226 - 232.

KURUMA, I., BARTHOLINI, G., TISSOT, R. & PLETSCHER, A. (1972)
Comparative investigation of inhibitors of extracerebral dopa decarboxylase in man and rats. J. Pharm. Pharmac., 24, 289 - 294.

LAIS, L. T. & BRODY, M. J. (1975)
Mechanism of vascular hyperresponsiveness in the spontaneously hypertensive rat. Circulation Res., 36 - 37, suppl. I, I - 216 - I - 222.

LAVERTY, R. (1961)
Increased vascular reactivity in rats with genetic hypertension. Proc. Univ. Otago Med. Sch., 39, 23 - 24.

LAVERTY, R., McGREGOR, D. D. & McQUEEN, E. G. (1967)
Vascular reactivity in experimental hypertension. N. Z. med. J., 67, 303 - 309.

LAVERTY, R. & SMIRK, F. H. (1961)
Observations on the pathogenesis of spontaneous inherited hypertension and constricted renal-artery hypertension in rats. Circulation Res., 9, 455 - 464.

LEDINGHAM, J. M. (1951)
The nature of the hypertension occurring in the nephrectomized parabiotic rat. Clin. Sci., 10, 423 - 439.

LEDINGHAM, J. M. (1971)
Mechanisms in renal hypertension. Proc. R. Soc. Med., 64, 409 - 418.

LEE, R. E. & HOLZE, E. A. (1951)
Peripheral vascular hemodynamics in the bulbar conjuctiva of subjects with hypertensive vascular disease. J. Clin. Invest., 30, 539 - 546.

LEENEN, F. H. H., DE JONG, W. & DE WIED, D. (1973)
Renal venous and peripheral plasma renin activity in renal hypertension in the rat. Am. J. Physiol., 225, 1513 - 1518.

LENEL, R., KATZ, L. N. & RODBARD, S. (1948)
Arterial hypertension in the chicken. Am. J. Physiol., 152, 557 - 562.

LENTZ, K. E., SKEGGS, L. T. WOODS, K. R., KAHN, J. R. & SHUMWAY, N. P. (1956)
The amino acid compostion of hypertensin II and its biochemical relationship to hypertensin I. J. exp. Med., 104, 183 - 191.

LEVI-MONTALCINI, R. (1964)
Growth control of nerve cells by a protein factor and its antiserum. Science, 143, 105 - 110.

LEVI-MONTALCINI, R. & ANGLETTI, P. U. (1966)
Immunosympathectomy. Pharmac. Rev., 18, 619 - 628.

LEVI-MONTALCINI, R. & ANGLETTI, P. U. (1968)
Nerve growth factor. Physiol. Rev., 48, 534 - 569.

LEVY, B. & AHLQUIST, R. P. (1962)
A study of sympathetic ganglionic stimulants. J. Pharmac. exp. Ther., 137, 219 - 228.

LEWIS, G. P. & REIT, E. (1965)
The action of angiotensin and bradykinin on the superior cervical ganglion of the cat. J. Physiol., Lond., 179, 538 - 553.

LEWIS, P. J., REID, J. L. CHALMERS, J. P. & DOLLERY, C. T. (1973)
Importance of central catecholaminergic neurones in the development and maintenance of renal hypertension. Clin. Sci. Mol. Med., 45, 115s - 118s.

LEYSSAC, P. P. (1964)
The *in vivo* effect of angiotensin on the proximal tubule reabsorption of salt in rat kidneys. Acta physiol. scand., 62, 436 - 438.

LIEBEFOTT, G. (1953)
Die pathologie der nebennieren. Dtsch. Ges. Path., 36, 21 - 30.

LIUZZI, A., FOPPEN, F. H. & ANGLETTI, P. U. (1974)
Adrenaline, noradrenaline and dopamine levels in brain and heart after administration of 6-hydroxydopamine and guanethidine. Biochem. Pharmac., 23, 1041 - 1044.

LJUNDQVIST, A. (1969)
Intrarenal vascular alterations and the persistence of experimental hypertension. Acta path. microbiol. scand., 76, 561 - 574.

LOKHANDWALA, M. F., BUCKLEY, J. P. & JANDHYALA, B. S. (1975)
Effect of methyldopa treatment on peripheral sympathetic nerve function in the dog. Europ. J. Pharmac., 32, 170 - 178.

LOOMIS, D. (1946)
Hypertension and necrotizing arteritis in the rat following renal infarction. Archs Path., 41, 231 - 267.

LORING, J. & GORACZ, G. (1954)
New method of inducing experimental hypertension in the rat. Acta physiol. hung., 5, 489 - 494.

LOUIS, W. J. & DOYLE, A. E. (1966)
The relationship between the vascular responses to the peptides of angiotensin and pitressin. Clin. Sci., 31, 255 - 263.

LOUIS, W. J., DOYLE, A. E., ANAVEKAR, S. N. & CHUA, K. G. (1973)
Sympathetic activity and essential hypertension. Clin. Sci. Mol. Med., 45, 119s - 121s.

LOWE, R. D. & SCROOP, G. C. (1970)
Effects of angiotensin on the autonomic nervous system. Am. Heart J., 79, 562 - 567.

LOWENSTEIN, J., BOYD, G. W., RIPPON, A. E., JAMES, V. H. T. & PEART, W. S. (1972)
Increased aldosterone in response to sodium deficiency in the angiotensin II - immunized rabbit. In Hypertension '72. ed. by J. Genest and E. Koiw, pp. 481 - 489. Springer-Verlag, Berlin.

LUDWIG, C. (1847)
Beitrage zur kenntniss des einflusses der respirationsbewegungen auf den blutlauf im aortensysteme. Arch. Anat. Physiol. wiss. Med., 6, 242 - 302.

LUNDGREN, Y. (1974)
Regression of structural cardiovascular changes after reversal of experimental renal hypertension in rats. Acta physiol. scand., 91, 275 - 285.

LUPU, A. N., MAXWELL, M. H., KAUFMAN, J. J. & WHITE, F. N. (1972)
Experimental unilateral renal artery constriction in the dog. Circulation Res., 30, 567 - 574.

LUPU, A. N., SAMBHI, M. P. & MAXWELL, M. H. (1967)
Renal artery constriction. An experimental study. Circulation, 36, suppl. II., II - 176.

McCUBBIN, J. W. (1958)
Carotid sinus participation in experimental renal hypertension. Circulation, 17, 791 - 797.

McCUBBIN, J. W., DE MOURA, R. S., PAGE, I. H. & OLMSTED, F. (1965)
Arterial hypertension elicited by subpressor amounts of angiotensin. Science, 149, 1394 - 1395.

McCUBBIN, J. W., GREEN, J. H. & PAGE, I. H. (1956)
Baroreceptor function in chronic renal hypertension. Circulation Res., 4, 205 - 210.

McCUBBIN, J. W. & PAGE, I. H. (1963)
Renal pressor system and neurogenic control of arterial pressure. Circulation Res., 12, 553 - 559.

McGREGOR, D. D. & SMIRK, F. H. (1968)
Vascular responses in mesenteric arteries from genetic and renal hypertensive rats. Am. J. Physiol., 214, 1429 - 1433.

McGREGOR, D. D. & SMIRK, F. H. (1970)
Vascular responses to 5-hydroxy-tryptamine in genetic and renal hypertensive rats. Am. J. Physiol., 219, 687 - 690.

McKENNY, J. M. (1974)
Antihypertensive drug therapy. J. Am. pharm. Ass., NS14, 204 - 220.

MACDONALD, G. J., BOYD, G. W. & PEART, W. S. (1975)
The effect of an angiotensin blocker, sarcosyl$^{1}$-alanyl$^{8}$-angiotensin II (P113) on two kidney hypertension in the rat. Clin. exp. Pharmac. Physiol., Suppl. 2, 89 - 91.

MAHOMED, F. A. (1974)
The etiology of Bright's disease and the prealbuminuric stage. Br. med. J., 1, 585 - 586.

MAISTRELLO, I. & MATSCHER, R. (1969)
Measurement of systolic blood pressure of rats: Comparison of intra-arterial and cuff values. J. appl. Physiol., 26, 183 - 193.

MAITRE, L. & STAEHELIN, M. (1963)
Effect of α-methyl-DOPA on myocardial catecholamines. Experientia, 19, 573 - 575.

MALLOV, S. (1959)
Comparative reactivities of aortic strips from hypertensive and normotensive rats to epinephrine and levarterenol. Circulation Res., 7, 196 - 201.

MALMFORS, T. (1965)
Studies on adrenergic nerves. Acta physiol. scand., 64, suppl. 248, 1 - 93.

MARTIN, C. J. (1905)
The determination of arterial blood pressure in clinical practice. Br. med. J., 1, 865 - 870.

MASON, R. C. & EWALD, B. H. (1965)
Studies on compensatory renal hypertrophy. I. Effect of unilateral ureteral ligation and transection. Proc. Soc. exp. Biol. Med., 120, 210 - 214.

MASSON, G. M. C. (1966)
Renin-induced hypertension. In Aktuelle Probleme der Nephrologie. ed. by H.P. Wolff and F. Kruck, pp. 111 - 121. Springer-Verlag, Berlin.

MASSON, G. M. C., CORCORAN, A. C. & PAGE, I. H. (1949)
Experimental vascular disease due to desoxycorticosterone acetate and anterior pituitary extract. J. Lab. clin. Med., 34, 1416 - 1426

MASSON, G. M. C., HAZARD, J. B., CORCORAN, A. C. & PAGE, I. H. (1950)
Experimental vascular disease due to desoxycorticosterone and anterior pituitary factors. II. Comparison of pathologic changes. Archs Path., 49, 641 - 664.

MASSON, G. M. C., KASHII, C., MATSUNAGA, M. & PAGE, I. H. (1964)
Hypertensive vascular disease produced by homologous renin. Science, 145, 178 - 180.

MASSON, G. M. C., KASHII, C., MATSUNAGA, M. & PAGE, I. H. (1966)
Hypertensive vascular disease induced by heterologous renin. Circulation Res., 18, 219 - 227.

MASSON, G. M. C., MIKASA, A. & YASUDA, H. (1962)
Experimental vascular disease elicited by aldosterone and renin. Endocrinology, 71, 505 - 512.

MASSON, G. M. C., PAGE, I. H. & CORCORAN, A. C. (1950)
Vascular reactivity of rats and dogs treated with DCA. Proc. Soc. exp. Biol. Med., 73, 434 - 436.

MASUYAMA, Y., SATO, R., YAMANAKA, Y., TANAKA, S., NISHIO, I., YOSHITOSHI, Y., ETO, M. & ITOKAWA, T. (1969)
Renin content of kidneys in various types of experimental hypertension in rats. Israel J. med. Sci., 5, 594 - 597.

MENDLOWITZ, M. (1967)
Vascular reactivity in essential and renal hypertension in man. Am. Heart J., 73, 121 - 128.

MENDLOWITZ, M. (1973)
Vascular reactivity in systemic arterial hypertension. Am. Heart J., 85, 252 - 259.

MENDLOWITZ, M. & NAFTCHI, N. E. (1958)
Work of digital vasoconstriction produced by infused norepinephrine in primary hypertension. J. appl. Physiol., 13, 247 - 251.

MENDLOWITZ, M., NAFTCHI, N. E., GITLOW, S. E. & WOLF, R. L. (1965)
Vascular responsiveness in hypertensive and hypotensive states. Geriatics, 20, 797 - 807.

MENDLOWITZ, M., NAFTCHI, N. E., WEINREB, H. L. & GITLOW, S. E. (1961)
The effect of prednisone and prednisolone on reactivity of the digital vessels to l-norepinephrine in normotensive and hypertensive subjects. J. appl. Physiol., 16, 89 - 94.

MENDLOWITZ, M., NAFTCHI, N. E., WOLF, R. L. & GITLOW, S. E. (1962)
Vascular reactivity in the patient with essential hypertension and hypertension of renal origin. Am. J. Cardiol., 9, 680 - 684.

MENEELY, G. R. & BALL, C. O. T. (1958)
Experimental epidemiology of chronic sodium chloride toxicity and the protective effect of potassium chloride. Am. J. Med., 25, 713 - 725.

MENEELY, G. R., TUCKER, R. G., DARBY, W. J. & AUERBACH, S. H. (1953)
Chronic sodium chloride toxicity in the albino rat. II. Occurrence of hypertension and of a syndrome of edema and renal failure. J. exp. Med., 98, 71 - 79.

MICHELAKIS, A. M., CAUDLE, J. & LIDDLE, G. W. (1969)
In vitro stimulation of renin production by epinephrine, norepinephrine and cyclic AMP. Proc. Soc. exp. Biol. Med., 130, 748 - 752.

MILLER, R. E., VANDER, A. J., KOWALCZYK, R. S. & GEELHOED, G. W. (1968)
Aldosterone secretion and plasma renin during renin infusion and acute salt depletion. Am. J. Physiol., 214, 228 - 231.

MITCHELL, J. R. & OATES, J. A. (1970)
Guanethidine and related agents. I. Mechanism of the selective blockade of adrenergic neurons and its antagonism by drugs. J. Pharmac. exp. Ther., 172, 100 - 107.

MOGIL, R. A., ITSKOVITZ, H. D., RUSSELL, J. H. & MURPHY, J. J. (1969)
Renal innervation and renin activity in salt metabolism and hypertension. Am. J. Physiol., 215, 693 - 697.

MOHAMMED, S., GAFFNEY, T. E., YARD, A. C. & GOMEZ, H. (1968)
Effect of methyldopa, reserpine and guanethidine on hindleg vascular resistance. J. Pharmac. exp. Ther., 160, 300 - 307.

MOLTENI, A. & BROWNIE, A. C. (1972)
Incidence of salt-induced hypertension in rats from different stocks. J. Med., Basel, 3, 193 - 198.

MOORE, H. C. & BILICZKI, F. P. (1968)
Failure to maintain steroid hypertension during pregnancy in the rat. J. Path. Bact., 95, 281 - 287.

MORIN, G., CORRIOL, J., NAQUET, R., RICCI, G. F. & BERARD-BADIER, M. (1954)
Caracteres et mechanismes de l'hypertension arterielle produite par la stimulation du cortex cerebral pericrucial chez le chat. J. Physiol., Paris., 46, 476 - 478.

MUELLER, P. S. & HORWITZ, D. (1962)
Plasma free fatty acid and blood glucose responses to analogues of norepinephrine in man. J. Lipid Res., 3, 251 - 255.

MUELLER, R. A. & THOENEN, H. (1970)
Effect of 6 hydroxydopamine hydrobromide (6HD) and adrenalectomy on the development of desoxycorticosterone trimethylacetate (DOC)-NaCl hypertension in rats. Fedn. Proc. Fedn. Socs. exp. Biol., 29, 546.

MUIRHEAD, E. E., LEACH, B. E. & ARMSTRONG, F. B. (1973)
Angiotensin-salt hypertension. Clin. Sci. Mol. Med., 45, 257s - 261s.

MULL, R. P. & MAXWELL, R. A. (1967)
Guanethidine and related adrenergic neuronal blocking agents. In Antihypertensive Agents. ed. by E. Schlittler, pp.115 - 149. Academic Press, New York and London.

MUSACCHIO, J. M., GOLDSTEIN, M., ANAGNOSTE, B., POCH, G. & KOPIN, I. J. (1966)
Inhibition of dopamine-β-hydroxylase by disulfiram in vivo. J. Pharmac. exp. Ther., 152, 56 - 61.

MUSCHOLL, E. (1966)
Autonomic nervous system: Newer mechanisms of adrenergic blockade. A. Rev. Pharmac., 6, 107 - 128.

MUSCHOLL, E. (1972)
Adrenergic false transmitters. Handb. exp. Pharmak., 33, 618 - 660.

MUSCHOLL, E. & MAITRE, L. (1963)
Release by sympathetic stimulation of α-methylnoradrenaline stored in the heart after administration of α-methyldopa. Experientia, 19, 658 - 659.

MYERS, M. G., REID, J. L. & LEWIS, P. J. (1974)
The effect of central serotonin depletion on DOCA-saline hypertension in the rat. Cardiovascular Res., 8, 806 - 810.

NAGAOKA, A., KIKUCHI, K. & ARAMAKI, Y. (1969)
Depressor responses of the spontaneously hypertensive rats to the anti-hypertensive agents. Jap. J. Pharmac., 19, 401 - 408.

NAKAMURA, K., GEROLD, M. & THOENEN, H. (1971)
Experimental hypertension of the rat: reciprocal changes of norepinephrine turnover in heart and brainstem. Naunyn-Schmiedebergs Arch. Pharmac., 268, 125 - 139.

NG, K. K. F. & VANE, J. R. (1967)
Conversion of angiotensin I to angiotensin II. Nature, Lond., 216, 762 - 766.

NG, K. K. F. & VANE, J. R. (1968 a)
Conversion of angiotensin I to angiotensin II in vivo. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak., 259, 188 - 189.

NG, K. K. F. & VANE, J. R. (1968 b)
Fate of angiotensin I in the circulation. Nature, Lond., 218, 144 - 150.

NICHOLAS, T. E. (1971)
Responses of mean arterial pressure to pressor agents and diuretics in renal hypertensive and salt hypertensive rats. Br. J. Pharmac., 42, 179 - 192.

NICKERSON, M. & NOMAGUCHI, G. M. (1948)
Locus of the adrenergic blocking action of dibenamine. J. Pharmac. exp. Ther., 93, 40 - 51.

NIJKAMP, F. P. & DE JONG, W. (1975)
α-Methylnoradrenaline induced hypotension and bradycardia after administration into the area of the nucleus tractus solitarii. Europ. J. Pharmac., 32, 361 - 364.

NIJKAMP, F. P., EZER, J. & DE JONG, W. (1975)
Central inhibitory effect of α-methyldopa on blood pressure, heart rate and body temperature of renal hypertensive rats. Europ. J. Pharmac., 31, 243 - 249.

NOSAKA, S. & WANG, S. C. (1972)
Carotid sinus baroceptor functions in the spontaneously hypertensive rat. Am. J. Physiol., 222, 1079 - 1084.

OATES, H. F., STOKES, G. S. & STOREY, B. G. (1975)
Plasma renin concentration in hypertension produced by unilateral renal artery constriction in the rat. Clin. exp. Pharmac. Physiol., 2, 289 - 296.

OATES, J. A., GILLESPIE, L., UNDENFRIEND, S. & SJOERDSMA, A. (1960)
Decarboxylase inhibition and blood pressure reduction by α-methyl-3,4-dihydroxy-dl-phenylalanine. Science, 131, 1890 - 1891.

OKAMOTO, K. (1969)
Spontaneous hypertension in rats. Int. Rev. exp. Path., 7, 227 - 270.

OKAMOTO, K. & AOKI, K. (1963)
Development of a strain of spontaneously hypertensive rats. Jap. Circul. J., 27, 282 - 293.

OKAMOTO, K., AOKI, K., NOSAKA, S. & FUKUSHIMA, M. (1964)
Cardiovascular diseases in the spontaneously hypertensive rat. Jap. Circul. J., 28, 943 - 952.

OKAMOTO, K., NOSAKA, S., YAMORI, Y. & MATSUMOTO, M. (1967)
Participation of a neural factor in the pathogenesis of hypertension in the spontaneously hypertensive rat. Jap. Heart J., 8, 168 - 180.

OKAMOTO, K., YAMORI, Y. & NAGAOKA, A. (1974)
Establishment of the stroke-prone spontaneously hypertensive rat (SHR). Circulation Res., 34 - 35, suppl. I., I - 143 - I - 153.

OLMSTED, F., CORCORAN, A. C. & PAGE, I. H. (1951)
Blood pressure in the unanaesthetized rat. Circulation, 3, 722 - 726.

OONO, Y. (1966)
Vascular reactivity in experimental hypertension: Responses of hind-quarters preparation of rats. Jap. Circul. J., 30, 267 - 280.

OPARIL, S. & HABER, E. (1972)
Conversion of angiotensin I to II in vivo and in vitro. In Control of Renin Secretion. ed. by T. A. Assaykeen, pp. 151 - 158. Plenum Press, New York and London.

OSTROVSKY, D., PAPSIN, F. R. & GORNALL, A. G. (1968).
Renal-adrenal interrelationships in experimental hypertension. Can. J. Physiol. Pharmac., 46, 179 - 188.

OWEN, D. A. A. (1969)
Studies on the interaction between angiotensin and the sympathetic nervous system. Ph. D. Thesis, University of Aston in Birmingham, England.

PAGE, I. H. (1939)
Production of persistent arterial hypertension by cellophane perineph-ritis. J. Am. med. Ass., 113, 2046 - 2048.

PAGE, I. H. (1940)
Demonstration of liberation of renin into blood stream from kidneys of animals made hypertensive by cellophane perinephritis. Am. J. Physiol., 130, 22 - 28.

PAGE, I. H. (1974)
Arterial hypertension in retrospect. Circulation Res., 34, 133 - 142.

PAGE, I. H. & BUMPUS, F. M. (1961)
Angiotensin. Physiol. Rev., 41, 331 - 390.

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 - 42.

PAGE, I. H., KANEKO, Y. & McCUBBIN, J. W. (1966)
Cardiovascular reactivity in acute and chronic renal hypertensive dogs. Circulation Res., 18, 379 - 387.

PAGE, I. H. & McCUBBIN, J. W. (1963)
Mechanisms by which ganglioplegics and atropine enhance cardiovascular responsiveness. Am. J. Physiol., 205, 1 - 9.

PAGE, I. H. & McCUBBIN, J. W. (1965)
The physiology of arterial hypertension. In Handbook of Physiology, Section 2, Circulation, Volume III. ed. by W. F. Hamilton, pp. 2163 - 2208. American Physiological Society, Washington, D. C.

PAGE, I. H. & McCUBBIN, J. W. (1968)
Renal Hypertension. Year Book Medical Publishers, Chicago.

PAGE, I. H. & OLMSTED, F. (1961)
Haemodynamic effects of angiotensin, norepinephrine and bradykinin continuously measured in unanaesthetised dogs. Am. J. Physiol., 201, 92 - 97.

PALS, D. T. & FULTON, R. W. (1968)
Interrelationships between angiotensin and vascular adrenergic receptors. Am. J. Physiol., 214, 506 - 512.

PANASEVICH, R. E., BELAIR, E. J., TRIVEDI, M. C. & YELNOSKY, J. (1969)
A study concerned with the failure of some rats treated with DOCA and saline to develop hypertension. Archs int. Pharmacodyn. Ther., 182, 198 - 205.

PARDO, E. G., VARGAS, R. & VIDRIO, H. (1965)
Antihypertensive drug action. A.Rev. Pharmac., 5, 77 - 98.

PEART, W. S. (1965)
Renin-angiotensin system. Pharmac. Rev., 17, 143 - 182.

PEART, W. S. (1966)
Catecholamines and hypertension. Pharmac. Rev., 18, 667 - 672.

PEART, W. S. (1969)
The renin-angiotensin system. A history and review of the renin-angiotensin system. Proc. R. Soc., Series B, 173, 317 - 325.

PEART, W. S. (1975)
Renin-angiotensin system. New Engl. J. Med., 292, 302 - 306.

PEART, W. S., ROBERTSON, J. I. S. & GRAHAME-SMITH, D. G. (1961)
Examination of the relationship of renin release to hypertension produced in the rabbit by renal-artery constriction. Circulation Res., 9, 1171 - 1184.

PFEFFER, J. M., PFEFFER, M. A. & FROHLICH, E.D. (1971)
Validity of an indirect tail-cuff method for determining systolic arterial pressure in unanaesthetized normotensive and spontaneously hypertensive rats. J. Lab. clin. Med., 78, 957 - 962.

PHELAN, E. L. (1966)
Cardiovascular reactivity in rats with spontaneous inherited hypertension and constricted renal-artery hypertension. Am. Heart J., 71, 50 - 57.

PHELAN, E. L. (1968)
The New Zealand strain of rats with genetic hypertension. N. Z. med. J., 67, 334 - 344.

PHELAN, E. L., ERYETISHIR, I. & SMIRK, F. H. (1962)
Observations on the responses of rats with spontaneous hypertension and control rats to pressor drugs and to hexamethonium. Circulation Res., 10, 817 - 824.

PHELAN, E. L. & WONG, L. C. K. (1968)
Sodium, potassium and water in the tissue of rats with genetic hypertension and constricted renal artery hypertension. Clin. Sci., 35, 487 - 494.

PICKENS, P. T., DUNSTAN, H. P., BUMPUS, F. M. & PAGE, I. H. (1965)
Measurement of plasma renin activity in hypertension. Hypertension, 13, 90 - 96.

PICKERING, G. W. (1955)
High Blood Pressure. J. and A. Churchill, Ltd., London.

PICKERING, G. W. (1968)
High Blood Pressure. Second Edition. J. and A. Churchill, Ltd., London.

PICKERING, G. W. & PRINZMETAL, M. (1938)
Experimental hypertension of renal origin in the rabbit. Clin. Sci., 3, 357 - 368.

PLUMMER, A. J. (1967)
Experimental hypertension in animals and its use in screening for antihypertensive compounds. In Antihypertensive Agents. ed. by E. Schlittler, pp.67 - 114. Academic Press, New York and London.

POISEUILLE, J. L. M. (1828)
Recherches sur la force du coeur aortique. These, No. 166, Paris.

POPOVIC, V. & POPOVIC, P. (1960)
Permanent cannulation of aorta and vena cava in rats and ground squirrels. J. appl. Physiol., 15, 727 - 728.

PORTER, C. C., TOTARO, J. A. & BURCIN, A. (1965)
Relationship between radioactivity and norepinephrine concentrations in the brains and hearts of mice following administration of labelled methyldopa or 6-hydroxydopamine. J. Pharmac. exp. Ther., 150, 17 - 23.

PORTER, C. C., TOTARO, J. A. & LEIBY, C. M. (1961)
Some biochemical effects of α-methyl-3,4-dihydroxyphenylalanine and related compounds in mice. J. Pharmac. exp. Ther., 134, 139 - 145.

POYSER, R. H., SHORTER, J. H. & WHITING, R. L. (1974)
The production of hypertension and the effects of some antihypertensive agents in the conscious unrestrained cat. Br. J. Pharmac., 51, 149P.

PROSKAUER, G. G., NEUMANN, C. & GRAEF, I. (1945)
The measurement of the blood pressure in rats with special reference to changes in temperature. Am. J. Physiol., 143, 290 - 296.

RAPP, J. P. & DAHL, L. K. (1971 a)
Adrenal steroidogenesis in rats bred for susceptibility and resistance to the hypertensive effect of salt. Endocrinology, 88, 52 - 65.

RAPP, J. P. & DAHL, L. K. (1971 b)
18-hydroxy-deoxycorticosterone secretion in experimental hypertension in rats. Circulation Res., 28 - 29, suppl. II., II - 153 - II - 159.

RAPP, J. P. & DAHL, L. K. (1972)
Mendelian inheritance of 18- and 11 β-steroid hydroxylase activities in the adrenals of rats genetically susceptible or resistant to hypertension. Endocrinology, 90, 1435 - 1446.

RATHER, L. J. (1951)
The nature and significance of changes in adrenal cytology, weight and cortical/medullary ratio in experimental renal hypertension and clinical hypertension. J. exp. Med., 93, 573 - 586.

REDLEAF, P. D. & TOBIAN, L. (1958)
Question of vascular hyper-responsiveness in hypertension. Circulation Res., 6, 185 - 193.

REID, I. A., SCHRIER, R. W. & EARLEY, L. E. (1972)
Effect of beta-adrenergic stimulation on renin release. In Control of Renin Secretion. ed. by T. A. Assaykeen, pp.49 - 64. Plenum Press, New York and London.

REMINGTON, J. W., CARTLAND, G. F., DRILL, V. A. & SWINGLE, W. W. (1944)
Purification and bioassay of tissue extracts capable of lowering the blood pressure of hypertensive rats. Am. J. Physiol., 140, 627 - 635.

RIVA-ROCCI, S. (1896)
Un nuovo sfigmomanometro. Gazz. med. Torino, 47, 981 - 1001.

ROJO-ORTEGA, J. M. & GENEST, J. (1968)
A method for production of experimental hypertension in rats. Can. J. Physiol. Pharmac., 46, 883 - 885.

ROMERO, J. C., HOOBLER, S. W., KOZAK, T. J. & WARZYNSKI, R. J. (1973)
Effect of antirenin on blood pressure of rabbits with experimental renal hypertension. Am. J. Physiol., 225, 810 - 817.

ROMERO, J. C., MAK, S. W. & HOOBLER, S. W. (1974)
Effect of blockade of angiotensin-I converting enzyme on the blood pressure of renal hypertensive rabbits. Cardiovascular Res., 8, 681 - 687.

ROSAS, R., ROJO-ORTEGA, J. M., GANTEN, D., BOUCHER, R. & GENEST, J. (1975)
The renin-angiotensin system in rats made hypertensive by ligation of the kidney poles. Proc. Soc. exp. Biol. Med., 148, 562 - 567.

ROSECRANS, J. A., WATZMAN, N. & BUCKLEY, J. P. (1966)
The production of hypertension in male albino rats subjected to experimental stress. Biochem. Pharmac., 15, 1707 - 1718.

ROSENFELD, S., THOMAS, H. V. & DRURY, D. R. (1954)
Effects of renal denervation on cerebral hypertension in the rabbit. Am. J. Physiol., 178, 392 - 398.

ROSZKOWSKI, A. P. (1961)
An unusual type of sympathetic ganglion stimulant. J. Pharmac. exp. Ther., 132, 156 - 170.

ROWBERG, A., FRANKLIN, D. & VAN CITTERS, R. L. (1969)
Nontraumatic method for measurement of blood pressure in animals with tails. J. appl. Physiol., 27, 301 - 302.

ROYCE, P. C. (1967)
Role of renal uptake of plasma protein in compensatory renal hypertrophy. Am. J. Physiol., 212, 924 - 930.

RYTAND, D. A. (1938)
Pathogenesis of arterial hypertension in coarctation of the aorta. Proc. Soc. exp Biol. Med., 38, 10 - 11.

SANAN, S. & VOGT, M. (1962)
Effects of drugs on the noradrenaline content of brain and peripheral tissues and its significance. Br. J. Pharmac. Chemother., 18, 109 - 127.

SAPIRSTEIN, L. A., BRANDT, W. L. & DRURY, D. R. (1950)
Production of hypertension in the rat by substituting hypertonic sodium chloride for drinking water. Proc. Soc. exp. Biol. Med., 73, 82 - 85.

SCHMID, P. G. & ABBOUD, F. M. (1974)
Neurohumoral control of vascular resistance. Archs intern. Med., 133, 935 - 945.

SCHMITT, H. & SCHMITT, H. (1967 a)
Modifications by catecholamines of pressor responses to angiotensin in pithed rats. Revue can. Biol., 26, 265 - 267.

SCHMITT, H. & SCHMITT, H. (1967 b)
Interrelations entre catecholamines et angiotensine. C. r. Seanc. Soc. Biol., 161, 753 - 756.

SCHROEDER, H. A. (1942)
Arterial hypertension in rats. I. Methods. J. exp. Med., 75, 513 - 526.

SCHROEDER, H. A. (1953)
Historical. In Hypertensive Diseases: Causes and Control. p. 35. H.Kimpton, London.

SCHWARTZ, J., BLOCH, R., KIENY, R., JURASCHECK, F. & FONTAINE, J. L. (1967)
Variations des taux de renine plasmatique et de la sensibilite a l'action pressive de l'angiotensine chez le chien hypertendu par defrenation ou par stenose de l'artere renale. Path. Biol., Paris, 15, 35 - 39.

SCROOP, G. C. & WHELAN, R. F. (1968)
Vascular reactivity studies in hypertension. Aust. J. exp. Biol. med. Sci., 46, 555 - 561.

SELYE, H. (1948)
Hypertension as a disease of adaption. Recent Prog. Horm. Res., 3, 343 - 361.

SELYE, H., HALL, C. E. & ROWLEY, E. M. (1943)
Malignant hypertension produced by treatment with desoxycorticosterone acetate and sodium chloride. Can. med. Ass. J., 49, 88 - 92.

SELYE, H. & PENTZ, E. L. (1943)
Pathogenetical correlations between periarteritis nodosa, renal hypertension and rheumatic lesions. Can. med. Ass. J., 49, 264 - 272.

SELYE, H. & STONE, H. (1943)
Role of sodium chloride in production of nephrosclerosis by steroids. Proc. Soc. exp. Biol. Med., 52, 190 - 193.

SELYE, H. & STONE, H. (1946)
Pathogenesis of the cardiovascular and renal changes which usually accompany malignant hypertension. J. Urol., 56, 399 - 419.

SEN, S., SMEBY, R. R. & BUMPUS, F. M. (1967)
Isolation of a phospholipid renin inhibitor from kidney. Biochemistry, Wash., 6, 1572 - 1581.

SEN, S., SMEBY, R. R. & BUMPUS, F. M. (1968)
Antihypertensive effect of an isolated phospholipid. Am. J. Physiol., 214, 337 - 341.

SEN, S., SMEBY, R. R. & BUMPUS, F. M. (1969)
Plasma renin activity in hypertensive rats after treatment with renin preinhibitor. Am. J. Physiol., 216, 499 - 503.

SEN, S., SMEBY, R. R. & BUMPUS, F. M. (1972)
Renin in rats with spontaneous hypertension. Circulation Res., 31, 876 - 880.

SHIBATA, S., KURAHASHI, K. & MORI, J. (1971)
Responsiveness to catecholamines, potassium, barium, angiotensin, nicotine and other agents in the vascular smooth musculature of the spontaneously hypertensive rat. Satellite Symp. 'Vasculature Smooth Muscle' of XXV International Congress of Physiological Sciences. p. 29.

SHIPLEY, R. E. & TILDEN, J. H. (1947)
A pithed rat preparation suitable for assaying pressor substances. Proc. Soc. exp. Biol. Med., 64, 453 - 455.

SHORE, P. A., BUSFIELD, D. & ALPERS, H. S. (1964)
Binding and release of metaraminol: mechanism of norepinephrine depletion by alpha-methyl-M-tyrosine and related agents. J. Pharmac. exp. Ther., 146, 194 - 199.

SHULER, R. H., KUPPERMAN, H. S. & HAMILTON, W. F. (1944)
Comparison of direct and indirect blood pressure measurements in rats. Am. J. Physiol., 141, 625 - 629.

SIVERTSSON, R. (1970)
The hemodynamic importance of structural vascular changes in essential hypertension. Acta physiol. scand., suppl. 343, 1 - 56.

SKEGGS, L. T., LENTZ, K. E., SHUMWAY, N. P. & WOODS, K. R. (1956)
The amino acid sequence of hypertensin II. J. exp. Med., 104, 193 - 197.

SKEGGS, L. T. & LEONARDS, J. R. (1946)
A new endpoint for blood pressure determinations in the rat's tail. Proc. Soc. exp. Biol. Med., 62, 294 - 296.

SKEGGS, L. T., MARSH, W. H., KAHN, J. R. & SHUMWAY, N. P. (1955)
Amino acid composition and electrophoretic properties of hypertensin I. J. exp. Med., 102, 435 - 440.

SKELTON, F. R. (1953)
Production of hypertension, nephrosclerosis and cardiac lesions by methylandrostenediol treatment in the rat. Endocrinology, 53, 492 - 505.

SKELTON, F. R. (1955)
Development of hypertension and cardiovascular-renal lesions during adrenal regeneration in the rat. Proc. Soc. exp. Biol. Med., 90, 342 - 346.

SKELTON, F. R. (1956)
Adrenal regeneration hypertension and factors influencing its development. Archs intern. Med., 98, 449 - 462.

SKELTON, F. R. (1969)
Production of hypertensive vascular disease in the rat by methyltestosterone. Lab. Invest., 21, 129 - 137.

SKINNER, S. L., McCUBBIN, J. W. & PAGE, I. H. (1946)
Control of renin secretion. Circulation Res., 15, 64 - 76.

SMIRK, F. H. (1967)
The pathogenesis of hypertension. In Antihypertensive Agents. ed. by E. Schlittler, pp.1 - 65. Academic Press, New York and London.

SMIRK, F. H. (1970)
The neurogenically maintained component in hypertension. Circulation Res., 26 - 27, suppl. II., II - 55 - II - 63.

SMIRK, F. H. & HALL, W. H. (1958)
Inherited hypertension in rats. Nature, Lond., 182, 727 - 728.

SMIRK, F. H., HAMILTON, M., DOYLE, A. E. & McQUEEN, E. G. (1958)
Treatment of hypertensive heart failure and of hypertensive cardiac overload by blood pressure reduction. Am. J. Cardiol., 1, 143 - 153.

SMITH, J. C. (1966)
Pharmacologic interactions with 4-(m-chlorophenylcarbamoyloxy)-2-butynyltrimethylammonium chloride, a sympathetic ganglion stimulant. J. Pharmac. exp. Ther., 153, 276 - 284.

SMITH, S. E. (1960)
The pharmacological actions of 3,4-dihydroxy-phenyl-α-methylalanine (α-methyldopa), an inhibitor of 5-hydroxytryptophan decarboxylase. Br. J. Pharmac. Chemother., 15, 319 - 327.

SMOOKLER, H. H. & BUCKLEY, J. P. (1969)
Relationships between brain catecholamine synthesis, pituitary adrenal function and the production of hypertension during prolonged exposure to environmental stress. Int. J. Neuropharmac., 8, 33 - 41.

SOBIN, S. S. (1946)
Accuracy of indirect determinations of blood pressure in the rat. Am. J. Physiol., 146, 179 - 186.

SOURKES, T. L. (1954)
Inhibition of dihydroxyphenylalanine decarboxylase by derivatives of phenylalanine. Archs Biochem. Biophys., 51, 444 - 456.

SOURKES, T. L. & RODRIGUEZ, H. R. (1967)
α-Methyldopa and other decarboxylase inhibitors. In Antihypertensive Agents. ed. by E. Schlittler, pp.151 - 189. Academic Press, New York and London.

SPECTOR, S., FLEISH, J. H., MALING, H. M. & BRODIE, B. B. (1969)
Vascular smooth muscle activity in normotensive and hypertensive rats. Science, 166, 1300 - 1301.

SPIELMAN, W. S., DAVIS, J. O., FREEMAN, R. H. & JOHNSON, J. A. (1974)
Stimulation of aldosterone by a heptapeptide fragment of angiotensin II in the rat. Fedn. Proc. Fedn. Am. Socs. exp. Biol., 33, 254.

STANTON, H. C. & COOPER, C. M. (1966)
Comparison of effects of antihypertensive agents on normotensive rats and rats with "metacorticoid" and adrenal regeneration hypertension. Cardiovascular Res. Cent. Bull., 5, 16 - 28.

STANTON, H. C. & COOPER, C. M. (1967)
Antihypertensive effects of drugs measured in unanesthetized rats with established adrenal regeneration hypertension. Archs int. Pharmacodyn. Ther., 168, 1 - 13.

STANTON, H. C. & WHITE, J. B. Jr. (1964)
Hypotensive actions of drugs on unanesthetized normotensive and "metacorticoid" hypertensive rats determined by a direct recording technique. Archs int. Pharmacodyn. Ther., 154, 351 - 363.

STILL, W. J. S. & DENNISON, S. M. (1969)
The pathogenesis of the glomerular changes in steroid-induced hypertension in the rat. Lab. Invest., 20, 249 - 260.

STONE, C. A. & PORTER, C. C. (1966)
Methyldopa and adrenergic nerve function. Pharmac. Rev., 18, 569 - 575.

STURTEVANT, F. M. (1953)
Response of metacorticoid hypertension to bistrium, apresoline, veriloid and serpentina. Proc. Soc. exp. Biol. Med., 84, 101 - 102.

STURTEVANT, F. M. (1956)
Studies on vascular reactivity in normotensive and metacorticoid hypertensive rats. Am. Heart J., 52, 410 - 418.

STURTEVANT, F. M. (1958)
The biology of metacorticoid hypertension. Ann. intern. Med., 49, 1281 - 1293.

SVENSSON, T. H. & WALDECK, B. (1969)
On the significance of central noradrenaline for motor activity: Experiments with a new dopamine β-hydroxylase inhibitor. Europ. J. Pharmac., 7, 278 - 282.

TAQUINI, A. C. Jr. (1963)
Neurogenic component of peripheral resistance in renal hypertension. Circulation Res., 12, 562 - 567.

TAQUINI, A. C. Jr., BLAQUIER, P. C. & BOHR, D. F. (1961)
Neurogenic factors and angiotensin in etiology of hypertension. Am. J. Physiol., 201, 1173 - 1175.

TAUBERGER, G. & KUHN, P. (1971)
Untersuchungen der zentralnervosen sympathicusdampfenden wirkungen von α-methyl-dopa. Naunyn-Schmiedebergs Arch. Pharmac., 268, 33 - 43.

THOENEN, H. (1972)
Chemical sympathectomy: A new tool in the investigation of the physiology and pharmacology of peripheral and central adrenergic neurons. In Perspectives of Neuropharmacology. ed. by S. H. Snyder, pp. 301 - 338. Oxford University Press.

THURANSZKY, K. (1966)
Continuous blood pressure measurement in non-anaesthetised animals. Acta physiol. hung., 29, 33 - 40.

THURAU, K., SCHNERMANN, J., NAGEL, W., HORSTER, M. & WAHL, M. (1967)
Composition of tubular fluid in the macula densa segment as a factor regulating the function of the juxtaglomerular apparatus. Circulation Res., 20 - 21, suppl. II., II - 79 - II - 89.

TIGERSTEDT, R. & BERGMAN, P. G. (1898)
Niere und krieslauf. Skand. Arch. Physiol., 8, 223 - 271.

TOBIAN, L. (1960)
Interrelationship of electrolytes, juxtaglomerular cells and hypertension. Physiol. Rev., 40, 280 - 322.

TOBIAN, L. (1974)
Hypertension and the kidney. Archs intern. Med., 133, 959 - 967.

TOBIAN, L. & REDLEAF, P. (1957)
Effect of hypertension on arterial wall electrolytes during desoxycorticosterone administration. Am. J. Physiol., 189, 451 - 454.

TOBIAN, L., TOMBOULIAN, A. & JANECEK, J. (1959)
Effect of high perfusion pressures on the granulation of juxtaglomerular cells in the isolated kidney. J. Clin. Invest., 38, 605 - 610.

TORCHIANA, M. L., LOTTI, V. J., CLARK, C. M. & STONE, C. A. (1973)
Comparison of centrally mediated hypotensive action of methyldopa and dopa in cats. Archs int. Pharmacodyn. Ther., 205, 103 - 113.

TORCHIANA, M. L., PORTER, C. C., WATSON, L. S. & STONE, C. A. (1965)
Relationship of cardiovascular and antihypertensive effects of methyldopa with α-methylnorepinephrine concentrations in the hearts of rats. Pharmacologist, 7, 145.

UEDA, H., KATAYAMA, S. & KATO, R. (1972)
Area prostrema-angiotensin-sensitive site in brain. In Control of Renin Secretion. ed. by T. A. Assaykeen, pp. 109 - 116. Plenum Press, New York and London.

UEDA, H., NISHIMURA, H. & YASUDA, H. (1967)
Experimental vascular lesions in desoxycorticosterone hypertension in rats. Jap. Heart J., 8, 42 - 57.

VANDER, A. J. (1963)
Inhibition of distal tubular sodium reabsorption by angiotensin II. Am. J. Physiol., 205, 133 - 138.

VANDER, A. J. (1967)
Control of renin release. Physiol. Rev., 47, 359 - 382.

VANDER, A. J. & MILLER, R. (1964)
Control of renin secretion in the anesthetised dog. Am. J. Physiol., 207, 537 - 545.

VAN ZWIETEN, P. A. (1973)
The central action of antihypertensive drugs, mediated via central α-receptors. J. Pharm. Pharmac., 25, 89 - 95.

VAPAATALO, H., HACKMAN, R., ANTTILA, P., VAINIONPAA, V. & NEUVONEN, P. J. (1974)
Effects of 6-hydroxydopamine on spontaneously hypertensive rats. Naunyn-Schmiedebergs Arch. Pharmac., 284 - 1 - 13.

VEYRAT, R., BRUNNER, H. R., MANNING, E. L. & MULLER, A. F. (1967)
Inhibition de l'activite de la renine plasmatique par le potassium. J. Urol. Nephrol., Paris, 73, 271 - 275.

VON BASCH, S. S. (1881)
Ueber die messung des blutdrucks am menschen. Z. klin. Med., 2, 79 - 96.

VON RECKLINGHAUSEN, H. (1901)
Ueber blutdruckmessung beim menschen. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak., 46, 78 - 132.

WAKERLIN, G. E. (1958)
Antibodies to renin as proof of the pathogenesis of sustained renal hypertension. Circulation, 17, 653 - 657.

WAKERLIN, G. E. & JOHNSON, C. A. (1941)
Reductions in blood pressures of renal hypertensive dogs by hog renin. Proc. Soc. exp. Biol. Med., 46, 104 - 112.

WALDMEIER, P., HEDWALL, P. R. & MAITRE, L. (1975)
On the role of α-methyldopamine in the antihypertensive effect of α-methyldopa. Naunyn-Schmiedebergs Arch. Pharmac., 289, 303 - 314.

WALKER, W. G., RUIZ-MAZA, F. & HORVATH, J. S. (1972)
Demonstration of free (unbound) angiotensin II in immunized rabbits. Proc. 5th Int. Cong. Nephrol., p. 115.

WALLIN, B. G., DELIUS, W. & HAGBARTH, K. E. (1973)
Comparison of sympathetic nerve activity in normotensive and hypertensive subjects. Circulation Res., 33, 9 - 21.

WATHEN, R. L., KINGSBURY, W. S., STOUDER, D. A., SCHNEIDER, E. G. & ROSTORFER, H. H. (1965)
Effects of infusion of catecholamines and angiotensin II on renin release in anesthetized dogs. Am. J. Physiol., 209, 1012 - 1024.

WESTERMANN, E., BALZER, H. & KNELL, J. (1958)
Hemmung der serotoninbildung durch α-methyl-dopa. Naunyn-Schmiedebergs Arch. exp. Path. Pharmak., 234, 194 - 205.

WILLARD, P. W. & BECKHELM, G. A. (1968)
Differential effect of hypotensive drugs on renal and steroid hypertensive rats. Archs int. Pharmacodyn. Ther., 173, 11 - 15.

WILLARD, P. W. & FULLER, R. W. (1969)
Functional significance of the sympathetic nervous system in production of hypertension. Nature, Lond., 223, 417 - 418.

WILSON, C. & BYROM, F. B. (1939)
Renal changes in malignant hypertension. Experimental evidence. Lancet, 1, 136 - 139.

WILSON, C. & BYROM, F. B. (1941)
The vicious circle in chronic Bright's disease. Experimental evidence from the hypertensive rat. Q. Jl. Med., 10, 65 - 93.

WILSON, J. W. (1965)
A comparative study of experimentally produced hypertensive disease and its effects on the cardiovascular system of cats (Felis Domesticus) as related to hypertensive disease in humans. Ph. D. Thesis, University of Kentucky, U.S.A.

WOLINSKY, H. (1972)
Long-term effects of hypertension on the rat aortic wall and their relation to concurrent aging changes. Circulation Res., 30, 301 - 309.

WOLINSKY, H. (1973)
Comparative effects of castration and antiandrogen treatment on the aortas of hypertensive and normotensive male rats. Circulation Res., 33, 183 - 189.

YU, R. & DICKINSON, C. J. (1965)
Neurogenic effects of angiotensin. Lancet, 2, 1276 - 1277.

ZAIMIS, E. (1964)
Pharmacology of the autonomic nervous system. A. Rev. Pharmac., 4, 365 - 400.

ZAIMIS, E. (1965)
The immunosympathectomized animal: a valuable tool in physiological and pharmacological research. J. Physiol., Lond., 177, 35P - 36P.

ZAIMIS, E., BERK, L. & CALLINGHAM, B. A. (1965)
Morphological, biochemical and functional changes in the sympathetic nervous system of rats treated with nerve growth factor-antiserum. Nature, Lond., 206, 1220 - 1222.

ZANCHETTI, A., GUAZZI, M. & BACCELLI, G. (1966)
Influence of sleep on circulation in normal and hypertensive animals. In Antihypertensive Therapy. Principles and Practice. An International Symposium. ed. by F. Gross, pp. 74 - 95. Springer-Verlag, Berlin.

ZIMMERMAN, B. G. (1967)
Evaluation of peripheral and central components of action of angiotensin on the sympathetic nervous system. J. Pharmac. exp. Ther., 158, 1 - 10.

ZIMMERMAN, B. G. (1973)
Involvement of angiotensin-mediated vasoconstriction in renal hypertension. Life Sci., 13, 507 - 515.