RENIN ACTIVITY

IN

HUMAN HYPERTENSION

Thesis submitted for the Degree of
Doctor of Philosophy

by

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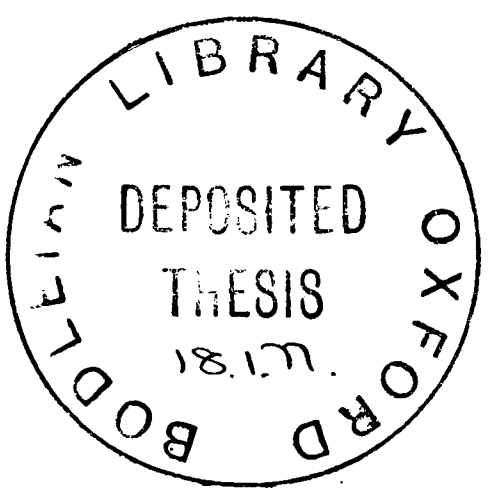

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SYMBOLS AND UNITS OF MEASUREMENT

Symbol	Variable	Units
HT	Hypertensive patient	
C	Control subjects	
RAS	Renin-angiotensin system	
PRA	Plasma renin activity	ng/ml/hr
PRA S	Supine plasma renin activity	ng/ml/hr
PRA E	Erect plasma renin activity	ng/ml/hr
PRA L	Post-frusemide (lasix) plasma renin activity (frusemide = lasix)	ng/ml/hr
ΔS-E	Absolute change in PRA from supine to erect	ng/ml/hr
ΔS-L	Absolute change in PRA from supine to lasix	ng/ml/hr
ΔE-L	Absolute change in PRA from erect to lasix	ng/ml/hr
%S-E	Percentage change in PRA from supine to erect	%
%S-L	Percentage change in PRA from supine to lasix	%
%E-L	Percentage change in PRA from erect to lasix	%
SBP	Systolic blood pressure	mm Hg
DBP	Diastolic blood pressure	mm Hg
MBP	Mean blood pressure	mm Hg
UNa 24	24 hour urinary sodium excretion	mmol/24 hr
UK 24	24 hour urinary potassium excretion	mmol/24 hr
NaE	Total exchangeable sodium	mmol/kg body wt

Symbol	Variable	Units
TBK	Total body potassium	mmol/kg body wt
PV	Plasma volume	$l/1.73m^2$ body surface area

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ABSTRACT

This thesis is concerned with the renin-angiotensin system in essential hypertension.

Humoral agents have been sought in the causation of high blood pressure for many years. The role of the renin-angiotensin system in hypertension remained controversial, but, gradually, the components and functions of this complex hormonal system were elucidated. Derangements of the system were confirmed in various examples of secondary hypertension, but the role of the renin-angiotensin system in essential hypertension remains unclear.

The belief that essential hypertension represents an homogeneous group of patients has been questioned in recent years, and this has led to the attempt to separate essential hypertensive patients into renin subgroups. One of the most intensively investigated has been that with low-renin, but whether or not this group represents a distinct entity is uncertain. An important hypothesis attributes this 'syndrome' to an excess of an unknown mineralocorticoid.

This work was undertaken to reappraise the role of renin in essential hypertension. Two major questions were considered: Is the activity of the renin-angiotensin system similar in essential hypertension and in the general population? Does low-renin hypertension exist as a separate entity attributable to mineralocorticoid excess? In

answering these questions many variables other than renin, including age, sex, blood pressure, sodium, potassium, plasma volume and aldosterone, and their interrelationships were considered.

Plasma renin activity is an index of the activity of the renin-angiotensin system, and the method used in its measurement has been described in considerable detail.

Volunteers to have their arterial pressure measured were sought amongst the employees of A.E.R.E. Harwell and two general practices in Wantage and Didcot. From them two samples of people willing to co-operate were chosen: 89 with arterial pressures above 100 mm Hg and 89 of similar age and sex with diastolics below 90 mm Hg. All subjects with high blood pressure who had received treatment or who had symptoms from it were excluded. Investigations showed that subjects with high pressures conformed to the diagnosis of essential hypertension.

RENIN IN ESSENTIAL HYPERTENSION

Blood was withdrawn for the measurement of plasma renin activity after the subject had been lying down for 2 hours (supine), after being up and around for 2 hours (erect), and 1 hour after an intravenous injection of frusemide (1 mg/kg). The renin responsiveness or change in plasma renin activity after stimulation was expressed in absolute and in percentage terms. In essential hypertension plasma renin and its responsiveness were found to be suppressed compared to control subjects.

Reasons for the reduction in plasma renin and in renin responsiveness in essential hypertension are uncertain, but various factors are known to affect renin and were considered. Plasma, urinary and total body potassium, plasma, urinary and total exchangeable sodium, and plasma volume were studied but did not account for the difference. Age was shown to correlate inversely with renin and its responsiveness in patients with hypertension and in controls, but the groups were age matched. The effects of previous antihypertensive drugs on renin levels were excluded by rejecting previously treated patients. Females tended to have lower plasma renin activity and responsiveness than males, but those with hypertension were sex matched with the controls. However, blood pressure was shown to be inversely related to plasma renin and its responsiveness in both groups.

Thus, reduced plasma renin activity and responsiveness appears to be a general feature of essential hypertension. The suppression of renin is probably a result of the combined effect of many factors on renin release, but blood pressure itself has been shown to be an important contributor.

LOW-RENIN HYPERTENSION

An analysis of the distributions of plasma renin and renin responsiveness showed the logarithms of the renin variables to be normally distributed with no evidence of bimodality. This suggests that the low-renin state forms part of a continuum in hypertension rather than a distinct diagnostic entity.

An attempt was made to separate the subjects with high arterial pressure into renin subgroups in a statistically acceptable way, while utilizing basal, stimulated and renin responsiveness data. The distributions of plasma renin and renin responsiveness were divided into three equal subgroups (lower, mid and upper) on the understanding that if a low-renin subgroup existed it would be contained in the lower third of the distributions.

Plasma renin correlated significantly with responsiveness; in view of our method of defining low-renin hypertension, the decreased responsiveness in the low-renin subgroup could not be used to support its existence as a separate entity. This strengthens the argument against the use of stimuli to define a low-renin subgroup in essential hypertension.

The hypothesis that an unknown mineralocorticoid is involved in the aetiology of low-renin hypertension did not withstand critical examination. Total exchangeable sodium, plasma volume, total body potassium and 24 hour urinary potassium excretion were measured in the three renin subgroups of essential hypertension; the characteristic findings of an excess of an aldosterone-like mineralocorticoid were absent.

It is proposed that low-renin hypertension represents no more than the lower end of the normal distribution of renin in essential hypertension. However, many factors including age and blood pressure may modify the renin status and need to

be taken into account in the interpretation of individual values.

PART I

INTRODUCTION

The following chapter reviews the renin-angiotensin system from its history, through its components and their function and onto its involvement in essential hypertension, and more especially low-renin hypertension.

The aims of the work are outlined.

CHAPTER 1

INTRODUCTION

HISTORICAL REVIEW OF THE RENIN-ANGIOTENSIN SYSTEM

In 1828 Richard Bright reported the hypertension might be of kidney origin. The concept of ductless glands and internal secretions was introduced in 1848 by Claude Bernard and extended by Brown-Sequard who, in 1892, proposed that the kidney had an internal secretion. Working on these ideas, Tigerstedt and Bergman in 1898 reported the discovery of the first hypertensive chemical principle, which they called renin (100). They showed that crude saline extracts of rabbit kidneys injected into other rabbits produced a pressor response.

This work remained in dispute and unconfirmed for many years. However, in 1914 Volhard and Fahr reaffirmed the role of the kidney in hypertension (105). The next major advance came in 1934 when Goldblatt and co-workers called attention to a renal mechanism in blood pressure control by their classical experiment demonstrating that hypertension could be produced by renal artery constriction, and suggested that this was due to the release of a pressor substance into the circulation (37). This led three separate groups (49,53,74) to repeat and confirm the studies of Tigerstedt and Bergman.

The next breakthrough occurred in 1939 when Page (70) and Braun-Menendez and co-workers (12) independently reported

that renin was not by itself a pressor substance, but acted as an enzyme to release a pressor peptide, now called angiotensin. Goormaghtigh proposed the renal juxta-glomerular cells as the source of renin, following his description of increased granularity of these cells in hypertension (40,41).

Then the nature of components of the renin angiotensin system was greatly clarified. In 1954 Skeggs and co-workers (90) were able to separate two forms of angiotensin - the decapeptide, angiotensin I and the octapeptide, angiotensin II. The isolation and composition of angiotensin was achieved by Peart (71a) and Skeggs et al (57) in 1956. In 1957 the synthesis of angiotensin was achieved by Bumpus and associates (21).

Thus, a complex hormonal system had been elucidated. The next few years saw clarification of the physiological role of the renin-angiotensin system in blood pressure and body fluid homeostasis. However, the role of this system in essential hypertension remains unclear.

COMPONENTS AND FUNCTIONS OF THE RENIN-ANGIOTENSIN SYSTEM

The Renin-Angiotensin System is a complex hormonal system operating through a series of enzymatic reactions to produce the effector hormone, Angiotensin II, which participates in the regulation of arterial blood pressure,

body electrolytes and fluid volume. A description of the components and functions of this system follows:

RENIN

Renin is a proteolytic enzyme which has not yet been obtained in a stable pure form. It is synthesised and stored in cytoplasmic granules by modified smooth muscle cells in the media of the afferent arteriole of the renal glomerulus (24a, 24b, 35). This structure was originally termed the juxtaglomerular apparatus (39), but this term has been widened to embrace not only the granular afferent arteriolar cells (juxtaglomerular cells) but also a group of specialised cells at the origin of the distal convoluted tubule that form the macula densa. Renin-like enzymes have been extracted from a variety of organs, but there is little evidence that they normally contribute to the levels of circulating renin or that they have any physiological role (66).

Renin has no known physiological action of its own and its biological effects are explained in terms of a sequence of enzymatic reactions that lead to the generation of an active polypeptide hormone, angiotensin II.

A number of mechanisms appear to govern the release of renin by the kidney (27), and it is possible that they interrelate. The important stimuli to renin secretion are changes in blood pressure and body fluid volume, mediated through changes in renal perfusion pressure and the

concentration or amount of sodium in the urine. Two intrarenal mechanisms have been proposed for the mediation of renin secretion by the kidney, and a number of other mechanisms have been suggested that may or may not operate via one of these two intrarenal receptors.

The baroreceptor theory proposes a stretch receptor in the afferent arteriole of the glomerulus which detects changes in pressure or stretch of the vessel and adjusts renin output accordingly. Strong support for this hypothesis has come from the work of Blaine, Davis and colleagues (8,9) using their non-filtering, denervated kidney model in adrenalectomised dogs. They showed that when the effects of the macula densa, the autonomic nervous system, and circulating catecholamines were excluded, there was still an increase in renin secretion associated with a decrease in perfusion pressure and on restoration of the perfusion pressure the plasma renin activity was restored to control levels.

The macula densa hypothesis of renin release proposes a receptor in the macula densa area of the distal convoluted tubule, which is sensitive to changes in tubular sodium. The work of Vander and Miller showed that mannitol and diuretics could prevent the increase in renin secondary to aortic constriction, suggesting that an increase in sodium load to the macula densa blocks renin release (103). Thurau's micropuncture studies demonstrated that iso - or hypertonic saline injected into the distal convoluted tubule caused

collapse of the proximal tubule, suggesting that an increase in sodium concentration at the macula densa stimulates renin release (98). Thus changes in distal tubular sodium delivery appears to act as a stimulus to renin release independent of perfusion pressure. It is unclear whether the stimulus to renin release is an increase or decrease in distal sodium delivery.

The central and autonomic nervous systems and catecholamine hormones can be considered together as an extrarenal mechanism influencing renin release. Autonomic nerves are found in close association with the vascular and tubular components of the juxtaglomerular apparatus (111,64). Stimulation of these nerves leads to renin release (51), while interruption of these nerves results in a reduced renin response to stimulation (63). The central nervous system via the sympathetic nerves has been shown to control renin secretion : stimulation of the medullary area near the obex leads to renin release (71), while stimulation of the hypothalmic area has the opposite effect (115). The role of the circulating catecholamines is less well defined. It has been shown that infusion of catecholamines into the renal artery will result in renin release (106), but it is difficult to dissociate this effect from the indirect effects of catecholamines on the vascular system. Adrenergic receptors have been proposed in the control of renin release, but their nature, specificity and location remain to be fully

defined. Beta-adrenergic blocking agents have been shown to impair the renin response to a variety of stimuli (109), but the response to alpha-adrenergic blocking agents is variable (66). The evidence now points to the beta-receptor mechanism as being dominant in the adrenergic control of renin release.

Various humoral agents regulate renin secretion and, apart from those already mentioned, potassium and anti-diuretic hormone will be discussed. Changes in potassium balance produce changes in renin secretion. Brunner and co-workers have demonstrated an inverse relationship between plasma renin activity and dietary induced changes in plasma potassium (15). However, large changes in potassium intake are required to produce a measurable effect on renin activity in man. A tubular mechanism of renin release by potassium has been suggested by experiments using the non-filtering kidney model (88). Antidiuretic hormone has been shown to inhibit renin release, and as this effect persists in the non-filtering kidney without affecting arterial pressure or renal blood flow, it suggests a direct effect of antidiuretic hormone on the juxtaglomerular cells (88).

Renin secretion is controlled by several feedback loops. The indirect pathway operates via the renin induced changes in blood pressure and fluid volume acting back on the renal blood vessels and tubules to modify perfusion pressure and distal tubular sodium, and hence renin secretion.

The direct feedback loop is mediated by angiotensin II acting directly on the juxtaglomerular cells to alter renin release without alteration in perfusion pressure or distal tubular sodium, and is supported by experimental evidence in sheep (10). Another possible feedback mechanism operates within the individual nephron and involves the direct stimulation of the juxtaglomerular cells by the macula densa in response to tubular sodium changes, to regulate renin secretion (99).

Studies suggest that renin has a half-life in the circulation of about 15-20 minutes (80). Renin is excreted in the urine but this apparently accounts for less than one percent of that which might be filtered (13). However, recent studies indicate that the liver is the major site of renin inactivation (47).

RENIN SUBSTRATE

Renin substrate is an $\alpha 2$ globulin of molecular weight 58,000, which is produced by the liver. There are several different forms of renin substrate, all having the same amino-acid composition but differing in carbohydrate composition (91). The mechanisms governing the role of production of renin substrate are incompletely understood, but various physiological and pathological states can alter substrate concentration. Renin substrate has been reported to occur normally in human plasma at a concentration of the order of 1,500 ng of angiotensin liberated per millilitre of plasma (85).

RENIN - RENIN SUBSTRATE REACTION

Neither renin nor renin substrate have any direct physiological effect, but renin reacts specifically with its substrate hydrolysing the leucyl^{10} - leucyl^{11} bond in substrate to yield the decapeptide angiotensin I (92). Renin attacks all forms of renin substrate at similar rates, and all yield the same angiotensin I molecule (92,91). This reaction occurs pedominantly in circulating blood. In normal human blood the enzyme kinetics appear to be first order in that the velocity of the renin-substrate reaction is dependent on substrate concentration (42). The pH optimum of this reaction is species dependent, but is between pH 5.5 and 6.5 for the reaction of human renin with human substrate (72). The existence of activators or inhibitors in plasma that can modify the activity of the enzyme, renin, are very doubtful (76).

ANGIOTENSIN I

Angiotensin I is a decapeptide hormone with the following amino-acid sequence
ASP-ARG-VAL-TYR-ILE-HIS-PRO-PHE-HIS-LEU (57), and is the product of the reaction of renin with renin substrate. Angiotensin I is physiologically inactive and it can be considered a prohormone which serves as a substrate to converting enzyme to yield angiotensin II.

CONVERTING ENZYME

Converting enzyme, a dipeptidylcarboxypeptidase, reacts specifically with its substrate, angiotensin I, to remove the terminal histidyl-leucine group (57), and leave the physiologically active octapeptide, angiotensin II. Most of this conversion takes place very rapidly in the lungs where the concentration of converting enzyme is exceedingly high (78), and to a lesser extent in circulating blood, kidney and other organ vascular beds (2).

ANGIOTENSIN II

Angiotensin II is the principal effector hormone of the renin-angiotensin system. It is an octapeptide which has the amino-acid sequence ASP-ARG-VAL-TYR-ILE-HIS-PRO-PHE (57), and is the product of the reaction of angiotensin I with converting enzyme. The important actions of angiotensin II are the direct vasoconstrictor effect, the regulation of aldosterone production, the indirect pressor effect via the central and sympathetic nervous systems and the alteration in renal function.

Angiotensin II is the most powerful naturally occurring vasoconstrictor substance known, and acts directly on the peripheral arteriolar or precapillary vessels. The pressor responsiveness to angiotensin depends on various factors including vascular tone, fluid balance, nervous stimulation and drugs. It has been shown that the infusion of relatively

small doses of angiotensin II into man frequently produces a progressive rise in blood pressure (3). Physiologically, angiotensin II plays a major role in maintaining circulating homeostasis in normal animals through its direct effects on arteriolar smooth muscle and its aldosterone-stimulating effects.

Angiotensin II is a potent stimulus for adrenal cortical secretion of aldosterone (3). It is uncertain where in the biosynthetic pathway for aldosterone that angiotensin exerts this aldosterone-stimulating action. Through aldosterone, angiotensin II regulates salt and water balance.

Angiotensin acts on the autonomic and central nervous systems to produce indirect vasoconstrictor effects (30). Although angiotensin has been shown to interact with hormones of the autonomic nervous system, the contribution of the autonomic nervous system to the action of angiotensin remains unclear. Various sites in the central nervous system have been demonstrated which, when stimulated, alter vascular tone (71,115), but it seems likely that these effects are mediated by the sympathetic nervous system.

The kidney is acted upon by angiotensin II to produce various changes in renal function. It is a very powerful renal vasoconstrictor resulting in a decrease in renal blood flow and a lesser reduction in glomerular filtration rate (34). Angiotensin II has variable effects on sodium and water excretion: lower doses produce sodium retention and higher doses natriuresis (58).

Angiotensin II receptors have been proposed as mediators of the biological response of the hormone (38). However, there is doubt about the physiological relevance of these receptor sites (29), and positive identification awaits firmer demonstration of a relationship between angiotensin binding and a metabolic response.

The biological half-life of angiotensin II is very short (about 15-20 secs.) (55). The hormone being removed from the circulation by enzymatic digestion by angiotensinases.

ANGIOTENSINASES

Plasma and tissue peptidases capable of destroying or inactivating angiotensin have been called angiotensinases. Tissue binding and removal mechanisms perhaps involving cellular angiotensinases are the main route of angiotensin II metabolism (7) and the inactivation in circulating blood is probably less important.

THE RENIN-ANGIOTENSIN SYSTEM IN ESSENTIAL HYPERTENSION

Investigators have sought humoral agents in the causation of hypertension for many years. One of the first reports of such work was in 1936 when Pickering transfused blood from hypertensive patients to normal controls, but was unable to produce a pressor response (73). However, angiotensin was eventually incriminated in the pathogenesis of hypertension by Skeggs and co-workers who reported a great increase in this humoral agent in patients with malignant hypertension, but the levels in essential hypertension overlapped with a group of normal people (89). Much work and controversy concerning renin in various forms of hypertension followed, but most of the explanation for this confusion appears to have been due to inadequate methods of measurement of renin and angiotensin, and the heterogeneous groups of patients studied.

However, derangements of the renin-angiotensin system were confirmed in malignant hypertension (54), primary hyperaldosteronism (24) and renovascular hypertension (33). These disorders are some of the more dramatic syndromes in which abnormalities of renin are easily detected, but together they comprise only a small proportion of the large hypertensive population. This lead to the question of whether more subtle abnormalities of the renin-angiotensin system are involved in the large population of hypertensive subjects called essential hypertensives. The recent availability of reliable

methods for the measurement of renin and angiotensin have made it possible to check whether this system is normal in uncomplicated essential hypertension.

In 1972 Laragh and co-workers introduced a new approach to assessing the RAS by constructing an index of normality whose continuity would reflect the linear responsiveness of this system (16). By subjecting normal volunteers to various levels of salt balance they were able to construct nomograms giving normal values for renin activity against 24 hour urinary sodium excretion over a wide range of physiological variation. They found that the scatter of renin values was considerably wider for essential hypertensive subjects than for the normal volunteers, permitting their classification into high, normal and low categories. In their series, 27 percent of patients showed reduced plasma renin activity (low renin hypertension), in 16 percent this parameter was abnormally high (high renin hypertension) and in the remaining 57 percent it fell within the normal range. Support for this classification of essential hypertensive patients into renin subgroups has come from pathophysiological and epidemiological evidence (17), from studies of the natural history of the condition with consideration of renin as a risk factor (16,18), from suggestions that the aetiologies of the subgroups may be different (93) and from work suggesting that renin levels may dictate the response to hypotensive therapy (1,20). Overall, this approach to renin in essential

hypertension and its implications are very controversial, but have stimulated much research, particularly in the field of 'low-renin hypertension'.

LOW-RENIN HYPERTENSION

In 1964, Conn and co-workers showed that plasma renin is suppressed in primary hyperaldosteronism and suggested the measurement of renin as an index of this syndrome (23). It was soon realised and originally reported by Helmer that many of these patients with low plasma renin levels had normal aldosterone secretion and essential hypertension (48). Thus the concept of 'low-renin hypertension' came into being, and since then many cases have been reported.

The existence of low-renin hypertension as a distinct disease entity with an aetiology different from essential hypertension has been questioned in an excellent review of this subject by Dunn and Tannen (32). Much of the conflicting data in this field can be accounted for by the lack of a universally accepted definition of what low-renin hypertension constitutes. The two criteria most frequently used in defining the condition are the absolute renin levels and the responsiveness to a renin elevating stimulus: in essential hypertension a renin value or response below some arbitrary range is usually designated low-renin hypertension. Therefore, to decide whether or not low-renin hypertension represents a distinct disease entity requires the consideration of the absolute renin levels and the renin responsiveness.

The low salt diet has become the 'standard' stimulus to renin release (32), but is unpractical as an out-patient test for large numbers of patients. A variety of other stimuli including frusemide have been used to assess renin responsiveness. Most workers using frusemide as a renin stimulant have given it orally (51a,22a,105a,31a,113) while others have injected it intravenously (17,31,67,1a). The results are conflicting as to the value of frusemide in separating a low-renin subgroup of essential hypertension. The confusion over the use of frusemide can be largely accounted for by the differences in the route of administration, the dosage, the time of sampling, and the posture at sampling. However, recently, Kaplan et al (52a) using intravenous frusemide (40 mg) and sampling at 30 and 60 minutes in the upright posture have shown close correlations between the post-frusemide plasma renin activity and the PRA measured after a low salt diet and have shown these two methods of renin stimulation to identify the same group of 'low-renin patients'.

In this study, intravenous frusemide was used as a rapid, simple and safe out-patient test of renin responsiveness. To achieve adequate stimulation, larger doses of frusemide than in most previous work (17,31,67,52a) were given (1 mg/kg body weight), and blood samples were taken in the upright posture. The effectiveness of this regime and the optimal time of blood sampling for PRA were confirmed in a pilot study. The renin responses in over 85 per cent of subjects were found to be maximal one hour after intravenous frusemide, and this time interval was adopted in all further renin investigations.

The mechanism of frusemide induced renin release is unclear. The work of Meyer et al (61a), Vander et al (103a)

and Baillie et al (4a) suggests an intrarenal mechanism unrelated to fluid volume or to serum sodium, but possibly dependent on sodium changes within the renal tubules or macula densa cells.

Apart from questioning the existence of low-renin hypertension and considering the multiple definitions of what different workers mean by the term, other inadequacies appear in much of the published work in this field. Variations in the methods of measurement of renin and angiotensin occur and limitations of some of the commonly used methods have been reported by Sealey and Laragh (86). The conditions of sampling are important: blood specimens for renin measurement may be taken at varying intervals after lying, standing or stimulation, at varying times of the day, in fasting conditions or completely randomly in different people in the same series. The stimuli, used to confirm that the low renin levels in these patients fail to respond when challenged, are many and variable (32), and it is uncertain not only whether they are comparable but also whether they identify the same group of non-responders. Laragh's group have adopted a new approach, as discussed above, by suggesting the evaluation of renin on random sodium intake in relationship to urine sodium excretion on the assumption that this reflects dietary sodium intake (16). Primary aldosteronism as a cause of the low and unresponsive renins in these patients needs to be excluded by the measurement of aldosterone levels, to fulfil the 'definition' of low-renin hypertension. The reproducibility in defining the low-renin state has received little attention, as it has been shown that a

significant proportion of patients on retesting do not have reproducible profiles (16,25). Renin values are influenced inversely by age, with a preponderance of low-renin hypertensives among older patients (102). Blood pressure itself has a direct effect on renin secretion (60), and we have demonstrated a significant inverse relationship between diastolic blood pressure and plasma renin activity. Previous drug therapy has been shown to alter the renin profile (59,4), and lead to patients being wrongly grouped as low-renin hypertensives. Sex and race need to be taken into account, since it has been reported that females and black patients have a greater incidence of low-renin hypertension. A consideration of these factors in future work in this field should lead to a better understanding of low-renin hypertension.

The adherents to the belief in low-renin hypertension as a distinct entity have suggested numerous aetiological mechanisms (32). However, most attention has been focused on a volume-expansion type of hypertension due to the increased secretion of some unidentified mineralocorticoid (93,87). The idea arose from the finding of hypertension and suppressed plasma renin in primary aldosteronism, and it was thought that low-renin hypertension represented an analogous condition with a mineralocorticoid, other than aldosterone, responsible. To date, no convincing evidence of volume expansion has been produced; rather, careful studies of plasma volume,

extracellular fluid and exchangeable sodium spaces have shown these parameters to be similar in all essential hypertensives (83,56). Support for a volume-expansion type of hypertension has come from the consistent observation that low-renin hypertensive patients respond to diuretic agents with a significantly greater antihypertensive effect than normal-renin hypertensives (1). Many mineralocorticoids have been proposed in the aetiology of low-renin hypertension, but have not been supported by further study and direct identification. Failing direct identification the most impressive support for mineralocorticoid involvement would be a favourable response to adrenalectomy. The finding of adrenal pathology and the response to surgery have, in general, been variable. Gunnells and co-workers (44) showed a favourable response to surgery in their low-renin patients, but this was a heterogeneous group of hypertensives including patients with primary aldosteronism. The favourable blood pressure response in the low-renin group to spironolactone (peripheral mineralocorticoid antagonist) and amino-glutethamide (inhibitor of mineralocorticoid synthesis) have been interpreted as support for an aetiological role of a mineralocorticoid, but the similar response to other diuretic agents opposes this (93,1,112). Further evidence against the unidentified mineralocorticoid theory in low-renin hypertension, includes the normal potassium status (16,93) and normal aldosterone secretion (or excretion) (1,112), which

should be suppressed by the mineralocorticoid induced volume expansion. Thus, although much of the evidence points against mineralocorticoid involvement in low-renin hypertension, the issue remains unsettled.

Interest in the measurement of plasma renin activity in essential hypertension has been stimulated by a recent claim that those patients with low-renin hypertension were less at risk from cerebrovascular accidents and myocardial infarcts, than patients in the normal and high-renin subgroups (16,18). This aroused considerable controversy with most other workers not being able to substantiate these findings (32). Brunner and Laragh have partially withdrawn their stand by no longer claiming a reduced incidence of cerebrovascular accidents in this condition (19). It is clear that the prognostic significance of renin measurements in patients with essential hypertension will only be resolved when large, carefully controlled prospective studies are completed, but at present the association between renin and prognosis is doubtful.

Diuretics appear to be especially useful as antihypertensive agents in those patients with essential hypertension who exhibit low plasma renin levels (1), but the mechanism of their favourable effect is uncertain. Conversely, Buhler et al have found that the blood pressure decrement after propranolol therapy to be least in the low-renin group and greatest in the high-renin group (20). The

significance of these findings are that they may provide useful guidelines in planning treatment.

Overall, the field of low-renin hypertension is a much researched and controversial one from which positive answers are still awaited.

Doubt pervades the whole subject of renin in essential hypertension: the role of renin is uncertain; the value of the division in renin subgroups is uncertain; the basis for any such division is uncertain. But promises of the establishment of low-renin hypertension as a definite entity, and of the confirmation of renin as a guide to prognosis and therapy encourage researchers to continue in this confused field.

AIMS OF THIS WORK

This work was undertaken to study the renin-angiotensin system in patients with uncomplicated essential hypertension. Great care was taken to avoid some of the inadequacies in published work, which have been discussed above.

The first problem was to decide whether or not the renin-angiotensin system was 'normal' in essential hypertension. To answer this, patients with previously untreated essential hypertension were compared with age and sex matched controls, in order to detect differences in basal and stimulated renin values.

Are patients with essential hypertension a homogeneous group, or can they be meaningfully subgrouped according to renin values? This question is tackled by a consideration of the distributions of the renin values and the bases of previous such divisions.

A detailed look is taken at 'low-renin hypertension'. The responsiveness of this subgroup to renin elevating stimuli is contrasted with that of other patients with essential hypertension. The possibility of mineralocorticoid involvement in the aetiology of this condition was studied by measurement of total body electrolytes and fluid volumes.

Finally, the effects on plasma renin of age, sex, blood pressure, sodium, potassium and fluid volumes were studied. By defining these effects, a more meaningful interpretation can be placed on isolated renin measurements.

PART II

METHODS

The succeeding chapters deal with the screening, clinical and laboratory methods that were used in this study. The measurement of plasma renin activity has been described in considerable detail to emphasise the complexity of the assay and the care taken to achieve accurate results.

CHAPTER 2

SCREENING AND CLINICAL METHODS

SCREENING FOR HYPERTENSION

In this study of the renin-angiotensin system in hypertension, large numbers of previously untreated essential hypertensive patients were required. Because hypertension is usually symptomless, a form of population screening was necessary to realise these numbers.

It was decided to study the employees at U.K. Atomic Energy Research Establishment (Harwell) and associated laboratories (7,587 people), and the people in the general practices at Wantage (7,000 town patients) and Didcot (10,000 patients). An invitation was sent to all of these people, between the ages of 21-70 years, to attend a blood pressure screening session.

The blood pressure was measured using the Random-Zero sphygmomanometer (114) while the patient was seated and at rest. Diastolic blood pressure was taken at the disappearance of the fifth Korotkoff sound. If the diastolic blood pressure was greater than 100 mm Hg the patient was seen twice more at weekly intervals, and the blood pressure on these occasions was measured using the London School of Hygiene and Tropical Medicine sphygmomanometer (77). The blood pressures were measured by the same team of workers at each screening session.

Those people whose diastolic pressure was persistently greater than 100 mm Hg were considered to have hypertension

and were eligible for inclusion. However, patients were excluded if their systolic pressure exceeded 220 mm Hg, their diastolic pressure exceeded 130 mm Hg, they had ever received any form of hypotensive therapy, they were taking any type of medication, they had any complication of hypertension, or if they were unwilling to co-operate in the research programme. Thus the sample represented mild, uncomplicated and untreated essential hypertension.

CONTROL SUBJECTS

To contrast the effects of hypertension on the renin-angiotensin system and to establish the norm, a group of control subjects from the same screening population was chosen for study.

The control subjects had diastolic blood pressures taken, under the same conditions as the hypertensives, on two occasions at least one week apart, of less than 90 mm Hg. They were not suffering from any known disease nor were they taking any form of medication. One control subject was matched for age and sex with each hypertensive patient.

HISTORY, EXAMINATION AND ROUTINE INVESTIGATIONS

A full history, medical examination and routine investigations were undertaken on all hypertensives and controls. The history included personal details, a

systemic enquiry, past medical history, obstetric details, family history and drug enquiry. All systems were thoroughly examined. The routine investigations covered: urine - microscopy, chemistry, culture, electrolyte estimation, creatinine clearance, and measurement of vanilyl-mandelic acid; blood - full blood count and measurement of urea, electrolytes and S.M.A. profile; radiology - chest x-ray and intravenous pyelography (hypertensives only); and electrocardiography.

Secondary causes and complications of hypertension and any other occult disease processes in hypertensives or controls were detected and those affected were withdrawn.

RENIN INVESTIGATIONS

The renin investigations on all patients and control subjects took place in the medical centre at A.E.R.E. Harwell. Subjects with hypertension and controls arrived at 08.45 hours and after emptying their bladders they rested supine from 09.00 to 11.00 hours. At 11.00 hours venous blood was taken for the measurement of plasma renin activity (supine PRA). For the next two hours subjects remained upright and were allowed to walk about. At 13.00 hours blood was taken (erect PRA) and frusemide (1 mg/kg body weight) was injected intravenously. At 14.00 hours blood was again taken (post-frusemide PRA) and the subjects were allowed to leave.

Plasma aldosterone was measured in blood taken at 11.00 hours after resting supine for 2 hours.

All subjects collected their urine during the 24 hours preceding the renin investigations for the measurement of sodium and potassium.

RADIO-ISOTOPIC INVESTIGATIONS

All subjects underwent a series of radio-isotopic tests. Total body potassium, total exchangeable sodium, and plasma volume were measured on the same day, and within three weeks of the renin investigations.

ETHICS AND CONSENT

The protocol of this study was approved by the Ethics Committees of the Radcliffe Infirmary, U.K.A.E.R.E. (Harwell), and the Medical Research Council.

Fully informed consent was obtained from both hypertensive and control subjects.

CHAPTER 3

LABORATORY METHODS AND MATERIALS

PLASMA RENIN ACTIVITY (PRA)

Renin cannot be measured directly, but its activity can be estimated by assay of the rate of formation of angiotensin I from the reaction of renin with renin substrate. The angiotensin I, generated during a controlled incubation of plasma, is quantitated by radioimmunoassay. During the incubation procedure the inhibition of converting enzyme prevents the conversion of angiotensin I to angiotensin II and the inhibition of angiotensinases prevents the destruction of formed angiotensin I. This measurement is referred to as plasma renin activity and not plasma renin concentration, as the capacity of the plasma to generate angiotensin I is dependent on the concentrations of both renin and renin substrate. This indirect method is an accurate and sensitive way of assessing the renin-angiotensin system.

The method of assay used was that reported originally by Sealey, Gerten-Banes and Laragh (85), but in view of its complexity and the difficulty in obtaining accurate, reproducible results, it is described in detail.

MATERIALS

(1) 0.1M TRIS BUFFER pH 7.5:

This was prepared by dissolving 12.114 gms TRIS (HYDROXYMETHYL)METHYLAMINE (BDH chemicals), 3.0 gms lysozyme

(Sigma Chemical Co.), 3.0 gms bovine serum albumin (fraction V, Armour), 35 mg phenylmercuric acetate (Sigma Chemical Co.) and 2 gms neomycin sulphate (Burroughs Wellcome) in distilled water, mixing, adjusting the pH to 7.5 with glacial acetic acid, making the volume up to one litre with distilled water, filtering twice through a Bruchner funnel and storing cold in siliconised sterile bottles. This Tris buffer was used as diluent throughout the radioimmunoassay procedure. The albumin was added to the buffer to inhibit absorption of angiotensin onto glassware and plastic, and bacterial growth was retarded by the addition of neomycin and phenylmercuric acetate.

(2) ANTIBODY:

The specific angiotensin I antibody was supplied by Professor John Laragh, New York, and the same stock serum was used throughout the renin assays. Approximately each month 50 µl of the stock serum was diluted 100 times with Tris buffer, divided into aliquots of about 300 µl and stored in small siliconised ampoules at -20°C. One of these 1/100 dilution aliquots was used per assay, and the unused contents discarded. A final antibody concentration of 1/100,000 was used - for the usual 120 tube (60 pairs) assay 250 µl 1/100 antibody was added to 250 mls of ^{125}I Angiotensin I-Tris mixture.

(3) ASP-1-ILEU-5-ANGIOTENSIN I: (Beckman)

The standard angiotensin I was supplied freeze-dried in vials of 1 mg, was claimed to be 100% pure, and was stored at 4°C. The content of the vial was reconstituted in 20 mls of sterile distilled water containing 0.2% neomycin sulphate, giving a concentration of 50 µg/ml. This solution was divided into 2 ml aliquots and stored in sterile siliconised bottles at -20°C. Once a month a 50 µg/ml bottle was thawed and a 10 µg/ml solution was made up using 1 ml of the 50 µg/ml solution and 4 mls Tris buffer. The remaining 50 µg/ml solution was discarded. The 10 µg/ml solution was divided into 200 µl aliquots and stored at -20°C. One of these aliquots was used per assay and the unused contents thrown away. Each new batch of 10 µg/ml aliquots was checked against the previous batch in an assay before the new batch came into general use. On the day of the radioimmunoassay an aliquot of the 10 µg/ml solution was thawed and diluted to 1, 4, 15 and 20 ng/ml in Tris buffer, and the remains of these dilute solutions after assay were discarded.

(4) ^{125}I-ANGIOTENSIN I: (Lepetit)

The labelled angiotensin I was supplied freeze-dried in bottles (1 µCi on calibration date) and was claimed to be purified, free of fragments and di-iodinated molecules. These bottles were stored at 4°C and were reconstituted with 10 mls sterile distilled water. This solution was stored at 4°C and used within one week of reconstitution. The volume of

this solution required for an assay varied according to the strength (age) of the label. For a normal 120 tube (60 pairs) assay, between 2.5 and 4 ml of labelled angiotensin I solution were used, and this was made up to 260 mls with Tris buffer.

(5) ^{125}I-ANGIOTENSIN-ANTIBODY MIXTURE:

Antibody dilution curves were plotted with each new batch of labelled angiotensin I to ensure that the antibody dilution of 1/100,000 binds approximately 50 percent of the radioactive angiotensin.

The antibody dilution experiment was performed by making up 30 mls of Tris/radioactive angiotensin mixture, (0.3 mls of new labelled-angiotensin was added to 29.7 mls Tris), and leaving to stir in an icebath. A stock ampoule of 1/100 antibody was diluted 100 times (200 µl 1/100 antibody was added to 1.8 mls Tris) to give a 1/1000 antibody dilution. 100 µl 1/1000 antibody was added to each of six tubes (labelled a to f) containing increasing amounts of Tris (Table 1), mixed thoroughly and 200 µl transferred from each tube into a corresponding series of duplicate tubes A,A^1 to F,F^1. 200 µl Tris was added to each of two tubes, X,X^1, in lieu of antibody solution. 1.8 mls Tris/radioactive angiotensin mixture was added to each tube in the series (A,A^1 to F,F^1 and X,X^1), mixed thoroughly and left in a refrigerator at 4°C for 18 hours. After equilibration 500 µl dextran-coated charcoal was added to each tube except tubes X and X^1

TABLE 1. ANTIBODY DILUTION EXPERIMENT

1st Series

Tube	Volume Tris (µl)	Volume 1/1000 Antibody (µl)	Concentration of Antibody
a	300	100	1/4,000
b	500	100	1/6,000
c	700	100	1/8,000
d	900	100	1/10,000
e	1,100	100	1/12,000
f	1,300	100	1/14,000

2nd Series

Tubes	Volume Added to each Tube (from 1st Series) (µl)	Volume Tris/ ^{125}I Angiotensin (ml)	Charcoal (µl)	Final Concentration Antibody	Comments
A,A[1]	200	1.8	500	1/40,000	
B,B[1]	200	1.8	500	1/60,000	
C,C[1]	200	1.8	500	1/80,000	
D,D[1]	200	1.8	500	1/100,000	
E,E[1]	200	1.8	500	1/120,000	
F,F[1]	200	1.8	500	1/140,000	
X,X[1]	(200 µl Tris)	1.8	(500 µl Tris)	-	Total counts

which are the total counts tubes. (500 µl Tris was added to tubes X and X^1 in lieu of charcoal.) Tubes were then centrifuged at 2000 rpm for 20 minutes at $4^{\circ}C$, supernatants decanted into labelled tubes and counted with tubes X and X^1, and a background tube (2.5 mls Tris) for 10 minutes each in a gamma counter. Radioactivity was measured in counts per minute, the background was subtracted and each result was expressed as a percentage of the mean total counts (tubes X,X^1). This should result in 1/100,000 antibody dilution (tubes D and D^1) giving around 50% binding. These results were plotted graphically. Each new batch of radioactive angiotensin I was checked in this way.

For the usual 120 tube (60 pairs) assay, 2.5 to 4.0 mls of the ^{125}I-angiotensin solution was made up to 260 mls with Tris buffer, and 250 µl 1/100 antibody was added to 250 mls of this mixture to give a final antibody dilution of 1/100,000, and approximately 50% binding of the labelled angiotensin to the antibody.

(6) DEXTRAN-COATED CHARCOAL:

A suspension was prepared by adding 1.0 gm charcoal (carbon decolorizing purified, Norit SX-1, Hopkin and Williams) and 0.1 gm dextran (T70, Pharmacia) to 100 mls chilled, distilled water mixed thoroughly with a magnetic stirrer in an icebath for about ½ hour before use. This suspension was prepared fresh for each assay. Each new batch of charcoal was checked for the optimum amount of charcoal that would give

a non-specific binding, of labelled angiotensin to protein in the Tris buffer, of less than 5 percent, but not so much that there was a large difference in counts when the charcoal was added 5 minutes apart to the duplicates of the standard curve.

(7) DIISOPROPYLFLUOROPHOSPHATE (DFP): (Fluorochem)

This inhibitor was diluted 1/20 with isopropyl alcohol and stored at $4^{o}C$ in a sealed container. Because of its extreme toxicity all handling of DFP took place with gloved hands, in a fume cupboard and with 5N sodium hydroxide readily available to neutralise any contamination.

(8) NEOMYCIN SULPHATE: (Burroughs-Wellcome)

Neomycin sulphate was supplied as a 10% solution which was stored at $4^{o}C$ and filtered before use. A new supply was obtained each month as its anti-bacterial activity waned.

(9) ETHYLENEDIAMINETETRAACETIC ACID (EDTA):

Blood specimens were taken directly into Vacutainers (Becton Dickinson, No. 4794) containing 0.17 ml of 15 percent potassium EDTA. This gave a final EDTA concentration of 3 mM in the blood specimen.

(10) HYDROCHLORIC ACID (Hcl): (BDH Chemicals)

Chilled Hcl (1N, 0.5N and 0.1N) was added dropwise through a blunt 19 gauge needle on a 1 ml syringe to the incubation

mixture to adjust the pH to 5.7. The smallest possible volume was used, noted and taken into account as a dilution factor during the calculations.

(11) STANDARD PLASMAS:

A large volume of normal human blood (±500 ml) was taken into heparin and the plasma separated and treated with an excess of charcoal to remove all the endogenous angiotensin. The charcoal was removed by cold centrifugation and filtration through glass wool. The plasma was diluted 1:4 with Tris buffer, EDTA added to give a final concentration of 3 mM, 0.1 gm neomycin sulphate (10%) added per 100 mls of plasma, and 1 ml DFP (1/20 dilution in isopropyl alcohol) per 100 mls of plasma was added, and mixed thoroughly at 4°C. This mixture was divided into two lots and synthetic angiotensin I added to give a high (12.3 ng/ml) and a low (4.3 ng/ml) standard plasma. Large numbers of 200 µl aliquots of each standard were ampouled and the stock solution and ampoules stored at -20°C. As supply demanded the stock standard plasmas were thawed in ice and more aliquots ampouled. One ampoule of each plasma was used per assay, and the unused contents were discarded.

(12) TEST TUBES:

For incubation and radioimmunoassay, 10 x 44 mm plastic tubes (Luckhams, No. PT/0944) were used.

(13) pH METER: (Electronic Instruments, No. 7020)

(14) SHAKER WATER BATH: (Gallenkamp, No. 1H 350)

(15) DEEP FREEZER: (Ace)

(16) DILUTER:

An automated pipetting station (Micromedic Model No. 25006) was used.

(17) REFRIGERATED CENTRIFUGE: (MSE, model MISTRAL 4L; head No. 34123-602; metal support and 24 place twinblock No. 43155-101/104).

(18) GAMMA COUNTER: (Packard Instruments, Model No. 5320)

(19) DISPENSING PIPETTES: (Oxford Instruments)

(20) NEEDLES:

The needles used were 19 gauge, 1½ inch, point No. 40/11 (Becton-Dickinson, No. 05187-0-11).

COLLECTION OF BLOOD

Blood was collected, as a sterile procedure, into chilled 20 ml EDTA Vacutainers, which were inverted to mix the contents and placed into crushed ice. The blood or plasma was never

allowed to reach room temperature after this time. After centrifugation at 2000 rpm at 4°C for 20 minutes, the plasma was separated and stored in plastic tubes at -20°C until incubation for generation of angiotensin I.

INCUBATION (Table 2)

Plasma was thawed in an icebath at 0°C, and 2 mls from each sample was accurately transferred under sterile conditions to another clean, sterile plastic tube for incubation. EDTA was used as the anticoagulant, and in the plasma the concentration was at least 3 mM, sufficient to completely inhibit converting enzyme and some angiotensinases (65). DFP was added to this mixture to inhibit the other angiotensinases (72), using a 1 ml syringe with a 19 gauge blunt needle to deliver 2 drops (±40 µl). To inhibit bacterial growth during incubation, 40 µl of neomycin sulphate (10%) were added, and thoroughly mixed. The pH was adjusted to 5.7 with drops (from 19 gauge blunt needle on a 1 ml syringe) of 1N, 0.5N or 0.1N hydrochloric acid. The minimum volume of acid possible was added and the volume recorded. The plasma was mixed between each addition of acid and the pH meter was checked for drift by frequent reference to chilled pH 7.03 buffer. The plasma was divided into 3 aliquots using sterile siliconised pasteur pipettes. One aliquot was kept as a blank, i.e. frozen without incubation, and the other two aliquots were incubated in a shaker waterbath at 37°C, one

TABLE 2. INCUBATION FOR GENERATION OF ANGIOTENSIN I

2 ml plasma containing 3 mM EDTA

40 µl 10 percent neomycin sulphate

2 drops DFP (1/20 dilution in isopropyl alcohol)

Hcl (1N, 0.5N, 0.1N) to pH 5.7

3 and 16 hours incubation at 37°C

Freeze

Radioimmunoassay

for 3 hours, and the other for 16 hours. Linear generation of angiotensin I was demonstrated for up to 20 hours of incubation. After incubation the reaction was stopped by rapid freezing and the specimen stored at -20°C until the angiotensin generated was quantitated by radioimmunoassay.

ANGIOTENSIN I RADIOIMMUNOASSAY

Principles of Radioimmunoassay (6): Angiotensin I was measured by utilizing (a) its affinity for highly specific antibodies, and (b) competition between radioactive and unlabelled angiotensin for binding sites on the antibodies. The angiotensin to be quantitated was added to a mixture of radioactive angiotensin and antibody in proportions such that half of the radioactive angiotensin was bound to the antibody and the other half remained free. The extent to which radioactive angiotensin I was displaced from the antibody by the unknown sample was a measure of the amount of angiotensin in the sample. The exact amount of angiotensin I in the unknown sample was determined by comparing the displacement caused by addition of known unlabelled angiotensin I standards.

Throughout the assay all glassware and tubes were sterile and siliconised, and all solutions were kept chilled. For a standard assay of 120 tubes (60 pairs), 2.5 to 4 mls ^{125}I-angiotensin I (depending on strength/age of label) were made up to 260 mls with Tris buffer in a conical flask and left mixing in an icebath. From this mixture 10 mls were removed for measurement of nonspecific binding of angiotensin to

proteins in the buffer solution. The appropriate amount of antibody was added to the Tris/labelled angiotensin mixture - normally that which gave a final antibody dilution of 1/100,000 and enough to combine with about 50 percent of the radioactive angiotensin. For a standard 120 (60 pairs) tube assay, 250 µl 1/100 antibody was added to the mixture, which was left mixing in an icebath for 15 minutes before use. The standard angiotensin solution (10 µg/ml) was diluted on the day of the assay to 100 ng/ml and the standards used in the radioimmunoassay (1, 4, 15, 20 ng/ml) were prepared using serial dilution (Table 3). The standard plasmas (an ampoule of the high and low standard) and the plasmas which had previously been incubated were thawed in preparation for assay. (Before radioimmunoassay some samples were diluted in Tris buffer when the renin value was expected to be very high, but for most samples no predilution was necessary.)

The standard curve (Table 4) was set up to include 10 tubes in duplicate. The first seven tubes in duplicate (1,1A to 7,7A) contained different amounts of angiotensin I standard. The remaining tubes measured total counts (8,8A), the nonspecific binding of angiotensin to protein in the buffer solution (9,9A) and the percentage binding of radioactive angiotensin to the antibody (10,10A). The next four tubes in duplicate (11,11A to 14,14A) were for different volumes (10 and 20 µl) of the high and low standard plasmas (Table 5). The remaining tubes (15,15A to 60,60A) contained the unknown plasma samples. For each such specimen 3 tubes

TABLE 3. DILUTION OF STANDARD ANGIOTENSIN SOLUTIONS FOR RADIOIMMUNOASSAY

Volume Added	of Stock Solution	+ Volume of Tris		Gives Final Concentration of Standard Solution
0.1 ml	10 µg/ml	+ 0.9 ml	=	100 ng/ml
2.0 ml	100 ng/ml	+ 8.0 ml	=	20 ng/ml
3.0 ml	20 ng/ml	+ 1.0 ml	=	15 ng/ml
1.0 ml	20 ng/ml	+ 4.0 ml	=	4 ng/ml
1.0 ml	4 ng/ml	+ 3.0 ml	=	1 ng/ml

TABLE 4. STANDARD CURVE IN RADIOIMMUNOASSAY

Tube No.	Angiotensin Concentration (ng/ml)	Volume Added (µl)	Angiotensin (ng)	Buffer + ^{125}I-Angiotensin (ml)	Buffer + Antibody + ^{125}I-Angiotensin (ml)	Charcoal (ml)	Comment
1,1A	1	10	.01	-	2	0.5	
2,2A	1	20	.02	-	2	0.5	
3,3A	4	10	.04	-	2	0.5	
4,4A	4	20	.08	-	2	0.5	
5,5A	15	10	.15	-	2	0.5	
6,6A	15	20	.30	-	2	0.5	
7,7A	20	20	.40	-	2	0.5	
8,8A	-	-	-	-	2	-*	Total Counts
9,9A	-	-	-	2	-	0.5	Nonspecific binding
10,10A	-	-	-	-	2	0.5	Zero Standard

*0.5 ml Tris buffer added in lieu of charcoal

TABLE 5. STANDARD PLASMAS AND UNKNOWN PLASMAS IN RADIOIMMUNOASSAY

Tube No.	Standard Plasma	Unknown Plasma	Volume (µl)	Buffer + Antibody + ^{125}I-Angiotensin (ml)	Charcoal (ml)	Comment
11,11A	Low	-	10	2	0.5	
12,12A	Low	-	20	2	0.5	
13,13A	High	1	10	2	0.5	
14,14A	High	1	20	2	0.5	
15	-	x (blank)	10	2	0.5	Repeated for each unknown in assay
15A	-	x (blank)	20	2	0.5	
16,16A	-	x (incubated)	10	2	0.5	
17,17A	-	x (incubated)	20	2	0.5	

in duplicate (e.g. 15,15A, 16,16A and 17,17A) were assayed - the first a blank measurement (unincubated) and the other two containing different volumes (10 and 20 µl) of the same specimen after incubation (Table 5).

The Micromedic diluter was used to dispense the various volumes required. Two millilitres of the radioactive angiotensin/antibody mixture was added to all tubes in the assay except tubes 9 and 9A, which contained 2 mls of labelled angiotensin/Tris mixture without the antibody. The Micromedic diluter was also used to dispense 10 and 20 µl volumes of the various solutions (standard angiotensin, standard plamsa, and unknown plasmas) into the tubes containing the 2 mls labelled angiotensin/antibody mixture, as set out in Tables 4 and 5. The pattern of tubes 15,15A to 17,17A was repeated for each unknown plasma being assayed. After all these additions the tubes were mixed and placed in a refrigerator at 4°C for 18 hours.

When blood had been taken from subjects in the supine position the specimen was incubated for 16 hours, but bloods taken erect or post-frusemide were assayed after 3 hours incubation, in the expectation of higher PRA values.

After 18 hours equilibration at 4°C the angiotensin bound to antibody was separated from free angiotensin by absorption of the free peptide onto dextran-coated charcoal. Tubes were removed from the refrigerator and placed into an icebath. From the continuously stirring charcoal mixture

500 µl was added to all tubes (except 8,8A, to which 500 µl Tris buffer were added to measure total counts), starting at 1 to 60, and ending with the duplicates 1A to 60A. This order was chosen to combat the slow tendency of charcoal to absorb small amounts of bound angiotensin. The charcoal step was performed as rapidly as possible, and the tubes then thoroughly mixed and centrifuged together at 2000 rpm for 20 minutes at $4^{o}C$. After centrifugation the supernatant (bound angiotensin) was decanted into labelled, fresh tubes, and counted in a gamma counter for 10 minutes per tube, using one tube containing 2.5 mls Tris buffer as the background.

Calculation of results (Table 6): The bound counts in each standard curve, standard plasma and unknown plasma sample were divided by the mean counts from the zero standard tubes 10 and 10A (B/B_O) and the logit Y was calculated from the formula $\frac{B/B_O}{1-B/B_O}$. The standard curve of the logit Y against ng angiotensin I was drawn (Figure 1) and the amount of angiotensin in each standard plasma and unknown sample was derived from the standard curve. The amount of angiotensin in each unknown sample was multiplied by the dilution factor (Chem DF) resulting from the addition of neomycin, DFP and acid to the specimen during incubation. The value was adjusted to ng/ml by multiplying the 10 µl specimens by 100 and the 20 µl specimens by 50 (RIA DF), and the mean of the duplicates calculated (Table 7). If the duplicates differed from the mean by less than 20%, the result was divided by the number of

Table 6

CALCULATION OF PLASMA RENIN ACTIVITY

Sample No.	Counts Bound (B) (CPM)	B/B_O	$\frac{B/B_O}{1-B/B_O}$ (=Y)	Angiotensin (ng)	Mean	Normal Range
	Standard Curve					
1	827	.939	15.39	0.01		
1A	837	.950	19.00	0.01		
2	787	.893	8.35	0.02		
2A	807	.916	10.90	0.02		
3	725	.823	4.65	0.04		
3A	739	.839	5.21	0.04		
4	597	.678	2.11	0.08		
4A	616	.699	2.32	0.08		
5	471	.535	1.15	0.15		
5A	483	.548	1.21	0.15		
6	329	.373	0.59	0.30		
6A	340	.386	0.63	0.30		
7	283	.321	0.47	0.40		
7A	283	.321	0.47	0.40		
8	1822	total counts				
8A	1797					
9	17	1.05% nonspecific binding				
9A	20					
10	873	48.76% binding (zero standard, B_O)				
10A	888					
	Standard Plasmas				Mean	Normal Range
11	724	.822	4.62	0.038	0.039	0.034-0.052
11A	721	.818	4.49	0.039		
12	632	.717	2.53	0.070	0.075	0.069-0.105
12A	611	.694	2.27	0.079		
13	568	.645	1.82	0.098	0.102	0.098-0.148
13A	554	.629	1.70	0.105		
14	407	.462	0.86	0.210	0.210	0.196-0.294
14A	407	.462	0.86	0.210		

Background 12

Note:
(i) Background is subtracted from all counts bound (B)
(ii) Angiotensin values of standard plasmas are read off graph (Figure 1) constructed from standard curve data
(iii) Normal range for standard plasmas is calculated from a previously determined mean $\pm$ 20 per cent.
(iv) The calculations for this Table are taken from an actual assay.

FIGURE 1

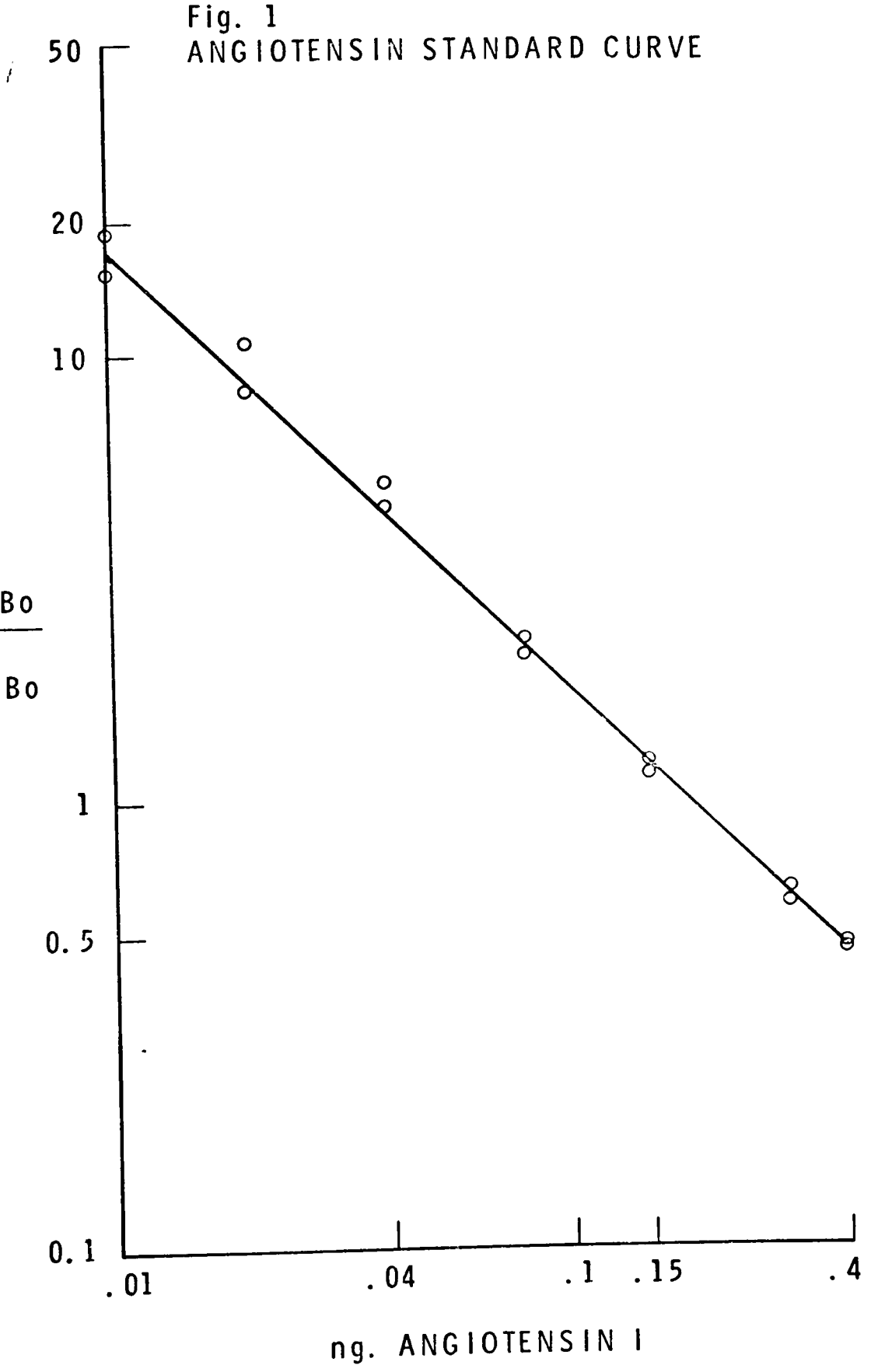


Fig. 1
ANGIOTENSIN STANDARD CURVE
50
20
10
1
0.5
0.1
B/Bo
1-B/Bo
.01
.04
.1
.15
.4
ng. ANGIOTENSIN I

TABLE 7. CALCULATION OF PLASMA RENIN ACTIVITY OF UNKNOWN PLASMAS

Sample No.	Counts Bound (B) (CPM)	B/B_O	$\frac{B/B_O}{1-B/B_O}$ (=Y)	Angiotensin (ng)	Chem DF	RIA DF	ng AI/ml Generated (3 or 16 hr)	Mean	Error (%)	PRA (ng AI/ml/hr)
15	869	.986	70.43	-	-	-	-	-	-	-
15A	862	.978	44.45	-	-	-	-	-	-	-
16	434	.493	0.97	0.187	1.10	100	20.57 (16 hr)		0.7	
16A	429	.487	0.95	0.192	1.10	100	21.12 (16 hr)	20.42	3.4	1.28
17	291	.330	0.49	0.375	1.10	50	20.63 (16 hr)		1.03	
17A	301	.342	0.52	0.352	1.10	50	19.36 (16 hr)		5.2	
18	863	.980	49.00	-	-	-	-	-	-	-
18A	844	.958	22.81	-	-	-	-	-	-	-
19	652	.740	2.85	0.063	1.09	100	6.87 (3 hr)		3.3	
19A	658	.747	2.95	0.061	1.09	100	6.65 (3 hr)	6.65	0	3.22
20	522	.593	1.46	0.124	1.09	50	6.76 (3 hr)		1.6	
20A	534	.606	1.54	0.116	1.09	50	6.32 (3 hr)		4.9	

Background 12

Note:
(i) Background is subtracted from all counts bound (B)
(ii) Angiotensin values are read off graph (Figure 1) constructed from standard curve (Table 6)
(iii) Chem. DF = dilution factor due to addition of DFP, neomycin and Hcl
(iv) RIA DF = dilution factor for volume of plasma added to radioimmunoassay
(v) Any PRA value less than 1 ng/ml/hr is repeated after a 16 hour incubation
(vi) The calculations in this Table are taken from an actual assay

hours of incubation to express the plasma renin activity as nanograms angiotensin generated per millilitre plasma per hour of incubation. If any PRA value was less than 1 ng/ml/hr then the specimens were reassayed after a 16 hour incubation. The 16 hour incubation enabled very low plasma renin activities to be measured: a plasma renin activity of 0.1 ng/ml/hr would lead to 1.6 ng of angiotensin I being generated in 16 hours, and this could easily and reproducibly be measured in the present assay. The situation of a plasma sample showing no detectable plasma renin activity has never occurred in our use of this assay technique.

Quality control: Continuous checks were made of the accuracy and reproducibility of the assay. If the duplicates varied by more than 20% the assay was repeated. The two standard plasmas were run with each assay (Table 6), and dilutions of these samples were such that they spanned the range of the standard curve (43,86,123 and 245 pg). If the result of either sample was outside $\pm$20% of a previously determined mean (low standard 10 µl range = 34-52 pg, low standard 20 µl range = 69-105 pg, high standard 10 µl range = 98-148 pg, high standard 20 µl range = 196-294 pg) the assay was repeated. The run was also discarded if the nonspecific binding (tubes 9 and 9A) rose above 5% of the total counts. The results were not accepted unless the duplicates of the unknown plasma samples varied by less than 20%, and both duplicates fell on the standard curve. The value of the blank was not subtracted from the results in the incubated specimens, but if it was more than 10% of the incubated value

the result was rejected and the specimen reincubated for 16 hours.

The calculation procedure was automated with the gamma counter information being recorded onto punch-tape which, when fed into a calculator, plotted the standard curve and calculated the plasma renin activity.

TOTAL BODY POTASSIUM

The measurement of total body potassium is limited by the complexity of the equipment required and very few centres are able to perform these measurements. Accordingly, the measurements in this study were undertaken by Dr. D. Newton and colleagues at U.K.A.E.R.E. (Harwell). A general description of the method used follows:

The estimation of whole-body potassium by body radioactivity measurement relies on the detection of gamma-rays emitted by ^{40}K (present in natural potassium to the extent of 0.0118%) with characteristic energy 1.46 MeV. The ^{40}K associated with a typical subject's potassium content of 140 grams emits approximately 30,000 quanta per minute.

DETECTION OF GAMMA RADIATION

Six scintillation counters, each comprising a 15 cm diameter x 9 cm thick thallium-activated sodium iodide crystal viewed by a 12.5 cm diameter photomultiplier tube, are arranged at intervals above and below the mid-line of the supine subject. The signals from these detectors are amplified and mixed, and their combined response is processed by a 256

channel pulse height analyser which displays it in the form of an energy spectrum covering the range 0.02 - 3.50 MeV. Only those quanta (about 1.6 percent of the total) which are emitted in the direction of a counter and which escape total absorption or energy degradation in the body are potentially useful, and, in practice, because only ~38 percent of these quanta deposit their full energy in the crystal, the overall efficiency of detection is reduced to ~0.6 percent. Thus, a subject containing 140 grams of potassium would give rise to ~180 counts per minute in the characteristic spectral peak at 1.46 MeV, and the ^{40}K is easily detected if adequate shielding (in this instance 10 cm lead) is provided to reduce the detector background (i.e., its response to ambient gamma-radiation) to levels comparable with or lower than this counting-rate.

CALIBRATION

A procedure is required to translate the response observed from the subject, after subtraction of the measured background counting-rate, into an estimate of his potassium content. The simplest approach is to fill a "phantom" (typically a series of polyethylene vessels of circular or elliptical cross-section which can be assembled to simulate the human form) with a solution containing a known amount of a potassium salt, and to measure the response from this phantom. This procedure may introduce a systematic error of as much as ± 10 percent,

depending on how well the phantom matches the subject's physique. This may arise because (i) the geometrical arrangement of activity in the subject relative to the detectors will not be identical with that in the phantom, and (ii) the subject may absorb more or fewer of the emitted quanta than does the phantom.

The use of other whole-body counter arrangements (e.g. those in which the supine subject is scanned from head to foot by a pair of vertically opposed detectors, or by a coplanar array of four or six detectors) would reduce variations in the geometry factor and might diminish the size of these errors, but all of these alternative procedures involve certain disadvantages in other respects, compared with an array of stationary detectors such as that employed at Harwell. Moreover, the use of more elaborate calibration techniques, involving the administration of a suitable radionuclide to the subject, can virtually eliminate the systematic error. The most appropriate nuclide is ^{42}K, whose decay with a half-life of 12.5 hours is accompanied by the emission of 1.52 MeV gamma-rays, i.e. radiation of energy very similar to that from ^{40}K. An ingested dose of ^{42}K equilibrates with the body's potassium within 12 hours, to an extent sufficient for these purposes, so that in its geometrical arrangement within the subject, and in the attenuation of its quanta in the body, it is virtually identical with the ^{40}K. Thus a body radioactivity measurement made 12 hours or later after the oral administration of a

known quantity of ^{42}K will, after allowance for measured urinary excretion, enable one to derive a calibration factor applicable to the subject's ^{40}K, with an estimated accuracy of $\pm$ 1 percent. In practice, despite the very low radiation dose involved, it is rarely justifiable to administer ^{42}K to a subject solely in order to derive his individual whole-body potassium with this precision. However, calibration factors derived from ^{42}K studies in a limited number of subjects can be correlated with simple physical parameters such as weight and height, and such correlations, when applied to spectra recorded from subjects for whom no ^{42}K calibration is available, may enable their whole-body potassium to be calculated with a standard error not exceeding $\pm$ 3 percent in most cases.

An alternative approach is possible in subjects who have received a known dose of ^{24}Na, administered for other purposes. ^{24}Na emits gamma-rays of energy 1.38 MeV, again similar to the 1.46 MeV quanta of ^{40}K. Although on a microscopic scale the distribution of sodium in the body differs from that of potassium, the two elements may reasonably be assumed to be distributed between the various anatomical regions in essentially the same fashion, and so it should be possible to derive reliable calibration data for an individual's ^{40}K from body radioactivity measurements following injection of ^{24}Na. This approach has been validated by administering (on separate occasions), both ^{42}K and ^{24}Na to six subjects, and comparing the calibration factors derived by the two methods.

In this study, where the subjects received ^{24}Na for the measurement of total exchangeable sodium, accurate calibration factors for each individual's ^{40}K were derived, and the overall estimated standard error was less than 2 percent.

TOTAL EXCHANGEABLE SODIUM (NaE)

Total exchangeable sodium (24 hour) is a measurement of the metabolically active pool of sodium in the body (50), and is quantitated using a standard isotope dilution technique (104). A modified form of this method, performed by UK AERE (Harwell) was used in this study, and a general description of the technique follows:

The isotope for injection was prepared by diluting the $^{24}Na^{+}$ (Radiochemical Centre, Amersham) to a 3 µCi/ml solution, with sterile normal saline. ($^{24}Na^{+}$ was supplied as 3 mCi/2.5 mls which was made up to 10 mls with saline, and 1 ml of this solution was further diluted in 100 mls saline, giving the 3 µCi/ml solution which was stored in sterile bottles.)

A small volume ($\pm$ 1 ml) of this 3 µCi/ml solution was drawn up into a sterile disposable syringe which was then very accurately counted, 40 cm from a 15 cm crystal, in a gamma counter. This method, taking into account the geometry of the syringe by measuring the activity at a considerable distance from an enormous crystal, was validated at UK AERE

(Harwell). After intravenous injection the empty syringe was recounted and the amount of $^{24}Na^+$ injected calculated. All injections were given between 10.00 and 14.00 hours to recumbent patients on a normal diet. The urine of the subjects was collected from 0-23 hours and from 23-25 hours (spot urine) after the injection, for measurement of stable ($^{23}Na^+$) and radioactive sodium excretion. At 24 hours equilibration was complete and the ratio of active to stable sodium in the urine reflects that in the body fluids, i.e. the specific activities were equal.

Knowing the $^{24}Na^+$ in the body (amount injected minus the amount excreted 0-23 hours in the urine), and in the 'spot urine' (23-25 hours), and the stable sodium ($^{23}Na^+$) in the 'spot urine', the stable sodium (NaE) in the body was calculated as follows:

At equilibration

$$\frac{^{23}Na^+ \text{ in body (mEq)}}{^{23}Na^+ \text{ in urine (mEq/ml)}} = \frac{^{24}Na^+ \text{ in body (CPM)}}{^{24}Na^+ \text{ in urine (CPM/ml)}}$$

$$\therefore\ ^{23}Na^+ \text{ in body (mEq)} = \frac{^{24}Na^+ \text{ in body (CPM)}}{^{24}Na^+ \text{ in urine (CPM/ml)}} \times {}^{23}Na^+ \text{ in urine (mEq/ml)}$$

$$= \text{24 hour total exchangeable sodium (mEq)}$$

The technique measures the 24 hour total exchangeable sodium (NaE) in milliequivalents with an estimated standard error of less than 5 percent.

PLASMA VOLUME (PV)

Plasma volume is measured using a standard isotope dilution technique (95). A modified form of this method, performed by UK AERE (Harwell) was used in this study, and a general description of the technique follows:

^{131}I human serum albumin is supplied (Radiochemical Centre, Amersham) as a 200 µCi solution which was diluted to 400 mls with normal saline containing human serum albumin (1% w/v) to give a 0.5 µCi/ml stock solution. The solution was stored in sterile bottles.

A small volume ($\pm$ 2 mls) of the 0.5 µCi/ml solution was drawn into a sterile disposable syringe, which was very accurately counted, 40 cm from a 15 cm crystal, in a gamma counter. This method, taking into account the geometry of the syringe by measuring the activity at a considerable distance from an enormous crystal, was validated at UK AERE (Harwell). After intravenous injection the empty syringe was recounted and the amount of ^{131}I injected calculated. All injections were given between 12.30 hours and 16.00 hours to recumbent patients on a normal diet.

Venous blood samples (20 mls) were taken into Heparin, 10, 20 and 30 minutes after the intravenous injection of the isotope. The plasma was separated and 5 mls of plasma from each specimen was counted in a gamma counter.

The plasma volume was calculated by simple proportion:

$$\text{Plasma volume (ml)} = \frac{\text{amount } ^{131}\text{I injected (CPM)}}{\text{concentration } ^{131}\text{I in plasma (CPM/ml)}}$$

The plasma volume was expressed as the mean of the calculations made on the three samples (10, 20 and 30 minutes). The measurement has an estimated standard error of less than 3 percent.

PLASMA ALDOSTERONE

Plasma aldosterone was measured according to the method of Mayes, Furuyama, Kem and Nugent (61) by Dr. Charles Horth at G.D. Searle & Co., High Wycombe.

PLASMA AND URINARY SODIUM AND POTASSIUM

Sodium and potassium were measured in plasma and urine by the standard method of flame photometry, with lithium as the internal standard, by the Biochemistry Department, Radcliffe Infirmary, Oxford.

STATISTICS

Standard statistical methods were used to analyse the data in this study.

Means are generally given $\pm$ standard error of mean (S.E.M.)

Significance levels of the quantitative data were assessed by Student's unpaired t-tests, and of the qualitative data by χ^2 tests. (In view of the large number of subjects, the t-test was considered an acceptable test of significance in situations where the distributions were not normal.)

Pearson's correlation coefficients were used to measure the mutual relationship between two variables. Partial correlation coefficients were used to measure the association between two variables while adjusting for the effects of additional variables.

Distributions were assessed by measurement of Kurtosis (peakedness) and of Skewness. Positive Kurtosis values indicate that the distribution is more peaked in the middle than a normal distribution, and negative values less peaked. Positive Skewness values indicate that the distribution is skewed to the right (extremely high scores are farther away from the mean than extremely low scores), and negative values to the left.

PART III

RESULTS

The succeeding chapters deal with the results of this study in essential hypertensive patients and matched normotensive control subjects. Renin and its relationship to other variables have been examined in considerable detail, but this has necessitated an abundance of statistics. Consequently, the content of these chapters will unavoidably make turgid reading.

The Tables and Figures are inserted at the end of each chapter.

CHAPTER 4

POPULATION STUDIED

The results of screening for hypertension in three populations from South Oxfordshire (employees at UK AERE (Harwell) and associated laboratories, town patients in a Wantage general practice, and patients in a Didcot general practice) between the ages of 21 and 70 years are shown. There was some overlap between these populations in that a number of people resident in Wantage and Didcot worked at Harwell.

HYPERTENSIVE PATIENTS

Patients were designated hypertensive when the mean diastolic blood pressure exceeded 100 mm Hg. Only previously untreated, uncomplicated essential hypertensive patients were admitted to the study.

At UK AERE (Harwell) and associated laboratories there were 7,587 employees between 21 and 70 years of age. In response to an invitation to have their blood pressure checked, 3,507 people (46.2%) attended, and of these 76 people (2.2%) were hypertensive and eligible for inclusion in the study. However, 7 patients were unwilling to participate in this programme and opted for general practitioner management (Table 8).

One Wantage general practice has about 12,000 people

registered as patients, and 7,000 of these are labelled town patients, i.e. they live in Wantage and the adjacent village of Grove. It was decided to limit the study to town patients between the ages 21-70 years, and after the general practitioners had eliminated obviously unsuitable people, 3,866 invitations were sent to people to have their blood pressure measured in their local Red Cross hall. Some letters (680, 18%) were returned as the person was unknown, had moved from the area, or had died. The remaining 3,186 people received at least three letters, but only 841 people (26.4%) attended before the programme was terminated. There were 23 people with hypertension who were eligible for inclusion in the study. However, of these, 9 had already been 'detected' at UK AERE, where they worked, and 7 preferred general practitioner care to participation in this study (Table 8).

In the Didcot general practice (± 10,000 people), 5,964 people were aged between 21 and 70 years and were invited to have their blood pressure measured. We were unable to trace 1,598 (27%) of these people. The remaining 4,366 received at least three letters, but only 2,429 (55.6%) attended, and of these 32 (1.3%) were found to be hypertensive and considered eligible for the study. However, 5 of these patients worked at UK AERE, where they had already been 'detected', and 14 hypertensives were unhappy about participating in the study and were referred to their own general practitioners (Table 8).

Thus, 117 previously untreated hypertensive patients

(1.73%) were found, and 89 of these (69 patients from UK AERE, 7 from Wantage, and 13 from Didcot) agreed to participate in the research project.

NORMOTENSIVE CONTROL SUBJECTS

The normotensive controls had mean diastolic blood pressures of less than 90 mm Hg, and were apparently healthy. They were chosen from the same populations as the hypertensive subjects with whom they were matched for age and sex. Thus, 89 normotensive controls were studied.

MEDICAL DETAILS

No subject had ever received hypotensive therapy, and no other drugs were being taken. In none was there clinical or biochemical evidence of Cushing's syndrome, phaeochromocytoma, primary aldosteronism, or co-arctation of the aorta. No patient had papilloedema, retinal haemorrhages or exudates, and none had a renal bruit or proteinuria. Excretion urography was normal and a plasma urea concentration was less than 7.0 $mmol.l^{-1}$ in all. Pregnant women, and those in whom there was evidence of any concurrent disease process or of previous stroke or myocardial infarction were excluded from the study. All the hypertensives were considered to have mild, uncomplicated essential hypertension and the controls to be normotensive and healthy.

SUMMARY

In a hypertension screening programme, of the 15,139 people who were contacted, 6,777 (44.8%) attended for blood pressure measurement. 117 previously untreated hypertensive patients (1.73%) were discovered, and of these 89 mild, uncomplicated essential hypertensives were included in the study.

89 age and sex matched normotensive control subjects from the same population were also investigated.

Details of the age, sex, and race will be discussed in a later chapter.

TABLE 8. RESULTS OF HYPERTENSION SCREENING PROGRAMME

Population	People eligible and contacted	Number screened (%)	Number hypertensive (%)	Number already 'detected'	Number of new hypertensives 'detected'	Number unwilling to participate	Number entered in study
UK AERE (Harwell) and associated laboratories	7,587	3507 (46.2%)	76 (2.2%)	0	76	7	69
Wantage general practice (town patients)	3,186	841 (26.4%)	23 (2.7%)	9	14	7	7
Didcot general practice	4,366	2429 (55.6%)	32 (1.3%)	5	27	14	13
Total	15,139	6777 (44.8%)	131 (1.9%)	14	117 (1.7%)	28	89

CHAPTER 5

PLASMA RENIN ACTIVITY AND ITS RESPONSIVENESS IN ESSENTIAL HYPERTENSIVE PATIENTS AND NORMOTENSIVE CONTROL SUBJECTS

In this chapter, differences in renin and its responsiveness between hypertensives and controls are examined, together with the distributions of these variables and how they interrelate. Finally, the basis on which the patients are divided into renin subgroups is considered and applied in later chapters.

BASAL AND STIMULATED PLASMA RENIN ACTIVITY (PRA)

The basal PRA measured after lying supine for 2 hours (supine PRA) was similar in hypertensive patients and control subjects (Table 9 and Figure 2).

The stimulated PRA's were significantly different between these two groups: erect PRA (after standing for 2 hours) was less in hypertensives than in controls, $P<0.005$; post-frusemide PRA (1 hour after frusemide 1 mg/kg intravenously) was lower in hypertensives than in controls, $P<0.001$ (Table 9 and Figure 2).

RENIN RESPONSIVENESS

The absolute renin responsiveness or the change in PRA

from specimens taken supine to those taken erect (ΔS-E), from supine to post-frusemide (ΔS-L), and from erect to post-frusemide (ΔE-L), showed the hypertensive patients to respond less compared to control subjects. The ΔS-E responsiveness in hypertensives was lower than in controls ($P<0.005$). The change ΔS-L revealed a greater difference ($P<0.001$) with hypertensives having lower values than controls. Similar results ($P<0.001$) were found for ΔE-L responsiveness in hypertensive patients compared to control subjects (Table 10 and Figure 3).

The percentage renin responsiveness was also calculated to take into account the tendency for the absolute rise to depend on the initial value. (For example, an increase from 0.50 to 1.00 ng/ml/hr would give an absolute increase of 0.50 ng/ml/hr with a percentage increase of 100%, while an increase from 3.00 to 6.00 ng/ml/hr would give the same percentage increase, but with a considerably greater (3.00 ng/ml/hr) absolute change.) The per cent change from supine to erect PRA (%S-E responsiveness) in hypertensives was less than in control subjects ($P<0.005$). The difference between these groups was more significant ($P<0.001$) when the per cent changes supine to lasix (%S-L) and erect to lasix (%E-L) were examined (Table 11 and Figure 4).

RENIN CORRELATIONS

It is important to know how the three sets of variables - plasma renin activity, absolute and percentage renin responsiveness - relate within and between themselves, and the Pearson correlation coefficient (r) was used to assess this.

The correlations between supine, erect and post-frusemide plasma renin activity were highly significant ($P<0.001$) in both hypertensive and control subjects. The absolute renin responsiveness (ΔS-E, ΔS-L, ΔE-L) was closely interrelated ($P<0.001$) in both groups. When the results were expressed as percentages, significant correlations ($P<0.001$) were found between %S-L and %S-E, and between %S-L and %E-L, but %S-E was unrelated to %E-L in both hypertensives and control subjects (Tables 12 and 13).

Renin values (PRA supine, erect and lasix) correlated significantly with all measures of absolute responsiveness, but not consistently with percentage responsiveness. The quanta within hypertensive and control subjects which correlated best with all other measures of renin status were:

(i) absolute value of post-frusemide PRA

(ii) absolute change in PRA supine to frusemide (ΔS-L), and

(iii) percentage change in PRA supine to frusemide (%S-L).

The relevance of these relationships will be discussed in relation to the separation of patients into renin subgroups and the choice of parameters for the comparison of renin to other variables.

DISTRIBUTIONS

The distributions of the renin values (PRA supine, erect, lasix), of the absolute renin responsiveness (ΔS-E, ΔS-L, ΔE-L) and of the percentage renin responsiveness (%S-E, %S-L, %E-L) were studied. Some hypotheses that low-renin hypertension is a separate entity would suggest that the distribution of renin and its responsiveness might be bimodal.

The data showed the distributions to be skewed to the right, but the logarithm (to the base 10) of the renin values appeared to normalise most of the distributions without any suggestion of bimodality (Figures 5,6,7,8,9,10,11,12,13 and 14). Statistical testing for normal distribution is of questionable value, but skewness and kurtosis (peakedness) indices give some indication of whether the distributions are significantly different from a normal distribution. Among the hypertensive patients, the distributions of these renin parameters are shown to be similar to a normal distribution ($P>0.05$) except for the absolute and percent change in PRA from supine to frusemide. In the control group, the logarithmic transformation of the renin values did not completely normalise the data (Tables 14 and 15).

RENIN SUBGROUPS

Patients with essential hypertension have been divided

into subgroups (low, normal, high) according to plasma renin levels. Many methods for such divisions exist, but almost all lack any statistical basis.

Laragh and co-workers group patients according to a nomogram relating 24 hour urinary sodium excretion to plasma renin activity (16). We were unable to reproduce such a nomogram plotting both supine and erect PRA against urinary sodium. The relationships between urine sodium and PRA for hypertensives and controls are shown in Figures 15 and 16.

An attempt was made to study renin subgroups characterised in a statistically acceptable way and incorporating basal, stimulated and renin response data. The distributions of the 9 variables (PRA supine, erect and lasix; absolute responsiveness ΔS-E, ΔS-L, ΔE-L; percentage responsiveness %S-E, %S-L, %E-L) were divided into equal thirds on the understanding that if a distinct 'low-renin' subgroup exists, it would be contained within the lower third of the distribution, while a 'high-renin' subgroup within the upper third. The subgroups for each variable are shown in Tables 16 and 17.

Each of these lower, mid and upper renin subgroups were compared one with all others when looking for differences between them. The results of the comparison of volume, sodium, potassium and sex between the subgroups will be reported in later chapters.

SUMMARY

Plasma renin activity and its responsiveness were suppressed in hypertensive patients compared to matched normotensive control subjects.

Both within and between the different methods of assessing renin activity (absolute renin levels, absolute renin responsiveness and percentage renin responsiveness) most values were highly correlated in both hypertensive and control subjects. The most frequently correlated variable in each group was the absolute post-frusemide PRA, the ΔS-L absolute responsiveness and the %S-L percentage responsiveness.

Plasma renin activity and renin responsiveness were normally distributed when expressed logarithmically without any suggestion of bimodality, in the hypertensive patients.

The Laragh nomogram relating PRA to urine sodium was not reproducible with our data.

Hypertensive and control patients were separated into groups by dividing the distributions of basal and stimulated renin levels and renin responsiveness into equal thirds, designated lower, mid and upper renin subgroups. Differences in various parameters between the subgroups will be considered in later chapters.

TABLE 9. BASAL AND STIMULATED PLASMA RENIN ACTIVITY (ng/ml/hr) IN ESSENTIAL HYPERTENSIVE PATIENTS AND MATCHED NORMOTENSIVE CONTROL SUBJECTS

Parameter	Group	Mean	N	t	f	P
Supine PRA	HT	1.29	89	1.42882	176	NS
	C	1.45	89			
Erect PRA	HT	2.70	89	2.92356	176	<0.005
	C	3.55	89			
Lasix PRA	HT	3.87	89	4.82103	176	<0.001
	C	6.29	89			

Note: HT = hypertensive patients, C = normotensive control subjects
Lasix PRA = post-frusemide PRA
t = Student's unpaired t-test, f = degrees of freedom

TABLE 10. ABSOLUTE RENIN RESPONSIVENESS (ng/ml/hr) IN ESSENTIAL HYPERTENSIVE PATIENTS AND MATCHED NORMOTENSIVE CONTROL SUBJECTS

Parameter	Group	Mean	N	t	f	P
ΔS-E	HT	1.40	89	2.95102	176	<0.005
	C	2.12	89			
ΔS-L	HT	2.55	89	5.19295	176	<0.001
	C	4.90	89			
ΔE-L	HT	1.22	89	4.96743	176	<0.001
	C	2.74	89			

Note: S-E = change in PRA from supine to erect, S-L = change from supine to post-frusemide, E-L = change from erect to post-frusemide
HT = hypertensive patients, C = control subjects
t = Student's unpaired t-test, f = degrees of freedom

TABLE 11. PERCENTAGE RENIN RESPONSIVENESS IN ESSENTIAL HYPERTENSIVE PATIENTS AND MATCHED NORMOTENSIVE CONTROL SUBJECTS

Parameter	Group	Mean	N	t	f	P
%S-E	HT	109.63	89	3.05800	176	<0.005
	C	175.93	89			
%S-L	HT	201.66	89	5.05003	176	<0.001
	C	398.51	89			
%E-L	HT	41.56	89	4.72853	176	<0.001
	C	81.39	89			

Note: S-E = change in PRA from supine to erect, S-L = change from supine to post-frusemide, E-L = change from erect to post-frusemide
HT = hypertensive patients, C = control subjects
t = Student's unpaired t-test, f = degrees of freedom

TABLE 12. RENIN CORRELATIONS IN HYPERTENSIVE PATIENTS

	Supine PRA	Erect PRA	Lasix PRA	ΔS-E PRA	ΔS-L PRA	ΔE-L PRA	%S-E PRA	%S-L PRA	%E-L PRA
Supine PRA		r= 0.52 P< 0.001	r= 0.47 P< 0.001	r= 0.24 P< 0.026	r= 0.27 P< 0.015	r= 0.19 P= NS	r=-0.05 P= NS	r=-0.06 P= NS	r=-0.02 P= NS
Erect PRA	r= 0.52 P< 0.001		r= 0.86 P< 0.001	r= 0.95 P< 0.001	r= 0.81 P< 0.001	r= 0.35 P< 0.001	r= 0.80 P< 0.001	r= 0.61 P< 0.001	r=-0.01 P= NS
Lasix PRA	r= 0.47 P< 0.001	r= 0.86 P< 0.001		r= 0.82 P< 0.001	r= 0.98 P< 0.001	r= 0.78 P< 0.001	r= 0.70 P< 0.001	r= 0.82 P< 0.001	r= 0.42 P< 0.001
ΔS-E PRA	r= 0.24 P< 0.026	r= 0.95 P< 0.001	r= 0.82 P< 0.001		r= 0.83 P< 0.001	r= 0.33 P< 0.002	r= 0.93 P< 0.001	r= 0.71 P< 0.001	r=-0.01 P= NS
ΔS-L PRA	r= 0.27 P< 0.015	r= 0.81 P< 0.001	r= 0.98 P< 0.001	r= 0.83 P< 0.001		r= 0.80 P< 0.001	r= 0.77 P< 0.001	r= 0.90 P< 0.001	r= 0.45 P< 0.001
ΔE-L PRA	r= 0.19 P= NS	r= 0.35 P< 0.001	r= 0.75 P< 0.001	r= 0.33 P< 0.002	r= 0.80 P< 0.001		r= 0.30 P< 0.004	r= 0.74 P< 0.001	r= 0.80 P< 0.001
%S-E PRA	r=-0.05 P= NS	r= 0.80 P< 0.001	r= 0.70 P< 0.001	r= 0.93 P< 0.001	r= 0.77 P< 0.001	r= 0.30 P< 0.004		r= 0.81 P< 0.001	r= 0.01 P= NS
%S-L PRA	r=-0.06 P= NS	r= 0.61 P< 0.001	r= 0.82 P< 0.001	r= 0.71 P< 0.001	r= 0.90 P< 0.001	r= 0.74 P< 0.001	r= 0.81 P< 0.001		r= 0.53 P< 0.001
%E-L PRA	r=-0.02 P= NS	r=-0.01 P= NS	r= 0.42 P< 0.001	r=-0.01 P= NS	r= 0.45 P< 0.001	r= 0.80 P< 0.001	r= 0.01 P= NS	r= 0.53 P< 0.001	

Note: r = Pearson's correlation coefficient
ΔS-E, ΔS-L, ΔE-L are the absolute responsiveness measures.
%S-E, %S-L, %E-L are the percentage responsiveness measures

TABLE 13. RENIN CORRELATIONS IN CONTROL SUBJECTS

	Supine PRA	Erect PRA	Lasix PRA	ΔS-E PRA	ΔS-L PRA	ΔE-L PRA	%S-E PRA	%S-L PRA	%E-L PRA
Supine PRA		r= 0.65 P< P.001	r= 0.63 P< 0.001	r= 0.30 P< 0.004	r= 0.46 P< 0.001	r= 0.46 P< 0.001	r=-0.32 P< 0.003	r=-0.35 P< 0.001	r=-0.03 P= NS
Erect PRA	r= 0.65 P< 0.001		r= 0.84 P< 0.001	r= 0.92 P< 0.001	r= 0.77 P< 0.001	r= 0.51 P< 0.001	r= 0.38 P< 0.001	r= 0.22 P< 0.038	r=-0.02 P= NS
Lasix PRA	r= 0.63 P< 0.001	r= 0.84 P< 0.001		r= 0.73 P< 0.001	r= 0.96 P< 0.001	r= 0.89 P< 0.001	r= 0.26 P< 0.013	r= 0.34 P< 0.001	r= 0.30 P< 0.004
ΔS-E PRA	r= 0.30 P< 0.004	r= 0.92 P< 0.001	r= 0.73 P< 0.001		r= 0.73 P< 0.001	r= 0.40 P< 0.001	r= 0.64 P< 0.001	r= 0.45 P< 0.001	r=-0.19 P= NS
ΔS-L PRA	r= 0.46 P< 0.001	r= 0.77 P< 0.001	r= 0.96 P< 0.001	r= 0.73 P< 0.001		r= 0.89 P< 0.001	r= 0.36 P< 0.001	r= 0.49 P< 0.001	r= 0.38 P< 0.001
ΔE-L PRA	r= 0.46 P< 0.001	r= 0.51 P< 0.001	r= 0.89 P< 0.001	r= 0.40 P< 0.001	r= 0.89 P< 0.001		r= 0.11 P= NS	r= 0.35 P< 0.001	r= 0.61 P< 0.001
%S-E PRA	r=-0.32 P< 0.003	r= 0.38 P< 0.001	r= 0.26 P< 0.013	r= 0.64 P< 0.001	r= 0.36 P< 0.001	r= 0.11 P= NS		r= 0.87 P< 0.001	r= 0.09 P= NS
%S-L PRA	r=-0.35 P< 0.001	r= 0.22 P< 0.038	r= 0.34 P< 0.001	r= 0.45 P< 0.001	r= 0.49 P< 0.001	r= 0.35 P< 0.001	r= 0.87 P< 0.001		r= 0.36 P< 0.001
% E-L PRA	r=-0.03 P= NS	r=-0.016 P= NS	r= 0.30 P< 0.004	r=-0.19 P= NS	r= 0.38 P< 0.001	r= 0.61 P< 0.001	r= 0.09 P= NS	r= 0.36 P< 0.001	

Note: r = Pearson's correlation coefficient
ΔS-E, ΔS-L, ΔE-L are the absolute responsiveness measures
%S-E, %S-L, %E-L are the percentage responsiveness measures

TABLE 14. SKEWNESS AND KURTOSIS IN THE ASSESSMENT OF THE DISTRUBUTION IN HYPERTENSIVE PATIENTS

Parameter	Normal data		Log Data	
	Skewness	Kurtosis	Skewness	Kurtosis
Plasma renin activity				
Supine	1.466	4.192	-0.330	0.417
Erect	2.802	12.863	0.126	0.069
Lasix	1.796	3.397	-0.039	-0.110
Absolute renin responsiveness				
ΔS-E	3.753	20.084	-0.191	-0.136
ΔS-L	2.164	4.699	-0.690	1.472
ΔE-L	2.496	6.562	-0.206	-0.213
Percentage renin responsiveness				
%S-E	3.075	12.565	-0.296	0.191
%S-L	2.074	4.026	-0.709	3.067
%E-L	2.197	6.655	-0.795	-0.242

Note: S-E = change from supine to erect, S-L = change from supine to lasix, and E-L = change from erect to lasix.

TABLE 15. SKEWNESS AND KURTOSIS IN THE ASSESSMENT OF THE DISTRIBUTION IN CONTROL SUBJECTS

	Normal data		Log data	
Parameter	Skewness	Kurtosis	Skewness	Kurtosis
Plasma renin activity				
Supine	1.036	1.038	-0.492	0.353
Erect	0.699	-0.032	-0.871	0.941
Lasix	1.642	5.034	-0.110	2.118
Absolute renin responsiveness				
ΔS-E	0.990	0.274	-1.657	4.884
ΔS-L	1.840	6.102	-1.576	3.261
ΔE-L	2.446	9.149	-1.460	3.232
Percentage renin responsiveness				
%S-E	2.364	6.416	-0.924	2.680
%S-L	1.894	4.471	-0.686	1.409
%E-L	0.614	0.359	-1.711	3.216

Note: S-E = change from supine to erect, S-L = change from supine to lasix, and E-L = change from erect to lasix.

TABLE 16. RANGE OF VALUES IN RENIN SUBGROUPS IN HYPERTENSIVE PATIENTS

Parameter	Renin Subgroups Lower (n=29)	Mid (n=30)	Upper (n=30)
Plasma renin activity (ng/ml/hr)			
Supine	0.35-0.99	1.04-1.33	1.36- 4.11
Erect	0.48-1.67	1.71-2.75	2.75-14.17
Lasix	0.63-2.10	2.20-4.15	4.16-14.68
Absolute renin responsiveness (ng/ml/hr)			
ΔS-E	0.07-0.55	0.55-1.29	1.29-12.58
ΔS-L	0.03-1.14	1.22-2.38	2.39-13.09
ΔE-L	0.00-0.29	0.31-1.25	1.26- 7.76
Percentage renin responsiveness (%)			
%S-E	6-46	52-105	114-791
%S-L	2-84	90-168	168-994
%E-L	0-15	15- 48	48-299

Note: S-E = change from supine to erect, S-L = change from supine to lasix, and E-L = change from erect to lasix.

TABLE 17. RANGE OF VALUES IN RENIN SUBGROUPS IN CONTROL SUBJECTS

Parameter	Renin subgroups		
	Lower (n=29)	Mid (n=30)	Upper (n=30)
Plasma renin activity (ng/ml/hr)			
Supine	0.17-0.95	0.95-1.59	1.67- 4.45
Erect	0.36-2.39	2.43-4.04	4.26- 9.09
Lasix	0.47-4.34	4.41-6.86	6.92-23.57
Absolute renin responsiveness (ng/ml/hr)			
ΔS-E	0.01-1.31	1.31-2.20	2.29- 6.29
ΔS-L	0.13-3.43	3.47-5.63	5.69-17.46
ΔE-L	0.00-1.68	1.71-2.91	2.92-15.15
Percentage renin responsiveness (%)			
%S-E	2- 89	94-178	184- 953
%S-L	17-245	246-414	420-1782
%E-L	0- 62	62- 94	97-245

Note: S-E = change from supine to erect, S-L = change from supine to lasix, and E-L = change from erect to lasix.

FIGURE 2

PLASMA RENIN ACTIVITY IN HYPERTENSIVES AND MATCHED CONTROLS

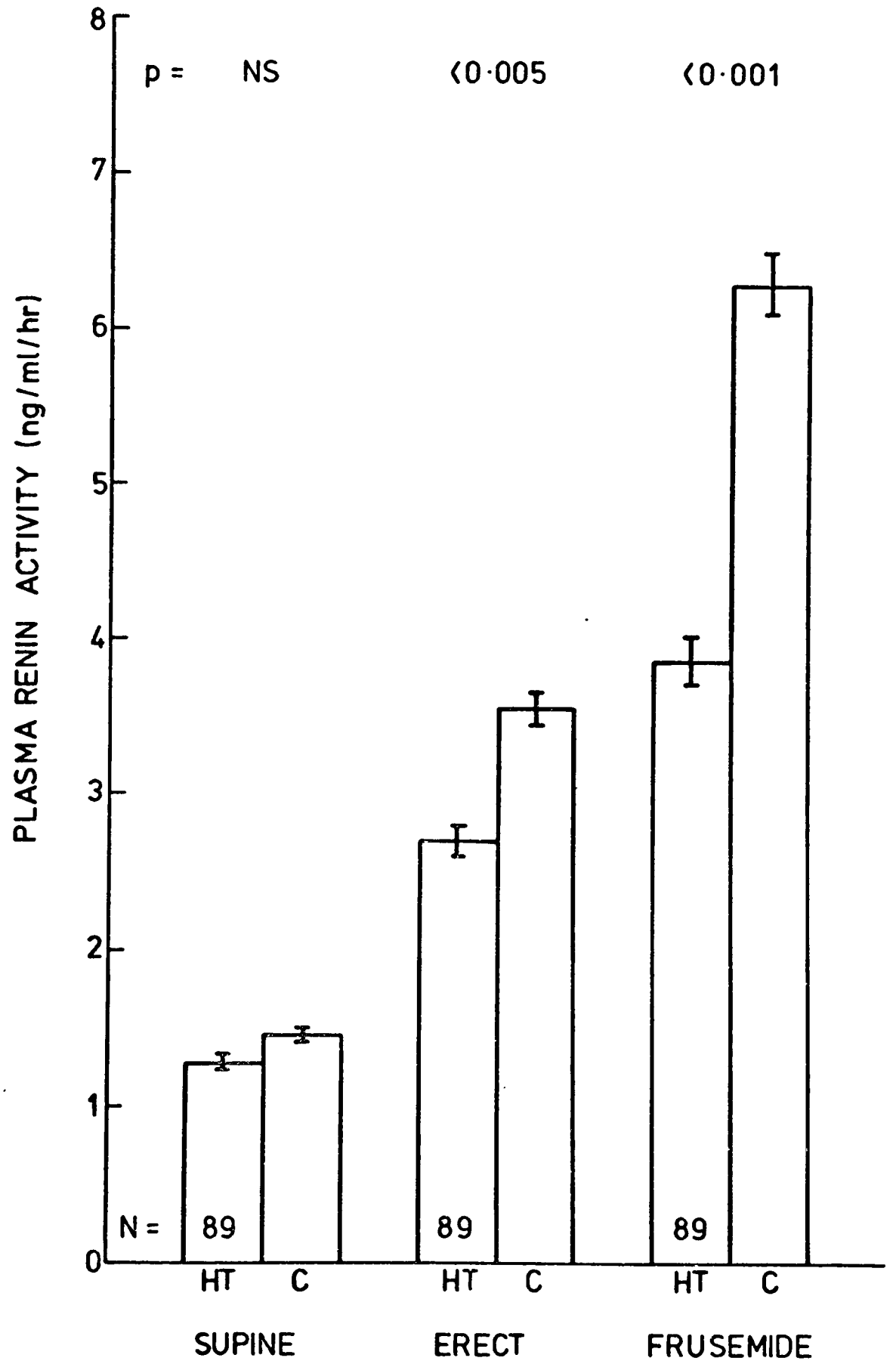


FIGURE 3

ABSOLUTE CHANGE IN PLASMA RENIN ACTIVITY
IN HYPERTENSIVES AND MATCHED CONTROLS

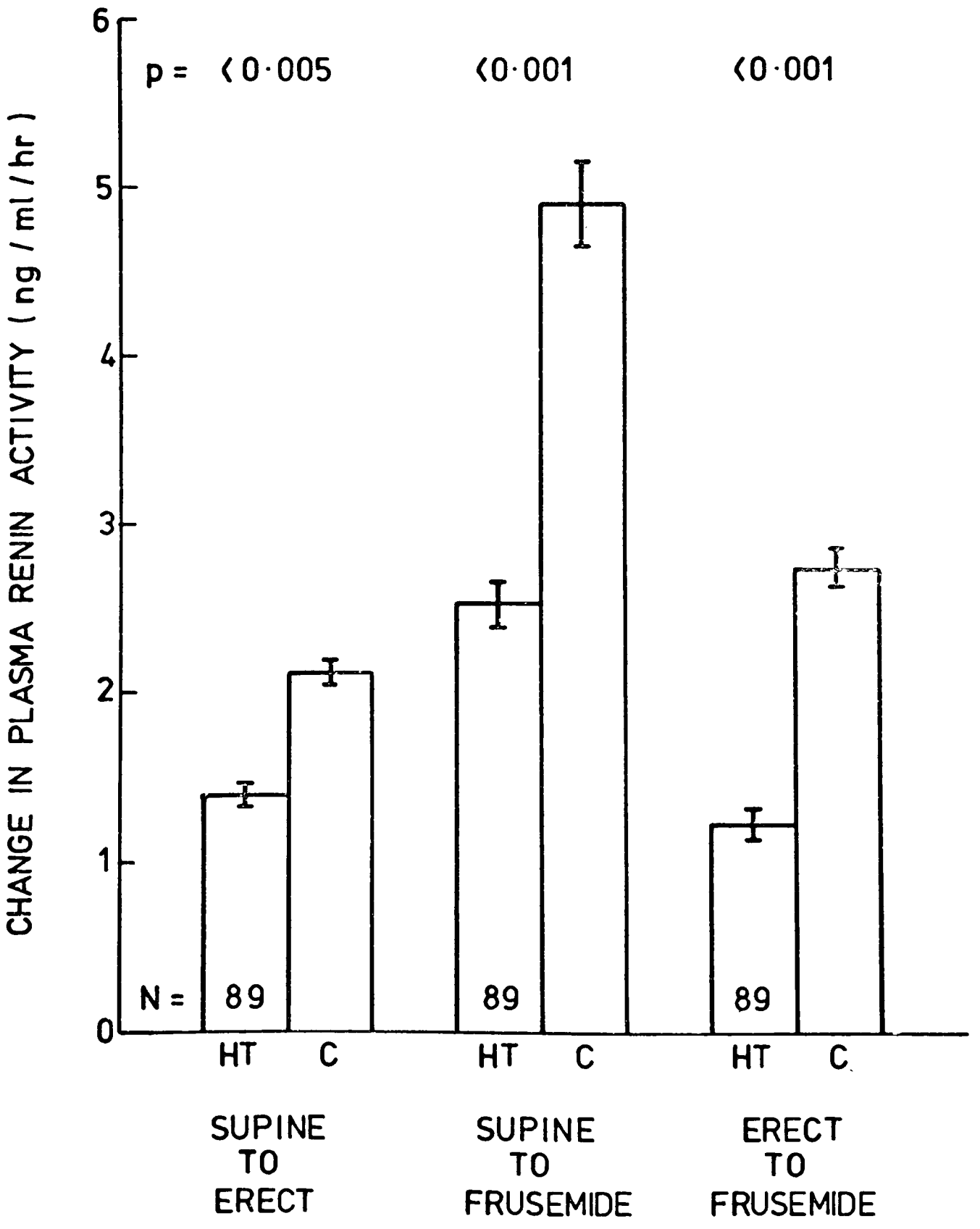


FIGURE 4

PERCENTAGE CHANGE IN PLASMA RENIN ACTIVITY IN HYPERTENSIVES AND MATCHED CONTROLS.

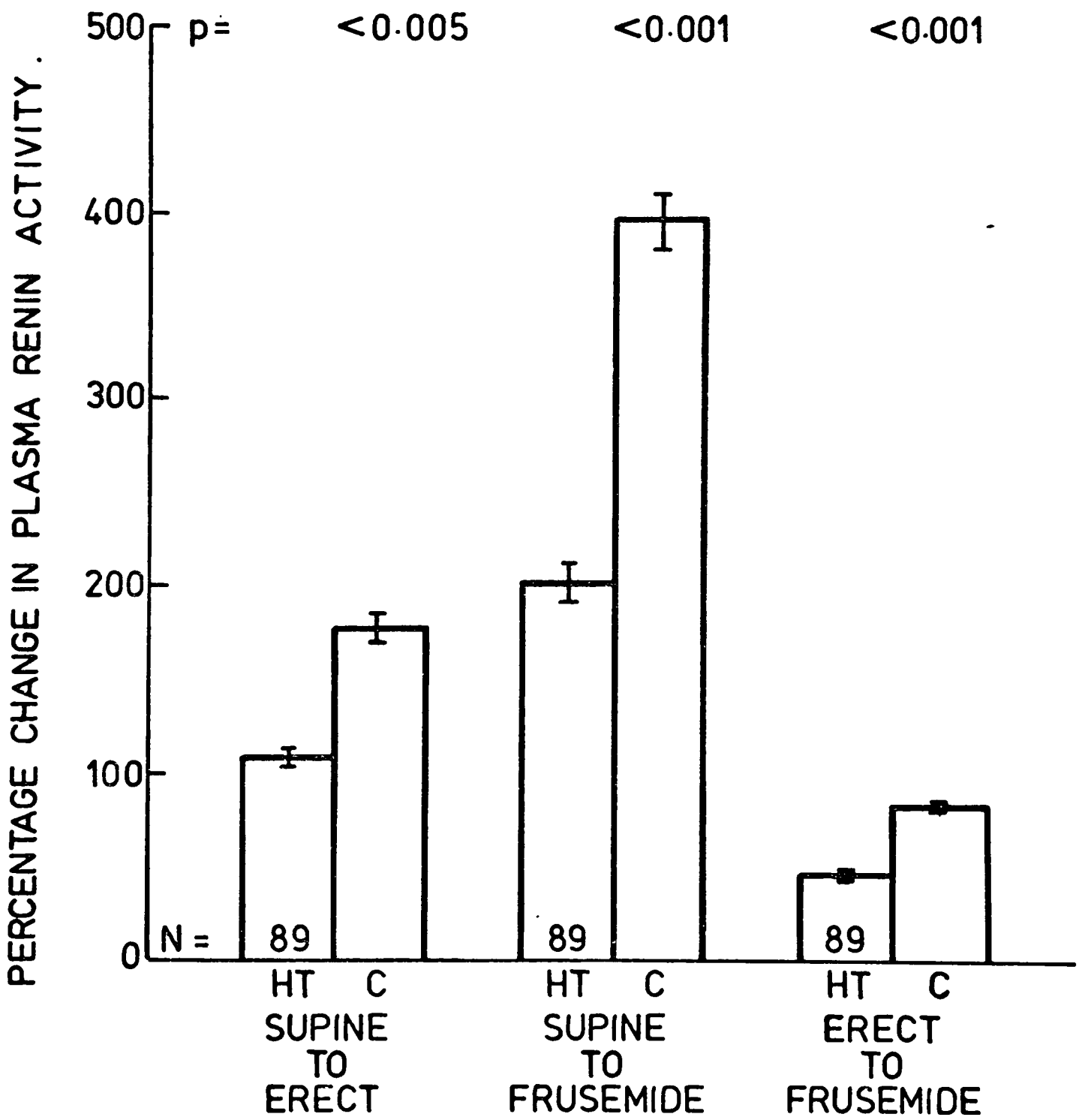


FIGURE 5

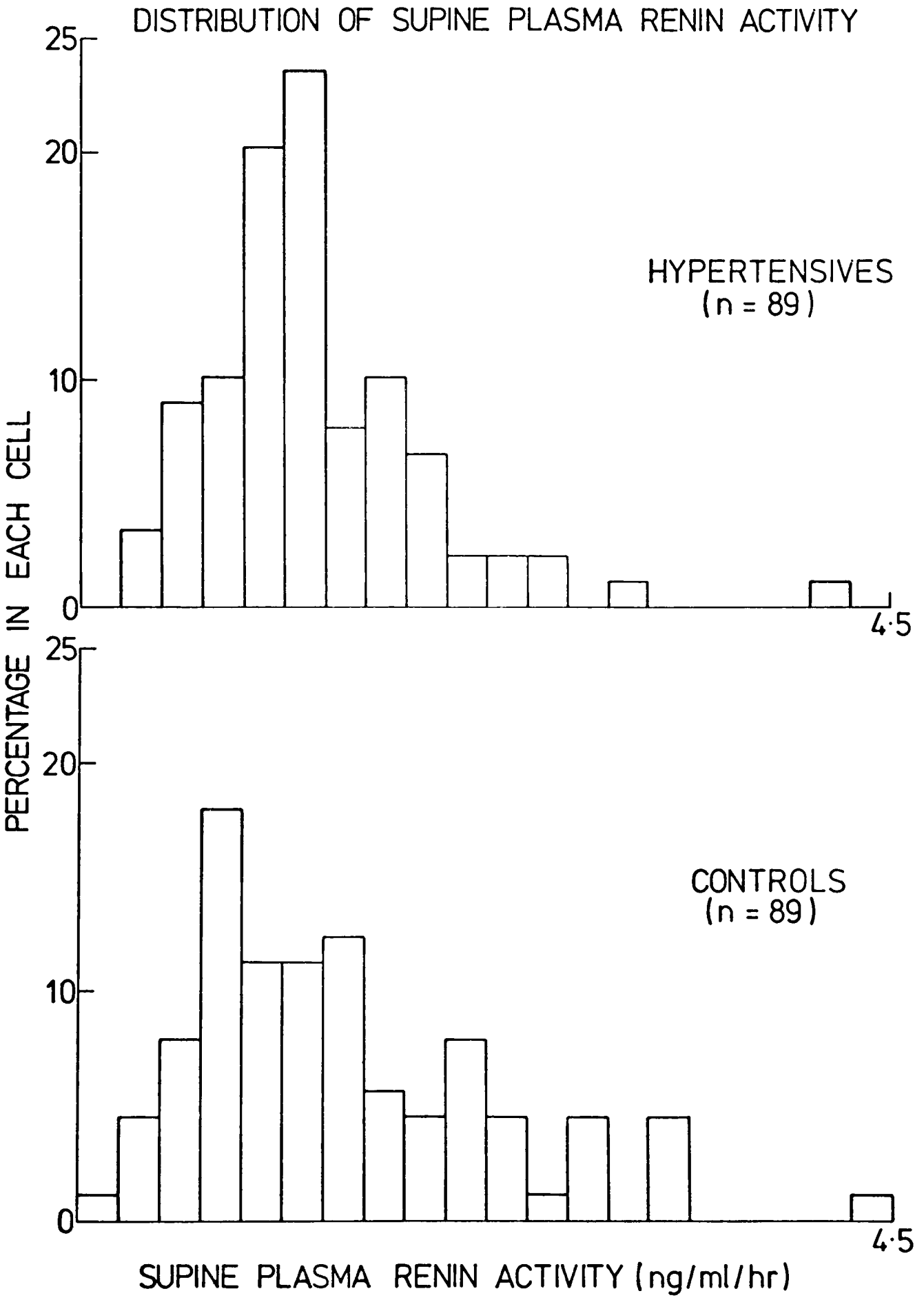

DISTRIBUTION OF SUPINE PLASMA RENIN ACTIVITY
HYPERTENSIVES
(n = 89)
CONTROLS
(n = 89)
PERCENTAGE IN EACH CELL
25
20
10
0
4·5
SUPINE PLASMA RENIN ACTIVITY (ng/ml/hr)

FIGURE 6

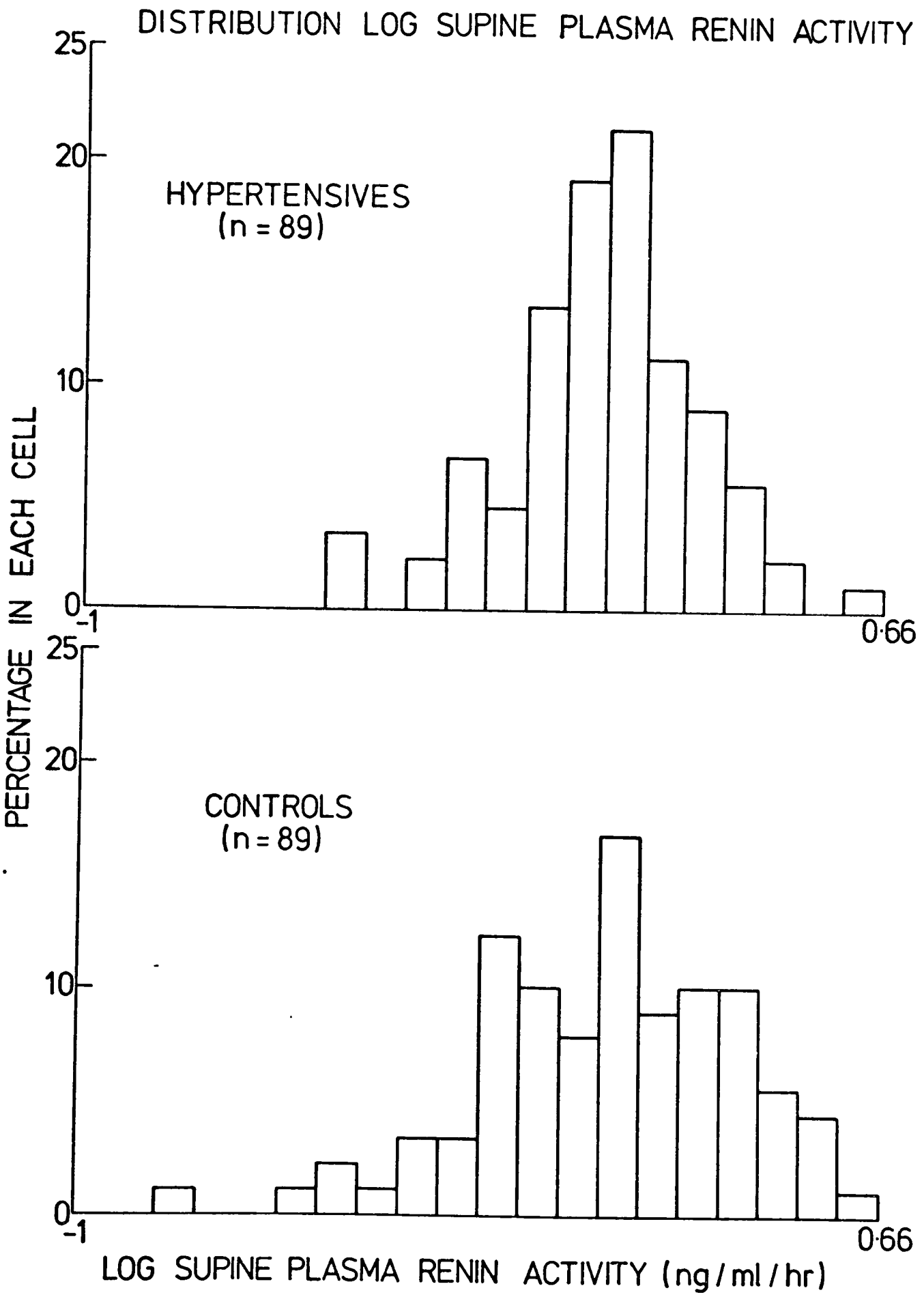

DISTRIBUTION LOG SUPINE PLASMA RENIN ACTIVITY
HYPERTENSIVES
(n = 89)
CONTROLS
(n = 89)
PERCENTAGE IN EACH CELL
25
20
10
0
-1
0·66
LOG SUPINE PLASMA RENIN ACTIVITY (ng/ml/hr)

FIGURE 7

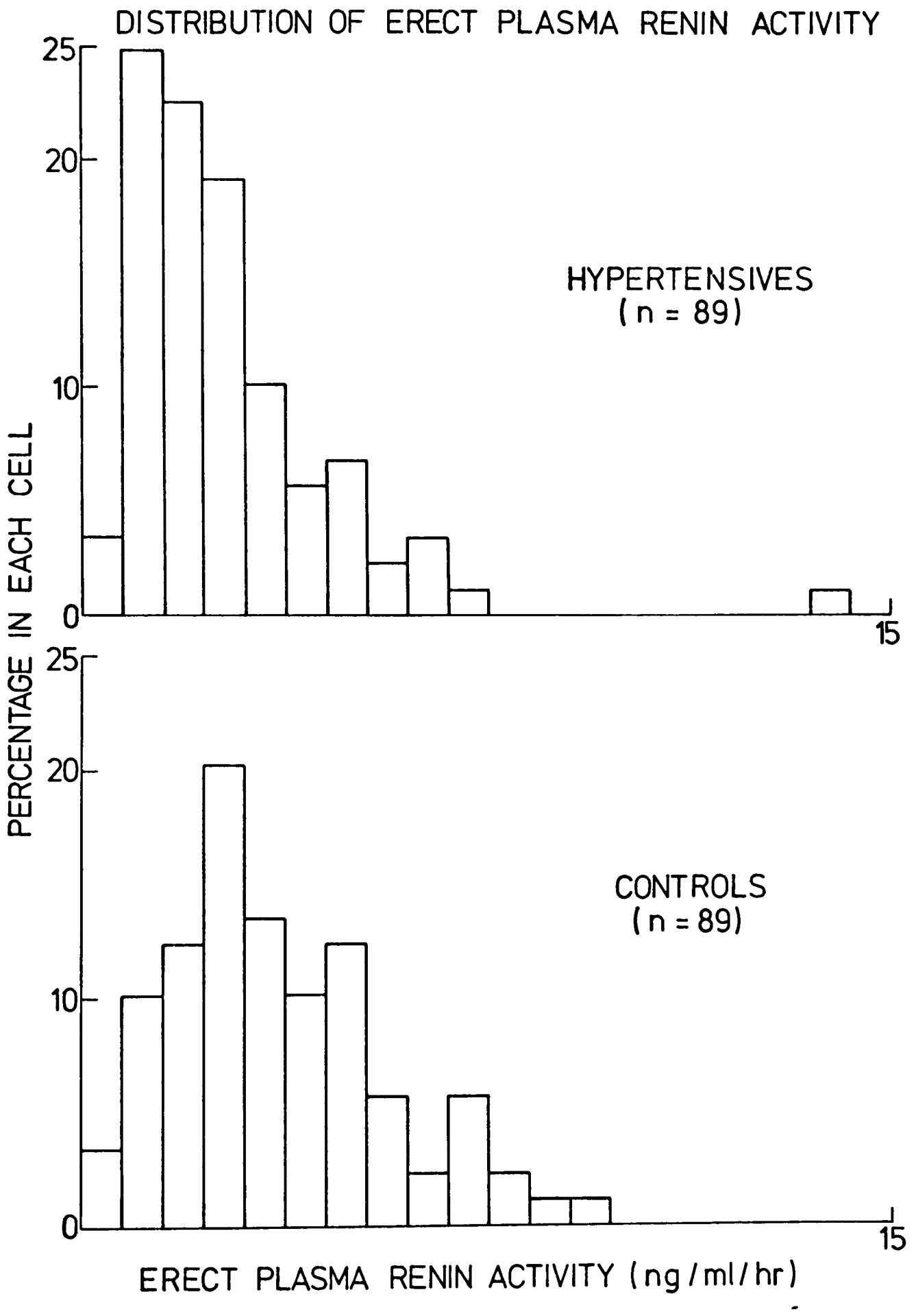

DISTRIBUTION OF ERECT PLASMA RENIN ACTIVITY
HYPERTENSIVES
(n = 89)
CONTROLS
(n = 89)
PERCENTAGE IN EACH CELL
25
20
10
0
15
ERECT PLASMA RENIN ACTIVITY (ng/ml/hr)

FIGURE 8

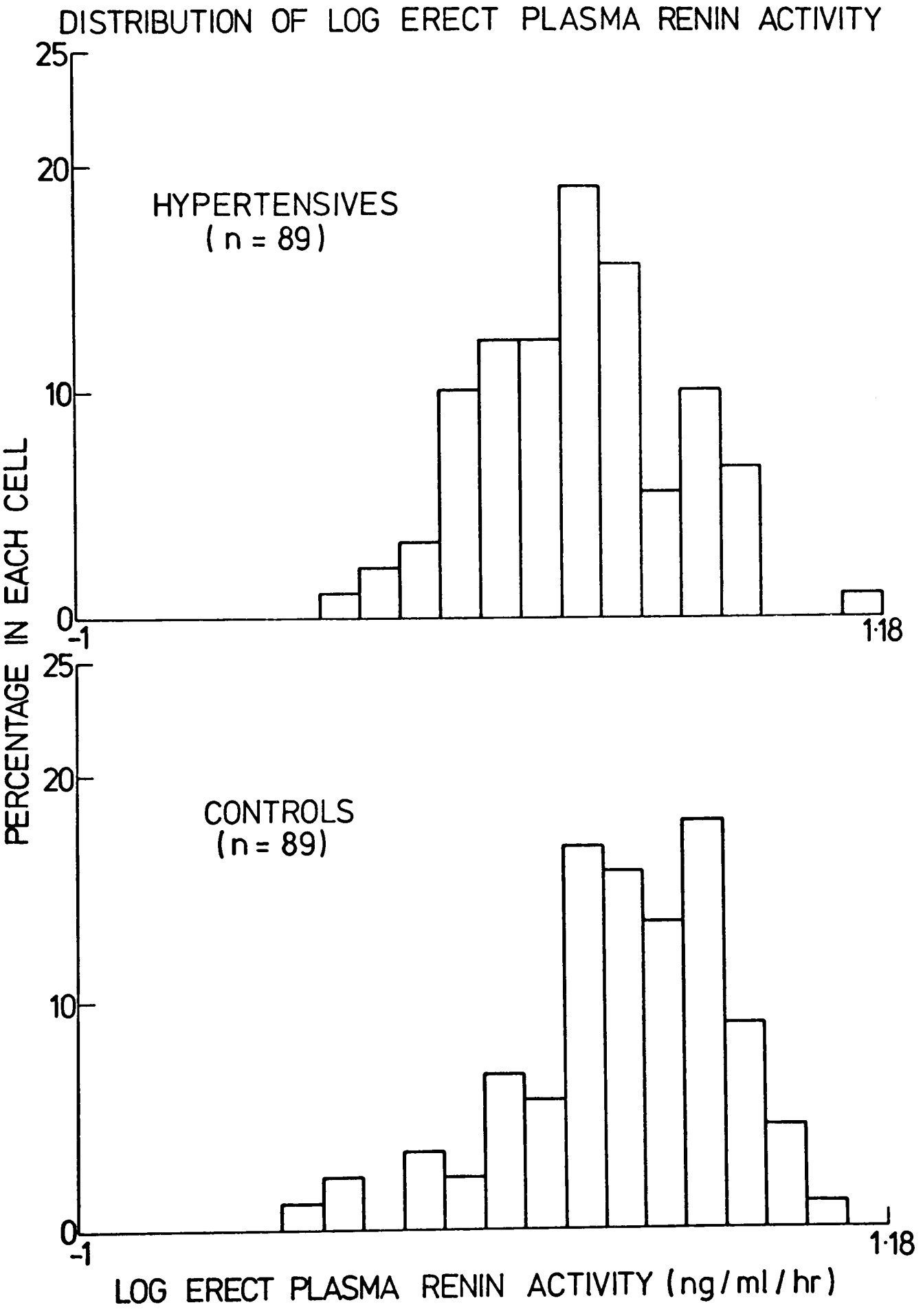

DISTRIBUTION OF LOG ERECT PLASMA RENIN ACTIVITY
HYPERTENSIVES
(n = 89)
CONTROLS
(n = 89)
PERCENTAGE IN EACH CELL
25
20
10
0
-1
1·18
LOG ERECT PLASMA RENIN ACTIVITY (ng/ml/hr)

FIGURE 9

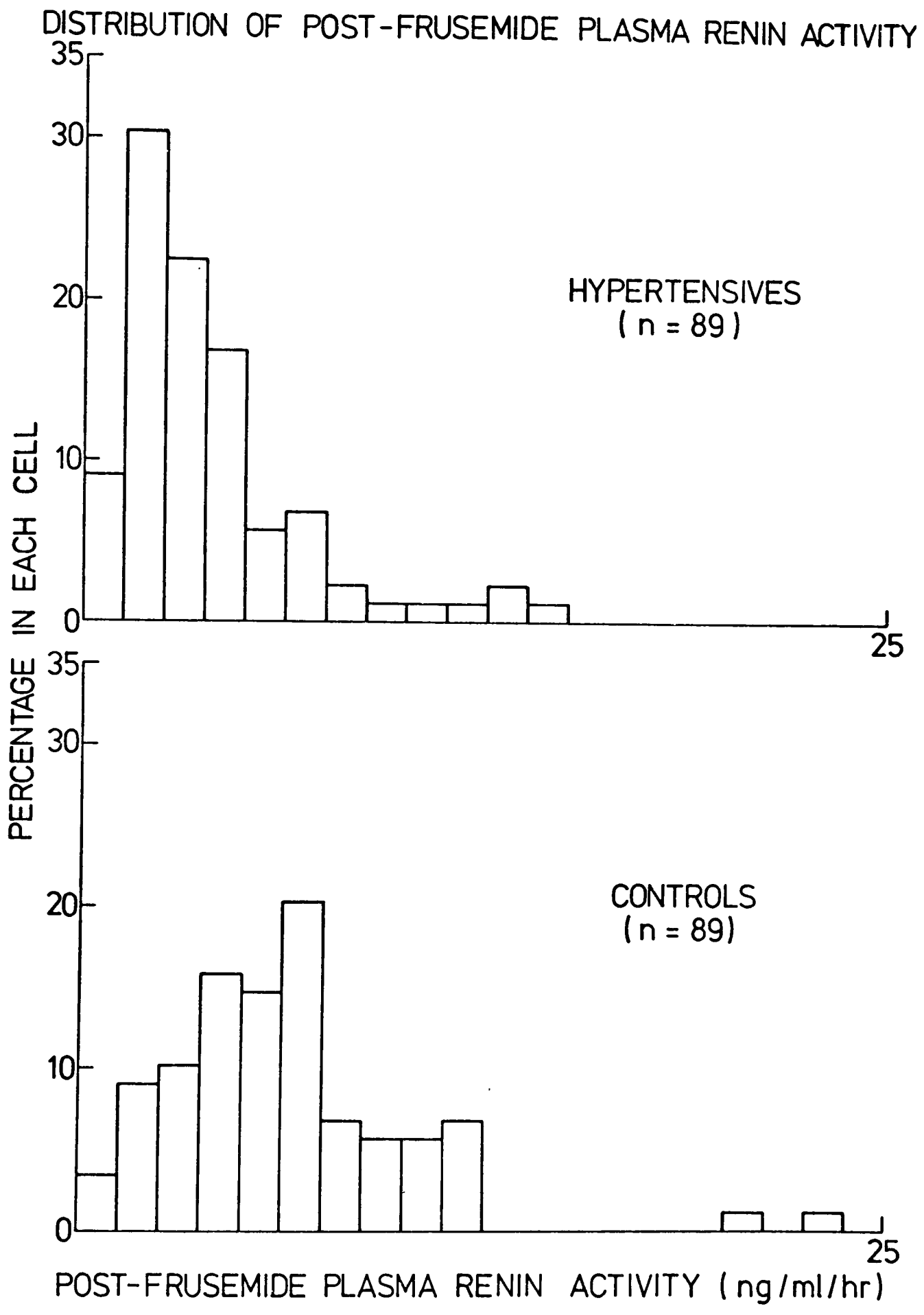

DISTRIBUTION OF POST-FRUSEMIDE PLASMA RENIN ACTIVITY
HYPERTENSIVES
(n = 89)
CONTROLS
(n = 89)
PERCENTAGE IN EACH CELL
35
30
20
10
0
25
POST-FRUSEMIDE PLASMA RENIN ACTIVITY (ng/ml/hr)

FIGURE 10

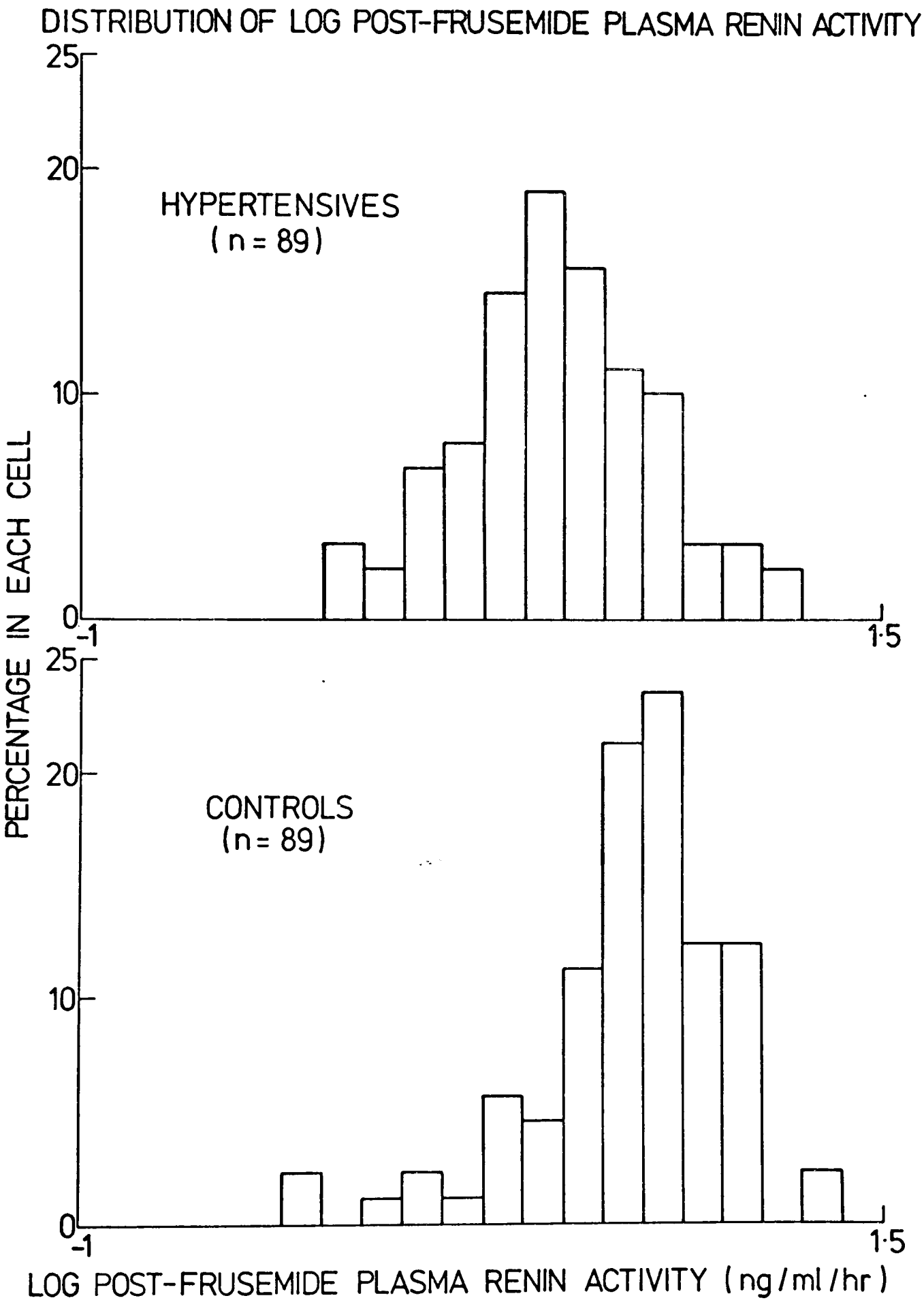

DISTRIBUTION OF LOG POST-FRUSEMIDE PLASMA RENIN ACTIVITY
HYPERTENSIVES
(n = 89)
CONTROLS
(n = 89)
PERCENTAGE IN EACH CELL
25
20
10
0
-1
1·5
LOG POST-FRUSEMIDE PLASMA RENIN ACTIVITY (ng / ml / hr)

FIGURE 11

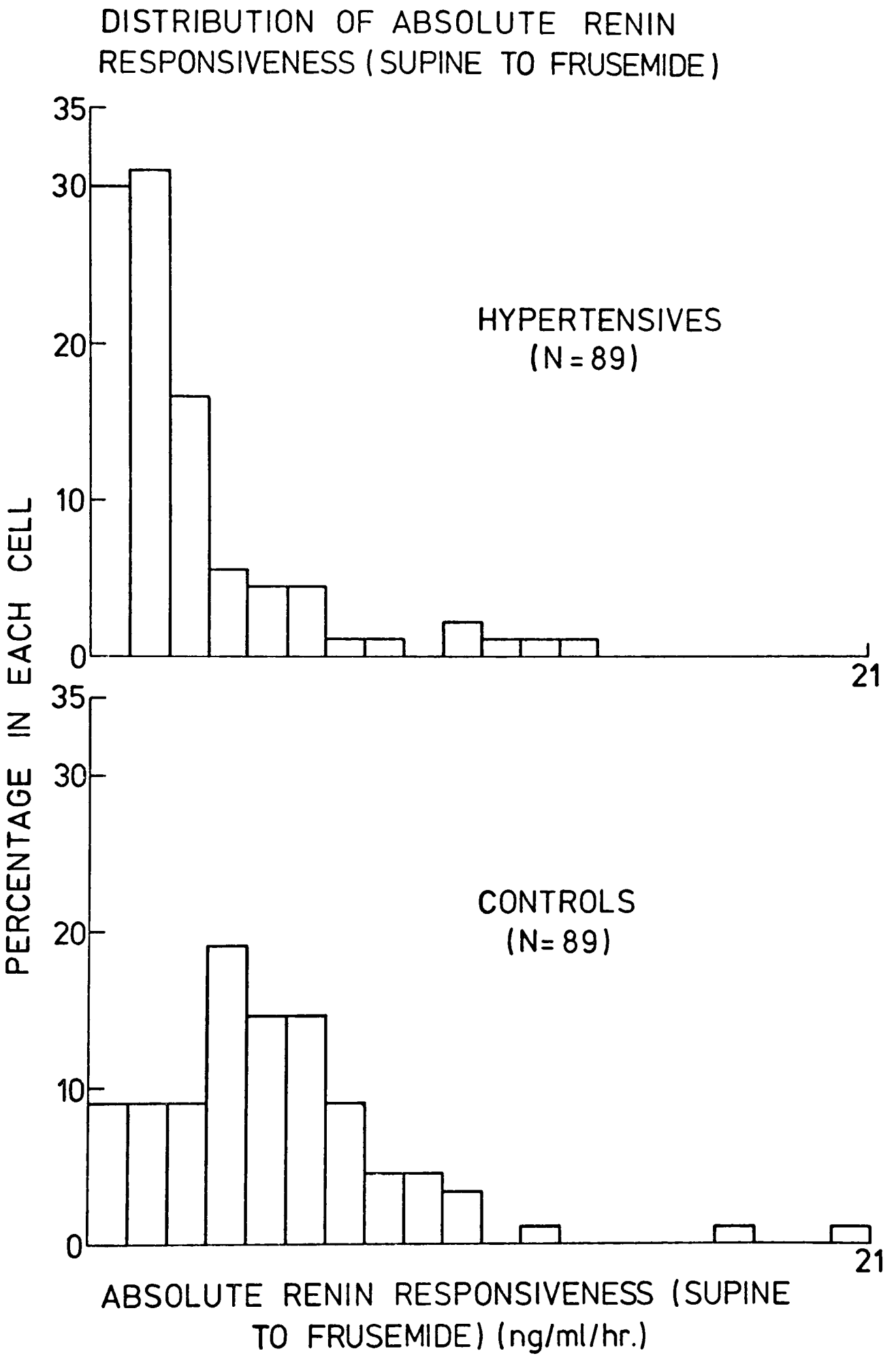

DISTRIBUTION OF ABSOLUTE RENIN RESPONSIVENESS (SUPINE TO FRUSEMIDE)
HYPERTENSIVES (N = 89)
CONTROLS (N= 89)
PERCENTAGE IN EACH CELL
35
30
20
10
0
21
ABSOLUTE RENIN RESPONSIVENESS (SUPINE TO FRUSEMIDE) (ng/ml/hr.)

FIGURE 12

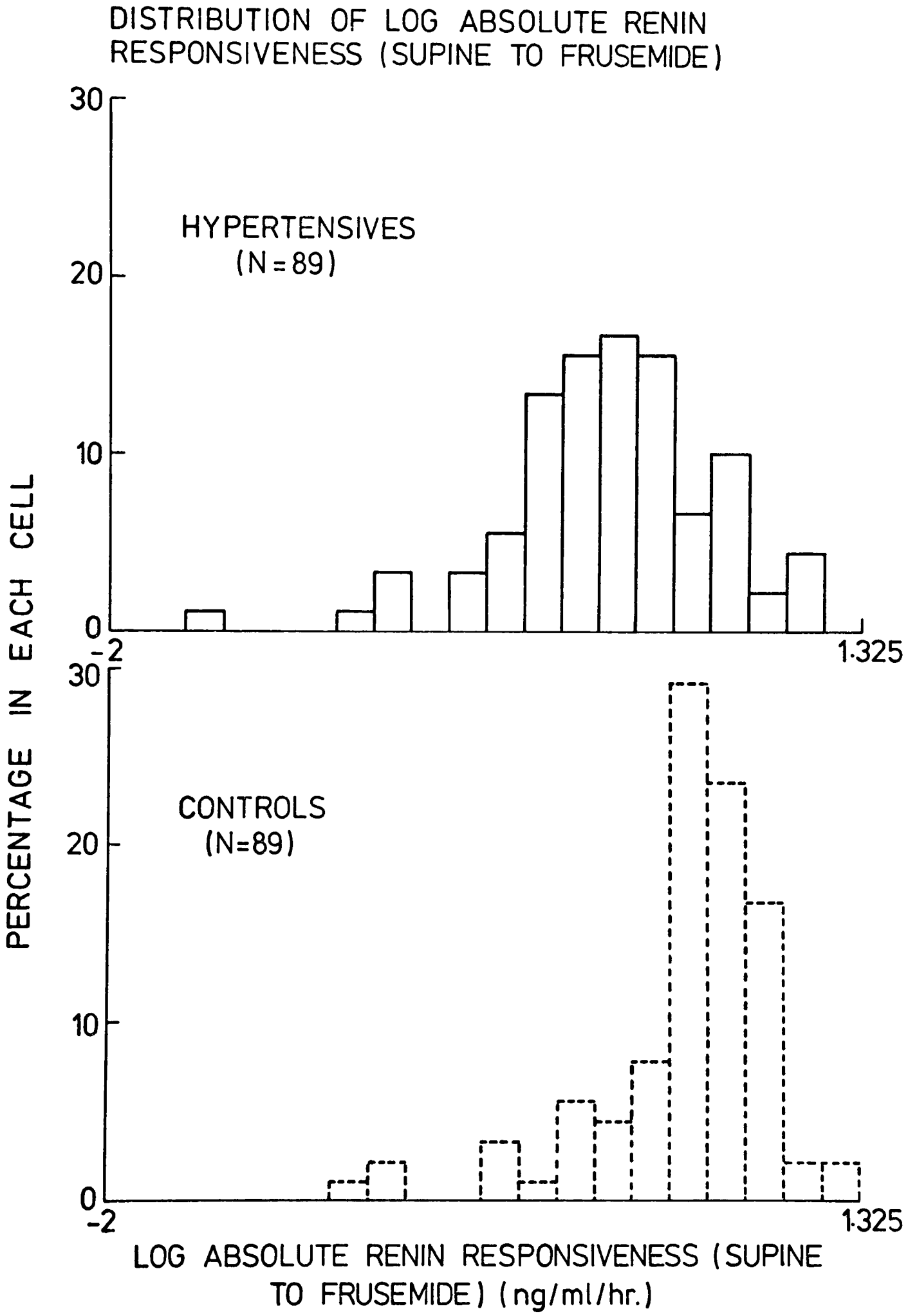

DISTRIBUTION OF LOG ABSOLUTE RENIN RESPONSIVENESS (SUPINE TO FRUSEMIDE)
HYPERTENSIVES (N=89)
CONTROLS (N=89)
PERCENTAGE IN EACH CELL
30
20
10
0
-2
1·325
LOG ABSOLUTE RENIN RESPONSIVENESS (SUPINE TO FRUSEMIDE) (ng/ml/hr.)

FIGURE 13

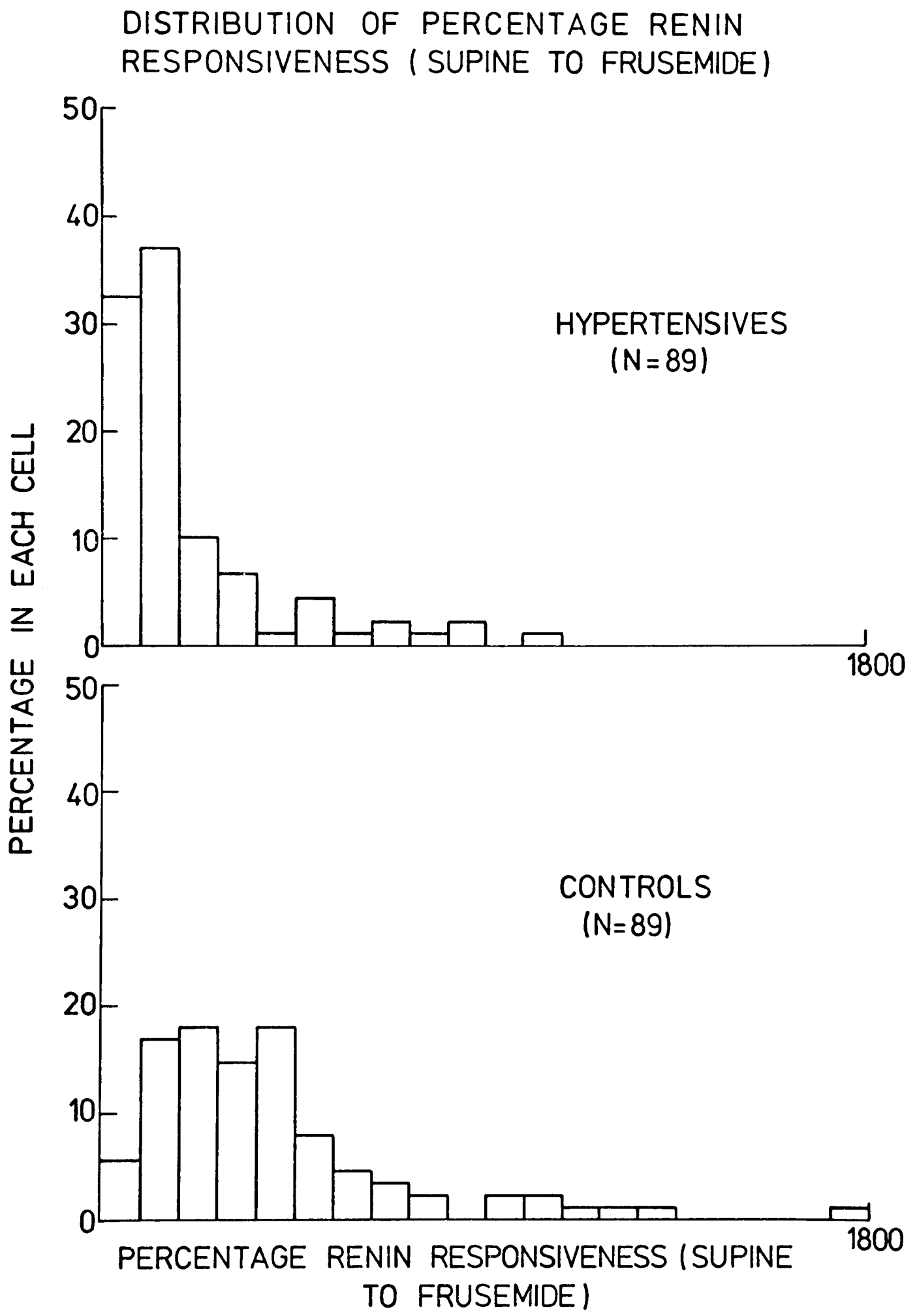

DISTRIBUTION OF PERCENTAGE RENIN RESPONSIVENESS (SUPINE TO FRUSEMIDE)
HYPERTENSIVES
(N=89)
CONTROLS
(N=89)
PERCENTAGE IN EACH CELL
0
10
20
30
40
50
1800
PERCENTAGE RENIN RESPONSIVENESS (SUPINE TO FRUSEMIDE)

FIGURE 14

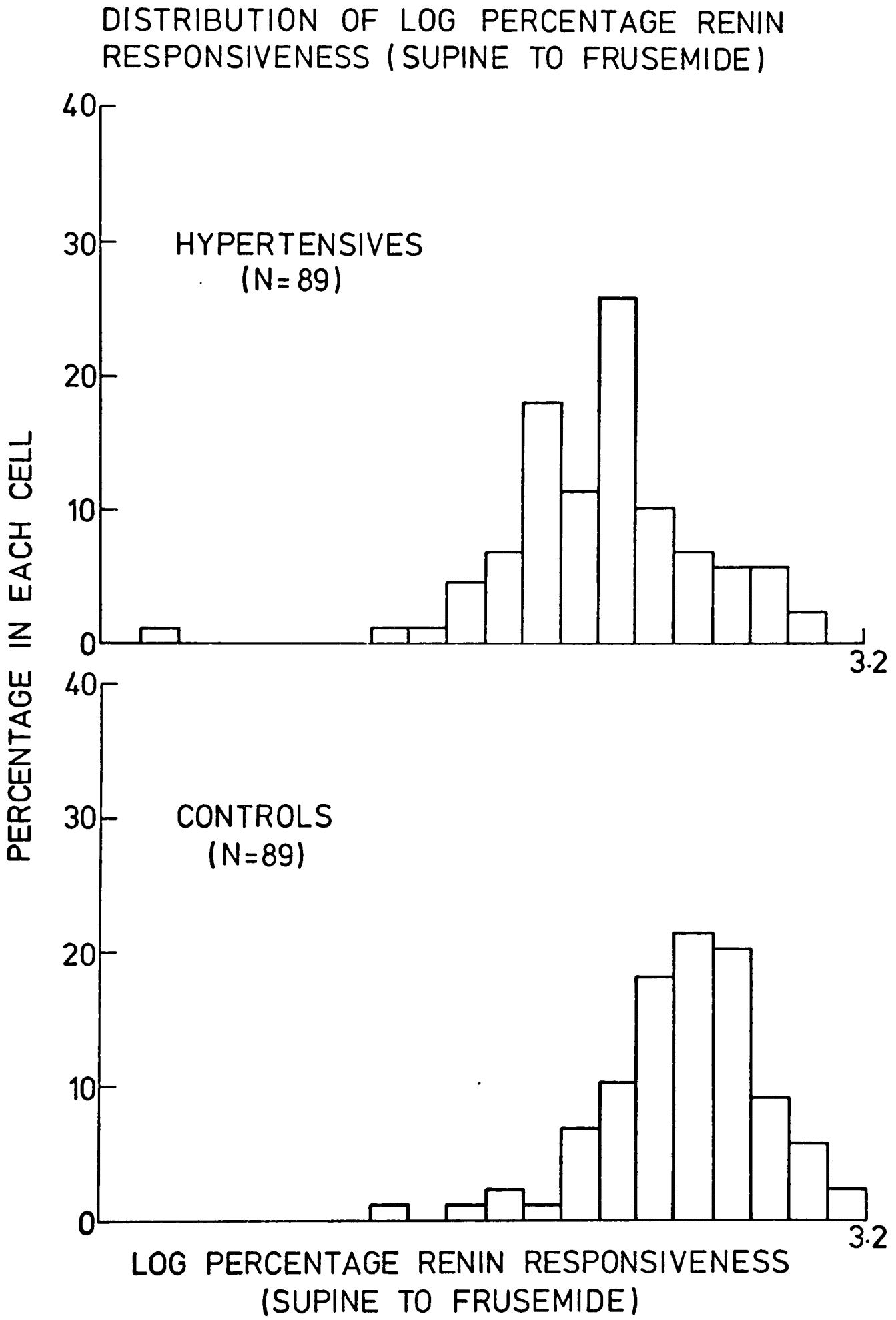

DISTRIBUTION OF LOG PERCENTAGE RENIN RESPONSIVENESS (SUPINE TO FRUSEMIDE)
HYPERTENSIVES
(N=89)
CONTROLS
(N=89)
PERCENTAGE IN EACH CELL
40
30
20
10
0
3·2
LOG PERCENTAGE RENIN RESPONSIVENESS
(SUPINE TO FRUSEMIDE)

FIGURE 15

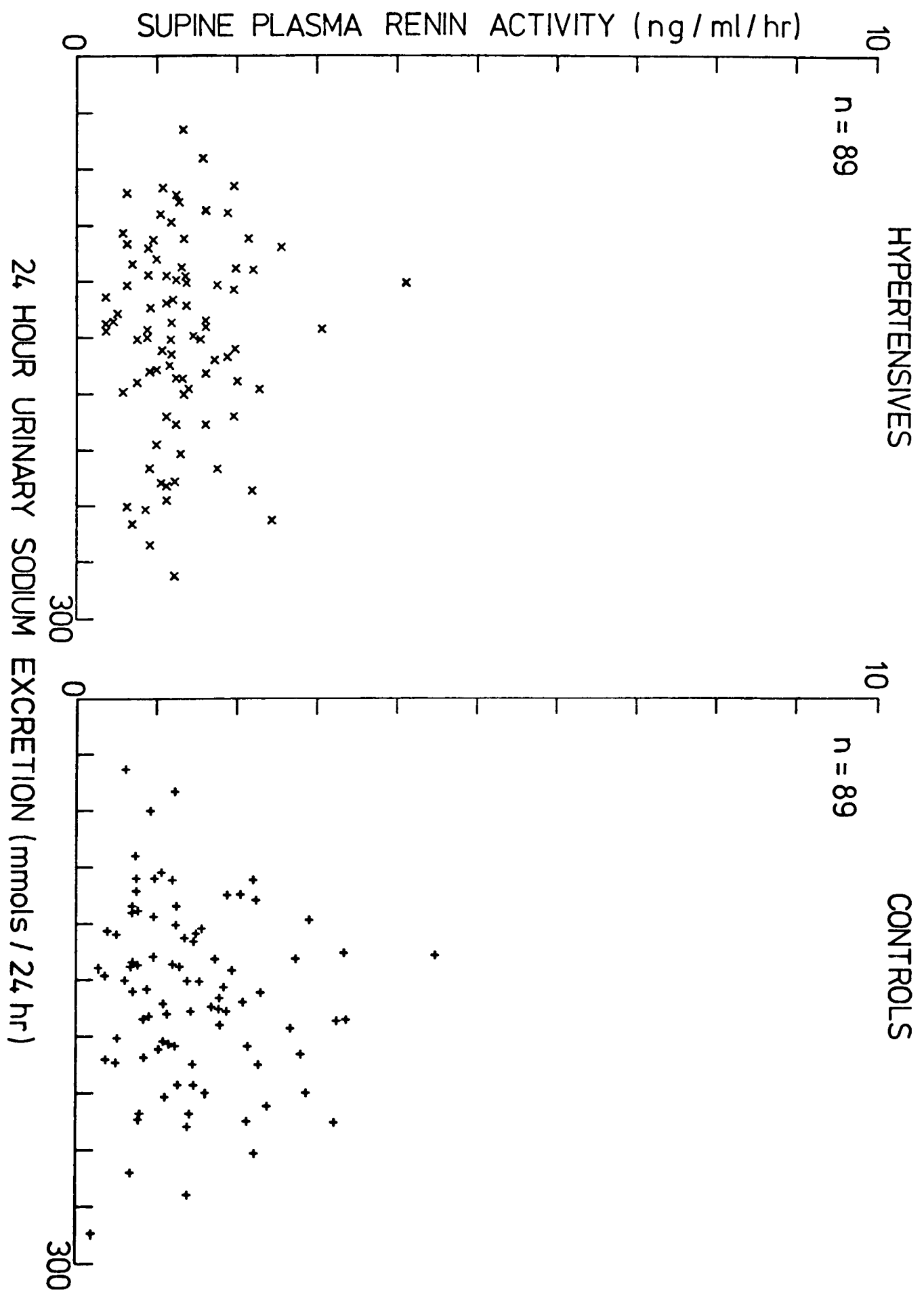
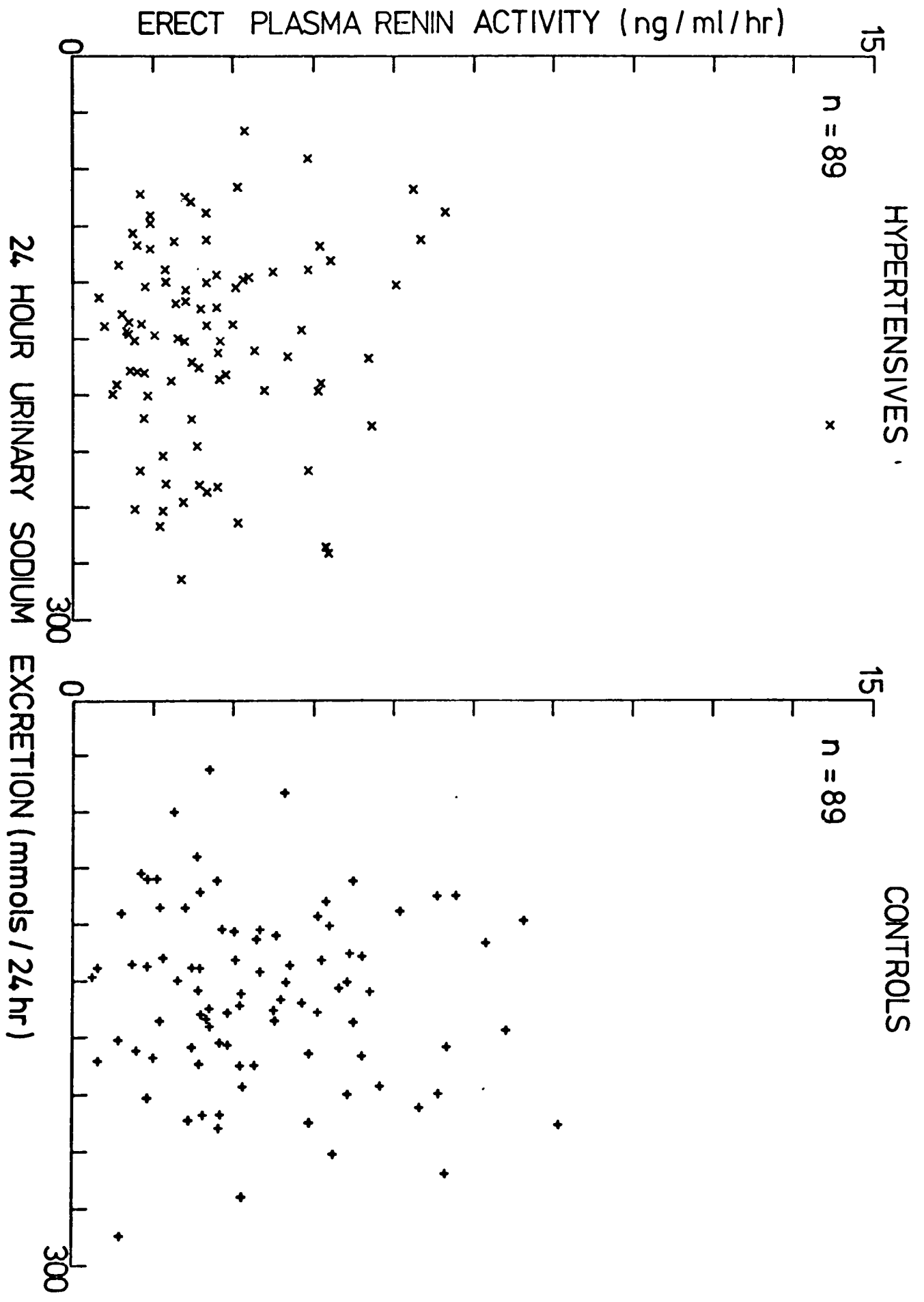

SUPINE PLASMA RENIN ACTIVITY (ng / ml / hr)
0
10
n = 89
HYPERTENSIVES
24 HOUR URINARY SODIUM EXCRETION (mmols / 24 hr)
300
0
10
n = 89
CONTROLS
300

FIGURE 16


ERECT PLASMA RENIN ACTIVITY (ng/ml/hr)
0
15
n = 89
HYPERTENSIVES
24 HOUR URINARY SODIUM EXCRETION (mmols/24 hr)
0
300
n = 89
CONTROLS

CHAPTER 6

RENIN: EFFECTS OF AGE AND SEX

In this chapter the age, sex and race of the 89 essential hypertensive patients and their matched controls will be discussed. The relationship of renin to age and sex is examined, together with an appraisal of the claim (32) that the low-renin subgroup may contain a predominance of females.

AGE

The age (mean $\pm$ SEM) of the hypertensive patients was 52.23 $\pm$ 0.80 years, which was similar to that of the control subjects (51.88 $\pm$ 0.75).

The paucity of female volunteers in the 60-plus age group necessitated 3 hypertensives in the 7th decade being matched with 3 females in the 6th decade, but in no case was the age discrepancy greater than 5 years. This accounts for the lack of identical mean ages in hypertensives and controls.

The scatter throughout the age range of 30 to 67 years is shown in decades in Table 18. There is a preponderance of numbers in the 5th and 6th decades. The mean age in each decade is shown.

The activity of the renin-angiotensin system tends to fall with age. Plasma renin activity (erect and lasix) and absolute renin responsiveness (ΔS-E, ΔS-L, ΔE-L) are significantly inversely correlated ($P<0.001$) with age, but

supine plasma renin activity correlates less well in controls and not at all in hypertensive patients. The percentage responsiveness is also inversely related to age in hypertensives, but not in control subjects (Tables 19 and 20, Figures 17 and 18).

As blood pressure influences renin levels (see next chapter), the relationship between renin and age was reanalysed taking blood pressure into account in a partial correlation. The results in most cases show that the correlation coefficients between renin and age are marginally lower with slightly weaker significance values (Table 21).

RACE

All the hypertensive and control subjects in this study were Caucasians.

SEX

There are 71 males and 18 females in the hypertensive group with the same number in the control group. The distribution of the males and females according to the age decades is shown in Table 18. There are fewer females in the study because most of the hypertensives came from UK AERE (Harwell), which has an excess of male exployees. The virtual absence of

young female hypertensive patients was due to the exclusion of cases taking oral contraceptive tablets.

Renin activity, however assessed, tended to be lower in females than in males, but the trend was significant in only two situations. The post-frusemide PRA (absolute value) was higher in males in both hypertensive and control groups ($P<0.05$) and the difference between the supine and post-frusemide PRA was also significantly higher in male hypertensives ($P<0.05$) but not in the control group (Tables 22 and 23).

When the effects of hypertension on renin were analysed for each sex separately, the results for males did not differ from those obtained when both sexes were taken together, i.e. renin activity was lower in the hypertensive group in all parameters except supine PRA. When women were analysed alone, the results were again similar to those obtained when both sexes were taken together, except for the erect PRA, which was not significantly lower in hypertensive than in control females (Tables 24 and 25).

The suggestion that the low-renin subgroup might contain more female patients was examined. The lower renin subgroup was defined according to the nine renin parameters (supine, erect and lasix PRA, and the absolute and percentage changes between these). The number of women in each of the 9 lower renin subgroups were not different from the expected number in 6 of them, but were increased in the groups defined by the

post-lasix PRA, the ΔS-L, ΔE-L results (Table 26).

SUMMARY

The mean age of the hypertensives was similar to that of the control subjects.

Most of the subjects were between 40 and 60 years of age.

There was a significant inverse correlation between age and renin.

The relationship between age and renin was independent of the effects of blood pressure on renin.

All hypertensive and control subjects were Caucasians.

There were 71 males and 18 females in the hypertensive group, with the same number in the control group.

Females tended to have lower renin levels and responsiveness than males, but this difference was only significant for lasix PRA and ΔS-L absolute responsiveness.

Comparisons between male hypertensives and male controls, or female hypertensives and female controls, were similar to hypertensives and controls considered as a whole.

An increased number of female subjects was found in the lower renin subgroup only when the patients were separated according to lasix PRA, ΔS-L or ΔE-L absolute responsiveness.

TABLE 18. AGE AND SEX DISTRIBUTION OF HYPERTENSIVE AND CONTROL SUBJECTS

Decade	Date of birth	Mean age (years)	Females (No.)	Males (No.)	Total (No.)
3rd	1955-1946	30	0	1	1
4th	1945-1936	36	0	3	3
5th	1935-1926	46	3	26	29
6th	1925-1916	55	10 (13)	34	44 (47)
7th	1915-1906	64	5 (2)	7	12 (9)
Total	1955-1906	52	18	71	89

Note: The figures in brackets are the number of controls where these differ from the number of hypertensives.

TABLE 19. CORRELATIONS BETWEEN RENIN AND AGE IN 89 HYPERTENSIVE PATIENTS

	Age	
	r	P
Plasma renin activity		
Supine	-0.1920	<0.071
Erect	-0.4414	<0.001
Lasix	-0.4820	<0.001
Absolute renin responsiveness		
ΔS-E	-0.4364	<0.001
ΔS-L	-0.4772	<0.001
ΔE-L	-0.3485	<0.001
Percentage renin responsiveness		
%S-E	-0.3637	<0.001
%S-L	-0.3800	<0.001
%E-L	-0.2291	<0.031

Note: S-E = change in PRA from supine to erect, S-L = change from supine to lasix, and E-L = change from erect to lasix.

r = Pearson's correlation coefficient
P = significance level

TABLE 20. CORRELATIONS BETWEEN RENIN AND AGE IN 89 NORMOTENSIVE CONTROL SUBJECTS

	Age	
	r	P
Plasma renin activity		
Supine	-0.2669	<0.011
Erect	-0.4048	<0.001
Lasix	-0.4434	<0.001
Absolute renin responsiveness		
ΔS-E	-0.3738	<0.001
ΔS-L	-0.4349	<0.001
ΔE-L	-0.3711	<0.001
Percentage renin responsiveness		
%S-E	-0.1209	<0.259
%S-L	-0.1566	<0.143
%E-L	-0.0695	<0.517

Note: S-E = change in PRA from supine to erect, S-L = change from supine to lasix, and E-L = change from erect to lasix.

r = Pearson's correlation coefficient
P = significance level

TABLE 21. PARTIAL CORRELATIONS BETWEEN RENIN AND AGE ALLOWING FOR BLOOD PRESSURE IN 89 HYPERTENSIVE AND 89 NORMOTENSIVE CONTROL SUBJECTS

Correcting for	Group	Age versus: Supine PRA	Erect PRA	Lasix PRA	Absolute Responsiveness ΔS-E	ΔS-L	ΔE-L	Percentage Responsiveness %S-L	%S-L	%E-L
Screening SBP	HT	r=-0.17	-0.41	-0.44	-0.41	-0.43	-0.29	-0.34	-0.33	-0.16
		P< 0.113	0.001	0.001	0.001	0.001	0.006	0.001	0.002	0.138
	C	r=-0.24	-0.37	-0.39	-0.34	-0.38	-0.31	-0.05	-0.08	-0.02
		P< 0.025	0.001	0.001	0.001	0.001	0.004	0.638	0.434	0.860
Screening DBP	HT	r=-0.17	-0.42	-0.45	-0.41	-0.45	-0.32	-0.35	-0.35	-0.19
		P< 0.118	0.001	0.001	0.001	0.001	0.003	0.001	0.001	0.075
	C	r=-0.24	-0.36	-0.39	-0.33	-0.38	-0.31	-0.05	-0.10	-0.07
		P< 0.024	0.001	0.001	0.002	0.001	0.003	0.638	0.365	0.497
Lying SBP	HT	r=-0.19	-0.44	-0.49	-0.44	-0.48	-0.35	-0.36	-0.38	-0.22
		P< 0.078	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.040
	C	r=-0.25	-0.39	-0.41	-0.37	-0.40	-0.32	-0.09	-0.12	-0.02
		P< 0.019	0.001	0.001	0.001	0.001	0.002	0.399	0.257	0.821
Lying DBP	HT	r=-0.20	-0.45	-0.50	-0.45	-0.49	-0.35	-0.36	-0.38	-0.23
		P< 0.066	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.035
	C	r=-0.27	-0.42	-0.43	-0.38	-0.42	-0.33	-0.11	-0.13	-0.04
		P< 0.011	0.001	0.001	0.001	0.001	0.002	0.323	0.213	0.685
Standing SBP	HT	r=-0.19	-0.44	-0.49	-0.44	-0.48	-0.34	-0.36	-0.38	-0.22
		P< 0.081	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.040
	C	r=-0.25	-0.39	-0.40	-0.36	-0.39	-0.30	-0.09	-0.11	-0.00
		P< 0.019	0.001	0.001	0.001	0.001	0.004	0.396	0.291	0.957
Standing DBP	HT	r=-0.19	-0.45	-0.50	-0.44	-0.50	-0.38	-0.37	-0.41	-0.25
		P< 0.072	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.018
	C	r=-0.28	-0.41	-0.43	-0.37	-0.42	-0.34	-0.09	-0.13	-0.07
		P< 0.007	0.001	0.001	0.001	0.001	0.001	0.393	0.213	0.502

Note: HT = hypertensive, C = control; SBP = systolic blood pressure, DBP = diastolic blood pressure (mm Hg)

TABLE 22. EFFECTS OF SEX ON RENIN IN ESSENTIAL HYPERTENSIVE PATIENTS

Parameter	Sex	Mean	Std. Dev.	SEM	N	t	f	P
PLASMA RENIN ACTIVITY (ng/ml/hr)								
Supine	F	1.11	0.51	0.12	18	1.46219	87	NS
	M	1.34	0.63	0.07	71			
Erect	F	1.93	0.80	0.19	18	1.93995	87	NS
	M	2.89	2.07	0.25	71			
Lasix	F	2.46	1.47	0.35	18	2.35505	87	<0.05
	M	4.21	3.06	0.36	71			
ABSOLUTE RESPONSIVENESS (ng/ml/hr)								
ΔS-E	F	0.82	0.50	0.12	18	1.63801	87	NS
	M	1.54	1.84	0.22	71			
ΔS-L	F	1.35	1.24	0.29	18	2.17285	87	<0.05
	M	2.86	0.86	0.34	71			
ΔE-L	F	0.58	0.82	0.19	18	1.88579	87	NS
	M	1.38	1.75	0.21	71			
PERCENTAGE RESPONSIVENESS								
%S-E	F	82.33	47.76	11.26	18	1.10151	87	NS
	M	116.49	128.85	15.30	71			
%S-L	F	122.56	105.38	24.84	18	1.85454	87	NS
	M	221.68	216.04	25.64	71			
%E-L	F	25.28	27.04	6.37	18	1.95647	87	NS
	M	50.97	53.87	6.39	71			

Note: Std. Dev. = standard deviation; SEM = standard error of mean; t = Student's unpaired t-test; f = degrees of freedom; F = female, M = male; S-E = change in PRA from supine to erect, S-L from supine to lasix, E-L from erect to lasix

TABLE 23. EFFECTS OF SEX ON RENIN IN NORMOTENSIVE CONTROL SUBJECTS

Parameter	Sex	Mean	Std. Dev.	SEM	N	t	f	P
PLASMA RENIN ACTIVITY (ng/ml/hr)								
Supine	F	1.15	0.39	0.09	18	1.70825	87	NS
	M	1.52	0.89	0.10	71			
Erect	F	2.74	1.69	0.40	18	1.87376	87	NS
	M	3.72	2.03	0.24	71			
Lasix	F	4.70	1.78	0.42	18	2.02851	87	<0.05
	M	6.69	4.04	0.45	71			
ABSOLUTE RESPONSIVENESS (ng/ml/hr)								
ΔS-E	F	1.59	1.36	0.32	18	1.60794	87	NS
	M	2.25	1.59	0.19	71			
ΔS-L	F	3.55	1.47	0.35	18	1.96607	87	NS
	M	5.25	3.57	0.42	71			
ΔE-L	F	1.96	0.87	0.21	18	1.57224	87	NS
	M	2.93	2.58	0.31	71			
PERCENTAGE RESPONSIVENESS								
%S-E	F	106.66	74.93	17.66	18	1.84908	87	NS
	M	188.00	182.12	21.61	71			
%S-L	F	311.06	112.93	26.62	18	1.35843	87	NS
	M	420.67	336.33	39.92	71			
%E-L	F	85.89	40.60	9.57	18	0.41949	87	NS
	M	80.25	53.11	6.30	71			

Note: Std. Dev. = standard deviation; SEM = standard error of mean; t = Student's unpaired t-test; f = degrees of freedom; F = female, M = male; S-E = change in PRA from supine to erect, S-L from supine to lasix, E-L from erect to lasix

TABLE 24. PLASMA RENIN ACTIVITY AND ITS RESPONSIVENESS IN MALE HYPERTENSIVE AND CONTROL SUBJECTS

Parameter	Group	Mean	Std. Dev.	SEM	N	t	f	P
PLASMA RENIN ACTIVITY (ng/ml/hr)								
Supine	HT	1.34	0.63	0.07	71	1.41210	140	NS
	C	1.52	0.89	0.11	71			
Erect	HT	2.89	2.07	0.25	71	2.52338	140	<0.02
	C	3.75	1.99	0.24	71			
Lasix	HT	4.21	3.06	0.36	71	4.11807	140	<0.001
	C	6.69	4.04	0.48	71			
ABSOLUTE RESPONSIVENESS (ng/ml/hr)								
ΔS-E	HT	1.55	1.85	0.22	71	2.42697	140	<0.02
	C	2.25	1.60	0.19	71			
ΔS-L	HT	2.85	2.87	0.34	71	4.43964	140	<0.001
	C	5.27	3.58	0.42	71			
ΔE-L	HT	1.38	1.75	0.21	71	4.17413	140	<0.001
	C	2.93	2.59	0.31	71			
PERCENTAGE RESPONSIVENESS								
%S-E	HT	116.49	129.07	15.32	71	2.69820	140	<0.01
	C	188.00	182.23	21.63	71			
%S-L	HT	226.51	217.99	25.87	71	4.08203	140	<0.001
	C	420.68	336.33	39.92	71			
%E-L	HT	53.97	50.75	6.02	71	3.28171	140	<0.005
	C	80.25	53.17	6.31	71			

Note: Std. Dev. = standard deviation; SEM = standard error of mean; t = Student's unpaired t-test; f = degrees of freedom; HT = hypertensive, C = control; S-E = change in PRA from supine to erect, S-L from supine to lasix, E-L from erect to lasix.

TABLE 25. PLASMA RENIN ACTIVITY AND ITS RESPONSIVENESS IN FEMALE HYPERTENSIVE AND CONTROL SUBJECTS

Parameter	Group	Mean	Std. Dev.	SEM	N	t	f	P
PLASMA RENIN ACTIVITY (ng/ml/hr)								
Supine	HT	1.11	0.51	0.12	18	0.32324	34	NS
	C	1.15	0.39	0.09	18			
Erect	HT	1.93	0.79	0.19	18	1.86086	34	NS
	C	2.74	1.69	0.40	18			
Lasix	HT	2.46	1.47	0.35	18	4.12252	34	<0.001
	C	4.70	1.78	0.42	18			
ABSOLUTE RESPONSIVENESS (ng/ml/hr)								
ΔS-E	HT	0.82	0.50	0.12	18	2.25181	34	<0.05
	C	1.59	1.36	0.32	18			
ΔS-L	HT	1.35	1.24	0.29	18	4.84669	34	<0.001
	C	3.55	1.47	0.35	18			
ΔE-L	HT	0.58	0.82	0.19	18	4.89172	34	<0.001
	C	1.96	0.87	0.21	18			
PERCENTAGE RESPONSIVENESS								
%S-E	HT	82.33	47.76	11.26	18	2.29233	34	<0.05
	C	128.33	70.48	16.61	18			
%S-L	HT	122.56	105.38	24.84	18	5.17768	34	<0.001
	C	311.06	112.93	26.62	18			
%E-L	HT	25.28	27.04	6.37	18	5.27139	34	<0.001
	C	85.89	40.60	9.57	18			

Note: Std. Dev. = standard deviation; SEM = standard error of mean; t = Student's unpaired t-test; f = degrees of freedom; HT = hypertensive, C = control; S-E = change in PRA from supine to erect, S-L from supine to lasix, E-L from erect to lasix.

TABLE 26. INCIDENCE OF FEMALES IN LOWER RENIN SUBGROUPS IN ESSENTIAL HYPERTENSIVE PATIENTS

Grouped according to:	Lower renin subgroup		Other hypertensives			
	No. in group	No. of females	No. in group	No. of females	χ^2	P
PLASMA RENIN ACTIVITY						
Supine	29	8	60	10	1.07	NS
Erect	29	8	60	10	1.07	NS
Lasix	29	10	60	8	4.00	<0.05
ABSOLUTE RESPONSIVENESS						
ΔS-E	29	5	60	13	0.25	NS
ΔS-L	29	10	60	8	4.00	<0.05
ΔE-L	29	10	60	8	4.00	<0.05
PERCENTAGE RESPONSIVENESS						
%S-E	29	6	60	12	0.00	NS
%S-L	29	8	60	10	1.07	NS
%E-L	29	8	60	10	1.07	NS

Note: S-E = change in PRA from supine to erect, S-L from supine to lasix, E-L from erect to lasix; χ^2 = significance test with 1 degree of freedom.

FIGURE 17

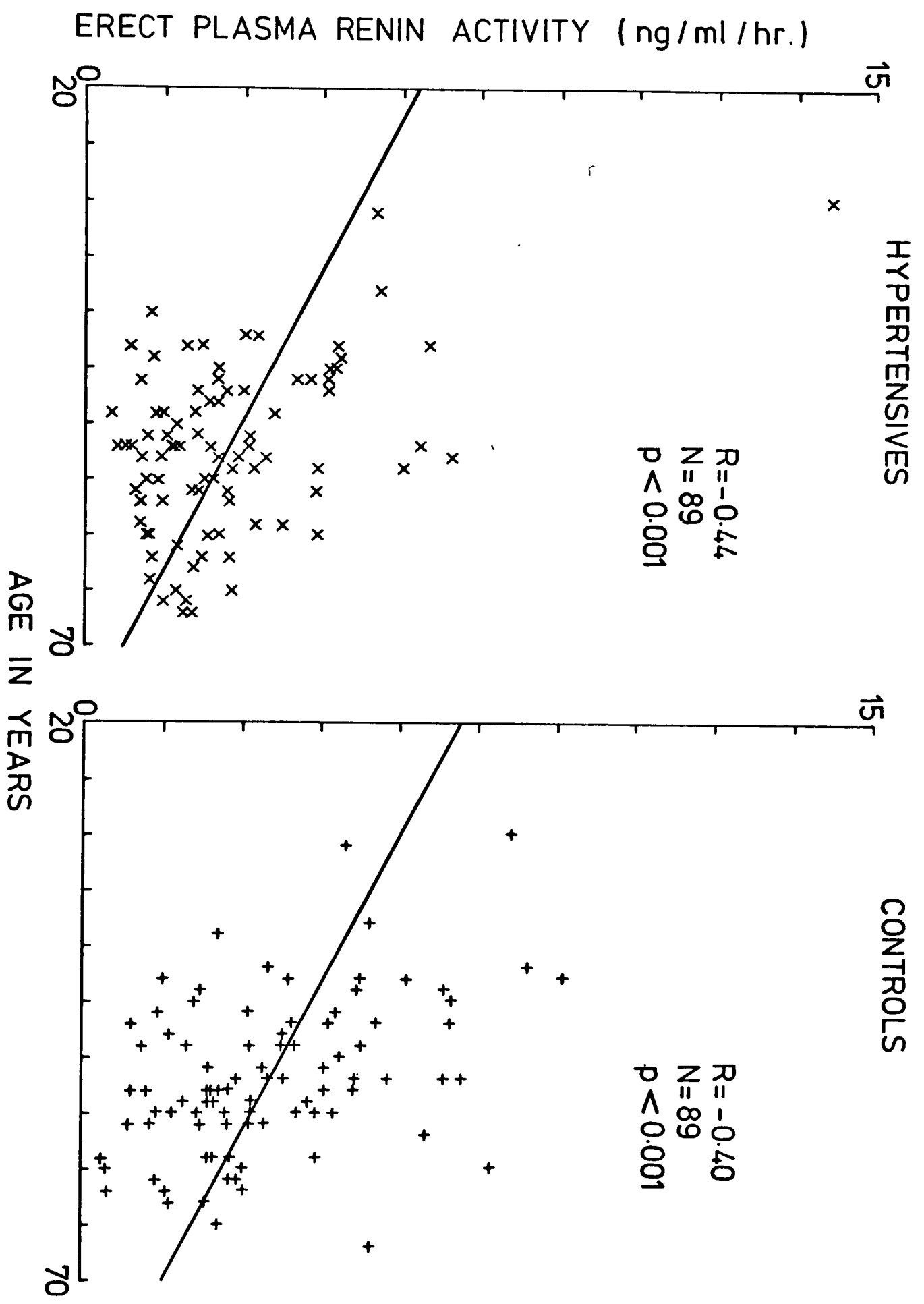
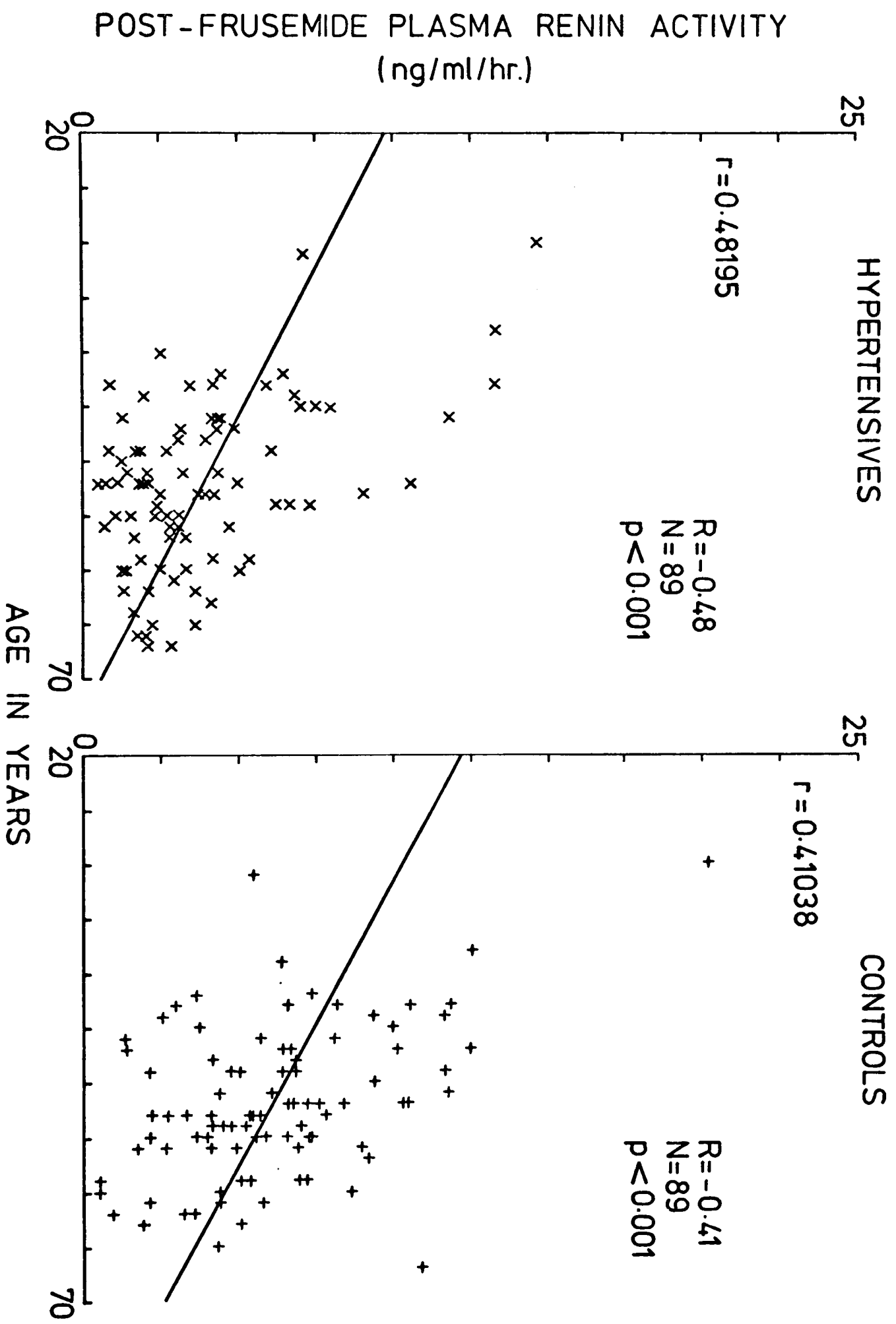

ERECT PLASMA RENIN ACTIVITY (ng/ml/hr.)
0
20
15
70
AGE IN YEARS
HYPERTENSIVES
R=-0.44
N=89
P<0.001
CONTROLS
R=-0.40
N=89
P<0.001

FIGURE 18


POST-FRUSEMIDE PLASMA RENIN ACTIVITY
(ng/ml/hr.)
0
20
25
r=0·48195
HYPERTENSIVES
R=-0·48
N=89
P<0·001
AGE IN YEARS
70
0
20
25
r=0·41038
CONTROLS
R=-0·41
N=89
P<0·001
70

CHAPTER 7

RENIN: EFFECTS OF BLOOD PRESSURE

This chapter describes the blood pressure characteristics of the essential hypertensive and control subjects. The effects of blood pressure on plasma renin and its responsiveness are discussed, together with the influence of age on this relationship.

BLOOD PRESSURE

Screening blood pressures are the mean of three measurements taken seated and at rest, at the first attendance at a screening session, and at two subsequent visits one week apart. The lying and standing blood pressures were recorded on the fourth visit, and were taken after 5 minutes in the recumbent and in the erect position. Values for systolic (SBP), diastolic (DBP) and mean blood pressure (MBP) are reported. The diastolic blood pressure was recorded at the disappearance of the 5th Korotkoff sound. The mean blood pressure was calculated from the addition of 1/3 pulse pressure to the diastolic blood pressure. All blood pressure readings are expressed in millimetres of mercury (mm Hg), and apart from the initial screening blood pressure, which was recorded by a Random-Zero sphygmomanometer, all blood pressures were measured using the London School of Hygiene sphygmomanometer.

In all situations the systolic, diastolic and mean blood pressures were signficantly greater ($P<0.001$) in the hypertensives than in the control subjects. The lying systolic blood pressure (mean $\pm$ SEM) was 161.53 $\pm$ 2.05 mm Hg in the hypertensives and 123.01 $\pm$ 1.28 mm Hg in the controls, while the lying diastolic blood pressure was 104.24 $\pm$ 0.85 mm Hg in the former and 77.98 $\pm$ 1.38 mm Hg in the latter. Within hypertensive or control groups, there were no statistical differences between systolic, diastolic and mean blood pressures taken lying, standing or at screening (Table 27).

RENIN AND BLOOD PRESSURE

The relationships of blood pressure to renin were studied. There were significant inverse correlations between renin and its responsiveness and most of the blood pressure measurements in both hypertensive and control subjects (Tables 28 and 29, and Figures 19, 20, 21 and 22).

Plasma renin activity (supine, erect, lasix): supine PRA did not correlate with any of the blood pressures in either hypertensives or controls. The most significant correlations occurred with lasix PRA, in both groups. The correlation coefficients for the hypertensives and control subjects combined were distinctly higher than for the two samples considered separately (Figures 20 and 22).

Absolute renin responsiveness (ΔS-E, ΔS-L, ΔE-L): the least number of correlations occurred with ΔS-E, but the lasix variable (ΔS-L) again showed greatest number of significant relationships in both hypertensive and control subjects.

Percentage renin responsiveness (%S-E, %S-L, %E-L): in the control group, percentage responsiveness related poorly with blood pressure. The same applied to %S-E responsiveness in hypertensives, but %S-L and %E-L responses correlated well with almost all blood pressure measurements.

RELATIONSHIP OF RENIN TO BLOOD PRESSURE CORRECTED FOR AGE

Plasma renin activity and its responsiveness have been shown to vary inversely with age. In order to examine this effect on the inverse relationship between renin and blood pressure, the data were re-analysed allowing for the effects of age in a partial correlation.

Age was shown to be contributing significantly to the renin-blood pressure relationship. In control subjects the significance of all but 7 correlations was lost when the effects of age were allowed for. Those which remained significant were lasix PRA with standing mean blood pressure ($P<0.031$), ΔS-E response with standing diastolic ($P<0.018$), and mean ($P<0.032$) blood pressure, ΔS-L response with standing systolic ($P<0.05$) and mean ($P<0.023$) blood pressure, %S-E response with screening mean blood pressure ($P<0.044$), and %E-L with standing systolic blood pressure ($P<0.030$) (Table 30). The standing blood pressure measurements are shown to correlate more frequently with the renin parameters than screening or lying readings in control subjects.

In the hypertensive group, age was also shown to be influencing the renin-blood pressure relationship, but not to the same extent as in control subjects. The renin parameters involving lasix retained their inverse relationships with blood pressure although in most cases the significance levels were slightly weaker. Erect PRA remained significantly correlated with lying systolic, diastolic and mean blood pressures, and with standing systolic blood pressure (Table 31).

SUMMARY

Screening, lying and standing blood pressures were recorded in all subjects.

The systolic, diastolic and mean blood pressures were significantly greater in hypertensives than in control subjects.

In most situations there were significant inverse correlations between renin (and its responsiveness) and blood pressure.

Age was shown to be contributing significantly to the renin-blood pressure relationships in hypertensive and control subjects.

TABLE 27. BLOOD PRESSURES IN HYPERTENSIVE AND CONTROL SUBJECTS

Parameter	Group	Mean	Std. Dev.	SEM	N	t	f	P
SCREENING BLOOD PRESSURE (mm Hg)								
Systolic	HT	163.49	19.48	2.07	89	14.61618	176	<0.001
	C	126.07	14.16	1.50	89			
Diastolic	HT	106.56	7.82	0.83	89	24.19441	176	<0.001
	C	77.58	8.09	0.86	89			
Mean	HT	125.34	10.24	1.09	89	21.62272	176	<0.001
	C	93.94	9.16	0.97	89			
LYING BLOOD PRESSURE (mm Hg)								
Systolic	HT	161.53	19.32	2.05	89	15.54417	176	<0.001
	C	123.01	12.09	1.28	89			
Diastolic	HT	104.24	8.06	0.85	89	22.76830	176	<0.001
	C	77.98	12.98	1.38	89			
Mean	HT	123.55	11.13	1.18	89	21.24942	176	<0.001
	C	93.44	13.54	1.44	89			
STANDING BLOOD PRESSURE (mm Hg)								
Systolic	HT	157.38	19.83	2.10	89	14.30722	176	<0.001
	C	120.42	14.18	1.50	89			
Diastolic	HT	108.08	7.50	0.80	89	22.23507	176	<0.001
	C	82.17	8.04	0.85	89			
Mean	HT	124.38	10.19	1.08	89	20.80880	176	<0.001
	C	94.88	8.67	0.92	89			

Note: HT = hypertensives, C = controls

TABLE 28. CORRELATIONS OF RENIN WITH BLOOD PRESSURE IN 89 ESSENTIAL HYPERTENSIVE PATIENTS

Parameter		Plasma Renin Activity			Absolute Responsiveness			Percentage Responsiveness		
		Supine	Erect	Lasix	ΔS-E	ΔS-L	ΔE-L	%S-E	%S-L	%E-L
Screening	SBP	r=-0.13 P< 0.231	-0.24 0.026	-0.34 0.001	-0.22 0.035	-0.34 0.001	-0.33 0.002	-0.15 0.153	-0.29 0.006	-0.34 0.001
	DBP	r=-0.15 P< 0.169	-0.23 0.033	-0.27 0.010	-0.21 0.051	-0.26 0.014	-0.23 0.031	-0.13 0.243	-0.20 0.058	-0.21 0.046
	MBP	r=-0.15 P< 0.161	-0.26 0.013	-0.35 0.001	-0.25 0.020	-0.34 0.001	-0.32 0.002	-0.16 0.129	-0.29 0.007	-0.32 0.002
Lying	SBP	r=-0.15 P< 0.153	-0.23 0.027	-0.30 0.004	-0.21 0.045	-0.29 0.006	-0.26 0.014	-0.14 0.179	-0.26 0.016	-0.23 0.027
	DBP	r=-0.01 P< 0.906	-0.24 0.022	-0.30 0.004	-0.27 0.010	-0.32 0.002	-0.24 0.022	-0.23 0.032	-0.29 0.006	-0.17 0.114
	MBP	r=-0.13 P< 0.220	-0.28 0.009	-0.34 0.001	-0.27 0.011	-0.34 0.001	-0.28 0.007	-0.19 0.069	-0.29 0.005	-0.23 0.031
Standing	SBP	r=-0.19 P< 0.080	-0.24 0.023	-0.31 0.003	-0.21 0.051	-0.30 0.005	-0.28 0.008	-0.14 0.206	-0.26 0.013	-0.27 0.010
	DBP	r=-0.11 P< 0.321	-0.02 0.859	-0.16 0.142	-0.06 0.594	-0.19 0.069	-0.26 0.014	-0.09 0.423	-0.25 0.018	-0.23 0.030
	MBP	r=-0.07 P< 0.544	-0.16 0.125	-0.28 0.009	-0.16 0.130	-0.28 0.007	-0.30 0.004	-0.13 0.226	-0.29 0.006	-0.28 0.008

Note: SBP = systolic blood pressure, DBP = diastolic blood pressure, MBP = mean blood pressure;
r = Pearson's correlation coefficient, P = significance level

TABLE 29. CORRELATIONS OF RENIN WITH BLOOD PRESSURE IN NORMOTENSIVE CONTROL SUBJECTS

		Plasma Renin Activity			Absolute Responsiveness			Percentage Responsiveness		
Parameter		Supine	Erect	Lasix	ΔS-E	ΔS-L	ΔE-L	%S-E	%S-L	%E-L
Screening	SBP	r=-0.16 P< 0.137	-0.26 0.013	-0.28 0.009	-0.25 0.018	-0.27 0.010	-0.22 0.035	-0.21 0.052	-0.22 0.037	-0.14 0.177
	DBP	r=-0.16 P< 0.130	-0.31 0.003	-0.31 0.004	-0.31 0.004	-0.29 0.005	-0.23 0.029	-0.23 0.029	-0.21 0.053	-0.00 0.999
	MBP	r=-0.19 P< 0.081	-0.31 0.003	-0.33 0.002	-0.30 0.004	-0.32 0.002	-0.27 0.011	-0.24 0.021	-0.24 0.021	-0.09 0.405
Lying	SBP	r=-0.14 P< 0.179	-0.21 0.050	-0.23 0.028	-0.19 0.073	-0.22 0.040	-0.20 0.063	-0.11 0.291	-0.14 0.206	-0.15 0.157
	DBP	r=-0.08 P< 0.448	-0.15 0.156	-0.23 0.030	-0.15 0.162	-0.23 0.028	-0.24 0.023	-0.10 0.366	-0.16 0.144	-0.16 0.135
	MBP	r=-0.03 P< 0.774	-0.12 0.255	-0.17 0.107	-0.14 0.193	-0.18 0.093	-0.17 0.105	-0.12 0.283	-0.15 0.168	-0.12 0.250
Standing	SBP	r=-0.15 P< 0.161	-0.23 0.027	-0.30 0.004	-0.22 0.039	-0.31 0.003	-0.29 0.006	-0.13 0.241	-0.18 0.086	-0.24 0.023
	DBP	r=-0.00 P< 0.998	-0.24 0.026	-0.24 0.022	-0.30 0.004	-0.26 0.015	-0.19 0.075	-0.21 0.054	-0.17 0.114	-0.01 0.897
	MBP	r=-0.09 P< 0.420	-0.28 0.009	-0.31 0.003	-0.30 0.004	-0.32 0.002	-0.27 0.011	-0.19 0.074	-0.19 0.074	-0.11 0.314

Note: SBP = systolic blood pressure, DBP = diastolic blood pressure, MBP = mean blood pressure
r = Pearson's correlation coefficient, P = significance level

TABLE 30. RELATIONSHIP OF RENIN TO BLOOD PRESSURE ALLOWING FOR AGE IN 89 NORMOTENSIVE CONTROL SUBJECTS

Parameter		Plasma Renin Activity			Absolute Responsiveness			Percentage Responsiveness		
		Supine	Erect	Lasix	ΔS-E	ΔS-L	ΔE-L	%S-E	%S-L	%E-L
Screening	SBP	r=-0.06	-0.12	-0.13	-0.12	-0.12	-0.10	-0.18	-0.18	-0.13
		P< 0.579	0.277	0.235	0.283	0.252	0.352	0.100	0.094	0.234
	DBP	r=-0.07	-0.19	-0.18	-0.20	-0.17	-0.12	-0.20	-0.17	-0.02
		P< 0.502	0.077	0.094	0.069	0.121	0.259	0.056	0.120	0.828
	MBP	r=-0.08	-0.17	-0.18	-0.16	-0.17	-0.14	-0.22	-0.20	-0.07
		P< 0.443	0.118	0.091	0.126	0.115	0.190	0.044	0.059	0.528
Lying	SBP	r=-0.06	-0.09	-0.11	-0.08	-0.10	-0.10	-0.08	-0.09	-0.14
		P< 0.560	0.415	0.305	0.474	0.373	0.366	0.456	0.390	0.203
	DBP	r=-0.03	-0.08	-0.17	-0.08	-0.17	-0.19	-0.08	-0.13	-0.15
		P< 0.774	0.458	0.122	0.444	0.114	0.078	0.469	0.216	0.161
	MBP	r=-0.04	-0.01	-0.07	-0.04	-0.08	-0.09	-0.09	-0.11	-0.11
		P< 0.688	0.900	0.543	0.698	0.480	0.409	0.409	0.289	0.307
Standing	SBP	r=-0.08	-0.13	-0.20	-0.12	-0.21	-0.21	-0.10	-0.15	-0.23
		P< 0.483	0.236	0.057	0.272	0.050	0.054	0.365	0.166	0.030
	DBP	r=-0.05	-0.18	-0.18	-0.25	-0.20	-0.14	-0.19	-0.15	-0.03
		P< 0.627	0.094	0.085	0.018	0.057	0.202	0.076	0.168	0.817
	MBP	r=-0.02	-0.19	-0.23	-0.23	-0.24	-0.20	-0.17	-0.16	-0.09
		P< 0.880	0.076	0.031	0.032	0.023	0.067	0.117	0.136	0.380

Note: SBP = systolic blood pressure, DBP = diastolic blood pressure, MBP = mean blood pressure
r = partial correlation coefficient, P = significance level

TABLE 31. RELATIONSHIP OF RENIN TO BLOOD PRESSURE ALLOWING FOR AGE IN 89 ESSENTIAL HYPERTENSIVE PATIENTS

Parameter		Plasma Renin Activity			Absolute Responsiveness			Percentage Responsiveness		
		Supine	Erect	Lasix	ΔS-E	ΔS-L	ΔE-L	%S-E	%S-L	%E-L
Screening	SBP	r=-0.08	-0.13	-0.24	-0.12	-0.24	-0.26	-0.07	-0.22	-0.30
		P< 0.464	0.218	0.022	0.266	0.023	0.014	0.525	0.040	0.005
	DBP	r=-0.10	-0.13	-0.18	-0.11	-0.17	-0.16	-0.05	-0.13	-0.17
		P< 0.338	0.216	0.096	0.294	0.122	0.147	0.650	0.225	0.113
	MBP	r=-0.10	-0.15	-0.24	-0.14	-0.24	-0.24	-0.07	-0.21	-0.27
		P< 0.366	0.159	0.022	0.209	0.026	0.024	0.507	0.054	0.010
Lying	SBP	r=-0.14	-0.23	-0.30	-0.20	-0.29	-0.25	-0.13	-0.25	-0.22
		P< 0.189	0.035	0.004	0.059	0.006	0.019	0.238	0.020	0.035
	DBP	r=0.00	-0.25	-0.32	-0.28	-0.34	-0.24	-0.23	-0.30	-0.16
		P< 0.980	0.021	0.003	0.008	0.001	0.024	0.034	0.005	0.129
	MBP	r=-0.11	-0.25	-0.33	-0.24	-0.32	-0.26	-0.17	-0.27	-0.21
		P< 0.305	0.018	0.002	0.023	0.002	0.015	0.125	0.011	0.049
Standing	SBP	r=-0.18	-0.23	-0.32	-0.20	-0.30	-0.27	-0.12	-0.26	-0.26
		P< 0.101	0.028	0.003	0.068	0.005	0.011	0.269	0.016	0.013
	DBP	r=-0.10	-0.05	-0.21	-0.09	-0.25	-0.30	-0.12	-0.30	-0.25
		P< 0.367	0.645	0.048	0.394	0.017	0.005	0.272	0.005	0.017
	MBP	r=-0.06	-0.17	-0.30	-0.17	-0.31	-0.32	-0.13	-0.31	-0.29
		P< 0.562	0.106	0.004	0.112	0.003	0.003	0.211	0.004	0.007

Note: SBP = systolic blood pressure, DBP = diastolic blood pressure, MBP = mean blood pressure
r = partial correlation coefficient, P = significance level

FIGURE 19

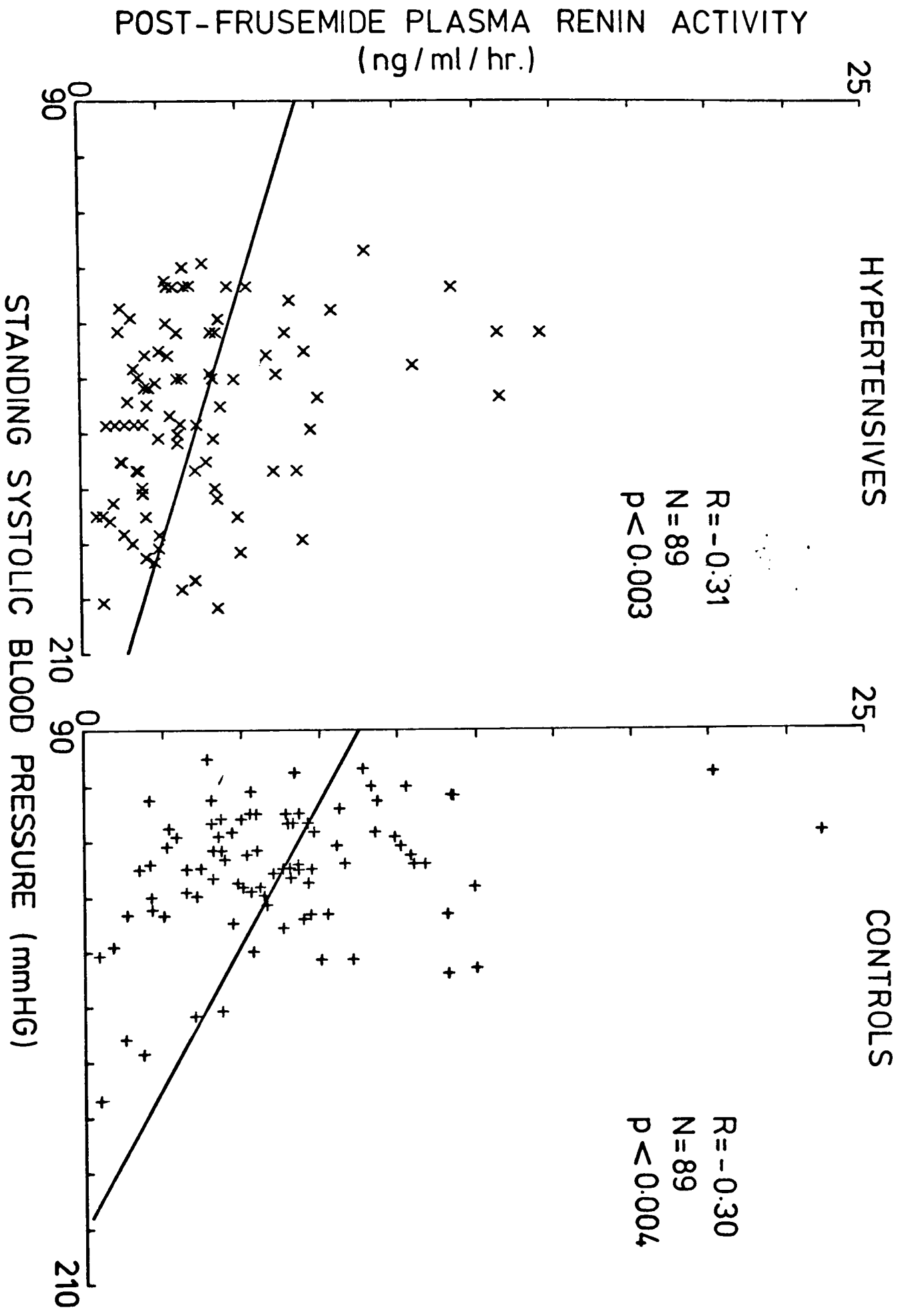

POST-FRUSEMIDE PLASMA RENIN ACTIVITY
(ng/ml/hr.)
0
25
90
210
STANDING SYSTOLIC BLOOD PRESSURE (mmHG)
HYPERTENSIVES
R=-0.31
N=89
P<0.003
CONTROLS
R=-0.30
N=89
P<0.004

FIGURE 20

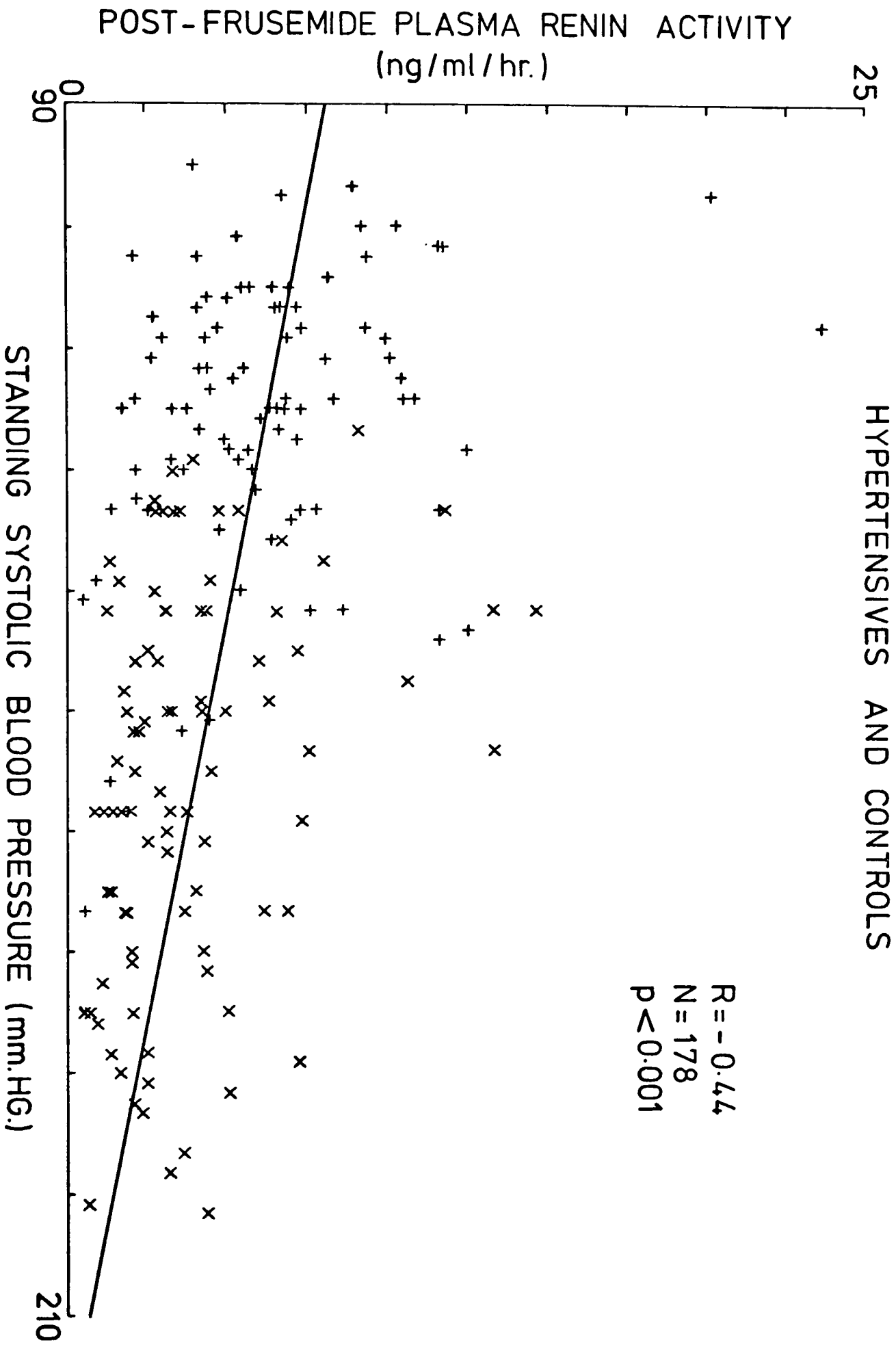

POST-FRUSEMIDE PLASMA RENIN ACTIVITY
(ng/ml/hr.)
0
25
90
210
STANDING SYSTOLIC BLOOD PRESSURE (mm.HG.)
HYPERTENSIVES AND CONTROLS
R=-0.44
N=178
P<0.001

FIGURE 21

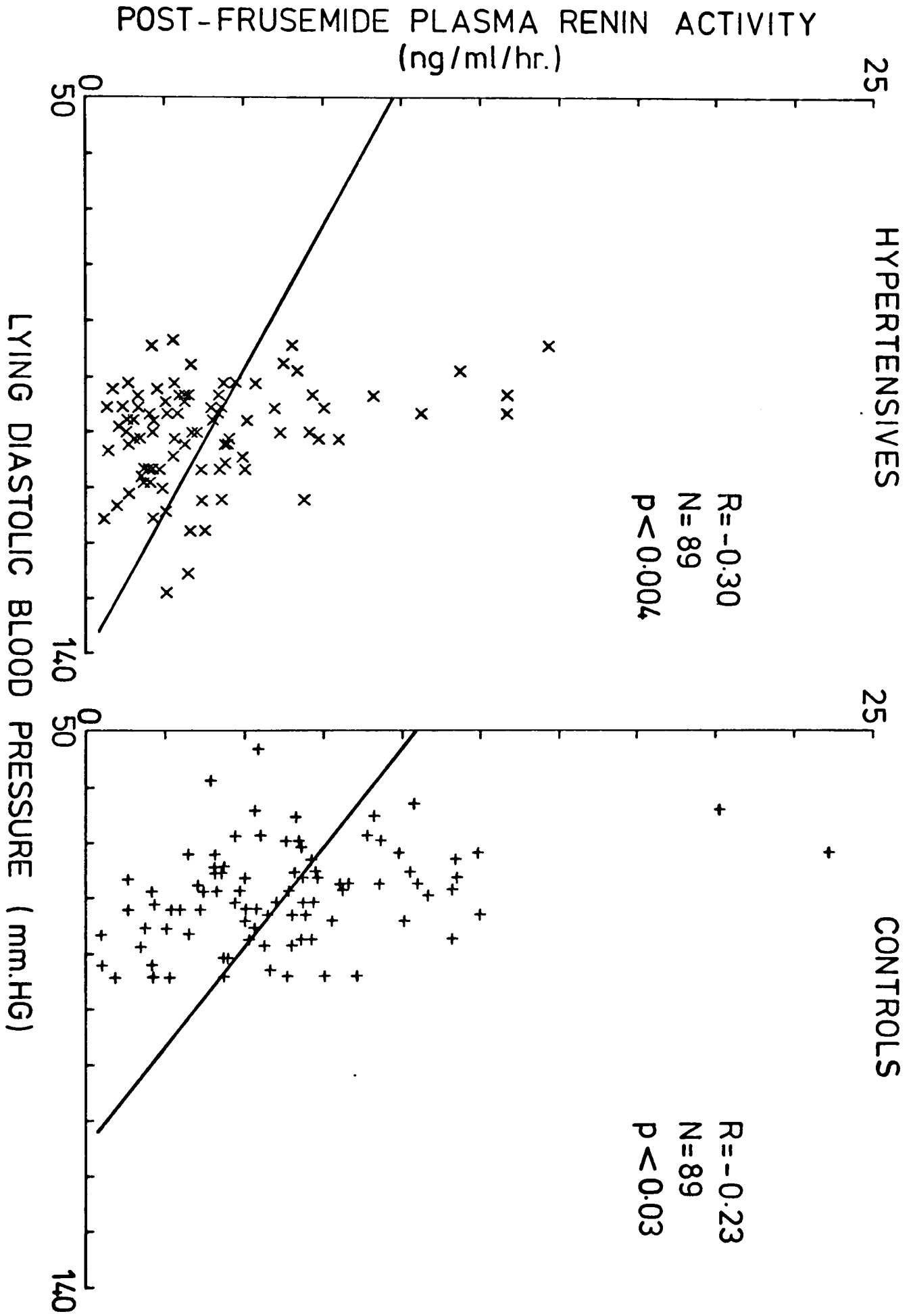
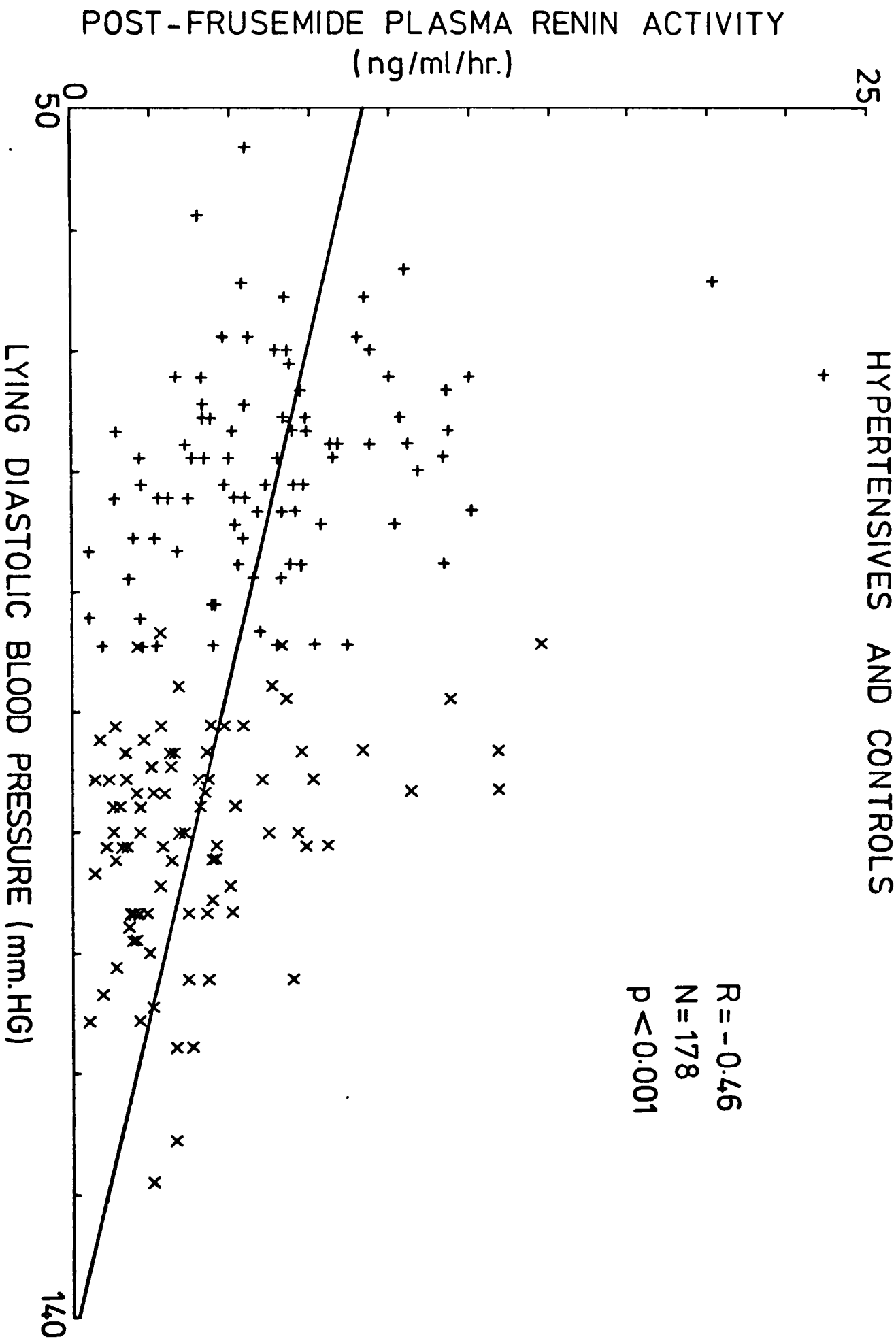

POST-FRUSEMIDE PLASMA RENIN ACTIVITY
(ng/ml/hr.)
0
25
HYPERTENSIVES
R=-0.30
N=89
P<0.004
LYING DIASTOLIC BLOOD PRESSURE (mm.HG)
50
140
CONTROLS
R=-0.23
N=89
P<0.03

FIGURE 22


POST-FRUSEMIDE PLASMA RENIN ACTIVITY
(ng/ml/hr.)
0
25
50
140
LYING DIASTOLIC BLOOD PRESSURE (mm.HG)
HYPERTENSIVES AND CONTROLS
R = -0.46
N = 178
P < 0.001

CHAPTER 8

RENIN: EFFECTS OF SODIUM, POTASSIUM, PLASMA VOLUME AND ALDOSTERONE

This chapter deals with the relationship of renin to body fluid and electrolyte levels. The plasma, urinary and whole body sodium and potassium concentrations are considered together with measurements of plasma volume and plasma aldosterone. The mineralocorticoid theory of low-renin hypertension (32) is examined in relation to the presence or absence of volume expansion, sodium retention and potassium depletion in this subgroup.

SODIUM (Na)

The plasma sodium concentration (mean $\pm$ SEM) was similar in hypertensive (139.90 $\pm$ 0.53 mmol.$^{-1}$) and control subjects (139.82 $\pm$ 0.32 mmol.$^{-1}$)(Table 32). In the hypertensive group, renin and its responsiveness were unrelated to plasma sodium levels. In the control subjects, significant inverse correlations were shown between renin activity and plasma sodium (supine PRA x Na $r = -0.23$, $P<0.031$; erect PRA x Na $r = -0.26$, $P<0.016$; lasix PRA x Na $r = -0.21$, $P<0.045$), but the renin responsiveness was unrelated to plasma sodium (Table 33). In hypertensive and control subjects, plasma sodium was unrelated both to 24 hour urinary sodium excretion (UNa24) (hypertensives - plasma Na x UNa24 $r = 0.04$, $P<0.690$;

controls - plasma Na x UNa24 r = 0.12, P<0.268), and to total exchangeable sodium (NaE) (hypertensives - plasma Na x NaE r = 0.04, P<0.676; controls - plasma Na x NaE r = 0.10, P<0.355).

There was no difference in the 24 hour urinary sodium excretion (UNa24) between hypertensive and control subjects (Table 34). Also, there was no linear relationship between urinary sodium and plasma renin or its responsiveness in either group (Table 35). Attempts to reproduce the hyperbolic relationship between plasma renin and 24 hour urinary sodium according to the Laragh nomogram (16) were unsuccessful (Figures 15 and 16). The 24 hour urinary sodium excretion did not correlate with total exchangeable sodium in either hypertensives (r = -0.04, P<0.731) or control subjects (r = -0.14, P<0.181).

Total exchangeable sodium (NaE) was expressed as millimoles of sodium per kilogram body weight. Hypertensive patients were shown to have a significantly lower (P<0.005) total exchangeable sodium (39.73 $\pm$ 0.61 $mmol.kg^{-1}$) than the control subjects (42.13 $\pm$ 0.56 $mmol.kg^{-1}$) (Table 36). This can partly be explained by the difference (P<0.005) in body weight (mean $\pm$ SEM) between hypertensive (77.26 $\pm$ 1.11 kg) and control subjects (72.11 $\pm$ 1.08 kg) (Table 37). Renin and its responsiveness were shown to be unrelated to total exchangeable sodium in both hypertensives and controls, except in one situation. In the control subjects ΔE-L absolute responsiveness correlated with total exchangeable sodium (r = 0.26,

P<0.015), but in view of the lack of significance with other renin parameters, this is probably due to chance (Table 38).

The mineralocorticoid hypothesis of low-renin hypertension (32) was tested by seeking expansion of total exchangeable sodium in the lower compared to mid and upper renin subgroups in the essential hypertensive patients. The hypertensive patients were divided into lower, mid and upper renin subgroups, according to the 9 renin parameters, as described in Chapter 5 and shown in Table 16. There were no differences in total exchangeable sodium between any of the subgroups, no matter how they were separated (Table 39). Thus, the hypertensive patients in the lower renin subgroup did not have an excess total exchangeable sodium to account for their lower renin values.

POTASSIUM (K)

There was no difference in the plasma potassium concentration (mean $\pm$ SEM) between hypertensive (3.88 ± 0.04 $mmol.l^{-1}$) and control subjects (3.96 ± 0.03 $mmol.l^{-1}$) (Table 40). Renin and its responsiveness were not related to plasma potassium in hypertensive or control groups (Table 41). Also, there was no relationship in either hypertensive or control subjects between plasma potassium and 24 hour urinary potassium excretion (hypertensives - r = 0.04, P<0.704;

controls - r = 0.05, P<0.653) or between plasma potassium and total body potassium (hypertensives - r = 0.05, P<0.673; controls - r = 0.02, P<0.823).

The 24 hour urinary potassium excretion (UK24) was significantly lower (P<0.05) in hypertensive than in control subjects (Table 42). The urine potassium did not correlate with renin or its responsiveness (Table 43). There was a significant relationship between 24 hour urine potassium and total body potassium in control subjects (r = 0.24, P<0.021), but not in the hypertensive patients (r = 0.21, P<0.051).

Total body potassium (TBK) was expressed as millimoles of potassium per kilogram body weight. The total body potassium was similar in hypertensive and in control subjects (Table 44). There were significant correlations between total body potassium and renin measurements. In the hypertensive group, TBK was related to erect and post-frusemide PRA, as well as to ΔS-E and ΔS-L absolute responsiveness. In the control subjects significant relationships between TBK and supine PRA, lasix PRA and ΔE-L responsiveness were shown (Table 45). However, total body potassium was significantly inversely correlated (P<0.001) with age in both hypertensives (r = -0.40) and control subjects (r = -0.41), and when the effects of age were removed from the renin-total body potassium relationships in a partial correlation, all the significant relationships were lost (Table 46).

The total body potassium and 24 hour urinary potassium excretion of the essential hypertensive patients in the 3 renin subgroups were studied to test the mineralocorticoid theory of low-renin hypertension (32) which, if correct, should be associated with potassium depletion in these patients. The hypertensive patients were divided into lower, mid and upper renin subgroups, according to the 9 renin parameters, as described in Chapter 5 and shown in Table 16. The patients in the lower renin subgroup when separated according to erect PRA, lasix PRA ΔS-L, ΔE-L and %S-L responses had a significantly lower total body potassium than patients in the other subgroups (Table 47). The 24 hour urinary potassium excretion was similar in lower, mid and upper renin subgroups except in one situation. When divided into sub-groups according to %S-L response, the mid renin patients excreted significantly more potassium than the upper renin hypertensives (Table 48).

PLASMA VOLUME (PV)

The plasma volume was expressed as litres per 1.73 square metres body surface area. There was no difference in the plasma volume between hypertensive and control subjects (Table 49). Renin and its responsiveness did not correlate with plasma volume in either hypertensive or control subjects (Table 50).

The mineralocorticoid theory of low-renin hypertension (32) was again tested to see whether the low renin patients were volume expanded compared to other essential hypertensives. The hypertensive patients were separated into the 3 renin subgroups, as before, and no differences in plasma volume were detected between the lower, mid or upper subgroups (Table 51). In particular, the lower renin hypertensive patients were not volume expanded to account for their lower renin levels.

PLASMA ALDOSTERONE

Plasma aldosterone (pg/ml) was significantly less ($P<0.01$) in control than in hypertensive subjects, but all values were within the reported normal range (0-400 pg/ml) for the assay (Table 52). In the hypertensive group no correlations between renin and aldosterone were shown, but in the control subjects aldosterone was significantly related to supine PRA, %S-L and %E-L responsiveness (Table 53).

Plasma aldosterone in the low, mid and upper renin subgroups was compared to ensure that it was not increased in hypertensive subjects with the lowest plasma renins. There was no significant difference in plasma aldosterone levels between the 3 renin subgroups except in two situations. When separated into subgroups according to supine PRA and ΔE-L absolute response, the lower renin hypertensives had lower plasma aldosterone levels than the mid renin subgroup ($P<0.05$) (Table 54).

SUMMARY

The plasma sodium concentration was similar in hypertensive and control subjects. Renin and its responsiveness were unrelated to plasma sodium in hypertensives, but in control subjects absolute renin levels correlated inversely with plasma sodium. In hypertensives and controls, plasma sodium was unrelated to either 24 hour urinary sodium excretion or total exchangeable sodium.

The 24 hour urinary sodium excretion was similar in hypertensive and control subjects and did not correlate with renin or its responsiveness. Attempts to reproduce the Laragh nomogram relating renin to urine sodium were unsuccessful. There was no relationship between 24 hour urine sodium and total exchangeable sodium in either group.

Total exchangeable sodium was significantly lower in hypertensive than in control subjects, but this may be partly explained by the hypertensives being significantly heavier than controls. Renin and its responsiveness were unrelated to total exchangeable sodium in both groups.

The plasma potassium concentration was not different in hypertensive and control subjects and was unrelated to renin, to 24 hour urinary potassium excretion or total body potassium.

The 24 hour urinary potassium excretion was significantly lower in hypertensives, but did not correlate with renin or its responsiveness in either group. In control subjects,

24 hour urine potassium was significantly related to total body potassium.

Total body potassium was similar in hypertensive and control subjects. Renin and its responsiveness correlated significantly with total body potassium in both groups, but these significant relationships were lost when a correction for age was made. There was a highly significant inverse relationship between total body potassium and age in hypertensives and controls.

Plasma volume was similar in hypertensive and control subjects, and unrelated to renin measurements.

Plasma aldosterone was significantly lower in control than in hypertensive patients. Plasma aldosterone was unrelated to renin or its responsiveness in hypertensive patients but was significantly related to supine PRA, %S-L, and %E-L responsiveness in control subjects.

The mineralocorticoid theory of low renin hypertension was tested. Essential hypertensive patients in the low renin subgroups did not show increased total exchangeable sodium or plasma volume. Total body potassium was significantly lower in 5 of the 9 low renin subgroups, but 24 hour urinary potassium excretion in the low renin subgroups was similar to mid and upper renin subgroups. In all but two situations plasma aldosterone concentrations were not different between lower, mid and upper renin subgroups in the essential hypertensive patients.

TABLE 32. PLASMA SODIUM IN ESSENTIAL HYPERTENSIVE AND NORMOTENSIVE CONTROL SUBJECTS

Parameter	Group	Mean	Std. Dev.	SEM	N	t	f	P
Plasma sodium ($mmol.l^{-1}$)	HT	139.90	3.33	0.53	89	0.16362	176	NS
	C	139.82	3.06	0.32	89			

Note: HT = hypertensive, C = control subject
t = Student's unpaired t-test, f = degrees of freedom, P = significance level
Std. Dev. = standard deviation, SEM = standard error of mean

TABLE 33. RELATIONSHIP BETWEEN RENIN AND PLASMA SODIUM IN 89 ESSENTIAL HYPERTENSIVE AND IN 89 NORMOTENSIVE CONTROL SUBJECTS

Parameter	Group	Plasma Renin Activity			Absolute Responsiveness			Percentage Responsiveness		
		Supine	Erect	Lasix	ΔS-E	ΔS-L	ΔE-L	%S-E	%S-L	%E-L
Plasma sodium	HT	r= 0.02	0.02	-0.05	0.02	-0.06	-0.15	0.01	-0.03	-0.10
		P< 0.868	0.834	0.653	0.870	0.600	0.149	0.963	0.796	0.334
	C	r=-0.23	-0.26	-0.21	-0.19	-0.17	-0.13	-0.06	-0.04	-0.01
		P< 0.031	0.016	0.045	0.069	0.119	0.233	0.591	0.742	0.938

Note: HT = hypertensive, C = control subjects
r = Pearson's correlation coefficient, P = significance level

TABLE 34. 24 HOUR URINARY SODIUM EXCRETION IN ESSENTIAL HYPERTENSIVE AND NORMOTENSIVE CONTROL SUBJECTS

Parameter	Group	Mean	Std. Dev.	SEM	N	t	f	P
Urinary sodium excretion ($mmol.24\ hr^{-1}$)	HT	150.79	53.04	5.62	89	0.94801	176	NS
	C	157.91	47.03	4.99	89			

Note: HT = hypertensive, C = control subject
t = Student's unpaired t-test, f = degrees of freedom, P = significance level
Std. Dev. = standard deviation, SEM = standard error of mean

TABLE 35. RELATIONSHIP BETWEEN RENIN AND 24 HOUR URINARY SODIUM EXCRETION IN 89 ESSENTIAL HYPERTENSIVE AND 89 NORMOTENSIVE CONTROL SUBJECTS

Parameter	Group	Plasma Renin Activity			Absolute Responsiveness			Percentage Responsiveness		
		Supine	Erect	Lasix	ΔS-E	ΔS-L	ΔE-L	%S-E	%S-L	%E-L
UNa24	HT	r=-0.05	-0.02	-0.06	-0.01	-0.05	-0.09	0.02	-0.01	-0.04
		P< 0.648	0.882	0.586	0.963	0.627	0.389	0.880	0.923	0.716
	C	r= 0.09	0.08	0.06	0.05	0.06	0.03	0.03	0.07	-0.01
		P< 0.428	0.451	0.563	0.643	0.560	0.762	0.770	0.544	0.939

Note: UNa24 = urinary sodium excretion (mmol.24 hr^{-1})
HT = hypertensive, C = control subjects
r = Pearson's correlation coefficient, P = significance level

TABLE 36. TOTAL EXCHANGEABLE SODIUM IN ESSENTIAL HYPERTENSIVE AND NORMOTENSIVE CONTROL SUBJECTS

Parameter	Group	Mean	Std. Dev.	SEM	N	t	f	P
Total exchangeable sodium ($mmol.kg^{-1}$)	HT	39.73	5.79	0.61	89	2.89366	176	<0.005
	C	42.13	5.25	0.56	89			

Note: Total exchangeable sodium is expressed as mmol/kg body weight
HT = hypertensive, C = control subjects
t = Student's unpaired t-test, f = degrees of freedom, P = significance level
Std. Dev. = standard deviation, SEM = standard error of mean

TABLE 37. BODY WEIGHT IN ESSENTIAL HYPERTENSIVES AND NORMOTENSIVE CONTROL SUBJECTS

Parameter	Group	Mean	Std. Dev.	SEM	N	t	f	P
Body weight (kg)	HT	77.26	10.50	1.11	89	3.32257	176	<0.005
	C	72.11	10.15	1.08	89			

Note: HT = hypertensive, C = control subject
t = Student's unpaired t-test, f = degrees of freedom, P = significance level
Std. Dev. = standard deviation, SEM = standard error of mean

TABLE 38. RELATIONSHIP BETWEEN RENIN AND TOTAL EXCHANGEABLE SODIUM IN 89 ESSENTIAL HYPERTENSIVE AND 89 NORMOTENSIVE CONTROL SUBJECTS

		Plasma Renin Activity			Absolute Responsiveness			Percentage Responsiveness		
Parameter	Group	Supine	Erect	Lasix	ΔS-E	ΔS-L	ΔE-L	%S-E	%S-L	%E-L
NaE	HT	r=-0.04	-0.09	-0.06	-0.12	-0.08	-0.00	-0.15	-0.13	-0.00
		P< 0.679	0.411	0.563	0.270	0.472	0.986	0.165	0.230	0.989
	C	r= 0.03	0.06	0.19	0.05	0.18	0.26	0.07	0.11	0.16
		P< 0.751	0.585	0.071	0.654	0.088	0.015	0.536	0.300	0.137

Note: NaE = total exchangeable sodium ($mmol.kg^{-1}$)
HT = hypertensive, C = control subjects
r = Pearson's correlation coefficient, P = significance level

TABLE 39. TOTAL EXCHANGEABLE SODIUM IN LOWER, MID AND UPPER RENIN SUBGROUPS IN ESSENTIAL HYPERTENSIVE PATIENTS

Subgrouped into:	Total Exchangeable Sodium ($mmol.kg^{-1}$) Renin subgroups			
	Lower (L) (n=29)	Mid (M) (n=30)	Upper (L) (n=30)	Significant differences between subgroups
according to:	Mean ± SEM	Mean ± SEM	Mean ± SEM	
Supine PRA	38.99 ± 1.11	41.07 ± 1.06	39.32 ± 0.90	NS (not significant)
Erect PRA	40.20 ± 1.21	40.21 ± 1.05	39.07 ± 0.84	NS
Lasix PRA	40.84 ± 1.23	39.59 ± 1.01	39.10 ± 0.84	NS
ΔS-E response	40.79 ± 1.04	39.52 ± 1.15	39.19 ± 0.90	NS
ΔS-L response	41.48 ± 1.15	38.64 ± 0.97	39.43 ± 0.93	NS
ΔE-L response	40.69 ± 1.21	39.79 ± 0.99	39.05 ± 1.19	NS
%S-E response	40.22 ± 0.81	39.65 ± 1.29	39.54 ± 0.94	NS
%S-L response	40.75 ± 1.07	38.63 ± 1.11	39.79 ± 0.94	NS
%E-L response	40.16 ± 1.05	39.37 ± 1.06	40.34 ± 0.98	NS

Note: The subgroups are equivalent to the lower third, mid third and upper third of the distribution of the renin variables
S-E = change in PRA from supine to erect, S-L from supine to lasix, and E-L from erect to lasix
ΔS-E, ΔS-L and ΔE-L are measures of absolute responsiveness
%S-E, %S-L and %E-L are measures of percentage responsiveness

TABLE 40. PLASMA POTASSIUM IN ESSENTIAL HYPERTENSIVE AND NORMOTENSIVE CONTROL SUBJECTS

Parameter	Group	Mean	Std. Dev.	SEM	N	t	f	P
Plasma potassium ($mmol.l^{-1}$)	HT	3.88	0.33	0.04	89	1.54500	176	NS
	C	3.96	0.28	0.03	89			

Note: HT = hypertensive, C = control subject
t = Student's unpaired t-test, f = degrees of freedom, P = significance level
Std. Dev. = standard deviation, SEM = standard error of mean

TABLE 41. RELATIONSHIP BETWEEN RENIN AND PLASMA POTASSIUM IN 89 ESSENTIAL HYPERTENSIVE AND IN 89 NORMOTENSIVE CONTROL SUBJECTS

Parameter	Group	Plasma Renin Activity			Absolute Responsiveness			Percentage Responsiveness		
		Supine	Erect	Lasix	ΔS-E	ΔS-L	ΔE-L	%S-E	%S-L	%E-L
		r=-0.12	-0.07	-0.04	-0.04	-0.01	0.01	0.01	-0.00	0.01
		P< 0.276	0.506	0.744	0.720	0.915	0.952	0.959	0.980	0.916
		r=-0.14	-0.08	-0.10	-0.03	-0.07	-0.10	0.11	0.10	-0.10
		P< 0.189	0.461	0.329	0.797	0.515	0.344	0.287	0.363	0.354

Note: HT = hypertensive, C = control subjects
r = Pearson's correlation coefficient, P = significance level

TABLE 42. 24 HOUR URINARY POTASSIUM EXCRETION IN ESSENTIAL HYPERTENSIVE AND NORMOTENSIVE CONTROL SUBJECTS

Parameter	Group	Mean	Std. Dev.	SEM	N	t	f	P
Urinary potassium excretion ($mmol.24\ hr^{-1}$)	HT	60.05	19.13	2.03	89	2.20194	176	<0.05
	C	66.09	17.67	1.87	89			

Note: HT = hypertensive, C = control subjects
t = Student's unpaired t-test, f = degrees of freedom, P = significance level
Std. Dev. = standard deviation, SEM = standard error of mean

TABLE 43. RELATIONSHIP BETWEEN RENIN AND 24 HOUR URINARY POTASSIUM EXCRETION IN 89 ESSENTIAL HYPERTENSIVE AND 89 NORMOTENSIVE CONTROL SUBJECTS

			Plasma Renin Activity			Absolute Responsiveness			Percentage Responsiveness		
Parameter	Group		Supine	Erect	Lasix	ΔS-E	ΔS-L	ΔE-L	%S-E	%S-L	%E-L
UK24	HT	r=	0.01	-0.08	-0.15	-0.09	-0.16	-0.18	-0.07	-0.12	-0.13
		P<	0.955	0.471	0.173	0.387	0.137	0.084	0.525	0.245	0.228
	C	r=	0.07	-0.02	0.02	-0.08	-0.01	0.05	-0.10	-0.05	-0.01
		P<	0.489	0.834	0.875	0.468	0.946	0.671	0.349	0.649	0.908

Note: UK24 = urinary potassium excretion (mmol.24 hr^{-1})
HT = hypertensive, C = control subjects
r = Pearson's correlation coefficient, P = significance level

TABLE 44. TOTAL BODY POTASSIUM IN ESSENTIAL HYPERTENSIVE AND NORMOTENSIVE CONTROL SUBJECTS

Parameter	Group	Mean	Std. Dev.	SEM	N	t	f	P
Total body potassium ($mmol.kg^{-1}$)	HT	44.39	6.05	0.64	89	1.75893	176	NS
	C	45.96	5.81	0.62	89			

Note: Total body potassium is expressed as mmol/kg body weight
HT = hypertensive, C = control subjects
t = Student's unpaired t-test, f = degrees of freedom, P = significance level
Std. Dev. = standard deviation, SEM = standard error of mean

TABLE 45. RELATIONSHIP BETWEEN RENIN AND TOTAL BODY POTASSIUM IN 89 ESSENTIAL HYPERTENSIVE AND 89 NORMOTENSIVE CONTROL SUBJECTS

			Plasma Renin Activity			Absolute Responsiveness			Percentage Responsiveness		
Parameter	Group		Supine	Erect	Lasix	ΔS-E	ΔS-L	ΔE-L	%S-E	%S-L	%E-L
TBK	HT	r=	0.15	0.23	0.26	0.21	0.25	0.20	0.18	0.21	0.15
		P<	0.168	0.032	0.014	0.050	0.020	0.055	0.095	0.052	0.161
	C	r=	0.28	0.13	0.23	0.04	0.20	0.25	-0.11	-0.04	0.13
		P<	0.007	0.209	0.033	0.727	0.058	0.018	0.284	0.724	0.226

Note: TBK = total body potassium ($mmol.kg^{-1}$)
HT = hypertensive, C = control subjects
r = Pearson's correlation coefficient, P = significance level

TABLE 46. RELATIONSHIP BETWEEN RENIN AND TOTAL BODY POTASSIUM CORRECTED FOR AGE IN 89 ESSENTIAL HYPERTENSIVE AND 89 NORMOTENSIVE CONTROL SUBJECTS

		Plasma Renin Activity			Absolute Responsiveness			Percentage Responsiveness		
Parameter	Group	Supine	Erect	Lasix	ΔS-E	ΔS-L	ΔE-L	%S-E	%S-L	%E-L
TBK	HT	r= 0.08	0.06	0.08	0.04	0.07	0.07	0.04	0.06	0.06
		P< 0.470	0.572	0.452	0.716	0.531	0.493	0.732	0.559	0.549
	C	r= 0.20	-0.04	0.06	-0.14	0.03	0.12	-0.18	-0.11	0.11
		P<0.062	0.733	0.606	0.206	0.786	0.284	0.091	0.294	0.302

Note: Above relationships are corrected for age in a partial correlation
r = correlation coefficient, P = significance level
HT = hypertensive, C = control subjects
TBK = total body potassium ($mmol.kg^{-1}$)
S-E = change in PRA from supine to erect, S-L from supine to lasix, E-L from erect to lasix

TABLE 47. TOTAL BODY POTASSIUM IN LOWER, MID AND UPPER RENIN SUBGROUPS IN ESSENTIAL HYPERTENSIVE PATIENTS

Subgrouped into:	Total body potassium ($mmol.kg^{-1}$) Renin Subgroups			
	Lower (L) (n=29)	Mid (M) (n=30)	Upper (U) (n=30)	Significant Differences between subgroups
according to:	Mean ± SEM	Mean ± SEM	Mean ± SEM	
Supine PRA	42.36 ± 0.96	44.13 ± 1.20	45.34 ± 1.12	NS (not significant)
Erect PRA	42.58 ± 0.93	43.47 ± 1.23	45.75 ± 1.08	L<U, P<0.05
Lasix PRA	41.20 ± 1.03	44.27 ± 1.06	46.27 ± 1.06	L<M, P<0.05; L<U, P<0.005
ΔS-E response	43.71 ± 1.03	42.67 ± 1.16	45.46 ± 1.09	NS
ΔS-L response	41.21 ± 1.08	44.53 ± 1.02	46.01 ± 1.06	L<M, P<0.05; L<U, P<0.005
ΔE-L response	41.43 ± 1.21	45.55 ± 0.98	44.78 ± 1.01	L<M, P<0.02; L<U, P<0.005
%S-E response	44.07 ± 1.04	42.51 ± 1.19	45.30 ± 1.06	NS
%S-L response	42.35 ± 1.11	43.95 ± 1.23	45.47 ± 0.92	L<U, P<0.05
%E-L response	42.79 ± 1.29	43.77 ± 1.04	45.53 ± 0.96	NS

Note: The subgroups are equivalent to the lower third, mid third and upper third of the distribution of the renin variables
S-E = change in PRA from supine to erect, S-L from supine to lasix, E-L from erect to lasix
ΔS-E, ΔS-L, ΔE-L are measures of absolute responsiveness
%S-E, %S-L, %E-L are measures of percentage responsiveness
L<U means the potassium in the lower subgroup was significantly less than that in the upper subgroup, and P = significance level

TABLE 48. 24 HOUR URINARY POTASSIUM EXCRETION IN LOWER, MID AND UPPER RENIN SUBGROUPS IN ESSENTIAL HYPERTENSIVE PATIENTS

Subgrouped into:	24 hour urinary potassium excretion (mmol) Renin subgroups			
	Lower (L) (n=29)	Mid (M) (n=30)	Upper (U) (n=30)	Significance differences between subgroups
according to:	Mean ± SEM	Mean ± SEM	Mean ± SEM	
Supine PRA	58.62 ± 4.22	61.40 ± 3.37	59.93 ± 2.98	NS (not significant)
Erect PRA	58.76 ± 3.78	61.13 ± 3.39	59.07 ± 3.35	NS
Lasix PRA	59.38 ± 4.16	62.53 ± 3.15	57.07 ± 3.11	NS
ΔS-E response	63.31 ± 3.89	56.03 ± 3.08	60.77 ± 3.82	NS
ΔS-L response	60.38 ± 3.84	63.40 ± 3.45	56.23 ± 2.90	NS
ΔE-L response	60.17 ± 3.87	64.33 ± 3.47	55.50 ± 3.14	NS
%S-E response	61.93 ± 3.85	58.67 ± 3.14	59.60 ± 3.61	NS
%S-L response	58.41 ± 3.30	65.77 ± 4.16	55.63 ± 2.84	M>U, P<0.05
%E-L response	62.28 ± 3.66	60.90 ± 3.21	57.03 ± 3.69	NS

Note: The subgroups are equivalent to the lower third, mid third and upper third of the distribution of the renin variables
S-E = change in PRA from supine to erect, S-L from supine to lasix, E-L from erect to lasix
ΔS-E, ΔS-L, ΔE-L are measures of absolute responsiveness
%S-E, %S-L, %E-L are measures of percentage responsiveness
M>U means that the urine potassium in the mid subgroup was significantly greater than that in the upper subgroup, and P = significance level

TABLE 49. PLASMA VOLUME IN ESSENTIAL HYPERTENSIVE AND NORMOTENSIVE CONTROL SUBJECTS

Parameter	Group	Mean	Std. Dev.	SEM	N	t	f	P
Plasma volume (1/1.73 m^2)	HT	2.96	0.33	0.04	89	1.55842	176	NS
	C	2.89	0.30	0.03	89			

Note: Plasma volume is expressed as litres/1.73 m^2 body surface area
HT = hypertensive, C = control subjects
t = Student's unpaired t-test, f = degrees of freedom, P = significance level
Std. Dev. = standard deviation, SEM = standard error of mean

TABLE 50. RELATIONSHIP BETWEEN RENIN AND PLASMA VOLUME IN 89 ESSENTIAL HYPERTENSIVE AND 89 NORMOTENSIVE CONTROL SUBJECTS

		Plasma Renin Activity			Absolute Responsiveness			Percentage Responsiveness		
Parameter	Group	Supine	Erect	Lasix	ΔS-E	ΔS-L	ΔE-L	%S-E	%S-L	%E-L
PV	HT	r= 0.04	0.05	-0.02	0.04	-0.03	-0.11	-0.00	-0.04	-0.05
		P< 0.727	0.662	0.837	0.741	0.746	0.307	0.972	0.686	0.657
	C	r= 0.04	-0.08	-0.06	-0.12	-0.05	-0.02	0.03	0.07	0.03
		P< 0.743	0.439	0.599	0.272	0.622	0.842	0.757	0.535	0.786

Note: PV = plasma volume (litres/1.73 m^2 body surface area)
HT = hypertensive, C = control subjects
r = Pearson's correlation coefficient, P = significance level

TABLE 51. PLASMA VOLUME IN LOWER, MID AND UPPER RENIN SUBGROUPS IN ESSENTIAL HYPERTENSIVE PATIENTS

Subgrouped into:	Plasma volume (litres/1.73 m^2 body surface area) Renin subgroups			
	Lower (L) (n=29)	Mid (M) (n=30)	Upper (U) (n=30)	Significance differences between subgroups
according to:	Mean ± SEM	Mean ± SEM	Mean ± SEM	
Supine PRA	3.00 ± 0.06	2.88 ± 0.05	3.00 ± 0.06	NS (not significant)
Erect PRA	3.00 ± 0.06	2.95 ± 0.05	2.94 ± 0.06	NS
Lasix PRA	2.96 ± 0.06	2.97 ± 0.05	2.94 ± 0.06	NS
ΔS-E response	2.99 ± 0.06	2.90 ± 0.06	2.98 ± 0.06	NS
ΔS-L response	2.94 ± 0.06	2.97 ± 0.05	2.95 ± 0.06	NS
ΔE-L response	2.95 ± 0.06	2.97 ± 0.05	2.96 ± 0.06	NS
%S-E response	2.99 ± 0.05	2.96 ± 0.07	2.94 ± 0.06	NS
%S-L response	2.94 ± 0.06	2.99 ± 0.06	2.91 ± 0.05	NS
%E-L response	2.97 ± 0.06	3.02 ± 0.06	2.90 ± 0.05	NS

Note: The subgroups are equivalent to the lower third, mid third and upper third of the distribution of the renin variables
S-E = change in PRA from supine to erect, S-L from supine to lasix, E-L from erect to lasix
ΔS-E, ΔS-L, ΔE-L are measures of absolute responsiveness
%S-E, %S-L, %E-L are measures of percentage responsiveness

TABLE 52. PLASMA ALDOSTERONE IN ESSENTIAL HYPERTENSIVE AND NORMOTENSIVE CONTROL SUBJECTS

Parameter	Group	Mean	Std. Dev.	SEM	N	t	f	P
Plasma aldosterone	HT	105.69	62.70	6.65	89	2.71755	176	<0.01
	C	86.53	22.19	2.35	89			

Note: HT = hypertensive, C = control subjects
t = Student's unpaired t-test, f = degrees of freedom, P = significance level
Std. Dev. = standard deviation, SEM = standard error of mean

TABLE 53. RELATIONSHIP BETWEEN RENIN AND PLASMA ALDOSTERONE IN 89 ESSENTIAL HYPERTENSIVE AND 89 NORMOTENSIVE CONTROL SUBJECTS

		Plasma Renin Activity			Absolute Responsiveness			Percentage Responsiveness		
Parameter	Group	Supine	Erect	Lasix	ΔS-E	ΔS-L	ΔE-L	%S-E	%S-L	%E-L
Plasma aldosterone	HT	r=-0.05	0.08	0.05	0.11	0.07	-0.01	0.12	0.09	-0.00
		P< 0.663	0.435	0.630	0.296	0.535	0.942	0.281	0.382	0.977
	C	r= 0.26	0.10	0.022	-0.00	-0.04	-0.05	-0.10	-0.23	-0.24
		P< 0.013	0.355	0.836	0.969	0.681	0.664	0.367	0.031	0.022

Note: Plasma aldosterone expressed as pg/ml
HT = hypertensive, C = control subjects
r = Pearson's correlation coefficient, P = significance level

TABLE 54. PLASMA ALDOSTERONE IN LOWER, MID AND UPPER RENIN SUBGROUPS IN ESSENTIAL HYPERTENSIVE PATIENTS

Subgrouped into:	Plasma aldosterone ($pg.ml^{-1}$) Renin subgroups			
	Lower (L) (n=29)	Mid (M) (n=30)	Upper (U) (n=30)	Significant differences between subgroups
according to:	Mean ± SEM	Mean ± SEM	Mean ± SEM	
Supine PRA	86.17 ± 7.46	116.23 ± 10.61	103.47 ± 11.09	L<M, P<0.05
Erect PRA	98.62 ± 7.44	96.57 ± 6.85	111.63 ± 12.23	NS (not significant)
Lasix PRA	98.38 ± 8.04	95.90 ± 6.17	98.38 ± 12.22	NS
ΔS-E response	96.17 ± 7.21	101.97 ± 7.66	108.60 ± 11.95	NS
ΔS-L response	88.59 ± 7.71	101.33 ± 6.73	112.80 ± 12.13	NS
ΔE-L response	92.14 ± 8.72	114.43 ± 6.49	100.10 ± 11.44	L<M, P<0.05
%S-E response	98.93 ± 7.08	97.53 ± 7.64	111.13 ± 12.02	NS
%S-L response	96.97 ± 7.60	102.80 ± 8.68	106.40 ± 11.11	NS
%E-L response	97.75 ± 9.06	102.30 ± 7.55	106.80 ± 10.86	NS

Note: The subgroups are equivalent to the lower third, mid third and upper third of the distribution of the renin variables
S-E = change in PRA from supine to erect, S-L from supine to lasix, E-L from erect to lasix
ΔS-E, ΔS-L, ΔE-L are measures of absolute responsiveness
%S-E, %S-L, %E-L are measures of percentage responsiveness
L<M means the plasma aldosterone in the lower subgroup was significantly less than that in the mid subgroup, P = significance level

PART IV

GENERAL DISCUSSION

The following chapter discusses the results of this study and the conclusions that were drawn.

CHAPTER 9

GENERAL DISCUSSION

Essential hypertension is a qualitative term which has come to be applied to individuals who have arterial pressures above some arbitrary value and no known disease to account for the raised pressure (75). This has stimulated research workers to attempt to explain why the arterial pressure is higher in some people than others. Hereditary and environmental factors are likely to be causal, but this has not prevented the search for humoral factors in the aetiology of essential hypertension. The role of the renin-angiotensin system in hypertension, as described in the introduction, remained controversial for many years. Gradually, the components and functions of this complex hormonal system were elucidated, and it was agreed that plasma renin was not raised in essential hypertension. However, the role of the renin-angiotensin system remains unclear. The recent trend to separate essential hypertensive patients into renin subgroups has revived interest in the concept that these patients might not be an homogeneous hypertensive group.

This work was undertaken to reappraise the role of renin in essential hypertension. Two major questions were considered: Is the activity of the renin-angiotensin system similar in essential hypertensive and control subjects? Does low-renin hypertension exist as a separate entity attributable to mineralocorticoid excess? In answering these

questions many variables, other than renin, including age, sex, blood pressure, sodium, potassium, plasma volume and aldosterone, and their interrelationships were studied.

A screening programme for the detection of hypertension was necessary to obtain the large numbers of patients required for this study in a reasonably short period of time. From such undertakings in Scotland (45) and in Wales (62), 10-20% of the patients screened were expected to have high blood pressure. However, of the 6,777 people from Harwell, Wantage and Didcot who had their blood pressures measured, only 1.73% were hypertensive. The major reason for this difference lies in the very strict criteria for inclusion in this study that were used. All patients who had ever received anti-hypertensive treatment or were taking any form of medication were rejected because of the effect of drugs on renin levels (59,4). For the same reason patients suffering from any complication of hypertension or from any other disease process were not included. The quality of medical care in the general practices screened must also have contributed to the paucity of hypertensives detected. The general practices were chosen because of the enthusiasm and co-operation of the local doctors, and it is likely that many of the hypertensive patients in their practices had already been detected and treated. Another possibility for the poor detection rate is that the people of rural Oxfordshire have a lower incidence of high blood pressure than elsewhere! Further epidemiological studies are needed to explain this issue.

RENIN IN ESSENTIAL HYPERTENSION

The renin status of a group of previously untreated uncomplicated essential hypertensive patients was studied in detail and compared to a group of healthy normotensive control subjects drawn from the same population.

Plasma renin and its responsiveness to stimulation were found to be suppressed in patients with essential hypertension compared to control subjects. This was originally proposed by Doyle and Jerums (31), and together with Padfield et al (67) we confirmed these findings (97). In both the Doyle and the Padfield studies some of the patients had received hypotensive therapy, and their control subjects were not age and sex matched. The present work extends our earlier findings, except where we originally reported a difference in supine (basal) plasma renin activity between hypertensive and control subjects, this is no longer the case, but all the other measures of renin and responsiveness remain significantly different. From these studies it may be concluded that a reduction of renin and its responsiveness appears to be a general feature of uncomplicated essential hypertension.

The reasons for the reduced plasma renin and renin responsiveness to stimulation in essential hypertension are quite uncertain, but renin is known to be affected by a variety of influences other than sodium (27,103,98,16), potassium (15,84) and plasma volume (33), including age (82,46, 79,69,107,102), and previous drug treatment (59,4), factors

which have not often been taken into account in previous work (32). In addition, arterial pressure itself may influence plasma renin activity (11,82,60,110).

Sodium (27,103,98,16), potassium (15,84) and plasma volume (33) are known to influence renin levels and were considered in an attempt to explain the suppression of renin and its responsiveness in hypertensives compared to control subjects. Plasma and urinary sodium were similar in the two groups, but total exchangeable sodium was lower in the hypertensive subjects. This difference cannot account for the renin suppression in hypertensives because lower sodium levels should elevate rather than suppress renin. The difference in total exchangeable sodium between the groups is largely explained by the fact that the hypertensives weighed significantly more than the controls, as this would influence the total exchangeable sodium when expressed as milli-equivalents per kilogram body weight. The work of Lebel et al (56) showed that total exchangeable sodium, expressed with reference to the 'leanness index' (ratio of height^3/weight) was similar in hypertensive and control subjects. Plasma potassium and total body potassium were similar in the two groups, but the 24 hour urine potassium was lower in hypertensive subjects. Again this trend is in the wrong direction to account for the renin suppression in hypertensives. As the difference in urine potassium is quite small, dietary factors alone may be responsible for this difference. The

plasma volume in hypertensives was similar to that in control subjects, and this is in keeping with the findings of Schalekemp et al (83).

In the course of considering sodium, potassium and plasma volume in the above context, their interrelationships were also studied. The 24 hour urine sodium and the total exchangeable sodium did not correlate with renin or its responsiveness. Plasma sodium was inversely related to supine, erect and post-frusemide plasma renin activity in the controls but not in the hypertensive subjects. There were no correlations between plasma, urinary and total exchangeable sodium. Plasma potassium, urinary potassium and total body potassium (corrected for age) were unrelated to renin or renin responsiveness. Plasma volume was also unrelated to renin or its responsiveness. While accepting that renin release is influenced by changes in sodium, potassium and plasma volume, in this study plasma renin and responsiveness do not appear to be directly related to these factors in either hypertensives or controls. Perhaps this demonstrates the complexity of the control of plasma renin and that plasma levels are the product of multiple interrelating variables.

Several studies have considered the relationship between plasma renin and age, but agreement that this relationship is present in both hypertensives and normotensive controls is lacking (82,46,79,69,107,102). In our study a significant inverse relationship between age and plasma renin activity

was found in both hypertensive and normotensive subjects. Padfield et al (69) suggested that the apparent effects of age on renin are mediated by raised arterial pressure: this is not supported in the present study. When the effects of blood pressure itself are allowed for, there remains a significant inverse relationship between age and renin in both normotensive and in hypertensive subjects. Our findings of age-dependent changes in absolute renin responsiveness accord with those of Hayduk (46). Many suggestions as to the cause of the inverse age-renin relationship have been made, and most concern changes in renal function. Schalekamp and colleagues have shown increasing renovascular resistance with age (82) and an inverse relationship between renovascular resistance and plasma renin concentration (81). Friedman et al showed a decrease of functional size of the kidney with age (36). Weidmann suggests decreased sympathetic nervous system activity with age (107) might be contributing to this effect, but recent work does not support this view (28). The common theme to most suggestions seems to relate to progressive structural changes in renal blood vessels with ageing: a reduction in afferent arteriolar distensibility would result in a fall in renin secretion and its responsiveness. Although these inverse relationships are clearly shown in both hypertensive and control subjects in the present study, they cannot account for the lower plasma renin levels and renin responsiveness in the hypertensives, because they were age matched with the control subjects.

Whereas antihypertensive drug treatment may have influenced the renin levels (59,4) in the Doyle(31) and in the Padfield (67) studies, and partially accounted for lower renin levels and responsiveness in hypertensives, they could not be incriminated in this work, where such patients were excluded.

In females, plasma renin and its responsiveness tended to be slightly lower than in males. This finding may be due to the effects of the female sex hormones on the renin-angiotensin system. In the present study, where the patients were sex matched with normotensive controls, the effects of sex could not have contributed to the lower renin levels in hypertensive patients. However, this stresses the importance of accurate matching for sex in comparative studies of the renin-angiotensin system.

Doyle and Jerums, attempting to explain lower renin levels in hypertensive patients have speculated (31) that the adrenal glomerulosa might be more sensitive to the effects of angiotensin in hypertensives than in normal patients, resulting in stimuli producing a normal rise in aldosterone in spite of a smaller rise in renin. The suppression of renin may be a feedback suppression via aldosterone. They go on in the same paper to offer another possible explanation: suppression of plasma renin in hypertensives might be due to a natriuretic process occuring proximal to the macula densa. The increased distal tubular sodium might suppress plasma renin

and blunt the response to renin elevating stimuli. However, these hypotheses have not been substantiated, and seem unlikely.

Previous data on experimental and most forms of human hypertension have usually found no simple correlation between renin response and blood pressure levels (93,110,108,52,43). However, Bloomfield and colleagues (11) who studied the effect of hospitalisation and low salt diet on blood pressure and renin response, found an inverse relation of renin levels to blood pressure. Similar findings were reported by Schalekamp et al (82) from their renal haemodynamic studies. The inverse relationship between renin and blood pressure was extended to normotensive control subjects by Lucas et al (60), but they were unable to reproduce the relationship in hypertensives. Weidmann and colleagues (107) were able to show significant although weak inverse renin-blood pressure interrelations in normotensive subjects on a normal sodium intake and after mild sodium depletion. Our study shows significant inverse relationships between blood pressure and renin in both hypertensive and control subjects, considered separately or together; the separation of "hypertensives" from "normotensives" being an arbitrary one (75). However, age was found to be contributing significantly to these renin-blood pressure relationships, especially in the control subjects where the relationships were almost entirely lost, but also in the hypertensives, where most of the relationships were weakened but remained significant. The mechanism of the blood

pressure dependent changes in renin release is probably a direct effect of blood pressure on the juxtaglomerular cells in the afferent arteriole. Renin release has been shown to be inhibited by increased pressure in blood vessels close to the glomerulus (27,101). This has been extended to humans with essential hypertension by Lowenstein et al (59a) who have shown the increased arterial pressure is transmitted beyond the arterioles and associated with raised glomerular pressure.

Any of the many factors affecting renin release (27) could be responsible for the suppression of plasma renin and its responsiveness in essential hypertensive subjects. After a consideration of some of the more important of these factors it seems likely that a major contributor to the reduced renin levels in essential hypertension is the effect of raised blood pressure on the kidney.

LOW-RENIN HYPERTENSION

The belief that essential hypertensives represent an homogeneous group of patients has been questioned in recent years. The development of sensitive assay techniques for the measurement of plasma renin has lead to the search for alterations in renin metabolism in essential hypertension. One of the most intensively investigated fields has been low-renin hypertension. About 25% of patients with apparently essential hypertension have been shown to have low plasma

levels of renin which are not associated with aldosterone excess and are often unresponsive to stimulatory manoeuvres (32), but whether or not this group, termed low-renin hypertension, represents a distinct entity remains unclear. Research in this field has been stimulated by the practical importance of claims that low renin patients have a lower incidence of vascular complications (16) and that they show an unusually favourable response to diuretic therapy (1,32).

Confusion has arisen in the literature on this subject, partly from the variability of criteria for the diagnosis of low-renin hypertension (68,32). Almost all the methods for the division of low-renin hypertensives from the essential hypertensive population involve the use of some arbitrary renin level and lack all statistical basis. The Glasgow workers in this field distinguish low-renin hypertension from essential hypertension by using the arbitrary level of 6 units of plasma renin concentration as their dividing line, but report a normal range of 4 to 20 units (56). Laragh and colleagues devised a nomogram of plasma renin against 24 hour urinary sodium excretion, and found the hypertensives to have a greater scatter of renin values than normal subjects, permitting the arbitrary classification of essential hypertensive patients into low, normal and high renin subgroups (16).

This study was designed to question the existence of low-renin hypertension as a separate entity, and to examine the hypothesis that mineralocorticoid excess is involved in the

aetiology of the condition (93,87,32).

In this work, considerable attention was paid to the possible methods of separation of low-renin hypertension from essential hypertension. Various renin parameters were studied (basal levels - supine PRA; stimulated values - erect PRA and post-frusemide PRA; absolute renin responsiveness - absolute change in PRA from supine to erect, supine to post-frusemide and erect to post-frusemide; and percentage renin responsiveness - percentage change in PRA from supine to erect, supine to post-frusemide and erect to post-frusemide) in an attempt to encompass many of the measurements used by other workers in subdividing the hypertensive patients.

Each of these 9 renin variables was examined in the essential hypertensive patients in an attempt to establish whether or not their frequency distributions suggested the existence of a distinct subpopulation with low renin levels. The distributions were shown to be skewed to the right, but the logarithms of the renin variables were not statistically different from a normal distribution with no suggestion of bimodality. Only the supine to lasix renin variables failed to fulfil the tests of normality. This might be peculiar to the effect of frusemide on renin release, the mechanism of which is unknown (67), because after stimulation by the erect posture, normal distributions were obtained. This is in agreement with the plasma renin concentration findings of Padfield et al (69), and the plasma angiotensin II findings

of Beevers et al (5). This suggests that the low-renin state forms part of a continuum in hypertension rather than a distinct diagnostic entity.

In this study an attempt was made to separate essential hypertensive patients into renin subgroups in a statistically acceptable way, while utilising basal, stimulated and renin responsiveness data. It was decided to divide the distributions of each of the nine renin parameters (PRA - supine, erect, post-frusemide; absolute renin responsiveness - ΔS-E, ΔS-L, ΔE-L; and percentage renin responsiveness - %S-E, %S-L, %E-L) into three equal renin subgroups (lower, mid and upper) on the understanding that if a low renin subgroup existed it would be contained in the lower third of the distribution, while a high-renin group would be in the upper third. Thus, nine different low-renin subgroups were defined and studied in an attempt to see if any of them housed a different population from other hypertensive patients.

Because of its widespread use, we attempted to reproduce the Laragh nomogram (16) in order to use their method of separating hypertensive patients into renin subgroups. In our study there was no difference in the scatter of renin values between hypertensives and controls, and the nomogram could not be reproduced. By not altering the dietary sodium we never expected to obtain the tail of high renin values with low urinary sodium excretion. However, in the range of urine sodium from 100 to 200 millimoles, in which most of our

patients fell, no separation could be made. In view of this finding, and the lack of any statistical basis in its creation, it was decided not to persevere with this method of subdividing essential hypertensive patients. Recently, Woods et al (113) have proposed an improvement to this nomogram division.

A consideration of low-renin hypertension requires that aldosterone excess, which could account for the 'syndrome' in these patients, be excluded. In our study, the essential hypertensive patients were found to have significantly greater plasma aldosterone levels than the control subjects. This finding is in keeping with the work of Sambhi, Crane and Genest (79), who explain the difference by studies showing a decreased metabolic clearance rate of aldosterone in patients with essential hypertension. Our studies of plasms aldosterone in the three renin subgroups demonstrated no excess of this hormone in the low renin patients.

Stimulation of the renin mechanism is claimed to sharpen the distinction between low and normal renin hypertension (22). These procedures have variously involved the use of low sodium diets, various periods of ambulation and the administration of diuretics (32). With the exception of two studies (93,25) there is little to suggest that stimuli assist in identifying a low-renin group (67,69). On the other hand, some of the popularity for the use of stimuli may be accounted for by the inaccuracy of many methods in measuring low renin levels.

However, despite the development of sensitive renin assay techniques capable of measuring very low renin levels, and despite doubt that stimuli help in separating a low-renin group, stimulation of the renin mechanism continues to be used and needs to be considered in any appraisal of renin subgrouping. In the present study the stimuli of erect posture and intravenous frusemide (lasix) were used.

Low-renin hypertensives are said to be less responsive to renin elevating stimuli than other essential hypertensive patients (32). Padfield et al (67) showed renin responsiveness to be independent of basal renin values. In contrast, our study and that of Hayduk et al (46) showed significant correlations between absolute renin levels and renin responsiveness. By virtue of this relationship and our method of classification, the low renin groups were expected to be, and were, less responsive than mid or upper renin subgroups. This strengthens the opposition to the use of stimuli in defining a low-renin subgroup.

It has been suggested that the low-renin subgroup of hypertensives may contain a preponderance of females (32). Our study failed to support this in six of the nine low-renin subgroups. In view of our findings that females tend to have lower renin levels and responsiveness than males, any study containing an excess of females may be biased towards this theory.

An important hypothesis of the aetiology of low-renin

hypertension suggests that this condition forms a distinct subgroup attributable to an excess of an unknown mineralocorticoid (93,22,87). It is generally agreed that primary hyperaldosteronism is associated with renin suppression (24,23), sodium retention (56), plasma volume expansion (96) and potassium depletion (24). Findings of this sort lead to the idea that excess of a mineralocorticoid other than aldosterone, might be responsible for low-renin hypertension. This hypothesis was tested by studying the indirect effects of mineralocorticoid excess on sodium, potassium and plasma volume in the low, mid and upper renin subgroups.

No differences in total exchangeable sodium between any of the renin subgroups, irrespective of the renin parameter used to subgroup them, were shown. The depression of renin cannot, therefore, be explained by abnormal sodium retention. These findings support those of Lebel et al (56). Plasma volume, in our study, was not expanded in the low-renin subgroup compared to the other renin subgroups, and is in agreement with the work of Schalekamp and colleagues (83). Dunn and Tannen (32) concluded that when all the evidence from plasma volume, extracellular fluid volume and exchangeable sodium spaces are considered, there is little evidence of volume expansion in low-renin hypertension, and our data accord with this view. The total body potassium in our study was inversely correlated with renin and its responsiveness in the hypertensive patients. Hence the low-renin subgroups, comprising the lower third of the distribution of the renin

parameters, were expected to, and did, show lower total body potassium levels. The unexpected positive correlation between renin and total body potassium can be accounted for by the similar effects of age on renin and on total body potassium: when the effects of age were removed from the renin-total body potassium relationship in a partial correlation, the significant associations were lost. To ensure that a mineralocorticoid was not partly responsible for these lower total body potassium levels, the 24 hour urinary potassium excretion was examined in the three renin subgroups, and no difference was shown between the lower and the other renin subgroups. From these studies of potassium no support for mineralocorticoid involvement in low-renin hypertension emerged.

Almost all indirect evidence is now firmly opposed to the idea of excess mineralocorticoid being involved in the aetiology of low-renin hypertension. However, this concept still has its supporters: Sennett, Liddle and colleagues (87) have recently reported the direct identification of excess mineralocorticoid (16 β hydroxydehydroepiandrosterone) in the urine of low-renin hypertensive patients. This work awaits confirmation, and if correct, awaits the explanation of how this mineralocorticoid is raising the blood pressure and suppressing renin without sodium retention and volume expansion - the mechanism generally agreed for primary aldosteronism.

Mechanisms other than mineralocorticoid excess have been

proposed to account for low-renin hypertension. Swales (94) has suggested that the vascular changes of nephrosclerosis result in a reduction of afferent arteriolar distensibility with impairment of basal renin secretion and responsiveness. Schalekamp et al (81,83) and Brown et al (14) favour the hypothesis that low-renin hypertension is a stage in the development of essential hypertension with a renal lesion as its cause.

Overall, much of the evidence is against the idea that low-renin hypertension is a separate entity. Low-renin hypertension is only distinguishable from essential hypertension by means of arbitrary criteria, and the frequency distribution studies do not support the idea of a sub-population. The role of mineralocorticoids with aldosterone-like activity have been excluded by sodium, potassium and plasma volume studies. I believe that low-renin hypertension is neither a distinct subdivision of essential hypertension nor a separate entity. This accords with the view of some other workers (67,56,83). It is proposed instead, that low-renin hypertension represents no more than the lower end of the normal distribution of renin in the essential hypertensive population. However, many factors, including blood pressure and age, may modify the renin status and need to be taken into account in the interpretation of individual values.

CONCLUSION

The reduced plasma renin activity in essential hypertensive patients appears to be a general feature of this condition. The suppression of plasma renin is probably a result of the combined effect of many factors on renin release, but blood pressure itself has been shown to be an important contributor.

Low-renin hypertension is neither a distinct subdivision of essential hypertension nor a separate entity attributable to mineralocorticoid excess. It is proposed instead that low-renin hypertension represents no more than the lower end of the normal distribution of renin in the essential hypertensive population, but many factors including blood pressure and age may modify the renin status.

Plasma renin is influenced by a number of factors: by taking account of some of these factors including age, sex, blood pressure, sodium, potassium and fluid volume, a more meaningful interpretation can be placed on isolated renin measurements.

PART V

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