THE ROLE OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM IN NORMAL AND HYPERTENSIVE PREGNANCY.

by

Ronald J. Weir

M.B., Ch.B. (Glasgow), M.R.C.P. (Glasgow).

A thesis submitted to the University of Glasgow for the degree of Doctor of Medicine.

September 1972

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

DR. RONALD J. WEIR

MEDICAL APPOINTMENTS HELD SINCE GRADUATION

House Officer in Surgery - August 1960 to January, 1961.
(Mr. John Hutchison, Stobhill Hospital, Glasgow).

House Officer in Medical Paediatrics - February 1961 to July 1961.
(Professor J. Hutchison, Royal Hospital for Sick Children, Glasgow).

House Officer in Medicine - August 1961 to February 1962.
(Dr. Olav Kerr, Western Infirmary, Glasgow).

Senior House Officer in Medicine - March 1962 to March 1963.
(Dr. Olav Kerr, Western Infirmary, Glasgow).

Registrar in Medicine - April 1963 to September 1964
(Vale of Leven Hospital, Dunbartonshire).

Registrar in Medicine - October 1964 to April 1965.
(Dr. A.A.F. Peel, Victoria Infirmary, Glasgow).

Resident in Biochemistry - July 1965 to June 1966
(Toronto General Hospital, Toronto, Ontario).

Registrar in Medicine - July 1966 to January 1969
(Department of Medicine, Victoria Infirmary, Glasgow).

Research Fellow (McCunn Fellowship) - February 1969 to January 1970
and

Senior Medical Registrar - February 1970 - present appointment
(MRC Blood Pressure Unit, Western Infirmary, Glasgow).

THE ROLE OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM IN NORMAL AND HYPERTENSIVE PREGNANCY

R.J. WEIR

Statement regarding my personal contribution to these studies

Owing to their number and complexity, it would have been impossible for one person to have performed all the laboratory techniques utilised in these studies. I have had to rely, therefore, on the willing co-operation of my laboratory colleagues (see page 1) to carry out the biochemical estimations. I did, however, contribute to the bioassay measurements of renin and renin-substrate which, in our unit, are performed by a team of workers. I also collaborated with Mr. M. Tree in the development of the method for measuring plasma renin-substrate.

My major personal contribution to the studies described in this Thesis was to design and set up the investigations, to supervise the clinical aspects (choice of cases, history taking, physical examination, blood pressure measurement and blood sampling), to co-ordinate the laboratory data and to analyse the results. The drawing of the graphs (apart from Figures 2 to 5) and the writing of the Thesis were entirely my personal work.

THE ROLE OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM IN NORMAL AND HYPERTENSIVE PREGNANCY.

R.J. Weir.

SUMMARY

In view of the potent vaso-pressor action of angiotensin II and the powerful sodium-retaining effect of aldosterone, an investigation has been made of the role of these substances in normal and hypertensive pregnancy.

Initially, the renin-angiotensin-aldosterone system was examined in a large number of normal pregnant women. This study mainly involved the estimation of the plasma concentrations of renin, renin-substrate, angiotensin II and aldosterone. Measurements were also made of plasma and urinary electrolytes; plasma proteins, colloid osmotic pressure and osmolality; blood urea; serum creatinine; plasma volume and haematocrit - some of these factors having been shown to be associated with changes in the renin-angiotensin-aldosterone system in non-pregnant situations. Many of these substances were measured concurrently and a number were estimated serially during pregnancy and the puerperium. Plasma concentrations of cortisol, corticosterone, 11-deoxycorticosterone (DOC) and progesterone were also measured in a number of women.

The circulating levels of renin, renin-substrate, angiotensin II and aldosterone were found to be increased above the normal non-pregnant range in many of the women studied. The mean plasma concentrations of renin-substrate, angiotensin II and aldosterone increased significantly from the first to the third trimester, whereas mean plasma renin

concentration fell significantly over the same period although it remained above the normal non-pregnant range. Following delivery of the foetus and placenta, plasma renin, angiotensin II and aldosterone concentrations returned to normal non-pregnant levels within two weeks. The administration of the oestrogen stilboestrol to suppress lactation was thought to account for the continuing high level of renin-substrate up to six weeks post partum. The plasma concentrations of cortisol and 11-deoxycorticosterone were generally elevated in the last trimester but most of the values for plasma corticosterone concentration were within the normal non-pregnant range throughout gestation.

A significant positive correlation was found between plasma renin and aldosterone and between plasma renin-substrate and angiotensin II in the first trimester but not thereafter, while plasma renin-substrate and aldosterone showed the only significant correlation when cases at all stages of pregnancy were analysed. Therefore, in spite of the marked changes which occur, the relationships between the four major components of the renin-angiotensin-aldosterone system appear to be more complex in pregnancy than in other physiological and clinical situations. A speculative explanation for this lack of relationship might be the presence in the circulation of "chorionic renin" which could be inactive in the generation of angiotensin II but which might possibly be involved in the secretion of aldosterone by another pathway as yet unidentified.

The present study has shown that in normal pregnancy a positive correlation exists between plasma sodium and aldosterone concentrations, suggesting that the increased aldosterone secretion is a primary event

influencing sodium balance rather than a secondary effect of established sodium depletion. Limited balance studies in two women showed no evidence of marked sodium retention or excretion at any stage of gestation. However, it is possible that the raised circulating levels of aldosterone may be part of the maternal adaptive process to prophylactically conserve sodium in the face of a tendency to sodium loss caused by the increased glomerular filtration rate, the diversion of fluid and electrolytes to the foeto-placental unit and the natriuretic and anti-aldosterone effect of increased progesterone secretion.

In a small study, symptoms in early pregnancy did not appear to be related to concurrent plasma levels of renin, renin-substrate, angiotensin II, aldosterone or sodium but they did show a negative correlation with plasma potassium concentration. Hypokalaemia may therefore contribute to some of the symptoms in the first trimester.

The renin-angiotensin-aldosterone system was also investigated in women who had developed hypertension (B.P. 140/90 or over) with albuminuria after the 24th week of pregnancy. The results were compared with those from normal pregnant women matched for age, parity and gestation. The mean plasma concentrations of renin, renin-substrate, angiotensin II and aldosterone were all significantly lower in the hypertensive compared to the normotensive group. Plasma electrolytes did not differ significantly but blood urea and serum creatinine were both increased in the women with hypertension.

A study of the effect of oestrogen-progestogen oral contraceptives on the renin-angiotensin-aldosterone system was thought to be pertinent to the larger study of pregnancy. Normotensive women taking oestrogen-progestogen oral contraceptives demonstrated an increase in plasma renin-substrate concentration similar to that found in normal pregnancy and this was shown to be due to the oestrogenic component. Plasma concentrations of renin, angiotensin II and aldosterone showed no significant change. In women who developed a raised blood pressure (140/90 or over) while taking oestrogen-progestogen oral contraceptives, plasma renin and renin-substrate concentrations were similar to those in the normotensive women, while mean plasma angiotensin II concentration showed a slight rise of borderline statistical significance.

In normal pregnancy, therefore, profound changes occur in the renin-angiotensin-aldosterone system which may be related to the maintenance of maternal fluid and electrolyte homeostasis. These changes are less marked in women with hypertensive disease of pregnancy and it seems possible that in this condition the system is being suppressed by an excess of circulating mineralocorticoid which has yet to be identified.

CONTENTS

Acknowledgements

In view of the large number of laboratory prodedures involved, some of them very intricate and time consuming, the studies presented here of necessity involved close collaboration with laboratory staff.

I would therefore like to gratefully acknowledge the help of:-

Dr. R. Fraser, Miss Jessie Young, Miss Sheena Thompson, Mr. M. Tree, Dr. G. Disterdieck, Dr. J. Morton, Mr. G. McElwee Mr. A. Wilson and Mr. R. Rae of the MRC Blood Pressure Unit, Western Infirmary, Glasgow.

Dr. R. Logan and staff of the Department of Biochemistry, Queen Mother's Hospital, Glasgow.

Dr. I. Coutts of the Department of Obstetrics, Royal Maternity Hospital, Glasgow.

I would like to thank the many women who volunteered to take part in these studies and also the following clinicians who were supervising their care:-

Professor I. Donald and staff of the Department of Obstetrics, Queen Mother's Hospital, Glasgow.

Professor M. MacNaughton and staff of the Department of Obstetrics, Royal Maternity Hospital, Glasgow - especially Dr. Gilian McIlwaine.

Dr. A. Giles and staff of the Department of Obstetrics, Stobhill Hospital, Glasgow - especially Dr. C. Kraszewski.

Dr. D. Painton and staff of the Department of Obstetrics, St. Mary's Hospital, London.

Dr. L. Naismith and staff of the Glasgow Clinic of the Family Planning Association.

I am grateful to the Nursing Staff of the MRC Blood Pressure Unit, Western Infirmary and the Queen Mother's Hospital, Glasgow for their assistance in supervising the urine collection and to Mrs. Sheila Shakeshaft who made up the controlled diets.

Dr. I.G. O'Muircheartaigh and Dr. M. Titterington of the Department of Statistics, University of Glasgow helpfully arranged the computer programmes for part of the statistical analysis.

This work was carried out while I was holding appointments as Research Fellow and Senior Medical Registrar at the MRC Blood Pressure Unit, Western Infirmary, and I gratefully acknowledge the financial support of the Medical Research Council, James McCunn Trust and Family Planning Association during this period.

Mr. G. Donald and his staff of the Medical Illustrations Department, Western Infirmary were very helpful in the preparation of the figures used in this text, and I am indebted to the secretaries of the MRC Blood Pressure Unit, especially to Mrs. Evelyn Cruikshank for her unstinting efforts in the typing of the manuscript.

In particular, I have appreciated the steady encouragement and stimulating criticism of Dr.J.J. Brown, Dr. R. Fraser, Dr. A.F. Lever and Dr. J.I.S. Robertson, the constant support of Dr. J.D.C. Kerr and Professor G.M. Wilson and the unflagging patience of my wife and family.

Publication of Work Contained in this Thesis

Material from part of this work has been reported in the following papers:-

WEIR, R.J., PAINTIN, D.B., FRASER, R., ROBERTSON, J.I.S., TREE, M., and YOUNG, J. (1970). Renin, angiotensin and aldosterone relationships in normal pregnancy. Proc. R. Soc. Med. 63: 1101.

WEIR, R.J., TREE, M. and FRASER, R. (1970). Effect of oral contraceptives on blood pressure and on plasma renin, renin-substrate and corticosteroids. J. clin. Path. 23: Suppl. 3: 49.

ROBERTSON, J.I.S., WEIR, R.J., DÜSTERDIECK, G.O., FRASER, R. and TREE, M. (1971). Renin, angiotensin and aldosterone in human pregnancy and the menstrual cycle. Scott. med. J. 16: 163.

WEIR, R.J., TREE, M., FRASER, R., CHINN, R.H., DAVIES, D.L., DÜSTERDIECK, G.O., ROBERTSON, J.I.S., HORNE, C.H.W. and MALLINSON, A.C. (1971). The effect of combined oestrogen-progestogen oral contraceptives, and of their separate components, on plasma levels of renin, renin-substrate, angiotensin and aldosterone, and on blood pressure. Proc. 3rd Inter. Congr. Hormonal Steroids, Hamburg, 1970. Excerpta Medica Inter. Congr. Series No. 219, p. 929.

WEIR, R.J., PAINTIN, D.B., BROWN, J.J., FRASER, R., LEVER, A.F., ROBERTSON, J.I.S. and YOUNG, J. (1971). A serial study in pregnancy of the plasma concentrations of renin, corticosteroids, electrolytes and proteins: and of haematocrit and plasma volume. J. Obstet. Gynaec. Br. Commonw. 78: 590.

WEIR, R.J., DÜSTERDIECK, G.O., FRASER, R. and TREE, M. (1971). Changes in blood pressure and in plasma renin, renin-substrate, angiotensin, and aldosterone concentrations in women taking contraceptive steroids. Nebenwerkungen Contraceptiver Steroide Symposium, Berlin, 1970, Westkruez-Verlag, p. 106.

WEIR, R.J., TREE, M. and McELWEE, G. (1972). Changes in blood pressure and in plasma renin, renin-substrate and angiotensin II concentrations in women taking contraceptive steroids. Proc. 4th Inter. Congr. Endocrinology, Washington 1972. In press.

SECTION I

INTRODUCTION

RENIN-ANGIOTENSIN-ALDOSTERONE RELATIONSHIPS IN NON-PREGNANT SITUATIONS

LABORATORY METHODS

CHAPTER 1

INTRODUCTION

"The discovery of the toxin in toxaemic cases by means of a perfected physiological chemistry will point to its antidote". Professor Amand Routh, Presidential Address to the Obstetrical Society of London, 1911.

Hypertension in pregnancy remains one of the major causes of maternal and foetal mortality. The fact that numerous investigators over the years have failed to unravel its cause lends fascination to this condition, which has aptly been called "die Krankheit der Theorien" - the disease of theories (Boyd, 1958).

In recent years, one of the theories in vogue has been that raised blood pressure in pregnancy might be associated with changes in the renin-angiotensin-aldosterone system, angiotensin II being a powerful vasoconstrictor and aldosterone having a potent sodium-retaining effect. However, although the relationships between plasma renin, renin-substrate, angiotensin II and aldosterone have now been defined in a number of physiological and pathological situations, their relationships in normal pregnancy have yet to be clearly elucidated, while their role in pregnancy hypertension has remained mainly speculative.

In the MRC Blood Pressure Unit at the Western Infirmary, Glasgow, methods have been developed for measuring plasma concentrations of renin, renin-substrate, angiotensin II, aldosterone and other mineralocorticoids. The purpose of the work presented here was to apply those methods to a detailed study of the physiological relationships of renin, renin-substrate, angiotensin II and aldosterone in normal pregnancy and to follow this by an examination of their possible pathological role in pregnancy hypertension.

Women taking oestrogen-progestogen oral contraceptives could be considered as a partial model for the effects of the steroid sex hormones secreted during pregnancy. As hypertension may be induced by these preparations, it was thought that an investigation of the changes in the renin-angiotensin-aldosterone system in women taking contraceptive steroids might be applicable to the larger problem of hypertensive disease of pregnancy.

CHAPTER 2

RENIN-ANGIOTENSIN-ALDOSTERONE RELATIONSHIPS IN NON-PREGNANT SITUATIONS.

Renin, renin-substrate and angiotensin.

Renin was found as a pressor substance present in extracts of renal cortex by Tigerstedt and Bergman (1898) and Pickering and Prinzmetal (1938) and is thought to be secreted by the juxtaglomerular cells of the kidney (see Brown, Davies, Lever and Robertson, 1966a). A renin-like substance has also been demonstrated in salivary glands and arterial walls (see Brown et al, 1966a), and in uterus, placenta and amniotic fluid (see Chapter 6.D).

In the plasma renin reacts with an alpha-2 globulin (renin-substrate, angiotensinogen) to form a decapeptide, angiotensin I, which is subsequently converted to an octapeptide, angiotensin II (Figure 1). The octapeptide is then broken down into peptide fragments by blood and tissue peptidases (angiotensinases) (see Page and Bumpus, 1961; Lee, 1969). In most situations it appears likely that changes in the concentration of the enzyme renin control the level of circulating angiotensin (Figure 2) but theoretically it is possible that substrate concentration may also be a factor limiting the rate of formation of angiotensin. Evidence for this has been given by Helmer and Judson (1967) and Skinner, Lumbers and Symonds (1969; 1972), but it has been disputed by Gould, Skeggs and Kahn (1966.

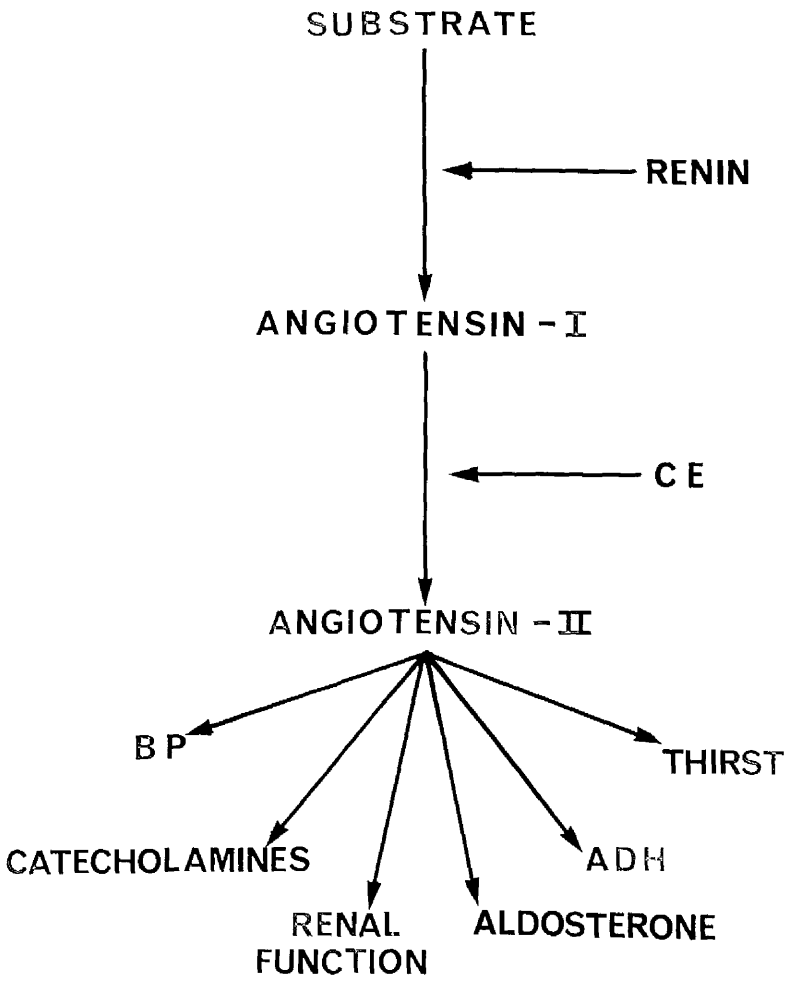


Figure 1. The renin-angiotensin system.

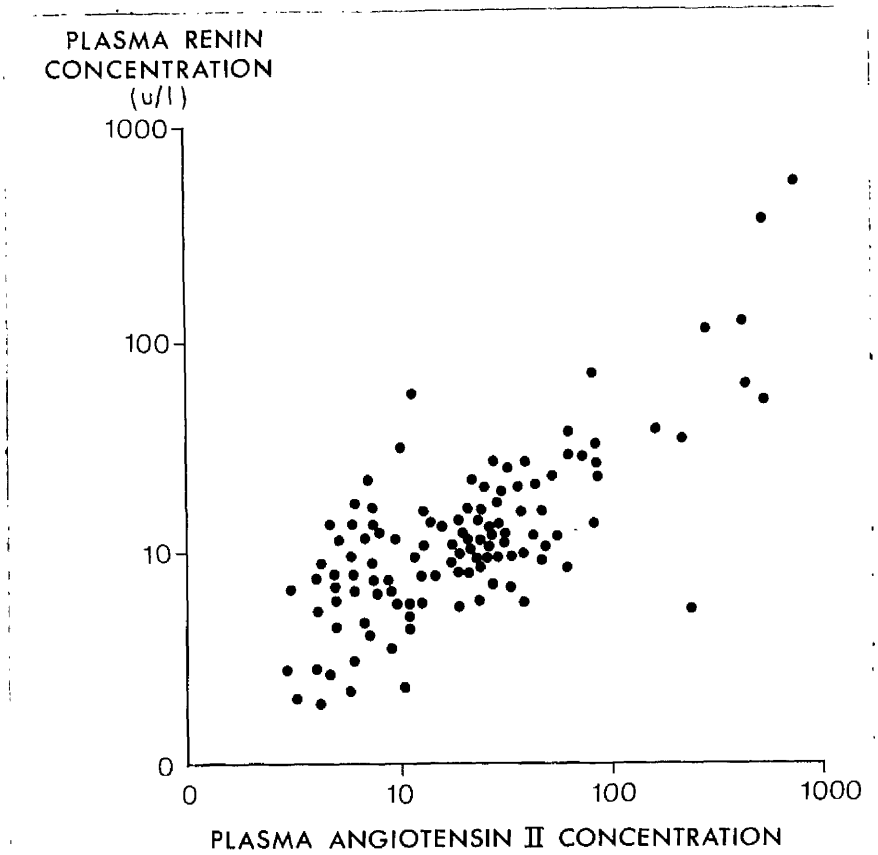


Figure 2

Renin-angiotensin II relationship in cases of hypertension

r = 0.82

p = <0.001

(data from MRC Blood Pressure Unit - to be published).

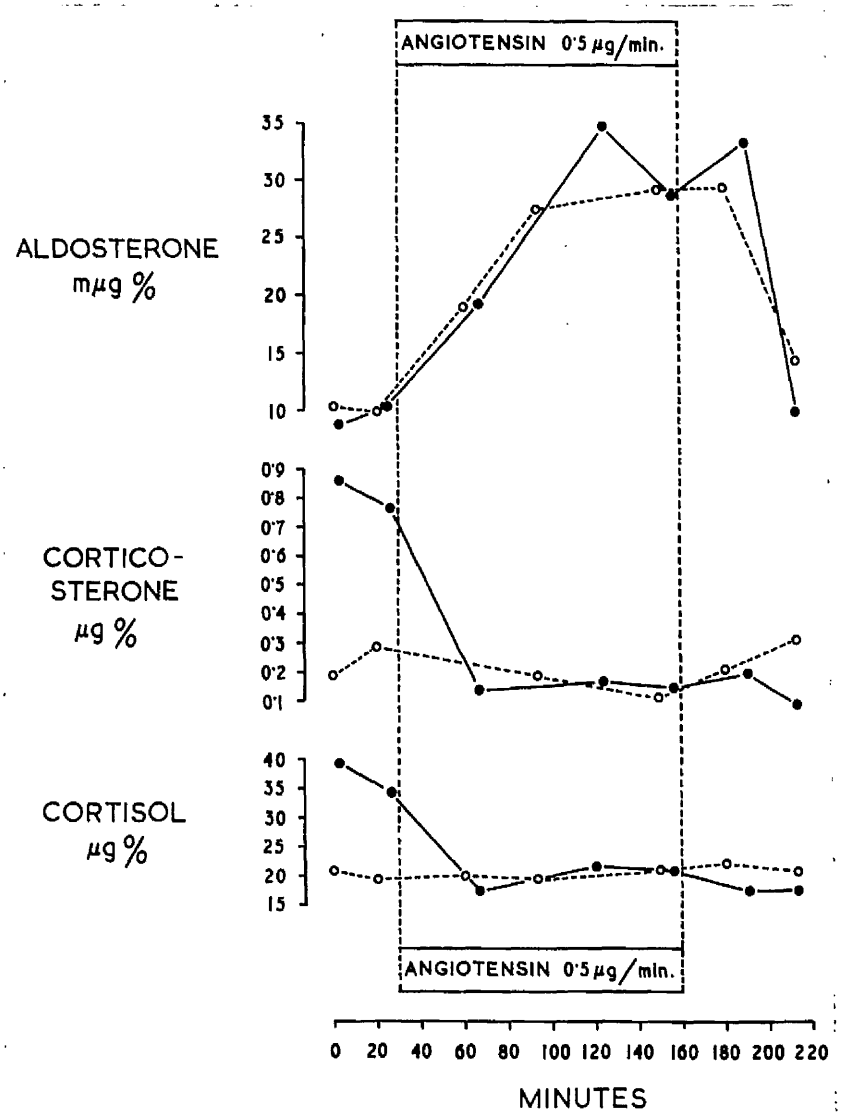


Figure 3

Effect of angiotensin II infusion

(from Fraser et al 1965)

The role of these substances both in health and disease remains incompletely understood, but as far as is known, renin itself has no direct physiological effect, its various actions being mediated by angiotensin. Although the decapeptide angiotensin I may have pharmacological effects on certain tissues (Carrière and Biron, 1970) and the 2 -8 heptapeptide fragments may stimulate aldosterone secretion (Blair-West, Coghlan, Denton, Funder, Scoggins and Wright, 1971) the octapeptide form, angiotensin II, has the major physiological and pharmacological actions.

Angiotensin II has a profound influence on the control of electrolyte and water metabolism and of blood pressure. It may promote sodium retention and potassium excretion indirectly by its ability to stimulate aldosterone secretion (see next section). In addition to its vasoconstrictor and pressor effect, it alters the excretion of sodium and water by the kidneys and may also stimulate the release of antidiuretic hormone and catecholamines and provoke thirst (see Brown et al, 1966a; Brown, Fraser, Lever and Robertson, 1968; Lee, 1969; Fitzsimmns and Simons, 1969; Bonjour and Malvin, 1970; Brown, Chinn, Gavras, Leckie, Lever, McGregor, Morton and Robertson, 1972b). Although many of these effects are inter-related and are involved in the overall fluid and electrolyte balance of the body, it is its effect on aldosterone secretion which will be considered in detail in this study.

Renin, angiotensin and aldosterone.

Aldosterone is a very potent steroid hormone secreted by the zona glomerulosa cells of the adrenal cortex. It was first isolated in 1953 (Simpson, Tait, Wettstein, Neher, von Euw and Reichstein). Its main effect is on the renal tubules where it promotes sodium reabsorption and causes increased potassium excretion. It also decreases the Na:K ratio of saliva, sweat and gastro-intestinal secretions (see Fraser, Brown, Chinn, Lever and Robertson, 1969; Brown, Fraser, Lever and Robertson, 1972b).

The control of aldosterone secretion has been discussed in detail by Fraser et al (1969) and Brown et al (1972b), with particular reference to stimulation by ACTH, sodium depletion, potassium loading and renin/angiotensin.

Although it still remains to be demonstrated that the concentrations of renin or angiotensin II in the plasma are within a range capable of affecting aldosterone secretion (Blair-West, Coghlan, Denton, Funder and Scoggins, 1972; Boyd, Adamson, Arnold, James and Peart, 1972; Brown et al, 1972b; Davis, 1972), there is strong circumstantial evidence to suggest that aldosterone production may be governed, at least in part, by changes in circulating levels of renin and angiotensin II (see Brown et al, 1968; Fraser et al, 1969; Brown et al, 1971a; 1972b). Angiotensin II may also effect the rate of clearance of aldosterone from the circulation (see Fraser et al, 1969).

A relationship between aldosterone and angiotensin II in man has been reported by several workers who have demonstrated increases in aldosterone secretion and excretion (Genest, Nowaczynski, Koiw, Sandor and Biron, 1960; Laragh, Angers, Kelly and Lieberman, 1960; Biron, Koiw, Nowaczynski, Broullet and Genest, 1961; Ames, Borkowski, Sicinski and Laragh, 1965), and in plasma concentration (Fraser, James, Brown, Isaac, Lever and Robertson, 1965) following systemic infusion of angiotensin II (Figure 3).

Correlated changes in concurrent plasma renin/angiotensin II and aldosterone concentrations have been shown in several situations, particularly those associated with sodium depletion (Figure 4), (Fraser et al, 1965; Fraser, James, Brown, Davies, Lever and Robertson, 1966; Fraser et al, 1969). A positive correlation between plasma renin/angiotensin and aldosterone concentration has also been demonstrated in cases of renal hypertension and hypertension associated with chronic renal failure (Figure 5) (Brown, Dusterdieck, Fraser, Lever, Robertson, Tree and Weir, 1971b).

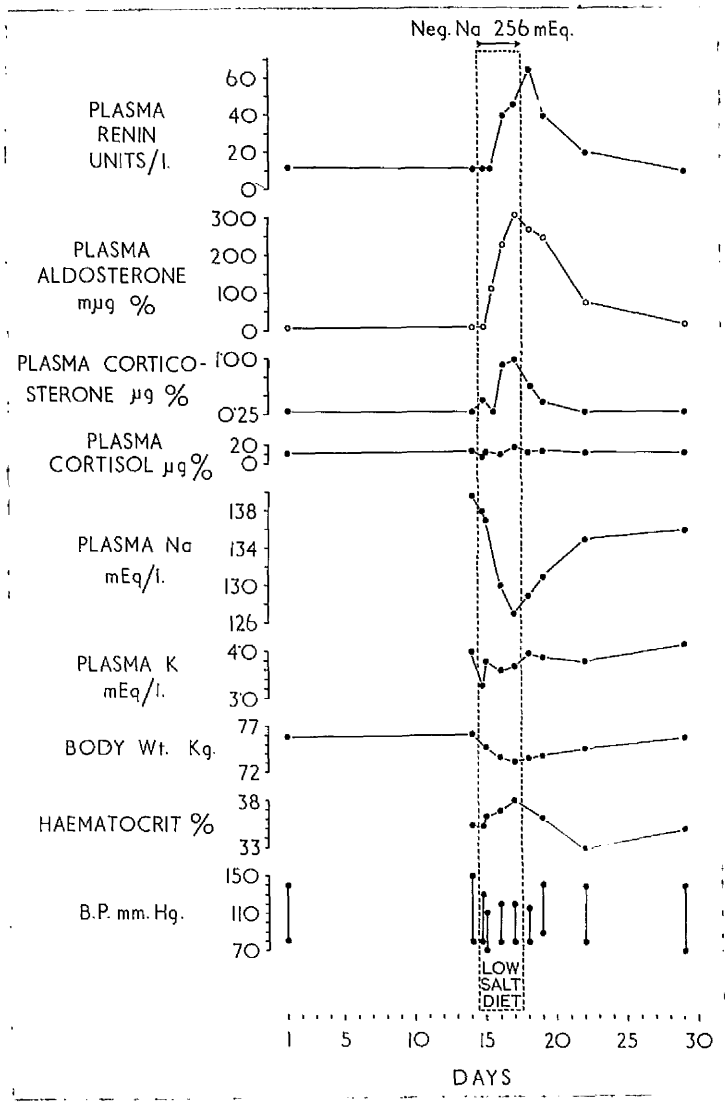


Figure 4

Effect of sodium deprivation

(from Fraser et al 1966).

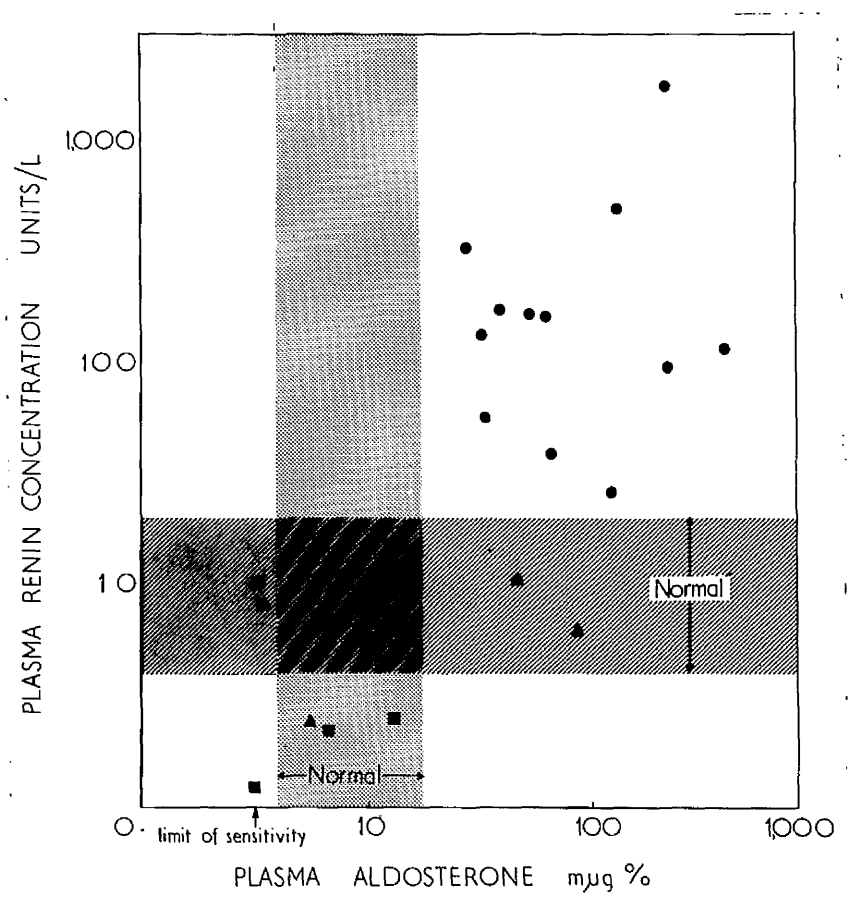


Figure 5

Renin-aldosterone relationship in cases of chronic renal failure with hypertension

(from Brown et al 1971b)

CHAPTER 3

LABORATORY METHODS

The substances analysed in the studies to be described were measured as follows:-

Plasma renin concentration.

By the enzyme kinetic method of Brown, Davies, Lever, Robertson and Tree (1964a).

In this technique renin is extracted from plasma by adsorption on diethylaminoethylcellulose, eluted, and acidified to remove traces of angiotensinases. An important function of this process is the removal of substrate, activators and inhibitors. It is then incubated with a standard preparation of angiotensinase-free substrate obtained from ox serum. The angiotensin formed is measured by its pressor effect on an anaesthetised rat preparation compared to standard octapeptide angiotensin II. The initial velocity of angiotensin formation in the incubation mixture is in these circumstances a measure of the renin concentration of the plasma.

Recoveries are low (40%) but consistent. Replicate estimations from a stock plasma pool during the period of this study showed a mean of 24.97 units/L (S.D. $\pm$ 3.98), n = 62. The normal range in non-pregnant subjects is 4 - 20 units/L.

A number of other methods measure plasma renin activity rather than concentration (see Lee, 1969). These techniques usually involve destruction or inactivation of angiotensinases, followed by incubation of the plasma sample for a fixed period, usually 3 hours. The angiotensin formed is then a measure of

the renin activity of the sample. As it may be affected by substrate present in the plasma, it gives a different kind of information compared with the method for renin concentration. When plasma renin substrate concentration can be measured separately, estimation of plasma renin concentration is likely to be more informative than measurement of plasma renin activity.

Estimation of plasma renin concentration or activity may be affected by any activator or inhibiter remaining in the incubated sample and by failure to estimate losses of renin or substrate during the process of angiotensinase destruction or inactivation. Also, some of the techniques fail to detect renin activity in a proportion of normal subjects.

Plasma renin substrate concentration.

By the method of Tree (1972).

The essence of this technique, as in a number of previously reported methods (see Lee, 1969), is the conversion of the substrate to angiotensin I using a high concentration of added renin, at the same time preventing the degradation of the formed angiotensin by means of prior inhibition of plasma peptidases.

The blood collected at venepuncture is immediately mixed with an angiotensin inhibitor solution containing ethylene diamine tetra-acetic acid (EDTA), o-phenanthroline-monohydrate and neomycin buffer. The mixture is then incubated with a standard excess amount of human renin. The angiotensin formed is measured by its pressor effect on an anaesthetised rat

preparation compared to standard decapeptide angiotensin I. The amount of angiotensin formed in the incubation mixture is then a measure of the concentration of renin substrate in the plasma.

Recovery of substrate through the method is 94%. Replicate estimations of 40 samples measured in 2 batches had coefficients of variation of 8.4% and 10.7%. The range in 30 normal non-pregnant females was 0.45 - 1.28 uM.

Differences in technique are likely to contribute to the wide variation in plasma renin substrate concentration which have been reported previously for normal subjects (see Tree, 1972). Most of the methods described give no details of recovery data, only a few show that conversion of substrate to angiotensin is complete, and none confirms that the assayed angiotensin is in the same molecular form as the standard.

Plasma angiotensin II concentration.

By the radioimmunoassay method of Dusterdieck and McElwee (1971).

In this technique angiotensinases and converting enzyme are inhibited by adding the sampled blood quickly to an inhibitor solution containing ethylene diamine tetra-acetic acid (EDTA) and o-phenanthroline in neomycin buffer. The angiotensin is then extracted by Dowex (H^+) particles, eluted with ammonia and incubated with rabbit antiserum containing antibodies against Asn^1-Val^5-angiotensin II. Asn^1-Val^5-angiotensin II iodinated with $Na^{125}I$ is used as the radioactive label.

Recoveries from blood for this technique were 83% (S.D. ± 11, n = 44). Replicates within one assay varied about their mean by a standard deviation of ± 10%, n = 16. Peripheral venous plasma concentrations in 33 normal adults ranged from 5 to 39 pg/ml.

The results obtained by this method are similar to those of other radio-immunoassay techniques using extracted plasma, but lower than those found with unextracted plasma (see Easterdieck and McIlwee, 1971). This difference may be due to cross-reaction of antiserum with renin substrate in the unextracted samples. Bioassay methods, which are in general less satisfactory than radioimmunoassay techniques, have shown either similar normal levels to those found here (Mulrow, 1964a) or considerably higher values (Massani, Finkelman, Worcel, Agrest and Paladini, 1966).

Plasma aldosterone, corticosterone and cortisol concentrations. By a modification of the double isotope derivative technique of Fraser and James (1968).

In this method, known quantities of the (^{14}C) steroids under investigation are added as tracers to the plasma before extraction. The extract is then acetylated with (^{3}H) acetic anhydride and the acetates subsequently subjected to preliminary chromatographic purification with a thin-layer silica gel system. The labelled acetates of aldosterone, corticosterone and cortisol are then purified separately by paper chromatography and the final residues counted in a liquid scintillation spectrometer.

Recoveries, replicate estimations from a stock plasma pool and normal ranges are shown:-

	Recovery of added steroid %	Replicate estimations Mean	±S.D.	n	Normal non-pregnant ranges
Aldosterone (mug/100ml)	118, 102	12.01	0.98	46	< 18.0
Corticosterone (ug/100ml)	95.2, 98.1	0.46	0.04	22	0.13 - 2.30
Cortisol (ug/100ml)	100.3, 99.5	6.32	0.19	22	6.0 - 20.0

In comparison to isotope derivative methods, other chemical techniques in general do not provide as great a degree of sensitivity in the measurement of the low concentrations of steroid hormones found in peripheral plasma. Even with the isotope derivative method used in this study, the sensitivity is limited (i.e. to about 4 mug/100 ml for aldosterone). This is mainly due to the low specific activity of (3H) acetic anhydride.

A simpler way of estimating aldosterone is by measurement of its daily excretion into the urine by fluorimetric, colorimetric or isotopic methods. These techniques, however, have two main disadvantages: (1) As a metabolite, urinary free aldosterone or its 3-oxoconjugate represents only a minor proportion of the total daily secretion of aldosterone; and (2) The quantity of a single metabolite may vary disproportionately to the total secretion rate.

Secretion rate measurements are theoretically more acceptable, since they give an indirect estimate of the actual production of steroids by the adrenal gland integrated over a 24-hour period. They are, however, more difficult to perform than excretion rate assays (see Fraser et al, 1969).

Although plasma concentration has the disadvantage of being a measurement at one point in time, it is preferable to excretion and secretion rate measurements in that sampling is relatively easy and less time consuming and can be repeated at short intervals. In addition, it can be correlated with other substances estimated in plasma taken at the same time.

Plasma 11-deoxycorticosterone (DOC) concentration.

By the method of Wilson and Fraser (1971).

In this method (^{3}H) DOC is added to the plasma before extraction. Two thin-layer chromatographic systems are employed, and the amount of DOC in the sample is measured by means of gas-liquid chromatography with electron capture detection.

After addition of 5, 10, 15 and 20 ng. DOC to samples of a normal plasma pool, the means of duplicate recoveries were 93%, 100%, 92% and 94% respectively. Replicate assays carried out on samples from three different pools of normal plasma gave mean DOC concentrations (in ng/100 ml. $\pm$ S.D.) of 18.1 $\pm$ 2.32 (n = 7), 15.0 $\pm$ 3.16 (n=8) and 6.7 $\pm$ 1.08 (n = 7). Plasma samples from 20 normal ambulant subjects (14 male, 6 female)

taken between 10.00 and 11.00 a.m., gave concentrations ranging from 4.1 to 17.2 ng/100 ml.

This method has the advantages of gas-liquid chromatography which is an extremely effective purification technique, and of electron capture detection which possesses a high level of sensitivity. The use of a detector by-pass valve largely overcomes the problem of contamination (see Wilson and Fraser, 1971). The normal range found by this method is very similar to that reported for a double isotope derivative technique (James, Rippon and Arnold, 1969).

Other measurements.

Plasma progesterone concentration - Modification of the competitive protein-binding method of Martin, Cooke and Black (1970).

Plasma* and urinary sodium Plasma* and urinary potassium	- Eppendorf flame photometer in first study; autoanalyser in subsequent studies.
Plasma osmolality*	- Advanced osmometer.
Blood and urinary urea Serum and urinary creatinine	- Autoanalyser.
Plasma proteins	- Biuret method in first study; paper electrophoresis in subsequent studies.
Urinary proteins	- Albustix (Ames Ltd.) in first study; in subsequent studies; turbidometric method in most cases; Esbach's reagent in some cases with marked proteinuria.

Colloid osmotic pressure	- Rowe colloid osmometer
Plasma volume*	- Method using Evans Blue, as described by Hytten and Paintin (1963).
Urinary oestriol	- Autoanalyser (method of Barnard and Logen, 1970).

CLINICAL METHOD.

Diet, posture, time of sampling and clinical details of the cases will be given later for each study.

Blood pressure was measured using a standard sphygmomanometer and cuff.

Blood samples were taken from an antecubital vein in every case.

STATISTICAL ANALYSIS.

Analysis of the data in the first study (Chapter 5.A) was carried out by an English Electric KDF 9 digital computer programmed for multiple regression analysis. In subsequent studies a Hewlett-Packard 9810A Desk Calculator was used, apart from a step-wise regression calculation carried out in the second study (Chapter 5.B) by an IBM 360/50 computer. Where appropriate, results between matched pairs were compared using the Wilcoxon test for pair differences (Documenta Geigy Scientific Tables, Seventh Edition, p. 192).

*The mean of duplicate measurements was used for statistical analysis.

SECTION II

NORMAL PREGNANCY.

CHAPTER 4

LITERATURE REVIEW.

A. PLASMA RENIN, RENIN-SUBSTRATE AND ANGIOTENSIN II IN NORMAL PREGNANCY.

The distinction between plasma renin activity and plasma renin concentration has already been drawn (see Chapter 3). As plasma renin-substrate is markedly increased during pregnancy (see Chapter 5), estimation of plasma renin activity in this situation is likely to be less meaningful than measurement of both plasma renin concentration and substrate concentration when sampled concurrently.

Previously reported results using these two different methods of measuring plasma renin will therefore be discussed separately.

PLASMA RENIN ACTIVITY.

The disadvantages of this method, which have been mentioned earlier (Chapter 3), should be kept in mind when interpreting the numerous published reports of changes in plasma renin activity in normal pregnancy (Fasciolo, de Vito, Romero and Cucchi, 1964; Winer, 1965; Genest, de Champlain, Veyrat, Boucher, Tremblay, Strong, Koiw and Marc-Aurèle, 1965; Helmer and Judson, 1967; Goelhood and Vander, 1968; Boonshaft, O'Connell, Hayes and Schreiner, 1968; Gordon, Parsons and Symonds, 1969; Lumbers, 1970;

Schmidt and Rosenthal, 1971; Kokot and Cekański, 1972, Skinner et al, 1972).

Due to variations in technique, posture, diet and time of sampling, it is not possible to make a direct comparison of the levels of plasma renin activity found by these different groups. However, all authors reported a general increase in plasma renin activity in normal pregnancy, apart from one report (Maebashi, Aida, Yoshinaga, Abe, Miwa and Watanabe, 1964) in which no significant difference from the normal non-pregnant range was demonstrated. Consistent increases were found by two groups (Geelhoed and Vander, 1968; Boonshaft et al, 1968), whereas in the other series a varying number of women had levels within the normal non-pregnant range. Parity did not appear to influence the changes in plasma renin activity in any of the series reported.

In two studies including early pregnancy (Genest et al, 1965; Helmer and Judson, 1967), increased plasma renin activity was found as early as 4 weeks and 8 weeks gestation. However, the progressive rise in plasma renin activity between the 4th and 21st week demonstrated by Genest and his colleagues (1965) was not confirmed over the same period by Helmer and Judson (1967); Gordon and his group (1969) found no significant difference between gestational periods from the 13th week to term; and Schmidt and Rosenthal (1971) found the mean value for the last 10 weeks to be lower than that for the preceding

10 weeks of pregnancy. A possible disadvantage of these studies is that the measurements were made on individual women at different stages of pregnancy. Serial measurements during pregnancy in the same women might be more meaningful.

Effect of Posture and Diet.

A normal physiological increase of plasma renin activity was shown to occur in pregnant women who changed from the supine to the upright posture (Boonshaft et al, 1960). A larger increase occurred in women who took a low salt diet (Boonshaft et al, 1960), but there was a considerable overlap with the range found in women on an unrestricted diet (Genest et al, 1963; Gordon et al, 1969). No significant difference was apparent in women treated with thiazide diuretics (Helmer and Judson, 1967).

Effect of Labour.

Reports on the effects of labour are conflicting, one showing an increase in plasma renin activity (Geelhoed and Vander, 1968) and one a decrease (Helmer and Judson, 1967).

Changes in the Puerperium.

After delivery of the foetus and placenta, plasma renin activity remained high in some cases for up to 7 days, but had fallen to normal non-pregnant levels by 2 weeks post-partum (Helmer and Judson, 1967; Geelhoed and Vander, 1968).

PLASMA RENIN CONCENTRATION.

Although a more accurate indication of circulating levels of renin in pregnancy, plasma renin concentration has been much less frequently studied than plasma renin activity in this situation.

In a study of 50 normal women at various stages of pregnancy, plasma renin concentration was found to be raised in 20 cases (Brown, Davies, Doak, Lever and Robertson, 1963). The samples were taken in either the sitting or lying position with the women on an unrestricted diet. Increased circulating renin levels occurred at all stages of pregnancy, several of the highest values being in the first trimester. There was no progressive rise in plasma renin concentration during pregnancy, in contrast to the plasma renin activity measurements of Genest and his colleagues (1963).

In a subsequent study (Brown, Davies, Doak, Lever and Robertson, (1966b) serial estimations of plasma renin concentration were carried out at intervals throughout pregnancy in seven normal women on an unrestricted diet. In each case the plasma renin levels were above the normal non-pregnant range (4 to 20 units/litre) on most occasions, one woman having a concentration of 38 units/litre as early as 5 weeks after the last menstrual period. As in the preceding study of a large number of individual cases (Brown et al, 1963), these serial measurements in a small number of women showed no consistent change in plasma renin

concentration from early pregnancy to term.

Using the same method, my colleagues and I have confirmed those findings. The results of our earlier reports (Weir, Paintin, Robertson, Tree, Fraser and Young, 1970a; Weir, Paintin, Brown, Fraser, Lever, Robertson and Young, 1971a) will be described with those of our subsequent larger study in Chapter 5.

Our results have also been confirmed in a smaller study by Lumbers (1970), who demonstrated increased plasma renin concentrations in the last 8 weeks of pregnancy in all 6 of the women studied by her.

Serum renin concentration was shown to be above the normal non-pregnant range in 7 cases when measured in 14 normal women on an uncontrolled diet between 16 and 39 weeks' gestation (Gould et al, 1966). The authors did not comment on the levels at different stages of pregnancy, but on analysing their results, I have found that the highest values occurred in the last trimester. No adequate comparison with the previous studies is possible as the renin concentration was measured in serum, a different method was used, the number of individual cases studied was small, and no serial estimations were made.

Effects of posture and diet.

As far as is known, there have been no reports on the effects of changes of posture or of sodium balance on plasma renin concentration in pregnant women.

Effects of delivery.

Using a method similar to our own, Skinner, Lumbers and Symonds (1968) found elevated plasma renin concentrations at delivery in each of 4 normotensive women. No other maternal plasma samples were obtained in what was mainly a study of renin-like activity in foetal and maternal tissues (see Chapter 6, D).

Changes in the puerperium.

Plasma renin concentrations measured in 10 women within two months of parturition were found to be in the normal non-pregnant range (Brown et al, 1963). In 3 women with increased plasma renin concentrations in late pregnancy, serial estimations showed a fall after delivery of the foetus and placenta, but in one case the renin level was still above the normal non-pregnant range after 4 days (Brown et al, 1966b). No marked change occurred post partum in 3 women who had plasma renin concentrations within the normal non-pregnant range before delivery.

PLASMA RENIN SUBSTRATE CONCENTRATION.

Disadvantages of some of the methods used to measure plasma renin substrate concentration have been mentioned previously (see Chapter 3).

In spite of those possible disadvantages, reports of plasma renin substrate concentration in normal pregnancy have shown a general consistency:-

Authors	Normal non-pregnant range.	Range in normal pregnancy.	Time of gestation.
Pickens et al (1965) (ng/ml.)	510 - 1010 (mean 729) n = 29	1930 - 3240 (mean 2613) n = 9	Last trimester
Gould et al (1966) (ng/ml.)	465 - 880 n = 23	2230 - 3860 n = 14	Not stated
Helmer & Judson (1967) (ng/ml.)	mean 425	560 - 2912 (mean 1444) n = 22	8 weeks - term
Lumbers (1970) (µg/ml.)	< 2.0 n = 5	0.5 - 7.8 n = 7	32 weeks-term
Weir et al (1970a) Robertson et al (1971) (mols x 10^{-6}, ml)	0.48 - 1.27 (mean = 0.79) n = 27	1.0 - 5.0 n = 40	6 weeks - term
Skinner et al (1972) (ug/ml.)	mean = 1.3 S.D. ± 0.5 n = 9	1.2 - 12.0 n = 34	4 weeks - term

The results described by Pickens and his colleagues (1965) are very similar to those of Gould and her associates (1966). Helmer and Judson (1967), however, reported a rather lower range in normal pregnant women. This could be due to the inclusion of data from the early weeks of pregnancy, but their mean of 425 ng/ml. for normal non-pregnant subjects is also lower than that quoted in other series. This could be explained by a low recovery of angiotensin as a result of incomplete inactivation of angiotensinases.

The earliest measurement in Helmer and Judson's study was at 8 weeks gestation, when a plasma renin substrate concentration of 830 ng/ml. was shown. Higher circulating levels of substrate were found later in pregnancy, but a progressive rise of plasma renin substrate during pregnancy could not be assumed from these data as only 4 measurements were made before 20 weeks' gestation. However, a similar trend was seen in the preliminary results from our own studies (Weir et al, 1970a; Robertson, Weir, Düsterdieck, Fraser and Tree, 1971 - to be described with the subsequent larger study in Chapter 5) and this has recently been statistically confirmed by Skinner and his group (1972).

Effects of Posture and Diet.

As far as is known, plasma renin-substrate concentration is not affected by changes in posture or of electrolyte balance in the normal non-pregnant state (see Lee, 1969), and pregnant women are unlikely to show a different response. In one study

(Helmer and Judson, 1967) thiazide medication in 7 women did not appear to influence the results.

Changes during labour and in the puerperium.

In 4 cases studied by Helmer and Judson (1967) a transient rise in plasma renin substrate concentration occurred after delivery. In a further 16 women the substrate level remained high for up to 5 days after delivery in most cases but had fallen to normal non-pregnant limits after 2 weeks.

BLOOD AND PLASMA ANGIOTENSIN CONCENTRATION.

Two studies of circulating angiotensin levels in pregnancy have been reported - that of Massani, Sanguinetti, Gallegos and Raimondi (1967) and preliminary results of our own study (Robertson et al, 1971). Details of our results which were obtained with a radioimmunoassay method, are given later (Chapter 5).

Massani and her colleagues used a chemical extraction procedure combined with a bioassay which did not distinguish between angiotensin I and II. Using arterial whole blood samples from women who had rested overnight, they found a range of 124 to 290 pg/ml (mean = 200 $\pm$ 22 S.E.) during the third trimester in 9 normal pregnant subjects. This compared with a range of 0 to 193 pg/ml. (mean = 120 $\pm$ 24 S.E.) in 7 non-pregnant normotensive patients. Thus, although there was an

overlap between the groups, the difference in mean angiotensin blood levels was significant ($p < 0.05$). Samples taken from 8 women between 3 and 5 days after delivery showed a fall in angiotensin in 4 and a rise in 4 cases, the mean level (219 ± 45 S.E. range 68 to 373 pg/ml) not being significantly different from the late pregnancy estimation.

For technical reasons it is likely that those figures overestimated the level of angiotensin II. Another criticism is that the control group consisted of patients suffering from a variety of diseases which could possibly have influenced the blood angiotensin concentration.

Blood Angiotensinases.

Various peptidases present in blood are capable of inactivating circulating angiotensin and are therefore known as "angiotensinases". The term however does not necessarily imply a specific action on angiotensin. Since variations in angiotensinase activity might affect the survival and hence the concentration of angiotensin, a number of measurements of angiotensinase activity have been made in blood from normal pregnant women.

In 1947, Page found a progressive increase in plasma angiotensinase activity during gestation, the levels in 11 women in the second half of pregnancy being up to 10 times the levels found after delivery. A gradual increase in angiotensinase

activity from 2 months to term was also shown by Berger and Langhans (1967) and Morandini and Mangioni (1969). High levels in late pregnancy have been confirmed in a number of other studies (Hickler, Lauler and Thorn, 1963; Nagatsu, Gillespie, George, Folk and Glenner, 1965; Talledo, 1967, and Lubash, Bard and Kline, 1969).

In contrast, other reports have shown no increase in angiotensinase activity even in late pregnancy (Klaus, 1962; Landesman, Biron, Castellanos, la Russe and Wilson, 1963; Klaus and Biron, 1964). Differences in sampling and in methodology may possibly explain this discrepancy.

Vascular and Renal Responses to Angiotensin Infusion.

A reduction in the pressor response to intravenous infusion of angiotensin is characteristic of pathological conditions associated with high circulating levels of renin and presumably angiotensin (Kaplan and Silah, 1964; Brown et al, 1966a; Lubash, Muleson, Alicandri, Garfinkel, Sickiorski and McConnaughey, 1971; Chinn and Düsterdieck, 1972).

It is not surprising, therefore, that the blood pressure response to angiotensin infusion has been shown to be consistently lower in the last trimester of pregnancy as compared with the non-pregnant state (Raab, Schroeder, Wagner and Gigee, 1956; Abdul-Karim and Assali, 1961; Chesley, Talledo, Bohler and Zuspan, 1965; Talledo, Rhodes and Livingstone, 1966), normal pregnant women at this stage of gestation requiring about 2.5 times more

angiotensin than in the first trimester when the blood pressure response is similar to non-pregnant subjects (Luukkainen, Kiseale, Jarvinen and Viranko, 1964). Page's suggestion (1949) that this might be due to increased circulating levels of angiotensinases is supported by the lower pressor responses nearer term (Abdul-Karim and Assali, 1961), when plasma angiotensinases are at their highest (see previous section).

Angiotensin II may reduce hepatic, splanchnic and peripheral blood flow in non-pregnant situations (de Bono, Lee, Mottram, Pickering, Brown, Keen, Peart and Sanderson, 1963; Brown, 1963; Emerson, 1966). In normal pregnancy the blood vessels appear to be less responsive to this effect of angiotensin, as Lumbers (1970) found that infused angiotensin II caused a smaller reduction in blood flow to the hands in pregnant compared to non-pregnant women.

Pregnancy has also been shown to modify the renal responses to angiotensin infusion, the urine flow and sodium excretion being reduced to a lesser degree in pregnant women in the last trimester compared to non-pregnant controls (Chesley, 1963; Chesley, Wynn and Silverman, 1963). Changes of posture apparently have no effect on this diminished response (Chesley, Sloan and Wynn, 1964). Luukkainen and his colleagues (1964) have shown a decrease in sodium excretion and creatinine clearance after angiotensin infusion in women between 9 and 15 weeks' gestation, but their results are open to question as no significant change occurred in their non-pregnant controls.

B. ALDOSTERONE AND OTHER MINERALOCORTICOIDS IN NORMAL PREGNANCY.

The advantages and disadvantages of estimating aldosterone excretion, secretion and plasma concentration have been discussed in Chapter 3. To date, most of the studies in pregnancy have involved measurement of excretion and/or secretion rates. Before discussing these, it will be helpful to outline the possible effects of pregnancy on the metabolic clearance of aldosterone.

METABOLIC CLEARANCE OF ALDOSTERONE.

The ratio of urinary free aldosterone to the secretion rate of aldosterone was shown to be 0.29 $\pm$ 0.04 (S.D.) in 6 normal pregnant women in the last trimester (Jones, Lloyd-Jones, Riondel, Tait, Tait, Bulbrook and Greenwood, 1959). This was similar to the ratio of 0.24 $\pm$ 0.07 (S.D.) in the non-pregnant group. Jones and his associates therefore suggested that, although there appeared to be a change in the pattern of aldosterone metabolism in pregnancy (see next section), there was little difference in the overall rate of metabolism. These results were subsequently confirmed by the same group (Tait, Little, Tait and Flood) 1962) in a comparison between 7 non-pregnant and 9 pregnant women in the last trimester. Similar clearance rates have also been demonstrated in a more recent study of

7 pregnant women near term and of 2 cases at 20 weeks' gestation (Bayard, Ames, Tapper, Waldon, Kowarski and Migeon, 1970a).

Tait and Little (1968) reported that there was a decrease in splanchnic clearance of aldosterone in pregnancy and they suggested that an increase in extra-splanchnic clearance occurred, associated with the foetus and placenta.

ALDOSTERONE EXCRETION.

Following the isolation of aldosterone (Simpson et al, 1953) attempts were made to implicate this hormone in the pathogenesis of pre-eclampsia and eclampsia (see Chapter 7). Naturally, normotensive pregnant women were also studied. Initial reports suggested that these women had urinary levels similar to non-pregnant subjects (Gordon, Chart, Hagedorn and Shipley, 1954; Venning, Singer and Simpson, 1954), but improvement in techniques later revealed increased aldosterone excretion during normal pregnancy (Barnes and Gulligan, 1956; Venning and Byrenfurth, 1956).

Using a more reliable method, Martin and Mills (1956) found urinary aldosterone to be increased in 35 out of 35 normal pregnant women, and as pregnancy progressed there was a tendency for the aldosterone values to increase, although the scatter was greater. Similar results were reported by other groups (Koenoreh, Wolff and Beer, 1957; Binsler and Rigby, 1957; Venning, Primrose, Caligaris and Byrenfurth, 1957).
1957)

Two later reports have shown an alteration in the pattern of aldosterone metabolites excreted in the urine in pregnancy as compared with the non-pregnant state (Jones et al, 1959; Tait and Little, 1968). Those workers found that the addition of a mild acid to the urine resulted in a greater release of aldosterone in pregnant compared with non-pregnant women and they considered that this was due to increased formation of the 3-oxo 4-ene conjugate of aldosterone in pregnancy. They suggested that this change in the pattern of metabolism of aldosterone in pregnancy might account, to some extent, for the increased urinary values found in earlier studies in which the analytical procedures measured aldosterone hydrolysed from the 3-oxo 4-ene conjugate.

Although those findings were not confirmed by another study (Watanabe, Meeker, Gray, Sims and Solomon, 1963), it remains possible that urinary aldosterone measurements in pregnancy may not accurately reflect changes in aldosterone secretion or plasma concentration.

ALDOSTERONE SECRETION.

Measurements of aldosterone secretion rate by isotope dilution methods have been made by a number of groups (next page).

The normal non-pregnant ranges quoted by the first three of those groups were very similar and the values for normal

Aldosterone Secretion Rate (ug/day).

Authors	Normal Non-pregnant Range	Normal Pregnancy	Time in Gestation	Posture	Diet
Jones et al (1959)	72 - 315 n = 6	248 - 1100 n = 6	32 - 38 weeks	Ambulant	Unrestricted
van de Wiele et al (1960)	100 - 400 n - not given	1040 - 2250 n = 3	Last trimester	Not stated	Unrestricted
Watanabe et al (1963)	85 - 216 n = 10	307 - 2912 n = 54	26 - 40 weeks	Not stated	Na = 80 - 190 mEq/day (3 unrestricted)
Thomas & Flynn (1964)	Not given	180 - 453 n = 4	Not stated	Not stated	Not stated
Bayard et al (1970a)	Not given	16 - 90 n = 5	38 - 40 weeks	Supine for 10 hours	Unrestricted

pregnancy were also approximately the same.

Watanabe and his colleagues (1963), however, found a larger variation in secretion rates, which may be a reflection of the larger number of women studied by them. They found an aldosterone secretion rate of 287 ug/day in one women at 15 weeks' gestation, while in another case results at 20 and 22 weeks were 892 and 953 ug/day respectively. The mean secretion rate increased from 859 ug/day to 1586 ug/day between 26 weeks and term, but a marked fluctuation occurred between those periods. In 2 other subjects, with a constant dietary sodium intake of 160 mEq/day, serial measurements of aldosterone secretion showed a considerable variation in the last trimester, an increase of 1048 ug/day occurring in one woman between 34 and 36 weeks, while in the other a decrease of 431 ug/day was found between 31 and 33 weeks.

Although Thomas and Flynn (1964) found lower aldosterone secretion rates than in the preceding studies, they claimed that those levels were increased, but did not quote a normal non-pregnant range for comparison.

The study by Beyard and his associates (1970a) showed even lower aldosterone secretion rates in late pregnancy (38 to 40 weeks) but once again the authors did not quote their own range for normal non-pregnant subjects. One woman studied by them at 20 weeks' gestation had a secretion rate within the late pregnancy range. They suggested that the discrepancy between

their results and those demonstrated previously might be due to the effects of posture on aldosterone secretion, as their patients had been supine for at least 10 hours before the study. This is supported by one report (Balikian, Brodie, Dale, Melby and Tait, 1968) which showed an 8-fold increase in aldosterone secretion rate in non-pregnant subjects on changing from the lying to the standing position. On the other hand, it is not supported by Sims (1964) who found that a period of recumbency of 4 to 5 days duration in 5 normal pregnant women did not reduce aldosterone secretion.

PLASMA ALDOSTERONE CONCENTRATION.

Four reports of plasma aldosterone concentrations in normal pregnancy have been published to date (Stark, 1967; Bayard et al, 1970a; Weir et al, 1970a, 1971a). The results of our two studies will be described in Chapter 5.

In a brief report, Stark (1967) demonstrated no difference in mean plasma aldosterone concentration between pregnant and non-pregnant women, but no further details are available.

Using a double isotope derivative technique, Bayard and his colleagues (1970a) measured plasma aldosterone concentrations in 5 normal pregnant women between 38 and 40 weeks' gestation. On an unrestricted diet, plasma aldosterone ranged from 1.7 to 5.0 mug/100 ml (mean = 3.5 mug). In one other woman

at 20 weeks gestation, plasma aldosterone concentration was 4.7 mug/100 ml. The authors suggested that those levels were about twice as high as the values for non-pregnant women (mean 2.16 ug/100 mg) reported by another group (Balikian et al, 1968). Strangely they do not compare their results with a non-pregnant range from their own laboratory, yet in a previous study by the same group (Bayard et al, 1970b) four normal non-pregnant subjects on an unrestricted diet had a range of 1.7 to 16.2 mug/100 ml. (mean = 6.1 mug/100 ml) after lying for one hour. These levels increased to 5.8 to 17.0 mug/100 ml (mean = 9.4 mug/100 ml) on standing. Unless a further fall in plasma aldosterone concentration might be expected to occur after lying for more than 10 hours, then the non-pregnant values were higher than those found by the same authors in pregnancy, but they make no comment about this.

ALDOSTERONE AND ELECTROLYTE BALANCE IN PREGNANCY.

Aldosterone is a very potent mineralocorticoid and, as was outlined in Chapter 2, its major physiological role appears to be in the control of electrolyte and fluid balance. The relationship between aldosterone and electrolyte balance in pregnancy has therefore been the subject of a number of studies.

In 1957 Rinsler and Rigby found a correlation between aldosterone excretion and the urinary Na:K ratio in the last

trimester, but two other reports failed to confirm this, nor did they demonstrate a correlation between plasma sodium concentration and aldosterone excretion in late pregnancy (Buchborn, Koczorek and Wolff, 1957; Jones et al, 1959). Watanabe and his colleagues (1963) took the endogenous creatinine clearance as an index of the quantity of sodium presented to the renal tubules by glomerular filtration, and found no correlation between this endogenous creatinine clearance and the aldosterone secretion rate in women taking 120 to 190 mEq sodium daily in the last trimester.

The effect of sodium depletion.

An increase in aldosterone excretion in normal pregnant women has been reported following sodium restriction (Barnes and Quilligan, 1956; Kumar, Feltham and Gornall, 1959) and the administration of a thiazide diuretic (Ehrlich, Lugibihl, Taylor and Janulis, 1967).

This normal physiological response to sodium deprivation and sodium loss in pregnancy was confirmed by Watanabe and his group (1963), who found that the aldosterone secretion rate in 3 women in the last trimester increased from a mean of 1576 ug/day to 7843 ug/day when the sodium intake was reduced from a mean of 150 mEq/day to 8.0 mEq/day for 2 weeks. They considered that this 5-fold increase in aldosterone secretion in pregnancy was greater than would be expected in the non-pregnant subject given the same degree of sodium deprivation. In one pregnant woman

studied by Bayard and co-workers (1970a), a "low sodium diet" was associated with a plasma aldosterone concentration of 29.4 mug/100 ml compared with the range of 1.7 to 5.0 mug/100 ml in 5 normal pregnant women on an unrestricted diet, but details of the "low sodium diet" were not given.

The effect of sodium loading.

Watanabe and his colleagues (1963) investigated the effect of sodium loading in pregnancy in 4 women in the last trimester, by increasing the sodium intake from a mean of 135 mEq/day to 190 mEq/day for 4 weeks. The aldosterone secretion rate rose in one of these women from 999 mug/day to 1208 mug/day, but fell in the remaining three, the overall mean showing a reduction from 1105 mug/day to 747 mug/day. Those results were confirmed by Stark and Lehmann-Achilles (1964) who found a 5-fold decrease in aldosterone excretion rate in 11 women in late pregnancy given an intravenous infusion of 18 g. sodium chloride for 10 days; and by two other studies which demonstrated a drop of at least 50% in aldosterone excretion in 4 pregnant women given over 12 g. sodium chloride daily (Ehrlich, Laves, Lugibihl and Landau, 1962; Ehrlich, Lugibihl, Laves and Janulis, 1966).

The effect of increased potassium.

Normal pregnant women given an increased intake of potassium (4.0 to 4.5 g/day) excreted similar quantities of aldosterone

as pregnant women given a normal potassium diet of 2 g/day (Barnes and Gulligan, 1956).

The effect of aldosterone inhibition.

Ehrlich (1971) has shown that heparinoid-induced inhibition of aldosterone secretion in pregnant women is associated with a rise in sodium excretion but has no effect on urine potassium.

It is apparent from the foregoing reports that, although the excretion and secretion of aldosterone in pregnancy is considerably higher than in non-pregnant situations, there appears to be a normal physiological response to both sodium deprivation and sodium loading, certainly in the later months of gestation. As far as is known, there has been no report of a study of aldosterone and sodium balance in the earlier months of pregnancy.

OTHER MINERALOCORTICOIDS.

Many reports have been published showing an increased plasma concentration of 17-hydroxycorticosteroids, mainly cortisol, during pregnancy, particularly in the last trimester (e.g. Gemzell, 1955; Bolliss, Browne, Bound and Stoinbeck, 1955; Robinson, Bernhard, Grubin, Wenner, Sevelow and Silber, 1955; Martin and Mills, 1958; Jailer, Christy, Longson, Wallace and Gordon, 1959; Bro-Rasmussen, Dans, Lundwall and Trolle, 1962;

Friedman and Beard, 1966; Goldberg, Lewenthal, Gottfried and Ben-Aderet, 1966; Neilsen, Binder and Starup, 1969; Kopelman and Levitz, 1970). This is mainly due to increased protein-binding in the plasma (Doe, Fernandez and Seal, 1964; de Moor, Steeno, Brosens and Hendrix, 1966; Keane, Pearson and Walker, 1969a; Rosenthal, Slaunwhite and Sandberg, 1969), only Daughaday (1958) failing to show this. A slight rise in inbound plasma cortisol has also been demonstrated (Doe, Dickinson, Zinneman and Seal, 1969; Keane et al, 1969a; O'Connell and Welsh, 1969; Rosenthal et al, 1969; Burke and Roulet, 1970) and an increased excretion of urinary free cortisol has been shown (Jailer et al, 1959; Thomas and Flynn, 1964; Gottfried, Lewenthal and Goldberg, 1968; Burke and Roulet, 1970).

As the purpose of the present study was to examine predominately mineralocorticoid activity in pregnancy, these reports on plasma and urinary cortisol levels are not presented in any detail here. They are well reviewed elsewhere (Forsham, 1967; MacNaughton, 1967; Hytten and Leitch, 1971).

Other adrenal hormones with greater mineralocorticoid activity have been less well reported.

Tobian (1949) measured urinary formaldehydogenic corticosteroids as an "index of deoxycorticosterone" and found raised levels in 18 normal pregnant women compared with 10 normal non-pregnant females. Exley and Norymberski (1964a) demonstrated an increased excretion of 17-deoxycorticosteroids (metabolites of corticosterone) in normal pregnancy, but no

metabolites of 11-deoxycorticosterone were found in a further study (Exley and Norymberski, 1964b). In urine pooled from several women in late pregnancy, Dässler (1969) reported higher levels of 11-deoxycortisol than in non-pregnant subjects.

Plasma corticosterone concentration (ug/100 ml) has been measured in normal pregnancy by four groups:-

Authors	Normal Non-pregnant Range.	Normal Pregnancy.	Time in Gestation.
Morris & Williams (1953)	4.0 - 10.5 n - not given	7.0 - 18.0 n = 4	Last trimester
Stewart et al (1961)	0.0 - 2.0 (mean 0.40) n = 27	0.0 - 2.0 (mean 0.67) n = 9	36th week
Martin & Martin (1968)	0.0 - 2.3 (mean 0.6±0.6 SD) n = 33	0.0 - 2.86 (mean 1.3±0.7 SD) n = 18	Late pregnancy
Schweitzer et al (1969)	Not stated	Mean 1.4±1.6 SD	Term

Stewart and his colleagues (1961) found no significant increase, but Martin and Martin (1968), using a similar fluorimetric method, did demonstrate a significant rise. Both of these studies, as well as the earlier one of Morris and Williams (1953), showed a considerable overlap with the normal non-pregnant range.

"Submicrogram" levels of plasma 11-deoxycorticosterone were reported in 7 normal pregnant women at term (Schweitzer,

Branchaud and Giroud, 1969), but no details of normal non-pregnant values were given.

C. COMMENTS ON LITERATURE REVIEW.

There is little doubt that pregnancy is associated with marked changes in the renin-angiotensin system. In spite of considerable differences in technique, plasma renin substrate has been shown to be elevated in almost all cases studied. Plasma renin activity and concentration are raised in many women, but a substantial number show levels within the normal non-pregnant range. Circulating levels of angiotensin II have been less well studied, but they appear to be increased in most women, although they also may remain in the normal non-pregnant range.

Many of these other investigations were not designed to study the renin-angiotensin system per se, and report only on changes in the individual components of the system, i.e. on renin, renin-substrate or angiotensin alone. In few, have the plasma renin and renin-substrate concentrations been measured together, and in none has the major end-product of the system, angiotensin II, been measured concurrently. Comments about the inter-relationships of renin and renin-substrate with angiotensin in pregnancy have therefore been based mainly on theoretical considerations.

Many pregnant women show increases in aldosterone secretion, excretion and plasma concentration. Assuming that the relationships established in non-pregnant situations might also hold true in pregnancy, a number of reports have implicated the renin-angiotensin system as the stimulus to this increase in aldosterone

production. However, no direct relationship has yet been demonstrated by concurrent measurement of the four factors involved, i.e. renin, renin-substrate, angiotensin II and aldosterone.

The studies to be described were therefore set up to investigate these questions:

(1) What changes take place in the plasma concentrations of angiotensin II and aldosterone (and also in plasma levels of other corticosteroids, e.g., corticosterone, DOC) during pregnancy?

(2) What effect has pregnancy on the relationships between plasma renin, renin-substrate, angiotensin and aldosterone when these are measured concurrently?

(3) How early in pregnancy do changes take place in the circulating levels of these substances, and how soon after pregnancy do they revert to the normal non-pregnant range?

(4) What stimulates the increases in renin, renin-substrate, angiotensin II and aldosterone in pregnancy? Is there a relationship to the changes in electrolytes, proteins, haematocrit, plasma volume, renal function and progesterone which also occur?

CHAPTER 5

THE ROLE OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM IN NORMAL PREGNANCY

5.A. A SERIAL STUDY IN PREGNANCY OF THE PLASMA CONCENTRATIONS OF RENIN, CORTICOSTEROIDS, ELECTROLYTES AND PROTEINS ; AND OF HAEMATOCRIT AND PLASMA VOLUME

(The results of this study have been published previously - see Weir et al, 1970a and 1971a).

The plasma concentrations of electrolytes, renin, haemoglobin the proteins, together with plasma volume, osmolality, colloid osmotic pressure and packed cell volume, were measured concurrently and serially throughout pregnancy in 15 women, some of the variables measured having been considered to be possibly concerned with the control of renin release and plasma renin concentration (see Brown et al, 1966a, 1966c, 1968; Vander, 1967; Brown, Lever, Robertson, Hodge, Lowe and Vane, 1967; Neilson and Miller, 1968; Hosie, Brown, Harper, Lever, MacAdam, MacGregor and Robertson, 1970; Brown, Davies, Johnson, Lever and Robertson, (1970). Plasma concentrations of aldosterone, corticosterone and cortisol were also measured at the same time in 7, 6 and 6 of the women respectively.

CLINICAL MATERIALS AND METHODS

No abnormality was found in any of the 15 women on initial clinical examination. All had blood pressures of 140/80 mmHg

or less prior to the 28th week of gestation. All but one (case 8, Table 1 - Appendix) were primigravid. One (case 14) had a twin pregnancy. Their mean age was 26.5 years (range 21 - 36); mean height 162.1 cm (range 145.0 - 167.5); and mean weight at the 16th week 60.2 kg (range 51.7 - 73.4) (see Table 1).

The women attended as out-patients at the 16th, 28th, 34th and 38th weeks of gestation, between 09.00 hours and 10.00 hours, having fasted overnight, but having eaten their normal diet to that time. At each visit they were weighed, the legs were examined for oedema, and the urine was tested for protein by the Albustix method (Ames Ltd.).

The women then rested supine for 30 minutes, the blood pressure was measured with a clinical sphygmomanometer, and a sample of peripheral venous blood was taken.

Laboratory and statistical methods have been outlined in Chapter 3.

RESULTS

All the women delivered healthy infants. The mean birth weight of the singletons was 3.37 kg (range 2.86 - 4.34 kg); the twins weighed 2.49 kg and 2.61 kg. Detailed results in individual women are shown in Table 1.

Proteinuria, blood pressure and weight.

In no case was proteinuria detected at any stage. In

12 women little change occurred in the systolic and diastolic pressures during pregnancy (Fig. 6). In 3 cases (13 - 15) the blood pressure increased in the last 6 weeks of pregnancy to exceed 140/90 mmHg, and case 14 also developed peripheral oedema. Since this rise in blood pressure would be regarded by some authorities as abnormal, data from cases 13 - 15 were not included in the statistical analysis and are plotted separately in Figures 6 to 8.

All the women showed a steady rise in weight from the 16th to the 38th week, with considerable variation between individuals (Table 1). If the twin pregnancy is excluded, the average weight gain was 9.68 kg (range 5.9 - 15.4 kg). The woman with twins (case 14), who developed marked oedema with increased blood pressure in the last trimester, had a total weight gain of 18.1 kg.

Plasma volume.

In every case the plasma volume increased during pregnancy (Table 1, Figure 6) the mean value being highest at 36 weeks ($t = 3.46$; $0.001 < p < 0.01$) as compared with 16 weeks. Two of the 3 cases whose blood pressure increased had higher than average values of plasma volume, but the individual results were within the range observed in the other subjects.

Total plasma proteins and colloid osmotic pressure.

Plasma proteins fluctuated markedly in individual women (Table 1), a significant rise in the mean concentration occurring

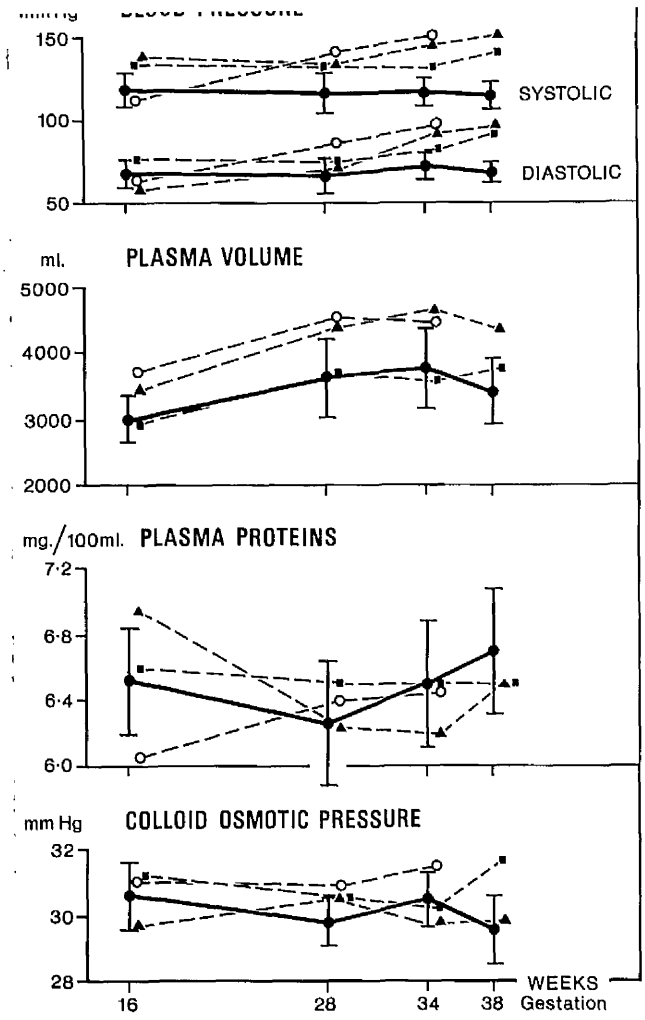


Figure 6.

See text.

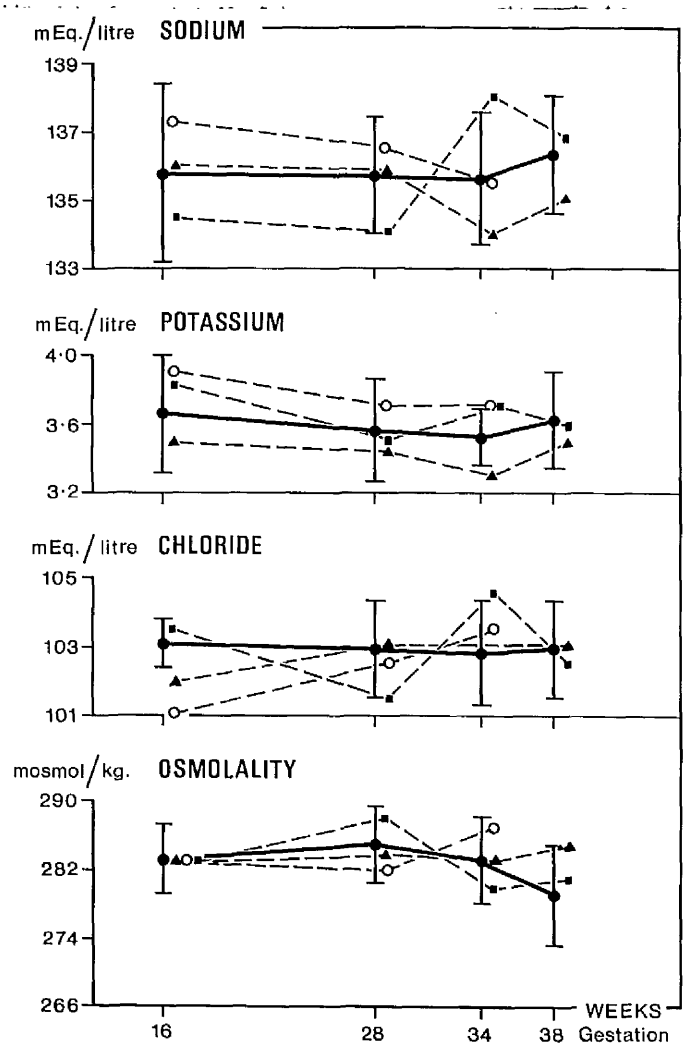


Figure 7.

See text.

between the 26th and 38th weeks ($t = 3.14$, $0.01 < p < 0.02$, Figure 6).

The individual and mean colloid osmotic pressure varied considerably with no clear pattern (Table 1, Figure 6).

Haemoglobin and haematocrit.

In conformity with previous studies (see Hytten and Leitch, 1971) throughout pregnancy haematocrit and haemoglobin concentration were frequently lower than for normal non-pregnant females.

Plasma electrolytes and osmolality.

No significant change was observed as pregnancy proceeded in the plasma concentrations of sodium, potassium or chloride. Similarly, no significant change in mean plasma osmolality occurred (Table 1, Figure 7).

Plasma renin concentration.

In 10 of the 15 women, the plasma renin values were above the non-pregnant range in each estimation, but a marked difference was apparent both between women and, in some cases, between different periods of gestation in the same woman (Table 1, Figures 8 and 9). Ten estimations out of a total of 53 in the whole series lay within the normal non-pregnant range. The fall in the mean plasma renin concentration from 16 to 38 weeks was not significant ($t = 1.06$, $p > 0.1$, Figure 8). In the 3 women whose blood pressure increased in late pregnancy the range

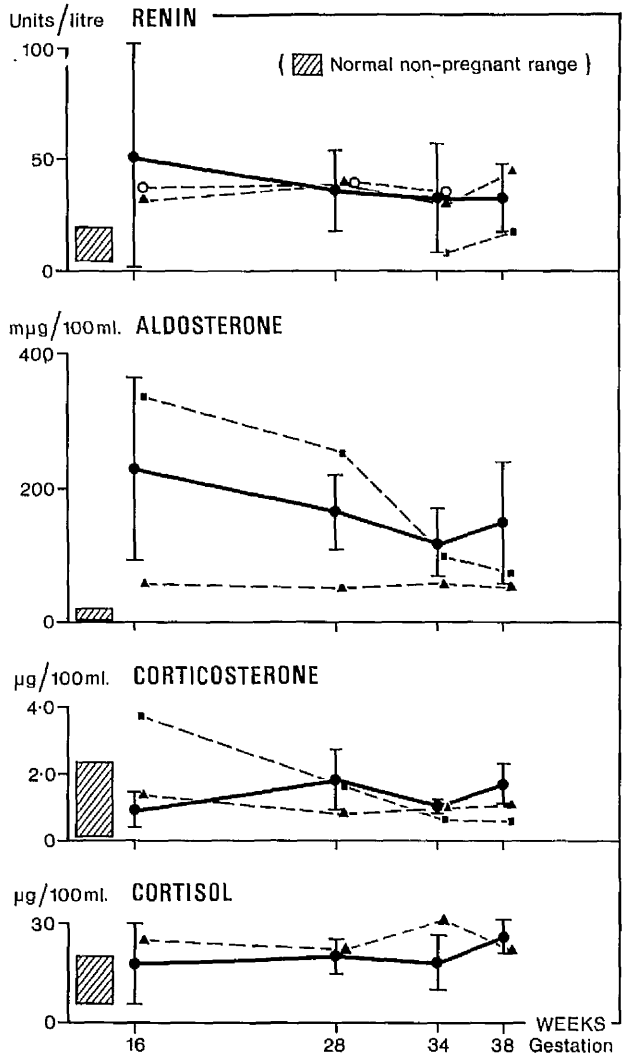


Figure 8.

See text.

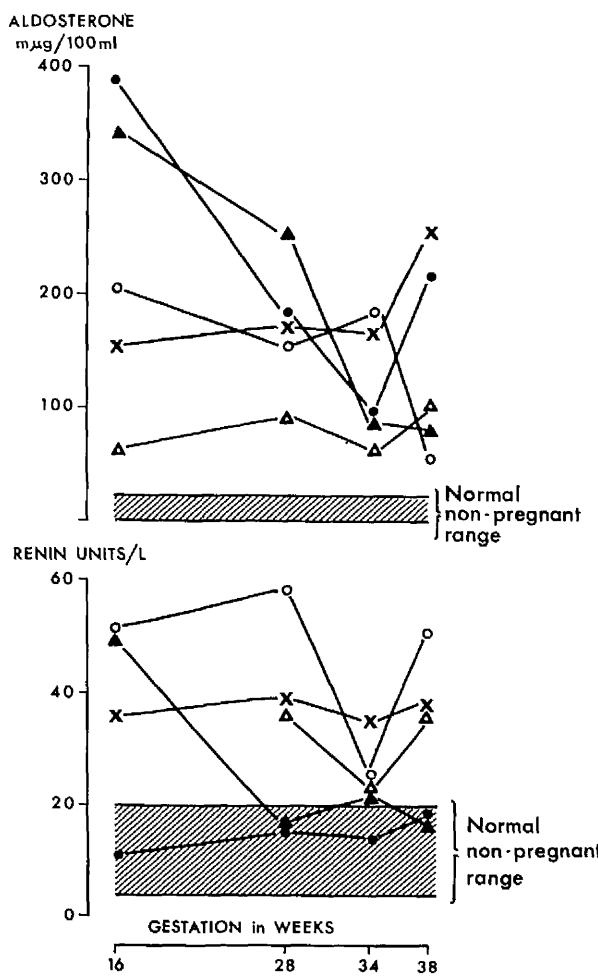


Figure 9.

Plasma renin and aldosterone in 5 normal pregnancies.

of plasma renin concentration was not remarkably different from that in the other 12 (Table 1, Figure 8).

Plasma aldosterone concentration.

Plasma aldosterone concentration was measured in 7 of the women, including 2 whose blood pressure rose latterly. The results were considerably higher than the normal non-pregnant range in each of the total of 28 estimations, the highest mean value being seen at 16 weeks (Table 1, Figure 8). The individual levels in the women whose blood pressure did not increase are shown in Figure 9. Although the mean aldosterone concentrations for these 5 women decreased from 16 to 34 weeks (Figure 8), this change was not significant by Student's t-test ($t = 2.07$, $0.05 < p < 0.1$) or by the Wilcoxon test for pair differences ($2\alpha = 0.10$). Plasma aldosterone was measured in 2 of the 3 women whose blood pressure rose in the last trimester. In one, the results were within the range found in those whose pressures did not increase. The other had rather lower values, with very little variation between the 5 serial estimations, although all were well above the non-pregnant upper limit (Figure 8).

Plasma corticosterone concentration.

Apart from elevated values at 28 weeks in 2 cases and at 16 weeks in another, the remainder of the 24 estimations in the 6 women studied, including 2 patients whose blood pressure increased latterly, showed plasma corticosterone concentrations within the normal non-pregnant range (Table 1, Figure 8). No consistent pattern was noted.

Plasma cortisol concentration.

In 4 of the 5 persistently normotensive women, the plasma cortisol concentration rose from 16 to 38 weeks (Table 1), whereas it fell slightly in one woman. No significant change in the mean value between 16 and 38 weeks was found ($t = 1.25$, $p > 0.1$) (Figure 8). All values were above the normal non-pregnant range at 38 weeks, whereas only 8 of 18 estimations were so elevated in earlier pregnancy. Plasma cortisol was measured in only one of the women whose blood pressure rose in the last 6 weeks and in this case it remained above the normal non-pregnant range throughout pregnancy.

Correlations

All the factors recorded in Table 1 (apart from those obtained in the 3 women whose blood pressure rose in the last trimester) were subjected to multiple regression analysis, both as absolute values and as a percentage change from the initial value. No statistically significant correlation was found between any of the factors measured. In particular, the plasma concentrations of renin and aldosterone were not significantly related; as shown in Figure 9, wide disparities between individual plasma renin and aldosterone concentrations occurred. It is particularly noteworthy that some of the highest plasma aldosterone levels were observed in a woman in whom plasma renin concentration remained throughout within

the normal non-pregnant range.

The results of this study will be discussed together with the results of the next 3 studies in Chapter 6.

5B. THE EFFECT OF NORMAL PREGNANCY ON PLASMA CONCENTRATIONS OF RENIN, RENIN-SUBSTRATE, ANGIOTENSIN II AND ALDOSTERONE AND ON THE RELATIONSHIPS BETWEEN THESE SUBSTANCES; INCLUDING CHANGES IN PLASMA PROGESTERONE, PLASMA AND URINARY ELECTROLYTES, PLASMA OSMOLALITY, BLOOD UREA, SERUM CREATININE AND CREATININE CLEARANCE.

CLINICAL METHOD OF STUDY.

A small number of healthy women studied early in pregnancy had been admitted for termination of pregnancy under the Abortion Act, 1968. Otherwise, all the women had normal full-term pregnancies with no complications across delivery or in the puerperium. No case was included where a raised blood pressure (i.e. 140/90 or over) was present at any stage of gestation.

A total of 112 women were studied at least once during pregnancy or post-partum. The parity of these women was as follows:- para 0 - 55; para 1 - 30; para 2 - 11; para 3 or over - 16. Their ages ranged from 17 to 41 years (mean 26.0). Two women had twin pregnancies.

In early and mid-pregnancy blood samples were taken mainly from women attending as out-patients, whereas in later pregnancy 50% of the women were in-patients at the time of sampling. Post-partum samples were taken from in-patients in the first two weeks after delivery and from outpatients later in the puerperium. The in-patients remained in bed in the morning before blood pressure measurement and venepuncture, which took place between 8.30 and 9.30 a.m. The outpatients, who attended

routine antenatal and postnatal clinics, usually in the morning, were rested supine for 30 minutes before the blood pressure was measured and the blood sample taken.

With the numbers of women involved, it was considered impracticable to control electrolyte balance precisely. All the women were therefore allowed to take an unrestricted diet.

To exclude a possible major disparity between inpatient and outpatient estimations, plasma renin and renin-substrate concentrations were measured in 7 women on 2 occasions each, less than 2 weeks apart - as an inpatient on one occasion and as an outpatient on the other. No significant difference was found by the paired t-test, either for plasma renin concentration ($t = 0.083$, $p > 0.1$) or for plasma renin-substrate concentration ($t = 0.759$, $p > 0.1$). Insufficient data were available to make the same comparisons for plasma angiotensin II and aldosterone concentrations, but it was possible to statistically compare the aldosterone results between a number of inpatients and a number of outpatients at the 30th week of gestation. Once again, no significant difference was found ($t = 0.426$, $p > 0.1$, $n = 10$).

Of the total of 112 women studied, all 4 components of the renin-angiotensin-aldosterone system were measured concurrently in 18 women, 3 components in 30 women, 2 components in 26 women and single measurements were made in 40 women. Details of these and of the numbers of other factors measured are included with the results. In 10 cases, serial measurements of at least one

component were made from the early weeks of pregnancy to term and post-partum.

LABORATORY METHODS.

These have been outlined in Chapter 3.

RESULTS.

The concentrations of each substances have been tabulated for each of the trimesters of pregnancy, and for weeks 1 to 2 and 6 to 12 of the puerperium. Where more than one result was available for each period, the mean of those was taken for statistical analysis. Individual results and/or means and standard deviations for each trimester and post-partum period are shown on accompanying graphs. The results for each period have been compared by measuring the t-statistic and this, together with the statistical significance, is tabulated for each substance.

In the case of plasma renin, renin-substrate, angiotensin II and aldosterone concentrations, the serial changes from early pregnancy to the post-partum period are illustrated for 6 women. This is accompanied by detailed graphs of the changes in a larger number of women in early pregnancy and serial changes across delivery and post-partum.

Where concurrent measurements have been made, the correlation coefficient has been tabulated for all the estimations during pregnancy as well as for each trimester and post partum period.

Where this has reached statistical significance, graphs are shown illustrating the two relevant substances plotted against one another, with the appropriate regression line.

Plasma renin concentration.

Individual results and means for each trimester and post-partum period are shown in Table 2 (Appendix) and Figure 10.

Comparison of the results at those difference stages was as follows:-

Comparison	t-statistic	Degrees of freedom	Significance p
I - II	1.605	54	> 0.1
II - III	0.866	59	> 0.1
I - III	3.374	67	< 0.01
III - 0.0 0.2	6.400	61	< 0.001
P.P. 0-2 - P.P. 6-12	3.334	40	< 0.01

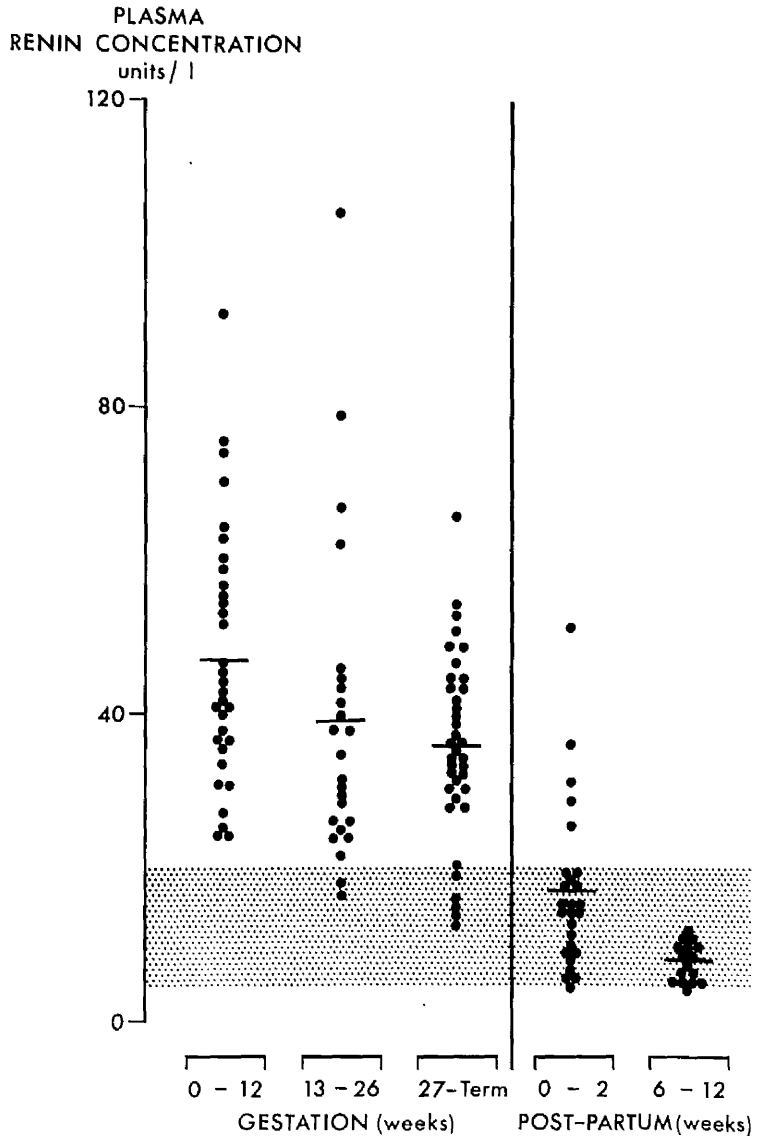


Figure 10.

Plasma renin concentration in normal pregnancy and post-partum.

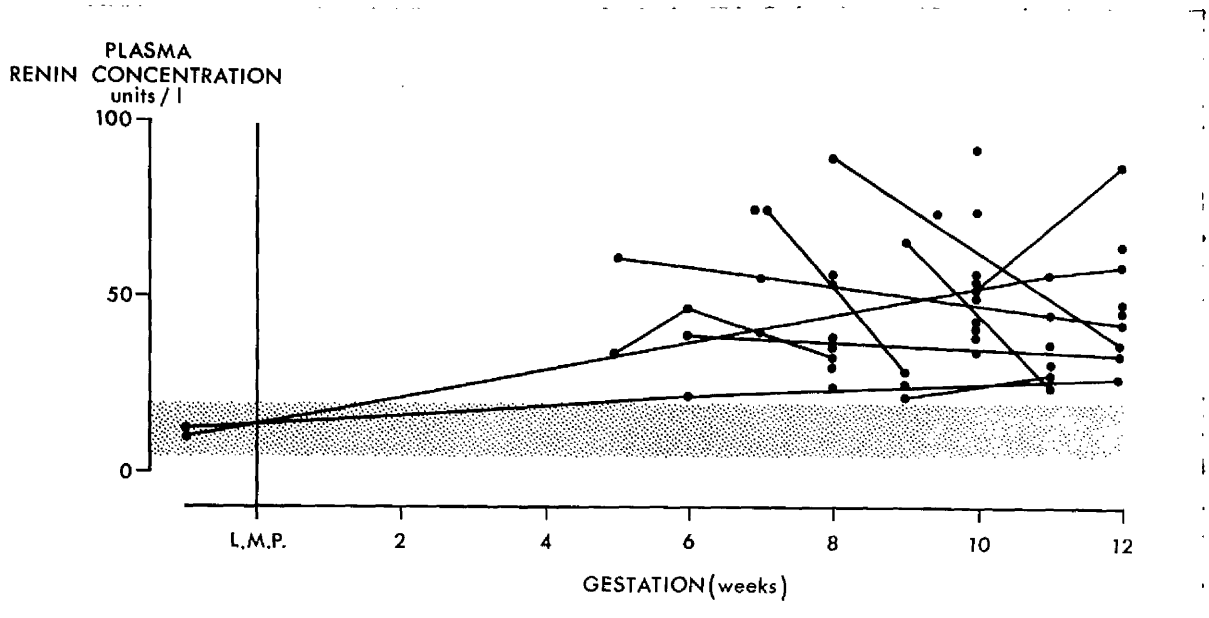


Figure 11. Plasma ronin concentration in early pregnancy.

In every case in early pregnancy, plasma renin concentration was elevated above the normal non-pregnant range, the highest value being 92 units/l. As shown in Figure 11, those increases were apparent as early as 5 weeks after the last menstrual period, i.e. approximately 3 weeks after conception. The serial readings in Figure 11 show that in these early weeks plasma renin concentration fluctuated considerably within the one individual and there was also a marked variation between individual women.

During pregnancy there was a significant fall in plasma renin concentration, although all but 6 of the women studied still had values above the normal non-pregnant range in the last trimester. This fall was less noticeable in the serial study of 6 women during pregnancy (Figure 12).

A marked fall in plasma renin concentration occurred after delivery of the foetus and placenta, although it was still elevated in some cases at the 6th day post-partum (Figure 13). By the 6th week post-partum, plasma renin concentration had dropped further,

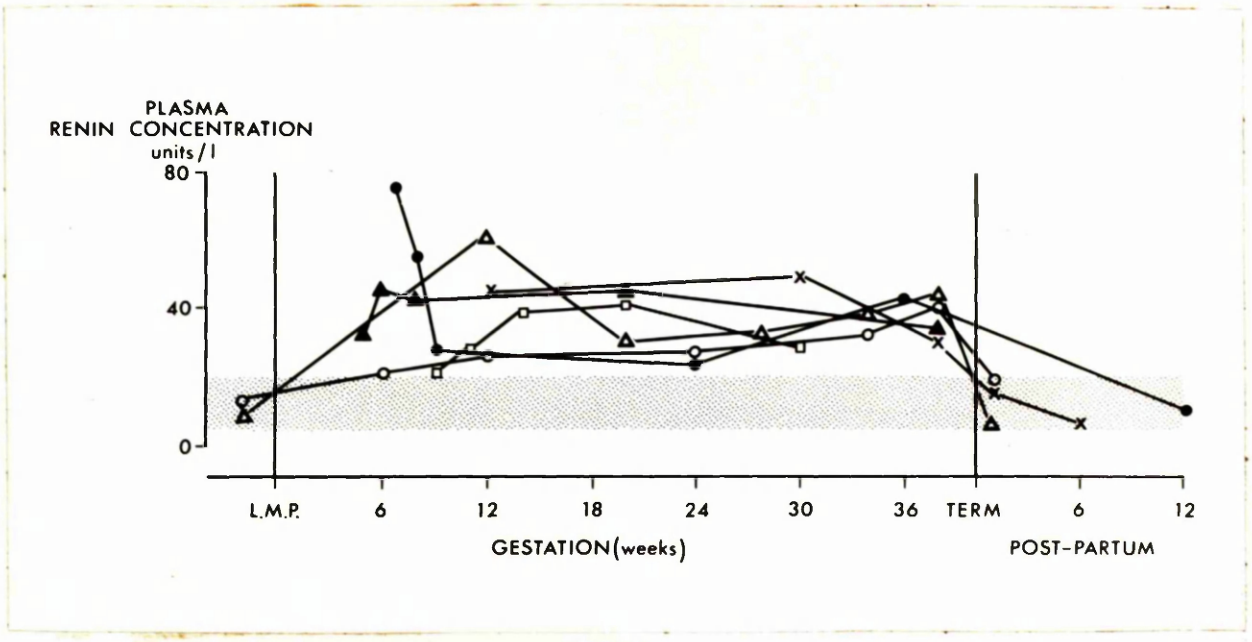


Figure 12. Serial measurements of plasma renin concentration in 6 pregnant women.

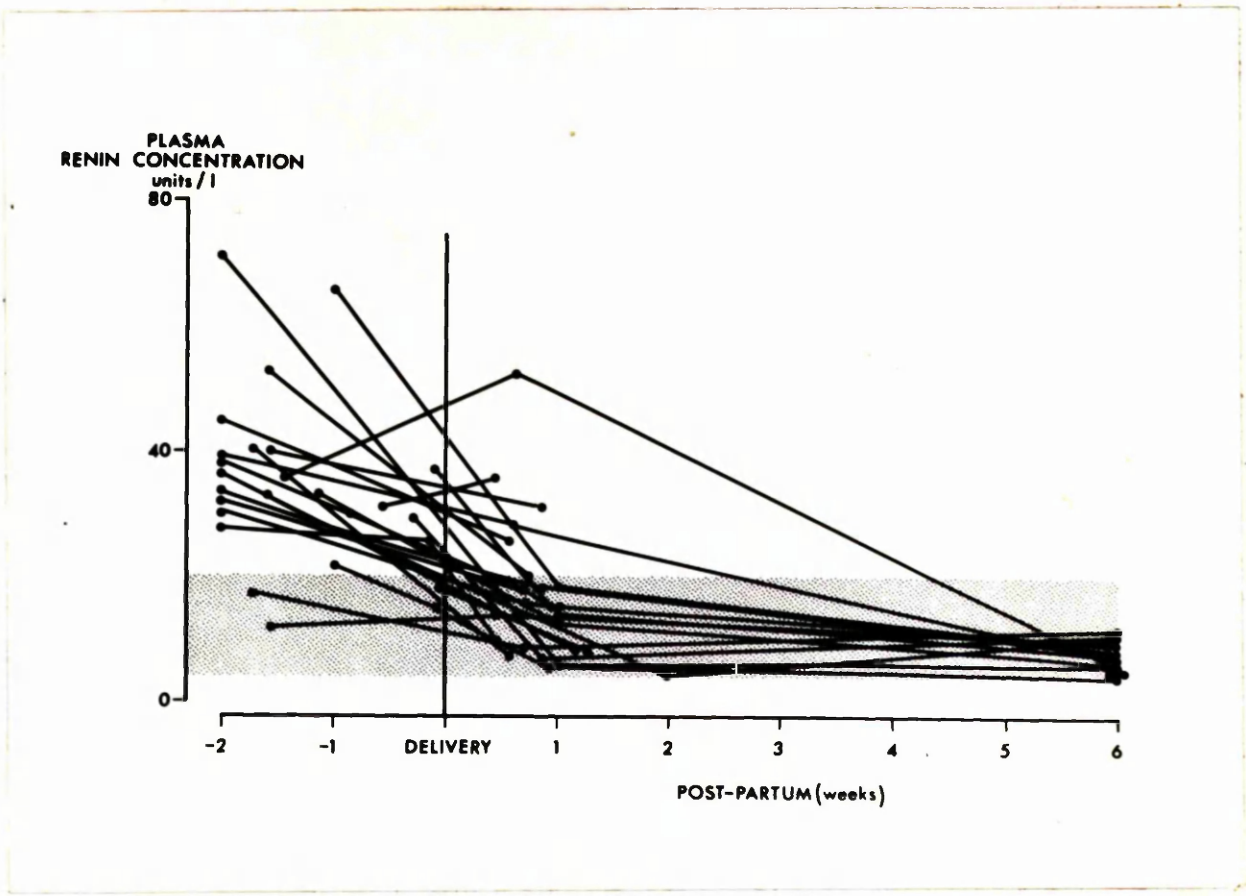


Figure 13. Serial measurements of plasma renin concentration across delivery and post-partum.

all the cases studied having levels in the lower half of the normal non-pregnant range (Figure 13).

Plasma renin-substrate concentration.

Individual results and means for each trimester and post-partum period are shown in Table 3 and Figure 14.

Comparison of the results at these different stages was as follows:-

Comparison	t-statistics	Degrees of freedom	Significance p
I - II	6.759	72	< 0.001
II - III	4.675	84	< 0.001
I - III	10.844	84	< 0.001
III - P.P. 0-2	2.104	76	< 0.05
P.P. 0-2 - P.P. 6-12	4.081	47	< 0.001

Plasma renin substrate concentration showed a marked and progressive increase throughout pregnancy (Figure 14). The earliest elevation found was at 6 weeks' gestation (Figure 15), and substrate levels increased thereafter, although in a few cases they remained within the normal non-pregnant range during the first 12 weeks. All were above the normal non-pregnant range in the third trimester, the highest level reached being 6.93 uM.

In addition to reflecting the general increase throughout pregnancy, the serial study showed a slight fall in 4 cases in the last few weeks of gestation (Figure 16).

Figure 14.

Plasma renin-substrate concentration in normal pregnancy and post-partum.

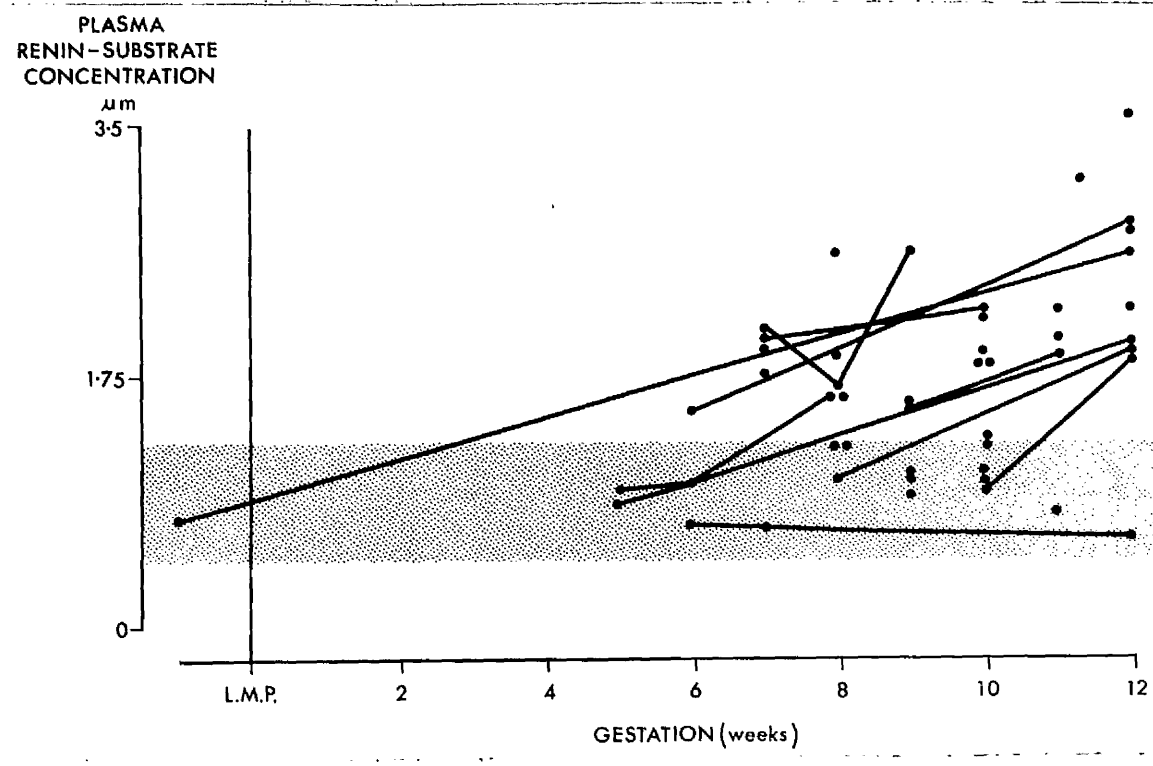


Figure 15. Plasma renin-substrate concentration in early pregnancy.

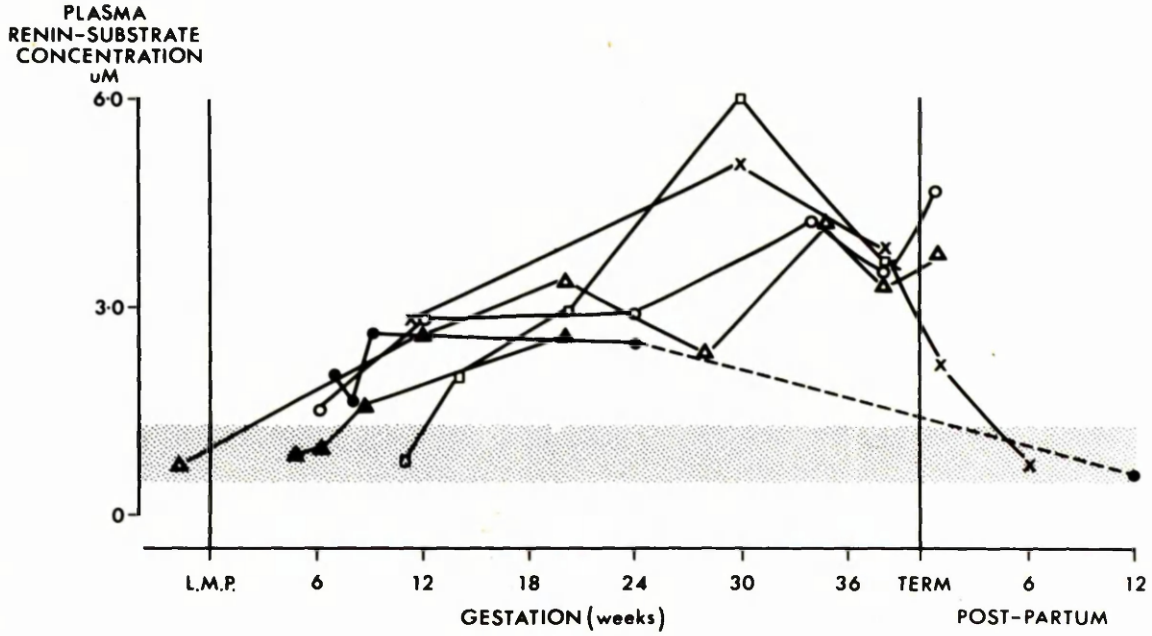


Figure 16. Serial measurements of plasma renin-substrate concentration in 6 pregnant women.

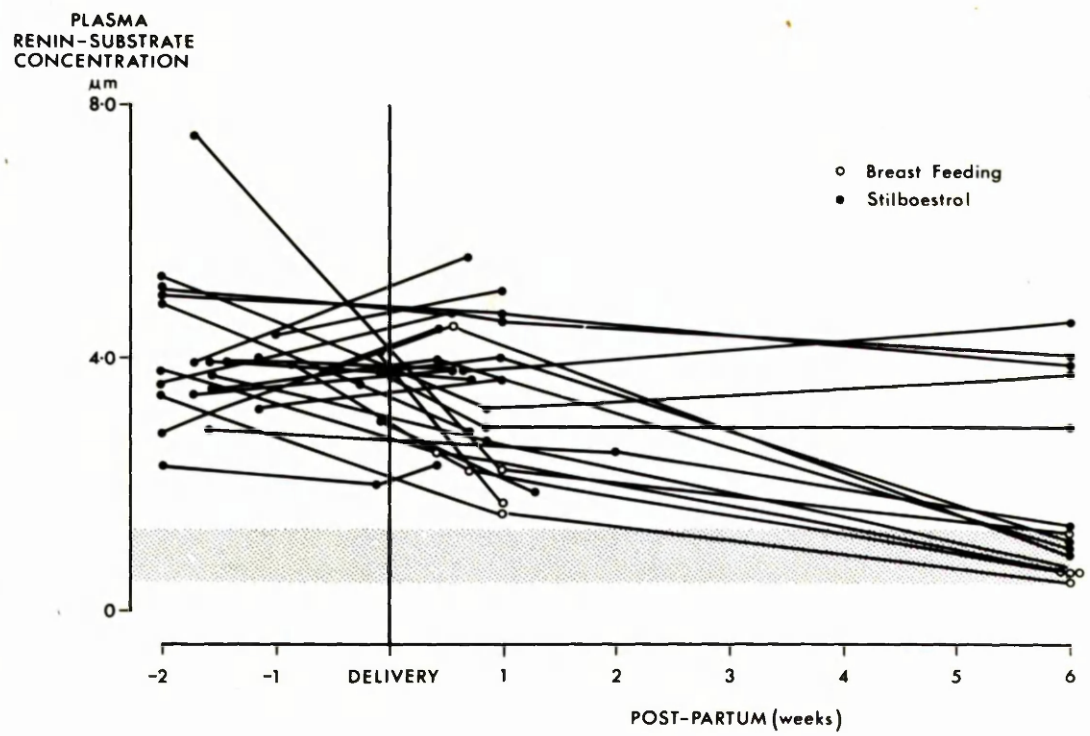


Figure 17. Serial measurements of plasma renin-substrate concentration across delivery and post-partum.

Mean plasma renin substrate concentration showed a small but significant decrease after delivery of the foetus and placenta, but this was less noticeable on serial readings (Figure 17), and in no case had it returned to non-pregnant levels by 2 weeks post-partum. Even at the 6th week in the puerperium, a number of women had plasma concentrations of renin substrate well above the normal non-pregnant range.

Women who did not breast-feed their infant were given the synthetic oestrogen, stilboestrol, to suppress lactation. As oestrogens may affect circulating levels of renin substrate (see Chapter 12), it was thought that this might explain, at least in part, the persistence of high concentrations after delivery. Women who breast-fed and women who were given stilboestrol have therefore been denoted by 2 different symbols in Figure 17. Plasma renin substrate concentrations in the women who breast-fed their infants were, in general, lower in the week after delivery and were within the normal non-pregnant range by the 6th week post-partum, the statistical difference between the 2 groups at these times being $t = 2.494$, $p < 0.02$ and $t = 1.711$, $p > 0.1$ respectively.

Plasma angiotensin II concentration.

Individual results and means for each trimester and post-partum period are shown in Table 4 and Figure 18.

Comparison of the results at those different stages was as follows:-

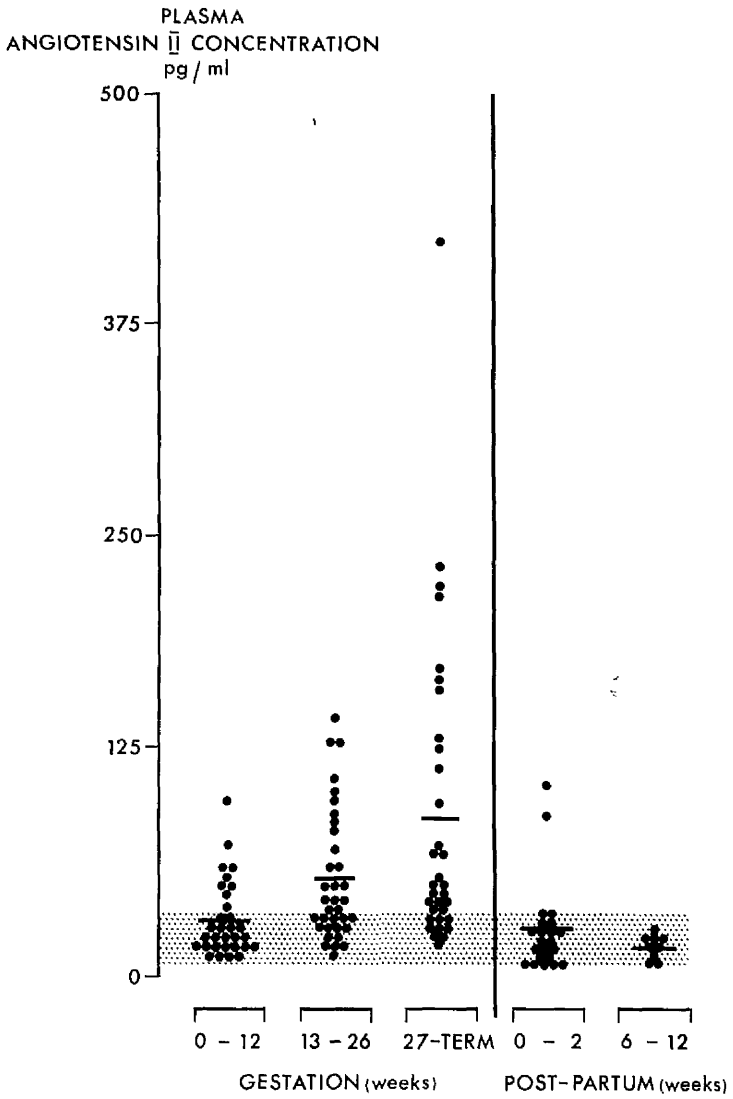


Figure 18.

Plasma angiotensin II concentration in normal pregnancy and post-partum.

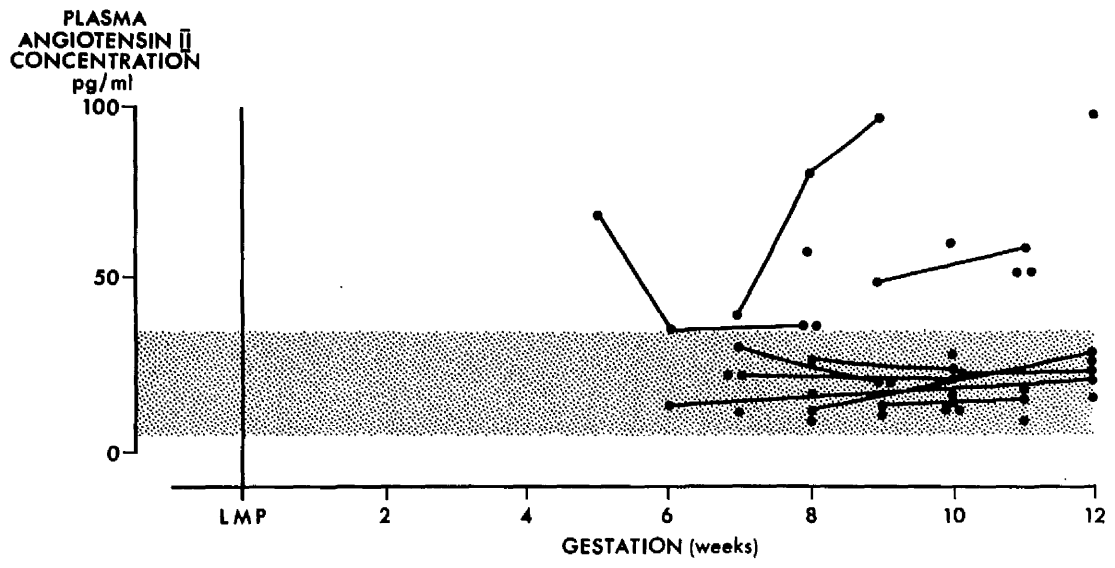


Figure 19. Plasma angiotensin II concentration in early pregnancy.

Comparison	t-statistics	Degrees of freedom	Significance p
I - II	2.983	64	< 0.01
II - III	2.179	66	< 0.05
I - III	3.599	62	< 0.001
III - P.P.	3.306	53	< 0.01
P.P. - P.P. 0-2 6-12	1.189	29	> 0.1

Mean plasma angiotensin II concentration showed a progressive increase during pregnancy, but in all trimesters a number of women had levels within the normal non-pregnant range. Increased levels were found as early as the 5th week of gestation (Figure 19). Some women showed very marked increases and variations throughout pregnancy whereas others showed very flat curves within the normal non-pregnant range from early pregnancy through to the puerperium (Figure 20). The highest level recorded was 415 pg/ml., in the third trimester.

By the second week after delivery, plasma angiotensin II concentration had returned to the normal non-pregnant range in all but 2 women, those two cases showing normal levels 4 weeks later (Figure 21).

Plasma aldosterone concentration.

Individual results and means for each trimester and post-partum period are shown in Table 5 and Figure 22.

Comparison of the results at those different stages was as follows:-

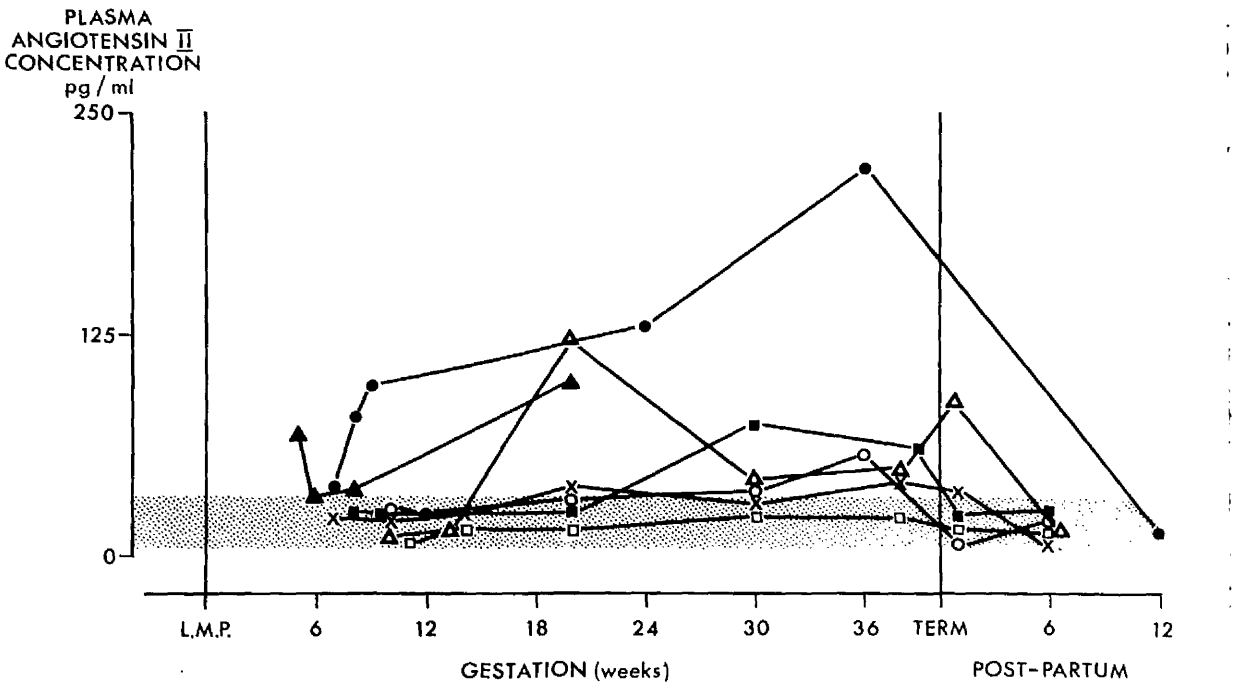


Figure 20. Serial measurements of plasma angiotensin II concentration in 6 pregnant women.

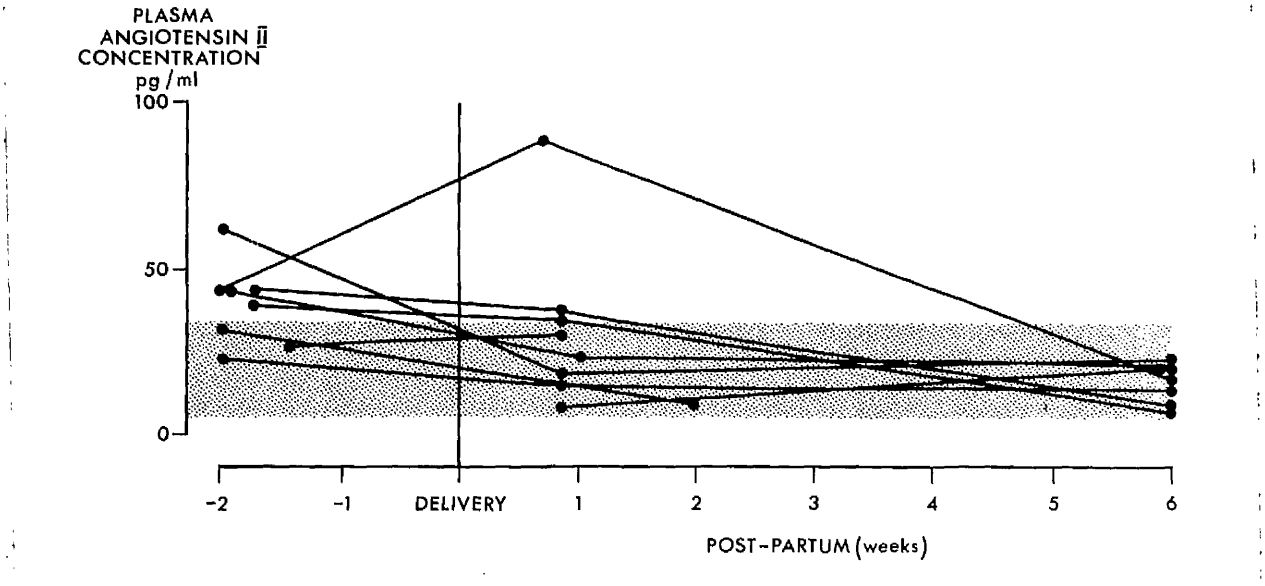


Figure 21. Serial measurements of plasma angiotensin II concentration across delivery and post-partum.

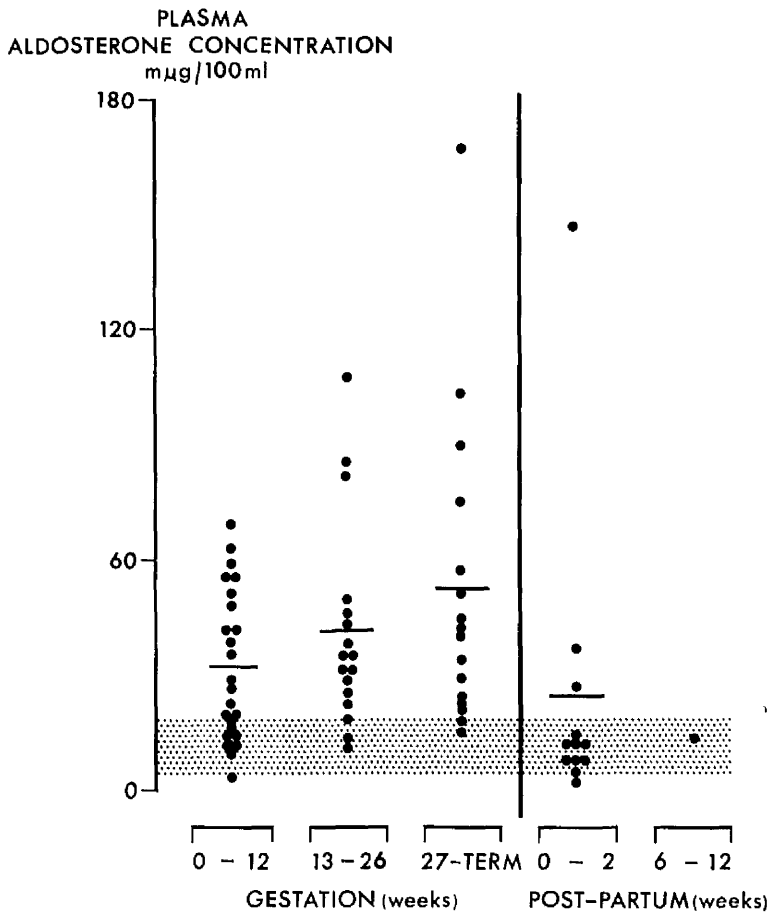


Figure 22.

Plasma aldosterone concentration in normal pregnancy and post-partum.

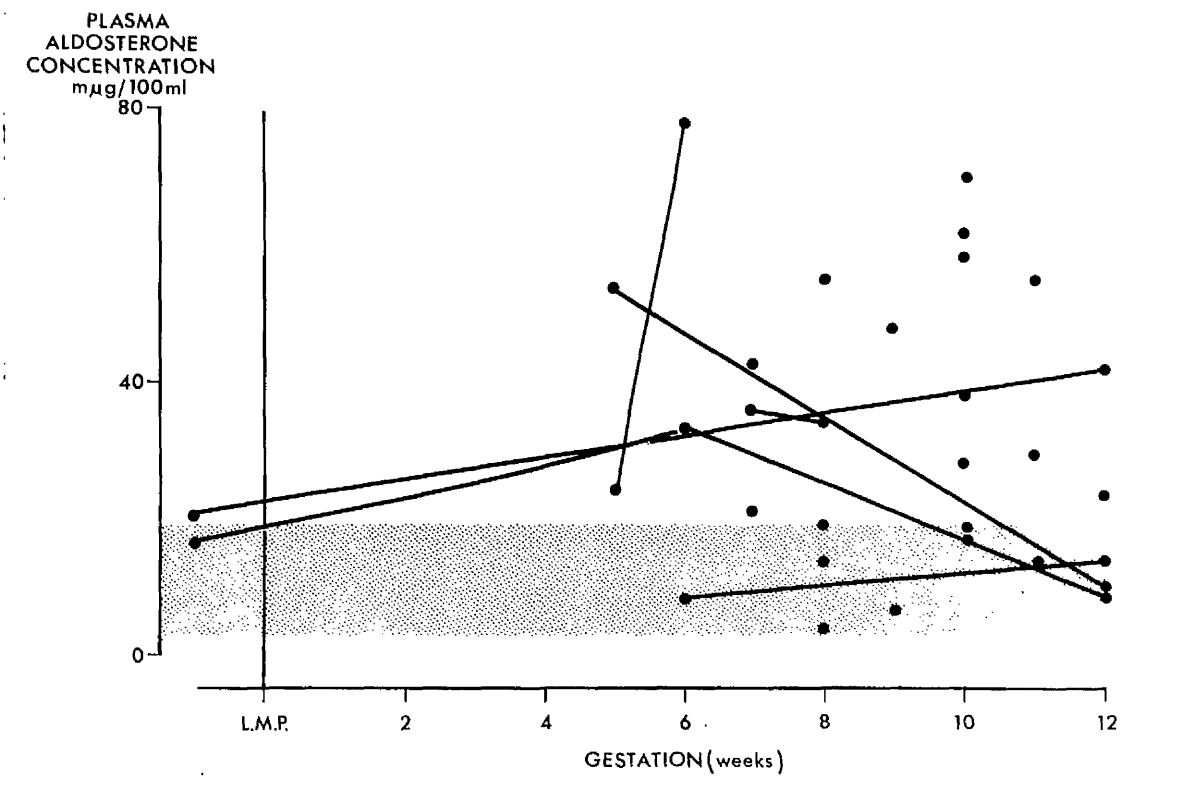


Figure 23. Plasma aldosterone concentration in early pregnancy

Comparison	t-statistic	Degree of freedom	Significance p
I - II	1.209	39	> 0.1
II - III	0.909	31	> 0.1
I - III	2.045	38	< 0.05
III - P.P. 0-2	1.820	26	$0.05 < p < 0.1$.
P.P. 0-2 - P.P. 6-12	-	-	-

Mean plasma aldosterone concentration showed a significant increase from early to late pregnancy, only 2 women having levels within the normal non-pregnant range in the last trimester.

High concentrations were found as early as the 5th week of gestation, but even in these early weeks, there was a considerable variation in levels, not only between different women but also from week to week in the same woman (Figure 23). Those differences were also present in the women followed serially throughout pregnancy, plasma aldosterone concentration rising markedly to 218 mug/100 ml. at the 34th week of gestation in one case, while it remained within or slightly above the normal non-pregnant range in another case (Figure 24).

Plasma aldosterone concentration fell in all but one of the cases in which it was measured serially across delivery (Figure 25), and most of the results were within the normal non-pregnant range within 2 weeks post-partum (Figure 22). In the one case where plasma aldosterone concentration rose from 117 mug/100 ml. at the 38th week to 147.2 mug/100 ml.

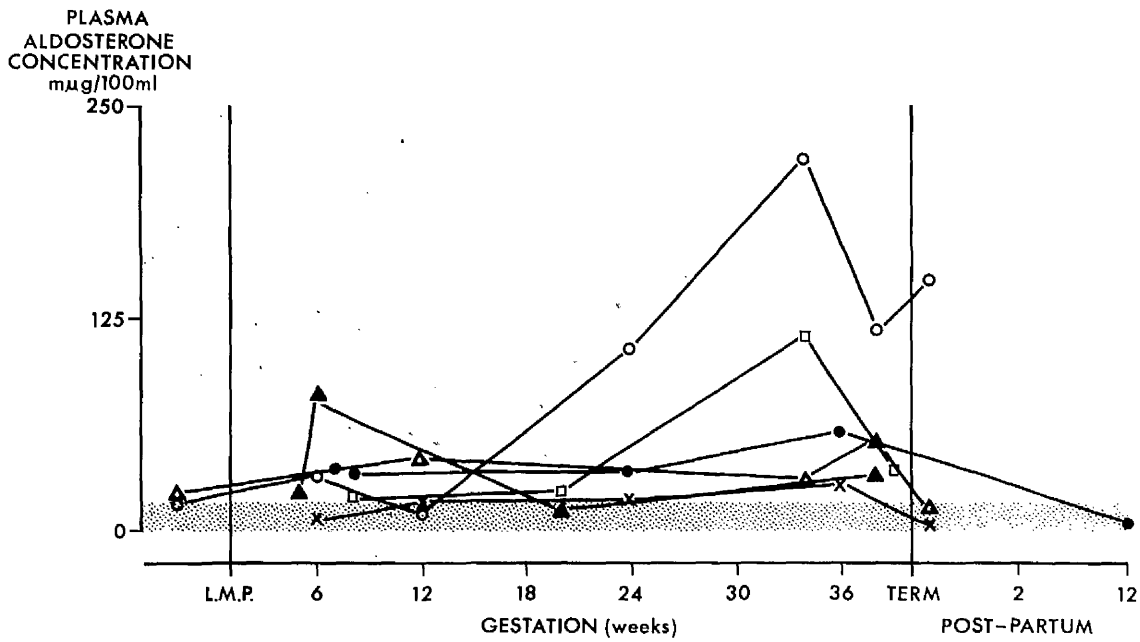


Figure 24. Serial measurements of plasma aldosterone concentration in 6 pregnant women.

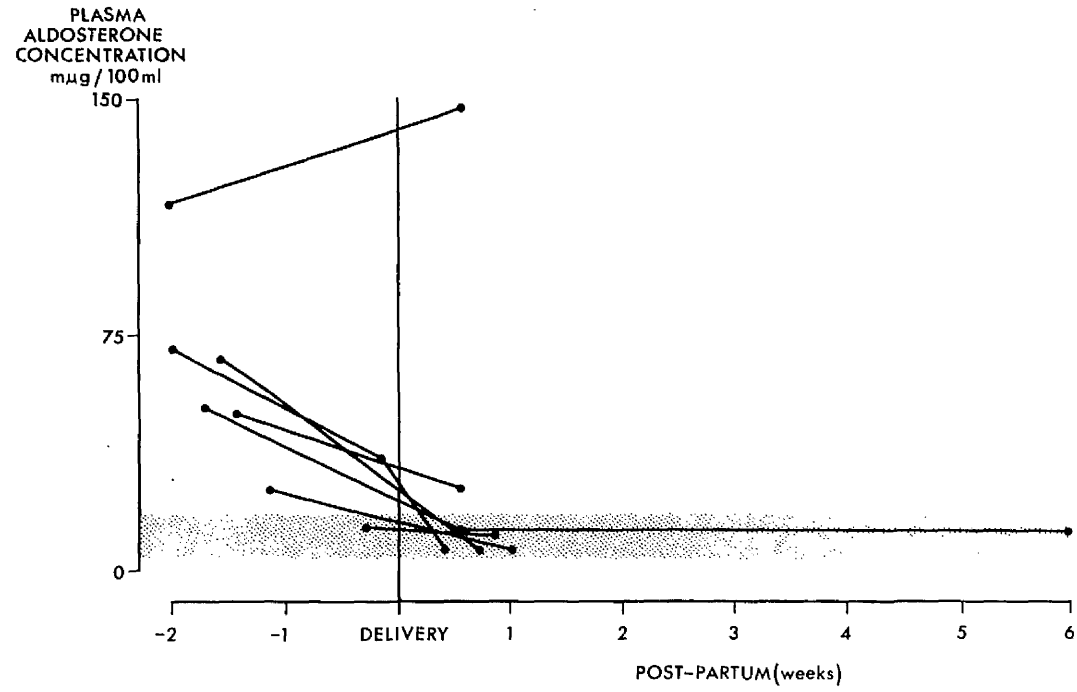


Figure 25. Serial measurements of plasma aldosterone concentration across delivery and post-partum.

4 days after delivery, no apparent cause for this change was found.

Plasma progesterone concentration.

Insufficient estimations were available to separate into trimesters. Serial changes in 9 women are shown in Figure 26. A progressive increase occurred in every case where it was measured beyond 12 weeks' gestation.

Plasma sodium and potassium concentrations and plasma osmolality.

Individual results with means and standard deviations for each trimester and post-partum period are shown in Tables 6 to 8 and Figure 27.

Comparison of the results at those different stages was as follows:-

	Comparison	t-statistic	Degree of freedom	Significance p
Plasma Sodium Concentration	I v II	0.593	59	> 0.1
	II v III	2.095	69	< 0.05
	I v III	1.668	76	> 0.1
	III v P.P. 0-2	2.339	69	< 0.05
	P.P. 0-2 v P.P. 6-12	0.462	40	> 0.1
Plasma Potassium Concentration	I v II	0.564	61	> 0.1
	II v III	0.297	70	> 0.1
	I v III	0.348	77	> 0.1
	III v P.P. 0-2	4.950	71	< 0.001
	P.P. 0-2 v P.P. 6-12	2.654	41	< 0.01

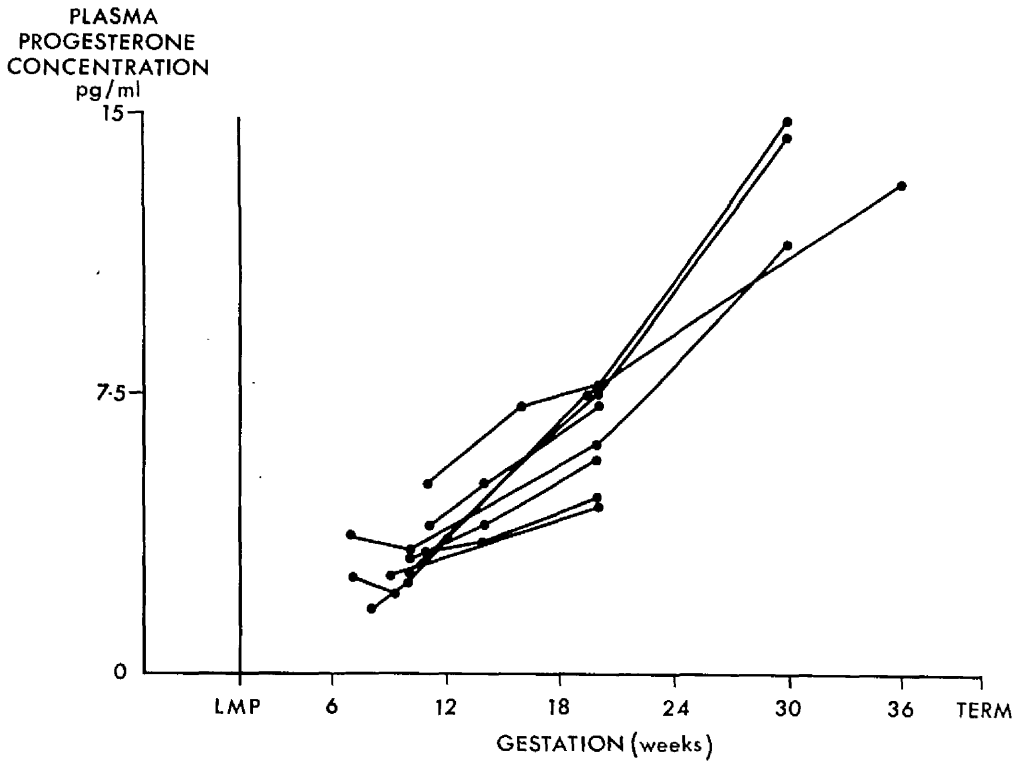


Figure 26. Plasma progesterone concentration in normal pregnancy.

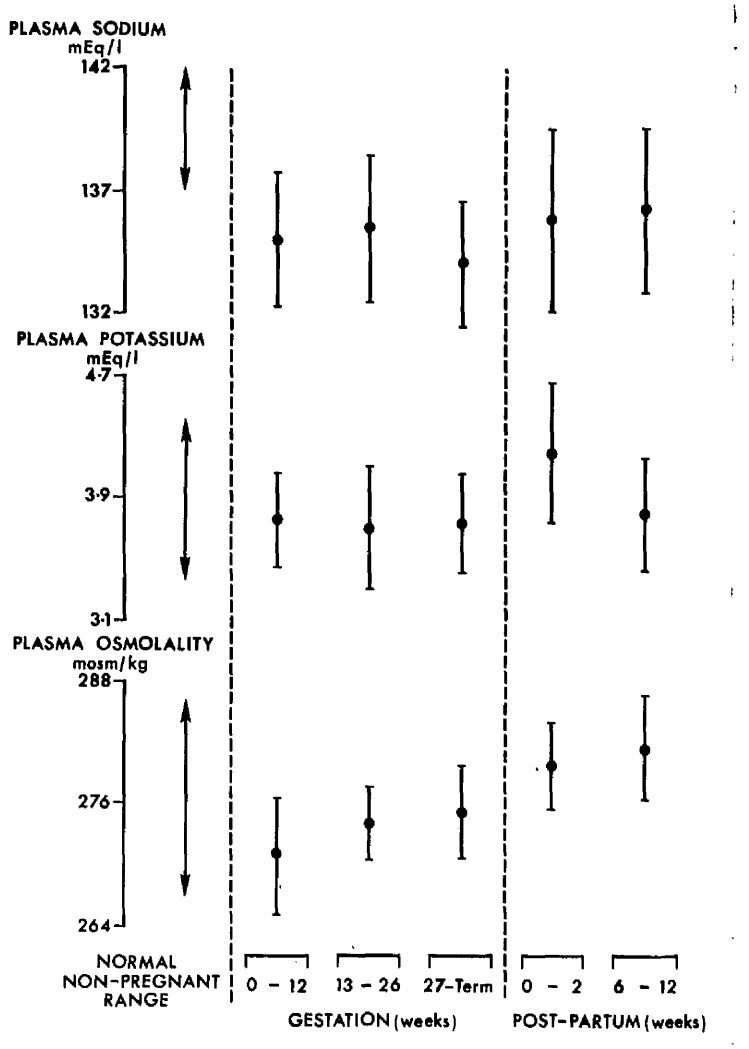


Figure 27

Plasma sodium, potassium and osmolality in normal pregnancy and post-partum

	Comparison	t-statistic	Degree of freedom	Significance p
Plasma Osmolality	I v II	1.680	87	> 0.1
	II v III	0.592	46	> 0.1
	I v III	2.326	47	< 0.05
	III v P.P. 0-2	3.964	56	< 0.001
	P.P. 0-2 v P.P. 6-12	1.074	35	> 0.1

Plasma sodium concentration was below the normal non-pregnant range in most women at each period of gestation, the mean concentration showing little change from early to mid-pregnancy, but a slight drop in late pregnancy. After delivery, a small rise occurred, but many values were still below the normal non-pregnant range between the 6th and 12th week post-partum.

Mean plasma potassium concentration remained in the lower part of the normal non-pregnant range with little fluctuation throughout pregnancy. A rise occurred in the first 2 weeks after delivery, with a subsequent fall to previous levels by the 6th to 12th week post-partum.

Mean plasma osmolality was also in the lower part of the normal non-pregnant range in early pregnancy. However, a significant increase had occurred by late pregnancy and a further increase took place after delivery. At none of the periods in which it was measured was it outwith the normal non-pregnant range.

Urine sodium and potassium concentrations.

These were calculated from urine collected over a 24 hour period.

Individual results with means and standard deviations for each trimester and post-partum period are shown in Tables 9 and 10.

Comparison of the results at those different stages was as follows:-

	Comparison	t-statistic	Degrees of freedom	Significance p
Urine Sodium Concentration	I v II	2.836	7	< 0.05
	II v III	1.894	25	< 0.05
	I v III	0.610	24	> 0.1
	III v P.P. 0-2	1.281	45	> 0.1
	P.P. 0-2 v P.P. 6-12	0.830	33	> 0.1
Urine Potassium Concentration	I v II	0.549	6	> 0.1
	II v III	0.970	24	> 0.1
	I v III	0.195	24	> 0.1
	II v P.P. 0-2	0.529	45	> 0.1
	P.P. 0-2 v P.P. 6-12	0.850	33	> 0.1

Apart from the increase in urine sodium concentration during the second trimester, no significant changes in urinary electrolyte excretion occurred during pregnancy or after delivery.

Blood urea, serum creatinine and creatinine clearance.

Individual results with means and standard deviations for each trimester and post-partum period are shown in Tables 11 to 13 and Figure 28.

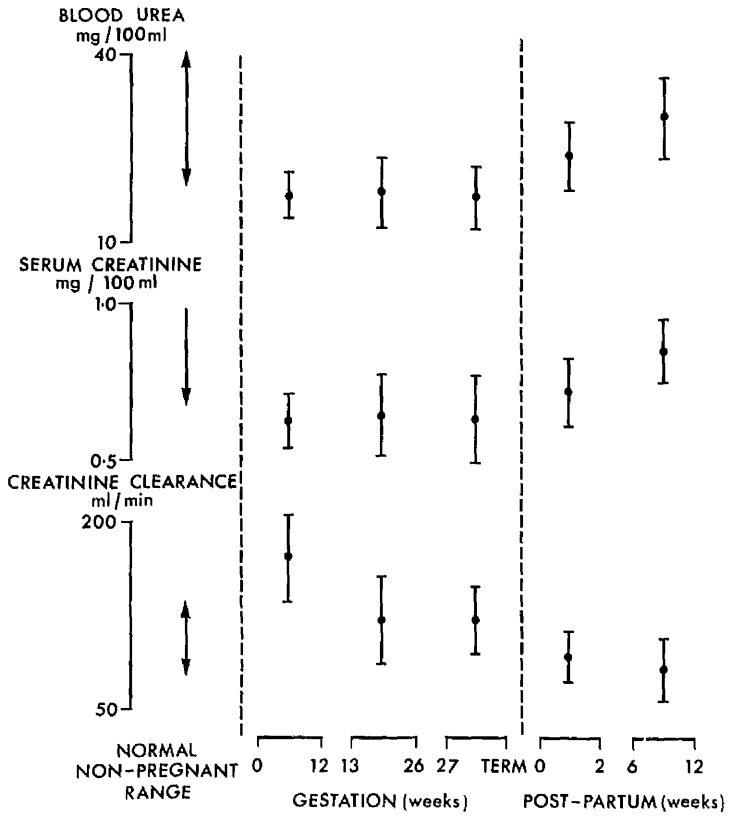


Figure 28. **Blood urea, serum creatinine and creatinine clearance in normal pregnancy and post-partum.**

Comparison of the results at these different stages was as follows:-

	Comparison	t-statistic	Degrees of freedom	Significance p
Blood Urea Concentration	I v II	0.419	44	> 0.1
	II v III	0.748	65	> 0.1
	I v III	0.326	63	> 0.1
	III v P.P. 0-2	5.631	70	< 0.001
	P.P. 0-2 v P.P. 6-12	3.168	42	< 0.001
Serum Creatinine Concentration	I v II	0.481	24	> 0.1
	III v III	0.290	48	> 0.1
	I v III	0.231	46	> 0.1
	III v P.P. 0-2	2.352	59	< 0.05
	P.P. 0-2 v P.P. 6-12	4.017	38	< 0.001
Creatinine Clearance	I v II	2.250	9	< 0.05
	II v III	0.269	26	> 0.1
	I v III	3.731	27	< 0.001
	III v P.P. 0-2	3.992	44	< 0.001
	P.P. 0-2 v P.P. 6-12	0.615	29	> 0.1

Blood urea and serum creatinine concentrations were in the lower part of the normal non-pregnant range in early pregnancy and remained so with little fluctuation to late pregnancy. After delivery, both increased significantly to the middle area of the non-pregnant range.

Mean creatinine clearance fell from early to mid-pregnancy but this was based on a small number of estimations. A further fall occurred after delivery but remained within the normal non-pregnant range.

Correlations.

Where concurrent measurements have been made, the correlation coefficient has been tabulated for all the estimations during pregnancy (Table 14). Correlations involving renin, renin substrate, angiotensin II and aldosterone have also been calculated for each trimester and post-partum, and these are shown in Table 15.

Positive correlations statistically significant at the 5% level where found between:-

renin and angiotensin	- post-partum (Figure 29).
renin and aldosterone	- first trimester (Figure 30)
renin substrate and angiotensin II	- first trimester (Figure 31)
renin substrate and aldosterone	- all pregnancy samples (Figure 32)
renin substrate and progesterone	- all pregnancy samples (Figure 33)
angiotensin II and progesterone	- all pregnancy samples (Figure 34)
angiotensin II and osmolality	- all pregnancy samples (Figure 35)

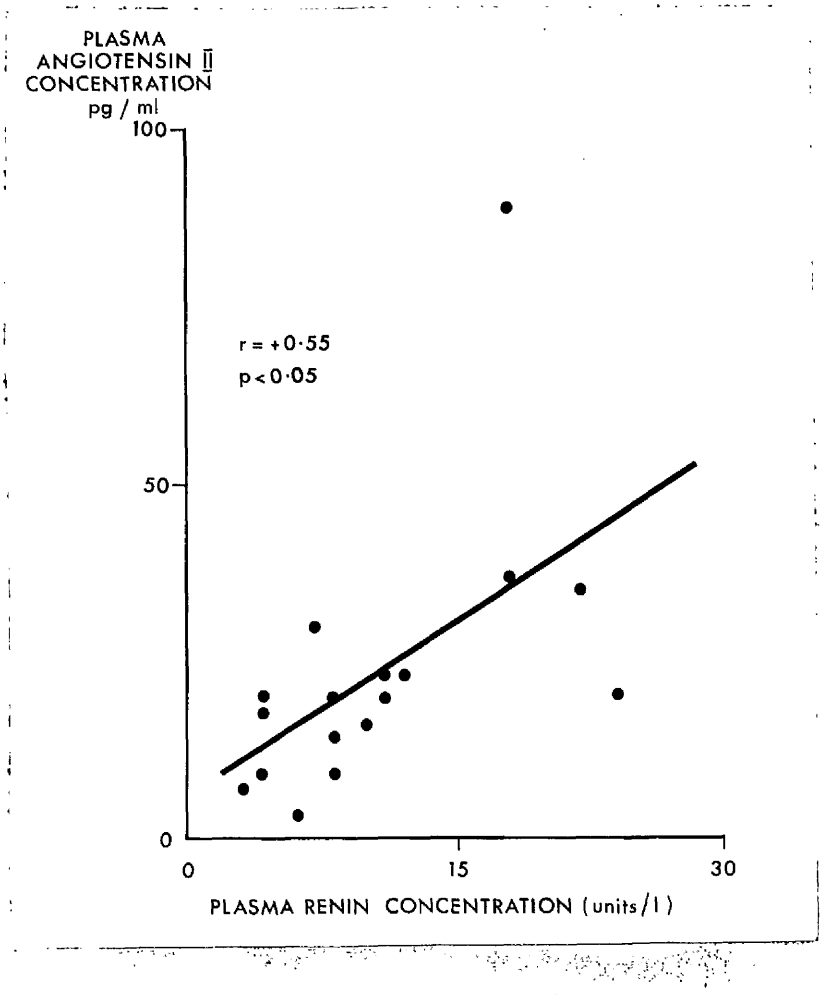


Figure 29

Plasma renin - angiotensin II relationships in the puerperium.

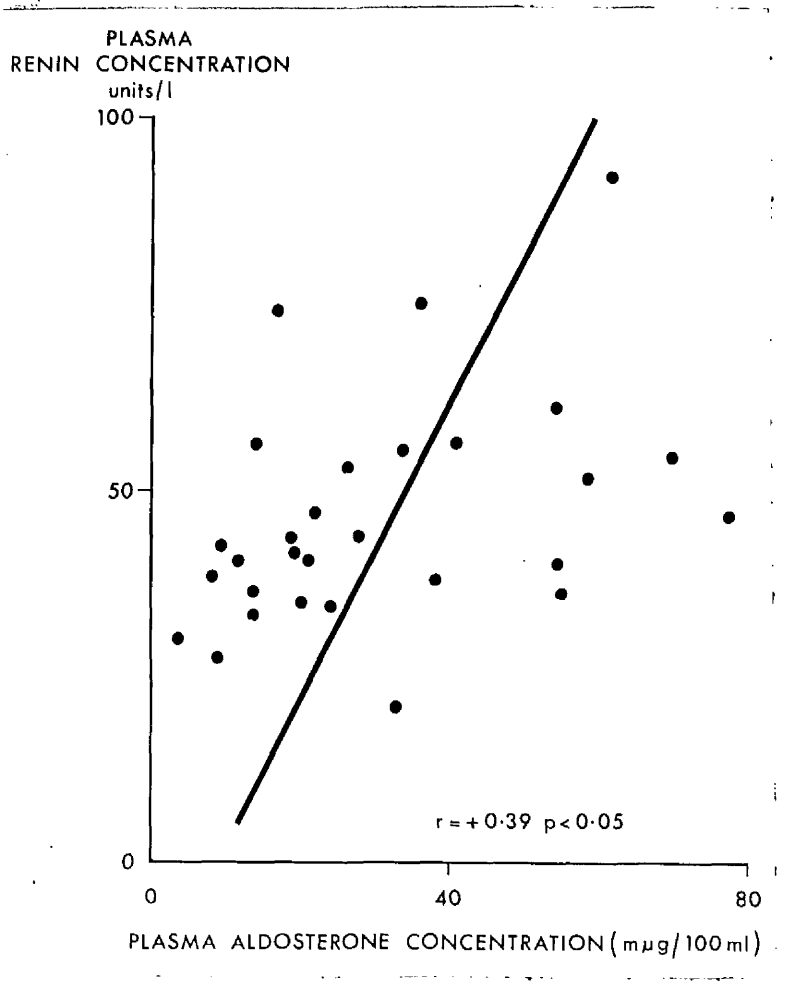


Figure 30

Plasma renin-aldosterone relationship in the first trimester

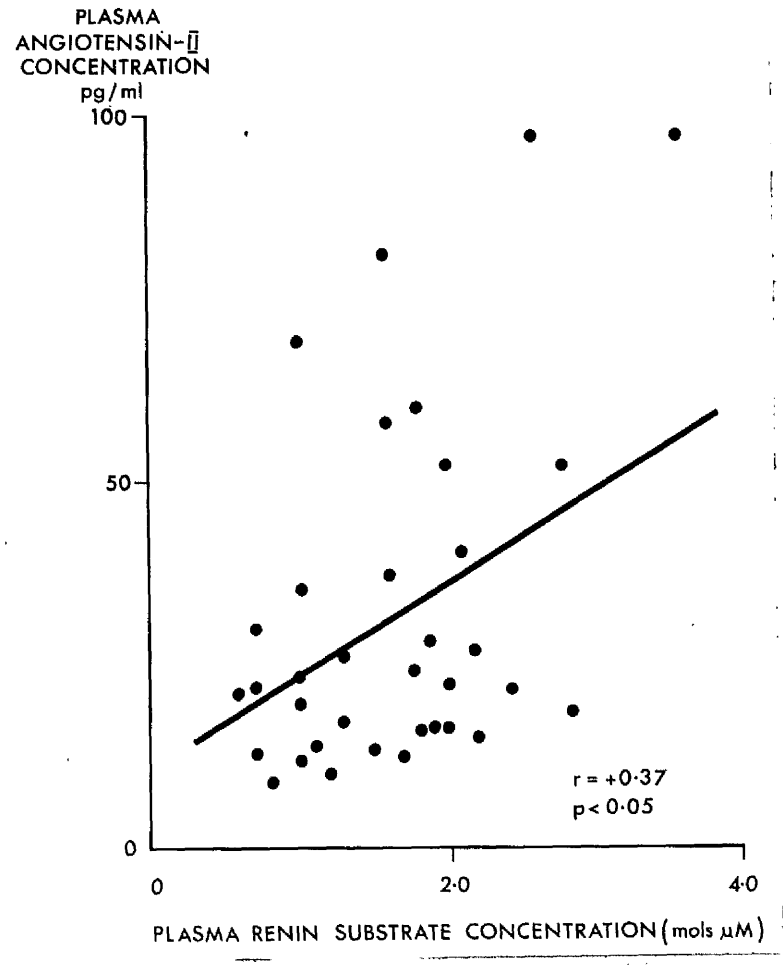


Figure 31

Plasma renin-substrate - angiotensin II relationship in the first trimester

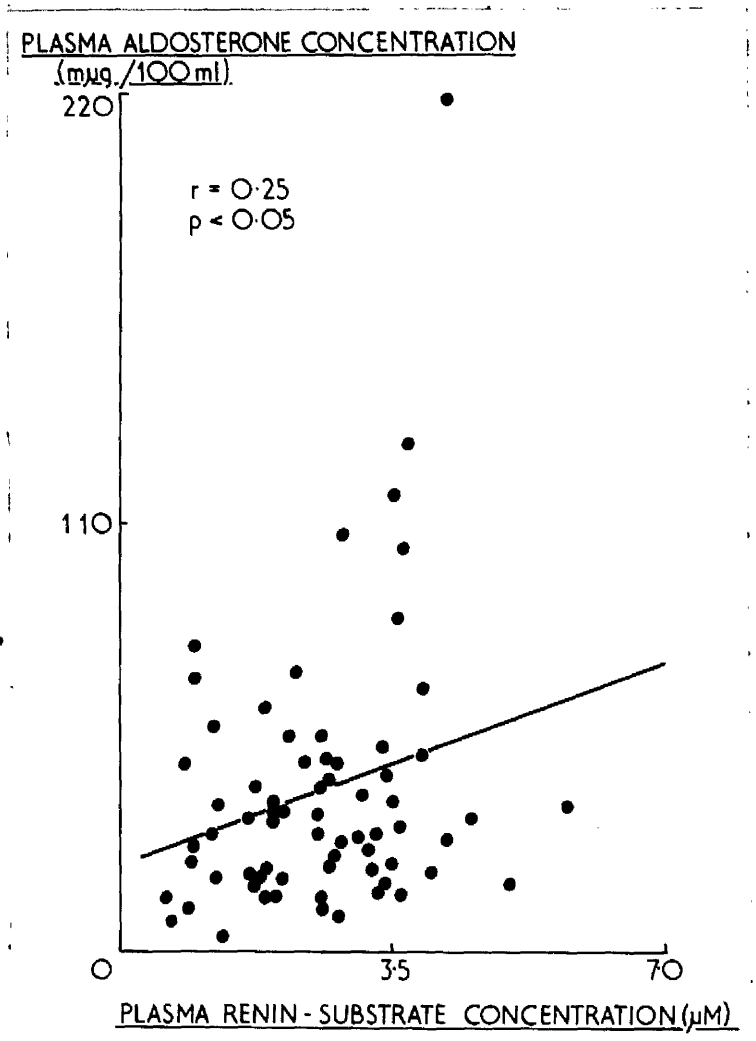


Figure 32

Plasma renin-substrate - aldosterone relationship in pregnancy

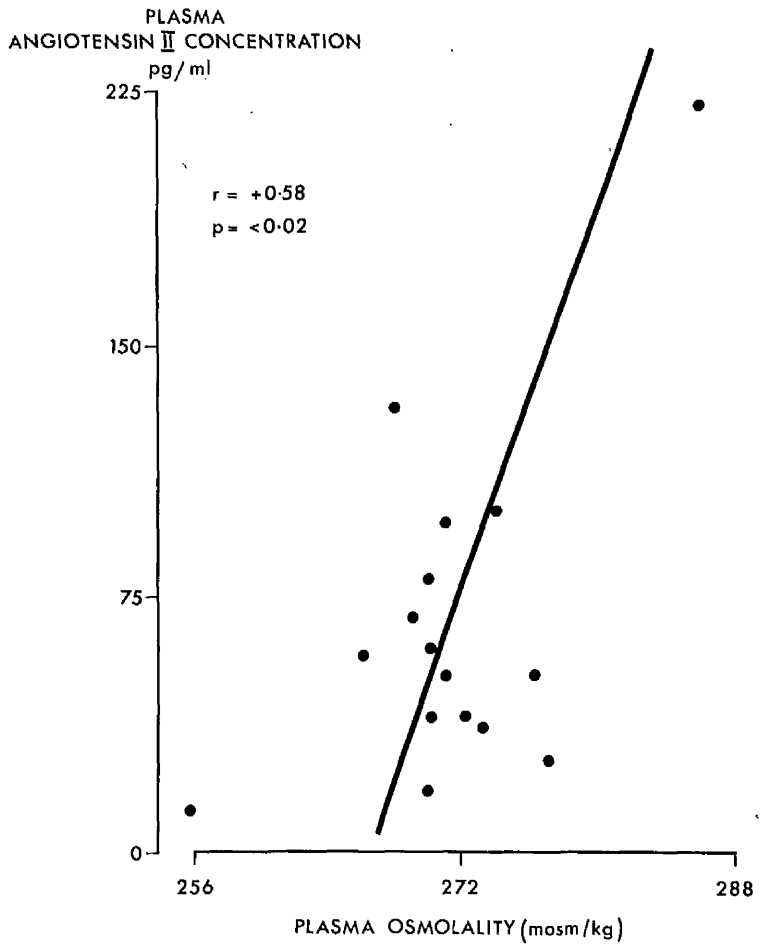


Figure 35.

Plasma angiotensin II - osmolality relationship in pregnancy.

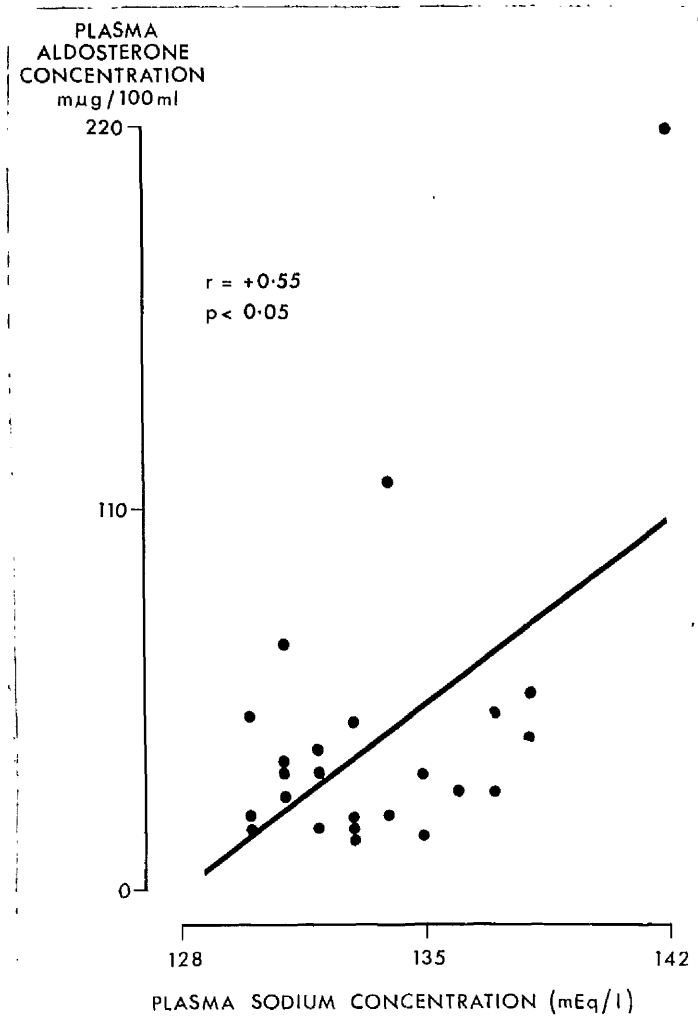


Figure 36.

Plasma aldosterone - sodium relationship - third trimester.

aldosterone and plasma sodium – third trimester (Figure 36)

aldosterone and plasma sodium – all pregnancy samples (Figure 37)

aldosterone and urinary potassium – all pregnancy samples (Figure 38)

plasma sodium and osmolality – all pregnancy samples

A negative correlation statistically significant at the 5% level was found between:-

renin-substrate and creatinine clearance – all pregnancy samples (Figure 39

renin and sodium – post-partum (Figure 40).

Positive correlations which almost reached statistical significance (i.e. those between 5% and 10% levels) were:-

renin substrate – post-partum

aldosterone and plasma sodium – second trimester

aldosterone and plasma osmolality – all pregnancy samples

progesterone and plasma bicarbonate – all pregnancy samples

plasma potassium and osmolality – all pregnancy samples

Step-wise regression was calculated where the following measurements had been made concurrently during pregnancy:-

Plasma renin, renin-substrate, angiotensin II, aldosterone, sodium and potassium, i.e. variables = 2 to 6, n = 19.

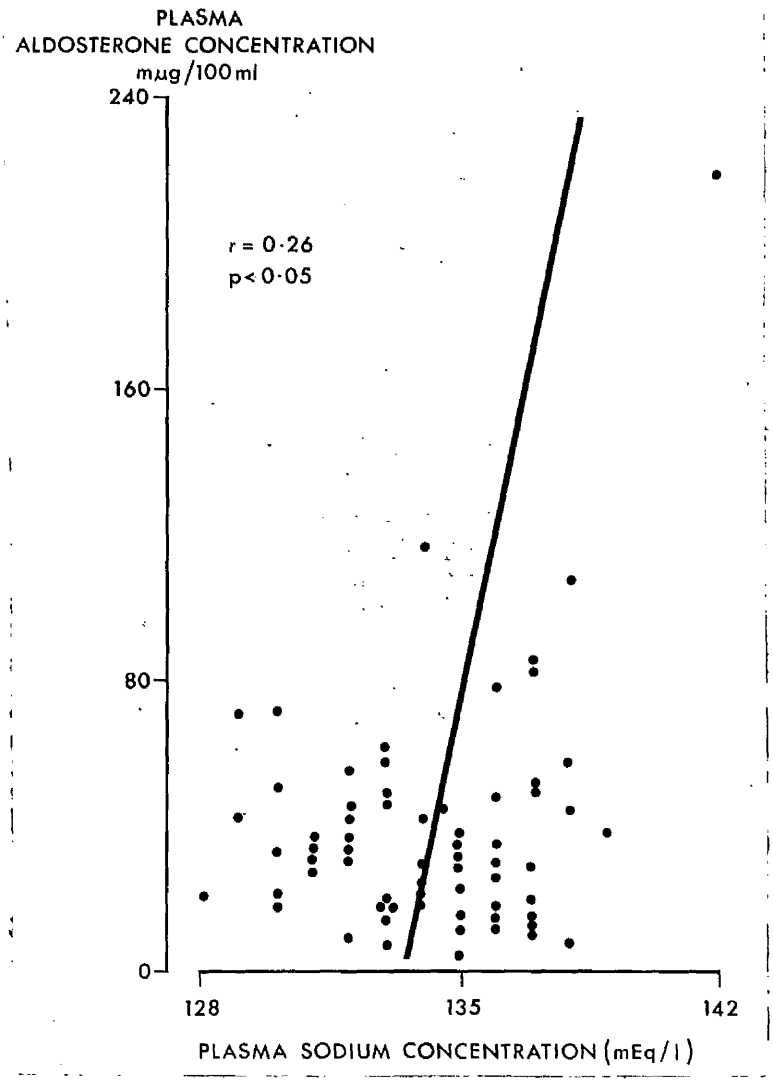


Figure 37.

Plasma aldosterone - sodium relationship - all pregnancy cases.

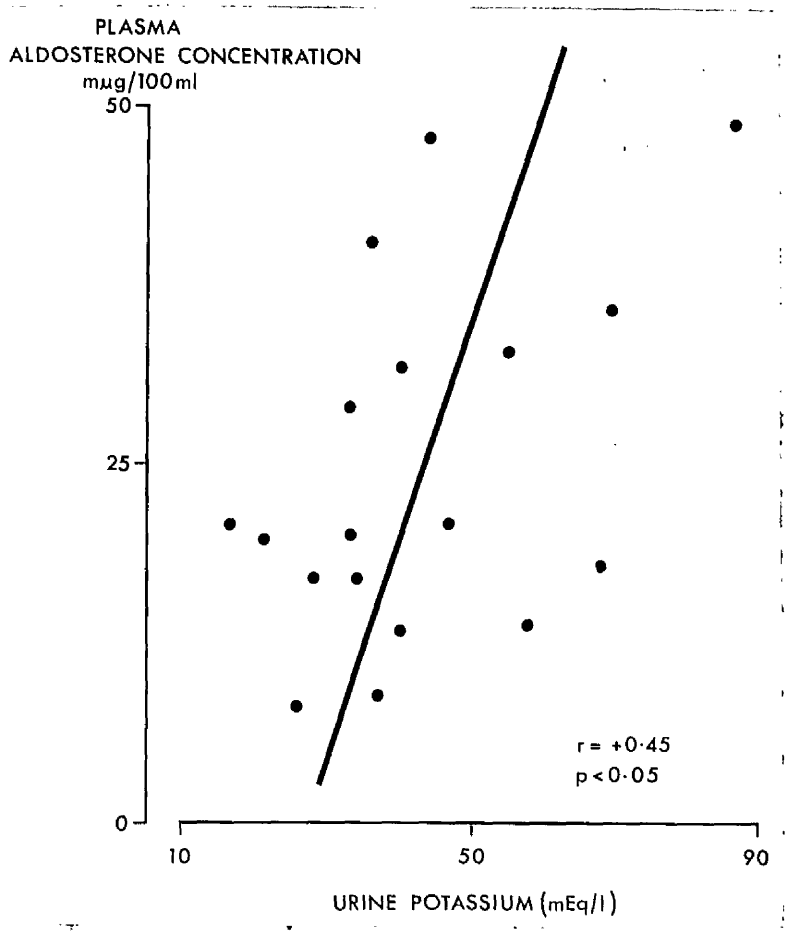


Figure 38.

Plasma aldosterone urine potassium relationship in pregnancy.

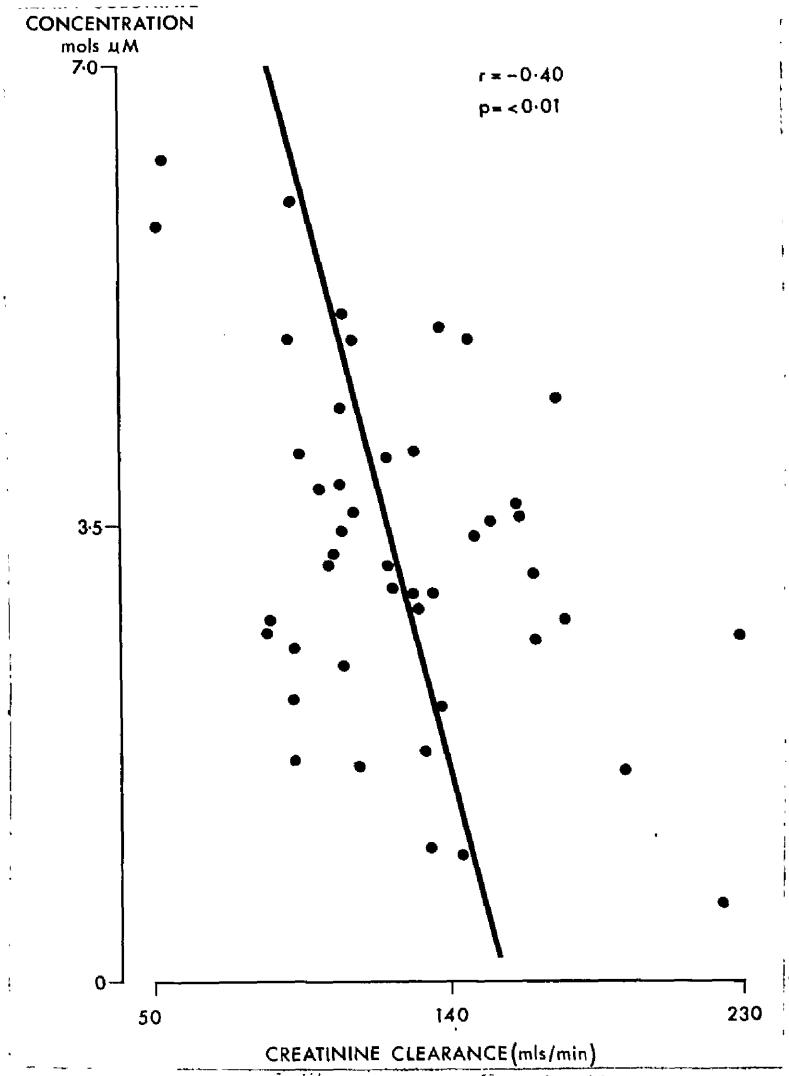


Figure 39.

Relationship of plasma renin-substrate to creatinine clearance in normal pregnancy.

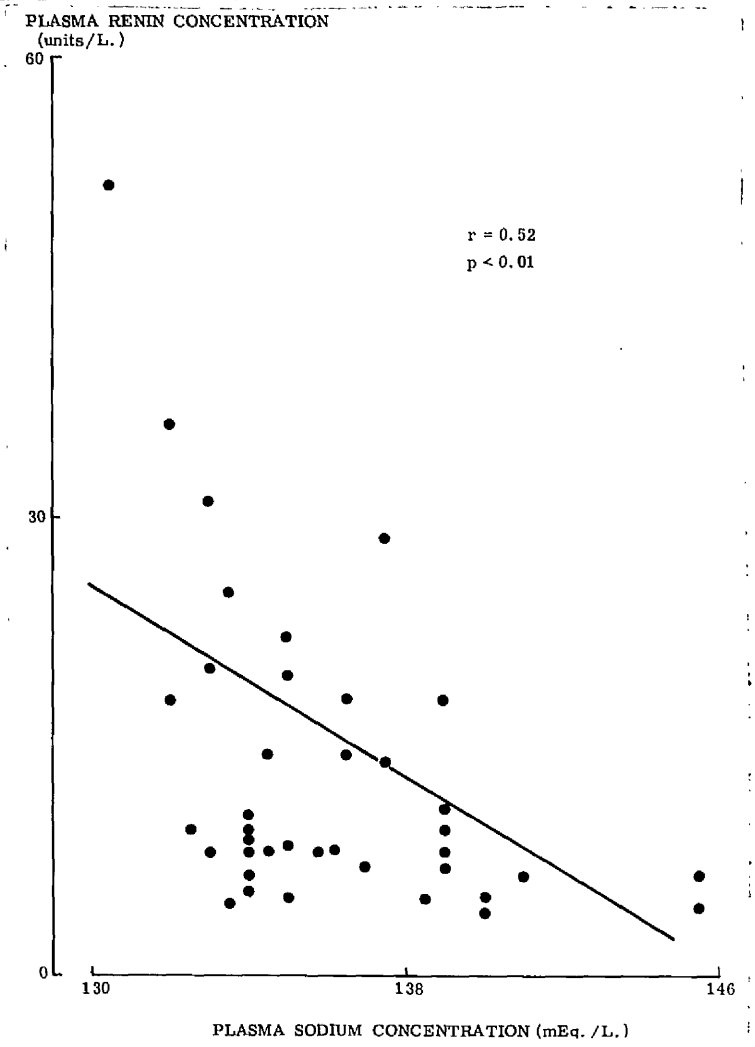


Figure 40.

Plasma renin-sodium relationship in the puerperium.

This showed a significant correlation (5% level) between plasma aldosterone concentration and the plasma concentrations of angiotensin II, potassium and renin substrate.

All the results from this study will be discussed later in Chapter 6.

5.C. CHANGES IN PLASMA 11 DEOXYCORTICOSTERONE (DOC) CONCENTRATION DURING PREGNANCY AND THE PUERPERIUM.

Clinical Study.

Measurements of plasma DOC concentration alone were made in 29 women at various stages of pregnancy and in 10 women in the first week post-partum. Parity in the women sampled during pregnancy was as follows:- para 0 - 14; para 1 - 11; para 2 - 2; para 3 or over - 2. All were single pregnancies. Ages ranged from 17 to 33 years, mean 24.6 years. Blood pressure was normal in every case and there were no other clinical abnormalities.

Blood samples were taken at antenatal or post natal clinics after the women had lain supine for 30 minutes. All the women were on an unrestricted diet.

Laboratory method.

This has been described in Chapter 3.

Results.

Individual results and means for each trimester and post-partum are shown in Table 16 and Figure 41.

Comparison of the results at these different stages was as follows:-

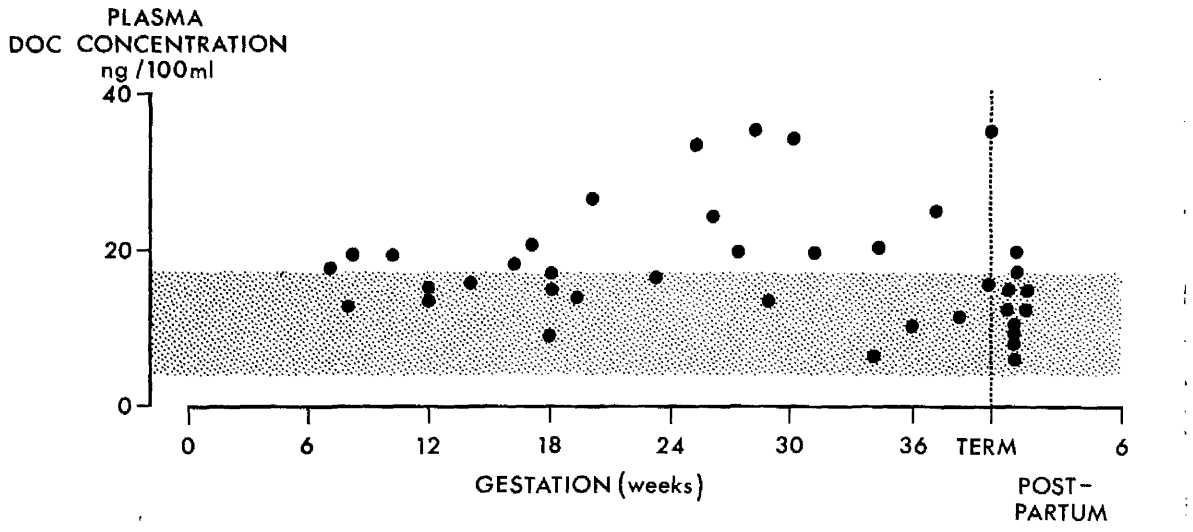


Figure 41. Plasma DOC concentration in normal pregnancy.

Comparison	t-statistic	Degrees of freedom	Significance p
I v II	1.161	15	> 0.1
II v III	0.362	21	> 0.1
I v III	1.099	16	> 0.1
III v P.P.	2.344	20	< 0.05

Plasma DOC concentration was therefore increased during pregnancy and fell quickly to normal in the puerperium. No significant difference was apparent between different stages of gestation, but the numbers involved were rather small, especially in early pregnancy.

These results will be discussed in more detail in Chapter 6.

5.D. A STUDY OF SODIUM AND POTASSIUM BALANCE IN NORMAL PREGNANCY

Clinical Study and Methods.

Two women volunteered to take a diet containing a known amount of sodium and potassium over a number of days, and at the same time collected all the urine passed in 24 hour aliquots for electrolyte estimations. This regime was carried out on an out-patient basis in both women in early and mid-pregnancy and in one of the women in late pregnancy. One woman was also tested 12 weeks post-partum.

Both women were in good health and blood pressure remained normal throughout. Details are shown in Table 17.

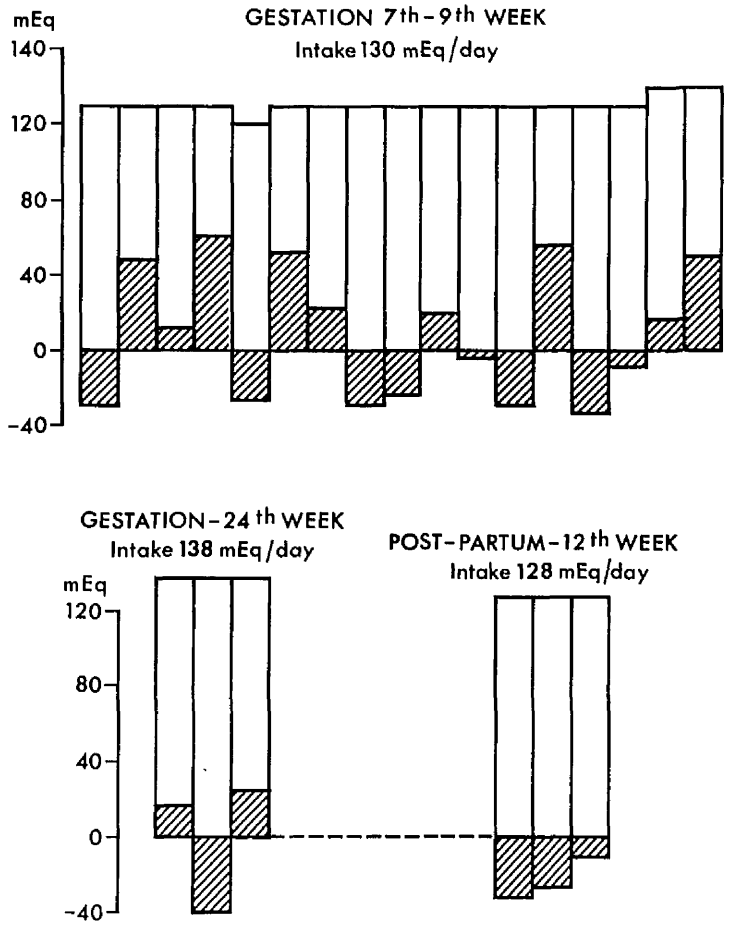
Plasma samples were taken with the women fasting and in the supine position for at least 30 minutes. Estimations carried out are shown in Table 17. Methods for these measurements have been described in Chapter 3.

Results.

These are illustrated in Table 17 and Figures 42 to 44.

Small positive sodium balances occurred in early pregnancy in both women and in mid- and late pregnancy in Case 2. A negative balance was present in Case 1 at the 12th week post-partum.

Small negative potassium balances were present during gestation in both women.

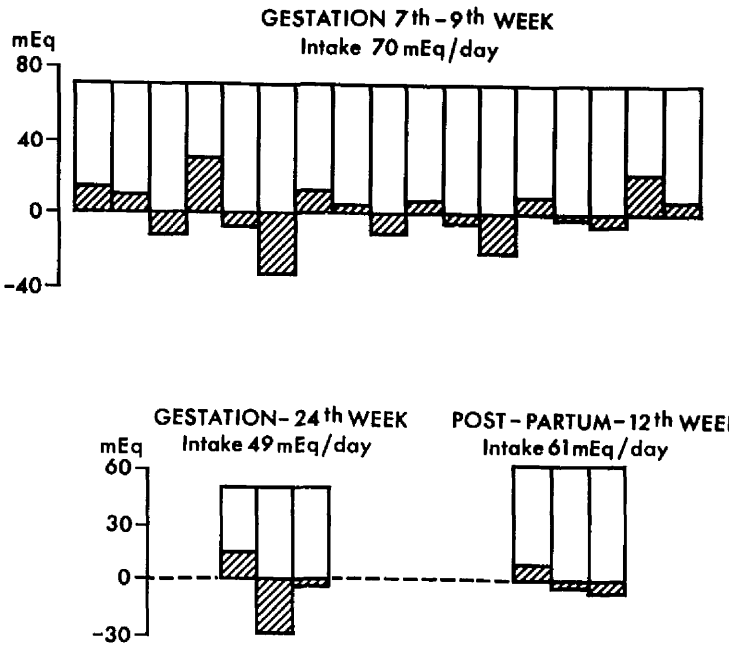


Case 1. SODIUM BALANCE

Figure 42.

Case 1.

Sodium balance



Case 1. POTASSIUM BALANCE

Figure 43.

Case 1.

Potassium balance.

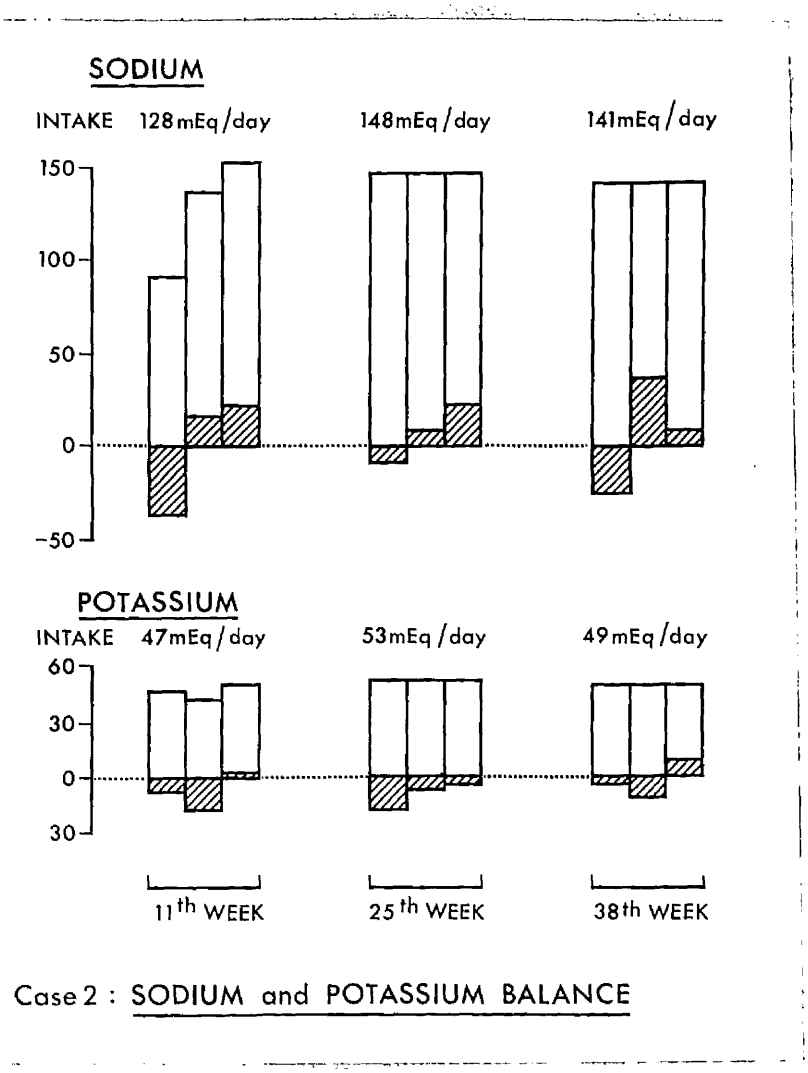


Figure 44. Case 2. Sodium and potassium balance.

Bearing in mind that faeces were not included in the analysis and that, apart from the early study in Case 1, the diet was controlled for only 3 days, too much should not be read into those results. However, they do suggest that further longer studies would be worthwhile. This may be difficult to achieve, as those two women had considerable trouble in adhering to their fixed diet (e.g. early pregnancy study in Case 2), a problem which was aggravated by nausea and other symptoms especially in early pregnancy.

Plasma electrolyte concentrations in Case 2 were unremarkable, but a very low sodium concentration was present in early pregnancy in Case 1. This was associated with increased concentration of plasma renin, renin substrate, angiotensin II and aldosterone.

These results will be discussed in more detail in Chapter 6.

CHAPTER 6

DISCUSSION

First of all, the changes in each substance described in Chapter 5 will be evaluated with reference to the results previously published by other workers. The effect of pregnancy on the relationships between renin, renin substrate, angiotensin II and aldosterone will then be discussed and compared to the relationships found in non-pregnant situations. Lastly will be sections dealing with possible stimuli to the reported changes in the renin-angiotensin-aldosterone system and the role of these changes in the maternal physiological adaptation which occurs in pregnancy.

6.A. CHANGES IN INDIVIDUAL SUBSTANCES DURING PREGNANCY AND POST-PARTUM.

Plasma renin concentration.

The fall in plasma renin concentration between early and late pregnancy is in agreement with the results reported by Skinner and his colleagues (1972), who also found very high levels in the early weeks of gestation. Their results, however, were higher than those found in the present series particularly in early pregnancy. This difference in the degree of increase could possibly be due to differences in posture, Skinner's patients being seated at the time of sampling whereas the women in the present series had been recumbent for at least 30 minutes.

As reported before (Brown et al, 1966b), plasma renin concentration took a number of days to return to normal after delivery of the foetus and placenta, while all the estimations were in the lower part of the normal non-pregnant range by the 6th week post-partum. The implications of these results will be discussed later.

Plasma renin substrate concentration.

The progressive rise in plasma renin substrate concentration was very striking, although a small number of women showed only slight increases above the normal non-pregnant range. These raised circulating levels of substrate are similar to the levels described in previous reports, which were discussed in Chapter 4.A.

Preliminary results of the present study had suggested that plasma renin substrate concentration might remain fairly consistent in individual women from about the 20th week onwards (Weir et al, 1970a). This has not been borne out by subsequent results (see Figure 16).

The delayed fall after delivery of the foetus and placenta may be due to the effect of the synthetic oestrogen, stilboestrol, given to suppress lactation. By the 6th week post-partum, all the women who had not been given stilboestrol had levels within the normal non-pregnant range, whereas high concentrations were still present in some of the other women. This effect of oestrogens will be discussed in more detail in Chapter 12.

Plasma angiotensin II concentrations.

Like renin-substrate, plasma angiotensin II concentration showed a progressive increase from early to late pregnancy. In a number of cases, however, values remained within the normal non-pregnant range throughout, in contrast to striking increases to very high levels in others (Figures 19 and 20). The overall range in the third trimester was wider than that reported by Massani and her colleagues (1967) and the mean was lower. As mentioned in Chapter 4.A, it is likely that Massani's method over-estimated the level of circulating angiotensin II.

The changes after delivery of the foetus and placenta were similar to those of renin, in that after one week most, and after 6 weeks all, the results were within the normal non-pregnant range.

Plasma aldosterone concentration.

In the preliminary study (Chapter 5.A) no significant change in mean plasma aldosterone concentration occurred from 16 to 39 weeks' gestation. In the subsequent larger study (Chapter 5.B), however, a significant rise in mean concentration occurred between early and late pregnancy (Figure 22). This increase during pregnancy is in agreement with the results of the secretion and excretion rate studies discussed in Chapter 4.B.

In the earlier investigation (Chapter 5.A) plasma aldosterone concentration was considerably higher than the normal non-pregnant range in every case in which it was measured, and many of the

results were above 100 mug/100 ml. (Figure 9). In contrast, over the same period of gestation a number of the results in the subsequent larger study (Chapter 5.B) remained within the normal non-pregnant range (Figures 22 and 24) and in only 3 cases did the levels rise above 100 mug/100 ml. One difference between the 2 groups was that the women studied in the earlier series were resident in London whereas those of the later series were from Glasgow. Dietary habits, and in particular the sodium intake, might possibly explain the differences between the 2 groups.

The increases in plasma aldosterone found by Bayard et al (1970a) were distinctly less than in either of the series we have studied; furthermore the secretion rates they reported were also much lower than in the studies by Jones et al (1959) and Watanabe et al (1963). Bayard and his colleagues tentatively ascribed this difference to the 10 hours recumbency assumed by their patients before the measurements were made. However, a recent study in our laboratory has shown a mean of 10.82 mug/100 ml. (S.D. $\pm$ 2.98) in 13 normal non-pregnant women after 12 hours' recumbency, this range being very similar to that for all normal non-pregnant subjects (see Chapter 3).

The degree of elevation of plasma aldosterone above the normal non-pregnant range in the present series was of a similar order to the increases in aldosterone secretion rate found in pregnancy by Jones et al (1959) and Watanabe et al (1963).

The results described in Chapter 5.B were also in accord with those reported by both those groups of workers who had found a proportion of results in or close to the normal non-pregnant range. Concordance between the magnitude of increase in aldosterone secretion rate and plasma aldosterone concentration is not unexpected, since there is general agreement that the overall metabolic clearance rate of aldosterone is not greatly altered in pregnancy (Jones et al, 1959; Watanabe et al, 1963; Tait et al, 1962; Bayard et al, 1970a,). Also, there is no evidence of significant protein-binding of aldosterone in pregnant women (Meyer, Layne, Tait and Pincus, 1961).

Plasma cortisol concentration.

The present results (Table 1, Figure 8) have shown smaller increases in total plasma cortisol than those previously reported (Stewart,et al, 1961; Bro-Rasmussen et al, 1962; Friedman and Beard, 1966; O'Connell and Welsh, 1969; Burke and Roulet, 1970). This may be due, at least in part, to differences in specificity. With the exception of those of Bro-Rasmussen et al (1962), for which no evaluation of specificity is available, and of Burke and Roulet (1970) who used a competitive protein binding method, those previous results were obtained by fluorimetry, which has been shown to give higher values than the double isotope derivative technique (James, Townsend and Fraser, 1967).

Plasma corticosterone concentration.

With few exceptions, plasma corticosterone concentrations remained within normal non-pregnant limits (Table 1, Figure 8), the range in pregnancy being similar to those of earlier reports (Stewart et al, 1961; Martin and Martin, 1968). The results found by Morris and Williams (1953) were higher, but their normal non-pregnant concentrations were also above those of this and the other 2 studies mentioned.

Plasma 11 deoxycorticosterone (DOC) concentration.

As far as is known, only one study of plasma DOC concentration in pregnancy has been published (Schweitzer et al, 1969). The authors merely mention that submicrogram levels were found in 7 normal pregnant women and did not give details of their results or of their normal non-pregnant values.

The data from the present study (Figure 41) have shown a pattern during pregnancy which is similar to that found for plasma cortisol concentration. Mean levels rose from early to late pregnancy at which stage 7 out of the 12 cases had concentrations above the normal non-pregnant range.

Plasma progesterone concentration.

The progressive increase in plasma progesterone concentration from early pregnancy to term (Figure 26) is in accord with the results of other studies (Eton and Short, 1960; Greig, Coyle, Cooper and Walker, 1962; Wiest, 1967; Yannone, McCurdy and Goldfien, 1968).

Plasma electrolytes and osmolality.

The reduced plasma sodium concentrations in early and mid-pregnancy (Figure 27) have confirmed the results of Newman (1957), Herbinger and Wichmann (1967) and MacDonald and Good (1971). Unlike those earlier reports and our own earlier study (Figure 7), the larger number of estimations illustrated in Figure 27 showed a further small but statistically significant fall in the third trimester, a finding which had been noted by Brandstetter and Schüller (1959).

Plasma potassium concentration showed less fluctuation than sodium during pregnancy, although in general the results were in the lower part of the normal non-pregnant range (Figure 27). This small decrease in pregnancy is in accord with previous reports (Newman, 1957; Herbinger and Wichmann, 1967; MacDonald and Good, 1971), although Brandstetter and Schuller (1959) had found no change.

MacDonald and Good (1971) reported a fall in plasma osmolality 2 to 4 weeks after ovulation and Robertson (1968) demonstrated a fall in plasma osmolality in the first 8 weeks of pregnancy, with no further change to term. The normal non-pregnant range associated with the technique used in the present study was considerably lower than that quoted by Robertson, but the low levels found in early pregnancy (Figure 27) are in accord with the previous reports. The earlier study (Figure 7) did not show such low levels at mid-pregnancy, but this may have been due to the smaller number of estimations carried out.

Unlike the results of our preliminary study and the previously reported series, an increase in mean plasma osmolality in the third trimester was found in the larger study (Figure 27).

The changes in plasma osmolality during pregnancy showed a small but statistically significant correlation with the changes in plasma sodium, less so with plasma potassium concentration (Table 14). MacDonald and Good (1971) have commented that plasma osmolality correlated less well with plasma sodium and potassium in pregnant than in non-pregnant women. They suggested that some plasma electrolytes may be osmotically inactive due to protein binding and that this becomes less as the plasma protein pattern changes during pregnancy.

No attempt has been made in the present study to compare the results of primigravid with multigravid women. MacDonald and Good (1972), however, found greater changes in plasma sodium in multigravidae and suggested that this might be due to the greater expansion of plasma volume which occurs in these women.

Blood urea, serum creatinine and creatinine clearance.

Creatinine clearance is a measure of glomerular filtration, although less accurate than inulin clearance. The results of this study (Figure 28) have confirmed those of previous reports that creatinine clearance is increased during pregnancy, particularly in the early months and that it returns quickly to normal levels after delivery (see Hytten and Leitch, 1971).

The lowered serum levels of creatinine (Figure 28) are a reflection of the increased clearance and are in accord with those reported by Sims and Krantz (1958) and less variable than those described by Kuhlback and Widholm (1966).

The reduced blood urea levels (Figure 28) are a reflection of the increased urea clearance found in pregnancy (see Hytten and Leitch, 1971) and have also been described by Sims and Krantz (1958).

Haemoglobin, haematocrit, plasma volume, plasma proteins and colloid osmotic pressure.

The changes in haemoglobin, haematocrit, plasma volume and total plasma proteins (Table 1, Figure 6) were generally similar to those which have been reported earlier (see Hytten and Leitch, 1971).

Mean colloid osmotic pressure (Figure 6) was slightly lower and showed a less consistent fall between the 16th and 38th week than that reported by Robertson (1968). Hytten and Leitch (1971) have illustrated a close relationship between serum albumin concentration and colloid osmotic pressure in pregnancy. This relationship is likely to be the reason for the divergence between colloid osmotic pressure and total plasma proteins found in the present study between the 28th and 38th weeks the increase in total proteins at this time being due to rising alpha and beta globulins which have little effect on colloid osmotic pressure (see Hytten and Leitch, 1971).

6.B. RENIN, RENIN-SUBSTRATE, ANGIOTENSIN AND ALDOSTERONE RELATIONSHIPS IN PREGNANCY.

Although the precise role of renin and angiotensin in relation to the stimulation of aldosterone secretion in man remains a matter of controversy, there is no doubt that, using the methods of the present study, positively correlated changes in concurrent plasma renin/angiotensin II and aldosterone concentrations have been demonstrated in a variety of situations (see Chapter 2 and Figures 4 and 5). These include sodium depletion induced by diuretics or dietary sodium restriction, renal hypertension and chronic renal failure (Fraser et al, 1965, 1966 and 1969; Brown et al, 1971b). By contrast, a significant inverse relationship has been shown between the high plasma aldosterone and low plasma renin concentrations of "primary" hyperaldosteronism (Brown, Chinn, Düsterdieck, Fraser, Gleadle, Lever, Robertson and Tree, 1969a; Ferriss, Brown, Fraser, Kay, Lever, Neville, O'Muircheartaigh, Robertson and Symington, 1970).

Before discussing the possibility that, in pregnancy, the renin-angiotensin system might be a regulator of aldosterone secretion, it is important to clarify the relationships between plasma renin, renin substrate and angiotensin II concentrations in this situation.

The relationships between renin, renin-substrate and angiotensin II.

The increases in plasma renin substrate occurring in pregnancy are within a range which theoretically could influence

the amount of angiotensin formed by a given concentration of the enzyme renin, and the dependences of reaction rate on the prevailing substrate concentration has been demonstrated for all substrate levels encountered in pregnancy (Helmer and Judson, 1967; Skinner et al, 1972). It is not surprising, therefore, that no correlation has been found in pregnancy between the plasma concentrations of renin alone and of angiotensin II, although they have a normal relationship post-partum (Figure 29).

In view of this, it seemed possible that in pregnancy angiotensin II might be related either to the product of renin x renin-substrate or to the level of renin-substrate alone. The present results, however, have shown no correlation between the product of plasma renin x renin-substrate and plasma angiotensin II at any stage of gestation.

Could, then, the amount of angiotensin II generated be governed alone by the increased amounts of substrate available in pregnancy? No evidence for this was found when all the estimations made in pregnancy were analysed. However, on separating the data into trimesters, a positive relationship was demonstrated between the plasma concentrations of renin substrate and angiotensin II in early pregnancy (Table 15, Figure 31). Both substances increased progressively throughout gestation and why this direct relationship was not apparent in later pregnancy is not clear. Following delivery, there appeared to be a possible relationship, although this did not reach statistical significance

at the 5% level (Table 15).

If, then, it is the substrate which is controlling the generation of angiotensin II in early pregnancy, why are the very high plasma concentrations of renin not also influencing the reaction at this stage? A clue may be found in the paper by Lumbers (1971). She found that most, but not all, of the renin in amniotic fluid is inactive at pH 7.5 and is only activated by acid treatment below pH 4.0. The method for estimating plasma renin concentration in the present study (Brown et al, 1964a) and the method used by Skinner (1967) both involve such an acidification.

It is possible, therefore, that these methods, in addition to measuring the active enzyme renin, also measure a renin-like enzyme which is inactive at the pH found in physiological situations. Skinner and his colleagues (1972) have suggested that this inactive component may be derived from foetal chorion (see later in this chapter). If the high concentrations of "renin" measured by these methods in pregnancy plasma are due, at least in part, to this inactive enzyme, this might explain the lack of apparent effect on angiotensin II production.

Increased circulating angiotensin II levels may, in some circumstances, exert a direct inhibiting effect on renin secretion (Genest et al, 1965; Vander and Geelhoed, 1965; Klaus and Holzmann, 1967). This feedback mechanism could be one explanation for the fall in plasma renin concentration in the face of increasing plasma concentrations of substrate and angiotensin II

from early to late pregnancy. It is possible also that the increased angiotensin II concentrations may have a stimulating effect on the production of renin substrate, but at present this remains speculative.

Throughout pregnancy in this study, plasma angiotensin II concentration was within normal non-pregnant limits in a greater proportion of women than plasma renin or renin-substrate concentrations. The presence of an inactive component in the renin concentration being measured could be the explanation for this. On the other hand, it could mean that angiotensin II production is blocked or that the clearance of angiotensin II is increased during pregnancy. To date there is no available evidence to support or refute these possibilities.

The relationship between the renin-angiotensin system and aldosterone.

In contradistinction to other situations discussed earlier (Chapter 2), no relationship has been found between concurrent plasma renin and aldosterone concentrations in mid and late pregnancy. This is true both of the present study and also of the smaller study previously reported (Weir et al, 1970a). Moreover, a marked disparity existed between the concurrent renin and aldosterone levels in individual women at those periods (Figure 9).

In early pregnancy, however, there was a significant correlation between plasma renin and aldosterone concentrations

(Figure 30). In view of the lack of relationship between plasma renin and angiotensin II levels at this stage, the possible reasons for which have been discussed in the previous section, it would seem unlikely that in early gestation renin is stimulating aldosterone solely via angiotensin II. The possibility therefore exists that in pregnancy renin might also be effecting aldosterone secretion by a pathway distinct from angiotensin II. This raises the question as to whether the postulated renin-like enzyme of pregnancy mentioned earlier might perhaps be inactive in the generation of the octapeptide angiotensin II, but active in the stimulation of aldosterone secretion by some other intermediate product. Although it is partly measured in the assay of angiotensin II, such a product might be 2-8 heptapeptide, which has been shown to stimulate aldosterone secretion by Blair-West and his associates (1971).

In our preliminary report (Weir et al, 1970a), a significant positive correlation was found when the aldosterone concentration was plotted against the product of the concurrent renin and renin-substrate concentrations. However, subsequent study of a larger number of women at all stages of pregnancy has now shown no relationship between the plasma renin x renin substrate product and plasma aldosterone concentration.

It was shown in the previous section that changes in plasma renin-substrate concentration occurring in pregnancy might be important in determining the effective plasma concentration of angiotensin. It seemed possible therefore that renin-substrate

might also be related to aldosterone in pregnancy. In the preliminary report of this study (Weir et al, 1970a), no such relationship was demonstrable in the second half of pregnancy, and measurements from the enlarged study have confirmed this for each stage of gestation. However, when all the estimations throughout pregnancy were analysed together, a significant relationship emerged (Figure 32). This positive correlation between plasma renin substrate and aldosterone concentrations during pregnancy, taken with the positive correlation between plasma renin substrate and angiotensin II concentrations certainly in early pregnancy (Figure 31), would be in accord with the hypothesis that circulating angiotensin II may be affecting aldosterone secretion, at least in part. The parallel changes in mean plasma concentrations of angiotensin II and aldosterone during pregnancy and the puerperium (Figures 18 and 22) are also suggestive, albeit indirectly, that a relationship might exist between them, and this is supported to some extent by the fact that both showed a correlation with plasma osmolality (Table 14, Figure 35).

Surprisingly, however, on statistically analysing the paired data from individual women, no significant correlation between plasma angiotensin II and aldosterone concentrations has been found at any stage of gestation (Tables 14 and 15). Unlike some other clinical situations, then, in pregnancy the amount of angiotensin circulating in the blood does not appear to be the major factor controlling aldosterone secretion.

Other substances acting separately or together, perhaps with angiotensin, have been implicated in the control of aldosterone secretion in non-pregnant states (see Fraser et al, 1969; Brown et al, 1972b). The effect of these factors in pregnancy will therefore be discussed in the next section.

6.C. FACTORS AFFECTING THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM IN PREGNANCY.

Although the relationships between circulating renin, renin substrate, angiotensin II and aldosterone are less clear-cut in pregnancy than in other situations, marked changes take place in all four components during gestation and the puerperium. A number of factors are known to influence the plasma concentrations of these components in the non-pregnant state. It is therefore pertinent to discuss the effect of these factors on the renin-angiotensin-aldosterone system in pregnancy, as well as their possible modifying influence on the inter-relationships within the system.

FACTORS INVOLVED IN ALDOSTERONE SECRETION.

1. The renin-angiotensin system.

In the previous section it was shown that plasma aldosterone concentration did not appear to be related to plasma angiotensin II concentration, whereas it was positively correlated with plasma renin substrate concentration over the whole of pregnancy and with plasma renin concentration in the first trimester.

As the overall metabolic clearance rate of aldosterone in pregnancy appears not to be significantly different from that in non-pregnant subjects (see Chapter 4.B.), it is likely that the secretion rates of the hormone in pregnant women are reflected

by its concentration in the plasma. It follows that circulating angiotensin II either has no influence on aldosterone secretion in pregnancy or that its effect is being modified by other factors yet to be discussed.

2. Pituitary ACTH.

The evidence in favour of physiological stimulation of aldosterone secretion by ACTH is conflicting (see Fraser et al, 1969 and Brown et al, 1972b). It is also uncertain whether ACTH output is increased in normal pregnancy (see Forsham, 1967; Hytten and Leitch, 1971). The marked dissociation found in this study between plasma aldosterone and cortisol concentrations when measured concurrently (see Chapter 5.A.) indicate that the increased aldosterone secretion is unlikely to be the result of stimulation by ACTH.

3. Human placental lactogen (HPL; human chorionic somatomammotrophin)

This peptide of placental origen, resembles pituitary growth hormone in its action, and its plasma concentration appears to increase steadily throughout pregnancy (see Hytten and Leitch, 1971). A preliminary study by Melby and his colleagues (1966) showed that the intravenous administration of HPL stimulated aldosterone secretion in normal non-pregnant females and in males. No confirmation of this report has yet been obtained, and a relationship between the increased plasma concentration of HPL and aldosterone in pregnancy remains to be investigated.

4. Electrolytes.

In non-pregnant subjects aldosterone secretion is increased by sodium depletion and also by potassium loading. These changes could be mediated by the renin-angiotensin system (see Chapter 2). Alternatively, they could be brought about by a direct effect of changes in plasma concentration on the adrenal cortex. In support of this is the rise of plasma aldosterone associated with hyponatraemia induced by severe sodium depletion (Fraser et al, 1966). However, it is also well established that aldosterone secretion is affected by electrolyte status before any change occurs in plasma electrolyte concentration (Brown et al, 1972b). It therefore seems more likely that alterations in electrolyte balance may stimulate aldosterone production indirectly.

Physiological quantities of ACTH have been shown to have little effect on plasma aldosterone concentration in normal subjects taking an unrestricted diet. However, when the subject is sodium depleted, ACTH causes a marked rise in aldosterone secretion (James, Landon and Fraser, 1967). Alterations in electrolyte balance may therefore be important in modifying the response of aldosterone to other trophic factors.

(a) Alterations in sodium balance.

If an increase in circulating aldosterone constitutes a normal response to sodium deprivation or depletion, its occurrence in pregnancy suggests a similar basis.

Plasma sodium concentration has been shown to be low throughout pregnancy (Table 6, Figure 27). A correlation with plasma aldosterone concentration has also been demonstrated

(Table 14, Figure 37). Surprisingly, however, this correlation was positive rather than negative as found in other hyponatraemic states. Although statistically significant, there was a very wide scatter on plotting the plasma concentrations of sodium against aldosterone during pregnancy, suggesting that this might be a spurious result (Figure 37). However, on dividing the data into trimesters, a more strongly positive correlation was obtained in late pregnancy (Table 15, Figure 36), while in mid-pregnancy a less marked, but still positive, correlation was found which was significant at the 10% but not at the 5% level (Table 15).

Plasma aldosterone concentration, therefore, appears to be positively correlated to plasma sodium concentration in pregnancy apart from the early months of gestation. Further support for this observation was obtained by finding that both plasma sodium concentration and plasma aldosterone concentration correlated in a positive direction with plasma osmolality, the correlation with sodium being significant at the 2% level and with aldosterone being less significant at the 10% level (Table 14).

Why should the relationship between the plasma concentrations of aldosterone and sodium at these stages in pregnancy be opposite to that of other hyponatraemic states? Could it be due to a difference in overall balance? In non-pregnant situations a low plasma concentration of sodium is usually a reflection of severe bodily sodium depletion with the exception of antidiuretic

hormone excess. Studies in later pregnancy, however, have shown no evidence of maternal sodium depletion. On the contrary, retention of sodium has been demonstrated, the quantities retained being approximately similar to those required by the foetus and placenta and by the increased extracellular fluid (MacGillivray and Buchanan, 1958; Plenti and Gray, 1959; MacGillivray, 1961).

The balance studies reported earlier (Chapter 5.D) were curtailed for practical reasons, especially because of nausea and other symptoms in early pregnancy. The results of these studies are therefore of limited value. However, they lend support to the data observed by the above workers, in that there was no evidence, even in early pregnancy, for maternal sodium depletion, and that, if anything, there appeared to be a slightly positive sodium balance (Table 17, Figures 42 to 44).

The foregoing data suggest that the increase in aldosterone secretion in pregnancy is not stimulated by sodium depletion or hyponatraemia. Rather, it seems more likely that, like primary hyperaldosteronism, the raised plasma aldosterone is the initial event, which results in sodium retention and a positive correlation with plasma sodium concentration, albeit at lower levels than those found in non-pregnant subjects. This effect of aldosterone on sodium balance will be discussed in more detail later (Chapter 6.E), when the overall role of the renin-angiotensin-aldosterone system in pregnancy will be examined.

(b) Alterations in potassium balance.

Total exchangeable potassium is increased in normal pregnancy although the amount of potassium per kg. body weight is less than in non-pregnant females (MacGillivray and Buchanan, 1958; MacGillivray, 1961). The present study has shown no evidence of hyperkalaemia - in fact, the plasma potassium concentration during pregnancy was mainly in the lower part of the normal non-pregnant range, a possible result of haemodilution. However, when the changes in plasma potassium were taken in conjunction with changes in plasma renin substrate and angiotensin II concentrations, a positive correlation with plasma aldosterone emerged (Chapter 5.B). The amount of circulating potassium may therefore be influencing the action of angiotensin II on aldosterone secretion in pregnancy.

The correlation between the urinary excretion of potassium and plasma aldosterone concentration is likely to be a reflection of the potassium excretory effect of aldosterone on the renal tubules, and is therefore an indication that the increased circulating aldosterone in pregnancy is in an active form.

FACTORS INVOLVED IN RENIN SECRETION.

The association between the plasma concentrations of renin and sodium in non-pregnant subjects has been discussed in Chapter 2, renin secretion being stimulated by sodium depletion and inhibited by sodium loading. This relationship

also seems to exist in the puerperium, where an inverse correlation has been found between the falling renin and rising sodium levels (Table 15).(Figure 40).

During gestation, a relationship between the hyponatraemia and the increased circulating renin might have been expected. However, no such correlation has been obtained at any stage. The reasons advanced for the failure of the hyponatraemia to stimulate aldosterone secretion could be put forward in the case of renin secretion. It also seems possible that other factors may be playing a part in renin release during pregnancy.

Like plasma sodium concentration, plasma osmolality is reduced, especially early in pregnancy (Figure 27), and failure to correlate with plasma renin concentration could be due to similar factors.

It has also been suggested that changes in blood volume or haematocrit may affect renin release and hence circulating renin levels (Brown et al, 1966c; Nielson and Møller, 1968), but no relationship was found in this study between plasma renin and plasma volume or haematocrit (Chapter 5.A). Similarly, changes in plasma protein concentration and colloid osmotic pressure have been shown to have no correlation with plasma renin concentration (Chapter 5.A).

Stimulation of renin secretion secondary to arterio-venous shunting of blood in the placenta (Mulrow, 1964b) or pressure on

the renal arteries by the gravid uterus (Forsham 1967) have also been postulated, but seem unlikely to be operative as early as the fifth week of pregnancy, when a marked increase in renin has already been noted (Figure 11).

The failure of circulating renin to correlate with any of the stimuli established in other situations might be due to the interaction of a number of these factors during pregnancy. On the other hand it could be taken as evidence that the plasma renin concentration being measured contains a proportion of inactive renin derived possibly from chorion (Chapter 6.B). The possible role of non-renal sources of renin in human pregnancy will be discussed in Chapter 6.D.

FACTORS INVOLVED IN RENIN-SUBSTRATE PRODUCTION.

Increases in plasma renin-substrate concentration similar to those found in pregnancy have been found in women taking combined oestrogen-progestogen oral contraceptives (see Chapter 12, Figure 58). As the secretion of oestrogens and progesterone increases steadily from early gestation (see Hytten and Leitch, 1971), one or other, or both, of these hormones could be the cause of these increases in plasma levels of renin-substrate.

Helmer and Griffith (1952) found that rat plasma renin-substrate was increased by oestrogen administration and this has since been confirmed in man (Helmer and Judson, 1967; Newton, Sealey, Ledingham and Laragh, 1968). Progestogen alone

had no effect on rat substrate (Helmer and Griffith, 1952), but the progestogen norethynodrel, which also has some oestrogenic properties, produced a small rise in human plasma substrate concentration (Newton et al, 1968).

In an experiment to be described later (Chapter 12), women given an oestrogen (mestranol) showed marked increases in plasma renin-substrate concentration, whereas those given a progestogen (ethynodiol diacetate) showed no response (Figure 63).

It seems likely, therefore, that it is the increased oestrogen secretion in pregnancy which stimulates the rise in plasma renin-substrate concentration.

Oestrogens and combined oestrogen-progestogen oral contraceptives have been shown to produce an increase in cortisol-binding globulin (Sandberg and Slaunwhite, 1959; Layne, Meyer, Vaishwanar and Pincus, 1962; Doe, Mellinger, Swaim and Seal, 1967; Keane et al, 1969a), thyroxine-binding globulin (Doe et al, 1967; Keane, Pegg and Johnson, 1969b) and in alpha-2 macroglobulin and transferrin (Horne, Howie, Weir and Goudie, 1970). As renin-substrate is in the alpha-2 globulin fraction, it seems possible that the raised concentration in pregnancy is associated with increased hepatic globulin synthesis stimulated by heightened oestrogenic activity.

FACTORS INVOLVED IN ANGIOTENSIN PRODUCTION.

The octapeptide angiotensin II is generated via the

decapeptide angiotensin I by the action of the enzyme renin on its protein substrate in the plasma. Factors which affect circulating levels of renin and renin-substrate would therefore be expected to influence the amount of angiotensin II present in the plasma. Such factors have already been discussed.

The presence or absence of inhibitors or activators of the renin - renin-substrate reaction might also influence the amounts of angiotensin I and II generated, as might alterations in the enzymes involved in the conversion of angiotensin I to angiotensin II. Little is known about these factors in human pregnancy.

Variations in "angiotensinase activity" might affect the survival, and hence the concentration, of angiotensin II in blood. As has been described earlier (Chapter 4.A), angiotensinase activity is generally increased in pregnancy, but its influence on the circulating levels of angiotensin II in this situation has not been clearly assessed. Also, an alteration in metabolic clearance of angiotensin II has not yet been shown in pregnancy.

6.D THE SIGNIFICANCE OF RENIN IN THE UTERUS AND FOETO-PLACENTAL UNIT AND OF ALDOSTERONE IN THE FOETUS.

In the human, high concentrations of renin, or an enzyme closely resembling it, have been found in amniotic fluid (Brown, Davies, Doak, Lever, Robertson and Tree, 1964b; Skinner et al, 1968; Lambers, 1971) (Figure 45) and in chorion, amnion, decidua, placenta and myometrium (Skinner et al, 1968). Renin has also been detected in the human foetal kidney (Ljungqvist and Wagermark, 1966).

Uterine renin increases during pregnancy (Geelhoed, Vander and Carlson, 1970) but considerably less renin is detectable in human uterine muscle than seems to be present in that of certain other mammals, such as the rabbit (Skinner et al, 1968; Ferris, Gorden and Mulrow, 1967; Ryan, 1970). As a result of tissue culture studies Symonds, Stanley and Skinner (1968) concluded that in man this intra-uterine renin was mainly of chorionic origin from where there was ready access to amniotic fluid.

Brown and his colleagues (1964b) found generally slightly higher concentrations of renin in umbilical vein plasma than in maternal peripheral venous plasma at term whereas in two similar studies Skinner et al (1968; 1972) found no consistent differences between the plasma renin concentrations at those two sites.

Although not certain, it seems likely that in late pregnancy the high levels of renin in the maternal blood are derived from the

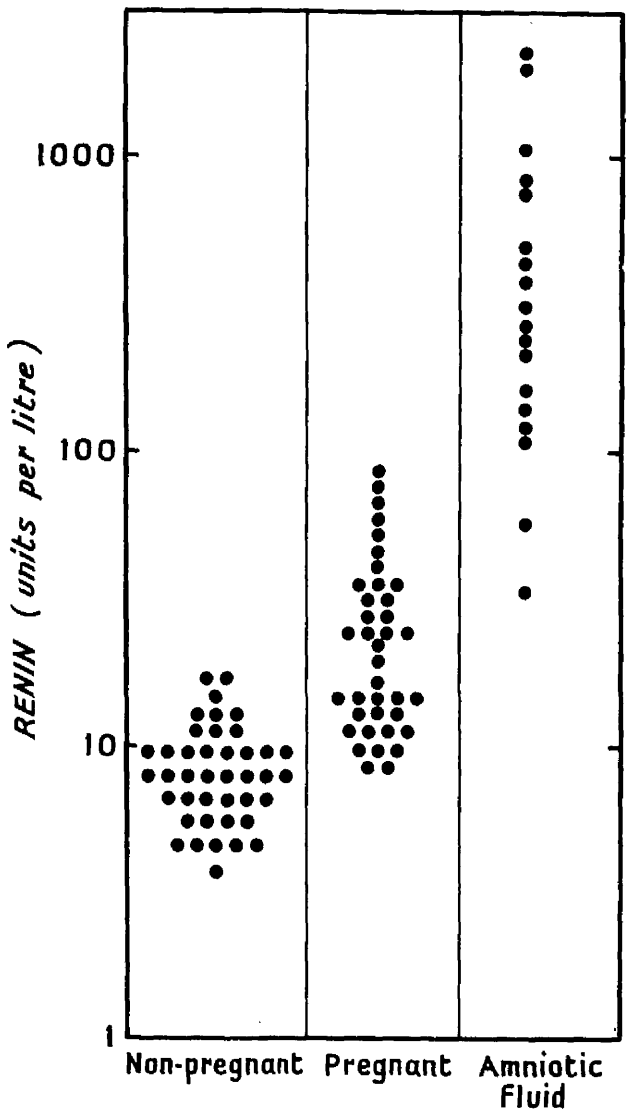


Figure 45 Renin in amniotic fluid and in plasma of pregnant and non-pregnant women. (from Brown et al 1964b).

maternal kidneys rather than from the uterus or its contents. Skinner et al (1968) postulated that it would be difficult for the human chorion to contribute to the maternal circulation through the thick decidual layer. Confirmation of this has been obtained by two recent studies which demonstrated lower renin activity and concentration in uterine compared to peripheral venous plasma in women at term (Kokot and Celiński, 1972; Skinner et al, 1972). Moreover, elevated levels of renin may persist in the maternal peripheral blood for several days after delivery (Figure 13), whereas the half-life of endogenous renin, at any rate in the anephric subject, is of the order of 120 minutes (Brown, Curtis, Lever, Robertson, de Wardener and Wing, 1969b).

These arguments, however, do not necessarily apply to the early weeks of pregnancy, at which time measurement of uterine venous renin concentration has not yet been reported. Skinner and his associates (1972) have shown that measurement of plasma renin concentration in pregnancy might include a physiologically inactive renin-like enzyme with characteristics similar to those of chorionic and amniotic fluid renin (Lumbers, 1971). They have also pointed out that chorionic gonadotrophin can pass into the maternal circulation during the phase of cytotrophoblastic proliferation (Jones, Belfs and Stern, 1944) and have suggested that chorionic renin might do likewise. As discussed in Chapter 6.B, this might explain the very high plasma renin concentrations in early pregnancy and the apparent

lack of correlation between circulating levels of renin and angiotensin II in this situation.

The function of intrauterine renin remains uncertain; possibly it may be concerned with the regulation of sodium and fluid transfer to the foetus. The rudimentary counter-current vascular system of the human placenta resembles, in some respects, the more elaborate arrangements in the kidney as described by Kriz and Lever (1969); it is at least possible on present evidence that in both situations renin, via angiotensin, controls the velocity of blood flow, and hence the efficiency of fluid and electrolyte exchange. Another possibility is that the renin-angiotensin system may have a role to play in parturition, as angiotensin II has been shown to have an oxytocic effect (Paiva and Paiva, 1960). Some support for this is suggested by the finding that 2 women with histories of poor uterine contractions had lower uterine renin concentrations than other women (Goolhoed et al, 1970).

Plasma renin-substrate concentration has been found to be lower in foetal than maternal plasma (Skinner et al, 1972) but to date no reports of angiotensin concentration in foetal blood have been published.

The human foetal adrenal is capable of coverting corticosterone to aldosterone in vitro by the 16th week of gestation (Dufau and Villee, 1969). At term, significant amounts of circulating aldosterone in the foetus appear to be secreted by the foetal adrenal while only a small proportion seems to cross the placenta

from the mother (Bayard et al, 1970a). The levels in foetal plasma are generally higher than in maternal plasma, but respond in a similar fashion to changes in maternal sodium intake (Beitins, Bayard, Levitsky, Ances, Kowarski and Migeon, 1972). Whether this disparity in aldosterone concentration between mother and foetus is associated with placental transfer of sodium remains to be investigated.

6.E. THE ROLE OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM IN PREGNANCY.

From the results of these and previously reported studies, it is now well established that profound changes occur in the circulating levels of renin, renin-substrate, angiotensin II and aldosterone during normal pregnancy. At the same time, the mother is adapting to the demands of the enlarging uterus, placenta and foetus by marked alterations in hormonal, fluid and electrolyte balance. What, then, is the physiological role, if any, of the renin-angiotensin-aldosterone system in preserving maternal homeostasis during this time?

It has been shown that, rather than sodium depletion acting as a stimulus to aldosterone secretion as in many non-pregnant situations, in pregnancy the increase in aldosterone production appears to be the prime mover in the control of sodium balance. That is, the increase in plasma aldosterone concentration may occur prophylactically in the early weeks to prevent the development of maternal sodium depletion as gestation advances.

A number of potential sources of maternal sodium depletion have been identified. Taken singly they might not have a dramatic effect on sodium balance, but their cumulative effect as pregnancy proceeds could result in a marked sodium deficit unless compensated by a powerful sodium-retaining agent such as aldosterone.

1. It is known that the glomerular filtration rate is markedly increased as early as the 12th week (Sims and Krantz, 1958; Hytten and Leitch, 1971). This would lead to increased sodium excretion unless compensated by increased tubular sodium reabsorption, promoted possibly by increased circulating aldosterone. Watanabe et al (1963) were unable to demonstrate a correlation between glomerular filtration and aldosterone secretion rates in later pregnancy, but by that stage other events to be described could have obscured a simple relationship.

2. During pregnancy, the increases in plasma oestrogens and progesterone parallel those of plasma renin-substrate, angiotensin II and aldosterone concentrations (see Hytten and Leitch, 1971; and also Chapter 5.B, Figures 14, 18, 22, 26, 33 and 34).

Progesterone inhibits the action of aldosterone on the renal tubule (Landau and Lugibihl, 1958, 1961), and increases the urinary excretion of sodium when given to non-pregnant women in doses comparable to the rate of secretion in normal pregnancy (Landau, Bergenstal, Lugibihl and Kascht, 1955; Landau, Lugibihl, Bergenstal and Dimick, 1957). In normal pregnant women the administration of progesterone has been shown to stimulate aldosterone secretion (Laidlaw, Ruse and Gornall, 1962) and a correlation between urinary pregnanediol excretion and aldosterone secretion has been found (Jones et al, 1959). A transient natriuresis and an increase in aldosterone secretion rate have also been demonstrated during

the administration of oestriol and oestradiol to non-pregnant subjects (Katz and Kappas, 1967).

However, in attempting to relate these events to changes in renin and aldosterone it should be borne in mind that progesterone (Figure 26) and oestrogens have been shown to increase slowly in early pregnancy, with a progressive rise to term (Short and Eton, 1959; Greig et al, 1962; Yannone et al, 1968; Brown, 1956; Klopper and Billewicz, 1963; Samaan, Bradbury and Goplerud, 1969) and they would therefore be expected to have a greater effect on sodium balance, renin and angiotensin in late rather than early pregnancy.

3. During pregnancy, there is an added requirement for approximately 950 mEq of sodium, of which about 530 mEq are needed for the growing uterus, foetus and placenta (Hytten and Leitch, 1971). Although it is unlikely that diversion of sodium to the foeto-placental unit would be sufficient to deplete the mother in the first weeks of gestation, a cumulated deficit could occur in later pregnancy unless counterbalanced by an increased dietary salt intake or by increased renal tubular reabsorption of sodium.

If a rise in circulating aldosterone is required to prevent the onset of maternal sodium depletion due to these factors, what is the initial stimulus to the aldosterone secretion? A speculative sequence of events is shown in Figure 46.

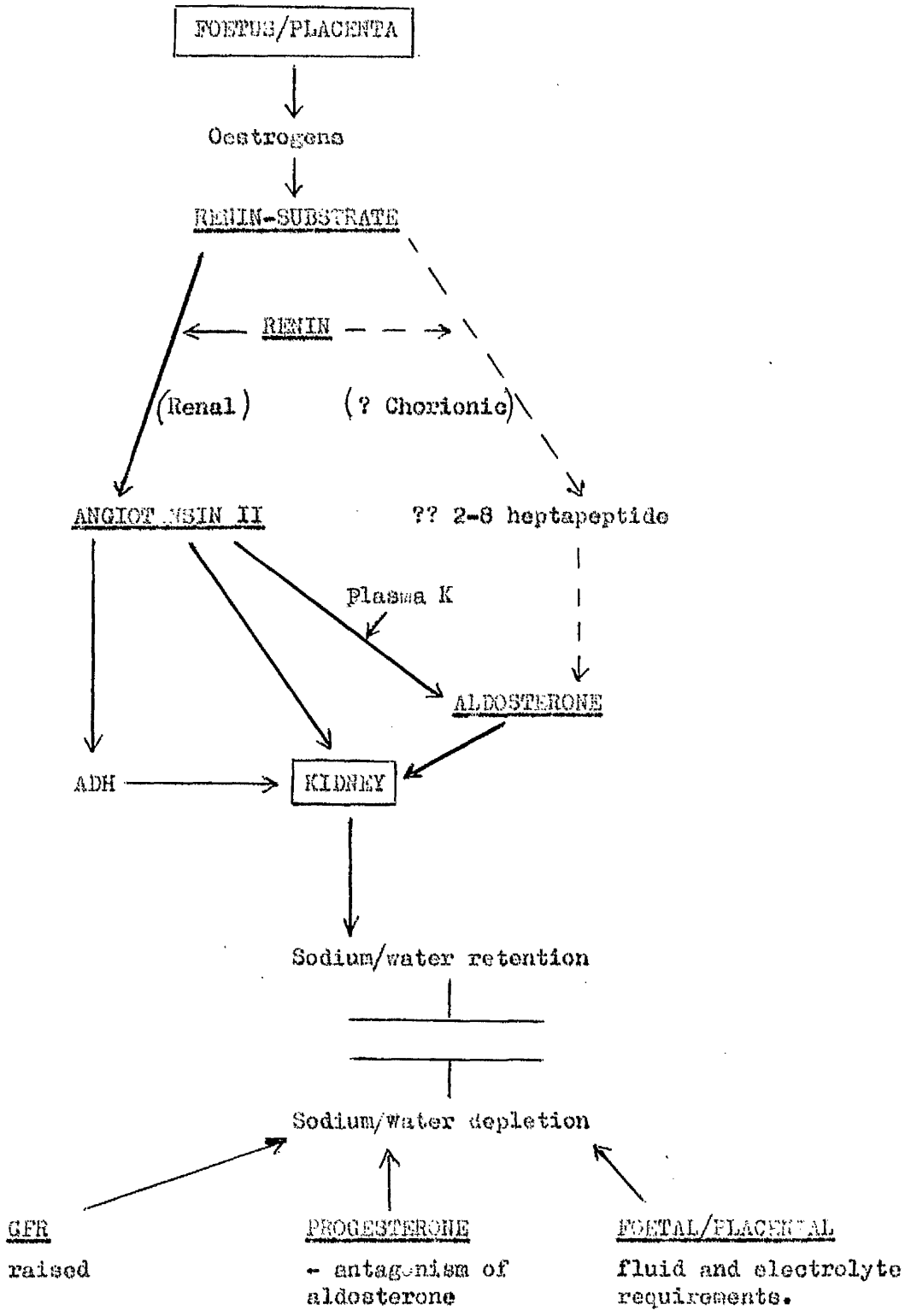


Figure 46

Speculative role of renin-angiotensin-aldosterone system in pregnancy.

The raised production of oestrogens from the early weeks of gestation may stimulate increased liver synthesis of renin-substrate. At the same time a rise in renin secretion occurs, possibly mainly from the chorion. The increased renin-substrate results in elevated levels of circulating angiotensin II.

Modifying influences, especially plasma potassium concentration, may explain the lack of direct correlation between the plasma levels of angiotensin II and aldosterone. Nevertheless, in view of the strong correlations in other situations, the absence of such a direct relationship in pregnancy is remarkable. It may mean that in pregnancy, in addition to its effect on the adrenal cortex, angiotensin II may also have a major influence on fluid balance by stimulating the secretion of antidiuretic hormone (Bonjour and Malvin, 1970) or by itself exerting an intrarenal antidiuretic effect (Brown et al, 1972a). To date, antidiuretic hormone levels have not been measured in pregnancy.

The direct relationship between renin and aldosterone in the absence of a relationship between renin and angiotensin II has led to the earlier speculation that circulating "chorionic renin" may act on substrate to form, for example, 2 - 8 hepta-peptide which has been stated to stimulate aldosterone secretion (Blair-West, et al, 1971). This, of course, remains hypothetical.

The combined effects of water retention (mediated by angiotensin II with or without antidiuretic hormone) and sodium retention (mediated by aldosterone) would result in increased plasma volume, which has been shown to be necessary for adequate development of the foetus (Hytten and Leitch, 1971). Increased renal blood flow and glomerular filtration rate would be a consequence of this. Greater retention of water than sodium would cause increased plasma water content (Paaby, 1959; Hytten and Leitch, 1971) and a lowering of plasma electrolyte concentration and osmolality.

The renin-angiotensin-aldosterone system appears to be functionally active in normal pregnancy and there is evidence, albeit mainly circumstantial, that it may have an important role in the maintenance of maternal electrolyte and fluid balance.

However, a puzzling situation arises when women with little or no adreno-cortical function become pregnant. There is no evidence for significant placental secretion of aldosterone (Baulieu, de Vigan, Bricaire and Jayle, 1957; Laidlaw, Cohen and Gornall, 1958; Christy and Jailer, 1959; Krieger, Gabrilove and Soffer, 1960), and these women therefore have negligible circulating levels of the hormone. Unless they increased their dietary sodium considerably, such women might be expected to require increased amounts of exogenous

mineralocorticoid to maintain sodium balance during their pregnancy. That this is not so has been suggested by a study of women following bilateral total adrenalectomy (Barber, Graber and O'Rourke, 1966). These women had quite normal pregnancies without requiring an increase in their replacement doses of glucocorticoid or mineralocorticoid. They were taking salt in their diet ad lib.

In 2 women with adrenocortical insufficiency studied in late pregnancy, serum electrolytes were within the normal pregnant range and aldosterone secretions were very low or undetectable (Drucker, Hendrix, Laragh, Christy and Van de Viele, 1963). When given a normal sodium intake of 91 mEq daily, one of the women had a negative sodium balance while the other had a positive balance when given an increased intake of 237 mEq sodium daily. Negligible effects on electrolyte excretion were observed when large quantities of aldosterone were administered intravenously to these women before delivery, whereas post-partum the normal sodium retaining effect of aldosterone was restored. The insensitivity to aldosterone in pregnancy was thought to be due to the antagonistic effect of progesterone.

In the absence of aldosterone, progesterone does not appear to cause a natriuresis (Landau et al, 1955; Landau and Lugibihl, 1958, 1961). The tendency to sodium depletion may, therefore, be less in pregnant women with adrenocortical

insufficiency than in normal pregnant women, and they may be able to cope more easily with the requirements for additional sodium by increasing their dietary salt intake.

Concurrent measurements of plasma renin, renin-substrate, angiotensin II and aldosterone concentration and of electrolyte and fluid balance during gestation in women with adrenocortical insufficiency might help to elucidate further the fascinating problem of the role of the renin-angiotensin-aldosterone system in normal human pregnancy.

CHAPTER 7

AN INVESTIGATION OF A POSSIBLE RELATIONSHIP BETWEEN THE SYMPTOMS OF EARLY PREGNANCY, PLASMA ELECTROLYTES AND THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM.

McCance (1936) gave a detailed account of the symptoms experienced by normal subjects during severe experimental sodium depletion. Prominent features included nausea, aberrations of taste, a peculiar metallic taste sometimes mistaken for thirst but not relieved by drinking water, muscular cramps, and mental and physical lassitude. In Chapter 2 it was seen that sodium depletion is associated with increased plasma concentrations of renin, angiotensin and aldosterone. It is uncertain to what extent the symptoms of sodium chloride deficiency are due to sodium chloride lack as such, or alternatively to the associated hormonal changes. For example, angiotensin has been shown to cause polydipsia in rats (Fitzsimons and Simons, 1969) and the associated rise in circulating angiotensin has been suggested as a possible cause of the intense thirst which sometimes accompanies severe renal failure in man (Brown et al, 1969b).

As has been seen, pregnancy is a situation where there are adequate causes for a marked tendency to sodium depletion (see Chapter 6.B). Moreover, several biochemical features associated with sodium depletion may be present from early pregnancy, namely, increases in the plasma concentrations of renin,(Figure 11), angiotensin II (Figure 19) and aldosterone

(Figure 23). Finally, many of the known symptoms of severe sodium depletion (nausea, aberrations of taste, a metallic sensation in the mouth unrelieved by drinking, thirst, cramp, mental and physical tiredness), are so common in pregnancy, especially in the early weeks, as to be dismissed often without comment. These similarities of biochemical features and symptoms seemed to be sufficiently striking to merit further examination.

Clinical methods.

28 normal pregnant women were investigated before the 12th week of gestation. Six of these women had been admitted for termination of pregnancy under the Abortion Act (1968), and blood samples were taken from them between 8.30 and 9.30 a.m. The remainder of the women had blood samples taken at morning or afternoon visits to the antenatal clinic or Ward. All the women were on an unrestricted diet and had been recumbent for at least 30 minutes before the samples were taken.

All the women were given a questionnaire to complete regarding their symptoms at the time of sampling. This is illustrated in Figure 47. The symptoms were then arbitrarily scored, one point being given for each symptom present, as shown. Where the patient indicated a marked severity of a symptom, 2 points were allocated. The total score was entered in the protocol before the results of the biochemical tests were known.

SYMPTOMS OF EARLY PREGNANCY

Figure 47

	NO	YES	
NAUSEA	☐	☐	MORNING ☐
VOMITING	☐	☐	EVENING ☐
THIRST	☐	☐	RELIEVED BY DRINKING — YES ☐ NO ☐
ABNORMALITY OF TASTE	☐	☐	NO TASTE FOR FOOD ☐ METALLIC TASTE ☐
CRAVINGS	☐	☐	FOR WHAT? :-
EXCESSIVE SALIVA	☐	☐	
PHYSICAL WEAKNESS	☐	☐	
MENTAL TIREDNESS	☐	☐	
CRAMPS	☐	☐	

OTHER SYMPTOMS:-

Biochemical and statistical methods.

These were as described in Chapter 3.

Results.

The correlations between symptoms and biochemical changes were as follows:-

	Plasma	n	r	Significance p
Symptoms	Renin	27	0.062	> 0.1
Symptoms	Substrate	28	0.010	> 0.1
Symptoms	Angiotensin II	22	0.283	> 0.1
Symptoms	Aldosterone	20	0.068	> 0.1
Symptoms	Sodium	27	0.270	> 0.1
Symptoms	Potassium	27	-0.530	< 0.01
Symptoms	Osmolality	15	0.309	> 0.1

No relationship was found between the overall symptoms of early pregnancy and changes in plasma concentrations of renin, renin-substrate, angiotensin II and aldosterone; neither were plasma sodium and osmolality related to the indidence of symptoms.

A significant negative correlation, however, was obtained between the severity of symptoms and plasma potassium concentration (Figure 48).

Further analysis of the results showed no evidence that plasma angiotensin II concentration was greater in women who complained of thirst alone than in women who did not admit to this symptom.

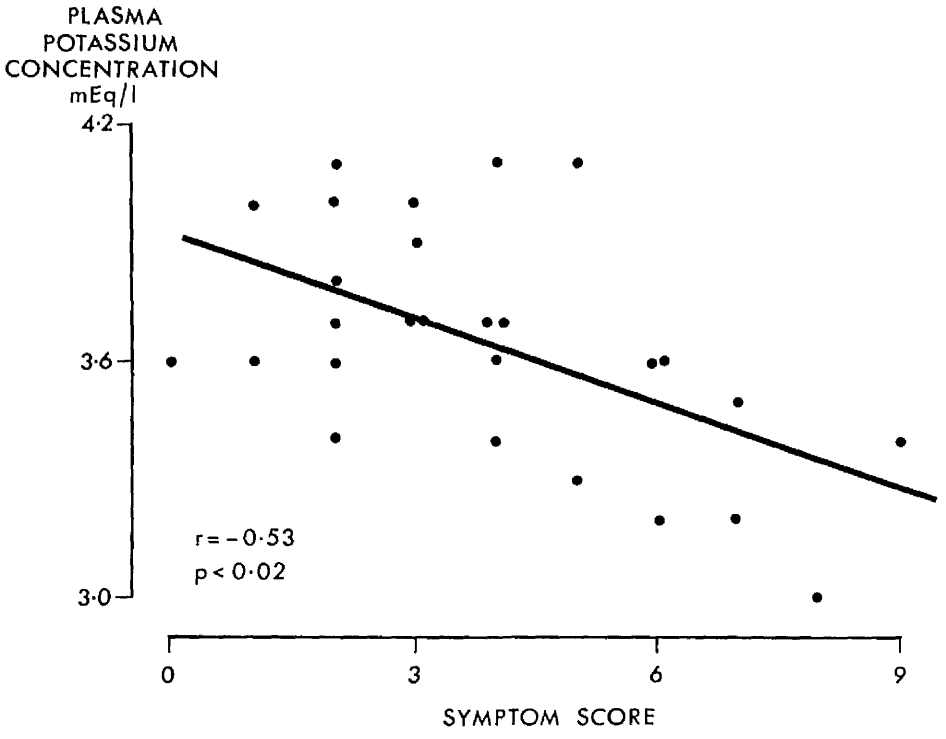


Figure 48. Relationship between plasma potassium concentration and symptoms in early pregnancy.

Discussion.

Bearing in mind the possible inaccuracies in the method of scoring, none of the components of the renin-angiotensin-aldosterone system correlated significantly with the number or severity of symptoms. In particular, plasma angiotensin II concentration did not appear to be related to the presence or degree of thirst.

The fact that plasma sodium concentration showed no correlation with symptoms does not necessarily imply that sodium depletion was not present. It is, however, in keeping with the results described in Chapter 5.D (Figures 42 and 44) in which there was no evidence of a negative sodium balance in early gestation.

None of the women studied complained of vomiting or diarrhoea. It is unlikely, therefore, that the hypokalaemia was due to these complications. On the other hand, at least some of the symptoms could be attributed to the lowered plasma potassium concentration.

In pregnancy, a relationship exists between urinary potassium excretion and plasma aldosterone concentration (Figure 38). Although no direct correlation has been shown between plasma levels of aldosterone and potassium (Table 14) or between plasma aldosterone and symptoms, it seems possible that some of the symptoms of pregnancy may be due to hypokalaemia induced by the increased circulating levels of aldosterone.

SECTION III

HYPERTENSIVE DISEASE OF PREGNANCY

CHAPTER 8

LITERATURE REVIEW.

THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM IN HYPERTENSIVE DISEASE OF PREGNANCY (PRE-ECLAMPSIA)

Definitions of what is called variously "pre-eclampsia" "toxaemia of pregnancy", "specific hypertensive disease of pregnancy", "pregnancy hypertension", differ widely from one centre to another. Where different components of the renin-angiotensin-aldosterone system have been estimated, adequate control subjects have been studied in parallel in only a few of the reported series. The methods for measuring these various components have also differed and satisfactory comparisons are therefore sometimes difficult to make (see Chapter 3).

PLASMA RENIN ACTIVITY.

This has been variously reported to be increased, unchanged or decreased in women with hypertensive disease of pregnancy. In 1944 Dexter and Haynes reported renin-like material in the blood of 3 women with hypertension of sudden onset in late pregnancy, but found no evidence of this substance in 5 pregnant women with a gradual onset of raised blood pressure. Their results, however, were not compared with normal pregnancy where an increase in plasma renin activity and concentration frequently occurs (see Chapter 4.A; 5.A and B; Figures 8, 10, and 12).

Pressor material in blood from patients with pre-eclampsia was also claimed by Hunter and Howard (1961) and Tatum and Mule' (1962) but in neither study was this definitely related to renin or angiotensin activity. Maebashi and his colleagues (1964) found increased plasma renin activity in pre-eclampsia as compared with normal pregnancy, but this finding is difficult to evaluate as the same workers found no difference in renin activity between pregnant and non-pregnent normal women (see Chapter 4.A). In a prospective study, Gordon and co-workers (1969) claimed that mean plasma renin activity between the 13th and 27th week of gestation was higher in 10 women who later developed toxaemia of pregnancy than in 48 women where pregnancy was uneventful. This difference, however, was not statistically significant.

Winer, in a brief abstract (1965), mentioned 3 patients with toxaemia of pregnancy where plasma renin activity was no higher than in normal pregnancy but no further details were given. Similar levels of renin activity in normotensive and hypertensive pregnant women were also reported in a more recent study in which no correlation was found between blood pressure and plasma renin activity (Schmidt and Rosenthal, 1971).

In contrast to the foregoing reports, Helmer and Judson (1967) reported the mean plasma renin-activity in pregnant women with "elevated blood pressure" to be lower than in normal pregnant women in spite of the use of thiazides in most of the hypertensive cases.

PLASMA RENIN CONCENTRATION.

The difference between the measurement of plasma renin activity and plasma renin concentration has been discussed in Chapter 3. Fewer studied of plasma renin concentration in hypertensive disease of pregnancy have been reported.

In a comparison between 52 women with hypertension during the third trimester of pregnancy and 60 women at a comparable stage of normal pregnancy (Brown, Davies, Doak, Lever, Robertson and Trust, 1965; 1966d), no significant difference in plasma renin concentration was found in those with raised blood pressure alone. However, the plasma renin concentration was significantly lower in the women with very high blood pressure plus proteinuria. This lower plasma renin concentration in the more severe cases of pre-eclampsia was also noted in 9 American negro women when compared with the levels in 7 normal pregnant controls (Bonar, Brown, Davies, Langford, Lever and Robertson, 1966).

In contrast, Brown and his associates (1966d) found that in 7 women in whom hypertension was a complication of rhesus iso-immunisation or hydatidiform mole the mean plasma renin concentration was considerably higher than the value for normal pregnancy.

PLASMA RENIN-SUBSTRATE CONCENTRATION.

As far as is known, only one adequate study has been reported

to date in which plasma renin-substrate concentration has been measured in pregnant women with raised blood pressure in the last trimester (Helmer and Judson, 1967). In 9 such women the circulating levels of renin-substrate did not differ from those found in normal pregnancy, the means being 1872 ng/ml, and 1736 ng/ml respectively. This was in keeping with an earlier finding in one case of hypertension in pregnancy of a plasma renin-substrate level within the normal pregnant range (Gould et al, 1966).

BLOOD ANGIOTENSIN CONCENTRATION.

The paper by Massani and her colleagues (1967) appears to be the only report of blood angiotensin concentration in hypertensive pregnant women. In 5 patients with raised blood pressure in late pregnancy they found a mean angiotensin concentration of 230 ng/l (S.D. $\pm$ 29), which was not significantly different from the mean of 9 normotensive pregnant controls (200 ng/l, S.D. $\pm$ 22).

Blood angiotensinases.

Reports of 'angiotensinase' activity in peripheral blood in pre-eclampsia have shown conflicting results. Compared with normal pregnancy at the same stage of gestation, angiotensinase activity in pre-eclampsia has been reported as lower (Page, 1947; Talledo, 1968), higher (Landesman et al, 1963; Berger and Langhans, 1967) or variable (Hickler et al, 1963). It is possible that these variations represent technical aberrations as much as genuine in vivo physiological differences.

Vascular response to angiotensin infusion.

It has already been shown that the pressor response to angiotensin infusion is reduced in normal pregnancy (see Chapter 4.A). Read and his associates (1956) found that pre-eclamptic women had greater pressor responses than normotensive pregnant women and this was subsequently confirmed by other workers (Chesley, 1966a; Talledo et al, 1966; Talledo, Chesley and Zuspan, 1968), the response to angiotensin infusion being similar to normal non-pregnant subjects.

ALDOSTERONE EXCRETION AND SECRETION.

In 1946 Venning suggested that an increased excretion of mineralocorticoids occurred in toxaemia of pregnancy. This appeared to be confirmed when the presence of a "sodium-retaining factor" in urine from women with pregnancy toxaemia was reported (Chart, Sibley and Gordon, 1951; Venning et al, 1954). However, although Barnes and Gilligan (1956) demonstrated aldosterone excretion rates in women with pre-eclampsia to be greater than in normal pregnant women, they surprisingly found that the urinary aldosterone levels were higher in the milder pre-eclamptic patients rather than in the severer forms.

In contrast to the earlier reports, Martin and Mills (1956) did not show any increase in aldosterone excretion in pre-eclampsia compared with normotensive pregnancy and several

other workers subsequently found urinary aldosterone values to be lower in the pre-eclamptic patients (Koszorek et al, 1957; Kinsler and Rigby, 1957; Venning et al, 1957; Kumar et al, 1959).

Aldosterone secretion rates were measured in 2 women with toxaemia of pregnancy by van de Weile et al (1960), and, in spite of a low salt diet, both showed lower values (320 and 710 ug/day) than 3 normal pregnant women on unrestricted diets (1040 - 2250 ug/day). Thomas and Flynn (1964) also found lower aldosterone secretion rates in 9 women with pre-eclampsia, although the mean values (mild PET 232 ug/day, severe PET 247 ug/day) were not significantly different from normotensive pregnancy (352 ug/day).

Both of those reports were confirmed by another study (Watanabe, Meeker, Gray, Sims and Solomon, 1963) which showed aldosterone secretion rates in 3 women with severe pre-eclampsia to be 100, 160 and 540 ug/day in contrast to a previously reported normal range for the same period of gestation of 390 to 2640 ug/day (Watanabe et al, 1963). Six women with mild pre-eclampsia had values within the normotensive pregnant range, while two had secretion rates of 330 and 380 ug/day. All the women in this study were taking a fixed sodium intake of 2G/day.

In general, then, aldosterone secretion appears to be lower in women with hypertensive disease of pregnancy compared to normal pregnant women, the earlier reports of higher urinary excretion most likely being due to the inadequate techniques then available.

PLASMA ALDOSTERONE CONCENTRATION.

Apart from one very high level in eclampsia mentioned in a brief paper by Stark (1967), as far as is known, there has been no other report to date of changes in plasma aldosterone concentration in women with raised blood pressure in pregnancy.

OTHER MINERALOCORTICOIDS.

In view of the raised plasma concentrations of 17-hydroxy-corticosteroids in normal pregnancy (see Chapter 4.B) a number of workers have studied women with pre-eclampsia to determine if this condition might be associated with even higher levels of these hormones, especially of unbound cortisol. However, apart from one report (Watanabe, 1961) none of those studies has shown greater plasma concentrations of total 17-hydroxycorticosteroids in hypertensive pregnant women (Assali, Garst and Voskian, 1955; Martin and Mills, 1958; Meyer, 1965; Friedman and Beard, 1966; Graves and Agersborg, 1966; Kopelman and Levitz, 1970) and one showed lower concentrations (Thomas and Flynn, 1964), compared with normal pregnancy.

Reduced cortisol binding was claimed by de Moor et al (1966) and this appeared to confirm the report by Meyer (1965) who had found less cortisol binding and greater "free" unbound cortisol in 15 pre-eclamptic women compared with 3 normal pregnant women in the last trimester. Two other studies, however, showed

no difference between normotensive and hypertensive pregnancies (Thomas and Flynn, 1964; Kopelman and Levitz, 1970).

Tobian (1949) found greater urinary levels of formaldehydogenic corticosteroids (these include metabolites of deoxycorticosterone) in women with marked oedema and with hypertension in pregnancy than in normal pregnant subjects. Venning, Singer and Simpson (1954) confirmed this in milder forms of pregnancy toxaemia but in severe pre-eclampsia they found lower values. Armstrong (1958) claimed no difference in deoxycorticosterone excretion between normotensive and hypertensive pregnant women, but examination of his data shows lower urinary levels of this hormone in the hypertensive group.

As far as is known, no measurements of plasma deoxycorticosterone have been made in women with hypertensive disease of pregnancy.

COMMENT.

Apart from plasma renin-activity, which has produced conflicting results, few measurements of the plasma concentrations of renin, renin substrate and angiotensin II have been reported in women with hypertensive disease of pregnancy. In particular, there is a dearth of information about plasma aldosterone concentration in this condition. Also, where these different components have been estimated in hypertensive pregnant women, comparison with a control group of normotensive pregnant women has not always been satisfactory.

Therefore, having established, at least in part, the physiological changes which take place in the renin-angiotensin-aldosterone system during normal pregnancy (see Chapters 5 and 6), the next step was to examine the possible pathological role of the system in hypertensive disease of pregnancy.

CHAPTER 2

A STUDY OF PLASMA RENIN, RENIN-SUBSTRATE, ANGIOTENSIN II AND ALDOSTERONE CONCENTRATIONS, AND POSSIBLE RELATED FACTORS, IN HYPERTENSIVE DISEASE OF PREGNANCY.

CLINICAL METHODS.

For the purposes of this study, hypertensive disease of pregnancy was defined as a rise of blood pressure to 140/90 or over on at least 2 separate occasions after the 24th week of gestation in a woman whose blood pressure had been consistently less than this before the 24th week of pregnancy. These blood pressure measurements were taken after a 30 minute period of recumbency.

A measure of the severity of hypertensive disease of pregnancy was the development of significant albuminuria in the last trimester in the absence of urinary tract infection or previous history of renal tract disease. Significant albuminuria was defined as a concentration of albumin in the urine of at least 1.0g/litre when measured by Esbach's albuminometer or of at least 0.3g/litre when measured by a turbidometric method.

Parity and the presence or absence of oedema were not included in the definition.

A total of 21 women met the requirements of this definition of hypertensive disease of pregnancy with albuminuria. Obstetric

and clinical details were as follows:-

Case	Age	Parity	Gest^{n}.	B.P.	Oedema	Albumin g/l
J.B.	35	3^{+0}	38	200/110	0	0.3
M.C.	28	0^{+2}	35	184/116	0	1.5
M.C.	23	0^{+0}	31	164/120	++	8.0
C.C.	20	0^{+0}	36	150/100	+	0.3
L.G.	17	0^{+0}	36	170/110	+	8.0
S.K	38	3^{+0}	28	180/120	+	6.0
C.L.	19	0^{+0}	37	170/100	++	4.0
S.R.	20	1^{+0}	32	150/106	+	6.0
H.S.	28	4^{+0}	28	170/94	++	1.2
A.W.	35	0^{+3}	28	154/110	+	2.0
A.H.	36	1^{+0}	30	160/110	0	1.0
J.B.	29	0^{+3}	34	190/120	+	0.4
S.B.	26	0^{+0}	37	140/95	+	1.0
J.C.	18	0^{+0}	36	140/95	+	0.5
H.H.	20	0^{+0}	38	180/110	++	0.35
M.J.	25	1^{+0}	35	170/110	+	1.7
E.K.	26	0^{+0}	39	140/100	0	1.8
A.McI.	27	2^{+0}	38	130/90	++	0.35
M.N.	37	0^{+0}	33	170/130	++	0.9
S.R.	25	2^{+0}	32	150/110	0	0.38
H.F.	26	0^{+0}	38	160/90	+	0.33

All were in-patients at the time of blood sampling, being treated by bed-rest with or without amytal sedation. None were

receiving hypotensive drugs or diuretics. All were taking an unrestricted diet. Blood samples were taken between 8.30 and 9.30 a.m. after at least 30 minutes recumbency.

From the women with normal blood pressure in pregnancy who have been described in Chapter 5.B, cases were taken retrospectively to match as near as possible the women in the hypertensive group. As it was not technically possible to make all the estimations under study in each woman, numbers were found to be too small for a comparison of 2 complete groups where all the estimations had been performed in every case. Therefore, a matched control group was selected for each substance investigated, the main aim being to have as close matching as possible for age, parity and time of gestation. Closeness of matching was tested by the paired t-test and in no instance was the difference between the 2 groups of a statistical significance less than the 5% level. To prevent bias, the results of the estimations under study were not known at the time of matching.

Comparison between the 2 groups for each substance measured was by the paired t-test and the Wilcoxon test for pair differences. An attempt was also made to see whether the correlations found in normal pregnancy were also present in hypertensive disease of pregnancy and whether any other significant correlations were present in this condition.

LABORATORY AND STATISTICAL METHODS.

These have been described in Chapter 3.

RESULTS.

These are illustrated and described for each substance or group of substances separately, and are followed by an analysis of the statistical correlations between substances which have been measured concurrently.

Plasma renin concentration.

The individual results for each matched pair of women are shown in Table 18, with data of age, parity and gestation. Means and standard deviations of both groups are illustrated in Figure 49.

Statistical comparison of the results from the 20 matched pairs was as follows:-

Comparison	Paired t-test	Significance p
Age	0.080	> 0.1
Gestation	1.997	> 0.05
Renin	-3.053	< 0.01

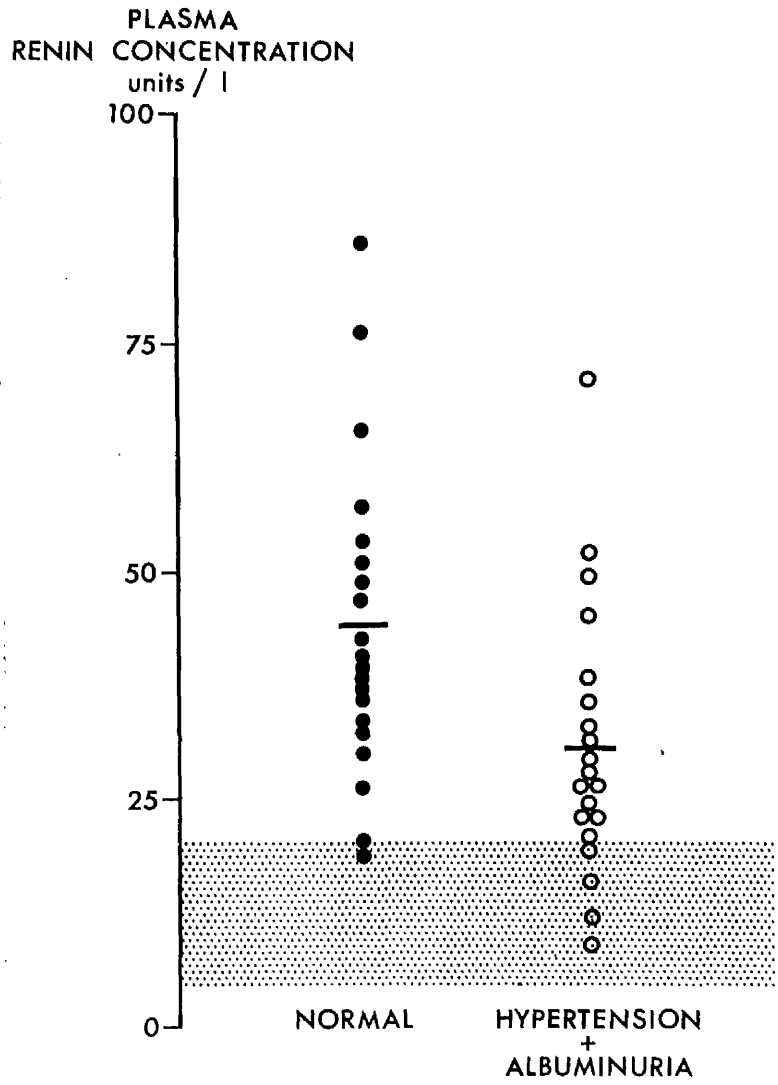


Figure 49.

Plasma renin concentration in hypertensive disease of pregnancy.

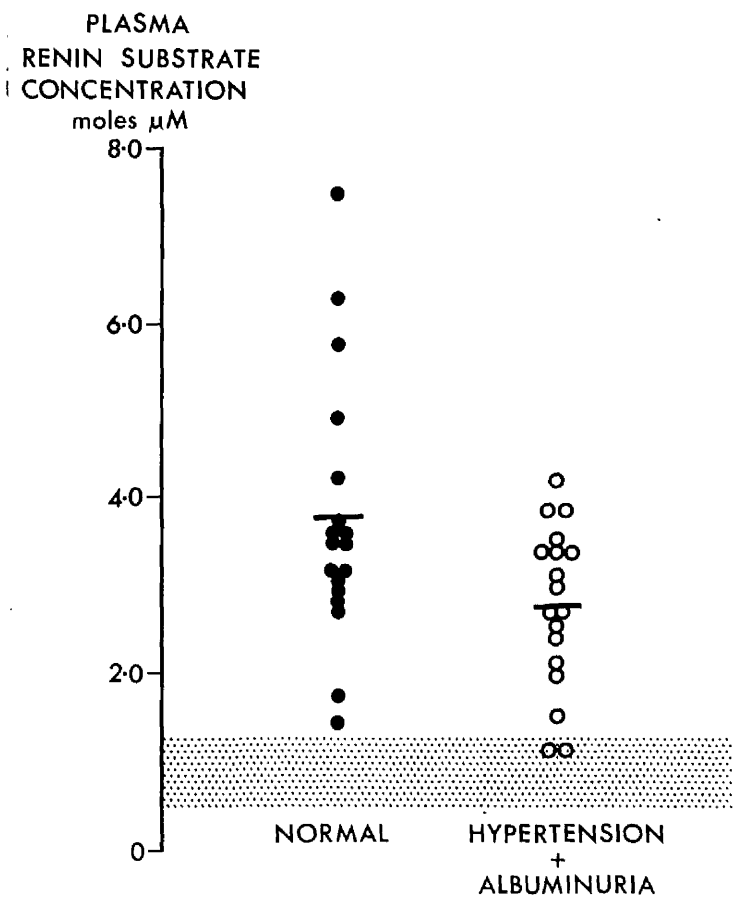


Figure 50.

Plasma renin-substrate concentration in hypertensive disease of pregnancy.

Plasma renin concentration was significantly lower in the women with hypertensive disease of pregnancy than in the matched control group of normotensive pregnant women, both by the paired t-test (above) and the Wilcoxon test for pair differences ($p < 0.01$). However, all but 4 of the results were above the normal non-pregnant range.

Plasma renin-substrate concentration.

The individual results for each matched pair of women are shown in Table 19, with data of age, parity and gestation. Means and standard deviations of both groups are illustrated in Figure 50.

Statistical comparison of the results from 18 matched pairs was as follows:-

Comparison	Paired t-test	Significance p
Age	1.257	> 0.1
Gestation	0.145	> 0.1
Renin-Substrate	-2.556	< 0.02

A significantly lower plasma renin substrate concentration was found in the hypertensive compared to the normotensive pregnant women, both by the paired t-test above and by the Wilcoxon test for pair differences ($p < 0.01$). In all except 2 women, however, the plasma substrate levels were above those for normal non-pregnant women.

Plasma angiotensin II concentration.

It was possible to satisfactorily match only 8 pairs of women in whom plasma angiotensin II concentration was measured. The individual results for each matched pair are shown in Table 20, with data of age, parity and gestation. Means and standard deviations of the two groups are illustrated in Figure 51.

Statistical comparison of the results from the 8 matched pairs was as follows:-

Comparison	t-statistic	Significance p
Age	1.698	> 0.1
Gestation	0.379	> 0.1
Angiotensin II	-2.598	< 0.05

In the women with hypertensive disease of pregnancy, plasma angiotensin II concentrations were within the normal non-pregnant range in every case studied, and were significantly lower than the levels found in normal pregnant women, both by the paired t-test (above) and by the Wilcoxon test for pair differences ($p < 0.01$).

Plasma aldosterone concentration.

The individual results for each matched pair are shown in Table 21, with data for age, parity and gestation. Means and standard deviations of both groups are illustrated in Figure 52.

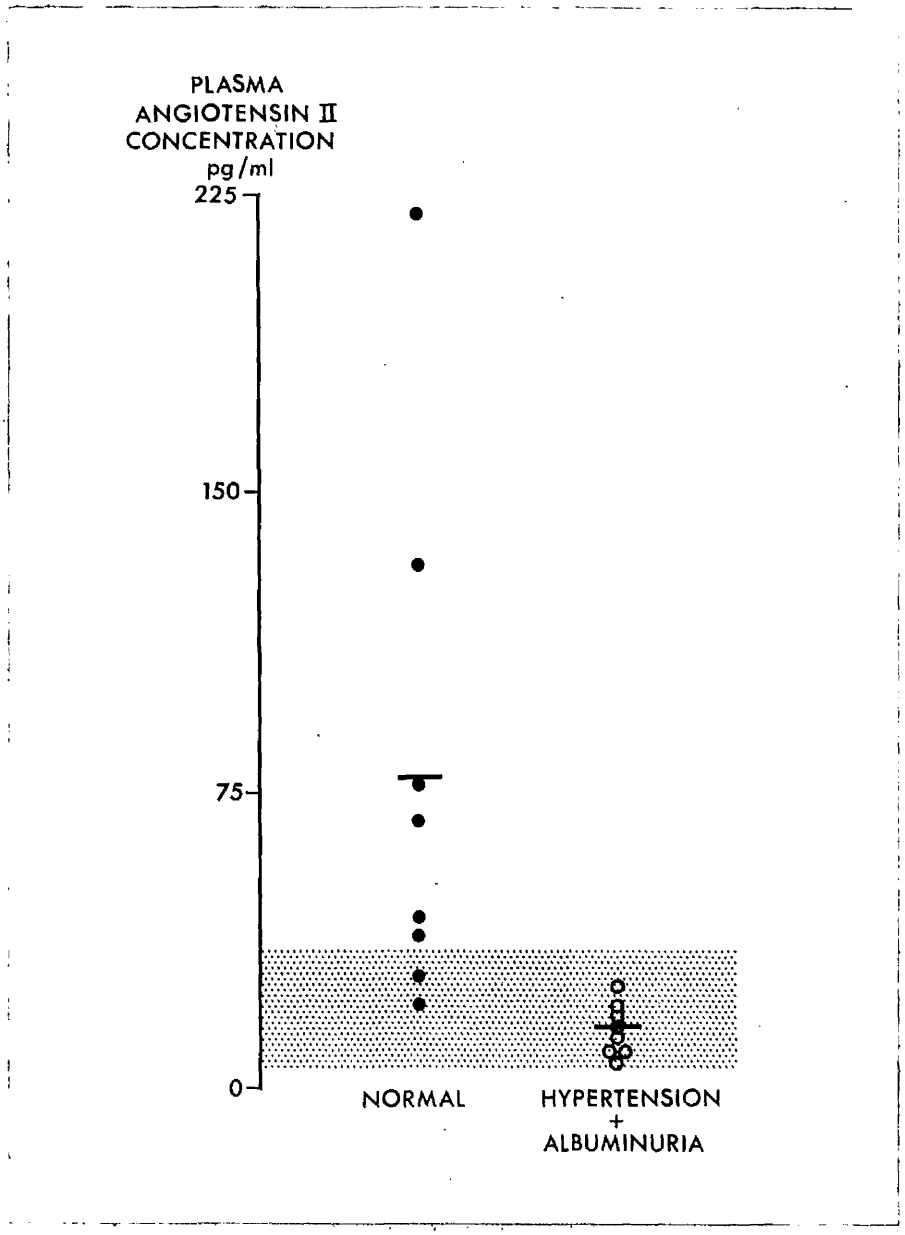


Figure 51. Plasma angiotensin II concentration in hypertensive disease of pregnancy.

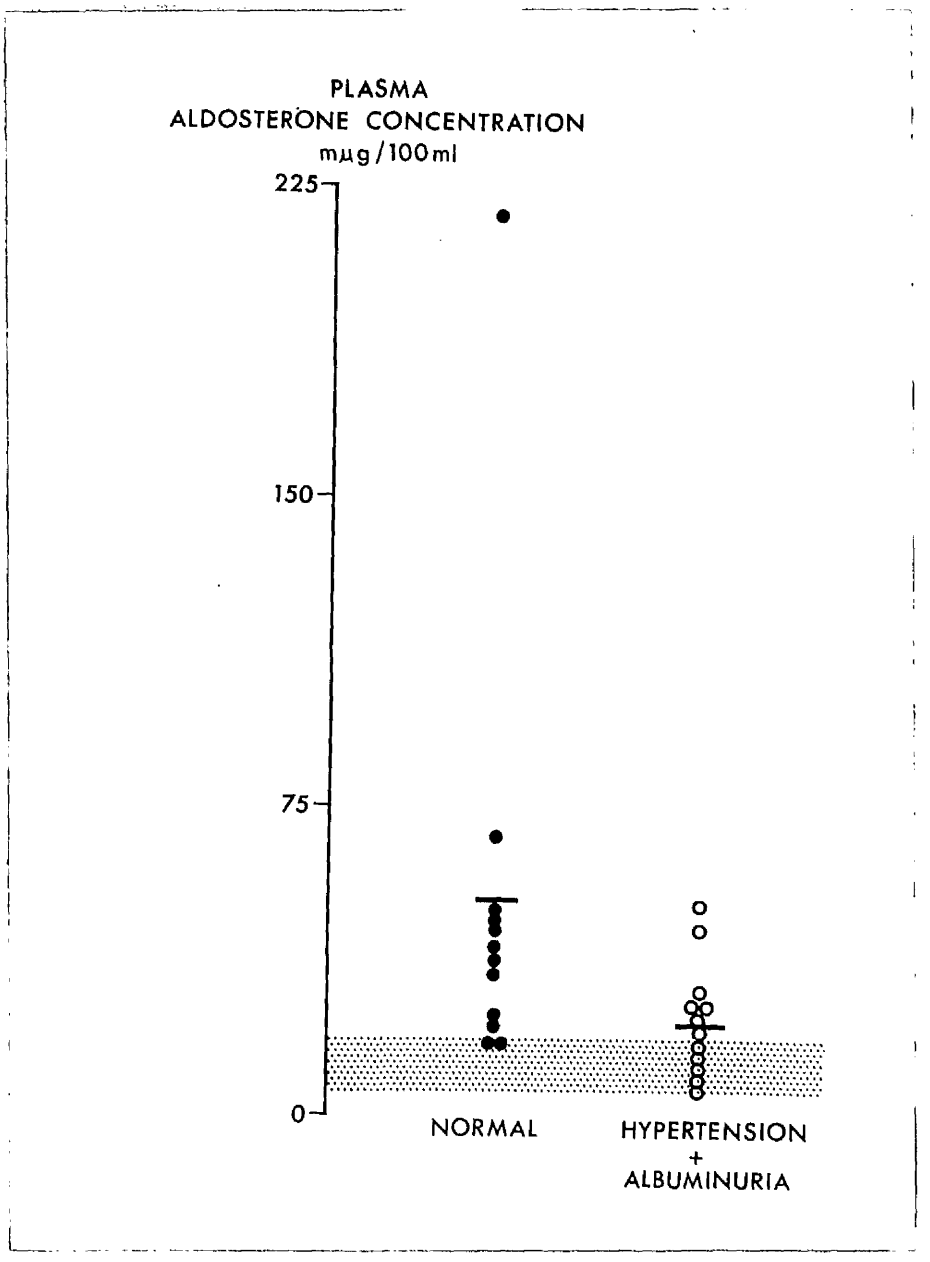


Figure 52. Plasma aldosterone concentration in hypertensive disease of pregnancy.

Statistical comparison of the results from the 12 matched pairs was as follows:-

Comparison	Paired t-test	Significance p
Age	2.103	> 0.05
Gestation	1.897	> 0.05
Aldosterone	-1.667	> 0.1

These women were less satisfactorily matched for age and gestation than for the other estimations, but these were the best matches possible among the cases where plasma aldosterone concentration had been measured. Although the paired t-test was not significant at the 10% level, the Wilcoxon test for pair differences was significant at the 5% level ($p < 0.05$).

Seven of the women with hypertensive disease of pregnancy had plasma concentrations of aldosterone above the normal non-pregnant range, but the mean was considerably lower than that of the normal pregnant women.

Plasma deoxycorticosterone (DOC) concentration.

To date only one estimation of plasma deoxycorticosterone concentration has been made in a woman with hypertensive disease of pregnancy. This result was within the normal pregnant range for the last trimester (16.0 ng/100 ml).

Plasma sodium and potassium concentrations and osmolality.

The individual results for each matched pair of women are shown in Tables 22 to 24, with data of age, parity and gestation. Means and standard deviations of both groups are illustrated in Figure 53.

Statistical comparisons of the results were as follows:-

Plasma sodium concentration (n = 14)

Comparison	Paired t-test	Significance p
Age	1.325	> 0.1
Gestation	0.354	> 0.1
SODIUM	+0.254	> 0.1

Plasma potassium concentration (n = 15)

Comparison	Paired t-test	Significance p
Age	1.417	> 0.1
Gestation	0.163	> 0.1
POTASSIUM	+1.906	$0.05 < p < 0.1$

Plasma osmolality (n = 9)

Comparison	Paired t-test	Significance p
Age	2.135	> 0.05
Gestation	1.000	> 0.1
OSMOLALITY	+2.918	< 0.02

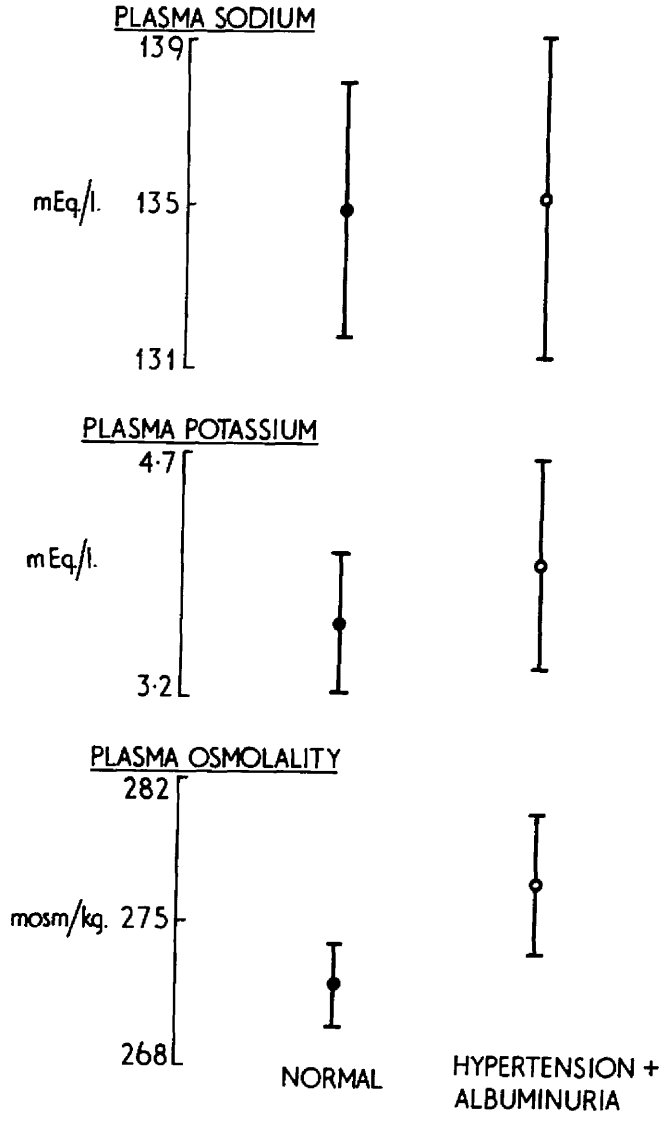


Figure 53 Plasma sodium, potassium and osmolality in hypertensive disease of pregnancy

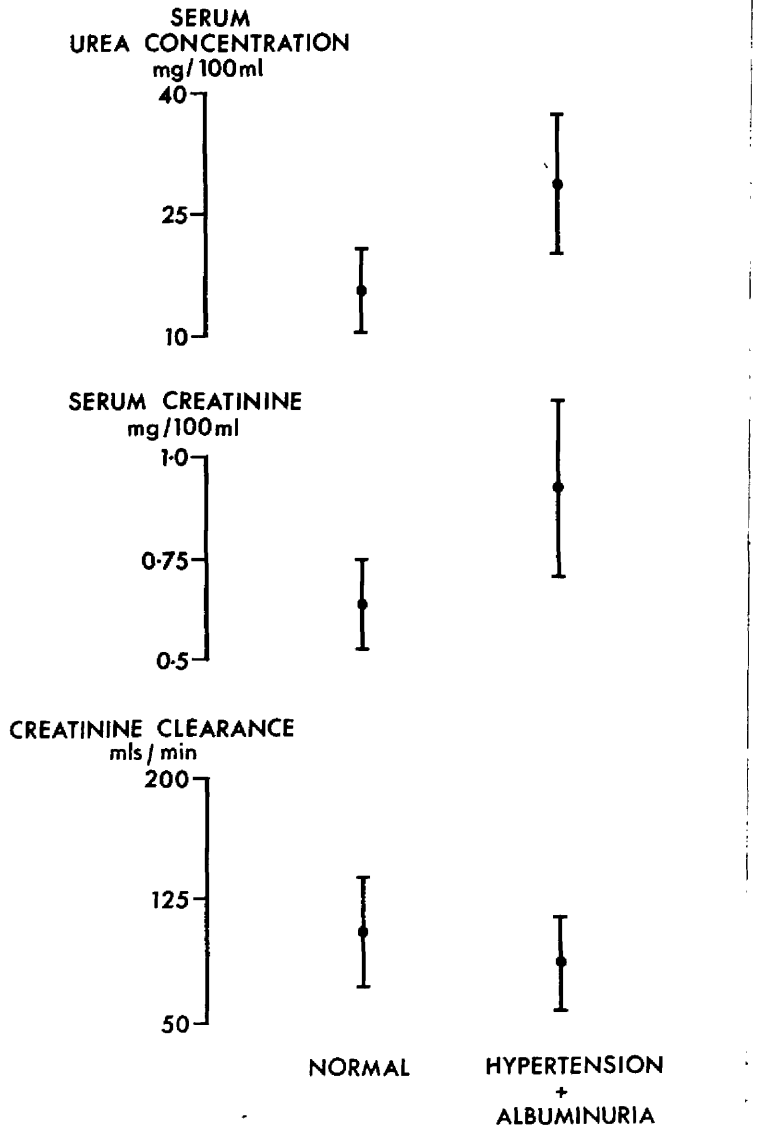


Figure 54 Blood urea, serum creatinine and creatinine clearance in hypertensiv disease of pregnancy.

Plasma sodium concentration was not significantly different in the hypertensive compared to the normotensive group, either by paired t-test (above) or by the Wilcoxon test for pair differences ($p > 0.1$). Most of the results were in the normal pregnant range, that is, lower than values found in normal non-pregnant women.

Nine of the 15 hypertensive women showed higher plasma potassium concentrations than the normotensive women and the mean was higher, but this difference did not reach statistical significance at the 5% level, either by the paired t-test or by the Wilcoxon test for pair differences. Most of the results were in the normal non-pregnant range.

Plasma osmolality, however, did show a statistical difference between the 2 groups by paired t-test (above). This was confirmed by the Wilcoxon test for pair differences ($p < 0.01$). All 9 women with hypertensive disease of pregnancy showed higher plasma osmolality than the normal pregnant women, but their levels were still well within the normal non-pregnant range.

Blood urea and serum creatinine concentrations and creatinine clearance.

The individual results for each matched pair of women are shown in Tables 25 to 27, with date of age, parity and gestation. Means and standard deviations are illustrated in Figure 34.

Statistical comparisons of the results were as follows:-

Blood urea concentration (n = 11)

Comparison	Paired t-test	Significance p
Age	2.018	> 0.05
Gestation	0.000	> 0.1
UREA	+3.697	< 0.001

Serum creatinine concentration (n = 10)

Comparison	Paired t-test	Significance p
Age	1.629	> 0.05
Gestation	0.918	> 0.1
CREATININE	+2.655	< 0.05

Creatinine clearance (n = 8)

Comparison	Paired t-test	Significance p
Age	1.705	> 0.1
Gestation	1.580	> 0.1
CREAT. CLEAR.	-1.416	> 0.1

Both blood urea and serum creatinine concentrations were significantly higher in women with hypertensive disease of pregnancy than in normal pregnant women. This was confirmed by the Wilcoxon test for pair differences ($p < 0.01$ and $p < 0.05$ respectively). All the results, however, were within the normal

non-pregnant range.

Creatinine clearance showed no significant difference by either test, a wide range of values being found, as in the normal pregnant women.

Urine sodium, potassium and oestriol excretion.

The individual results for each matched pair of women are shown in Tables 28 to 30, with data of age, parity and gestation.

Statistical comparisons of the results were as follows:-

Urine sodium and potassium excretion (n = 8)

Comparison	Paired t-test	Significance p
Age	1.705	> 0.1
Gestation	1.580	> 0.1
URINE SODIUM	+0.698	> 0.1
URINE POTASSIUM	+0.345	> 0.1

Urine oestriol excretion (n = 7)

Comparison	Paired t-test	Significance p
Age	1.326	> 0.1
Gestation	1.660	> 0.1
OESTRIOL	+0.009	> 0.1

The urinary excretion of sodium, potassium and oestriol showed no significant difference in hypertensive compared to normotensive pregnant women, whether by the paired t-test or Wilcoxon test for pair differences.

Correlations.

Table 31 illustrates the correlation coefficients between substances measured concurrently in women with hypertensive disease of pregnancy.

Positive correlations, statistically significant at the 5% level, were found between:-

plasma renin substrate and angiotensin II (Figure 55)

and at the 10% level between:-

plasma renin substrate and aldosterone

plasma renin and plasma sodium

A negative correlation statistically significant at the 5% level was found between:-

plasma renin substrate and serum creatinine (Figure 56)

and at the 10% level between:-

plasma aldosterone and creatinine clearance.

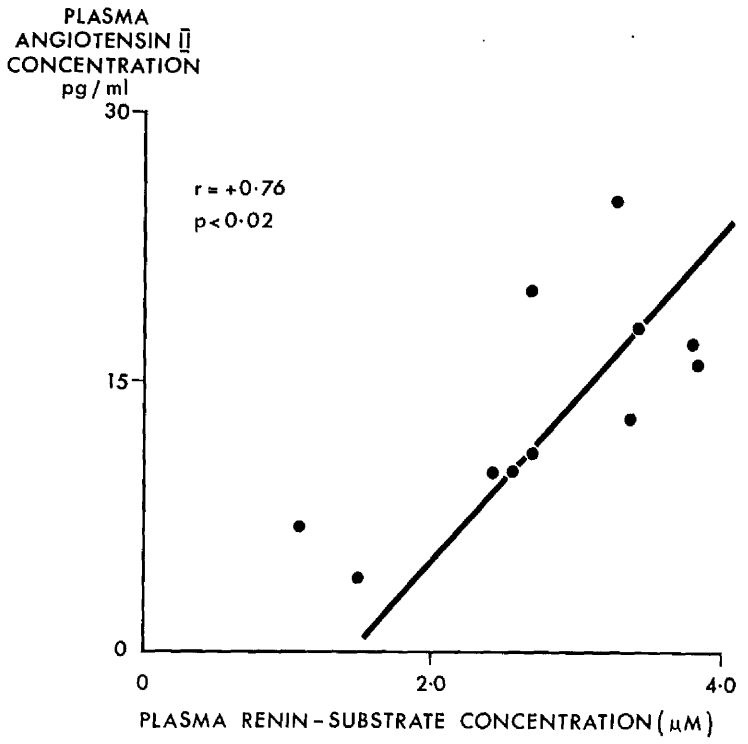


Figure 55

Plasma renin substrate - angiotensin II relationship in hypertensive disease of pregnancy.

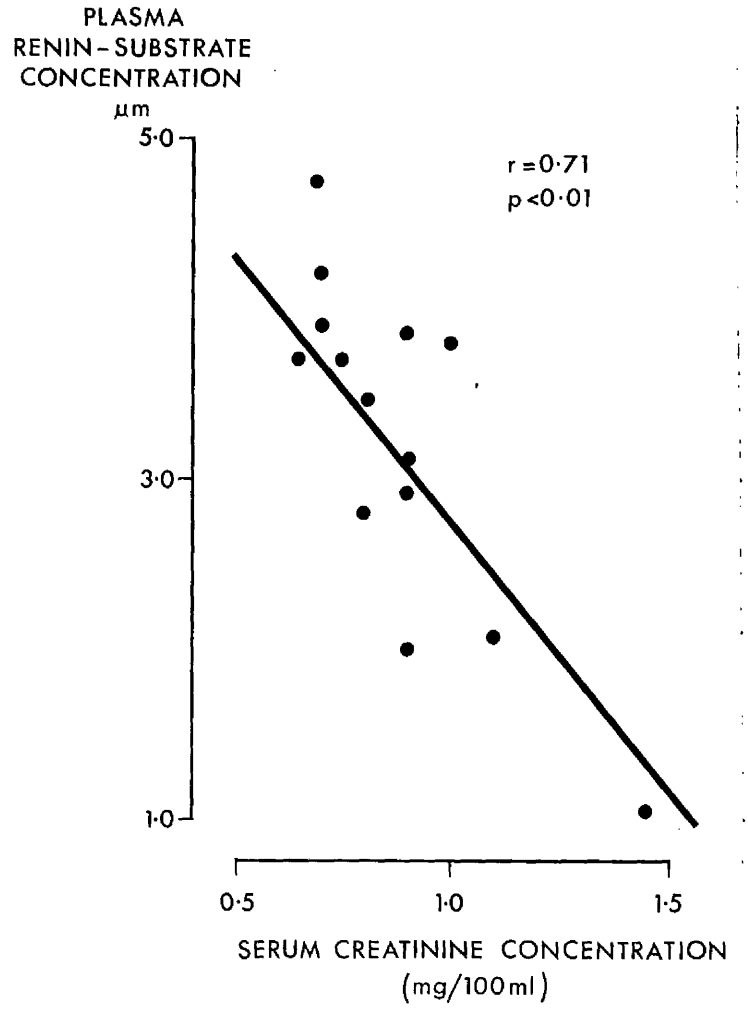


Figure 56

Plasma renin-substrate - serum creatinine relationship in hypertensive disease of pregnancy.

CHAPTER 10

THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM IN HYPERTENSIVE DISEASE OF PREGNANCY.

In this chapter, the changes in each substance described in Chapter 9 will be evaluated with reference to the results previously published for hypertensive disease of pregnancy and will be compared with those found in normal pregnant women. The significance of the differences found between normotensive and hypertensive pregnancy will then be discussed, with a critical assessment of the postulated role of the renin-angiotensin-aldosterone system in the pathogenesis of hypertensive disease of pregnancy.

10.A. CHANGES IN INDIVIDUAL SUBSTANCES IN HYPERTENSIVE DISEASE OF PREGNANCY.

Plasma renin concentration.

The significant reduction in plasma renin concentration in women with late pregnancy hypertension and albuminuria compared to normal pregnant women (Figure 49) is in agreement with the results previously reported using a similar method (see Chapter 8). There was, however, a considerable overlap between the two groups. The range in the 20 normal pregnant women appeared to be representative of that found in 37 normal women analysed in the last trimester in the larger study of normal pregnancy (Figure 10), the means being 35.4 units/l (S.D. $\pm$ 12.0) and 43.8 units/l (S.D. $\pm$ 17.3) respectively.

Brown and his colleagues (1966d) demonstrated very high plasma concentrations of renin in cases of rhesus isoimmunisation of hydatidiform mole complicated by hypertension. No such case became available for analysis during the time of the present study. However, in one case of hydatidiform mole and one of choriocarcinoma, both women being normotensive, plasma renin concentrations were 21.1 units/l and 25.4 units/l respectively. Also, 12 normotensive women with rhesus isoimmunisation had circulating renin levels in the last trimester ranging from 15.6 to 52.9 units/l (mean = 38.1, S.D. $\pm$ 11.1). These results are very similar to those found in normal pregnancy (Figure 10).

Plasma renin-substrate concentration.

The plasma concentrations of renin-substrate in women with hypertension in pregnancy have been shown in previous reports to be similar to those of normal pregnant women (see Chapter 8). The presence or absence of proteinuria was not mentioned in those cases, but it is interesting that, of 2 cases with eclampsia, one had plasma renin substrate levels much lower than the normal pregnant range (Helmer and Judson, 1967). In the present study, significantly lower plasma renin substrate concentrations were found in the hypertensive compared to the normotensive pregnant women (Figure 30). The distinction between these results and those previously reported may be due to the fact that albuminuria was present in all the hypertensive women studied here. As with plasma renin concentration, there was a wide overlap with the normal pregnant subjects, who had values similar to those in the

larger normal third trimester group described earlier (Figure 14), the means of those 2 normal groups being 3.73 uM (S.D. ± 1.52) and 3.98 uM (S.D. ± 1.07), respectively.

Plasma angiotensin II concentration.

All 5 hypertensive pregnant women reported by Massani and her colleagues (1967) had blood angiotensin levels within the range found in 9 normal pregnant women at the same stage of gestation. Two of the hypertensive cases had proteinuria greater than a trace. As mentioned in Chapter 4.A, those results may have been influenced by technical factors in the method employed.

In the present study of 8 cases of hypertension with albuminuria in late pregnancy, a wide disparity was present when the plasma angiotensin II levels were compared to those found in 8 normal pregnant women in the last trimester. In every hypertensive woman except one, circulating levels of angiotensin II were lower than in the normal women, and all were within normal non-pregnant limits (Figure 51). The 8 normal pregnant controls had values within the range found for 33 normal women in the last trimester (Figure 18) the means being 78.2 pg/ml (S.D. ± 67.4) and 88.2 pg/ml (S.D. ± 87.1) respectively.

Plasma aldosterone concentration.

Compared to the other components of the renin-angiotensin-aldosterone system which have been measured, plasma aldosterone concentration has shown a less marked distinction between hypertensive and normotensive pregnant women. Although the

mean for the hypertensive group was considerably lower than for the normal pregnant group, the latter could be influenced by one very high result (Figure 52), and this result apart, many of the values overlapped one another. However, this one high reading in the normal group could also be affecting the t-test, and when the matched pair including this result was withdrawn from the statistical analysis, the paired t-test became significant at the 5% level (t = 2.351). That plasma aldosterone concentration is lower in the hypertensive women is also suggested by the significant difference obtained for all the pairs by the Wilcoxon test for pair differences. The mean for the normal group was very similar to that for the rather larger number of normal women in the third trimester reported earlier (Figure 22), but a very wide scatter of results was present in both (means 50.9 mug/100 ml, S.D. $\pm$ 54.7 and 51.7 mug/100 ml, S.D. $\pm$ 40.5 respectively).

Apart from one very high value in eclampsia mentioned in a brief paper by Stark (1967), as far as is known, there has been no other previous report of plasma aldosterone concentration in hypertensive disease of pregnancy. However, the lower circulating levels found in the hypertensive women in the present study are in agreement with the secretion rate studies previously described (see Chapter 8).

Plasma 11-deoxycorticosterone (DOC) concentration.

Only one women with hypertensive disease of pregnancy has so far been studied in our laboratory, and as far as is known, no other reports of plasma 11-deoxycorticosterone concentration

have been published. This one case had levels within the normal pregnant range for the last trimester.

Plasma sodium and potassium concentration and osmolality.

Three previous reports (Dieckmann and Pottinger, 1956; Johnson, McGaughey and Thornton, 1961; Lister, 1962) demonstrated no difference in serum or plasma sodium concentrations between women with normal and women with raised blood pressure in the last trimester. The results of the present study have confirmed this (Figure 53), the plasma sodium levels in both groups being lower than the normal non-pregnant range. MacGillivray (1967) reported a mean serum sodium of 136.5 mEq/l in pre-eclamptic patients but did not give figures for normal pregnancy.

Plasma potassium concentration was generally higher in the hypertensive compared to the normotensive women, and most of the results were in the normal non-pregnant range (Figure 53). Although this difference was only significant at the 10% level, the higher plasma potassium concentrations are in agreement with the findings of Lister (1962) who had carried out metabolic studies in a small number of women.

As far as is known, measurements of plasma osmolality have not previously been reported in hypertensive disease of pregnancy. The results of the present study have shown a significantly higher mean plasma osmolality in this condition when compared to normal pregnant women (Figure 53). However, the values in both groups were within the normal non-pregnant range.

Blood urea and serum creatinine concentration and creatinine clearance.

As discussed in Chapter 6.A, the increase in clearance by the kidney during normal pregnancy is associated with reduced circulating levels of urea and creatinine (Figure 28). Glomerular filtration rate is increased to a lesser extent in women with hypertension in pregnancy (Chesley and Duffus, 1971) and the correspondingly smaller increase in urea and creatinine clearance would be expected to result in higher blood levels of these substances than in normal pregnancy. The higher blood levels have been confirmed by the results of the present study (Figure 54), but the difference in creatinine clearance between normotensive and hypertensive women was not significant, this being in accord with Klopper's failure to find a difference in creatinine excretion (1964). All the cases of hypertensive disease of pregnancy had blood urea and serum creatinine concentrations within the normal non-pregnant range.

Urinary sodium and potassium excretion.

In the present study, no difference in sodium or potassium excretion was found in the hypertensive compared to the normotensive pregnant women (Tables 28 and 29). As the electrolytes were measured in urine collected for 24 hours, a distinction between the 2 groups may have been affected by differences in posture, the hypertensive women being subjected to more bed rest than their more ambulant normal counterparts. Klopper (1964) found that sodium excretion was less in hypertensive than in normotensive pregnant women when in the semi-recumbent position, but obtained no difference when the women were lying on their sides.

The data presented here are inadequate to determine whether sodium retention does or does not occur to a greater extent in hypertensive disease of pregnancy than in normal pregnancy. Studies of total exchangeable sodium are more likely to give an answer, but there is a considerable conflict of evidence from these, Chesley (1966b) in particular favouring increased sodium retention, while MacGillivray (1961 and 1967) considers that the severe pre-eclamptic patient without oedema has a similar amount of sodium as a normal pregnant woman.

Urinary oestriol excretion.

The urinary excretion of oestriol is widely used as an index of adequate foeto-placental function, although its interpretation must be treated with some caution (see Hytten and Leitch, 1971). In the present study, women with hypertension and albuminuria in late pregnancy had urinary oestriol levels similar to normal pregnant women at the same stage of gestation. This is in accord with the results described by Samaan and his colleagues (1969), but at variance with those of another study (Hoyo, Scott, Oakey and Stitch, 1969) which showed a correlation between subnormal levels of urinary oestriol and the degree of albuminuria in pre-eclamptic women.

10.B. RENIN, RENIN-SUBSTRATE, ANGIOTENSIN II AND ALDOSTERONE RELATIONSHIPS IN HYPERTENSIVE DISEASE OF PREGNANCY.

The only positive correlation to emerge which was significant at the 5% level was the relationship between the plasma concentrations of renin-substrate and of angiotensin II. In the study of normal pregnancy, a similar relationship had been observed in the first trimester (Figure 31), but, for unexplained reasons, not subsequently. A less significant correlation at the 10% level, was however present post-partum in those normal cases. As discussed in Chapter 6.B, this association between the circulating levels of renin-substrate and angiotensin II is in agreement with the theoretical aspects of the renin - renin-substrate reaction.

Like normal pregnancy, the plasma levels of renin did not correlate with the circulating levels of angiotensin II. Thus the pattern established in normal pregnant women seems to be seen also in women with hypertension in pregnancy. That is, it seems likely that the plasma concentrations of angiotensin II are being generated by the amount of available substrate rather than by the concentration of the enzyme renin.

The possibility has been discussed earlier (see Chapter 6.B) that the renin being measured in the plasma of normal pregnant women might contain a proportion of physiologically inactive "chorionic renin", and that this might explain the lack of relationship with plasma angiotensin II concentration. This may also apply to women with pregnancy hypertension. As

far as is known, no measurements of renin in chorion or amnion have been reported in cases of hypertensive disease of pregnancy.

Although the correlation between the plasma concentration of renin-substrate and aldosterone was positive only at the 10% significance level in the hypertensive cases, this is in accord with the more significant relationship demonstrated in normal pregnancy (Figure 32). Once again, this gives rise to the puzzle as to why renin substrate is related to both angiotensin II and aldosterone in the plasma, yet these two substances have not shown a correlation. A speculative explanation for this has been given in Chapter 6.B and this could also be applicable to pregnancy hypertension.

The plasma concentrations of renin and aldosterone correlated only in the first trimester of normal pregnancy (Figure 30) but not subsequently. It is not surprising, therefore, that no relationship has been found in women with hypertension in late pregnancy.

In view of the low level of significance, it is tempting to consider the possible association between plasma renin and plasma sodium concentration in hypertensive disease of pregnancy as spurious. It is interesting to note, however, that this was a positive relationship, which is unexpected in view of experience in non-pregnant situations (see Chapter 2). This might be regarded as a further indication, albeit rather tenuous, that the renin-angiotensin-aldosterone system is the prime mover in the control of sodium balance, rather than that sodium depletion is the initial stimulus to aldosterone secretion.

The fact that the circulating levels of the components of the system are all lower than in normal pregnancy, could then indirectly suggest that sodium retention may be less in the hypertensive women than in normal women, thus aggravating even further the existing controversy about sodium balance in pre-eclampsia.

No adequate explanation is available as to why the hypertensive cases did not show the relationships between the plasma concentrations of aldosterone and sodium and between plasma angiotensin II concentration and osmolality found in normal pregnancy (Figures 36, 37 and 35).

The negative correlation between the plasma renin substrate and serum creatinine concentrations raises the possibility that the kidney may be affecting the amount of circulating substrate. Bilateral nephrectomy may lead to an increase of circulating renin-substrate in some situations and a decrease in others (see Brown et al, 1971b). This variable effect of changes in renal function may also be seen in pregnancy. While an increase in creatinine clearance was associated with a lowered serum creatinine and a decrease in plasma renin-substrate concentration in normal pregnant women, a lowered serum creatinine was accompanied by an increase in plasma renin-substrate levels in hypertensive pregnancy. The explanation for these opposite effects is not known.

10.C THE POSSIBLE ROLE OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM IN THE PATHOGENESIS OF HYPERTENSIVE DISEASE OF PREGNANCY.

In view of the abrupt onset of marked hypertension in some women in late pregnancy and the dramatic fall to normal levels after delivery of the placenta, the possible secretion of a potent circulating pressor agent has been postulated on many occasions.

Perhaps the commonest and most longstanding theory to explain the changes in toxaemia of pregnancy has been that of uterine and placental ischaemia (Young, 1914; Baker, 1929; Page, 1939; van Dowdijk, Bastiaanse, 1954). Reduced uterine blood flow has been demonstrated by Assali and Morris (1964) and reduction in both chorio-decidual and myometrial blood flow by Dixon, Browne and Davey (1965). These functional changes are consistent with the pathological changes of infarcts, haematomas, proliferative endarteritis and degeneration which are found more frequently in hypertensive than in normotensive pregnancies (Dixon and Robertson, 1958; Brosens, 1964; Salvatore, 1968).

Several reports have shown that interference with the placental circulation in animals can result in an elevation of blood pressure which does not appear to be dependent upon intact kidneys (Ogden, Hildebrand and Page, 1940; Gyöngyössy and Kelentey, 1958; Kumar, 1962; Berger and Cavanagh, 1963; Modari, 1969). Sophian (1955) and Franklin and Winstone (1955)

have claimed that stretching of the uterine muscle could result in a "utero-renal reflex" with renal cortical ischaemia and subsequent release of renin. Jeffcoate (1966) however, considered this theory to be unlikely and pointed out that simple hydramnios was not usually associated with hypertension.

More acceptable is the theory of pressor release following placental ischaemia. In rabbits a circulating pressor substance has been demonstrated following reduction of the placental circulation (Berger and Boucek, 1964). In women with pre-eclampsia Hunter and Howard (1961) found a pressor material in amniotic fluid while Tatum and Mulé (1962) showed a slight rise in blood pressure on re-infusing after delivery blood drawn from women with hypertension in late pregnancy. Neither of those studies, however, showed adequate comparison with normal pregnancy. Nevertheless it did seem possible that the renin-like substance found in the foeto-placental unit in normal pregnancy (see Chapter 6.D) might be present in greater quantities in the circulation of women with hypertensive disease of pregnancy.

An increase in the circulating levels of angiotensin or of another pressor material is therefore an attractive hypothesis, whether due to placental or renal ischaemia or to other factors. It has been shown conclusively by the present study, however, that plasma angiotensin II concentrations are not increased in hypertensive disease of pregnancy. On the contrary, all the hypertensive cases studied had levels within the normal non-pregnant range, whereas most normal pregnant women had

considerably elevated levels (Figure 51). Plasma concentrations of renin, renin-substrate and aldosterone have also been shown to be significantly lower in hypertensive disease of pregnancy than in normal pregnancy (Figures 49, 50 and 52).

The lower plasma angiotensin II concentration in pregnancy hypertension does not necessarily imply that it is incapable of affecting blood pressure. In states of sodium excess the pressor effect of angiotensin II is enhanced (see Brown et al, 1971a). If, then, hypertensive disease of pregnancy is a condition in which excessive sodium retention occurs, the lower circulating levels of angiotensin II might be having a greater effect on blood pressure than the higher levels found in normal pregnancy. As has been discussed earlier in this chapter, however, the existence of greater sodium retention in hypertensive pregnant women is a matter of controversy. Also, in the present study no direct relationship was found between the changes in plasma angiotensin II concentration and the changes in blood pressure (Table 31).

As pressor amines have likewise been shown not to be increased in hypertensive disease of pregnancy (Pickering, 1968), the long-postulated existence of a circulating humoral pressor agent remains as elusive as ever.

On the basis of the raised pressure, oedema and possible sodium retention in pre-eclampsia, a number of reports have speculated on the role of the mineralocorticoid aldosterone in this condition. Excretion and secretion data, however, have demonstrated lower rates in hypertensive than in normal pregnant women (see Chapter 8). In spite of the secretion rate results,

it seemed possible that pregnancy hypertension might be associated with impaired metabolic clearance of aldosterone with alterations in the circulating levels of the hormone. No abnormality of metabolic clearance has been found in normal pregnant women (Tait et al, 1962) but, as far as is known, clearance studies in hypertensive disease of pregnancy have not been reported. The present study has now shown that the plasma concentrations of aldosterone are not higher in hypertensive than in normotensive pregnant women (Figure 52). In fact, like the other components of the renin-angiotensin system, the levels in the hypertensive patients were lower than in normal pregnancy, although this was less marked in the case of aldosterone.

Sims (1965) was unable to demonstrate any change in adrenaline induced contractility of rabbit aorta when aldosterone in physiological concentration was added to the media. He thus failed to confirm the theory that women with pre-eclampsia had an increased sensitivity to pressor agents due to the action of aldosterone on the arterial wall.

If the circulating levels of aldosterone are not greater in hypertensive disease of pregnancy, could there be an excess of other mineralocorticoids? This had been shown previously not to be the case for plasma cortisol concentration (see Chapter 8) and the present results have shown no evidence of greater plasma concentrations of corticosterone in two women or of 11-deoxycorticosterone in one woman with this condition.

Horrobin and Lloyd (1970) have suggested that progesterone could cause all the facets of pre-eclampsia. In general, however, progesterone secretion has been found to be normal or low

in pregnancy hypertension (Eton and Short, 1960; MacNaughton, 1967; Klopper, 1969). Oestrogen secretion also is usually normal or low in this condition (Roy, Harkness and Kerr, 1963; MacNaughton, 1967; Nachtigall, Bassett, Hogsander and Levitz, 1968; Klopper, 1969).

Decreased production of oestrogen and progesterone may possibly be a reflection of impaired placental function in women with hypertensive disease of pregnancy. The fact that the plasma concentrations of renin, renin-substrate, angiotensin II and aldosterone are also reduced is suggestive that they too may be influenced by placental function. On the other hand, it is also possible that they are being suppressed by an excess of circulating mineralocorticoid which has yet to be identified.

On the basis of this study, the renin-angiotensin-aldosterone system does not appear to be a major factor in the pathogenesis of this still mysterious and sometimes lethal affliction.

SECTION IV

ORAL CONTRACEPTIVES

In normal women combined oestrogen-progestogen oral contraceptives may be associated with alterations in the renin-angiotensin-aldosterone system. Changes in blood pressure also occur and it has been postulated that these may be related to increases in circulating levels of the vaso-pressor angiotensin II and/or the sodium-retaining hormone aldosterone.

In view of the possible similarity to the changes which occur in pregnancy, it was thought worthwhile to examine the changes in plasma concentrations of renin, renin-substrate, angiotensin II and aldosterone in women taking oestrogen-progestogen oral contraceptives, and to assess their relationship to the changes in blood pressure which may also occur.

The results of these studies have been published previously (see Weir et al, 1970b; 1971b; 1971c; 1972).

CHAPTER 11

LITERATURE REVIEW:

RENIN, RENIN-SUBSTRATE, ANGIOTENSIN AND MINERALOCORTICOIDS IN WOMEN TAKING OESTROGEN-PROGESTOGEN ORAL CONTRACEPTIVES.

Plasma renin-activity and renin-concentration.

The difference between the terms renin-activity and renin concentration have been discussed in Chapter 3, where the advantage of measuring the concentrationsof renin in the plasma was emphasised.

Plasma renin-activity is raised in the majority of women taking oestrogen-progestogen oral contraceptives (Newton et al, 1968; Crane and Harris, 1969, Weinberger, Collins, Dowdy, Nokes and Leutscher, 1969; Saruta, Saade and Kaplan, 1970; Crane, Harris and Winsor, 1971; Cain, Walters and Catt, 1971). In contrast, Skinner and his colleagues (1969) found the plasma renin concentration to be reduced in 6 and unchanged in 2 women during the first month of oestrogen-progestogen therapy. This disparity between renin activity and concentration was subsequently confirmed in 2 other studies, no significant change in plasma renin concentration having occurred in 51 women who had taken oestrogen-progestogen oral contraceptives for up to 30 weeks (Saruta et al, 1970), while a mean fall to 52% of the control value occurred during treatment in 15 women studied by Cain and his associates (1971).

The women in those studies remained normotensive throughout, but Saruta and his colleagues reported in the same paper a further

11 cases in whom a rise in blood pressure occurred while taking an oral contraceptive. In those women, plasma renin concentration increased from a mean of 6.8 units/ml to 9.1 units/ml after 14 weeks, the difference between the normotensive and hypertensive group being statistically significant at this time, although it was not significant after 18 to 30 weeks.

Plasma renin-substrate concentration.

A number of reports have shown that plasma renin substrate concentration is consistently raised in women taking oestrogen-progestogen oral contraceptives:-

Authors	Normal range	On Oral Contraceptives	Duration of Oral Contraceptives
Helmer and Judson (1967) (ng/ml)	Mean = 425	742 - 2240 mean 1617 (n = 7)	Not given
Skinner et al (1969) (ug/ml^{-1})	0.9 - 2.0	2.3 - 5.3 (n = 8)	4 days - 3 weeks
Saruta et al (1970) (ng/ml)	Mean = 1048 S.D. ± 263	Mean = 2555 S.D. ± 416	2 weeks
		Mean = 2886 S.D. ± 590 (n = 51)	18 - 30 weeks
Cain et al (1971) (ug/ml^{-1})	1.0 - 3.0 (approximate - read from graph)	2.5 - 11.0 (approximate - read from graph) (n = 13)	8 - 12 weeks

The results quoted by Helmer and Judson are lower than the others for both users and non-users of oral contraceptives, but

the degree of increase is similar in all 4 series. Skinner and his colleagues found the maximum effect to be from 4 days to 2 weeks after starting the oral contraceptives, while normal results were obtained 2 to 4 weeks after stopping treatment. In the larger and longer study by Saruta and his coworkers, 5 of the 51 women had initially high blood pressures which were not affected by the oral contraceptives.

Three other reports (Newton et al, 1968; Weinberger et al, 1969; Crane et al, 1971) also showed marked increases in plasma renin substrate concentration but can be criticised on the grounds that all the measurements were made on hypertensive women with no normotensive controls. However, 11 women who developed a raised blood pressure while taking oral contraceptives (Saruta et al, 1970) showed greater increases in plasma renin substrate concentration (Mean = 2850 ng/ml, S.D. $\pm$ 321) at 2 weeks and 3500 ng/ml, S.D. $\pm$ 616 between 18 and 30 weeks) compared to the 51 normotensive women already described. As with the plasma renin concentration, there was a statistically significant difference in the early weeks after starting oral contraceptives, but not beyond 14 weeks.

Blood angiotensin concentration and angiotensinase activity.

In a study of blood angiotensin II concentrations in normal and hypertensive subjects (Catt, Ziumet, Cain, Cran, Best and Coghlan, 1971) the angiotensin II levels in normotensive women receiving oral contraceptives were found to be elevated to 3 and 4 times the normal value. This was subsequently confirmed in a more detailed study (Cain et al, 1971) in which the plasma angiotensin II

concentrations in 3 women rose from between 3.5 and 4.5 ng/100 ml before, to a mean of 12.5 ng/100 ml (S.D. ± 2.7) 5 days after starting treatment. Ten days after the oral contraceptive was withdrawn, the levels had returned to the normal range. During more prolonged treatment in 10 women, the same authors found a mean concentration of 8.5 ng/100 ml (S.D. ± 2.9) at the end of the first and second cycles compared to a pre-treatment mean of 2.7 ng/100 ml (S.D. ± 1.0), the blood angiotensin II levels falling to normal within one month of stopping the oral contraceptive. No difference was demonstrated between the effects of Ovulen (ethynodiol diacetate 1.0 mg; ethinyloestradiol 0.05 mg) and Lyndiol (lynestrenol 2.5 mg; Mestranol 0.075 mg).

In contrast, a preliminary report from our laboratory showed a variable response in 2 women given oral contraceptives, one showing a rise and one a fall in plasma angiotensin II concentration (Weir et al, 1971b). This apparent discrepancy will be discussed in more detail in Chapter 12.

Increased angiotensinase activity induced by combined oestrogen-progestogen and sequential oral contraceptives has been described (Lubash et al, 1969) but has not yet been confirmed.

Aldosterone excretion and secretion and plasma concentration.

In 1962, Layne and his associates reported an increase in aldosterone excretion in 7 normotensive women treated with Enovid (Norethynodrel 5.0 mg Mestranol 0.025 mg) for 20 days, compared with 9 normal women on no treatment. Aldosterone

secretion was also increased (mean 203 ug/day S.E. ± 31.7) compared with the control group (mean 76 ug/day S.E. ± 9.2). The authors did not comment on diet or posture.

Less consistent results were obtained by Newton et al (1968) who found elevated aldosterone excretion in 4 out of 8 hypertensive patients taking oral contraceptives, the urinary aldosterone returning to normal in 3 of these women after stopping the treatment.

In another study (Crane and Harris, 1969) 2 out of 15 women on an unrestricted diet showed a reduced aldosterone excretion rate but the mean value for the group was increased significantly compared with normal controls. Dietary sodium restriction for 3 days produced a normal increase in urinary aldosterone both during and after the oral contraceptive treatment. The same workers later demonstrated a significant decrease in mean aldosterone excretion several months after stopping oral contraceptives (Crane et al, 1971).

Sixteen women studied by Weinberger et al (1969) showed increased mean levels of urinary aldosterone, although only 5 had higher levels while taking oral contraceptives compared to 3 months after stopping the drug. Those women had a normal response to a low sodium intake and to the administration of a thiazide diuretic while taking the combined preparation.

As far as is known, apart from our own studies (Weir et al, 1970b, 1971b) to be discussed in Chapter 12, no reports of plasma aldosterone concentration in women taking oral contraceptives have

so far been described.

Other corticosteroids.

A consistent increase in plasma cortisol induced by oral contraceptives has been reported by many workers (Layne et al, 1962; Metcalf and Beaven, 1963; Wallach, Garcia, Kistner and Pincus, 1963; Dodek, Segre and Klaiber, 1965; Starup, Sele and Baus, 1966; Nielsen et al, 1969; O'Connell and Welsh, 1969). Most of those authors attributed the raised plasma cortisol levels to increased protein-binding and this has been confirmed by other workers (Layne and Meyer, 1965; de Moor et al, 1966; Keane et al, 1969a). However, Burke (1969) has also reported a small rise in non-protein-bound cortisol during oral contraceptive therapy.

As far as is known, plasma concentrations of other more potent mineralocorticoids such as 11-deoxycorticosterone have not been reported in women taking contraceptive steroids.

CHAPTER 12

A STUDY OF THE EFFECTS OF OESTROGEN-PROGESTOGEN ORAL CONTRACEPTIVES ON PLASMA RENIN, RENIN-SUBSTRATE, ANGIOTENSIN II AND ALDOSTERONE CONCENTRATIONS IN NORMAL WOMEN.

LABORATORY METHODS.

These have been described in Chapter 3.

CLINICAL METHODS.

A group of normal women were studied before and between 2 and 6 weeks after starting oestrogen-progestogen oral contraceptives. Diet was unrestricted and blood samples were taken in the sitting position at morning or evening clinics, at the same time in the menstrual cycle when possible.

The plasma concentrations of renin were measure in 20, renin substrate in 22, angiotensin II in 18 and aldosterone in 12 women. No significant changes in blood pressure, weight or plasma electrolyte concentrations occurred during the short period of the study.

RESULTS.

Plasma renin concentration.

As shown in Figure 57, mean plasma renin concentration fell from 12.7 units/l (S.D. $\pm$ 8.7) to 8.6 units/l (S.D. $\pm$ 6.5). This decrease was not statistically significant (paired $t = -1.552$, $p > 0.1$). The reason for the increased levels in

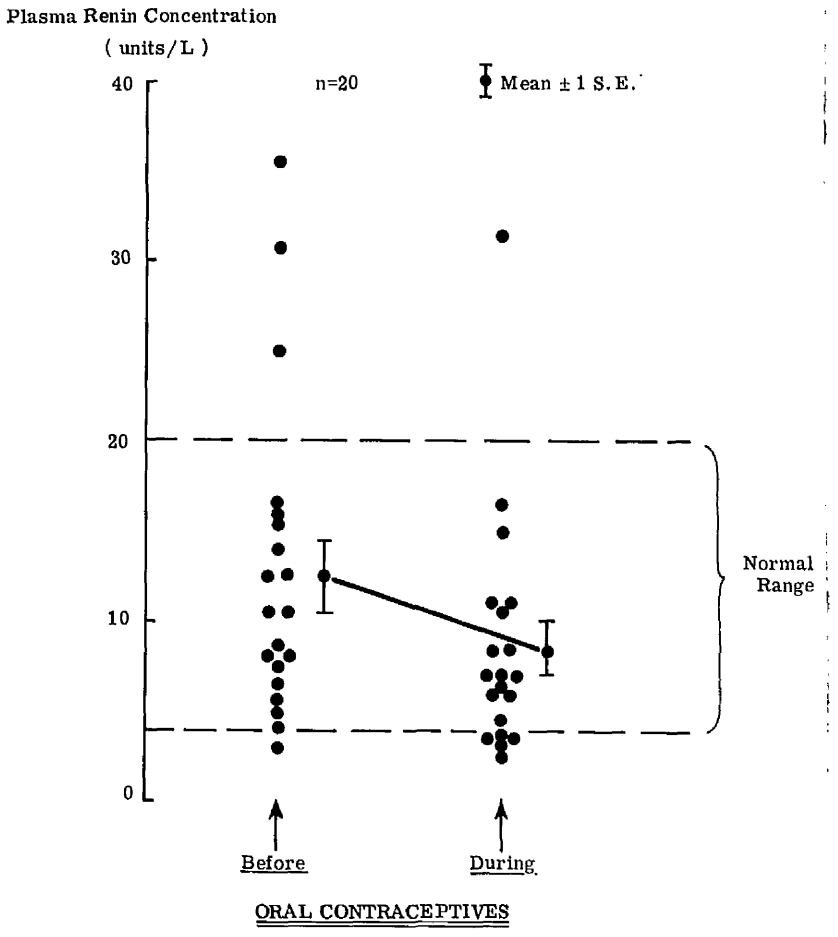


Figure 57

Plasma renin concentration in women taking oestrogen-progestogen oral contraceptives

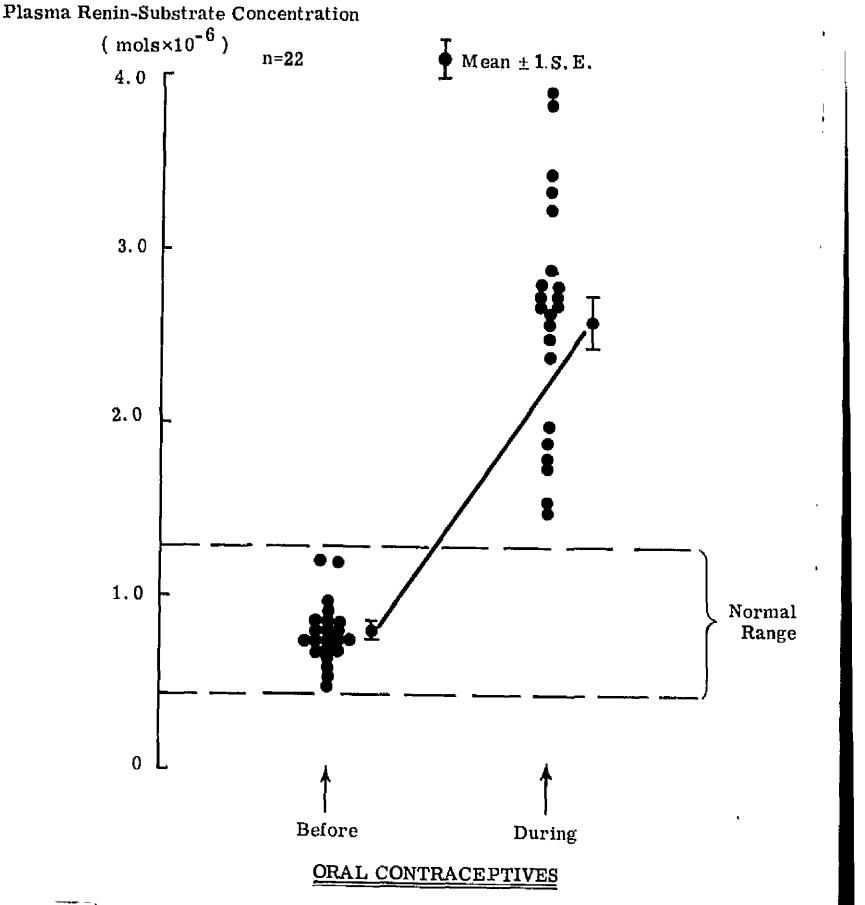


Figure 58

Plasma renin-substrate concentration in women taking oestrogen-progestogen oral contraceptives.

3 women before treatment was unclear. Those women were apparently normal and were sampled in the earlier part of the menstrual cycle when plasma renin concentration is slightly lower than in the luteal phase (Brown, Davies, Lever and Robertson, 1964c; Skinner et al, 1969). Also, the normal range quoted for this method in our laboratory includes values from women at every stage in the menstrual cycle. When the results from those 3 women were excluded from the analysis, the small decrease in plasma renin concentration induced by oral contraceptives became even less significant.

In 5 women in whom plasma renin concentration was measured up to 9 months after starting treatment the mean showed an insignificant change of 0.2 units/l, compared with the value after one month.

Plasma renin-substrate concentration.

This increased to above the normal range in every woman taking oral contraceptives (Figure 58), the mean rising from 0.78 uM (S.D. $\pm$ 0.17) to 2.62 uM (S.D. $\pm$ 0.68). This difference was highly significant (paired $t = 11.686$, $p < 0.001$).

Unlike plasma renin levels, those of plasma renin-substrate continued to increase up to 9 months after starting treatment (Figure 59).

Plasma angiotensin II concentration.

The response of plasma angiotensin II concentration was very variable (Figure 60). The mean level showed a slight increase from 13.1 pg/ml (S.D. $\pm$ 8.8) before to 15.8 pg/ml

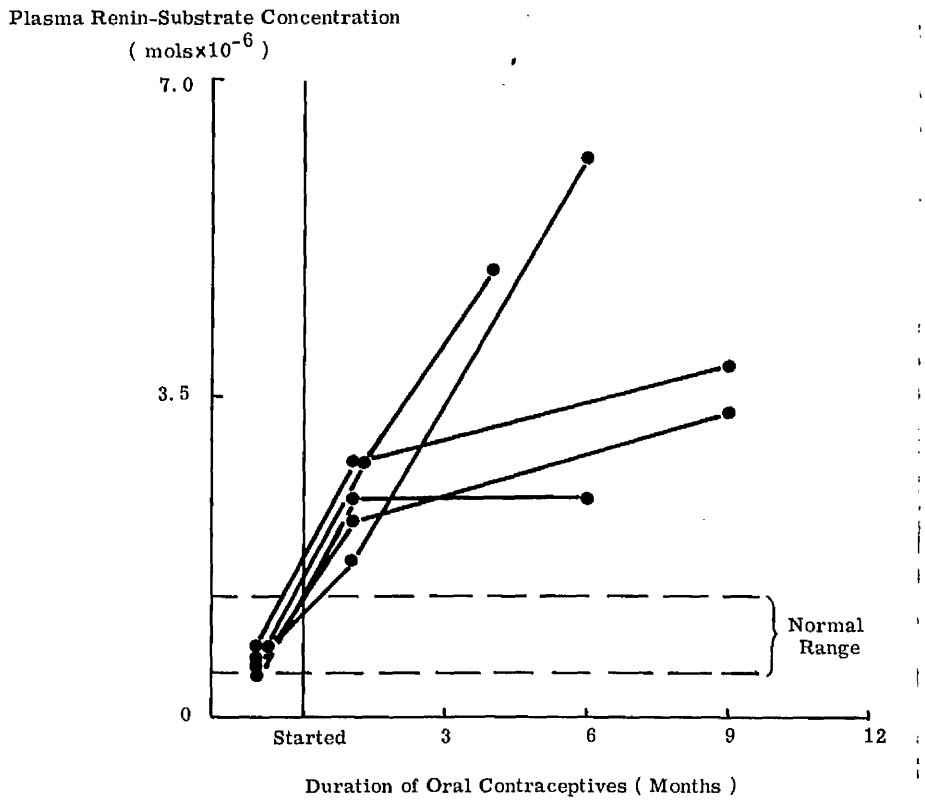


Figure 59 Effect of oestrogen-progestogen oral contraceptives on plasma renin-substrate concentration

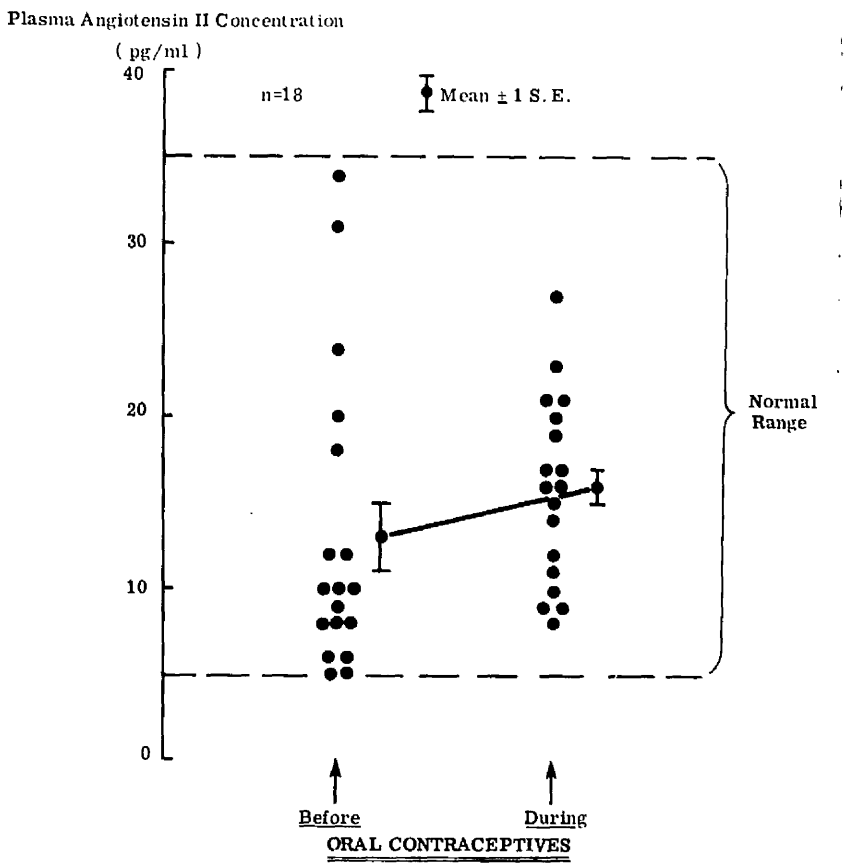


Figure 60

Plasma angiotensin II concentration in women taking oestrogen progestogen oral contraceptives

(S.D. ± 5.4) after starting oral contraceptives. This difference was not statistically significant (paired t = 1.285, p > 0.1). None of the women showed increases above the normal range.

Of two women followed while taking oral contraceptives for up to 6 months, one showed a rise of 8 pg/ml and another a fall of 6 pg/ml in circulating angiotensin II levels.

Plasma aldosterone concentration.

Like angiotensin II, plasma aldosterone concentrations showed no significant change (paired t = 1.004, p > 0.1) after oral contraceptives had been started (Figure 61). The means before and after starting treatment were 7.7 mug/100 ml (S.D. ± 2.8) and 8.6 mug/100 ml (S.D. ± 3.2) respectively. In no case did the plasma levels rise above the normal range.

In 4 women followed for up to 9 months, plasma aldosterone concentration rose to 20 mug/100 ml in one, fell to 4 mug/100 ml in another and remained unchanged in 2 women.

Correlations.

The foregoing estimations were measured concurrently in a number of women taking oral contraceptives. The correlation coefficients for those concurrent measurements are shown in Table 32.

Bearing in mind the small numbers involved, there was no evidence of any statistically significant relationship between the individual components of the renin-angiotensin-aldosterone system in women taking oral contraceptives.

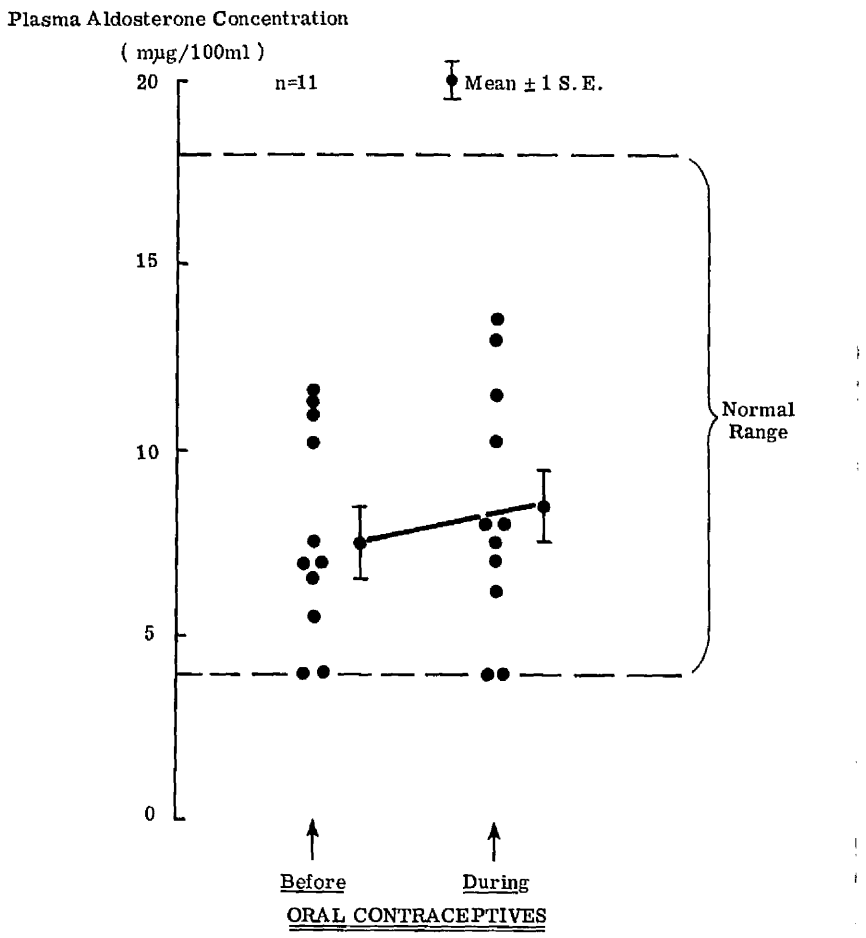


Figure 61. Plasma aldosterone concentration in women taking oestrogen-progestogen oral contraceptives.

DISCUSSION.

The increase in plasma renin-substrate concentration found in this study are similar to those described in other reports (see Chapter II).

The lack of significant change in plasma renin concentration has confirmed the findings of Saruta and his colleagues (1970) but the fall in circulating levels of renin reported by others (Skinner et al, 1969; Cain et al, 1971) was not seen in the present study, in which a similar laboratory method was used. This discrepancy might have been due to sampling at different phases of the menstrual cycle, plasma renin concentration being increased during the luteal phase in normal women (Brown et al, 1964c; Skinner et al, 1969). However, the present study did not show a significant difference in mean levels between the follicular and luteal phases in women either before or after starting oral contraceptives ($t = 0.044$, $p > 0.1$ and $t = 1.019$, $p > 0.1$ respectively.

The results for angiotensin II demonstrated in this study (Figure 60) are at considerable variance with those reported from another laboratory using a broadly similar radio-immunoassay method (Catt et al, 1971; Cain et al, 1971). The reason for this difference is not clear. Although most of the women in the present study were sampled at the same stage of their menstrual cycle before and after starting oral contraceptives, a small number were seen at different stages. In this laboratory, a study of one woman, who was not taking

oral contraceptives, showed a very marked rise in plasma angiotensin II concentration during the luteal phase (Figure 62), and this appeared to confirm the report by Sundsfjord and Aakvaag (1970). However, the present study did not show any significant difference in mean levels between the follicular and luteal phases in women either before or after starting oral contraceptives ($t = 0.480$, $p > 0.1$ and $t = 0.871$, $p > 0.1$ respectively). This latter finding is in accord with the results of Cain and his colleagues (1971), who found a small but insignificant rise in the luteal phase. It seems unlikely, therefore, that variations in time of sampling during the menstrual cycle could influence the present results significantly.

Variations between morning and evening samples are also unlikely to have affected the results, as it has been shown that circulating levels of angiotensin II did not differ between 11.00 a.m. and 4.00 p.m. (Cain et al, 1971). Posture has a significant effect, recumbent women having significantly lower levels than ambulant whether taking oral contraceptives or not (Cain et al, 1971). This postural effect might be expected to give higher results in the present study in which the women were sitting when the samples were taken, in comparison with the 2 hour recumbency used by the other group. The opposite results have been obtained, however, and the discrepancy cannot therefore be explained on this basis.

Most of the studies of aldosterone secretion and excretion have involved women with raised blood pressure while taking oral

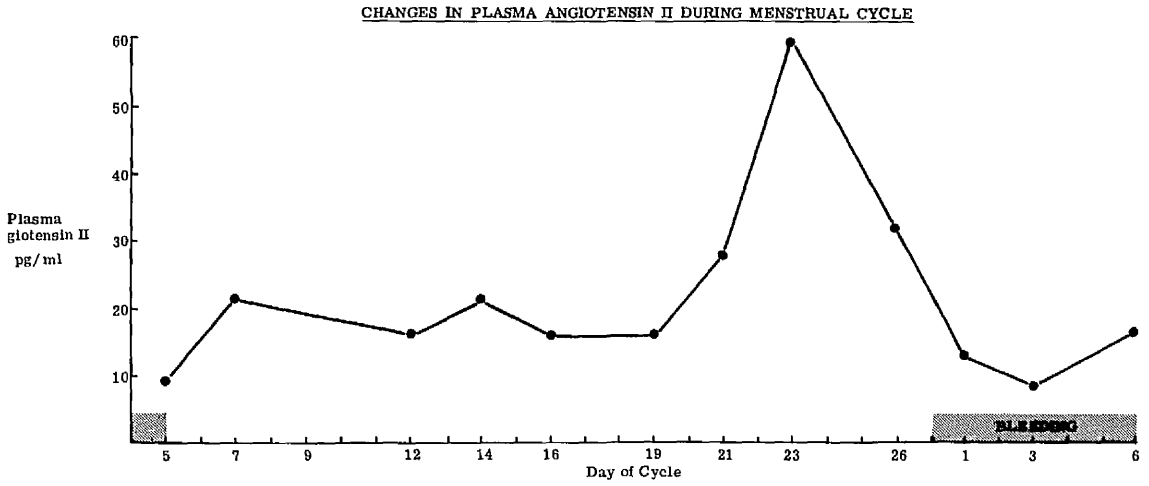


Figure 62. Changes in plasma angiotensin II during the menstrual cycle.

contraceptives. In one study of normotensive women (Layne et al, 1962), aldosterone secretion and excretion were elevated during oral contraceptive administration. The unchanged plasma concentrations found in the present study are at variance with this report. Changes in metabolic clearance may explain this difference, but this has so far not been investigated in women taking oral contraceptives. The increase in aldosterone binding protein induced by oestrogen-progestogen oral contraceptives (Meyer et al, 1961; Layne et al, 1962) would not explain the low plasma levels in the face of apparently increased secretion and excretion.

Renin, renin-substrate, angiotensin and aldosterone relationships in women taking oral contraceptives.

The increases of plasma renin-substrate concentration induced by oral contraceptives were similar to those which occurred in pregnant women (Figures 58 and 14). In pregnancy, a significant relationship has been demonstrated between the plasma concentrations of renin-substrate and angiotensin II in the first trimester (Figure 31) and between renin-substrate and aldosterone in overall pregnancy (Figure 32). Neither of those relationships has been found in women taking oral contraceptives. A significant correlation has also been shown between the plasma concentrations of renin and aldosterone in early pregnancy (Figure 30), but not in women given oral contraceptives.

As mentioned in Chapter 6.B, raised circulating levels of angiotensin II may inhibit renin secretion. This may be one explanation for the fall in plasma renin concentration from the first to the third trimester (Figure 10). It has also been suggested as a reason for the decrease in plasma renin concentration in some women while taking oral contraceptives (Skinner et al, 1969). This postulate, however, is dependent on the confirmation of raised angiotensin II blood levels being found in women given these preparations. As has been discussed in the previous section, the effect of oral contraceptives on plasma angiotensin II concentration is controversial, and the results of the present study do not lend support to this theory.

The reason for the apparent failure of the increased substrate levels to influence angiotensin II generation and possibly aldosterone secretion in this situation must await further investigation.

Cause of rise in plasma renin-substrate concentration.

The main effect of the combined oestrogen-progestogen oral contraceptive on the renin-angiotensin-aldosterone system appears to be the increase in plasma renin-substrate concentration (Figure 58).

Helmer and Griffith (1952) found that rat plasma renin-substrate was increased by oestrogen administration and this has since been confirmed in man (Helmer and Judson, 1967; Newton et al, 1968). Progestogen alone had no effect on rat

plasma renin-substrate (Helmer and Griffith, 1952) but the progestogen norethynodrel produced a small rise in human plasma renin- substrate concentration (Newton et al, 1968).

Recently a small study was carried out in our laboratories to determine the effect on plasma renin, renin-substrate and aldosterone concentrations when the oestrogen and progestogen components of the oral contraceptive Ovulen were administered separately (Weir et al, 1971b). Two groups, each of six healthy women taking a normal diet, were given orally either 50 ug mestranol (oestrogen) or 1 mg ethynodiol diacetate (progestogen) daily for 21 days. Samples were taken in the morning from the antecubital vein while sitting, 1 week before, 3 weeks after starting and 3 weeks after stopping therapy. The plasma was analysed for renin, renin-substrate and aldosterone concentrations. Plasma renin-substrate concentration increased markedly in all 6 women taking mestranol, in contrast to the group taking ethynodiol diacetate in which there was no significant change (Figure 63). The 6 women taking mestranol showed no change in mean plasma renin concentration, but a mean rise did occur in those taking ethynodiol diacetate ($t = 2.86$, $0.02 < p < 0.05$, Figure 63). The significance of this rise is difficult to interpret, as the mean plasma renin concentration before treatment was lower in this group compared to the mestranol group. In no case did the plasma renin concentration exceed the upper limit of the normal range. Plasma aldosterone

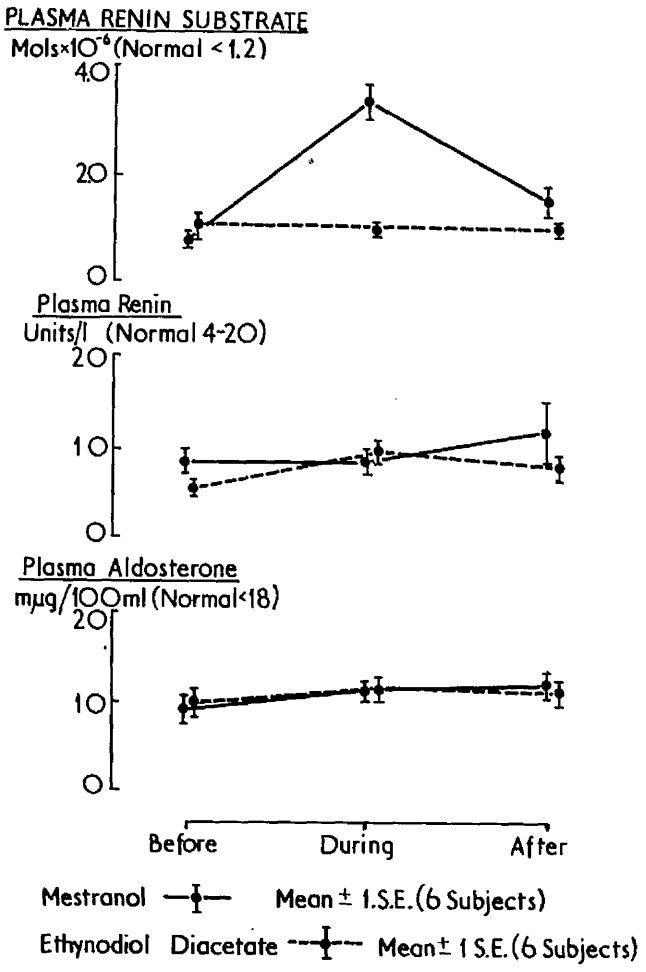


Figure 63 Changes in plasma renin, renin-substrate and aldosterone in women given mestranol or ethynodiol diacetate.

concentration also remained within the normal range in every case and no significant change occurred in either group (Figure 63).

This experiment has confirmed the marked increase in plasma renin-substrate concentration in women given an oestrogen (mestranol) alone, with no change in the women taking a progestogen (ethynodiol diacetate) alone. It seems likely, therefore, that it is the oestrogen component of the combined preparation which stimulates the rise in plasma renin-substrate concentration, and the slight increase induced by norethynodrel (Newton et al, 1968) may have been due to the oestrogenic properties of this compound.

The possibility has been discussed earlier (see Chapter 6.C) that elevated circulating substrate might be due to increased hepatic globulin synthesis stimulated by oestrogen.

CHAPTER 13

A STUDY OF THE RELATIONSHIP OF RENIN, RENIN-SUBSTRATE ANGIOTENSIN II AND ALDOSTERONE TO CHANGES IN BLOOD PRESSURE IN WOMEN TAKING OESTROGEN-PROGESTOGEN ORAL CONTRACEPTIVES.

Small increases in systolic and diastolic blood pressures have been reported in women taking combined oestrogen-progestogen oral contraceptives (Walters and Lim, 1970; Carmichael, Taylor and Ayers, 1970; Weir, Briggs, Browning, Mack, Naismith, Taylor and Wilson, 1971d; Spellacy and Birk, 1972; Weir, Tree and McElwee, 1972) (Figures 64 and 65). It is also now well established that combined oestrogen-progestogen oral contraceptives may sometimes induce or aggravate hypertension (see Weir et al, 1971d and 1972; Crane et al, 1971; Wallace, 1971; Spellacy and Birk, 1972) (Figure 66).

Similar changes in plasma renin and renin-substrate concentrations and in aldosterone secretion and excretion have been described in normotensive and hypertensive women while taking oral contraceptives (Laragh, Sealey, Ledingham and Newton, 1967; Weinberger et al, 1969; Crane et al, 1971). Sarata and his colleagues (1970), however, reported greater concentrations of plasma renin and renin-substrate in women with a rise of blood pressure while taking oral contraceptives, compared to women whose blood pressure remained unchanged.

The present study was designed to assess the possible relationship of changes in the renin-angiotensin-aldosterone

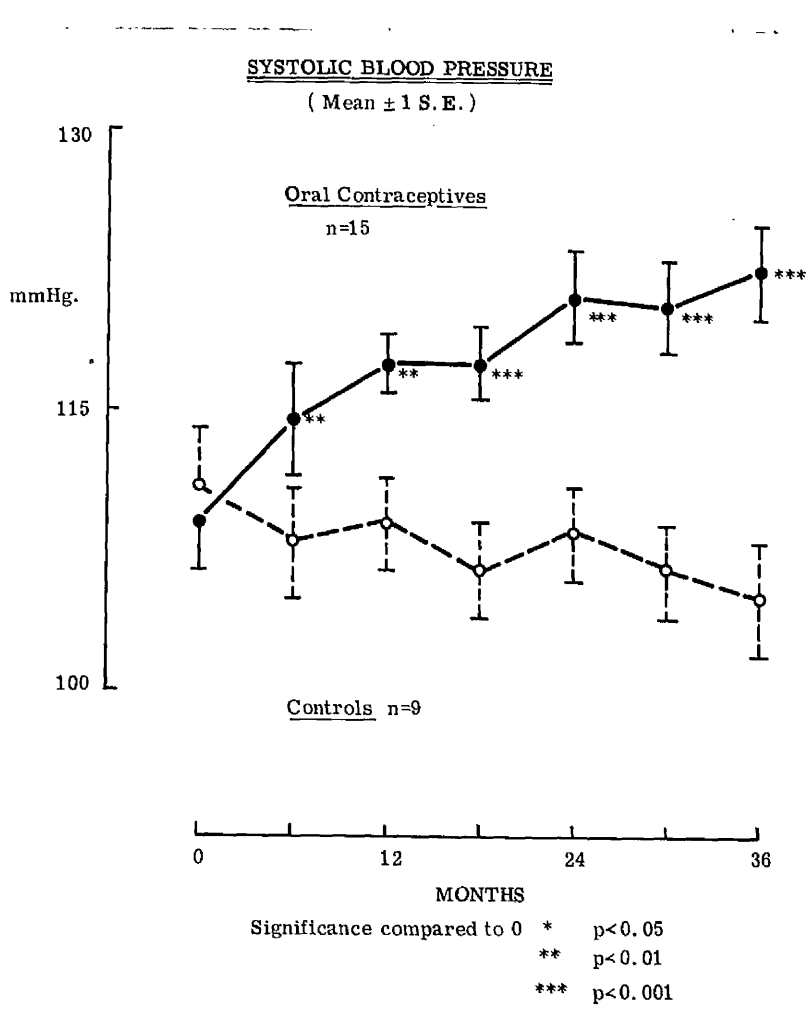


Figure 64

Changes in systolic blood pressure in women taking oestrogen-progestogen oral contraceptives

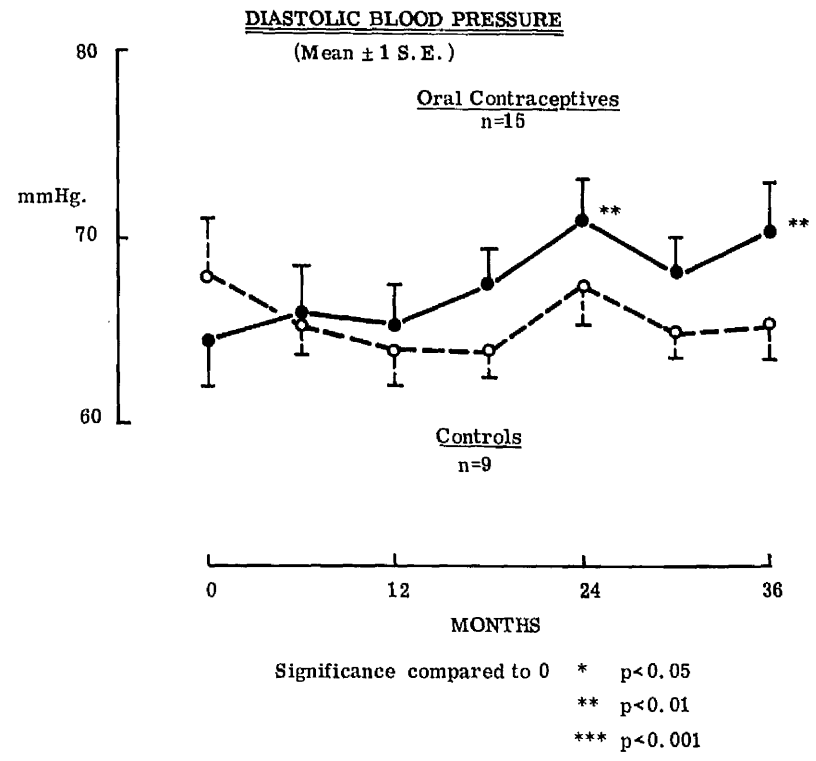


Figure 65

Changes in diastolic blood pressure in women taking oestrogen-progestogen oral contraceptives

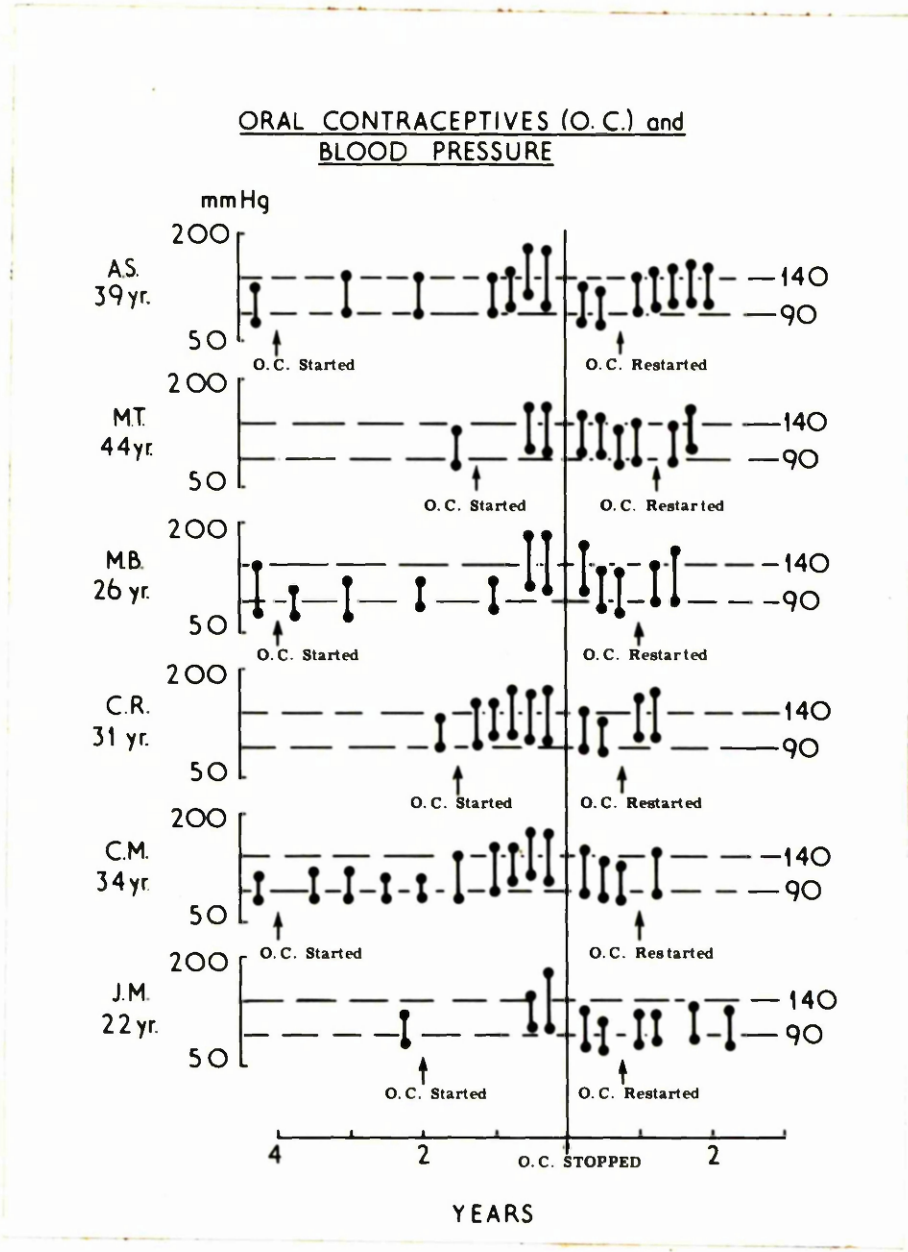


Figure 66 **Hypertension associated with oestrogen-progestogen oral contraceptives (o.c.).**

system to changes in blood pressure in women taking oestrogen-progestogen oral contraceptives.

LABORATORY METHODS.

These have been described in Chapter 3.

CLINICAL METHODS.

Twelve women were studied in whom persistent hypertension (i.e. B.P. 140/90 or over) had developed while taking combined oestrogen-progestogen oral contraceptives, the mean pressure of the group being 164/104 (systolic S.D. ± 11.0 mmHg, diastolic S.D. ± 5.6 mmHg). In each case blood pressure fell below 140/90 within 1 to 3 months after stopping the pill. The brands of oral contraceptives being taken when hypertension was first discovered were Minilyn (5 cases), Gynovlar, Minovlar and Orthonovin 1/50 (2 cases each) and Volidan (1 case). These had been taken for periods varying from 3 months to 10 years (mean 29.1 months). Mean age was 28.4 years (S.D. ± 7.0).

The measurements from this hypertensive group were compared with those from 22 normotensive women taking oral contraceptives. The results from these normotensive women were reported in Chapter 12. The mean blood pressure of this group was 113/68 systolic S.D. ± 13.5 mmHg, diastolic S.D. ± 8.8 mmHg). Mean age was 24.5 years (S.D. ± 5.4). Oral contraceptives had been taken for one month in all but one case (6 months) and the brands used at the time of sampling were Ovulen-100 (7 cases), Minovlar

(5 cases), Norinyl-1 (4 cases), Minilyn (3 cases), Orthonovin 1/50 (2 cases) and Gynovlar (1 case). In both groups diet was unrestricted. Blood samples were taken from the antecubital vein while the women were sitting. Time of sampling in the menstrual cycle was not standardised, as no significant difference had been found between the follicular and luteal phases in the normotensive women (see Chapter 12).

RESULTS.

As shown in Table 33 and Figures 67 and 68, there was no significant difference in mean plasma concentrations of renin or renin-substrate between the hypertensive and normotensive groups. Mean plasma angiotensin II concentration, however, was higher in the hypertensive women, although the difference did not reach statistical significance at the 5% level. Two of these women showed increases of plasma angiotensin II concentration to above the upper limit of the normal range (Figure 69). Plasma aldosterone was not measured,

DISCUSSION.

Mechanism of the changes in blood pressure.

In women taking oestrogen-progestogen oral contraceptives the mechanism of the increase in blood pressure is not clear. Increased sodium and fluid retention, possibly due to excess circulating aldosterone, has been postulated, but no relationship

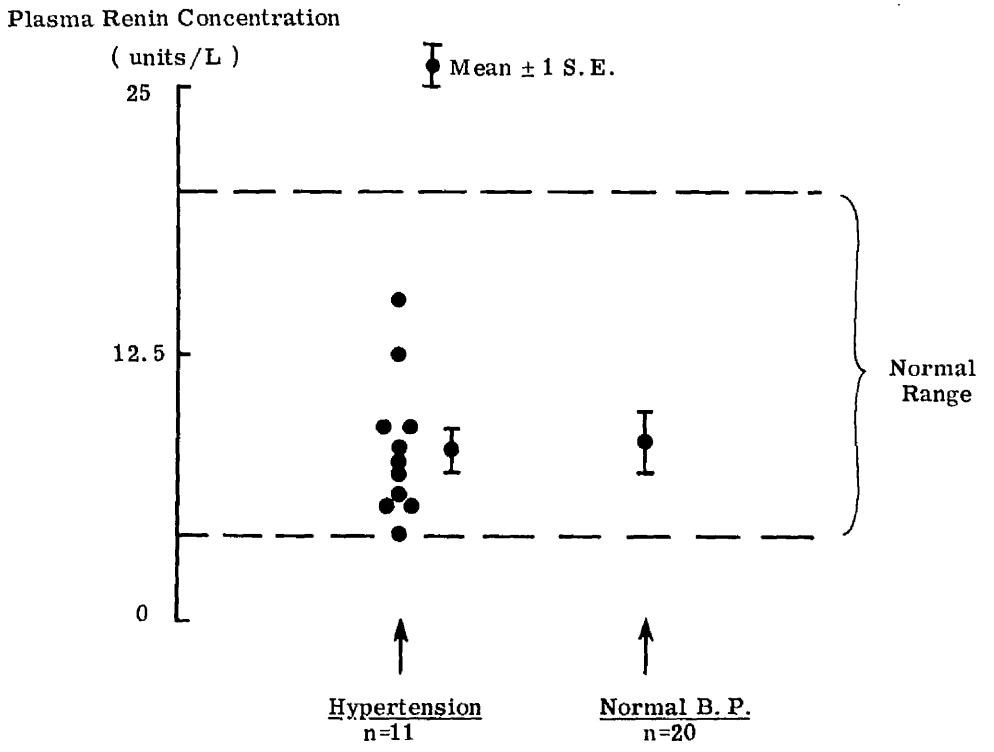


Figure 67 Plasma renin concentration in women with raised blood pressure while taking oral contraceptives

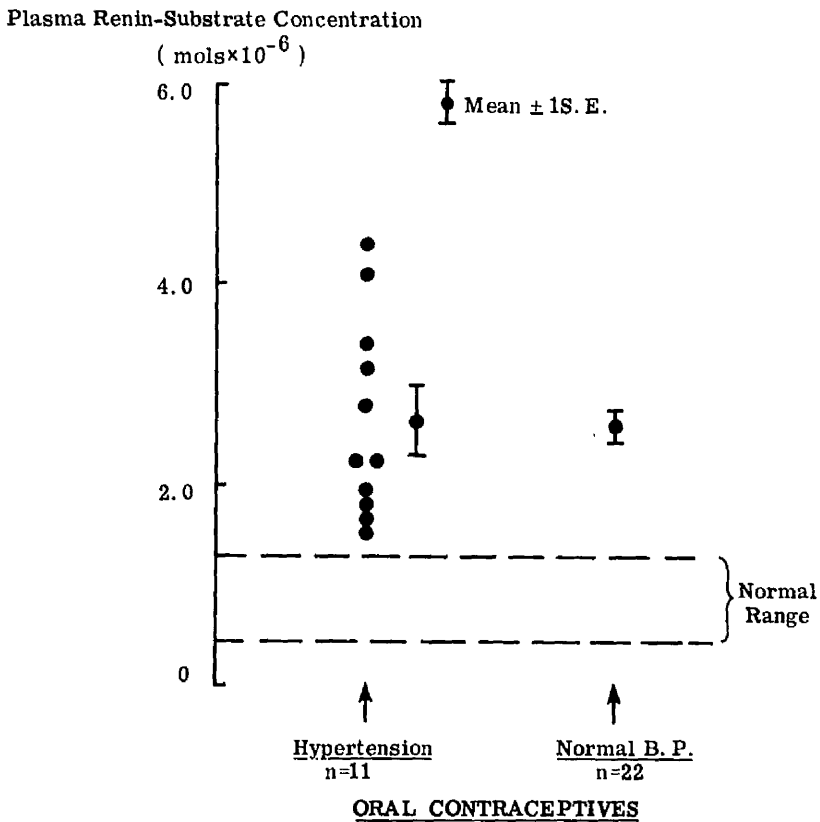


Figure 68

Plasma renin-substrate concentration in women with raised blood pressure while taking oral contraceptives

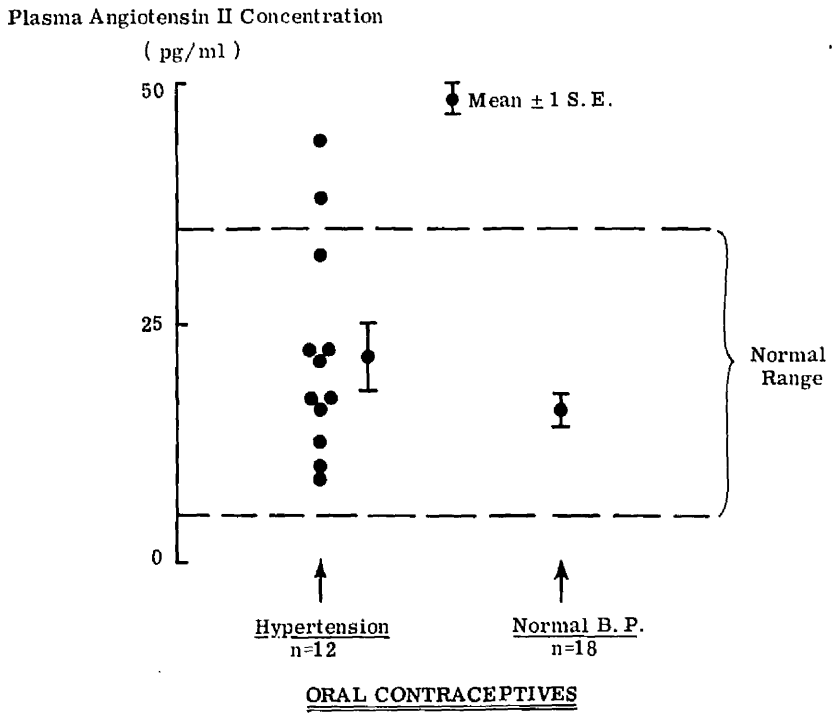


Figure 69

Plasma angiotensin II concentration in women with raised blood pressure while taking oral contraceptives

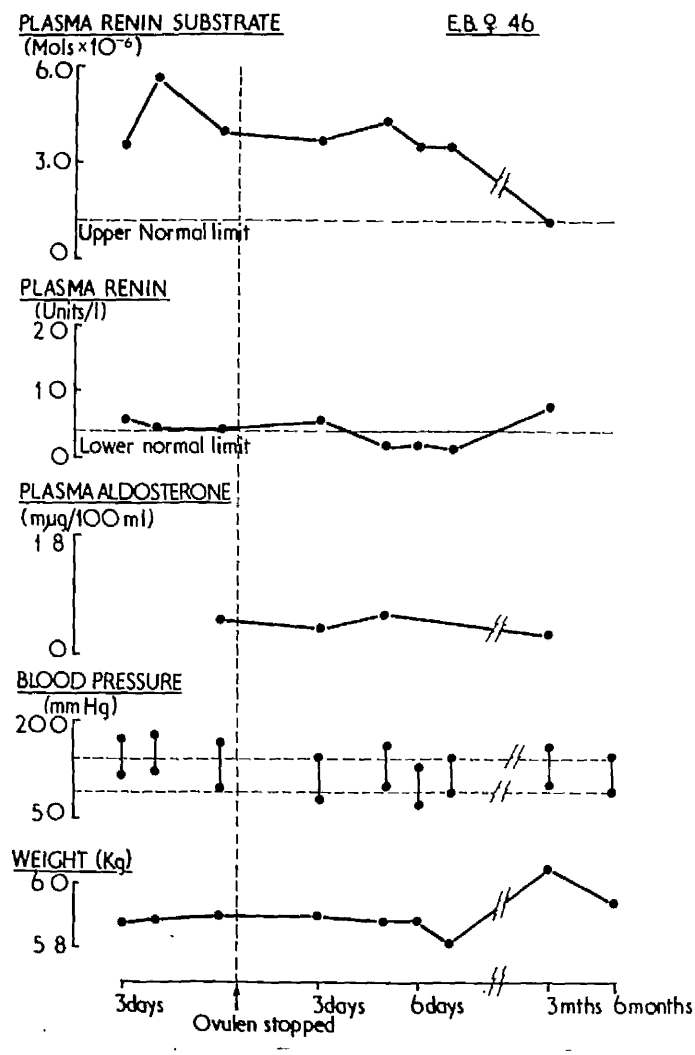


Figure 70

The effect on plasma renin, renin-substrate and aldosterone following the withdrawal of an oral contraceptive in a hypertensive patient.

has been demonstrated between change in blood pressure and change in weight in those women (Weir et al, 1971b). Also, total exchangeable sodium and potassium, total body water, and plasma aldosterone concentration did not change significantly in one hypertensive woman whose blood pressure fell significantly after withdrawal of the oral contraceptive (Weir et al, 1971b) (Figure 70). This, however, does not exclude a role for sodium and water retention in producing the initial increase of blood pressure in those cases (see Ledingham and Cohen, 1963; Coleman and Guyton, 1969).

Lim, Lumbers, Walters and Whelan (1970) demonstrated an increase in systolic blood pressure during intravenous infusion of oestrogen and suggested that it may be the oestrogen component which causes the raised blood pressure in women taking oral contraceptives, by inducing a rise in plasma volume, cardiac output and stroke volume (Walters and Lim, 1970). In support of this a raised blood pressure during oral oestrogen treatment has been reported (Crane et al, 1971; Spellacy and Birk, 1972), although Gov and MacGillivray (1971) using smaller doses demonstrated no change. However, no evidence has so far been found that oral contraceptives with a higher dose of oestrogen have a greater effect on blood pressure than those with a lower dose (Weir et al, 1971d; 1972).

Horrobin and Lloyd (1970) demonstrated that progesterone could cause small increases in systolic blood pressure in rabbits. We have confirmed this in a small pilot study of 3 rabbits given 50 mg intramuscular progesterone compared to 2 controls

given intramuscular ethyloleate (unpublished data). However, a recent report (Winter et al, 1972) has failed to demonstrate this effect. No increase in blood pressure has been found in humans given a progestogen either intramusculalry (MacKay, Khoo and Adam, 1971) or orally (Spellacy and Birk, 1972) and there is no evidence so far that the progestogenic potency of oral contraceptives is related to the changes in blood pressure (Weir et al, 1972).

Relationship of plasma renin, renin-substrate and angiotensin II concentrations to changes in blood pressure.

As the changes in the renin-angiotensin system were at first demonstrated in women with hypertension while taking oral contraceptives, it was thought that these changes might induce the rise in blood pressure (Laragh et al, 1967). However, it has since been shown that the changes in plasma renin, renin-substrate and angiotensin concentration occur irrespective of the changes in pressure (see Chapters 11 and 12).

In Chapter 12, the possibility of a feed-back mechanism was discussed as an explanation of the normal or reduced plasma renin concentration in the face of raised plasma renin substrate concentration and possible augmented angiotensin II levels in women taking oral contraceptives. An impairment of this suppression of renin has been postulated as a cause of the raised blood pressure in some cases (Laragh et al, 1967; Skinner et al, 1969; Saruta et al, 1970).

When compared to a normotensive group, Saruta and his colleagues (1970) reported that hypertensive women had greater

plasma concentrations of renin at 10 and 14 weeks and of renin-substrate at 2 and 6 weeks after starting oral contraceptives, but not thereafter. In spite of the fact that plasma renin-activity was not significantly different between the 2 groups, the authors suggested that their results lent support to the hypothesis of impaired feed-back suppression of renin as a cause of the raised blood pressure.

Bearing in mind the difference in duration of oral contraception in the 2 groups, the present study has shown the plasma concentrations of renin and renin-substrate to be similar in normotensive and hypertensive women (Figures 67 and 68). However, in the hypertensive group 2 women showed increases of plasma angiotensin II concentration to above the normal range (Figure 69). and the mean level of circulating angiotensin II was higher, although this was of borderline statistical significance. It remains doubtful whether this small change in plasma angiotensin II concentration has any significant bearing on the changes in blood pressure.

In animal experiments no evidence has been found for an increase in sensitivity to infused angiotensin in rats treated with an oestrogen-progestogen combination (Woods, 1967; Douglas, Hull and Langfords, 1970), progesterone alone (Chesley and Topper, 1967; Hettiaratchi and Pickford, 1968) or oestrogens alone (Hettiaratchi and Pickford, 1968). Similarly, in a study of two hypertensive women, no change in sensitivity to intravenous angiotensin occurred after withdrawal of the oral contraceptive

in spite of a significant fall in blood pressure in one case (Weir et al, 1971b).

On the basis of the results of this study and of previous reports it seems unlikely that the renin-angiotensin-aldosterone system is playing a significant role in the occasional development of hypertension in women taking oestrogen-progestogen oral contraceptives.

SECTION V

CONCLUSIONS.

CHAPTER 14.

CONCLUSIONS

The studies presented here were stimulated by the reports of previous workers, which had suggested that alterations in the renin-angiotensin-aldosterone system might be involved in the aetiology of hypertensive disease of pregnancy.

On reviewing the literature it was found that, although some of the individual components of the system had been studied in some detail, the inter-relationships in this system had not been clearly defined in normal pregnancy. Also, the circulating levels of angiotensin II and aldosterone, the probable active end-products of the renin - renin-substrate reaction, had not been adequately measured in normal pregnant women.

Detailed studies of the changes in the plasma concentrations of renin, renin-substrate, angiotensin II and aldosterone were therefore undertaken in a large number of normal pregnant women at all stages of gestation and the puerperium. In view of the known relationships in certain non-pregnant situations, an attempt was made to correlate the components of the renin-angiotensin-aldosterone system one with another and also with other metabolic and hormonal changes in pregnancy, especially sodium balance.

Statistical correlations between circulating substances do not necessarily imply a cause and effect relationship. Also, the statistical significance of a true biological correlation may be reduced by a dispersal of the data due to technical and biochemical factors. However, fairly simple direct correlations have been demonstrated in

non-pregnant subjects, and it might be expected that the very marked changes in plasma concentrations of renin, renin-substrate, angiotensin II and aldosterone in pregnancy would give similarly concise relationships.

The results of the studies described in this thesis have shown, however, that this is not the case and have revealed much more complex inter-relationships in the pregnant women than have been encountered in other situations. It seems very likely that the normal physiological relationships of the renin-angiotensin-aldosterone system are being modified by the profound alterations in hormonal balance which occur in pregnancy, for example in progesterone and oestrogen secretion. The rapid proliferation of the chorionic and trophoblastic cells may be exerting a hitherto unknown influence on renin and aldosterone secretion in the very early days of pregnancy, and elucidation of the role of these substances may come from more intensive investigation of the primitive foeto-placental unit. The actions of angiotensin and aldosterone in maternal-foetal fluid and electrolyte exchange also requires detailed examination. Their role in overall maternal fluid and electrolyte balance is still controversial, but it does seem likely that they are playing, at least a small part in the adaptive processes required for adequate foetal nutrition and well being.

In spite of a superficial similarity, it has been found that the changes induced by oestrogen-progestogen oral

contraceptives do not mimic those found in pregnancy, apart from the increases in plasma renin-substrate concentration. The oestrogen-progestogen oral contraceptive has therefore proved deficient as a possible model of the pregnant state but the effects of the oestrogen component have thrown light on the origin of the raised renin-substrate levels. Raised blood pressure in women who are taking oral contraceptives does not appear to be due to increased circulating levels of angiotensin II or aldosterone, but this remains to be confirmed by further studies of these uncommon patients.

If the physiological relationships in normal pregnancy are complex, the pathological relationships in hypertensive disease of pregnancy are no less so. The changes in circulating levels of renin, renin-substrate, angiotensin II and aldosterone have now been investigated in detail and all the components of the system have been found to be lower than those occurring in normal pregnancy. Whether these decreased levels of angiotensin II and aldosterone may be more active in the presence of the altered fluid, electrolyte and hormonal balance of pregnancy remains to be answered but this seems unlikely.

The present study has shown that increased blood levels of the pressor agent angiotensin II and of the mineralocorticoid aldosterone can no longer be implicated in the pathogenesis of hypertensive disease of pregnancy. Their lowered plasma concentrations may be a reflection of reduced placental function.

On the other hand, it is possible that the renin-angiotensin-aldosterone system is being suppressed by an excess of circulating mineralocorticoid which has yet to be identified. Examination of the role of plasma DOC and AMI in the aetiology of pregnancy hypertension appears to be the next step in the search for Professor Amand Routh's "toxin" of 60 years ago. Over these years many substances have been examined by sophisticated laboratory techniques, only to be eventually discarded, and the enigma of pre-eclampsia remains as challenging as ever.

SECTION VI

BIBLIOGRAPHY

and

APPENDIX

BIBLIOGRAPHY

AARSKAUG, T. (1956).
Acta endocr., Copnh., 22: 344.

ABDUL-KARIM, R. and ASSALI, N.S. (1961).
Am. J. Obstet. Gynec. 82: 846.

AMES, E.P., BORKOWSKI, A.J., SINCLAIRE, A.M. and LARAGH, J.H. (1963).
J. clin. Invest. 42: 1171.

ASSALI, N.S. and MORRIS, J.A. (1964).
Obstet. gynec. Surv. 19: 925.

ASSALI, N.S., GARST, J.D. and VOSKIAN, J. (1955).
J. Lab. clin. Med. 46: 389.

BALIKIAN, H.M., RHODES, A.R., HALE, S.L. MELBY, J.C. and
HART, J.F. (1969). J.clin.Endocr.Metab. 28: 1630.

BARDEN, H.R.E., GRADEN, E.A. and O'ROURKE, J.J. (1966).
Obstet. and Gynec. 27: 414.

BARNARD, W.P. and LAGAN, E.V. (1970).
Clinica chim. Acta 29: 401.

BARNES, A.C. and QUILLIGAN, E.J. (1956).
Am. J. Obstet. Gynec. 71: 670.

BAULIEU, E.E., de VIGAN, H., HERCAIRE, H. and JAYLE, M.F. (1957).
J. clin. Endocr. Metab. 17: 1473.

BAYARD, F., ANCES, I.G., WAPNER, A.J., WELDEN, V.V., KOWARSKI, A.
and MIGEON, C.J. (1970a).
J. clin. Invest. 49: 1539.

BAYARD, F., KOWARSKI, A., WELDEN, V.V. and MIGEON, C.J. (1970b).
J. Lab. clin. Med. 75: 347.

BAYLISS, R.I.S., BROWNE, J.C.McC., HUNT, E.F. and STRINDBERG, A.E.,
(1955).
Lancet. 1: 62.

BEITINS, I.Z., BAYARD, F., LEVITSKY, L., ANCES, I.G., KOWARSKI, A.
and MIGEON, C.J. (1972).
J. clin. Invest. 51: 586.

BEKER, J.C. (1929).
Am. J. Obstet. Gynec. 18: 368.
BERGER, M. and BOUCEK, R.J. (1964).
Am. J. Obstet. Gynec. 89: 230.
BERGER, M. and CAVANAGH, D. (1963).
Am. J. Obstet. Gynec. 87: 293.
BERGER, M., LANGHANS, J. (1967).
Am. J. Obstet. Gynec. 98: 215.
BIRON, P., KOIW, E., NOWACZYNSKI, W., BROUILLET, J. and
GENEST, J. (1961).
J. clin. Invest. 40: 558.
BLAIR-WEST, J.R., COGHLAN, J.P., DENTON, D.A., FUNDER, J.W.
and SCOGGINS, B.A. (1972).
In "Control of Renin Secretion", ed. Assaykeen, T.A.
New York and London : Plenum Press, p. 167.
BLAIR-WEST, J.R., COGHLAN, J.P., DENTON, D.A., FUNDER, J.W.,
SCOGGINS, B.A. and WRIGHT, R.D. (1971).
J. clin. Endocr. Metab. 32: 575.
BONAR, J., BROWN, J.J., DAVIES, D.L., LANGFORD, H.G., LEVER, A.F.,
ROBERTSON, J.I.S. (1966).
J. Obstet. Gynaec. Br. Commonw. 73: 418.
BONJOUR, J.P. and MALVIN, R.L. (1970).
Am. J. Physiol. 218: 1555.
de BONO, E., LEE, G. de J., MOTTRAM, F.R., PICKERING, G.W.,
BROWN, J.J., KEEN, M., PEART, W.S. and SANDERSON, P.H. (1963).
Clin. Sci. 25: 123.
BOONSHAFT, B., O'CONNELL, J.M.B., HAYES, J.M., SCHREINER, G.E. (1968).
J. Clin. Endocr. Metab. 28: 1641.
van BOUWDIJK BASTIAANSE, M.A. (1954).
Am. J. Obstet. Gynec. 68: 151.
BOYD, G.W., ADAMSON, A.R., ARNOLD, M., JAMES, V.H.T. and
PEART, W.S. (1972).
Clin. Sci. 42: 91.

BOYD, W. (1958).
In "Pathology for the physician". Sixth Edition.
Henry Kimpton, London. p. 156.

BRANDSTETTER, F. and SCHULLER, E. (1959).
Arch. Gynäk. 193: 420.

BRO-RUSSMUSSEN, F., BUUS, O., LUNDWALL, F. and TROLLE, D. (1962).
Acta endocr., Copnh. 40: 571.

BROSENS, I. (1964).
J. Obstet. Gynaec. Br. Commonw. 71: 222.

BROWN, J.B. (1956).
Lancet. 1: 704.

BROWN, J.J. (1963).
Mem. Soc. Endocr. 13: 303.

BROWN, J.J., DAVIES, D.L., DOAK, P.B., LEVER, A.F., ROBERTSON, J.I.S. (1963).
Lancet. 2: 900.

BROWN, J.J., DAVIES, D.L., LEVER, A.F., ROBERTSON, J.I.S. and TREE, M. (1964a).
Biochem. J. 93: 594.

BROWN, J.J., DAVIES, D.L., DOAK, P.B., LEVER, A.F., ROBERTSON, J.I.S. and TREE, M. (1964b).
Lancet. 2: 64.

BROWN, J.J., DAVIES, D.L., LEVER, A.F. and ROBERTSON, J.I.S. (1964c).
Br. med. J. 2: 1114.

BROWN, J.J., DAVIES, D.L., DOAK, P.B., LEVER, A.F., ROBERTSON, J.I.S. and TREST, P. (1965).
Lancet. 2: 1219.

BROWN, J.J., DAVIES, D.L., LEVER, A.F., and ROBERTSON, J.I.S. (1966a).
Postgrad. Med. J. 42: 153.

BROWN, J.J., DAVIES, D.L., DOAK, P.B., LEVER, A.F., and ROBERTSON, J.I.S. (1966b).
J. Endocr. 35: 575.

BROWN, J.J., DAVIES, D.L., LEVER, A.F., MACPHERSON, D. and ROBERTSON, J.I.S. (1966c).
Clin. Sci. 30: 279.

BROWN, J.J., DAVIES, D.L., DOAK, P.B., LEVER, A.F., ROBERTSON, J.I.S. and TRUST, P. (1966d).
J. Obstet. Gynaec. Br. Commonw. 73: 410.

BROWN, J.J., LEVER, A.F., ROBERTSON, J.I.S., RODGE, R.L., LOWE, R.D. and VANE, J.R. (1967).
Nature, Lond. 215: 853.

BROWN, J.J., FRASER, R., LEVER, A.F., and ROBERTSON, J.I.S. (1968).
In Recent Advances in Endocrinology, Eighth Edition.
Editor V.H.T. James, London; Churchill, p. 271.

BROWN, J.J., CHINN, R.H., DÜSTERDIECK, G.O., FRASER, R., GLEADLE, R.H., LEVER, A.F., ROBERTSON, J.I.S. and TREE, M. (1969a).
Proc. R. Soc. Med. 62: 1258.

BROWN, J.J., CURTIS, J.R., LEVER, A.F., ROBERTSON, J.I.S. de WARDENER, H.E. and WING, A.J. (1969b).
Nephron, 6: 329.

BROWN, J.J., DAVIES, D.L., JOHNSON, V.W., LEVER, A.F. and ROBERTSON, J.I.S. (1970).
Am. Heart J. 80: 329.

BROWN, J.J., FRASER, R., LEVER, A.F. and ROBERTSON, J.I.S. (1971a).
Abstr. World Med. 45: 549.

BROWN, J.J., DÜSTERDIECK, G., FRASER, R., LEVER, A.F., ROBERTSON, J.I.S., TREE, M. and WEIR, R.J. (1971b).
Br. med. Bull. 27: 128.

BROWN, J.J., CHINN, R.H., GAVRAS, H., LECKIE, B., LEVER, A.F., McGREGOR, J., MORTON, J. and ROBERTSON, J.I.S. (1972a).
In "Hypertension 1972". Springer-Verlag, Berlin, p. 81.

BROWN, J.J., FRASER, R., LEVER, A.F., and ROBERTSON, J.I.S. (1972b).
Clin. Endocr. Metab. 1: 397.

BUCHBORN, E., KOCZOREK, Kh.H. and WOLFF, H.P. (1957).
Klin. Wschr. 35: 452.

BURKE, C.W. (1969).
Br. med. J. 2: 798.

BURKE, C.W. and ROULET, F. (1970).
Br. med. J. 1: 657.

CAIN, M.D., WALTERS, W.A., and CATT, K.J. (1971).
J. clin. Endocr. Metab. 33: 671.

CARMICHAEL, S.M., TAYLOR, M.M. and AYERS, C.R. (1970).
Obstet.and Gynec. 35: 371.

CARRIÈRE, S. and DIRON, P. (1970).
Am. J. Physiol. 219: 1642.

CATT, K.J. ZIMMET, P.Z., CAIN, M.D., CRAN, E., BEST, J.B. and COGHLAN, J.P. (1971).
Lancet. 1: 459.

CHARE, J.J., SHIPLEY, E.G. and GORDON, E.S. (1951).
Proc. Soc. exp. Biol. Med. 78: 244.

CHESLEY, L.C. (1963).
Am. J. Obstet. Gynec. 87: 410.

CHESLEY, L.C. (1966a).
Clin. Obstet. and Gynec. 9: 871.

CHESLEY, L.C. (1966b).
Am. J. Obstet. Gynec. 95: 127.

CHESLEY, L.C. and DUFFUS, G.M. (1971).
Obstet. and Gynec. 38: 1.

CHESLEY, L.C. and TEPPER, I.H. (1967).
J. clin. Endocr. Metab. 27: 576.

CHESLEY, L.C., SLOAN, D.M. and WYNN, R.M. (1964).
Am. J. Obstet. Gynec. 90: 281.

CHESLEY, L.C., TALLEDO, E., BOHLER, C.S. and ZUSPAN, F.P. (1965).
Am. J. Obstet. Gynec. 91: 837.

CHESLEY, L.C., WYNN, R.M. and SILVERMAN, N.I. (1963).
Circulation Res. 13: 232.

CHINN, R.H. and DÜSTERDIECK, G. (1972).
Clin. Sci. 42: 489.

CHRISTY, N.P. and JAILER, J.W. (1959).
J. clin. Endocr. Metab. 19: 263.

COLEMAN, T.G. and GUYTON, A.C. (1969).
Circulation Res. 25: 153.

CRANE, M.G. and HARRIS, J.J. (1969).
J. clin. Endocr. Metab. 29: 558.

CRANE, M.G., HARRIS, J.J. and WINSOR, W. (1971).
Ann. intern. Med. 74: 13.

DÄSSLER, C.G. (1969).
Acta endocr., Copnh. 61: 198.

DAUGHADAY, W.H. (1958).
Archs. intern. Med. 101: 286.

DAVIS, M.O. (1972).
New Engl. J. Med. 286: 100.

DEXTER, L. and HAYNES, F.W. (1944).
Proc. Soc. exp. Biol. Med. 55: 288.

DIECKMANN, W.J. and POTTINGER, R.E. (1956).
Am. J. Obstet. Gynec. 71: 596.

DIXON, H.G. and ROBERTSON, W.B. (1958).
J. Obstet. Gyn. Br. Emp. 65: 803.

DIXON, H.G., BROWNE, J.C.McC. and DAVEY, D.A. (1963).
Lancet. 2: 369.

DODEK, O.I., SEGHE, E.J. and KLAIDER, E.L. (1965).
Am. J. Obstet. Gynec. 93: 173.

DOE, R.P., DICKINSON, P., ZINNEMAN, H.H. and SEAL, U.S. (1969).
J. clin. Endocr. Metab. 29: 757.

DOE, R.P., FERNANDEZ, R. and SEAL, U.S. (1964).
J. clin. Endocr. Metab. 24: 1029.

DOE, R.P., HELLINGER, G.T., SWAIM, W.R., and SEAL, U.S. (1967).
J. clin. Endocr. 27: 1081.

DOUGLAS, B.H., HULL, R.P. and LANGFORD, H.G. (1970).
Proc. Soc. exp. Biol Med. 133: 1142.

DRUCKER, W.D., HENDRIKX, A., LARAGH, J.H., CHRISTY, N.P., and van de WIELE, R.L. (1963)
J. clin. Endocr. Metab. 23: 1247.

DUFAU, M.L., and VILLEE, D.B. (1969).
Biochim. biophys. Acta. 176: 637.

DÜSTERDIECK, G. and McELWEE, G. (1971).
Europ. J. Clin. Invest. 2: 32.

EHRLICH, E.N. (1971).
Am. J. Obstet. Gynec. 109: 963.

EHRLICH, E.N., LAVES, A.D., LUGIBIHL, K. and LANDAU, R.L. (1962).
J. Lab. clin. Med. 59: 588.

EHRLICH, E.N., LUGIBIHL, K., LAVES, M. and JANULIS, M. (1966).
J. clin. Endocr. Metab. 26: 1160.

EHRLICH, E.N., LUGIBIHL, K., TAYLOR, C. and JANULIS, M. (1967).
J. clin. Endocr. Metab. 27: 836.

EMERSON, T.E. (1966).
Am. J. Physiol. 210: 953.

ETON, B. and SHORT, R.V. (1960).
J. Obstet. Gynaec. Br. Commonw. 67: 785.

EXLEY, D. and NORYMBERSKI, J.K. (1964a).
J. Endocr. 29: 293.

EXLEY, D. and NORYMBERSKI, J.K. (1964b).
J. Endocr. 29: 303.

FASCIOLO, J.C., de VITO, E., ROMERO, J.C. and CUCCHI, J.N. (1964).
Con. med. Ass. J. 90: 206.

FERRIS, T.F., GORDEN, P. and MULROW, P.J. (1967).
Am. J. Physiol. 212: 698.

FERRISS, J.B., BROWN, J.J., FRASER, R., KAY, A.W., LEVER, A.F., NEVILLE, A.M., O'MUIRCHEARTAIGH, I.G., ROBERTSON, J.I.S. and SYMINGTON, T. (1970).
Lancet. 2: 995.

FITZSIMMONS, J.T. and SIMONS, B.J. (1969).
J. Physiol. Lond. 203: 45.

FORSHAM, P.H. (1967).
In "The Human Adrenal Cortex". Ciba Foundation Study Group No. 27. Ed. G.E.W. Wolstenholme and R. Porter. Churchill, London, p. 48.

FRANKLIN, K.J. and WINSTON, N.E. (1955).
J. Obstet. Gynaec. Br. Emp. 62: 29.

FRASER, R. and JAMES, V.H.T. (1968).
J. Endocr. 40: 59.

FRASER, R., BROWN, J.J., CHINN, R.H., LEVER, A.F. and ROBERTSON, J.I.S. (1969).
Scott. Med. J. 14: 420.

FRASER, R., JAMES, V.H.T., BROWN, J.J., DAVIES, D.L., LEVER, A.F., and ROBERTSON, J.I.S. (1966).
J. Endocr. 35: 511.

FRASER, R., JAMES, V.H.T., BROWN, J.J., ISAAC, P., LEVER, A.F., and ROBERTSON, J.I.S. (1965).
Lancet. 2: 989.

FRIEDMAN, M. and BEARD, R.W. (1966).
J. Obstet. Gynaec. Br. Commonw. 73: 123.

GEELHOED, G.W. and VANDER, A.J. (1968).
J. clin. Endocr. Metab. 28: 412.

GEELHOED, G.W., VANDER, A.J. and CARLSON, J. (1970).
Proc. Soc. exp. Biol. Med. 133, 479.

GEMZELL, C.A. (1953).
J. clin. Endocr. Metab. 13: 898.

GENEST, J., de CHAMPLAIN, J., VENRAT, R., BOUCHER, R., TREMBLAY, G.Y., STRONG, C.G., KOIW, E. and MARC-AURELE, J. (1965).
Hypertension. 13: 97.

GENEST, J., NOWACZYNSKI, W., KOIW, E., SANDOR, T. and BIRON, P. (1960).
In Essential Hypertension. Edited by K.D. Bock and P.T. Cottier, Berlin: Springer-Verlag, p. 126.

GOLDBERG, S., LEWENTHAL, H., GOTTFRIED, I. and BEN-ADERET, N. (1966).
Am. J. Obstet. Gynec. 95: 892.

GORDON, E.S., CHART, J.J., HAGEDORN, B.S. and SHIPLEY, E.G. (1954).
Obstet. and Gynec. 4: 39.

GORDON, R.D., FISHMAN, L.M. and LIDDLE, G.W. (1967).
J. clin. Endocr. Metab. 27: 385.

GORDON, R.D., PARSONS, S. and SYMONDS, E.M. (1969).
Lancet. 1: 347.

GOTTFRIED, I., LEWENTHAL, H. and GOLDBERG, S. (1968).
Am. J. Obstet. Gynec. 102: 924.

GOULD, A.B., SKEGGS, L.T. and KAHN, J.R. (1966).
Lab. Invest. 15: 1802.

GOW, J. and MACGILLIVRAY, I. (1971).
Br. med. J. 2: 73.

GRAVES, L.K. and AGERSBORG, H.P.K. (1966).
Obstet. and Gynec. 28: 258.
GREIG, M., COYLE, M.G., COOPER, W. and WALKER, J. (1962).
J. of Obstet. Gynaec. Br. Commonw. 69: 972.
GYÖNGYÖSSY, A. and KELENTEY, B. (1958).
J. Obstet. Gynaec. Br. Emp. 65: 617.

HELMER, O.M. and GRIFFITH, R.S. (1952).
Endocrinology. 51: 421.
HELMER, O.M. and JUDSON, W.E. (1967).
Am. J. Obstet. Gynec. 99: 9.
von HERRINGER, W. and WICHMANN, H. (1967).
Gynaecologia (Basel) 163: 1.
HETTIARATCHI, E.S.G. and PICKFORD, M. (1968).
J. Physiol., Lond. 196: 447.
HEYS, R.F., SCOTT, J.S., OAKEY, R.E. and STITCH, S.R. (1969).
Obstet. and Gynec. 33: 390.
HICKLER, R., LAULER, D. and THORN, G. (1963).
J. clin. Invest. 42: 633.
HODARI, A.A. (1969).
Am. J. Obstet. Gynec. 97: 597.
HORNE, C.H.W., HOWIE, P.W., WEIR, R.J. and GOUDIE, R.B. (1970).
Lancet. 1: 49.
HORROBIN, D.F. and LLOYD, I.J. (1970).
J. Obstet. Gynaec. Br. Commonw. 77: 253.
HOSIE, K.F., BROWN, J.J., HARPER, A.M., LEVER, A.F., MACADAM, R.F., MACGREGOR, J. and ROBERTSON, J.I.S. (1970).
Clin. Sci. 38: 157.
HUNTER, C.A. and HOWARD, W.F. (1961).
Am. J. Obstet. Gynec. 81: 441.
HYTTEN, F.E. and LEITCH, I. (1971).
The Physiology of Human Pregnancy. Second Edition. Blackwell Scientific Publications, Oxford.
HYTTEN, F.E. and PAINTIN, D.B. (1963).
J. Obstet. Gynaec. Br. Commonw. 70: 402.

JAILER, J.W., CHRISTY, N.P., LONGSON, D., WALLACE, E.Z. and
GORDON, W.E.L. (1959).
Am. J. Obstet. Gynec. 78: 1.

JAMES, V.H.T., LANDON, J. and FRASER, R. (1967).
Mem. Soc. Endocr. 17: 141.

JAMES, V.H.T., RIPPON, A.E. and ARNOLD, M.L. (1969).
Acta endocr. Copnh. Suppl. 138: 12.

JAMES, V.H.T., TOWNSEND, J. and FRASER, R. (1967).
J. Endocr. 36: xxviii.

JEFFCOATE, T.N.A. (1966).
Proc. R. Soc. Med. 59: 397.

JOHNSON, W.L., McGAUGHEY, H.S. and THORNTON, W.N. (1961).
Surg. Forum. 12: 422.

JONES, K.M., LLOYD-JONES, R., RIONDEL, A., TAIT, J.F., TAIT, SAA.S.,
HULBROOK, R.D. and GREENWOOD, F.C. (1959).
Acta endocr., Copnh. 30: 321.

JONES, J.E.S., DELFS, E., and STRAN, H.M. (1944).
Bull. John Hopkins Hosp. 75: 359.

KAPLAN, N.M., and SILAH, J.G. (1964).
J. clin. Invest. 43: 659.

KATZ, F.H. and KAPPAS, A. (1967).
J. clin. Invest. 46: 1768.

KEANE, P.M., PEARSON, J. and WALKER, W.H.C. (1969a).
J. Endocr. 43: 571.

KEANE, P.M., PIGG, P.J., and JOHNSON, E. (1969b).
J. clin. Endocr. Metab. 29: 1126.

KLAUS, D. (1962).
Klin. Wchsr. 40: 701.

KLAUS, D. and BIRON, P. (1964).
Nature. 204: 381.

KLAUS, D. and HEINZMANN, A. (1967).
Klin. Wchsr. 45: 657.

KLOPPER, A. (1964).
Lancet. 2: 565.

KLOPPER, A. (1969).
In "Foetus and Placenta". Ed. A. Klopper and E. Diczfalusy.
Blackwell Scientific Publications. Oxford and Edinburgh. p. 471.

KLOPPER, A. and BILLEWICZ, W. (1963).
J. Obstet. and Gynaec. Br. Commonw. 70: 1024.
KOCZOREK, Kh. R., WOLFF, H.P. and BEER, M.L. (1957).
Klin. Wchnschr. 35: 497.
KOKOT, F. and CIEKAŃSKI, A. (1972).
J. Obstet. Gynaec. Br. Commonw. 79: 72.
KOPELMAN, J.J. and LEVITZ, M. (1970).
Am. J. Obstet. Gynec. 108: 925.
KRIEGER, D.T., GABRILOVE, J.L., and SOFTER, L.J. (1960).
J. clin. Endocr. Metab. 20: 1493.
KRITZ, W. and LEVER, A.F. (1969).
Am. Heart J. 78: 101.
KUHLBÄCK, B. and WIDHOLM, O. (1966).
Scand. J. clin. Lab. Invest. 18: 654.
KUMAR, D. (1962).
Am. J. Obstet. Gynec. 84: 1325.
KUMAR, D., PELTHAM, L.A.W. and GOINALL, A.G. (1959).
Lancet. 1: 541.

LAIDLAW, J.C., COHEN, M. and GOINALL, A.G. (1958).
J. clin. Endocr. Metab. 18: 222.
LAIDLAW, J.C., MUSE, J.L. and GOINALL, A.G. (1962).
J. Clin. Endocr. Metab. 22: 161.
LANDAU, R.L., and LUGIBIHL, K. (1958).
J. clin. Endocr. 18: 1237.
LANDAU, R.L. and LUGIBIHL, K. (1961).
Recent Prog. Horm. Res. 17: 249.
LANDAU, R.L., BERGENSTAL, D.M., LUGIBIHL, K., and KASCHT, M.E. (1955).
J. Clin. Endocr. 15: 1194.
LANDAU, R.L., LUGIBIHL, K., BERGENSTAL, D.M., and DIMICK, D.F. (1957).
J. Lab. clin. Med. 50: 613.
LANDESMAN, R., BIRON, P., CASTELLANOS, R., LARUSSA, R. and WILSON, K.H. (1963).
Obstet. and Gynec. 22: 316.
LARAGH, J.H., ANGERS, M., KELLY, W.G. and LIEBERMAN, S. (1960).
J. Am. med. Ass. 174: 234.

LARAGH, J.H., SEALEY, J.E., LEDINGHAM, J.G.G. and NEWTON, M.A. (1967).
J. Amer. med. Ass. 201: 918.

LAYNE, D.S. and MEYER, C.J. (1965).
Metabolism. 14: 489.

LAYNE, D.S., MEYER, C.J., VAISWANAR, P.S., PINCUS, G. (1962).
J. clin. Endocr. Metab. 22: 107.

LEDINGHAM, J.M. and COHEN, R.D. (1963).
Lancet. 2: 979.

LEE, M.R. (1969).
Renin and Hypertension. A modern synthesis.
Lloyd-Luke (Medical Books) Ltd., London.

LIM, Y.L., LUMBERS, E.R., WALTERS, W.A.W. and WHELAN, R.F. (1970).
J. Obstet. Gynaec. Br. Commonw. 77: 349.

LISTER, U.M. (1962).
J. Obstet. Gynaec. Br. Commonw. 69: 761.

LJUNGQVIST, A. and WAGENMARK, J. (1966).
Acta path. microbiol. scand. 67: 257.

LUBASH, G.D., BAND, R.H. and KLINE, J.A. (1969).
Am. J. Med. Sci. 257: 155.

LUBASH, G.D., MUTESAN, G.E., ALICANDRI, C.L., GARFINKEL, D.J., SIEKIERSKI, E.C. and McCONNAUGHEY, C.K. (1971).
Experientia. 27: 68.

LUMBERS, E.R. (1970).
Aust. J. exper. Biol. med. Sci. 48: 495.

LUMBERS, E.R. (1971).
Enzymologia. 40: 329.

LUUKKAINEN, T., EISALO, A., JÄRVINEN, P.A. and VIRANKO, M. (1964).
Acta obstet. gynec. scand. 43: 28.

McCANCE, R.A. (1936).
Proc. R. Soc. (Series B) 119: 245.

MACDONALD, H.N. and GOOD, W. (1971).
J. Obstet. Gynaec. Br. Commonw. 78: 798.

MACDONALD, H.N. and GOOD, W. (1972).
J. Obstet. Gynaec. Br. Commonw. 79: 441.

MACGILLIVRAY, I. (1961).
In "Water and Electrolyte metabolism. Edited by C.P. Stewart and T. Strengers. Amsterdam: Elsevier, p. 124.

MACGILLIVRAY, I. (1967).
Scott. med. J. 12: 237.

MACGILLIVRAY, I. and BUCHANAN, T.J. (1958).
Lancet. 2: 1090.

MACKAY, E.V., KHOO, S.K. and ADAM, R.R. (1971).
Aust. N.Z. J. Obstet. Gynaec. 11: 148.

MACNAUGHTON, M.C. (1967).
Am. J. Obstet. Gynec. 97: 998.

MAEBASHI, M., AIDA, M., YOSHINAGA, K., ABE, K., MIWA, I. and WATANABE, N. (1964).
Tohoku J. exp. Med. 84: 55.

MARTIN, B.T., COOKE, B.A. and BLACK, W.P. (1970).
J. Endocr. 46: 369.

MARTIN, M.M. and MARTIN, A.L.A. (1968).
J. clin. Endocr. Metab. 28: 137.

MARTIN, J.D. and MILLS, I.H. (1956).
Br. med. J. 2: 571.

MARTIN, J.D. and MILLS, I.H. (1958).
Clin. Sci. 17: 137.

MASSANI, Z.M., FINKIELMAN, S., WORCEL, M., AGREST, A. and PALADINI, A.C. (1966).
Clin. Sci. 30: 473.

MASSANI, Z.M., SANGUINETTI, R., GALLEGOS, R. and RAIMONDI, D. (1967).
Am. J. Obstet. Gynec. 99: 313.

MELBY, J.C., DALE, S.L., WILSON, T.E. and NICHOLS, A.S. (1966).
Clin. Res. Proc. 14: 283.

METCALF, M.G. and BEAVEN, D.W. (1963).
Lancet. 2: 1095.

MEYER, C.J. (1965).
Steroids, Suppl. 1: 199.

MEYER, C.J., LAYNE, D.S., TAIT, J.F. and PINCUS, G. (1961).
J. clin. Invest. 40: 1663.

de MOOR, P., STEENO, O., BROSENS, I. and HENDRIKX, A. (1966).
J. clin. Endocr. Metab. 26: 71.

MORANDINI, G. and MANGIONI, C. (1969).
Acta med. scand. 185: 311.

MORRIS, C.J.O.R. and WILLIAMS, D.C. (1953).
Biochem. J. 54: 470.

MULROW, P.J. (1964a).
Can. med. Ass. J. 90: 277.

MULROW, P.J. (1964b).
In discussion "Aldosterone: a symposium"
Edited by E.E. Baulieu and P. Robel, Oxford: Blackwell. p. 511.

NACHTIGALL, L., BASSETT, M., HOGSANDEE, U. and LEVITZ, M. (1968).
Am. J. Obstet. Gynec. 101: 638.

NAGATSU, I., GILLESPIE, L., GEORGE, J.M., FOLK, J.E. and
GLENNER, G.G. (1965).
Biochem. Pharmac. 14: 853.

NEWMAN, R.L. (1957).
Obstet. and Gynec. 10: 51.

NEWTON, M.A., SEALEY, J.E., LEDINGHAM, J.G.G. and LARAGH, J.H. (1968).
Am. J. Obstet. Gynec. 101: 1037.

NIELSEN, M., DAMGJAER, BINDER, C. and STARUP, J. (1969).
Acta endocr., Copnh. 60: 473.

NIELSON, I. and MØLLER, I. (1968).
Acta med. scand. 183: 381.

O'CONNELL, M. and WELSH, G.W. (1969).
J. clin. Endocr. Metab. 29: 563.

OGDEN, E., HILDEBRAND, G.J. and PAGE, E.W. (1940).
Proc. Soc. exp. Biol. Med. 43: 49.

PAAVY, P. (1959).
Acta obstet. gynec. scand. 38: 297.
PAGE, E.W. (1939).
Am. J. Obstet. Gynec. 37: 291.
PAGE, E.W. (1947).
Am. J. med. Sci. 213: 715.
PAGE, E.W. and BUMPUS, F.M. (1961).
Physiol. Rev. 41: 331.
PAIVA, T.B. and PAIVA, A.C.M. (1960).
J. Pharmac. exp. Ther. 130: 177.
PICKENS, P.T., BUMPUS, F.M., LLOYD, A.M., SMITH, R.R. and PAGE, I.H. (1965).
Circulation Res. 17: 438.
PICKERING, G. (1968).
High Blood Pressure. Second edition. Churchill, London.
PICKERING, G.W. and PRINZMETAL, M. (1938).
Clin. Sci. 3: 211.
PIENTL, A.A. and GRAY, M.J. (1959).
Am. J. obstet. Gynec. 78: 472.

RAAB, W., SCHROEDER, G., WAGNER, R. and GIGEE, W. (1956).
J. clin. Endocr. Metab. 16: 1196.
RINSLER, M.G. and RIGBY, B. (1957).
Br. med. J. 2: 966.
ROBERTSON, E.G. (1968).
J. Reprod. Fert. 16: 323.
ROBERTSON, J.I.S., WEIR, R.J., DÜSTERDIECK, G.O., FRASER, R. and TREE, M. (1971).
Scott. med. J. 16: 163.
ROBINSON, H.J., BERNHARD, W.G., GRUBIN, H., WANNER, H., SEVERON, G.W. and SILDER, R.H. (1955).
J. clin. Endocr. Metab. 15: 317.
ROSENTHAL, H.A., SHAUNWHITE, W.R. and SANDBERG, A.A. (1969).
J. clin. Endocr. Metab. 29: 362.

ROUTH, A. (1911).
Proc. R. Soc. Med. 5: Obstet. Gynaec. Sec.: 1.
ROY, E.J., HARKNESS, R.A. and KERR, M.G. (1963).
J. Obstet. Gynaec. Br. Commonw. 70: 597.
RYAN, J.W. (1970).
Biochem. J. 116: 159.

SALVATORE, C.A. (1968).
Am. J. Obstet. Gynec. 102: 347.
SAMAAN, N.A., BRADBURY, J.T. and GOPLERUD, C.P. (1969).
Am. J. Obstet. Gynec. 104: 781.
SANDBERG, A.A. and SLAUNWHITE, W.R. (1959).
J. clin. Invest. 38: 1290.
SARUTA, T., SAADE, G.A. and KAPLAN, N.M. (1970).
Archs. intern. Med. 126: 621.
SCHMIDT, A.W. and ROSENTHAL, J. (1971).
Arch. Gynak. 210: 39.
SCHWEITZER, M., BRANCHAUD, C. and GIROUD, C.J.P. (1969).
Steroids. 14: 519.
SHORT, R.V. and ETON, B. (1959).
J. Endocr. 18: 418.
SIMPSON, S.A., TAIT, J.F., WETTSTEIN, A., NEHER, R., van EUW, J.V.
and REICHSTEIN, T. (1953).
Experientia. 9: 333.
SIMS, E.A.H. (1964).
In discussion "Aldosterone: a symposium." Ed. E.E. Baulieu
and P. Robel. Oxford. Blackwell. p. 512.
SIMS, E.A.H. (1965).
A. rev. Med. 16: 221.
SIMS, E.A.H. and KRANTZ, K.E. (1958).
J. clin. Invest. 37: 1764.
SKINNER, S.L. (1967).
Circulation Res. 20: 391.
SKINNER, S.L., LUMBERS, E.R. and SYMONDS, E.M. (1968).
Am. J. Obstet. Gynec. 101: 529.

SKINNER, S.L., LUMBERS, E.R. and SYMONDS, E.M. (1969).
Clin. Sci. 36: 67.

SKINNER, S.L., LUMBERS, E.R. and SYMONDS, E.M. (1972).
Clin. Sci. 42: 479.

SOPHIAN, J. (1955).
J. Obstet. Gynaec. Br. Commonw. 62: 37.

SPELLACY, W.N. and BIRK, S.A. (1972).
Am. J. Obstet. Gynec. 112: 912.

STARK, G. (1967).
Arch. Gynäk. 204: 259.

STARK, G. and LEHMANN-ACHILLES, J. (1964).
Acta endocr., Copnh. 47: 469.

STARUP, J., SELE, V. and BUUS, O. (1969).
Acta endocr., Copnh. 53: 1.

STEWART, C.P., ALBERT-RECHT, F. and OSMAN, L.M. (1961).
Clinica chim. Acta 6: 696.

SUNDSFJORD, J.A. and AAKVAAG, A. (1970).
Acta endocr., Copnh. 64: 452.

SYMONDS, E.M., STANLEY, M.A. and SKINNER, S.L. (1968).
Nature, Lond. 217: 1152.

TAIT, J.F. and LITTLE, B. (1968).
J. clin. Invest. 47: 2423.

TAIT, J.F., LITTLE, B., TAIT, S.A.S. and FLOOD, C. (1962).
J. clin. Invest. 41: 2093.

TALLEDO, O.E. (1967).
Am. J. Obstet. Gynec. 97: 571.

TALLEDO, O.E. (1968).
Am. J. Obstet. Gynec. 101: 254.

TALLEDO, O.E., RHODES, K. and LIVINGSTON, E. (1966).
Am. J. Obstet. Gynec. 96: 141.

TALLEDO, O.E., CHESLEY, L.C. and ZUSPAN, F.P. (1968).
Am. J. Obstet. Gynec. 100: 218.

TATUM, H.J. and MULE, J.G. (1962).
Am. J. Obstet. Gynec. 83: 1028.
THOMAS, J.P. and FLYNN, T.G. (1964).
Clin. Sci. 26: 69.
TIGERSTEDT, R. and BERGMAN, P.G. (1898).
Scand. Arch. Physiol. 8: 223.
TOBIAN, L. (1949).
J. clin. Endocr. Metab. 9: 319.
TREE, M. (1972).
J. Endocr. In press.

VANDER, A.J. (1967).
Physiol. Rec. 47: 359.
VANDER, A.J. and GEELHOED, G.W. (1965).
Proc. Soc. exp. Biol. 120: 399.
VENNING, E.H. (1946).
Endocrinology. 38: 203.
VENNING, E.H. and BYRENFURTH, I. (1956).
J. clin. Endocr. Metab. 16: 426.
VENNING, E.H., PRIMROSE, T., CALIGARIS, L.C.S. and BYRENFURTH, I. (1957).
J. clin. Endocr. Metab. 17: 473.
VENNING, E.H., SINGER, B. and SIMPSON, G.A. (1954).
Am. J. Obstet. Gynec. 67: 542.

WALLACE, M.E. (1971).
Aust. N.Z. J. Med. 1: 49.
WALLACH, E.E., GARCIA, C.R., KISTNER, R.W. and PINCUS, G. (1963).
Am. J. Obstet. Gynec. 87: 991.
WALTERS, W.A.W. and LIM, Y.L. (1970).
J. Obstet. Gynaec. Br. Commonw. 77: 1007.
WATANABE, M., MEHER, C.I., GRAY, M.J., SIMS, E.A.H. and SOLOMON, S. (1963).
J. clin. Invest. 42: 1619.

WATANABE, M., MEEKER, C.I., GRAY, M.J., SIMS, E.A.H. and SOLOMON, S. (1965).
J. clin. Endocr. Metab. 25 1665.

WATANABE, T. (1961).
J. Jap. Obstet. Gynec. Soc. 8: 29.

WEINBERGER, M.H., COLLINS, R.D., DOWDY, A.J., NOKES, G.W. and LUETSCHER, J.A. (1969).
Ann. intern. Med. 71: 891.

WEIR, R.J., PAINTIN, D.B., ROBERTSON, J.I.S., TREE, M., FRASER, R. and YOUNG, J. (1970a).
Proc. R. Soc. Med. 63: 1101.

WEIR, R.J., TREE, M., and FRASER, R. (1970b).
J. clin. Path. 23: Suppl. 3: 49.

WEIR, R.J., PAINTIN, D.B., BROWN, J.J., FRASER, R., LEVER, A.F., ROBERTSON, J.I.S., and YOUNG, J. (1971a).
J. Obstet. Gynaec. Br. Commonw. 78: 590.

WEIR, R.J., TREE, M., FRASER, R., CHINN, R.H., DAVIES, D.L., DÜSTERDIECK, G.O., ROBERTSON, J.I.S., HORNE, C.H.W. and MALLINSON, A.C. (1971b).
Proc. 3rd Inter. Cong. Hormonal Steroids, Hamburg 1970.
Excerpta Medica International Congress Series No. 219, p. 929.

WEIR, R.J., DÜSTERDIECK, G.O., FRASER, R. and TREE, M. (1971c).
Nebenwirkungen contraceptiver Steroide Symposium, Berlin, 1970.
Westkreuz-Verlag, p. 106.

WEIR, R.J., BRIGGS, E., BROWNING, J., MACK, A., NAISMITH, L., TAYLOR, L. and WILSON, E. (1971d).
Lancet. 1: 467.

WEIR, R.J., TREE, M., and McELWEE, G. (1972).
Proc. 4th Inter. Cong. Endocr., Washington, 1972. In press.

van de WEILE, R.L., GURPIDE, E., KELLY, W.G., LARAGH, J.H., and LIEBERMAN, S. (1960).
Acta endocr., Copnh. Suppl. 51: 159.

WIEST, W.G. (1967).
Steroids. 10: 279.

WILSON, A. and FRASER, R. (1971).
J. Endocr. 51: 557.
WINER, B.M. (1965).
J. clin. Invest. 44: 1112.
WINTER, M., VELDHUYZEN, B. and DORHOUT MEES, E.J. (1972).
Lancet. 1: 1263.
WOODS, J.W. (1967).
Lancet. 2: 653.

YANNONE, M.E., McCURDY, J.R. and GOLDFIEN, A. (1968).
Am. J. Obstet. Gynec. 101: 1058.
YOUNG, J. (1914).
Proc. R. Soc. Med. 7 Obstet. Gynaec. Sec: 307.

APPENDIX

Tables 1 to 33.

		16	64·0	138/78	2570	13·8	41·4	135·5	3·5	102·5	284	6·9	29·8	59	—	—	—
0	164·0	28	68·5	128/80	3100	11·6	35·8	136·5	4·0	102·0	282	5·9	30·2	24	—	—	—
		34	70·3	130/88	3170	13·6	40·2	138·0	3·7	103·0	286	7·0	30·3	25	—	—	—
		38	72·2	132/84	3080	14·3	40·8	136·5	4·0	100·5	289	6·2	29·4	28	—	—	—
		16	53·5	112/74	2690	13·0	36·5	136·0	4·0	101·5	279	6·4	31·8	25	—	—	—
0	158·5	28	56·9	114/74	2900	11·8	34·8	136·0	3·9	103·0	285	6·9	29·2	54	—	—	—
		34	58·9	106/76	3500	9·9	32·9	134·0	3·4	103·0	285	6·9	32·5	20	—	—	—
		38	59·4	122/72	2910	11·0	34·5	134·0	3·4	103·0	285	7·2	30·5	38	—	—	—
		16	51·9	118/68	3210	10·5	32·8	138·0	3·6	102·0	284	6·2	30·7	36	154	38·0	0·37
0	158·5	28	55·3	98/62	3860	10·7	32·4	137·0	3·7	103·0	285	5·9	30·4	39	164	29·0	0·74
		34	58·0	114/68	3710	12·2	36·5	137·0	3·6	99·5	287	6·0	30·6	33	164	29·0	0·84
		38	59·8	112/72	3820	13·1	39·3	137·0	3·4	103·0	283	7·1	31·1	35	267	34·0	2·20
		16	53·1	122/62	2520	11·3	35·6	135·0	3·7	103·0	283	5·9	30·5	52	202	6·8	0·81
0	167·0	28	59·9	98/64	3300	11·3	33·9	133·5	3·3	103·5	290	6·5	30·0	59	133	18·1	2·50
		34	61·7	118/66	3630	12·0	34·7	136·0	3·6	104·0	286	6·6	31·6	29	185	7·2	1·14
		38	63·3	112/68	3850	12·1	33·3	138·5	3·6	103·5	284	6·4	29·1	48	60	21·5	2·10
		16	51·7	110/62	2720	11·7	38·0	131·5	3·3	103·0	280	6·2	30·1	190	—	—	—
0	153·5	28	60·4	120/72	2880	11·8	38·4	136·5	3·6	103·5	273	6·4	28·7	63	—	—	—
		34	63·1	120/72	3290	12·0	38·4	137·0	3·5	103·5	272	6·4	29·7	93	—	—	—
		38	65·1	112/72	2990	12·9	39·4	137·0	3·7	100·0	273	6·3	27·8	41	—	—	—
		16	73·4	112/48	3540	11·9	34·3	138·0	3·9	104·0	288	6·6	28·8	21	—	—	—
0	145·0	28	79·8	126/58	4300	11·1	33·9	136·5	3·4	102·5	290	6·4	30·1	31	—	—	—
		34	82·5	120/64	3320	10·4	33·6	135·5	3·6	103·5	283	6·2	30·0	29	—	—	—
		38	85·7	112/62	3580	12·1	37·1	135·0	3·4	103·5	282	6·6	27·9	47	—	—	—
		16	57·1	130/70	2640	12·8	37·0	133·5	4·0	103·0	278	6·5	32·2	41	—	—	—
3	162·5	28	61·6	126/62	4690	12·7	36·6	134·0	3·8	104·0	285	6·7	28·7	52	—	—	—
		34	63·7	124/76	5030	12·2	37·7	132·0	3·4	103·0	282	6·5	30·7	70	—	—	—
		16	71·1	122/76	2850	13·9	38·7	137·0	3·7	—	291	6·8	30·3	12	393	13·7	—
0	167·5	28	77·8	110/78	3540	13·1	38·0	135·0	3·4	99·0	281	5·7	29·2	15	190	12·6	—
		34	81·0	118/74	4190	12·4	38·2	135·0	3·4	103·0	281	6·9	29·3	14	98	15·4	—
		38	81·0	112/68	3240	13·2	37·2	135·0	3·7	102·5	281	7·2	28·6	19	222	21·9	—
		16	62·5	114/62	2960	11·7	36·7	140·0	4·0	103·5	284	6·6	30·4	36	—	—	—
0	158·5	28	68·5	124/62	3530	11·9	36·7	139·0	3·8	102·5	283	6·4	30·7	37	—	—	—
		34	70·1	122/72	3680	12·6	35·3	138·0	3·7	103·5	272	6·5	30·4	31	—	—	—
		38	72·6	118/68	3240	11·2	33·8	138·0	4·1	104·5	274	6·4	30·2	23	—	—	—
		16	61·7	116/75	3350	12·0	33·9	135·5	3·3	102·5	282	6·2	31·1	—	63	18·0	1·6
0	165·0	28	66·5	112/60	3280	9·7	33·9	135·5	3·4	101·5	285	6·0	29·3	36	90	21·0	1·5
		34	68·3	110/68	3630	9·8	34·3	137·0	3·5	102·5	287	6·2	29·8	28	65	22·0	1·0
		38	67·6	98/60	3810	12·0	36·0	138·0	3·4	103·5	273	6·4	31·1	35	102	26·0	0·78
		16	56·7	110/72	2690	11·7	34·2	132·0	3·3	104·0	282	7·0	32·0	—	—	—	—
0	165·0	28	61·9	128/80	3500	8·6	30·3	133·0	3·1	104·0	290	6·0	30·7	22	—	—	—
		34	64·0	110/76	3050	10·7	32·2	135·0	3·4	104·0	284	6·7	31·3	16	—	—	—
		38	65·1	110/73	2620	11·4	34·8	136·0	3·4	103·0	271	6·7	30·3	—	—	—	—
		16	70·3	138/60	3460	10·9	33·8	136·0	3·5	102·0	283	6·9	29·7	32	61	25·4	1·39
0	164·0	28	78·3	132/70	4400	11·9	34·9	136·0	3·4	103·0	284	6·2	30·6	39	54	22·4	0·88
		34	81·0	144/92	4640	12·3	36·6	134·0	3·3	—	283	6·2	29·8	32	58	30·9	1·11
		38	83·0	150/96	4340	12·3	37·2	135·8	3·5	103·0	285	6·5	29·9	45	54	21·7	1·08
		16	62·6	112/62	3710	11·2	34·4	137·5	3·9	101·0	283	6·0	31·0	35	—	—	—
0	162·5	28	74·4	140/85	4500	12·6	36·0	136·5	3·7	102·5	282	6·4	30·9	39	—	—	—
twins		34	80·7	150/94	4460	12·5	37·0	135·5	3·7	103·5	287	6·4	31·5	33	—	—	—
		16	58·5	134/76	2960	12·0	35·4	134·5	3·8	103·5	283	6·6	31·1	—	339	—	3·7
0	158–5	28	64·8	132/74	3670	11·0	33·3	134·0	3·5	101·5	288	6·5	30·6	—	253	—	1·6
		34	67·4	132/82	3540	11·0	34·8	138·0	3·7	104·5	280	6·5	30·3	9	100	—	0·7
		38	68·1	140/94	3760	12·3	39·4	136·5	3·6	102·5	281	6·5	31·7	19	74	—	0·6
		16	60·2	117/67	2980	12·1	36·3	136·0	3·7	103·0	283	6·5	30·6	52	232	17·6	0·92
s 1–12	160·5	28	65·7	115/66	3590	11·3	34·9	135·5	3·6	103·0	285	6·3	29·8	37	163	19·7	1·83
		34	68·1	116/72	3730	11·6	35·9	135·5	3·5	103·0	283	6·5	30·5	34	121	17·8	0·96
		38	69·8	114/67	3410	12·4	36·7	136·5	3·6	103·0	279	6·7	29·6	33	148	25·8	1·67

nents available for 38 weeks gestation.

TABLE 2. Plasma Renin Concentration (Units/l)

Gestation (Weeks)					Post Partum	
0 – 12		13 – 26	27 – 40		0 – 2	6 – 12
75.4	54.6	105.0	43.1	53.8	19.3	9.5
63.0	64.0	29.8	33.7	14.5	6.7	9.7
44.9	41.6	39.6	35.2	44.1	10.0	5.0
37.6	27.0	78.7	36.0	40.8	14.7	6.5
40.4	59.8	37.8	32.0	43.9	7.1	5.4
48.5		24.7	48.6	39.3	14.4	4.4
24.5		16.8	27.8	30.5	15.0	10.9
24.0		45.5	29.5	45.6	5.5	6.9
24.1		26.0	34.2	42.9	28.4	10.3
55.9		29.0	13.7	50.2	5.2	8.1
70.3		31.0	37.0		9.5	9.2
34.0		26.0	30.0		14.0	9.2
36.3		41.0	38.0		13.0	7.9
42.0		37.5	18.7		4.3	4.8
46.8		17.8	53.2		8.1	6.5
53.0		67.1	53.8		17.8	5.5
55.8		62.1	15.8		31.0	
30.4		34.7	32.1		14.0	
92.0		44.0	11.9		18.0	
56.4		28.1	35.9		8.1	
51.5		24.4	32.8		51.3	
39.7		25.4	30.8		19.9	
36.7		22.1	27.8		14.9	
30.2		45.3	65.3		36.0	
44.0			48.7		25.0	
74.0			40.0		11.7	
35.5			20.0			
Mean	47.1	39.1	35.4		16.6	7.5
± 1 S.D.	16.6	20.8	12.0		10.7	2.1
n	32	24	37		26	16

TABLE 3. - Plasma Renin Substrate Concentration (μM)

Gestation (Weeks)						Post Partum	
0 - 12		13 - 26		27 - 40		0 - 2	6 - 12
2.09	1.78	2.81	4.05	4.74	3.98	2.77	0.60
3.11	1.89	2.74	3.30	2.75	3.82	3.71	2.88
0.99	1.00	3.10	2.08	3.65	5.07	2.90	3.92
1.46	2.20	2.41	2.64	3.92	4.10	1.69	3.97
1.24	1.18	2.28	3.44	4.26	4.48	1.98	0.80
1.69	2.67	3.60	2.89	4.39	3.72	4.68	0.97
1.30	2.82	2.43	2.96	3.60	3.58	4.56	1.08
2.16	2.86	3.83	1.64	3.46	5.28	4.56	1.21
0.90	1.81	1.91	2.84	3.69	4.85	3.99	1.28
1.26	0.68	3.05	4.98	6.10	4.98	2.27	0.67
0.79		2.62		4.97	3.33	3.84	0.85
1.56		2.55		3.85	3.90	2.32	0.84
1.39		2.58		3.21	3.64	2.51	3.75
1.10		2.71		2.94	3.05	1.61	4.63
2.19		3.45		4.03	3.10	5.09	0.76
1.80		3.31		3.92	4.26	2.67	2.16
5.55		3.73		2.09	3.94	2.63	2.02
2.11		2.70		3.52	4.84	3.18	0.81
1.74		3.56		3.90	5.75	3.57	2.74
1.90		2.54		2.90	3.43	3.76	1.31
0.84		1.19		2.84	6.95	3.71	
1.99		2.76		1.76	4.45	2.25	
1.26		2.70		4.72		4.42	
2.62		2.13		1.40		1.96	
0.65		3.82		3.73		3.77	
1.62		3.22		3.85		4.71 3.78	
2.84		5.20		3.25		1.37 6.59	
1.76		2.97		3.98		3.41	1.86
D. 0.73		0.81		1.07		1.29	1.31
37		37		49		29	20

TABLE 4

Plasma Angiotensin II Concentration (pg/ml)

Gestation (Weeks) 0 – 12		13 – 26		27 – term		Post Partum 0 – 2	6 – 12
22	16	33	26	38	127	37	9
20	27	20	18	132	53	23	7
54	47	10	145	29	232	35	18
30	37	27	60	37	18	23	20
10		51	85	39	49	20	25
25		27	38	42	43	17	14
15		29	132	21		9	20
22		70	25	37		30	16
17		103		70		17	3
15		17		25		4	
11		28		67		4	
25		16		47		4	
9		28		27		15	
28		37		220		5	
24		30		14		30	
29		52		30		25	
14		40		218		24	
14		132		159		106	
97		14		173		18	
73		38		415		9	
12		100		26		89	
18		37		167		7	
52		80		67			
17		111		48			
58		93		29			
52		49		115			
60		59		97			
30.3		53.1		88.2		24.9	14.4
S.D. 21.2		37.5		87.1		25.7	6.8
31		35		33		22	9

TABLE 5

Plasma Aldosterone Concentration (mug/100 ml).

Gestation (Weeks)			Post Partum	
0 - 12	13 - 26	27 - term	0 - 2	6 - 12
18.6	10.7	57.0	7.4	13.3
22.3	35.0	103.1	11.0	
35.0	82.0	23.1	10.3	
42.1	12.3	14.0	11.0	
61.7	21.3	20.0	37.0	
47.6	34.8	17.0	7.0	
14.2	29.6	21.0	25.5	
58.5	30.2	41.6	7.2	
54.9	107.3	89.9	147.2	
4.0	85.2	50.7	12.9	
27.8	48.8	167.3	3.9	
17.0	17.8	40.1	2.9	
13.5	45.1	28.6		
70.0	29.5	75.9		
50.8	24.0	33.4		
8.7	43.5	44.0		
42.0	37.0			
20.9				
10.9				
19.1				
27.4				
38.4				
11.0				
55.0				
32.1	40.8	51.7	23.6	
S.D. 19.4	26.8	40.3	40.1	
24	17	16	12	

TABLE 6

Plasma Sodium Concentration (mEq/l).

Gestation (Weeks)						Post-Partum	
0 - 12		13 - 26		27 - term		0 - 2	6 - 12
135	135	135	132	132	131	135	139
135	136	135		139	132	135	134
139	136	137		135	130	135	135
135	139	140		140	134	136	134
137	134	139		138	135	140	135
139	135	136		137	135	135	139
137	132	138		135	131	145	139
135	134	137		136	136	132	134
135		138		135	128	134	134
137		138		138	135	137	131
132		140		137	134	137	138
137		137		135	137	134	134
138		135		138	130	134	139
137		136		136	132	137	140
135		137		132	131	139	141
135		134		132	135	132	
129		137		135	132	138	
141		138		138	135	146	
135		131		132		135	
135		137		132		137	
137		134		135		135	
136		132		134		139	
135		128		135		136	
132		130		135		130	
137		131		132		135	
						132	
135		135		134		135	
135.0		135.4		134.0		135.7	136.3
2.7		3.1		2.7		3.7	3.1
34		27		44		27	15

Plasma Potassium Concentration (mEq/l).

Gestation (Weeks)						Post-Partum	
0 - 12		13 - 26		27 - term		0 - 2	6 - 12
3.8	3.6	3.8	3.2	3.7	3.9	3.8	3.5
4.0	4.0	3.5	4.2	3.4	3.1	4.3	3.4
4.0	3.7	3.6		4.0	3.7	4.3	3.0
4.3	3.4	3.2		3.9	3.7	3.4	4.4
3.7	3.2	3.8		4.0	4.2	3.8	3.4
3.8	3.7	3.5		3.9	4.0	3.9	4.0
4.0	3.8	3.8		3.7	3.5	4.6	3.8
3.9	3.1	3.5		3.9	3.6	3.3	3.7
4.1	3.4	3.7		3.6	3.0	4.9	4.2
4.3		3.9		3.7	3.6	4.6	3.7
4.1		4.5		3.3	3.2	3.4	4.0
3.8		3.8		3.6	4.0	4.3	4.1
4.1		3.4		4.2	3.4	4.2	3.7
3.3		3.4		3.6	3.7	3.9	4.0
3.5		3.8		3.3	3.6	4.0	
4.0		3.1		3.5	3.6	4.5	
3.7		3.7		4.0	3.6	4.1	
3.7		3.6		3.7	4.6	4.4	
3.6		3.6		4.2		4.8	
3.8		3.8		3.7		4.0	
3.0		3.5		3.9		4.5	
4.1		3.7		3.9		3.7	
4.0		3.5		4.5		3.9	
3.7		3.8		3.5		5.0	
3.6		3.2		3.4		4.2	
3.4		3.4		3.7		3.7	
						4.0	
						3.8	
						4.0	
3.75		3.70		3.72		4.18	3.78
0.32		0.41		0.33		0.46	0.37
35		28		44		29	14

Plasma Osmolality (mosm/kg)

Gestation (Weeks)				Post-Partum	
0 – 12	13 – 26	27 – term		0–2	6–12
271	270	286	272	273	287
271	268	293	276	285	272
256	275	277	277	273	281
270	278	280	273	276	280
280	272	275	275	278	282
271	279	271	275	286	275
271	274	275	271	277	279
266	273	274	279	275	278
276	271	275	[illegible]	279	281
270	278	271		278	285
272	272	270		278	286
277	272	285		280	279
271	279	273		280	293
271	276	275		282	
275		271		282	
		275		275	
		272		278	
		272		288	
		272		281	
		275		285	
		276		282	
		270		282	
		271		286	
		276		275	
		278			
		272			
271.2	274.1	274.8		279.7	281.4
5.4	5.5	4.9		4.2	5.4
15	14	34		24	15

Urine Sodium Concentration (mEq/l).

Gestation (Weeks)			Post-Partum	
0 - 12	13 - 26	27 - term	0 - 2	6 - 12
66	125	73	121	178
92	152	168	119	54
72	165	73	45	195
126	142	66	140	145
	169	106	124	128
		115	97	124
		96	121	144
		77	107	77
		204	167	122
		89	88	107
		174	177	
		130	95	
		97	84	
		93	75	
		77	88	
		132	65	
		68	104	
		66	137	
		48	135	
		56	183	
		106	129	
		132	111	
			157	
			127	
			102	
89.0	130.2	102.0	115.9	127.2
27.1	24.9	40.7	33.8	42.2
4	5	22	25	10

TABLE 10

Urine Potassium Concentration (mEq/l).

Gestation (Weeks)			Post-Partum	
0 - 12	13 - 26	27 - term	0-2	6-12
26	68	33	60	61
40	86	52	55	35
37	17	33	20	18
69	40	55	58	46
		58	25	44
		43	28	41
		32	41	50
		39	30	52
		50	55	37
		44	41	37
		76	62	
		58	43	
		49	48	
		38	62	
		47	45	
		51	42	
		32	38	
		23	42	
		31	63	
		58	87	
		41	46	
		34	41	
			35	
			37	
			39	
43.0	52.7	44.4	46.5	42.1
D. 18.3	30.4	12.4	14.6	11.6
4	4	22	25	10

TABLE 11

Blood Urea Concentration (mg/100 ml).

Gestation (Weeks)				Post-Partum	
0 - 12	13 - 26	27 - term		0 - 2	6 - 12
19	19	19	16	16	35
17	14	15	20	33	30
14	14	13	23	22	28
18	9	12	19	32	28
13	18	18	16	21	20
13	17	14	17	19	31
15	13	14	25	23	32
18	10	17	30	30	34
25	15	16	18	25	26
13	18	22	11	31	22
16	20	16	21	15	27
22	21	13	21	20	42
16	24	17	23	25	23
20	16	7	17	22	22
17	16	25	17	25	40
22	16	20	16	28	
20	23	23	14	25	
17	24	10		28	
14	11	15		25	
24	25	11		28	
17	18	18		14	
17	22	13		25	
	34	22		27	
	19	15		20	
				17	
		17		24	
		15		22	
				30	
				19	
17.6	18.2	17.2		23.7	29.3
3.5	5.5	4.6		5.1	6.5
22	24	43		39	15

Serum Creatinine Concentration (mg/100 ml).

Gestation (Weeks)				Post-Partum	
0 - 12	13 - 26	27 - term		0 - 2	6 - 12
0.57	0.80	0.60	1.20	0.70	0.80
0.70	0.90	0.80	0.80	0.60	1.00
0.50	0.80	0.50	0.60	0.70	0.95
0.55	0.50	0.55	0.57	0.70	1.00
0.60	0.60	0.52	0.70	0.65	0.75
0.55	0.50	0.70	0.50	0.70	0.70
0.65	0.60	0.51	0.75	0.60	0.90
0.65	0.69	0.70	0.75	0.60	0.90
0.75	0.80	0.65	0.65	0.50	0.80
0.80	0.60	0.75	0.60	0.55	0.80
0.50	0.50	0.60		0.60	0.85
0.65	0.65	0.70		0.80	0.80
	0.65	0.60		0.80	0.70
	0.50	0.57		0.60	0.90
		0.40		0.80	0.90
		0.65		0.85	
		0.62		0.70	
		0.59		0.75	
		0.67		0.80	
		0.50		0.60	
		0.62		0.95	
		0.60		0.70	
		0.60		0.75	
		0.50		0.80	
		0.48		0.90	
		0.60			
0.62	0.64	0.63		0.71	0.85
0.09	0.13	0.14		0.11	0.10
12	14	36		25	15

TABLE 13

Creatinine Clearance (mls/min).

Gestation (Weeks)			Post-Partum	
0 - 12	13 - 26	27 - term	0 - 2	6 - 12
192	94	151	78	63
157	123	159	42	56
223	138	92	99	84
131	167	136	87	87
133	86	107	75	112
176		111	107	53
		86	105	108
		116	87	94
		74	91	
		139	71	
		112	82	
		104	128	
		132	91	
		52	65	
		126	142	
		102	67	
		154	112	
		112	69	
		93	66	
		134	81	
		147	108	
		107	79	
		162	87	
168.7	121.6	117.7	87.8	82.1
S.D. 35.7	33.0	28.3	22.3	22.8
6	5	23	23	8

TABLE 14 . Correlations - All Pregnancy Samples.

		n	r	Significance	
Renin	Substrate	145	0.119	$p > 0.1$	
Renin	Angiotensin II	65	0.071	$p > 0.1$	
Renin	Aldosterone	68	0.064	$p > 0.1$	
Substrate	Angiotensin II	77	0.033	$p > 0.1$	
Substrate	Aldosterone	72	+0.245	$p < 0.05$	*
Angiotensin II	Aldosterone	32	0.009	$p < 0.1$	
Progesterone	Renin	25	0.001	$p > 0.1$	
Progesterone	Substrate	25	+0.685	$p < 0.001$	*
Progesterone	Angiotensin II	26	+0.551	$p < 0.01$	*
Progesterone	Aldosterone	10	0.483	$p > 0.1$	
Renin x Substrate	Angiotensin II	65	0.123	$p > 0.1$	
Renin x Substrate	Aldosterone	63	0.141	$p > 0.1$	
Plasma Sodium	Renin	140	0.028	$p > 0.1$	
Plasma Sodium	Substrate	138	0.037	$p > 0.1$	
Plasma Sodium	Angiotensin II	72	0.014	$p > 0.1$	
Plasma Sodium	Aldosterone	65	+0.257	$p < 0.05$	*
Plasma Potassium	Renin	142	0.084	$p > 0.1$	
Plasma Potassium	Substrate	140	0.142	$p > 0.1$	
Plasma Potassium	Angiotensin II	71	0.141	$p > 0.1$	
Plasma Potassium	Aldosterone	67	0.138	$p > 0.1$	

TABLE 14 - continued

		n	r	Significance	
Osmolality	Renin	98	0.017	$p > 0.1$	
Osmolality	Substrate	98	0.098	$p > 0.1$	
Osmolality	Angiotensin II	16	+0.578	$p < 0.02$	*
Osmolality	Aldosterone	50	0.258	$0.05 < p < 0.1$	
Urea	Renin	127	0.022	$p > 0.1$	
Urea	Substrate	125	0.110	$p > 0.1$	
Urea	Angiotensin II	52	0.036	$p > 0.1$	
Urea	Aldosterone	45	0.101	$p > 0.1$	
Creatinine	Renin	106	0.061	$p > 0.1$	
Creatinine	Substrate	102	0.062	$p > 0.1$	
Creatinine	Angiotensin II	16	0.329	$p > 0.1$	
Creatinine	Aldosterone	51	0.022	$p > 0.1$	
Creat. Clear.	Renin	50	0.050	$p > 0.1$	
Creat. Clear.	Substrate	45	-0.493	$p < 0.01$	*
Creat. Clear.	Angiotensin II	13	0.012	$p > 0.1$	
Creat. Clear.	Aldosterone	21	0.119	$p > 0.1$	
Urine Sodium	Renin	46	0.124	$p > 0.1$	
Urine Sodium	Substrate	44	0.186	$p > 0.1$	
Urine Sodium	Angiotensin II	5	0.593	$p > 0.1$	
Urine Sodium	Aldosterone	21	0.081	$p > 0.1$	
Urine Potassium	Renin	45	0.019	$p > 0.1$	
Urine Potassium	Substrate	43	0.076	$p > 0.1$	
Urine Potassium	Angiotensin II	4	0.824	$p > 0.1$	
Urine Potassium	Aldosterone	20	+0.446	$p < 0.05$	*
Plasma Sodium	Progesterone	30	0.002	$p > 0.1$	
Plasma Potassium	Progesterone	29	0.026	$p > 0.1$	
Serum Urea	Progesterone	30	0.266	$p > 0.1$	

TABLE 14 - continued

		n	r	Significance	
Plasma Bicarb.	Renin	13	0.264	$p > 0.1$	
Plasma Bicarb.	Substrate	12	0.008	$p > 0.1$	
Plasma Bicarb.	Angiotensin II	22	0.346	$p > 0.1$	
Plasma Bicarb.	Aldosterone	2	-	$p > 0.1$	
Plasma Bicarb.	Progesterone	6	0.746	$0.05 < p < 0.1$	
Urine Oestriol	Renin	34	0.059	$p > 0.1$	
Urine Oestriol	Substrate	32	0.170	$p > 0.1$	
Urine Oestriol	Angiotensin II	-	-	-	
Urine Oestriol	Aldosterone	15	0.266	$p > 0.1$	
Plasma Sodium	Osmolality	91	+0.267	$p < 0.02$	*
Plasma potassium	Osmolality	93	0.191	$0.05 < p < 0.1$	

* Statistically significant at 5% level.

TABLE 15 . Correlations - for each trimester.

Trimester			n	r	Significance	
I	Angiotensin	Renin	37	0.0026	$p > 0.1$	
II	"	"	23	0.1230	$p > 0.1$	
III	"	"	18	0.0774	$p > 0.1$	
P.P.	"	"	17	+0.5536	$p < 0.05$	*
I	Angiotensin	Substrate	38	+0.3705	$p < 0.05$	*
II	"	"	25	0.1584	$p > 0.1$	
III	"	"	16	0.2168	$p > 0.1$	
P.P.	"	"	10	+0.6057	$0.05 < p < 0.1$	
I	Angiotensin	Renin x Substrate	35	0.3210	$p > 0.1$	
II	"	"	22	0.1738	$p > 0.1$	
III	"	"	11	0.1468	$p > 0.1$	
P.P.	"	"	9	0.4774	$p > 0.1$	
I	Angiotensin	Aldosterone	19	0.0187	$p > 0.1$	
II	"	"	10	0.2374	$p > 0.1$	
III	"	"	6	0.1651	$p > 0.1$	
P.P.	"	"	2	-	-	
I	Aldosterone	Renin	28	+0.3857	$p < 0.05$	*
II	"	"	18	0.3038	$p > 0.1$	
III	"	"	30	0.1542	$p > 0.1$	
P.P.	"	"	13	0.2057	$p > 0.1$	
I	Aldosterone	Substrate	30	0.1075	$p > 0.1$	
II	"	"	17	0.3240	$p > 0.1$	
III	"	"	24	0.1293	$p > 0.1$	
P.P.	"	"	13	0.4408	$p > 0.1$	
I	Aldosterone	Renin x Substrate	28	0.2133	$p > 0.1$	
II	"	"	17	0.0816	$p > 0.1$	
III	"	"	23	0.1350	$p > 0.1$	
P.P.	"	"	13	0.3322	$p > 0.1$	

TABLE 15 - continued

Correlations - for each trimester

Trimester			n	r	Significance	
I	Aldosterone	Plasma Sodium	30	0.2220	$p > 0.1$	
II	"	"	17	+0.4154	$0.05 < p < 0.1$	
III	"	"	24	+0.5505	$p < 0.01$	*
P.P.	"	"	11	0.1126	$p > 0.1$	
I	Aldosterone	Plasma Potassium	31	0.0087	$p > 0.1$	
II	"	"	16	0.2699	$p > 0.1$	
III	"	"	23	0.1388	$p > 0.1$	
P.P.	"	"	13	0.2080	$p > 0.1$	
I	Aldosterone	Osmolality	15	0.1477	$p > 0.1$	
II	"	"	12	0.3630	$p > 0.1$	
III	"	"	21	0.2067	$p > 0.1$	
P.P.	"	"	9	0.5272	$p > 0.1$	
I	Renin	Substrate	45	0.0856	$p > 0.1$	
II	"	"	34	0.1319	$p > 0.1$	
III	"	"	78	0.0385	$p > 0.1$	
P.P.	"	"	30	0.0357	$p > 0.1$	
I	Renin	Sodium	41	0.1160	$p > 0.1$	
II	"	"	29	0.2150	$p > 0.1$	
III	"	"	72	0.0784	$p > 0.1$	
P.P.	"	"	26	0.5224	$p < 0.01$	*
I	Renin	Potassium	41	0.0635	$p > 0.1$	
II	"	"	29	0.1459	$p > 0.1$	
III	"	"	74	0.1632	$p > 0.1$	
P.P.	"	"	28	0.3258	$0.05 < p < 0.1$	

TABLE 15 - continued

Correlations - for each trimester

Trimester			n	r	Significance
I	Renin	Osmolality	17	0.0970	p > 0.1
II	"	"	13	0.1169	p > 0.1
III	"	"	64	0.0892	p > 0.1
P.P.	"	"	25	0.0942	p > 0.1
I	Angiotensin	Sodium	34	0.2757	p > 0.1
II	"	"	23	0.1792	p > 0.1
III	"	"	15	0.2651	p > 0.1
P.P.	"	"	6	0.2880	p > 0.1
I	Angiotensin	Potassium	33	0.1599	p > 0.1
II	"	"	23	0.1629	p > 0.1
III	"	"	15	0.1219	p > 0.1
P.P.	"	"	6	0.2626	p > 0.1

* Statistically significant at 5% level.

TABLE 16. Plasma DOC Concentration (ng/100 ml).

Gestation (weeks)			Post-partum
0 - 12	13 - 26	27 - term	0 - 2 weeks
19.0	26.3	19.8	17.3
14.0	24.4	34.7	10.1
19.0	14.6	20.0	20.1
15.0	20.6	20.0	11.5
13.0	17.1	35.0	8.8
15.0	16.3	10.0	14.8
	33.3	12.0	14.7
	9.1	35.0	11.6
	16.0	7.0	9.2
	18.0	16.0	6.1
	15.0	11.0	
		25.0	
15.8	19.2	20.5	12.4
2.6	6.7	10.1	4.3
6	11	12	10

I v II	t = 1.1613	(15)	$p > 0.1$
II v III	t = 0.3621	(21)	$p > 0.1$
I v III	t = 1.0904	(16)	$p > 0.1$
III v P.P.	t = 2.3443	(20)	$p < 0.05$

TABLE 17. Sodium and potassium balance study.

Case	Age	Parity	Gest. (weeks)	Duration of Test. (days)	Mean Sodium (mEq/day)			Mean Potassium (mEq/day)			Mean Plasma Sodium mEq/l	Mean Plasma Pott. mEq/l	Renin units/l	Sub.^{s} µM.	A.II pg/ml	Aldo mug/100
					Intake	Output	Balance	Intake	Output	Balance						
1	36	1 $^{+o}$	7-9	17	130.0	119.4	+11.6	70.0	71.1	-1.1	132.2	3.53	53.0	2.11	73.0	35.
			24	3	138.4	138.9	-0.5	49.1	57.1	-8.0	136.5	3.40	24.7	2.55	132.0	35.
			12 P.P.	3	128.0	150.6	-22.6	61.0	60.3	+0.7	139.0	3.55	9.5	0.60	16.0	5.7
2	30	I^{+o}	11	3	128.2	127.0	+ 1.2	46.9	54.4	-7.5	141.0	3.65	30.4	2.86	18.0	-
			25	3	148.0	141.2	+ 6.8	53.0	62.3	-9.3	137.0	3.80	16.8	-	14.0	82.
			38	3	141.0	135.0	+ 6.0	49.0	51.3	-2.3	138.0	3.85	-	3.65	14.0	103.

Table 18 . Plasma Renin Concentration (units/l).

Hypertension + Albuminuria					Normal				
Case	Age	Parity	Gest^{n}.	Renin	Case	Age	Parity	Gest^{n}.	Renin
L.G.	17	0	36	20.7	E.P.	17	0	36	26.2
J.C.	18	0	36	35.3	E.W.	19	0	36	36.1
C.L.	19	0	37	11.7	W.M.	30	0	38	57.0
H.H.	20	0	38	48.6	N.Z.	21	0	38	85.8
S.R.	20	1	32	38.0	M.B.	21	1	32	65.3
S.B.	26	0	37	32.8	C.B.	26	0	30	18.9
M.C.	28	0	35	9.4	J.M.	28	0	30	20.1
J.B.	29	0	34	25.9	V.H.	26	0	30	33.1
E.K.	26	0	39	52.0	G.G.	25	0	40	52.9
H.Y.	26	0	38	23.7	D.C.	25	0	40	36.8
M.J.	25	1	35	30.6	K.F.	25	1	34	39.0
S.R.	25	2	32	26.0	S.S.	26	2	32	46.6
H.S.	28	3+	28	20.4	J.H.	28	2	26	37.5
A.McI.	27	2	38	29.5	S.B.	30	2	36	32.0
A.W.	35	0	28	44.8	M.K.	35	0	24	43.3
M.N.	37	0	34	16.4	S.T.	28	0	30	75.7
A.H.	36	1	30	22.6	A.McD.	35	1	33	51.3
S.K.	38	3+	28	71.3	B.B.	40	2	30	48.7
J.B.	35	3+	38	23.2	M.C.	41	2	34	40.0
C.C.	20	0	36	28.3	E.T.	20	0	36	30.0
Mean	26.7		34.4	30.6		26.8		33.2	43.8
± 1 S.D.	6.6		3.6	14.8		6.7		4.4	17.3

Table 19. Plasma Renin-Substrate Concentration (uM).

Hypertension + Albuminuria					Normal				
Case	Age	Parity	Gest.n	Renin Substrate	Case	Age	Parity	Gest.n	Renin Substrate
L.G.	17	0	36	2.47	E.P.	17	0	36	1.69
J.C.	18	0	36	5.38	E.W.	19	0	36	3.50
C.L.	19	0	37	2.69	M.McD.	20	0	38	2.80
M.C.	23	0	31	3.84	J.M.	21	0	30	3.64
H.H.	20	0	38	1.07	M.Z.	21	0	38	4.88
S.R.	20	1	32	2.70	M.D.	21	1	32	3.08
S.B.	26	0	37	3.46	M.M.	25	0	38	3.47
J.B.	29	0	34	2.01	C.B.	26	0	30	3.10
E.K.	26	0	39	3.10	G.G.	25	0	40	5.75
H.Y.	26	0	38	2.96	D.C.	25	0	40	4.15
M.J.	25	1	35	4.19	K.F.	25	1	34	6.25
S.R.	25	2	32	3.44	S.S.	26	2	32	3.55
H.S.	28	3+	28	1.50	J.B.	28	2	26	3.73
A.W.	35	0	28	2.56	S.T.	28	0	30	2.73
M.N.	37	0	34	2.65	M.K.	35	0	36	3.05
A.H.	36	1	30	1.11	A.McD.	35	1	28	2.93
S.K.	38	3+	28	3.80	B.B.	40	2	30	1.40
C.C.	20	0	36	3.40	E.T.	20	0	36	7.48
Mean	26.0		33.8	2.76		25.4		33.9	3.73
± 1 S.D.	6.7		3.7	0.93		6.1		4.3	1.52

Table 20 . Plasma Angiotensin II Concentration. (pg/ml)

Hypertension + Albuminuria					Normal				
Case	Age	Parity	Gest^{n}.	Angio.II	Case	Age	Parity	Gest^{n}.	Angio.II
C.L.	19	0	37	20	M.F.	19	0	30	132
J.C.	18	0	36	25	W.M.	20	0	38	67
L.G.	17	0	36	10	K.McQ.	21	0	38	21
M.C.	23	0	31	16	L.W.	23	0	30	28
M.C.	28	0	35	18	J.M.	28	0	38	43
A.W.	35	0	28	10	M.K.	35	0	24	38
A.H.	36	1	30	7	P.F.	36	1	36	220
C.C.	20	0	36	13	J.M.	21	0	30	77
Mean	24.5		33.6	14.9		25.4		33.0	78.2
±1 S.D.	7.6		3.4	6.0		6.8		5.2	67.4

Table 21 . Plasma Aldosterone Concentration (mug/100 ml).

Hypertension + Albuminuria					Normal				
Case	Age	Parity	Gest^{n}.	Aldo.	Case	Age	Parity	Gest^{n}.	Aldo.
L.G.	17	0	36	9.0	E.P.	17	0	36	33.0
S.R.	20	1	32	25.5	M.B.	21	1	32	40.1
M.C.	28	0	35	44.0	S.T.	28	0	36	44.0
E.K.	26	0	39	15.7	G.O.	25	0	40	36.4
H.Y.	26	0	38	7.0	P.H.	23	0	34	21.0
M.J.	25	1	35	13.0	P.H.	23	1	34	218.0
S.R.	25	2	32	26.0	M.Q.	22	2	34	67.1
H.S.	28	3+	28	12.0	C.C.	31	3+	24	45.1
A.McI.	27	2	38	23.0	J.G.	24	3+	30	17.0
A.W.	35	0	28	22.0	M.K.	35	0	24	17.8
S.K.	38	3+	28	29.0	A.K.	32	3+	26	21.5
J.B.	35	3+	38	50.0	D.McD.	31	3+	38	50.1
Mean	27.5		33.9	23.0		26.0		32.3	50.9
±1 S.D.	6.1		4.2	13.3		5.4		5.3	54.7

Table 22. Plasma Sodium Concentration (mEq/l).

Hypertension + Albuminuria					Normal				
Case	Age	Parity	Gest^{n}.	Sodium	Case	Age	Parity	Gest^{n}.	Sodium
H.H.	20	0	38	139	N.E.	21	0	38	129
S.B.	26	0	37	136	M.M.	25	0	38	139
J.B.	29	0	34	133	C.B.	26	0	30	136
H.Y.	26	0	38	130	V.H.	26	0	38	136
M.J.	25	1	35	135	K.F.	25	1	34	134
S.R.	25	2	32	131	S.S.	26	2	32	133
A.McI.	27	2	38	138	S.D.	30	2	36	132
M.N.	37	0	34	128	M.K.	35	0	36	137
J.B.	35	3+	38	134	D.McD.	31	3+	38	130
M.C.	28	0	35	142	S.T.	28	0	36	138
M.C.	23	0	31	135	L.W.	23	0	30	135
S.K.	38	3+	28	139	E.C.	32	3+	29	133
H.S.	28	3+	28	134	C.C.	31	3+	29	138
A.W.	35	0	28	139	J.M.	28	0	30	138
Mean	28.7		33.9	135.2		27.6		33.9	134.9
±1 S.D.	5.5		3.9	3.9		3.8		3.7	3.1

Table 23. Plasma Potassium Concentration (mEq/l).

Hypertension + Albuminuria					Normal				
Case	Age	Parity	Gest^{n}.	Potas^{m}.	Case	Age	Parity	Gest^{n}.	Potas^{m}.
H.H.	20	0	38	3.8	N.Z.	21	0	38	2.7
S.B.	26	0	37	3.2	M.M.	25	0	38	4.2
J.B.	29	0	34	4.3	J.B.	26	0	30	4.0
H.Y.	26	0	38	4.0	V.H.	26	0	38	3.9
M.J.	25	1	35	3.9	K.F.	25	1	34	3.5
S.R.	25	2	32	4.3	S.S.	26	2	32	3.5
A.McI.	27	2	38	2.7	S.B.	30	2	36	3.3
M.N.	37	0	34	3.9	M.K.	35	0	36	3.3
E.K.	26	0	39	4.4	D.C.	25	0	40	3.5
J.B.	35	3+	38	3.8	D.McD.	31	3+	38	3.4
M.C.	28	0	35	4.0	S.T.	28	0	36	4.1
M.C.	23	0	31	4.5	L.W.	23	0	30	3.6
S.K.	38	3+	28	3.9	E.C.	32	3+	29	4.3
H.S.	28	3+	28	5.6	C.C.	31	3+	29	3.6
A.W.	35	0	28	4.1	J.M.	28	0	30	4.0
Mean	28.5		34.2	4.03		27.5		34.3	3.66
$\pm$1 S.D.	5.5		4.0	0.63		3.7		3.9	0.42

Table 24

Plasma Osmolality (mosm/kg)

Hypertension + Albuminuria					Normal				
Case	Age	Parity	Gest.^{n}	Osmol.^{y}	Case	Age	Parity	Gest.^{n}	Osmol.^{y}
H.H.	20	0	38	278	E.P.	17	0	38	273
S.D.	26	0	37	277	E.W.	19	0	36	275
J.B.	29	0	34	279	E.T.	20	0	32	272
H.Y.	26	0	38	272	M.McD.	20	0	37	271
M.J.	26	1	35	278	K.F.	25	1	34	272
S.R.	25	2	32	275	S.S.	26	2	32	270
A.McI.	27	2	38(T)	282	S.B.	30	2	36(T)	273
M.N.	37	0	34	281	N.Z.	21	0	36	268
E.K.	26	0	39	274	D.C.	25	0	40	273
Mean	26.8		36.1	277.1		22.6		35.7	271.9
±1S.D.	4.5		2.4	3.5		4.2		2.6	2.0

TABLE 25

Blood Urea Concentration (mg/100 ml.)

Hypertension + Albuminuria					Normal				
Case	Age	Parity	Gest^{n}.	Urea	Case	Age	Parity	Gest^{n}.	Urea
H.H.	20	0	38	25	N.Z.	21	0	38	16
J.B.	29	0	34	34	C.B.	26	0	30	21
H.Y.	26	0	38	14	M.M.	25	0	38	14
M.J.	25	1	35	30	K.F.	25	1	34	14
S.R.	25	2	32	20	S.S.	26	2	32	12
A.McI.	27	2	38	20	S.B.	30	2	36	17
M.N.	37	0	34	35	S.T.	28	0	36	18
E.K.	26	0	39	42	D.C.	25	0	40	26
M.C.	23	0	31	32	E.T.	20	0	32	15
S.K.	38	3+	28	36	E.C.	32	3+	29	9
A.W.	35	0	28	28	V.H.	26	0	30	10
Mean	28.3		34.1	28.7		24.0		34.1	15.6
±1 S.D.	5.9		4.0	8.3		8.5		3.8	4.9

TABLE 26

Serum Creatinine Concentration (mg/100 ml)

Hypertension + Albuminuria					Normal				
Case	Age	Parity	Gest^{n}.	Creat.	Case	Age	Parity	Gest^{n}.	Creat.
H.H.	20	0	38	1.45	N.Z.	21	0	38	0.50
J.B.	29	0	34	0.90	M.McD.	20	0	34	0.70
H.Y.	26	0	38	0.55	G.B.	22	0	39	0.70
M.J.	25	1	35	0.70	K.F.	25	1	34	0.70
S.R.	25	2	32	0.80	S.S.	26	2	32	0.60
A.McI.	27	2	38	0.75	S.B.	30	2	36	0.70
M.N.	37	0	34	1.10	E.W.	19	0	36	0.60
E.K.	26	0	39	0.90	D.C.	25	0	40	0.80
M.C.	25	0	31	0.90	E.T.	20	0	32	0.60
S.K.	38	3+	28	1.00	E.C.	32	3+	29	0.45
an	27.6		34.7	0.91		24.0		35.0	0.63
S.D.	5.7		3.6	0.25		4.4		3.5	0.11

TABLE 27

Creatinine Clearance (mls/min)

Hypertension + Albuminuria					Normal				
Case	Age	Parity	Gest^{n}.	Creatn.	Case	Age	Parity	Gest^{n}.	Creat.
H.H.	20	0	38	75	N.Z.	21	0	36	147
J.B.	29	0	34	69	MMcD	20	0	32	52
H.Y.	26	0	38	142	G.B.	22	0	39	107
M.J.	25	1	35	92	K.F.	25	1	38	94
S.R.	25	2	32	84	S.S.	26	2	32	162
A.McI.	27	2	38	100	S.B.	30	2	36	92
M.N.	37	0	34	34	D.C.	25	0	28	86
E.K.	26	0	39	86	E.T.	20	0	32	112
Mean	26.9		36.0	85.2		23.6		34.1	106.5
±1 S.D.	4.8		2.6	30.4		3.5		3.7	34.9

TABLE 28

URINE SODIUM CONCENTRATION

(mEq/l)

Hypertension + Albuminuria					Normal				
Case	Age	Parity	Gest^{n}.	Urine Sodium	Case	Age	Parity	Gest^{n}.	Urine Sodium
H.H.	20	0	38	22	N.Z.	21	0	36	56
J.B.	29	0	34	82	M.McD.	20	0	32	97
H.Y.	26	0	38	94	G.B.	22	0	39	66
M.J.	25	1	35	101	K.F.	25	1	38	79
S.R.	25	2	32	112	S.S.	26	2	32	138
A.McI.	27	2	38	152	S.B.	30	2	36	168
M.N.	37	0	34	170	D.C.	25	0	28	113
E.K.	26	0	39	124	E.T.	20	0	32	68
Mean	26.9		36.0	107.1		23.6		34.1	98.1
$\pm$ 1 S.D.	4.8		2.6	45.3		3.5		3.7	39.2

TABLE 29

Urine Potassium Concentration.

(mEq/l)

Hypertension + Albuminuria					Normal				
Case	Age	Parity	Gest^{n}.	Urine Potassium	Case	Age	Parity	Gest^{n}.	Urine Potassium
H.H.	20	0	38	15	N.Z.	21	0	36	58
J.B.	29	0	34	61	M.McD.	20	0	32	49
H.Y.	26	0	38	37	G.B.	22	0	39	55
M.J.	25	1	35	51	K.F.	25	1	38	55
S.R.	25	2	32	36	S.S.	26	2	32	29
A.McI.	27	2	38	68	S.B.	30	2	36	52
M.N.	37	0	34	80	D.C.	25	0	28	43
E.K.	26	0	39	49	E.T.	20	0	32	32
ean	26.9		36.0	49.6		23.6		34.1	46.6
S.D.	4.8		2.6	20.5		3.5		3.7	11.0

TABLE 30

URINE OESTRIOL EXCRETION

(ug/24 hr).

Hypertension + Albuminuria					Normal				
Case	Age	Parity	Gest.^{n}	Oestriol	Case	Age	Parity	Gest.^{n}	Oestriol
H.H.	20	0	38	23,600	N.Z.	21	0	36	10,000
J.B.	29	0	34	5,600	E.T.	20	0	32	10,500
H.Y.	26	0	38	28,750	G.B.	22	0	39	16,000
M.J.	25	1	35	6,750	K.F.	25	1	38	14,500
S.R.	25	2	32	5,500	S.S.	26	2	32	23,600
A.McI.	22	2	38	27,000	S.B.	30	2	36	16,500
M.N.	37	0	34	5,300	D.C.	25	0	28	11,100
ean	27.0		35.6	14,643		24.1		34.4	14,600
1 S.D.	5.2		2.4	11,157		3.4		3.9	4,775

Hypertensive Disease of Pregnancy

Correlations

		n	r	Significance	
Renin	Substrate	22	0.039	p > 0.1	
Renin	Angiotensin II	10	0.106	p > 0.1	
Renin	Aldosterone	18	0.060	p > 0.1	
Substrate	Angiotensin II	11	+0.756	p < 0.02	*
Substrate	Aldosterone	19	+0.416	0.05 < p < 0.1	
Angiotensin II	Aldosterone	8	0.177	p > 0.1	
Renin x Substrate	Angiotensin II	9	0.227	p > 0.1	
Renin x Substrate	Aldosterone	18	0.055	p > 0.1	
Plasma Sodium	Renin	20	+0.390	0.05 < p < 0.1	
Plasma Sodium	Substrate	18	0.089	p > 0.1	
Plasma Sodium	Angiotensin II	6	0.664	p > 0.1	
Plasma Sodium	Aldosterone	17	0.106	p > 0.1	
Plasma Potassium	Renin	21	0.047	p > 0.1	
Plasma Potassium	Substrate	19	0.267	p > 0.1	
Plasma Potassium	Angiotensin II	6	0.550	p > 0.1	
Plasma Potassium	Aldosterone	16	0.276	p > 0.1	
Serum Urea	Renin	17	0.353	p > 0.1	
Serum Urea	Substrate	16	0.069	p > 0.1	
Serum Urea	Angiotensin II	3	0.778	p > 0.1	
Serum Urea	Aldosterone	17	0.416	p > 0.1	
Serum Creatinine	Renin	16	0.354	p > 0.1	
Serum Creatinine	Substrate	15	-0.710	p < 0.01	*
Serum Creatinine	Angiotensin II	2	-	-	
Serum Creatinine	Aldosterone	11	0.272	p > 0.1	
Creat. Clear.	Renin	14	0.188	p > 0.1	
Creat. Clear.	Substrate	12	0.110	p > 0.1	
Creat. Clear.	Angiotensin II	0	-	-	
Creat. Clear.	Aldosterone	9	-0.664	0.05 < p < 0.1	

TABLE 31 - continued

Hypertensive Disease of Pregnancy

Correlations

		n	r	Significance
Urine Sodium	Renin	14	0.171	$p > 0.1$
Urine Sodium	Substrate	12	0.594	$p > 0.1$
Urine Sodium	Angiotensin II	0	-	-
Urine Sodium	Aldosterone	9	0.107	$p > 0.1$
Urine Potassium	Renin	14	0.135	$p > 0.1$
Urine Potassium	Substrate	12	0.192	$p > 0.1$
Urine Potassium	Angiotensin II	0	-	-
Urine Potassium	Aldosterone	9	0.442	$p > 0.1$
Urine Oestriol	Renin	12	0.450	$p > 0.1$
Urine Oestriol	Substrate	11	0.491	$p > 0.1$
Urine Oestriol	Angiotensin II	0	-	-
Urine Oestriol	Aldosterone	9	0.435	$p > 0.1$
Urine Protein	Renin	19	0.173	$p > 0.1$
Urine Protein	Substrate	18	0.188	$p > 0.1$
Urine Protein	Angiotensin II	11	0.208	$p > 0.1$
Urine Protein	Aldosterone	17	0.3415	$p > 0.1$
Systolic	Renin	19	0.207	$p > 0.1$
Systolic	Substrate	18	0.362	$p > 0.1$
Systolic	Angiotensin II	11	0.004	$p > 0.1$
Systolic	Aldosterone	17	0.294	$p > 0.1$
Diastolic	Renin	19	0.173	$p > 0.1$
Diastolic	Substrate	18	0.103	$p > 0.1$
Diastolic	Angiotensin II	11	0.041	$p > 0.1$
Diastolic	Aldosterone	17	0.364	$p > 0.1$

Statistically significant at 5% level.

TABLE 32

Correlations in women taking oral contraceptives.

Comparisons		r	Degrees of freedom	Significance
Renin	Substrate	0.228	17	$p > 0.1$
Renin	Angiotensin	0.130	5	$p > 0.1$
Renin	Aldosterone	0.391	8	$p > 0.1$
Angiotensin	Substrate	0.043	8	$p > 0.1$
Angiotensin	Renin x Substrate	0.135	5	$p > 0.1$
Angiotensin	Aldosterone	-	-	-
Aldosterone	Substrate	0.201	8	$p > 0.1$
Aldosterone	Renin x Substrate	0.378	8	$p > 0.1$

TABLE 33

	Renin (units/l)		Substrate (uM)		Angiotensin II (pg/ml)	
	BP raised	BP normal	BP raised	BP normal	BP raised	BP normal
n	11	20	11	22	12	18
Mean	8.2	8.6	2.66	2.62	21.7	15.8
± 1 S.E.	1.0	1.5	0.30	0.14	3.1	1.3
t-statistic	0.005		0.070		1.974	
p	> 0.1		> 0.1		0.05 < p < 0.1.	