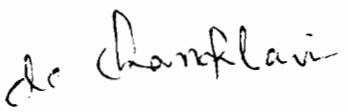
Studies on renin-angiotensin system in humans.



STUDIES ON RENIN-ANGIOTENSIN SYSTEM IN VARIOUS HUMAN PHYSIOLOGICAL AND PATHOLOGICAL STATES

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To my wife,

whose great understanding and

encouragement made this work possible.

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I - INTRODUCTION

The corner-stone of the Renin-Angiotensin System was laid down almost seven decades ago when Tigerstedt and Bergman gave the name "Renin" to the pressor substance they had discovered in kidney extracts (1). However, this discovery was forgotten for many years before its importance was recognized. This recognition came in 1934 when Goldblatt demonstrated the role of the kidney in the pathogenesis of a certain type of hypertension (2) and especially after it appeared that the renal pressor substance might be involved in the pathogenesis of experimental and human hypertension. Thereafter, for many years, the renin-angiotensin system became closely and almost exclusively associated with the field of hypertension.

This interest in hypertension, concomitantly, resulted in increased knowledge of the chemistry, and enzymology of the components of this system (3). It was then found in the years 1939-1940 that the active compound of the system was not renin but an octapeptide which is known today as angiotensin II (4, 5, 37). Although the role of the renin angiotensin system has been extensively studied in various hypertensive diseases, much controversy still exists today. The lack of knowledge concerning the purification and isolation of proteins and polypeptides, and, the lack of sensitive chemical or physico-chemical methods for the measurement of renin and angiotensin are largely responsible for this stagnation.

Recently a new field of action was opened to the renin-angiotensin system, namely the control of aldosterone and, by extension, the regulation of sodium. This emerged simultaneously from two major series of observations. Studies dealing with the control of aldosterone secretion have produced evidence in favor of its humoral control by a substance originating

from the kidney (6). Simultaneously, in 1960, it was demonstrated by Genest and coworkers (7) that angiotensin II markedly, and quite specifically, stimulated aldosterone excretion in man, independently of its pressor effect. Thereafter, the knowledge of the two fields of research became united and gave impetus to a tremendous increase in interest.

Although the aldosterone-stimulating effect of angiotensin had been confirmed by many workers when the present work was initiated, little was known concerning the role of endogenous renin and angiotensin in the physiological control of aldosterone and in the pathological states of hyperaldosteronism.

The development, in our laboratory, of two sensitive, specific and reproducible methods for the determination of plasma angiotensin and renin activity by Boucher and coworkers (8) enabled us to undertake this study:

Peripheral plasma renin activity was measured in various physiological conditions generally associated with increased aldosterone excretion and secretion such as during sodium restriction and depletion, during potassium loading, during fasting, and during normal pregnancy. Renin-activity and arterial angiotensin levels were also measured in pathological conditions such as generalized edema and hypertensive diseases of various etiologies.

In order to elucidate the possibility of a humoral feed-back on renin secretion, the effect of various infusions on the level of peripheral renin activity was sought for.

Finally, a new procedure for the determination of plasma angiotensinase activity was developed and a preliminary study of this parameter in various conditions was initiated.

II - HISTORICAL REVIEW

A - CHEMISTRY OF THE RENIN-ANGIOTENSIN SYSTEM.

1. RENIN:

a) Discovery:

The pressor property of kidney extracts was first reported in 1898 by Tigerstedt and Bergman (1) and by Livon (9). Nevertheless, the discovery of renin is attributed to Tigerstedt and Bergman since these investigators named and characterized the pressor material they had extracted from the normal rabbit kidneys. During the same period, the then current practice of injecting crude organ extracts into animals led several other workers to report the pressor effect of kidney extracts (10-14). However, because crude methods of extraction were used, depressor, as well as pressor, responses were observed. In the hands of some investigators, the depressor effect was even more pronounced (15, 16). Although the previous experiments of Tigerstedt and Bergman were repeated by Bingel and Strauss in 1909 (12), this discovery did not attract investigators of that generation and it fell into oblivion for many years.

Goldblatt's work (2) on experimental renal hypertension in 1934, again focussed attention on the kidney and stimulated intensive research on the role of the kidney in the pathogenesis of experimental and human hypertension. Renin became popular only after 1937, when it appeared that the renal pressor substance described by Tigerstedt and Bergman might be involved in the pathogenesis of experimental renal hypertension.

The vasopressor properties of normal kidney extracts were very soon reconfirmed (17-20) and its role in human hypertensive diseases was suspected (17, 18, 21). Thus it was felt that renin might be the answer to the

riddle of human hypertensive diseases.

The combined work of Page and Helmer, and Braun-Menéndez' group led to a better understanding of the chemical and enzymatic reactions involved in the biological action of renin and so established the concept of a renin-angiotensin system.

b) Biochemical properties:

Early studies gave an accurate account of the general properties of renin. This substance was found to be heat labile (1, 12, 19, 22, 23), non dialyzable (1, 12, 19, 22), acid and alkali labile (19) and insoluble in organic solvents (1, 12, 19, 22). Further purification studies by Katz and Goldblatt (23) revealed that renin is salted out with ammonium sulfate at pH 6.0 in concentration between 1.4 and 2.6 M, that it is more resistant to acid and alkali in cold temperature, and that it migrates toward the anode at pH 7.6 in electrophoretic studies. Several color reactions characteristic of either amino acids or peptide linkage were tested on crude renin preparation in order to elucidate the chemical structure of renin (24, 23). However, the presence of impurities in the various preparations diminished greatly the value of these studies. Hass and coworkers (25) studied the most highly purified renin preparation available by ultra-violet spectroscopy and they claimed three different active configurations which appeared easily reversible from one form to another. The molecular weight of renin has been grossly estimated on Sephadex column to be approximately between 42,000 and 49,000 (127). From these properties, renin may be considered to be a protein although it has not been obtained in pure form.

c) Purification:

The advances in the purification of renin seem to have reached a pla-

teau in the past twelve years.

The first methods for the purification of renin were crude and non specific since investigators were using the general properties of proteins for its extraction (24, 26, 27). The discovery of more specific properties of renin based on salt fractionation, on pH, and on temperature permitted the elaboration of better means of purification (20, 22, 23, 28, 29). The best purification of renin to date was achieved in 1953 by Haas and collaborators (30) with a method based on autolysis of renal tissue, extraction with water, precipitation with sodium tungstate and ammonium sulfate, serial fractionation with acetone, ethanol and ammonium sulfate, and dialysis. With this method, they were able to achieve a 56,000 fold purification of renin with a specific activity of 780 units/mg of dry substance. This degree of purity is many times greater than the one obtained with any previous or subsequent methods. Unfortunately, at this stage renin is still contaminated with slight angiotensinase activity, but recent modifications of the method with the use of ethylene diamine tetraacetic acid (EDTA) yield a renin preparation of a lesser purity but practically angiotensinase-free (31).

Although some degree of purification of renin was obtained with DEAE cellulose chromatography (32, 33), or with electrophoresis combined with kaolin adsorption and immunological precipitation of impurities (34), no better purification was achieved. Recently, Chandra, Skelton and Bernardis (35) were able to isolate renin in a well defined zone by multiple phase centrifugation including ultra centrifugation. The implications of this discovery in the purification of renin have not yet been evaluated.

d) Actions:

Since the numerous actions of renin are known today to be accomplish-

ed through the liberation of angiotensin, these will be reported and discussed in the section of this thesis reserved for the pharmacological actions of angiotensin.

i) As an enzyme:

The experiment of Kohlstaedt, Helmer and Page in 1938 (36), showing that renin has little or no vasoconstrictor action when perfused through isolated organs in Ringer's solution, is probably the first indication in the literature that renin "per se" was a vasoinactive substance and that the pressor effect observed in presence of plasma or whole blood was probably the result of some enzymatic reactions between renin and other constituents of the plasma. The enzymatic nature of renin was clearly established after the simultaneous work of Page and Helmer in United States (5) and Braun-Menéndez and coworkers in Argentina (37). These investigators showed that a substance in the plasma was activated by renin to form a vasopressor substance which has now been designated as angiotensin (37). The substance necessary for the pressor action of renin was called "renin-activator" by the Cleveland group, and "hypertensinogen" by the Buenos-Aires group. The renin-activator was believed to be a protein-like substance since it was destroyed by boiling (36) and was later reported to migrate with the $\alpha 2$-globulin fraction in electrophoretic systems (38). The liver was designated as the major source of this substance since only removal or injury of the liver resulted in a decrease in its concentration (39, 40). This substance, now called angiotensinogen, was extensively studied by Skeggs' group (41). These workers isolated and demonstrated the existence of 3 major and 2 minor forms of hog renin substrate. They could purify 3 of these forms and analyze their chemical composition. These studies established that the various

substrates are gluco-proteins having a molecular weight of about 57,000. The fourteen terminal amino acids of the polypeptide chain are now identified (41). Once these facts were known, the specific sites of breakdown of substrate by renin could be localized. Renin breaks specifically a leucyl-leucine bond in the polypeptide chain of the substrate liberating a decapeptide called Angiotensin I (41). The relationship between angiotensinogen and renin is further demonstrated by the observation that the substrate decreases after injection of renin (42-45) and increases after nephrectomy (42, 46).

Cross injection of renin between various species and cross incubation of renin with substrates coming from various species revealed that in certain circumstances renin was almost or completely inactive (47). This suggests that there probably exist various forms of renin as well as various forms of substrate.

ii) As a pressor agent:

It is well established today that the pressor effect of renin is accomplished by the liberation of angiotensin. However, the dose-pressor response curves of renin and angiotensin differ greatly because of the prolonged half-life of renin compared to the very short one of angiotensin. The usual pressor curve induced by a renin injection is characterized by a rapid rise in blood pressure followed by a slow decrease in 30 to 60 minutes, depending upon the dose given (48).

iii) As an antigen:

The antigenicity of renin was recognized as early as 1940 by Johnson and Wakerlin (49) when they prepared antibodies to renin by injecting hog renin into dogs. This property was shown to lower blood pressure in experimental hypertension (50). At first it appeared that the antigenicity was

closely related to pressor activity (52) but a recent work has clearly established that these two functions are separate since antigenic properties can be destroyed without any loss in pressor effect and vice versa (53). Important advances in that field were made recently by Deodhar, Haas and Goldblatt (54) when they succeeded in inducing the development of antirenin by means of injections of acetylated homologous renin, in dogs, rabbits and rats. The antirenin produced with acetylated dog renin in the dog, proved to be effective also, with a high degree of efficiency, against human renin. According to these investigators, these findings open the way to its application in the treatment of human hypertensive diseases (51).

e) Methods of measurement:

Since most groups of research workers have developed their own methods for renin measurement or have modified other's methods, it is impossible and rather confusing to cover them all. We shall mention the most commonly used methods and those most recently described. Older methods have been reviewed extensively by Braun-Menéndez and associates (47).

These methods of measurement can be divided into two major groups: the direct and indirect methods.

The direct methods consist of the injection into an animal of a solution containing partially purified renin and the measurement of its concentration by the rise in blood pressure. In this type of methodology, renin utilizes the substrate and other factors of the injected animal to induce its pressor effect by the production of angiotensin. Since the concentration of renin is low in blood and kidney, relatively large amounts of tissue and blood are required to yield a measurable activity. Many methods were proposed but very few were specific enough to give positive and reproducible results. Of all these, the most specific method is probably the one des-

cribed by Haas, Lamfron and Goldblatt (55) which is a simplification of their previous procedure of purification (30). One unit of renin, as suggested by Goldblatt and his group, is equal to that quantity of renin which produces a blood pressure rise of 30 mm Hg in the dog (48).

The indirect methods appraise the concentration of renin by measuring the quantity of angiotensin produced in vitro during a certain period of time by the incubation of renin and other factors of the plasma under specific conditions of pH and temperature. However, the kinetic formation of angiotensin is not solely dependent upon renin concentration, but is the result of the interaction of numerous factors present in the plasma. For this reason, the results obtained with these methods can hardly be extrapolated in terms of renin concentration, and, in this laboratory, the appellation of "renin-like activity" or "renin-activity" is considered to be in better conformity with the nature of the information given by these indirect methods.

Several indirect methods have been developed in the past 25 years but most of the older methods were neither sensitive nor reproducible. The availability of standard synthetic angiotensin, the use of new means of purification and greater knowledge concerning the kinetics of the renin-angiotensin system permitted Helmer (56), Boucher (8), Fasciolo (57), Hoobler (58), Skinner (59), Lever (33) and their respective coworkers to develop new and more sensitive indirect methods. Most of them use whole plasma (8, 57-59) or dialyzed plasma (56) for their incubation without trying to purify renin, but Lever and his associates prefer to purify renin partially before incubating it with an excess of ox substrate (33). In all methods the incubations are done at a constant temperature of 37° ± 1°C for 30 minutes (58), one hour (59, 56), two hours (57), for various incubation times varying from one to three hours (8, 33) or up to 96 hours (64). The amount of angioten-

tensin produced is finally estimated on a rat pressor-assay (8, 33, 57-59), on pithed cat, or on strip of aorta (56). The major limiting factor of these methods is the presence of angiotensinases in the incubation media. The sensitivity and recovery of these methods will thus be proportional to the efficiency in inhibiting or preventing the action of angiotensinases. Numerous means were proposed to lessen or to eliminate the angiotensinase activity during the incubation period. Most investigators adjust the incubation media at a pH varying between 5.1 to 5.7 (8, 33, 56-58) since, at this pH, the optimum activity of renin is obtained while the angiotensinase activity is greatly reduced. Others inactivate angiotensinase by acidification of the plasma below pH 4.0 at room temperature for 20 to 30 minutes before incubation (33, 57), but renin can also be partly inactivated by this procedure. EDTA was reported to inhibit completely the plasma angiotensinases (60) but incomplete inhibition was observed in a certain number of cases in this laboratory (8, see data on the effects of EDTA on "angiotensinase-activity" in the experimental section of this thesis). None of these procedures used alone or in association were found adequate for a complete and consistant inhibition of the angiotensinase-activity in the incubation media (72). Boucher (8) found that the addition of a resin (Dowex 50W-X2) to the media was the best mean of protecting angiotensin from the destruction by angiotensinase during incubation. This latter method is described in detail in the section of this thesis on methodology. Lever and associates (33) claimed also that no angiotensinase activity was present in their incubation media, but the long and complicated methodology necessary for the preparation of the various constituents of their enzymatic incubation contribute to give them a mean recovery of only 40% when renin is added at the start of the procedure.

Therefore, depending upon the care taken in the sampling of the blood, the efficiency in inhibiting angiotensinase during the formation of angiotensin and the purification of the final product, these methods can be more or less sensitive, reproducible and specific. The marked differences between the various methods and the lack of standardized units in the expression of results prevent quantitative comparison between the results reported by the various groups of investigators.

f) Metabolism and fate of renin:

When a large amount of renin is injected I.V. into the dog, 50% disappears after 10 minutes (44) and almost none is recoverable from the circulation after 30 minutes (39) or 60 minutes (44). The same rate of disappearance has been observed in the rat but renin remained in circulation up to two hours when higher doses were given (61). After nephrectomy in the dog, endogenous renin activity disappeared from peripheral blood in the following 30 minutes (62).

What is thus the fate of renin? Is it destroyed, stored or is it excreted? The presence in the blood of proteolytic enzymes able to destroy renin should easily solve the question but no neutralizing or destructive properties against renin could be demonstrated in blood from jugular vein, carotid artery, hepatic vein or renal vein (39). Moreover, it has been recently shown by Haas and collaborators (53) that renin is very resistant to proteolytic enzymes.

There is some evidence that the kidney might be an important factor controlling the destruction and/or excretion of renin. The disappearance rate of renin is prolonged in nephrectomized and in uremic dogs (39). Houssay and coworkers (39) noted that after intravenous injection of renin a fraction of it appears in the urine. Recently, Brown and his group (63) also reported

a large amount of "Renin-like" substance spontaneously occurring in the urine of normal subjects.

Renin-activity was also measured in various organs and fluids. It was detected in the lymph (65, 66), in amniotic fluid (67), in placenta (68-70), in the wall of aorta, in liver, in veins and, to a lesser extent, in some other vascular organs (70). However, since renin rapidly disappears from the circulation after nephrectomy (62, 71, 72), the extra-renal sources of renin are consequently excluded. The small amounts detected in extra-renal organs and structures might represent a storage of renin. In conclusion, it would seem, at first, that renin is metabolized mainly by the kidney, stored in various vascular structures or organs and probably excreted in urine but much more evidence is needed to clarify this question.

2. ANGIOTENSIN.

a) Discovery:

The demonstration of the enzymatic nature of renin initiated the search for the substance resulting from the action of renin on substrate. This was achieved with the simultaneous discovery of angiotensin by two groups of investigators. After incubating renin with plasma in vitro, Page and Helmer (5, 73) were able to isolate a potent vasoconstrictor substance which was heat stable, water and alcohol soluble and dialyzable. They proposed the name "angiotonin" for this pressor material. At the same time, in Argentina, Braun-Menéndez and his group were trying to elucidate the nature of the humoral mechanism involved in the production of experimental renal hypertension. They observed that the ischemic kidney was pouring into the circulation a potent pressor substance which was present in acetone extracts of the plasma (4, 37). They were also able to isolate this substance after

allowing renin to act in vitro on plasma. They called it "Hypertensin". Later, both groups agreed to standardize their nomenclature by proposing the hybrid word of angiotensin for the pressor substance and angiotensinogen for the substrate (74). In this thesis, the term "angiotonin" will be used to designate partially purified preparations, and the name "angiotensin" will be reserved for synthetic preparation or highly purified material.

b) Chemistry:

Early studies revealed that the chemical properties of angiotonin differed greatly from those of renin since angiotonin was found heat stable and dialyzable (5, 42). It was also found highly soluble in water, acetic acid and alcohol, partly soluble in acetone, insoluble in organic solvents, acid stable and alkali labile (5, 42). Page and Helmer observed that it was a reducing substance destroyed rapidly by strong oxidizing agents (5).

i) Isolation and purification:

First attempts to purify angiotensin resulted in only partially purified extracts (5, 29, 47, 76, 77). However, the development of new techniques of purification such as chromatography on paper, resin column and countercurrent distribution led quite rapidly to its purification during the years 1954-55. Purification of horse angiotensin was achieved by Skeggs (78), bovine angiotensin by Peart (80, 79) and hog angiotensin by Bumpus (3, 89, 90) and their respective coworkers. No purification of human angiotensin has yet been reported. The existence of two forms of angiotensin was suspected by Page and Helmer (81) in 1940. These investigators found that the injection of angiotonin into a rabbit's ear perfused with Locke-Ringer's solution caused vasoconstriction but the second and third injections were ineffective. They observed that the addition of normal plasma or plasma fraction restored

the ability of angiotonin to induce constriction. Although they did not prove the enzymatic nature of the conversion of the inactive form of angiotonin to the active form, they concluded that a factor present in the blood, an angiotensin activator, was necessary to convert angiotonin into a pressor substance. This hypothesis became obvious after the isolation and purification, by Skeggs and his group (82), of two distinct forms of angiotensin: angiotensin I, a decapeptide which is the inactive form and angiotensin II, an octapeptide which is the active form. These workers also established that the decapeptide is rapidly converted into the octapeptide form through the splitting of two terminal amino acids by a chloride ion dependent enzyme which they called converting enzyme, present in the plasma (82, 83). Using spirally cut strips of rabbit aorta, Helmer (84) was also able to demonstrate the existence of two forms of angiotensin. One form, angiotensin II, caused a contraction of the strip whereas the other form, angiotensin I, was inactive. When incubated with plasma or purified converting enzyme, angiotensin I induced a contraction of the strip, thus illustrating the enzymatic conversion of the inactive form into the active form. Angiotensin I and II are equally pressor when injected intravenously into animals, since in these conditions, angiotensin I is rapidly converted to angiotensin II by the excess of the converting enzyme present in the plasma of intact animals (84-86).

ii) Structure and synthesis:

Horse angiotensin I was analyzed by Skeggs and his group in 1955. They found a decapeptide presenting the following sequence (87):

1	2	3	4	5	6	7	8	9	10
ASP	ARG	VAL	TYR	ILEU	HIS	PRO	PHE	HIS	LEU

Angiotensin II was soon reported to be an octapeptide identical to the decapeptide except for the absence of the two terminal amino acids HIS-LEU (83).

Bumpus, Schwarz and Page found an identical sequence in hog angiotensins (89, 90). Elliot and Peart found identical amino acid sequence in bovine angiotensin except for valine in position 5 (79, 91). Since human angiotensin has not yet been purified, its amino acid sequence is unknown, but there is an indication that it is an amide form because it migrates at the same rate than the synthetic amide form in high or low voltage electrophoretic systems and on paper chromatography (8).

The advances achieved by Du Vigneault during his studies on the synthesis of polypeptides permitted and stimulated the work of two major groups, Bumpus' in United States and Schwyzer's in Switzerland. Due to the imagination and determination of these workers, naturally occurring angiotensins and many of their analogues were synthesized (92-100). One of these, α-asparagine 1 valine 5 angiotensin was shown to have an activity identical to that of the natural angiotensin (96) and it is now produced on a large scale and used by many investigators as a standard. Most of the synthetic analogues of angiotensin are less active than the natural compounds (94, 97), probably indicating that the natural compounds already represent the ideal configuration for an optimal activity. One exception now escapes the limits of this hypothesis. Since its synthesis (99), the β L angiotensin II was found 1.5-2 times more active than natural angiotensin in nephrectomized rats. However, it is postulated that this increase in activity is not dependent upon the effectiveness of the spatial configuration of this compound at the site of action but rather upon an increased resistance to proteolytic enzymes, namely amino peptidases (100-102). The study of these various analogues permitted further understanding of the constitual components responsible for the biologic activity of angiotensin II. These requirements for biological activity were summarized by Bumpus et al (103) as follows:

1- A free C-terminal carboxyl group

2- A phenyl group as a side group of the amino acid in position 8

3- A phenolic group on the amino acids in position 4

4- Proline in position 7

5- At least 6 amino acids from the C-terminus

6- Possibly a definite degree in spatial configuration

7- Possibly the imidazole of histidine in position 6

Bumpus and his associates (94) proposed an alpha-helix structure for angiotensin. Paiva et al (104) completely disagreed on that point. They stated that angiotensin shows a random conformation in aqueous solution and they concluded that a predetermined spatial arrangement of the molecule is not necessary for its biological activity. However, it is the opinion of Riniker (100) that further studies are required in this field to determine whether the effect of biologically active peptides is dependent upon the chemical structure of the peptide back bone or only on the correct spatial arrangement of a number of functional groups and side chains.

c) Measurement of angiotensin:

Measurement of the blood level of angiotensin has given rise to much controversy among various investigators. Since circulating angiotensin is found in minute amounts (in the order of few milli-micrograms per 100 ml of plasma) and since it is rapidly destroyed by the proteolytic enzymes present in the plasma, one can easily understand that the most meticulous care must be taken during the procedures to avoid its inactivation. The isolation of angiotensin requires numerous manipulations, so that only a method with a very high recovery rate will be able to give consistent and reproducible results.

The first methods described for angiotensin measurement consisted mainly

of bioassay of crude extracts obtained after heat precipitation of plasma (105, 106) and acidification (105) or dialysis of blood (107). Although some positive results were obtained in conditions known today to present with very high angiotensin levels, in most cases these methods were unable to detect any pressor substance because of their lack of specificity and sensitivity.

The first attempt at purification came with the method of Kahn and coworkers (108) when they proposed further purification of the crude ethanol extract by chromatography on alumina. This procedure gave them a recovery rate of 50 to 60%. Scornik and Paladini (109) extracted angiotensin from whole blood by ethanol precipitation and they introduced the use of Dowex 50W-X2 resin for the final purification of angiotensin. They obtained a mean recovery of 63% with this procedure. When Boucher and his coworkers tried to reproduce the previous procedure, they failed to obtain a similar recovery and they often detected the presence of depressive substances in the end product. This led them to develop a more specific method of extraction (110). They extracted angiotensin from whole blood by ethanol precipitation and used the Dowex resin for the purification of angiotensin according to the method of Scornik and Paladini, but they developed a different system for the elution of angiotensin from the resin and they introduced the use of a paper chromatographic system for the separation and purification of both forms of angiotensin. However, this method was time consuming and required a large amount of blood. Further modifications and improvements in the previous procedure permitted the achievement of a faster method of measurement which returned the previous sensitivity and specificity. This latter procedure, recently described by Boucher and coworkers (8), differs from the previous one as follows: Angiotensin is extracted from plasma in-

stead of whole blood, many steps are carried out in the cold room. A different chromatographic system (But:H_2O;CH_3CO_2H 45:50:10 v/v) is used in the final purification step. The use of cold prevents any significant formation or destruction of angiotensin during the process of purification. The mean recovery of angiotensin is 92% when added to the plasma and 83% when added to whole blood. Mulrow (111) also developed a method for the extraction of angiotensin from the plasma and although he advocated the use of successive column chromatography on Dowex 50W-X2 and I.R.C. 50 for the purification of angiotensin, he obtained a mean recovery of only 46% with his method. Finally, Morris and Robinson (112) described a method of extraction based on successive ethanol, methyl alcohol and ether precipitations. The final purification is achieved by the use of a paper chromatographic system identical to the one used by Boucher (110). A mean recovery of 85% was reported with this procedure.

The measurement of angiotensin is generally done by rat bioassay and the pressor activity is compared with that of synthetic valine 5 angiotensin II amide (Ciba preparation) (8, 109-112). All the methods previously reported measure both forms of angiotensin except for the methods described by Boucher et al (110, 8) and by Morris (112) which separate the two forms of angiotensin and measure only angiotensin II. Since in vivo, angiotensin I is rapidly converted to angiotensin II, no distinction can be made in assay on whole animals (82, 85).

When assay is done on isolated preparations in which the converting enzyme is missing a distinction is found between the two forms of angiotensin, as demonstrated by Helmer, using strips of aorta (84). It is also possible to obtain a good separation of the two forms of angiotensin by paper chromatography (110, 112-114).

d) Metabolism:

In vivo, the short pressor response induced by the injection of angiotensin points to a rapid inactivation of this substance. In vitro studies confirmed that angiotensin is rapidly inactivated during incubation with various tissues (47, 115). This suggests that angiotensin is probably destroyed by a group of different non specific enzymes present in tissues and blood. The binding of angiotensin to circulating proteins or to tissues might also represent another mean of inactivation but this is purely hypothetical and not yet proven. Wolf and his collaborators (116,117) have labelled synthetic angiotensin II with Iodine^{131} and studied its disappearance rate in human blood. They reported a half life of many hours up to 15 hours in normotensive and hypertensive subjects. This observation is quite surprising because the in vivo and in vitro biological half life of angiotensin is a matter of a few minutes. Although these investigators claimed that their product was stable and identical to angiotensin II (116), this substance was found to be quite unstable in the hands of other investigators who suggested that these workers were measuring the disappearance rate of the degradation products of angiotensin II rather than angiotensin itself (118, 119).

Khairallah, Bumpus et al (60, 120) have studied the fate and distribution of tritiated angiotensin in rats. In order to differentiate the active octapeptide from its metabolic fragments, they measured the radioactivity after an electrophoretic separation of their extracts. They found that in normal rats immediately following the infusion of a large dose, the active labelled peptide was primarily distributed to the adrenal, and secondarily to kidney and uterus. Thirty minutes after the cessation of infusion, only metabolically inactive fragments were found. In nephrectomized rats imme-

diately following an infusion of II^3 angiotensin, the distribution was more random, except in the liver where the radioactivity was greater. Also in such rats, angiotensin disappeared more slowly from the blood. They concluded from their studies that the high concentration of radioactivity in the kidneys and adrenals suggests that these organs either have a role in the metabolic pathway or are target organs of angiotensin (60).

Other workers have studied the angiotensin "clearance" in various organs (112, 121, 122). From these studies, it appeared that the major portion of angiotensin entering the liver (112, 121) and hind limbs (112, 121) is cleared from the blood while very little is retained by lung and heart (112, 121).

The knowledge concerning the biological fate of angiotensin is meager indeed and this chapter remains open to further investigation.

3. ANGIOTENSINASES.

Page and Helmer (5) and Braun-Menéndez et al (37) observed in their early studies that the formation of angiotensin was decreased when the incubation of renin with its substrate or plasma was prolonged. This phenomenon was later attributed to the destruction of angiotonin by a protein present in the plasma. At first, this enzyme appeared specific and was named angiotonin inhibitor (81) or hypertensinase (123, 124). On the light of present knowledge, this destruction now appears to be rather non-specific and probably involves the interaction of many proteolytic enzymes or mechanisms. So far, much evidence has been reported for the existence of several different angiotensinases and it would seem more acceptable to refer to "angiotensinase-activity" instead of concentration when measuring their activity.

a) General properties:

The properties of angiotensinases are summarized in the book of Braun-

Menéndez and coworkers (47). Angiotensinases are thermo-labile, non dialyzable and acid labile. The optimum pH of action is between 7.0 and 8.0 in the plasma, red blood cells and many tissues (42, 56, 60, 125, 126) but it was found to be pH 4.0 in the kidney (125).

Angiotensinase activity has always been a constant source of frustration to investigators dealing with renin and angiotensin purification or measurements. Many means have been suggested to inhibit that activity, but so far no specific inhibitor has been found. The action of the angiotensinases could be completely or partially overcome by acid treatment at pH 4.0 (42) by the use of charcoal (80), by acetylation or benzoylation (128), by diisopropylfluorophosphate (128) and by the ammonium salt of ethylene diamine tetraacetic acid (60, 8).

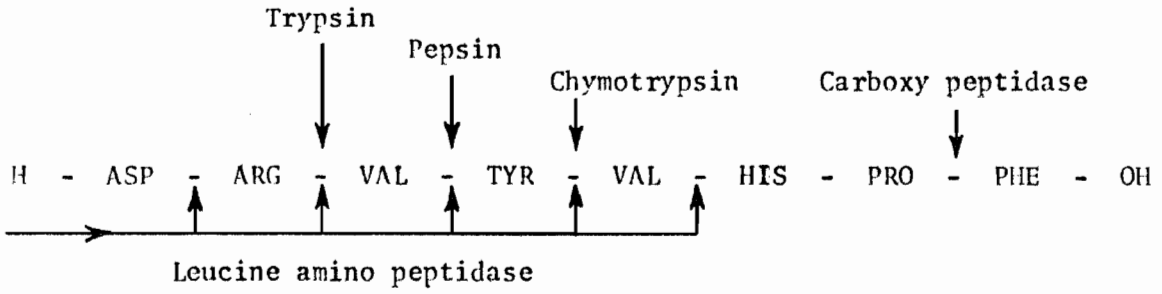
b) Nature:

The first indication that more than one angiotensinase existed, came after the observation by Helmer, Kohlstaedt and Page (125) that, in several tissues, the optimum pH for angiotensinase was 7.0-8.0 while it was 4.0 in the kidney. After the work of Khairallah, Bumpus and colleagues (102), it appeared that a peptidase with a high degree of specificity was present in plasma and red cells. This enzyme, called angiotensinase A, has an optimum pH between 7.5-8.0, is completely inhibited by EDTA and is reactivated by the addition of calcium. In contrast, other tissue angiotensinases are not completely inhibited by EDTA (60). Further studies with synthetic angiotensin analogues have demonstrated that angiotensinase A required an α L aspartic acid or an α L asparagine in position 1 for its activity (60, 129), thus indicating that this enzyme probably cleaves the peptide bond between the amino acids in position 1 and 2. Regoli, Riniker and Bunner (130) concluded, from similar studies, that the plasma contains at least two forms of angiotensinase,

the most important being an amino peptidase which is probably identical to angiotensinase A and the other one being an endopeptidase able to cleave angiotensin into two tetrapeptides.

Recently, purification of angiotensinase has been tried with various electrophoretic systems. These studies revealed that, in normal plasma, the maximum peak of activity is located in the α^1 globulin fraction (131, 132). Moreover, Klaus and Biron (132) also reported that when an increased angiotensinase activity was present in the plasma, they could detect some activity in the β^2-globulin and in the albumin fractions. This last distribution is not specific for only one enzyme but would support the existence in the plasma of various enzymes able to destroy or inactivate angiotensin.

In vitro experiments have revealed that angiotensin can be inactivated by various proteolytic enzymes. Angiotensin is inactivated by trypsin (4, 115, 133), pepsin (42, 115, 134), polypeptidases (115, 134), amine oxidase and tyrosinase (135). Riniker and Schwyzer (115) have studied the site of action of many enzymes on synthetic valine 5 angiotensin II amide and they have summarized their findings in the following schema:



Thus it would appear most probable that the angiotensin inactivation is accomplished by the interaction of various enzymes rather than by a single specific enzyme.

c) Methods of measurement:

There is no direct method for the measurement of angiotensinase concentration. Most investigators like Fasciolo (124), Dexter (29), Page (136), Hickler (137), Landesman (138) and their associates deduce the activity or concentration of angiotensinase by estimating the disappearance rate of the pressor activity of a known quantity of angiotonin or angiotensin incubated in presence of plasma, blood or homogenized tissues. However, the findings reported with these methods are conflicting and these discrepancies might be explained by some technical points which render them neither satisfactory nor reproducible:

1) All these methods imply prolonged incubation times varying from 20 minutes to 4 hours at 37° C. This also favors the production of angiotensin if renin is present in the incubation media. In these conditions, the destruction of angiotensin can hardly be adequately estimated if angiotensin is replaced as soon as it is destroyed.

2) These methods do not measure the true activity since, in all, the plasma or organ extract is diluted before incubation.

3) None of these methods use siliconized glassware for the processing of the incubation so that part of the angiotensin may be adsorbed on the glass. Since this adsorption is not constant (See Experimental data reported in this thesis on angiotensinase-activity), variations can be found in the disappearance rate of angiotensin without any changes in the angiotensinase activity. For these reasons the previous methods for the estimation of angiotensinase-activity are not satisfactory.

Others have proposed different means for the measurement of angiotensinase-activity. For instance, Wolf and coworkers (798) estimate the half-life of angiotensin by the removal of a radioactive iodine from the angiotensin

molecule; and Klaus (140, 797) measures the amount of valine freed during incubation of angiotensin with angiotensinase as an index of inactivation. None of these two methods give results which parallel the decrease in biological activity.

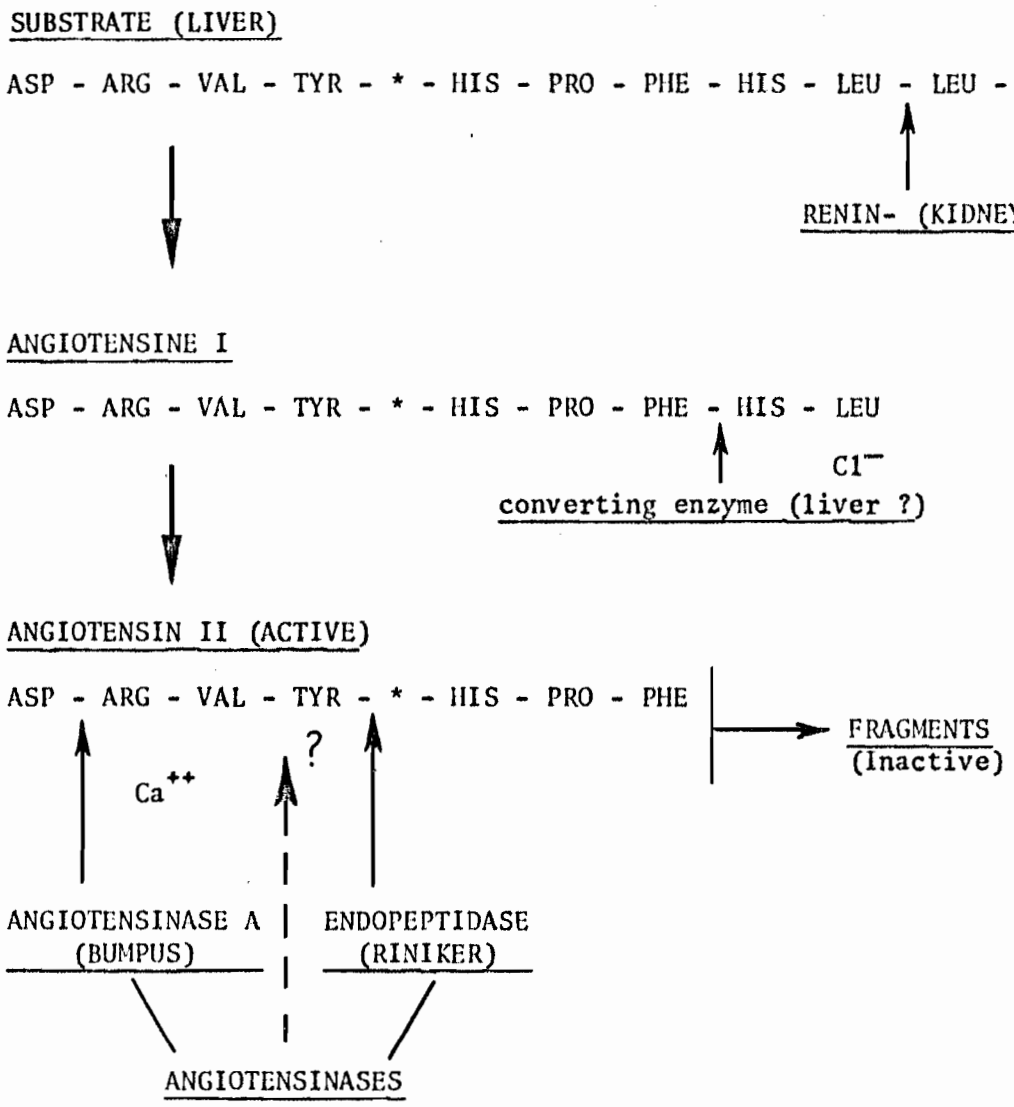
The various and contradictory results reported by these investigators will be discussed later in this thesis.

d) Concentration in various tissues:

Angiotensinase-activity was evaluated in various tissues and was reported in all instances to be many times greater than in the plasma or whole blood (124). Some organs, however, appeared more active than others, especially intestine and kidney (124, 142, 135) which were found to be respectively 1,200 and 800 times more active than plasma (124). Hemolyzed blood contained 20-30 times more angiotensinase than non-hemolyzed blood (124). No difference was found between arterial and venous blood (143), and lymph was reported to be less active than plasma (144). Recently, Hollander and associates (145) stated that venous tissue destroys angiotensin almost instantly and is more active than kidney tissue.

4. THE ENZYMATIC REPRESENTATION OF THE RENIN-ANGIOTENSIN SYSTEM.

As the research progresses in this field, the renin-angiotensin system becomes more and more complex. The existence of facilitators or inhibitors acting on the various enzymatic reactions of this system are suspected and postulated by many investigators but not yet directly proven. It is believed by some investigators that we may be facing another enzymatic system which resembles in complexity the coagulation process. The present status of the renin-angiotensin system may be schematized as follows:



* This position is filled with Isoleucine in horse and hog, and with Valine in ox.

The production of angiotensin II is under the influence of many factors. Changes in one or more of the various constituents of this system would result either in an increase or a decrease in the plasma concentration of angiotensin II. For instance, a decrease in either the concentration of renin, or the substrate, or the converting enzyme; or an increase in angiotensinase-activity would result in a decreased concentration of angiotensin II. The

opposite changes would increase its concentration.

Therefore it would appear most likely that the liberation or the production of angiotensin is determined by the interaction of numerous active compounds, renin probably being the most important.

The enzymatic reactions underlying angiotensin formation and destruction were estimated to be of the first order kinetics (56, 146, 147).

B - SOURCE OF RENIN.

The kidney is probably the only source of renin in the body. However, the exact site of renin formation in the kidneys has been intensively sought for during the past 25 years and this aspect still remains neither completely, nor definitely established. Although the exact location is still debated a great deal of indirect evidence has been put forth over the years to indicate with certitude that the formation of renin takes place somewhere in the juxtaglomerular apparatus. Goormaghtigh (148) was the first, in 1939, to notice marked histological changes characterized by hyperplasia, hypertrophy and vacuolization of the afibrillar cells at the vascular pole of the glomeruli in canine ischemic kidneys. Goormaghtigh also noted the presence of secretory granules in the cytoplasm of these afibrillar cells. Since an increase in the secretion of renin was reported in similar conditions, he thus postulated that these cells were the source of renin (149, 150).

Numerous studies have since confirmed that the juxtaglomerular cells contain renin or precursors of renin.

1. DESCRIPTION OF THE JUXTAGLOMERULAR APPARATUS.

The juxtaglomerular apparatus is chiefly composed of the following three anatomical structures:

1) The juxtaglomerular granular epitheloid cells.

2) The macula densa.

3) The Lacis cells (pseudo meissnerian cells of Goormaghtigh).

a) Juxtaglomerular cells:

The juxtaglomerular cells were first described in mice by Ruyter in 1925 (151). Oberling reported the existence of this structure in man in 1927 (152) and a few years later Goormaghtigh gave a very accurate description of these cells and related structures in human kidneys (153). These cells are located in the media of arterioles in close proximity to the glomerulus. They are differentiated from the surrounding smooth muscle cells by their clear afibrillar cytoplasm which often contains granules (149, 148, 154). Electron microscopic studies revealed that they contain zymogen-like granules, numerous mitochondria, a rich endoplasmic reticulum with abundant RNA granules and increased Golgi elements, all of which being compatible with a secretory nature (155).

b) Macula densa:

The macula densa is that specialized area of cells of the distal convoluted tubule which is always in close proximity to the vascular pole of the glomerulus. This structure was first described in 1932 by Zimmermann (156) as being composed of groups of tall columnar epithelial cells having their nuclei very close to one another. These cells have different tinctorial properties from other tubular cells which are less basic. McManus (154, 157) pointed out that the position of the Golgi apparatus is reversed in cells of the macula densa and that the basement membrane is absent in this portion of the tubule. He suggested that this arrangement might permit substances to pass back and forth between these two types of cells. These observations were confirmed in studies with the electron microscope (155, 158). The close relationship found between the enzymatic activity (glucose-6-phosphate dehy-

drogenase, 6 phosphogluconate dehydrogenase) of the macula densa, the granularity of the juxtaglomerular cells and the renin content of the kidney (159-162) gives strong support to the combined participation of both structures in the formation of renin. Reeves et al (163) also reported recently a close correlation between the hydropic changes or height of the macula densa and the number of juxtaglomerular cells in cirrhotic patients. The macula densa has now become a major point of interest in the study of the mechanisms underlying the control of renin secretion. It is now speculated that the activity of the juxtaglomerular cells might be under the control of some constituents of the urine by the intermediary of the macula densa (158, 159, 164, 165, 166).

c) Lacis cells:

These cells are small interstitial cells located in the space limited by the macula densa and the two branches of the glomerular arterioles. They were once described by Goormaghtigh (153) as neural elements having the aspect of meissnerian cells. Zimmermann (156) gave the name of Polkissen to the group of cells formed by these elements and the juxtaglomerular cells. Oberling and Hatt (158) believed that the Lacis cells are continuous with the mesangial cells of the glomerulus and they postulated that these might be transformed into granular epithelioid cells during the periods of hyperactivity. These workers also described a complex network of membranes surrounding these structures which would play a most important role in the transmission of information regarding the osmotic changes in the urine to the juxtaglomerular cells. At the present time, the functions of these elements remain purely hypothetical.

2. LOCALIZATION OF RENIN IN THE KIDNEY.

a) Anatomical:

In early studies, renin was detected in the renal cortex and not in the medulla (1, 19, 169). Friedman (167) observed that there was no renin in the aglomerular kidney of the marine Midshipman fish, while it was present in other glomerular kidneys of fresh water fish and hog. He later reported that renin could no longer be extracted from glomerular kidneys after selective necrosis of proximal tubules by the administration of tartrate (168). The pressor activity was detected only in areas of the kidney containing glomeruli (169). The glomeruli of the outer part of the cortex are reported to contain more renin than those in the inner part (170, 171).

Cook and Pickering in 1958 (172), developed an ingenious mean for the separation of the glomeruli from the rest of the renal tissue by injecting magnetic iron oxide into the renal artery and then by exposing the ground kidneys to an electromagnet. With this method they found that the glomerular fraction (magnetic) contained much more renin than the tubular fraction. Further microdissection studies enabled them to observe that the glomeruli with attached fragments contain more renin than those without (173). By cutting the glomerulus in half, they were able to demonstrate that renin was present in the glomerular segment containing the afferent and efferent arterioles and the macula densa while no pressor activity could be detected in the fragment to which the proximal tubule was attached (170).

Another group of workers went further in microdissection studies. Bing and Wigerg (174) reported that the isolated glomerular tuft contains no renin. Bing and Kazimierczak (175) found, like Cook and Pickering (173, 170), that the glomerulus with its attached fragments contains renin which appears to be located especially at the vascular pole. Recently, they were able to achieve a good dissection and separation of the JGC from the macula densa (176).

They could thus measure the renin content of these two structures separately and they found that renin was chiefly present in the macula densa and part of the distal convoluted tubule whereas the isolated afferent arterioles were sometimes devoid of any pressor activity (176). They concluded that renin is formed in the macula densa and that part of it is distributed to the afferent arterioles.

b) Histological:

Another group of studies has demonstrated the secretory nature of the juxtaglomerular cells and identified its secretion product as renin or very closely related compounds.

The first line of indirect evidence was established by the finding of a very close correlation between the granularity of the JGC and the renin content of the kidney. A parallel increase in these two parameters was observed in ischemic kidneys (160, 177, 178), during dietary sodium restriction (179) and in animals having high serum antirenin titers (180). A parallel decrease was noted in the contralateral "untouched" kidney, in DOC hypertension (160, 177, 178) and during high sodium intake (178).

Studies with fluorescent antirenin antibodies permitted a more precise localization of renin. The first study by Nairn and coworkers (181) led to the observation that the fluorescent antibodies localized almost exclusively to the glomerular tuft while much less were fixed by the juxtaglomerular structures. However, the use of a very impure renin extract, containing other kidney proteins in the preparation of their antibodies appears to invalidate their results (182). Modifications of their technique and the use of a purer renin preparation permitted to Edelman and Hartroft (183) to observe that renin antibodies were adsorbed and fixed exclusively by the granules of the juxtaglomerular cells and not by the glomerular tuft nor by the macula densa (182-184).

Marshall and Wakerlin (185) reported, in 1949, that the solubility characteristics of canine renin and those of JGC granules were identical. Recent studies with ultracentrifugation of kidney homogenates gave more support to the presence of renin in these granules. Cook (186) found that the pressor activity is associated with granules approximating mitochondria in size (186). In another study, Chandra, Skelton and Bernardis (35) were able to isolate the pressor activity in a well defined "liquid pellet" which revealed, on electron microscopy, small granules of 4-5 μ in diameter, that they postulated to be sub-particular constituents of the juxtaglomerular cell granules.

In summary, although the definitive proof is lacking, it can be concluded that renin or close precursors are present in the juxtaglomerular cells and that these cells and/or those of the macula densa participate in the synthesis of renin.

3. SIGNIFICANCE OF THE GRANULARITY OF JUXTAGLOMERULAR CELLS.

After the recognition of a close correlation between the renin content of kidney and the granularity of the juxtaglomerular cells, it was easily assumed by some investigators that the granularity was representative of the dynamics of renin production. However, one must not forget that they may also represent the storage of renin without necessarily indicating its secretion rate.

Renin secretion does not always parallel the renin content or the granularity of the JGC. In rats treated with DOC, salt or cortisol, Masson et al (187) reported that the liberation of renin soon decreased after the onset of treatment while the renin content remained unchanged or decreased much later. After a large hemorrhage, in rats, Gross et al (165) observed that the concentration of renin in the blood increased rapidly without any

detectable changes in the renal renin content. During the first days of sodium depletion, Miller and Hartroft (188) noted that a degranulation of the JGC and a decrease in the renin content occurred whereas an increase in renin secretion is reported by others in similar circumstances. Tremblay (519), in our department, could observe a degranulation of the JGC during acute ureteral clamping in dogs while the liberation of renin increased in the renal vein.

On the other hand, a parallelism was postulated between the granularity of the JGC and the liberation of renin in several chronic conditions (189, 190). Recently, a significant correlation could be established, in our department, between the renal venous renin-activity and the juxtaglomerular granularity in patients having a renal artery stenosis (191). Nevertheless, it seems safer to assume that the juxtaglomerular granularity would be an index of the secretion of renin only in chronic conditions but not in acute situations. This must be kept in mind when studies of the JGC are reported later on.

Much confusion has arisen from the various ways of expressing the activity of the JGC. Some investigators appraise the degree of granularity of these cells while others estimate their cellularity or number. Since it is obvious from the study of Turgeon and Sommers (192) that the granularity and the number of the JGC are not always parallel, the interpretation of the various studies reporting either the granularity or the number of cells as an index of the activity of the renin-angiotensin system is becoming a challenge. Most investigators express the granularity as an index (JGI) according to the criteria suggested by Hartroft and Hartroft (193). Despite these criteria, great subjectivity remains in the interpretation of findings. This probably explains the wide range of variation encountered in the JGI of simila

control animals between the various groups of workers using the same technique or even between different studies coming from the same investigator.

More recently, Turgeon and Sommers (192) suggested a classification of the juxtaglomerular cells in four different types according to their state of secretory activity. They express their results as the total juxtaglomerular cell count per 25 glomeruli with the percentage of various types of cells. Although their classification of various types of cells can be criticized, the classification of the three first types of cells is quite representative of the state of granularity and, as a whole, this technique gives a good account of the activity of the JGA since it appraises its hyperplasia as well as its granularity.

C - PHARMACOLOGICAL EFFECTS OF ANGIOTENSIN.

The injection of partly purified renin and angiotonin preparations in animals, led many investigators to report variable and conflicting effects before 1959. Since then, the achievement of synthetic angiotensin and its availability have produced standardization in the pharmacological studies so that the effects could be better recognized and described. The main objection to these studies is that synthetic angiotensin might differ in structure and action from the natural human angiotensin. This objection remains valid since the purification and analysis of human angiotensin have not yet been achieved. Nevertheless, there is good indirect evidence that both natural and synthetic angiotensin behave similarly in vivo. So far, no difference has been reported in vivo between their actions, or between their chemical properties (3,). Both induce an identical pressor curve in animals, both are inactivated by trypsin and they behave similarly in various electrophoretic and paper chromatographic systems (110). However, Helmer (194) reported recently that, although no difference could be found in vivo between asparginyl valine-5-

angiotensin (Ciba preparation) and natural angiotensin, the synthetic asparginyl valine-5-angiotensin is much less active on strips of aorta than the natural compounds.

Since it is now recognized that the pharmacological actions of renin are accomplished through the formation of angiotensin, pertinent studies with renin will also be reported in this chapter.

1. RESPONSIVENESS TO ANGIOTENSIN.

The responsiveness to angiotensin and its variations under certain conditions have been the subject of many studies and discussions during the past years. This aspect was investigated largely for the purpose of understanding the basic mechanism involved in the development or maintenance of hypertension. However, the finding of normotensive subjects with high blood levels of angiotensin and renin-activity raises many questions regarding the factors controlling the reactivity to angiotensin. Since this aspect is indirectly related to the present work, more attention will be given to it.

a) Tachyphylaxis:

There is much confusion about the meaning of the term "tachyphylaxis". It appears that a part of the controversy can be attributed to the fact that some investigators use the term tachyphylaxis to describe a state of decreased responsiveness. To our mind, these two phenomena are different and must be defined before proceeding.

According to Dorland's medical dictionary (475), tachyphylaxis is a phenomenon characterized by decreasing responses to a given substance following consecutive injections of the same substance made at short intervals. No definition has really been given for the terminology of "Decreased Responsiveness". However, looking at the various studies, it appears that this phenomenon is a variable state of responsiveness rather than a progressively

decreasing responsiveness as in tachyphylaxis. Tachyphylaxis would imply immune mechanisms or limiting factors such as receptor sites and would be specific to a substance while "decreased responsiveness" would be nonspecific and determined by many variable factors.

A tachyphylaxis to renin was first observed by Tigersted and Bergman (1) at the time of its discovery. Later work with crude renin extracts confirmed this first observation (12, 81, 254). Using a more purified renin preparation, Haas and Goldblatt (44, 45) found no tachyphylaxis unless a very high dose was given. The role of renin in the pathogenesis and maintenance of renal hypertension was questioned after the report by a few investigators (255, 256, 663) that prolonged renin infusion failed to maintain high blood pressure and that within short periods, blood pressure returned to normal levels. However, Bock and Gross (257) reported that renin infusion at low rate maintained high blood pressure, but when renin was infused at much higher rate, blood pressure rapidly returned to nearly basal levels after a steep initial rise. Haas and Goldblatt (44, 45) using the most purified renin preparation available, demonstrated clearly that renin infusion at medium rate, in dogs, maintained a rise in blood pressure for the period of infusion and that the blood pressure remained higher than pre-infusion levels up to five hours following the cessation of the infusion. In their study (45), the infusion of large amounts of renin resulted in an initial steep rise in blood pressure followed by a decrease to a high blood pressure plateau which was maintained constant throughout the infusion period. They also reported that the pressor response to a single injection of renin was decreased during the infusion period. This decreased responsiveness was not specific for renin, but was also observed with angiotensin and nor-epinephrine. Furthermore, they showed that the administration of a sympathomimetic drug

(Methoxamine) to normal animals produced an identical decreased responsiveness to the same substances. Thus, they concluded that this was not tachyphylaxis but only a nonspecific state of decreased responsiveness. However, they also observed that injection of a very high dose of renin resulted in the establishment of a state of complete refractoriness to subsequent injections of renin. This latter phenomenon might be explained by the exhaustion of the substrate since it has been reported that the concentration of the substrate decreases after injection of large doses of renin (42, 44).

In their early studies with crude angiotonin preparation, Page and Helmer reported that a tachyphylaxis phenomenon developed after many repeated doses (81) but this extract was impure and contained other substances which could account for this phenomenon. However, when synthetic angiotensin is used, in small and medium repeated doses, or by continuous infusion, it does not elicit tachyphylaxis (200, 238, 247, 252, 257). Furthermore, a sustained pressor response could be maintained for days, weeks and months with the same infusion dose (85, 258, 259). Marx and coworkers (260) reported that rats chronically treated with angiotensin in oil, sub-cutaneously, developed a steady rise in their blood pressure. Katz et al (261) could reproduce the renal vascular lesions of malignant hypertension by injecting rats subcutaneously with angiotensin in water, daily for 7-14 days.

On the other hand, Bock and Gross (664) claimed that tachyphylaxis occurs when large doses of angiotensin are injected. During prolonged infusion of large doses of angiotensin into the rabbit, Brown et al (258) observed a progressive decrease in blood pressure after a few hours. A so-called "cross tachyphylaxis" is reported to occur between renin and angiotensin. Page and Helmer (81) first observed that large doses of renin reduced and finally abolished the pressor response to angiotonin. Later, Page et al (85) showed

that this phenomenon was highly specific for both synthetic and natural angiotensin since no decreased responsiveness occurred with nor-epinephrine. The same phenomenon was reported by Bock and Gross (257) and recently by Haas and Goldblatt (45) after infusion of a large dose of renin. This refractoriness to angiotensin occurs even when blood pressure had returned to normal levels while nor-epinephrine responsiveness was unaffected. The specificity of this phenomenon for angiotensin is unexplained. During infusion of renin at lower dose, Haas and Goldblatt (45) observed a decreased responsiveness not only to single doses of angiotensin and renin, but also to nor-epinephrine.

In isolated heart preparation, Beaulnes and coworkers (242, 262) observed a tachyphylaxis phenomenon with high doses of angiotensin. They further observed that the administration of nor-epinephrine restored the original activity of angiotensin.

Therefore, it can be concluded from this review that angiotensin and the purest renin preparation do not produce tachyphylaxis when given at low or medium dose either in repeated doses or by continuous infusion. However, high doses appear to induce tachyphylaxis.

b) Factors modifying the responsiveness to renin and angiotensin.

Changes in the pressor responsiveness to angiotensin and renin have been observed in many clinical states, in many experimental conditions and following the administration of various drugs. So far, these observations are well documented and have been reproduced consistently in many instances. However, the degree of responsiveness can hardly be evaluated since the criteria and the doses injected varied with different investigators.

In man, a decreased responsiveness or a resistance to the pressor action of angiotensin has been observed in such physiological or pathological

conditions as pregnancy (263, 264), decompensated cirrhosis of the liver (267, 266), malignant hypertension (266), Addison's disease (268), primary juxtaglomerular hyperplasia (269) and during sodium restriction or depletion (265, 266).

Johnston and Jose (270) also noted a decreased responsiveness in cardiac, cirrhotic and nephrotic edematous patients. Since most of their patients were receiving natriuretic drugs, the lack of responsiveness might also be attributed to the natriuretic treatment. In contrast to the previous reports (267, 266, 270), Pazourek et al (271) did not find any significant difference between the pressor response to angiotensin in a group of cirrhotic patients versus a group of normal subjects.

In animals, a decreased responsiveness is reported in pregnant rats (272), in dogs following vena cava constriction, (273), in natriuretic treated dogs (274), in sodium depleted sheep (275) and dogs (273), in potassium depletion (276), in adrenalectomized rats (272, 277, 278), after sympathectomy (201, 279), in shock conditions secondary to injury of the central nervous system (280) and during anesthesia (44). However, other investigators found no change in the responsiveness of pregnant rats (281) or after adrenalectomy (282-284).

A decreased responsiveness to injected renin was also observed in pregnancy (281, 285, 286), in shock (287) and in adrenalectomized animals (282, 277, 284).

An increased responsiveness to angiotensin in man is reported during salt loading, in essential hypertension and in primary hyperaldosteronism (266). In animals, nephrectomy (1, 81, 282), pithing or destruction of the central nervous system (5), denervation of carotid baro-receptors (288, 289) and spinal cord section (203, 288, 290) also enhance the pressor responsiveness to renin and angiotensin.

Inconsistent results are reported regarding the responsiveness to norepinephrine in identical conditions. Some investigators claimed that there are specific changes in the responsiveness to angiotensin (265, 267), while others reported changes in the responsiveness to both substances (266, 274, 275). This last finding would suggest that the responsiveness to angiotensin is related to a generalized phenomenon rather than to a single specific factor. From the various studies done so far on the mechanism of the pressor response to vasoconstrictor substances, it appears that many factors are probably involved in the establishment of the state of responsiveness.

Most of the clinical entities in which a decreased responsiveness occur are associated with a certain degree of sodium imbalance. Conditions such as sodium restriction and depletion, edematous states, malignant hypertension, pregnancy, vena cava constriction and hemorrhagic shock are generally accompanied by an increase in aldosterone secretion.

Thus, is aldosterone involved in this mechanism? Katz et al (291) reported a decreased responsiveness to single doses or continuous infusion of angiotensin in rabbits treated for two days with large doses of aldosterone. Ostrovsky and Gornall (272) were unable to observe a decreased responsiveness in rats acutely treated with aldosterone, but they did observe a slight decreased responsiveness in rats previously treated with aldosterone for weeks. Although these studies suggest a partial inhibition by aldosterone of the pressor response to angiotensin, this mechanism would explain neither the decreased responsiveness encountered in adrenalectomy (272, 277, 278) nor the increased responsiveness in primary aldosteronism (266).

The influence of other steroids was also investigated. Silva (292) and Masson et al (293) found no change in the pressor response to renin after acute treatment with desoxycorticosterone but, they observed a slightly in-

creased responsiveness during chronic treatment. Furthermore, Masson et al later reported (294-297) a marked potentiation of the pressor response to renin in rats treated with steroids and salt. Under these conditions, the chronic administration of renin resulted in the development of a malignant type of hypertension associated with necrotic vascular lesions. Gross and Lichtlen (298) also observed an increased responsiveness to renin and pressor peptides following treatment with desoxycorticosterone and salt. On the other hand, Friedman (277) remarked that the sensitivity was restored in adrenalectomized rats by the injection of "cortin". Ostrovsky and Gornall (272) could restore the response to nor-epinephrine in adrenalectomized rats by treating them with aldosterone, whereas the response to angiotensin and renin was restored only with corticosterone. The decrease in renin substrate reported by Gaudino (299) and Helmer and Griffith (665) in bilaterally adrenalectomized rats might partly account for the decreased responsiveness to renin but would not explain the decreased sensitivity to angiotensin. In the same manner, the increase in renin substrate reported after nephrectomy by Collins and Harakal (300) and Blaquier (301) would explain the increased sensitivity to renin but not that to angiotensin. Helmer and Griffith further observed that mineralo-corticoids or gluco-corticoids restored the substrate value in adrenalectomized rats. Also, they reported that ACTH, fresh adrenal cortical extracts and estrogens could increase the renin substrate in normal rats and humans (302, 665).

Recent findings in humans, which will be discussed later in this thesis, show either high endogenous angiotensin or renin activity levels in many conditions reported to have a decreased responsiveness to infused angiotensin while low endogenous levels are found in some conditions shown to have an increased responsiveness. Assuming that angiotensin responsiveness is deter-

mined by the circulating endogenous angiotensin and/or by renin levels, Kaplan and Silah (303) proposed a clinical test based on angiotensin responsiveness to differentiate between various types of hypertension. Their test appeared to be especially effective in the differentiation between renovascular hypertension and essential hypertension. In their study, the renovascular hypertensive patients were significantly more resistant to angiotensin than the patients with essential hypertension, but their responsiveness was not statistically different from that of normal subjects. This is difficult to reconcile with the basic assumption made by these workers, since most investigators agree to the finding of a significant increase in renin activity or angiotensin levels in most patients with renovascular hypertension. Therefore, their responsiveness should be significantly decreased when compared to normal if it is dependent upon endogenous angiotensin and renin levels. Another observation against the exclusive control of angiotensin responsiveness by endogenous angiotensin or renin levels can be added to the previous objection. Investigators who use nephrectomized rats for the bioassay of angiotensin are familiar with the wide range of sensitivity encountered in these rats identically treated, anesthetized with the same dose of nembutal and previously nephrectomized for the same period of time. Some of these rats give a pressor response of less than 10 mm Hg to injections of 5 nanograms of angiotensin while others show a response of more than 30 mm Hg to the same dose. Moreover, in the same rat, great variations occur during the day. They may even become unresponsive for a while and then later recover a good sensitivity. These changes, at least, cannot be attributed to variations in the renin or angiotensin levels. Therefore, it appears doubtful at present that the responsiveness to angiotensin is determined only by endogenous angiotensin or renin levels.

The vascular response to a pressor substance appears greatly dependent upon the ionic content of the vascular muscle cell. This is suggested by the recent studies of Friedman and Friedman (206) in which they clearly demonstrated that the peripheral resistance can be modified by changes in ionic gradient between the cell and its surroundings. They perfused rat tails "in situ" with different solutions containing various concentrations of electrolytes and they could demonstrate that the peripheral vascular resistance increased either after a lowering of the Na and/or K gradient or simply by shifting Na from environment to cell. They also observed that the vascular resistance was decreased when intracellular sodium was lost. The studies of Bohr (304) on rabbit aorta showed that the rate-limiting factor in the response to angiotensin is determined by the membrane excitability. Ionic concentration is the main determinant of this excitability. For instance, Bohr reported that the angiotensin response was depressed by an increase in calcium concentration. Gross and Lichtlen (298) also reported that an increase in sodium, potassium and water content of arterioles induces an increased responsiveness to adrenergic substances, renin and pressor peptides. Recently, Takahashi et al (305) measured the electrolyte content of aorta, psoas muscle and cardiac muscle in rats rendered hypertensive, nephrotic and cirrhotic, by various experimental means. They found characteristic changes in the ionic content of arteries and muscles of these rats. The sodium content was consistently increased in most of these conditions, the potassium content being increased only in experimental conditions associated with hypertension.

In the light of this group of studies, it can be postulated that similar ionic changes occurring at the cellular level in such clinical entities as secondary hyperaldosteronism and hypertension, might possibly account for

changes in the responsiveness to angiotensin. Therefore, this factor must be kept in mind as one of the most important in the determination of the response to pressor substances.

Finally, another factor must be considered before ending this section. In the course of their studies, Page and McCubbin (306) observed great variations from time to time in the responsiveness of their animals to angiotensin. They believed that the state of responsiveness to pressor drugs is greatly dependent upon the nervous influences controlling the hemodynamic state. This was confirmed by their observation that no more variation occurred in the responsiveness of their animals following cervical spinal cord section. Furthermore, the pressor responsiveness increased gradually in the following 24 hours then stabilized at that level.

The same group had also reported previously that the denervation of baroreceptors and vagotomy resulted in a marked increase in the responsiveness to angiotensin (289, 288). According to their findings, the administration of ganglionic blocking agents markedly enhanced the responsiveness to angiotensin and nor-epinephrine (307, 306). The association of these drugs with the denervation of baroreceptors enhanced it evermore (288). Prado and Carlini (308) confirmed that the ganglionic blocking agent T.E.A.C. (tetra ethyl ammonium chloride) increased the responsiveness to angiotensin. Page and McCubbin (307, 306) also noted that atropine administered to unanesthetized animals enhanced the pressor responsiveness to nor-epinephrine and angiotensin, the responsiveness to nor-epinephrine being greater. Haas and Goldblatt (45, 309) were unable to confirm an increase in angiotensin responsiveness after atropinization or following the administration of ganglionic blocking agents in unanesthetized dogs although they observed an increased responsiveness to nor-epinephrine. In anesthetized animals, atropine has no

effect on pressor responsiveness (201,203 , 306). Zimmermann (279) reported a decreased responsiveness to angiotensin and none to nor-epinephrine following spinal cord section and acute sympathectomy in dogs. He postulated that the reduction in the response to angiotensin depended upon the degree of neurogenic tone possessed initially by the vascular bed. Haas and Goldblatt (45) found that a sympathomimetic agent (methoxamine) decreased the responsiveness to renin, angiotensin and nor-epinephrine. Sympatholytic and parasympatholytic drugs had no effect on the responsiveness (203).

Thus, the responsiveness to angiotensin appears to be dependent on many important factors which, at first, seem quite unrelated. However, if one considers that aldosterone and angiotensin are closely related to sodium and potassium balance and that the ionic concentration is closely related to membrane potential and nervous transmission, an interrelationship seems less unlikely. The interaction of all these factors probably constitutes the basis for variation of pressor response.

2. CARDIOVASCULAR EFFECTS.

a) On blood pressure:

The pressor effect of renin and angiotensin has received the most attention since it is the only way of detecting their presence or measuring their concentration. Their typical pressor response curves were recognized early, even with crude material (1, 4, 5, 26, 195, 196). In recent years, numerous studies done with synthetic valine-5 angiotensin II amide (Ciba) have clearly demonstrated that this substance is actually the most potent pressor substance known (197-200). The pressor curve response of angiotensin is characterized by a sharp initial rise followed by a smaller compensatory fall and then a return to normal within 4 to 6 minutes (85). During the pressure rise, the cardiac rate and cardiac output decrease while the peripheral re-

sistance rises and the pulse pressure tends to increase slightly. It raises the diastolic pressure to a greater extent than the systolic pressure (201).

The various parameters related to the general circulation have been extensively studied in animals and in man, and in isolated organ preparations, either after single injected doses or during constant infusion of angiotensin at various dose levels. It would be tedious and unnecessary for the purpose of this thesis to report these studies in detail. Only the general outlines of these studies will be reported.

b) On vessels:

When Bingel and Strauss (12), in 1909, stated that kidney extracts constricted vessels of extremities as well as those of intestine, kidneys and excised arteries, they almost summarized the actions of angiotensin on arteries as it is known today.

Finnerty et al (200) found that synthetic angiotensin is ten times more potent than nor-epinephrine in normal subjects and two to three times more potent in shocked patients. Haddy et al (202) demonstrated that it is the most potent known vasoconstrictor substance on hind limb vessels. Angiotonin and angiotensin injected to whole animals constrict arteries without necessarily increasing the systemic blood pressure (203, 202). Angiotensin is also active on denervated vascular beds (202, 204) and on isolated arteries (84, 205, 304). Helmer (194) observed that the magnitude of contraction of aortic strips is greater in presence of natural angiotensin II than with any other synthetic analogues. For instance, he reported (194) that the synthetic angiotensin II (hypertensin - Ciba), commonly used for pharmacological studies and for standardization of angiotensin measurement, had only one fourth the activity of natural angiotensins on strips of rabbit aorta. Because of its potent action on arteries, angiotensin induces a marked increas

in peripheral resistance and in the resistance of various organs (198-200, 207-209). Studies on various regional circulations have shown that the action of angiotensin varies greatly from one vascular bed to another, being more active on kidney and mesenteric arteries and less active on adrenal and brain vessels (207). According to several workers, its action is limited to small arteries and it would appear to be inactive on veins (202, 210, 211, 213). In that respect, angiotensin would differ from nor-epinephrine which is active on both veins and arteries. The lack of veno-constriction claimed by some investigators served to explain the maintenance of a constant blood volume during angiotensin infusion while, on the other hand, the decrease in blood volume during nor-epinephrine infusion was attributed to the occurrence of venoconstriction with the latter substance (202, 210, 214, 215).

However, more recent studies greatly differ on that point. Wood and coworkers (216) reported that venous constriction also occurs in normal man during angiotensin infusion. Nickerson and Sutter (217) observed a similar decrease in blood volume after equipressor infusion of either angiotensin or nor-epinephrine in dogs. These workers concluded that angiotensin induces constriction of veins and they explained the negative findings of other investigators in this regard, by pointing out that no method employed so far had effectively measured constriction of the veins and venules under 0.5 mm of diameter so that the action of angiotensin at that level could not be detected. The recent study of Emerson and coworkers (218) supports the previous statement since they observed a constriction of small veins in cats, monkeys and dogs receiving an angiotensin infusion.

c) Other hemodynamic effects:

Koch-Weser (219) recently reviewed 32 different reported studies concerning the action of angiotensin on the cardiac output in man or animals. In

19 of these, the cardiac output was decreased while no change was noted by the remaining 13. Others reported that the cardiac output decreased slightly during the first minute after a single injection of angiotensin, but rapidly returned to normal in the following few minutes (201, 220). During continuous angiotensin infusion for 30 minutes into anesthetized dogs, Binnion and Hatcher observed a decrease in cardiac output, but when norepinephrine was infused in combination with angiotensin a transient decrease occurred in cardiac output and stroke work (208). Olmsted and Page (221) studied the effect of prolonged pressor infusion (2-29 days) of angiotensin into unanesthetized dogs on many hemodynamic parameters. They found that the cardiac output was decreased and that the pulse pressure and peripheral resistance were increased during the first days of infusion, but these values tended to return toward control values after five days of infusion unless the infusion rate was increased.

The actions of angiotensin on regional circulation have also been investigated extensively. During angiotensin infusions a decreased blood flow and an increased resistance occur in the skin (197), in hind limbs (202, 210, 222), in mesenteric and hepatic circulation (202,224 , 225, 226, 232) and especially in the kidney (200, 203, 207, 223, 227). On the other hand, blood flow is reported to increase in adrenal, in brain, in spleen (207) and in skeletal muscle (197). More discrepancies exist about the effects of angiotensin on the myocardial blood flow and resistance. In perfused hearts and heart-lung preparations, angiotensin always increases coronary vascular resistance and diminishes blood flow (47, 222, 228, 229). However, less consistent findings are reported during in vitro studies on animal and man. In these studies, the coronary blood flow is found increased (201, 207, 230, 23, or unchanged (198, 232), while the coronary resistance is reported increased (198, 220, 222, 232, 233), or unchanged (201, 207).

Angiotensin appears to have no direct effect on the pulmonary circulation and the hemodynamic changes reported in this circulation during angiotensin infusion are believed to be secondary to the systemic effects (212, 215, 234). Eckert and Rose in 1959 (235), reported convincing evidence for the lack of activity of angiotensin on the pulmonary vessels. In their study, they substituted a constant pump to the left ventricle and with this experimental design they were able to calculate that all the changes observed in the pulmonary circulation during angiotensin infusion were secondary to the changes elicited in the systemic circulation.

d) Cardiac muscle:

Myocardial work is generally found increased (198, 201, 215, 220, 228) 231) while the cardiac rate is decreased (1, 196, 199, 229, 236-238) during renin, angiotonin or angiotensin administration. Few others have reported no significant changes in the ventricular work (208) or in the pulse rate (201, 219). The bradycardia observed with angiotensin and renin is generally believed to be secondary to a vagal reflex initiated by the rise in blood pressure. The fact that bradycardia can be abolished by atropine (239), by denervation of the carotid sinus or by vagotomy (240) favors this last hypothesis.

Recently, Beaulnes and his co-workers (241-244) demonstrated that angiotensin has antiarrhythmic properties in experimental fibrillation either in vitro or in vivo heart preparations. They found that angiotensin, like quinidine, induces a lengthening of the refractory period but in this respect, it is 10,000 times more potent than quinidine.

Angiotensin has also a positive inotropic effect on isolated papillary muscle (219, 220), on isolated cat atria (219, 245-247), on isolated rabbit heart (222, 242) and on dogs and human heart, as suggested by the calculati

of the stroke work (215). Angiotensin increases the oxygen consumption by the heart concomitantly with the increase in muscular activity (228). Koch-Weser (219) observed that the inotropic effect on isolated papillary muscle is greater with angiotensin than with nor-epinephrine at low concentrations, but at high doses nor-epinephrine has a more pronounced musculotropic effect. Downing (246) reported in his studies on anesthetized cats that angiotensin II does not have any positive inotropic effect on the well compensated heart, but it does on the exhausted ventricle. On the other hand, in the isolated cat cardiac muscle preparation, Nueten and Dresse (247) noted that angiotensin was as active on exhausted as on fresh cardiac muscle.

e) Clinical use of angiotensin in shock:

From the studies previously reported, it appears at first that angiotensin would be more reliable than nor-epinephrine in the management of shock. Angiotensin is more potent than nor-epinephrine in raising blood pressure. It increases blood pressure in conditions where epinephrine and nor-epinephrine are no longer effective (197, 248). Furthermore, it does not produce the deleterious necrotizing effects on tissues (204, 249) nor the ectopic impulses in the myocardium reported to occur with nor-epinephrine. The cardiotonic effect of angiotensin (215, 233) also favors its use.

Certain disadvantages have been pointed out, however. Holmes and Fowler (220, 233) cautioned against its use in patients with coronary artery disease since they observed that angiotensin is a potent constrictor of the coronary arteries. Nickerson and Sutter (217) found the same degree of decrease in blood volume with angiotensin or nor-epinephrine infused at equipressor doses. Moreover, McQueen and Morrison (250) pointed out that angiotensin produces a greater depression of the renal function than does nor-epinephrine. Recently, Byrom (251) reported that a single large dose of angio-

tensin causes acute medial necrosis of the large renal arteries in the rat and that repeated doses may cause glomerular necrosis.

From the clinical standpoint, the results obtained in the treatment of shock with angiotensin were identical to those reported with the use of nor-epinephrine (249, 252), and better than with other pressor drugs (253). In a comparative study of the effect of various pressor drugs in peptone shock in cats, Mijan and Blanco (248) reported that angiotensin restores the blood pressure to normal while epinephrine and nor-epinephrine are not so effective.

Angiotensin can thus be used in the treatment of shock, but in most of these conditions it has no advantage over nor-epinephrine.

f) Mechanisms of action of angiotensin:

The observation that angiotensin is active on denervated vascular beds, isolated aortic strip and isolated heart preparation led to the general assumption that angiotensin owes its pharmacological properties to a direct stimulation of smooth muscle. However, in the past few years, fast growing experimental evidence suggests that the muscular activity is not the sole mechanism involved and that a nerve-mediated action might also play a role. The first suggestion for a central nervous mechanism involved in the pressor action of angiotensin was put forth by Bickerton and Buckley (310). In dogs, in which the head perfused by a donor remained connected to the trunk only by nerves, these investigators showed that angiotensin given into the carotid artery caused a peripheral vaso-constriction and increased blood pressure of the trunk and limbs of the animal. Since in these experiments, only nervous connections were maintained between the two parts of animal, a nerve mediated action of angiotensin is strongly suggested. Furthermore, Liverty (311) observed in the perfused innervated hind-limb of a rat, that angiotensin given

to the remainder of the animal, induced vaso-constriction or vasodilatation in the vascular isolated limb. Small doses induced vasodilatation whereas high doses caused constriction; both of these responses were abolished by section of the nerves, illustrating once more the nervously mediated action of angiotensin.

Kaneko, McCubbin and Page (312) presented the first evidence for an action of angiotensin on the release of catecholamines from adrenal medulla after sensitization by ganglion stimulating agents. Since they also observed the same phenomenon with other pressor substances and after the clamping of the adrenal artery, they attributed the discharge of catecholamine to the hypoxia secondary to the vaso constrictor effect of these drugs. Feldberg and Lewis (313) demonstrated in eviscerated cats that angiotensin, injected into the coeliac artery, is the most potent releasor of catecholamines. In that preparation, they found that the major part of the pressor response is mediated through the release of catecholamine since adrenalectomy almost abolished the pressor response. In the light of their discovery, they also postulated that the same action on the release of catecholamines might also occur locally in the vessel.

While studying the action of angiotensin on the isolated heart, Beaulnes (314) noted that dicholoroisoproterenol (a beta receptor blocking agent) and tyramine, which releases catecholamine from its storage site, both partially blocked the stimulating action of angiotensin. When the preparation was incubated with nor-epinephrine, the sensitivity to angiotensin was completely restored. He thus postulated that angiotensin probably acted partially by releasing catecholamines and that its action was on type beta receptors.

Benelli, Della Bella and Gandini (315) recently demonstrated the direct action of angiotensin on the peripheral sympathetic nerves. They showed th

angiotensin strongly potentiated the response of isolated muscle or organ to nerve stimulation probably by promoting a greater output of nor-epinephrine at the peripheral nerve ending. It is not known at present to what extent the nervous system is involved in the mechanism of action of angiotensin. It seems that the "direct" action of angiotensin on the vessel is related to the presence of some specific receptors. The findings that angiotensin has a greater effect on certain arteries and not on veins (210), that the effect of angiotensin is partially blocked by some specific receptor inhibitors and enhanced by others (201, 242, 310) and finally that the biological activity of angiotensin in vivo and in vitro is closely related to its structural configuration (92, 129, 194), all point to a specific site of action.

The contraction of muscle or the metabolic effects of angiotensin might be initiated by changes in ionic content of the cell since Friedman and Friedman (201) found that angiotensin, like other vaso constrictor substances, shifts sodium into, and potassium out of the vascular muscle cells.

3. ACTION ON SMOOTH MUSCLE IN VITRO.

The studies of Luduena (459) on various smooth muscle preparations have demonstrated that angiotensin is a strong musculotropic substance acting on almost every organ investigated. However, this investigator found that it was most active on rat and rabbit uterus, being less active on the small intestine of dog, guinea pig and rabbit, and the uterus of guinea pig. Since angiotensin acts on the uterus which is a non-innervated organ, it is clearly demonstrated that it has a direct musculotropic effect. This musculotropic action of angiotensin is probably present in the contraction of other innervated smooth muscles but much evidence has accumulated also for a nerves mediated mechanism.

In 1948, Collins (316) reported that the ganglion blocking agent tetraethylammonium chloride potentiates the contraction of the guinea pig ileum by angiotonin. Prado and Carlini (308) later confirmed their findings with synthetic angiotensin, but they also found that pentolinium and hexamethonium did not alter the response of these muscles. Ross, Ludden and Stone (318) observed that atropine and morphine, which prevent the action of acethylcholine, also effectively block the action of angiotensin on the guinea pig ileum, while ganglionic blocking agents, with the exception of mecamylamine and pempidine at high doses, did not block the action of angiotensin. They concluded that angiotensin acts on the post-ganglionic cholinergic mechanism of the ileum. Robertson and Rubin (319) produced the most direct proof for the involvement of a cholinergic mechanism in the mode of action of angiotensin on the rabbit and guinea pig ileum. They showed that the contractile response was enhanced by anticholinesterase agents, abolished by botulinum toxoid and inhibited up to 65 to 85% by atropine. They thus postulated that angiotensin might release acetylcholine by a direct or indirect mechanism. In their studies on guinea pig ileum, Khairallah and Page (320) reported that atropine, morphine and a paralyzing dose of nicotine decreased the response to angiotensin. On the other hand, these drugs were without effect on the uterine muscle. They concluded that angiotensin acts on isolated intestinal muscle mainly by stimulating the ganglion cells in Auerbach's plexus, that only 25-30% of its action was due to direct muscle stimulation and that, on uterine muscle, angiotensin had only a direct myotropic action. Pursuing their work, these investigators (321) later reported that most of the adrenergic blocking agents inhibited the muscular response of either guinea pig ileum or uterus. They also found that epinephrine, and to a lesser extent, nor-epinephrine and isoproterenol inhibited the contract-

ion of intestinal as well as uterine muscles. They suggested that the changes in sensitivity reported in their studies (321) were probably due to a direct action of the drugs on ionic shift across the cell membrane, thus altering intracellular content of sodium and potassium. Nevertheless, the mediation of a cholinergic mechanism in the action of angiotensin on intestinal muscle remains probable.

4. ACTION ON THE KIDNEY.

Many studies have been undertaken on the renal effect of renin and angiotensin in the last 25 years. This has led to quite confusing and contradictory findings between different species and in various diseases. Most of the mechanisms involved remain obscure and badly understood probably because some links are still missing in the complex field of urine formation. Nevertheless, it appears that the renin-angiotensin system might have a role in this function.

a) Hemodynamic effects:

As early as 1938, Merrill et al (322) observed that alcoholic extracts of hog renin decreased the renal blood flow of dogs. Two years later, Corcoran and Page (323) reported that semi-purified renin decreased renal blood flow but increased the inulin clearance of the dog kidney transplanted under the skin. During the pressor response to renin, a marked constriction of the vessels and a lengthening of the circulation time of the kidney was visualized on angiography, even after blood pressure had returned to normal levels (227). Angiotensin was found by many investigators to have a marked vaso constrictor action on the renal vessels, even more pronounced than on any other vascular beds (200, 207, 223, 238, 324). This potent vascular action leads to a decrease in both glomerular filtration rate and renal blood flow, but it decreases the renal blood flow more, so that the filtration fraction is generally

increased. This last observation implies that angiotensin would probably be more active on the efferent than on the afferent arterioles. However, McQueen and Morrison (250) observed a marked decrease in G.F.R. and R.B.F. during angiotensin infusions but they could not find any significant change in the filtration fraction.

b) Effect on urine formation:

i) Renal excretion of water and electrolytes:

Credit for the first observation of the action of renin on the kidney probably belongs to Bingel and Claus, who, in 1910 (325), remarked that, in lightly anesthetized rabbit, the administration of crude renal extracts increased urine flow and kidney volume. The first documented and systematic study of this action of renin was reported by Pickering and Prinzmetal in 1940 (326). They found that, in normally hydrated unanesthetized rabbits, intravenous administration of crude kidney extracts caused a diuresis and natriuresis, while in over hydrated animals, renin caused first an antidiuresis followed by a diuresis. They concluded that the antidiuresis was due to the action of renin on glomerular vessels while the diuretic effect was probably due to a direct action of renin on renal tubular cells. The natriuretic and diuretic effect of renin was later confirmed in the rabbit by Brandt (327), Hughes-Jones (328) and their collaborators. In dog, renin increased urine flow (322) or decreased it (329, 330). In normally hydrated rats, renin consistently increased the diuresis and natriuresis (293, 329, 371), but Croxatto et al (371) also reported that in over-hydrated rats, the diuresis was first decreased by renin. Hughes-Jones et al, in 1949 (328), suggested that the action of renin on the kidney was mediated through the production of angiotensin, since they were able to obtain the same effects with angiotonin. Later studies with synthetic angiotensin in animals confirmed most of the previous findings with renin.

According to Peter's studies (340, 341), angiotensin is a potent natriuretic agent in rats, having 17,000 to 35,000 times the potency of hydrochlorothiazide. This effect was also reported even when subpressor doses were used. Angiotensin usually induces an antidiuresis and antinatriuresis in dogs (165, 259, 324, 332-334), although a natriuresis is also reported in anesthetized dogs (335-338), after buffer nerve section (337) or during mannitol diuresis and stop flow experiments (338). Urquhart et al (259) reported that a continuous angiotensin infusion in normal dogs can maintain a sodium retention only for 1 to 2 days but thereafter an escape occurs and the sodium is no longer retained. They later found (339) that in the dog with experimental arterio-venous fistula, sodium was consistently retained as long as the infusion was given, even for periods of ten days.

Healy et al (334) recently reported that the timing and dose given could explain some of the discrepancies reported with the action of angiotensin on the kidney. They showed that at low rate in dogs, angiotensin retains sodium but when dosage was increased, a natriuresis occurs but persists only for the first 50 minutes.

In normal man, angiotensin consistently induced antinatriuresis and antidiuresis simultaneously with a decrease in G.F.R. and R.B.F. (238, 263, 342-349). In pregnancy, the same changes are observed but to a lesser degree than in normal non-pregnant women (263, 331). Nijensohn (350) first observed a natriuresis in hypertensive subjects following angiotonin administration. This was confirmed with synthetic valine-5 angiotensin in essential hypertension and other hypertensive diseases by the groups of Peart (345, 351, 352), Genest (353), Del Greco (354) and Vagnucci (335). However, the renal response to angiotensin in coarctation of aorta is identical to that of normal subjects (355). The natriuretic effect of angiotensin is reversible and the

antinatriuretic effect can be restored in renal hypertensive patients whose blood pressure has returned to normal after appropriate surgical or drug treatment (351). Jones and Barraclough (356) reported, in two patients with renal artery stenosis, that angiotensin is antinatriuretic in the stenotic kidney while it is natriuretic in the contralateral kidney. One of these patients was cured by surgical repair of his stenosis, and thereafter, the antinatriuretic response to angiotensin was also restored. Recently, Brown, Matthew and Robertson (357) also studied the effect of angiotensin on separate kidneys in patients with unilateral renal artery stenosis. They found a diuresis in the two kidneys in 8 out of 16 patients, bilateral antidiuresis in five and an antidiuresis in the stenotic kidney with a diuresis in the opposite kidney in three other instances. When a diuresis was observed in both kidneys, it was less marked on the stenotic side, and when bilateral antidiuresis was noted, it was more pronounced in the stenotic kidney. In decompensated cirrhosis of the liver, angiotensin is a potent natriuretic and diuretic agent according to Laragh (267, 358), Schroeder (359) and Pazourek (271) and their associates. However, in the nephrotic syndrome, no natriuresis occurs with angiotensin (271). In sodium depleted normal subjects, Laragh (267) reported a very slight increase in urinary sodium after angiotensin infusion, but, on the other hand, Del Greco (354) observed that a low sodium intake, in hypertensive patients with glomerulonephritis, prevents the natriuretic effect of angiotensin from occurring. In adrenalectomized patients, angiotensin induces sodium retention and decreases the diuresis (317, 360-362) while in adrenalectomized patients with hypertension, it increases sodium excretion (362).

Potassium excretion is only slightly modified or unchanged during angiotensin infusion (271, 340, 349, 351, 363). Gantt and Carter (364) re-

ported that potassium, calcium, phosphorus and magnesium follow the same pattern of excretion as sodium during angiotensin infusion. The water clearance generally follows the excretion of sodium, so that only slight variations in urinary osmolality occur (349, 348).

ii) Mechanism of antidiuresis:

As was previously discussed, angiotensin is a potent vaso constrictor of renal vessels, and in most instances, it induces a decrease in renal blood flow, and glomerular filtration rate. These hemodynamic changes could probably explain the antidiuresis encountered in normal subjects and dogs, since slight changes in G.F.R. can lead to marked changes in sodium excretion (366, 367). On the other hand, equipressor doses of nor-epinephrine which induce similar hemodynamic changes do not produce a similar sodium retention (7, 346).

Another mechanism for sodium retention could be realized by the intermediary of aldosterone as suggested by Genest et al (7, 346), after their finding that angiotensin is a potent stimulus for aldosterone production. However this would not explain why angiotensin induces a decrease in sodium excretion within 10 minutes after the beginning of an infusion while aldosterone requires usually a much longer period to act (349). Moreover, angiotensin produces a sodium retention in adrenalectomized patients (319, 360-362). Nevertheless, it is possible that aldosterone might intervene in the sodium retention during prolonged angiotensin infusion. This is supported by the observation that, in rats, the natriuretic effect of angiotensin markedly decreases at the end of the first hour of infusion despite the continuation of the infusion at the same rate (340). Similarly, in dogs, angiotensin given at a high rate (100 ng/kg/min.) produces a natriuresis at the beginning of infusion, but after 50 minutes, the sodium is retained (334). Finally, Horky et al (361) observed that sodium excretion remained low for a short period after

they stopped the angiotensin infusion in their normal subjects while sodium retention stopped immediately at the end of infusion in their subjects with adrenal insufficiency. Since angiotensin induces an increase in aldosterone secretion which persists for many hours after stopping the injection, the last observation would favor the participation of aldosterone in the sodium retention, at least in the post-infusion period.

The action of angiotensin does not seem to be mediated by a release of antidiuretic hormone. In man, Barbour et al (348) compared the action of angiotensin and vasopressin and they found that vasopressin greatly decrease the urinary volume and greatly increases the osmolality without changing the total amount of sodium excreted, while, angiotensin decreases urinary volume to a lesser extent, only slightly modifies the osmolality but markedly decreases the total sodium excretion. Moreover, Gill et al (349) and Del Greco (36 recently reported that angiotensin was equally active in patients with diabetes insipidus.

iii) Mechanism of increased diuresis:

In the group of animals and patients in which angiotensin induces an increase in the natriuresis and diuresis, the R.B.F. and the G.F.R. are generally little or not modified. Pazourek et al (271) even observed a marked initi increase in G.F.R. during infusion of angiotensin into cirrhotic patients (27 McGiff and Itskovitz (368, 369) also reported that, in ischemic canine kidney, angiotensin increases renal blood flow in contrast to normal kidney in which it induces a marked decrease in renal blood flow. Thus, it appears that the renal vessels, in these conditions, are less sensitive to the constrictor action of angiotensin. Consequently, the glomerular filtration rate is more easily influenced by other hemodynamic changes.

Would, therefore, an increased blood pressure or perfusion pressure be

responsible for an increase in sodium excretion during angiotensin infusion, in certain pathological conditions or in various animal species? The experiments of Selkurt (366) and Thurau et al (370) regarding the hemodynamic influences on the kidney function would fit the present situation. These workers reported that increased mean perfusion pressure even without an increase in G.F.R. can increase urine flow and sodium excretion. It can be postulated that angiotensin might produce a diuresis and natriuresis by a similar mechanism. Against this hypothesis, some workers have reported a natriuretic effect even with subpressor doses of angiotensin (328, 340, 353), or after the blood pressure had returned to normal (328), but these observations do not take into consideration the possibility that local hemodynamic changes can occur independently of changes in systemic blood pressure. Furthermore, Lauler et al (336), perfusing dog kidney with a pump at a constant pressure identical to that reached with angiotensin infusion, found a natriuresis of lesser magnitude than with angiotensin alone. They concluded that these findings supported a direct tubular action for angiotensin, but, the changes due to a rise in perfusion pressure might be different from the local hemodynamic changes induced by angiotensin. Thus, the renal hemodynamic effects cannot be excluded from the mechanism involved in the natriuresis effected by angiotensin.

Schroeder et al (359) reported recently that the natriuresis observed in cirrhotic patients was not due to tubular ischemia or to the redistribution of blood within the kidney during angiotensin infusion, since the extraction of paraaminohippurate remained over 84% in these patients. One of the most puzzling and contradictory finding in this mechanism is that the presence of adrenals is of the greatest importance for the appearance of the natriuretic effect of angiotensin. Since aldosterone possesses a marked sodium retain-

activity, one would expect that the natriuretic effect of angiotensin would be partially blocked in presence of adrenal tissue while it would be enhanced in its absence. Experimental evidence has revealed an opposite mechanism. In 1952, Croxatto et al (371) observed that the diuretic effect of renin could be abolished by adrenalectomy. Masson et al (372) reported one year later that corticosteroids restored the diuretic effect of renin in adrenalectomized animals. Croxatto et al (373) recently reported that aldosterone greatly enhances the diuretic and natriuretic effect of renin. Peters (374) observed the same phenomenon with angiotensin in the rat. In his studies, the natriuretic effect of angiotensin gradually decreased during the week following adrenalectomy. Aldosterone partially restored the natriuretic effect of angiotensin during the first days, but was without effect after the first week following adrenalectomy. At this later time, the natriuretic effect was partially restored by prednisolone or to an even greater extent by the combined administration of prednisolone and aldosterone. Thus from these findings, it seems that increased endogenous levels of aldosterone might possibly enhance the natriuretic effect of angiotensin. This could explain the natriuretic response in cirrhotic patients. However, the same natriuretic response was not reported in nephrotic syndrome, which is also associated with hyperaldosteronism (271).

The potent natriuretic and diuretic effect of angiotensin infusions in several animal species and in some human conditions without any consistent changes in glomerular filtration rate led many investigators to postulate a direct tubular action (338, 340, 351, 358, 375). In his previous studies, Peters (341, 340) concluded that angiotensin had a direct tubular action on sodium transport since natriuresis occurred independently of changes in G.F.R. or pressor response. He later found that angiotensin did not influence the

distal reabsorption of water or the tubular permeability to water during a hypotonic diuresis (374). Since he reported that aldosterone enhanced the natriuretic action of angiotensin, Peters postulated that angiotensin would inhibit the process by which aldosterone acts on the reabsorption of sodium. Langford and Pickering (375) reported that relatively low doses of angiotensin produced an antidiuresis in the rabbit but when the dose was increased, a marked natriuresis and diuresis resulted. When angiotensin was given during full water diuresis, little change occurred in urine flow but sodium excretion increased greatly, thus suggesting a decrease in the reabsorption of sodium. Vander (338) also reported a decreased tubular sodium reabsorption during mannitol diuresis in dogs infused with angiotensin into the renal artery. In his cirrhotic patients, Laragh (358) calculated that the ratio of Na filtered to Na excreted increased greatly during angiotensin infusion despite a constant G.F.R., thus demonstrating that angiotensin decreases the renal tubular reabsorption of sodium. The first direct approach of an action of angiotensin on sodium transport was provided by Leyssac and his coworkers (376) when they reported that angiotensin almost completely inhibited the active sodium transport in isolated kidney slices. However, recently, Leyssac (377) admitted that they could not reproduce their own previous findings. Some studies have already been pursued along this line but so far none of these could demonstrate a direct effect on the transport of sodium. Barbour et al (378) failed to find any effect of angiotensin on the transport of sodium by toad skin. Bonting et al (379) were unable to find any inhibitory effect of angiotensin on the Na-K ATPase activity in the kidney cortex or medulla in vitro. Bergeron (380) reported recently that angiotensin had no effect on the ionic transport in intact erythrocytes. He also found that it did not inhibit the ATPase activity of a nuclear fraction of

mammalian kidney cortex homogenate nor the ATPase activity of the microsomal fraction of dog kidney homogenate.

In summary, the action of angiotensin on the kidney appears to be related mostly to its action on kidney vessels, and to its hemodynamic effects. The presence of the adrenal cortex seems to be important in inducing and maintaining antidiuresis, but appears also to be permissive in the production of natriuresis. A direct tubular action of angiotensin is suspected from the experiments previously reported but no direct proof has been produced at present.

c) Effect on proteinuria:

Pickering and Prinzmetal (326) first observed that the natriuretic and diuretic effect of renin was accompanied by a significant degree of proteinuria. Brandt and Gruhn (327) confirmed this observation and they suggested that this phenomenon was secondary to a diminished tubular reabsorption of proteins. Addis et al (381) reported that, in rats, the proteinuria was associated with the excretion of fibrinogen but was not accompanied by any evidence of damage to the glomerulus. They thus suggested that the proteinuria was due to an increase in glomerular permeability. They also reported later (382) that adrenalectomy inhibited the proteinuria. Sellers et al (383) further established that the proteinuric effect of renin was secondary neither to its proteinic nature nor to the rise in blood pressure since intramuscular injection of renin induced proteinuria and since, following adrenalectomy, a pressor dose of renin did not produce proteinuria. Recently, Deodhar, Cuppage and Gableman (384) also established that the proteinuric effect of renin was not dependent on its proteinic nature, or on the increase in blood pressure but nevertheless was dependent on its biological activity. Biologically inactive renin given at high dose did not induce proteinuria, while "active"

renin given at non pressor dose produced proteinuria. They could also obtain a significant degree of proteinuria in their animals after the administration of angiotensin II. They confirmed that adrenalectomy prevented the proteinuria but they found that pretreatment with DOCA or aldosterone overcame the inhibition of the proteinuria. After intravenous injections of saccharated iron oxide they could demonstrate that the permeability of the glomerular capillary basement membrane to these particles was increased during renin infusion. Electron microscopic studies of the kidney at the time of maximal proteinuria showed focal flattening and fusion of epithelial foot processes as well as swelling and vesicle formation in endothelial and epithelial cells of the glomeruli. These renal "nephrotic like" lesions seen with electron microscope were previously reported by Fisher and Masson (385) after renin, and by Langford (386) after angiotensin administration.

In conclusion, renin and angiotensin, independently of their pressor action can alter the glomerular membrane permeability and induce proteinuria. This property of angiotensin suggests that it might modify the permeability of other membranes.

5. EFFECT ON PLASMA AND TISSUE ELECTROLYTES:

Foglia and Moglia reported in 1940 (387) that "angiotonin" increased plasma potassium, but recently Gantt and Carter (364) did not find any significant change in the plasma concentration of Na, K, Ca, P, and Mg after an angiotensin infusion. Read (388) reported that angiotonin caused a potassium decrease and a small sodium increase in the isolated rat uterus. Friedman et al (389) observed a decrease in extracellular sodium and fluid volume while infusing angiotonin in nephrectomized rats. They concluded that angiotonin promoted the movement of sodium and water into the intracellular compartment. In their latest work (206) they conclude that angiotensin, like

other vasoconstrictor substances shifts sodium into and potassium out of the vascular smooth muscle cells.

As previously discussed, angiotensin does not appear to exert a direct action on the active ATPase in the kidney, but nevertheless, this does not exclude such an action in the muscular cell so that the permeability of the cell membrane would be modified.

6. ADRENAL CORTEX:

a) In vivo:

The discovery of the aldosterone stimulating effect of angiotensin came after a series of indirect observations indicating the kidney-adrenal relationships. In 1951, Deane and Masson (390) reported that encapsulation of the kidney, a procedure which results in hypertension, presumably through release of renin, or injections of crude renin preparation, both produced an enlargement of the zona glomerulosa in rat adrenal glands. Toussaint et al (391) and Hartroft and coworkers (193) found, in 1953, that sodium loading decreased both the number of juxtaglomerular cells and the width of the zona glomerulosa. Later Hartroft and coworkers were able to establish a relationship between sodium intake, the juxtaglomerular granularity and the width of the zona glomerulosa in the rat (392, 393) and in postmortem human material (394). Tobian et al confirmed these results (189, 395).

In 1958, following their finding of an increased aldosterone excretion in many patients having essential hypertension, Genest and his coworkers undertook the study of various factors which could influence aldosterone excretion. They had already demonstrated that nor-epinephrine, epinephrine and reserpine had no effect on the aldosterone excretion (421) when they studied the action of the newly synthesized angiotensin II on this parameter in 1959. This led them to report the first direct evidence that angiotensin

was a potent aldosterone stimulating substance and also probably the major factor controlling aldosterone production (7, 346, 363, 397-400). This effect appeared to be independent of the pressor activity since subpressor doses elicited the same stimulation while infusion of other vaso-active substances at pressor rates failed to do so. Laragh et al (401) later reported that angiotensin strongly increased the aldosterone secretion rate in humans, thus confirming the previous findings of Genest et al. Laragh et al also found that in normal subjects, continuous intravenous infusion of angiotensin for periods up to 11 days maintained a high aldosterone secretion rate (402). However, in cirrhotic patients, angiotensin failed to further increase aldosterone secretion suggesting that, in these patients, the aldosterone secretion was already maximally stimulated (267). The latter phenomenon has also been observed by Bryan et al (403) in two patients with a syndrome of hyperaldosteronism secondary to primary juxtaglomerular hyperplasia. In addition to the increase in aldosterone, Genest et al reported in 2 out of 4 subjects a small increase in the excretion of cortisone, cortisol and their tetrahydro derivatives during angiotensin infusion. Nowaczynski and his coworkers (404) reported the increase of unidentified substances referred to as compounds III, IX and X during similar infusions.

The findings by the groups of Davis (405, 406) and Ganong (111, 407-409) that crude kidney extracts increased the aldosterone secretion rate of hypophysectomized-nephrectomized dogs, while liver (405, 414) and spleen (408, 414) extracts failed to do so, gave further support to the hypothesis that the "aldosterone stimulating hormone" was originating from the kidney. Semi-purified renin preparations had the same effects on aldosterone secretion (273, 409-414). Moreover, Ganong et al (415) showed that this effect was specific since renin failed to increase aldosterone after incubation with

plasma containing renin antibodies. Finally, Mulrow and Ganong (111, 416) confirmed the aldosterone-stimulating effect with the administration of small doses of angiotensin II into hypophysectomized-nephrectomized dogs. They also found (416) in dogs, that high doses of angiotensin increased both glucocorticoids and aldosterone while small doses increased only aldosterone secretion as opposed to ACTH which, in small doses, stimulated glucocorticoid secretion only. Other studies in animals have also reported important increases in the secretion of corticosterone (410, 413, 414) and 17-hydroxycorticosteroids (412-414, 417) simultaneously with a rise in aldosterone secretion during angiotensin infusion.

Further demonstration of a specific aldosterone stimulating effect of angiotensin was provided by studies in which angiotensin and other substances were directly infused into the adrenal artery. Ganong, Mulrow and coworkers (418) showed that angiotensin was more active when infused directly into the adrenal artery of dogs than when given intravenously. Hog renin, human kidney extracts, and dog renin were also effective when given into the artery while erythropoietin and β-carboline were without effect (419). Blair-West et al (420) observed an increase in the aldosterone secretion of transplanted sheep adrenal gland after direct infusion of angiotensin II into the artery, while only slight increases occurred with vasopressin and bradykinin and no effect followed infusions of acetylcholine, nor-epinephrine, serotonin and oxytocin. In these preparations, Blair-West et al (420) did not find a simultaneous increase in cortisol and corticosterone unless large doses were given while Ganong and Mulrow (418) reported a slight increase in 17-hydroxysteroids. ACTH infused into isolated adrenal glands of sheep increased corticosterone and cortisol secretion consistently while it increased aldosterone secretion only transiently (420).

Rats behave differently from other animals in their adrenal response to angiotensin. Eilers and Peterson (422) reported that renin and angiotensin were ineffective in stimulating rat aldosterone secretion. Similarly, Marieb and Mulrow (423, 424) could not detect any increase in the aldosterone secretion rate of these animals even during continuous angiotensin infusion lasting 7 to 12 days. Margoulie et al (425) reported that a single dose (5μ gr/100 gr) of angiotensin injected intra-peritoneally induced a transitory increase in corticosteroid secretion but chronic treatment for 2 to 12 days, neither caused a hyperplasia nor increased the enzymatic activity of the zona glomerulosa such as was observed during a period of low sodium diet. Contrary to these negative findings, Hungerford et al (426), Marx et al (260), and Lamberg et al (427) found a hyperplasia of the zona glomerulosa in rats, after chronic injections of angiotensin in oil for 3-5 weeks (260, 427) or in water for two weeks (426). Furthermore, the zona glomerulosa of these adrenal glands presented a marked increase in enzymatic activity (260, 427) involving especially Δ5-3β hydroxysteroid dehydrogenase (260), glucose-6-phosphate dehydrogenase (260, 427) and succinic dehydrogenase (427), whereas the enzymatic activity in the other zones remained unchanged.

In 1953, Sevy and Ohler (428) observed a decreased ascorbic acid content in the adrenals of normal but not of hypophysectomized rats, after administration of crude hog renin. No changes were noted after the administration of angiotonin (428). Carr and Bartter (429) found no decrease in adrenal ascorbic acid content after infusion of angiotensin into hypophysectomized rats while treatment with ACTH depleted its content. Although Margoulie et al (425) could not detect any decrease in ascorbic acid, they did report a significant decrease in the cholesterol content of intact rat adrenal after a single injection of angiotensin.

b) In vitro:

Many conflicting results are reported concerning the action of angiotensin on the adrenal tissue "in vitro". Some of these discrepancies might be explained by differences in conditions of incubation and in the dose of angiotensin added to the medium. Since angiotensin is rapidly destroyed by many enzymes and since "in vitro" incubations are pursued usually for many hours, it thus appears probable that in some of these studies, angiotensin was inactivated during the period of incubation.

Renin has been consistently found to be without effect on aldosterone production during incubation with rat adrenals (430, 431) or with beef adrenal slices (431). However, Kaplan and Bartter (431) found that angiotensin II significantly increased the synthesis of aldosterone, corticosterone and cortisol during incubation with beef adrenal slices but they could not reproduce these findings with rat adrenals. They suggested that angiotensin was acting early in the steroidogenesis since angiotensin increased aldosterone only when allowed to incubate with cholesterol. This would indicate an action as early as that of ACTH in the steroidogenesis, but nevertheless it would imply a different mechanism since angiotensin is more active on the production of aldosterone and corticosterone than ACTH (431) and has no effect on the phosphorylase activity (431, 432). Carballeira (433) found no consistent change in the incorporation of progesterone-4-C^{14} into aldosterone under the influence of angiotensin incubation with rat adrenal. He and Tsang (433), working with beef adrenal slices, found similar results. Also, they could not reproduce the work of Kaplan and Bartter (431) using cholesterol-4-C^{14} as precursor. Statchenko (434) was also unable to demonstrate any effect of angiotensin on aldosterone production in rat or beef adrenals. However, she found a marked increase in the production of corticosterone.

Kumagai et al (435) reported recently the first study done on human adrenals thus far. They incubated adrenals from normal subjects, and patients with primary hyperaldosteronism and Cushing's disease, for 2 hours at 37°C with 25 micrograms of angiotensin for the first hour, adding the same amount for the second hour. When incubated with normal adrenals, angiotensin increased two-fold the production of aldosterone and about threefold the production of corticosterone, but had no effect on cortisol and cortisone production. ACTH was less active for aldosterone production, equally active for corticosterone and definitely more active than angiotensin for cortisol and cortisone production when incubated with normal adrenal gland. When angiotensin was incubated with adrenal tissue from patients with primary aldosteronism, it increased the production of aldosterone only. On the other hand, ACTH increased all steroids except aldosterone during incubation with adrenals from patients with Cushing's disease due to adrenal adenoma.

Two groups of studies have shown that the capacity of adrenals to synthesize aldosterone was increased in rats previously treated with angiotensin. In the study of Glaz and Sugar (436), the adrenal production of aldosterone was increased in rats treated with angiotensin 24 hours previously. However, the addition of angiotensin to the adrenal slices did not increase the synthesis of aldosterone. Marx et al (260) also reported that the adrenals of their rats chronically treated with angiotensin in oil for four weeks exhibited an increased production of aldosterone when incubated for one hour.

Angiotensin is a potent aldosterone stimulating substance in vivo in all animals except rats. Its action in vitro is far from being clearly established and its mechanism is unknown. Despite the lack of unanimity regarding the "in vitro" findings, much evidence supports the possibility of a direct

action on the adrenal gland. Its action on the various biosynthetic intermediaries leading to aldosterone production is not completely clarified and understood at present, so that the action of angiotensin cannot be clearly defined. Nevertheless, the discovery of its powerful action on aldosterone secretion in man and dogs has opened a new fascinating field of research. Numerous studies in progress are currently establishing the renin-angiotensin system as a vital link in the maintenance of electrolyte homeostasis.

7. ADRENAL MEDULLA:

The action of angiotensin on the adrenal medulla is the latest described and probably one of the most promising. This action was previously discussed in the section of this thesis dealing with the mechanism of action of angiotensin on muscle. Kaneko, McCubbin and Page (312) discovered the ability of vasoconstrictor drugs to evoke an adrenal medullary discharge after sensitization by ganglion stimulating agents. This effect was found to be specific not only for angiotensin, but also for nor-epinephrine, serotonin, vasopressin, phenylephrine, metarominol and barium chloride as well. This sensitization was not prevented by atropine, ergotomine, spinal cord section or tachyphylaxis. Since they also observed adrenal medullary discharge after constriction of the adrenal artery, these workers concluded that the release was not due to a stimulating action on the medullary cells but was due to the hypoxia secondary to vasoconstriction. Feldberg and Lewis (278, 437) put forth the most convincing evidence for the action of angiotensin on the release of catecholamines. Their findings arose from experiments on a special animal preparation especially designed for the study of release of catecholamines. Eviscerated cats were injected via the coeliac artery and the release of catecholamines was estimated by the contraction of denervated nictitating membrane. Angiotensin has no musculotropic effect on this membrane but when Feldberg and Lewis injected

angiotensin into the coeliac artery, they noticed a marked contraction of the nictitating membrane, suggesting a release of catecholamines. Furthermore, they could demonstrate that angiotensin was the most potent catecholamine releasing substance. They calculated grossly that 1 mol of angiotensin was able to release 1000 mol of catecholamines, being more potent than acetylcholine in this respect. Adrenalectomy abolished the contraction of the nictitating membrane and greatly reduced the systemic pressor response to angiotensin, thus indicating that part of the pressor response to angiotensin is due to a release of catecholamines. Very recent studies by Poisner and Douglas (438), and Robinson (439) confirmed the previous study. Moreover, it was reported that calcium was necessary for that mechanism of action (438). Vincent et al (440) were unable to detect any significant change in the excretion of vanillyl mandelic acid after 6 hours infusion of angiotensin or nor-epinephrine.

8. ON INTERMEDIARY METABOLISM:

Very few investigations with angiotensin have been undertaken with regard to carbohydrate and fat metabolism.

Foglia and Moglia (387) reported in early studies that crude angiotonin preparation produced an increase in blood glucose of dogs. This effect was abolished by adrenalectomy. Synthetic angiotensin was also reported to increase blood glucose in dogs (231, 441), rabbits and humans (441). Heidenreich et al (441) concluded that the rise in blood glucose was not due to the release of catecholamines because the action of angiotensin on blood glucose was faster and more transient than the one encountered with adrenaline and also because the action of adrenaline was antagonized by ergotamine while the action of angiotensin was not. Johnson and Bruce (209), on the other hand, observed no change in blood glucose, lactic acid, pyruvate, or free fatty acid concentration during infusion of angiotensin in normal subjects.

9. OTHER EFFECTS:

Katz et al observed that chronic injections of angiotensin dissolved in water into rats produced an increase in renin content of the kidney (261) and an increase in J.G.I. after one week of treatment (442). The J.G.I. returned to normal after 2 weeks of treatment (442). On the contrary, Kuhn, Hartroft and Pitcock (443) found a degranulation of the J.G.C. and a decrease in extractable renin of the kidney during the first twelve days of chronic injection of a crude kidney extract into rats but both parameters increased over normal values after twelve days of treatment. Marx et al (260) reported that the J.G.I. fell to zero in their rats treated for four weeks with angiotensin in oil while the J.G.I. was within normal values in rats treated with angiotensin in water for 2 weeks. The rats treated with angiotensin in oil gained weight more slowly than normal control rats.

Hungerford and Panagiotis (426) found that daily sub-cutaneous injections of 6 micrograms of angiotensin to rats for twelve days, resulted in a severe lipid depletion in the pineal gland. Since the activity of the pineal gland has been related in some instances to the state of sodium balance, aldosterone secretion and the width of the zona glomerulosa, they concluded that the pineal gland was probably involved in the sodium regulating process. However, this conclusion appears to be overstated since pinealectomy does not modify the normal response of aldosterone secretion to various stimuli (595, 596).

D - BLOOD PRESSURE AND THE RENIN-ANGIOTENSIN SYSTEM.

1. NORMAL BLOOD PRESSURE:

Since many previous studies failed to report the occurrence of pressor material (renin ?) in the renal venous or systemic blood of normal animals

and men (143, 444-447), the physiological role of the renin-angiotensin system in the maintenance of normal blood pressure was considered dubious by many investigators. However, these negative findings could be attributed to the lack of specificity and sensitivity of the various methods used. Recent studies with the measurement of renin-activity, which is a very sensitive means of appraising the activity of the renin-angiotensin system, revealed that almost all normal subjects had some activity in their peripheral or renal blood (57, 147, 448-453). In contrast, the angiotensin blood levels were found very low or undetectable in many normal subjects (8, 108, 454-456). The occurrence of renin in normal kidneys has been consistently reported even with crude extraction methods (1, 12, 18-20, 457). Schaffenburg, Haas and Goldblatt (458) in 1960, measured and compared the renin content of normal kidneys in various species. They found that renin was present in all normal kidneys studied, although concentrations varied greatly between species. Renin was more concentrated in rabbits, less concentrated in rats, beefs, hogs, cats, chickens, dogs, horses, and finally, was at lowest concentration in human kidneys. The presence of granulations in the juxtaglomerular cells was also reported in the kidneys of normotensive humans (192, 394). Pitcock et al (394) reported that the J.G.I. was more elevated in men than in women.

Therefore, it appears that a basal level of renin secretion is present in normotensive subjects. However, nobody has ever been able to establish a correlation between the level of blood pressure and the renin-angiotensin system in normotensive subjects or animals. Moreover, as was reported in the experimental section of this thesis, many normotensive subjects have higher than normal levels of renin-activity and angiotensin while many hypertensive subjects have lower or undetectable levels. If the renin-angiotensin system was the sole mechanism in the maintenance of normal blood pressure, nephrect-

omy would result in a marked decrease in blood pressure but the experimental data from such procedures in normotensive animals do not support this hypothesis. Yet, this does not exclude the interaction of the renin-angiotensin system with other mechanisms in the maintenance of blood pressure.

Nevertheless, the renin-angiotensin system appears to assist some physiological mechanism in the hemodynamic adjustment encountered during exercise or changes in posture. Helmer (147) reported recently in 5 out of 7 normal subjects submitted to exercise on a bicycle ergometer for 20 minutes, a significant increase in plasma renin-activity. Cohen et al (451), using the method of Boucher et al (8) found a slight but significant increase in the mean renin activity of normal subjects in standing position for four hours compared to normal subjects who remained in reclining position for more than 12 hours. They could not reproduce the findings of Helmer during exercise on a bicycle ergometer.

2. EXPERIMENTAL HYPERTENSION:

a) Goldblatt's type of hypertension:

Katzenstein (460) in 1905 made the first observation that renal ischemia, secondary to partial or total constriction of the renal artery, produces an increase in blood pressure. Furthermore, this investigator also observed that the re-establishment of the circulation through a totally ischemic kidney induces a rise in blood pressure. However, this most interesting observation was forgotten for many years. The kidney became a major point of interest only after 1934 when Goldblatt and coworkers (2) demonstrated a sustained elevation of blood pressure in dogs following partial narrowing of the renal arteries. These workers described a reproducible method for the production of chronic renal ischemia and this gave rise to a great deal of investigation by permitting the study of experimental hypertensive diseases in

many mammalian species.

Soon, it was postulated that this type of hypertension was produced by a substance liberated by the ischemic kidney into the circulation. Dicker (461) and later Taquini and coworkers (462) observed that the re-establishment of the circulation through a totally ischemic kidney was accompanied by a rise in blood pressure. Houssay and Fasciolo, in 1937 (463), were the first to demonstrate that renal experimental hypertension was mediated through a humoral mechanism. After grafting an ischemic kidney into the neck of a nephrectomized dog, they observed a rise in the blood pressure of the animal within a few minutes. Thereafter, the old and almost forgotten discovery of Tigerstedt and Bergman (1) was reconsidered with great interest. Numerous studies concerning the role of the renin-angiotensin system in experimental and human hypertension were initiated thereafter. The findings reported in Goldblatt's type of hypertension are quite consistent from one group to another and the most recent methods of measurement do not greatly modify the previous findings reported with crude methods of extraction. During the acute phase of the development of hypertension, renin increases progressively during the first days in the peripheral blood and renal vein of the clamped kidney (133, 143, 444, 445, 464-469). This is also supported by the finding of a rise in blood angiotensin (106, 107, 109, 470) during the same period. The renin content of the clamped kidney is generally found increased (17, 18, 160, 458, 463, 465, 471-473), while in the contralateral "normal" kidney, renin is almost absent (17, 18, 177, 178, 472-474). Hyperplasia and hypergranularity of the J.G.C. is generally seen in the ischemic kidney (148, 160, 177, 178, 476-480), while the opposite kidney is almost completely degranulated (160, 177, 178, 477, 479, 480). When the hypertension becomes chronically established after a few weeks, blood levels of renin (445, 464, 467-469, 481) and of angiotensin (109, 482, 483) and the renin content of the

ischemic kidney (465, 471) return to undetectable levels or are markedly decreased. In rats hypertensive for 7 weeks, Hartroft et al (479) found many normal or below-normal J.G.I. in the clamped kidney. Among the more recent studies, only that of Peart and Brown (446) failed to find any increase in blood renin during the acute phase of hypertension, but later the same workers reported that renin was increased in blood (484) and in lymph (66) of experimental renal hypertensive animals.

In summary, the renin-angiotensin system appears to play an important initiating role in the development of hypertension following renal artery constriction, but during the chronic phase of hypertension, its role is much less clear. However, since most of the methods used previously for the measurement of renin were rather crude, slight increases over the normal levels might have been undetected. Indirect evidence for the role of the renin-angiotensin system in chronic "Goldblatt" hypertension was supported by the findings of Wakerlin (50), Deodhar (54) and their respective coworkers that the administration of antirenin antibodies lowers the blood pressure of these animals. However, many other observations have accumulated over the years to indicate that the renin-angiotensin system is probably neither the sole nor the most important mechanism involved in the development of chronic hypertension.

After total or partial nephrectomy, hypertension was reported to persist for a few days in renal hypertensive rats, rabbits and dogs (485-488). Hartroft (479), Tobian (480) and Fisher (160) and their coworkers, reported an increased J.G.I. in the clamped kidney of animals who did not overdevelop hypertension. Many investigators claimed that they were unable to demonstrate any correlation between the increase in blood pressure and the renin content of the kidneys (474, 489, 490). Along this line of evidence,

Regoli et al (491, 492) reported that if the unclamped kidney is excised in renal hypertensive rats, the level of blood pressure remains unchanged despite a progressive decrease in renin content to normal values within three weeks in the remaining clamped kidney. More strikingly, they also showed that when unilateral nephrectomy is done before clamping the remaining kidney, the same degree of hypertension develops within the same time than in non nephrectomized rats, but no change can be detected in the renin content of the ischemic kidney. The same group (493) further demonstrated that a normal concentration of renin-like material is circulating in unilaterally nephrectomized hypertensive rats while it is greatly increased in non nephrectomized hypertensive animals.

It thus appears, that the renin-angiotensin system is probably not the sole mechanism involved in the maintenance of high blood pressure, at least in chronic experimental renal hypertension. Many hypotheses have been proposed to explain the chronic phase of experimental hypertension, but only a few of them have received support from experimental studies.

One of these hypotheses proposes that after a rather prolonged sustained hypertension due to an increased renin secretion, an antipressor mechanism would be "blurred out" and subsequently less, or no, renin would be necessary to maintain a high blood pressure. In their book, Braun-Menéndez et al (47) summarized many observations suggesting a protective effect by the kidney against high blood pressure: first, renin is more potent in nephrectomized animals; second, removal of the normal kidney in renal hypertensive dogs results in a further increase in blood pressure and finally, the return of blood pressure to normal levels after removal of the ischemic kidney is more rapid in animals having their contralateral kidneys than in bilaterally nephrectomized animals. Grollman, William and Harrison (285, 494) in 1940,

prepared a renal extract which, when injected to hypertensive animals, lowered the blood pressure. The antihypertensive function of the kidney was further demonstrated by the observation that bilateral nephrectomy resulted in the development of hypertension (495-497), but it was believed by others to be due mainly to overhydration and positive sodium balance in most of these conditions (498). Recently, Muirhead et al (499), Hickler et al (500) and Milliez et al (501) have isolated from the kidney medulla a potent vasodepressor material which they claimed to be the active component of the antihypertensive mechanism of the kidney. None of these groups has purified their material to a sufficient degree for characterization.

Despite the lack of a direct demonstration of the physiological role of a renal antihypertensive mechanism of the kidney, its existence appears most likely in the light of the recent findings of Tobian, Schonning and Seefeldt (502). These investigators have demonstrated that when a normal isolated kidney was connected to the circulatory system of rats with renal hypertension, a marked lowering in blood pressure occurred after an hour. When the isolated kidney was connected to the same type of rats, but with a variable resistance in order to regulate the kidney perfusion pressure at a normal level, no drop occurred in the rats' blood pressure. They thus concluded that the antihypertensive mechanism of the kidney was controlled by perfusion pressure, being stimulated by high perfusion pressure while being inhibited under normal perfusion pressure.

Other findings suggested that the chronic phase of hypertension might be mediated through the nervous system. Reed et al (503) observed that the blood pressure of chronic hypertensive animals was lowered by a sympatholytic agent while this same agent was inactive on the blood pressure during the acute phase of hypertension. McCubbin and Page (504) reported that angiotensin

potentiates the response to factors releasing catecholamines. Since the response to tyramine is increased in both acute and chronic renal hypertension, they postulated that very small amounts of angiotensin, difficult to detect by present assay methods, would continue to have a potentiating effect on the sympathetic nervous system during chronic renal hypertension. Lawrence and Dickinson (505) reported recently that, although denervation of carotid sinus in normal animals causes only a transitory increase in blood pressure, it greatly potentiates and facilitates the establishment of hypertension in rabbits following renal artery constriction. This observation appears to be especially significant since most of their animals with renal artery constriction did not develop hypertension unless the carotid sinus was previously denervated.

It can also be postulated that after a prolonged and sustained secretion of renin, the establishment of irreversible ionic changes in the wall of arteries might be responsible for the maintenance of high blood pressure afterwards. The relationship between electrolytes and hypertension has been reviewed and studied extensively by Tobian (189) and Dahl (508, 509). From these reviews, it appears that the increase in sodium content of the arterial wall is the common denominator of chronic hypertensive diseases. However, Tobian (189) pointed out that the level of blood pressure correlates better with the intracellular potassium content than with the sodium content. Takahashi (305) supported this point recently by reporting that potassium, but not sodium, is the most important ion in the development of hypertension. He based his conclusion upon the observation that in such experimental conditions as aminonucleoside nephritis, or during carbon tetra-chloride administration, the sodium content alone increases in the aortic wall of rats without any increase in blood pressure, while in experimental renal hypertension, only the

potassium content of the aorta increases in the first two weeks following clamping, during the development of hypertension. Nevertheless, these studies are not entirely convincing since hypertension depends more on the small arterioles than on large vessels like the aorta. Thus, until confirmation of the previous findings, it would seem safer to keep in mind the well documented correlation established by Dahl (508, 509) between hypertension and the sodium intake in animals and man. In addition to this factor, Dahl (509) also put forward the concept of a genetic background in the determination of a greater sensitivity to sodium in certain animal species or in certain stains of a same species. This factor is certainly of utmost importance and might explain the lack of hypertension in certain animals after clamping of the renal artery despite a hypergranularity of the juxtaglomerular apparatus (160, 479, 480) and also the lack of hypertension in a great number of human subjects reported to have a narrowing of the renal artery at postmortem examination.

The adrenal steroids might represent an important permissive factor in the development of hypertension since adrenalectomy prevents the development of experimental hypertension (472, 506, 507). Masson and his coworkers summarized many of their previous experiments which support the potentiation of renin pressor action by steroids (295). They showed that administration of semi-purified renin to normal animals increased blood pressure but did not result in the development of hypertensive disease. On the other hand, they found that the administration of semi-purified renin to uninephrectomized rats pretreated with desoxycorticosterone, cortisone, cortisol or aldosterone and salt causes an acute syndrome characterized by water retention, severe hypertension and vascular disease.

Although a large number of studies have been conducted on the Goldblatt

type of hypertension, the final conclusion cannot be drawn on this form of hypertension. In many investigations, the renin-angiotensin system appeared to be involved to a large extent especially in the acute phase of hypertension but a less clear association was reported during the chronic stage. It seems very difficult to separate the role of the renin-angiotensin system from other factors: the sympathetic nervous system, the antihypertensive mechanism, the ionic balance, the genetic background and the adrenal function. A participation of all these factors would most suitably explain the development and the maintenance of this type of hypertension.

b) Other types of experimental renal hypertension:

The development of hypertension was also reported in animals following coarctation of the aorta, silk and cellophane perinephritis, figure of eight ligatures around the kidney, renal infarct and ureteral ligature. Following coarctation of the aorta above the renal arteries in dogs, Scornik and Paladini (510) were unable to detect any increase in angiotensin blood levels. Nolla-Panades et al (511) did not find any change in the juxtaglomerular cell granularity in identical conditions. An increased level of pressor material in blood and in the involved kidney was reported during experimental perinephritis (444, 512, 513) and in unilateral cellophane perinephritis (489), while the pressor material content was reported to be decreased in the opposite kidney. A hypergranularity of the juxtaglomerular cell was also observed in the cellophane perinephritic kidney during the early stage of hypertension (514).

Omae et al (515, 516) remakred that grafts of a figure of eight-ligatured kidney or of an infarcted kidney in nephrectomized animals release less pressor material than normal kidneys similarly transplanted. Sokabe and Grollman (517) reported an absence of pressor material released from the kid-

ney with figure of eight ligatures one week after renal injury. In uninephrectomized rats with figure of eight ligatures on the remaining kidney, Hartroft (479), Fisher (160) and coworkers found a decreased granularity of the juxtaglomerular cells in the injured kidney while Garber (518) observed an increase in the activity of J.G.C. during the early stage of hypertension. However, in the late stage of hypertension, Garber (518) noted a decreased activity.

During chronic ureteral ligation in dogs, the renin content of the involved kidney was found increased (18) and hyperplasia of the juxtaglomerular apparatus was observed (148). Normal or increased J.G.I. were reported in rats (160, 479) submitted to similar conditions. Twenty to 120 minutes after acute ureteral ligature, Tremblay (519) in our department, found a degranulation of the juxtaglomerular cells associated with an increase in the liberation of renin in the renal vein of dogs. Vander and Miller (166) also reported that renin-activity increased rapidly in renal venous blood following acute ureteral clamping in dogs.

3. HUMAN HYPERTENSION:

The early findings involving the renal pressor system in the development of experimental renal hypertension were most stimulating and promising for the understanding and the treatment of human hypertensive diseases. The first studies in man, reported by Harrison et al (18) and Prinzmetal and Friedman (17) in 1936, strongly suggested that the renal pressor system was involved in human hypertension since they found an increase in the pressor material extracted from the kidneys of various hypertensive humans. In 1939, Corcoran and Page (520) claimed that they were able to reproduce the intrarenal hemodynamic changes encountered in essential hypertension by infusing crude angiotonin and renin into animals. They thus believed that the renal

pressor system might play a role in human hypertension. However, most of the subsequent studies, using nonspecific methods failed to demonstrate the occurrence of increased pressor substances in the blood of patients presenting any kind of hypertensive disease (345, 445, 521-523). The only positive findings in the early literature were reported by Page in 1940 (444) when he detected an increased pressor substance in the blood of patients with essential hypertension, malignant hypertension and chronic nephritis. Later, a pressor substance was found in the blood of patients suffering from acute glomerulonephritis (524, 525) and during acute eclampsia or pre-eclampsia (524).

However, the evidence for a role of the renin-angiotensin system in hypertensive diseases remained scanty for many years. The advent of more sensitive and specific methods for the measurement of angiotensin and renin created a renewal of interest in the renin-angiotensin system and initiated a new series of studies on the problem of human hypertension. In 1952, Kahn and coworkers (108) reported that arterial angiotensin levels were within normal range in most of their cases of essential hypertension, but they found a marked increase in all cases of malignant hypertension. However, most of their patients in the latter group presented a certain degree of congestive heart failure, a condition which is known today to be accompanied by a high level of angiotensin (See experimental data on congestive heart failure). In 1961, Genest et al (526, 527) confirmed the results of Kahn et al in essential hypertension, but disagreed with their findings in malignant hypertension, since they found normal levels in this group of patients. They also found a significant increase of angiotensin levels in renal vein or peripheral blood in hypertension associated with renal artery stenosis and low or undetectable levels in primary hyperaldosteronism. Morris et al (112, 455, 528) were unable to detect any circulating angiotensin in essential and malignant hyper-

tension, in hypertension associated with pyelonephritis and in primary hyperaldosteronism, but they found some in all their cases of renal artery stenosis and coarctation of the aorta. Langford et al (529, 530) using Palladini's method reported an increased angiotensin levels in the renal venous blood of most of their patients with renal artery stenosis. Mulrow et al (532) reported that all their patients with renal artery stenosis had peripheral blood levels of angiotensin within their normal range.

Helmer and Judson (147, 533-536) detected the presence of a pressor substance in the blood of patients with malignant hypertension, renal artery stenosis and in few cases of essential hypertension, pyelonephritis and coarctation of the aorta. The dialyzed plasma of these patients induced a rise in blood pressure when injected to pithed cats and a contraction of rabbit aortic strip "in vitro". They later identified this material with renin. In 1962, they proposed (56) a modified version of their first method for the measurement of renin-activity. Their positive findings stimulated many other investigators to re-appraise the role of the renin-angiotensin system in human hypertension. However, the use of different methods for the measurement of renin activity, rendered almost impossible the comparison of results among the different studies. These recent findings will be reported in the experimental section of this thesis dealing with the investigation of hypertensive disease.

The juxtaglomerular apparatus is normal in most cases of essential hypertension (192, 537-539) except in the more severe cases presenting nephrosclerosis (192, 537-540). In malignant hypertension, the juxtaglomerular apparatus is frequently hyperplastic (192, 537, 540) with increased granularity (537) or with decreased granularity (192). In most ischemic kidneys secondary to renal artery stenosis, the J.G.C.C. and J.G.I. are markedly increased (191, 192, 538, 539, 541-543), while in such conditions, normal J.G.C.C. and J.G.

are generally associated with failure of corrective surgery regarding the cure of hypertension. Tremblay et al (191) recently reported that the granularity of the juxtaglomerular apparatus by itself is not a sufficient criterion for the prognosis of surgery, since patients presenting arteriolosclerosis of the kidney are not usually improved by surgery despite a greatly increased J.G.C.C. and/or J.G.I. in the involved kidney. In primary aldosteronism, the juxtaglomerular apparatus is normal (538, 539). It is increased in the acute phase of glomerulonephritis (537) but normal during the chronic phase (192, 538). It is also normal or increased in chronic pyelonephritis (192, 538). Finally, an increased granularity and hyperplasia is reported in eclampsia (537) and a hyperplasia with normal granularity in pheochromocytoma (192).

4. HYPOTENSION AND SHOCK:

Saperstein et al (548) in 1940, observed that the administration of hypertensinogen to dogs in hemorrhagic shock resulted in an elevation of blood pressure. They later found (549) that an increased pressor material was circulating in the peripheral blood of these animals. Hamilton and Collins (105, 237, 550) soon confirmed that after hemorrhage, a pressor substance increased in the renal vein and peripheral blood of dogs. They also observed (550) that the mortality of nephrectomized animals was greater than that of non-nephrectomized animals after identical blood loss. Huidobro and Braun-Menéndez (551) showed that short periods of profound hypotension of only 4-11 minutes were sufficient to produce a liberation of renin by the kidney. Dexter et al (552) reported that a renin-like substance increased progressively in blood of shocked dogs and that renin returned to normal after blood transfusion. They also observed that a significant decrease in angiotensinogen occurred in many of these animals. They concluded that the

renal pressor system was a compensatory mechanism in shock and that death occurred in these conditions when the organism was unable to synthesize angiotensinogen as rapidly as it was converted to hypertensin by renin. Leloir et al (553) and Collins and Hamilton (105) noticed also that angiotensinogen concentration decreased in blood of animals following hemorrhage. Mikasa and Masson (554) have reported that grafting of a normal kidney to a nephrectomized rat in hemorrhagic hypotension is followed by a prompt but temporary return of the blood pressure to normal. Shipley et al (556) found in the blood of cats dying from hemorrhagic shock or acute poisoning, a sustained pressor principle which was later identified as renin.

Recently, Scornik and Palladini (510) demonstrated a significant increase of angiotensin blood level in dogs following hemorrhage. Gross and his group (493) observed that 15 minutes after hemorrhage, a renin-like substance increased in the blood of rats while the renin content of kidneys remained unchanged. Taquini et al (71) also reported recently that the renin content of the kidney remained unchanged after 30 minutes of marked hypotension in rats. No renin or angiotensin appears in the blood following nephrectomy in animals submitted to hemorrhage (287, 510, 551, 552), thus suggesting the renal origin of renin. However, the existence of other pressor substances from extrarenal sources was reported in shock conditions. Page et al (555) detected in dog plasma a vasoconstrictor substance not originating from the kidney or adrenal during shock induced by hemorrhage, by limb tourniquets or by burns.

Hypergranularity of the juxtaglomerular cells was observed during hypotension following hemorrhage in rabbit (557) or following acute administration of various hypotensive drugs in rats (518). However, no increase in number or granularity of the juxtaglomerular cells was found in humans who died of circulatory collapse (558).

E - ALDOSTERONE.

1. DISCOVERY:

Many years before the discovery of aldosterone, an amorphous fraction of the adrenal cortex was known to have a potent sodium retaining effect when injected to animals. This fraction was even recognized as the most important for the maintaining of life after adrenalectomy. In 1950, Deming and Luetscher (559) using a rat bioassay, reported the presence of increased sodium retaining activity in urine from nephrotic patients. However, the numerous attempts to isolate and purify this substance failed until 1952. At that time, Zaffaroni and Bush developed new paper chromatographic systems and applied them to the separation of steroids. These chromatographic systems, combined with the use of a bioassay, led Tait and Simpson to isolate aldosterone in 1952. Its structure was then soon determined through the combined work of two groups: Tait and Simpson in England and Wettstein and Reichstein in Switzerland.

Giroud and coworkers (590) established that aldosterone synthesis was located in the zona glomerulosa. The main biosynthetic pathway was described through progesterone, desoxycorticosterone and corticosterone.

When administered to normal subjects, aldosterone has a half-life of 15 to 30 minutes. It is metabolized mainly by the liver in which it is inactivated by oxidation into tetrahydroderivatives and by combination with glucoronic acid. Five to fifteen percent of the secreted aldosterone passed into the urine as free aldosterone or conjugated with glucuronic acid.

2. ACTION OF ALDOSTERONE:

Aldosterone is the most potent sodium retaining hormone isolated, being 20-30 times more potent than desoxycorticosterone and at least 100 times more potent than cortisone.

The administration of aldosterone to man and animals results in an increased reabsorption of sodium and increased excretion of potassium (560). The stimulation of sodium transport is believed to occur mainly at the distal portion of the kidney tubule through an ion exchange mechanism (561, 562). During prolonged administration to normal subjects, aldosterone induces a marked sodium retention by the kidney, only for a period of 5 to 7 days after which an escape occurs while the increase in potassium excretion continues.

Blair-West et al (563) reported that administration of aldosterone decreases sodium and increases potassium in the saliva of sheep. They were able, later (564), to correlate these changes with the aldosterone secretion rate of their animals. Crabbé and Thorn (560) found less marked changes in human saliva following administration of aldosterone.

Aldosterone can promote active transport of sodium by the toad bladder (565, 566) while other steroids were found less active or inactive. Aldosterone probably acts through specific receptor sites. Certain substances having a structural similarity to aldosterone were found to antagonize the action of aldosterone. These substances, which are inert in the absence of aldosterone, block its action probably by binding to the same receptor sites. This is likely since lesser amounts of tritiated aldosterone are found in the wall of the toad bladder after treatment with aldosterone antagonists (565).

There is a delay of up to one hour before the appearance of the renal tubular effects of injected aldosterone. This was clearly shown by Barger, Berlin and Tulenko (567) and confirmed by Ganong and Mulrow (568) who injected d-aldosterone directly into one renal artery and observed the characteristic effect in both kidneys some 30-60 minutes later. The same delay is also observed in vitro when aldosterone is applied to the serosal side of a toad

bladder preparation. Recently, Porter and Edelman (569, 569a) and Crabbé and Deween (570) put forward evidence that aldosterone acts through the stimulation of protein synthesis. This mechanism of action would explain the delay observed following an injection of aldosterone. This delayed onset of action would also exclude aldosterone as mediating the early fall in sodium excretion observed in standing position and after hemorrhage. Nevertheless, the discovery of this mechanism of action may, in future studies, help to shed light on the basic mechanism of electrolyte transport.

3. CONTROL OF ALDOSTERONE:

The mechanisms controlling aldosterone secretion have been sought for intensively for many years but remained the subject of much discussion and controversy. The studies of several workers have failed to unify the mechanism of control of aldosterone under a single factor. The discovery of the humoral control of aldosterone by the renin-angiotensin system brought a new concept to this area of research. As the work progresses, more and more evidence is accumulating to designate the renin-angiotensin system as the major mechanism controlling aldosterone secretion. In order to give a proper perspective concerning the role of the renin-angiotensin system in the control of aldosterone, the other mechanisms of control will be briefly reviewed.

a) Role of nervous receptors:

Even before aldosterone was identified and isolated, Peters (571, 572) put forward the concept of volume-sensitive receptors regulating the peripheral blood level of the still unidentified sodium-retaining hormone of the adrenal cortex. A large body of experimental data had accumulated to support the view that expansion of extracellular fluid volume was associated with a decrease in urinary aldosterone excretion and natriuresis while contraction of body fluids led to an increased aldosterone output and sodium retention (6, 573, 575).

Bartter and his associate (575) established in their extensive studies in man that the changes in urinary aldosterone were dependent upon body fluid volume and independent of changes in extracellular and intracellular ion and water concentration. This hypothesis has met some opposition from other studies. For instance, Cox, Singer and Verel (576) found no obvious relationship between total extracellular fluid and the increase in urinary aldosterone but their results did not rule out the possibility that plasma volume might control aldosterone secretion. Bartter et al (575) were also aware of the difficulty in explaining the increased aldosterone secretion in patients with nephrosis, cirrhosis with ascites, and cardiac failure since these conditions are associated with an increase in extracellular fluid. They postulated that in these conditions, a decrease in effective volume within the vascular compartment might be the stimulus.

Thereafter, many attempts were made to localize and identify the site of the receptors controlling aldosterone. Barger (577) claimed that sodium retention was secondary to an increase in sympathetic activity resulting from a fall of pressure in the carotid sinus and aortic arch. Subsequently, Farrell (573) suggested the right atrium and Bartter et al (578) proposed the common carotid artery and carotid sinus as possible loci for these receptors.

Carpenter and associates (579) failed to reproduce the increase in aldosterone secretion reported by Bartter et al (578) following a decrease in the blood flow of carotid artery. Bartter and his group (578) also claimed that the aldosterone stimulating effect of the constriction of vena cava in normal dog was prevented by the previous removal of all visible nerves from the thyrocarotid arterial junction, but not by denervation of the carotid sinus. On the other hand, Carpenter et al (579) showed that hyperaldosteronism persisted in dogs with chronic constriction of the thoracic inferior vena cava des-

pite extensive stripping and denervation of all upper arterial tree. Biglieri and Ganong (580) questioned the hypothesis of Bartter and coworkers by showing that after hypophysectomy, carotid artery compression failed to increase aldosterone secretion.

b) Role of the pituitary gland:

In 1940, Swann (581) was able to postulate the existence of a separate, non pituitary mechanism for the control of mineralo-corticoid activity of the adrenal gland. He observed that after hypophysectomy the zona glomerulosa producing aldosterone does not atrophy, whereas the zona fasciculata producing cortisol does. This observation was also confirmed by Deane and Creep (582). McLean et al (583) stated that a patient without his adrenal glands dies of sodium deficiency if deprived of salt, whereas a patient without his pituitary gland conserves salt normally.

On the other hand, hypophysectomy results in a marked decrease of aldosterone secretion in a normal animal or in an animal in secondary hyperaldosteronism. In his review, Davis (6) summarized most of the work done on that subject. His own experiments and those of others clearly establish that aldosterone secretion decreases to 10-20% of control levels following hypophysectomy and that a normal secretion is restored after infusion of ACTH. Most of these studies strongly suggest that ACTH exerts an influence on aldosterone secretion. Nevertheless, it was also reported that hypophysectomy does not prevent the increase in aldosterone secretion in dogs following hemorrhage (405, 411), constriction of aorta (584), or inferior vena cava constriction (6). Barbour et al (411) reported that hypophysectomy is without effect on aldosterone secretion in sodium restricted dogs. Recently, Slater (585) pointed out that most of the studies reporting a marked fall in aldosterone following hypophysectomy, were done in heavily anesthetized dogs acutely

stressed by surgery so that most of the acute effects reported could be attributed to the originally high level of aldosterone due to the stress. To avoid this factor, he measured adrenocorticoid secretion many hours before and after surgery and found in these conditions that, following hypophysectomy, aldosterone secretion does not change despite a sharp fall in the rate of cortisol and corticosterone secretion (456).

The administration of ACTH to normal human subjects is followed by a moderate and transitory increase in the urinary output of aldosterone (586, 587) but the rise is considerably smaller than that of Porter-Silber chromogens (586). This action of ACTH on aldosterone secretion or excretion was confirmed many times but it is generally agreed that this action is only transitory and that it is much more consistent in increasing corticosterone and cortisol secretion. Nevertheless, two recent reports regarding the action of ACTH on aldosterone production are quite puzzling, and if consistently confirmed, might indicate the importance of the pituitary gland in the control of aldosterone secretion under special conditions. Blair-West and coworkers (588) observed that ACTH infused directly into the adrenal artery, is a stronger aldosterone stimulating substance in salt depleted sheep than in salt repleted animals. On the other hand, Muller et al (589) reported that, in the stressed dog, infusion of ACTH can selectively increase aldosterone production without any change in corticosterone and hydrocortisone secretion.

Giroud et al (590) observed that the addition of ACTH to the incubation medium of beef adrenal slices resulted in a marked increase in the production of cortisol, but not in aldosterone production. However, recent studies by Kaplan (431) and Jouan (591) with "in vitro" incubation strongly support the action of ACTH on the biosynthesis of aldosterone in vitro but to a lesser degree than on the biosynthesis of corticosterone and hydrocortisone.

c) Role of the pineal gland and nervous system:

Farrell has greatly emphasized the role of the pineal gland and the adjacent region of the brain in the regulation of aldosterone secretion. This hypothesis was proposed in 1958 (592) and was supported when he found that extracts of beef diencephalon stimulated the secretion of aldosterone in decerebrated dogs (593). This factor was isolated and called adrenoglomerulotropin (594). Unfortunately all the following studies failed to reproduce these findings even in the hands of Farrell himself. Furthermore, it was reported that pinealectomy did not alter the usual response of aldosterone secretion to salt depletion and repletion (595, 596) or to inferior vena cava constriction. Davis and coworkers (597) observed that acute and chronic hypothalamic lesions were without effect on aldosterone secretion unless median eminence was injured. Recently, Farrell (598) was able to isolate inhibitory factors in pineal gland extracts. These substances were isolated on a florisil column and the major component was found to be ubiquinone or coenzyme Q. Farrell also claimed that he was able to inhibit the increase in aldosterone secretion in intact dogs after hemorrhage simply by infusing ubiquinone intravenously. These findings await confirmation.

Other parts of the central nervous system were also proposed in the control of aldosterone secretion. Despite the fact that an increase in aldosterone was reported after stimulation of various cerebral areas, their physiological role appears dubious. Davis and his group (599) showed that complete mid-brain transection was completely ineffective in influencing aldosterone secretion and failed to diminish the response to caval constriction. They later reported (405) that complete decapitation failed to block the response in aldosterone secretion. Nevertheless, Bartter and coworkers (600) reported recently that the nervous system has an inhibitory effect on aldosterone since

they demonstrated a very significant increase in aldosterone, corticosterone and cortisol secretion rate when mid-collicular section was done in hypophysectomized and nephrectomized dogs.

d) Role of electrolytes:

Although a decrease in sodium intake is consistently accompanied by a rise in aldosterone secretion and excretion, it is doubtful that this stimulus is mediated through changes in plasma sodium concentration since this parameter is not modified even after a prolonged period of sodium depletion (564). Several studies suggested that the aldosterone stimulation or inhibition is more related to changes in plasma potassium concentration than to changes in sodium. Potassium loading was reported to increase the width of the zona glomerulosa (609) and aldosterone excretion or secretion (422, 601, 603-608, 616). However, other studies did not reveal a significant change in aldosterone excretion following potassium loading (610-612). Gann et al (613) could not detect any effect during peripheral intravenous infusion of KCl into intact dogs but they reported a striking increase in aldosterone secretion when potassium was given through the carotid artery. On the basis of these findings, they suggested the existence of an intracranial area sensitive to changes in potassium concentration. Laragh and Stoerk (606) postulated that the level of plasma potassium is the primary determinant of the rate of aldosterone secretion. They also observed that, in dogs submitted to low sodium diets, no increase occurred in urinary aldosterone unless small amounts of potassium were added to the diet. This last observation was later confirmed with aldosterone secretion in man (265).

Changes in K balance are also often accompanied by changes in sodium balance and some investigators have suggested that these changes in sodium balance might be the mechanism by which potassium influences aldosterone (585, 614).

Recently, Gann et al (615) were able to induce changes in aldosterone excretion by modifying the dietary potassium, independently of changes in Na balance, intravascular volume, or arterial pressure.

The local effect of electrolytes on the adrenal gland was demonstrated by the perfusion of isolated adrenals in animals with solutions containing various concentrations of electrolytes. Denton (603, 616), Blair-West (564), Davis (607) and Bartter (600) and their coworkers showed that perfusion of the gland with solutions containing higher than normal potassium concentration with or without low sodium content, increased aldosterone secretion. Bartter et al (600) further observed that perfusion with caesium and rubidium also increased aldosterone secretion. It thus appears that ionic changes, especially those involving potassium or the Na/K ratio, might act locally on the adrenal. This mechanism of action is not yet elucidated and its relationship to other factors modifying aldosterone secretion is not clear at present.

e) Role of the kidney:

Transplanted and denervated adrenals respond to inferior vena cava constriction by an important increase in aldosterone production, thus indicating a humoral mechanism (617). In 1959, the simultaneous work of Davis in United States and Denton, in Australia produced direct evidence for a specific aldosterone stimulating hormone circulating in the peripheral blood of animals. Yankopoulos, Davis, Kliman and Peterson (618) demonstrated by cross-circulation experiments, that the blood of dogs with secondary hyperaldosteronism caused by inferior vena cava constriction produced a significant increase in the aldosterone output when perfused through the isolated adrenal gland of another animal. Denton, Goding and Wright (603, 616), using the same technique, obtained the same evidence in sheep submitted to sodium restriction. The source of this aldosterone-stimulating hormone was the next point to cla-

rify. By a series of ablation experiments, Davis et al (405) implicated the kidney as the source of aldosterone-stimulating hormone. They showed that ablation of anterior pituitary, pineal body, the head and the liver, failed to block the response to hemorrhage. In contrast, they found that removal of the kidneys in hypophysectomized animals resulted in a marked fall in aldosterone to very low rates and the usual response to acute blood loss failed to occur in these animals. The next step was obvious for Davis and his group (619): they injected crude saline extracts of kidney and observed an increase in aldosterone secretion. By fractionation of the kidney extracts, they later (273) found that the fraction having an aldosterone stimulating effect was the one containing renin, thus associating the aldosterone stimulating hormone with renin.

Meanwhile, Genest and his group (7) had demonstrated that angiotensin was a most potent aldosterone stimulating substance in man. Since 1956, this latter group has also been investigating the factors stimulating the aldosterone excretion. Since they had reported previously that aldosterone excretion was elevated in many hypertensive subjects, they had oriented their studies on the effects of blood pressure on the aldosterone excretion. They undertook, in 1957, a study on the effects of various pressor agents on aldosterone excretion and found that nor-epinephrine, epinephrine and reserpine were without effect. Pursuing their investigation with synthetic angiotensin, they obtained a striking stimulation of aldosterone excretion and they soon realized that this effect was not related to the increase in blood pressure since subpressor doses of this substance produced a similar effect. This discovery introduced the renin-angiotensin system into the field of action of aldosterone, namely, the control of body fluids and electrolytes. Soon, these findings received confirmation from several workers (111, 408, 409, 411, 413, 617, 619, 620).

This mechanism of control has a great physiological implication, since nephrectomy results in a marked decrease in aldosterone secretion and since the kidneys are necessary in the development of experimental secondary hyperaldosteronism. Nephrectomy prevents the increase in aldosterone secretion following hemorrhage (111, 405, 408, 409, 620), in animals submitted to a low sodium diet (619), following vena cava constriction (413, 619, 621), in low output heart failure (273), and in high output heart failure secondary to aortocaval fistula (622).

Eilers and Peterson (422) and Blair-West and coworkers (564, 588) claimed recently that bilateral nephrectomy did not result in a significant decrease in aldosterone secretion in their sodium depleted animals. Gann and Travis (546) reported that one to two hours after bilateral nephrectomy, their dogs responded normally to a constriction of the inferior vena cava while the aldosterone response to the constriction of the carotid artery was abolished. However, in these studies, the measurement of aldosterone after nephrectomy was done quite rapidly after the ablation of the kidneys (422, 546, 564, 568). Since high circulating renin levels were probably present prior to nephrectomy and since it is not known how long renin remains in the circulation following nephrectomy, it is possible that renin was still circulating and acting during the study periods. It should also be recalled that, after stopping of infusion of angiotensin, the stimulation of aldosterone production persists for up to 48 hours. These last studies (422, 546, 564, 568) are thus not convincing and do not rule out the role of the kidney in the control of aldosterone secretion.

F - THE REGULATION OF SODIUM.

The remarkable osmotic and volume stability of our "milieu intérieur"

necessitates the existence of very efficient and very sensitive control mechanisms. Under "normal" salt intake, the kidney must reabsorb 99.5 percent of the filtered load of sodium in order to maintain homeostatis. Moreover, it is well known that salt balance can be preserved in subjects maintained for long periods on a low sodium diet. Therefore, the mechanism by which the kidney is able to detect and correct the changes in salt balance are of the greatest importance in the maintenance of life.

The rate of sodium excretion may be modified either by changes in glomerular filtration or in rate of tubular reabsorption, or by a combination of these two mechanisms. For many years, a major role was attributed to the glomerular filtration rate in the control of salt excretion. For instance, it was well demonstrated that even slight changes in G.F.R. can lead to large changes in sodium excretion in the dog (366, 370). However, numerous clinical, as well as experimental, observations accumulated to indicate that disturbances in glomerular filtration rate cannot always explain the rate of sodium excretion and retention under various conditions. After several days of sodium deprivation, a rapid infusion of hypertonic saline increased the G.F.R. but the rate of urinary sodium excretion still remained below normal (623, 624). Davis and Howell have shown that dogs with constriction of the thoracic inferior vena cava can have an elevated G.F.R. in the presence of maximal sodium retention (625, 626). Barger showed that dogs with valvular lesions and normal renal hemodynamics retain sodium after a salt load and this without any change in the glomerular filtration rate (627). Similar discrepancies were also reported in clinical conditions characterized by edema and sodium retention. Although it is generally recognized that the G.F.R. is decreased in cardiac failure (628, 629), in nephrotic syndrome the G.F.R. was often found to be normal (630) or increased (631) and, in many cirrhotic

patients with ascites, the filtration rate was also reported normal (632, 633). Moreover, others have reported (634, 635) that the G.F.R. could remain low during the period of diuresis in congestive heart failure.

Thus, changes in tubular reabsorption of sodium must also be considered to explain the variations in sodium excretion, at least under certain circumstances. Investigation of this aspect has been stimulated in recent years by the discovery of hormonal influences on the tubular reabsorption of sodium. Hemodynamic influences cannot be ruled out and it appears that the interrelationship between these two mechanisms would better explain the maintenance of sodium balance under normal conditions or during the development of edema. Since this work is chiefly concerned with the hormonal control of urinary sodium, the evidence for the role of aldosterone and the renin angiotensin system in this mechanism will be reviewed briefly.

1. ROLE OF ALDOSTERONE.

a) In sodium retention:

As early as the 1930's, adrenalectomy or adrenal insufficiency was associated with changes in the concentration of plasma sodium and potassium (636-638). It was soon observed that treatment with cortical extracts or DOC corrected the electrolyte disturbances of Addison's disease (639-641). Before aldosterone was identified, Deane, Shaw and Creep, in 1948 (642), noted that the width and the enzymatic activity of the adrenal zona glomerulosa of rats increased following either sodium deprivation or high potassium intake while it decreased after a high sodium intake. They postulated that the salt regulating factor of the adrenal probably came from the zona glomerulosa and escaped the control of the pituitary since they observed identical changes in hypophysectomized animals. Confirmation of these findings was reported in rats (643, 644) and in patients maintained on a rice diet (644). More recent-

ly, it was reported that the enzymatic activity of glucose-6-phosphate dehydrogenase increased markedly in the zona glomerulosa of rats following sodium depletion (645, 646).

The first direct indication of a relationship between sodium balance and aldosterone was established by Luetscher, Axelrod and Johnson (647, 648) in 1954, when they reported an increase in urinary aldosterone in subjects maintained on a low sodium intake. They also observed that aldosterone excretion was inversely proportional to the urinary sodium. Similar findings have been reported many times with aldosterone excretion (564, 575, 604, 610, 612, 649-654), aldosterone secretion (265, 595, 619) and plasma aldosterone levels (655). However, in correlating urinary excretion of aldosterone with the time of the renal response to sodium deprivation, Crabbé, Ross and Thorn (656) were unable to find a significant relationship between these two parameters. They observed that a decrease in urinary sodium occurred before the increase in aldosterone and that sodium retention persisted during the refeeding period following a low sodium diet despite a normal urinary aldosterone excretion. Similar discrepancies were also observed between aldosterone secretion rate and sodium excretion during low sodium intake and during the circadian rhythm (629, 657). Moreover, it is also reported that Addisonian or adrenalectomized patients maintained on an adequate steroid replacement therapy can manipulate their rate of sodium excretion in an apparently normal fashion after quick changes of posture or after sequestration of blood in the legs.

Therefore, mechanisms other than the sodium retaining function of aldosterone must be postulated to explain the acute changes in urinary sodium. The delay observed in the response of aldosterone to sodium deprivation might also indicate that some intermediary mechanisms are involved in the stimulation of aldosterone secretion.

b) In edema formation:

Many years ago, Widal, Ambard and their collaborators recognized the importance of sodium chloride in the development of edema. The concept of Starling regarding the equilibrium between osmotic and hydrostatic forces of vascular and interstitial fluids was used to explain the changes in the distribution of volume encountered in edematous conditions. Although this concept could very well explain the shift of fluid from one compartment to another, it could not explain the marked over-all body retention of sodium and water associated with edematous conditions. Further investigations indicated that changes in neither oncotic pressure nor glomerular filtration rate could always explain the observed fluctuation of edema.

The excretion and secretion rates of aldosterone are increased in most nephrotic (265, 671-673) and cirrhotic patients (265, 653, 672-675) and in about 50% of cardiac patients with edema (265, 559, 653, 677, 678). Recently, also the plasma levels of aldosterone were found increased in similar patients (679). The absence of high aldosterone secretion rate in some cardiac patients is hard to explain since these patients present all the characteristics and symptoms of secondary hyperaldosteronism. One possible explanation would be that these subjects were studied during improvement of their condition. It is reported that, during the treatment and relief of edema by natriuretic drugs and sodium restriction, the aldosterone secretion of cardiac patients falls rapidly to normal (678, 680). One other possibility would be that aldosterone is destroyed less rapidly in that condition so that a normal secretion rate would maintain a state of hyperaldosteronism. This last hypothesis is most probable in the light of the recent findings that the half-life of aldosterone is prolonged whenever the liver is congested or damaged as found in congestive heart failure and cirrhosis (679-683).

The most conclusive studies demonstrating the major role of aldosterone in the production of edema will be reviewed briefly. Davis and coworkers (684) observed that the excessive fluid retention which can be induced in dogs by constricting the thoracic inferior vena cava (an excellent stimulus to aldosterone production (685) failed to occur following adrenalectomy and could be mimicked only if DOC was given in very large amounts. Singer (686) reported that the secretion of aldosterone was increased during aminonucleoside nephrosis in rats. She also observed that this increase preceded the development of edema. Deane and collaborators (687) noted, in similar nephrotic rats, a progressive hypertrophy of the zona glomerulosa, especially on the fifteenth day, at the time of maximal aldosterone secretion and just preceding the maximum ascites accumulation. Giroud and his collaborators (688) produced the most remarkable and convincing piece of work concerned with the physiopathological role of aldosterone in edema formation during aminonucleoside nephritis. They clearly demonstrated that the increase in aldosterone secretion was closely associated with the development of edema and that adrenalectomy prevented the occurrence of edema. Adrenalectomized rats receiving a normal replacement dose of aldosterone did not develop edema despite the presence of all the biochemical changes characteristic of nephrosis. In these rats, edema and ascites developed only after the administration of excessive doses of aldosterone.

Why is it that under certain conditions such as congestive heart failure, hepatic cirrhosis and nephrosis, high aldosterone secretion rates are associated with edema formation whereas in other conditions such as primary aldosteronism, malignant hypertension, and in some cases of hypertension associated with renal artery stenosis, edema does not develop despite similar secretion rates? When prolonged infusions of aldosterone are given to normal

man and animals, edema does not appear because an "escape" occurs in the tubular action of aldosterone on Na retention after a period of time which depends on the salt intake and the dose of steroid administered. Under special circumstances, which appear to be hemodynamic in nature, and for unknown reasons, this escape phenomenon ceases to exist and identical prolonged aldosterone infusions lead to the formation of edema. This phenomenon is not specific to aldosterone since it has also been observed with DOC, sodium loading and angiotensin. Mach and Muller (678) demonstrated this phenomenon during aldosterone infusion to cardiac patients who had previously been rendered free of edema. Barger et al (689) observed in dogs with experimental congestive heart failure that saline infusinns did not induce natriuresis whereas it did so in normal animals. Davis and his group reported that dogs with inferior vena cava constriction (690) or with an arterio-venous fistula (339) do not escape from the sodium retaining effect of DOC, aldosterone and angiotensin. Continuous infusion of these substances resulted in an almost complete sodium retention and the development of edema and ascites. Removal of the caval ligature induced a natriuresis and loss of edema despite continuation of treatment. They showed also that changes in G.F.R. and R.B.F. could not account for that sodium retention. They finally concluded that another factor was necessary in addition to these substances to maintain the sodium retaining effect, and they postulated that this factor was either a humoral agent or some undefined renal functional change. This factor or factors are of utmost interest in the understanding of the basic mechanism of edema formation.

2. ROLE OF THE RENIN-ANGIOTENSIN SYSTEM.

a) In sodium retention:

The absence of renin in the kidney of marine fish (658, 659) and its presence in the kidney of fresh water fish (167, 658) constitute the first

indirect indication that the renin-angiotensin system might be related to the conservation of electrolytes. Dunihue (660) reported a hypergranularity of the juxtaglomerular cells in adrenalectomized rats. Toussaint (391), Hartroft (193) and coworkers showed that sodium loading produced a decrease in both the number of the granular cells and the width of the zona glomerulosa. Hartroft and his group (193, 393, 661, 662) were later able to demonstrate in sodium depleted rats, dogs and cats, an increase in the granularity of the juxtaglomerular cells associated with an increase in the width of the zona glomerulosa. In man, Pitcock and Hartroft (394) found that the J.G.I. was inversely proportional to plasma sodium and proportional to the width of the zona glomerulosa. Gross and coworkers first reported that the renin content of kidneys decreased in rats maintained on a high sodium intake (531, 602) and increased during low sodium intake (602). More recently, Pitcock et al (179) established a close relationship between the increase in renin content of the kidney and the granularity of the juxtaglomerular cells. In salt depleted rats, Demopoulos et al (178) reported the opposite relationship under salt loading condition. Hartroft (667) was unable to detect any alteration in the J.G.I. of rats submitted to either an excess or a deficiency in dietary potassium. Tobian et al (668) observed a hypergranulation of the juxtaglomerular cells in rats treated with chlorothiazide for one month. All these findings constitute indirect evidence since it has not yet been proven that the juxtaglomerular granularity and the renin content really parallel the secretion rate of renin or its blood concentration. The reports concerning the lack of aldosterone stimulation in rats following prolonged infusion of angiotensin raise some doubt about the physiological role of the renin-angiotensin system in the conservation of sodium in this species (422-424).

On the other hand, the aldosterone-stimulating effect of angiotensin has been well established and confirmed many times in other species. (See previous section concerning the action of angiotensin on adrenal cortex). However, the physiological significance of this mechanism was less clearly demonstrated because of the lack of sensitive means to measure the circulating levels of renin or angiotensin.

The findings reported to date with the measurement of angiotensin are neither convincing nor consistent. Barbour et al (669) reported a slight but significant increase in the arterial angiotensin levels of dogs fed a low salt diet. In man, Mulrow et al (456) observed an increase in angiotensin blood levels in only four out of their twelve sodium depleted subjects. This finding led them to conclude that the renin-angiotensin system does not play an important role in the conservation of sodium in man.

In 1962, Winer (670) reported that the administration of diuretics to normotensive subjects was followed by an increase in the pressor activity of the peripheral plasma but they did not characterize their pressor substance.

b) In secondary hyperaldosteronism:

Very little evidence for the role of the renin-angiotensin system in the pathogenesis of edema and secondary hyperaldosteronism was provided before the present work was initiated. Using a crude method, in 1946, Merrill, Morrison and Brannon (447) found a significant amount of pressor material in the renal venous blood of 8 out of 11 patients with chronic heart failure. Since the relationship between the renin-angiotensin system and the adrenal cortex was not known at that time, they concluded that the pressor material was probably responsible for the active vasoconstriction found in cardiac patients. Schwartz et al (691) found that the renin content of kidneys of cirrhotic patients who had died while edematous was significantly greater than normal.

A hypergranularity of human juxtaglomerular cells was observed in such conditions as heart diseases with edema (692) and liver diseases with ascites (163).

Davis et al (273) demonstrated that the renin content and the J.G.I. of the kidneys of dogs with inferior vena cava constriction and ascites were significantly increased. Tobian and coworkers (396) found a very significant degree of correlation between the amount of ascites and the increase in J.G.I. in rats in aminonucleoside nephrosis. On the other hand, Gross and his coworkers (693) reported that the renin content of the kidneys of rats with aminonucleoside nephrosis did not differ from that of normal rats and they concluded that there was no relationship between edema formation and renin. In conclusinn, the role of the renin-angiotensin system in the regulation of body sodium and in the formation of edema is not clearly established by the preceding studies. There is no study reported establishing a correlation between the blood levels of renin or angiotensin, sodium balance and aldosterone secretion and excretion. Such a study might either establish or rule out a physiological role for the renin-angiotensin system in the regulation of sodium. The present work was concerned in great part with that kind of study.

G - FACTORS CONTROLLING THE RENIN-ANGIOTENSIN SYSTEM.

With the recent recognition that the renin-angiotensin system plays a major role in the control of the aldosterone secretion, it has become of paramount interest to identify the nature of the factor(s) controlling the production and liberation of renin by the kidneys. In reviewing the literature, it appeared that very few studies have been undertaken to shed light on this most important problem. For many years it has been generally accepted that changes in blood pressure, blood flow or pulse pressure were the trigger me-

chanisms for the release of renin.

1. HEMODYNAMIC CHANGES:

Kohlstaedt and Page (694) first, claimed that the decrease in pulse pressure was the stimulus for the release of pressor material by the kidney. However, more recently, workers from the same laboratory could not reproduce these first findings. As a matter of fact, Skinner, McCubbin and Page (695) reported that mild suprarenal aortic constriction which decreased pulse pressure but which did not reduce mean perfusion pressure and blood flow through the kidney, did not liberate pressor material into the renal vein blood.

In 1959, Tobian, Tomboulian and Janecek (696) suggested that changes in mean renal arterial pressure might be the major factor controlling the release of renin by the juxtaglomerular cells. While perfusing isolated kidney at various pressure, they observed that, when perfusion was done at high pressure, the granulation of the juxtaglomerular cells fell significantly, whereas no change was observed when the kidney was perfused at normal mean pressure. They postulated that, by their situation in the wall of afferent arterioles, the juxtaglomerular cells would act as a baroreceptor or stretch-receptor. A decrease in perfusion pressure would result in an increased secretion of renin which would restore and maintain an adequate perfusion pressure, while an increased perfusion pressure would shut off the production of renin. Skinner, McCubbin and Page (59, 695, 697) recently produced support for this hypothesis. They demonstrated that the rate of renin secretion may vary inversely with the level of mean arterial pressure independently of renal blood flow and pulse pressure. A slight decrease in mean renal perfusion pressure of only 5 to 40 mm Hg increased renin secretion within 60 seconds while a rise in perfusion pressure had the reverse effect. These changes cannot be attributed to ische-

mia alone since a decrease in the oxygen concentration of the blood without changes in the perfusion pressure is without effect on the release of renin (57, 551, 698, 699).

In the past years, Tobian's theory of a "stretch receptor" at the site of the juxtaglomerular cells has received much attention. Tobian himself claimed that all the conditions characterized by an increase or a decrease in renin secretion could be explained on the basis of his theory (190). Nevertheless, some findings still remain unexplained by this hypothesis. Taquini et al (71) found no change in the kidneys' renin content of animals in shock for 30 minutes. In uni-nephrectomized rats, the renin content of the kidney and the release of renin do not increase after clamping the remaining kidney (491-493). No increase in the juxtaglomerular index occurs following clamping of aorta above both renal arteries (511). Moreover, the role of renin in restoring the perfusion pressure is dubious since the blood pressure failed to increase in many rats following constriction of one renal artery despite a marked increase in the J.G.I. of the clamped kidneys (480). Therefore, other hypotheses have to be considered.

2. TUBULAR FLUID COMPOSITION:

Changes in perfusion pressure with or without variations in G.F.R. can alter the chemical content of the urine (366, 367, 370, 700, 701). The macula densa, which is in close contact with the distal tubular fluid, has also close histological connections with the juxtaglomerular cells (155, 158). Moreover, a close functional relationship was established recently by the finding of a correlation between the enzymatic activity of the macula densa, the granularity of the juxtaglomerular cells (159, 160, 702, 703) and the pressor activity of the kidney (160). These findings led many investigators to postulate that the macula densa might be actively involved and might pro-

bably be the initiating factor in the mechanism leading to the formation and secretion of renin (153, 158, 164). This structure is ideally located to receive information from the urinary chemical content and then to secondarily influence the activity of the juxtaglomerular cells. Guyton, Langston and Navar (704) after deriving a series of equations and calculations from experimental studies, concluded that the autoregulation of blood flow into the kidney was most probably controlled by an osmotic feed-back at the site of the macula densa. Almost all the experimental data from other studies and from their own fitted very well into this mathematical concept. However, they could not completely rule out the possibility of a sodium feed-back as well. The most striking evidence for the urinary "chemical" control of renin and against the baroreceptor theory was put forth recently by Vander and Miller (166). These investigators found that increasing the urinary osmotic load by osmotic diuretics, acetazolamide and chlorothiazide blocked the increase in renin release following suprarenal aortic constriction. The changes in urinary sodium and osmolality were accompanied by slight or no variation in the renal blood flow and G.F.R. The data were interpreted to support the view that an "intrarenal structure responsive to sodium variations, perhaps at the macula densa area" was involved in the control of renin secretion. Just recently, Leyssac (705) measured the intrarenal capillary and intratubular pressure by micropuncture. He found that in the clamped kidney, the proximal intratubular and peritubular capillary pressure were identical but that the distal intratubular pressure was decreased up to fifty percent. Therefore, he postulated that a decrease in intratubular pressure might increase the production of renin and angiotensin by a chemical or electrical mechanism at the site of macula densa. All these recent studies are fascinating and promising since they offer new concepts regarding the basic mechanism involved in elec-

trolyte and volume control and in autoregulation of the kidney circulation.

3. HUMORAL SUBSTANCES:

The possibility of a "humoral" feed-back within the renin-angiotensin system remains to be clarified. To date, the only direct evidence in favor of such a mechanism was suggested by the studies of Masson and his coworkers. They postulated that the adrenal mineralocorticoids might represent the feed-back substances regulating the renin-angiotensin system. They observed that the secretion of pressor material by the kidney, as estimated grossly by the rise of blood pressure following graft of kidneys to be studied into nephrectomized rats, decreased rapidly after the administration of DOC and salt before the development of hypertension (187, 515, 706) and while the renin content of the kidney remained unchanged. Similarly, but more gradually, cortisone or cortisol administration decreased the liberation of pressor material by the kidney (187, 706). ACTH had no effect on either the secretion of pressor substances (706) or the renin content of the kidney (178, 706).

Many indirect observations were reported to be in favor of the inhibitory effect of adrenal steroids on the renin-angiotensin system. A disappearance of renin in the kidney is consistently reported in rats treated with DOC and salt (160, 177, 472, 531, 708-710) as well as an almost complete degranulation of the juxtaglomerular cells (160, 177, 178, 193, 480, 711, 712). Since hypertension occurs under these conditions, it is very difficult to determine which of the various possible factors, hypertension, salt or DOC, is directly involved in the inhibition of renin. When DOC is administered alone, no changes are noted in J.G.I. (713), in blood renin (466, 714) or in the renin content of the kidney (531). Moreover, DOC failed to decrease the J.G.I. in rats maintained on a low salt intake (193, 480, 711, 715) and even the J.G.I. was reported to increase during such an experimental procedure (193). On the other

hand, others reported a slight decrease in the renin content (187) or in the J.G.I. (712) of rats during chronic treatment with DOC alone. Nevertheless, it appears that DOC in itself, is practically devoid of any consistent inhibitory function on the renin-angiotensin system.

A decrease in the renin content of the kidney was also reported in rats treated with aldosterone and salt (710). Fisher and Tamura (716) observed a decrease in both the J.G.I. and the zona glomerulosa of their rats injected sub-cutaneously with aldosterone in oil for three weeks. However, the blood pressure of their rats rose following this chronic administration of aldosterone and in addition they could not exclude an inhibition by the retention of sodium alone. Fukuchi et al (712) noted only a slight decrease in the J.G.I. in rats treated with aldosterone in water for two weeks while the animals remained normotensive. Genest reported (527, 717-721) undetectable or very low arterial angiotensin levels in six patients with proven primary hyperaldosteronism due to adreno-cortical adenoma. He thus proposed that the measurement of angiotensin and, preferably, renin-activity in the blood of hypertensive patients would be the best means to differentiate between hyperaldosteronism due to adreno-cortical adenoma and that due to renal artery stenosis. This assumption was confirmed and supported by the finding of undetectable levels of renin-activity after 3 hours of incubation in similar patients by Conn et al (722, 723) and Genest et al (724). Brown and coworkers (725) recently reported one case of primary aldosteronism with very low levels of renin-activity. All the previous findings strongly support a marked inhibition of the renin-angiotensin system either following prolonged treatment with aldosterone and salt or under prolonged high endogenous secretion of aldosterone by a tumor. However, it is not possible to determine to what extent aldosterone alone is involved in this inhibition, since salt is retained

in large amounts and plasma volume is increased in these conditions.

The increase of pressor material in the blood of adrenalectomized animals (493, 602), the increase in the renin content of their kidneys (157, 277, 602, 708) and the increased granularity of the J.G.C. (392, 480, 518, 557, 660, 662, 726) all pointed to a hyperactivity of the renin-angiotensin system after bilateral adrenalectomy. Chemical inhibition of the steroidogenesis by Metopiron (729) or amphenone (518) is also accompanied by an increase in J.G.I. These conditions are associated with a loss of sodium and a decrease in blood volume and again the changes cannot be attributed solely to the removal of adrenal steroids.

Masson, Mikasa and Yasuda (707) have reported that crude hog kidney extracts and semipurified hog renin injected for four days into rats, resulted in a marked decrease in renal secretion of pressor material while the renin content of the kidney remained normal. However, from this last study, they concluded that renin was not involved in this inhibition since a greater inhibition was observed with crude kidney extract than with concentrated semipurified renin preparation. They thought that the active factor was lost during the purification process of renin.

From the more recent literature, the action of nor-adrenaline on the renin angiotensin system appears of great interest despite the few observations reported so far. Scornik and Paladini (510) observed that infusions of norepinephrine in dogs increase slightly but significantly the angiotensin arterial level. Wathen et al (730) reported recently that infusions of nor-epinephrine into the renal artery decreased G.F.R. and R.B.F. and increased the liberation of renin by the kidney. When nor-epinephrine was infused into a systemic vein, no change occurred during the infusion, but renin increased after stopping the infusion. These findings combined with the recent discovery that

angiotensin is a potent catecholamine releasing factor, constitute the basis for an interrelationship between catecholamines and the renin-angiotensin system.

4. NERVOUS SYSTEM:

Although "denervated" kidneys are reported to secrete renin in response to various stimuli, the role of the nervous system cannot be ruled out completely since after denervation the nervous receptors and terminal sites remain in the kidney. In his original description of the juxtaglomerular apparatus in man, Goormaghtigh (153) described a group of small cells resembling the tactile corpuscles of Meissner situated between the afferent and efferent arterioles. Furthermore, he observed a fine nervous network around the juxtaglomerular segments. He thus concluded that there exists a rich innervation of the juxtaglomerular area. Demuylder (731) confirmed the existence of a rich network of nervous fibers surrounding the specialized juxtaglomerular structures. Recently, Barajas (574) made an electron microscopic study of the innervation of the juxtaglomerular apparatus in monkeys and rats, and he found numerous non-myelinated nerves fibers associated with the afferent and efferent glomerular arterioles in monkey kidney and to a lesser extent in rat kidney. He also observed that the dilated vasiculated nerve processes lie adjacent to smooth muscle cells and granular cells in the arteriolar wall. Recently, Gilmore (727) reviewed many studies and reported his own findings on the contribution of nervous system to the control of renal function. From this study, it appeared that the nervous system had a determinant role on the kidney hemodynamics and secondarily on excretion of electrolytes. It can be postulated that the hemodynamic changes initiated by the nervous system might influence the activity of the juxtaglomerular complex. Along this line of investigation also, Taquini and coworkers (71) reported that the renin content

of denervated rat kidneys decreases markedly, and Tobian and collaborators (732) found a decrease in the J.G.I. of the unilateral denervated kidneys of rats.

In conclusion, it appears that many factors might be involved in the control of the renin-angiotensin system. Many mechanisms have been proposed but most of them taken separately can account for all the findings reported so far. This aspect is of utmost importance since in it lies probably the understanding of the exact role of the renin-angiotensin system.

H - INTRODUCTION TO THE EXPERIMENTAL WORK.

From the preceding review, it is obvious that a great deal of investigation has already been conducted on the renin-angiotensin system. However, this system has been mainly investigated in connection with hypertensive diseases and, despite the large amount of work done, much discrepancy persists. Its relationship to aldosterone and electrolyte balance has been more recently established and few investigations along this line have been pursued so far.

The present work makes use of new, sensitive, and reproducible procedures for the determination of plasma angiotensin, renin-activity and angiotensinase-activity. The main purpose of this study was to investigate the physiological and physiopathological role of the renin-angiotensin system in various hypertensive diseases, in the control of aldosterone, in the maintenance of sodium homeostasis and in the accumulation of sodium in states of secondary hyperaldosteronism. Since it has been postulated that the renin-angiotensin system is involved in the control of aldosterone and sodium metabolism, the factors regulating the secretion of renin are gaining important physiological significance. Therefore, part of the present investigation was devoted to the study of the effects of various factors on the secretion of renin.

III - INVESTIGATIVE SECTION

METHODOLOGY

A - CLINICAL MATERIAL AND PROCEDURES.

The major purpose of this work was to study the role of the renin-angiotensin system in humans. For this reason, all the studies to be reported have been limited to man. The extrapolation from animal studies to man is often risky or dangerous because of species differences. However, it must be understood that studies in man may also be limited by various factors. In some instances, it would have been of great interest to pursue further the experimentation, but this could not have been done without really exceeding the limits of the medical ethics. The total amount of blood drawn, the medical condition, the well-being, and the good will of the patients and subjects studied were always taken into consideration and were limiting factors in many of these studies.

Since many factors may modify the activity of the renin-angiotensin system, the subjects were studied under conditions as near normal as possible, i.e. when unanesthetized and without medication, except in a few instances which will be pointed out. The subjects, material, diets and diagnosis will be described in detail before each experimental section.

Over three hundred normotensive and hypertensive patients or subjects were used for these studies. Most of them were studied during hospitalization in the metabolic ward of the Hôtel-Dieu Hospital, except pregnant women who were studied at Maisonneuve Hospital.

Blood was always withdrawn in the morning between 8:30 a.m. and 9:30 a.m. unless serial determinations were done during the day, the subject or patient being in recumbent position for at least half an hour before each blood with-

drawal. The amount of blood withdrawn varied between 50 and 75 ml when either angiotensin or renin-activity alone were measured, but 100-125 ml were necessary when both were measured on the same sample.

During study, patients were either under calculated diets, which will be described in each instance, or under unrestricted diets.

B - BIOCHEMICAL METHODS.

1. DETERMINATION OF BLOOD ANGIOTENSIN LEVELS.

The method for angiotensin measurement, previously described by Boucher et al (251) in 1961, was highly specific with an almost quantitative recovery of the added angiotensin, but was also laborious, time consuming and required large amounts of blood. This led Boucher and coworkers (8) to develop a new method which is more simple, more rapid, requires less blood and is as specific and as sensitive as the previous one. The latter method only was used for the measurement of angiotensin blood levels reported in this thesis.

This procedure has been published (8) and can be summarized as follows: Fifty to 75 ml of blood are withdrawn rapidly under partial vacuum from femoral or brachial artery. The blood is collected through a siliconized copper coil immersed in crushed ice into a glass flask containing 10 ml of the ammonium salt of ethylene-diamine-tetraacetic acid (EDTA) 3.8% solution, pH 6.5 and continuously stirred with a magnetic agitator. During this procedure, blood is cooled to 4-8°C within 10 seconds. Thereafter, the blood is centrifuged in a refrigerated centrifuge for 10 minutes and 25-30 ml of plasma are adjusted to pH 6.0 with 0.1 N HCl. The plasma is transferred to a Dowex 50W-X2 (NH_4^+) chromatographic column (1 cm x 10 cms) equilibrated at pH 6.0 and maintained at 0-5°C. The column is washed with 15 ml of 0.2 N ammonium acetate

(pH 6.0), then with 20 ml of acetic acid 10% v/v and finally with 20 ml of distilled water. These eluates are discarded. All the preceding steps are carried out at a temperature of 0-5°C in a cold room in order to prevent the destruction of angiotensin by angiotensinase(s).

At this point, the column is transferred to room temperature since the angiotensinase activity is completely eliminated by the previous procedures. Angiotensin is eluted with 25 ml of 0.1 N diethylamine followed by 25 ml of 0.2 N ammonium hydroxide into a flask containing 1 ml of concentrated acetic acid and a color indicator. The eluate is thereafter evaporated to dryness.

The dry residue is dissolved into 2 ml of 80% aqueous ethanol and evaporated to dryness five times to remove all traces of ammonium acetate. Finally, the residue is acidified with a few drops of 1 N HCl and then dissolved and evaporated to dryness twice with 0.5 ml of 50% aqueous ethanol.

The residue is quantitatively transferred to a Whatman No. 2 chromatographic paper strip 15 cms wide, on a straight line near the edge of the paper. After a minimum of two hours equilibration in the chromatographic jar, the ascending chromatographic separation is done with a solution of n-butanol-water-acetic acid (45:50:10 v/v) during 10 hours, at room temperature. During that time, with this system, the solvent front migrates 20 cms and the R_f value of angiotensin is 0.27. Since minor variations of temperature and humidity may alter this R_f value, three strips, 3.6 cms wide, are cut: one corresponding to the R_f value, one below and one above. (Usually, the angiotensin is located in the strip corresponding to the R_f value).

These strips are eluted by descending capilarity with 30 ml of a mixture containing 95% aqueous ethanol, water and 1 N HCl in a ratio of 500:495:5 v/v. The eluates are evaporated to dryness and then redissolved in 2 ml of 80% aqueous ethanol and reevaporated to dryness. This operation is repeated five

more times in order to remove all traces of HCl.

The dry residue is finally dissolved in 0.4 ml of 20% ethanol and assayed on a rat preparation and compared with an angiotensin standard: valine-5 angiotensin II amide (Ciba preparation). Rats weighing 120 to 150 grams, bilaterally nephrectomized 24 hours previously, are anesthetized. Both jugular veins are cannulated for injection of unknown and standard. One carotid is cannulated for the recording of blood pressure and a tracheotomy is performed. The only rats used are those which respond by at least a 10 mm Hg blood pressure rise on intravenous injection of 5 ng angiotensin standard. The solution to be assayed is injected in increasing amounts until a rise in blood pressure of at least 5 mm Hg is obtained. The same rise is sought with angiotensin standard and the procedure is pursued according to the four points assay. Volumes not greater than 0.08 ml of unknown are given to the rat since larger amounts might provoke a pressor rise due to the intravascular volume expansion. Pressor response of less than 5 mm Hg with 0.08 ml of unknown are considered "undetectable". With this type of assay the lower limits of sensitivity is of the order of 5 nanograms (millimicrograms) per 100 ml of blood. The accuracy of this assay is of the order of 90% when repeated measurements are made. The results are expressed in nanograms per 100 ml of plasma.

Since the blood sample is diluted in 10 ml of anticoagulant, it is necessary to calculate the correction for this factor in order to determine the concentration in the undiluted plasma. This is realized with the following formula:

$$\underset{(\text{ng/100 ml of plasma})}{\text{ANGIOTENSIN}} = \frac{\text{Angiotensin found in the extract} \times 100}{\text{Volume of diluted plasma } \left[1 - \frac{1000}{\text{Vol. blood } (100-\text{Ht})}\right]}$$

Eight recovery experiments with angiotensin in blood gave a mean recovery of 83%, with quantities varying from 0.01 to 1 microgram. Seven experiments

in plasma alone gave an average recovery of 92%. Only a small fraction of the blood angiotensin is lost through adsorption on erythrocytes. In recovery experiments performed on the red cell fraction of the sample, only 6-10% of the total angiotensin added to the whole blood is recovered. Arginine vasopressin, nor-epinephrine, bradykinin and tyramine, when added to the blood, are not recovered by this method.

The specificity of this procedure is based on the following similarities between the isolated material and the synthetic valine-5 angiotensin II, aspartic-β-amide:

1. Identical R_f values in two different paper chromatographic systems.
2. Inactivation by trypsin.
3. Identical pressor response curve in the rat assay.
4. Identical migration rate in one paper electrophoretic system at high and low voltage.

2. DETERMINATION OF RENIN-ACTIVITY.

The procedure used for the determination of renin-activity has been developed by Boucher et al in our laboratory. All the basic experiments leading to its elaboration were published in detail (8).

The method is essentially based on the summation of all the factors involved in the renin-substrate reaction leading to the formation of angiotensin, under carefully controlled conditions of pH, temperature and time of incubation. The conditions of incubation are maintained as close as possible to those of plasma in vivo except that the angiotensinase activity is prevented throughout the period of incubation. Attempts to find adequate and specific chemical inhibitor of the angiotensinase activity have been unsuccessful so far. Boucher found that the addition of Dowex resin 50W-X2 (NH_4^+) to the incubation media was the most suitable means for protecting angiotensin from

the action of proteolytic enzymes during the time of incubation. Angiotensin is adsorbed by the resin and is so protected from the angiotensinase activity until the end of the procedure. This procedure may be summarized as follows:

Venous or arterial blood is obtained in the same way as described in the angiotensin method. It is collected with 10 ml of EDTA as anticoagulant, and is centrifuged immediately in a refrigerated centrifuge for 10 minutes. If the plasma is not to be processed on the same day, it can be kept frozen for many days without any loss of activity.

Cold plasma is adjusted to pH 5.5 by addition of 1 N HCl and filtered on glass wool. This pH was found to be the optimum for the activity of renin. Ten ml of plasma are used for each incubation. These incubations take place in siliconized Erlenmeyer flasks (50 ml) to which 4 ml of moist Dowex 50W-X2 (NH_4^+) are added before the beginning of incubation. Usually, three incubations are done for each blood sample at three time intervals : 60 minutes, 120 minutes and 180 minutes. When insufficient plasma is available, 120 and 180 minute incubations only are done. The incubations take place in a constant temperature bath at 37°C under vigorous shaking (about 200 strokes per minute) so that the resin is maintained in suspension in the plasma throughout the period of incubation.

Following incubation, the mixture is transferred onto a glass column already containing 1 ml of Dowex 50W-X2 (NH_4^+) resin. The resin with the adsorbed angiotensin is retained on the column while the plasma passes through. The column is first washed with 15 ml of ammonium acetate (pH 6.0), and then with 20 ml of 10% acetic acid (v/v) followed by 15 ml of distilled water. These three eluates are discarded. Angiotensin is eluted from the column with 15 ml of 0.1 N diethylamine followed by 15 ml of ammonium hydroxide and collected in a flask containing a small amount of concentrated acetic acid

and a color indicator. The evaporation and the sublimation of the eluate is processed in the same manner as for the angiotensin procedure. Since the ratio of impurities to angiotensin is smaller in this procedure, chromatography is not usually done. After sublimation of the ammonium acetate, the dry residue is dissolved in 1 ml of 20% aqueous ethanol and assayed directly on the rat preparation previously described. The sensitivity of this procedure permits the detection of amounts of renin of less than 0.0004 dog unit.

After correction for the dilution caused by anticoagulant at time of sampling, the results are expressed in nanograms of angiotensin liberated per liter of plasma per minute of incubation (ng/L/min.). Since the production of angiotensin is almost always linear for the first three hours of incubation, the mean value of the two different incubations done on each sample is given as the final result. The results obtained with this method are highly reproducible.

The addition of amounts of angiotensin varying from 0.1 to 5 µg to the incubation media at zero time in nine different experiments showed that the mean average recovery is 84% (range 70% to 98%), after three hours of incubation. Thus, the protective effect of the resin is almost complete during the incubation. A series of plasma incubations conducted in parallel with, or without, the Dowex resin, but in presence of EDTA, showed a partial inhibition of angiotensinase activity in some plasmas and a complete inhibition in others. Such an observation is in accordance with the results obtained after the study on angiotensinase-activity (See experimental section on angiotensinase) and strongly supports the hypothesis that at least two different angiotensinases are present in some plasmas. These findings emphasize the importance of using the Dowex resin for a more adequate and constant protection in this type of procedure.

The pressor substance produced during the incubation behaves similarly to the synthetic angiotensin II and shows the same criteria of specificity as those described in the method for angiotensin determination. The vasopressor lipid discovered by Khairallah and Page (676) in plasma incubated at 37°C is not adsorbed on the Dowex resin in the condition described. Moreover, catecholamines, bradykinin and tyramine are not recovered by this procedure.

Several factors, such as the amount of substrate, the presence of different types of substrate, inhibitors, activators, ionic concentration and other variables, might influence the reaction catalized by renin. Moreover, little is known concerning the kinetics of the converting enzyme catalyzing the transformation of angiotensin I into angiotensin II. For all these reasons, the term of "renin-activity" was preferred to that of "renin concentration" for expressing the results obtained with this procedure.

3. DETERMINATION OF ANGIOTENSINASE ACTIVITY.

Since this procedure was developed entirely by the author, it will be reported in detail in the experimental section.

4. DETERMINATION OF URINARY ALDOSTERONE.

The method used for the determination of urinary aldosterone was that of Nowaczynski, Genest and Koiw (728). Purification of aldosterone is achieved through the use of three different chromatographic systems, and final determination is made with the isonicotinic hydrazide reaction and ultraviolet light absorption at 240 mμ. The results are expressed in μg per 24 hours. The normal range obtained with this method varies between 2 and 12 μg per 24 hours.

5. DETERMINATION OF URINARY ELECTROLYTES.

The urinary and plasma sodium and potassium were measured by flame pho-

tometry. The results are expressed in mEq excreted per 24 hours or in µEq/kg/min. for urinary electrolytes and in mEq per Liter of blood for plasma electrolytes.

RESULTS

The present work has been subdivided in various sections according to the experimental or pathological conditions. In each of these sections, the material and procedures will be given in detail and a separate discussion will follow the results of each separate study.

The renin-activity and angiotensin levels will be reported simultaneously when available. Undetectable levels under the present conditions of methodology will be termed zero. The angiotensinase-activity will be reported in a separate section in which the methodology will also be described in detail.

Finally, a general discussion will be presented at the end of the experimental section.

A - THE RENIN-ANGIOTENSIN SYSTEM IN VARIOUS PHYSIOLOGICAL CONDITIONS.

1. RENIN-ACTIVITY AND ANGIOTENSIN LEVELS IN NORMAL SUBJECTS:

a) Arterial angiotensin levels:

Subjects: Arterial angiotensin plasma levels were determined in 20 normal subjects and venous angiotensin levels in 9 others. Most of these were inmates of the Montreal Jail. A case history and a physical examination of each subject was done at the time of blood sampling. Only normotensive and healthy subjects, on unrestricted diet and without any pertinent past medical history were used as controls. The subjects were put in a recumbent position for at least half an hour before blood was drawn from brachial artery or vein.

Results: Details of individual data concerning the arterial plasma an-

giotensin levels are grouped in Table I. The mean arterial plasma angiotensin levels for the twenty subjects studied was 6.25 nanograms per 100 ml of plasma ±9.3 S.D. and ±2.0 S.E. Fourteen of the twenty subjects had undetectable plasma levels. The range varied from undetectable to 35 nanograms per 100 ml of plasma. Angiotensin was undetectable in the venous plasma of all but one of the nine subjects so studied, Table II.

b) Peripheral plasma renin-activity levels:

Subjects: Renin-activity was measured in the arterial blood of 20 normal subjects and in the venous blood of 7 others. In addition, ten subjects presenting various diseases had simultaneous sampling of arterial and venous blood. Parallel incubations were done on these two blood samples in order to determine if there exists a difference between arterial and venous renin-activity levels.

Results: The details of individual values are given in Table III. The plasma renin-activity level measured in 27 normal subjects was 9.5 ± 6.7 S.D. and ± 1.3 S.E. No significant difference was found between the mean value estimated in arterial blood and in venous blood. The range of these values varied from undetectable to 32 ng/L/min. Only 3 out of 27 subjects had undetectable levels after three hours of incubation under the conditions previously described. Although the mean value in men was slightly higher than the mean value for women, no significant statistical difference was found between both groups.

In order to further investigate if there was any arterio-venous difference, the renin-activity was measured in arterial and venous blood drawn simultaneously from ten different subjects and patients. The results of these experiments are reported in Table IV. No significant difference could be detected between arterial and venous renin-activity levels in the same subject.

TABLE I

ARTERIAL ANGIOTENSIN LEVELS IN NORMAL SUBJECTS

SUBJECTS	AGE	BLOOD PRESSURE (mm Hg)	ANGIOTENSIN (ng/100 ml Plasma)
1. P.B.	20	128/70	0
2. A.M.	35	120/74	0
3. W.M.	26	140/72	0
4. J.T.	39	120/70	0
5. Y.D.	20	120/62	0
6. E.M.P.	27	120/60	0
7. E.B.	52	120/70	0
8. R.N.	19	120/70	0
9. P.P.	29	120/60	0
10. J.G.B.	23	118/60	0
11. J.B.	42	110/62	0
12. R.C.	18	120/80	0
13. A.D.	25	130/70	0
14. C.D.	31	115/55	0
15. C.C.	23	150/82	10
16. A.B.	27	120/80	10
17. J.H.	22	120/60	15
18. C.G.	32	132/70	25
19. R.P.	36	120/72	30
20. M.L.	23	110/60	35
		MEAN (20 subjects) : 6.25 ±9.3 S.D. ±2 S.E.	

TABLE II

PERIPHERAL VENOUS ANGIOTENSIN LEVELS IN NORMAL SUBJECTS

SUBJECTS	AGE	BLOOD PRESSURE (mm Hg)	ANGIOTENSIN (ng/100 ml Plasma)
1. R.C.	34	118/74	0
2. J.M.C.	37	118/54	0
3. J.G.P.	27	120/64	0
4. F.S.	35	124/76	0
5. W.S.	39	120/80	0
6. W.D.	53	140/72	0
7. A.S.	37	136/92	0
8. R.C.	33	120/70	0
9. S.B.	26	144/74	55

TABLE III

PLASMA RENIN ACTIVITY IN PERIPHERAL ARTERIAL OR VENOUS BLOOD OF NORMAL SUBJECTS

ARTERIAL BLOOD

NAME	SEX	AGE	BLOOD PRESSURE (mm Hg)	RENIN ACTIVITY (Ng/L/min.)	
1. S.H.	F	19	120/70	0	
2. P.J.	F	20	110/70	0	
3. H.M.	F	45	130/90	0	
4. L.C.	F	22	130/80	1	
5. G.A.	F	29	120/84	5	
6. B.E.	F	24	140/80	5	
7. B.C.	F	35	140/80	6	
8. M.A.	F	54	130/90	6	
9. M.W.	F	21	120/80	7	
10. M.C.	F	22	120/70	7	
11. F.D.	F	16	118/80	8	
12. V.C.	M	35	100/80	8	
13. F.L.	M	33	134/82	10	
14. J.B.R.	F	25	120/70	13	} 10
			116/76	6	
15. L.B.	F	46	140/70	13	
16. M.L.	M	23		24	
				5	} 14
				13	
17. M.M.	M	19	140/80	15	
18. I.V.	F	36	120/80	16	
19. G.B.	F	19	120/60	18	
20. M.F.	F	31	120/60	32	

VENOUS BLOOD

21. D.M.	F	52	150/85	12	
			120/70	0	} 7
			120/75	9	
22. D.A.	M	32		9	
23. J.de C.	M	26	120/80	10	
24. R.V.	M	41	125/85	6	
				12	} 10
				11	
25. D.F.	M	34	130/90	15	
				7	} 11
26. R.T.	F	23	110/60	12	
27. S.L.	F	46	115/65	16	

... 125c

TABLE III (continued)

PLASMA RENIN ACTIVITY IN PERIPHERAL ARTERIAL OR VENOUS BLOOD OF NORMAL SUBJECTS

STATISTICAL ANALYSIS

GROUP	NUMBER	MEAN	ST. DEVIATION	ST. ERROR
Arterial blood	20	9.0	± 7.6	± 1.7
Venous blood	7	10.5	± 2.8	± 1.0
Men (Art. and Ven.)	8	10.9	± 2.4	± .85
Women (Art. and Ven.)	19	8.9	± 7.8	± 1.8
Total (Art. and Ven.)	27	9.5	± 6.7	± 1.3

TABLE IV

RENIN ACTIVITY* IN SIMULTANEOUS ARTERIAL AND VENOUS BLOOD SAMPLES

CASE NO.:	1	2	3	4	5	6	7	8	9	10
ARTERIAL:	12	7	18	47	15	41	64	76	11	22
VENOUS:	11	7	17	46	12	44	68	65	9	16

* In Ng/L/min.

c) Discussion:

The angiotensin blood levels were undetectable in most of the normal subjects studied and especially when measured in the venous blood. These results are in accordance with the findings of other investigators using different methods of measurement (108, 454-456). These studies do not rule out the possibility that angiotensin is circulating in normal subjects because most methods used are limited by their sensitivity and specificity. For instance, the lower limit of sensitivity for the method used in the present study is 10 ng. per 100 ml of plasma. Therefore, it is possible that angiotensin might be present in arterial blood at levels below these limits of sensitivity. Angiotensin is such a potent substance that only traces of it might be sufficient for metabolic activity.

In contrast, most normal subjects presented a certain degree of renin activity in their peripheral blood except in three instances. These findings are also in accordance with the results reported by other investigators using different methods of measurement (57, 147, 448-453). This indicates that renin is secreted in normal subjects, thus suggesting that the renin-angiotensin system is probably a physiological mechanism.

There is no significative difference between the levels of renin-activity in arterial and in venous blood. Although the mean renin-activity is slightly

more elevated in man than in woman, this difference is not statistically significant.

All values, but one, found in normal subjects occur between undetectable levels and 24 ng/L/min. The value at 32 ng/L/min. exceeds the superior limit of normal distribution (true at 99%) that can be expected with the present values. This distribution is grossly estimated by adding 3 times the standard deviation to the mean value, which would give a superior limit at 29.5 ng/L/min.

2. DAILY AND DIURNAL VARIATIONS IN RENIN-ACTIVITY LEVELS:

The measurement of renin activity would be of little use if too great variations were to occur from day to day or during the day in the same subject under normal conditions.

In order to evaluate this factor, two or more determinations of renin-activity were done in each of 11 patients, under the same conditions of diet, with intervals of one to eight days between samplings. The blood samples were always taken at the same hour in the morning.

Moreover, 5 other subjects had 2 or more determinations of renin-activity within the same day.

Results: The details of the renin-activity levels measured at varying intervals in 11 subjects, are reported in Table V. The variations are strikingly slight from day to day in each subject, either on an unrestricted or on a constant diet. Consecutive determinations at an interval of only one day showed no greater variation than 12 ng when levels were below 35 ng/L/min. When samples were separated by a few days, slightly greater variations could be observed. For instance, in patient no. 3, a difference of 20 ng. was found between two determinations done at an interval of four days, but in this patient, the levels of renin activity were high. Nevertheless, in most

TABLE V

DAILY VARIATIONS OF RENIN-ACTIVITY*

NAME DAYS:	1	2	3	4	5	6	7	8	DIET
1. W.M.	12	14							Unrestricted
2. F.G.	0	0							"
3. J.M.	87				107				"
4. M.S-J.	23						8	13	
5. A.A.	17	21							135 mEq.Na 90K
6. C.G.	35	30	24				30	25	" "
7. D.M.B.	12	0	9			15	9		" "
8. A.M.	13	15	10						" "
9. P.T.	7	11							" "
10. R.B.	10					12			" "
11. D.F.	15	8							" "

* In Ng/L/min.

TABLE VI

DIURNAL VARIATIONS OF THE RENIN-ACTIVITY*

NAME TIME:	9:00 a.m.	NOON	4:00 p.m.	8:00 p.m.	DIET
1. L.M.	0	0	0	0	Unrestricted
2. Y.B.	20	26	8	28	10 mEq.Na 90 K
3. L.B.	38	33	31	37	" "
4. J.R.	58	-	-	58	Unrestricted
5. R.F.	26	-	-	16	"

* In Ng/L/min.

cases, the variations were neither significant nor marked when blood samples were drawn at short intervals during unrestricted or constant diet.

The results of the determinations done within 12 hours also showed very slight variations, as illustrated in Table VI.

Discussion:

These results indicate that the renin-activity levels are quite stable in the same subject under constant conditions of diet within a few days, or within a few hours interval. Slight variations were observed in few subjects but these were within a narrow range, not exceeding 20 ng/L/min. Therefore, larger variations induced by various factors may be considered significant. Thus, the study of the factors modifying the secretion of renin can be undertaken with security concerning the interpretation of results.

3. VARIATIONS OF RENIN ACTIVITY UNDER VARIOUS SODIUM INTAKES.

The aldosterone stimulating effect of angiotensin has been repeatedly demonstrated in man and in various animal species. Moreover, many indirect observations have been accumulated showing a relationship between sodium balance, the granularity of the juxtaglomerular cells and the renin content of the kidney. All these observations strongly suggest a major role for the renin-angiotensin system in the regulation of aldosterone secretion and the maintenance of sodium balance. However, no direct demonstration of such a mechanism had been reported before this work was initiated.

Since changes in dietary sodium are often associated with inverse variations in aldosterone excretion and secretion, the administration of various sodium containing diets to human subjects and the simultaneous study of the variations in plasma levels of renin-activity and in aldosterone excretion or secretion probably represent the best experimental device to investigate the participation of the renin-angiotensin system in the normal physiological me-

chanisms.

Such a study was initiated in human subjects and was conducted in partial collaboration with Dr. Robert Veyrat.

Preliminary reports of these studies have already been published or reported (719, 724, 733-737).

a) Subjects and clinical procedure:

Fifteen normotensive and seventeen patients with benign essential hypertension and without any degree of cardiac failure, received various amounts of dietary sodium. Plasma renin-activity was measured at repeated intervals during the course of these various diets. The following daily sodium intakes were given: a) addition of 10 gm of sodium to an unrestricted diet, b) unrestricted diet alone, c) 100 mEq of sodium and d) 10 mEq of sodium, with or without administration of natriuretic agents. When on calculated diets, the subjects received a constant potassium intake at 90 mEq/day. All the patients were hospitalized during the course of these experiments and were maintained on a carefully controlled metabolic balance. Urinary aldosterone, sodium and potassium excretions were determined in many of these cases as well as plasma sodium and potassium concentration at the time of sampling of the blood for renin-activity. With the exception of four subjects (Fig. 7 and 9) who had serial renin-activity determinations during the day, all blood samples were drawn between 8:30 and 9:30 a.m. with the subject in recumbent position.

b) Results:

Details concerning blood level variations of renin-activity during the course of these studies are given in Tables VII, VIII and IX. The levels of renin-activity found during the control period and those measured two or three days after the onset of sodium restriction, or the addition of a daily sodium load to a normal diet, are illustrated in Fig. 1. As seen, the restriction of

EFFECTS OF SALT RESTRICTION, DEPLETION, OR LOAD ON PLASMA RENIN ACTIVITY

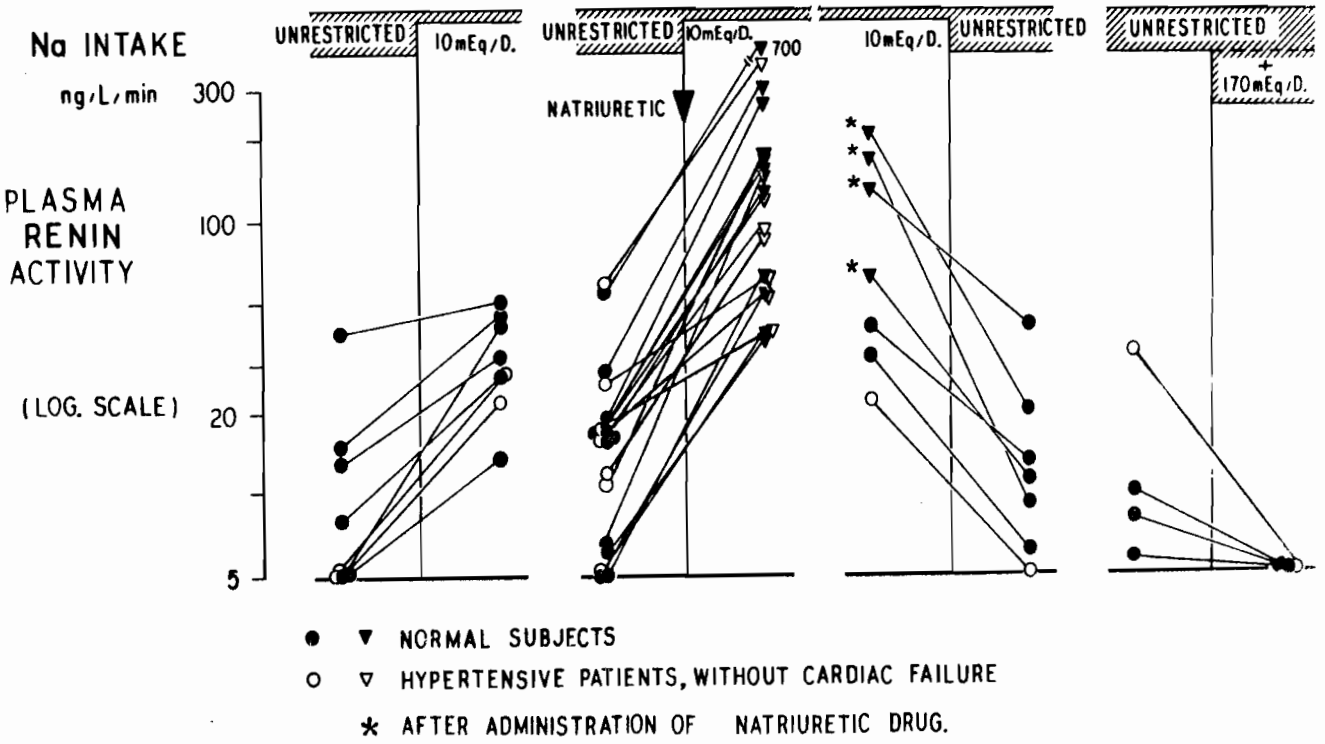


Fig. 1

Summary of 31 experiments concerning the effects of various sodium intakes on the plasma renin-activity two or three days after each change in dietary sodium. During sodium restriction to 10 mEq, renin-activity consistently increased. When a natriuretic agent was added to a sodium restricted diet, a greater increase in renin-activity occurred. The restoration of a normal sodium intake after sodium depletion or restriction was accompanied by a decrease in renin-activity towards control values. Salt loading decreased renin-activity to very low or undetectable levels.

sodium is accompanied by a significant increase in renin-activity in all instances. The increase is more pronounced when the administration of a natriuretic agent is made at the onset of sodium restriction. When the sodium depleted subjects are re-fed with a normal amount of sodium, the high blood renin-activity levels return rapidly to normal levels. Finally, when the subjects are given a sodium load, renin-activity falls to low or undetectable levels. Some individual studies, which are representative of each of these groups, will be reported in detail in the following pages.

i) Effects of sodium restriction:

In all eight subjects given a daily diet containing 10 mEq Na and 90 mEq K, the transition to a low sodium intake was accompanied by a significant increase in renin-activity. This increase could be observed on the third day of the diet but was greater in subjects studied on the fourth day (Table VII, Fig. 1).

The detailed study of three subjects in whom urinary sodium and aldosterone excretion were determined simultaneously with the measurement of renin-activity, is reported in Fig. 2. In these subjects, the renin-activity increased up to three times the control value after three days of sodium depletion. In all the renin-activity increased while the urinary sodium markedly decreased. In only one subject (J.B.R.), the changes in urinary aldosterone followed the same pattern as the variations in renin-activity, both increasing during sodium depletion and both returning to control pre-dietary values upon refeeding with a normal sodium intake. In the two others, the aldosterone excretion was not significantly modified during the period of sodium depletion.

ii) Effects of sodium depletion:

A sodium depletion was realized in 20 different instances by combining the administration of natriuretic agents with the intake of a low sodium diet

TABLE VII

EFFECT OF SODIUM RESTRICTION ON RENIN ACTIVITY

SUBJECT	AGE	SEX	B.P.*	DIET**	PLASMA Na mEq/L	URINARY Na mEq/day	RENIN ACTIVITY ng/L/min.	ALDOSTERONE EXCRET. µgm/day
1. M.L.	23	M	N	100 mEq Na (3) 10 mEq Na (3) Unrestrict.(4)			5 41 13	 29
2. H.A.	49	M	Ht	Unrestricted 100 mEq Na (3) 10 mEq Na (2) Unrestrict.(4)			0 13 22 5	 16
3. J.B.R.	25	F	N	Unrestricted 10 mEq Na (3) Unrestrict.(4)	141 142 139	66 4 	13 40 6	14 24 14
4. M.M.	19	M	Ht	Unrestricted 10 mEq Na (3)			15 45	36 45
5. M.V.	20	F	Ht	50 mEq Na (3) 10 mEq Na (3)	143 145	25 5	39 50	11 12
6. F.D.	16	F	N	Unrestricted 10 mEq Na (3)	140 136	158 49	8 27	6 6
7. Y.B.	36	M	Ht	Unrestricted 10 mEq Na (2)			0 22	
8. M.S.G.	51	F	N	Unrestricted 10 mEq Na (2)		170 35	0 15	

* Patients were classified as hypertensive if diastolic pressure was above 90 mm Hg.

** The number between brackets indicates the duration (days) of each diet prior to sampling.

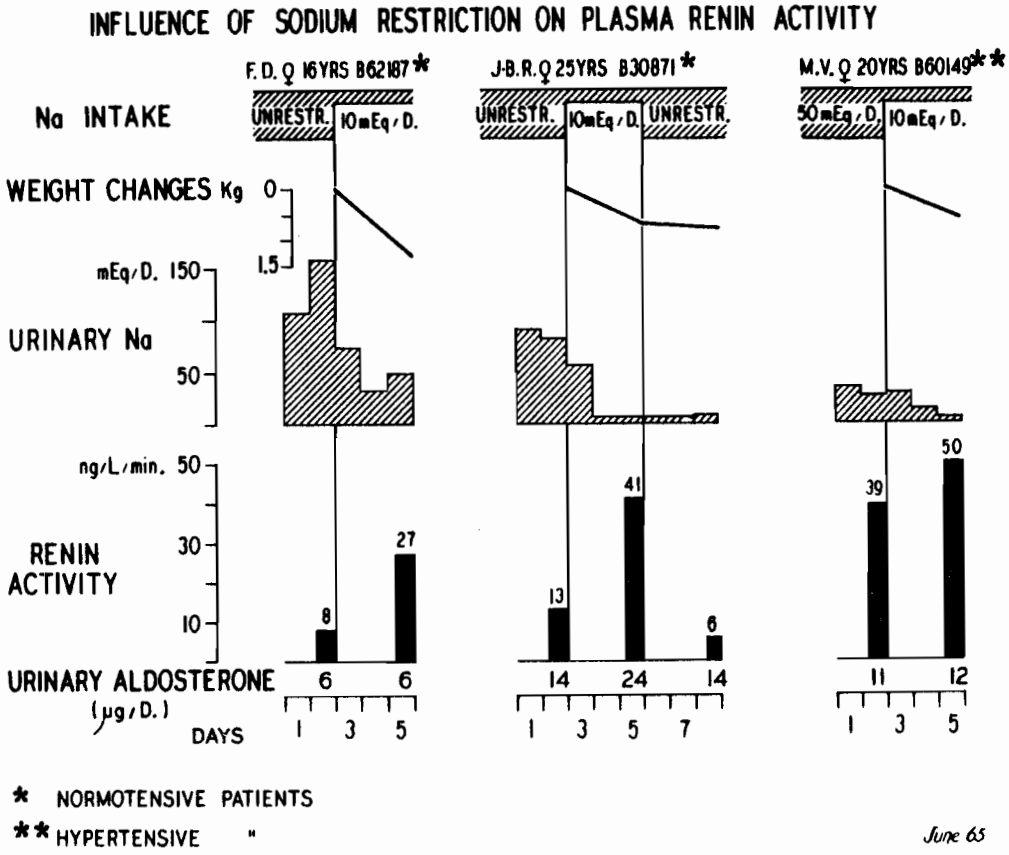


Fig. 2

The transition to a diet severely restricted in sodium (10 mEq/day) was accompanied in all three subjects by an increase in renin-activity. This increase occurred concomitantly with a marked decrease in urinary sodium and a loss of weight. In subject J.B.R., urinary aldosterone excretion followed the same pattern as the renin-activity levels. In this subject, the reestablishment of a normal diet was accompanied by a decrease in plasma renin-activity and urinary aldosterone excretion to control value within three days.

at 10 mEq per day. In most instances, this regimen induced a greater weight loss, a more consistent increase in urinary aldosterone and a greater increase in renin-activity (Fig. 1,Table VIII). After 2 or 3 days of this procedure, the renin-activity reached levels as high as 16 times the control value. The levels continued to increase when the diet was pursued for longer periods (up to six days). The reestablishment of the normal dietary sodium intake is accompanied by a rapid decrease to lower or normal levels (Fig. 1, Table VIII).

One detailed study of a normal subject is shown in Figure 3. In that subject, the renin-activity increased simultaneously with aldosterone excretion during the six-day period of sodium depletion and both parameters returned to normal shortly after a normal sodium refeeding. In that study, the blood renin-activity levels varied inversely with changes in urinary sodium. In two hypertensive subjects who were studied according to the same protocol, a similar pattern of response in renin activity was noted (Fig. 4).

Two normotensive subjects were submitted to a sodium depletion of longer duration and their renin-activity was measured at shorter intervals during the establishment of the sodium depletion and after the return to normal sodium intake (Fig. 5 and 6). In both subjects, an increase in renin-activity could be observed 24 hours after the initiation of the sodium restriction and these levels rose progressively to very high levels in the following days. In one of these subjects, R.V. (Fig. 5), the increase in renin-activity preceded the rise in aldosterone excretion by approximately 24 hours while in the other subject, S.L. (Fig. 6), renin-activity and urinary aldosterone increased simultaneously. On the sixth day of the sodium depletion, these two subjects were also given Metarypone (Ciba, SU-4885) for a period of three days in order to inhibit their adreno-cortical function. During that treatment the urinary aldosterone fell markedly, but the blood levels of renin-activity remained elevated and did not appear to be significantly modified.

TABLE VIII

EFFECT OF SODIUM DEPLETION* ON RENIN-ACTIVITY

SUBJECT	AGE	SEX	B.P.	DIET**	PLASMA Na mEq/L	URINARY Na mEq/day	RENIN ACTIVITY ng/L/min.	ALDOSTERONE EXCRET. ugm/day
9. R.V.	40	M	N	Unrestricted		163	6	14
				10 mEq Na (1)		129	34	15
				" " " (2)		41	103	13
				" " " (3)		10	110	45
				" " " (5)		3	157	65
				" " " (10)		1	170	96
				Unrestrict.(1)		6	53	69
				" (2)		10	34	66
				" (3)		45	34	40
				" (4)		200	13	12
				" (8)		210	9	14
10. S.L.	46	F	N	Unrestricted		166	16	16
				10 mEq Na (1)		61	54	30
				" " " (2)		16	112	42
				" " " (5)		4	192	66
				" " " (10)		1	210	80
				Unrestrict.(5)		217	20	28
11. R.V.	40	M	N	Salt load (3)	139	273	2	14
				10 mEq Na (3)	137	10	50	33
				" " " (6)	137	7	63	61
				Unrestrict.(3)	140	90	11	13
12. I.V.	36	F	N	Unrestricted			16	
				10 mEq Na (2)			129	
				Unrestrict.(3)			41	
13. J.R.	28	F	Ht	Unrestricted		144	58	26
				Unrestrict.(12h)		134	58	18
				10 mEq Na (12h)		300	97	27
				" " " (24h)		110	184	21
				" " " (48h)		12	376	56

... 131b

TABLE VIII (continued)

SUBJECT	AGE	SEX	B.P.	DIET**	PLASMA Na mEq/L	URINARY Na mEq/day	RENIN ACTIVITY ng/L/min.	ALDOSTERONE EXCRET. µgm/day
14. R.F.	34	F	N	Unrestricted		108	16	26
				Unrestrict.(12h)		100	26	25
				10 mEq Na (12h)		160	35	37
				" " " (24h)		54	106	19
				" " " (48h)		13	118	50
15. W.M.	36	M	Ht	Unrestricted	132	248	12	12
				Unrestrict.(1)	136	236	14	
				10 mEq Na (3)	136	6	80	25
				" " " (6)	137	6	85	12
16. A.L.	61	M	Ht	Unrestricted	143	110	19	17
				10 mEq Na (3)	141	9	38	10
				" " " (6)	130	2	268	32
17. J.M.	60	M	Ht	Unrestricted	136	132	17	18
				100 mEq Na (3)	140	78	41	16
				10 mEq Na (3)	136		94	12
18. G.B.	19	F	N	Unrestricted	139	80	18	14
				10 mEq Na (3)	139	15	168	19
19. M.A.	54	F	N	Unrestricted		49	6	
				10 mEq Na (3)		12	39	
20. L.L.	50	F	Ht	Unrestricted		72	9	
				10 mEq Na (8h)		308	0	
				" " " (16h)		93	13	
				" " " (24h)		52	36	
				" " " (36h)		48	56	
21. H.C.	31	F	Ht	Unrestricted		70	12	
				10 mEq Na (4h)		440	9	
				" " " (8h)		285	12	
				" " " (12h)		150	9	
				" " " (16h)		72	17	
				" " " (24h)		52	40	
				" " " (30h)		46	55	

... 131c

TABLE VIII (continued)

SUBJECT	AGE	SEX	B.P.	DIET**	PLASMA Na mEq/L	URINARY Na mEq/day	RENIN ACTIVITY ng/L/min.	ALDOSTERONE EXCRET. µgm/day
22. P.B.	35	F	Ht	Unrestricted 10 mEq Na (3)			17 54	
23. L.C.	20	F	N	Unrestricted 10 mEq Na (2)	140	80 8	53 700	
24. L.F.	35	M	N	Unrestricted 10 mEq Na (2)	139 135	 35	28 313	
25. C.T.	50	F	Ht	Unrestricted 10 mEq Na (2)	140	 20	5 54	
26. L.B.	40	M	Ht	Unrestricted 10 mEq Na (2)			7 38	
27. R.T.	23	F	N	Unrestricted 10 mEq Na (2)			12 180	
28. Q.F.	64	M	Ht	Unrestricted 10 mEq Na (2)			26 65	

* Obtained by the administration of a natriuretic agent (Chlorthalidone 100 mg) on the first day (subjects 9-21) and on both the first and second days (subjects 22-28) of the low sodium diet at 10 mEq Na.

** The number between brackets indicates the duration [hours (h) where written, otherwise days] of each diet prior to sampling.

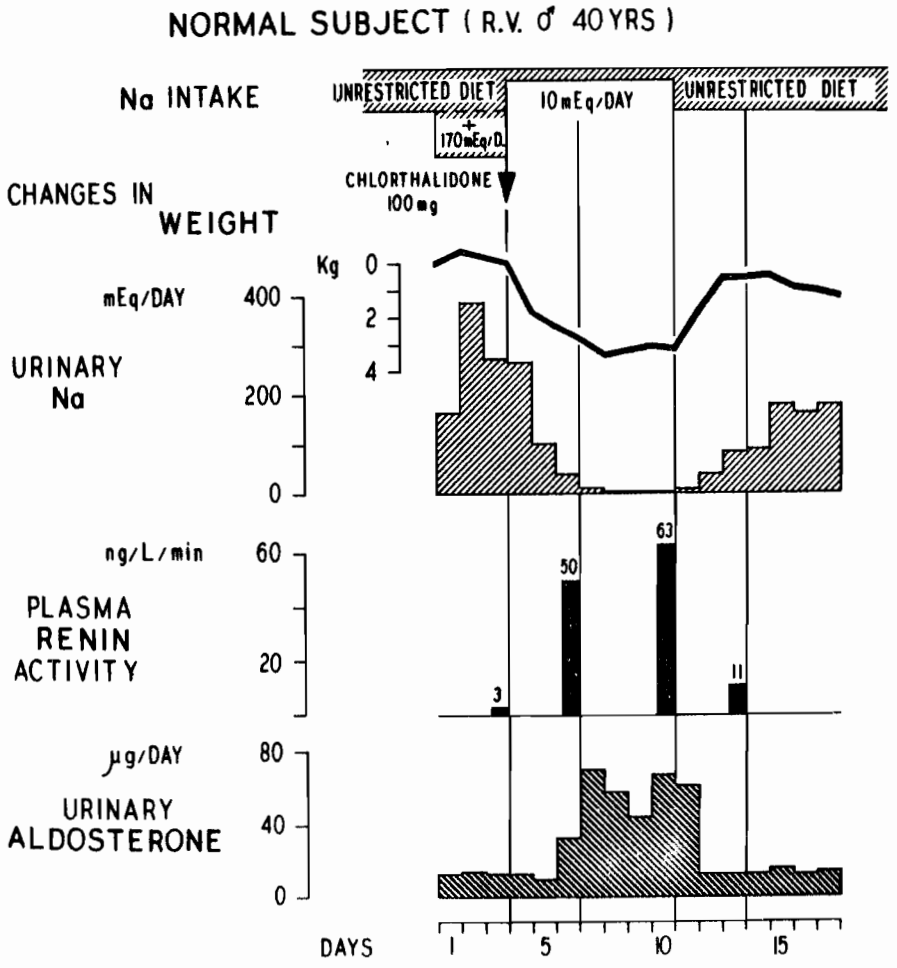


Fig. 3

Effect of sodium depletion (induced by the administration of a natriuretic agent on the first day of the transition to a low sodium diet) on plasma renin-activity and urinary aldosterone in a normal healthy subject. On the third and sixth day of this diet, both renin-activity and urinary aldosterone were found to be increased, while urinary sodium was markedly decreased. Three days after the return to unrestricted diet, renin-activity and aldosterone excretion decreased to normal while sodium increased gradually in the urine.

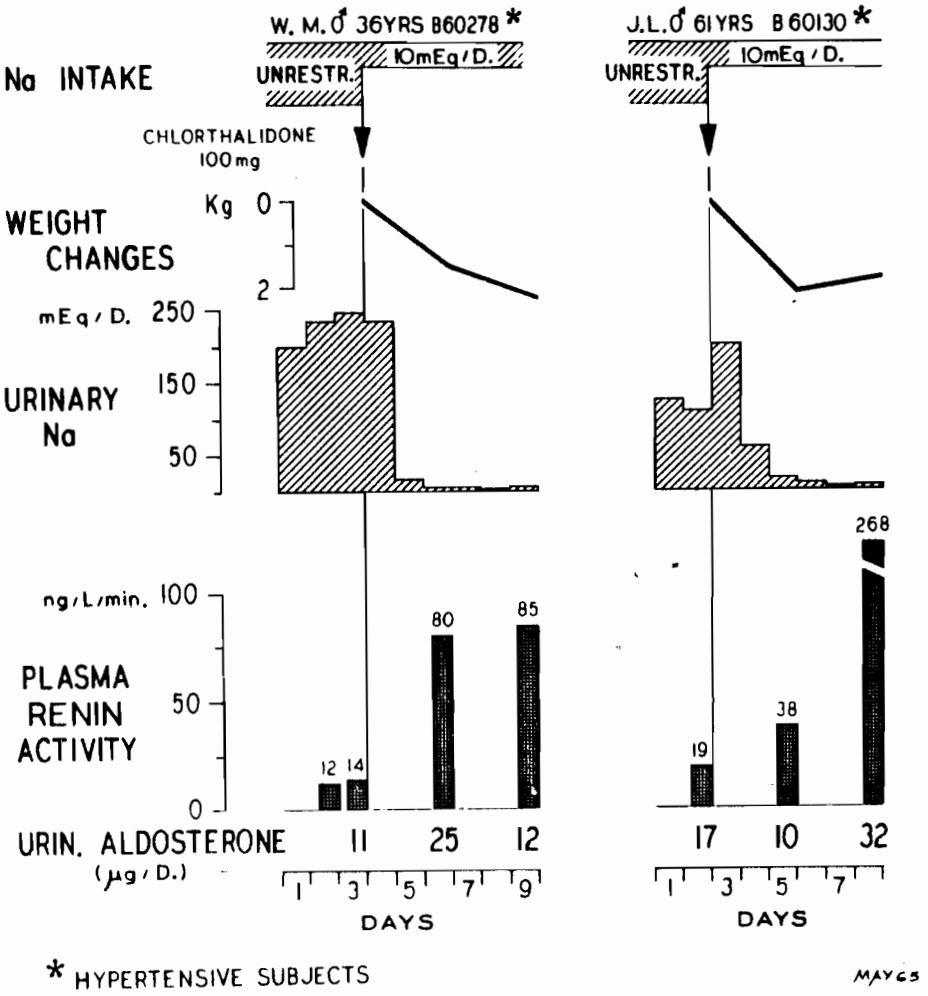


Fig. 4

After three days of sodium depletion, renin-activity increased in these two hypertensive patients. The levels were higher after six days of this diet. These variations occurred while urinary sodium fell markedly in the urine.

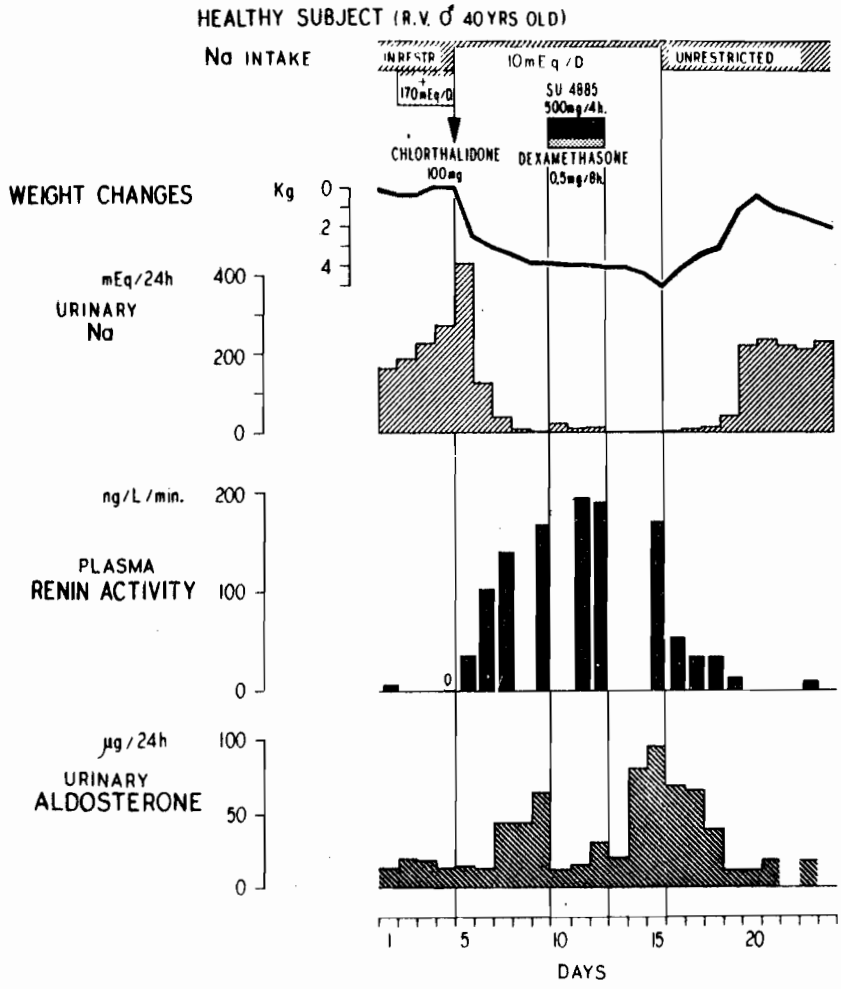


Fig. 5

Effect of sodium depletion and inhibition of adrenocortical function on renin-activity in a normotensive subject. Twenty-four hours after the onset of sodium depletion, the increase in renin activity had already been initiated. Renin-activity continued to rise progressively thereafter to reach a plateau around the sixth day of sodium depletion. At that time, the inhibition of the adrenocortical function by Metyrapone (SU-4885, Ciba) for 3 days, did not appear to modify the response in renin-activity. Note that in this study, the increase in renin-activity preceded the increase in urinary aldosterone by approximately 36 hours. At the end of sodium depletion, the decrease in renin-activity also preceded the decrease in urinary aldosterone.

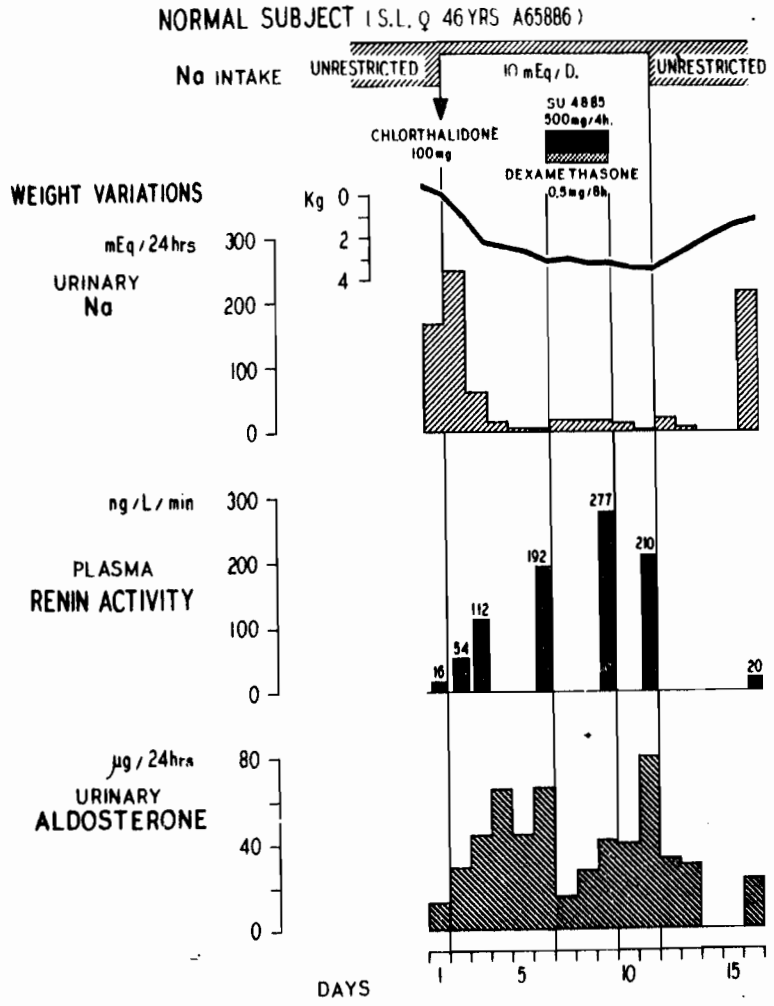


Fig. 6

Similar experiment than that reported in Fig. 5. An increase in plasma renin-activity and urinary aldosterone occurred simultaneously 24 hours after the onset of sodium restriction and depletion. The renin-activity levels continued to rise progressively during the following days. The inhibition of adrenocortical function on the sixth day of sodium depletion did not significantly modify the pattern of response of the renin activity. The return to unrestricted diet is accompanied by a decrease in renin-activity and aldosterone excretion.

In two other subjects, urinary sodium and aldosterone as well as plasma renin-activity were determined at 12 hour intervals during the first two days of sodium depletion, in order to look for the sequential response of renin-activity, aldosterone and sodium excretion during the establishment of a sodium depletion (Fig. 7). In one of these, an increase in renin-activity appeared 12 hours after the onset of sodium restriction and the administration of natriuretic agents while in the other who received two doses of the natriuretic drug, the renin-activity increased only 24 hours after the beginning of the sodium depletion. In these studies, a significant increase in urinary aldosterone occurred only 12-24 hours after the rise in renin-activity was well established. The sequential response of renin-activity and aldosterone is more clearly illustrated in Figure 8 where the response of another subject (Fig. 5) is added to the latter studies.

In order to investigate the early phase of the response of renin-angiotensin system to the stimulus of sodium depletion or restriction and its relationship to changes in urinary sodium, blood renin activity and urinary sodium were measured at eight hour intervals in one subject and at four hour intervals in one other (Fig. 9). These subjects were kept in a recumbent position for the entire duration of the experiment to prevent as much as possible the variations in sodium excretion that could be mediated through changes in position. In these two subjects, the beginning of the rise in renin-activity occurred only after the cessation of the natriuretic effects of the drug administered 16 to 24 hours after the start of sodium depletion. During the natriuretic period, the renin-activity decreased in one subject and remained low in the other. This study clearly illustrates that the renin-activity remains low as long as the diuresis and natriuresis persist and that renin-activity started to increase about the 16th hour, when the urinary sodium was de-

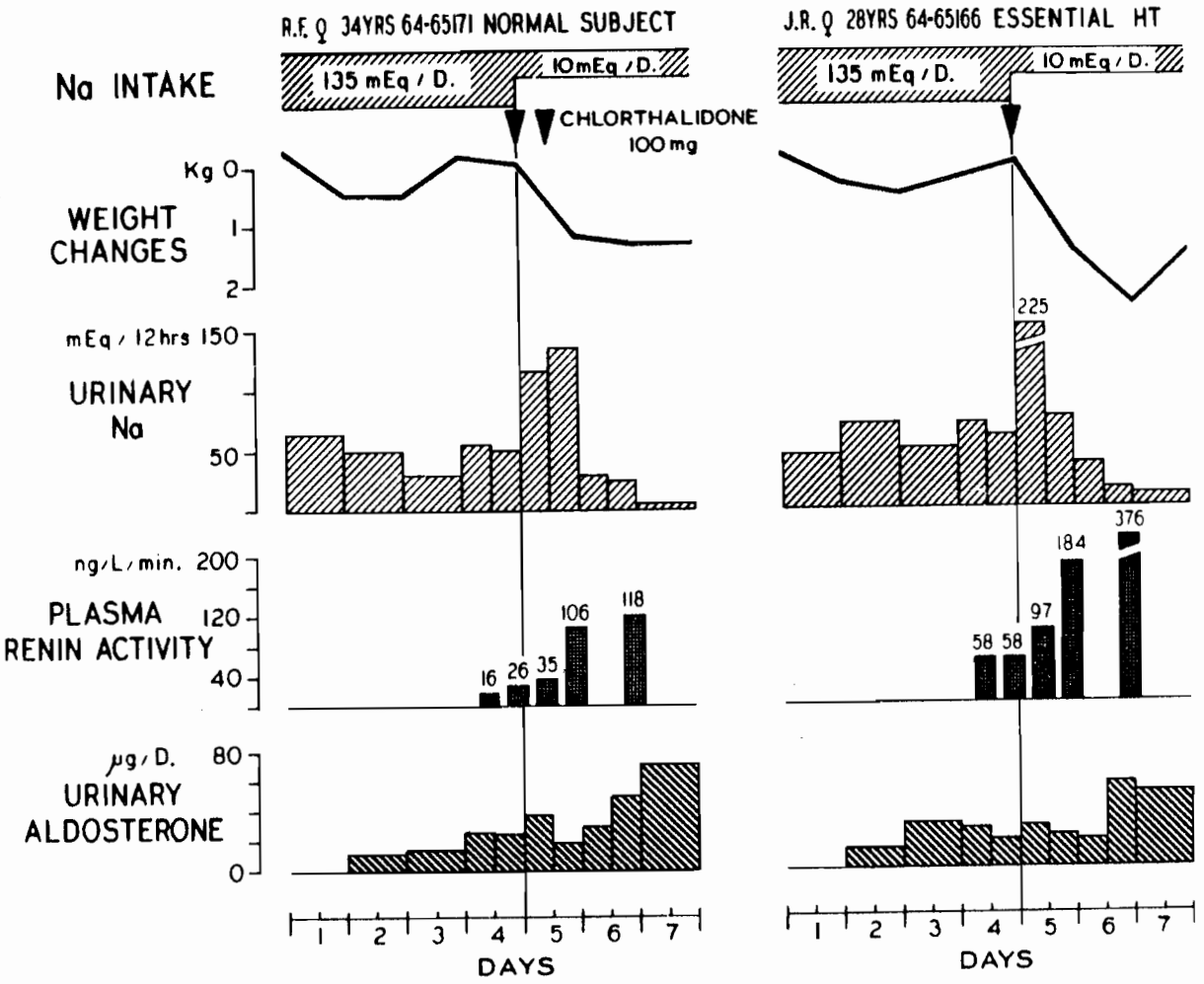


Fig. 7

Sequential response of blood renin-activity and urinary aldosterone during the establishment of a sodium depletion. In these two subjects, the urinary sodium and aldosterone and the plasma renin-activity were measured at 12 hour intervals during the first two days of sodium depletion. In subject R.F., the renin-activity increased 24 hours after the beginning of the diet when the period of increased natriuresis was over. The aldosterone excretion rate increased significantly 12 to 24 hours later. In subject, J.R., the renin-activity was found increased 12 hours after the onset of sodium depletion and at a time when the natriuretic effect of Chlorthalidone was over. Again urinary aldosterone increased with a delay of 24 hours over the increase in renin-activity.

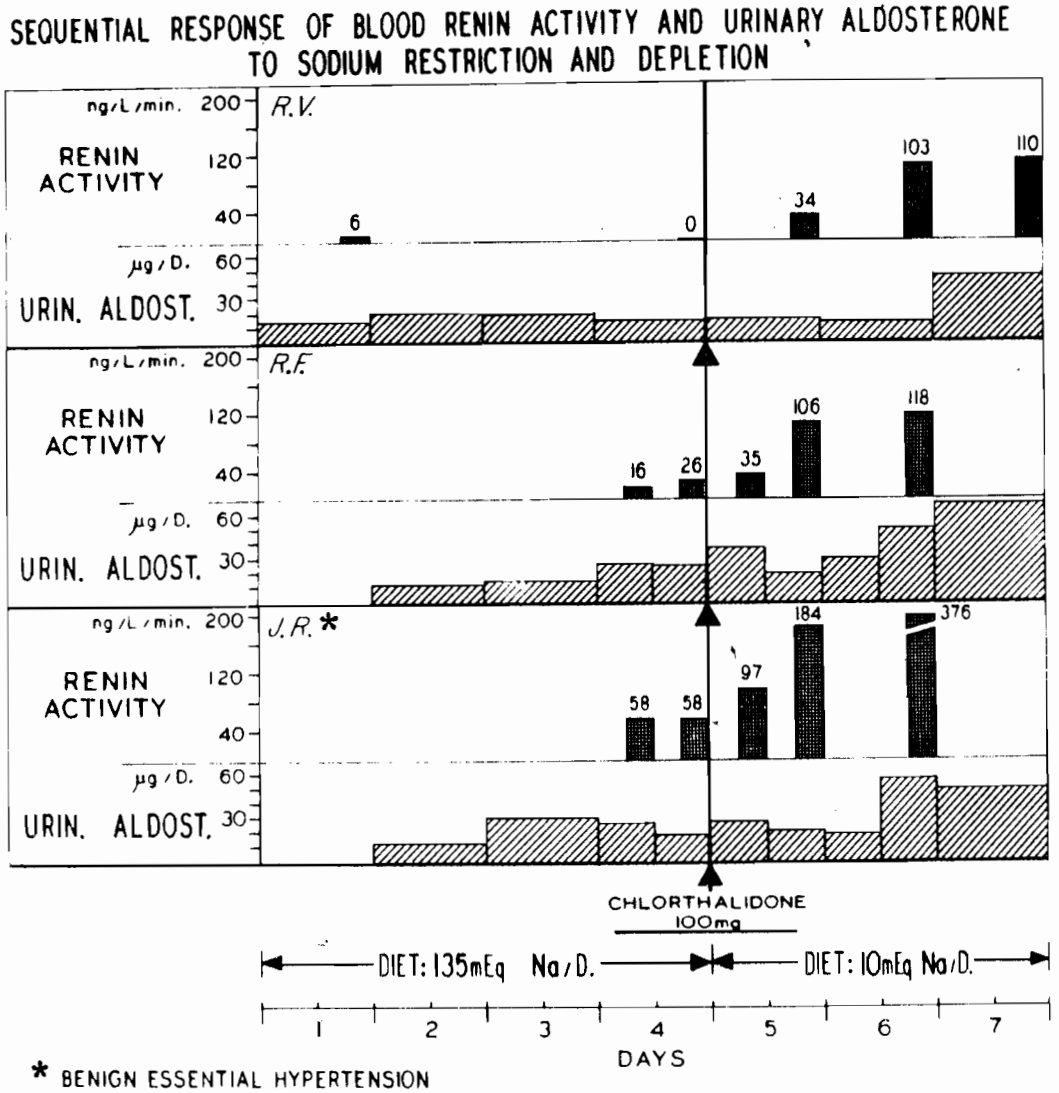


Fig. 8

Sequential response of blood renin-activity and urinary aldosterone during sodium depletion in three patients. In these, the rise in renin-activity preceded the increase in aldosterone excretion by 12 to 24 hours.

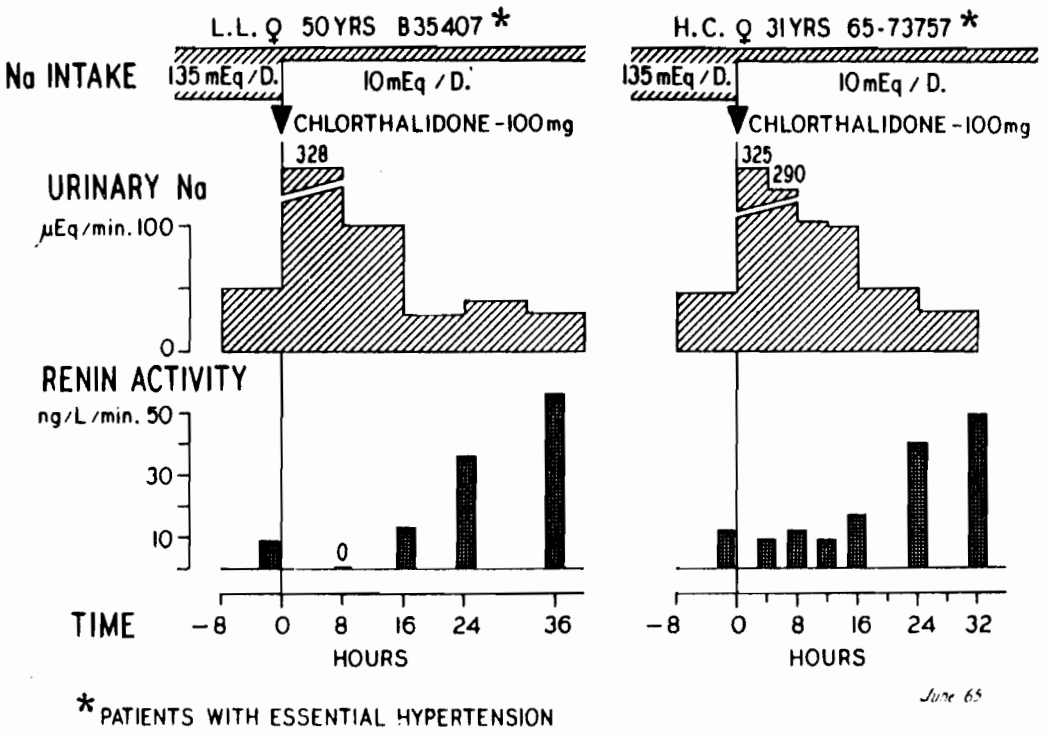


Fig. 9

This Figure illustrates the sequential response of renin-activity and urinary sodium during the establishment of sodium depletion induced by the administration of a natriuretic drug (Chlorthalidone) and low sodium intake. In subject, L.L., the determination of plasma renin-activity and urinary sodium done at 8 hour intervals revealed that plasma renin-activity increased significantly between 16 and 24 hours after the administration of the natriuretic drug when the natriuretic effect of the drug was over and when urinary sodium had already began to decrease. In this subject, the renin-activity fell to undetectable levels at the time of maximum natriuresis. In subject H.C., in whom the same parameters were measured at 4 hour intervals, the same pattern of response was observed. The increase in renin-activity occurred between the 16th and 24th hour following the onset of treatment only when sodium began to decrease in the urine.

creasing. In both subjects, the increase in renin-activity was well established after 24 hours and became quite high after 32 and 36 hours. It is of interest to observe that these two subjects, having approximately the same weight and almost the same blood renin-activity at the start of the diet, also showed almost identical variations in their renin-activity under similar conditions.

iii) Effects of salt loading:

When 10 gm of sodium were added to a normal unrestricted diet, the renin-activity fell to very low or undetectable levels (Fig. 1, Table IX). Three of these studies are illustrated in Figure 10. In all of them, plasma renin-activity decreased significantly to very low levels after three days of this diet, while urinary sodium increased markedly.

iiii) Relationship between peripheral renin-activity and sodium intake and/or excretion:

The correlation of plasma renin-activity (as measured after 2 or 3 days of administration of various diets) with the amount of sodium intake, is illustrated in Fig. 11. For this analysis, eighty determinations of peripheral renin-activity done in several subjects placed under dietary conditions varying from salt load to sodium depletion were divided into five major groups according to the amount of sodium ingested. The mean renin-activity of each of these groups differs significantly from each other group as shown by the statistical analysis reported in Table X.

TABLE IX

EFFECT OF HIGH SODIUM INTAKE* ON RENIN-ACTIVITY

SUBJECT	AGE	SEX	B.P.	DIET**	PLASMA Na mEq/L	URINARY Na mEq/day	RENIN ACTIVITY ng/L/min.	ALDOSTERONE EXCRET. µgm/day
29. F.L.	33	M	N	Unrestricted	140	85	10	
				Salt load (3)		323	4	
30. V.C.	35	M	Ht	Unrestricted	140	220	8	23
				Salt load (4)		290	2	10
31. R.V.	40	M	N	Unrestricted		163	6	14
				Salt load (3)		271	0	14
32. J.G.B.	19	M	Ht	Unrestricted		82	33	
				Salt load (3)		405	0	
33. Y.B.	21	M	N	Salt load (3)			8	
34. A.B.	66	M	Ht	Salt load (3)			7	
35. R.V.	40	M	N	Salt load (3)	139	273	2	

* Obtained by adding 10 gm Na to unrestricted diet.
** Between brackets is indicated: the number of days of salt loading diet prior to sampling.

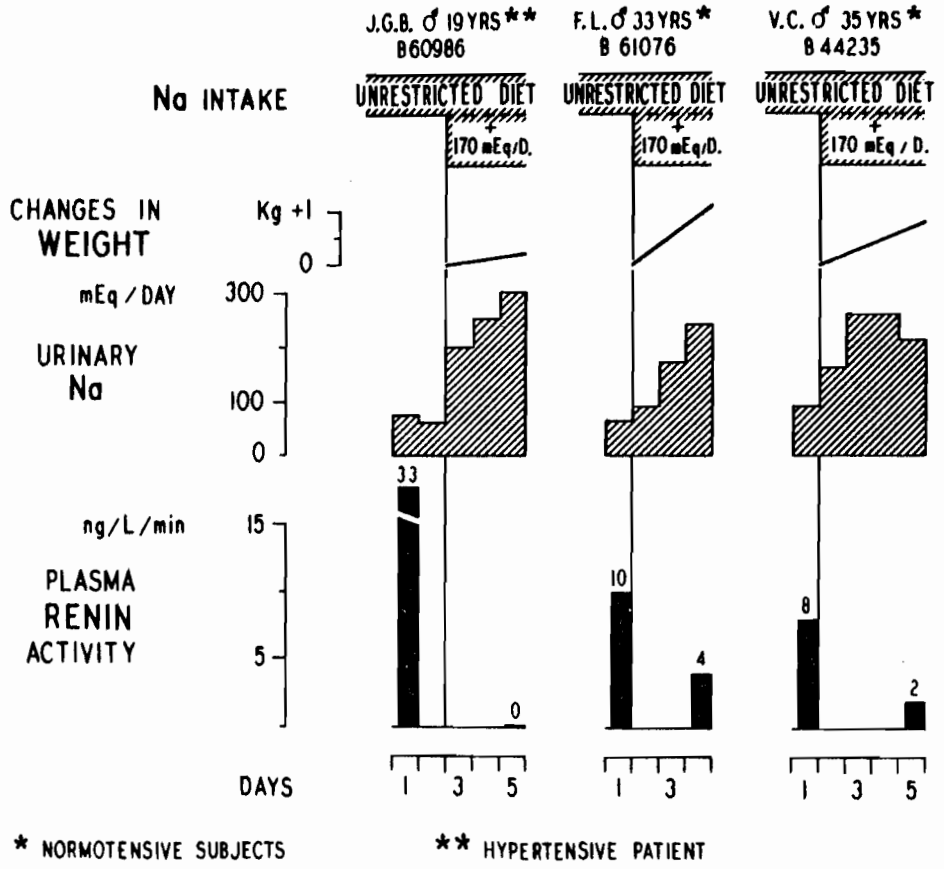


Fig. 10

Variations of plasma renin activity in three subjects given 170 mEq Na per day for three days in addition to an unrestricted diet. In all instances, the renin activity decreased markedly while urinary sodium increased.

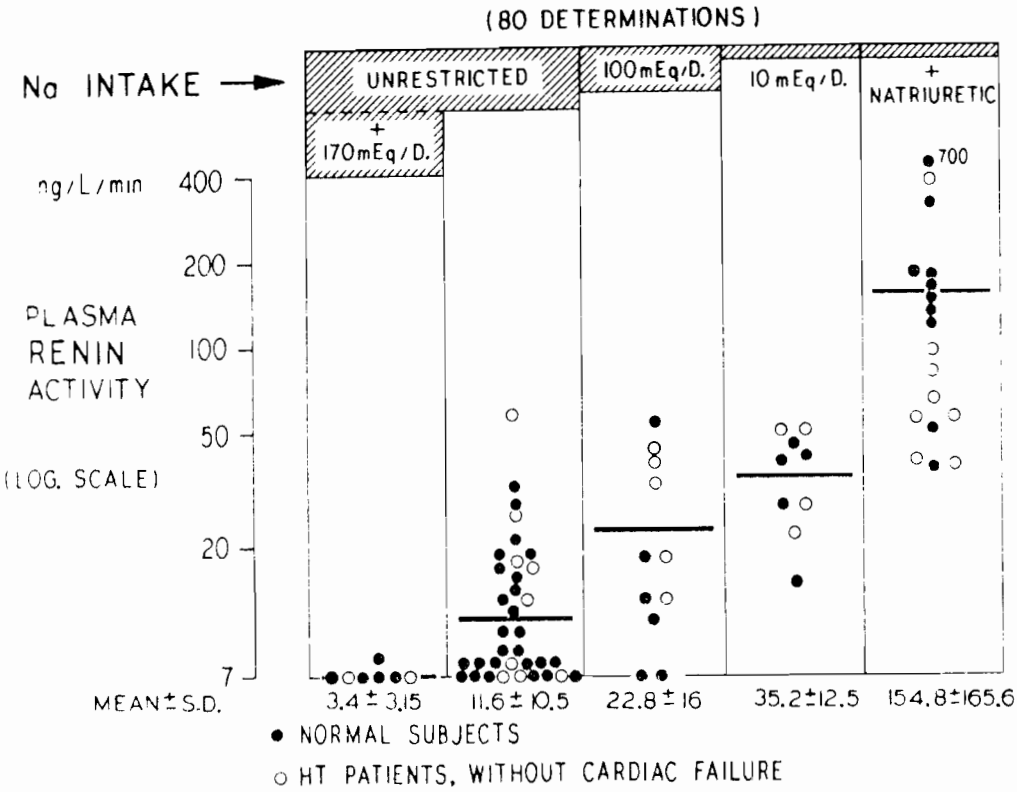


Fig. 11

Illustration of the close relationship existing between the plasma levels of renin-activity determined after 2 or 3 days of a given diet and the amount of sodium intake. The renin-activity increases in proportion to the severity of the sodium restriction reaching the highest levels when natriuretics were added to the sodium restriction.

TABLE X

SIGNIFICANCE OF THE DIFFERENCES OF RENIN ACTIVITY* IN SUBJECTS OR PATIENTS RECEIVING DIFFERENT AMOUNTS OF SODIUM PER DAY

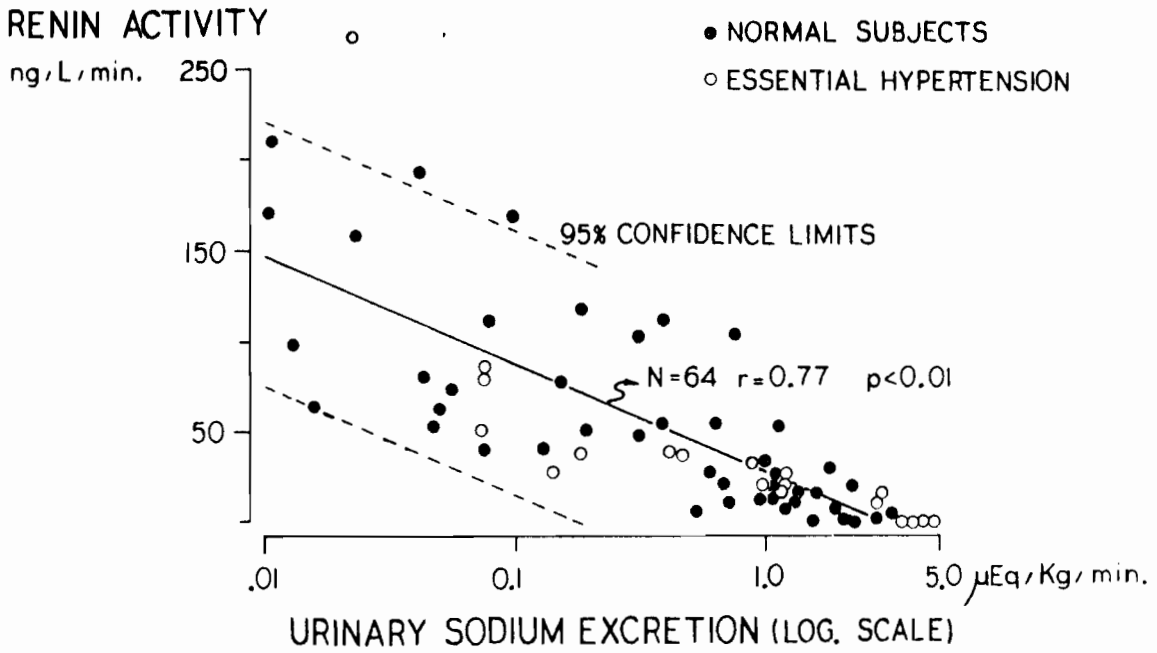
DIET	NUMBER	MEAN Ng/L/min.	ST. DEV.	S.E.M.	"p" BETWEEN GROUPS
SALT LOAD	7	3.4	± 3.15	± 1.2	
					$p < 0.05$
UNRESTRICTED	35	11.6	± 10.5	± 1.75	
					$p = 0.05$
100 mEq Na DIET	11	22.8	± 16.05	± 4.8	
					$p = 0.05$
10 mEq Na DIET	9	35.2	± 12.57	± 4.19	
					$p < 0.02$
10 mEq Na DIET PLUS NATRIURETIC	18	154.8	± 165.6	± 38.96	

* Determined after 2 or 3 days of administration of specified diets and expressed in ng/L/min.

The levels were lowest under salt loading conditions and increased gradually with increasing severity of sodium restriction and depletion. The values are quite widely distributed in the group of subjects on unrestricted diet, but this is understandable since this kind of a diet also implies a wide range of sodium intake. In the light of these findings, the 100 mEq diet which was used by many investigators as a "normal" sodium intake during basal metabolic studies, appears to be a lower-than-normal sodium intake since the mean level of the renin-activity obtained under such conditions is significantly higher than that found in subjects receiving an unrestricted diet.

A correlation of greater significance was obtained when the peripheral renin-activity levels were plotted against the logarithm of urinary sodium expressed in μmEq/Kg/min. (Fig. 12). The statistical analysis of these data obtained by the measurement of the regression line showed a very significant inverse linear correlation between peripheral renin-activity and urinary sodium for the 64 determinations of this study ($r = 0.77$, "p" < 0.01). A simi-

RELATIONSHIP BETWEEN RENIN ACTIVITY AND URINARY SODIUM EXCRETION IN MAN



REGRESSION LINE $y = -59.67 \log x + 27.88$

NOV '64

Fig. 12

Correlation of peripheral plasma renin-activity and the logarithm of urinary sodium excretion in μEq/Kg/Min. obtained in 64 instances. A very significant regression coefficient ($r = 0.77$, "p" < 0.01) was found between these two parameters. The renin-activity is elevated when urinary sodium is low and is decreased when urinary sodium is high.

lar correlation could be calculated with the logarithm of the sodium concentration ($r = 0.77$, "p" < 0.01) (Fig. 12a). No correlation could be found between plasma renin-activity and the urinary osmolality, the urinary potassium, or the plasma sodium and potassium. The lack of correlation with plasma Na is best illustrated by the finding of an undetectable renin-activity in a patient who had a profound dilution hyponatremia at 106 mEq/L. Two days later, after correction, the plasma sodium concentration had returned to normal levels and the renin-activity remained at undetectable level.

c) Discussion:

The present work constitutes the first direct evidence for a close relationship between sodium balance and the activity of the renin-angiotensin system. The actual findings of a gradual, linear, and significant elevation of the renin-activity during the transition from salt loading conditions to a progressively severe sodium depletion, and, also the close inverse relationship found between plasma renin-activity and the urinary sodium excretion strongly suggest an important role for the renin-angiotensin system in the physiological control of body sodium. Moreover, the increase in renin-activity preceding the rise in aldosterone excretion reported in at least three subjects, suggest that the renin-angiotensin system might be the regulator of aldosterone secretion under these dietary conditions.

Simultaneously to the first report of this work and to the subsequent publications (719, 724, 733-737), Brown and his coworkers (449, 738-740) reported a significant increase in renin-activity in 6 normotensive subjects maintained on a low sodium intake for 6 days. Only half of them showed a significant increase after 3 days of this diet. In our study, all the subjects showed a significant increase after three days of salt depletion. As in our study, Brown and coworkers observed a significant decrease in renin-

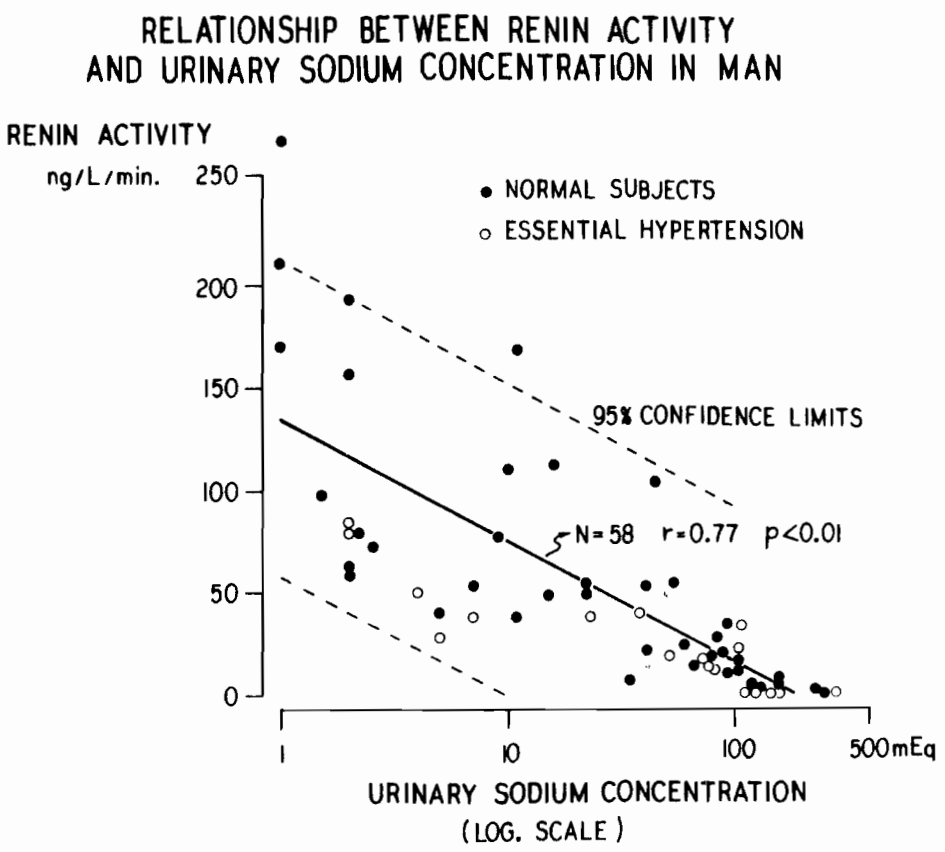


Fig. 12a

Correlation between the plasma renin-activity and the logarithm of the urinary sodium concentration in mEq/Liter. A very significant regression coefficient was found between these parameters.

activity of 6 other subjects after salt loading. Fasciolo et al (57) also reported a significant increase in the blood renin-activity of 5 normal subjects submitted to a severe sodium restricted diet. Machashi (741) recently confirmed the previous findings by reporting that a sodium restriction at 2 grams of sodium per day increased the plasma renin-activity of most of his normotensive and benign hypertensive subjects. Ajzen and coworkers (742), using Helmer's method (56) have just reported that the renin-activity increased significantly in the renal venous blood of sodium depleted subjects concomitantly with an increased granularity of the J.G.C. of the kidneys of these same subjects. However, none of these studies had reported the simultaneous determination of aldosterone excretion or secretion. With the exception of Brown's very recent publication (740), nobody had reported the simultaneous measurement of urinary electrolytes. All these studies strongly support our findings that the renin-activity varies inversely with the amount of sodium intake. Moreover, the unanimity in the conclusions of all these studies strongly suggest a participation of the renin-angiotensin system in the mechanism of sodium homeostasis.

The addition of a natriuretic agent to a low salt diet provokes a greater sodium loss and more pronounced sodium depletion. This state was shown to be accompanied by a more rapid and more marked increase in renin-activity than with salt depletion alone. This increase cannot be attributed to the natriuretic substance alone since the renin-activity begins to increase only after the maximum effect of the drug is over, as illustrated in Fig. 9. Moreover, the step-wise rise of renin activity continues during the subsequent days in all the subjects (Table VIII), while the natriuretic is no longer active.

In many instances, the aldosterone excretion was found increased during the period of sodium depletion or restriction simultaneously with an increase

in renin-activity. In three subjects in whom renin-activity and aldosterone excretion were measured at closer intervals (Fig. 8), the rise in renin-activity clearly preceded the rise in aldosterone secretion, thus indicating that the changes in aldosterone secretion are secondary to the changes in renin-activity in these conditions. On the other hand, aldosterone appeared to have little acute effect on the renin-activity since the complete inhibition of the adrenocortical function did not significantly modify the response of the renin-activity (Fig. 5, 6). During the inhibition of the adrenocortical secretion, only a slight increase in urinary sodium occurred despite a marked decrease in aldosterone excretion, thus indicating that mechanisms other than aldosterone were responsible for sodium retention at that moment.

Can angiotensin participate directly in the retention of sodium without the intermediary of the adrenal cortex? This would seem possible in the light of studies reporting that angiotensin infusion can produce an antinatriuresis in adrenalectomized patients (317, 360-362). The possibility of such an active participation of the renin-angiotensin system in the conservation of sodium is also suggested by the finding of Hartroft (667) that injections of serum containing anti-renin antibodies into sodium depleted dogs resulted in the appearance of a marked natriuresis.

The mechanism by which the changes in dietary sodium affect the secretion of renin is at the present time the subject of much speculation. Since sodium restriction is generally associated with a decrease in plasma volume, while sodium loading is associated with an increased volume, it was postulated that the changes in renin secretion might be mediated through the changes in plasma volume. Bartter and coworkers (575) showed that experimental alterations of intravascular volume were followed by inverse changes in aldosterone production. Moreover, according to Tobian (696), the juxtaglomerular cells

located in the media of the juxtaglomerular arterioles would act as a stretch or a presso-receptor regulating renin secretion. For instance, a decrease in sodium intake would induce a decrease in plasma volume which, by decreasing the stretch inside the afferent arteriole, would stimulate the juxtaglomerular cells to liberate renin. Subsequently, the increased levels of angiotensin would stimulate the secretion of aldosterone.

Many other workers also suggested the participation of the macula densa in such a mechanism (158, 164, 166, 704, 705). The anatomical location of the cells of the macula densa between the tubular fluid and the juxtaglomerular cells, the close histological connection between both structures and the close relationship between the enzymatic activity of the macula densa and the granularity of the J.G.C. constitute the main indirect evidence for the participation of the macula densa, under the influence of urinary ionic changes, in the control of renin-secretion. So far, changes in osmolality, in sodium concentration or in the intratubular pressure at the site of macula densa have been postulated for the stimulus of renin secretion, but none of these hypotheses have been clearly demonstrated. The striking inverse relationship between the urinary sodium excretion or concentration and the plasma levels of renin-activity found in our studies would favor the latter hypothesis. It cannot be concluded from the present work whether the changes in urinary sodium are secondary to the increase in renin-activity or aldosterone, or whether the renin secretion depends on the urinary sodium. Nevertheless, there is some indication that the changes in urinary sodium might precede the changes in renin secretion. As illustrated in studies in which more fractionated urinary collections were done, no increase in plasma renin-activity occurred so long as there existed a good natriuresis. Only when the urinary sodium decreased significantly did an increase in renin-activity occur. Recently, Vander

and Miller (166) also demonstrated a very similar relationship between urinary sodium and the plasma renin-like activity of dogs in which the aorta was clamped above the renal arteries. In their studies, they could significantly decrease the renin-activity of their animals simply by increasing the osmotic load or sodium content of the tubular fluid without changing the renal hemodynamics, thus indicating that renin secretion might be controlled by the urinary ionic content, probably at the site of the macula densa. Since the correlation found in the present work, is almost identical to theirs, our findings would support their hypothesis. These findings by two different groups, in two different species, under different experimental conditions, is most striking.

The marked discrepancies between renin-activity levels and the blood pressure are well illustrated in the present study. Very high levels were found in normotensive subjects during sodium depletion with no change in blood pressure. A decrease in the pressor responsiveness to angiotensin has been reported in sodium depleted subjects and animals (265, 266, 274, 275). This would explain why markedly elevated endogenous renin-activity levels do not produce hypertension in these subjects. The mechanism by which the decrease in responsiveness occurs is not known. Many factors which could influence the responsiveness to pressor drugs were extensively discussed in the part of this thesis on the responsiveness to angiotensin.

Whatever the stimulus of renin release, or the mechanism of action of renin and angiotensin, the present findings strongly suggest that the renin-angiotensin system might play an important role in the maintenance of sodium balance.

4. VARIATIONS OF RENIN-ACTIVITY DURING POTASSIUM LOADING.

Much controversy still exists regarding the role of potassium in the regulation of aldosterone excretion and secretion. Many investigators have re-

ported an increase in aldosterone production (422, 601, 603-608) after oral or I-V potassium administration, but others could not confirm these findings (610-613). In man, a high potassium intake has been reported to increase aldosterone excretion (615) and secretion (265), while a low potassium intake has been reported to prevent the increase in aldosterone secretion during sodium depletion (265). On the other hand, it was reported that very large changes in potassium balance were necessary to induce only slight changes in aldosterone excretion when the variations in sodium balance were corrected throughout the administration of potassium (585). In rats given a potassium load, Hartroft (667) could not detect any alteration of the J.G.I.

Since renin-activity had never been studied under such conditions, subjects were put on a high potassium intake in order to find out if the changes in aldosterone reported in such conditions were mediated through the liberation of renin.

a) Subjects and clinical procedure:

Four subjects were maintained for three days under a control diet at 135 mEq sodium and 90 mEq potassium. Thereafter, these subjects were given, in addition to the control diet, a potassium load of 100-120 mEq per os (Kaon 20 mEq potassium/cc) for 3 to 4 days. During that time, urinary aldosterone, sodium and potassium, and plasma sodium, potassium and renin-activity were measured daily.

b) Results:

The details of these studies are reported in Table XI. Only one of the four subjects studied showed a significant and progressive increase in renin-activity during the time of potassium loading. The study of this subject is illustrated in Figure 13. The renin-activity increased progressively while the potassium increased in urine and in plasma. Simultaneously a slight in-

TABLE XI

EFFECTS OF HIGH POTASSIUM INTAKE ON PLASMA RENIN-ACTIVITY

NAME	AGE	SEX	DAY	DIET	PLASMA K mEq/L	URINARY Na mEq/day	URINARY K mEq/day	RENIN ACT. Ng/L/min.	ALDOST. µgr/day
1. A.A.	60	M	1	135 Na 90 K		70	34	21	12
			2	" " " "	4.2	76	55	17	11
			3	135 Na 200K		100	82	40	17
			4	" " " "		--	--	51	--
			5	" " " "		76	150	76	21
			6	" " " "	5.4	46	155	60	18
			7	135 Na 90 K		28	65	42	11
			8	" " " "	4.0	34	84	12	19
2. D.McB.	52	F	1	135 Na 90 K	3.5	--	--	12	--
			2	" " " "		81	32	0	4
			3	135 Na 200K		128	190	9	12
			4	" " " "		69	184	18	28
			5	" " " "	5.0	69	191	11	25
			6	135 Na 90 K		37	80	14	14
			7	" " " "		65	89	15	8
			8	" " " "	4.0	110	88	9	13
3. A.M.	52	F	1	135 Na 90 K		82	27	*	--
			2	" " " "	4.0	80	75	*	--
			3	135 Na 200K		108	118	14	--
			4	" " " "		108	160	10	--
			5	" " " "	5.2	101	121	13	--
			6	135 Na 90 K		131	91	15	--
			7	" " " "	3.7	121	52	10	--
4. C.G.	28	F	1	135 Na 90 K	3.5	140	68	33	--
			2	" " " "		139	120	--	--
			3	" " " "		110	80	30	--
			4	135 Na 200K		67	110	24	--
			5	" " " "	3.8	38	81	33	--
			6	" " " "		62	150	32	--
			7	135 Na 90 K		84	88	--	--
			8	" " " "		69	73	33	--
			9	" " " "	3.5	100	80	24	--

* Samples lost.

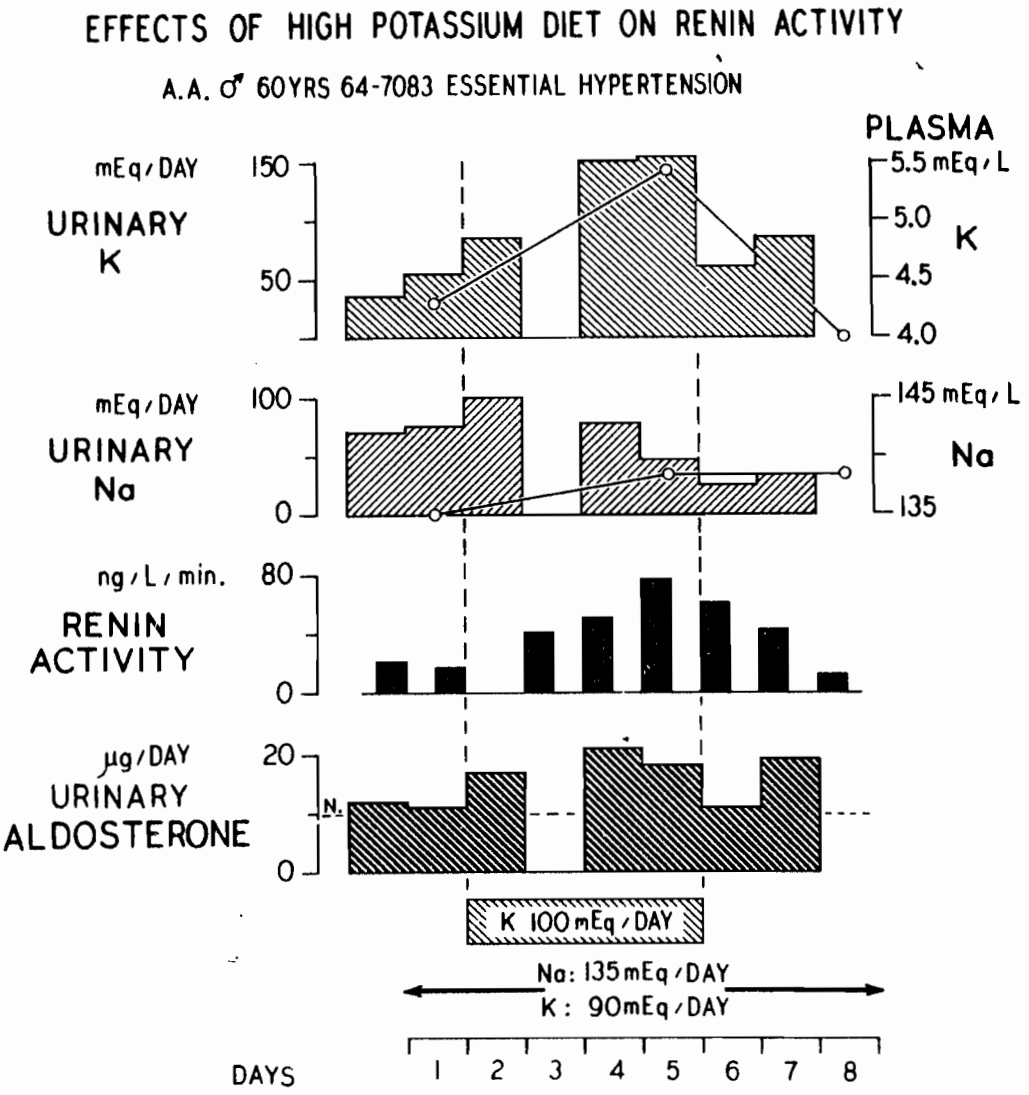


Fig. 13

Effect of potassium load on plasma renin-activity in a hypertensive subject. The administration of a supplement of 100 mEq potassium for four days was accompanied in this subject by a slight increase in aldosterone excretion and a progressive increase in plasma renin-activity. Meanwhile, the urinary and plasma potassium concentration increased markedly and the urinary sodium decreased significantly. When the potassium load was stopped, the plasma renin-activity gradually returned to normal. In three other subjects who were submitted to the same regimen for three days, the renin-activity did not change significantly.

crease was observed in the aldosterone excretion. However, during this same period, a decrease occurred in sodium excretion.

In the other three subjects, no significant increase could be noted in blood renin-activity during similar studies. In subject D.McB., a slight increase occurred in aldosterone excretion but the renin-activity did not change significantly.

The administration of a potassium load to the subjects was frequently accompanied by side effects: nausea, vomiting, diarrhea and loss of appetite, were the most commonly encountered. In subjects No. 2,3,4, the potassium load had to be stopped after the third day of diet because of some of these side-effects. A few other subjects which are not reported here presented nausea and vomiting on the first day of the high potassium intake and therefore could not be used for prolonged studies. The subject A.A., is the only one who tolerated his diet without any side effect and also the only one who showed a significant increase in renin-activity.

c) Discussion:

With the exception of one subject, the administration of a potassium load did not significantly modify the plasma renin-activity. The lack of significant increase in renin-activity in three of these subjects is in accordance with the findings of Hartroft (667) who did not detect any change in the granularity of the juxtalgomerular cells of rats given a potassium load.

5. VARIATIONS OF RENIN-ACTIVITY DURING FASTING.

It was reported that a low potassium diet prevents the rise in aldosterone excretion and secretion in sodium depleted animals and man (265, 606). It is thus of interest to investigate whether the response of renin-activity can be blocked in presence of a simultaneous potassium depletion. Since fasting represents the ideal state of sodium and potassium depletion, renin-activity was

measured during the first week of fasting.

a) Subjects and clinical procedure:

Six obese subjects were submitted to complete fasting for 7 days and were allowed to drink only distilled water during that period. Three days of control diet at 135 mEq Na and 90 mEq K preceded the fasting period. Renin-activity was measured usually on the second, the fourth or fifth, and last day of fasting. Plasma and urinary sodium and potassium were measured daily.

b) Results:

The results are reported in detail in Table XII and illustrated in Fig. 14. Although renin-activity did not change significantly on the second day of fasting, a significant increase was observed on the last day of fasting in all but one subject. During that period the subjects lost a mean weight of 6 kilograms and urinary sodium and potassium levels fell to very low levels.

c) Discussion:

These findings combined with those reported precedingly in the section on high potassium intake suggest that the changes in renin-activity are not dependent upon the potassium intake and appear to be quite specific to the changes in sodium intake and/or excretion. Aldosterone secretion rate was recently reported to be elevated during the first and second week of fasting (744). This is in agreement with the present findings of an increase in renin-activity and would indicate that the increase in aldosterone secretion is probably mediated by the renin-angiotensin system.

6. RENIN-ACTIVITY IN NORMAL PREGNANCY.

Several studies have indicated that the aldosterone excretion (745-750) and secretion rate (751-753) are greatly increased in pregnancy, chiefly in the last trimester. Although the G.F.R. and the filtered sodium increase as

TABLE XII

EFFECTS OF FASTING ON RENIN-ACTIVITY

NAME	AGE	SEX	DAY OF FASTING	WEIGHT	PLASMA Na mEq/L	PLASMA K mEq/L	URINARY Na mEq/24 hr	URINARY K mEq/24 hr	RENIN Ng/L/min.
1. F.S.	28	F	0	114.5	143	3.9	154	42	22
			2	113.3			120	36	18
			5	110.5	139	4.1	47	58	52
			8	106.4	139	3.2	3	27	170
2. M.P.	19	F	0	81.6	140	2.9	75	90	37
			2	80.6			46	37	10
			4	78.9	142	3.9	45	40	74
			8	77.0	137.5	3.6	30	28	80
3. B.O.	16	F	0	69.0	141	3.6	107	57	26
			2	67.0			57	32	14
			5	64.1			19	45	47
			8	63.0	138	3.9	1	8	78
4. R.B.	37	F	0	81.9	140	3.7	97	69	23
			2	80.3			35	34	30
			4	78.2	138	4.1	34	26	18
			8	75.7	136.5	3.7	9	25	58
5. P.A.R.	16	F	0	80.1	146	4.1	98	69	10
			2	79.2			20	30	43
			8	75.8	141	3.8	5	17	0
6. L.R.	36	F	0	119.0	143	4.5	41	46	0
			2	117.9			53	28	10
			5	114.3	139	3.9	23	23	11
			8	112.0	140	3.6	27	40	24

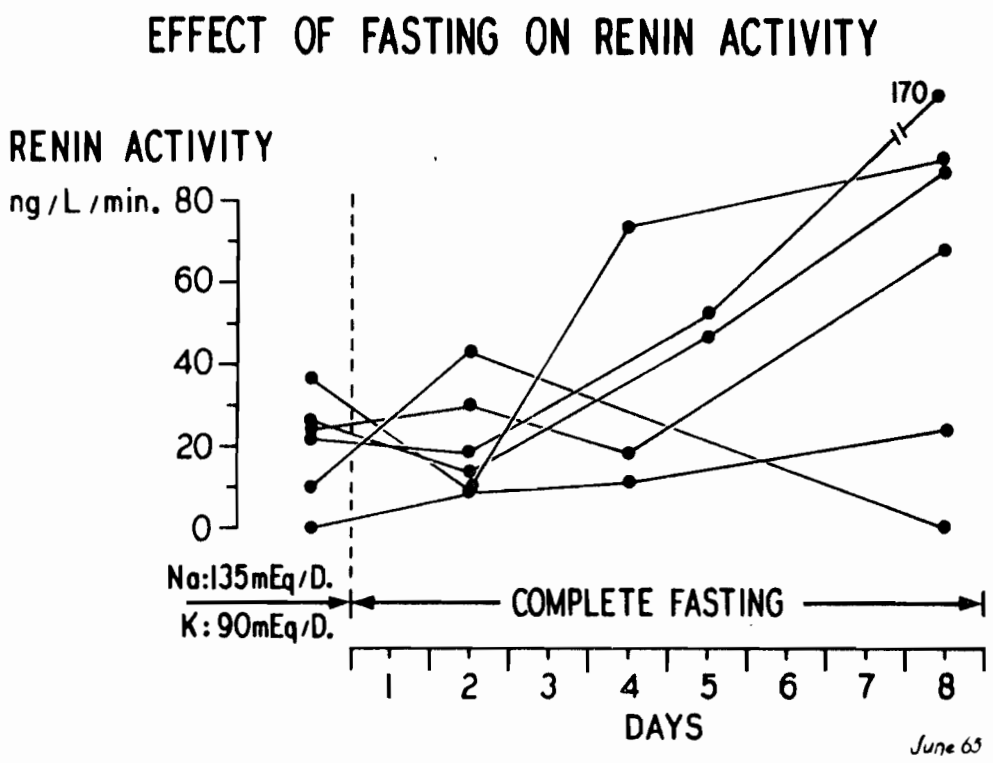


Fig. 14

This Figure illustrates the response of renin-activity measured at different day intervals during the first week of fasting in six obese subjects. On the second day of fasting the renin-activity levels were not significantly changed in most subjects. On the eighth day of fasting, the renin-activity was significantly increased in 5 out of 6 subjects. In the other subject, the renin-activity rose on the second day of fasting and then fell to undetectable levels. The reasons for this are now known.

much as 50% during pregnancy (754, 755), sodium and water are retained consistently. The total body sodium and water increase (756-758) and an expansion of the extracellular and plasma volume occurs (759-761) as the pregnancy progresses.

The factors which are responsible for the increase in aldosterone secretion rate during pregnancy are not completely understood. Several studies suggested that this increase in aldosterone might be attributed to an antagonism between the actions of progesterone and aldosterone on the renal tubule (762, 763). The increase in plasma progesterone levels (764, 765) parallels quite closely the curve of the increase in aldosterone excretion and secretion.

After the close correlation between sodium balance and the renin-activity previously reported, it was of interest to determine if the changes in aldosterone secretion and sodium retention during pregnancy could be mediated by the renin-angiotensin system. When this work was undertaken, no one had studied this aspect in normal pregnancy.

a) Subjects and clinical procedure:

Thirty-six normotensive pregnant women had one determination of plasma renin-activity at different periods of pregnancy, ranging from the fourth to the fortieth week. Most women were taking a normal unrestricted diet at the time of sampling and had no positive medical history or associated diseases. These findings are compared to 15 normotensive women who were having normal menstrual cycles.

b) Results:

The mean renin-activity of 15 non-pregnant normotensive women was found at 9.0 ng/L/min. ± 7.8 S.D., ± 2.0 S.E., with a range from undetectable to 32 ng/L/min. With the single exception of a value at 32 ng/L/min., all were below 18 ng/L/min.

Details concerning the pregnant women: age, weight gain, time of pregnancy, and gravidity, are given in Table XIII, along with the results of renin-activity measurement. The correlation of the levels of renin-activity with the time of pregnancy are illustrated in Fig. 15 and the statistical analysis of these findings are reported in Table XIV. For statistical analysis, the 36 patients were divided into four groups of nine weeks each: 4th to 12th; 13th to 21st; 22nd to 30th and 34th to 40th week. Each of these groups is significantly increased compared to the normal mean value for non-pregnant women. The statistical analysis between adjacent groups revealed that the mean level of renin-activity increases significantly from the fourth to the twenty-first week of pregnancy. Thereafter the mean level increases slightly, but not significantly in the following 9 weeks and decreases slightly but not significantly in the last part of the pregnancy.

In the group extending from the fourth to the thirteenth week, the mean level of plasma renin-activity is statistically increased compared to normal non-pregnant women. However, the values obtained at the beginning of that period were within the normal range and only the values obtained after the eighth week were above normal range. In the 31 women studied between the eighth week and the end of pregnancy, only 3 had values within the normal range (Fig. 15).

c) Discussion:

During the course of this work, two studies have been published on this subject. Brown and coworkers (766) reported higher than normal "renin" levels in many normotensive women at various stages of pregnancy and Fasciolo et al (57) found high renin-activity levels in 6 out of 6 pregnant women during the third trimester of pregnancy. Our study, which was presented elsewhere (724), is in accordance with the findings of Fasciolo (57) during the

TABLE XIII

BLOOD RENIN ACTIVITY IN NORMAL NORMOTENSIVE PREGNANT WOMEN

PATIENT	AGE	WEIGHT GAIN Kg.	GRAVIDA	WEEKS OF PREGNANCY	RENIN ACTIVITY*
1. A.N.	29	10.4	7	4½	11
2. J.D.	23	0	4	5	17
3. R.C.	26	0	4	6	9
4. P.I.	24	3.1	3	7½	20
5. E.D.	28	0	2	8	23
6. A.S.	39	-	13	9½	28
7. M.B.	20	0	4	12	39
8. R.C.	27	2.3	5	14½	33
9. A.M.	29	3.6	8	16	33
10. N.M.	23	2.7	2	16	46
11. M.B.	32	11.0	12	17	36
12. J.L.	27	6.4	2	17½	65
13. C.D.	29	1.6	7	18	17
14. J.B.	18	1.8	1	20	85
15. J.T.	15½	1.8	1	22	33
16. M.B.	19	7.0	1	22	67
17. J.D.	19	3.6	1	23½	76
18. L.B.	21	-	4	23½	48
19. J.L.	33	1.8	3	23½	42
20. J.B.	18	9.1	1	25	79
21. R.J.	22	8.0	4	26½	43
22. L.B.	29	7.0	1	27	77
23. J.L.	21	5.5	1	29	56
24. A.P.	29	6.0	5	29½	42
25. C.G.	23	8.0	4	30½	70**
26. G.C.	17½	15.0	1	32	16
27. A.B.	34	9.5	3	33	43**
28. J.B.	20	10.0	1	34	43
29. C.P.	21	12.0	3	34	47
30. J.C.	26	20.0	1	35	109**
31. J.C.	25	9.6	1	36	44
32. M.P.	23	10.4	4	37	42
33. J.B.	25	12.0	7	38	36
34. J.P.	22	7.2	2	38	78
35. J.G.	21	5.0	2	40	75
36. M.D.	26	2.3	3	40	20

* Ng/L/min.

** Patients told by their physician to "avoid salty foods".

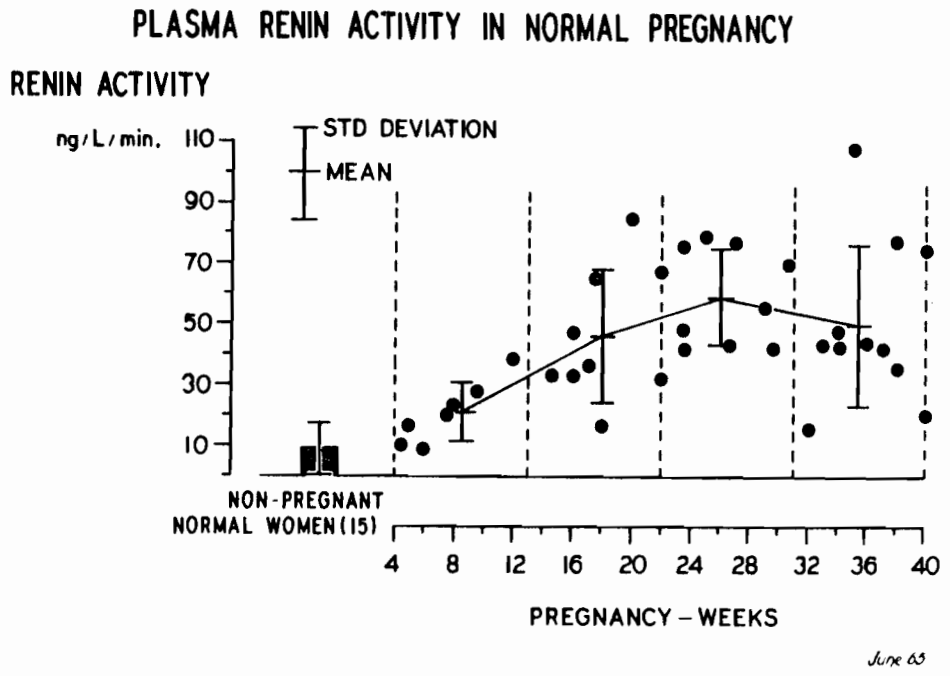


Fig. 15

The determination of plasma renin-activity in 36 normotensive pregnant women at different periods of pregnancy showed that the renin-activity increases progressively and significantly until the 22nd week of pregnancy. Thereafter, renin-activity reaches a plateau and remains elevated until delivery. In the second and third trimester only 3 out of 29 women had a plasma renin-activity within normal range. The subjects were separated in four groups, each corresponding to a period of 9 weeks. The mean level of each of these groups was significantly increased compared to the mean level of normal non-pregnant women.

TABLE XIV

STATISTICAL ANALYSIS OF RENIN-ACTIVITY LEVELS IN NORMAL PREGNANT WOMEN

	NO. SUBJECTS	MEAN	ST. DEV.	S.E. MEAN	"p" (VERSUS N)	"p" (BETWEEN GROUPS)
NON-PREGNANT NORMAL WOMEN	15	9.0	± 7.8	± 2.0		< 0.05
4th -12th WEEK	7	21	± 10.4	± 3.9	< 0.05	< 0.05
13th-21st WEEK	9	46.1	± 21.8	± 7.3	< 0.01	NOT SIG.
22nd-30th WEEK	9	59.2	± 16.2	± 5.4	< 0.01	" "
31st-40th WEEK	11	50.3	± 27.3	± 8.2	< 0.01	

last trimester but there is some discordance with those of Brown and coworkers (766). Although no quantitative comparison can be made between the results because of the marked differences in methodology, gross comparison can be obtained by comparing the number of subjects above the normal range of each procedure. Such an analysis between the two studies is reported in Table XV.

TABLE XV

RENIN ACTIVITY IN NORMAL PREGNANCY

	Present study 1964	Brown & al. 1963
NO. OF PREGN. WOMEN	36	38
NO. ABOVE NORMAL RANGE*		
1st TRIMESTER	1 out of 7	6 out of 8
2nd TRIMESTER	12 out of 13	6 out of 14
3rd TRIMESTER	14 out of 16	8 out of 16
TOTAL	27/36	20/38

* By the procedure used.

While in the present study a progressive increase was found until the end of the second trimester, where a plateau was reached, the study of Brown and coworkers reported a progressive decrease after the first trimester. In the first trimester, most of our subjects were within normal range except at the end of this period while most of their subjects were well over their normal range. During the second and third trimesters, all but three of our thirty-one subjects were above the normal range while more than half of their subjects were within their normal range.

Recently, Maebashi et al (767) could not find any higher than normal renin-activity values in eleven normal pregnant women studied during the third

trimester. However, these negative results might be partially explained by the very low recovery rate of their procedure which is only 30-35% (452). Winer (768), using Helmer's method (56) has very recently reported that the vasoconstrictor activity of the venous plasma of 21 normal pregnant women was increased 2 to 8-fold. The values were high from the sixth week until the end of pregnancy and after delivery these values fell gradually to normal control levels within a week. With the exception of the findings of Maebashi (767), our findings and those reported by Brown, Fasciolo and Winer, establish the increased activity of the renin-angiotensin system during the evolution of normal pregnancy.

The present study indicates a progressive and significant increase in plasma renin-activity during the first half of normal pregnancy. From the end of the first trimester until the end of pregnancy, the plasma renin-activity was higher than normal in most of the subjects studied. This pattern of increase resembles that reported for aldosterone excretion (745) and secretion (753), and that found with plasma progesterone during pregnancy (764, 765). The possibility of an antagonism between progesterone and aldosterone at the site of the renal tubule has also been postulated as the mechanism for the increase in aldosterone secretion during pregnancy. Some recent findings have suggested a relationship between progesterone and the renin-angiotensin system. Helmer (743) found that the administration of Enovid (norethynodrel) to humans resulted in an increase in renin-substrate. Winer (768) reported that daily administration of progesterone caused a twofold rise in plasma renin within four days in 3 female dogs. He also found that renin activity was low during the first few days of human menstrual cycle but increased gradually to reach a peak on the fourth week of the cycle during the luteal phase. Brown and his coworkers (769) had also reported a slight increase in the renin-activity of 5 out of 8

normal women during the luteal phase of the menstrual cycle. The participation of hormones other than progesterone in the stimulation of the renin-angiotensin system is not excluded since Helmer (147) reported that the administration of estrogens increased the concentration of the renin-substrate in normal rats and human subjects. Therefore, it is possible that progestational or estrogenic hormones might be the primary and direct cause of the increase in renin-activity during normal pregnancy.

An increase of renin is difficult to explain on the basis of the theory of a stretch receptor at the site of the afferent arteriole. As a matter of fact, the plasma volume is markedly increased during normal pregnancy (759 - 761) as well as the total body water (756-758). This should induce a decrease in renin secretion. Therefore, the findings of an increased renin secretion during pregnancy constitute an objection to the exclusive control of renin by the changes in plasma volume.

Other studies had suggested that an extrarenal (probably placental) source of renin might exist during pregnancy. Stakemann (68) has demonstrated a renin-like pressor substance in the placenta of cat, and Gross et al (69) and Skeggs et al (70) have reported similar findings in the rabbit. However, these findings do not eliminate the possibility that renin is stored in this organ. Brown and his associates (67) also reported very large amounts of renin-like substance in the amniotic fluid during the third trimester of pregnancy at a concentration much higher than simultaneous determinations in maternal plasma and umbilical vein plasma. However, they could not eliminate the foetal kidney as the source of this activity. In order to determine whether the uterus or placenta contributed a renin-like substance to the circulation, Winer (768) compared the renin levels in renal, uterine and peripheral vein plasma of dogs and he came to the conclusion that renin was secreted by the kidneys during

pregnancy since he found the renin levels highest in the renal vein blood. Thus, the secretion of renin by the placenta remains hypothetical.

The absence of hypertension in presence of high renin activity levels may be linked to the increased resistance to infused angiotensin and renin during pregnancy (264, 272, 281, 286) or to the antihypertensive effect of pregnancy on experimental hypertension in animals (770-773).

In conclusion, the present findings support the hypothesis that the increase in aldosterone secretion or excretion reported during pregnancy is probably mediated by the renin-angiotensin system.

B - THE RENIN-ANGIOTENSIN SYSTEM IN PATHOLOGICAL CONDITIONS.

1. ANGIOTENSIN LEVELS IN PATIENTS WITH HIGH PERIPHERAL RESISTANCE:

One of the most potent and consistent pharmacological actions of angiotensin is to produce a marked increase in peripheral resistance. In many cardiac diseases, the cardiac output is markedly decreased but the blood pressure is maintained at normal levels due to an increase in peripheral resistance. It is thus of interest to determine whether angiotensin is involved in the maintenance of a high peripheral resistance under these conditions.

a) Subjects and clinical procedure:

Fifteen normotensive, edema-free, cardiac patients with valvular disease were investigated. Aortic blood was drawn by a catheter during a routine procedure of cardiac catheterization and the concentration of angiotensin was measured. Simultaneously, the peripheral resistance of these patients was estimated according to Fick's calculation.

b) Results:

Details concerning these patients as well as the angiotensin levels and peripheral resistance are reported in Table XVI.

TABLE XVI

ANGIOTENSIN ARTERIAL LEVELS* AND PERIPHERAL RESISTANCE IN CARDIAC PATIENTS WITH VALVULAR DISEASES**

NAME	AGE	SEX	DIAGNOSIS	B.P.	PERIPHERAL RESISTANCE Dynes/Cm^{-5}	ANGIOTENSIN Ng/100 ml
1. M.B.	42	F	Mitral stenosis	145/90	1423	0
2. O.G.	31	F	Mitral disease	106/70	1555	0
3. H.B.	56	M	Mitral disease	110/74	1565	0
4. J.B.	43	M	Mitral stenosis	108/72	1641	0
5. R.D.	27	F	Mitral stenosis	112/66	1720	0
6. G.D.	33	F	Mitral disease	128/76	1820	38
7. P.P.	45	F	Mitral stenosis	112/65	1823	0
8. J.D.	31	M	Mitral stenosis	102/70	1837	42
9. G.D.	17	F	Mitral stenosis	122/66	1890	20
10. A.S.	53	F	Mitral stenosis	124/64	2112	36
11. T.M.	57	M	Mitral stenosis	146/90	2216	0
12. E.L.	48	F	Mitral disease	123/78	2303	26
13. G.C.	57	F	Mitral stenosis	124/70	2533	26
14. P.M.	45	F	Mitral disease	150/94	2831	150
15. C.B.	41	F	Mitroaortic dis	112/76	3272	46

* Determined in blood drawn from aorta.
** Without edema.

Although no linear relationship could be established between the angiotensin levels and the peripheral resistance, one observation stands out. Angiotensin was detectable only in patients with the highest peripheral resistance. With the technique used the mean normal peripheral resistance is 1250 dynes/Cm^{-5} with a range of 1000 to 1500. As seen in the Table XVI, only one of the patients studied had a peripheral resistance within normal range. If the patients are arbitrarily separated into two groups: those having a peripheral resistance below 1800 dynes and those having a peripheral resistance above, one can see that all the five patients in the first category had no detectable angiotensin in their plasma (Patients 1-5), while 8 out of 10 patients having higher peripheral resistance had detectable angiotensin levels (Patients 6-15). Moreover, 5 out of the 10 patients with peripheral resistance higher than 1800 dynes had angiotensin levels above normal range and the two highest angiotensin levels were found in patients having the highest peripheral resistance of that series (Patients 14 and 15).

c) Discussion:

Since angiotensin is more often increased in patients with very high peripheral resistance, this would suggest that angiotensin might be involved in the maintenance of a high peripheral resistance in some diseases associated with a low cardiac output. Therefore, it is possible that the renin-angiotensin system might participate in the maintenance of a normal blood pressure under such conditions.

2. ANGIOTENSIN AND RENIN-ACTIVITY IN EDEMATOUS CONDITIONS.

Edematous states are often associated with an increased excretion or secretion of aldosterone (265, 559, 653, 671-675, 677, 678). Moreover, a marked sodium retention consistently occurs in such conditions.

So far, very few studies on the renin-angiotensin system in such edematous conditions have been reported. In 1946, Merrill et al (447), using a crude method of measurement, reported the presence of pressor activity in the renal vein blood of 8 out of 11 patients with chronic congestive heart failure. The renin content (691) and the granularity of the juxtaglomerular cells (163) were reported to be increased in cirrhotic patients. A hypergranulation was also observed in the kidneys of patients with congestive heart failure (692).

Preliminary studies were done, using the procedure for the measurement of angiotensin blood levels, and this work was continued with the measurement of renin-activity when this method became available. Part of these studies were reported and published elsewhere (718, 719, 724, 733-735, 774, 775).

a) Subjects and clinical procedure:

Thirty-two patients having edema of various etiology were studied, in most instances, before any treatment was initiated, and also, in many of these, during the course of their treatment after partial or total relief of edema. The bad physical condition at the time of admission in several patients prevented sometimes adequate period of control before initiating the treatment. The treatment of edema was initiated in most subjects by a lowering of the dietary sodium intake (10 to 30 mEq per day) combined with the administration of one or two natriuretic agents (Hydrochlorothiazide, Chlorothiazide, Chlorthalidone, Acetazolamide, Meralluride or Fursemide (Hoechst)) with or without an aldosterone antagonist. In addition, the cardiac patients were digitalized if they had not been previously. The first blood sample of these patients was taken the day they were admitted to the hospital, before onset of treatment and while they were on an unrestricted diet. The second or subsequent samples were withdrawn during the course of treatment while under low sodium intake and natriuretic treatment and in most instances after marked relief of edema.

Gross evaluation of clinical edema was estimated according to the following criteria:

+ Slight pitting edema of the ankles and pretibial regions.

++ More marked pitting edema of the ankles and legs up to knee level.

+++ Marked edema of lower limbs with presacral edema.

++++ Anasarca.

b) Results:

i) Congestive heart failure:

Sixteen patients with congestive heart failure secondary to arteriosclerotic, valvular or hypertensive diseases were studied. Twelve of these had determinations of arterial angiotensin blood levels, before and during, or after, partial or total relief of edema (Table XVII) (Cases 1-12). Details concerning these patients: age, blood pressure, loss of weight, angiotensin blood levels, renin-activity, aldosterone, are reported in Table XVII. In all of these, a weight loss varying from five to twenty-three kilograms was obtained during the period of treatment. With the exception of patient A.B. (case no. 16), all these patients had a marked degree of edema, sometimes associated with ascites.

Thirteen out of the 14 determinations of arterial angiotensin and all five determinations of renin-activity were above normal range in these patients before treatment was initiated. The levels were as high as 850 ng per 100 ml of plasma for angiotensin, and up to 300 ng/~~%g~~ L/min. for renin-activity. After total or partial relief of edema by the administration of a low sodium diet and natriuretic agents, 10 out of the 12 patients so studied presented a marked decrease in their angiotensin levels, most of them to undetectable levels and only three values remaining above normal range (Table XVII and Figure 16). Before treatment, the mean angiotensin level of these patients

TABLE XVII

PLASMA ANGIOTENSIN AND RENIN-ACTIVITY LEVELS IN CONGESTIVE HEART FAILURE BEFORE AND AFTER RELIEF OF EDEMA

SUBJECT	AGE	B.P.*	DAY	EDEMA	ASCITES	R_x**	DIET	WEIGHT kg	ANGIOTENSIN ng/100ml pl.	RENIN ACT. ng/L/min.	ALDOST. EXCR. ugm/24 hrs.
1. A.G.	33M	N	1	+++	0		Unrestr.	49	142		
			7	0	0	D	Low Na	39.5	0		
2. O.F.	73M	Ht	1	+++	0		Unrestr.	77.7	82		
			5	0	0	DC	Low Na	72.7	190		
3. A.S.	54M	Ht	1	+++	0		Unrestr.	70.9	186		
			10	0	0	DC	Low Na	61.4	0		
4. A.O.	78M	Ht	1	+++	0		Unrestr.	66.0	260		
			6	0	0	DC	Low Na	56.8	74		
5. J.Y.†	65M	Ht	1	+++	0	D	Unrestr.	86.4	510		
			6	0	0	DC	Low Na	80.4	260		
6. C.A.	63F	Ht	1	+++	0	D	Unrestr.	70	12		13
			31	0	0	D	Low Na	63	32		15
7. E.M.	58F	N	1	++++	++	D	Unrestr.	77	850		
			6	++	0	DC	Low Na	68.9	220		
			15	+	0	DCS	" "	63.0	400***		
			28	+	0	D	" "	59.7	10		
8. R.R.	61M	N	1	+++	+++		Unrestr.	68	94		
			18	++	+	DCA	Low Na	63	25		
9. A.L.	53F	N	1	+++	++		Unrestr.	62.8	52		73
			14	++	+	DCSP	Low Na	58.3	0		44
10.H.A.	66F	N	1	+++	++		Unrestr.	63.6	130	43	
			5	++	0	DA	Low Na	57.2	0	0	
			12	0	0	DA	" "	51.2	0	0	
11.N.T.	47M	N	1	+++	+	D	Low Na	70.9	83	153	31
			12	+	0	DM	" "	60.0	0	58	39
			19	0	0	DM	" "	55.4		94	14

... 151b

TABLE XVII (continued)

SUBJECT	AGE	B.P.*	DAY	EDEMA	ASCITES	R_x**	DIET	WEIGHT kg	ANGIOTENSIN ng/100ml pl.	RENIN ACT. ng/L/min.	ALDOST. EXCR. µgm/24 hrs.
12.G.V.	55M	N	1	++++	++++		Unrestr.	72.7	61	300	
			2	++++	+++	DP	"	70.4		300	30
			7	++	++	DF	Low Na	64.3	39	110	23
			15	++	++	D	" "	65.0	0	8	14
			18	++	++	D	Unrestr.	65.4	0	13	11
			21	+++	+++	D	"	67.3		0	9
			22	+	++	DF	Low Na	62.7		0	9
			24	+	+	DF	" "	58.2		0	9
			28	0	0	DF	" "	53.0	0	0	10
13.J.L.	47F	Ht	1	+++	0		Unrestr.	93.2		73	
			8	0	0	DTS	Low Na	70.2		49	
14.G.D.	34M	N	1	++++	++	D	Unrestr.	50.9	176		
15.P.A.	36M	N	1	+++	+		Unrestr.	66.8	42		
16.A.B.	72F	N	1	++	0		Unrestr.			37	

* Patients were classified as hypertensive if diastolic pressure was above 90 mm Hg.

** The symbols used stand for the following medication:

D - Digitaline
C - Chlorothiazide
S - Spironolactone
A - Acetazolamide
M - Meralluride
P - Abdominal Paracentesis
F - Fursemide (Hoechst)
T - Chlorthalidone

*** Sample drawn during neurogenic shock, B.P. 70/x.

† Chronic pyelonephritis.

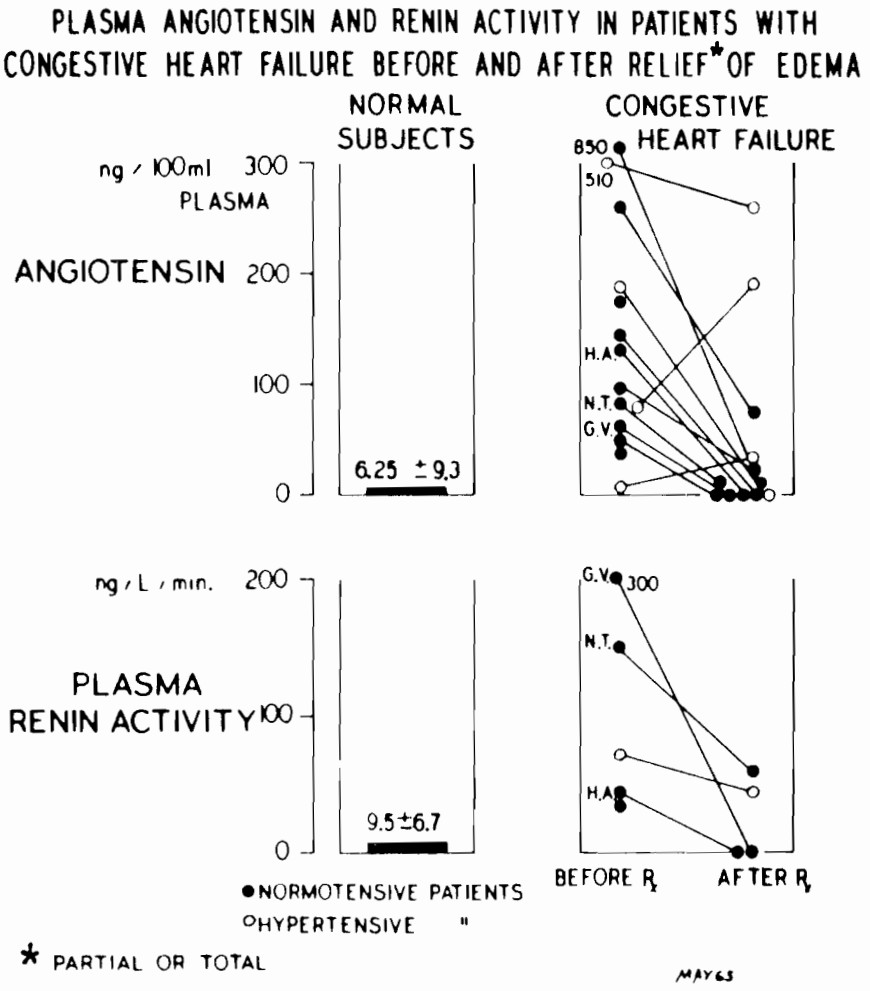


Fig. 16

Before treatment, plasma angiotensin and renin-activity levels were found to be above normal range in all but one of the 16 edematous cardiac patients studied. After treatment consisting of sodium restriction combined with the administration of natriuretic drugs, these levels fell markedly in all but 2 of the 13 patients so studied, after partial or total loss of edema. Most of the values returned to normal range during treatment. This response is opposite to that observed in normal and hypertensive subjects submitted to a low sodium intake and natriuretic treatment. Despite markedly elevated angiotensin and renin-activity levels, only five cardiac patients were hypertensive.

was 205 ng per 100 ml of plasma (± 245 S.D., ± 70 S.E.) while, after treatment, the mean level fell to 49 ng per 100 ml (± 73.5 S.D., ± 21 S.E.). Only two patients showed an increase in angiotensin levels after treatment, one increase being slight but not significant, and the other marked. Both had edema secondary to hypertensive cardiovascular disease.

The plasma renin-activity also decreased markedly in the four patients in whom this parameter was measured during improvement of their condition. This decrease was observed concomitantly with a decrease in angiotensin levels (Table XVII, cases 10, 11 and 12. Figure 16).

Before treatment, 3 out of 4 patients had an elevated urinary aldosterone. The lowest value at the upper limit of normal was found in the only subject having a normal angiotensin level (C.A. case #6, Table XVII). After treatment, the aldosterone excretion fell markedly in three of them.

Two patients of this group are illustrated in greater detail in Figures 17 and 18. In the first study (Fig. 17) is reported the measurement of arterial angiotensin levels during the treatment and evolution of a cardiac patient admitted to the hospital with anasarca. This normotensive patient had the most elevated angiotensin level (850 ng/100 ml plasma) found so far in this department prior to the onset of treatment, but after treatment and a considerable loss of edema, angiotensin returned to normal. During the course of her treatment, this patient experienced neurogenic shock associated with an increase in the arterial angiotensin level. The second study (Fig. 18) illustrates the parallelism between the changes in renin-activity, angiotensin and the aldosterone excretion rate during the treatment and relief of edema. In this edematous cardiac patient, renin-activity, angiotensin and aldosterone excretion rates, which were elevated at the onset of treatment, gradually returned to normal levels with the improvement of the cardiac function and the disappearance of edema.

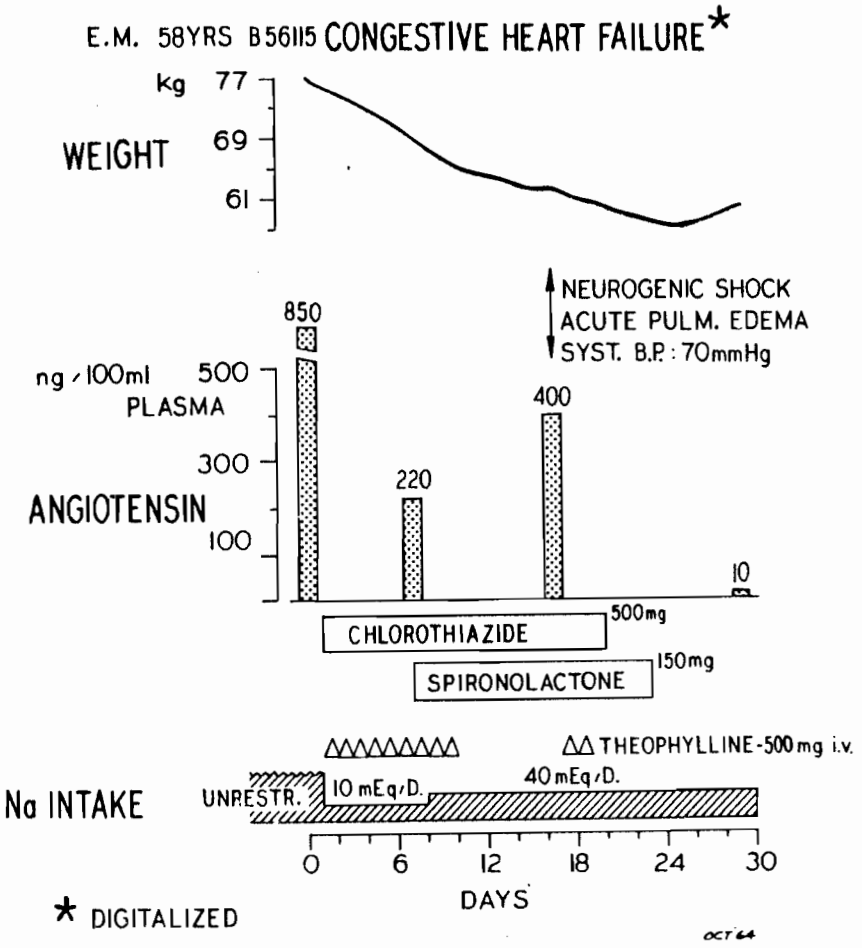


Fig. 17

This patient with mitral valvular disease was admitted to hospital with anasarca and subacute pulmonary edema. Arterial angiotensin was found at 850 ng/100 ml plasma prior to treatment. Digitalization, lowering of the dietary sodium and natriuretic treatment contributed successfully to the relief of edema. Six days after the onset of treatment, 8 kilograms of edema had been lost while the angiotensin concentration had decreased to 220 ng. On the 15th day of treatment, the patient suddenly developed shock. A determination of angiotensin at that moment showed an increase to 400 ng. After appropriate treatment, the patient recovered rapidly and continued to improve thereafter. On the 28th day of treatment, after the patient had lost 19 kilograms, angiotensin had returned to normal. With the exception of the episode of shock, the patient remained normotensive during the whole period of treatment.

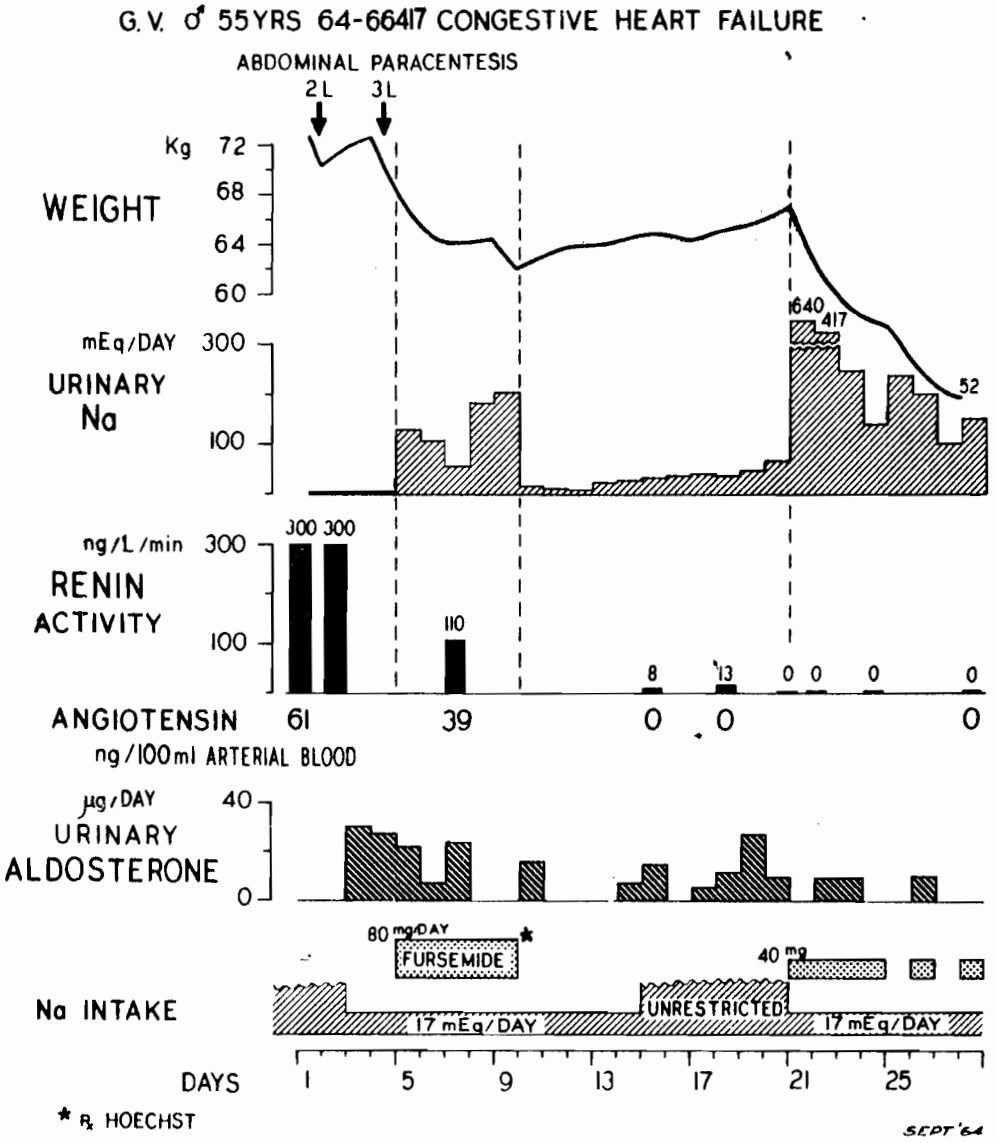


Fig. 18 «

This patient was admitted to the hospital with anasarca and subacute plumonary edema. Priot to treatment, renin-activity was found to be 300 ng/L/min. angiotensin 61 ng/100 ml and aldosterone excretion 300 µgm/day. An abdominal paracentesis (2L) done shortly after admission did not modify the level of renin-activity. After digitalization, low sodium intake and natriuretic treatment, the condition of the patient improved rapidly. On the 7th day of treatment, after a loss of 8 kilograms, renin-activity and angiotensin had decreased markedly but were still higher than normal. On the 15th day, renin-activity, angiotensin and aldosterone excretion were normal and they remained normal until the end of treatment. The patient remained normotensive during the course of treatment.

ii) Nephrotic syndrome:

Eight nephrotic patients were studied before and/or after treatment. In six of them, the nephrotic syndrome was secondary to membranous glomerulonephritis. In one patient (No. 1, Table XVIII), this condition was secondary to lupus erythematous and in one other (No. 6), the etiology remained undetermined. With the exception of the two latter patients who had a marked edema, all the others presented only slight to moderate edema.

Details concerning these patients as well as the levels of angiotensin, renin-activity and aldosterone excretion rate are given in Table XVIII. The variations of angiotensin and renin-activity during treatment are illustrated in Fig. 19. Six patients had a determination of arterial angiotensin before treatment. Only one of the six had level above the normal range at the onset of treatment and four had undetectable levels. In three subjects who had undetectable angiotensin levels before onset of treatment, angiotensin increased slightly to 20, 30 and 40 ng per 100 ml of plasma after treatment and relief of their edema. In one other patient who was studied only during treatment, high angiotensin levels were detected (Case no. 1, Table XVIII).

Six patients had measurements of renin-activity before and during the course of treatment. Three out of the six patients studied before the onset of treatment had plasma renin-activities above the normal range. In all five patients studied after relief of edema, the plasma renin-activity increased markedly in four of them, and to a lesser degree in the fifth one. This increase coincided with that of angiotensin in three instances (Cases no. 3, 4, 6, Table XVIII). Unexpectedly, the aldosterone excretion rate was found within normal range in three out of four patients before treatment, but it was above normal range in all five subjects in whom it was measured during treatment. Two such studies are illustrated in detail in Figures 20 and 21. The first

TABLE XVIII

PLASMA RENIN ACTIVITY AND ANGIOTENSIN LEVELS IN NEPHROSIS BEFORE AND AFTER RELIEF OF EDEMA

SUBJECT	AGE	B.P.	DAY	EDEMA	ASCITES	R_x*	DIET	WEIGHT kg	ANGIOTENSIN ng/100ml pl.	RENIN ACT. ng/L/min.	ALDOST. EXCR. µgm/24 hrs.
1. A.B.	27F	N	1	+++	++	SH	Low Na	50.8	216		25
			8	+++	++	SHO	" "	51.6	344		60
2. V.M.	24M	Ht	1	+	0		Unrestr.		25		
3. R.A.	29M	Ht	1	++	0		Low Na	63.1	0	49	40
			4	0	0	H	" "	58.7	20	194	28
4. P.B.	55F	N	1	++	0		Unrestr.	73.8	0	17	8
			25	0	0	HSO	Low Na	66.9	40	169	299
5. L.D.	19F	N	1	+	0		Unrestr.	51.0	260	84	5
			3	+	0		High Na	51.0	0	41	5
			6	0	0		Low Na	48.5		144	15
6. M.G.	69M	Ht	1	+++	+		Unrestr.	76.4	0	50	7
			23	++	0	HS	Low Na	72.5		128	37
			36	0	0	HS	" "	66.6	30	167	33
7. R.M.	15M	N	1	++	0		Unrestr.		0	0	
8. F.V.	44M	Ht	1	++	0		Unrestr.	64.5		0	
			4	+	0		Low Na	60.5	0	7	
			8	0	0	F	" "	58.0		11	
			13	0	0	F	" "	55.6	0	9	

* Symbols for medication:
S - Spironolactone
H - Hydrochlorothiazide
O - Prednisolone
F - Fursemide (Hoechst)

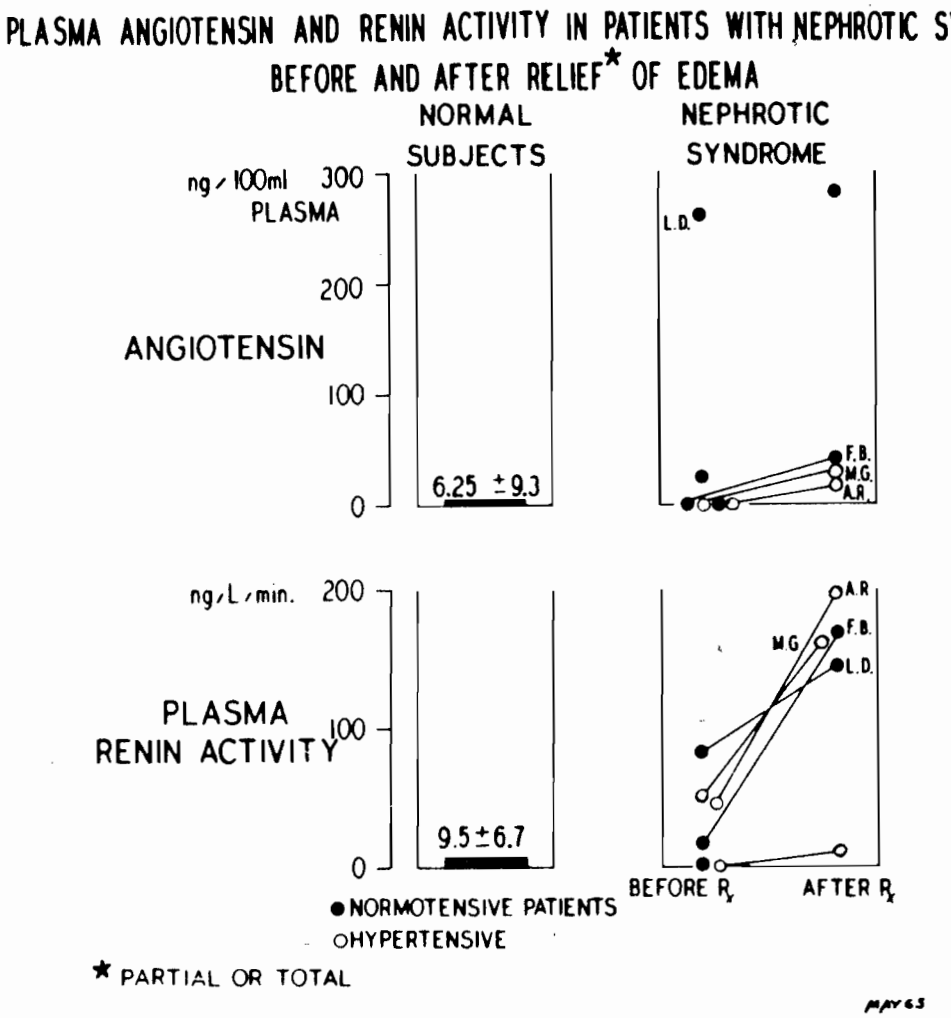


Fig. 19

Before treatment, only one out of 6 nephrotic patients had a higher-than-normal angiotensin levels while 3 of them had a high renin-activity. Treatment by sodium restriction and administration of natriuretics consistently increased the angiotensin and renin-activity levels. This response is contrary to that observed in cardiac patients and similar to that observed in normotensive and hypertensive subjects submitted to low sodium intake and natriuretic treatment.

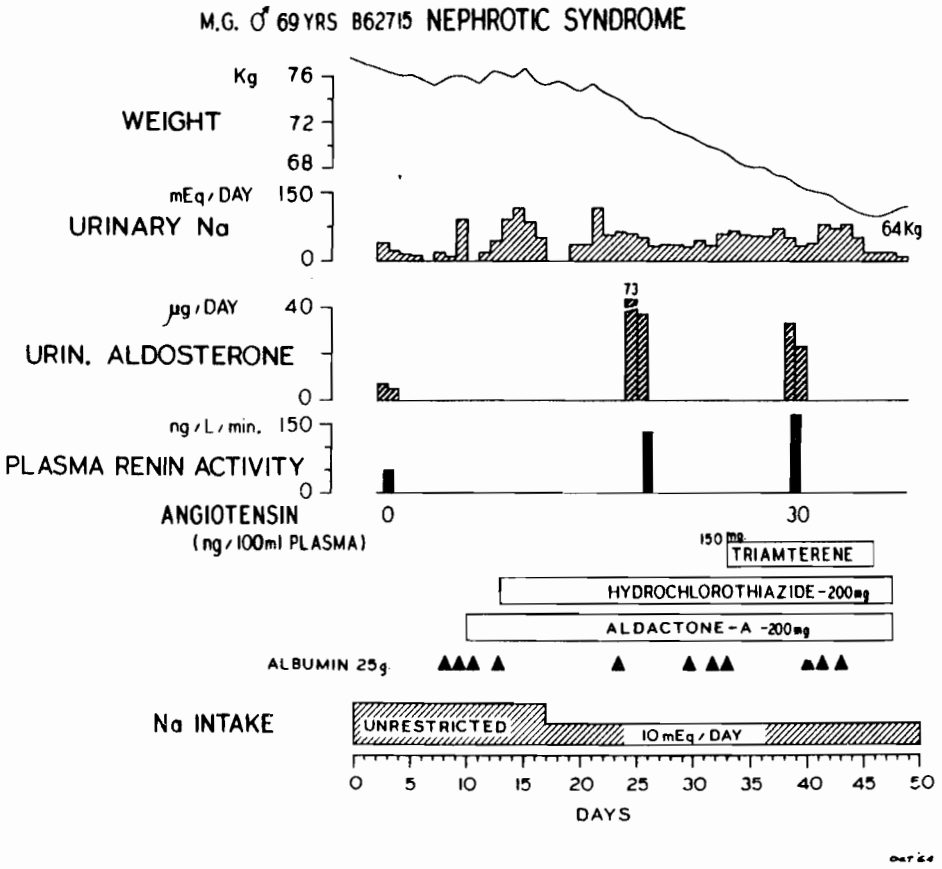


Fig. 20

This subject presented marked edema of the lower limbs and ascites due to a nephrotic syndrome of undetermined etiology. Before the onset of treatment, renin-activity was at 50 ng/L/min. and angiotensin was undetectable. The treatment was undertaken with a low sodium diet associated with diuretics, an aldosterone antagonist and occasional administrations of concentrated human albumin. On the 23rd day of treatment, renin-activity had increased to 130 ng/L/min., and to 170 ng/L/min. on the 36th day, while the patient was almost edema-free. Simultaneously, angiotensin increased slightly to 30 ng/100 ml. Meanwhile, aldosterone excretion increased above normal. This patient was also hypertensive and had a markedly decreased renal function (Blood urea: 70 mg, creatinine clearance: 35 ml/min.).

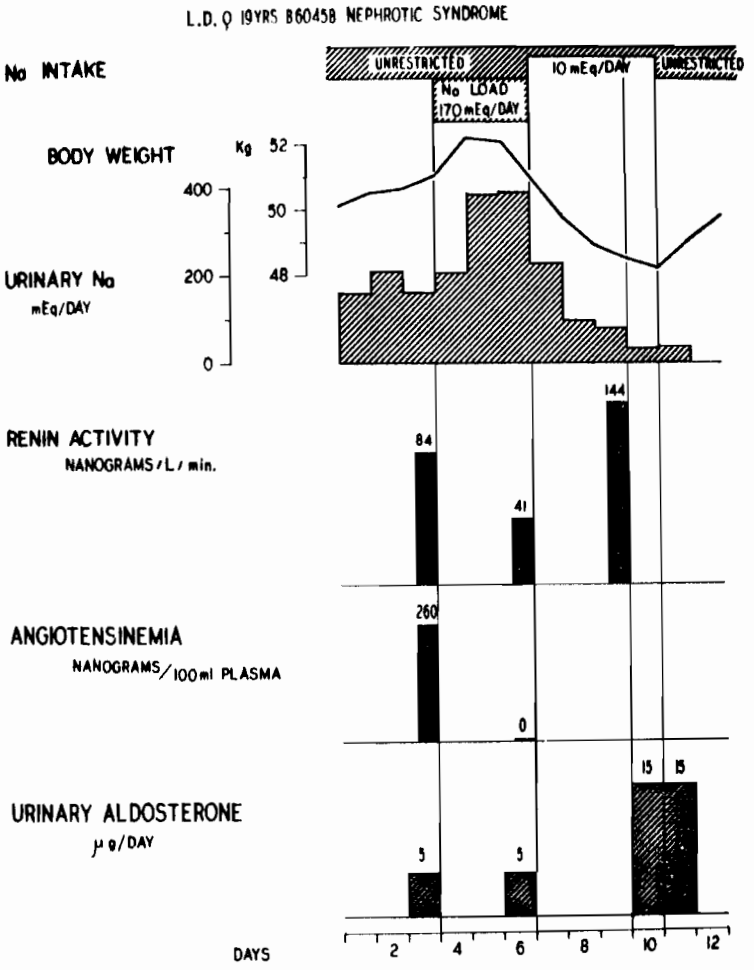


Fig. 21

This 19 year old patient was suffering from a nephrotic syndrome secondary to membranous glomerulonephritis. At the time of admission, she presented slight pitting edema of the ankle but both renin-activity and angiotensin were markedly elevated. When the patient was submitted to changes in dietary sodium, the renin-activity varied inversely to the sodium intake. A high sodium intake decreased renin-activity and angiotensin, but when sodium was restricted to 10 mEq/day, renin-activity increased markedly. The low aldosterone excretion rate found in this patient cannot be explained.

study (Fig. 20) reports the investigation and treatment of a 69 year old nephrotic subject admitted to the hospital with marked edema of the lower limbs associated with a large amount of ascites. Before the onset of treatment, the plasma renin-activity was higher than normal but angiotensin was undetectable. During treatment and successful relief of edema, the renin-activity increased to higher levels and angiotensin increased to the upper limit of normal. Meanwhile, aldosterone excretion, which was normal at onset of treatment, increased above normal. The second study (Fig. 21) illustrates the response of renin-activity and angiotensin to changes in dietary sodium in a nephrotic patient. This study shows that the response of renin-activity is similar to that reported in normal and hypertensive subjects. Prior to the experiment, the renin-activity and angiotensin levels were elevated. The administration of a salt load for three days markedly decreased the blood levels of renin-activity and angiotensin. On the other hand, when a low sodium diet was given, a marked increase in renin-activity occurred.

iii) Liver cirrhosis with ascites:

Eight patients with marked ascites secondary to decompensated liver cirrhosis were studied before and after treatment. In addition, many of them also presented marked edema of legs. Details concerning the renin-activity and angiotensin blood levels and aldosterone excretion are reported in Table XIX, and the changes in renin-activity and angiotensin during treatment are illustrated in Figure 22.

Before treatment, only one out of seven patients had an angiotensin value slightly above normal range while three out of four of these had a blood renin-activity well above normal range. After treatment and relief of edema and ascites, angiotensin and renin-activity levels did not vary significantly in many instances (patients 4 to 8, Table XIX). Only two out of the five patients in

TABLE XIX

PLASMA RENIN-ACTIVITY AND ANGIOTENSIN LEVELS IN CIRRHOSIS OF THE LIVER BEFORE AND AFTER RELIEF OF EDEMA

SUBJECT	AGE	B.P.	DAY	EDEMA	ASCITES	R_x*	DIET	WEIGHT kg	ANGIOTENSIN ng/100ml pl.	RENIN ACT. ng/L/min.	ALDOST. EXCR. µgm/24 hrs.
1. R.N.	56M	N	1	+	++		Unrestr.	59.0	0		
2. T.G.	56M	N	1	+++	++++		Unrestr.	69.1	0		
3. F.D.	52M	N	1	+++	++++	M	Low Na		490		142
4. A.D.	50M	N	1	+++	++++		Unrestr.	99	44		32
			6	+++	++++	MS	"	100	25		
5. A.S.L.	52F	N	1	+	++++		Unrestr.	48.7	0	174	
			2	+	+	P	"	43.2	15	175	
6. R.S.	36M	N	1	+++	++++		Unrestr.	77.3	20	68	340
			12	+	++	MSO	"	70.1	50	38	401
7. G.C.	51M	N	1	+++	++++		Unrestr.	83.6	30	116	
			28	+++	++++	O	Low Na	90.9	20	50	131
			43	++	+++	OSCP	" "	80.9	55	85	101
			55	+++	++++	OSC	" "	90.9	120	100	52
8. C.G.	57M	N	1	+++	++++		Unrestr.	86.0	0	23	
			3	++	+++		Low Na	83.4		20	
			6	+	++		" "	75.0	0	35	
			9	0	+		" "	69.8	0	23	
			12	0	0		" "	66.4		31	
			17	0	0		" "	63.7	0	29	

* Symbols for medication:
- M - Meralluride
- S - Spironolactone
- P - Abdominal Paracentesis
- O - Prednisolone
- C - Chlorothiazide

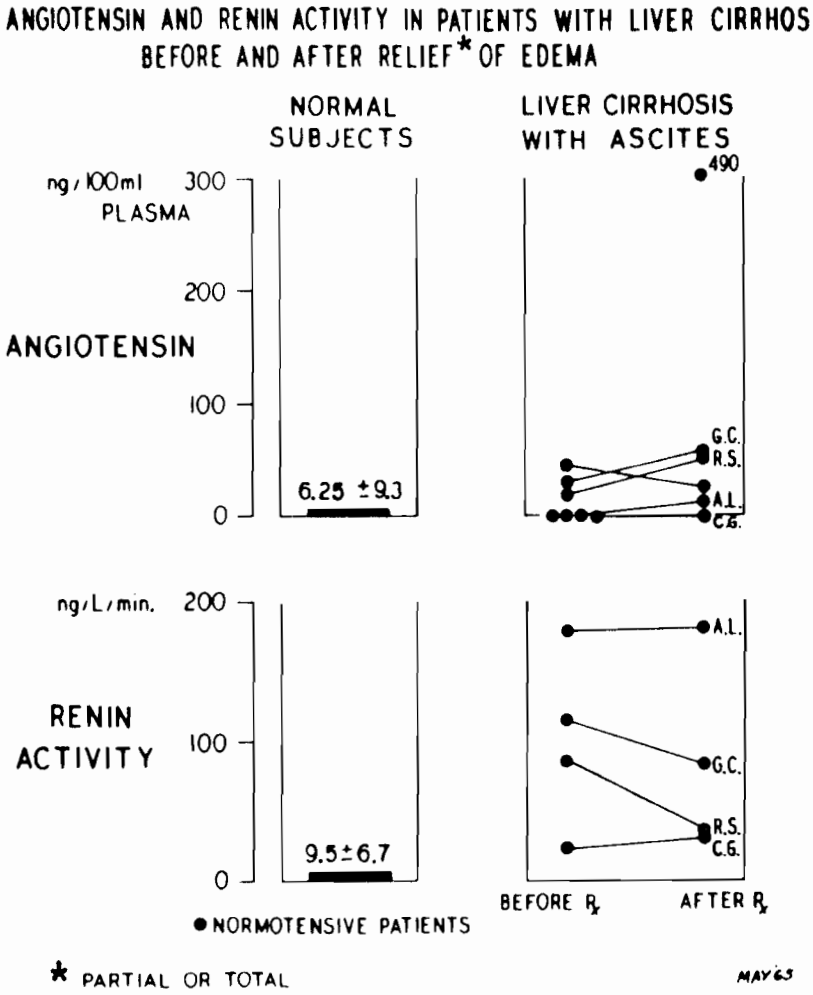


Fig. 22

Before treatment, all cirrhotic patients but one had angiotensin levels within the normal range while three out of four had a high plasma renin-activity. In this group of patients, the treatment of edema did not induce a typical response in renin-activity or angiotensin. In most cases, these parameters did not vary significantly or consistently.

whom angiotensin was measured after treatment showed a significant increase during treatment (patients 6 and 7) and only one out of the four patients in whom renin-activity was determined presented a significant decrease (patient 6). No significant or consistent changes occurred in any of the others. The renin-activity remained above normal range after treatment in the three patients in whom it has previously been found elevated.

In one patient (No. 3), who had been operated upon for a spleno-renal anastomosis 17 days previously, one angiotensin plasma level was found to be 490 ng/100 ml. One other patient (No. 5) was studied before and after an abdominal paracentesis of 5.6 liters. In this patient, who presented a markedly distended abdomen due to ascites, the relief of abdominal tension did not significantly modify the renin-activity and angiotensin plasma levels.

The aldosterone excretion rate was above normal range in 3 patients in whom this parameter was measured.

In figures 23 and 24 are illustrated the studies of two of these patients during the course of treatment. The first (Fig. 23) reports the changes in renin-activity, urinary aldosterone excretion and urinary sodium during successful treatment of edema and ascites in one case of Laennec's cirrhosis. The other study (Fig. 24) illustrates the lack of significant change in renin-activity and angiotensin levels repeatedly measured in one edematous, cirrhotic patient submitted to a sodium restriction without any other treatment. These levels remained remarkably stable despite considerable changes in urinary sodium and body weight.

c) Discussion:

These findings constitute a strong indication that the renin-angiotensin system might be involved in the pathogenesis of edema, particularly that associated with congestive heart failure.

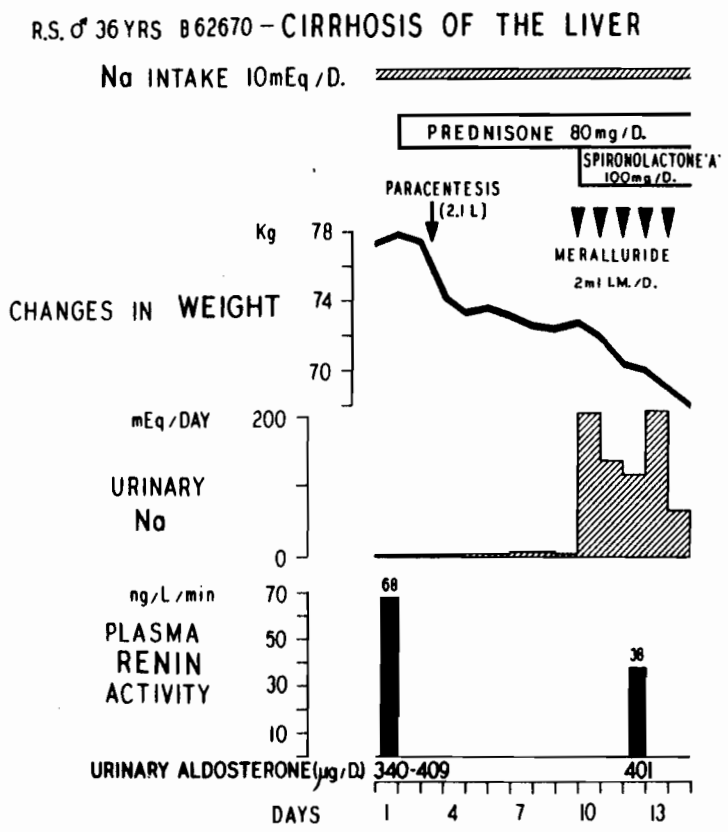


Fig. 23

This 36 year old patient with a Laennec's cirrhosis was admitted with marked edema of the legs and marked ascites. Before treatment, renin-activity was elevated. Treatment consisted of sodium restriction and the administration of natriuretic agents and prednisone. After 12 days of this treatment, 7 kilograms of edema were lost, renin-activity decreased but remained above normal range. Meanwhile urinary sodium increased markedly. The urinary aldosterone remained at very high levels.

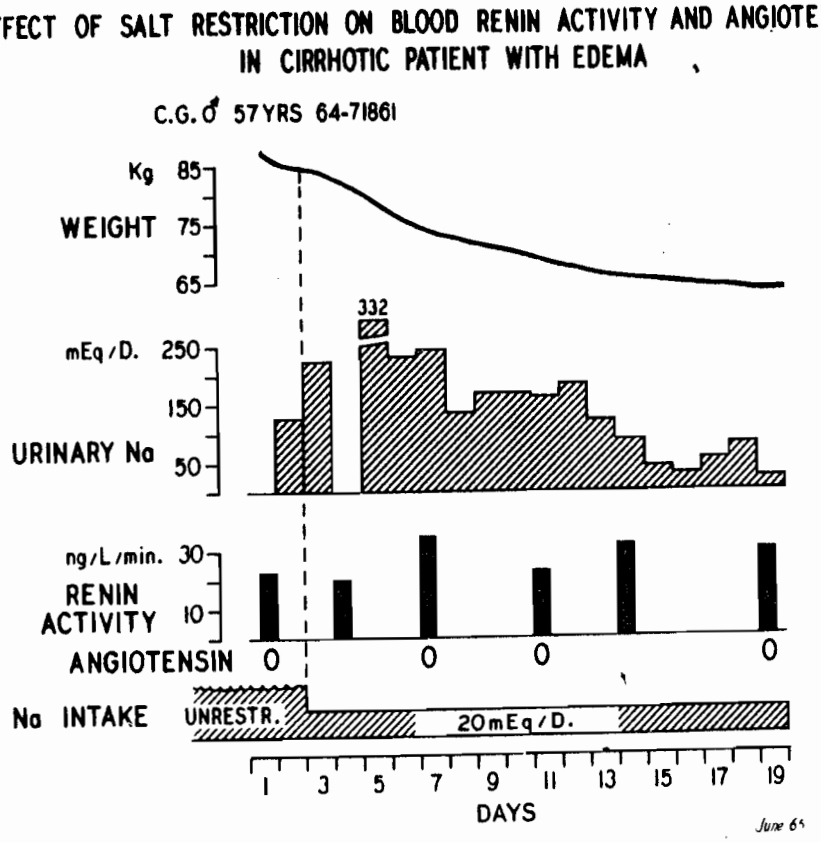


Fig. 24

This 57 year old patient with Laennec's cirrhosis had marked ascites and edema of the lower extremities at time of admission. The restriction of dietary sodium without any other therapy resulted in a weight loss of 23 kilograms and complete disappearance of edema and ascites less than 20 days after the admission. The renin-activity and angiotensin levels which were within normal range before the sodium restriction, were not significantly modified during the course of treatment despite the marked changes in body weight and urinary sodium.

During the period of maximum water and sodium retention, prior to any treatment, arterial angiotensin and renin-activity levels were consistently found elevated in all but one cardiac patient. Concomitantly with the loss of sodium and water during treatment, both renin-activity and angiotensin decreased in most subjects. Therefore in this group of patients a good correlation exists between the state of sodium and water retention and the levels of angiotensin and renin-activity. The changes in the two previous parameters also parallel the changes in aldosterone excretion rate in three of these subjects, illustrating once more the close relationship between the renin-angiotensin system and aldosterone.

The relationship between the renin-angiotensin system and the state of edema was less obvious in nephrotic and cirrhotic patients. Although the renin-activity levels were elevated in many of these subjects before treatment, angiotensin levels were low or undetectable in most of them at the same time. These discrepancies are difficult to explain and illustrate the lack of knowledge concerning the kinetics of the renin-angiotensin and the factors activating or inhibiting its enzymatic reactions. Nevertheless, it can be postulated that in these conditions there might exist a block in the transformation of the substrate to angiotensin II. The latter hypothesis was suggested recently by Loyke (776). This worker injected carbon tetrachloride into rats in order to produce liver injury. He found that after such a treatment, the converting enzyme was almost completely inhibited since he could detect only angiotensin I in these rats. He thus postulated that some similar mechanism would explain the lack of hypertensive disease in cirrhotic patients. Since the method used for the determination of angiotensin in this study measured only angiotensin II, such mechanism would explain the present findings in cirrhotic patients. It is also possible that changes in angiotensinases concentra-

tion might also contribute to the occurrence of these discrepancies. As a matter of fact, angiotensinase activity was reported to be increased in conditions associated with secondary hyperaldosteronism and especially in cirrhotic patients (137, 138, 777). Such an increase might explain the occurrence of low angiotensin levels despite a high renin-activity (see pp. 190, 190a).

During treatment, all nephrotic subjects presented an increase in angiotensin and renin-activity while sodium and water were eliminated. This type of response is similar to that of non-edematous patients and subjects submitted to sodium depletion, but is completely opposite to that observed in cardiac patients. The difference in response to treatment between cardiac and nephrotic patients might indicate a marked difference in the pathogenesis of edema in these two conditions. In cardiac diseases, renal hemodynamics are generally markedly altered due to a marked decrease in cardiac output. Therapy generally improves the cardiac output in these patients and consequently restores the kidney perfusion and filtration rates toward normal, thus probably suppressing the stimulus for renin secretion.

During treatment, most cirrhotic patients did not show any significant change in their angiotensin or renin-activity levels despite marked changes in body weight and urinary sodium. It would appear that in these patients, the usual stimuli for renin secretion had become ineffective.

Most of the edematous subjects with high renin-activity and high angiotensin levels were normotensive. This finding is of great interest, expecially in cardiac subjects in whom both angiotensin and renin-activity were found consistently and markedly increased. Since the cardiac output of these subjects is generally greatly decreased, the presence of high circulating angiotensin might account for the maintenance of a normal blood pressure by increasing the peripheral resistance.

Following our first report (774), many investigators reported similar studies in edematous patients. Fasciolo and his coworkers (57) found an increased renin-activity in 1 out of 2 cirrhotic patients and in 4 out of 9 cardiac patients. In the latter group of patients, only those untreated and having a marked edema had high renin-activity levels. Brown and collaborators (449) reported that 8 out of 9 cirrhotic patients with ascites had high renin-activity levels, while only 3 out of 9 without ascites had higher-than-normal values. Maebashi (741) found high blood renin-activity in 9 nephrotic patients with marked edema and in 2 cirrhotic patients with edema and ascites, but he could not detect any above normal value in 5 congestive heart failure patients. However, in this latter study, no mention of treatment or diet is made. As illustrated in our study, the treatment must be considered in the interpretation of these studies since opposite findings may be obtained depending whether the treatment was initiated or not. More recently, Kaley et al (778) published that the renin-activity was significantly increased in the lymph of patients with congestive heart failure. Davis and his coworkers (62, 65) reported an increased renin-activity in the renal venous blood, peripheral blood and lymph of dogs with caval constriction.

All the recent studies concerning the measurement of renin-activity in secondary hyperaldosteronism support our previous findings of high angiotensin levels in congestive heart failure patients and that of high renin-activity levels in most cardiac, cirrhotic and nephrotic patients with edema. No other study concerning the angiotensin levels in these conditions has been reported and no one else has studied the variations of renin-activity during treatment of these edematous states. The finding of high renin-activity in most edematous patients is also in agreement with the previous findings of an increased renin-content in the kidneys of cirrhotic patients (691) as well as with the

increase in the granularity of the juxtaglomerular cells in the kidneys of patients with congestive heart failure and cirrhosis (163, 692).

The role of aldosterone in the formation of edema has already been discussed in the review of the literature. The importance of aldosterone in the accumulation of edema in experimental aminonucleoside nephritis in rats was clearly demonstrated by the experiments of Singer, Giroud and their coworkers. A participation of the renin-angiotensin system in these experimental conditions has been suggested by Tobian et al (396) when they reported a correlation between the granularity of juxtaglomerular cells, the amount of ascites and the width of the zona glomerulosa of rats rendered nephrotic by the administration of aminonucleoside.

It was reported that chronic administration of aldosterone to cardiac patients, free of edema, resulted in a marked sodium retention leading to the development of edema (678). Usually in normal man, the administration of aldosterone causes a retention of sodium for the first few days but an escape occurs before the appearance of edema, and sodium balance is achieved despite the continuation of treatment. In cardiac patients, this capacity to escape the action of aldosterone is lost by an unknown mechanism. A similar phenomenon has been reproduced experimentally by the group of Davis in dogs in which an aorto-caval fistula was created (339). These dogs completely lost their capacity to escape the sodium retaining activity of aldosterone. When a prolonged infusion of this steroid was given to these dogs, a marked retention of sodium and water occurred and ascites and edema appeared after only a few days of this treatment. Moreover, they could reproduce the same phenomenon by infusing angiotensin instead of aldosterone. This last observation is the most direct evidence for the participation of angiotensin in the formation of edema, at least in conditions associated with hemodynamic disturbances.

The main determinant triggering edema formation might be the loss of this capacity to escape the sodium retaining action of angiotensin and/or aldosterone.

What may be the stimulus for renin secretion in these pathological conditions? In patients with congestive heart failure, both cardiac output and glomerular filtration rate are generally found markedly decreased. Although the total plasma volume is unchanged or increased in this condition, there is a redistribution of blood within the vascular tree. The venous side of the circulation gains volume at the expense of the arterial side so that the "effective circulating volume" is decreased (779). Therefore, pressor or stretch receptors such as the one postulated by Tobian in kidney arterioles might be stimulated by this decrease in arterial volume. However, as discussed previously, such hemodynamic changes can also induce a decrease in sodium excretion by the kidneys. Therefore, it appears that either changes in intrarenal hemodynamic factors or in electrolyte content of the tubular fluid might be the primary stimulus for the secretion of renin in "low output failure".

In cirrhosis of the liver, the mechanism of edema and ascites formation is believed to depend in great part on the increase in capillary pressure due to an increase in portal pressure and resistance (780-782). The role of the altered renal function in the mechanism of sodium retention in this condition is less clear than for cardiac patients. Papper (783) reviewing studies of thirteen groups of investigators, could not correlate the presence of ascites formation with any particular depression in either G.F.R. or R.B.F. Thus, in cirrhotic patients the renal hemodynamic factors are less obviously involved in the stimulation of renin secretion. Inconsistent changes in G.F.R. have also been reported during edema formation in nephrosis. As a matter of fact, an increase in G.F.R. and R.B.F. has been observed in many nephrotic subjects with edema (631, 784-786). Therefore, it is more difficult to relate the in-

creased renin secretion in cirrhotic and nephrotic patients to hemodynamic changes in the kidney.

Since many of the patients studied also had ascites, it was necessary to determine if the increase in intra-abdominal pressure could be a stimulus for the secretion of renin. However, this does not appear to be the case since in two patients in whom the renin-activity was measured before and after an abdominal paracentesis, no change occurred in plasma renin-activity (see case G.V., No. 12, Table XVII, and A.S.L., case No. 5, Table XIX).

In summary, in most subjects presenting marked edema, the renin-activity was found to be significantly increased while the arterial angiotensin was consistently increased only in cardiac patients. After treatment and relief of edema, these parameters decreased in cardiac patients, increased in nephrotic patients and did not change consistently in cirrhotic patients.

These findings suggest that the renin-angiotensin system may participate to the mechanism of edema formation and in the maintenance of a state of hyperaldosteronism in certain pathological conditions. Finally, the consistent finding of increased angiotensin levels in heart failure patients indicates that the renin-angiotensin system might account in part for the increased peripheral resistance reported in these patients.

3. RENIN-ACTIVITY IN HUMAN HYPERTENSIVE DISEASES.*

Despite the large number of studies done concerning the role of the renin-angiotensin system in the pathogenesis of human hypertensive diseases, no definite answer has been provided. In early studies much controversy existed among the findings of several groups of investigators. Recently, Helmer and Judson (56, 147, 533, 534, 536) measured the plasma renin-activity of patients with various forms of hypertensive diseases and found increased levels in many of these.

* Thanks are due to Dr. Cameron Strong for permitting to include 12 of his patients in this study.

A similar study was undertaken with the method of Boucher et al (8). Since the usual therapy of hypertensive diseases (low sodium intake, natriuretic drugs and various hypotensive drugs) can in itself constitute a stimulus for the activation of the renin-angiotensin system, so far as possible all forms of therapy were avoided in the study to be reported. Moreover, only patients without signs of congestive heart failure will be reported since this condition per se is generally accompanied by high renin-activity levels as reported in the previous section.

Part of this study has been published (718, 719, 724, 734, 735, 775).

a) Subjects and clinical procedure:

One hundred and nine patients with hypertension of various etiologies were investigated. All of these had a thorough investigation including renal angiography in most instances. Forty-nine of these were classified as having essential hypertension since no specific cause could be found for their increased diastolic pressure. In 24 instances, the hypertension was associated with renal parenchymatous diseases and in 28 others, with renal artery stenosis. In addition, 3 patients with malignant hypertension, 3 with coarctation of the aorta, 1 with primary aldosteronism and 1 with pheochromocytoma were also studied.

All these patients were either receiving an unrestricted diet or a diet containing 135 mEq Na at the time of the blood sampling. None of them was receiving natriuretic drugs at the time of study and for at least one week preceding the blood sample.

b) Results:

Details concerning these patients: age, fundi (Ghans classification), duration of hypertension, blood pressure and plasma renin-activity are given in tables XX, XXI, XXII and XXIII. The plasma renin-activity levels in ess-

ential hypertension, in hypertension associated with renal parenchymatous diseases and with renal artery stenosis are illustrated in Figure 25. The statistical analysis of these findings are presented in Table XXIV.

i) Essential hypertension (Table XX, Fig. 25):

Forty-nine hypertensive patients (30 men and 19 women) without edema, aged 19 to 66 years, had one or more renin-activity determinations during their hospitalization for investigation. Of this group, 6 had values above normal range and 15 had undetectable levels. Among the six patients having values above normal range, 3 of them had levels just slightly above normal range while the 3 others had significantly elevated levels. These three patients with highest renin-activity had a history of appearance of hypertension during the six months preceding. In this group of patients with essential hypertension, nine had an history of recent hypertension (six months or less) and of these nine, five had levels at 30 ng/L/min. or higher.

The mean value for the whole group of patients is 15.0 ng/L/min. (±21 S.D., ±3 S.E.) with a range from undetectable to 128 ng/L/min. This mean value is not significantly different from the mean normal value (Table XXIV).

No correlation could be found between the level of blood pressure and plasma renin-activity levels.

ii) Hypertension secondary to renal parenchymatous diseases (Table XXI, Fig. 25):

Twenty-four patients with proven renal parenchymatous diseases were investigated. Four of them had polycystic disease, 7 had chronic glomerulonephritis, 10 had chronic pyelonephritis and 3 had unilateral hydronephrosis secondary to ureteral stenosis. Of this group of patients, 6 had values slightly above normal range and 6 others had undetectable levels.

The mean value for the whole group of patients is 18.8 ng/L/min. (±14.5 S.D.

TABLE XX

RENIN ACTIVITY IN ESSENTIAL HYPERTENSION

NAME	AGE	SEX	FUNDI (GHANS)	DURATION OF HYPERTENSION	B.P.* mm Hg	RENIN ACTIVITY ng/L/min.
1. J.A.	54	M		4 years	210/120	0
2. Y.B.	36	M	A2 HO-1	3½ "	160/100	0
3. L.C.	53	F	A2 HO	2 "	220/130	0
4. G.D.	47	M	A1 HO	10 "	180/115	0
5. J.G.F.		M			200/125	0
6. L.L.	44	F	A2 H1	4 "	220/140	0
7. G.L.	56	M	A1-2 HO	5 "	205/118	0
8. G.L.	50	F	A1 H1	6 months	180/102	0
9. R.L.	53	F		2 years	200/120	0
10. D.M.	43	M	A1 HO-1	5 months	170/97	0
11. L.M.	54	M	A1 HO	8 years	165/100	0
12. R.P.	66	F	A1 H1	30 "	190/100	0
13. L.P.	55	M	A2-3 HO	10 "	170/110	0
14. W.R.	60	F	A2 HO	6 "	200/100	0
15. L.S.	50	M			160/110	0
16. A.P.	50	M	A1 HO	1½ "	170/85	6
					152/93	0 } 3
17. C.L.	59	M		5 "		4
18. C.T.	55	F	A1 HO	1½ "	160/90	5
19. H.A.	54	M	A1 HO	10 "	225/115	11
						0 } 6
20. M.A.	54	F	A2 HO	1 month	158/78	6
21. R.D.	37	F	A1 HO	1 year	195/110	6
22. L.B.	40	M	A3 H1	3 years	160/110	7
23. P.T.	52	F	A1 HO	3 months	200/110	7
					210/110	11 } 9
24. R.B.	32	F	A2 H1	19 years	240/140	10
					190/140	12 } 11
25. P.M.	21	M		2 "		11
26. H.C.	31	F	AO HO	2 "	133/94	12
27. R.L.	58	M	A1 HO	10 "		12
28. A.S.	55	F	A3 H1	14 "	170/110	12
29. W.M.	49	M	A1 HO	3 "	210/140	12
					218/128	14 } 13
30. C.L.	43	M	A1 H1	21 "	211/148	17
					170/120	8 } 14
					180/120	16
31. R.H.	58	F			190/100	16
32. H.M.	54	M	A1-2 HO	2 "	170/70	16
33. P.P.	48	F	A2 H1	10 "	190/110	18
34. A.A.	60	M		4 "	160/100	21
					192/120	17 } 19
35. J.A.L.	61	M	A1-2 HO	6 months	170/95	19
36. L.L.	50	M	A1-2 HO	17 years	214/148	20**

* At time of sampling.

** Sample drawn during aortography.

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TABLE XX (continued)

NAME	AGE	SEX	FUNDI (GHANS)	DURATION OF HYPERTENSION	B.P.* mm Hg	RENIN ACTIVITY ng/L/min.	
37. O.S.	26	F	AO HO	2 years	220/125	22	
38. S.D.	54	F	A2-3 H1	30 "	170/100	35	} 23
						10	
39. G.F.	64	M	A1-2 HO	3 "	158/110	26	
40. M.M.	23	F	A2 H1	1 "	210/138	31	} 26
					190/130	21	
41. J.B.	30	M	AO H1	2 months	140/90	30	
42. E.D.	29	M	AO HO	Recent	140/100	30	
43. G.C.	57	M	A1-2 HO	7 years	148/96	32	
44. J.G.B.	19	M	AO HO	3 "	174/100	33	
45. A.P.	49	M	A2 HO-1	5 "	184/104	33	
46. I.D.	62	M	A1 H1	5 "	208/135	35	
47. G.H.	52	M		6 months	160/100	38	
48. J.R.	28	F	AO HO	2 weeks	176/115	58	
49. R.C.	33	M	AO HO	2 months	150/100	128	

* At time of sampling.

TABLE XXI

RENIN-ACTIVITY IN HYPERTENSION SECONDARY TO RENAL PARENCHYMATOUS DISEASES

NAME	AGE	SEX	FUNDI (GUANS)	DURATION OF HYPERTENSION	B.P. mm Hg	RENIN ACTIVITY ng/L/min.
POLYCYSTIC DISEASE						
1. M.S-J.	18	M	AO HO	4 years	200/90	15
2. C.G.	28	F	AO HO	10 "	158/95	32
3. R.L.	19	M	AO HO	3 "	158/95	38
4. C.G.	38	F	A1 HO	11 "	156/94	42
CHRONIC GLOMERULONEPHRITIS						
1. L.C.	16	F	A1 H2	3 years	160/125	0*
2. G.G.	35	M				10*
3. A.C.	64	F	A2 HO		160/110	16*
4. R.T.	35	M	A2 H2	8 "	230/150	16*
5. C.D.	40	M	A1 H1	2 months	182/70	21*
6. T.D.	38	M	A2 H2	1½ year	180/80	35
7. G.L.	47	M	A2 H1	1½ "	165/105	37*
CHRONIC PYELONEPHRITIS						
1. F.G.	55	F	A2 H1	6 years	230/120	0*
2. A.L.	58	M	A2-3 HO	4 "	176/120	0*
3. G.M.	44	M	A1 HO	4 months	226/143	0
4. G.B.	38	F	A1 H1	14 years	202/110	10*
5. L.C.	20	M	AO H1	3 "	176/116	15
6. J.R.	57	M	A3 H1	2 "	260/140	16*
7. L.L.	37	F	A1 H2	30 "		17*
8. R.L.	44	F		6 "	200/110	26*
9. R.A.	37	M	A1 HO	6 months	260/165	30*
10.C.B.	25	F	AO HO	1½ year	200/130	40
HYDRONEPHROSIS						
1. A.P.	42	F	A1 HO	2 years	172/94	0
2. R.S.	52	M	A2 HO	Recent	200/116	0
3. M.D.	50	F	A1 H1	3 months	150/80	36

* Markedly decreased renal function. (Blood urea higher than normal, creatinine clearance decreased by 50% or more).

TABLE XXII

RENIN-ACTIVITY* IN HYPERTENSION ASSOCIATED WITH RENAL ARTERY STENOSIS

NAME	AGE	FUNDI (GHANS)	DURATION OF HYPERTENSION	B.P. mm Hg	GRADIENT**	RENIN ACTIVITY ng/L/min.	RESULT OF SURGERY
1. L.B.	56M	A2 H2	3 years		25	0	
2. L.L.	61F	A2 H0	18 "	190/100		0	
3. R.Q.	51F	A2-3 H1	28 "	180/105	50%	0	
4. A.R.	51F	A1-2 H1	21 "	150/90	60	0	Failure
5. P.R.	41F	A1 H1	7 "	186/100	105 L 85 R	10	"
6. E.M.	64M	A2 H0	3 "	170/110	90 L 25 R	15	"
7. B.L.	49M	A3 H2	12 "	190/150	20%	16	
8. R.M.	19M	A0 H1	8 "	160/110	100	17	Success
9. R.P.	37M	A1 H1	10 months	240/150	100	18	Failure
10.A.W.	48M	A2 H2	5 years	155/100	80%	22	"
11.W.R.	58F	A0 H0	7 months	200/110	20	31	Success
12.G.G.	44M	A2 H1	4 years	240/140	50	34	"
13.M.B.	26F		6 "	220/130	90	35	Failure
14.A.L.	39F	A1-2 H3	2 "	217/116		36	
15.A.R.	41M	A1 H1	1 "	160/110	Thrombosis	36	Success
16.A.B.	66F	A2 H0	1 "	235/115	"	97	Failure
17.M.H.	59F	A1 H0	1½ "	240/120	100	49	Success
18.M.D.	45M	A2 H0			140	50	Failure
19.E.P.	58M	A2-3 H1	1½ "	220/140	38	50	
20.R.G.	49M	A2 H1	7 "	175/115	50	50	
21.H.P.	49M	A2 H1	6 "	230/130	70	55	"
22.G.R.	36F	A2 H1	1 "	210/125	100	56	Success
23.D.M.	67F	A2 H1	Recent	162/92	Thrombosis	60	
24.F.A.	53M	A2 H0	2 years	250/120	75%	78	Failure
25.J.M.	52F	A1-2 H0	6 months	225/135	Thrombosis	97	"
26.E.V.	58M	A2 H1	3 "	167/104	Thrombosis	151	"
27.L.G.	48F		4 years	210/115	Thromb. L 75% R	244	
28.A.R.	35M			185/120	50	335	Success

* Measured in peripheral blood 7 to 15 days before surgery.

** Values obtained by direct measurement at operation or during renal artery catheterization at the time of selective angiography. Percentage values represent decreases in renal perfusion as estimated by angiographic appearance. L: Left; R: Right.

TABLE XXIII

RENIN-ACTIVITY IN OTHER HYPERTENSIVE DISEASES

NAME	AGE	SEX	FUNDI (GHANS)	DURATION OF HYPERTENSION	B.P. mm Hg	RENIN ACTIVITY ng/L/min.
MALIGNANT HYPERTENSION*						
1. J.B.	24	M	A2 H3	4 months	240/130	0**
2. C.C.	32	M	A1 H3	5 "	220/140	17**
3. G.D.***	59	F		25 years	230/115	639**
COARCTATION OF THE AORTA						
1. C.B.	13	F	AO HO		140/90	0
2. L.B.	38	M	A1 H2	10 years	250/160	6
3. C.B.	49	M			210/85	9
PRIMARY ALDOSTERONISM						
1. S.B.	36	F	A1 H1	15 years	200/130	0
					160/108	0
					134/84	0
					180/110	0
					SURGERY†	
					130/100	26
					115/70	0
					140/85	38
PHOECHROMOCYTOMA						
1. L.M.	30	F	A1 H1	3 years	210/140	14

* With neither edema nor heart failure.
** Severe renal insufficiency.
*** This patient was receiving Serpasil I-M.
† Removal of a 10 gm adrenal adenoma (Details, Fig. 26).

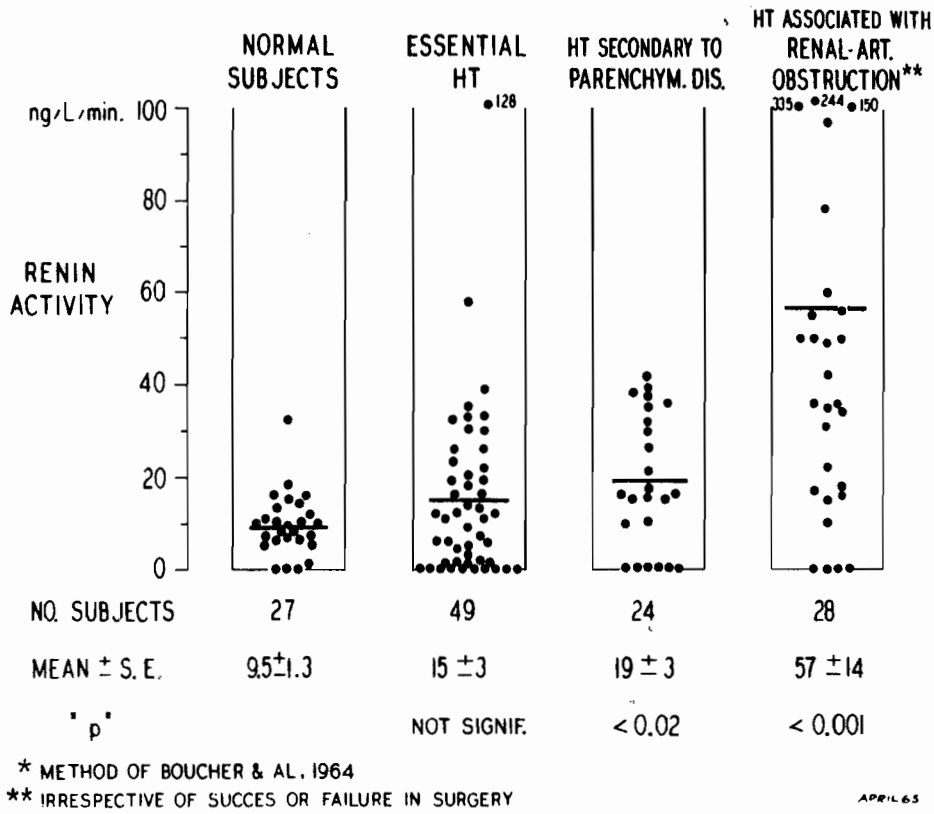


Fig. 25

Renin-activity in hypertensive patients compared to normal subjects. Only the mean value for patients with hypertension associated with renal parenchymatous diseases and with renal artery stenosis are significantly elevated. The details of statistical analysis are given in Table XXIV.

TABLE XXIV

STATISTICAL ANALYSIS OF MEAN RENIN-ACTIVITY* LEVELS OF VARIOUS HYPERTENSIVE DISEASES

	NORMAL	ESSENTIAL HYPERTENSION	RENAL PARENCHYMATOUS DISEASES					HYPERTENSION ASS.WITH REN.ART.STEN.
			POLYCYST.	CHR.GL.NEP.	PYELON.	HYDRON.	TOTAL	
NUMBER	27	49	4	7	10	3	24	28
MEAN:	9.5	15.0	31.8	19.3	15.4	12.0	18.8	56.5
STANDARD DEVIATION	±6.7	±21.0	±11.8	±13.2	±13.5	±20.7	±14.5	±74.4
STANDARD ERROR:	±1.23	±3.0	±5.9	±5.0	±4.3	±12.0	±3.0	±14.0
NUMBER AND % ABOVE NORM. RANGE	RANGE: 0-32 ng	6/49 12%	2/4 50%	2/7 28%	1/10 10%	1/3 33%	6/24 25%	17/28 60%
"p" VALUE: VERSUS NORMAL	--	not signif.	<0.05	not signif.	not signif.	--	<0.02	<0.01

* In ng/L/min.

±3 S.E.) with values ranging from undetectable to 42 ng/L/min. This mean value is significantly higher than the mean normal value at "p" < 0.05 (Table XXIV). However, in analyzing each group of disease separately, it appeared that only the group of patients with polycystic disease had a mean value significantly higher than normal ("p" < 0.05). Three of these four patients had a plasma renin-activity at the upper limit or above normal range. Despite higher renin-activity, the blood pressure of patients with polycystic disease was only slightly elevated while, on the other hand, most patients with chronic pyelonephritis had severe hypertension with only one value out of ten standing above normal range (Table XXI). Therefore, again, no correlation could be established between the level of blood pressure and renin-activity.

iii) Hypertension associated with renal artery stenosis (Table XXII, Fig. 25)

Twenty-eight patients presenting a significant stenosis or thrombosis of a renal artery were studied. The degree of the stenosis was estimated either by selective catheterization of the involved renal artery, or at the time of surgery by the means of a needle or by a gross estimation of the decrease in circulation of the involved kidney as seen during renal angiography.

In this group of patients, 17 out of 28 had higher-than-normal levels which were greatly increased in many instances. The mean level of these patients is 56.5 ng/L/min. (± 74 S.D., ±14 S.E.) with a range from undetectable to 335 ng/L/min. This mean value is very significantly increased with a "p" value < 0.01 when compared with the normal mean level (Table XXIV).

No direct relationship could be found with the degree of stenosis and the level of renin-activity. Nevertheless, all the six patients having a thrombosis of one main renal artery or of one of its branches had higher than normal renin-activity.

The mean duration of hypertension was 2.3 years ±2.3 S.D., for 15 pa-

tients with higher than normal renin-activity levels, while the mean time was 9.6 years ±9.0 S.D. for the eleven patients having their levels within the normal range (Difference significant "p" < 0.05).

The success or failure of the surgical repair of the stenosis or nephrectomy of the involved kidney could not be predicted from the level of the peripheral renin-activity alone prior to the surgery.

iiii) Renin-activity in other hypertensive diseases (Table XXIII):

Three patients with malignant hypertension but without edema or cardiac decompensation were studied. Two of them had values within the normal range but the third one had a very high renin-activity. All of these were in severe renal failure.

Three patients with coarctation of the aorta had levels within the normal range.

One patient with primary aldosteronism was studied before and after the removal of an adrenal adenoma. The study of this patient is illustrated in Figure 26. This patient was admitted to the hospital with hypokaliemic alkalosis. In addition, the renal angiography revealed multicystic kidneys and annular stenosis of the left renal artery. However, renin-activity remained undetectable in blood samples drawn on four different days. At operation, an adenoma of 10 grams was removed from the left adrenal cortex. Postoperatively, the blood pressure returned to normal, the symptoms and biochemical alterations rapidly disappeared and renin-activity was detected in two instances thereafter.

Finally, one patient with a pheochromocytoma was found to have a normal renin-activity.

c) Discussion:

With the exception of a few slightly elevated levels in essential hyper-

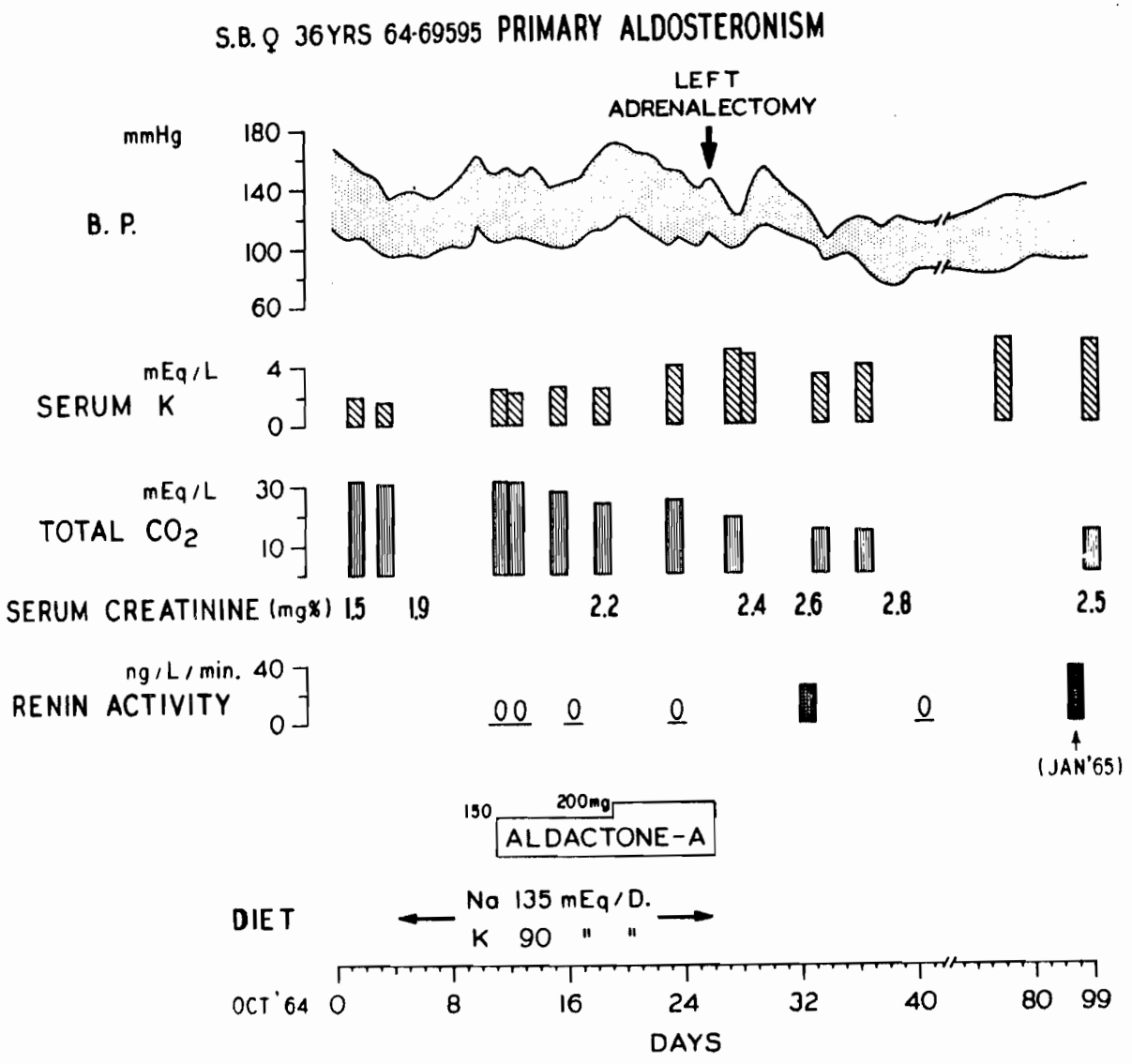


Fig. 26

This patient was admitted with a history of periods of severe weakness, nycturia and urinary tract infection. She presented hypokalemic alkalosis and decreased renal function. The renal angiogram revealed an annular stenosis of the left renal artery and multicystic kidney disease. Four determinations of plasma renin-activity did not yield any detectable level after three hour incubations. On that basis and despite the renal insufficiency and the presence of the renal vascular lesion, the patient was admitted to surgery and a 10 gram adenoma was removed from the left adrenal cortex. All symptoms and biochemical signs of primary aldosteronism disappeared after surgery.

tension and renal parenchymatous diseases, the renin-activity was found to be elevated almost exclusively in the group of patients presenting hypertension associated with a renal artery stenosis. These results are in accordance with the previous findings of Genest and coworkers in our department with the measurement of arterial angiotensin levels in similar groups of patients (527, 717, 718, 724).

With the exception of the studies of Helmer and Judson, all the other studies with renin-activity were not available at the time the present work was undertaken. Simultaneously to, or after, our first reports (718, 719, 724, 733, 734, 775), many other studies using various methods for the measurement of renin-activity were published. These results are compared with those of our study in Table XXV. In this table, the absolute values could not be compared since the marked differences in methodology prevented any quantitative comparison between the values obtained by various groups of investigators. However, a certain degree of comparison could be obtained by expressing the number of values above the normal range reported with each method.

Concerning essential hypertension, the results of several studies are strikingly similar. Helmer (147, 536) first reported renin-activity levels ranging from undetectable to increased levels in essential hypertension. In the present study, only 6 out of 49 subjects were found to have values above normal range and the mean, although slightly higher than the normal mean level, was not significantly increased. These findings are in agreement with those of Brown and coworkers (449) and those of Yoshinaga, Maebashi and coworkers (452, 741) in studies dealing with a comparable number of patients. These are also supported by the smaller series of Fasciolo (57) and of Warzynski (58) and their collaborators.

TABLE XXV

PERIPHERAL PLASMA RENIN ACTIVITY IN HUMAN HYPERTENSION. (Values above normal*)

AUTHORS	ESSENTIAL	MALIGNANT	REN. ART. STENOSIS	GL. NEPHR.	PYELON.	COARCT.	PRIMARY ALDOSTERONISM
Helmer (1960-1964) (147, 536)	↓ N ↑	↑	N or ↑	N or ↑	2/6	3/4	0/1
Maebashi et al (1963-64) (452, 741)	6/48	9/10	4/7	2/17		0/1	0/3
Brown et al (1964) (449, 725)	6/46		20/46				0/1
Fasciolo et al (1964) (57)	2/8		1/2				
Warzynski et al (1964) (58)	1/13		0/5				
Conn et al (1964) (722, 723)			3/3				0/3
PRESENT WORK (1963-65)	6/49	1/3	17/28	2/7	1/10	0/3	0/1

* As determined by each method used.

Approximately one third of the patients with essential hypertension had undetectable levels. This proportion is greater than that observed in normal control subjects. A similar observation was reported by Helmer (147) and Maebashi (741). These findings are of interest in the light of the recent findings of undetectable renin-activity in cases of true primary aldosteronism due to adrenal adenoma. Genest and his coworkers had observed that the arterial angiotensin levels were undetectable or low in true primary aldosteronism (527, 717-721). Moreover, he proposed that the measurement of angiotensin or renin-activity would be the best way to differentiate hyperaldosteronism due to adrenal adenoma from that due to renal artery stenosis. This hypothesis was given support by the recent studies of Conn (722, 723), Genest (724) and Brown (725) and their coworkers reporting undetectable or minimal renin-activity in true primary hyperaldosteronism. Conn even observed that the renin-activity did not increase in these patients even after a period of severe sodium restriction. Recently, Conn (787) reported the case of a patient classified as essential hypertension who was operated upon only on the basis of an undetectable renin-activity after a period of sodium depletion. Despite an aldosterone secretion rate only slightly above the upper limit of normal and in absence of hypokaliemic alkalosis, this patient was found to have a large adenoma of the adrenal cortex. The removal of this tumor resulted in relief of his hypertension. Therefore, patients with essential hypertension having undetectable renin-activity should be given special attention since their condition might be secondary to a state of pre-clinical and "hidden" primary aldosteronism. In one patient of our study (Fig. 26), the repeated finding of undetectable renin-activity led to the discovery of a 10 gm adrenal adenoma. This example illustrates the importance of a deeper investigation in each patient with low renin-activity.

These observations suggesting the existence of pre-clinical states of hyperaldosteronism in a number of patients with essential hypertension, give more support to the hypothesis suggested by Genest in 1956 that aldosterone and the adrenal cortex are involved in the pathogenesis of essential hypertension. This hypothesis was based on the repeated findings of an increased aldosterone excretion rate in 35% to 50% of patients with essential hypertension (399, 719, 788, 789). These findings were confirmed by several investigators (790-793) with aldosterone excretion but were not in accordance with the findings of a normal aldosterone secretion rate in most patients with essential hypertension (794, 795).

In the group of patients associated with renal parenchymatous diseases, the highest values were found in patients having polycystic kidney diseases. This might be secondary to local ischemia due to the compression exerted on the surrounding tissue by the renal cysts. Most of the patients with other parenchymatous renal diseases had renin-activity levels within normal range, despite severe hypertensive disease in some instances. These findings are in accordance with those of Helmer (147, 536) and Maebashi (741).

In our study, 60% of the patients with renal artery stenosis had a value above the normal range. These findings are in accordance with those reported by Helmer (147, 536), Yoshinaga and Maebashi (452, 741), Brown and coworkers (449), Fasciolo et al (57) and Conn et al (722, 723). Warzynski and his coworkers (58) did not find one value above normal range in the five patients of their study. These negative findings might be explained by the lack of specificity and sensitivity of their method.

Nevertheless, the determination of renin-activity in peripheral blood was not a sufficient criterion to foresee the prognosis of corrective surgery in many patients. In a study done in collaboration with Tremblay and Veyrat

in our department (796), it was found that the degree of arteriolosclerosis of the involved kidneys was another criterion to establish the prognosis of the surgery when associated with renal vein plasma renin-activity and J.G.I. In that study, Tremblay observed that the presence of arteriolosclerosis in the involved kidney was generally associated with failure of surgery while the absence of arteriolosclerosis combined with a significant pressure gradient and high renin-activity was associated with a cure or significant improvement of the illness after corrective surgery. According to the theory of stretch-receptor at the site of the afferent arteriole, the stimulus for renin secretion would be brought about by a decrease in renal perfusion pressure whether due to renal artery stenosis, renal polycystic disease or other renal conditions. However, in this study, some cases had a normal renin-activity even with a marked systolic gradient between the pressure into the aorta and the renal artery distal to the stenosis. Moreover, all the cases of coarctation of aorta had a normal renin-activity. Vander and Miller (166) recently suggested another possible stimulus in these conditions for the liberation of renin. They demonstrated that the increase in angiotensin-like activity induced by the constriction of aorta in dogs could be prevented or decreased simply by increasing the osmotic load of the kidneys despite the persistence of the decreased perfusion pressure. This last observation in experimental hypertension is of utmost interest since in human renovascular disease, a decrease in urinary sodium is often reported on the side of the involved kidney.

Only three patients devoid of cardiac insufficiency with malignant hypertension could be studied. Two of them had low or undetectable levels despite a marked increase in diastolic and systolic blood pressure. These results, although small in number, differ from those reported by Helmer (147) and

Maebashi (741) who found increased values in all their patients suffering from malignant hypertensive disease. These patients often present cardiac decompensation and edema and this might account for the difference in findings. Kahn and coworkers (108) had also previously reported markedly increased arterial angiotensin levels in ten patients with malignant hypertension, but they noted that many of them also presented a "certain degree of cardiac failure". The studies of Genest et al (526, 527) and Morris et al (33, 455, 528) showed low angiotensin levels in almost all cases of malignant hypertension.

Since no correlation could be found with the degree of hypertension, it remains difficult to assess the exact role of the renin-angiotensin system in the pathogenesis of hypertensive diseases. Our findings do not suggest that this system is active in the causation and maintenance of hypertension in most patients with essential hypertension, renal parenchymatous diseases, coarctation of the aorta and malignant hypertension. On the other hand, it appears to be related to the hypertensive process of most patients with renal artery stenosis and of those with polycystic renal diseases. The negative findings in many hypertensive patients do not exclude the possibility that the renin-angiotensin system might have been active in an earlier stage of the disease. In that respect, it is striking to note that in essential hypertension, the highest values were found in three patients with a recent history of hypertension and that patients with renal artery stenosis presenting a renin-activity above normal range have a lower mean duration of hypertension than those with a normal renin-activity. However, no definite conclusion can be drawn from this observation because the determination of the duration of hypertension is based only on the time when high blood pressure was noted for the first time and therefore, is a very crude means of appraising the beginning of the

disease. Nevertheless, the probability of error is approximately the same for all the subjects.

Studies with experimental hypertension have shown that many factors can be involved in the development of hypertensive diseases (see review of literature). Human hypertensive diseases might also involve the participation of many mechanisms as well. The renin-angiotensin system might be only one of these. The nervous system, the genetic background and the antihypertensive mechanisms of the kidneys cannot be excluded from the pathogenesis of hypertensive diseases at the present time. Perhaps blood pressure rises as the result of the interaction of many of these factors and mechanisms.

C - THE ACTION OF ANGIOTENSIN, ALDOSTERONE AND NOR-EPINEPHRINE ON RENIN-ACTIVITY.

The control of renin secretion has been chiefly investigated in regard to mechanical or hemodynamic factors. Although a few recent observations have indirectly suggested the possibility of a humoral mechanism in the control of renin secretion, this aspect has never been clarified. (See review of the literature on the mechanism of control of renin secretion).

The present work was undertaken in order to bring about a more direct study of the possibility of a humoral mechanism of control. For that purpose, the acute effects on renin activity of some substances likely to account for such a mechanism were sought for.

a) Subjects and clinical procedure:

Twelve normotensive or slightly hypertensive subjects and one nephrotic subject were given a total of twenty infusions of angiotensin, aldosterone, nor-epinephrine and dextrose 5%. Prior to the experiment, these subjects were placed on a low dietary sodium intake (10 mEq Na and 90 mEq K) for three days and received, in addition, a natriuretic agent (Chlorthalidone, 100 mg/day) on the first one or two days of that diet. This procedure was used to

obtain a high renin-activity before the beginning of the infusion. From the night preceding and for the whole duration of the experiment, the subjects remained in the recumbent position. The infusions were given by means of a constant rate infusion pump and during that period, the blood pressure was recorded every five minutes.

Ten I.V. angiotensin (Hypertensine, Ciba preparation) infusions in dextrose 5% were given at doses ranging from 1.6 ng/kg/min. to 50 ng/kg/min. The periods of infusion generally lasted 2 to 4 hours but in one instance the infusion was prolonged for a period of 30 hours. Four of these infusions were administered at pressor doses while the six others were given at sub-pressor rates.

Five d-aldosterone (Ciba preparation) infusions were given for 2 to 4 hour periods at very high doses (260 to 640 ng/kg/min.) corresponding to 10 to 50 times the normal aldosterone secretion rate. Finally, five nor-epinephrine (Levophed) infusions were administered for 2 to 4 hours at pressor rates (46 to 200 ng/kg/min.).

b) Results:

Details concerning doses, rates of infusion, pressor responses and the variations of the plasma renin-activity levels are given in Table XXVI.

i) Effects of angiotensin infusions:

During each of the ten angiotensin infusions, whether given at pressor or at sub-pressor rates, plasma renin-activity decreased markedly and significantly. The lowering of the renin-activity was noted as soon as 30 minutes after the beginning of the infusion and the return to pre-infusion values was observed within 20 minutes after the cessation of the infusion. In one patient who was infused for a period of 30 hours at various rates, the plasma renin-activity was found to vary inversely to the amounts of angiotensin in-

TABLE XXVI

EFFECTS OF ANGIOTENSIN, ALDOSTERONE AND NOR-EPINEPHRINE INFUSIONS ON RENIN-ACTIVITY (Ng/L/min.)

SUBJECT	AGE	B.P.	DOSE ng/kg/min.	RATE cc/min.	PRESSOR RESPONSE*	CONTROL PRE-INF.	DURING INFUSION 30 min.	DURING INFUSION 90-120 min.	DURING INFUSION 180-240 min.	POST-INFUSION 20 min.	POST-INFUSION 2 hrs.	POST-INFUSION 4 hrs.	POST-INFUSION 20 hrs.
ANGIOTENSIN													
1. G.M.	69	Ht	6.4	0.4	Nil	128			33**				102
2. L.F.	35	N	25	1.25	Nil	313			77				790
3. C.T.	50	Ht	8	0.5	↑10	77			40				
4. L.C.	20	N	15	0.5	Nil	1050			600				700
5. R.T.	23	N	35	1.5	Nil	180		50	5	175	400	400	410
6. G.F.	64	Ht	25	2.0	Nil	65		45	28		105		
7. G.F.	64	Ht	50	3.75	↑20	105		35			35		170
8. M.G.	23	N	33	1	Nil	180	118	38	Changed for aldosterone inf. (see case 14)				
9. J.G.	35	Ht	21	0.75	↑20	51	31	15	Changed for nor-epinephrine inf. (see case 18)				
10. G.T.		Ht	20	1.2	↑18	250		80	45				
ALDOSTERONE													
11. L.F.	35	N	300	1.0	Nil	790			450				
12. C.T.	50	Ht	260	1.0	Nil	50			45				77
13. L.C.	20	N	300	1.25	Nil	700			874				
14. M.G.	23	N	640	1.6	Nil	38***	94	117			95		
15. G.T.		Ht	550	1.35	Nil	255		142	250				
NOR-EPINEPHRINE													
16. L.C.	20	N	115	1.25	↑20	700			500				1000
17. M.S.G.	51	N	46	1	↑20	15		14	23				
18. J.G.	35	Ht	200	1.4	↑15	15***	77	75					130
19. R.H.	58	Ht	90	0.8	↑10	60		70	75				
20. G.G.	28	N	185	1.3	↑10	350		435	340	290			

* Increase in diastolic pressure (mm Hg).

** This sample was taken after 5 hours infusion and this infusion was continued for 30 hrs. Details Fig. 27.

*** Values after 2 hrs. angiotensin infusion.

fused (Figure 27). After five hours of infusion at constant rate, plasma renin-activity fell to one fourth of the initial level. When the dose of angiotensin was decreased by half, the renin-activity doubled and when the dose was again decreased by half the renin-activity returned to pre-infusion levels. At that time, a slight increase in the dose infused resulted in a slight decrease of the renin-activity.

The rapidity of the renin response to the infusion of angiotensin is illustrated in one study reported in Figure 28. This subject received an angiotensin infusion at two different sub-pressor rates for a period of 5 hours which resulted in a progressive and rapid decrease in renin-activity from 180 to 5 ng/L/min. Within 20 minutes following cessation of the angiotensin infusion, the plasma renin-activity returned to pre-infusion levels. In the following hours, there was a "rebound" to very high levels. During the period when renin-activity was very high, the patient remained recumbent because of marked orthostatic hypotension until the next morning.

The inhibitory effect of angiotensin on renin-activity is not due to the stress of the experiment, as shown in the experiment illustrated in Figure 29. In this experiment, two angiotensin infusions were given at different rates, separated by an infusion of 5% dextrose in water given through a three-way stopcock, without the patient's knowledge of the "switched-in" infusion. Plasma renin activity decreased progressively from 65 to 28 ng/L/min. during the first non-pressor infusion. The angiotensin infusion was then replaced by a 5% dextrose infusion and two hours later, the renin-activity rose to 105 ng/L/min. A second infusion of angiotensin at a higher rate was again substituted without the patient's knowledge and once more, a marked decrease in renin-activity occurred after only two hours of infusion.

As shown in Table XXVI, in all the other subjects, the infusion of angio-

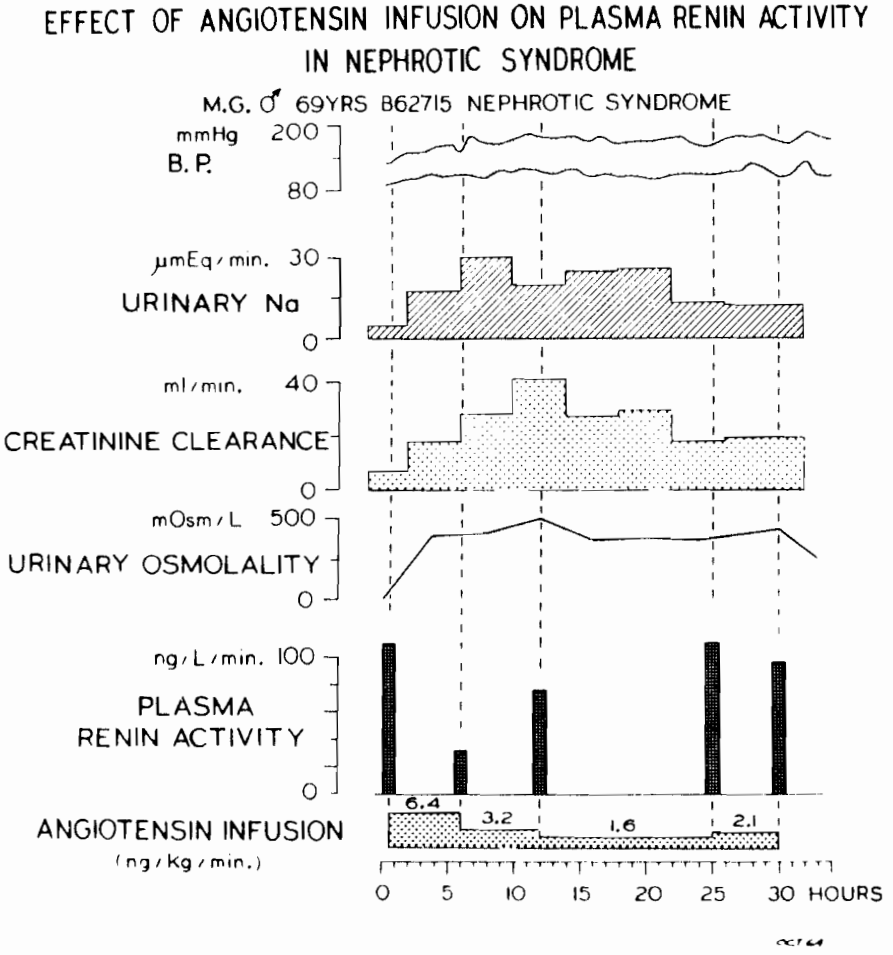


Fig. 27

This nephrotic subject received an angiotensin infusion at various doses during a period lasting 30 hours. During the first five hours of infusion, renin-activity fell from 128 to 33 ng/L/min. The rate of infusion was decreased by half and 7 hours later renin activity rose to 76 ng/L/min. The rate of infusion was again decreased by half and the renin-activity returned to pre-infusion levels 12 hours later. Finally, when the rate of infusion was slightly increased, the renin-activity slightly decreased 5 hours later. Natriuresis and endogenous creatinine clearance increased during the higher dose infusions.

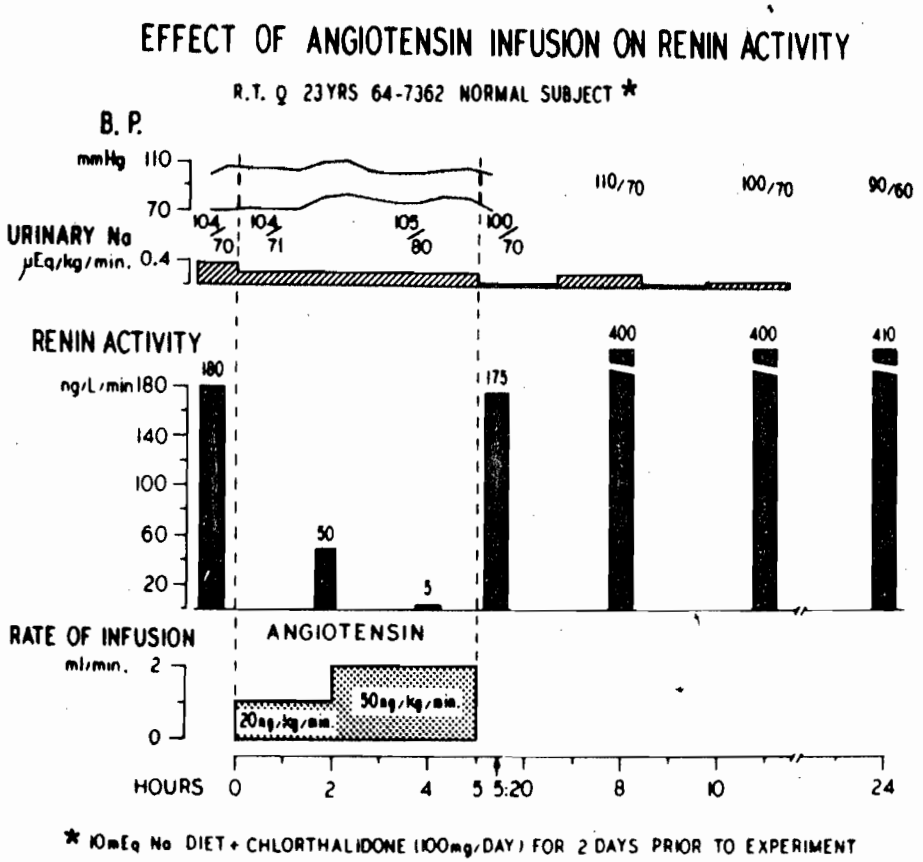
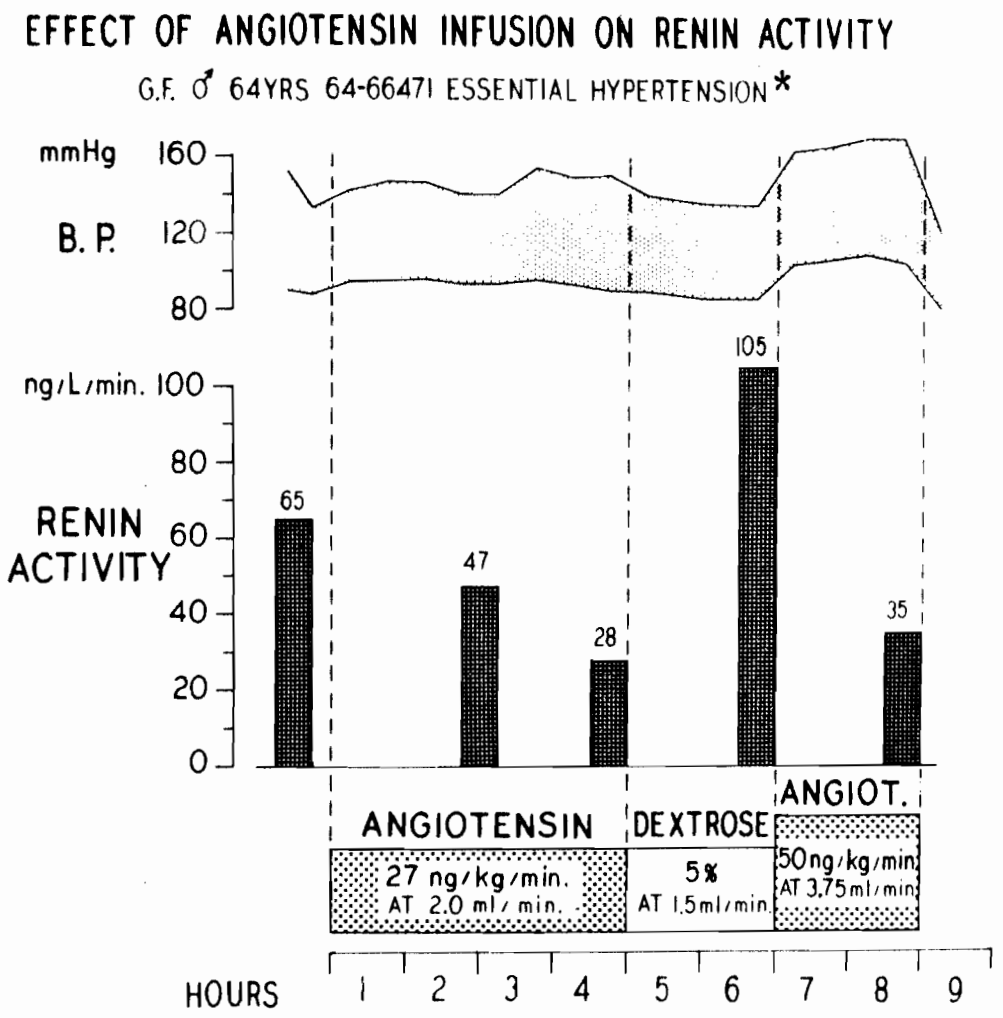


Fig. 28

In this sodium depleted subject, infusion of angiotensin at subpressor doses resulted in a marked decrease in renin activity from 180 ng/L/min., to 50 ng/L/min., after 2 hours of infusion and to 5 ng/L/min. after 4 hours of infusion. Twenty minutes after the cessation of infusion, the renin-activity returned to preinfusion levels. In the following three hours, the renin-activity rebounded to very high levels and stabilized at these levels for the following 16 hours. Note that during these large variations in renin-activity, the level of blood pressure did not change significantly. Note also that during the period of infusion the levels of urinary sodium excretion which were very low at the beginning, did not change significantly during and after infusion. Moreover, during the post-infusion period when renin-activity was markedly elevated, the subject had marked orthostatic hypotension for 18 hours.



* 10mEq Na DIET+CHLORTHALIDONE(100mg/DAY) FOR 2 DAYS PRIOR TO EXPERIMENT

Fig. 29

This experiment illustrates that the inhibitory action of angiotensin is not due to the stress of the infusion. In this subject, a four hour angiotensin infusion resulted in a gradual decrease in renin activity from 65 ng/L/min. to 28 ng/L/min. Thereafter, the infusion was changed for a dextrose infusion by means of a three way stopcock without the patient's knowledge and during the following two days of infusion, the renin-activity rebounded to 105 ng/L/min. When angiotensin was given again at higher rates, the renin-activity decreased to low levels.

tensin induced a marked decrease in renin-activity. Moreover, in all, the renin-activity rose to or above the pre-infusion control value after the cessation of the angiotensin infusion.

ii) Effects of aldosterone infusions:

Four aldosterone infusions given at very high doses in four different subjects had no consistent effect on renin-activity and the variations of renin-activity observed during the course of these infusions appeared neither significant nor marked (Table XXVI).

A fifth aldosterone infusion was given in one other subject following angiotensin infusion after renin-activity had been markedly decreased. In this study, which is illustrated in Figure 30, the angiotensin infusion was replaced by an aldosterone infusion without the patient's knowledge through a three-way stopcock. Although aldosterone was infused at a very high rate, this did not prevent the renin-activity from returning to high levels.

iii) Effects of nor-epinephrine infusions:

In four instances, nor-epinephrine infusions given at pressor rates failed to show any consistent or significant effect on renin-activity (Table XXVI).

A fifth infusion of nor-epinephrine at pressor rate was given in replacement of one angiotensin infusion after an inhibition of the renin-activity had occurred. As seen in Figure 31, the infusion of nor-epinephrine did not prevent the renin-activity from returning to high levels. In one other study illustrated in Figure 32, a nor-epinephrine infusion was given first and the renin-activity was not modified significantly. When this infusion was replaced by an angiotensin infusion, however, the renin-activity dropped by half at the end of a 90 minute infusion period. After the cessation of infusion, plasma renin-activity increased to high levels.

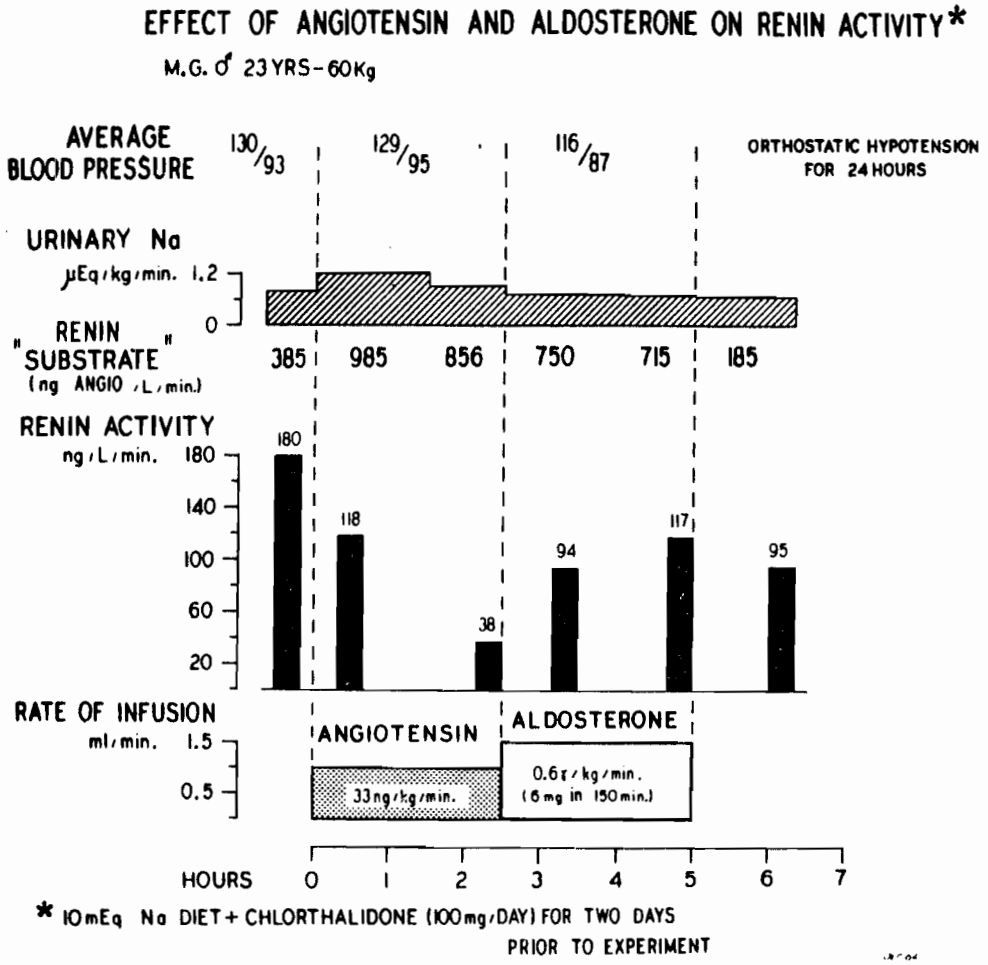


Fig. 30

In this experiment, an aldosterone infusion was substituted to an angiotensin infusion after a strong inhibition of the renin activity had been obtained. Although aldosterone was given at very high dose and at a higher infusion rate, this did not prevent the renin activity to return rapidly to high levels during the duration of the aldosterone infusion. On each blood samples, the "renin substrate" was grossly estimated by incubating 10 ml of plasma for three hours with an excess of human renin. These results indicate that the "substrate" is not the limiting factor in the variation of renin activity. Again, the changes in urinary sodium were minimal during the period of infusion.

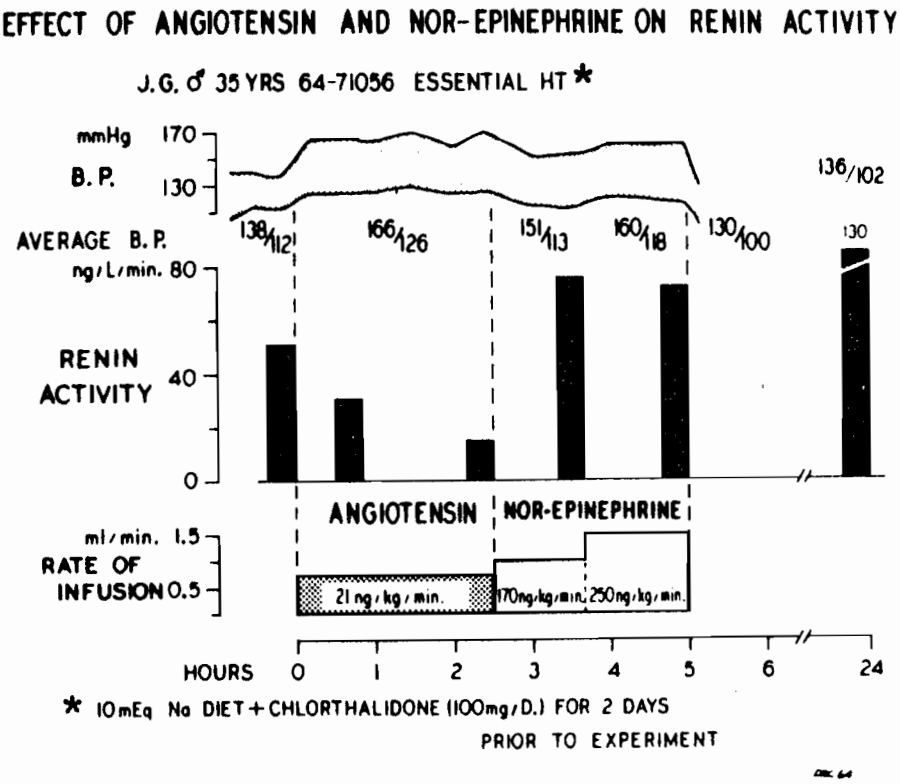


Fig. 31

In this experiment, a pressor infusion of angiotensin resulted in a gradual decrease in renin activity from 50 ng/L/min. to 15 ng/L/min. after 150 minutes. At this time the infusion was replaced by an infusion of norepinephrine also at pressor dose and at a higher infusion rate and this did not prevent the renin-activity from returning to high levels.

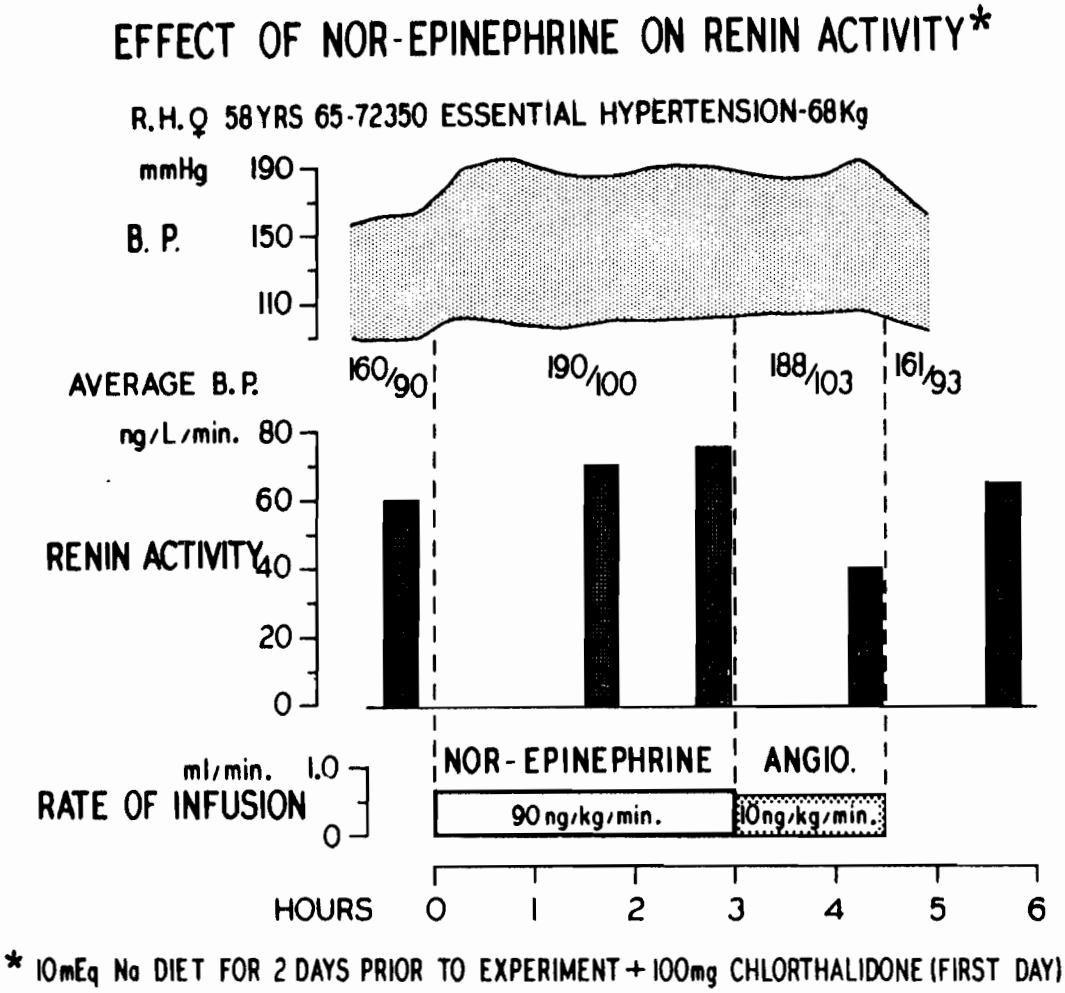


Fig. 32

This experiment illustrates that the inhibitory action of angiotensin is not secondary to its pressor action. Norepinephrine was first given at pressor dose for three hours. During this infusion, renin activity slightly increased. At the end of this infusion when angiotensin was infused at the same pressor rate, renin activity decreased by half within 90 minutes. After the cessation of the angiotensin infusion, the renin activity returned to preinfusion levels within 90 minutes.

c) Discussion:

From these findings, it appears that angiotensin exerts a powerful inhibition of renin release. Whether this mechanism works by the inhibition of the release of renin or by the blockage of its synthesis cannot be concluded from the present study.

This inhibitory action appears to be mediated neither by a vascular action nor by the aldosterone-stimulating effect of angiotensin. Since angiotonin and angiotensin were reported to produce a marked vasoconstriction on the kidney vessels even in the absence of a systemic increase in blood pressure (202, 203), there was a possibility that the effect of angiotensin on renin-activity might be mediated through this vascular action. However, pressor infusions of nor-epinephrine, which is also a potent vasoconstrictor of the kidney vessels, did not inhibit the renin-activity whereas sub-pressor infusions of angiotensin consistently and markedly decreased the renin-activity. Since angiotensin is a potent aldosterone-stimulating substance, it was necessary to determine if the action encountered with angiotensin could not be ascribed only to the increase in aldosterone secretion. However, acute infusions of aldosterone at doses corresponding to ten to fifty times the normal daily secretion rate were found to have no consistent effect on the renin-activity. Therefore, it seems unlikely that, in these acute experiments, the inhibition of renin-activity by angiotensin was mediated by aldosterone.

These findings are in contradiction with the hypothesis that aldosterone is involved in the inhibition of the renin-angiotensin system in patients with true primary hyperaldosteronism. However, there is a major difference between the present experiments and primary aldosteronism. The acute effect of one infusion of aldosterone differs greatly from the chronic effect of the excessive aldosternne secretion rate for periods of years in patients with primary

hyperaldosteronism. Nevertheless, it is possible that the hypervolemia reported in primary hyperaldosteronism might also be responsible in great part for the inhibition of the renin secretion and that aldosterone "per se" is not directly involved in the inhibitory process.

Although it appears that the mechanism of inhibition of angiotensin is not mediated through the aldosterone-stimulating effect or by the vasoconstrictor action on vessels, other indirect mechanisms cannot be excluded completely. For instance, angiotensin might produce changes in tubular fluid osmolality or ionic composition which could influence the secretion rate of renin. Since all subjects studied in the present work were already on severe sodium restriction prior to and during the experiment, little change occurred in urinary sodium during angiotensin infusion as illustrated in Figs 28 and 30. Moreover, in a nephrotic patient, as shown in Fig. 27, an increase in renin-activity occurred while the subject was showing an increase in creatinine clearance and during the period of natriuresis (Third sample). Nevertheless, minute changes at the site of the macula densa during angiotensin infusions cannot be eliminated as the primary factor in modifying the synthesis or release of renin.

Since the rates of infusion were generally less than 2 ml/min. and in many instances less than 1 ml/min., it is unlikely that the total amount of volume infused would be responsible for the changes in renin-activity. Moreover, similar infusions of aldosterone, nor-epinephrine and 5% dextrose in water, at identical rates failed to show any consistent effects on renin-activity.

The changes noted in renin-activity cannot be ascribed to diurnal variations since the same inhibition of renin-activity was not observed with aldosterone infusions and nor-epinephrine infusions given at the same period of

the day. Moreover, a previous study reported earlier in this thesis showed that renin-activity is quite stable throughout the day in patients maintained on a constant dietary sodium.

In some of the subjects who received angiotensin infusions, a marked orthostatic hypotension developed and persisted for many hours following the end of the infusion period. This aspect has not been investigated systematically, but this observation is of interest and deserves further investigation. Such an effect implies either an action of angiotensin on the peripheral sympathetic nervous system or on the central structures responsible for control of blood pressure in response to changes in position. This side-effect could be brought about by depletion of the peripheral stores of catecholamines or by the depletion of other neuro-transmitters in the central nervous system. Such an hypothesis fits into the concept of the relationship recently demonstrated between angiotensin and catecholamines.

This work suggests that angiotensin quite specifically and powerfully inhibits the renin-activity either by preventing its liberation or by inhibiting its synthesis in a manner that cannot be fully explained at present. It indicates that the mechanisms controlling renin secretion may be numerous and that the final secretion rate might result from the complex interaction of mechanical hemodynamic, and biochemical factors.

D - PLASMA ANGIOTENSINASE ACTIVITY.

In reviewing the medical literature, it is hard to define the role of angiotensinase(s) because of much confusion and conflicting results regarding the determination of angiotensinase activity in various diseases.

While in studies on cirrhotic and toxemic patients the angiotensinase activity was reported increased in most instances (137, 139, 777), many con-

flicting findings were reported in studies concerning hypertensive diseases (116, 137, 139, 544, 545, 547) and normal pregnancy (137, 139, 796). Most of these discrepancies may be due to the different methods used by the various workers. As an indication of the angiotensinase activity, Klaus et al (797) measure the liberation of free valine from the angiotensin molecule and Wolff and coworkers (798) calculate the loss of radioactivity of angiotensin I^{131} during incubation with plasma. However, these procedures give results which do not correlate with the loss of biological activity and therefore have little physiological meaning. Wood (547) incubates whole blood (30 ml) with a known amount of angiotensin for 10 minutes and appraises the angiotensinase activity by measuring the pressor response during a reinfusion of this blood into the patient. Such a procedure does not take into account the angiotensin responsiveness of the subject studied which, for instance, may be increased in patients with essential hypertension. Moreover, if renin is present in large amounts in the blood sample, a significant amount of angiotensin might be produced during incubation since large quantities of blood are used. Others utilize methods which are based upon the appraisal of inactivation of the pressor activity of angiotensin after incubation with plasma (29, 136-138). In all of these, angiotensin is incubated with diluted plasma so that the true physiological activity of angiotensinases may be decreased and modified. As a matter of fact, the half-life of angiotensin reported with these methods is relatively long compared to the half-life of angiotensin "in vivo". In the methodologies using longer incubation times (29, 136), the production of angiotensin during the period of incubation is most likely to occur especially with plasma containing large concentrations of renin. Although the physiological activity of the angiotensinases of the plasma occurs at 37°C "in vivo", Hickler and coworkers (137) measure the angiotensinase act-

ivity at approximately 25°C. Finally, none of the methods reported use siliconized glassware and therefore do not take into consideration the quantity of angiotensin that might be adsorbed during the procedure.

The main purposes of our study were, firstly, to develop a reproducible procedure for the estimation of angiotensinase activity in plasma under conditions as close as possible to those existing "in vivo" and, secondly, to investigate this parameter in various human diseases to determine if variations in angiotensinase activity might have any physiopathological significance.

1. METHODOLOGY.

a) Reagents and material:

- A solution of angiotensin (Hypertensin Ciba preparation) at a concentration of 2 µgm/ml., in ethanol 20% is used as a standard. This solution is kept at 0 to 5°C., in a siliconized flask.

- Glass centrifuge tubes (5 ml) are siliconized with a solution of 10% silicone in CCl4. and washed with distilled water 10 to 15 times. Thereafter, one ml (2 µgm) of the angiotensin solution is added to the tube and evaporated to dryness under an air jet. The siliconized tube containing 2 micrograms of dry angiotensin is closed with a rubber stopper until incubation with plasma.

- A half ml of the heparin solution (1 mg/ml) is added to a 40 ml polyethylene centrifuge tube and evaporated to dryness under an air jet.

b) Procedure:

i) Fifteen to 25 ml of blood are drawn from the brachial vein under vacuum into a 40 ml polyethylene tube containing 0.5 mg of dry heparin and immersed in crushed ice. The blood is centrifuged in a cold centrifuge at 13,000 R.P.M. for 10 minutes and the plasma is separated. Generally, the incubation takes place immediately following that step. Otherwise, the plasma can be kept in a deep freeze.

ii) For each incubation, 2 ml of cold plasma are added to a siliconized glass tube (5 ml) containing 2 µgm of dry angiotensin and kept in a cold room at 0 to 5°C. The tube is tightly closed with a rubber stopper and is transferred rapidly to a shaking bath oscillating at 40 to 50 strokes per minute and maintained at 37°C. Usually two incubations are done for each sample, one lasting 5 minutes and the other 10 minutes.

iii) At the end of the incubation period, 0.1 ml of saturated NaCl solution and 0.1 ml of glacial acetic acid are added to the medium and the incubation is stopped by immersing the tube in boiling water for approximately one minute. With this technique, a white granulated precipitate appears in the tube after 30 seconds of immersion in boiling water. When the tube is centrifuged, one milliliter of a clear supernate is obtained.

iiii) The angiotensin concentration of the supernate is estimated by a rat bioassay according to the four point assay. For comparison, the same standard solution of angiotensin is used.

Control incubations are done by transferring the tube, prepared to be incubated, immediately from the cold room to the boiling-water bath.

The results are expressed by the percentage of loss of angiotensin during the incubation time. For example, if after a 5 minute incubation the angiotensin concentration has decreased to 60% of the original concentration, the result will be expressed as a 40% loss or inactivation per 5 minutes of incubation.

c) Reliability of the method:

i) Recovery of angiotensin in siliconized and non-siliconized tubes:

Recovery experiments were done with plasma and saline in siliconized and non-siliconized glassware to determine the loss of angiotensin during the steps preceding or following the incubation and also to determine the degree

of the adsorption of angiotensin on non-siliconized glass. For that purpose, 2 ml of cold plasma or cold saline were added to siliconized or non-siliconized 5 ml centrifuge tubes containing 2 µgm of dry angiotensin kept in a cold room and then the tubes were immediately transferred to the boiling water bath.

After one minute in boiling water, the tubes were centrifuged and the angiotensin concentration was measured by rat assay. These results are reported in Table XXVII and expressed as the percentage of recovery.

TABLE XXVII

RECOVERY OF ANGIOTENSIN (2 µgm) IN SILICONIZED AND NON-SILICONIZED GLASSWARE DURING ANGIOTENSINASE PROCEDURE.

RECOVERY WITH 2 ml PLASMA		RECOVERY WITH 2 ml SALINE	
SILICONIZED	NON-SILICONIZED	SILICONIZED	NON-SILICONIZED
1. 100%	11. 60%	1. 100%	9. 75%
2. 100%	12. 75%	2. 100%	10. 90%
3. 100%	13. 90%	3. 100%	
4. 100%	14. 100%	4. 100%	
5. 100%	15. 75%	5. 100%	
6. 90%	16. 66%	6. 100%	
7. 100%		7. 100%	
8. 100%		8. 100%	
9. 100%			
10. 100%			

In all but one recovery experiment done in non-siliconized tubes a significant loss of angiotensin occurred either with plasma or saline. Moreover, the loss or adsorption of angiotensin was not constant and differed markedly

from one tube to one other. On the other hand, all the recovery experiments done in similar conditions with siliconized tubes showed a total recovery with both plasma or saline. Since these tubes were also immersed in boiling water, these experiments indicate that angiotensin is not lost during the precipitation of the plasma proteins.

ii) Pressor activity of the plasma during the period of incubation:

To determine if any endogenous angiotensin was produced during the short time of incubation used in this procedure, ten different plasma specimen were incubated at 37°C for periods of 30 and 15 minutes in siliconized tubes without angiotensin. The results of these experiments are reported in Table XXVIII.

TABLE XXVIII

PRESSOR RESPONSE OF PLASMA (2 ml) INCUBATED FOR 15 AND 30 MINUTES AT 37°C.

PLASMA NO.	TIME OF INCUBATION	PRESSOR-ACTIVITY
1.	30 min.	0
2.	30 "	0
3.	30 "	0
4.	30 "	0
5.	30 "	0
6.	30 "	0
7.	15 "	0
8.	15 "	0
9.	15 "	0
10.	15 "	0

Therefore, it seems that periods of only 5 to 10 minutes of incubation will not permit 2 ml of plasma to liberate a significant amount of pressor activity that might interfere with the activity of angiotensinase. In suppo-

sing that a plasma with a high renin-activity at 100 ng/l/min. is incubated in these conditions, one can calculate that only 1 ng will be liberated in the medium during a five-minute incubation period. This amount is negligible compared to the very large amount of angiotensin present in the medium during the determination of angiotensinase activity.

iii) Reproducibility:

To check the reproducibility of the results obtained with the present method, parallel incubations were done with plasma coming from the same source. This was repeated with 8 different plasmas and the results obtained are reported in Table XXIX.

TABLE XXIX

REPRODUCIBILITY OF ANGIOTENSINASE ACTIVITY* IN PARALLEL INCUBATIONS.

PLASMA NO.	5 min.	10 min.	15 min.
1. A	40%	60%	68%
B	40%	56%	68%
2. A		60%	65%
B		60%	65%
C		60%	60%
D		60%	65%
3. A		75%	80%
B		70%	85%
4. A		60%	
B		55%	
5. A		70%	
B		70%	
6. A		65%	
B		65%	
7. A		70%	
B		72%	
8. A		70%	74%
B		70%	75%

* Results are expressed as percentage of inactivation or loss of angiotensin.

As shown, the reproducibility of this method is satisfactory and the results do not vary more than 5% when one or more parallel incubations of plasma coming from the same source are done.

iiii) Effect of temperature on angiotensinase activity during storage of plasma:

Hickler and coworkers (137) have pointed out in their publication that the activity of the angiotensinases was destroyed when the plasma was stored at -17°C. This observation is quite surprising and differs greatly from what is generally observed with other enzymes. In order to check this point, one sample of plasma was separated into two aliquots. One of these was stored at 0-4°C., and the other at -17°C., for 6 weeks. At the end of that period, parallel incubations were done with plasma from each aliquot in the presence of 2 µgm of angiotensin. The results of this experiment are reported in Table XXX.

TABLE XXX

EFFECTS OF TEMPERATURE ON ANGIOTENSINASE ACTIVITY* DURING STORAGE

	INCUBATION NO.	INCUBATION TIME	
		10 min.	15 min.
PLASMA KEPT AT 0-4°C			
	1.	60%	65%
	2.	60%	65%
	3.	60%	60%
	4.	60%	65%
PLASMA KEPT AT -17°C			
	5.	75%	80%
	6.	70%	85%

* Values expressed in percentage of loss or inactivation of angiotensin.

From the preceding data, it is obvious that freezing temperature does not destroy the angiotensinase activity, but on the contrary, it seems to protect it. This finding is in agreement with those reported concerning the storage of other enzymes.

iiiii) Effect of EDTA on angiotensinase activity:

Bumpus and coworkers (102) claimed that EDTA was a specific inhibitor of plasma angiotensinase. This point was also investigated with the present methodology. For that purpose, blood coming from the same source was drawn in two different polyethylene tubes, one containing dry heparin and the other one, 7 mg of the ammonium salt of ethylene-diamino-tetraacetic acid (EDTA). This experiment was repeated with nine different blood samples. The results of these studies are reported in Table XXXI.

TABLE XXXI

EFFECT OF EDTA (AMMONIUM SALT) ON ANGIOTENSINASE ACTIVITY*

SAMPLE NO.	HEPARIN INCUBATION TIME 10 min.	15 min.	EDTA INCUBATION TIME 10 min.	15 min.
1.	50%	68%	0%	0%
2.	52%		0%	
3.	70%		40%	
4.	50%	70%	40%	40%
5.	48%	60%	0%	20%
6.	48%	70%	0%	8%
7.	68%	76%	0%	20%
8.	60%		60%	80%
9.	48%	73%	40%	

* The angiotensinase activity is expressed in percentage of angiotensin inactivation.

Although the angiotensinase activity was totally inhibited by EDTA in a few instances, it was only partially inhibited or unaffected in four other instances. After 15 minutes of incubation, the total inhibition persisted in only one sample, while in all the others which were presenting a total inhibition during a 10 minute incubation period, a slight inactivation occurred.

2. DETERMINATION OF PLASMA ANGIOTENSINASE-ACTIVITY IN VARIOUS CONDITIONS.

The results to be reported constitute preliminary observations in a limited number of subjects.

a) In normal subjects:

Ten normal subjects were studied for the determination of angiotensinase-activity after incubations of 5, 10 and 15 minutes. All these subjects were normotensive and did not have any history of serious illness. Most of them were eating unrestricted diets at the time of the blood sampling. The values of angiotensinase activity in these subjects are reported in details in Table XXXII.

These results show that the mean "in vitro" half-life of angiotensin in the plasma of normal subjects is about 5 minutes. The difference between the findings after 10 and 15 minute incubations is slight, indicating that a plateau is reached in the rate of inactivation between 10 and 15 minutes. The percentage of inactivation varies between 40% and 60% after 5 minutes of incubation, between 60% and 80% after 10 minutes and between 68% and 80% after 15 minutes of incubation.

The mean curve of inactivation is compared with that of other conditions in Fig. 33.

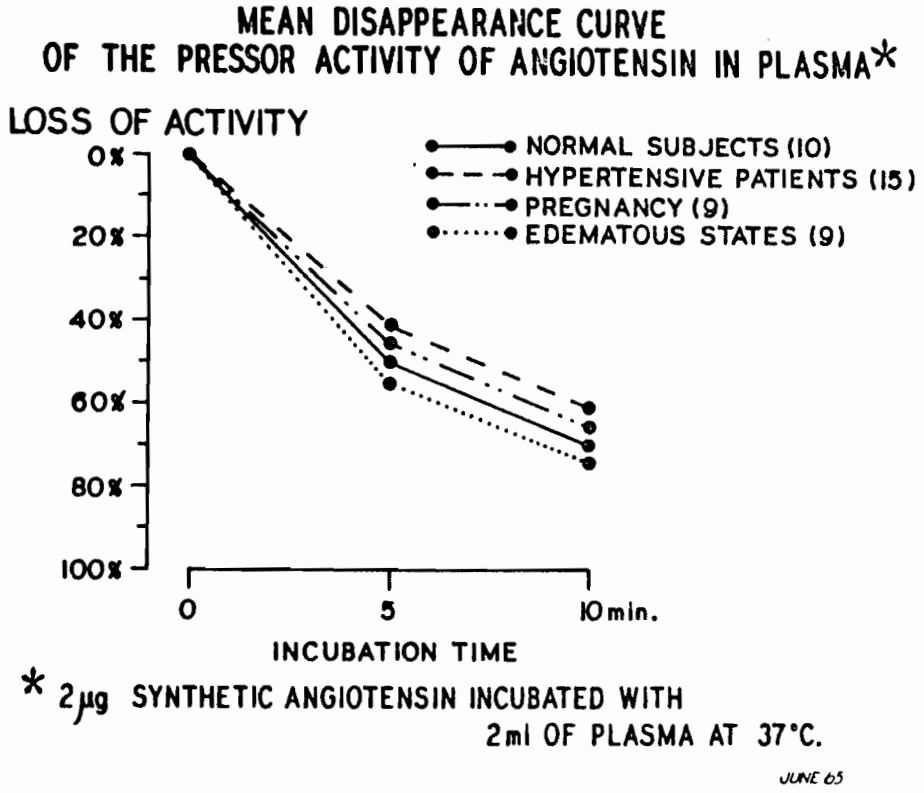


Fig. 33

This figure illustrates the mean curve of inactivation of pressor activity of angiotensin during the incubation with plasma from various groups of subjects. The number of subjects is given within brackets. The mean curve of hypertensive patients is the only one differing significantly from the normal curve ("p" < 0.05). Details of the statistics and the results of each subject are given in Tables XXXII, XXXIII, XXXIV and XXXV.

TABLE XXXII

ANGIOTENSINASE-ACTIVITY* IN NORMAL SUBJECTS.

NAME AND AGE	TIME OF INCUBATION		
	5 min.	10 min.	15 min.
1. G.T. 26		66%	68%
2. M.B. 52	a) 45%	60%	70%
	b) 50%	64%	73%
3. R.B. 32	50%	70%	70%
4. J.de C. 27	60%	76%	80%
5. R.B. 27	60%	80%	80%
6. C.G. 26	48%	70%	
7. M.O. 28	50%	70%	
8. L.B. 25	40%	70%	
9. M.D. 23	48%	70%	
10. G.G. 27	46%	60%	
NUMBER:	(9)	(10)	(5)
MEAN:	50.0%	69.4%	74%
STANDARD DEVIATION:	±6.4%	±5.8%	±5.6%
STANDARD ERROR:	±2%	±1.8%	±2.5%

* Expressed as percentage of angiotensin inactivation.

b) In hypertensive subjects:

Fifteen subjects with hypertension of various etiologies were studied in similar conditions. Since only a few subjects have been studied at present, these are reported as a single group in Table XXXIII.

These results indicate that, although most of the hypertensive patients fell within the normal range, the mean percentage of inactivation for incubation times at 5 and 10 minutes are significantly lower than normal with a

TABLE XXXIII

ANGIOTENSINASE-ACTIVITY* IN HYPERTENSIVE SUBJECTS.

NAME AND AGE	B.P. mm Hg	TIME OF INCUBATION 5 min.	10 min.	15 min.	DIAGNOSIS
1. H.A. 49	170/100	-	60%	70%	Essential Ht.
2. M.L. 58	190/100	40%	65%	80%	" "
3. D.M. 43	170/97	40%	72%	-	" "
4. R.B. 32	240/140	36%	-	70%	" "
5. A.P. 50	160/90	50%	68%	-	" "
6. A.L. 61	160/100	-	60%	70%	" "
7. A.M. 52	150/95	a) 50% b) 40% c) 40%	65% 62% 60%	75% 70% -	" "
8. J.M. 52	220/130	52%	70%	-	" "
9. R.H. 58	160/100	20%	50%	-	" "
10. O.S. 26	220/125		70%	-	" "
11. M.S-J.18	170/90	36%	48%	-	Polycystic Kid.
12. L.B. 56		46%	60%	-	Renal Art. Sten.
13. R.L. 31	200/125	-	48%	70%	" " "
14. J.P. 35		-	60%	-	" " "
15. D.M. 67	162/92	50%	70%	76%	" " Thromb.
NUMBER:		(9)	(14)	(7)	
MEAN:		41.5%**	61.0%**	73%	
STANDARD DEVIATION:		±9.4%	±8.1%	±3.7%	
STANDARD ERROR:		±3.1%	±2.2%	±1.4%	

* Percentage of angiotensin inactivation.
** Singificantly different from normal $P < 0.05$.

"p" value less than 0.05. In hypertensive patients, the range for 5 minute incubations stands between 20% and 52% but only 4 out of 9 had a percentage of inactivation above 45%, while in normal subjects 8 out of 9 were above that value. The range of values for 10 minute incubations was found between 48% and 72% with only 4 out of 14 at, or above, 70% of inactivation whereas 7 out of 10 normal subjects had values equal to, or above 70%. After the incubation period of 15 minutes, the values are almost identical with the values found in normal subjects. The mean curve given by these values is illustrated in Fig. 33 in comparison with other groups of subjects.

c) In pregnancy:

Nine normotensive pregnant women had a determination of the angiotensinase activity during their pregnancies. The values obtained are reported in Table XXXIV, as well as the time of pregnancy at which the blood sample was withdrawn.

TABLE XXXIV

ANGIOTENSINASE-ACTIVITY* IN NORMAL PREGNANT WOMEN

NAME AND AGE	WEEKS OF GESTATION	TIME OF INCUBATION 5 min.	10 min.
1. A.N. 29	4½	40%	74%
2. J.M.B. 32	17	50%	-
3. J.B. 18	22	50%	-
4. J.T. 15½	22	-	56%
5. L.B. 21	23½	40%	-
6. A.P. 29	29½	40%	60%
7. J.C. 25	36	40%	71%
8. G.P. 22	38	45%	60%
9. J.B. 26	40	60%	-
	NUMBER:	(8)	(5)
	MEAN:	45.6%	64.2%
	STANDARD DEVIATION:	±7.2%	±8.1%
	STANDARD ERROR:	±2.8%	±3.3%

* Percentage of angiotensin inactivation.

Although the number of subjects is limited in this study, it seems that the angiotensinase-activity does not increase with the progression of the pregnancy. The mean values of 5 and 10 minute incubations do not differ significantly from the mean values obtained with plasma of normal subjects. The mean curve is illustrated in Fig. 33.

d) In edematous states:

Three edematous patients with congestive heart failure, three with nephrotic syndrome and three others with liver cirrhosis and ascites were studied before treatment and in some instances, during treatment and after relief of edema. The values of angiotensinase-activity measured in these patients are reported in Table XXXV, as well as the renin activity at the time of sampling.

Each one of the previous patients were studied during the course of their treatment and had simultaneous determinations of their renin-activity. Most of them had more than one determination. From these results, it appears that most of these patients have an angiotensinase activity within the normal range at the exception of 1 cardiac (N.T.) and 2 cirrhotic patients (J.G.C., C.V.) who have higher than, or at the upper limit of normal. No direct relationship could be found between the renin-activity levels and angiotensinase-activity.

e) In salt depletion:

The finding that renin-activity increased during salt depletion without increasing the blood pressure led us to investigate whether angiotensinase-activity was involved in that mechanism.

Three patients were studied before and after sodium depletion induced by the restriction of sodium intake for three days (10 mEq Na, 90 mEq K diet) and the administration of a natriuretic drug (Chlorthalidone, 100 mg) on the

TABLE XXXV

PLASMA ANGIOTENSINASE-ACTIVITY AND RENIN-ACTIVITY IN EDEMATOUS STATES

NAME AND AGE	ANGIOTENSINASE ACTIVITY* TIME OF INCUBATION			RENIN ACTIVITY**
	5 min.	10 min.	15 min.	
CARDIAC				
G.V. 55		70%		300
	46%	70%	75%	8
	40%	66%		13
	40%	70%		0
N.T. 52	80%	85%		53
	64%	75%		58
J.H. 72	50%	60%	74%	19
	54%	65%	75%	21
	60%	64%	80%	33
NEPHROTIC				
R.A. 29	52%			49
	48%	70%		194
F.V. 44	40%	60%		0
	40%	60%	72%	7
	46%	66%	70%	11
M.G. 69	52%	80%		50
CIRRHOTIC				
J.G.C. 51	70%	85%		116
	81%	87.5%		50
	60%	70%	75%	100
R.S. 36	50%			68
	52%	80%		38
C.V. 57	40%	70%	85%	23
	60%	85%	88%	20
	60%	80%		35
	60%	80%		23
	55%			31
	56%			29

* Expressed as percentage of inactivation.
** Ng/L/min.

first day of the diet. The results of these studies are reported in Table XXXVI.

TABLE XXXVI

EFFECT OF SODIUM DEPLETION ON ANGIOTENSINASE-ACTIVITY*

NAME		ANGIOTENSINASE ACTIVITY* TIME OF INCUBATION		RENIN-ACTIVITY**
		5 min.	10 min.	
J.A.L.	BEFORE	60%	70%	24
	AFTER	50%	70%	268
G.G.	BEFORE	46%	60%	36
	AFTER	46%	56%	350
R.H.	BEFORE		50%	16
	AFTER		50%	56

* Expressed as percentage of inactivation.
** Ng/L/min.

These experiments clearly indicate that angiotensinase activity does not participate in the changes found in the activity of the renin-angiotensin system during sodium restriction or depletion. Once more, these studies show that the angiotensinase activity is not related to the level of renin-activity.

3. DISCUSSION:

a) Methodology:

The procedure developed for the determination of angiotensinase activity is based on the rate of inactivation of pressor activity of angiotensin during "in vitro" incubation with plasma under optimum conditions. This method differs from those previously discussed (29, 136-138) by the use of siliconized glassware, by the appraisal of activity in undiluted plasma and by a shorter

period of incubation at 37°C.

The mean biological half-life of angiotensin with this procedure was found to be about 5 minutes for normal plasma. This rate of inactivation is much greater than those reported with any other methodology and is closer to the rapid rate of inactivation observed "in vivo".

Angiotensin may be adsorbed on glass during manipulation. On the other hand, when the glassware is siliconized, the angiotensin can be manipulated and heated without any loss of activity (Table XXVII). Since the adsorption on non-siliconized glass is not constant, it was found necessary to siliconize the glassware during this procedure in order to eliminate an important source of error in the interpretation of the rate of inactivation.

The use of undiluted plasma presents a problem for such a methodology since one step of the procedure consists of stopping the reaction by boiling the plasma. Generally, after heating, the plasma forms in a thick coagulum from which it is almost impossible to separate a liquid supernate even after prolonged and high speed centrifugation. The addition of 0.1 ml of saturated NaCl solution and 0.1 ml of glacial acetic acid before boiling permitted the formation of a white granulated precipitate in the plasma from which it was easy to obtain a clear supernate even after low speed centrifugation.

The short time of incubation with a small volume of plasma does not allow significant production of pressor material by the plasma during the incubation period so that there is no interference with the manifestation of the activity of angiotensinases.

All these factors contribute to give to this method a high degree of reproducibility. Moreover, in this procedure are realized conditions which are closer to those existing "in vivo". Therefore, the results obtained are closer to the physiological activity of these enzymes.

b) Results:

The results reported with this procedure are still limited to a relatively small number of observations.

The finding that the ammonium salt of EDTA totally inhibits the angiotensinase-activity of some plasmas while it only partially inhibits the activity of others, strongly suggests that there probably exist many kind of angiotensinases, some of them inhibited by EDTA and some not. Moreover, many workers have shown that angiotensin can be inactivated by various proteolytic enzymes (See review of the literature on angiotensinase). Therefore, it is likely that the destruction of angiotensin in the plasma might also be assumed by the combined action of several non-specific proteolytic enzymes circulating in the blood. In that respect, the designation of the inactivation of angiotensin by the term "angiotensinase-activity" is preferable until the proof of the existence of a specific enzyme is brought forth.

A relatively large range of activity was observed in normal subjects. Most of the hypertensive, pregnant and edematous patients presented an angiotensinase-activity within the normal range. However, this activity was generally in the low normal range in many hypertensive subjects and definitely lower than normal in some of them. The measurement of angiotensinase-activity in hypertensive patients has given rise to much disagreement among the reports of the several groups of investigators. In essential hypertension, it was found to be normal (137, 139, 544, 545), increased (116, 137) or decreased (547). It was also reported to be normal (545) or increased (137) in renal artery stenosis, normal (545) or increased (137) in malignant hypertension, normal in pyelonephritis (137, 139) and increased in chronic glomerulonephritis (137).

The small number of patients reported in our study does not permit a

classification of these into various groups of hypertensive diseases. However, the present findings indicate that the angiotensinase activity is decreased in some hypertensive patients. The mean value of inactivation in this group is significantly lower than normal after 5 and 10 minute incubation periods. This preliminary information suggests that in some hypertensive patients, angiotensin might be more "active" because of a slower rate of inactivation. Therefore, it could be postulated that in these patients, a normal renin secretion might possibly induce a rise in blood pressure. Nevertheless, more work is needed along these lines before coming to a final conclusion.

In pregnancy, the angiotensinase activity was not significantly different from that of normal subjects. Our findings are in agreement with the findings of Klaus (132) and Biron (796), but disagree with those of Hickler et al (137) and Page (136) reporting an increased angiotensinase-activity in this condition.

In edematous patients, the angiotensinase activity was found elevated in 1 out of 3 cardiac patients, elevated or at the upper limit of normal in 2 out of 3 cirrhotic patients, but normal in all 3 nephrotic patients studied. The finding of an elevated angiotensinase activity in cirrhotic patients has already been reported by Klaus (132), Hickler (137), Biron (797) and their coworkers. This observation might explain the discrepancy, reported previously in the section of this thesis on edematous conditions, concerning the levels of renin-activity and angiotensin in cirrhotic patients. Such an increase in angiotensinases in this condition might also explain the low incidence of hypertensive diseases in these subjects.

Angiotensinase-activity is not apparently influenced by the levels of renin-activity since no correlation could be found between renin-activity and angiotensinase-activity. Moreover, the angiotensinase-activity does not

respond to the same stimuli as renin-activity. This is shown in experiments reporting the simultaneous determination of renin-activity and angiotensinase activity during sodium depletion (Table XXXVI). During this condition, the renin-activity increased markedly while the angiotensinase-activity did not change significantly.

In conclusion, although this study is only preliminary, there is some indication that the variations in angiotensinase-activity might be of some physiopathological significance in some hypertensive diseases and in diseases associated with disturbed liver function such as cirrhosis and congestive heart failure. Therefore, more studies are needed to confirm and clarify this finding and it is hoped that the present methodology will aid in the understanding of this aspect of the renin-angiotensin system by providing more consistent results.

GENERAL DISCUSSION

Little is known concerning the kinetics of the renin-angiotensin system and about the factors which enhance or inhibit the renin-substrate reaction, the converting enzyme, the inactivation of angiotensin and its binding at receptor sites.

The method used in our studies for the determination of renin-activity is based on the summation of all the factors present in plasma.

The present work was designed for the study of the physiological and pathological roles of the renin-angiotensin system in man. Conditions affecting sodium balance and those associated with hypertensive diseases constituted the two major fields of investigation. In general, our findings indicate that the activity of the renin-angiotensin system is more closely related to the state of sodium balance THAN to the level of blood pressure.

A very close inverse relationship was established between renin-activity and sodium intake or excretion. It was also demonstrated that in some instances, renin-activity was more closely related to sodium balance than was aldosterone and that the rise in renin-activity clearly preceded the rise in aldosterone in response to Na restriction or depletion in at least 3 instances. These observations strongly suggest that the renin-angiotensin system is an important factor in the regulation of sodium balance either by a direct action on the kidney tubules or by stimulating the secretion of aldosterone or both. This relationship with sodium appears to be quite specific since changes in dietary potassium do not significantly modify the response of renin-activity to sodium as illustrated by the studies with potassium loading or depletion by fasting.

In pregnancy, a condition associated with increased blood volume, sodium retention and increased aldosterone secretion, the renin-angiotensin system

is hyperactive. The renin-activity is also found increased in most conditions associated with generalized edema, especially when due to congestive heart failure. On the other hand, angiotensin is elevated almost exclusively in patients with cardiac failure and edema while in nephrotic and cirrhotic edema it is undetectable even in presence of an elevated renin activity. These discrepancies illustrate the complexity of the factors regulating the renin-substrate reactions and our lack of knowledge on this aspect. Nevertheless, our findings suggest an active participation of the renin-angiotensin system in the formation of edema. It is also probable that the renin-angiotensin system may be the factor maintaining the state of hyperaldosteronism encountered in most of these conditions. However, it is difficult to reconcile our findings of high angiotensin levels in most cardiac patients as well as low levels in most cirrhotic patients with the studies of others reporting a moderately increased aldosterone excretion and secretion in only 50% of cardiac patients and a markedly increased aldosterone excretion and secretion in most cirrhotic patients. During appropriate treatment and relief of edema, renin-activity and angiotensin vary in opposite directions in cardiac patients when compared to nephrotic patients. During improvement, cardiac patients showed a marked decrease in renin activity or angiotensin levels while the opposite was observed in nephrotic patients. These differences suggest a basic difference in the pathogenesis of edema in these two conditions.

Since angiotensin is detected in greater concentration in cardiac patients with low cardiac output and greatly increased peripheral resistance, it is possible that in these conditions angiotensin participates in the maintenance of "normal" blood pressure by increasing the peripheral resistance.

The only hypertensive patients who presented high renin activity with few exceptions, were those with renal artery stenosis and renal polycystic

diseases. Most patients with essential hypertension, parenchymatous renal diseases, coarctation of the aorta, and malignant hypertension had levels within the normal range. Moreover, no correlation could be established between level of blood pressure or severity of hypertension and level of renin-activity. These findings indicate that in most hypertensive diseases, the renin-angiotensin system does not appear to play a major role.

In the present study, many normotensive subjects were found to have markedly elevated renin activity levels during sodium depletion without any increase in blood pressure. This observation raises many questions concerning the role of the renin-angiotensin system in the control of blood pressure or in the pathogenesis of hypertension. However, these discrepancies cannot be resolved without a better understanding of the factors regulating pressor responsiveness or "vascular reactivity" to angiotensin. Search of the factors preventing the vaso-pressor effect of high angiotensin circulating levels is of fundamental importance.

The findings of the specific inhibitory effects of angiotensin on renin activity support the possibility of a humoral mechanism controlling renin secretion. There are many other mechanisms already postulated for the control of renin secretion. Among the other possible factors, the effect of volume or perfusion pressure at the site of the afferent arterioles is strongly supported. The existence of receptors sensitive to changes in osmolarity or in ionic content of the tubular fluid at the site of the macula densa has also been postulated in recent years and has received support from many indirect observations, one of them being the actual finding of a close inverse relationship between urinary sodium and peripheral renin activity. The recent findings of undetectable or minimal renin-activity in the blood of patients with primary hyperaldosteronism would also suggest the inhibition of the renin-angiotensin

system under these conditions. Finally, it is not possible to reject completely a role of the sympathetic nervous system in the control of renin secretion because of the repeated descriptions of numerous non-myelinated fibers around the juxtaglomerular cells and the recent findings of the significant decrease in renin content and J.G.I. in denervated kidneys. Therefore, it would appear that the control of renin may be the result of a complex interaction among all these factors.

The application of a new procedure for the determination of angiotensinase may help to shed light on this so controversal aspect of the renin-angiotensin system. The preliminary findings reported in this thesis indicate that the activity of angiotensinases might have some physiopathological significance in the pathogenesis of some hypertensive diseases.

The present findings strongly support important physiological and pathological roles for the renin-angiotensin system. Many aspects remain unclarified however, and require much more study. For instance, the mechanism of action of angiotensin, its relationship to the nervous system, the determinants of "angiotensin responsiveness", the kinetics of the renin-substrate reaction and the role of the macula densa in controlling renin secretion, all represent the large number of "unknowns" awaiting further investigation.

CONCLUSION

Measurement of arterial angiotensin, of peripheral blood renin-activity and of angiotensinase activity in various physiological and pathological conditions in man led to the following conclusions:

1) Peripheral plasma renin activity is detectable in normal subjects.

2) There is no difference between arterial and venous blood renin activity.

3) A close and significant inverse relationship exists between renin activity and sodium intake or excretion in normal and benign hypertensive patients. The renin activity levels increase with the severity of sodium depletion.

4) Renin-activity and aldosterone excretion vary in parallel in many physiological and pathological conditions in response to the same stimuli.

5) During the first and second trimester of pregnancy, there is a progressive and significant increase in renin activity which thereafter remains elevated until delivery.

6) In cardiac patients with valvular lesions and without edema or hypertension, angiotensin levels were detectable or elevated only in patients with greatly increased peripheral resistance.

7) In most of the edematous cardiac, cirrhotic patients and in half of the nephrotic patients, renin activity is elevated prior to treatment. Angiotensin levels were significantly and consistently increased only in cardiac edematous patients. During treatment and relief of edema, both renin activity and angiotensin levels decrease in cardiac patients, while they increase in nephrotic patients. In cirrhotic patients renin activity and angiotensin do not change consistently.

8) Renin activity is within normal range in 43 out of 49 patients with

essential hypertension, in 5 out of 7 with chronic glomerulonephritis, in 9 out of 10 with chronic pyelonephritis, in 3 out of 3 with coarctation of the aorta and in 2 out of 3 with malignant hypertension. On the other hand, this parameter is increased in most patients with renal artery stenosis and polycystic kidney diseases.

9) Angiotensin given intravenously for periods of 2 to 4 hours at pressor or non pressor rates markedly decreases renin-activity. Similar infusions of aldosterone at high doses and of nor-epinephrine at pressor rates are without effect.

10) The procedure developed for the determination of angiotensinase activity is reliable.

Preliminary findings reported with this procedure indicate that angiotensinase activity is decreased in some hypertensive patients and is increased in some patients with cirrhosis of the liver.

In summary, it appears that the renin-angiotensin system

a) has a major role in sodium regulation,

b) varies in parallel with aldosterone and is probably the major stimulant of aldosterone,

c) and is probably the mechanism by which blood pressure is increased in cases of true renovascular hypertension and polycystic kidney diseases.

CLAIM OF ORIGINALITY

The findings reported in this thesis are original insofar as they extend our knowledge and contribute to a better understanding of the physiological and pathological role of the renin-angiotensin system in the regulation of sodium in normal human conditions and in states accompanied by secondary hyperaldosteronism, by edema, or by increased blood pressure. Prior to this work, nothing was known concerning the following points: 1) The close inverse relationship between sodium intake or excretion and the peripheral renin-activity, 2) The levels of angiotensin and renin-activity in conditions of generalized edema and their variations in response to treatment, 3) The peripheral plasma renin-activity in normal pregnancy, 4) The specific inhibitory effect of angiotensin on renin secretion. Moreover no satisfactory and reliable methodology for the determination of angiotensinase activity was available.

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