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SODIUM AND WATER IN RATS MADE
HYPERTENSIVE BY SUBTOTAL NEPH-
RECTOMY.

The Ohio State University, Ph. D., 1960
Physiology

University Microfilms, Inc., Ann Arbor, Michigan

EXCRETION OF SODIUM AND WATER IN RATS MADE HYPERTENSIVE
BY SUBTOTAL NEPHRECTOMY

DISSERTATION

Presented in Partial Fulfillment of the Requirements
for the Degree Doctor of Philosophy in the
Graduate School of The Ohio State
University

By

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The Ohio State University
1960

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ACKNOWLEDGMENT

I wish to express my profound gratitude to Dr. Leo A. Sapirstein for the advice and encouragement which I received from him during my training at Ohio State University and afterwards. I also wish to acknowledge the help and instruction given by the various members of the Department of Physiology. I am also very grateful for the assistance by Dr. Edward J. Reininger who determined the blood pressures in this study.

TABLE OF CONTENTS

LIST OF TABLES	Page v
CHAPTER	
I INTRODUCTION	3
Preface	1
PART ONE: GENERAL CONSIDERATIONS	
II PHYSIOLOGICAL AND PATHOLOGICAL CONSIDERATIONS IN HYPERTENSION	7
Hypertension Defined	7
Peripheral Resistance and Cardiac Output	8
Clinical Manifestations	9
Pathology of Essential Hypertension	9
Etiological Factors	10
Neurogenic Mechanisms	11
Endocrine and Hormonal Factors	12
Renal Factors	17
PART TWO: REVIEW OF SODIUM-HYPERTENSION LITERATURE	
III SODIUM-HYPERTENSION RELATIONSHIPS	23
Relationship Between Dietary Sodium and Hypertension	23
Sodium Content of Plasma and Body Fluids in Hypertension	26
Renal Excretion of Sodium and Water in Hypertension	29
Relationship of Sodium Factors of the Adrenal Cortex to Hypertension	36
Conclusions	37
PART THREE: METHODS	
IV SODIUM AND WATER EXCRETION IN RATS MADE HYPER- TENSIVE BY SUBTOTAL NEPHRECTOMY	39
Introduction	39
Methods	39

TABLE OF CONTENTS (Continued)

CHAPTER		Page
V	THE CONCEPT OF "FREE SODIUM"	45
VI	RESULTS	50
	PART IV: DISCUSSION	
VII	THE SIGNIFICANCE OF ALTERATIONS OF "FREE SODIUM" IN HYPERTENSION	56
VIII	"FREE SODIUM" - A COMPARISON OF THE RESULTS OBTAINED IN THE PRESENT EXPERIMENT WITH DATA FROM OTHER EXPERIMENTS.	63
IX	POSSIBLE MODES OF ACTION OF SODIUM IN PRODUCING HYPERTENSION	73
	Sodium Retention Causes an Increased Extra- cellular Volume which Results in Hyperten- sion	75
	The Effect of Sodium of the Arteriole	81
X	NEUROGENIC FACTORS IN HYPERTENSION	89
XI	EFFECTS OF OSMOLARITY ON THE NEUROGENIC STAGE OF HYPERTENSION - A NEW HYPOTHESIS	94
	Intracarotid Injections of Hypertonic Solutions	96
	Changes of Body "Free Sodium" Only Affect Blood Pressure of Hypertensive Individuals	97
	The Extra-Renal Pressor System of Late Hypertension Depends Upon the Presence of a Renal Abnormality	100
	Conclusions	101
XII	SUGGESTIONS FOR ADDITIONAL STUDIES ON <u>HYPER-</u> <u>TENSION</u>	103
XIII	SUMMARY AND CONCLUSIONS.	106
	BIBLIOGRAPHY	109
	AUTOBIOGRAPHY	121

LIST OF TABLES

	Page
1. Urinary Sodium and Water Losses by Normal and Hypertensive Rats During a 12-13 Hour Metabolic Period	52
2. Urinary Sodium and Water Losses by Normal and Hypertensive Rats During a 6-Hour Metabolic Period After Loading With .3, .9 and 1.5% NaCl solution	53

EXCRETION OF SODIUM AND WATER IN RATS
MADE HYPERTENSIVE BY SUBTOTAL NEPHRECTOMY

PREFACE

When the experimental work reported in this thesis was started, many lines of evidence suggested that an increase in body sodium caused hypertension. At the time of publication of some of the results, the writer felt that this hypothesis had to be modified. Instead of considering that increased body sodium alone was the cause of hypertension, it was felt that the abnormal changes of hypertension would be better described by considering sodium metabolism in relation to that of water.

In the time that has elapsed since this original formulation, it has become apparent that the relationship between sodium and hypertension, whether sodium is considered alone or in relation to water is a weak one. Yet, weak as it is, a relationship appears to exist, and it seems premature to discard the hypothesis solely because the mechanism of the relationship is not understood.

For this reason, an attempt has been made in this thesis to review the literature bearing on the question of this relationship. The relevant existing information has been considered, and an attempt made to formulate new hypotheses consistent with what is known.

Judging from past experience in this field, it seems unlikely that the new hypotheses will be successful in accounting for all the phenomena of hypertension. They cannot be considered answers to the

problem but are merely suggestions for future research which may be profitable.

Probably a novel approach to the problem is necessary, one which depends upon finding answers to completely different questions from those that have been asked up to this time rather than seeking the answers to the older ones. The author asks the indulgence of the readers if from time to time he seems to be spending more energy in evaluating and synthesizing the work of others than in describing his own. This is the result of his feeling that instead of working with a weak hypothesis, uncritically, one should first attempt to revise the hypothesis. This involves becoming familiar with other aspects of the field before proceeding with further experimental work.

CHAPTER I

INTRODUCTION

The experimental work presented in this thesis was done in 1953. At that time I was working on the general problem of sodium excretion patterns of the normal rat under various concentrations and loads of sodium chloride. With this as a background, it seemed reasonable to study the patterns of sodium excretion in the hypertensive animal in which many lines of evidence (see below) suggested that an abnormality in this respect might exist.

The belief that further studies of sodium metabolism were necessary to describe the pathogenesis of hypertension was based mainly on three lines of evidence. Studies in the literature showed:

- (1) Dietary intake of salt affects hypertension. A high intake produces increased blood pressure while sodium restriction lowers blood pressure.
- (2) Increased concentrations of sodium have been found in the plasma and body tissues of hypertensive man and animals.
- (3) Sodium excretion by the kidney has been reported to be increased in hypertension.

Even these generalities are controversial and to clarify the status of the problem it will be necessary to review the literature in some detail.

In most of the reported work there is an implicit assumption that excessive body sodium is directly related to hypertension. The precise nature of this relationship has not been defined. Several logical possibilities exist; (1) Excess sodium in a part of or the whole body may be the sole and sufficient cause of hypertension (2) Sodium excess

may be a necessary but not sufficient condition for a chain of events leading to hypertension. The initiating factor being something else.

(3) Sodium excess may only be a consequence of hypertension which bears no important relationship to the etiology or pathophysiology of the disease process, similar to the sodium excess of edema due to heart failure. (4) The abnormalities in sodium metabolism may be incidental findings resulting from the experimental manipulations used to produce the hypertension.

Discarding (3) and (4) for the moment as trivial, the strongest formulation of (1) and (2) consistent with available evidence is as follows: Excess body sodium plays a role in the production and/or maintenance of hypertension. This hypothesis will be referred to in the remainder of this thesis as the "sodium hypothesis".

Despite the implicit acceptance of the "sodium hypothesis" by most of those who have worked in this field, there is substantial evidence which indicates that the elimination of sodium is in excess of normal in hypertension. The work reported in this thesis was begun in an attempt to resolve this seeming inconsistency.

As will be shown below, the apparent inconsistency disappears when the metabolism of sodium is described in relation to that of water rather than in absolute terms. It will be shown that most of the results reported previously as well as the experimental findings described here are consistent with the hypothesis that the excretion of sodium is defective when it is compared with the excretion of water in hypertension.

Though this modified "sodium hypothesis" fits the known facts better than the original one, some facts are not included in it. These will be discussed in a separate section.

The organization of the thesis is as follows:

Part I General physiological and pathological considerations in hypertension

Part II Review of sodium-hypertension literature

Part III Experimental Approach - Methods, Free Sodium, Results

Part IV Discussion

- A. "Free Sodium", a comparison of the results obtained in the present experiment with data from other experiments.
- B. "Free Sodium" and other factors involved in the mechanism of hypertension.
- C. Possible modes of action of sodium in producing hypertension.
- D. Osmolarity and its possible relationship to neurogenic hypertension.
- E. Suggestions for additional studies on hypertension.

PART ONE

GENERAL CONSIDERATIONS

CHAPTER II

PHYSIOLOGICAL AND PATHOLOGICAL CONSIDERATIONS IN HYPERTENSION

Hypertension Defined

Hypertension refers to an abnormal elevation of blood pressure and may be caused by many disease processes. Therefore, the term essential hypertension was introduced to designate a more or less specific disease entity in which the blood pressure is elevated in the absence of known causes of increased blood pressure such as pheochromocytoma, coarctation of the aorta, or renal disease, i.e. glomerulonephritis or pyelonephritis. Essential hypertension usually occurs in individuals past the fourth decade in life, although it is sometimes seen before this.

The fundamental hemodynamic alteration in essential hypertension is the finding of an increased peripheral resistance. The basal cardiac output remains unchanged (1). As a consequence of this increase in peripheral resistance, the systemic blood pressure is elevated. In practice, since cardiac output is not usually measured, the diagnosis of essential hypertension is made when the systolic pressure exceeds 150 mm Hg and the diastolic pressure exceeds 90 mm. Hg. It should be noted that the rise in diastolic pressure is of more significance than the change in systolic pressure since the latter may be increased by a variety of factors which decrease or have no effect on peripheral resistance such as changes in the elasticity of the

aorta with aging, aortic insufficiency, thyrotoxicosis, anemia, or arteriovenous aneurysms. The abnormal increase in diastolic pressure is considered to be more nearly related to increased peripheral resistance.

Peripheral Resistance and Cardiac Output

A detailed consideration of the methods of measurement of peripheral resistance and cardiac output is outside the scope of this paper. However, since these factors will be discussed later, a brief discussion is included. Peripheral resistance may be defined as the impedance to the flow of blood through the circulation. The arterioles represent the anatomical subdivision of the vascular tree where the pressure drop is greatest and hence the energy loss per unit of flow is also greatest at this point. According to Poiseuille's theorem for flow-pressure relationships in tubes the pressure varies inversely to the fourth root of the radius. From this relationship it is readily apparent that small changes in the total cross-sectional area of the arterioles will result in a large increase in pressure. The body is usually considered to be a system of organs in parallel and the total peripheral resistance is equal to the sum of the reciprocals of the resistances of the separate organs. The total peripheral resistance can be calculated by the following formula:

$$R = \frac{P_m \times 1332}{V} = \frac{\text{dynes sec}}{\text{cm}^5}$$

where R = Resistance, P_m denotes mean pressure in mm. Hg. and V = the cardiac output or volume flow per sec. 1332 is a correction factor for pressure measurement which relates mm. Hg. units to the cgs system.

The mean pressure can be measured with a mercury manometer. The cardiac output represents the amount of blood ejected from the left ventricle per unit of time. This can be measured by the direct Fick method in which mixed venous blood is sampled by means of right heart catheterization (2).

Clinical Manifestations

The clinical manifestations of essential hypertension are characterized by its complications which are congestive failure, cerebral vascular disease, and less commonly uremia.

Pathology of Essential Hypertension

In the absence of one of the complications of hypertension the pathological changes of hypertension consist of myocardial hypertrophy and arteriolar hyaline thickening. In most cases, the left ventricle is markedly hypertrophied and the heart weighs over 450-500 grams. The upper limit for a normal male is around 400 grams and for female it is about 50 grams less. In some cases increased heart weight is not observed.

There is also variation in the amount and distribution of arteriolar hyaline thickening. In long-standing cases of "severe hypertension" arteriolar thickening may be found in the kidney, adrenal, pancreas, liver, and gastrointestinal tract. A "fibrinoid change" in the small cerebral arteries designated "hypertensive arteritis" has been described by Feigin and Prose (3). The arterioles supplying skeletal muscles are usually normal in appearance. With less generalized involvement the most striking feature is the degree of renal involvement. In an autopsy series, Moritz and Oldt (4) found that

97 out of 100 cases with essential hypertension had characteristic renal involvement. These workers were in agreement with Goldblatt's conclusion that renal arteriolar thickening is a structural event which proceeds the onset of hypertension. Fishberg (5), and Goldring and Chasis (6), take the view that vasoconstriction is the primary cause for increased peripheral resistance and that this is not dependent upon previous anatomical changes. The dispute remains unresolved. It illustrates one of the main difficulties encountered in studying this disease for on the basis of present information it is frequently impossible to decide whether an observed phenomenon initiates hypertension or is the result of the hypertensive process.

Etiological Factors

The term Essential Hypertension is used to show that our understanding of the etiology of this disease process is still obscure. However, three etiological categories have received a great deal of attention. These are:

- (1) Neurogenic Mechanisms
- (2) Endocrine and Hormonal Factors
- (3) Renal Factors

Like most classifications this one is somewhat arbitrary and there is an overlap between categories. For example, neurogenic mechanisms frequently involve consideration of effects of epinephrine and nor-epinephrine and at this point there is a merging with endocrine and hormonal factors. In the same way release of renin from the kidney has an overlap between endocrine and renal factors.

The classification is helpful because it tends to separate the three main lines of research. The literature on each phase of the

problem is extensive and the material is chosen to include some of the fundamental contributions, yet at the same time there is an attempt to include the most recent work done. The purpose of this survey is to give a general idea of the scope and diverse nature of the problem of hypertension and to show some of the considerations leading to the hypothesis that sodium is closely related to hypertension.

(1) Neurogenic Mechanisms

Although nervous influences are probably quite important in essential hypertension, it is difficult to assess this aspect from an etiologic view. Hering (7) was able to produce hypertension by section of the aortic and carotid sinus nerves. The hypertension achieved by this means differs from that observed in man in several important respects. Following section of the "depressor nerves", the blood pressure is found to be increased but there are marked fluctuations in blood pressure and the hypertension is accompanied by a tachycardia. There is also an increase in cardiac output while peripheral resistance is generally not altered (8).

Numerous studies have been done on the effects of sympathectomy and ganglionic blocking agents. The clinical results following sympathectomy are unpredictable. In some cases permanent reduction in blood pressure has been accomplished but in most instances the blood pressure rises to its original level in a short time following the operation. The ganglionic blocking agents have been reported to be beneficial in reducing blood pressure clinically. Some of the experimental evidence and implications derived from studies on ganglionic blocking agents in experimental hypertension will be discussed in detail later.

(2) Endocrine and Hormonal Factors

Most of the work on endocrine and hormonal factors has centered around a search for a substance or substances which act as chemical mediators for the initiation or maintenance of hypertension. These studies have been concerned with the metabolic aspects of adrenal cortical steroids, catechol amines, and extracts from renal tissue with observed vasopressor qualities such as renin and vasoexcitator material (VEM). The studies dealing with the adrenal cortex depend primarily upon the sodium retaining effect and will be discussed later when sodium factors are considered.

A. The possible role of epinephrine and nor-epinephrine in hypertension.

Since the pressor effects of epinephrine and nor-epinephrine are well known, repeated attempts to show an increase in circulating levels of these substances in the plasma of hypertensives have been made. These studies have been uniformly disappointing and it is now generally conceded that hypertension cannot be explained on this basis. Mendlowitz et al. (9) recently tried to account for the failure to find sympathetic amines in the blood by suggesting that their effects may be explained on a local basis. Since 1-norepinephrine is the chemical mediator of sympathetic nerve discharge, it is entirely conceivable that this substance could exert its influence directly on vascular smooth muscle without being detected in the general circulation. Studies on digital circulation revealed increased neurogenic vasoconstriction in patients with essential hypertension. These patients also showed an increased digital vascular response to infused nor-epinephrine. Increased sensitivity to norepinephrine injections was

observed in normotensive patients who received premedication with cortisone, prednisone, or ACTH as well as in patients with Cushing's syndrome. This effect could be attributed to increased response by the vascular smooth muscle elements or to a delay in the inactivation of norepinephrine. Although the authors could not exclude the first possibility, they chose to consider the second. The precise biochemical pathway for the destruction of norepinephrine in vivo is not known; however, monoamine oxidase and o-methyl transferase are two enzymes which take part in norepinephrine inactivation. Iproniazid, a monoamine oxidase inhibitor was given to hypertensive patients to see if it could block the increased sensitivity to norepinephrine. The results were negative. Axelrod et al. (10) suggested that the existing facts might be explained if it could be established that o-methyl transferase activity is inhibited by adrenal steroids. If this hypothesis could be proved adrenal steroids might be the initiating cause of hypertension.

In summary, though a number of mechanisms through which alterations in catechol amine metabolism might produce hypertension have been described, no strong evidence is available to implicate them in the pathogenesis of hypertension.

B. Renin

The position of renin with respect to its possible role in hypertension will be considered next. Renin was isolated in 1898 by Tigerstedt and von Bergmann (11). They found that saline extracts of renal tissue produced a transient hypertension following intravenous injection in experimental animals. Further work has shown that renin is a proteolytic

enzyme which produces its effect by converting hypertensinogen, an α -2 globulin found in blood plasma, to hypertensin, a polypeptide which is the active pressor principle.

In the past there was a great deal of interest in the renin-pressor system and a vast literature exists on this subject. This subject has been extensively reviewed in Braun-Menendez book (12).

The evidence that renin is a major factor in essential hypertension is not convincing. This conclusion is based upon clinical and experimental observations. Studies on hypertensive patients failed to reveal any significant difference in the amounts of renin, hypertensinogen, or hypertensin in the plasma of these patients when compared with normotensive controls (13, 14). Although increased amounts of renin were found in hypertensive patients with acute glomerulonephritis and eclampsia (14), these diseases differ from essential hypertension by having an acute onset, presumably one in which there is actual renal damage to account for the release of renin.

Even in experimental hypertension where renin is known to be released by the kidney, the evidence does not support its having an active role in the production of hypertension. When dogs are made hypertensive by the Goldblatt technique, renin is released immediately following the operation. However, a sustained and permanent rise in blood pressure does not occur until two to three weeks after the operation. At this time assays of renin are very low or show it to be absent (15). From known facts on the pressor properties of renin, one cannot attribute the rise in blood pressure in experimental hypertension entirely to the presence of renin. There is widespread agreement that the renin-pressor system does not operate to maintain hypertension. The complete

absence or presence of exceedingly small amounts of renin seen in experimental studies at the time of development of hypertension would tend to discredit renin as an initiating factor in hypertension, yet there are some investigators who still feel that renin may be an important factor in initiating hypertension. Renin may have an effect on intrinsic regulation of renal function by causing changes in either hemodynamic relations or excretion patterns and the fact that renin is not observed in significant quantities in blood sampled from the renal vein does not absolutely exclude it from being important in the production of hypertension.

There are a number of studies which attempt to show that renin has a physiological function in maintaining blood pressure under normal conditions. These studies depend upon observations on histological changes in the granularity of cells in the juxtaglomerular apparatus. In a recent review of this subject, Hartroft et al. (16) worked out a hypothesis which would relate renin production with sodium metabolism in an attempt to implicate renin in "salt hypertension". The author starts with the assumption first propounded by Goormaghtigh (17) that renin is produced by the cells of the juxtaglomerular apparatus (J-G cells). Histological studies of these cells have shown that the granularity of these cells is increased during hypotension, and by sodium chloride deficiency and decreased with hypertension and hypernatremia. In those conditions in which the J-G granules are increased in size and amount, hyperplasia of the zona glomerulosa of the adrenal gland is also observed. This correlation resulted in the further assumption that renin (increased granularity of J-G cells) stimulates

adrenal hyperplasia which in turn results in sodium retention and as a consequence of sodium retention, hypertension develops. The authors realize that this scheme is quite hypothetical but suggest that it is a possible mechanism which should be considered.

In summary, although renin has a known pressor effect, it does not appear to participate in the production or maintenance of hypertension. Studies in experimental animals and hypertensive patients have failed to reveal significant amounts of renin in the blood during the late development and course of hypertension. Hartroft has suggested that (J-G) cells, the hypothetical site of renin formation may act to regulate sodium metabolism thru an adrenal effect in hypertension.

C. Vasoexcitor Material (VEM)

One last substance should be mentioned for historical interest. This substance is VEM (vasoexcitor material) of Zweifach and Shorr (18). According to theory, VEM is produced by ischemic kidneys and when it is released into the general circulation it has pressor properties which act mainly by potentiating epinephrine and norepinephrine. Zweifach has been unable to quantitate VEM levels with the severity of hypertension (19)

D. Conclusions

In this section proposed mechanisms of hypertension via chemical mediators have been considered. The suggestion that norepinephrine metabolism is of significance in hypertension remains to be proved. While more work is undoubtedly required on this aspect of the problem, it seems unlikely that hypertension is merely a disturbance of norepinephrine sensitivity.

The possibility that renin may initiate or maintain hypertension has been considered. The time of release of renin and blood levels present during hypertension render this suggestion unlikely.

The significance of Harcroft's proposal that J-G granularity is related to both renin production and sodium metabolism through regulation of adrenal cortical hormones cannot be evaluated because there are too many gaps in our information. This suggestion would appear to be unlikely since no proved relationship between renin and hypertension has been shown to exist.

Although VEM has been suggested as a factor in hypertension, no confirmatory evidence has been obtained by other investigators and the original observations have been placed in doubt by one of the original proponents of the hypothesis.

(3) Renal Factors

There has been a great deal of interest and experimental investigation centered around the kidney with the implication that it might be the primary cause for essential hypertension. This interest developed for several reasons. First, the histological finding of renal arteriolar thickening and sclerosis out of proportion to other organs would tend to implicate the kidney. These suspicions found further confirmation when hemodynamic studies were performed (20, 21). These studies showed:

- (1) Renal plasma flow is decreased in hypertension
- (2) Glomerular filtration is essentially normal at first but may be decreased with progressive disease
- (3) Tm for diodrast and p-aminohippuric acid (PAH) is decreased in hypertensive patients.

The plasma flow may be defined as the amount of plasma in cc. per minute which circulates through functional renal tissue as measured

by diodrast or p-aminohippuric acid (PAH) clearance. A decrease in renal plasma flow could be attributed to either organic vascular changes or physiological vasoconstriction. In any event, the decreased renal blood flow in the presence of an elevated renal arterial pressure indicates increased peripheral resistance of the kidney.

The glomerular filtration rate is the amount of plasma filtered by the glomerulus per minute. The observation that the glomerular filtration rate is frequently not reduced despite reduction of renal plasma flow is interpreted to indicate that there is constriction of the efferent arteriole in hypertension.

Ordinarily, at low concentrations the amount of PAH or diodrast excreted is virtually the same as that which is presented to the kidney (extraction ratio of about .90) and the clearance value may be interpreted as a measurement of the renal plasma flow. At high plasma concentrations the tubular excretory capacity of the kidney is exceeded. The maximum tubular excretory capacity or T_m for diodrast at saturation concentration is given by the formula:

$$T_{m_D} = U_D V - GFR \times P_D$$

where

- T_{m_D} = tubular maximum for diodrast (mgm/min)
- U_D = urinary concentration of diodrast (mgm/cc.)
- V = urinary volume per minute (cc.)
- GFR = glomerular filtration rate (cc./min)
- P_D = plasma concentration of diodrast (mgm/cc.)

In hypertension the T_m for diodrast and PAH is markedly decreased.

This, in effect, means that there is a defect in tubular function.

Furthermore, the defect in tubular function cannot be easily explained.

by primary alterations in glomerular function since the glomerular filtration is often normal under these conditions. It is of interest to note that the earliest histological change in renal biopsy material from hypertensive patients was in the form of tubular damage as evidenced by cloudy swelling of the tubular cells (22).

These observations on human subjects show that even in the early phases of hypertension derangements in both hemodynamic and excretory functions occur. In these studies, the demonstration of an excretory defect involves the use of materials which are given experimentally. In view of these results, one might expect to find excretory abnormalities of a "physiological" nature as well.

These studies with human subjects involving use of clearance techniques reveal a correlation between observed pathological changes and physiological function in the kidney. Furthermore, they show that excretory, as well as hemodynamic, changes are involved in hypertension. The second type of reasoning which supports the argument that the kidney is the primary cause of hypertension is that renal damage in other diseases, notably glomerulonephritis and pyelonephritis, is frequently found to be associated with hypertension.

Perhaps the greatest credit for present interest in the kidney as the responsible agent in hypertension goes to Goldblatt (23). In 1934 he reported that he was able to produce hypertension in the dog by partially occluding the renal artery with a clamp. The hypertension associated with partial occlusion of one renal artery was usually transient. However, by occluding both renal arteries or by occluding one renal artery and excision of the contralateral kidney, a permanent sustained hypertension was found to develop. This hypertension

serves as an excellent experimental model for the blood pressure is elevated and stable, the cardiac output is normal and no tachycardia is present. Since Goldblatt first introduced a practical means for producing hypertension in the dog, several modifications have been employed. These include perinephritic encapsulation by Page (24) and the figure of 8 ligature by Grollman (25). These modifications have the advantage of being applicable to small animals such as the rat and rabbit.

Once methods were available for the production of hypertension, various experiments were designed to demonstrate the role of the kidney in hypertension. In the course of these experiments it was found that once hypertension was established, elevation of the blood pressure persisted despite complete nephrectomy (26). By use of peritoneal lavage to prolong the life of experimental animals, Grollman demonstrated in dogs that the kidneys were not necessary for the production of hypertension (27). The hypertension which develops in the absence of kidneys was named "renoprival hypertension".

These observations on the effects of nephrectomy did much to change the focus of interest in experimental hypertension. Since kidneys are not necessary for the production or maintenance of hypertension, additional doubt was expressed concerning a renal-pressor system as an important factor in hypertension. Grollman (27) proposed that the kidney had a blood pressure reducing factor. Substantiating this argument Kolff and Page (28) found that hypertensive renoprival dogs in which kidneys were implanted in the neck from a donor animal showed a drop in blood pressure to normotensive levels. Hence, they

suggested that the kidney regulates blood pressure by elaboration of a hormonal substance which lowers the blood pressure. In the absence of kidneys or with renal damage, this function is impaired and hypertension results. Attempts to find this postulated substance have been unsuccessful. The other possibility, namely that some known renal excretory function is altered in hypertension will be considered in more detail subsequently.

PART II

REVIEW OF SODIUM-HYPERTENSION LITERATURE

CHAPTER III

SODIUM-HYPERTENSION RELATIONSHIPS

As has already been indicated in the preface the relationship between sodium and blood pressure is supported by various types of evidence. In this section, this evidence will be reviewed in more detail.

A. Relationship Between Dietary Sodium And Hypertension.

In 1904 Ambard and Beaujard (29) reported clinical improvement in hypertensive patients following restriction of sodium chloride in the diet. Eighteen years later Allen and Sherrill (30) were the first in this country to advocate salt restriction in the treatment of hypertension. Their findings were that sodium chloride restriction reduced the blood pressure to normal values in 34 of a total of 180 cases in which hypertension was present. In some of the remaining cases they reported clinical improvement even though the blood pressure was not decreased. They observed that the dietary level of sodium chloride had to be restricted to a daily intake of less than 0.5 gm. of sodium chloride before any therapeutic effect could be observed.

Although Kempner (31) reported in 1934 that a rice diet had beneficial effects on hypertension, a study by the Medical Research Council (32) in Britain in 1950 established that the effectiveness

of the Kempner diet was directly related to sodium restriction and was not dependent upon any special property of rice.

Animal experiments substantiate the effectiveness of salt restriction in the diet. Grollman and Harrison (33) and Danford et al. (34) have shown that dietary restriction of sodium chloride has a blood pressure lowering effect upon renal hypertensive rats. The former group did not obtain any reduction in the blood pressure of normotensive animals. Page and Lewis (35) failed to effect a reduction in blood pressure when sodium chloride was restricted in renal hypertensive dogs to 25 mgm. per day for a period of eight to nine months. It would seem that this failure to effect a reduction in blood pressure in the dog may be due to an unusual ability of the dog to conserve sodium. Supporting this idea, Frieden et al. (36) were able to show a reduction in blood pressure in hypertensive dogs by means of peritoneal lavage which decreased the plasma sodium concentration from 145 meq/l to 115 meq/l. Similar decreases in plasma sodium concentration of normal animals were without effect on blood pressure.

Many reports indicate that hypertension may be produced by the excessive ingestion of sodium chloride. McQuarrie et al. (37) noticed that hypertension developed in diabetic children who ate massive doses of sodium chloride as the result of a salt craving. Lenel et al. (38) were able to produce experimentally the rapid development of hypertension in chickens by giving isotonic or hypertonic drinking solutions. Saperstein, Brant, and Drury (39) produced

the same results in rats by the substitution of hypertonic sodium chloride (2%) solution for drinking water. However, contrary to the findings observed in chickens, when large quantities of isotonic saline were given to rats, hypertension did not result. Reports by Masson and Corcoran (40) and Mucio et al. (41) confirmed that hypertension can be established by the substitution of hypertonic saline for drinking water in rats. In a modification of this experiment, Meneely and Ball (42) reported that the degree of blood pressure elevation was proportional to the concentration of salt in the diet. The rats' lives were shortened on high salt diets. Sturtevant (43) reported that rats made chronically hypertensive by DCA administration showed no significant difference in blood pressure or survival rates if given tap water or .86% sodium chloride. These results are not inconsistent with those of Meneely and Ball who observed that very large increases in sodium chloride intake were necessary before effects on blood pressure and survival became pronounced.

Since increased ingestion of salt produces hypertension, the question comes up as to whether naturally occurring hypertension in humans is the result of increased salt intake. In connection with this question, Dahl and Love (44), and Dahl (45) have reported an increased desire for salt by hypertensive patients.

Rats apparently react differently. The hypertensive rat has an aversion to salt according to Abrams et al. (46). Fregly (47) also reported that the hypertensive rat dislikes drinking solutions high in salt. However, the hypertensive animal may take as much salt as the normal if it is offered as a hypotonic solution.

B. Sodium Content Of Plasma And Body Fluids In Hypertension.

Slight elevations in the plasma sodium levels have been reported in hypertensive subjects by Kylin and Elmquist (48), Holley et al. (49), Albert et al. (50) and Taquini et al. (51). Increases in sodium concentration in the plasma are small, averaging approximately 2-5 meq/l. Fregly et al. (52) reported sodium to be elevated only in young rats which were made hypertensive. The finding of an elevated plasma sodium is by no means diagnostic, since many hypertensives have plasma sodium concentration in the normal range, and in several studies with small groups the plasma sodium has been within normal range (in both man and rats) (53-55). In agreement with the findings of a slight increase, a study on the cerebro-spinal fluid of man showed sodium to be elevated by 7 meq/l. (56).

Tissues also seem to show increased sodium chloride content in hypertension. Eichelberger (57) first noticed an increase in sodium content in muscle of hypertensive dogs. Laramore and Grollman (58), using rats, found increases in the sodium content of most organs. The greatest increase was in the heart. At the same time the potassium levels were decreased and the water content was slightly elevated. The suggestion was made that the increased sodium was primarily intracellular in distribution, the sodium replacing potassium within the cell. Greene and Sapirstein (53) reported an increased total sodium content in renal hypertensive rats, but the potassium and nitrogen content of these animals was slightly elevated. They were unable to determine the location of the excess sodium. The total sodium was

1.2 to 1.5 times the control value and it was thought unlikely that the excess could be entirely extracellular in distribution since the plasma concentration of hypertensive animals was normal and no edema was present. They attributed the excess to the intracellular compartments but could not adequately explain the mechanism by which this could occur although they considered that the excess sodium may have replaced magnesium.

Grollman et al. (59) measured extracellular space with mannitol and radio-sulfate in dogs and radio-sulfate in humans and found that the extracellular fluid was expanded by approximately 20% in hypertensive subjects. Braun-Menendez (60) also found an increased extracellular fluid volume by 12.5% as measured by thiocyanate space in hypertensive rats. A slight increase in extracellular volume was noted in early renal hypertension by Iedingham (61). Significantly in late hypertension, there was no increase in extracellular volume indicating that hypertension can be maintained in the absence of overhydration. In this study, analysis of cardiac and skeletal muscle intracellular sodium content showed the sodium content of these organs to be decreased although in other organs it was unchanged.

Ross (62) measured the total readily exchangeable sodium in hypertensive patients and found it to be elevated. There was no significant increase in total body water and only 10.6% increase in the extracellular volume. Calculations on "intracellular sodium" showed that the hypertensive group had a concentration of 93.8 meq/l

as compared to 43.0 meq/l for the normal group. This author concluded that the principal increase in sodium content was in the intracellular compartment, although he could not rule out the possibility of the excess being the amount stored in bone where sodium is less readily exchangeable. There was not a good correlation between exchangeable sodium and blood pressure measurements. Another important finding was that a low sodium regime could cause lowering of blood pressure without alteration of the total exchangeable sodium. Ross, therefore suggested that the distribution of sodium chloride rather than its amount may be significant in the production and maintenance of hypertension.

Ross' interpretation of his findings is difficult to accept in view of the fact that others (63, 64) found no demonstrable increase in exchangeable sodium of hypertensive humans. Ross' finding that the intracellular sodium of the hypertensive is elevated is based on calculations in which the sodium of the extracellular space was subtracted from total exchangeable sodium. Clearly large errors in the calculation can result from small errors in the extracellular space measurement. The method used by Ross (sodium thiocyanate) for the extracellular fluid volume determination is of uncertain validity. Others who have measured extracellular fluid volume in hypertension have found it to be normal or elevated. Such a finding, coupled with the finding that exchangeable sodium is in the normal range in hypertension (53, 54) would suggest that intracellular sodium is reduced rather than increased in hypertension as has been suggested by Ledingham (61).

Tobian and associates have focused attention on the electrolyte content of large arteries in hypertensive human subjects and hypertensive rats. Increased concentration and amounts of sodium were found in the renal arteries of patients at autopsy and in the aortas of animals which were made hypertensive by subtotal nephrectomy and by DCA administration (65, 66). There was considerable variation in the values for sodium in these studies and the differences between normotensive and hypertensive groups in many instances were not impressive. The objection may be made that increases in sodium of arteries analyzed from an autopsy population studied on the average of six hours post-mortem may not be meaningful. Tobian's suggestion that increased sodium content in the arterioles may cause swelling, thus decreasing the lumen of the arteriole and increasing the peripheral resistance is based on the assumption that changes in arteriolar electrolytes are similar to those found in the arteries. This is by no means established, and even the findings on arterial electrolytes have been called into question (67).

C. Renal Excretion Of Sodium And Water In Hypertension

Studies on the renal excretion of sodium and water have almost invariably revealed that there is an abnormal pattern of excretion of these substances in hypertensive patients. Before considering these findings in detail, it should be stressed that excretion patterns will be determined as much by previous dietary intake as by the function of the kidney. For example, it would not appear proper to argue that excessive excretion of sodium by an individual

on a high salt intake represented inability of the kidney to conserve salt. In human hypertension, where evidence exists that the sodium intake is abnormally high (44, 45), such an interpretation would seem especially hazardous unless meticulous attention was paid to control of the diet in advance of the observations. Neither is it clear for how long a period such control is necessary, for knowledge of the time relationship of renal sodium excretion to dietary intake is, at best, fragmentary.

Despite the self-evident nature of this proposition, little attention has been paid in most of the studies reported in this section to this point. Inconsistencies in the conclusions reached in these studies may, in part, be explained on this basis.

In 1898 von Koranyi (68) reported that hypertensive patients elaborate a weakly concentrated urine. Chanutin and Ferris (69) produced hypertension in the rat by partial nephrectomy. Polyuria with a lowered specific gravity of the urine was observed. However, permanent damage to the kidneys resulted from the technique used as evidenced by the presence of albuminuria and nitrogen retention. Oster and Martinez (70) observed polyuria and polydipsia in hypertensive rats in which there was no overt renal failure. In these experiments the rise in blood pressure did not appear to be temporarily related to the alterations in renal function. Polydipsia and polyuria were well correlated with each other. However, polydipsia was seen to occur before, during, or following the rise in

blood pressure. The authors, therefore, supported the view that the abnormalities in excretion were a result of renal ischemia rather than of changes in blood pressure.

Stamler, Hwang and Kuramoto (71) reported sodium excretion to be normal in Goldblatt hypertensive dogs. The majority of evidence favors the view that sodium chloride excretion is increased in hypertension. Farnsworth (72, 73) found that an increased elimination of chloride in hypertensive patients was associated with a decreased tubular reabsorption of chloride. During basal conditions the chloride clearance was increased and was proportional to the urinary volume, while in normal patients the chloride clearance was constant and not related to urinary volume (73). Unfortunately in these studies the data was reported in terms of chloride-inulin clearance ratios. Interpretation of this data is difficult because no figures are given for chloride excretion with respect to urinary volume. As Homer Smith has pointed out in his criticism of this work, the increased chloride clearance in the hypertensive patient does not necessarily indicate abnormal renal mechanism for handling chloride. The differences in excretion of chloride may be due to increased ingestion of this substance. "Since hypertensive patients can keep themselves in salt balance on as low an intake as normal subjects, it is clear they suffer no excessive loss of chloride." (74).

Weston et al. (75) found the excretion of sodium chloride and water to be elevated in hypertensive patients without increased glomerular filtration rates. They were in agreement with Farnsworth

that the primary renal abnormality in hypertension is a tubular defect in reabsorption of sodium chloride. The hypertensive patient is a "salt waster."

Green, Wendell, Wall, and Learned (76) observed the sodium and water excretion was generally highest in those hypertensive patients in whom the blood pressure elevation was greatest. Since PSP and maximal concentrating capacity of the kidney were normal, they concluded that the excretory abnormalities observed were due to the increased arterial pressure rather than an intrinsic renal defect. Cottier et al. (55) also found sodium and water excretion elevated in hypertensive patients. In general, they found rough correlation between blood pressure levels and increased sodium excretion.

Green, Johanson, Bridges and Lehmann (77), showed that the duration of human hypertension also affects the electrolyte excretion. In early stages of disease, water and sodium excretion were increased. Later on, the sodium excretion returned to normal values, while the water excretion was still greater than the normotensive, although it is less than during early hypertension. They attributed the decreased excretory function in late hypertension to decreases in renal plasma flow and glomerular filtration which accompanied renal damage in late hypertension.

Baldwin et al. (78) reported increased natriuresis in hypertensive patients infused with 2.5% sodium chloride during the first hour of experiment.

They infused 2.5% sodium chloride in both hypertensive and normotensive subjects for three hours. In the first hour increased sodium and water excretion was observed in the hypertensive group. In the second and third hours sodium excretion was the same for both groups. No difference in excretion of sodium was observed when the hypertensive patients were placed on a low salt diet for several days preceeding the metabolic period. The authors interpreted these results to show that no renal tubular defect is present in hypertension since the hypertensive patients could excrete sodium normally when salt loaded following a low salt regime and during the later periods of salt administration. They suggested that the increased natriuresis observed during the first hour of infusion may have been due to increased body sodium in hypertension.

Braun-Menendez (79) reported increased chloride and water excretion in hypertensive rats loaded with .80% sodium chloride solutions. Hypertensive rats loaded with small quantities of sodium chloride by stomach tube were found by Friedman, Hinke and Hardwick (80) to have increased sodium and water excretion. They observed that the hypertensive animals reached their maximum rate of excretion earlier than the normotensive group. Sodium excretion was not affected by the anion used. Elevation of diastolic blood pressure appeared to be associated with increased sodium and water excretion but had no apparent effect upon the plasma sodium concentration. When rats were made hypertensive by desoxycorticosterone (DCA) administration comparable results were obtained in sodium and water excretion.

The effect of sodium chloride withdrawal on renal excretion of water and electrolytes has been studied by Perera and Blood (81) who reported a presumed retention of water in hypertensive individuals on a restricted diet. The hypertensive group lost less weight than controls. These results have never been confirmed and values for sodium excretion were not given. Renwick et al. (82) could not find any difference in sodium excretion when the diets of normotensive and hypertensive individuals were strictly controlled with respect to salt intake. When salt was withdrawn from the diet the excretion of sodium chloride and water did not differ significantly between the two groups indicating that ordinarily the hypertensive patient remains in balance with respect to sodium and water.

Birchall et al. (83) also were unable to detect differences in sodium and water excretion during basal conditions, but did show that the hypertensive individual was unable to inhibit a diuresis following infusion of hypertonic saline. The normal individual showed a marked diminution in urinary volume when saline was infused. The hypertensive excreted more sodium + water than the normotensive under these conditions. During dehydration the hypertensive did not show an increased tubular rejection of chloride in water. These authors reasoned that under normal conditions and dehydration the hypertensive is able to maintain his balance with respect to sodium chloride. Only when the hypertensive is subjected to excessive hypertonic sodium chloride load does the defective renal regulatory function become readily apparent and then he is a "salt waster" showing an increased tubular rejection

of Chloride and water. Hollander and Judson (84) similarly showed that the hypertensive is unable to inhibit diuresis even when anti-diuretic hormone was given.

Thompson et al. (85) were able to effect a reduction in sodium excretion one to two weeks following sympathectomy. Sodium excretion was not directly related to blood pressure levels following sympathectomy suggesting the possibility of a nervous influence on sodium excretion. Sodium and water excretion which were increased under hypertonic load prior to sympathectomy gradually returned in three to nine months to the original levels observed before the operation, as did the blood pressure.

ER (86) showed that heavy hydration in the form of a rapid infusion of 3-5% glucose at the rate of 25 ml/min results in a marked natriuretic effect for the hypertensive individual as compared to the control. In the hypertensive group the sodium excretion was proportional to the urinary volume. The normotensive group showed hydration increased urinary volume but had little effect upon sodium excretion. Under basal conditions the hypertensive excreted less sodium than the normal.

These results differ lightly from those of Brodsky and Graubarth (87) who studied excretion of water and electrolytes in hypertensive patients under hydropenic conditions with mannitol load. The hypertensive patient had a greater urinary volume and sodium excretion under basal conditions and this same tendency was noted after osmotic load with mannitol.

D. Relationship Of Sodium Factors Of The Adrenal Cortex To Hypertension

It has been suggested that hypertension may be related to a disturbance in adrenal hormones. However, the hypertension produced by adrenal steroids has been shown to be closely related to sodium and water metabolism. Friedman, Friedman, and Polley (88) demonstrated that the hypertensive effects of corticosterone acetate are potentiated by addition of various sodium salts. Conversely, Knowlton et al. (89) found that DCA-hypertension does not occur in rats in which sodium chloride intake is limited. In regard to experimental DCA-hypertension, Braun-Menendez (90) concludes, "... the presence of sodium in the diet or its administration in the drinking fluid is a condition sine qua non for the production of renal lesions and hypertension." On the other hand, adrenal cortex is not necessary for the production or maintenance of salt hypertension (91, 92). It has been shown that hypertensive animals which have been adrenalectomized will maintain their blood pressure if given sufficient salt. Adrenalectomized animals can also be made hypertensive by the use of salt. Genest et al. (93) have suggested that excess aldosterone is present in the urine of hypertensive patients and may contribute to the hypertensive state. They found 8.1 micrograms per 24 hours urinary excretion as compared with 1.4 micrograms for the normal individual. In malignant hypertension the excretion of aldosterone was only 6.3 micrograms. This last finding makes it appear unlikely that aldosterone levels are directly related to hypertension. Although adrenal corticoids may in special instances be related to hypertension, it appears unlikely that they can be regularly implicated.

E. Conclusions

Certain conclusions would seem to be justified on the basis of the experimental work just cited as follows:

(1) Increased sodium ingestion under certain experimental conditions will produce hypertension. Although there is some evidence to indicate that hypertensive patients do, in fact, eat more salt than the normal population, it has not been established whether this is sufficient to produce human disease.

(2) Dietary restriction of sodium chloride has been shown in experimental hypertensive animals to reduce blood pressure. This effect has been seen in hypertensive patients with less regularity. It is not known how sodium restriction actually causes a reduction in blood pressure nor is any information available to suggest a priori which cases will be refractory to treatment.

(3) Although small increases in the concentration of sodium in the plasma and increased total body sodium have been reported, the differences seem too small and irregular to be of physiological significance. It has not been proved that sodium levels in the plasma or body fluids bears any consistent relationship to hypertension. From this work it seems unlikely that increased sodium in the plasma and body fluids is a necessary condition for hypertension. The finding of increased concentration or amounts of sodium in some patients but not in others would suggest that a sodium effect may be time dependent, that is sodium levels may be important in some circumstances in hypertension but not in all. If such circumstances do exist, they have not yet been defined clearly.

PART III

METHODS

CHAPTER IV

SODIUM AND WATER EXCRETION IN RATS

MADE HYPERTENSIVE BY SUBTOTAL NEPHRECTOMY

Introduction:

The literature reviewed in Part II suggested the need for a study of sodium and water excretion in experimental hypertension in circumstances where voluntary intake of sodium was not a significant variable. To this end groups of hypertensive rats and normal rats were subjected to loads of sodium chloride and water, and the excretion of the loading substances was followed as a function of time.

In addition to following the excretion of the two substances individually, an attempt was made to describe the excretion patterns of sodium and water in relation to each other.

Methods

Female rats from the Harland Small Animal Farm weighing 90 to 100 grams at the time of operation were made hypertensive by the method of Grollman (25). Ether anesthesia was used. A midline vertical skin incision was performed in the lumbar area. The kidneys were exposed by making the usual muscle incision in the lumbar area. The superior and inferior pole of each kidney was tied firmly with silk thread. Sufficient tension was placed on the ligature before tying it to cause the normal shape of the kidney to be deformed. Special care was taken to avoid injury to the ureter and blood vessels emerging from the hilus. The upper and lower poles of each kidney were tied separately. This

modification was used instead of the usual figure of eight ligature to insure that the ureter and blood vessels would not be damaged. The kidneys were then replaced in their original position and the skin flap was closed with surgical skin clips. The animals appeared to tolerate the procedure well and approximately 70 percent of the animals operated upon developed hypertension.

After three months the blood pressures of the animals were determined by the foot plethysmograph method of Kersten et al (9⁴). The animal was placed in a specially designed holder which immobilized it and yet allowed a maximum degree of comfort. The head of the animal was covered to minimize any extraneous excitement. One hind foot was securely placed on a holder which contained a photoelectric cell. A light source was placed above the animal's foot. A mercury manometer was attached to a blood pressure cuff designed to be of optimal thickness for measuring the blood pressure in the rat. The blood pressure cuff was placed around the animal's ankle and was inflated to well above the systolic pressure of the animal. The pressure in the cuff was then lowered slowly. At the point when the systolic pressure is equal to the pressure in the cuff, blood enters the animal's foot, but venous occlusion prevents the blood from leaving the animal's foot. The increased blood in the animal's foot causes a decrease in the amount of light transmitted from the light source to the photoelectric cell placed below the foot causing a sharp reduction the electrical output of the cell. At this point the systolic pressure was read from the mercury manometer. The pressure determination by this method is considered to

be more precise than those employing the tail blood pressures. A careful attempt was made to measure the blood pressures when the animals were not excited. Animals which had systolic blood pressure readings of 140 mm. Hg. or more on three consecutive determinations and no lower blood pressure readings were considered to be hypertensive. The control animals were unoperated. Thirty hypertensive and 30 control animals were employed.

Two types of experiments without load were carried out. In one type the animals received both food and water ad libitum until the beginning of the period of observation. In the other type of experiment, a fast of 18 hours during which water was available preceded the period of observation.

In the loading experiments, the animals were fasted, but permitted access to water during an 18 hour period prior to the administration of the load. Sodium chloride solutions containing 0.3, 0.9 or 1.5% salt were injected by the intraperitoneal route just before the metabolic period. Each animal received 20 ml. of loading solution. The samples of urine were collected in 100 ml. graduated cylinders which were changed at the end of each collection period.

All urine collections were made in large metabolism cages. These cages were constructed to accomodate large paraffin-lined funnels which were placed below the cages. The side of the funnels were sloped at greater than 60 degrees to facilitate urine collection. A finely meshed, paraffin wire grating was placed in the bottom of the funnel to prevent feces from being collected with the urine.

In the experiments without load, groups of 10 rats were employed. Urine collections were made at two-hour intervals. In the loading experiments, the groups consisted of five to seven rats and urine collections were made at hourly intervals for six hours. The "wash out" sodium collected from the cages following the experiment was found to be negligible and, therefore, was not included in the results reported.

Plasma sodium determinations were made on 10 hypertensive and 10 normotensive rats using blood collected from the tails of lightly etherized rats which had been warmed in a draft of hot air. The animals had been fasted for 18 hours, but had been allowed water.

All sodium determinations were carried out by the direct reading method using a Perkin-Elmer 52-C flame photometer. In the procedure compressed air for the atomizer was supplied at a pressure of 10 lb./sq. in. and acetylene for the flame was at a pressure of 5 lb./sq. in.

The standards were made from chemically pure sodium chloride and ranged from .20 meq./l to 2.0 meq./l at regular intervals of .20 meq./l. The standards when measured show a roughly linear reading with approximately 5 units between each standard at the gain used. However, because the drift of the machine was great and unpredictable, it was not practical to construct a calibration curve. Instead each unknown sample was bracketed by the two closest standards and calculation of the unknown was made by interpolation. The known urine samples were diluted either 1-100 or 1-200 times. A sample calculation is included.

Assume that the unknown urine sample has been diluted 1-100 and read 37 on the meter and standards of .60 meq./l and .80 meq./l were found to read 35 and 40 respectively. Each division on the scale represents .04 meq./l and a reading of 37 represents two-fifths of the total spread between .60 meq./l and .80 meq./l standards or two-fifths of .20 meq./l = .08 meq. The value for the diluted urine would therefore be .68 meq./l. The urine, however, was diluted by a hundred times, therefore, the urinary sample in this case would be 68 meq./l. The direct reading method was found to be adequate for the accuracy of the sodium determinations required in this study. Duplicate determinations were found to differ by less than 3%.

The principle advantage of the internal standard method is to correct for fluctuations in flame intensity and interfering substances. In the procedure used, the air pressure and gas pressure were found to be sufficiently stable so that marked fluctuations in the flame were not observed. The problem of interfering substances is not a serious one for the determination of sodium by flame photometry (95).

The principle source of difficulty encountered in this determination was clogging of the atomizer, by minute particles contained in the diluted urine sample. When this occurred, there was usually a sudden deflection of the needle and the reading was too low. It was found that the most expedient method of making the determination was to bracket the unknown between the two nearest standard solutions. When the interpolated value of the unknown was the same in both directions, either starting with the lowest standard and going to the next higher

standard or starting with the higher standard and going to the lower standard, the determination was accepted as valid.

At the end of the study, the rats were autopsied. All the hypertensive animals had marked cardiac hypertrophy confirming the validity of the blood pressure measurements.

CHAPTER V

THE CONCEPT OF "FREE SODIUM"

While considering the results of many of the published experiments, it became apparent that the differences in sodium excretion between hypertensive and normotensive groups is small and usually the hypertensive group showed an increased urinary volume as well. It seemed necessary to devise a method which would relate sodium and water excretion in a way that would give a single value so that the two groups could be compared.

The need for a single term which would relate concentration and volume was recognized by West and Rapoport (96). They conceived of a way of relating urinary osmolarity with urinary volume. The term which expresses this relationship is called "Water Economy". The approach that West and Rapoport used will be considered at this time because it shows why concentration and volume should be considered together. In addition, derivation of the formula for free sodium stems from this approach.

West and Rapoport reasoned that when the osmolarity of the plasma is elevated, the excretion of a hypertonic urine acts in effect to save water for the animal. They were able to express this relationship quantitatively by relating the solute clearance to the urinary volume. The solute clearance is a term which expresses the volume of plasma per unit of time from which osmotically active material is completely removed by the kidney and excreted in the urine. It is determined by

dividing the total quantity of the solute excreted in the urine per unit of time by the concentration of solute in the plasma. The solute clearance is given by the following formula:

$$C_{os} = \frac{U_{os} V}{P_{os}}$$

where

- C_{os} = solute clearance (l./min.)
- U_{os} = urinary osmolarity (m. osm./l.)
- P_{os} = plasma osmolarity (m. osm./l.)
- V = urinary volume (l.)

The plasma and urinary osmolarity are obtained by freezing point determinations. A molal solution depresses the freezing point 1.86 degrees centigrade.

An example is given for illustrative purposes. Suppose that an animal is found to excrete 10 cc. of urine per minute with an osmolar concentration of 600 m. os/l and the plasma concentration is 300 m. os/l. The clearance of solute from the plasma is equal to $600/300 \times .010 = .020$ (l./min.) or 20 cc./min. In effect 20 cc. of plasma per minute is being cleared of osmotically active material. However to accomplish this 10 cc. of urine is also excreted. Water economy is the amount of water which is free to alter the body composition and is expressed as the difference between the solute clearance and the urinary volume.

$$\text{Water economy} = \text{solute clearance} - V$$

where

$$V = \text{urinary volume.}$$

Smith (97) has applied the name "Free Water Clearance" to this relationship. Its significance and derivation are the same as for "Water Economy".

In the preceeding example the "Water Economy" (solute clearance-volume) is $20 \text{ cc.} - 10 \text{ cc.} = 10 \text{ cc.}$ This means that under the conditions specified in the example, in which the kidney excretes a hyperosmolar urine, its effect is to free 10 cc. of plasma completely of osmotically active material. This 10 cc. is economized by the animal. It has the same effect as adding 10 cc. of water to the animal as far as dilution of osmotically active material within the body is concerned.

It is apparent by inspecting the formula for "Water Economy" that neither the volume nor the concentration of urine taken by itself can give an accurate estimation of the effectiveness of the kidney in regulating body fluids. A urine with a high osmolar concentration contained in a small volume will have little effect in saving water for the body since the actual amount of solute cleared will be small. In the same way, when the urinary volume is large and the osmolarity is close to that in the plasma, the osmotic clearance is small and the amount of plasma free to effect a change in the body composition will also be small.

The calculation of "Water Economy" is limited for our purposes by the fact that it does not allow one to evaluate the effect of specific urinary constituents. In the present study, sodium excretion was being considered. Realizing from the previous studies that the variations in sodium and water excretion in animals might be slightly but significantly altered in hypertension, a method for evaluation of these two parameters simultaneously was devised.

In considering the excretion of sodium, both the urinary sodium concentration and urine volume can be considered together in a single term which defines the excretion of sodium in excess of water in the urine. This term is designated free sodium. Free sodium represents the difference between the sodium content in the urine and that contained in a similar volume of plasma or extracellular fluid. The value of free sodium can be calculated from the following operation.

$$FNA = (UNA - PNA) V$$

where

- FNA = Free sodium (meq.)
- UNA = Urinary concentration of sodium (meq./l)
- PNA = Plasma concentration of sodium (meq./l)
- V = Urinary volume (l.)

The free sodium value is positive when a hypernatric urine is being formed and negative when a hyponatric urine is elaborated. A hypernatric urine can be considered to be composed of urine which is at the same concentration as an equal volume of plasma, i.e., the isonatric moiety plus an additional amount of sodium which is not obligated to maintain the plasma concentration. This amount of sodium is free to effect a change in body constituents, hence is designated free sodium.

A hyponatric urine can also be considered to consist of an isonatric moiety equal to the urinary volume plus an amount of sodium which has been removed from the urine. That amount which has been removed is free to effect a change in body composition and has a negative sign by convention.

Since the calculation of free sodium relates the urinary concentration to volume, the value for free sodium can be translated to "Water Economy" or "free water" moieties considered with respect to sodium. Assuming the plasma concentration for sodium to be 150 meq./l, 1 meq. of sodium is distributed to every 6.67 cc. $\frac{1000}{150}$ of water. Therefore, for every meq. of "free sodium", 6.67 cc. of plasma are "cleared" of sodium.

The same considerations which were applied to the analysis of "Water Economy" are pertinent to the discussion of "free sodium". Not only the composition of the urine, but also its amount, must be considered in evaluating the regulatory action of the kidney on the body's sodium water ratio. For example, a kidney which eliminates 1 ml. of urine containing 10 meq./l of sodium has conserved for the extracellular fluid 140 μ Eq. of sodium (assuming 1.0 ml. of extracellular fluid represents 150 μ Eq. of sodium); a kidney eliminating 5.0 ml. of urine at a sodium concentration of 20 meq./l has conserved 650 Eq. of sodium for the body. In the second case, there is a greater economy of sodium with respect to water even though the urinary excretion and concentration are higher.

CHAPTER VI

RESULTS

The results of two experiments without loading are described in Table 1A and B. Table 1A presents the urinary sodium and water losses by normal and hypertensive rats which had access to both food and water until the beginning of the period of observation. The sodium excretion of the hypertensive animals was slightly less than that of the controls in this experiment; the excretion of water was much greater in the hypertensive group. The urinary sodium concentration was always over 200 meq./l in the normal group and always less than 120 meq./l in the hypertensive group.

Table 1B describes the urinary sodium and water losses by rats when a fast preceded the period of observation. In this experiment, the hypertensives excreted almost 70% more sodium than the normals; the urine volume in the hypertensive group was almost four times that of the normals. The urine sodium concentration of the normals was over 200 meq./l in every period. In only one period was this value over 130 meq./l in the hypertensive group.

The results of nine experiments with different sodium and water loads are given in Table 2A, B, C. The results in all experiments were similar. Each value given in the table represents combined values from three experiments.

The pattern of sodium excretion in hypertensive animals does not show any striking differences from that shown by the normals. Loaded

with hypertonic or hypotonic saline, hypertensive animals eliminate a little more sodium than the normals; when they are loaded with isotonic saline, sodium excretion is at the normal level.

The urinary sodium concentration was lower in the hypertensives loaded isotonically or hypertonically than in their normal controls in 35 of 36 observations. On the other hand, in hypotonic loading, the urinary sodium concentration of the hypertensives was substantially above that of the normals during the first four hours.

Only in respect to water excretion was there a consistent difference between the excretory behavior of normal and hypertensive animals. In all the loading experiments, as well as in both types of unloaded experiments, the hypertensives invariably excreted more water than their controls.

The results of the loading experiments are represented in Figure 1. The ordinates in the figure are adjusted so that water and sodium will have the same value when the concentration of sodium in the urine is 150 meq./l. All values are cumulative.

The plasma sodium concentration in the hypertensive rats was found to be 149.5 ± 3.1 meq./l; in the normal controls, the value was 149.0 ± 2.9 meq./l.

TABLE I
URINARY SODIUM AND WATER LOSSES BY NORMAL AND HYPERTENSIVE
RATS DURING A 12-13 HOUR METABOLIC PERIOD

Hour	Hypertensive				Normal			
	Vol.	Na	U _{Na}	Free Na	Vol.	Na	U _{Na}	Free Na
	ml	mEq	mEq/l.	mEq	ml	mEq	mEq/l.	mEq
A. Ad libitum food and water intake until metabolic period (10 animals/group)								
2	10	.08	8	-1.42	4	.82	204	+.22
4	5	.56	111	-.19	2	.87	435	+.57
6	7	.74	106	-.31	3	.60	200	+.15
8	7	.55	78	-.50	1	.26	256	+.11
11	4	.36	90	-.24	1	.26	261	+.11
12	2	.16	80	-.14				
Total	35	2.45		-2.80	11	2.81		+1.16
B. 18-Hour fast with water ad libitum preceding metabolic period (10 animals/group)								
2	3	.46	153	+.01	1	.22	222	+.07
4	4	.36	90	-.24	1.5	.33	221	+.10
6	4	.36	90	-.24	1	.26	261	+.11
8	3	.35	117	-.10	1.5	.43	287	+.20
10	3	.39	130	-.06	1	.16		
12	10	.95	95	-.55	1	.32	322	+.17
Total	27	2.87		-1.20	7	1.72		+.65

TABLE 2

URINARY SODIUM AND WATER LOSSES BY NORMAL AND HYPERTENSIVE
RATS DURING A 6-HOUR METABOLIC PERIOD AFTER LOADING WITH
.3, .9 and 1.5% NaCl solution

Hour	Hypertensive				Normal			
	Vol.	Na	U Na	F Na by pe- riod	Vol.	Na	U Na	F Na by pe- riod
	ml	mEq	mEq/l.	mEq	ml	mEq	mEq/l.	mEq
A. 3% NaCl (19 animals/group)								
0								
1	21	.50	24	-2.7	4	.02	5	-.6
2	68	1.16	17	-9.0	75	.30	4	-11.0
3	67	1.20	18	-8.9	71	.36	5	-10.2
4	46	1.52	33	-5.4	35	.77	22	-4.5
5	14	.48	34	-1.6	6	.42	70	-.5
6	33	1.88	57	-3.1	37	3.96	107	-1.6
Total	249	6.74		-30.7	228	5.83		-28.4
B. .9% NaCl (19 animals/group)								
0								
1	3	.37	123	-.1	2	.15	75	-.1
2	9	1.21	134	-.2	10	1.57	157	+.1
3	27	3.97	147	-.1	12	2.38	198	+.6
4	31	4.22	136	-.5	25	3.80	152	+.1
5	12	1.66	138	-.1	16	2.53	158	+.1
6	45	6.44	143	-.4	50	7.60	152	+.1
Total	127	17.87		-1.4	115	18.03		+.9
C. 1.5 NaCl (17 animals/group)								
0								
1	42	9.58	228	+3.3	22	6.42	292	+3.1
2	41	11.40	278	+5.2	32	10.91	341	+6.1
3	22	6.34	288	+3.0	19	6.76	356	+3.9
4	21	5.86	279	+2.8	16	6.11	382	+3.7
5	13	3.67	282	+1.7	8	2.94	368	+1.7
6	15	4.59	306	+2.3	11	4.36	396	+2.7
Total	154	41.44		+18.3	108	37.50		+21.2

Fig. 1. Excretion of water and sodium by normal and hypertensive rats loaded with various salt solutions. The results are plotted on an isonatric scale. When the water and sodium ordinates are equal, the urine excreted contains 150 mEq/l. Na. Similarly, when the sodium ordinate lies above or below the water ordinate the urine is hyper- or hyponatric. Free sodium (see text) may be read directly from these plots by reading the difference between sodium and water ordinates on the sodium scale. Each point is the average of the values obtained in three experiments on a total of 17-19 rats. X Na; O water.

PART IV

DISCUSSION

CHAPTER VII

THE SIGNIFICANCE OF ALTERATIONS OF "FREE SODIUM" IN HYPERTENSION

In the present studies the hypertensive animal failed to show any consistent alteration in sodium excretion. However, when "free sodium" calculations were made, it became evident that the hypertensive animal excreted a urine with a less positive "free sodium" than the normotensive control. The sodium balance was presumed to be within normal range, but there was a disturbance in the sodium-water ratio such that the hypertensive animal excreted more water for the same amount of sodium. Inspection of the urinary volumes of hypertensive and normotensive animals confirms this observation.

One might conclude from this experiment that the basic defect is in the renal elimination of water rather than of sodium and that the hypertensive animal shows a derangement in water conservation. Oster and Martenez (70) observed polydipsia and polyuria in hypertension. In their experiment they felt that the polydipsia was secondary to polyuria. In the present experiment it was our opinion that the polyuria could not be a consequence of a defective thirsting mechanism. In those experiments in which water was withdrawn during the metabolic period in which no loading solution was given the hypertensive animals consistently excreted a larger urinary volume

in every period recorded. Since the metabolic period in which observations were made extended for over 12 hours, the increased urinary excretion could not be explained on the basis of a water diuresis following increased ingestion of water in the period immediately proceeding the collection of urine samples. In a water diuresis, the animal would presumably correct for an increased water load by excreting a larger quantity of water in the first few hours, but following this one would expect a return to a normal excretion pattern. The loading experiments provide a second reason for the belief that the abnormalities in excretion truly represent a renal defect. In these studies large quantities of loading fluid were given and any small alteration in body composition due to a defect in the thirsting mechanism would be insignificant in comparison to the conditions imposed by the load itself. The observed defect in "free sodium" excretion of the hypertensive animal was not considered a consequence of either increased thirsting or of changes in body composition proceeding the start of the experiment. The defect was considered to be a direct consequence of a renal abnormality.

Although water excretion is increased in hypertension, this does not mean that the defect is in water metabolism anymore than an increased sodium excretion per se indicates a defect in sodium metabolism. From the discussion on "free sodium" it should be apparent that neither sodium nor water metabolism taken by itself gives an indication of the status of fluids within the body. Only if both are considered together can one get a true impression of

the sodium-water relationship. One is reminded of the fact that when urinary "free sodium" is positive, the kidney has excreted sodium in excess of water and the effect will be to lower the plasma sodium concentration. When urinary "free sodium" is negative, the effect is to increase plasma sodium concentration. In the present experiments the hypertensive animals always excreted a more "negative" free sodium than the normotensive group. Compared to the normotensive group, the hypertensive group always showed a tendency to increase the plasma sodium concentration above that of the normotensive group (as judged by "free sodium" relationships). Although the differences were not large between the groups, the trends were consistent.

The possibility is raised that these small differences in the excretion of "free sodium" may be of physiological importance in hypertension. The proposition will be considered that excretion of water in excess of sodium results in a rise in sodium concentration in the body fluids in hypertension. This, in itself, may be of considerable more importance than an absolute increase in the total amount of sodium present within the body in hypertension. This may be restated as follows: An increase in a sodium to water ratio actually represents an excess of "free sodium" within the body in hypertension even if body sodium has decreased. If this is considered, certain factors that could not otherwise be explained begin to make sense. For instance, if one considers the hypothesis ~~that~~ hypertension is the result of increased body sodium or an increase in the total body fluids, one is puzzled by the failure

of edematous patients to become hypertensive. When it is realized that increased salt retention is associated with increased water retention, the net result is that the plasma concentration of sodium is unchanged and the "free sodium" in the body is not increased.

The results of studies on hypertensive patients in general indicate an increased plasma sodium concentration (48-51). If sodium-water relationships are considered together and the plasma concentration of the normotensive patient is used as the standard value, the hypertensive shows a relative increase in "free sodium" within the body.

The statement by Abrams et al. (46) that the hypertensive animal shows an aversion to salt suggests the possibility that there is an increased body "free sodium" in hypertension. Richter (98) suggested that when an animal has a bodily excess or deficiency of a required substance it tends to restore the homeostatic relationship by selective diet. When Abrams data is analyzed, it becomes evident that this relationship of a sodium aversion in hypertension is not so clear. In his experiments with .17 M. sodium chloride used as a drinking solution, three of six groups showed decreased sodium ingestion. The other groups showed either the same or greater ingestion of sodium. These animals showed an increase in water ingestion of two to four times the normotensive animal. The results with sodium chloride can be considered to be inconclusive but the increased water intake was clearly present.

The experiment reported by Fregly (47) is particularly instructive and seems to show that the hypertensive rat does have an aversion to sodium chloride under appropriate circumstances. When graded drinking solutions containing sodium chloride were given to the hypertensive animal it was observed that with weak solutions the hypertensive animal drank more fluid than the normotensive control and as a consequence ingested more salt. With strong solutions the effect was just the opposite. The hypertensive animal drank less saline and showed a relative aversion to salt. This response of the hypertensive animal is entirely rational and is consistent with what might be expected from an animal with a slightly increased body "free sodium". Since a hyponatric solution has a negative free sodium, its effect, when ingested, is to lower plasma sodium. Despite the fact that the hypertensive animal drinks more sodium under these conditions, the effect is to lower the sodium concentration. In this situation the hypertensive animal goes into a positive sodium balance compared to the normotensive rat in order to achieve osmotic or isonatric homeostasis. The hypertensive rejects the hypernatric solution because body sodium concentration is already increased and by taking the same amount of sodium as the normotensive control, the net effect for the hypertensive would be to increase the "free sodium" of the body to even higher levels. Therefore, the proper adjustment is to take less of the hypernatric solution if osmotic homeostasis is to be maintained. Fregly's data are immutable on this point, though he emphasized a different aspect of this finding.

The observation by Dahl and associates (44, 45) that hypertensive patients prefer excessive amounts of sodium chloride supports the view that the hypertensive patient increases "free sodium" of the body by ingesting larger amounts of sodium chloride than the normotensive patient when given a free selection of salt in the diet. This work does not contradict the aversion to sodium found in rats. It might well be that hypertensive patients also have an aversion to drinking solutions high in sodium concentration.

The observation of Ellis and Grollman (99) that hypertensive patients and dogs have increased levels of antidiuretic hormone in plasma and urine samples is easily reconciled with the view that there is an increased body "free sodium" in hypertension. According to Verney (100) the stimulus for the release of antidiuretic hormone is increased osmolarity of body fluids. It can be presumed that since sodium is the principal cation contributing to the osmolarity of the extracellular fluid that an increase in free sodium would result in increased osmolarity of the extracellular fluid.

This point is of fundamental importance. The effects which are attributed to sodium may be a consequence of changes in the osmolarity of the body fluids rather than being due to any specific property of sodium. This view will be expanded in the following section.

The observation that antidiuretic hormone is increased in hypertension would seem to be of importance from a theoretical standpoint since antidiuretic hormone has pressor properties which may be of significance in raising blood pressure. In the time which has

elapsed since Ellis and Grollman reported this work, quantitative assay methods have become available for measuring antidiuretic hormone and it would be profitable to repeat this experiment. If antidiuretic hormone levels are increased in hypertension as suggested by Ellis and Grollman it is interesting to observe that the increased water loss associated with hypertension is not corrected, as one would anticipate.

Conclusions

(1) The hypertensive animals in the experiment reported in this thesis showed an excretory pattern in which the "free sodium" is less positive than the normotensive controls. The suggestion was made that the data is best interpreted as being primarily due to a renal abnormality rather than the result of pre-existing changes in body fluids.

(2) The excretory abnormalities observed favor the view that the hypertensive animal has an increased body "free sodium".

(3) The assumption of an increased "free sodium" in the body would explain the apparent aversion to salt of hypertensive rats under certain conditions, the increased plasma sodium in some hypertensive patients, and the increased levels of antidiuretic hormone reported in hypertensive patients.

(4) The effects attributed to increased "free sodium" may not be due to any specific property of sodium but rather to hyperosmolarity of body fluids.

CHAPTER VIII

"FREE SODIUM" - A COMPARISON OF THE RESULTS OBTAINED IN THE PRESENT EXPERIMENT WITH DATA FROM OTHER EXPERIMENTS

In the preceeding discussion the author developed the hypothesis that the hypertensive animal excretes a urine in which "free sodium" is less positive than in the normotensive control and that as a consequence of this renal disturbance an imbalance in the sodium-water ratio occurs within the body. This sodium-water defect in excretion is reflected as an increased body "free sodium."

In order to test this hypothesis the data from other studies on sodium excretion in hypertension was evaluated to see if urinary "free sodium" was always reduced in hypertension. The comparison showed that "free sodium" was frequently decreased but this relationship was not always present.

Few studies have been done on sodium excretion in experimental hypertension. Braun-Menendez' (101) description of water and chloride excretion in the fasting hypertensive rat showed that chloride excretion did not differ appreciably from that of the normotensive animal; the hypertensive showed considerable polyuria. Assuming that sodium and chloride excretion closely parallel each other, these results can be considered to be in essential agreement with the suggested hypothesis. There was a tendency of the hypertensive rat to excrete a less positive "free chloride" resulting in increased chloride retention within the body.

Most of the work on sodium excretion has been done on hypertensive patients rather than experimental animals. The review of the literature showed that in the majority of these studies the hypertensive individual excreted excess sodium. The study by Green, Wendel, Wall, and Learned (77) showed hypertensive patients to be "salt losers" but the salt losses were associated with disproportionately large losses of water in the hypertensive group. When "free sodium" calculations were made, the hypertensive group was seen to have a less positive "free sodium" than the normotensive patients.

In another report by Green, Johnson, Bridges, and Lehman (76) sodium excretion was found to be increased in the early stages of hypertension. Later, the sodium excretion approached normal values although urinary volume was still increased. The authors concluded that there was a disturbance in sodium metabolism only in early hypertension. "Free sodium" calculations showed the hypertensive individual to excrete less positive "free sodium" than the normotensive. The calculation failed to disclose any significant difference in "free sodium" excretion between early and late hypertension. This example illustrates the point that when sodium excretion is normal one may erroneously conclude that sodium metabolism is not impaired. However, the disturbance in sodium metabolism becomes apparent if it is related to water metabolism.

The data of Cottier et al. (55), Thompson et al. (85), and Baldwin et al. (78) are all subject to the same interpretation.

Although sodium is excreted in excess of the normotensive group in each of these studies, water is excreted in proportionately greater amounts than the sodium with the result that the hypertensive patients end up in a less positive "free sodium" balance.

Applying the same principles to chloride, the findings of Birchall et al. (83) were found to be similar in interpretation. Although chloride excretion was three times greater in the hypertensive than the normotensive subject, the "free chloride" status showed the hypertensive to be in less positive balance.

In other studies the relationship between hypertension and a less positive "free sodium" could not be confirmed. Using hypertensive rats, Friedman, Hinke, and Hardwick (80) showed that the hypertensive animal excreted more sodium and water. However, calculation of "free sodium" failed to reveal any difference between the two groups.

In this study relatively large amounts of sodium were given at a high concentration by stomach gavage. The results may be as much a consequence of the rate absorption from the stomach as to any renal abnormality. Furthermore, unilateral nephrectomized animals were used as controls.

In an experiment conducted by Braun-Menendez (79) in which .80% sodium chloride was given corresponding to 5% of the body weight, the hypertensive rats eliminated a greater percentage of water and sodium chloride than the controls. At the end of 24 hours the normal rats had excreted 55 and 61% of the ingested water and 80% of the sodium chloride while the hypertensive rats excreted more than 70%

of the water and 100% of the sodium chloride. "Free sodium" calculation on the results of this experiment showed that the hypertensive animals were in slightly positive "free sodium" balance. This study contained only four hypertensive rats and therefore cannot be considered to be conclusive.

In a study by Renwick et al. (82) in which sodium chloride was given to both hypertensive and normotensive patients under carefully controlled metabolic conditions there was no difference between groups when "free sodium" was calculated. Following restriction of sodium chloride, calculated "free sodium" still failed to reveal any difference between the groups.

In other studies, hypertensive individuals excreted a urine in which the "free sodium" was positive. Weston et al. (75) measured sodium excretion following loading with hypertonic saline. The hypertensive patients were "salt losers" excreting more sodium and water than the control patients. Calculations showed the hypertensive group to be in positive "free sodium" balance. Mannitol clearances were done simultaneously with the studies of sodium excretion.

It was observed that the patient's urinary volumes were abnormally high during the control periods. The plasma levels of mannitol were not given but it can be assumed that the altered "free sodium" excretion in this experiment may have been induced by mannitol loading.

When Ek's (86) results were reviewed, it was seen that the hypertensive patient excreted more sodium and less water than the normotensive patient following hydration with glucose and water. "Free sodium"

calculation again revealed the hypertensive patient to be in an apparent positive balance, compared to normals.

From the studies in which "free sodium" was calculated; it can be seen that most are essentially in agreement with the interpretation of the experimental evidence presented in this thesis suggesting that the hypertensive excretes a urine which has a less positive "free sodium" than the normotensive control.

It might be argued that the group of studies in which "free sodium" is the same for hypertensive and normotensive animals is small and the results are not entirely conclusive. The study by Braun-Menendez can legitimately be minimized because only four animals were used and exact figures of urinary excretion were not available.

In the study by Renwick et al. (82) sodium ingestion was restricted and perhaps the differences in "free sodium" excretion are best seen when the patients are allowed an ordinary intake of sodium or during sodium load.

The experiments by Ek and Weston et al. are complicated and the results are not easily evaluated. However, both measured sodium excretion following infusion of an osmotic diuretic. The discrepancy in "free sodium" excretion in these experiments can be adequately explained by assuming that osmotic diuretics increase "free sodium" excretion in hypertension. The manner in which osmotic diuretics may act on "free sodium" excretion will be considered next.

The study by Brodsky and Graubarth (87) using osmotic diuretics complicates the picture with respect to "free sodium" excretion in

hypertension. However, the information revealed by this study is quite informative from the standpoint of the general problem of renal regulation of water and electrolytes in hypertension.

Hypertensive patients were subjected to osmotic loads using mannitol. Sodium excretion and total osmolarity of the urine were measured simultaneously. This permitted a comparison of "free sodium" and "water economy" in the same experiment. The findings showed that hypertensives excreted two to two and one-half times more sodium in the urine than patients in the normotensive group. The urinary volume of the hypertensives was increased by less than 50% over the normotensive group. When "free sodium" was calculated, the hypertensive patient was found to excrete a urine with a more positive "free sodium" than the normotensive control. Nevertheless, the hypertensive patient showed an inability to concentrate the urine. The osmolarity of the urine collected from hypertensive patients averaged 455 m. osm./l. compared to 540 m. osm./l. for the normotensive group. When "water economy" was calculated by the authors, the value for the hypertensives was found to be less than 50% than that of the normal subjects. The authors calculated "renal work" and found that under osmotic load the hypertensive kidney was capable of doing less than 50% of the work that the normotensive kidney does.

The results of this study show that osmotic diuretics may act to increase "free sodium" excretion in hypertension. At the same time, however, disturbance in osmotic regulation resulting in a diminished ability to concentrate solutes was observed in the hypertensive.

It would appear that in the hypertensive the kidney is so altered that it is relatively unable to save water for the body. Shown by studies of "free sodium" and osmotic diuretics this defect could induce hyperosmolarity of the body fluids. When viewed from this standpoint, the hypertensive would always show a slight tendency toward hyperosmolarity of body fluids due to the increased urinary loss of "free water." However, this loss would be covered by the increased intake of water observed in hypertension. The homeostatic mechanisms are presumably finely adjusted so that no large disturbance in body osmolarity occurs. However, the hypertensive patient is always in a situation where hyperosmolarity of the body fluids may develop. If the observation that hypertensive patients have a polydipsia as well as a polyuria is taken into account, it is possible that some of the discrepancies in studies in electrolyte content in hypertension may be resolved by further study. Possibly an increased osmolarity of body fluids or an increase in the sodium to water ratio in hypertension could be demonstrated by restricting water for some time before the determination is made. In those studies in which water was not restricted the hypertensive patient might still have a small increase in osmolarity of body fluids or electrolytes over his normal value.

Since it is difficult to determine the absolute "normal" value for a given individual one has to depend upon a range of "normal" values. Small increases in body osmolarity of hypertensive individuals may be masked as a result of this difficulty.

The experiment by Brodsky and Graubarth shows that the basic renal defect in hypertension is in osmotic regulation rather than in some particular disturbance in sodium metabolism. However, since sodium chloride is the main inorganic solute in the urine under usual circumstances, the diseased kidney in hypertension is unable to concentrate sodium as well as the control. This is seen in the disturbance in free sodium excretion in hypertension. Although the defect in hypertension is an inability of the kidney to concentrate solutes, from a physiological standpoint it can be anticipated that this defect would be reflected in disturbances in sodium metabolism.

In many of the studies on sodium excretion, the inference has been drawn that the hypertensive patient is a "salt loser." However, insufficient attention has been focused upon the fact that the hypertensive patient also ingests more sodium. If this is correct, it would be necessary for the hypertensive patient to excrete more sodium in order to maintain a metabolic balance. The study by Renwich et al. supports the view that the increased excretion of sodium seen in hypertension is a consequence of an increased salt appetite by the hypertensive patient. In this study in which sodium diet was carefully regulated so that the hypertensive patients and normotensive patients received the same amount of sodium for a prolonged period of time, there was no difference in the amount of sodium excreted by the two groups.

Conclusions

(1) In a majority of the studies in which "free sodium" excretion was calculated, the "free sodium" excretion of hypertensive patients and animals was found to be less positive than in the normotensive controls.

(2) In a few of the studies there was either no difference in "free sodium" excretion or "free sodium" excretion was found to be increased in hypertension. These studies indicate that the "free sodium" disturbance is not primarily involved in the pathogenesis of hypertension.

(3) Work with osmotic diuretics indicates that the primary renal defect in hypertension is decreased ability of the kidney to concentrate solutes.

(4) The finding of a less positive "free sodium" excretion in hypertension in most studies is the physiological consequence of the reduced ability of the hypertensive kidney to do osmotic work.

(5) The loss of concentrating power by the hypertensive kidney results in a tendency toward hyperosmolarity of the body fluids. This is likely to result in increased concentration of the major osmotically active solute of the extracellular fluid, i.e. sodium.

(6) Increased "free sodium" excretion in studies using osmotic diuretics, does not indicate an increased ability of the hypertensive patient to concentrate the urine under these circumstances. The osmolar concentration and "water economy" were found to be diminished despite a reversal in "free sodium" excretion in hypertension when osmotic diuretics were employed.

(7) The increased renal excretion of sodium observed in essential hypertension is most likely a consequence of an increased salt appetite.

CHAPTER IX

POSSIBLE MODES OF ACTION OF SODIUM IN PRODUCING HYPERTENSION

Up to this point in the discussion, the author has attempted to show that some of the inconsistencies in the "sodium hypothesis" could be resolved by considering "free sodium" changes in the urine and body fluids. However, when one considers the problem of the sodium-water relationship to hypertension in a broader context, many areas remain in which our knowledge is either inconclusive or highly controversial. This is especially noticable when one deals with the question of the etiological role of sodium in hypertension. In many instances when the attempt is made to relate sodium to hypertension, the experimental data either do not support this hypothesis or are so weak that they can be used to support several hypotheses. It will be demonstrated that the validity of one of the cogent arguments for a relationship between sodium and hypertension does not depend upon the experimental data offered.

By reviewing some of the current theories and speculations, it will be seen that no argument for the etiological role of sodium in hypertension is strongly supported by existing data. In addition, several experiments would seem to show that sodium has a casual role, if any at all, in the production of hypertension.

The attempts to prove that sodium has an etiological role in hypertension may be considered under two main categories. 1) Sodium

retention causes an increased extracellular fluid volume which somehow results in hypertension and 2) An increase in body sodium or changes in the ratio of intracellular and extracellular sodium causes an increased peripheral resistance either by changing arteriolar tonus, vascular reactivity, or swelling of the arteriole. These ideas will now be considered in more detail.

(1) Sodium Retention Causes an Increases Extracellular Volume Which Results in Hypertension.

It has been suggested that a slight increase in circulating blood volume and extracellular fluid volume might be a factor in causing hypertension. Much of the literature reporting increases in the extracellular volume and in total body sodium has already been presented.

(See sodium-hypertension relationships.) The main points in the development of this hypothesis will be restated briefly at this time.

Braun-Menendez presented evidence that hypertension is often associated with an increase in the extracellular volume and blood volume. He found increased blood volume and extracellular fluid volume in rats made hypertensive by perinephritis, by administration of desoxycorticosterone (DCA), and in bilaterally nephrectomized rats which subsequently developed hypertension (60). Additional evidence supporting the view that sodium is increased in tissues and extracellular fluids is found in studies reported on hypertensive patients (37, 62), dogs (35), and rats (36, 53).

There are three main objections to the idea that an increase in the extracellular fluid volume or blood volume may play an important role in hypertension. These are as follows: (1) Not all studies show increased extracellular space in hypertension (63, 64). (2) Patients with increased extracellular fluid volume frequently do not have hypertension. (3) Many of the measurements of extracellular fluid volume were made with thiocyanate and radiosulfate. These substances are known to give erroneous values for the extracellular fluid volumes.

Despite these objections, increases in extracellular fluid and blood volume may be of importance under certain conditions in hypertension.

In one study, Ledingham (61) used inulin as a measure of the extracellular fluid volume in rats. Inulin gives a lower value than other substances which measure extracellular volume and is thought to be a more accurate measure of this space. His studies showed that although extracellular volume was increased in hypertension of short duration, it fell to normal as the duration of the hypertension increased. The author concluded that experimental hypertension could exist in the presence of a perfectly normal extracellular fluid volume. This finding seems to support the view that expansion of the extracellular space is not of great importance in hypertension since hypertension persists when the extracellular volume has returned to its normal value.

It is attractive to speculate on the other possibility that an increased extracellular fluid volume may be of significance in the development of hypertension. According to this view, an expansion of the extracellular volume would result in an increased blood volume since the two are closely related. It would be anticipated that this would facilitate venous return to the heart. By this mechanism cardiac output might be increased and the initial rise in blood pressure in early hypertension might be achieved without any increase in the peripheral resistance. Unfortunately, hemodynamic studies during the early stages of hypertension are lacking both clinically and experimentally so that it is not possible to subject this hypothesis to critical evaluation at the present time.

The formulation that an increased extracellular volume or blood volume results in increased cardiac output is admittedly oversimplified since the body is subject to many compensatory adjustments. Recently, hemodynamic studies have been performed using hypotensive drugs which indirectly support the view that hypertension is associated with expansion of plasma volume and extracellular fluid volume. Crosley et al. (102) showed that the effect of chlorothiazide, a natriuretic agent, seems to depend upon reduction of plasma volume as a result of extracellular fluid depletion. In this study, a decrease in arterial pressure after giving chlorothiazide was associated with a fall in the cardiac output. The right arterial pressure was reduced and this in the presence of a lowered cardiac output was interpreted to show a decrease in venous return. Fries (103) showed that restoration of the plasma volume with salt-free dextrose reversed the antihypertensive effect of chlorothiazide.

These results suggest that an increased extracellular fluid volume may be of importance in maintaining hypertension. It would seem to the author that the significance of this work lies in another direction. The finding that chlorothiazide reduces cardiac output suggests that sudden alterations in cardiac output may occur in late hypertension under basal conditions. In this connection, Varnauskus (104) found that in a large series of so-called essential hypertensive patients, 36% had peripheral resistance values within normal range. There was no correlation between peripheral resistance measurements and the severity of hypertension.

Although the hemodynamic studies by Crosely et al. and Fries suggest that increases in extracellular volume may be related to hypertension, direct measurements of the extracellular volume during treatment with antihypertensive agents do not support the view that the reduction of blood pressure is due to contraction of an abnormally increased extracellular volume. In a study on the mode of action of chlorothiazide and mercurial diuretics, Hollander and Chobian (105) found that chlorothiazide reduced the blood pressure in 14 of 20 hypertensive patients. Chlorothiazide had no effect upon the blood pressure of 20 normotensive patients used as controls in these studies. Both hypertensive patients and normotensive patients had the same changes in weight and sodium excretion. The sodium space and extracellular fluid volume were measured by radiosodium and radiosulphate. Neither of these measurements was significantly altered as the result of the antihypertensive drugs. When Ross (62) and Dole et al. measured sodium space in hypertensive individuals following salt restriction, no significant changes in sodium space was observed even when blood pressure was reduced.

On the basis of current studies, it can be stated that the extracellular fluid volume is not always increased in hypertension, and it is clear that hypertension can exist in the absence of an increase in the extracellular fluid volume. Expansion of the extracellular volume may be of etiological significance in the early phases of hypertension, but insufficient evidence is available to decide this point. Hemodynamic studies with natriuretic drugs seem to indicate that the hypertensive patient responds to diminution in blood volume by reducing the

cardiac output. However, when direct measurements of sodium space and extracellular volume are made these have not been shown to be lowered. There are two possibilities. Either extracellular fluid volume measurements are not of sufficient accuracy to reflect the physiological changes which are occurring, or the natriuretic agents may act to reduce cardiac output without affecting the extracellular volume.

The hemodynamic studies on the effects of natriuretic agents show that the hypertensive patient can quickly adjust blood pressure to normotensive levels by reducing the cardiac output. This response to natriuretic agents is not seen in normotensive individuals exhibiting comparable sodium and water losses. No explanation has been given for the difference in response between the two groups.

The observation that natriuretic agents lower cardiac output to subnormal values, suggests that normal or expanded extracellular volumes, may be of importance in maintaining the hypertensive state. However, these agents may have an affect upon the cardiac output regulated by a mechanism not related to changes in extracellular volume.

Even if increased cardiac output is responsible for elevation in blood pressure in the early phases of hypertension, it is difficult to show how increased cardiac output then causes alterations in the vascular bed to account for the increased peripheral resistance found in most cases of hypertension.

It has been proposed that increased blood pressure may cause arteriolar lesions which may increase peripheral resistance in time.

Wilson and Byrom (107) consider that the production of arterial lesions is due to the combined effect of hypertension and vasoconstriction.

While this hypothesis is an attractive one, there is insufficient information to decide this point at present.

Some evidence suggests that an increased extracellular volume is associated with hypertension, but at the present time, it must be concluded that direct evidence is lacking to implicate this as a necessary condition in the production or maintenance of hypertension. It is clear that further hemodynamic studies are necessary to decide whether or not changes in the extracellular volume have an etiological significance in hypertension.

(2) The Effect of Sodium of the Arteriole

In this section an attempt will be made to arrive at an understanding of how increases in sodium or changes in the ratio of intracellular and extracellular sodium may cause hypertension. Several possibilities have been suggested for the role of sodium, such as (A) sodium causes swelling of arteriolar walls, (B) sodium may have local effect causing an increase of vascular resistance, (C) it may increase vascular reactivity or (D) change arteriolar tonus.

A. Swelling of Arteriolar Walls

Tobian has suggested that increased sodium content in arteriolar walls has an osmotic effect which draws water into the blood vessels causing "waterlogging" of the arteriolar walls with narrowing of their lumina. Tobian and Binion (65, 66) found increases in the sodium content in the renal arteries of autopsied hypertensive patients and in the aortas of hypertensive rats. This work was used to support the hypothesis that there is narrowing of the lumina of the arterioles. Their data on the chemical content of aortas showed wide variation and in many instances the differences between normotensive and hypertensive animals was not appreciable. In addition, potassium content was also elevated in the study on hypertensive rats. Although much of the data supporting this argument is unconvincing, the chief difficulty lies in the fact that there is no way at present to subject this hypothesis to experimental test. Measurements on the aorta appear to substantiate the hypothesis, but there is no reason to postulate that

changes in composition of the aorta will be reflected as changes in the composition of the arteriole.

Attempts to confirm the findings of Tobian and associates by other investigators have not been successful. Daniel and Dawkins (67) found no difference in the chemical content of aortas of hypertensive rats from that observed in normotensive rats. Freed et al. found although there was essentially no difference in sodium concentration between hypertensive and normotensive aortas, the potassium content was increased in the hypertensive group. These negative findings do not invalidate the hypothesis since the area that must be considered is the arteriole.

One might expect that it would be possible to demonstrate swelling of arterioles by histological examination if the structural changes were sufficient to account for the necessary increase in peripheral resistance. However, hypertension often occurs with little evidence of generalized arteriolar thickening. This view also minimizes the importance of nervous factors in hypertension in regulating arteriolar tone. For these reasons, the author believes that it unlikely that swelling of the arteriole can account for the increased peripheral resistance seen in hypertension.

B. The Effect of Sodium on Vascular Resistance

The strongest argument in favor of the view that sodium excess is a necessary condition for hypertension would be the finding of a direct relationship between the administration of sodium and a demonstrable

increase in arteriolar resistance. The results of studies on the effect of sodium on arteriolar resistance have been particularly disappointing and give additional evidence that sodium is probably not involved in any simple manner in the etiology of hypertension. The effects of sodium have been studied by perfusion, by studies on vascular reactivity, and by its effect on contractility on smooth muscle strips. In the great majority of these studies, excess sodium has not been shown to produce an increased peripheral resistance.

Haddy (109) perfused the forelimb of the dog with varying concentrations of sodium chloride and found that hypertonic saline caused a decreased arteriolar resistance. Eliakin et al. (110) studied the effect of giving high concentrations of sodium intravenously to dogs. In this experiment, 20% saline was given in a dose of 1 ml/kgm. Immediately following the injection of saline, the blood pressure fell precipitously reaching levels as low as 5-10 mm. Hg. The blood pressure returned to normal within 60-90 seconds. This would seem to show that except for its immediate effect when the dogs were apneic, excess sodium has little or no effect upon blood pressure. In both of these experiments, the vascular response was measured immediately after loading. It might be anticipated that a permanent increase in arteriolar resistance would depend on a more chronic stimulus. These experiments do not exclude the possibility that sodium may increase arteriolar resistance following chronic administration of hypertonic saline solutions. The author is not aware of any studies which have been performed on the long term effects of sodium administration on

peripheral resistance. The occurrence of salt hypertension has not been shown to be independent of changes in the cardiac output. It would be necessary to show that chronic administration does not change peripheral resistance before one can definitely conclude that sodium does not increase the peripheral resistance in hypertension.

C. Sodium Effects Upon Vascular Reactivity

Although pressor agents, such as epinephrine and norepinephrine have not been shown to be increased in hypertension, the possibility should be considered that increased peripheral resistance could be a consequence of sensitization of the blood vessels by sodium which would, in turn, augment the response of norepinephrine. Most studies do not support this hypothesis. Friedman et al. (111) found that sodium had a slight depressor effect upon vascular reactivity to epinephrine in normotensive rats when isotonic saline and hypertonic sodium acetate were given.

Zsoter and Szabo (112) measured vascular reactivity in the rat following a high salt diet of 10 weeks duration. Vascular reactivity to epinephrine was increased as judged by direct visualization of the rat mesoappendix. The authors did not wish to speculate on the significance of these results since they felt that the rat mesoappendix may not be representative of other vascular beds.

Raab et al. (113) observed that sodium restriction reduced the pressor response to norepinephrine infusion in hypertensive patients. Dahl (114) was not able to confirm these findings, finding no effect.

On the basis of the evidence which has been reviewed, it would appear that excess sodium has an inconstant effect, but it does not seem to increase the effect of vasopressor drugs under normal conditions. It is not clear what effect excess sodium produces on the vascular reactivity of hypertensive animals. The experiments also fail to show what the long-term effects of sodium administration are on vascular reactivity. On the basis of available information, one can conclude that the evidence does not support the view that sodium increases vascular reactivity.

D. Cation Effects Upon Arteriolar Tonus

In a number of studies, the suggestion has been made that shifts in sodium between the extracellular fluid and intracellular fluid might be of significance in hypertension. It has also been suggested that sodium may replace potassium in the intracellular fluids and that this exchange may act to increase the peripheral resistance. These suggestions have been made because a simple relationship between sodium excess and hypertension does not appear to exist. However, as explanations become more sophisticated in this field, they also become more speculative. Friedman, Butt, and Friedman (115) proposed that blood pressure regulation depends upon exchanges of sodium and potassium between the intracellular and extracellular compartments. Elevation of blood pressure by pitressin and norepinephrine was claimed to result in a transfer of sodium and water into the intracellular compartment. Increased extracellular potassium was also observed. In these studies the changes in extracellular fluid were

measured by inulin space after nephrectomy to prevent renal excretion of inulin. The changes in potassium and sodium observed after elevation of blood pressure were small, (less than 2%) and were well within the expected error of the determinations.

In studies of smooth muscle tension using strips of rat colon, Friedman (118) found that hypertonic solutions reduced basal tension. However, as sodium equilibrated inside and outside the cells the tension increased. According to the concept presented in this paper, the tension in smooth muscle of vascular walls varies directly with the ratio of sodium concentration inside the cells to that outside.

In other reports, Friedman and associates (117, 118) have emphasized the level of potassium and potassium-sodium ratios in the regulation of blood pressure. In these studies, excess potassium in the diet caused a 25 mm. Hg. rise in blood pressure in normotensive rats. Potassium restriction lowered the blood pressure of hypertensive rats to normotensive levels. Force of this finding is reduced by the fact that cardiac output was not measured.

These studies suggest the possibility that electrolytes may influence vascular tonus. It is interesting to note that excess sodium usually causes a decrease in tonus while excess potassium causes increased tonus. The means by which these cations alter tonus is not clear and the situation is further complicated by conflicting evidence. McQuarrie (37) and Meneely (42) claim that potassium in large amounts has a protective action on salt hypertension.

Since no well-defined redistribution of intracellular electrolytes has been shown to exist during hypertension, there is considerable doubt that these observations of cation influences on vascular tonus can be applied to explain the increased peripheral resistance in the hypertensive state.

Conclusions

The situation with respect to the etiological role of excess sodium in hypertension remains poorly defined. The frequent observation that excess salt aggravates hypertension while sodium restriction is beneficial implies a connection between salt and hypertension. Yet, when it comes to describing the precise way in which sodium produces its effect, we find that our knowledge is deficient. In the previous discussion, it was shown that no simple or direct relationship between sodium and hypertension has been clearly established, at least as far as an understanding of the mode of action of sodium on blood vessels is concerned. The failure of studies to uncover a simple explanation for the effect of sodium on hypertension weakens the belief that sodium is causally related to essential hypertension. Another possibility is that sodium exerts its effect by some indirect method which has not been studied at this time. If sodium does have an effect upon hypertension, it is unlikely that it will be demonstrated by continuation of experiments along the lines of present investigation since we have already seen that there is much conflicting evidence already gathered

with no easy way of resolving the discrepancies. It would seem that a new approach is necessary. Perhaps sodium acts by a less direct mechanism than those that have been postulated.

CHAPTER X

NEUROGENIC FACTORS IN HYPERTENSION

In the preceeding discussion the evidence that sodium acts directly on the arteriole to increase vascular tone was analyzed and it was concluded that this was unlikely since in most of the studies if excess sodium had any effect, it was to decrease vascular tonus. This finding is not particularly surprising since it is generally accepted that nervous factors mediated thru the autonomic nervous system, are of prime importance in the regulation of vascular tonus.

One of the current views favors the idea that hypertension is maintained by a neurogenic mechanism. Some of the evidence supporting this hypothesis will be reviewed. In 1940 Dock (119) observed that destruction of the brain stem of hypertensive and normotensive rabbits resulted in a precipitous drop in blood pressure. Following this procedure the differences in blood pressure between the two groups of animals was abolished. This experiment was used to show that renin does not act to maintain blood pressure in hypertension. It was reasoned that if the hypertensive animals had a higher level of circulating pressor material it would be expected that they should have a higher blood pressure following this procedure. When renin was given to the

rabbits following destruction of the brain stem, there was a transitory increase in blood pressure indicating that the blood vessels were still capable of reacting to pressor substances.

Dock suggested that hypertension is regulated by the central nervous system. In hypertension the blood pressure regulating center is "reset" at a higher level which then becomes "normal" for the hypertensive state.

Glenn, Child, and Page (120) had previously shown that transection of the spinal cord immediately below the level of the phrenic nerve resulted in a rapid drop of blood pressure in hypertensive dogs which was followed by a slow rise in blood pressure. The blood pressure finally stabilized at levels which were slightly higher than the normotensive levels determined before hypertension was produced in these animals. This experiment shows that in the "spinal hypertensive dog" the blood pressure is maintained at normotensive levels. The increased blood pressure of hypertension would seem to be influenced by the blood pressure regulating center which has become "set" at a new level as suggested by Dock.

In another approach to neurogenic control in hypertension pharmacological agents were used to block nervous activity.

Sapirstein and Reed (121) observed that the sympatholytic and adrenolytic drug F 883 reduced blood pressures of chronically hypertensive rats to nearly normotensive levels. F 933 and adrenolytic drug was without effect showing that the drop in blood pressure was due to the ganglionic blocking action of F 883 rather

than its action on circulating pressor amines. Both drugs were without effect in early hypertension. In a similar type of experiment Reed, Sapirstein, Southard and Ogden (122) using nembutal in anesthetic doses and a sympatholytic drug, yohimbine, showed that these agents reduced blood pressure in chronic hypertensive rats. There was no significant response to these drugs in early hypertension.

In an experiment on dogs with hypertension of more than one year's duration Mandel, Greene, Sapirstein and Ogden (123) confirmed the results previously obtained with rats. Hypertensive dogs showed a reduction in arterial pressures following administration of nembutal in anesthetic doses.

In still another approach to this problem, Patton, Page and Ogden (124) in America and later Pickering (125) in England showed that nephrectomy in early hypertension had a blood pressure lowering effect while in chronic hypertension the blood pressure remained elevated despite nephrectomy.

Reed, Sapirstein, Southard, and Ogden (122) and later Ogden, Collings, and Sapirstein (126) suggested that the difference in response of hypertensive animals to ganglionic blocking agents, nembutal anesthesia, and nephrectomy in early and late hypertension could be explained by postulating a change of mechanism during the course of the disease with early hypertension being caused by a renal disturbance and maintained in the chronic stages by a neurogenic mechanism.

Caution must be used in the interpretation of the experimental evidence cited in postulating a change of mechanism in hypertension. . . . The reduced blood pressure observed in these studies may not have been a consequence of changes in peripheral resistance as was originally assumed. Since the time of the original studies on the effects of ganglionic blocking agents, nembutal anesthesia, and nephrectomy in early and late hypertension, hemodynamic studies have been reported which suggest that these agents may alter cardiac output rather than peripheral resistance.

A study by Fries et al. (127) on the hemodynamic effects of hexamethonium, a ganglionic blocking agent, showed that it acted to reduce blood pressure in hypertensive patients by decreasing the cardiac output. The peripheral resistance was not altered by this drug. It is not clear whether nephrectomy alters cardiac output. Grollman, Turner, Levitch, and Hill (128) reported that the cardiac output of nephrectomized dogs, subjected to intermittent peritoneal lavage, was not changed by this procedure. Krieger and Hamilton (129) found that "renoprival hypertension" was usually associated with an increased cardiac output rather than with changes in peripheral resistance. In this study, only two of seven animals developing hypertension after nephrectomy showed an increased peripheral resistance. Further studies may be required to clarify the effect of nembutal anesthesia on early and late hypertension. Simpson, Ezrow, Vidt, and Sapirstein (130) reported that nembutal causes a reduction of plasma volume as well as an increased hematocrit when given in anesthetic doses. These studies serve to

emphasize that some of the blood pressure responses previously ascribed to changes in peripheral resistance may be a consequence of alterations in cardiac output.

CHAPTER XI

EFFECTS OF OSMOLARITY ON THE NEUROGENIC STAGE OF HYPERTENSION- A NEW HYPOTHESIS

The hypothesis that chronic hypertension is mediated through a neurogenic mechanism is based upon the presupposition that ganglionic blocking agents and nembutal lower blood pressure by inhibiting nervous activity maintaining vascular tone. Thus the demonstration of a neurogenic mechanism, as formulated in the present hypothesis, depends upon showing that inhibition of the neurogenic mechanism results in a decreased peripheral resistance. Although the strength of this argument has recently been challenged, principally by the finding that a ganglionic blocking agent may alter cardiac output without changing peripheral resistance, the hypothesis that a neurogenic mechanism intervenes to maintain hypertension still offers the best explanation for the observed response to sympatholytic drugs, nembutal anesthesia, and nephrectomy in late hypertension.

Assuming that a neurogenic mechanism intervenes to maintain the hypertensive state once hypertension develops, one may question how a change of mechanism occurs. The author is not aware of any attempt to show a causal relationship between a renal mechanism in early hypertension and the neurogenic mechanism which maintains hypertension.

There is considerable evidence reviewed in this thesis which suggests that hypertension is associated with increased "free sodium" of the body. This may be the result of a renal abnormality, excessive salt intake, or a combination of the two factors. When the attempt is made to relate the excessive "free sodium" to hypertension, its role is vague. Present information does not strongly support the view that it acts directly on the arteriole to cause an increased peripheral resistance yet increased peripheral resistance is presumed to be the characteristic abnormality of hypertension. It would appear that the increase in total peripheral resistance seen in hypertension is caused by a neurogenic mechanism but the stimulus for this mechanism is not known. It is suggested that a positive body "free sodium" or increased osmolarity of the extracellular fluids may be the stimulus initiating the neurogenic mechanism. According to this view, increased osmolarity or body "free sodium" acts directly on osmoreceptors in a vasomotor regulating center within the brainstem. This activates the vasomotor regulating center to increase sympathetic tone. There are three main lines of evidence which support this hypothesis.

(1) Intracarotid injections of hypertonic saline increase systemic arterial blood pressure suggesting the presence of an osmoreceptor in the brainstem capable of regulating blood pressure.

(2) Changes of body "free sodium" affect blood pressure of the hypertensive individual but are usually without effect in the normotensive individual.

(3) The extra-renal pressor system of late hypertension depends upon the presence of a renal abnormality.

The significance of these observations and their relationship to the proposed hypothesis that the stimulus for the neurogenic component of hypertension is increased osmolarity of the extracellular fluid or increased body "free sodium" brought about by a renal abnormality in hypertension will now be discussed.

(A) Intracarotid Injections Of Hypertonic Solutions

Intracarotid injections of hypertonic solutions have been shown to increase arterial blood pressure. The result of a study by Holland, Sundsten, and Sawyer (131) showed that intracarotid injections of hypertonic saline and glucose solutions resulted in a transient lowering of blood pressure lasting a few seconds. This was followed by a second phase in which there was a pronounced elevation of blood pressure. During this second phase the mean arterial blood pressure was increased by 40-50 mm. Hg. and the peak was reached 15 seconds after the beginning rise was noted. The blood pressure fell to normal levels in less than one minute. This result was highly reproducible occurring in 46 of 48 rabbits given hypertonic solutions. Significantly no response was observed when isotonic saline was given by intracarotid injection or if hypertonic saline was given systemically. The authors were able to show that the response did not depend upon a baroreceptor mechanism since denervation of the carotid bodies, carotid sinuses, and bilateral vagotomy were without effect upon the response. They

also did not consider that the result was due to the release of neurohypophyseal hormones since they could show that these were released after the blood pressure effects had occurred. They also showed that midbrain transections did not appear to alter the response.

This isolated study needs confirmation. Objections to this experiment may be raised. The increased blood pressure response was only of short duration and may have been the result of a generalized effect upon the brain due to increased osmolarity of the medium bathing cerebral tissue rather than being due to a specific mechanism activated by osmoreceptors. The increase in blood pressure may have been a consequence of changes in cardiac output rather than due to increased peripheral resistance. Despite these objections, this report may be considered as tentative evidence which appears to support the hypothesis suggested by the author.

(B) Changes Of Body "Free Sodium" Only Affect Blood Pressure Of Hypertensive Individuals

A second line of reasoning which supports the author's hypothesis is based upon dietary studies with salt. Dietary restriction has a blood pressure lowering effect upon human subjects (30, 31, 32) and rats (33, 34). However, restriction of sodium chloride has no effect upon the blood pressure of normotensive animals (33). In the same way, the natriuretic agent, chlorothiazide will reduce blood pressure of hypertensive individuals but has no effect upon the blood pressure of normotensive individuals (105). The sodium effects seem to

be unique being capable of regulating blood pressure of the "hypertensive individual" while being without effect on the regulation of blood pressure under physiological conditions.

It is also of interest to note that in experimental studies (33, 36) when salt restriction is effective in lowering blood pressure, the blood pressure does not drop to some indeterminate point but rather it returns to the normotensive level. This suggests that in hypertension the vasomotor center is "set" at a new blood pressure level which becomes "normal" for the hypertensive animal but this "set" has a built-in memory device which allows the animal to return to its original blood pressure under appropriate circumstances.

These observations combined with those on salt hypertension are amenable to the following interpretation. A significant and sustained increase in the body "free sodium" or osmolarity results in an increased blood pressure thru stimulation of osmoreceptors located within the brainstem. These are mostlikely located in the lower pons or medulla oblongata according to Holland et al. Stimulation of these osmoreceptors results in an increased blood pressure, mediated thru the sympathetic nervous system. These osmoreceptors would differ from those of Verney in their location and by activating the sympathetic nervous system rather than the release of neurohypophyseal hormone.

Increasing the body osmolarity either by excessive salt ingestion or by a renal defect leading to excessive water elimination would lead to stimulation of osmoreceptors which, in turn, would cause an

increase in blood pressure levels. In hypertension sodium restriction, by reducing body osmolarity, would inactivate the osmoreceptor system and restore blood pressure to normotensive levels. Since osmolarity in the normotensive individual is adequately regulated by the usual renal mechanism, an increase in blood pressure would not be expected with a moderately increased salt diet. Grant and Reichman (132) demonstrated that relatively large amounts of ingested sodium have no effect upon the blood pressure of normotensive individuals. However, increasing the amount of salt ingested by a hypertensive individual with a renal defect in free sodium excretion would increase body free sodium. This would increase blood pressure. According to this view restriction of salt in normotensive individuals would not affect blood pressure since the osmoreceptors would not be affected by this procedure.

It is interesting to note that while the immediate effect of chlorothiazide is to decrease cardiac output (102), its long-term therapeutic effect is to decrease peripheral resistance. In a recent study of hypertensive patients by Conway and Lauwers (133) these investigators found that plasma volume and cardiac output were not significantly altered by chlorothiazide therapy but there was an average decrease in the peripheral resistance of 23.6% showing that a long term sodium effect can alter peripheral resistance. In this study, patients with splanchnicectomy (partial sympathectomy) responded to chlorothiazide therapy more regularly than unoperated patients,

showing that natriuretic agents may influence vascular tone and that the combined effect of reducing body "free sodium" and partial blocking of sympathetic outflow by surgical means results in an earlier and more consistent reduction in blood pressure.

It would seem strange if a hypothetical osmoreceptor's sole function were to produce "hypertension" in the animal. Possibly an osmoreceptor mechanism functions to increase sympathetic tone during dehydration. In this condition, blood volume is depleted while the osmolarity of body fluids is increased. An osmoreceptor acting to increase sympathetic tone could function to maintain blood pressure under most conditions of dehydration. The baroreceptor mechanism would cause increased vasoconstriction with severe depletion of blood volume.

(C) The Extra-Renal Pressor System Of Late Hypertension Depends Upon The Presence Of A Renal Abnormality

Byrom and Dodson (134) presented experimental evidence that under appropriate circumstances late hypertension is reversible. Hypertension was produced by placing a removable clip on the left kidney of the rat two weeks following excision of the right kidney. In these studies hypertension was maintained in a range of from 4-1/2 to 32 weeks. The clip on the remaining kidney was then removed. Following removal of the clip the blood pressure returned to the normal range within 24 hours in 33 of 34 animals studied. The rats continued to be normotensive for the remainder of the experiment. Observations on some of the animals extended for as long as 19 weeks following removal of

the clip. Floyer (135) confirmed the observation that hypertension may be abolished by removing the clip from the remaining kidney in late hypertension. Byrom and Dodson interpreted their findings to show that no extra-renal mechanism intrudes in chronic renal hypertension in the rat. It would seem to the author that these findings show that if late hypertension is maintained by a neurogenic mechanism, this mechanism is reversible and depends upon a renal excretory abnormality. According to the author's hypothesis, placing a clip on the kidney results in a disturbance in the "free sodium" excretion in a manner which results in increased body "free sodium" or increased osmolarity. It has been suggested that this is the stimulus for the neurogenic mechanism which maintains hypertension. When the clip is removed, the "protected" kidney functions normally and the increased body "free sodium" or osmolarity associated with hypertension is abolished. The neurogenic mechanism is inactivated and the animal's blood pressure returns to normotensive levels.

Conclusions

Although the precise mechanism causing hypertension is not well understood, experimental evidence suggests that hypertension is initiated by a renal disturbance and maintained in the chronic stages by a neurogenic mechanism. The author's hypothesis is that the renal abnormality in hypertension is characterized by the excessive elimination of water out of proportion to the urinary sodium excretion which results in an increased body "free sodium" or an increased

osmolarity of the extracellular fluid. This results in stimulation of osmoreceptors which regulate a neurogenic mechanism via the sympathetic nervous system to increase peripheral resistance by increasing arteriolar tone. Three types of evidence have been used to support this hypothesis. It has been shown that (1) intra-carotid injections of hypertonic saline cause increased arterial blood pressure (2) sodium restriction lowers blood pressure in the hypertensive state but has no effect upon normotensive individuals suggesting that reduction of body "free sodium" or extracellular osmolarity has an effect only on the stimulated osmoreceptor (3) studies on chronic uninephrectomized rats show that removal of the clip from the remaining kidney results in a rapid lowering of blood pressure to normotensive levels. This suggests that the neurogenic mechanism in chronic hypertension is reversible provided that normal renal function can be re-established.

CHAPTER XII

SUGGESTIONS FOR ADDITIONAL STUDIES ON HYPERTENSION

In the course of evaluating and synthesizing the material presented in this thesis, additional experiments occurred to the author which might help to clarify some of the existing problems. Experiments which might usefully be employed to verify hypotheses suggested in this thesis will be considered.

(1) The experimental data presented in this thesis was done on the rat. The assumption is made that in this animal hypertension is the consequence of increased peripheral resistance. No studies on cardiac output of the rat in renal hypertension have been performed as far as the author is aware. It would be useful to determine the peripheral resistance of the rat made hypertensive by various procedures to see if this animal does simulate the conditions in essential hypertension.

(2) The inference that hypertension is associated with hyperosmolarity of body fluids is based on the urinary finding of a less positive "free sodium" excretion in hypertension and increased sodium concentration in the plasma. Direct measurements of the osmolarity of the plasma of hypertensive individuals to see if this is increased should be performed.

(3) The assumption was made in this thesis that the stimulus for the neurogenic mechanism of hypertension is a renal abnormality

which develops in early hypertension. To prove this further studies on "free sodium" excretion during early hypertension are necessary. Studies on plasma osmolarity could be conducted at the same time to see if there is any change in early hypertension. These studies might give information which would make it possible to decide whether the renal abnormalities associated with experimental hypertension are causally related to hypertension, merely a consequence of damage to the kidney, or due to the increased blood pressure itself.

(4) Studies with osmotic diuretics show that these alter "free sodium" excretion in hypertension. Since mannitol is frequently used for renal clearance studies at the same time sodium determinations are being made, further studies to determine its effects on "free sodium" excretion on both normotensive and hypertensive individuals should be undertaken.

(5) The belief that sodium has an etiological role in hypertension is supported by the fact that hypertension can be produced experimentally by ingestion of excessive amounts of salt. This argument could be strengthened if it could be shown that salt hypertension causes an increased peripheral resistance. Measurements of cardiac output in salt hypertension are required.

(6) It has been suggested that late hypertension is maintained by a neurogenic mechanism. This hypothesis is based upon the finding that ganglionic blocking agents and nembutal anesthesia reduce blood pressure in late hypertension. These experiments have been questioned because recent hemodynamic studies indicate that the reduction in

blood pressure may be a consequence of reduced cardiac output rather than decreased peripheral resistance. These experiments should be repeated to determine whether the observed effects are due to changes in peripheral resistance. The studies showing a difference in blood pressure response in early and late hypertension following nephrectomy should also be repeated to see what happens to the peripheral resistance under these conditions.

(7) A single experiment has been reported which shows that intracarotid injections of hypertonic solutions increase arterial blood pressure in the rabbit. This study should be repeated to see if the results can be duplicated in other species. The suggestion that intracarotid injection of hypertonic solutions results in stimulation of osmoreceptors which activate a neurogenic mechanism via the sympathetic nervous system could be tested by seeing if the effects of intracarotid injection of hypertonic solutions on blood pressure are inhibited by ganglionic blocking agents or sympathectomy. Studies could also be performed to see if intracarotid injections of hypertonic solutions result in an increased total peripheral resistance.

(8) Following removal of the clip from the remaining kidney of the hypertensive rat, Wilson and Byrom found that blood pressures returned to normal levels. The author suggested that this was a consequence of abolishing the renal abnormality is "free sodium" excretion. Studies on "free sodium" excretion and osmolarity of body fluids following removal of the clip could be performed to see if the reduction in blood pressure is accompanied by sudden changes in "free sodium" excretion and body osmolarity.

CHAPTER XIII

SUMMARY AND CONCLUSIONS

Evaluation of the literature suggested that increased body sodium might be of etiological significance in the pathogenesis of essential hypertension. Studies on renal excretion of sodium and water have almost invariably revealed that there is an abnormal pattern of excretion of these in hypertensive patients. The possibility was considered that increased excretion of sodium might also be due to an increased sodium intake, since in human hypertension evidence exists that sodium intake is abnormally high. The present studies were begun to see if sodium excretion is increased in experimental hypertension. These studies showed:

(1) Rats made hypertensive by subtotal nephrectomy under hypertonic, isotonic and hypotonic load and under basal conditions had a normal sodium excretion. Water excretion was invariably increased.

(2) A method was devised to relate urinary sodium excretion with water excretion together in a single term which defines the excretion of sodium in excess of water in the urine. This term is designated "free sodium."

(3) Urinary "free sodium" calculations showed that the hypertensive animal excretes a less positive "free sodium" than the normotensive animal.

(4) The consequences of (3) to the animal is an increased body "free sodium."

(5) This increase in body "free sodium" could explain the increased plasma sodium concentration reported in hypertension, aversion to sodium of the hypertensive rat under appropriate conditions, and the reported increase in antidiuretic hormone in hypertension.

(6) Comparison of the "free sodium" excretion patterns in the present studies, with those of others, amply confirmed the tendency of the hypertensive subject to excrete a less positive urinary "free sodium."

(7) In the studies reported in this thesis sodium excretion was not increased. The increased sodium excretion frequently seen in human hypertension probably is the result of increased ingestion of sodium in human hypertension, since hypertensive animals and patients usually remain in sodium balance.

(8) The observed abnormalities on "free sodium" excretion in hypertension are considered to be a consequence of a renal defect in sodium and water metabolism.

(9) Studies on osmotic diuretics show that the renal defect in hypertension is a loss of ability to do osmotic work and is not a specific defect in sodium and water metabolism per se.

(10) The suggestion was made that the renal defect in osmotic regulation may have the consequence of producing a slight hyperosmolarity of the body fluids.

(11) The "sodium hypothesis" suggests that sodium may be of etiological significance in the production and/or maintenance of hypertension. However, no definite etiological relationship between sodium and hypertension could be established from review of literature. The suggestion that sodium may cause hypertension by increasing cardiac output or peripheral resistance were considered. Neither seemed adequate to account for the major abnormalities in hypertension.

(13) Past studies employing ganglionic blocking agents, nembutal, and nephrectomy have been used as evidences to suggest that a neurogenic mechanism may intervene to maintain the elevated blood pressure in late hypertension.

(14) A new hypothesis was postulated relating abnormalities in body "free sodium" to neurogenic hypertension. According to this view, increased osmolarity of body "free sodium" acts directly on "osmoreceptors" in a vasomotor regulating center within the brainstem. Increased osmolarity, according to this view, activates the vasomotor center to increase sympathetic tone.

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