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ALDOSTERONE SECRETION AND ITS RELATIONSHIP TO THE RENIN-ANGIOTENSIN SYSTEM IN THE RAT

William T. Cave, Jr.

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I. ALDOSTERONE

Introduction:[1]

The fatal consequence of the pathological destruction of the adrenal glands in man or of their experimental removal in animals was established 100 years ago. It was not until 1927, however, that the fall in plasma sodium and rise in plasma potassium concentrations which followed bilateral adrenalectomy were observed[2] and attention thereby drawn to the role played in sodium metabolism by the secretions of the adrenal glands. This was followed by the recognition that some of the symptoms of adrenal insufficiency could be alleviated by the administration of sodium chloride[3] and the beneficial affect of sodium chloride in the treatment of Addison's disease was established.

Attempts were consequently made to isolate the life-saving constituent elaborated by the adrenal gland. It was only after the failure of the medullary hormone adrenaline to maintain life in the adrenalectomized animal that it was recognized that the vital constituent originated in the cortex of the gland. In 1927 several groups of researchers independently reported[4] the preparation of adrenalcortical extracts claimed capable of prolonging the survival of adrenalectomized experimental animals. Analyses of concentrates of whole adrenal glands revealed that these contained a large number of steroid substances which originated in the adrenal cortex. The first steroid to be isolated which possessed recognized adrenal cortical activity was corticosterone in 1937.[5] Cortisone had been isolated the previous year but its biological activity had not been recognized at that time. By 1943 the number of steroids isolated from adrenal extracts had grown to 28.

When all the known crystalline steroids had been removed from adrenocortical extract there remained an 'amorphous fraction' which possessed great physiological activity as measured by its sodium retaining effect on

the kidney of the adrenalectomized dog[6] and by the survival test in rats. In particular the 'amorphous fraction' contained at least 50% of the sodium retaining activity of the original extract.

Molecular Identification and Synthesis:[7,8,9]

In 1952 Simpson, Tait and co-workers began to publish the results of a systematic investigation of the activity of the amorphous fraction of adrenal extract. By means of a sensitive bioassay and of the new chomatographic methods of steroid extraction,[11,12] they discovered the presence of a strong sodium retaining activity in one specific chromatographic fraction of hog adrenal extracts.[13] The same substance was found in dog adrenal vein blood and in monkey adrenal perfusate. These workers in collaboration with Wettstein and Neher of Ciba Ltd., and von Euw and Reichstein of the University of Basle isolated and crystallized the substance, aldosterone, in 1953.

The constitution of the new steroid was elucidated in 1954 by Reichstein and his collaborators and its chemical properties described. It is 18-corticosterone or corticosterone-18-aldehyde and has accordingly been given the name of 'aldosterone'. In solution this aldehyde form exists in equillibrium with the cyclohemiacetal form; the cyclohemiacetal form predominating.

Fig. 1.

"It will be seen that the compound does not possess a 17 hydroxy group and hence does not give the Porter-Silber reaction. It possesses an -α-ketol side-chain and hence reacts with blue tetrazolium and it is a delta-4,3 -αβ-3-unsaturated keytone and so gives a yellow soda florescence with ultra-violet light. Unlike other corticosteroids which give only one acetate in the twenty-one position, aldosterone can be acylated to give three compounds, the 18- and 21- monoacetates and the 18, 21 diacitate.[15]"

The amount of crystalline aldosterone available from adrenal glands, however was so small that the only prospect for full clinical investigation lay in obtaining material by synthesis. At the onset, the only feasable approach appeared to be total synthesis, and between 1955 and 1960, no less than five different total syntheses of aldosterone were reported. In spite of these efforts devoted to total synthesis it was realized that if methods could be developed for the oxygenation of the C-18 methyl group the partial synthesis would be of much greater value in making aldosterone in quantity. Tremendous efforts were directed along these lines and in the space of a few short months in 1960 four partial syntheses were reported.

Metabolism-Biosynthesis, Secretion and Excretion:[15,16]

Figure 2. deals with the biosynthesis of aldosterone. Aldosterone formation has been demonstrated by a variety of procedures from such steroid substrates as progesterone, desoxycorticosterone and corticosterone. Some discrepancies occur in the literature as to which one of the three steroids mentioned is the immediate precursor; however, the bulk of these investigations indicate that the likely

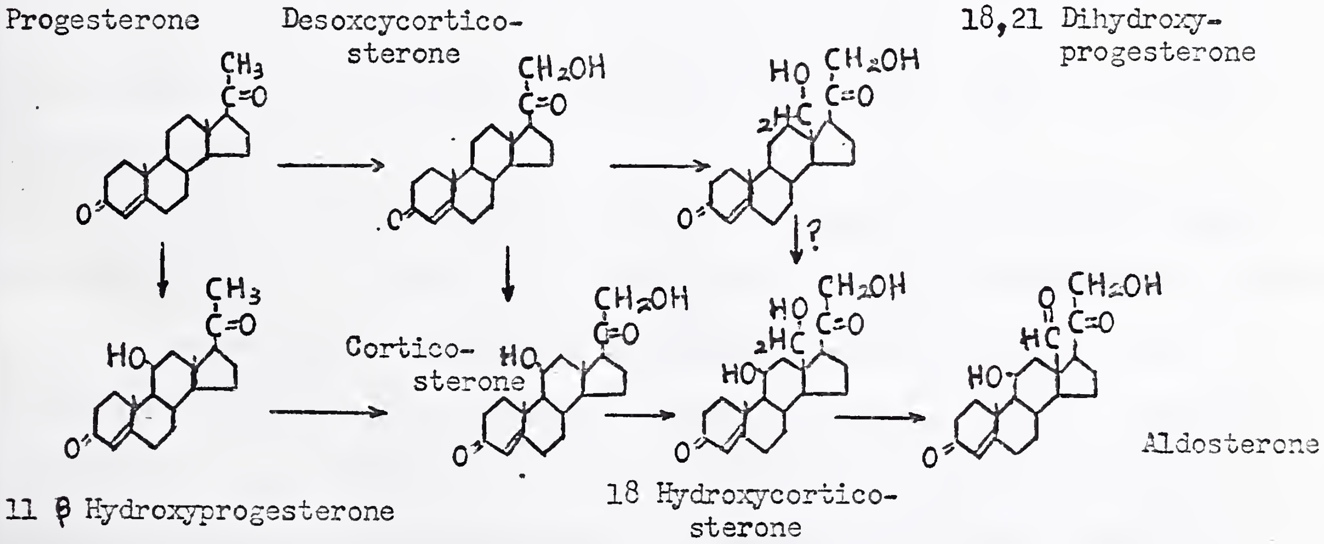


Figure 2.

(While hydroxylation at C-11, C-21 and C-17. occur in the mitochondria (C-11) and microsomes of the cells of the zona reticularis and fasciculata, the enzyme system for the C-18 oxidation is localized in the zona glomerulosa)

pathway is progesterone→ deoxycorticosterone→ corticosterone→ aldosterone. In terms of yield, progesterone by the perfusion technique or by incubation with adrenal capsule strippings[17,18] gave the largest conversion to aldosterone. However, the relative specific activity of the isolated aldosterone fom these steroids labeled with C^{14} was the lowest for progesterone and highest for corticosterone in one study,[19] whereas the specific activity was lowest for corticosterone and highest with progesterone as substrate in another study.[20] Deoxycorticosterone gave values intermediate in range in these cases. In the work of Wettstein deoxycorticosterone-21-C^{14} was converted to aldosterone in high yields, whereas progesterone was not, using adrenal homogenates. In this study the 18-aldehyde derivative of doxycorticosterone was isolated and this compound was suggested to be a direct intermediate in aldosterone biosynthesis. More detailed experiments using bovine capsule strippings by Ayres,[21] while not discounting the presence of other pathways, strongly suggest that the major conversion to aldosterone occurs by a sequence involving cholesterol→progesterone→deoxycorticosterone→corticosterone→aldosterone.

The mechanism for the formation of the 18-aldehyde (free or in hemiacetal form) in aldosterone remains to be demonstrated; however, the 18-hydroxyl derivatives of corticosterone and/or deoxycorticosterone have been synthesized in vitro from steroid precursors, progesterone, deoxycorticosterone, and corticosterone, and it has been suggested that the 18-hydroxy derivatives take the role of intermediates to aldosterone formation. It remains to be proved whether in biosynthesis the 18-hydroxyl group of 18-hydroxycorticosterone is converted to the 18-aldehyde form in aldosterone.

Normal men and women on a normal diet produce about 150 µg of aldosterone per day with no apparent sex difference. This agrees well with the dose of 150 to 200 µg reported necessary to maintain electrolyte balance in Addison's disease.

Low sodium diets call forth an important response and the mean value of 1800 µg that is obtained illustrates the dramatic increase. But this change was only a portion of the value of 5700 µg found for the combination of decreased sodium and increased potassium in the diet. High levels of potassium increased aldosterone about 1005 and high levels of sodium resulted in a secretion of one third that of normal. Nephrosis, hypertension, and congestive failure, and the last trimester of pregnancy cause dramatic increases.

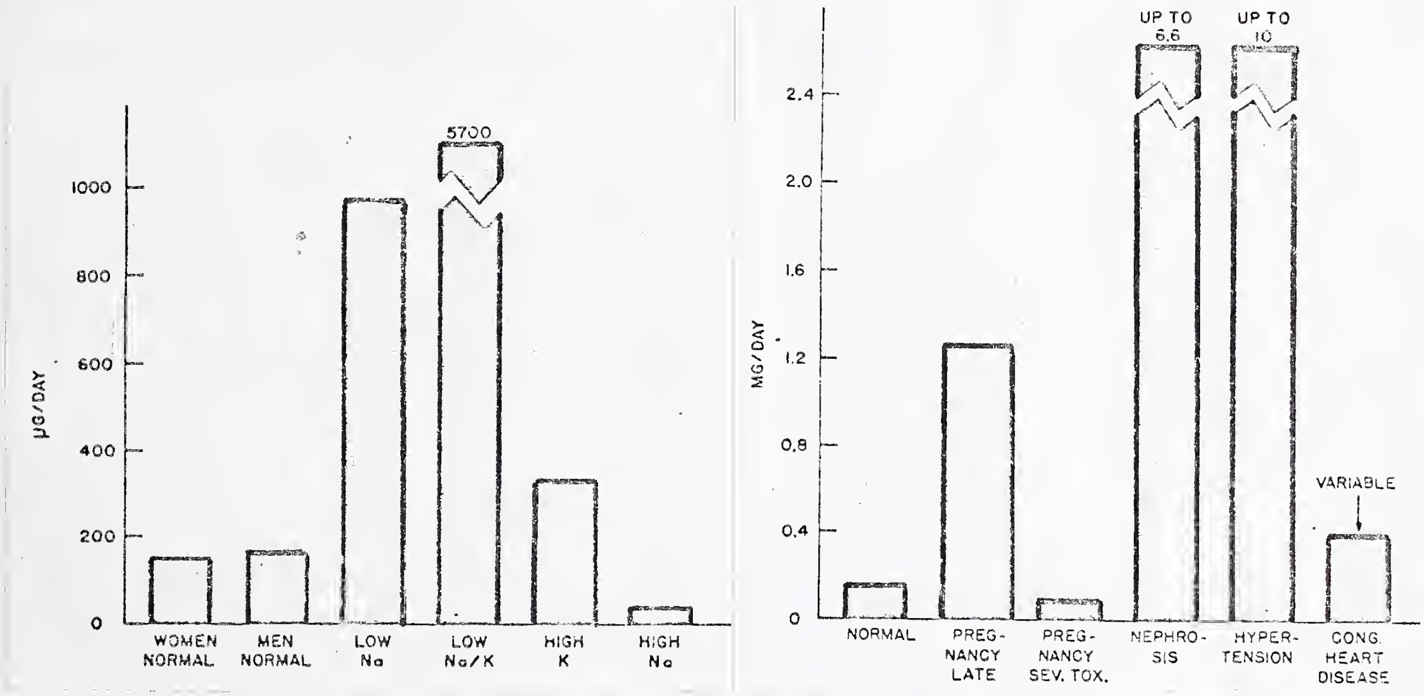


Figure 3. (from Ref. (4))

The results of human studies with 16 H^3 aldosterone indicate that its half life in peripheral blood is from 3 to 5 times shorter than that of 4 C^{14} hydrocortisone, This suggests that aldosterone is metabolized more rapidly than hydrocortisone.

The major metabolites of aldosterone in man are those excreted in urine, and released at pH 1, e.g. aldosterone itself; or by incubation with β glucuronidase, e.g. 3 -5 β tetrahydroaldosterone. From radioactive tracer studies it seems ~~on~~ 20% of secreted aldosterone is converted to the pH. 1 conjugate in normal subjects, themajor route being to the metabolites

conjugated with glucuronic acid after A ring reduction of the steroid molecule.

Biologic Effects of Aldosterone[22,23]

The major biologic role of aldosterone is to modify electrolyte transport, but the mechanism of this effect is as yet still uncertain. Its ability to cause renal sodium retention and increased potassium excretion in man, the adrenalectomized rat and the adrenalectomized dog[24] has been demonstrated. In its absence, depression of the salivary sodium-potassium ratio occurs; chiefly because of a decrease in sodium retention. The potency of exogenous aldosterone with respect to electrolyte metabolism is about 20 to 30 times that of deoxycoticosterone.

In the kidney aldosterone appears to affect tubular function, but no final statement can be made as to which parts of the nephron are under the influence of aldosterone. Stop-flow analysis[25] indicates that at least one site of regulatory influence is the distal tubule. The fact that aldosterone can reduce sodium excretion without altering urinary volume is compatable with such a site of action. Aldosterone by enhancing sodium reabsorption could secondarily enhance potassium and hydrogen ion excretion by an exchange process. When sodium intake is restricted, the increase in potassium excretion that usually follows aldosterone administration is significantly reduced. The phenomenon is explained by postulating that insufficient sodium reaches the location in the distal tubule where the exchange takes place.

There can be no doubt that in many instances potassium excretion parallels sodium reabsorption. However, exceptions do occur. For example, aldosterone can increase potassium excretion without altering sodium reabsorption in sodium loaded dogs.[26] In man, aldosterone has been observed to induce sodium reabsorption without increasing potassium excretion. The exchange process is a frequent

accompaniment but not an essential feature of aldosterone action.

Because of the morphological complexity of the mammalian kidney investigators have turned to the structurally simple isolated toad bladder for analysing the exact effect of aldosterone in sodium reabsorption. Aldosterone stimulates transport by the toad bladder. The transport of sodium in this organ is an active process that is directed against an electrochemical gradient and not accompanied by an exchange for hydrogen or potassium. Edelman and his associates have come forward with a most stimulating suggestion as to the mode of action of aldosterone. The following facts are pertinent to the suggestion: (1) Aldosterone increases sodium transport in isolated toad bladder after a latent period of about one hour; (2) actinomycin and puromycin inhibit the action of aldosterone; and (3) aldosterone increases uptake of H^3-uridine into R.N.A. of the toad bladder. It is suggested that aldosterone induces de novo synthesis of enzymes linking metabolic processes to sodium transport. The increase in sodium transport is considered to be brought about by an increased rate of D.N.A. mediated nuclear synthesis of R.N.A. which in turn increases the rate of synthesis of enzymes involved in sodium transport. The long latent period of action of aldosterone on the mammalian kidney is more readily explained by an influence on nuclear processes concerned with enzyme synthesis than by an action on a cell membrane usually considered to have no latency of onset.

The effects of long term exposure to excessive quantities of aldosterone are dramatically illustrated by Conn's syndrome or primary aldosteronism, a disease in which the adrenal cortex secretes an excess of the sodium retaining steroid. The patient is usually in sodium balance and the concentration of sodium in the plasma is normal or slightly elevated. However, potassium is excreted in excessive amounts despite the hypokalemia. The muscular weakness is a result of the marked loss of potassium. The aberrations in electrolyte and water balance are

complicated by pathological changes in the kidney characteristic of severe potassium deficiency regardless of cause — mainly an inability to form a concentrated urine.

When there is a tendency towards edema, for example, in hepatic cirrhosis and nephrosis, there is frequently an associated abnormally high rate of secretion of aldosterone. Since the primary cause is not the adrenal, the condition is described as secondary hyperaldosteronism. The nature of the stimulus to the adrenal cortex is a matter of controversy and experimental study.

II. REVIEW OF FACTORS REGULATING ALDOSTERONE SECRETION

The actual significance of the several individual physiologic mechanisms known to influence aldosterone secretion is at present, uncertain and often debated. The following review is designed to elucidate the various possible mechanisms[27,28,29] and to present the significant experimental data supporting each. It is hoped that this will provide an adequate background by which one can better understand the purpose of our experience and more critically evaluate the data.

Electrolytes

As mentioned in the previous section, varying the electrolyte composition of diets can elicit rather remarkable fluctations in aldosterone secretion. The two ions most studied in this regard are sodium and potassium because of the known effect of aldosterone on their metabolism.

It was initially thought that the concentration of sodium in plasma might be a regulating factor, and, in fact, perfusion of dog[30] and sheep[31] adrenals with plasma of low sodium concentration was found to increase aldosterone secretion in acute experiments. The observation, however, that the plasma sodium concentration was normal during sodium depletion in man made it seem unlikely that the plasma concentration 'per se' was a major stimulus to aldosterone secretion. This point was further substantiated by data which showed that when vasopressin and water were given to sodium depleted subjects, aldosterone secretion was reduced in spite of a fall in plasma sodium.

Another possible way in which the sodium ion might influence aldosterone secretion was suggested by Stuart-Harris et al[33], who noted that the calculated extracellular sodium decreased and increased 'pari passu' with changes in the extracellular volume to a much greater extent than the total exchangeable sodium.

He thus concluded that changes occurred in the electrolyte composition of cells during hydration and salt depletion, and that these intracellular ectrolyte changes rather than fluid volume might trigger the mechanism controlling the release of aldosterone. These observations and their conclusion can be criticized however, because of the many inaccuracies inherent in the measurement of total exchangeable sodium and the calculation of intracellular sodium.

In brief, then, at present, sodium concentration per se in the cell or in the plasma is not considered to be a critical factor in regulating aldosterone secretion.

In contrast to sodium, plasma potassium levels do seem influential in aldosterone regulation. Gann et al[33] were able to demonstrate in humans that a low serum potassium concentration, which followed potassium depletion, decreased aldosterone secretion while potassium loading increased it.

Increments in the plasma potassium concentration, as small as 1.3 mEq/L have been associated with an increased aldosterone secretion in hypophsectomized dogs[34]. Furthermore, this direct relationship between serum potassium concentration, as controlled by diet, and aldosterone secretion has been shown to be independent of changes in sodium balance, intra-vascular volume, or arterial pressure, and even occurred in the hypophysectomized, nephrectomized animal. Significantly also, it has been shown that acute infusions of KCl and K_2SO_4 into the arterial supply of isolated adrenals increased aldosterone secretion; thus, providing evidence for a direct effect of the potassium ion on the adrenals[35].

An interesting observation made by Gann[36] was that only the infusions of KCl into the carotid artery elicited increased aldosterone secretion, while similar infusions into the peripheral veins elicited no response despite high plasma levels

of potassium. This response did not seem related to ACTH release, as hypophsectomy did not abolish the effect. Because these intracarotid infusions did cause a significant degree of hypertension for some unknown reason, it is possible that this may have been a factor in initiating aldosterone release.

Data from sheep experiments have generally been similar to those from the dog, in that elevation of the potassium concentration of the blood perfusing a transplanted adrenal stimulated aldosterone secretion directly, while acute peripheral infusions of the same or higher ion concentration showed no effect.[37] Finally, data from the rat have been consistent with the observations in the dog and sheep.[38]

Thus, in summary, although the mechanism is not clear, dietary potassium loading does appear to increase aldosterone secretion while potassium depletion diminishes it. Although it is possible its effects are mediated through corresponding changes in potassium ion concentration, acute changes in peripheral plasma concentration seem to have little effect. It is also known, however, that in some species potassium concentration has a direct affect on adrenal tissue, and it might be postulated that ultimately adrenal cell potassium may be an important factor in aldosterone secretion. It is even possible that potassium concentrations influence a neural intermediary to produce its effect on alsosterone secretion.

<u>ACTH</u>

ACTH is another factor which, depending upon the species involved and the metabolic state of the adrenal, can produce a definite effect on aldosterone secretion. Man has been found to increase aldosterone secretion in response to acute administration of ACTH and this effect was enhanced by sodium depletion. However, the response to ACTH was of transient nature and diminished with continued administration. And even though hypophysectomy eventually caused a slow adrenal

atrophy histologically, acute hypophysectomy was not found to lower basal aldosterone levels or prevent the rise in secretion that followed sodium depletion.

It was only in patients with chronic deficiency of ACTH (e.g. with panhypopituitarism for several years) that the immediate increases in aldosterone, usually elicited by sodium deficiency, were reduced or abolished[30].

In the dog, although chronic administration of ACTH did not increase the size of the zona glomerulosa histologically, acute adminstration of ACTH stimulated aldosterone secretion, and hypophysectomy did, in 4 - 6 weeks, cause a mild atrophy in the zona glomerulosa. In fact, in the dog, ACTH is considered to play quite a significant regulatory role. Mulrow et al[40] have demonstrated a dose dependent response in which incremental doses of ACTH only stimulated increased glucocorticoid function initially, but once maximal glucocorticoid stimulation was reached, further increments of ACTH stimulated only aldosterone secretion. This stimulating action was further enhanced by sodium depletion. Another proof that ACTH has an important role comes from the studies of Davis[41] who showed that hypophysectomy in acutely stressed dogs caused a 76% - 97% decrease in aldosterone secretion within two hours. Acute hypophysectomy or suppression of ACTH with high doses of cortisone also decreased aldosterone secretion in dogs with experimental secondary aldosteronism, to values almost as low as those following nephrectomy plus hypophysectomy. Furthermore, acutely hypophysectomized dogs were unable to produce the normally expected increase in aldosterone secretion in response to vena cava constriction.

ACTH has a somewhat less significant role in the rat. Although acute ACTH administration has been found to stimulate aldosterone secretion and hypophysectomy to decrease it[43,44], and even though hypophysectomized rats do not respond to sodium deficiency with increases in aldosterone equal to the controls, it has been shown that acute hypophysectomy in sodium deficient rats does not decrease aldosterone

secretion. Moreover, when ACTH alone was administered to hypophysectomized rats in normal electrolyte balance, it increased alsosterone secretion only slightly and not to levels observed with sodium depletion.

Quite a different situation has been found in the frog, where aldosterone is the main steroid excreted. ACTH was found not only to stimulate aldosterone secretion, but, if suppressed with dexamethasone, the increased secretion levels normally found in certain conditions could not be maintained.

Thus, in summary, while ACTH is known to influence aldosterone secretion in nearly all species, its specific contribution is quite variable. Moreover, its mechanism of action is not clear. Generally, however, it is not felt to be, by itself, the major factor regulating aldosterone secretion but, rather to be important in facilitating the effects of other stimuli.

Neural Factors

In addition to the pituitary, other sections of the nervous system have been postulated to influence aldosterone secretion. In the dog, the major experimental subject for such studies, there certainly is no direct neural control to the adrenals. The adrenals can be transplanted and secrete aldosterone at a high rate and will respond to the general maneuvres known to increase aldosterone. The first suggestion of a C.N.S. humoral influence other than ACTH came from the observations of Rauschkolb and Farrell[45] who noted that removal of the brain above the mid-brain in hypophysectomized dogs resulted in an aldosterone secretion rate lower than in the hypophysectomized controls. Extracts of pineal and adjacent tissues were found to be active when infused intravenously.[46] Further purification revealed both a stimulating factor, thought to be 1-methyl- 6 - methoxy carboline[47] and an inhibitory factor thought to be ubiqinone. Subsequent experimentation, however, showed

that the effect of 1-methyl-6-methoxy carboline was limited exclusively to decerebrate dogs and did not stimulate aldosterone secretion in intact normals.[48] The significance of the influence of ubiquinone is uncertain.

Experiments of Bartter[49] have suggested that the peripheral nervous system in the dog may mediate some control over aldosterone secretion. He demonstrated that when the carotid artery was constricted below the thyrocarotid junction, an increase in aldosterone secretion occurred. This result was independent of peripheral arterial pressure and could be abolished by prior denervation of this junction. It was thus postulated that hemorrhage, thoracic inferior vena cava constriction, and carotid artery constriction below the neck, caused a decrease in pulse pressure and this was detected by nerves at the thyrocarotid junction which relayed the message to the brain to increase aldosterone.

Arguments against there being an important regulatory mechanism in the C.N.S. include the following. Ganong[50] pointed out that only lesions in the hypothalmus which destroyed the median eminence and therefore interfered with ACTH release, could cause a drop in aldosterone secretion. Similarly Davis[51] suggested that diencephalic lesions probably also had no effect on aldosterone secretion except by decreasing ACTH release.

In rats, pineal extracts have been claimed to cause histologic changes indicative of increased activity in the zona glomerulosa, and atrophy of this region was reported to follow pinealectomy. Moreover, there are in vitro studies which report that the addition of fresh pineal and diencephalic extracts to adrenal tissue incubations increased aldosterone production.[52] But, here again, controversy exists. Others have reported no effect of pineal extracts or pinealectomy on plasma or urinary electrolytes, water

intake, or size and histochemisry of the zona glomerulosa.[53] Moreover, it has been demonstrated that pinealectomy has not prevented zona glomerulosa hypertrophy secondary to sodium depletion.[54]

In sheep, the few studies that have been done indicate that, although a sheep with mid collicular brain sectioning, when sodium depleted, increased aldosterone secretion, repletion of the sodium deficit could not turn off the high levels of secretion as it did in normals.[55] Possibly this indicates the prescence of an inhibiting C.N.S. factor in normals.

In any event, the controversy on this subject and the difficulty in correctly interpreting the data are more than evident. At present it is probably reasonable to conclude that, although possibly the nervous system has some facilitative or inhibitive function, the evidence does not seem substantial enough to allot the C.N.S. a major role in the control of aldosterone secretion.

Renin Angiotensin

While it has long been recognized that the renin angiotensin system plays a role in blood pressure regulation it was not until Deane and Masson in 1951[56] observed that hypertrophy of the zona glomerulosa could be produced secondary to renin induced hypertension, that a further role was considered. In 1958,[57] Gross postulated that renin exerted a control function on the secretion of aldosterone. This hypothesis has been investigated by numerous experimenters and has been generally supported; but here also, the exact contribution of this mechanism in the physiologically normal animal is uncertain and, as with ACTH, it may well vary with the species involved.

In essence, the renin-angiotensin system has been elucidated as follows. Renin, an enzyme synthetized in the juxtaglomerular cells of the kidney, is

released into the blood to act upon a substrate in the α-2 globulin fraction termed angiotensin, thus forming the decapeptide angiotensin I, which is then changed by a converting enzyme in the blood to the octapeptide angiotensin II. Angiotensin II is considered the active principle and is rapidly inactivated by a number of peptidases in tissue and plasma.

Before examining the data of the effects of the proposed mcechanism on aldosterone secretion, it is helpful to discuss the renin release system.[58] It is known that acute reduction of renal perfusion pressure will increase renin release from the kidney; and also, that procedures which increase renin release do not influence the plasma sodium concentration at all, or do so in a variable manner. From these facts two interpretations of the renin mechanism have been proposed. Some favor the concept that the juxtaglomerular cells act as baroreceptors through which a decrease in mean arterial pressure is translated into renin release,[59] while others[60] emphasize the work of Vander and Millller[61] and postulate that the macula densa in the distal tubule can translate changes in the filtered sodium load into renin release from the juxtaglomerular cells.

The effects of the eventual products of renin release on aldosterone have been studied in several species. In man, infusions of angiotensin II have been shown to stimulate aldosterone secretion.[62] Conversely, patients with secondary hyperaldosteronism have been observed to have high blood levels of renin and angiotensin. Salt depletion, a stimulus know to increase alsosterone, has been observed to be associated with elevated peripheral plasma levels of renin while salt loading has been associated with depressed levels.

Not readily explained, however, is the observation that angiotensinII levels were not increased in patients who had been on a low sodium diet 5 - 7 days.[63]

Several bodies of evidence favor a role for the renin-angiotensin system in dogs. Infusions of saline extracts, or semi-purified renin extracts of dog kidneys, or angiotensin II, were all observed to stimulate aldosterone secretion.[64] Moreover, infusions of angiotensin II in relatively small doses directly into the adrenal artery supply elicited an increase in aldosterone secretion.[65] Beyond this, it has been observed that the constriction of the aorta above the renal artery, a known stimulus for renin production, caused an increase in aldosterone secretion while constriction below the renal artery which causes no release of renin also caused no aldosterone increase.[66] Another piece of evidence comes from Ganong & Mulrow,[67] who were further able to show that nephrectomy in hypophysectomized dogs not only lowered basal aldosterone secretion level, but prevented the normal rise in secretion seen during hemorrhage. Also it has been shown that increased renin and angiotensin II levels were found in sodium depleted dogs.[68] Using a immunological approach it has been shown that antibodies to hog renin block both its aldosterone stimulating properties and its pressor effect.

In rats, in contrast to the dog and man, experimental data favoring the renin-angiotensin system is not very strong. Some support for the system is found in the work of Gláz and Sugár[69] which showed that when the adrenals from rats which had received large intravenous doses of angiotensinII for three days, were removed and incubated, a significant increase in the capacity to synthesize aldosterone was observed. Another piece of evidence comes from the work of Masson[70] (not yet published) who has been able to cause a 2 - 3 fold increase in aldosterone secretion in uninephrectomized rats by giving chronic subcutaneous injections of large doses of hog renin. Furthermore there are data which show that the elevation in aldosterone that follows

vena cava constriction can be lowered by nephrectomy and that the decrease in aldosterone caused by nephrectomy plus hypophsectomy can be returned to normal by infusion of angiotensin II. In spite of these data there is a significant body of evidence against there being a major role for renin-angiotensin in the rat. For example, when Gláz and Sugár[71] added angiotensin II to the adrenal tissue in vitro they failed to produce the stimulating effect seen in vivo. Extensive experiments with infusions of renin or angiotensin II have been notably negative in their ability to influence increases in aldosterone secretion or show zona glomerulosa histologic changes, whether in the normal[72] or hypophysectomized rat.[73] Moreover, in experimental situations known to have high aldosterone secretion rates, such as following hemorrhage, or salt depletion of a hypophysectomized rat, nephrectomy has not been found to be effective in lowering the secretion rate. In fact, sodium depleted animals have maintained high levels of aldosterone secretion for up to 8 hours following nephrectomy.

From these data it is evident that the humoral renal - adreno cortical relationship of the rat may differ from that of other species and that observations made in rats may not be confirmed in other animals.

In brief, then, in the dog and man the experimental evidence supports the concept of the renin-angiotensin system as an important mechanism in the regulation of aldosterone. The data favoring this system in the rat is at best controversial and even though it may be possible in certain severe experimental conditions to elicit an aldosterone increase with renin, the contribution of the system in the normal physiologic state would not seem to be very great.

Summary

The experimental data presented show that the process by which

aldosterone is regulated is complex. Not only does there seem to be a variety of influences which can modify the equilibrium state, but the importance of each of these influences seems relative to the species involved. The C.N.S., (although well studied) does not seem to have an important role, however, it may have a minor facilitating or inhibiting ability. The potassium ion, ACTH, and the renin-angiotensin system are the major recognized influences. Potassium loading seeems to stimulate aldosterone secretion in all species although it is not the means by which many phenomena increase aldosterone. ACTH seems to have at least a minimal effect in the regulation of aldosterone secretion in most species and is especially important in the frog and the dog. Many consider ACTH to possess a facilitory function necessary in some degree for the full effect of other stimuli. The renin-angiotensin system is probably the most important means of influencing aldosterone secretion, especially in man and the dog. In other species, especially the rat, its role is less well defined. Finally, in light of the difficulty in constructing consistent explanations of all the experimental phenomena known to alter aldosterone metabolism with the above mentioned mechanisms, the possibility of other influences, as yet unrecognized, may exist.

III. EXPERIMENTAL WORK

Introduction

The question of whether the renin-angiotensin system is a major factor in regulating aldosterone secretion in the rat, as it seems to be in the dog and man, is not well answered. A study[75] in 1951, relating adrenocortical changes with various types of hypertension, provided data which would suggest that this mechanism does operate significantly. It was found that injections of partially purified renin into uninephrectomized rats did cause enlargement of the zona glomerulosa. Other studies, notably those of Eilers and Peterson,[76] and Marieb and Mulrow,[77] have shown that neither infusions of semipurified renin nor angiotensin II could elict increases in aldosterone response. One of the explanations for this discrepancy wasthat the injected renin in the original study caused a diuresis and the subsequent sodium loss thus caused zona glomerulosar enlargement (aldosterone secretion). In hope of evaluating this explanation specifically, but generally in hope of elucidating more accurately the function of the renin-angiotensin system in the rat, the following study was undertaken.

Methods

Charles River male rats were used. The following table identifies the group numbers, the numbers of animals tested and the number of animals used for each test.

Table I

			Number of Animals in Each Test			
Group	No. of Animals	Termination Date	Untreated Controls	Sodium Deficient	Denatured Renin	Renin
I	4	6/22/66	2			2
II	4	6/29/66		2		2
III	7	7/13/66	2		3	2
IV	4	8/5/66	1			3
V	7	8/22/66	1		1	5
VI	21	2/3/67	6	3	6	6

There was a variable weight range: groups I - III included rats from 244-253 gm.; groups IV - V from 121 - 200 gm.; and group VI from 193 - 307. They were fed a balanced metabolic diet[78] and given tap water to drink. Rats on a sodium deficient had exactly the same diet except for the omission of sodium chloride.

The renin used was a crude preparation extracted from rat kidneys according to a modified procedure of Haas and Goldblatt.[79,80] The renin in the samples given to the controls was denatured by heating it in a physiologic saline solution to 80 - 90 °C for more than one hour. Both active and inactive samples were evaluated qualitatively for activity by bioassay. In each experiment the selected doses of renin or denatured control were injected subcutaneously in 0.1cc. of physiologic saline, either twice daily (groups I - IV) or three times daily (groups V - VI). The duration of the injection periods varied from nine to twelve days. From the experience of others,[81] it has been shown that one gram of rat kidney tissue (approximately the weight of one normal rat kidney) is equivalent to 2.3 dog units.

Collection of adrenal vein blood for aldosterone secretion measurements was performed in the following manner. The animals were anesthetized with nembutal (5mg/100gm of body weight). They were given 2000 units of heparin parenterally into their tail veins prior to laparotomy. Through a long midline incision the left adrenal vein was exposed and then a 22 gauge needle with a short length of P.E. 50 tubing attached was inserted through the left renal vein and threaded into the adrenal vein. The venous blood drained through this tubing into a graduated centrifuge tube packed in ice. During the ensuing ten to forty minute collection period necessary to obtain an adequate sample (2-4ml. of whole blood) the viscera were covered with a warm saline moistened gauze. Immediately following collection, the sample volume was measured and centrifuged at 3,000 r.p.m. for fifteen minutes. The separated plasma volume was measured and the plasma then stored in a frozen

state until final aldosterone analysis at a later time. The aldosterone assay method used was the double isotope determination of Kliman and Peterson.[82]

In addition to venous blood samples, the organ weights of heart, kidney and adrenals were recorded for each animal. The adrenal glands were further preserved in 10% formalin. These glands were eventually sectioned and individual sections stained with hematoxolin and eosin, and sudan fat stain.

Metabolic studies were also performed on groups I - V. Each animal was maintained in an individual metabolic cage and a daily record was maintained for intake, output, and weight. Alternate daily urines were analyzed for sodium and potassium.

Blood pressure measurements were also taken on the rats in group V according to the method of Harrison et al.[83] The development of any persistent chronic hypertension was evaluated by daily measurements taken approximately eight hours after a sample dose of renin had been given.

Results

Organ Weights: (graph no. 1)

Initiallly small amounts of crude renin (o.16 mg in group I and 0.32 mg in groups II and III) were injected for approximately 10 days. Although the quantities chosen were known to produce an immediate short term pressure rise (at least 20 minutes) in the nephrectomized bioassay animal, no increase in the relative heart weights or adrenal weights of the treated animals as compared to the controls were noted. The amount of renin injected in the subsequent experiments was increased to 9.6 mg for group IV and to 20mg for groups V and VI. In groups IV and V a small consistent relative increase in heart weight (indicative of hypertensive cardiovascular strain) was suggested,

but in group VI using a similar dosage in slightly larger animals no significant heart weight differences were noted. It is possible that the adrenal weights of the sodium depleted animals significantly, exceed the rest; (for example, in group VI an average increase of 18 % was observed.)

Metabolic Studies: (graph no. 1, 2 &3)

Generally it was evident that the untreated controls gained weight most rapidly, (compared to the next largest weight gain, the percentage of extra gain for the untreated controls was as follows: group I, 59%, group III, 36%: group IV, 8%, group VI, 16%). With the exception of the rats given a sodium free diet, no significant comparative differences were noted in the other parameters. The animals receiving renin showed no evidence of a diuresis or excessive sodium loss. It is noteworthy (group II, graph No. 3) that the animals on a sodium free diet showed the anticipated marked decrease in urinary sodium (0.3 mEq as compared 1.6 mEq for renin treated animals) and consequent low Na/K ratio (0.26 as compared 1.25).

Adrenal Zone Widths: (graph no. 4)

No consistent increase in adrenal zone widths seemed evident in any of the renin treated animals. There is a suggestion of a slight increase over the controls in group V but this is not repeated in group VI. The expected increase in adrenal zone width of the sodium deficient rats, however, was present as shown in graph no. 4 for groups II & VI (group II increase was 12% and group VI increase was 51% as compared to the renin treated animals).

The values for both control and hypertrophied sections seem to be proportionally higher than other reported values for similar sections, but this was felt to be a consistent pattern of reading and that it was the relative changes which were important.

Aldosterone Secretion: (graph no.4)

Because of the technical difficulty involved in cannulating the adrenal vein, samples were not obtained from all experimental animals. From the results of those which were collected, only the sodium deficient animals showed an unequivocal increase over the control values. The absolute values for the control rats seem somewhat lower in group VI, but relationship between the denatured renin treated rats and the ones treated with active renin remained relatively constant throughout all groups.

Blood Pressure:

This parameter was only measured in group V. It was found that although there was often a slight blood pressure rise within the first hour after injection, it was usually absent eight hours after injection. No endogenous hypertension could be found after ten days of treatment.

Discussion

The availability, and economy of using rats make them in many ways ideal experimental models for study. Because of this, it would be extremely helpful to know definitively if the renin-angiotensin system, which seems to be a major factor in man, has a functional counterpart in the rat. If this were demonstrated, research on all aspects of aldosterone metabolism might advance more swiftly. If, however, this system were proven not to be functional in the rat, it would emphasize the need for alternate experimental models as well as clarify the interpretation of discrepant observations between the rat and man.

The data presented in these experiments confirms the stimulatory effect of sodium depletion on aldosterone secretion. It also provides evidence that our studies were sensitive enough to accurately detect changes in the

factors measured. The remainder of the data demonstrate that chronic daily injections of moderate doses of renin (up to 7-10 units) will not stimulate aldosterone secretion or cause histologic hypertrophy of the zona glomerulosa in normal rats. Furthermore, no metabolic changes were evident. Blood pressure measurements indicated that only an acute pressor response occurred after renin injections and no chronic hypertension was present at the end of treatment. Thus, these data support the conclusions of Eilers and Peterson,[84] and Marieb and Mulrow,[85] that in the normal rat, the renin-angiotensin mechanism does not seem to be an important mechanism for stimulating aldosterone secretion.

In contrasting our results with those of Masson,[86,87] it is worthwhile to note several significant points. First, in all his data from which it is claimed that chronic injections or renin do cause a definite increase in aldosterone secretion the test animal is uninephrectomized. This hardly seems to simulate the physiologic state. Secondly, the daily doses of renin injected, especially in his most recent work, are very large (80 units). The concomitant cardiac hypertrophy and hypertensive vascular lesions would seem to underline these as nonphysiologic levels since a sodium depleted animal will markedly increase his aldosterone secretion without any evidence of cardiac hypertrophy. Therefore, it is our contention that only under extremely unphysiologic circumstances can renin increase aldosterone secretion.

Summary

Chronic daily injections of renin in normal rats do not stimulate aldosterone secretion, cause hypertrophy of the sona glomerulosa, or alter electrolyte excretion. Support is provided for the view that the renin-angiotensin mechanism is not an important stimulus for aldosterone secretion in the normal rat.

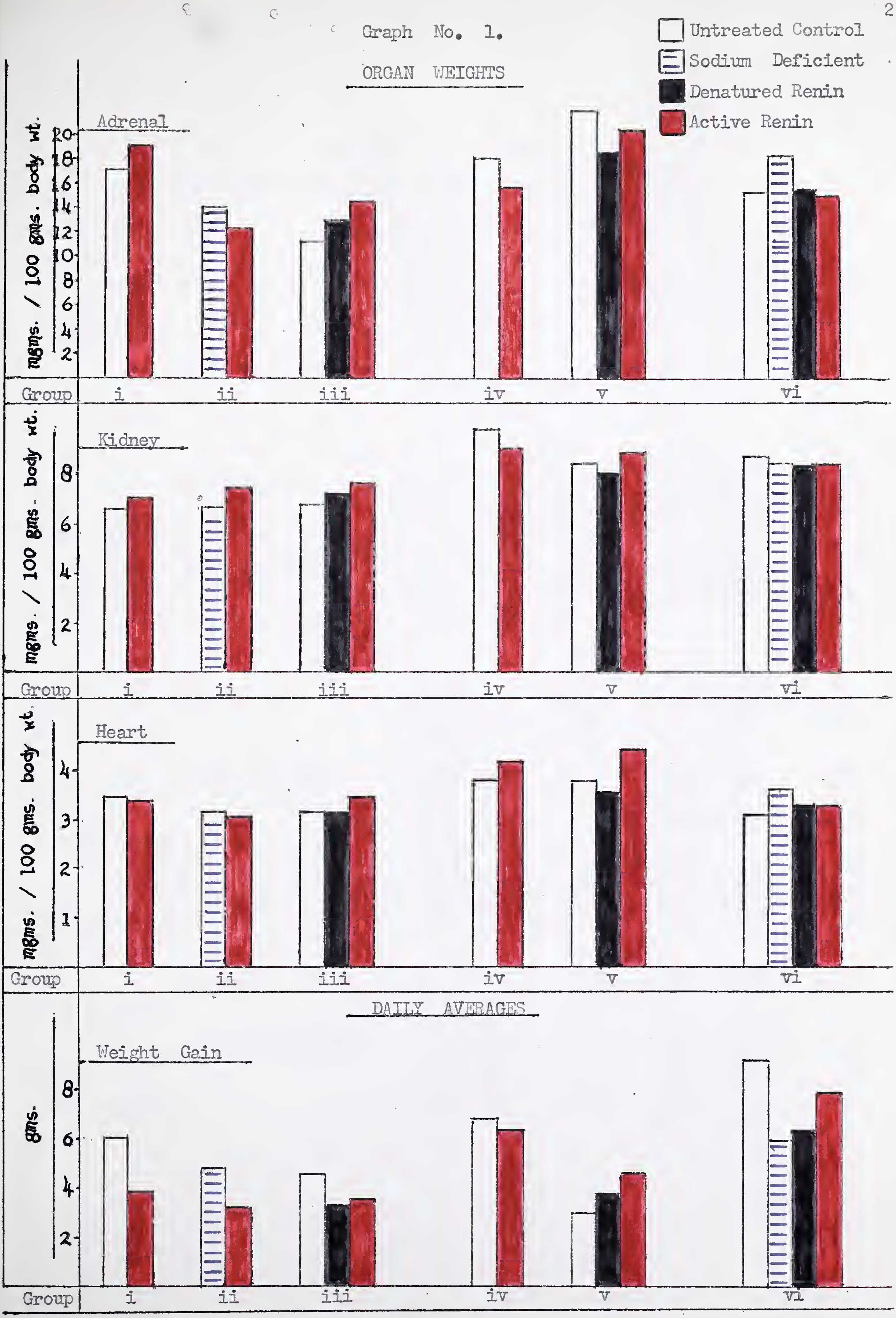
DATA
(for graph no. 1)

GROUP	DAYS	TYPE	BODY WEIGHT (gms)			TISSUE WEIGHTS (In mg/100 gm body wt.)		
			Initial	Final	Average Daily Gain	Adrenal	Kidney	Heart
I	9	Untreated	255	309	6.0	15.92	634.9	345.6
I	"	"	257	315	6.4	18.66	698.4	349.2
Group Average					6.2	17.29	666.7	347.4
I	9	Renin (.16 mg/day)	251	293	4.7	19.93	682.5	341.3
I	"		284	312	3.1	18.58	734.6	347.1
Group Average					3.9	19.26	708.5	344.2
II	9	Na^+ def.	283	325	4.7	12.06	697.8	336.0
II	"	"	309	353	4.9	15.86	680.4	302.5
Group Average					4.8	13.96	689.1	319.3
II	9	Renin (.32mg/day)	302	336	3.7	14.4	861.9	294.9
II	"	"	282	306	2.7	11.11	652.9	307.5
Group Average					3.2	12.24	757.4	301.2
III	12	Untreated	261	310	4.1	11.10	727.7	359.0
III	"	"	276	335	4.9	11.10	642.4	275.2
Group Average					4.5	11.10	685.1	317.1
III	12	Denatured Renin	244	283	3.3	14.98	744.9	368.9
III		"	236	276	3.3	16.96	726.1	312.3
III	"	"	256	295	3.3	7.73	728.8	316.9
Group Average					3.3	13.22	733.3	318.8
III	12	Renin (.32mg/day)	259	303	3.7	18.08	879.8	369.9
III	"	"	244	283	3.3	11.31	639.2	324.7
Group Average					3.5	14.70	759.5	347.3
IV	9	Untreated	138	192	6.8	18.23	983.9	384.7
Group Average					6.8	18.23	983.9	384.7
IV	9	Renin (9.6mg/day)	128	167	4.9	17.48	903.6	400.6
IV	"	"	130	191	7.6	14.76	948.2	425.7
IV	"	"	121	173	6.5	15.38	865.9	458.4
Group Average					6.3	15.87	905.9	424.9

DATA
(for graph no. 1)

GROUP	DAYS	TYPE	BODY WEIGHT (gms)			TISSUE WEIGHTS (In mg/100 gm body weight)		
			Initial	Final	Average Daily Gain	Adrenal	Kidney	Heart
V	9	Untreated	150	176	2.9	22.0	855.1	376.0
Group Average					2.9	22.0	855.1	376.0
V	9	Denatured Renin	138	171	3.7	18.5	819.2	351.0
Group Average					3.7	18.5	819.2	351.0
V	9	Renin(19.1mg/day)	145	200	6.1	27.3	740.0	349.0
V	4	"	142	163	4.2	18.7	1036.8	538.7
V	5	"	141	176	5.8	19.1	953.0	517.6
V	9	"	137	174	4.1	17.8	875.8	397.7
V	"	"	137	165	3.1	20.8	881.2	417.0
					4.6	20.7	897.4	444.0
VI	10	Untreated	205	299	9.4	11.97	864.3	272.7
VI	"	"	192	276	8.4	16.45	803.8	319.5
VI	"	"	193	280	8.7	16.93	909.2	326.7
VI	"	"	204	297	9.3	14.55	984.1	330.8
VI	"	"	208	307	9.9	13.36	854.1	277.6
VI	"	"	198	293	9.5	19.18	814.8	316.4
Group Average					9.2	15.40	871.7	307.2
VI	"	Na$^+$Def.	195	249	5.4	16.99	877.8	347.9
VI	"	"	207	280	7.3	17.14	918.9	349.6
VI	"	" "	194	245	5.1	20.90	813.5	405.3
Group Average					5.9	18.34	870.0	367.6
VI	"	Denatured Renin	195	264	6.9	18.11	860.3	324.3
VI	"	"	197	250	5.3	14.16	826.3	328.0
VI	"	"	202	260	5.8	16.23	901.3	348.5
VI	"	"	204	276	7.2	13.91	815.5	335.6
VI	"	"	194	248	5.2	15.48	848.0	353.1
VI	"	"	199	280	8.1	16.71	884.0	334.3
Group Average					6.4	15.77	855.9	337.3
VI	"	Renin(20mg/day)	199	293	9.4	20.34	884.6	333.6
VI	"	"	203	297	9.4	12.65	909.6	308.1
VI	"	"	200	270	7.0	12.04	850.5	312.3
VI	"	"	193	260	6.7	18.27	844.3	373.5
VI	"	"	193	267	6.9	14.91	902.9	368.7
VI	"	"	193	271	7.8	12.32	797.7	333.1
Group Average					7.9	15.09	864.9	338.2

Graph No. 1.


□ Untreated Control
▤ Sodium Deficient
■ Denatured Renin
🟥 Active Renin
ORGAN WEIGHTS
Adrenal
mgms. / 100 gms. body wt.
20
18
16
14
12
10
8
6
4
2
Group
i
ii
iii
iv
v
vi
Kidney
mgms. / 100 gms. body wt.
8
6
4
2
Group
i
ii
iii
iv
v
vi
Heart
mgms. / 100 gms. body wt.
4
3
2
1
Group
i
ii
iii
iv
v
vi
DAILY AVERAGES
Weight Gain
gms.
8
6
4
2
Group
i
ii
iii
iv
v
vi

DATA
(for graphs no. 2 & 3)

AVERAGE DAILY INTAKE - OUTPUT AND SALT EXCRETION

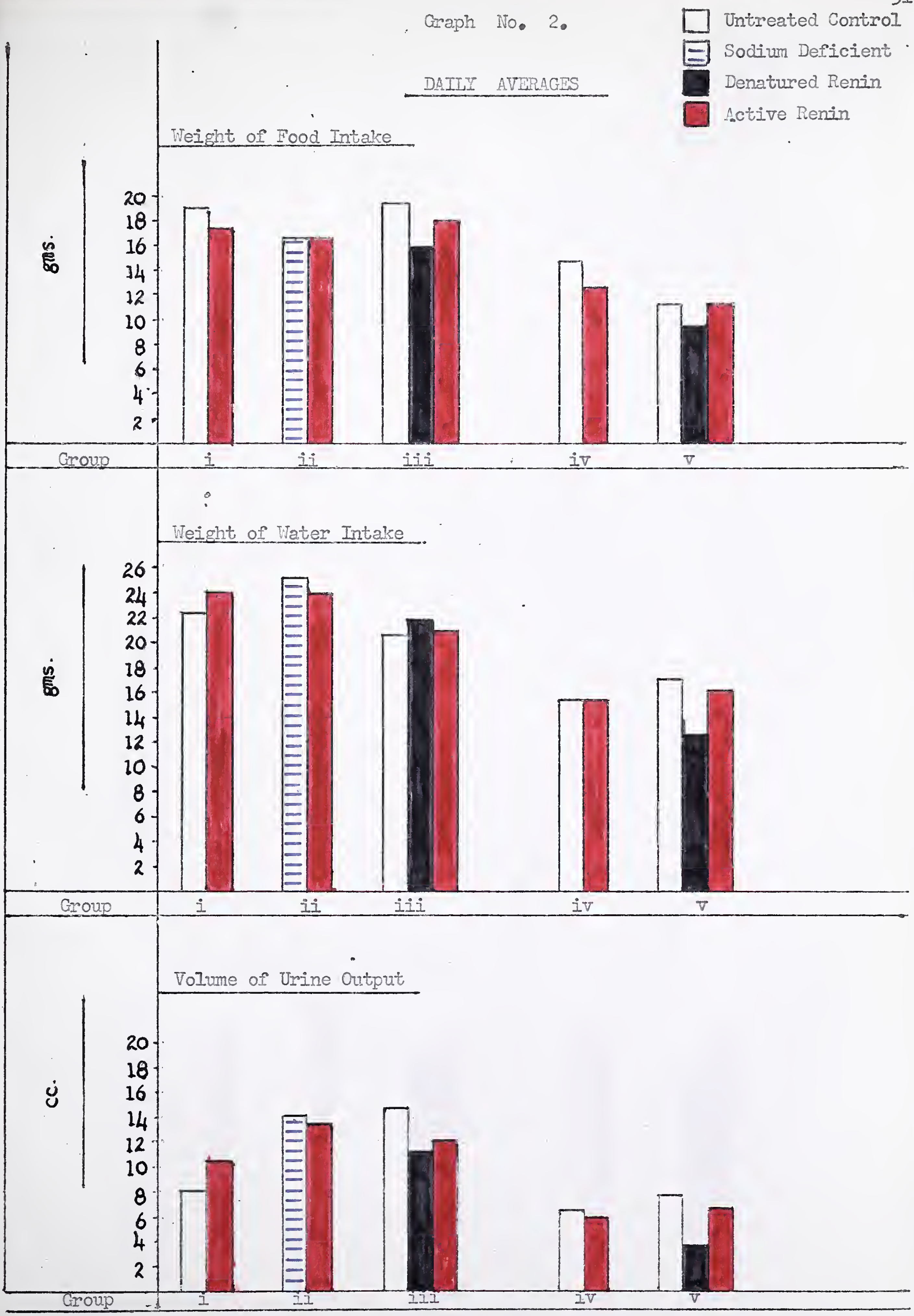
GROUP	DAYS	TYPE	FOOD Gm	WATER Gm	URINE cc	URINE		Na+/K+
						Na+ mEq	K+ mEq	
I	9	Untreated	18.3	21.7	6.8	1.218	1.112	1.19
I	"	"	18.6	22.7	9.3	1.330	1.170	1.13
Group Average			18.5	22.2	8.1	1.314	1.141	1.16
I	9	Renin (.16mg/day)	16.9	20.9	8.5	1.296	1.144	1.14
I	"	"	17.4	26.0	13.2	1.371	1.406	1.07
Group Average			17.2	23.5	10.9	1.334	1.275	1.11
II	9	Na+ def.	16.1	23.4	12.5	.364	1.242	0.32
II	"	"	16.9	26.7	16.0	.240	1.442	0.19
Group Average			16.5	25.1	14.3	.302	1.342	0.26
II	9	Renin(.32mg/day)	17.6	23.8	13.6	1.622	1.432	1.14
II	"	"	15.3	21.9	13.8	1.557	1.201	1.36
Group Average			16.5	23.5	13.7	1.590	1.317	1.25
III	12	Untreated	18.9	27.6	15.6	1.776	1.622	1.09
III	"	"	19.2	23.4	14.0	1.778	1.817	0.99
Group Average			19.1	20.5	14.8	1.777	1.720	1.04
III	12	Denatured Renin	15.6	22.0	11.8	1.440	1.387	1.03
III	"	" "	15.4	23.5	13.5	1.299	1.429	0.90
III	"	" "	15.7	19.8	9.1	1.399	1.319	1.06
Group Average			15.6	21.8	11.5	1.380	1.378	1.00
III	12	Renin (.32 mg/day)	18.9	21.8	12.9	1.862	1.771	1.08
III	"	"	16.7	19.8	11.2	1.537	1.566	0.99
Group Average			17.8	20.8	12.1	1.700	1.669	1.04
IV	9	Control	14.3	15.3	6.5	.991	.979	1.02
Group Average			14.3	15.3	6.5	.991	.979	1.02
IV	9	Renin (9.6mg/day)	11.7	15.3	6.3	.670	.572	1.22
IV	"	"	13.6	15.2	6.3	.895	.932	0.97
IV	"	"	11.2	14.1	5.3	.752	.764	0.99
Group Average		"	12.2	14.9	6.0	.772	.756	1.06

DATA
(for graphs no. 2 & 3)

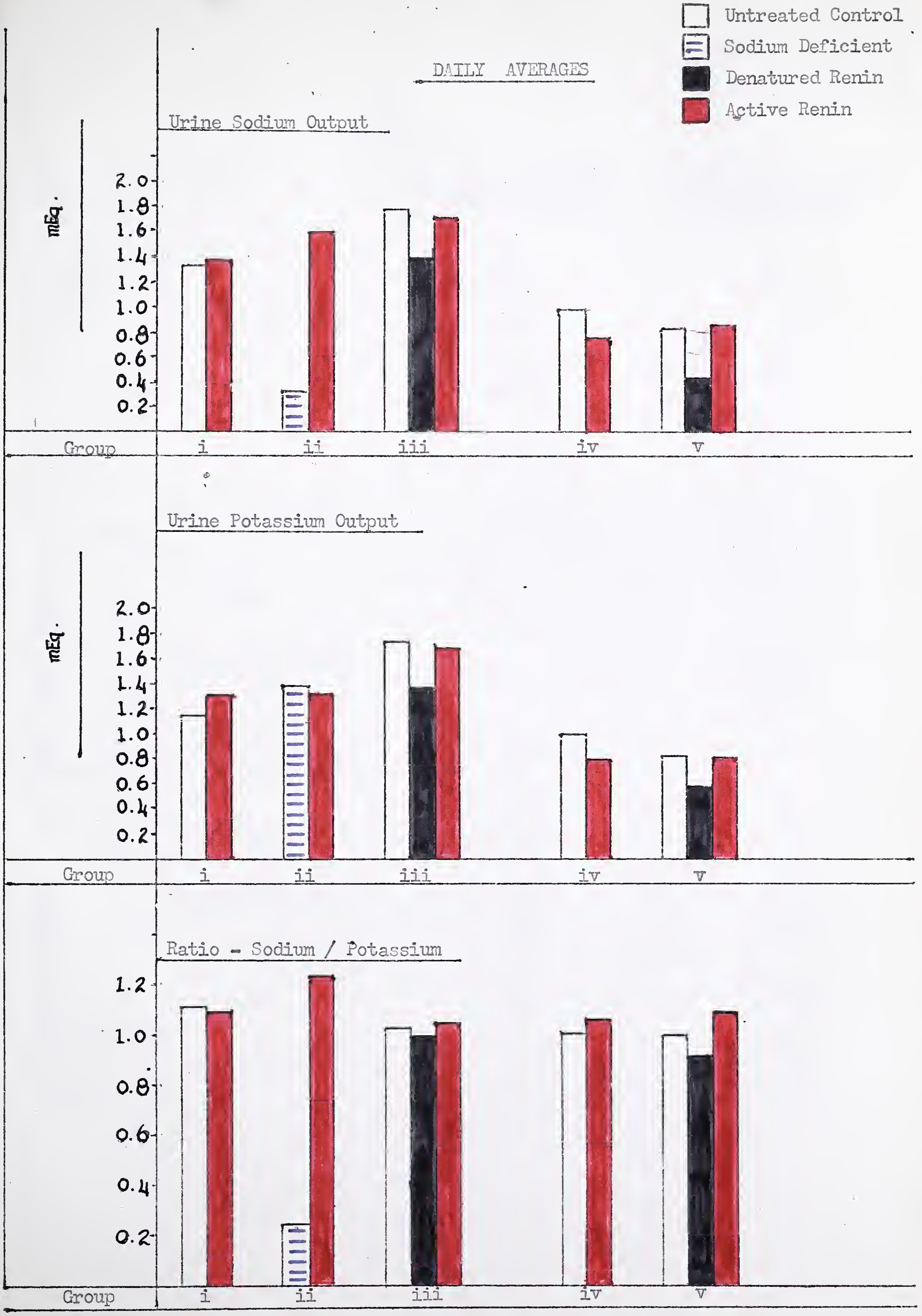
						URINE		
GROUP	DAYS	TYPE	FOOD Gm	WATER Gm	URINE cc	Na+ mEq	K+ mEq	Na+/K+
V	9	Untreated	11.1	16.9	7.7	.821	.830	1.01
Group Average			11.1	16.9	7.7	.821	.830	1.01
V	9	Denatured Renin	9.1	12.4	3.8	.513	.576	0.92
Group average			9.1	12.4	3.8	.513	.576	0.92
V	9	Renin(19.1mg/day)	12.1	17.3	7.9	.962	.748	1.26
V	4	" "	9.8	14.4	5.6	.675	.672	0.99
V	5	" "	13.1	14.3	7.3	.869	.871	1.00
V	9	" "	11.5	14.7	7.9	.965	.837	1.15
V	9	" "	10.3	14.0	5.3	.760	.711	1.07
Group Average			11.4	15.9	6.8	.846	.768	1.01

Graph No. 2.

DAILY AVERAGES



Graph No. 3.


DAILY AVERAGES
☐ Untreated Control
▤ Sodium Deficient
■ Denatured Renin
🟥 Active Renin
Urine Sodium Output
mEq.
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
Group
i
ii
iii
iv
v
Urine Potassium Output
mEq.
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
Group
i
ii
iii
iv
v
Ratio - Sodium / Potassium
1.2
1.0
0.8
0.6
0.4
0.2
Group
i
ii
iii
iv
v

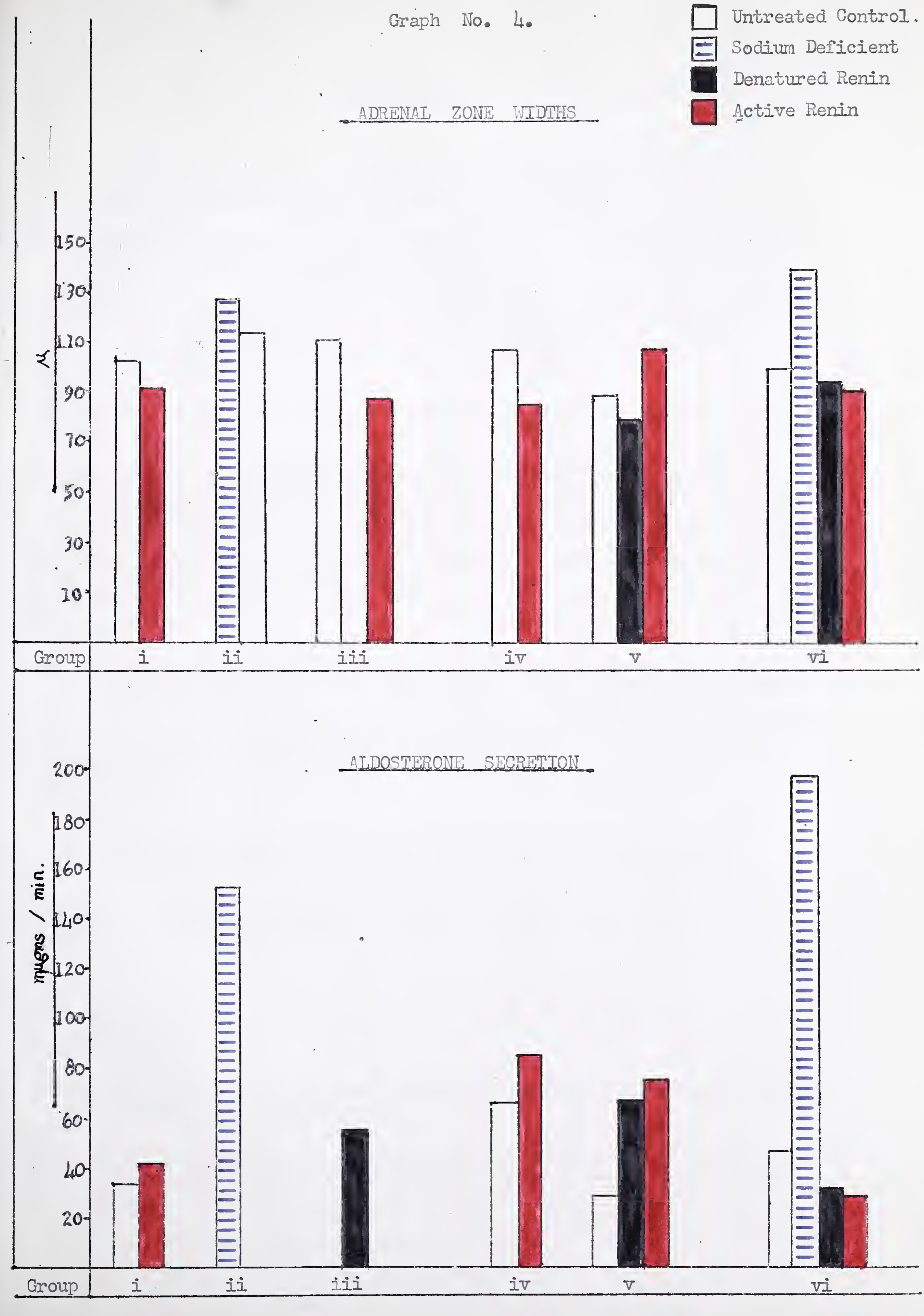
DATA
(for graph No.4)

GROUP	DAYS	TYPE	AV. ZONA GLOMERULOSA WIDTH microns	ALDOSTERONE SECRETION RATE mμ gm/min
I	9	Untreated	100	3.4
Group Average			100	3.4
I	9	Renin (.16mg/day)	92	-
I	"	"	-	4.2
Group Average			92	4.2
II	9	Na+ def.	-	12.9
II	"	" "	128	17.7
Group Average			128	15.3
II	9	Renin(.32mg/day)	115	-
Group Average			115	
III	12	Untreated	111	-
Group Average			111	
III	12	Denatured Renin	-	5.7
III	"	" "	-	5.5
Group Average				5.6
III	12	Renin(.32 mg/day)	87	-
Group Average			87	
IV	9	Untreated	107	6.6
Group Average			107	6.6
IV	9	Renin(9.6 mg/day)	97	8.7
IV	9	" "	76	6.4
Group Average			87	7.6
V	9	Untreated	89	2.8
Group Average			89	2.8
V	9	Denatured Renin	80	6.6
Group Average			80	6.6

DATA
(for graph no.4)

GROUP	DAYS	TYPE	AV. ZONA GLOMERULOSA WIDTH microns	ALDOSTERONE SECRETION RATE mμ gm/min.
V	9	Renin (19.1 mg/day)	93	
V	4	" "	106	
V	5	" "	119	
V	9	" "	116	7.4
V	9	" "	107	7.7
Group Average			108	7.6
VI	10	Untreated	94	4.2
VI	"	"	110	
VI	"	"	111	
VI	"	"	85	5.2
VI	"	"	100	
VI	"	"	100	
Group Average			100	4.7
VI	10	Na+ def.	138	23.8
VI	"	" "	130	14.4
VI	"	" "	150	
Group Average			139	19.1
VI	10	Denatured Renin	91	6.3
VI	"	" "	99	0.0
VI	"	" "	129	
VI	"	" "	88	
VI	"	" "	84	
VI	"	" "	84	
Group Average			96	3.2
VI	10	Renin 20.0mg/day	87	2.5
VI	"	" "	79	3.2
VI	"	" "	84	
VI	"	" "	90	3.4
VI	"	" "	109	
VI	"	" "	103	
Group Average			92	3.0

Graph No. 4.


☐ Untreated Control.
▤ Sodium Deficient
■ Denatured Renin
🟥 Active Renin
ADRENAL ZONE WIDTHS
μ
150
130
110
90
70
50
30
10
Group
i
ii
iii
iv
v
vi
ALDOSTERONE SECRETION
mμgms / min.
200
180
160
140
120
100
80
60
40
20
Group
i
ii
iii
iv
v
vi

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