FACTORS AFFECTING ADRENAL-REGENERATION HYPERTENSION IN THE RAT

by

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HISTORICAL INTRODUCTION

THE ADRENAL CORTEX IN HYPERTENSIVE VASCULAR DISEASE IN MAN

In 1855 Addison described a group of symptoms, among which was a weak thready pulse, which were associated with bilateral descruction of the adrenal glands (1, 2). These findings were the first indication that the adrenal was important in the regulation of blood pressure. In 1932, Cushing called attention to the presence of hypertension and other symptoms which were frequently associated with basophilic adenomas of the pituitary (3,4). Other workers have shown that "Cushings syndrome" was also associated with adrenal cortical tumours or hyperplasia and that the latter were actually present more frequently than pituitary changes. Schroeder (5) has thoroughly reviewed the literature on various endocrinopathies in relation to hypertension, and notes the frequency with which metabolic alterations indicative of adrenal cortical hyperfunction and high blood pressure are associated. Recently a new clinical syndrome has been described and called "Primary Aldosteronism" (250, 251, 252). In this disease an excessive excretion of aldosterone from an adrenal cortical tumour is invariably associated with hypertension.

In these diseases the relationship between hypertension and adrenal malfunction is not disputed. It was natural

then that attempts should be made to find a similar connection between the adrenal cortex and hypertension in the more common conditions essential and malignant hypertension.

PATHOLOGICAL CHANGES IN HYPERTENSION IN MAN

A large number of studies have been made of the adrenal glands in hypertension. Oppenheimer and Fishberg (7), Nuzum and Dalton (8), Fisher and Hewer (9), and Rinehart et al (10) in studies of the morbid anatomy in cases of hypertension describe a significant increase in the frequency with which adrenal cortical hyperplasia or tumours are found. In an extensive study of the adrenals in 9000 autopsies Russi et al (11) found an incidence of 1.45 percent of adrenal cortical adenomas. When a correlation was made between these findings and the symptoms shown by the patients, hypertension was found to be 7 times more common in patients with adenomata than it was in those without adrenal pathology. Schroeder (5) found that 96 percent of all subjects with adrenal adenomas, or focal or diffuse cortical hyperplasia, had cardiac hypertrophy with heart weights 50 g. or more above that which would be anticipated according to their body weight. Rinehart et al (10) described nodular hyperplasia of the adrenals as pathognomonic for essential hypertension, based on a comparison of 26 hypertensive and 100 control cases.

In equally impressive studies other writers, Dublin (12), Bruger et al (13), Commons and Callaway (14) have denied any association between the presence of adrenal cortical abnormalities and hypertension. Dempsey (15) found that the average weight of the adrenals in 102 cases of essential hypertension was not greater than that in control cases; and whereas 5 cases showed the picture of adenomatous hyperplasia as described by Rinehart, this lesion was also found in 4 control cases.

Dawson (16) in a recent study of the adrenals in hypertension has compared the adrenals from 90 cases of essential hypertension with those from 44 cases of renal hypertension and 90 normotensives. The average weight of the two adrenals were 15.3 g for those with essential hypertension, 15.7 g for those with renal hypertension and 11.8 g for the normotensives. The enlargement in the hypertensive groups was due to hyperplasia of the zona fasciculata associated with an irregular arrangement of the cells which were hypertrophied and vacuolated and contained abundant lipid. The zona glomerulosa was not affected in either form of hypertension. The interpretation of many of these studies is difficult because the authors have not defined strict criteria for hypertension and in some cases have failed to

appreciate that other conditions, notably infections and shock, may give rise to adrenal cortical hyperplasia.

Effect of Adrenal Steroids on Blood Pressure in Man

In 1939 Loeb et al (19) called attention to two patients with Addisons' disease whose arterial blood pressure exceeded normal limits in the course of treatment with desoxycorticosterone acetate (DCA). Since that time these observations have been confirmed by many investigators (20, 22, 23, 24, 25). It is evident from a study of these reports that overdosage with DCA in the treatment of Addison's disease may cause hypertension, edema, rapid gain in weight and in some cases congestive heart failure. These symptoms are especially marked when the sodium intake is high and they disappear when the dose of DCA is lowered and the sodium intake is restricted.

The effect of DCA on blood pressure has also been studied in normotensive and hypertensive subjects without adrenal disease. The administration of this steroid to normotensive subjects is associated over a period of weeks with a gradual rise in blood pressure with little or no salt or water retention (80, 26). This effect on blood pressure was reversed after withdrawal of the steroid therapy (80).

When DCA was administered to subjects with uncomplicated hypertensive vascular disease, a further elevation in blood pressure ensued (78, 76, 84). Goldman and Schroeder (81) were able to produce this effect by the intravenous injection of DCA, but not with a number of closely related steroids administered by the same route.

More recently cortisone has been employed in the study of the effects of steroids on blood pressure. In four hypertensive patients Perera et al (85) recorded declines in the blood pressure after the oral administration of 80 mg of this steroid daily. In another hypertensive patient treatment with 200 mg of cortisone daily was followed by a preliminary rise and then a sustained fall in blood pressure. Other studies have reported a slight reduction in blood pressure after cortisone treatment of hypertensive patients (86, 87). Unlike DCA cortisone had little effect on the blood pressure of normal man (27) under the conditions of these studies.

Studies similar to these with crude adrenal cortical extracts are of interest in the interpretation of the effects of cortisone and DCA. Whereas Goldman reported that adrenal cortical extract did not induce hypertension in animals (21), Pines found it caused a small decrease in the blood pressure

of hypertensive subjects when administered over a period of a month (28). In an extension of this study Perera (29) found that adrenal cortical extract blocked the pressor effects of DCA. These findings led Soffer (23) to conclude that the adrenal cortex produces a "blood pressure balancing factor" as well as a hypertensive factor. These studies with adrenal cortical extract are difficult to interpret. Such preparations were relatively crude and it is impossible at this time to determine what the doses were in terms of mineralocorticoid or glucocorticoid activity. Nevertheless it is perhaps significant that none of the authors who studied the effects of adrenal cortical extract in man, or as will be described later in experimental animals, were able to produce an increase in blood pressure with this material.

The interpretation of the findings reviewed above as evidence of a direct relationship between the adrenal cortex and hypertensive vascular disease is far from convincing. They do, however, show that under certain circumstances the administration of DCA may have profound effects on blood pressure. The doses used in most investigations must be regarded as pharmacological rather than physiological (30), and, of course, during the course of these studies it was not established that DCA was produced by the adrenal cortex.

The studies of Luft and Sjögren add emphasis to these reservations (82). Using relatively small doses of DCA they found no effect on the blood pressure of Addisonian patients except when concomitant organic renal disease was present. It would be of great value to know the effects of administration of aldosterone on blood pressure, however supplies of this material adequate for extensive studies of this nature are not yet available. When administered as replacement therapy in Addison's disease, return of the blood pressure to normal levels has been reported (31, 32), but as yet it is not known whether aldosterone in man is capable of producing high blood pressure, or aggravating an established hypertension.

Adrenal Cortical Steroid Excretion in Hypertension

The excretion of 17 ketosteroids in the urine may provide helpful information in the diagnosis of hyperfunction, or hypofunction of the adrenal cortex. These steroid metabolites arise solely from the adrenal cortex in the female, and from the adrenal cortex and the testes in the male. They are known to be excreted in grossly increased quantities in the presence of masculinizing tumours or hyperplasia of the adrenal cortex, and are either lacking or present in only small quantities in the urine in Addison's disease.

Bruger et al (33) found the excretion of 17 ketosteroids in hypertensive women was lower than normal. These results were confirmed by Selye (34). However the urinary excretion levels of 17 ketosteroids are only an incomplete measure of adrenal cortical function.

The urinary elimination of non-conjugated formaldehydogenic corticoids in hypertension has been studied by Corcoran and his associates (327). They found the urinary level to be considerably augmented in about 50% of all patients with essential or malignant hypertension. Ballan (17) confirmed these findings in the study of a large series of patients with essential hypertension. He found, however, that hypercorticoiduria did not occur when there were organic renal lesions associated with the hypertension, and suggested that the elimination of corticoids is impeded by the renal lesions. Tompsett and Oastler (35) using the method of Talbot to measure urinary corticosteroids found that patients with uncomplicated essential hypertension had excretory levels within the range for normals, while significant increases were noted in two cases of essential hypertension with obesity (35).

Other workers have also failed to duplicate the results of Corcoran et al (36, 37), but the number of cases they studied was small, which may explain their negative findings.

Raab studied the level of 17 hydroxycorticosteroids in the blood of patients with renal hypertension and found levels higher than the normal (38). He concluded that a fluctuating mode of secretion existed in the adrenals of patients with hypertension, this concept is compatible with the view of Corcoran (327) that an increased lability of corticoid excretion is part of the biochemical pattern of the disease.

Dobriner (18) in a most extensive study of adrenal steroid excretory patterns in various diseases, found that two abnormal steroids were excreted in patients with hypertension. One steroid, 9 etiocholenolone,was found in the urine of 3 of 6 hypertensive patients and only 2 of 24 normal subjects. Another unidentified compound was found in 5 cases of essential hypertension and only in traces in normal urines. Dobriner concluded that "dysfunction of the adrenals is an important factor in the pathogenesis of this disease".

The hypertensive properties of DCA which were first noted in Addisonian patients were also reproduced as noted on page 27 in experimental animals. These findings led Selye (205) to introduce the concept that hypertension represents one of the diseases of adaptation, and that "mineralocorticoids" play an etiologic role in this disease. Similar considerations led to studies of the level of sodium-retain-

ing substances in the urine of patients with hypertension. Later with the elucidation of the structure of aldosterone (40) and the development of methods of assay (41), the urinary excretion values of this steroid were also studied in essential and malignant hypertension.

Singer (42) in a study of four patients with essential hypertension found no evidence of an increase in the excretion of "salt-retaining lipids" in this disease.

More recently Genest et al (43, 44) using bioassay techniques, and expressing their results in terms of a decrease in the sodium excretion and sodium-potassium ratio of adrenalectomized rats, have found that patients with malignant or severe essential hypertension excrete significantly larger amounts of aldosterone than normal subjects. These findings, the association of hypertension and high urinary levels of aldosterone in primary aldosteronism (45, 46), and the similarity of effect of aldosterone and DCA on sodium excretion, tempt one to consider that aldosterone may be the elusive hypertensive agent responsible for the role of the adrenal cortex in hypertension. However there are several objections to such a hypothesis. Although hyperaldosteronism has been reported in Cushing's syndrome, essential and malignant hypertension and toxaemia of pregnancy, diseases in

which high blood pressure is almost always present, it has also been reported in nephrosis, cirrhosis of the liver, congestive heart failure, and normal pregnancy (44). The latter group are not characterized by persistent elevations in blood pressure.

To summarize: the analysis of urine for steroids arising from the adrenal has not yielded any definitive evidence as to the role of the adrenal cortex in hypertension. Most of the evidence although negative has been valuable in delineating certain functions of the adrenal gland, through their respective metabolites which are probably not involved in hypertension. However, one cannot help but feel from a study of these investigations that some derangement of adrenal metabolism exists in this disease, but the exact nature of the disturbance and whether it bears a casual relationship to hypertension is as yet unknown.

ELECTROLYTES IN HYPERTENSION

The role of steroids from the adrenal cortex in the regulation of electrolyte metabolism is well known and has been ably reviewed (30). The relationships between sodium and potassium metabolism, particularly the former, and the functions of the adrenal cortex have formed the basis for attempts to correlate abnormalities in electrolyte metabolism with the pathogenesis of hypertensive vascular disease.

Ambard and Beaujard in 1904 postulated that a condition of "dry retention" of salt existed in hypertension, and advocated a salt poor diet to counteract it (52). Allen and Sherill (64) and later Volhard (65) studied the effects of low salt diets in hypertension, their findings were in general agreement with the original study of Ambard and Beaujard (57) and they also recommended this therapy.

Until recent years the bulk of the clinical investigations in this field have centred around the more or less empirical use of low sodium diets in the treatment of hypertensive vascular disease without the support of unequivocal data demonstrating that an abnormality in electrolyte metabolism exists. The subject has as a result become highly controversial. Kempner (49, 50) was one of the first on

this continent to recommend a low sodium intake, by means of a rice diet, in the treatment of hypertension. He claimed a beneficial effect when patients were assessed by a number of criteria, such as blood pressure, heart size, eye vessels etc. as well as symptomatic relief. Corcoran and Page (54) found that roughly one quarter of a group of hypertensive patients were benefited by limiting the daily sodium intake to less than 0.5 g of sodium chloride. They attributed the beneficial effect of Kempner's rice diet to its low sodium content since the addition of salt to this diet negates its effect.

Bryant and Blecha (66) and also Dock (67) found that low salt regimes were of value in the treatment of hypertension, whereas the results from other groups using either low salt or rice diets have been disappointing (51). The subject of low salt diets and hypertension has been reviewed recently by Schroeder (51) and others (62, 63). In general it would appear that low sodium diets fail to exert an unequivocal effect on the blood pressure although many patients are symptomatically improved without a commensurate fall in blood pressure. Low potassium diets have resulted in slight though significant reductions in the blood pressure of hypertensive patients, however these diets prove to be

quite unpalatable and reduction in total caloric and protein intake may account for the effect on the blood pressure apart from the effect of low potassium (57).

In contrast to the beneficial effects of low sodium regimes, high sodium intakes have been shown to augment the hypertensive process in man and all forms of experimental hypertension in which it has been studied (52, 58, 59, 60, 61, 76). Dahl (72) has noted a positive correlation between the elevation of blood pressure and sodium chloride intake in hypertensive patients. It has also been shown that salt potentiates the pressor effect of DCA in Addisonian patients (76, 77), and that this effect disappears when the salt is withdrawn from the diet (76, 75).

The findings in respect to the apposing influence of high and low sodium intakes on hypertension have been inferred, when coupled with the sodium retaining activity of steroids from the adrenal cortex, as circumstantial evidence that an abnormal function may exist in this gland in hypertension. Recently, more direct evidence has been added to lend weight to this supposition.

A number of authors claim that more or less manifest derangements in salt metabolism exist in hypertension (69,

70, 71). Others have reported that the concentration of serum sodium is normal in hypertensive patients (107, 84). Frances Selye reported that the ratio of serum concentration of sodium to chloride was elevated in about one third of a group of hypertensive subjects (34). Dahl (56) found in a study of salt excretion in a series of 1364 men that those with low salt intake and excretion had a significantly lower incidence and those with a high salt intake and excretion a significantly higher incidence of hypertension than would occur by chance alone. Perera and Blood (53) have also provided evidence of abnormal electrolyte metabolism in hypertension. In a study of the effect of 24 hours of sodium deprivation they found that hypertensive patients failed to lose weight and fluid as did the control group. The deficit in the hypertensive group may have been due to secondary renal changes, however the change is consistent with the view that the adrenal cortex is implicated in this disease since the tubular reabsorption of salt and water is influenced by this gland. Other authors (72) studying sweat sodiums in hypertension find no differences in such values when hypertensive and control patients are compared, and doubt for this reason that there is any considerable increase in "electrolyte influencing steroids" in this disease.

The most convincing evidence of a derangement of electrolyte metabolism in hypertension has come from sodium and water tolerance tests. Green (204) showed that excretion of a salt and water load was more rapid in a group of hospitalized hypertensive patients than in patients with normal blood pressure, and that a direct relationship existed between the elevation of blood pressure and the rapidity of excretion of the load.

It cannot be denied that studies based on sodium intake or sodium excretion in the sweat or urine fail to give a comprehensive picture of sodium metabolism in the body. Recently Tobian and others (73, 74) have shown that the concentration of sodium is significantly greater in the muscles and arteries of patients with essential hypertension than in normotensive patients. Thus it may well be that the complementary effects of sodium and steroid hormones from the adrenal cortex are exerted at the cellular level and a change in the response of vascular organs to the effect of adrenal hormones may be the primary determinant of the effect of sodium on blood pressure.

THERAPY IN HYPERTENSION AS RELATED TO THE ADRENAL CORTEX

The possible relationships between the adrenal cortex and essential hypertension have prompted many attempts to modify the hypertensive state by decreasing the effective mass of the adrenal gland. The earlier approaches to surgical removal of the adrenal glands were obviously hampered by the fact that adequate replacement therapy was not available. In spite of this, beneficial results were claimed by a number of workers after unilateral adrenalectomy (105), bilateral subtotal adrenalectomy (91, 100, 101) or bilateral hemiadrenalectomy and sympathectomy (100, 101, 104). In a typical study Zintel (103) removed 88 to 98% of both adrenals as the only therapy in a group of 13 patients with essential hypertension. The average blood pressure before surgery was 228/142 and 4-12 months after surgery the average blood pressure was 167/108. The removal of the adrenals relieved symptoms and some of the signs as well as causing a variable reduction in blood pressure. In view of the effects of adrenal regeneration to be discussed later it would be interesting to know in the studies above what the long term responses to the surgery were, and whether or not regeneration of the adrenal cortex had occurred. X-irradiation of the adrenal region (93, 94) and bilateral adrenal denervation

have been attempted in an effort to modify the progress of essential hypertension but with inconsistent results.

Complete bilateral adrenalectomy has been performed on many patients with hypertension during recent years (88, 89, 90, 91, 92). Removal of the adrenals is followed by a fall in blood pressure to normal limits in about one third of the patients. Forty percent appear to derive no benefit whatever from the procedure, while in the remainder the progress of the disease is often interrupted with some regression of cerebral and cardiac complications even though no fall in blood pressure occurs. Substitution therapy with small maintenance doses of adreno-cortical extract or cortisone can maintain the patients in good condition without restoring the blood pressure to the previously abnormally high levels. Excessive administration of DCA produces unusually marked pressor responses in such individuals (95, 96, 88, 98).

Adrenalectomy appears to be least helpful in those patients with advanced renal disease or arteriosclerosis (104), other than that little is known about the selection of patients who are likely to have a good response to adrenalectomy.

It is obvious that if a drug was available with which

one could safely depress adrenal-cortical function it might allow a more accurate choice of the patients who would respond to adrenalectomy.

STUDIES IN ANIMALS

Studies on a number of different species of experimental animals have contributed a wealth of data to our knowledge concerning the role of the adrenal gland in hypertension. This was possible after procedures were developed by means of which hypertension could be induced in animals. The first methods developed involved different procedures which appeared to operate through a common mechanism to produce so called "renal" hypertension (133, 134, 135, 136). More recently it has been noted that bilateral nephrectomy in the dog would also produce a rise in blood pressure which has been termed "renoprival" hypertension (127). Ablation of the moderator nerves to the carotid sinus also caused the development of hypertension in dogs (139). In rats hypertension has been induced by stress in the form of auditory stimulation (115), the administration of steroids from the adrenal cortex (Table I), or enucleation of the adrenal gland (140). Ligation of the adrenal artery of the dog was also reported to result in hypertension (139). In spite of studies reported over almost three decades the exact role

of the adrenal in these phenomena is still not clearly understood.

Goldblatt (112) made the initial discovery that complete bilateral, but not subtotal, adrenalectomy interfered with the development or maintenance of experimental renal hypertension. This finding was subsequently confirmed by many others (110, 111, 112, 113, 128). It was also shown that hypophysectomy counteracted renal hypertension, an effect attributed by these authors to the atrophy and insufficiency of the adrenal cortex after the removal of the tropic influences of the pituitary (113, 114). The administration of DCA or adrenal cortical extract in these studies restored the blood pressure to the previous hypertensive levels.

In explanation of these findings it has been shown that bilateral adrenalectomy decreased the response to injections of renin (121, 122). The renin substrate hypertensinogen which is a globulin formed in the liver decreased or disappeared from the systemic blood of untreated adrenalectomized dogs, while adrenal cortical extract or DCA caused a return of the hypertensinogen to normal levels (123, 124).

Adrenalectomy also modified the hypertension induced in dogs by the intracisternal injection of kaolin (118) or elevations of blood pressure produced by the injection of dihydroxy phenylalenine in rats (119). Similarly hypertension in rats exposed to stress in the form of auditory stimulation was not maintained in the absence of the adrenals (115). The adrenal medulla was probably not directly involved in these responses to adrenalectomy since it was shown in renal hypertension in dogs that elevations in blood pressure still occurred after unilateral adrenalectomy, and on the opposite side adrenal demedullation and splanchnic nerve resection (129).

Zweifach and Shorr (125) have recently reported some studies which may throw light on the effect of adrenalectomy on experimental hypertension. These workers showed that whereas the mesemteric arterioles of intact rats with renal hypertension had an increased threshold response to pressor agents, similar changes were not observed after adrenalectomy. This inability of the terminal arterioles to respond to pressor agents after adrenalectomy may explain the failure of such animals to develop hypertension.

In contrast to the studies above, it has been shown by

a number of workers that under certain conditions the presence of the hypophysis or the adrenal gland is not absolutely essential for the production or maintenance of experimental hypertension. Turner and Grollman have shown that hypertension and cardiovascular-renal lesions produced by nephrectomy would develop in bilaterally adrenalectomized dogs (127), and Ragoff et al (120) claimed that the blood pressure remained high in three hypertensive dogs four to nine days after adrenalectomy. Renal hypertension has also been produced and maintained in the absence of the hypophysis in dogs (131). In rats it has been shown that a significant elevation of blood pressure would occur in adrenalectomized animals injected with rabbit antirat kidney serum (130) or in adrenalectomized animals subjected to renal encapsulation (132). In the latter study the degree of hypertension or the cardiovascular-renal lesions did not differ from that of rats with intact adrenals.

The development of hypertension in the absence of the adrenal gland has been accepted by some as definitive evidence that this gland is not involved in the etiology of this disease (127). This would not appear to be an unbiased conclusion. The development of hypertension is certainly a complex phenomenon to which many factors such as

the kidneys, electrolytes, adrenal cortical steroids etc. may contribute an etiologic role. The fact that hypertension can develop in the absence of the adrenal glands is not proof that these glands cannot participate in its development.

CORTICOID HYPERTENSION

Corticoid hypertension is so-called because it is provoked by an excess of adrenocortical hormones. Such an excess may be produced by injecting large doses of cortical hormones (Table 1), or by stimulating the adrenal cortex with pituitary extracts (199) or purified growth hormone (200). The early studies of corticoid hypertension with its theoretical clinical implications caught the attention of many workers in the field. The vast literature which has accrued as a result has been reviewed by a number of authors (201, 202, 203). The following are the major contributions which have been made in this field with particular attention placed on those aspects of corticoid hypertension which have a bearing on the hypertension which follows adrenal enucleation.

Kuhlman first observed in 1939 that the administration of 25 mg of DCA each day to dogs caused a significant increase in blood pressure (160), In 1940 Grollman induced

hypertension in rats with DCA (21) and Swingle (159) obtained transitory hypertension by administering DCA to normal and adrenalectomized dogs. Selye (165) showed that DCA would cause cardiac hypertrophy, nephrosclerosis and tissue edema in the chick and later found that chronic overdosage with this steroid would produce nephrosclerosis and hypertension in the rat (138). These findings have since been confirmed in many laboratories and for most species of experimental animals (Table 1). The implantation of pellets of DCA or chronic administration of this material is now an accepted procedure for producing experimental hypertension, and the factors which effect it have been studied extensively.

This hypertension, it was believed originally, could be explained by the renal lesions resulting from the procedure (144). However this view is no longer tenable since subsequent studies have revealed that the hypertension persists in the absence of the kidneys (145) and that it is promptly reversible upon the removal of the DCA implants (146, 159, 167).

Hypertension induced by DCA in rats will persist after it has been established for a variable period, even though DCA treatment is stopped and the adrenals are removed.

TABLE I. EFFECTIVENESS OF ADRENAL STEROIDS IN THE PRODUCTION OF HYPERTENSION

DESOXYCORTICOSTERONE

Experiments with successful production of hypertension

Experimental Animal	Dosage	Drinking Fluid	Reference
Unilaterally nephrectomized Rat	3 mg/100 g/day S.C.	1% Saline	Selye (153)
Unilaterally nephrectomized Cat	3 " " S.C.	1% "	Selye (153)
" " Guinea Pig	3 " " S.C.	1% "	Selye (153)
" " Mouse	3 " " S.C.	1% "	Selye (153)
Normal Dog	0.5 mg/day S.C.	H_2O + 2 g NaCl per day	Swingle et al.(159)
Normal Dog	20 mg/day S.C.	Water	Kuklman et al (160)
Normal Dog	20 " S.C.	Water	Rodbard and Freed(161)
Renal Hypertensive Dog	20 " S.C.	Water	" " " "
Normal Rat	0.5 mg/day S.C.	Water	Briskin et al (162)
Normal Rat	0.5 " S.C.	Water	Grollman et al (21)
Renal Hypertensive Rat	2.5 mg/day S.C.	0.2% Saline	Knowlton et al (166)
Adrenalectomized Dog	0.5 mg/day S.C.	Water + 2g NaCl per day	Swingle et al (159)
Adrenalectomized Dog	0.5 " S.C.	Water	Remington et al (164)

Table I cont....

Experimental Animal	Dosage	Drinking Fluid	References
Normal Chick	1 mg/day S.C.	Water	Selye (165)
Normal Rat	20 mg pellet I.M.	1% Saline	Sturtevant(171)(170)
Normal Rat	20 " " "	0.86% Saline	Green et al(173)(154)
Rats given antiplacenta serum	2.5 mg/day S.C.	0.2% Saline	Loeb et al (174)
Unilaterally nephrectomized Rat	1 mg/day S.C.	1% Saline	Gross et al (179)(180)

DESOXYCORTICOSTERONE

Experiments with failure to produce hypertension

Normal Rat	2.5 mg/day S.C.	0.2% Saline	Knowlton (166)
Normal Rat	2.5 " S.C.	0.2% "	Loeb (174)
Normal Rat	4 mg/day S.C.	Water	Brown-Menendez (193)
Normal Rat	5 mg/day S.C.	Water	Gaudino (194)
Normal Rat	0.5 mg/day I.M.	Water	Leatham and Drill(195)
Normal Rat	0.5 " "	Water	Selye (196)
Normal Rat	20 mg pellet I.M.	Water	Green (154)

Table I cont. ...

Experimental Animal	Dosage	Drinking Fluid	Reference
Adrenalectomized Rat	0.5 mg/day I.M.	Water	Leatham (195)
Hypophysectomized Rat	0.5 " I.M.	Water	Leatham (195)
Normal Dog	100 mg/day S.C.	H_2O + 10 g NaCl per day	Summers (176)
	CORTISONE		
	Experiments with successful production of Hypertension		
Rats with rabbit anti rat kidney serum nephritis	2.5 mg/day S.C.	0.85% Saline	Knowlton (186)
Adrenalectomized rats with rabbit anti rat kidney serum nephritis	2.5 " S.C.	0.85% "	Knowlton (186)
	No reports of failure to produce Hypertension		
	CORTICOSTERONE		
	Experiments with successful production of Hypertension		
Unilaterally nephrectomized rat	5 mg/day S.C.	1% Saline	Gross (191)
	Experiments with failure to produce Hypertension		
Normal Rat	1 mg/day S.C.	1% Saline	Selye (319)

Table I cont. ...

HYDROCORTISONE

Experiments with successful production of Hypertension

Experimental Animal	Dosage	Drinking Fluid	Reference
Normal Rat	2 mg/day S.C.	1% Saline	Friedman et al (313)
Normal Rat	1 " S.C.	1% "	Friedman et al (305)
Normal Rat	1 " S.C.	1% Saline	Masson et al (279)

No reports of failure to produce Hypertension

COMPOUND S

Experiments with successful production of Hypertension

Normal Rat	2.5 mg/day S.C.	1% Saline	Selye (185)
Adrenalectomized renal hypertensive rat	2.5 " S.C.	250 mg NaCl/100 g	Guadino (169)

Experiments with failure to produce Hypertension

Normal Rat	2 x 30 mg pellet I.M.	1% Saline	Deane (178)

ALDOSTERONE

Experiments with successful production of Hypertension

Unilaterally nephrectomized rat	0.5 mg/day S.C.	1% Saline	Gross (180)

Table I cont. ..

Experimental Animal	Dosage	Drinking Fluid	Reference
Normal Rat	0.5 - 1 mcg/day S.C.	Water	Gornall et al(181)(182)
Normal Rat	0.5 - 1 mcg/day S.C.	0.85% Saline	Kumar et al (183)
	Experiments with failure to produce Hypertension		
Unilaterally nephrectomized rat	0.04 mg/day S.C.	1% Saline	Gross (179)
Unilaterally nephrectomized rat	0.25 " S.C.	1% Saline	Gross (180)
Unilaterally nephrectomized rat	0.5 mcg/day S.C.	Water	Gaunt (184)
	PROGESTERONE		
	Experiments with successful production of Hypertension		
Normal Cat	1 mg/day S.C.	Water	Grollman (21)
	Experiments with failure to produce Hypertension		
Hypophysectomized renal hypertensive rat.	2 mg/day S.C.	1% Saline	Page (168)
	TESTOSTERONE		
	Experiments with successful production of Hypertension		
Normal Rat	1 mg/day S.C.	Water	Grollman (21)

Table I cont. ...

Experimental Animal	Dosage	Drinking Fluid	Reference
	TESTOSTERONE		
	Experiments with failure to produce Hypertension		
Renal hypertensive rat	2.5 mg/day S.C.	1% Saline	Page (168)
Normal Rat	10 mg/day S.C.	Water	Selye (187)
Normal Dog	50-75 mg/week S.C.	Water	Blackman (188)
	ESTRADIOL		
	Experiments with successful production of Hypertension		
Normal Rat	0.25 mg/day S.C.	Water	Grollman (21)
	Experiments with failure to produce Hypertension		
Renal hypertensive rat	0.17 mg/day S.C.	1% Saline	Page et al (168)
	ADRENAL CORTICAL EXTRACT		
	Experiments with successful production of Hypertension		
Hypophysectomized renal hypertensive rat.	1.0 cc daily	1% Saline	Page et al (168)
	Experiments with failure to produce Hypertension		
Normal Rat	not stated	Water	Grollman (21)

This self-sustaining post DCA hypertension has been termed "metacorticoid hypertension" (172). Corticoid hypertension is characterized by renal and cardiac hypertrophy, nephrosclerosis, widespread vascular lesions and marked changes in fluid and electrolyte metabolism.

HEART AND KIDNEY IN CORTICOID HYPERTENSION

As previously mentioned, the administration of DCA and sodium chloride was shown to result in cardiac hypertrophy in the chick (165) and the rat (138) by Selye. Many authors have confirmed this observation (161, 170, 172, 173), and the invariable presence of cardiac hypertrophy in hypertension is now used as a check on the reliability of the measurement of blood pressure. Renal hypertrophy will also develop after DCA administration provided sodium chloride is administered (206). In this respect the DCA appears to sensitize the animal to the sodium chloride since the administration of high levels of sodium chloride alone will cause renal hypertrophy in the rat.(207).

Removal of one kidney was first noted by Selye to enhance the effect of DCA in causing a blood pressure elevation (153). The mechanism of this "sensitizing" procedure is not clear, but DCA is believed to act by diminishing the capacity of the kidney to excrete sodium (208). The presence of renal lesions, serum nephritis (166) or experimental

perinephritis (174), which would favour the retention of sodium in the body, also facilitate the development of corticoid hypertension.

The characteristic lesions in this type of hypertension have been extensively described by Selye (205). In the kidney they consist primarily of nephrosclerosis with hyalinization of glomerular loops and transudation of hyaline material into the capsular space. The convoluted tubules are dilated and may contain hyaline casts. The vascular lesions in corticoid hypertension are widespread throughout the body, but are most marked in the small arteries and arterioles of the kidney, heart, mesenetery, pancreas, and brain. They consist of intimal thickening, often with occlusion of the lumen, sub intimal fibrinoid necrosis, hyalinization, and perivascular granulomatous proliferation (periarteritis nodosa). Degenerative and necrotic lesions in the parenchyma of these organs are usually associated with completely occluded arteries or arterioles.

The cardiovascular and renal lesions in corticoid hypertension, like the rise in blood pressure, appear to be associated with the sodium chloride rather than the DCA, and more particularly with the sodium ion. Chlorides other than sodium are ineffective in producing these lesions,

while other sodium salts share this action of sodium chloride (210, 212). Conversely rats maintained on a sodium free diet are entirely resistant to the hypertensive, nephrosclerotic and arteriosclerotic actions of DCA (211).

FLUID AND ELECTROLYTE METABOLISM IN CORTICOID HYPERTENSION

Studies in recent years have shown with increasing clarity that disturbances of salt and water metabolism may have a fundamental role in essential hypertension and experimental forms of the disease. The importance of sodium in DCA hypertension has been recognized since the first studies in this field. Lenel, Rodbard and Katz produced hypertension in the chicken by replacing the drinking water with isotonic or slightly hypertonic saline (151). However, in the rat 1% sodium chloride without DCA fails to induce hypertension (142, 211), and as mentioned earlier, in the absence of sodium, DCA does not cause hypertension or the characteristic associated lesions. It is now generally accepted that DCA in some manner sensitizes the animal to the effect of sodium since sodium chloride alone will produce hypertension in the chick (165, 151), and when given in 2% concentration in the drinking water will also cause hypertension and renal lesions in the rat (207). The necessity of a high level of sodium in the diet probably accounts for the failure of some authors

to produce hypertension with DCA (Table I) (166, 193, 194, 195). In other cases such failures are attributed to the use of small doses of DCA (195, 194), or to resistance to this steroid in the particular strain of rat used (177).

Although the necessity of a high intake of sodium for the production of DCA hypertension is well recognized, the role of this cation in the hypertensive process is only partly understood. Green (163) found that in rats implanted with a DCA pellet and given saline to drink there was initially a retention of sodium followed by polydipsia, polyuria and excessive sodium excretion. After the blood pressure became elevated, usually in 5-8 weeks, saline consumption and renal excretion of sodium and water decreased and a balance was reestablished. At this time the rats, if given free choice, would voluntarily decrease their intake of saline and increase their water intake. The reestablishment of an equilibrium with normal intake and excretion of sodium chloride and water was thought to indicate a permanent derangement of the mechanism for handling sodium and water in the body. Tolerance tests have proven that this was the case. Freidman et al (157) demonstrated that rats with established DCA hypertension eliminated a saline load more rapidly than did normal control rats,

though elevations of plasma sodium were not observed. Tobian and Binion (143) showed that increased amounts of sodium were present in the arterial wall in DCA hypertensive rats compared to normal controls. Similar studies by Daniel and Daniel (126) failed to show these differences in the stomach, liver or abdominal muscles. The brilliant studies of the group led by Friedman and Friedman have shown that dynamic shifts of cations between the cells and extracellular spaces play an important role in the regulation of blood pressure. When Pitressin was injected intravenously in the dog, sodium and water moved into the cells and potassium was extruded as the blood pressure became elevated, and shifts in the opposite direction occurred with decline of the blood pressure (109, 106). Tobian and Fox (108) found by measuring the electrolyte content of the arteries after a norepinephrine infusion that the pressor action of this drug was associated with a loss of potassium from the vessel wall and a gain in sodium. It is evident from this discussion that the role of sodium in DCA hypertension is complex and probably of primary importance in the development and maintenance of the elevated blood pressure. It is also clear that sodium balance studies in hypertension are of limited value without information on changes which might occur in the intracellular and extracellular compartments.

ALDOSTERONE

As mentioned earlier hypertension is an important component of the syndrome of primary aldosteronism, and an increased excretion of aldosterone may accompany essential hypertension (43, 99). The results of studies with this steroid in experimental animals are therefore of great interest. Gross (179) administered aldosterone for 32 days to unilaterally nephrectomized rats given saline. A dose of .04 mg per day did not produce hypertension, whereas an equivalent dose of DCA in terms of sodium retaining activity (1 mg per day) was quite effective. Kumar et al on the other hand claimed that aldosterone had a cumulative effect, and that small doses (0.0005 - 0.001 mg every 48 hours) would cause hypertension (182) and renal lesions (183) when administered for several months to male rats. The results in these studies were essentially the same in normal, adrenalectomized or salt-treated animals.

Gaunt et al (184) administered the same dose of aldosterone (0.0005 mg) every day to female rats and could not confirm the findings of Kumar et al. In further studies Gross (180) was able to produce hypertension in unilaterally nephrectomized rats given saline when aldosterone was administered at a dose of 0.5 mg daily, however this dose

is one thousand times greater than that required by Kumar et al (182) to produce the same effect. The discrepancies between the results of Gaunt et al (184) and Gornall et al (181) were explained by the latter group on the basis of the sex of the animals used. They showed that intact female rats did not exhibit the hypertensive response to aldosterone shown by the males while ovariectomized rats were equally responsive (97).

The results of the group led by Gornall on the hypertensive properties of aldosterone appear unconvincing for two reasons. First, neither male or female animals injected with aldosterone, and for which a hypertensive response was claimed, had renal or cardiac hypertrophy. An increase in size of the kidney and heart as mentioned earlier is invariably seen in DCA or renal hypertension in rats. Second, although in these studies aldosterone was administered for periods as long as six months, the rise in blood pressure seldom exceeded 150 mm Hg systolic. This increase in blood pressure would be considered by some authors (172) to be on the borderline of significance. It is well known that the blood pressure of the normal rats increases with age and as described later unilateral nephrectomy and saline administration will also provoke an in-

creased blood pressure in the rat. However like the hypertension produced by Gornall et al with aldosterone, that which is seen in ageing or which follows sensitization procedures is not accompanied by renal or cardiac hypertrophy.

SUMMARY

From this discussion it is evident that certain steroids, principally from the adrenal cortex, are capable of causing hypertension in experimental animals. It is evident also that sodium plays a major role in the development of the cardiovascular and renal lesions, cardiac and renal hypertrophy and increased blood pressure which are seen after the administration of the steroids. It appears likely in fact that the steroids exert these effects through an influence on sodium metabolism. However, there is no evidence of an excessive secretion of adrenal steroids in any form of experimental hypertension. Goldblatt (212) has stated that "until the disease (hypertension) with its secondary manifestations can be consistently reproduced by disturbing adrenal function, evidence that the adrenals are implicated as primary etiologic agents must be considered inadequate."

ADRENAL REGENERATION

In 1938 Ingle and Higgins (218) demonstrated that when one adrenal gland of the rat was transplanted to a different site of the same animal, and the opposite adrenal was removed, degeneration of the medulla and all the cortical zones except the glomerulosa took place. Following this degeneration a functional mass of cortical tissue regenerated from the glomerulosa cells. Regeneration began on the 3rd day following transplantation with complete restoration of a poorly differentiated cortex in 5-6 weeks (222). They also showed that an identical regeneration would occur if the adrenal gland was simply enucleated, and the adrenal capsule and adherent cortical tissue was left in its normal position. These observations have been confirmed by many authors (219, 215, 216, 218).

SOURCE OF REGENERATING ADRENAL CORTICAL TISSUE

According to Ingle and Higgins (218, 219) the cells which serve as a nucleus for the regeneration process originated both in the capsule and the glomerulosa zone. This concept, that capsular elements may give rise to cortical tissue was supported by the studies of Zuemer et al (224), Turner (225), Baxter (226), and Butcher (227).

More recently it has been questioned by Greep and Deane (229), and Brenner et al (228). In extensive studies they found no evidence of conversion of capsule into cortical cells following adrenal enucleation. They concluded that only the glomerulosa cells adhering to the capsule provided the seed for cortical proliferation, and that the capsule and its blood vessels serve as support and a source of nutrition for these glomerulosa cells. The role of the capsule in this respect is nevertheless essential. Williams (223) and Ingle and Higgins (222) have shown that no regeneration followed the transplantation of the enulceated portions of adrenal glands, which consisted of parts of the glomerulosa zone, the fasciculata and reticularis zones, and the medulla. These invariably degenerated completely.

Ingle and Higgins (218) also showed that if one adrenal of the rat is enucleated, and eight weeks later the contralateral adrenal is removed, then regeneration of cortical tissue will still ensue from the adrenal remnant. Thus cortical cells under these conditions are capable of prolonged survival and later regeneration, when removal of the other adrenal provides the stimulus.

CELLULAR CHANGES

The histological changes which occur in regenerating adrenal glands in the rat have an important bearing on the functional considerations which are discussed below. It is obvious from a study of histological observations after adrenal transplantation (218, 228) and adrenal enucleation (229), that the sequence of cellular changes which occur differ but little. After transplantation, the medulla, reticularis and fasciculata cells degenerate, and regeneration occurs from the capsule and glomerulosa cells. While following adrenal enucleation, regeneration occurs directly from the capsule and glomerulosa cells which are left intact. It is possible therefore to discuss the regenerative cellular changes after either transplantation or enucleation as a single process.

Immediately after transplantation of the adrenal gland the cells of the fasciculata, reticularis, and medulla degenerate. Polymorphonuclear leucocytes and macrophages enter the center of the implant and phagocytize the dying cells. Reabsorption of the central degenerated tissue begins three days after implantation and at 10-12 days the entire necrotic mass has disappeared (228).

Twenty-four hours after adrenal enucleation on the other hand, the central area from which most of the cortex and all the medulla has been removed is filled with a blood clot containing cellular debris, hemosiderin, and polymorphonuclear leucocytes. Between the third and eighteenth days this clot is reabsorbed and replaced by compact fibrous tissue which may in some areas become calcified (229).

Glomerulosa cells are the predominant cell type 3-6 days after adrenal transplantation (228), or 8 days after adrenal enucleation. These cells are crowded with many small lipid droplets which enlarge and coalesce to obscure the nuclei. After this period the glomerulosa cells lose this accumulation of lipid and mitosis begins. This mitosis is most abundant at about the eighteenth day (229). Regeneration of all the cortical zones takes place in centripetal fashion from the dividing glomerulosa cells. Fascicles are observed after the eighteenth day, and at 32 days the reticularis is clearly developed. Regeneration of the adrenal cortex is complete in 4-5 weeks.

FACTORS AFFECTING ADRENAL REGENERATION

Ever since Halsteds' studies on parathyroid transplantation (220) the concept has been variously considered

that there must be a deficiency of, or a physiologic need for, the principle of a given endocrine gland in order to have successful grafts or regeneration of it. Adrenal regeneration has been shown to be a response through the pituitary to an insufficiency of adrenal cortical secretions. Regeneration of the fasciculata and reticularis does not occur if the pituitary is removed (218, 241, 221), or if the contralateral adrenal is left intact (218). Similarly the regeneration of the adrenal cortex is restored in the absence of the pituitary if adrenocorticotropic hormone is administered (241), and is inhibited by the administration of adrenal cortical extract (218). Ingle and Higgins found that the size of the adrenal remnant remaining after enucleation influenced the mass of adrenal cortical tissue which regenerated (219). Wyman and Tum Suden (215) found that 14 days following homotransplantation of bisected adrenal glands to the trapezius muscle in rats, the total volume of regenerated adrenal cortical tissue was proportional to the number of transplants growing. At 21 and 90 days the total volume of regenerated tissue was about the same irrespective of the number of grafts, so that the volume of each transplant was inversely proportional to the number of grafts. These results suggested that up to 2 weeks after transplantation the amount of cortical secre-

tion from the regenerating adrenal was inadequate to establish a pituitary adrenal equilibrium.

It is mentioned above that adrenal cortical extract will inhibit adrenal regeneration. The administration of a single hormone will also influence adrenal regeneration. Greep and Deane (230) have shown that, in normal animals, DCA caused a suppression of secretory activity in the glomerulosa zone. In rats with regenerated adrenals the administration of DCA for one month caused disappearance of lipid droplets from the cells of the glomerulosa, and shrinking of the cells of this zone (229). These findings suggested that the salt-retaining hormones of the adrenal were secreted by the cells of this zone. Direct evidence that this was the case was provided by the elegant in vitro experiments of Giroud et al (231). These workers showed that following decapsulation the core of the rat adrenal gland produced insignificant amounts of aldosterone, while the capsule secreted large amounts of aldosterone when incubated. Histological study showed that decapsulation separated the glomerulosa zone and capsule from the fasciculata, reticularis, and medulla so that the role of aldosterone production was attributed to the glomerulosa. These observations were later confirmed by the study of slices of beef adrenal gland which were composed of glomerulosa cells

or reticularis and fasciculata cells (79).

It is well known that important functional interrelationships exist between the thyroid gland and adrenal cortex. In animals rendered hyperthyroid, the adrenal cortex enlarges and it shrinks in size in animals made hypothyroid by thyroidectomy or antithyroid drugs. Studies in this field have been summarized by Deane and Greep (232). Ingle and Higgins (233) showed that thyroxine had no effect on adrenal size in the absence of the pituitary. It seems likely that the hyperplasia of the adrenal cortex is a response by the adrenal to an increased requirement of cortical hormone in the hyperthyroid state. Support for this concept was provided by the demonstration that thyroxine administration increased the requirement of the adrenalectomized dog for adrenal cortical extract (233).

Wyman and tum Suden (216) found that gonadectomy at the time of homotransplantation of the adrenal gland in the rat did not interfere with regeneration of the adrenal cortex. This was confirmed by McPhail and Read (217) in the mouse although they felt that regeneration of the X-zone in this species was not complete. There is little information on the effect of sex steroids on adrenal regeneration. However, Read (241) has recently reported that stilboestrol

markedly inhibited regeneration of the adrenal gland following enucleation. The administration of large doses of adrenocorticotropic hormone did not reverse the stilboestrol inhibition.

FUNCTIONAL ASPECTS OF ADRENAL REGENERATION

Greep and Deane (229) showed in rats that 30 days after adrenal enucleation the blood sugar response to a prolonged fast was similar to that of normal animals. On the other hand, Brownell and Hartman (235) suggested that in mice there was an excessive secretion of glucocorticoids following adrenal regeneration. They based this conclusion on the finding that 30 days following adrenal enucleation the mice deposited almost three times as much glycogen in their liver in response to 24 hours fasting as did normal animals. These workers also suggested an excessive secretion of "fat factor" existed during adrenal regeneration in the mouse (236). They found the maximum output of this "fat factor" at seven days after adrenal enucleation.

Evans showed that in rats the liver glycogen deposition in response to exposure to low atmospheric pressure was absent at 3 days, and low 10-18 days following adrenal enucleation (236). It has also been shown that rats re-

cover their ability to tolerate exposure to stress as early as 7 days following adrenal enucleation and contralateral adrenalectomy (237). Such rats survived an intraperitoneal injection of 150 mg/kg of histamine, or exposure for four hours at 2 degrees C, procedures which uniformly caused death in adrenalectomized animals.

Apparently the recovery of the functions of the adrenal cortex influencing salt and water metabolism is much slower following adrenal enucleation than those which concern carbohydrate metabolism. Jones and Wright (238) have found that adrenalectomized rats, if offered free choice, will drink entirely saline rather than water twelve days after operation. Rats with enucleated adrenals drink mainly water 24 hours after operation, equal water and saline 48 hours after operation, and at 6 days they are back to a primarily water intake. These results suggest a rather quick recovery of the mineralocorticoid functions of the adrenal cortex. However, though this may be so under normal conditions, the same is not true under the stress of a water load. Normal rats excrete 90% of a water load within 11 hours and adrenalectomized rats only 13-15%. Rats with regenerating adrenal glands excrete little more than adrenalectomized animals up to 8 days after operation,

and even 53 days after operation the excretion is only half that of a normal rat (237). These studies were confirmed in part, Jones and Spalding (239) found that the handling of water loads was deficient up to 3 months after adrenal enucleation, and only approached the normal at 6 months.

Giroud (240) has recently studied the secretion of aldosterone and corticosterone into the adrenal vein of the rat at varying intervals after enucleation. A gradual increase in steroid secretion begins 3 days after operation, and the secretion of both aldosterone and corticosterone are within the normal range at 30 days.

It is evident from these studies that, following adrenal enucleation and contralateral adrenalectomy, regeneration of an adrenal cortex occurs which at about 30 days is normal both in function and histological appearance, with the exception of the response to a water load. There is no evidence in the rat of excessive excretion of either mineralocorticoid or glucocorticoid elements from the regenerating adrenal cortex. It would appear instead that in respect to mineral corticoids the regenerated gland was unable to respond in a normal manner to undue stress such as that imposed by a water load.

ADRENAL - REGENERATION HYPERTENSION

The first observation of hypertension accompanied by adrenal regeneration in the rat was made by Skelton (242). This author noticed in a study of the effect of methylandrostendiol on the blood pressure in the absence of the adrenals that an adrenalectomized control animal developed hypertension. Examination of this animal at autopsy revealed the presence of a regenerated adrenal on one side. This observation was investigated, and it was shown that unilaterally nephrectomized rats maintained on 1 per cent saline developed hypertension, cardiac and renal hypertrophy, and widespread cardiovascular-renal lesions following adrenal enucleation (242, 243). The hypertension and vascular changes developed during a 4-5 week period as the adrenal cortex regenerated. Skelton has shown that young rats (50-51 g) were more susceptible than older rats (218-244 g) to the development of this syndrome which was termed "adrenal-regeneration hypertension" (244).

Various factors which influence adrenal-regeneration hypertension have also been studied. It was mentioned earlier that adrenal regeneration per se does not develop after adrenal enucleation if the hypophysis is removed or

the contralateral adrenal is left intact. Similarly adrenal-regeneration hypertension does not develop following adrenal enucleation, unilateral nephrectomy, and the administration of 1% sodium chloride, if the contralateral adrenal is left intact (245) or the hypophysis is removed (245). It has also been shown that reduction of renal mass by unilateral nephrectomy is essential to the development of adrenal-regeneration hypertension (245). Although there is no increase in fluid intake in adrenal-enucleated rats over that of the control animals (242), and serum sodium and potassium values of hypertensive animals fall within the normal range (242), the administration of excessive amount of sodium chloride is essential to the development of adrenal-regeneration hypertension (245). Both hypertension and vascular lesions have been shown to develop in castrated and noncastrated rats of either sex, indicating that neither sex nor castration materially influences its development (245).

The morphological changes in rats with adrenal-regeneration hypertension are characterized in the kidney by arteriolar nephrosclerosis, fibrinoid degeneration of the glomeruli and arterioles and tubular dilatation with

hyaline casts. Arteriolar sclerosis was also found in the heart with fibrinoid necrosis of the vessel walls and perivascular fibroblastic proliferation. Similar vascular lesions in the brain were associated with neuronal degeneration and cystic infarcts. Periarteritis was seen in a small percentage of the rats with adrenal-regeneration hypertension (242, 243, 244, 255).

Skelton theorized on the basis of his experiments (242, 243) that adrenal-regeneration hypertension in rats was the result of adrenal cortical hyperfunction during regeneration. Specifically it was felt that there was increased secretion of some "mineralocorticoid either alone or together with glucocorticoids" (242). There is no support for this view in the earlier work on adrenal regeneration per se, in fact without any reduction in renal mass and on an intake of water, rats appear to have a deficiency of the factors controlling water and electrolyte metabolism (237,239).

Since the work in this thesis was commenced, a number of papers have appeared which confirm in general the observations of Skelton. Chart et al (246) showed that Hydralazine (Apresoline) or Reserpine would reduce the blood pressure of adrenal-regeneration hypertensive rats, while hydrocortisone or the adrenal-depressant drug Amphenone, at a

dose of 100 mg/kg orally had little effect on the blood pressure. Masson et al (247) also confirmed the development of adrenal-regeneration hypertension in the rat following adrenal enucleation and found that the administration of thyroxine to these animals significantly enhanced the degree of hypertension and cardiovascular renal lesions, and caused an increase in size of the regenerating adrenal gland. Skelton has recently reported that thyroparathyroidectomy (248), or the administration of diethylstilboestrol (248) would prevent the development of adrenal-regeneration hypertension, and that removal or re-enucleation of a regenerated adrenal after the development of hypertension would cause the blood pressure to return to normal (249). When the adrenal was completely removed the fall in blood pressure was permanent, while after re-enucleation of the adrenal the fall was transient, and the hypertension returned as the adrenal regenerated again (249).

SUMMARY OF THE HISTORICAL REVIEW

For the past half century evidence has accumulated from clinical investigations and animal experimentation that implicates the adrenal cortex in the etiology or pathogenesis of hypertensive vascular disease in man. It has been clearly

shown that absence of the adrenal gland in most circumstances is incompatible with the maintenance of hypertension except that induced by the administration of adrenal cortical steroids. Many of the steroids which are known to be synthesized in the adrenal cortex are capable of producing hypertension, or augmenting an established hypertension, in man or in experimental animals. They are particularly effective in this respect if their administration is accompanied by an increase in the intake of sodium. Although many of the recent studies are promising and point to important changes in electrolyte transfer at the cellular level as a possible mode of action, the exact role of the adrenal cortex and its steroids in the pathogenesis of hypertension remains obscure.

The recent demonstration that rats, if properly sensitized by unilateral nephrectomy and saline administration, would develop hypertension and cardiovascular-renal lesions following adrenal enucleation represents an important advance in this field. This is the first fully confirmed technique for the development of an "endocrine" hypertension in experimental animals without the use of exogenous steroids.

Study of this type of hypertension may possibly shed some light on the mechanisms by which the adrenal cortex participates in hypertension, and indicate the relative im-

portance of the various histological zones of the adrenal cortex and various endogenously produced adrenal steroids in this respect.

PURPOSE AND GENERAL OUTLINE OF THE STUDY

The purpose of the following work was to study some factors affecting the adrenal cortex in the hope that in adrenal-regeneration hypertension they might influence the regenerating adrenal gland and the blood pressure, and thereby add to our knowledge of the pathogenesis of this form of hypertension.

It was necessary at the outset to adapt and test a method for indirectly measuring the blood pressure of the rat which would allow these measurements to be made without anaesthesia. This would obviate interference from anaesthesia which is known to affect blood pressure, and also would not impose an undue stress on adrenalectomized animals or those in adrenal deficiency immediately after adrenal enucleation.

Experiments were first performed to confirm the development of hypertension during adrenal regeneration in the rat. With unilateral nephrectomy and the administration of saline as a procedure common to all groups, the

effects of bilateral adrenalectomy, unilateral adrenalectomy, and unilateral adrenalectomy with delayed adrenal enucleation were studied. In a number of experiments the administration of Amphenone, an adrenal depressant compound, was investigated in adrenal-regeneration hypertension. Since this compound possesses weak estrogenic properties (253), and also depresses the thyroid gland (254), experiments on the effects of estrogen and propylthiouracil on this hypertension were also performed. Further evidence on the specificity of action of Amphenone was obtained by giving the drug to animals with DOCA-induced hypertension.

In all of these studies data was recorded on blood pressure and body weights. At the completion of the studies, tissue weights and complete histological examinations of various tissues were made.

In order to obtain further information on the secretion of salt-retaining steroids by the regenerating adrenal glands, samples of adrenal effluent blood were collected from animals with adrenal-regeneration hypertension, normal controls on an intake of saline, normal controls on a water intake, and rats which had undergone adrenal hypertrophy after unilateral adrenalectomy. These samples were analysed

by Giroud and coworkers for aldosterone content.

In a further experiment a comparison was made in the rat between adrenal-regeneration hypertension and that produced by the administration of large doses of corticosterone.

Under the section of methods the techniques which were used are described in detail, occasional alterations which were made are described with the individual experiments. At the end of each section there is a discussion, and at the end of the entire experimental part a general discussion and summary.

METHODS

SELECTION AND CARE OF THE ANIMALS

The animals used in these studies were either hooded rats of the Long Evans strain or albino rats of the Wistar strain. The hooded rats were from a colony bred in this department. When these animals were no longer available, albino rats were obtained from Canadian Breeding Farms, St. Faustin, Quebec. Both strains were relatively free of chronic pyelonephritis which is endemic in many colonies. The presence of this disease would have resulted in a high incidence of spontaneous hypertension in the animals, and obvious discrepancies in the experimental results.

Male rats were used in all experiments except one. They weighed between 80 and 100 g at the start of each experiment, and were carefully selected for uniformity of weight and healthy appearance. They were fed Purina rat chow, to which they were allowed free access at all times.

The drinking fluid was either tap water or 1% saline as indicated in the indicidual experiments. The rats were kept in screened cages with not more than ten animals per cage. At the beginning of each study the animals were transferred to an air-conditioned room. The temperature in this room was maintained at 75 degrees F $\pm$ 5 degrees, and the humidity at approximately 50%. Blood pressures were taken

in the same room so that uniform conditions were maintained during the entire experimental period. A number was assigned to each animal by means of a system of ear marks, and the body weight of individual animals was taken each week using a Shadowgraph balance.

OPERATIVE TECHNIQUES

A number of different operations were employed; these were unilateral nephrectomy, adrenalectomy, and adrenal enucleation, either singly or together. All operations were performed under sodium pentobarbital anaesthesia (30 mg/kg intraperitoneally). Unilateral nephrectomy was performed through an incision in the right paralumbar fossa. After isolating the kidney from the surrounding fat tissue, a ligature of cotton thread was placed around the renal pedicle and the vein and artery were cut with scissors distal to the ligature. In most experiments the adrenal on the right side was also removed through the same incision. The adrenal was grasped with forceps, and separated by gentle traction from the connective and fat tissue which surrounded it. Care was taken to remove the adrenal intact, and avoid leaving remnants which would regenerate. On the left side the adrenal was either left intact, removed completely in the manner described above, or enucleated.

Adrenal enucleations were done by the method described by Ingle (218). After exposure of the gland through a paralumbar incision, it was grasped with forceps and a longitudinal incision made in the capsule. Gentle pressure was then exerted on the gland and its contents extruded, usually as a solid core. The capsule and adherent cortical cells were left in situ and served as a nucleus for regeneration of the cortex. After the surgical maneuvers were complete the incisions in the skin and abdominal muscles were closed with one layer of interrupted sutures of cotton thread. The animals were kept warm by means of a heat lamp until they had recovered from the anaesthesia.

MEASUREMENT OF BLOOD PRESSURE

The adaptation of a method for indirect measurement of the systolic blood pressure was a basic necessity for this study. The various methods which have been developed have been reviewed in The Rat in Laboratory Investigation (255), and more recently by Olmsted et al (256). The indirect methods of measuring the blood pressure of the rat are admittedly not as accurate as those involving direct arterial cannulation. However the latter methods have several inherent disadvantages. Hamilton (257) devised a high frequency manometer for use with direct cannulation

of the carotid artery or the aorta, but the use of anesthetics and the operative procedure in this technique may cause unpredictable disturbances in blood pressure (256, 258). Friedman (259) uses an elegant adaptation of this technique by direct cannulation of the femoral artery in the unanaesthetized rat, however, few workers are endowed with the high degree of technical skill required, and as a result the method has not been used by other workers.

All of the indirect methods of measuring blood pressure are based on the detection of arterial inflow distal to a slowly deflating cuff on the foot, tail, or ear of the animal. Detection of arterial inflow in the ear has previously been achieved by direct microscopic observation (260), and in the tail by a water plethysmograph (261, 262, 263), microphonic manometer (264), photoelectric transmittance (265), or by noting the appearance of hemorrhage after snipping a piece of the tail (266).

These methods suffer two major disadvantages, first the animals must be anaesthetized causing unpredictable changes in blood pressure (256, 258), and second because tail arterial inflow in the rat is small the whole animal or the tail must be heated. These procedures have been shown to result in significant increases in the blood

pressure (267, 268).

The older methods of detecting arterial inflow in the foot involved simply observing the change in colour which did not provide a clear endpoint (269), or microscopic observation of capillary inflow in the interdigital web (270) which required anaesthesia.

Recently Kirsten et al (271) described a photoelectric foot plethysmograph which was adapted for use in this study. This method appeared particularly suitable since no anaesthesia or heating was required. A period of training is an advantage in this method, since after acclimatization to the apparatus, the animals move less and there is less interference with pressure measurements. This training was automatic in these experiments as the blood pressure was measured three times weekly over periods of up to twelve weeks. Arterial inflow representing systolic blood pressure is detected by deflection of an ammeter needle connected by a direct current amplifier to a phototube which receive variations in transmitted light caused by volume changes in the foot.

Several difficulties were encountered with the use of this apparatus. The single volume control for the ammeter needle did not allow rapid adjustment of current flow after the artery was occluded.

TABLE II - Blood Pressures of Normal Animals, the Blood Pressure Having Been Taken on Five Animals in Triplicate and for Four Consecutive Weeks.

Animal No.	1st Week	2nd Week	3rd Week	4th Week	Averages
1	130	129	130	128	129.4
	128	130	131	128	129.4
	126	127	129	130	128.0
Average	128.0	128.7	130.0	128.7	128.8
Animal No. 2	128	130	112	111	120.2
	130	131	114	110	121.2
	130	128	114	110	120.3
Average	129.3	129.7	130.0	110.3	120.7
Animal No. 3	136	137	128	128	132.3
	138	136	130	128	133.0
	138	137	130	129	133.8
Average	137.3	136.7	129.3	128.3	132.9
Animal No. 4	140	140	140	138	139.5
	140	142	140	137	139.7
	142	142	139	137	140.0
Average	140.7	141.3	139.7	137.3	139.8
Animal No. 5	133	129	128	128	129.5
	128	129	128	130	128.8
	132	130	129	130	130.3
Average	131.0	129.3	128.3	129.3	129.5
Grand Averages	133.3	133.1	128.1	126.8	130.3

TABLE III. Analysis of Variance of Blood Pressure Data From Table II.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	F Ratio	Significance
Between Animals(5)	2,301	4	575	38	High Probability <.01
Between Weeks (4)	506	3	169	11	High Probability <.01
Between Replicates (3)	1	2	0.5	<1	None
Error	749	50	15*	1	--
Total	3,557	59			

Estimate of Error = $\pm$ 15 = $\pm$ 3.9%

Index of Precision = 96%

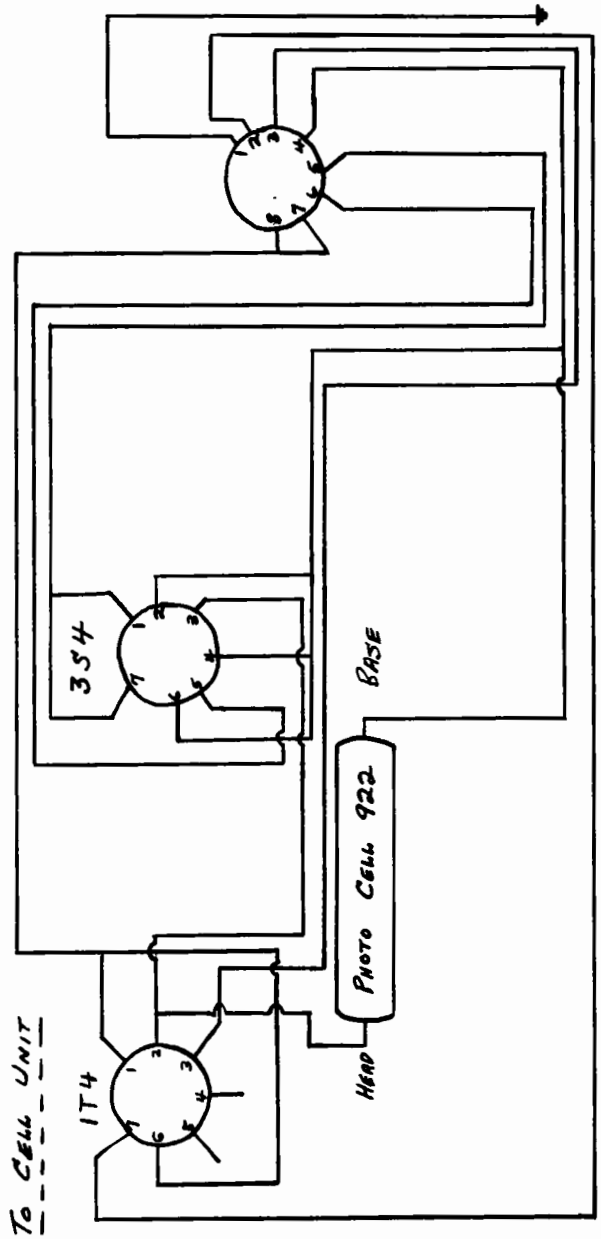

Fig. I. Wiring Diagram of Modified Photocell Unit

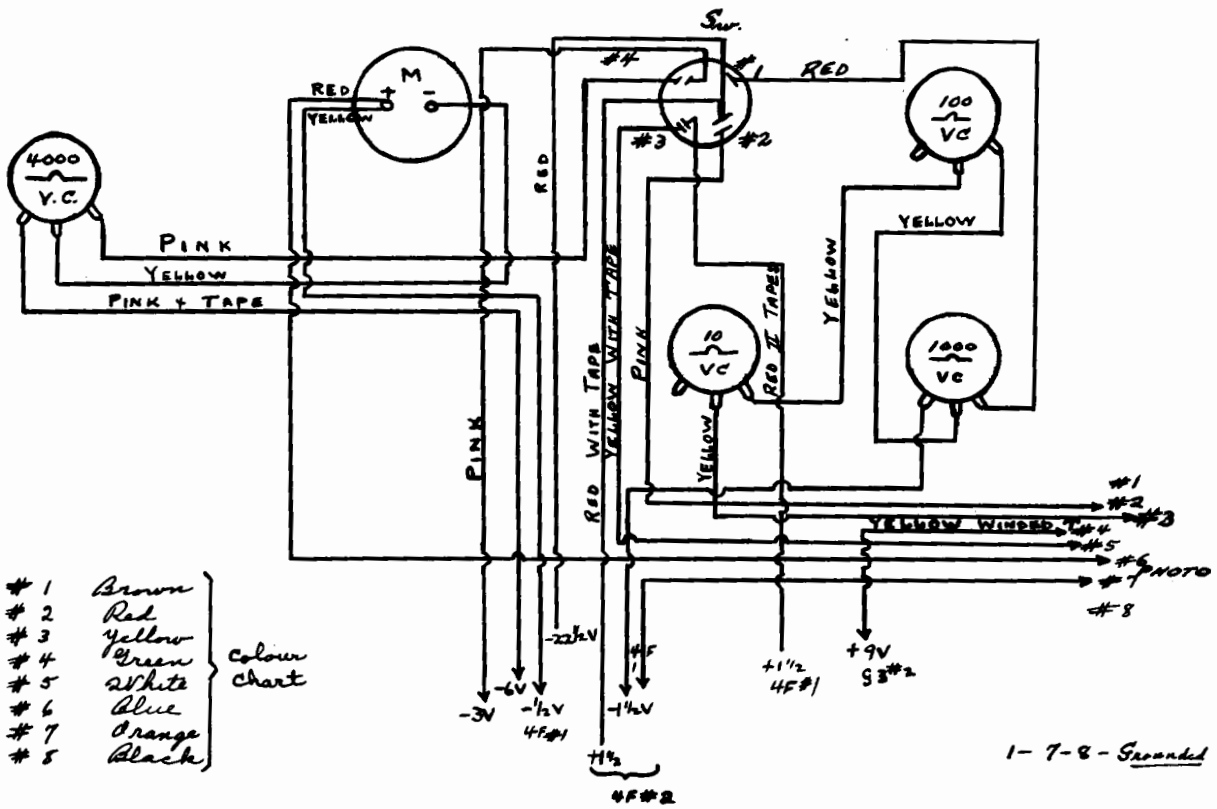

Fig. 2. Wiring Diagram of Amplifier of Modified Photocell Unit

This difficulty was overcome by rebuilding the apparatus and including two additional volume controls in parallel with the one in the original circuit. The wiring diagrams which include these modifications are shown in Figures I and 2. It was also noticed that the light source was subject to marked fluctuations as the batteries became weak, this also interfered with pressure measurements. The battery operated light was therefore replaced with a 3 volt lamp operated by alternating current.

As a measure of reliability of the method of testing the blood pressure an analysis of variance was done on recordings of blood pressure from 5 normotensive animals chosen at random from a control group (Table II.). A 4 week period was used and the analysis was done on a total of 60 blood pressure measurements. It may be noted from Table III that no significant difference was seen between replicate readings and that the Index of Precision was high. Significant differences were noted between the blood pressure of different animals in the group, and between measurements taken at weekly intervals.

COLLECTION OF ADRENAL EFFLUENT BLOOD

The technique employed for the collection of adrenal

vein blood was similar to that used by Bush (272), Singer and Stacke-Dunne (273), and Giroud (274). The rats were anaesthetized with sodium pentobarbital, 30 mg/kg administered intraperitoneally. They were then pinned by the feet to a cork board in the dorsal position. To facilitate respiration a tracheal cannula was inserted through a ventral incision in the neck. A midline incision was then made from the xyphoid cartilage to the pubis, and the abdominal viscera was exposed. To obtain a clear field in the region of the kidney the viscera were compressed to the right side by means of sponges moistened with warm isotonic saline. The left kidney and its vascular pedicle were then freed of peritoneum, fat, and connective tissue, and all small vessels which emptied into the renal vein, with the exception of the adrenal vein were cauterized. Ligatures were placed around the renal pedicle, one close to the vena cava and a second close to the kidney, the latter was tied to isolate the kidney. Heparin (500 units) was then administered intravenously into the sublingual vein or the right mammary vein. A fine glass cannula was then inserted through a tiny incision into the renal vein, and the junction of this vein with the vena cava was ligated. All the blood which emptied into the renal vein then came from the adrenal. The adrenal

effluent from the cannula was collected in 15 ml centrifuge tubes kept cool by dry ice. The bleeding period was approximately 60 minutes. The exact length of this period, the adrenal weight, body weight, and blood volume were recorded.

The samples of adrenal effluent blood were analysed for their aldosterone content by Dr. C.J.P. Giroud of the Montreal Children's Hospital using the Simpson-Tait assay (275).

HISTOPATHOLOGICAL TECHNIQUES

Upon the completion of each experiment, the animals were sacrificed using chloroform. Their organs were dissected free of fat, weighed on a Roller Smith torsion balance and fixed in 10% formalin or Bouin's solution. The following organs were examined histologically after paraffin sectioning and staining with haematoxylin and eosin-brain, heart, kidney, thyroid, stomach, intestine, mesentery, pancreas, liver and spleen. Kidneys, and in some cases, heart and mesentery, were stained with Cason's Trichrome (276) and Sudan IV in frozen section. Pituitary glands, and in some cases pancreas, were stained by the method of Rona and Morvay (277).

Adrenal glands were fixed in formalin, after fixation frozen sections were made and the remaining tissue embedded in paraffin for haematoxylin and eosin staining. Frozen sections were examined for birefringent material, and additional slices were stained with Sudan IV and haematoxylin.

STATISTICAL ANALYSIS

The statistical methods used in this study were those outlined by Gore (39). The standard error is given for mean values of tissue or body weights, and the 95% confidence limits are plotted on the graphs of blood pressure. These values were obtained as follows:

Standard Error

$$S.E. = \frac{S.D.}{\sqrt{n}}$$

where S.E. = Standard error

S.D. = Standard deviation

n = Number of individual measurements or size of the sample.

$$S.D. = \sqrt{\frac{\sum_{i=1}^{n} x_i^2}{n-1} - \frac{\left(\sum_{i=1}^{n} x_i\right)^2}{n(n-1)}}$$

where $\sum_{i=1}^{n} x_i^2$ = sum of the squares

$\left(\sum_{i=1}^{n} x_i\right)$ = square of the sum

x = individual measurements

Confidence Limits

The factor (a) for determination of the confidence limits was obtained from tables supplied by Gore (39).

Confidence Limits = a x S.E.

Usually a = 3 for 99% confidence limits and a = 2 for 95% confidence limits except when sample size was smaller than 7 or 8.

EXPERIMENTAL PROCEDURES AND RESULTS

PART 1 - STUDIES ON THE PATHOGENESIS OF ADRENAL-REGENERATION HYPERTENSION.

The studies which make up this series were designed first, to confirm the findings of Skelton (242) that rats become hypertensive, and develop widespread vascular lesions following adrenal-enucleation, contralateral adrenalectomy and unilateral nephrectomy with the administration of 1% saline in the drinking water. And second, by altering several of the variables in this technique, to study the pathogenesis of this form of hypertension.

Although the observations of Skelton have been confirmed in general by the studies of Chart et al (246), and Masson et al (247), there is not a unanimity of opinion on the subject. Grollman (278) questions the idea that the adrenal cortex plays a direct etiologic role in the genesis of adrenal-regeneration hypertension, and has stated that "no hypertensive principle is secreted by the regenerating adrenal nor is the hypertension a result of an imbalance in adrenal cortical activity".

Ingle and Higgins (218 showed that if one adrenal of the rat was enucleated, and eight weeks later the contralateral adrenal removed, then regeneration of cortical tis-

sue would still ensue from the adrenal remnant. This technique was applied to a study of adrenal-regeneration hypertension to allow a more direct approach to the relationship between the development of hypertension and actual adrenal regeneration.

Experimental Design -

Operative procedures and treatment of the animals.

Group I - Normal rats (15 animals). These were intact control rats given tap water to drink.

Group II - Adrenal-regeneration hypertension (15 animals) At the same operation these animals were unilaterally nephrectomized and adrenalectomized on the right side, and the left adrenal was enucleated. Saline 1% was substituted for the drinking water.

Group III - Bilateral adrenalectomy (25 animals). These animals were unilaterally nephrectomized on the right side and bilaterally adrenalectomized. Saline 1% was substituted for the drinking water.

Group IV - Adrenal hypertrophy (25 animals). These rats were unilaterally nephrectomized and adrenalectomized on the right side. Saline 1% was substituted for the drinking water.

Group V - Nephrectomy alone (25 animals). These rats were unilaterally nephrectomized on the right side and 1% saline was substituted for the drinking water.

Group VI - Delayed adrenal-regeneration hypertension (30 animals). At the first operation the right kidney was removed, the left adrenal was enucleated, and 1% saline was substituted for the drinking water. Five weeks later 5 animals from this group were sacrificed for study of the enucleated adrenal. The intact adrenal was removed from remaining 25 animals at this time.

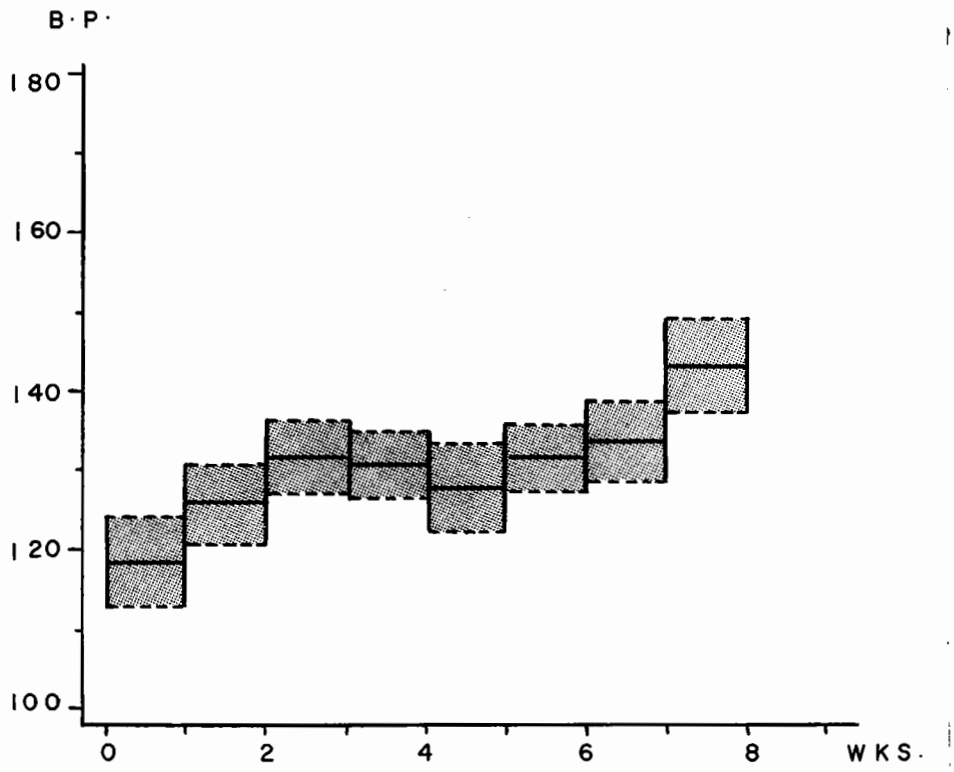

Fig. 3 - Normal intact control rats. In these graphs the solid horizontal lines represent the average weekly systolic blood pressure and the shaded areas the 95% confidence limits.

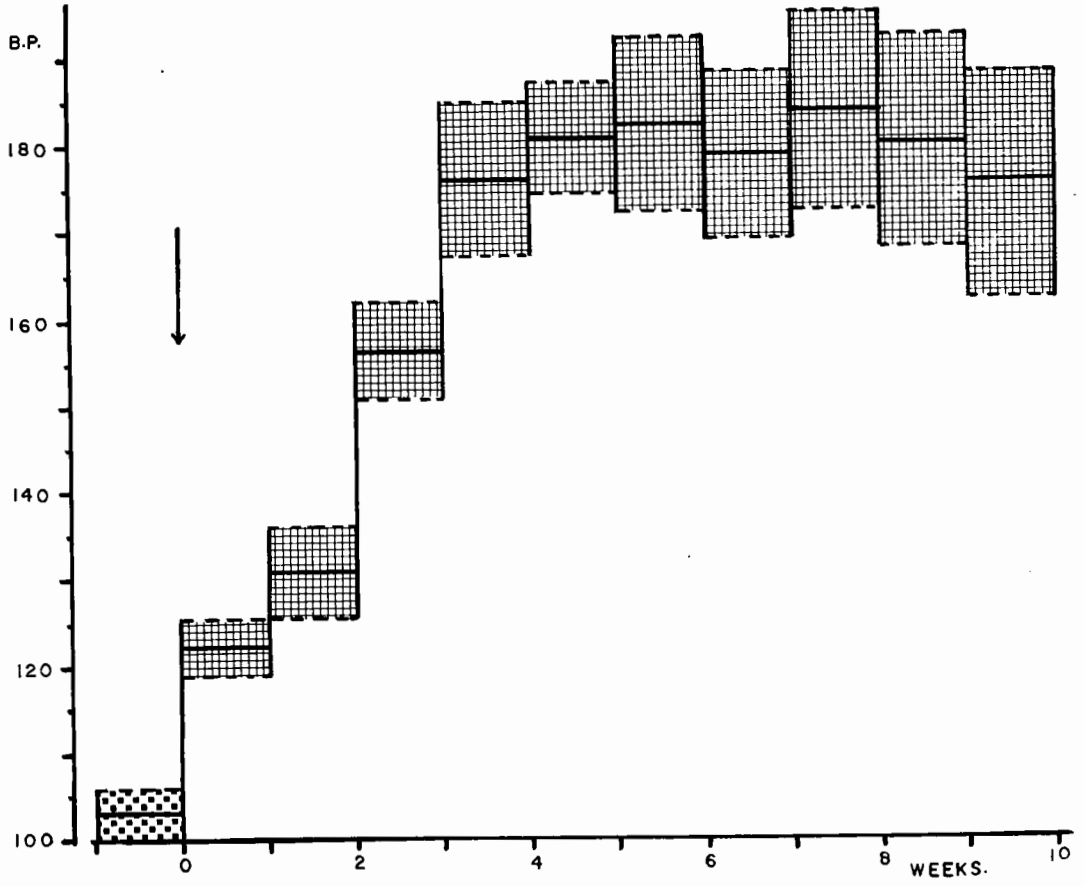

Fig. 4 - Hypertensive responses in animals of Group 2. Rats were surgically prepared at arrow.

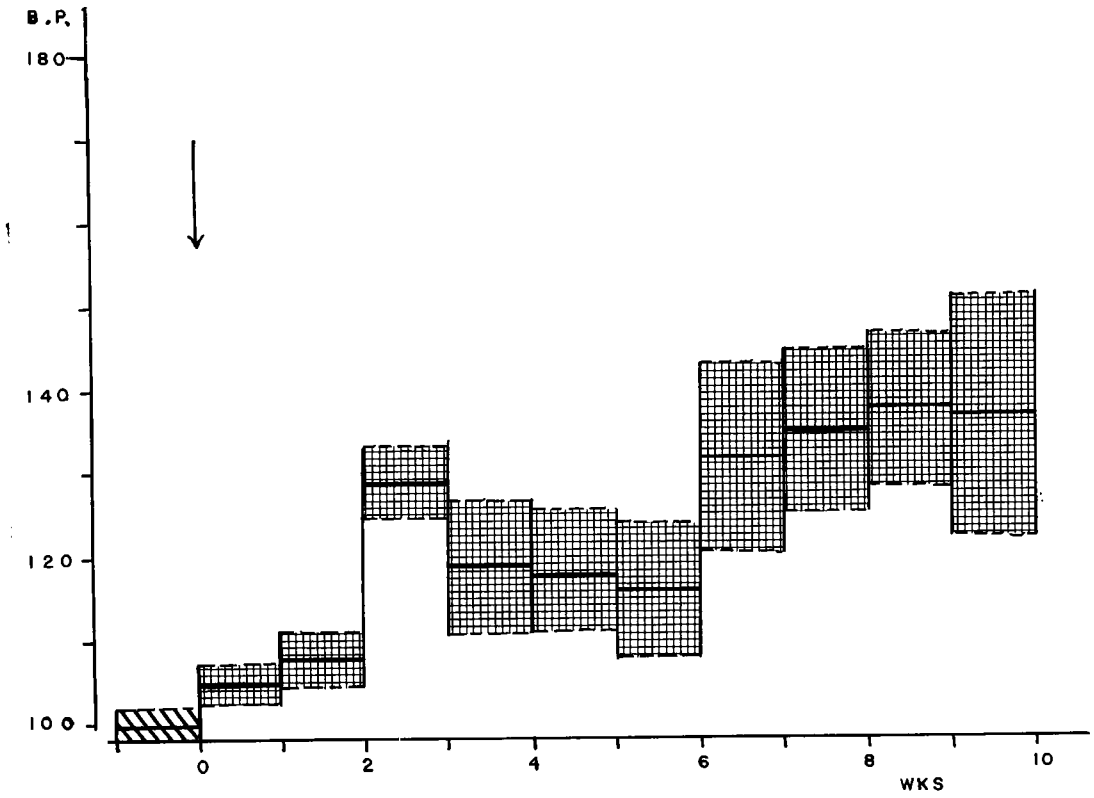

Fig. 5 - Mean weekly blood pressure of animals of Group III, which were bilaterally adrenalectomized and unilaterally nephrectomized at the arrow.

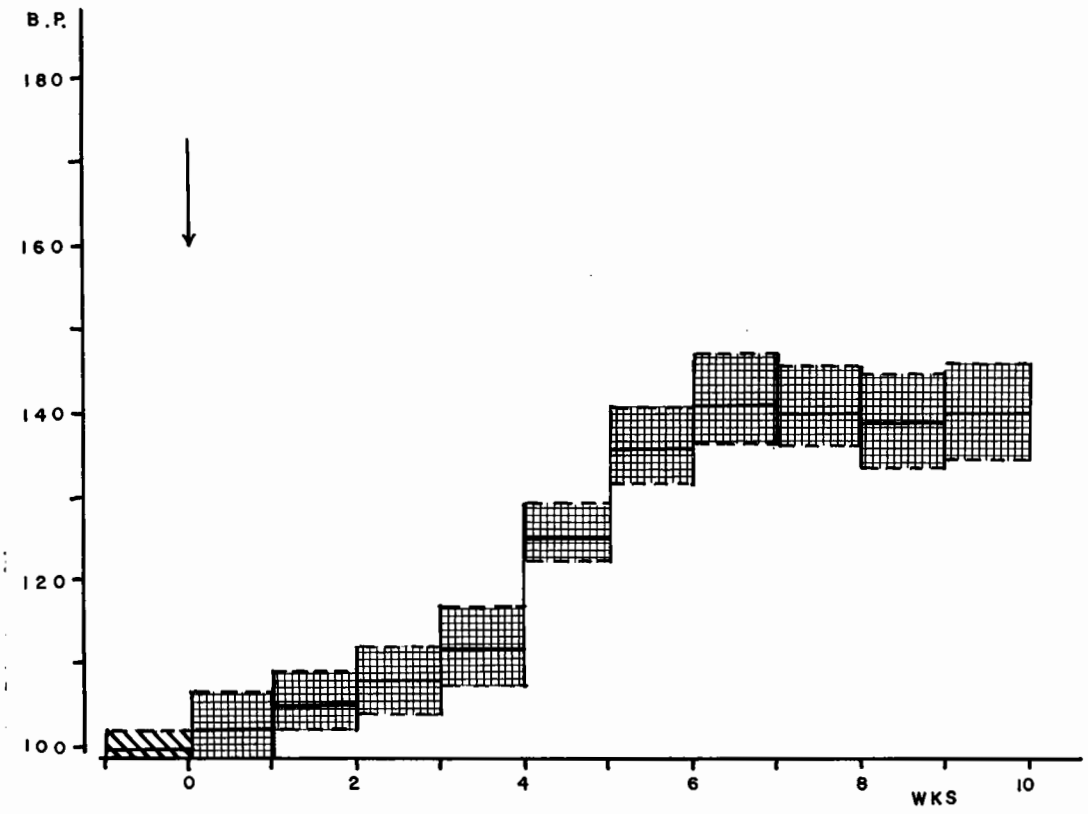

Fig. 6 - Mean weekly blood pressures of animals of Group IV which were unilaterally adrenalectomized and unilaterally nephrectomized at the arrow.

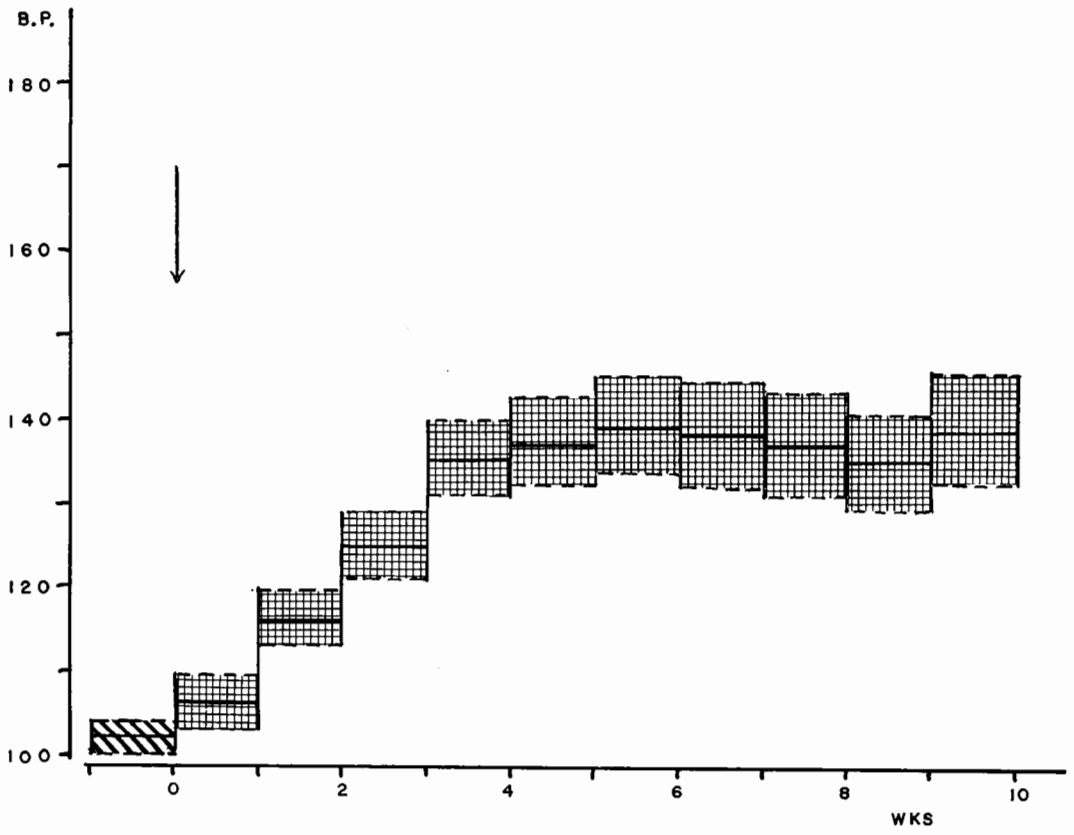

Fig. 7 - Mean weekly blood pressure of animals of Group V which were unilaterally nephrectomized at the arrow, adrenals were left intact.

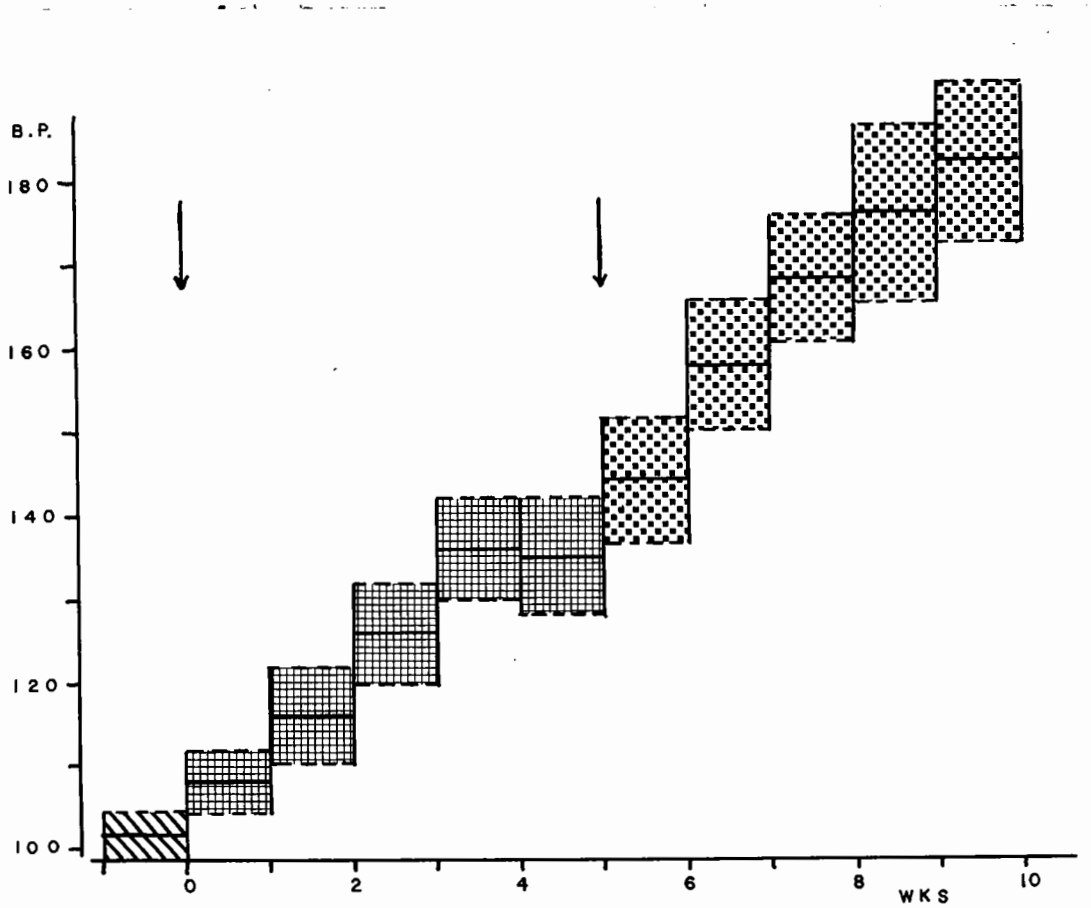

Fig. 8 - Mean weekly blood pressure of animals of Group VI. At left arrow right nephrectomy and right adrenal enucleation. At the right arrow, the contralateral intact adrenal was removed.

RESULTS

1. Blood Pressure

The changes in blood pressure of a group of normal intact animals in an 8-week period are seen in Figure 3. During this time the animals gained approximately 160 g. in weight. There is a gradual increase in mean blood pressure as the rats mature. Figure 4 illustrates the typical hypertensive response coincident with adrenal regeneration in the rat. The blood pressure rises during a four to five week period after enucleation of the adrenal, and remains at hypertensive levels for the remainder of the experiment. The rats of Groups III, IV and V were all exposed to the "sensitization" procedure, i.e. unilateral nephrectomy and a high salt intake during the entire experimental period. They were treated additionally in the case of Group III by bilateral adrenalectomy, and Group IV by unilateral adrenalectomy. It is evident from Figures 5, 6 and 7 that a rise in systolic blood pressure occurred in each of the three groups. The terminal blood pressure was close to 140 mm of mercury in all these animals. However it will be noted (Fig. 3) that a similar rise in blood pressure occurred in the intact control rats of Group 1, which were on normal sodium intake. Thus it must be con-

cluded that the "sensitization" procedures alone are inadequate to produce hypertension. Since as mentioned later the rats of these groups did not have renal or cardiac hypertrophy or cardiovascular-renal lesions, these results support the contention expressed in the literature that in the rat only blood pressures above 150 mm of mercury should be considered hypertensive. The blood pressures of the rats with delayed adrenal-regeneration hypertension, Group VI, are shown in Figure 8. During the first five weeks of this experiment, the animals showed only a slight rise in blood pressure to 132 mm of mercury which did not differ from that of the controls. However, following removal of the intact adrenal gland at the second operation, the mean blood pressure of these animals became elevated to levels which were clearly hypertensive. This elevation of blood pressure from 136 to 182 mm of mercury, occurred during the period when, as will be shown later, regeneration of cortical tissue was occurring from the adrenal remnant. The elevation of blood pressure occurred very rapidly in this group after the second operation. This may be related to the fact that resorption of the blood clot, and development of blood vessels to the capsule, had occurred during the first 5 weeks, so that regeneration

could begin immediately following the removal of the intact adrenal.

2. Body and Tissue Weights

The average body and tissue weights of each group are contained in Table IV. The smaller body weight of the normal control animals was due to the experimental period, being only 8 weeks in this case, and the animals being started at a slightly lower body weight. The relatively poor growth of bilaterally adrenalectomized rats is well known, and accounts for the lower mean body weight of the animals in Group II. It is not possible to compare the weights of the adrenal glands from the animals of the various groups. The single adrenals of the animals of Group IV which have undergone hypertrophy, or the regenerated adrenals of animals in Group II or Group VI, are significantly greater in weight than a single adrenal from animals of Groups I or V in which the adrenals remained intact.

At the time of the second operation on the animals of Group VI, the contralateral adrenals which were removed intact, had a mean weight of 38.4 $\pm$ 2.4 g. The adrenal remnants from five animals of this group which were sacri-

ficed at this time weighed less than 0.5 mg. In only four of these animals was it possible to recognize adrenal cortical tissue by histological examination. At the end of the experiment the regenerated adrenal glands of these animals had a mean weight of 32.5 ± 1.9 g.

The thymus glands of the animals of Group III show a significant hyperplasia, this would be expected following bilateral adrenalectomy. When calculated in terms of body weight there are no differences in the mean weight of the thyroid, pituitary, spleen, or brain of the animals from the various groups. The liver weights of the animals of Group VI were significantly greater than those of the remaining groups, passive congestion in the livers of a number of the animals of this group, it is felt, accounts for this increased weight.

The heart and kidney weights of the animals from Groups II and VI are significantly greater than the weight of these organs from the animals of the other groups. This renal and cardiac hypertrophy would be anticipated on the basis of the high blood pressure in these groups, and in fact, indirectly confirms the presence of hypertension.

TABLE IV - BODY AND TISSUE WEIGHTS

	Group I Normals	Group II Adrenal Regeneration Hypertension	Group III Bilateral Adrenal-ectomy	Group IV Unilateral Adrenal-ectomy	Group V Intact Adrenals	Group VI Delayed Adrenal-Regeneration Hypertension
Adrenal (mg)	18.5± 2.7	34.1 ± 1.2*	-	39.5± 1.0	24.7± 1.5	32.5 ± 1.9
Thymus (mg)	285 ± 21	333 ± 20	406± 3.4	340 ± 16	372 ± 24	327 ± 18
Thyroids (mg)	14.4± 2.2	25.7 ± 2.0	20.9±1.4	23.9± 0.9	21.8± 1.0	24.2 ± 0.9
Pituitary (mg)	7.0± 0.4	12.6 ± 9	11.6±0.6	11.1± 0.03	10.2± 0.3	11.5 ± 0.4
Spleen (g)	1.1± 0.4	0.62± 0.2	0.70±0.06	0.66± 0.02	0.64± 0.2	0.70 ± 0.02
Brain (g)	1.6± 0.02	1.82± 0.02	2.01±0.05	1.83± 0.02	1.84± 0.03	1.89 ± 0.03
Liver (g)	10.6± 0.4	12.7 ± 1.0	13.8±0.9	13.6± 0.03	14.3± 0.5	16.6 ± 0.5
Heart (g)	0.83±0.19	1.20± 0.08*	0.97±0.08	1.02± 0.02	1.02± 0.03	1.25 ± 0.04
Kidney (g)	1.71±0.06	2.31± 0.10*	1.84±0.12	2.11± 0.05	2.19± 0.05	2.63 ± 0.08
Body weight (g)	249 ± 13	343 ± 10	298 ± 9	328 ± 11	333 ± 8	346 ± 9

* Significant at the 95% level of confidence compared to normal animals of Group I corrected for differences in body weight.

3. Gross and Microscopic Pathological Changes

Gross examination of the animals of Group I did not reveal any evidence of pathology with the exception of several animals which had pneumonic lesions. Two animals from Group IV and one animal from Group VI showed evidence of acute bronchopneumonia at autopsy, however, this is a casual finding in laboratory rats, and it is felt, unrelated to the experimental conditions. A total of nine animals from the various groups died of pneumonia during the experimental period.

Group II - Adrenal-Regeneration Hypertension

Adrenal - The adrenal cortex of all animals had undergone regeneration with distinct zonal differentiation and a variable degree of nodularity. The zona glomerulosa was thin and composed of three or four layers of flattened, darkly stained cells containing some traces of lipoid and occasional mitotic figures. The zona fasciculata was well developed and the cells contained a moderate amount of lipoid, some of the cells of this zone were swollen and vacuolated. Venous engorgement was apparent between the cell columns. The zona reticularis was distinct and normal in appearance. Interspersed between the nodules of these adrenals, and filling the centre of the gland was a fibrous hyaline mass in

some areas of which calcium deposition was noted. A typical regenerated adrenal gland is shown in Figure 9.

Pituitary - A consistent change was noted in the basophils of the anterior pituitary of these hypertensive animals. These cells were increased in number and size compared to pituitaries of normal rats. From 10 to 20% of these basophils contained clearly delineated single, or less frequently, multiple vacuoles which were approximately equal in size to the nuclei. In these vacuoles some evenly distributed purple granules were seen. The pituitary from a rat with adrenal-regeneration hypertension is illustrated in Figure 10. No changes were seen in eosinophil or chromophobe cells.

Kidney - At autopsy a granular kidney was observed in one rat, this animal had a particularly severe nephrosclerosis. A more moderate grade of nephrosclerosis was observed in four other rats and only mild renal lesions were seen in the 10 remaining animals. The animal with the most severe renal lesions showed multiple scar formations in the kidney replacing destroyed tubules and glomeruli. Dilated tubules filled with cylindrical protein casts were seen around these scars. The glomeruli were partly enlarged and partly sclerotic. Marked hyalinization of glomerular loops was seen, these stained bright red with Cason's trichrome stain.

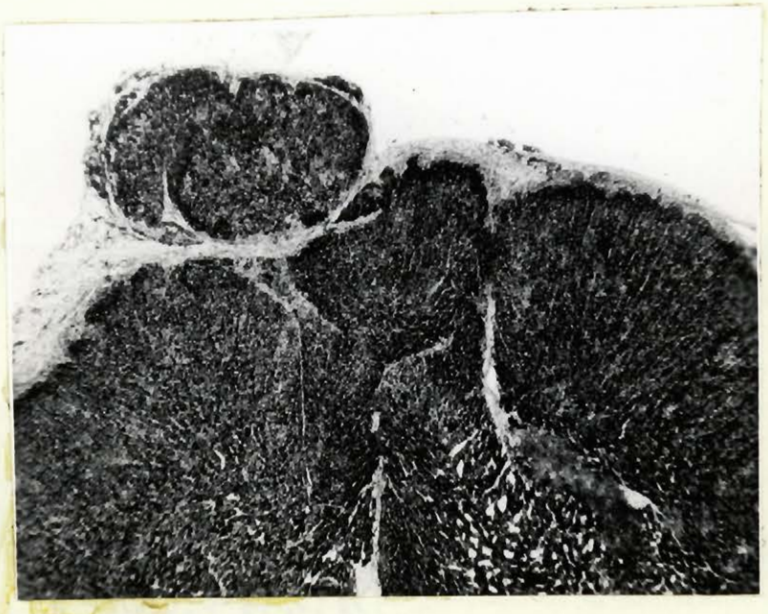

Fig. 9 - Regenerated adrenal cortex. Note the marked nodular structure and zonal differentiation. Sudan IV - hematoxylin X 40.

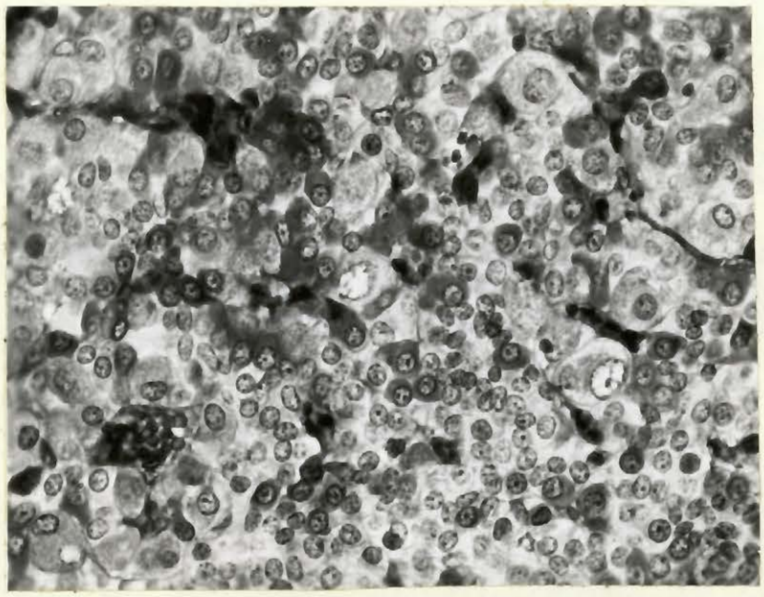

Fig. 10 - Pituitary of a rat with adrenal-regeneration hypertension. Small cells with scanty pale cytoplasm are chromophobes. Darkly stained cells are eosinophils. Swollen large cells with occasional vacuoles are basophils - Rona - Morvay Stain X 450.

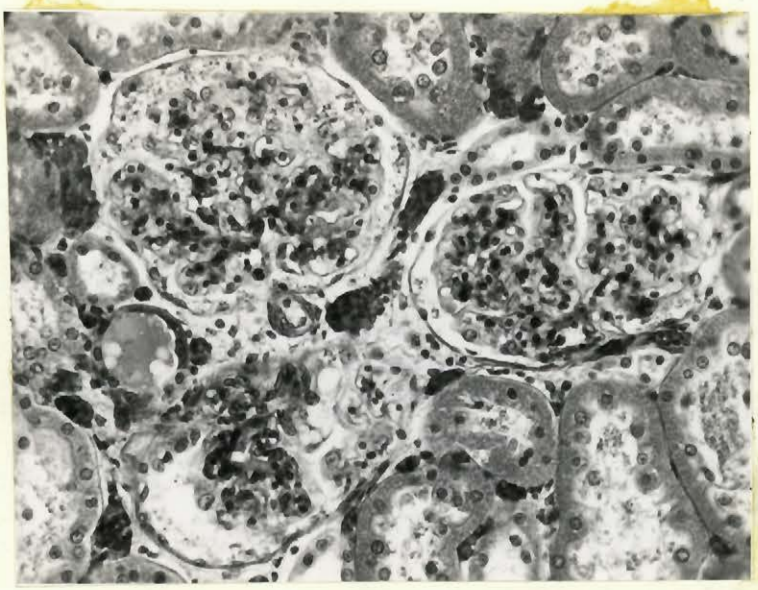

Fig. 11 - Kidney from rat of Group II with mild renal lesions. Only thickened basement membranes and mild hyaline changes are present. Masson's trichrome X 225.

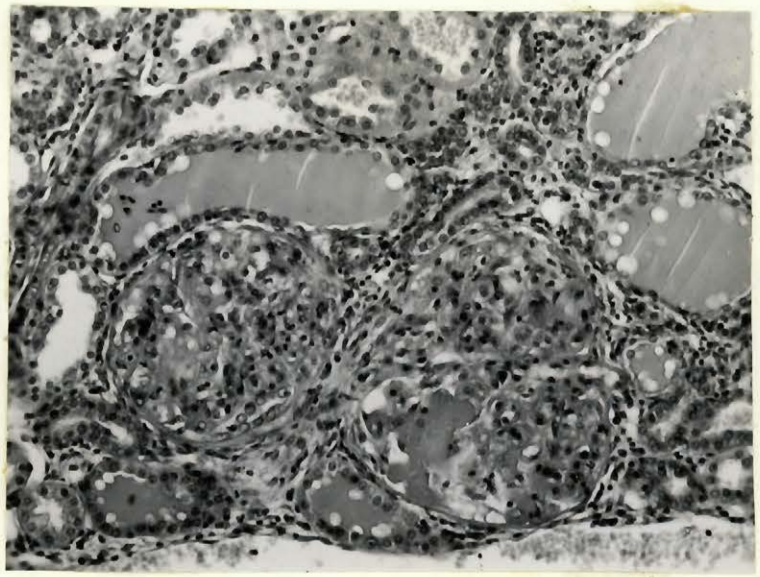

Fig. 12 - Kidney from rat of Group II with severe glomerular lesions. There is marked enlargement of glomeruli, focal necrosis of glomerular loops, thickening of basement membrane and proliferative endarteritis of afferent vessels. Tubules are dilated, lined with flattened cells, and contain protein casts. Hematoxylin and eosin X 175.

The glomeruli were bloodless due to occlusion or narrowing of the loops. Adhesions between the glomeruli and Bowman's capsule were marked in the shrunken, sclerotic glomeruli. The arterioles in these kidneys revealed a marked patchy, or sometimes diffuse, fibrinoid necrosis of the wall, thickening of which caused a narrowing of the lumen. Proliferation of subintimal cells caused complete occlusion in some areas. Around some arterioles a granuloma formation was observed, composed of leucocytes, lymphocytes and histiocytes.

The renal lesions were qualitatively similar, but more moderate in the other four animals which had nephrosclerosis. The remaining animals in the group had only mild renal lesions. These consisted of enlarged glomeruli with thickened basement membranes, anaemic capillary tufts and occasional hyalinized glomeruli. The cells of the proximal convoluted tubules were enlarged and contained large vacuoles. Hyaline cylinder formation was seen in many of the lumens. Examples of mild and severe renal lesions are shown in Figures 11, 12 and 13.

Vascular lesions - The rat which showed a severe nephrosclerosis also had equally severe vascular lesions. The

latter were also present in the four animals with moderate nephrosclerosis but absent in the remaining rats of the group. The character of the lesions was consistent with the arteriolar lesions described for the kidney. Arteriolar necrosis and productive endarteritis was observed in the adrenal capsule, thymus, mesentery and intestine, and particularly severe lesions were noted in the heart and brain. The occlusion of the smaller branches of the coronary arteries in the heart caused multiple star-shaped myocardial scars localized most frequently in the left ventricle. In the brain the vascular occlusions were associated with focal cerebral softening and encephalomalacia particularly of the cortex. These areas were surrounded by lipoid and hemosiderin laden microglial cells. A typical myocardial lesion is illustrated in Figure 14.

Morphological lesions were not seen in the pancreas, spleen, stomach, or thyroid gland.

Group III - Bilateral Adrenalectomy

A total of twelve animals from this group died during the experimental period. These animals, compared to those in the other groups, appeared to be particularly sensitive to the stress of the restraint necessary during the blood pressure measurements as several animals died while measurements

were being made. At autopsy four animals were found to have regenerated adrenal cortical tissue; it was impossible to determine whether this was the result of incomplete adrenal extirpation or hypertrophy of accessory adrenal tissue. One animal was found to have a severe hydronephrosis, and one had a mild chronic pyelonephritis. These animals were excluded from the group.

The thyroid glands were well preserved with the exception of those in three animals which had a slight interacinar fibrosis. No lesions were found in other organs, and no evidence of vascular lesions was detected.

Group IV - Unilateral Adrenalectomy

The only lesions found in the animals of this group were in the kidney. In two rats there were scattered foci of round cell infiltration without alterations in the renal parenchyma or evidence of vascular changes.

The single adrenal from these animals was enlarged and brownish-yellow in colour. The capsule was thickened with the zona glomerulosa of normal thickness, but containing cells which were darkly stained and poor in lipoid material. The zona fasciculata was wide and heavily infiltrated with lipoid material concentrated mostly at the periphery

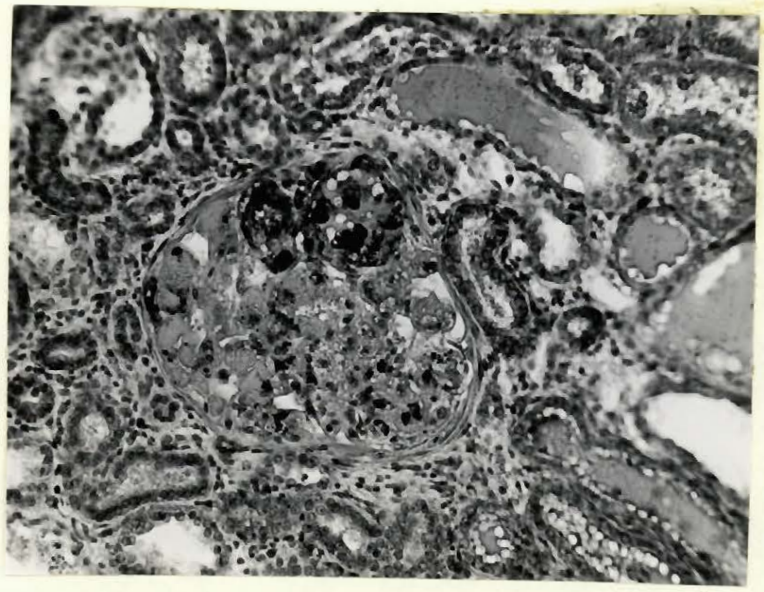

Fig. 13 - Kidney from rat of Group II. Lipoid deposition in necrotic glomeruli, this lipoid was double refractile, Sudan IV - hematoxylin X 175.

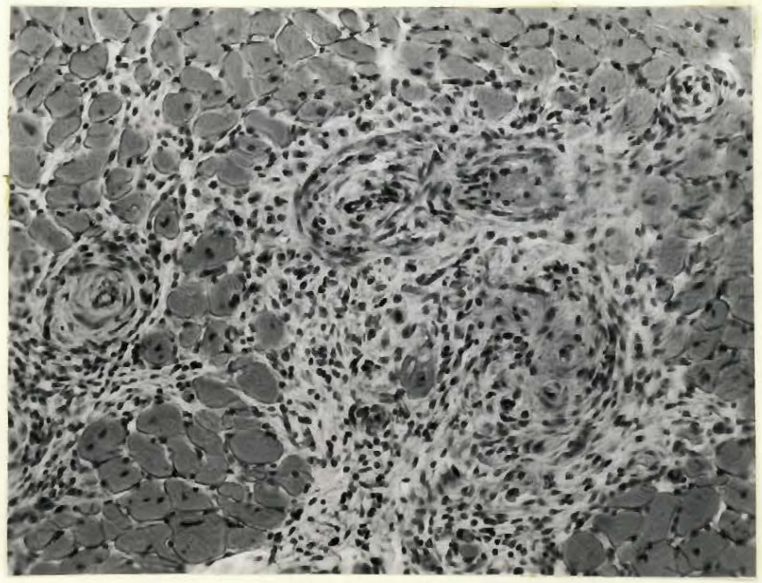

Fig. 14 - Productive endarteritis and myocardial scars in rat of Group II. Note the occlusion of some arterioles. The darkly stained streaks in the arterioles represent lipoid deposition. Sudan IV - hematoxylin stain X 175.

of this zone. The zona reticularis was normal in appearance.

Group V - Intact Adrenals

The animals from this group which had blood pressures above the average were found to have a moderate degree of chronic pyelonephritis without vascular involvement. No changes were detected in the other organs.

Group VI - Delayed Adrenal Regeneration

Adrenals - At the time of the second operation, the adrenal remnants from the five animals which were sacrificed and the adrenal glands which were removed from the remaining animals were preserved for examination. The intact right adrenal from these animals was enlarged and microscopically resembled the hypertrophid adrenal from the animals of Group IV. However, one difference was noticed in that there appeared to be an overall decrease in lipoid content in the zona fasciculata. The adrenal remnants after enucleation varied from small nests of undifferentiated cortical cells to circumscribed groups of cells which showed no zonal differentiation (Fig. 15). Fibrous tissue proliferation was evident in each remnant, with bands of connective tissue, occasionally calcified, separating the cortical cells. The vessel stems had thickened walls and narrowed lumens (Fig. 16).

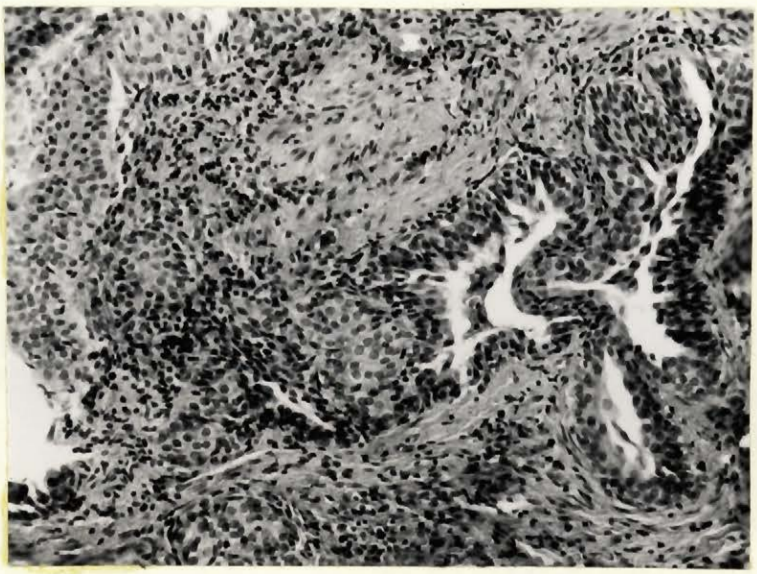

Fig. 15 - Adrenal remnant 5 weeks after enucleation. Solid bundles and some glandular structures composed of darkly stained small cells are visible, embedded in dense connective tissue. Hematoxylin and eosin stain X 200.

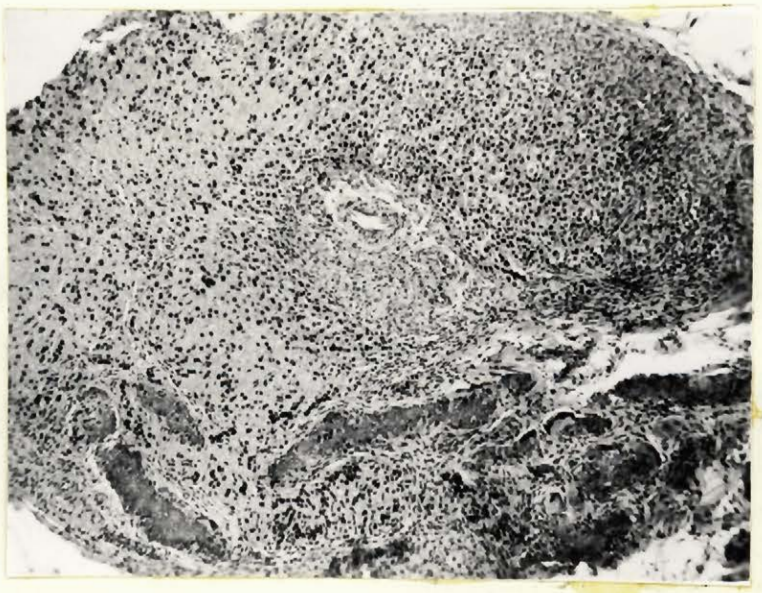

Fig. 16 - Adrenal remnant 5 weeks after enucleation, weight 0.5 mg. Note the marked fibrosis and calcification. Only a few small areas of atrophic cortical tissue remains. Hematoxylin and eosin X 100.

At the end of the ten week experimental period adrenal regeneration could be recognized in all rats. The general architecture of these adrenals showed a marked nodularity. For the most part, the adrenal cortical cells showed a clear zonal differentiation with an increase in the lipoid and double refractive material of the zona fasciculata and reticularis. However, in many areas, small islands of atrophic darkly stained cortical cells surrounded by thick fibrous tissue were seen. In general there were no differences in the appearance of the regenerated adrenal gland in this group of animals compared to those of the rats from Group II.

Kidney and Vascular Lesions - In this group of rats with delayed regeneration the renal and cardiovascular lesions were similar in nature to those described in Group II. However, there was a trend toward greater severity with a higher percentage of the animals showing involvement. The kidneys were enlarged, particularly so in four animals which had kidney weights much greater than the average. In these latter animals, the capsule stripped easily and the renal surface was smooth. On the cut surface one could recognize small, yellow, irregular and indistinct areas. One animal showed a marked hydronephrosis. On microscopic examination ten of the twenty-five animals had some arteriolar and glo-

merular lesions. Three of these were particularly severe, with arteriolar nephrosclerosis and generalized vascular necrosis. The glomeruli in these animals were enlarged and bloodless, with focal hyalinization of the tufts and occlusion of the lumina. Adhesions were present between the glomerular tufts and Bowman's capsule, and proliferation of the epithelium of the capsule formed crescents around the glomeruli. Only a few glomeruli remained intact. The walls of the afferent vessels and smaller vessels were thickened and focal homogenization of the walls, proliferation of subendothelial cells, and occlusion of the lumen were present. Fatty infiltration with double refractive material was seen in necrotized glomerular loops and in the walls of the vessels involved. The epithelium of the proximal convoluted tubules was swollen and granular, containing fine lipoid droplets. There was focal dilatation of nephrons which were lined by flattened darkly stained cells, and had protein-hyalin cylinders in their lumen. The interstitium of the kidney was edematous, and small scattered foci of lymphocytic infiltration were seen in the subcapsular areas of the cortex. The mucosa of the pelvis was well preserved with edematous swelling in the submucosa. These changes were qualitatively similar, but less severe in the remaining animals.

The hearts of two animals showed transverse yellowish streaks on gross examination, and microscopically areas of myocardial scar formation and necrosis of the muscle fibres. These myocardial changes were the result of obliterative endarteritis and necrosis of smaller branches of the coronary arteries. Vascular changes characterized by focal fibrinoid or hyaline change, swelling of the walls, proliferation of subintimal cells and perivascular inflammatory reaction were also found in the vessels of the mesentery (Fig. 17), brain and pancreas of these rats.

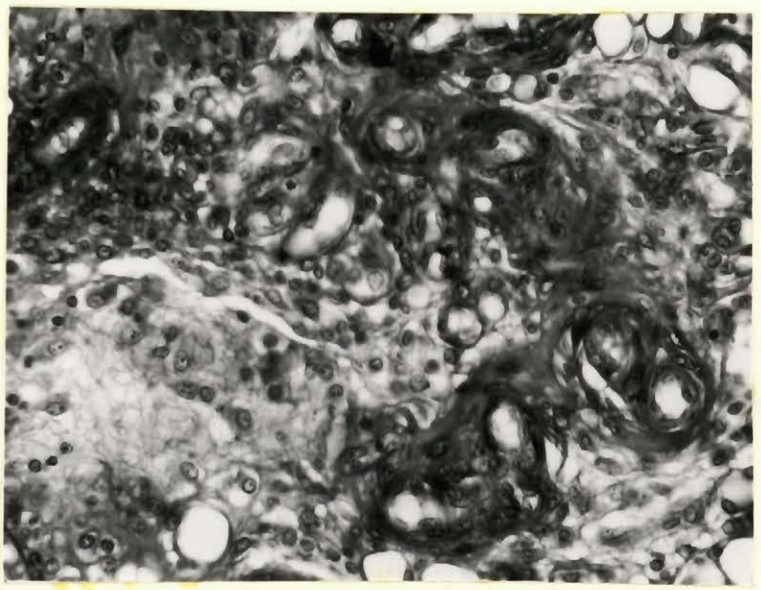

Fig. 17 - Endarteritis and fibrous thickening of the capallaries of the mesentery. Cason's Trichrome stain X 300.

DISCUSSION

The results from Group II (adrenal-regeneration hypertension) confirm the findings of Skelton (242, 243) that hypertension develops in unilaterally nephrectomized adrenal-enulceated rats on a high salt intake during the process of adrenal regeneration. In terms of the elevation of blood pressure there is a remarkable consistency between these data and those of Skelton (242), Chart et al (246), and Masson et al (247). The blood pressure reaches a plateau at hypertensive levels 4 to 5 weeks after enucleation of the adrenal gland, and remains on this plateau thereafter. It is interesting that this period coincides with that shown by Ingle and Higgins (218, 222) to be necessary for complete regeneration of the adrenal cortex from the capsule and adherent cortical cells remaining after enucleation. On a morphological basis our results differ somewhat from those of Skelton (243). The incidence of glomerular and arteriolar lesions in these experiments is less than those reported by this author, and no evidence of periarteritis nodosa has been found in the rats of either Group II (adrenal-regeneration hypertension) or VI (delayed adrenal-regeneration hypertension). Skelton has emphasized the greater sensitivity of younger rats to the development of hypertension

after adrenal enucleation (244). The body weight of the animals in this study was between 80 and 100 g at the beginning of the experiment, while those of Skelton weighed an average of 62 g. A second difference lay in the strain of rats used, whereas those of Skelton were Sprague Dawley, in this study the rats were Wistar strain. These differences may explain the lower frequency of pathological lesions in adrenal-regeneration hypertension in this experiment compared to the incidence described by Skelton (242, 243).

The final hypertensive level of approximately 180 mm Hg is somewhat less than the levels which are seen in hypertension produced by DCA, as described in the literature (162, 170, 173) or as shown in Group VI of Part II. This is partially due to variability within the hypertensive groups. Of the 15 animals in Group II only 9 developed a hypertension greater than 160 mm Hg, while the remainder ranged from this level down to normal. It was found on re-examination of the data of individual animals that there was a close correlation between the degree of hypertension and the severity of the cardiovascular lesions. Similarly animals which failed to become hypertensive in spite of regeneration of the adrenal did not have any cardiovascular

or renal lesions.

The treatment of Groups III (bilateral adrenalectomy), IV (adrenal hypertrophy), V (nephrectomy alone) and VI (delayed adrenal-regeneration hypertension) was similar in that all animals were unilaterally nephrectomized and given 1% sodium chloride in the drinking water. The responses of these groups to this procedure were also alike in that moderate increases in blood pressure were seen, but with failure to rise to hypertensive levels or to develop cardiac or renal hypertrophy and pathological lesions. Thus, adrenal insufficiency following bilateral adrenalectomy, adrenal hypertrophy which followed unilateral adrenalectomy, or adrenal enucleation per se failed to influence the blood pressure and the levels were comparable to those in Group V in which the adrenals were left intact. These results are in agreement with the findings of many workers who have demonstrated that the administration of excessive sodium chloride to the rat, with or without unilateral nephrectomy, is sufficient to cause elevation of the blood pressure (280, 281, 282). However, adrenal regeneration following the removal of the contralateral adrenal from the animals of Group VI, which had an enucleated adrenal on the opposite side, resulted in regeneration of the remnant and development of marked hypertension, renal and cardiac hypertrophy,

and vascular lesions. These results support the thesis that adrenal regeneration plays a primary role in the development of this hypertension.

This study has shown, as reported by Ingle and Higgins (222), that following adrenal enucleation the remnant, consisting of capsule and adherent cortical cells, survives over a period of 5 weeks and is capable of regeneration when the contralateral adrenal is removed. The histological picture of these adrenals does not differ significantly from the experiments in which the adrenal enucleation and contralateral adrenalectomy were performed simultaneously (Group II). Furthermore, this study shows that such delayed regeneration in unilaterally nephrectomized animals given saline to drink can provide the stimulus for the development of hypertension.

The histological observations of the regenerated adrenal cortices of animals with hypertension are perhaps worthy of comment. Skelton (242) theorized that adrenal-regeneration hypertension might be due to excessive mineralocorticoid secretion by the regenerating adrenal. It is known that the glomerulosa zone of the adrenal undergoes morphological changes in physiological states that involve

disturbance of electrolyte balance of the body. Decrease of the sodium potassium ratio which creates a demand for mineralocorticoid causes hypertrophy of this zone while increasing this ratio results in an atrophy of the glomerulosa zone (293). Furthermore Giroud (231) has shown that the glomerulosa zone is the site of aldosterone synthesis in the rat. If as stated above excessive mineralocorticoid secretion from the regenerating adrenal cortex is responsible for adrenal-regeneration hypertension then some evidence of hyperactivity of the glomerulosa zone might be expected. However, this was definitely not the case. The glomerulosa zone in the adrenals of hypertensive animals was invariably thin and composed of small atrophic cells with darkly staining nuclei and little lipid material closely resembling that described by Deane et al (283) in rats receiving desoxycorticosterone and a potassium deficient diet. The atrophic glomerulosa zone in these experiments might be explained in terms of the high intake of sodium chloride, resulting in a decreased demand for mineralocorticoids from this zone. Histological examination is admittedly not a reliable guide to the functional state of the various zones of the adrenal cortex, however, the presence of atrophy in the glomerulosa would certainly be evi-

dence against a theory of excessive activity in this zone.

These considerations led to the experiments in the next section. An attempt was made to influence the function of regenerating adrenal cortical tissue after enucleation, and determine whether alterations in function would be reflected in changes in blood pressure.

PART II

EFFECT OF AMPHENONE ON ADRENAL-REGENERATION AND DOCA HYPERTENSION

In Part I earlier findings of Skelton (242) have been confirmed, and additional experiments presented which add to the evidence pointing to a primary role of the adrenal cortex in adrenal-regeneration hypertension. During recent years it has been shown that amphenone (1,2-bis (p-aminophenyl) 2 methyl-propanone-1 dihydrochloride) has adrenal cortical depressant properties in both man and experimental animals. These findings are briefly reviewed below. The experiments reported in this section were initiated with the idea that such a compound, in allowing a degree of control over steroidogenesis by the adrenal cortex, might provide additional evidence of the role of this gland in adrenal-regeneration hypertension. If in this syndrome steroids from the regenerating adrenal cortex are responsible for the hypertension, then depression of steroidogenesis might be reflected in changes in the blood pressure of the animals.

Amphenone was first synthesized by Allen and Corwin in 1950 (284); it was studied as one of a series of anti-

estrogens by Hertz (285). Further studies revealed that amphenone had weak progestational properties (286) and an atypical folliculoid action (285). It does not support deciduoma formation in the rat, however, in this species it was observed to cause adrenomegaly (285) and thyromegaly (285, 288).

The action of amphenone on the thyroid gland is believed to be a direct one. The enlargement of this gland in the rat produced by 32 mg/day of amphenone is inhibited by treatment of the animals with thyroxine (287). Rats treated with amphenone also have a reduced thyroid uptake of I_{131} (288) and reduced oxygen consumption (290). Treatment of isolated rat thyroid glands in vitro also causes a reduction in the uptake of I_{131} (289).

The depressant effect of amphenone on thyroid function has been demonstrated in man (291), and it has been shown that this compound acts like a thiourea type goitrogen, blocking organic "binding" of radioactive iodine (292).

The effects of amphenone on the adrenal gland have been studied extensively. Hertz (285, 287), Coste F. (293), Hogness (288) and Vogt (294) have shown that this compound causes marked enlargement of the adrenal gland in the rat.

This increase in size is associated with a change from the normal reddish brown to a yellow colour with histologically an increase in the intracellular lipid of the fasciculata and reticularis zones. Both the width of these zones and the volume of their component cells were increased, while neither the width of the glomerulosa zone nor the cell volume or lipid content were affected by amphenone. Analysis of the adrenals from rats treated with amphenone revealed a threefold increase in cholesterol content (285), and a decrease in ascorbic acid (285, 295). These effects of amphenone on the adrenal in the rat are apparently mediated by the pituitary gland. Adrenal enlargement does not occur after hypophysectomy (285) or the administration of cortisone (285, 287), and exogenous adrenocorticotropic hormone (ACTH) will restore the enlargement of the adrenal, induced by amphenone therapy, which is lost after removal of the pituitary (285).

The effect of amphenone on adrenal cortical function has been studied in both experimental animals and man. In the perfused calf adrenal amphenone inhibited the biosynthesis of hydrocortisone, cortisone and corticosterone (296). Similarly hypophysectomized dogs treated with amphenone show a reduced response in terms of plasma 17 hydroxy cor-

ticoids,to stimulation by a standard dose of ACTH when compared to controls (285, 298).

In man, amphenone likewise exerts rather striking effects on adrenal function. In subjects with normal adrenal function amphenone produces a definite suppression of blood and urinary levels of 17-hydroxy corticoids (297,299) as well as 17-ketosteroids (297, 300) and aldosterone (301). Furthermore, amphenone inhibits adrenal steroidogenesis in patients with hyperadrenocorticism (297) and functioning adrenal carcinoma (297, 302, 303).

In contrast to these findings is the demonstration by Vogt (294) of an increased secretion of corticosterone after oral, but not intravenous, administration of amphenone to the rat. It is known from the studies of Pittman and Westfall (304) that, after oral administration of amphenone to man, the compound rapidly disappears from the plasma, also after cessation of amphenone therapy in man sharp rebound phenomena will occur (297, 301). In Vogts' study the interval between the last treatment with amphenone and the study of adrenal effluent was not stated. However, if the last treatment was on the day prior to the measurement of adrenal secretion of corticosterone then it is quite possible that her animals were in the phase of rebound hyper-

secretion.

In this series of experiments amphenone has been administered to rats at varying intervals after operation which produced adrenal-regeneration hypertension. The effect of amphenone in this form of hypertension was compared to that in hypertension induced in adrenalectomized rats given DCA. The changes in blood pressure are compared with those of a normal control group treated with amphenone, and a group without treatment having adrenal-regeneration hypertension.

EXPERIMENTAL DESIGN

Group I - Adrenal-regeneration hypertension (15 animals) These animals were unilaterally nephrectomized and adrenalectomized on the right side, and the left adrenal gland was enucleated. The drinking water was replaced with 1% saline at the time of operation.

Group II - Adrenal-regeneration hypertension treated with Amphenone at 5 weeks (8 animals). The operative procedure was the same as that described for Group I.

Group III - Adrenal-regeneration hypertension treated with Amphenone at 3 weeks (7 animals).

The operative procedure was the same as that described for Group I.

Group IV - Adrenal-regeneration hypertension, treated with amphenone at two weeks (9 animals). The operative procedure was the same as that described for Group I.

Group V - Normal intact (15 animals). These rats were given tap water to drink and were treated with Amphenone during the entire experimental period.

Group VI - DCA hypertension (12 animals). These rats were unilaterally nephrectomized on the right side, bilaterally adrenalectomized, and were given 1% saline to drink. They were injected subcutaneously with 1 mg of DCA in propylene glycol each day during the entire experimental period. Amphenone therapy was started 5 weeks after operation.

The animals receiving amphenone therapy were treated each day at a dose level of 200 mg/kg by gavage. This dose was recommended by Hertz (285). The material was dissolved in distilled water and given in a volume of 1 cc by stomach tube each morning.

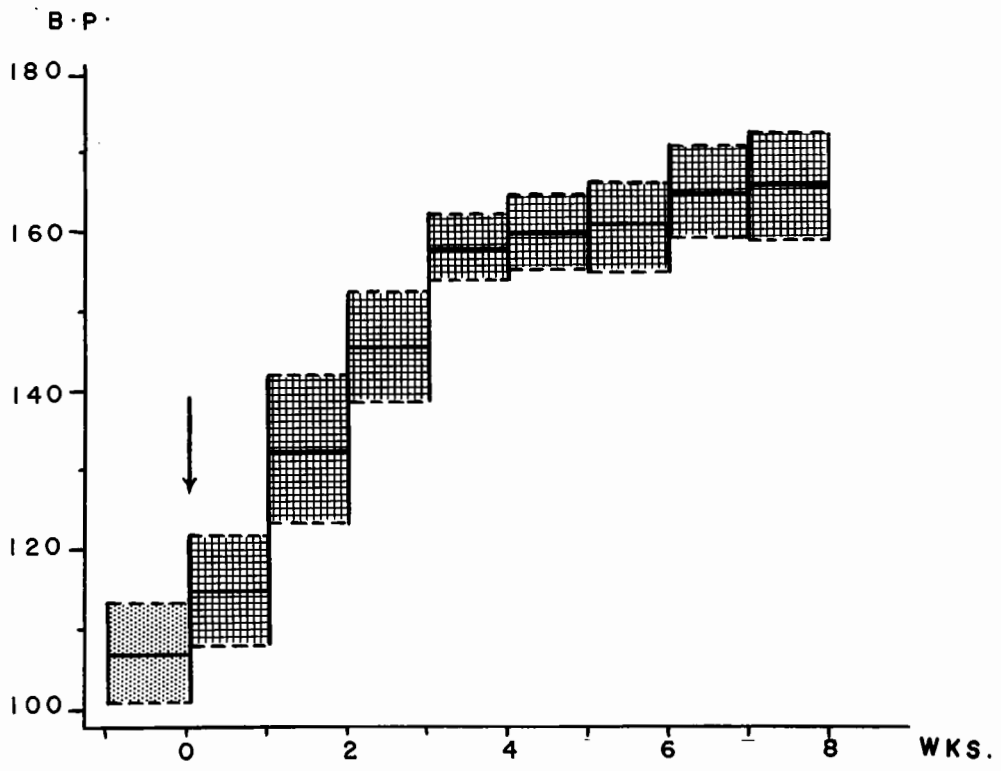

Fig. 18 - Mean weekly blood pressure of rats with adrenal-regeneration hypertension without treatment (Group I).

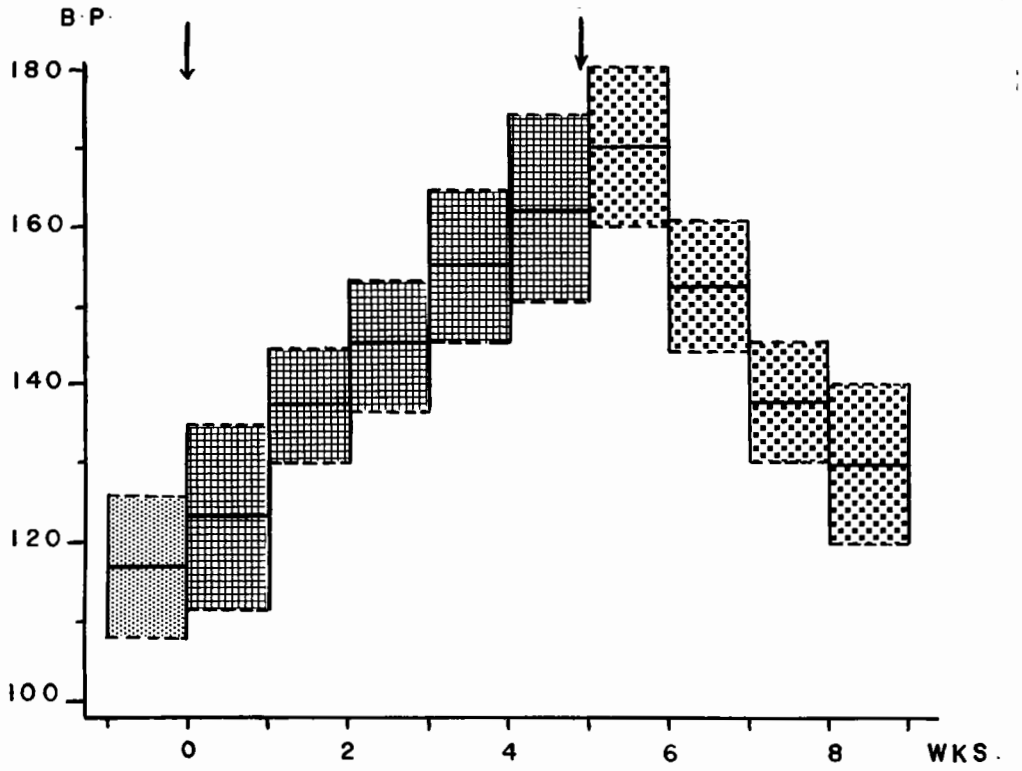

Fig. 19 - Mean weekly blood pressure, rats operated at left arrow to produce adrenal-regeneration hypertension, amphenone therapy started at right arrow, Group II.

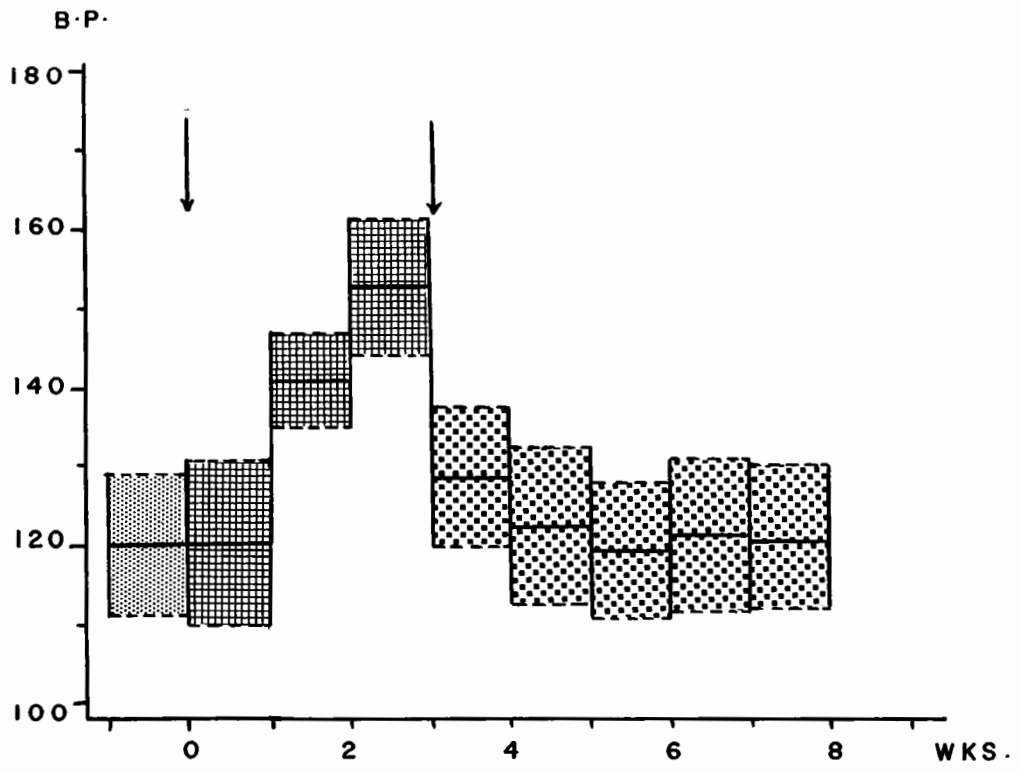

Fig. 20 - Mean weekly blood pressure of rats operated at left arrow to produce adrenal-regeneration hypertension, amphenone therapy started at right arrow, Group III.

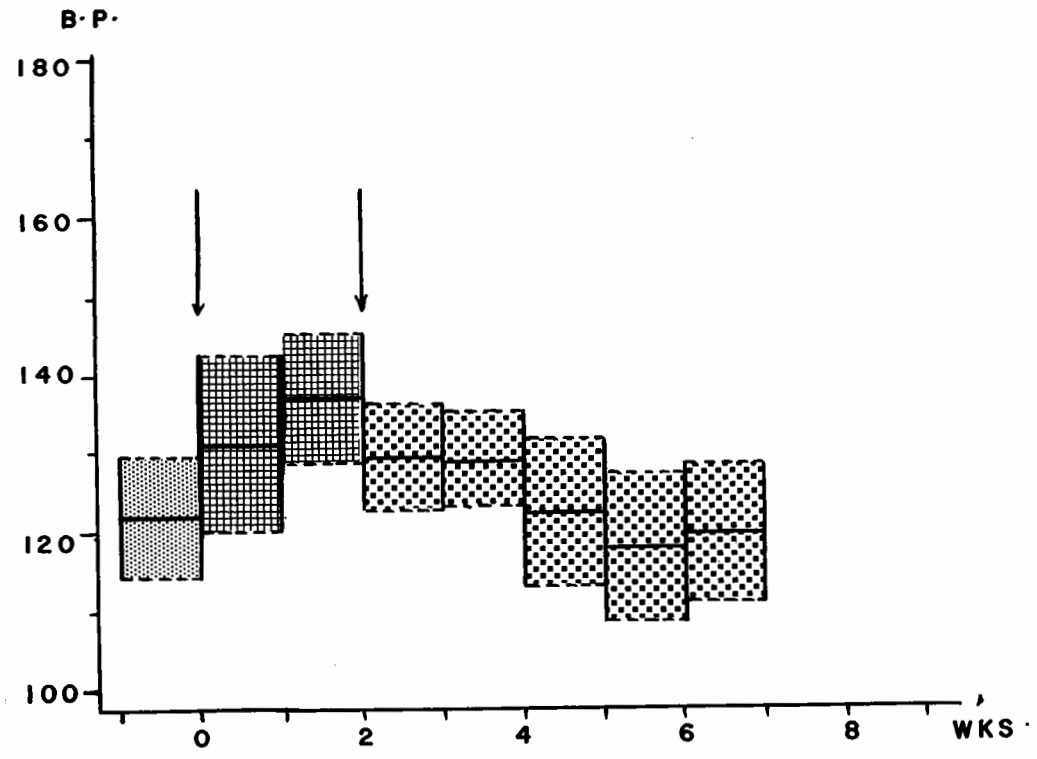

Fig. 21 - Mean weekly blood pressure, rats operated at left arrow to produce adrenal-regeneration hypertension, amphenone therapy started at right arrow, Group IV.

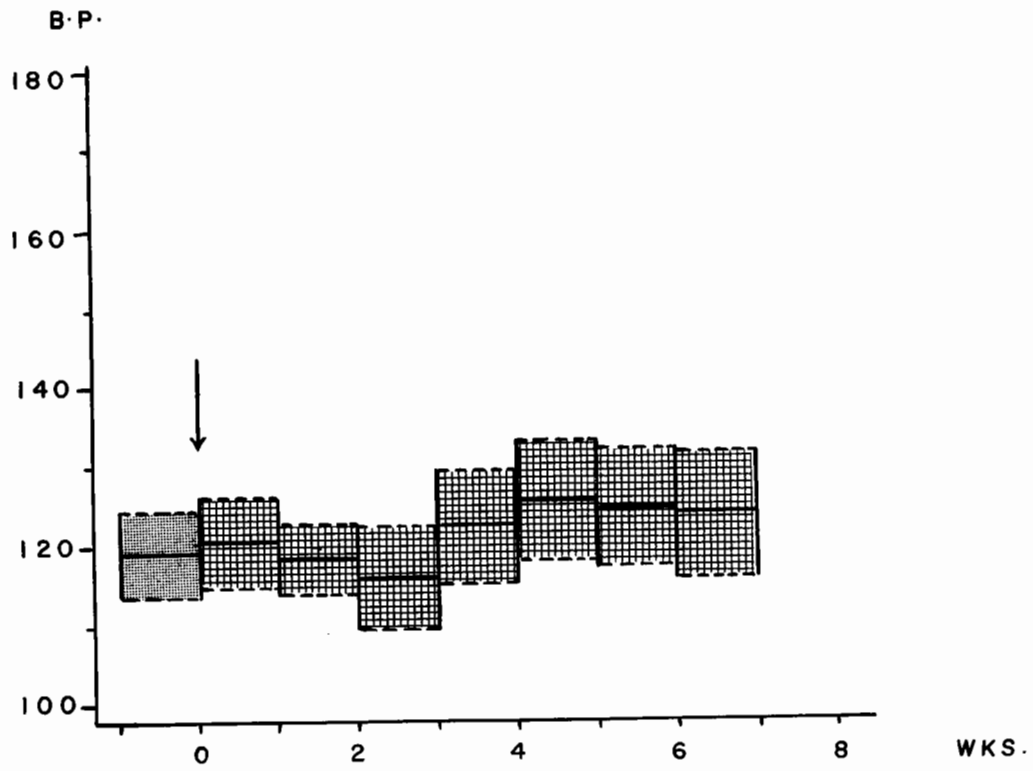

Fig. 22 - Mean weekly blood pressure, normal intact rats (Group V) treated, starting at the arrow, with amphenone.

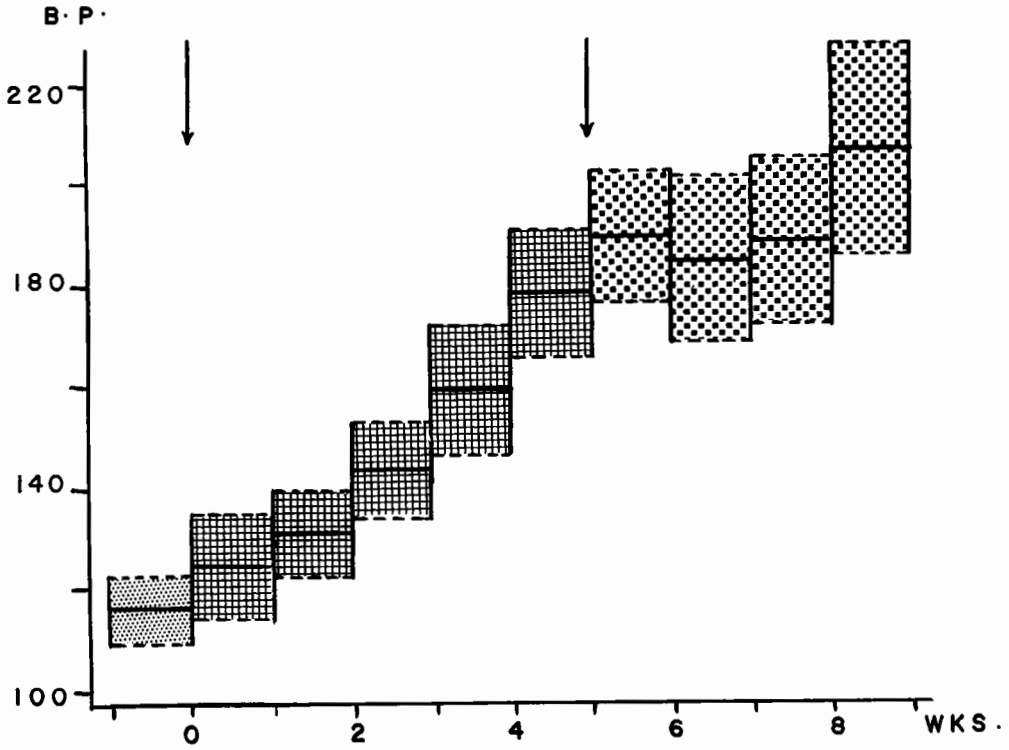

Fig. 23 - Mean weekly blood pressure, bilateral adrenalectomy and DCA administration at left arrow, amphenone therapy started at right arrow, Group VI.

RESULTS

1. Blood Pressure

The hypertensive response of the rats of Group I after adrenal enucleation, which is shown in Figure 18, is almost identical to that seen in Group II of Part I treated in the same manner. Figure 19 shows the effect of amphenone on the mean blood pressure of a group of rats with adrenal-regeneration hypertension. Treatment of these rats (Group II) was begun 5 weeks after adrenal enucleation when the mean blood pressure was 163 mm of mercury. During the succeeding four weeks there was a progressive fall in blood pressure to normotensive levels. When treatment with amphenone was instituted three weeks after adrenal enucleation (Group III, Fig. 20) when the mean blood pressure was 144 mm of mercury, or two weeks after adrenal enucleation (Group IV, Fig. 21) when the mean blood pressure was 137 mm of mercury, the return of the blood pressure to normal was more prompt and with continuation of therapy the blood pressures remained in the normal range.

Treatment of normal rats with amphenone (Group V) during a seven week period did not result in any change in mean blood pressure (Fig. 22). Although there might appear

to be a depressant effect (compare Fig. 3 and Fig. 22) when the mean blood pressure of this group and normal untreated controls are compared, these differences are not significant.

Figure 23 demonstrates the typical response of bilaterally adrenalectomized rats receiving saline to drink and given DCA. The hypertension which developed was more severe in terms of augmented blood pressure than adrenal-regeneration hypertension (Fig. 18). Amphenone therapy over a four week period had no effect on DCA hypertension.

2. Body and Tissue Weights

The average body and tissue weights are contained in Table V. The body weights of the animals at autopsy are difficult to compare since all the studies were not of the same duration. The animals treated with amphenone showed a depression of growth which was reflected in the lower weights of these rats at autopsy. This property of amphenone was also described by Hertz (285). Because of these differences in body weight the weights of the tissues in Table V are expressed/unit of body weight. Normal rats treated with amphenone show the enlargement of the thyroid, adrenal glands, and the liver, which has been reported by Hertz (285). The thyromegaly and hepatomegaly are seen in all groups treated with amphenone. It will be noted that

TABLE V. BODY AND TISSUE WEIGHTS

	Group	Thymus[1]	Thyroid	Adrenal	Pituitary	Heart	Liver	Kidney	Body Wgt.
I	Adrenal-Enucleated Untreated	148±11[2]	7 ± 1	12 ± 1	3 ± 1	425±15*	4.59±0.3	605±34*	233± 10
II	Adrenal-Enucleated Amphenone at 5 weeks	64± 8	16 ± 2*	12 ± 1	5 ± 1	313±23	7.10±0.8*	451±49	168± 15
III	Adrenal-Enucleated Amphenone at 3 weeks	97±17	17 ± 2*	10 ± 2	5 ± 1	312±13	13.4±0.4*	510±29	172± 12
IV	Adrenal-Enucleated Amphenone at 2 weeks	84± 11	15 ± 1*	12 ± 2	5 ± 1	326±27	7.70±0.2*	478±40	174± 6
V	Normal Amphenone treated	78± 4*	24 ± 1*	23 ± 1*	5 ± 1	306±22	7.95±0.6*	340±18	215± 9
VI	Adrenalectomized DCA and Amphenone	81± 12	19 ± 3*	-	4 ± 1	437±40*	8.22±0.1*	590±60*	233± 14

1. Tissue weights are expressed in mg., or in the case of the liver as g., per 100 g. of body weight.
2. Mean value and standard error.

* Significant at the 95% level of conficence compared to normal controls.

the weight of regenerated adrenals in the groups treated with amphenone, 12, 10, and 12 mg/100 g are not significantly different from the weight (12 mg/100 g) of this gland in the rats with a regenerated adrenal but without treatment. Thus, although amphenone exerted an effect on blood pressure in these animals presumably due to an influence on adrenal function, it did not interfere with adrenal regeneration in a morphological sense. The changes in heart size noted at autopsy are in agreement with the blood pressure findings. The untreated group with adrenal-regeneration hypertension, and the adrenalectomized animals given DCA and amphenone show the cardiac hypertrophy which was anticipated on the basis of their unrelieved hypertension. However, the heart size of adrenal enucleated groups treated with amphenone was within the range of normal control animals (Part I).

While no statement can be made about the size of the heart in these animals at the time of instituting therapy, it would be expected from the blood pressure of Group II (163 mm of mercury) that cardiac hypertrophy was present and was reversed by the amphenone therapy.

After unilateral nephrectomy and adrenal enucleation hypertrophy of the remaining kidney ensues, until at eight

weeks, the kidney under these circumstances is almost equal to the combined weight of the kidneys from normal controls. Amphenone has little effect on the renal weight of normal animals but appears to depress the renal hypertrophy which occurs after adrenal enucleation and unilateral nephrectomy. This renal hypertrophy persists in animals given DCA following adrenalectomy and then treated with amphenone. The failure of amphenone to influence the renal weight of normal animals or prevent the renal hypertrophy in the DCA group would suggest that the absence of compensatory hypertrophy in the adrenal regeneration groups cannot be attributed to a direct action of amphenone. It is felt that the lower renal weights like the lower cardiac weights are a reflection of the diminished blood pressure in the adrenal regeneration groups treated with amphenone. Similarly in Group II on the basis of the blood pressure at the start of amphenone therapy, it would be expected that renal hypertrophy was present, and that this enlargement of the kidney was reversed by amphenone therapy.

A thymolytic activity of amphenone was reported by Hertz (285). The data in these experiments in general confirm this finding as the thymic weights of groups treated

with amphenone are lower than those of control animals. However, in only two groups is the difference statistically significant because of wide variation between individual animals.

3. Gross and Microscopic Pathological Changes

Group I (adrenal-regeneration hypertension). The histopathological lesions seen in this group of animals did not differ appreciably from those described for the animals with adrenal-regeneration hypertension in Part I.

Group II (adrenal-regeneration hypertension treated with amphenone at 5 weeks). Group III (adrenal-regeneration hypertension treated with amphenone at 3 weeks) and Group IV (adrenal-regeneration hypertension treated with amphenone at 2 weeks).

These animals were treated with amphenone for either 4 or 5 weeks depending on the length of the experiment. No differences were noted in the histological appearance of tissues from these animals, for this reason they are discussed as one group.

Adrenals - Amphenone treatment as shown by adrenal weights and histology did not inhibit regeneration of the enucleated adrenal. However, there were definite modifications of the histological architecture of cortical cells. In contrast

to the animals of Group I (adrenal-regeneration hypertension) there was no distinct border between the different cortical zones. The regenerated cortex was uniformly composed of clear vacuolated cells, the cytoplasm of which as shown by Sudan III staining was densely filled with lipid material. The connective tissue proliferation and calcification in the middle of the regenerated adrenal was not altered by amphenone treatment. The comparison between regenerated adrenal cortex with and without amphenone treatment is shown in Figures 24 and 25.

Pituitary

The predominant change in the pituitary was enlargement and vacuolization of basophilic cells. This vacuolization of the basophils, which made them resemble thyroidectomy cells, occurred in fifty percent of the cells of the anterior pituitary in some animals, and in only a few scattered cells in others.

Thyroid

The enlarged thyroids were composed of dilated acini with many papillary infoldings which were almost devoid of colloid. Occasionally the epithelium which had undergone hyperplasia occluded the lumina of the acini. The epithelium was composed of tall columnar cells with nuclei arranged close to the basement membrane.

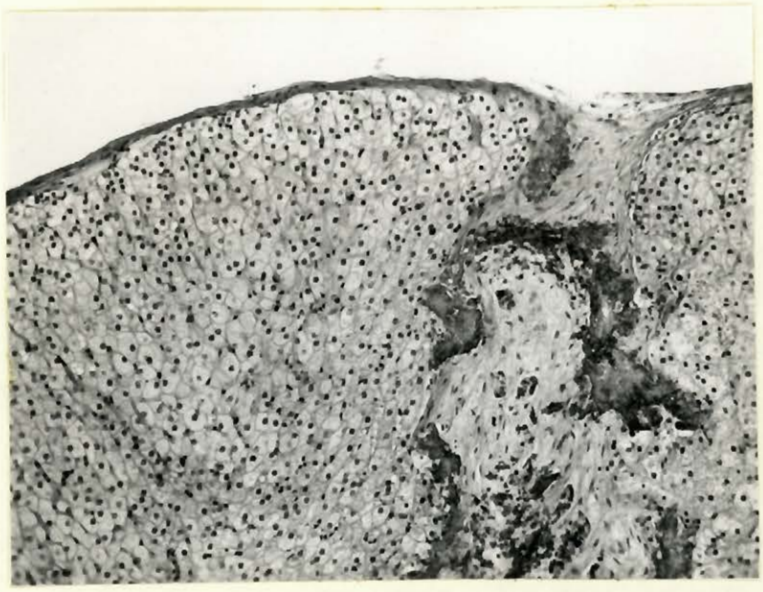

Fig. 24 - Regenerated adrenal cortical tissue 8 weeks after adrenal enucleation. Amphenone was administered during the last 5 weeks of this period. Marked nodularity is present. The pale appearance can be attributed to the enlargement of cortical cells and dissolution of lipids. When compared with Figure 25 the absence of zonal differentiation may be seen. Haematoxylin and eosin X 120.

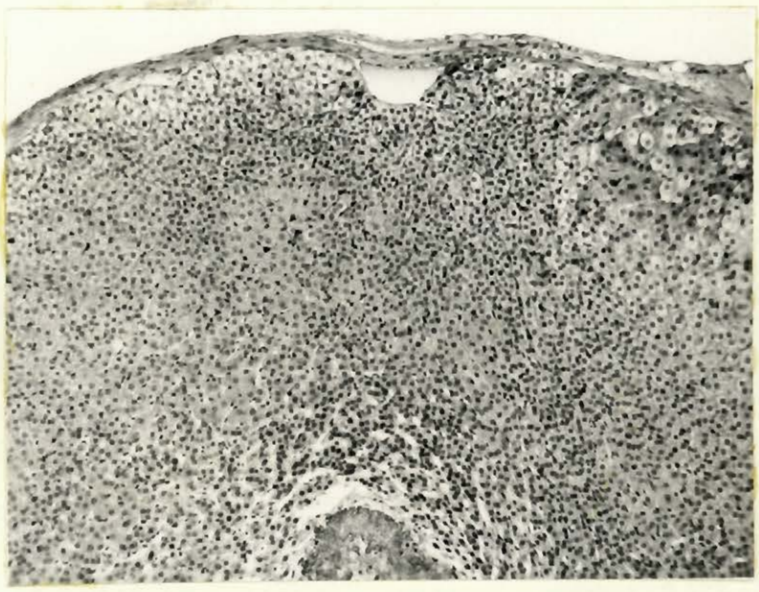

Fig. 25 - Regenerated adrenal cortical tissue 8 weeks after adrenal enucleation. Differentiation of the cortical zones can be distinguished. Haematoxylin and eosin X 120.

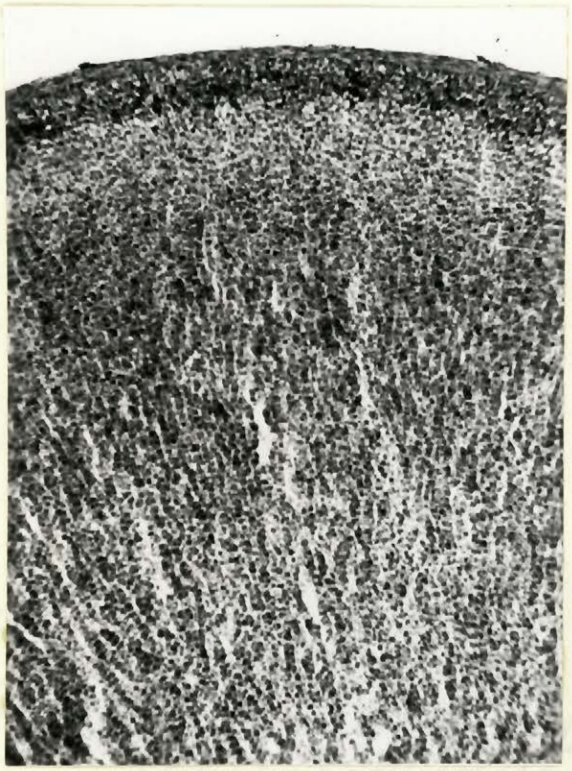

Fig. 26 - Normal adrenal cortex. Sudan III and Haematoxylin X 125.

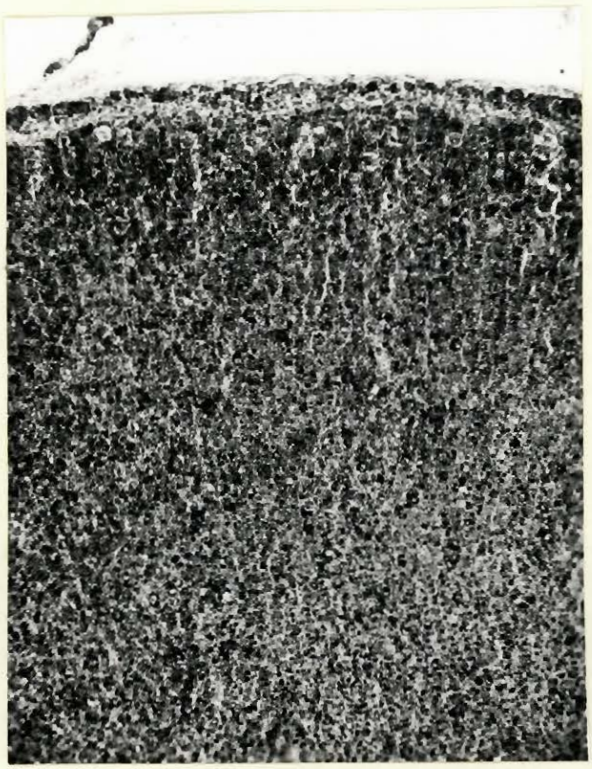

Fig. 27 - Adrenal cortex from intact animal treated for 7 weeks with amphenone. Heavy lipid accumulation may be seen in all zones with a lipid free layer of cells between the glomerulosa and fasciculata zones. Sudan III and Haematoxylin X 125.

Liver

The liver of these animals showed lesions similar to those described below in normal animals treated with amphenone.

Other Organs

No cardiovascular or renal lesions were found in any of those animals which were treated with amphenone. Careful examination of the kidneys failed to reveal scars of healed glomerular lesions.

Group V.(Intact Rats Treated with Amphenone)

Treatment of normal animals with amphenone caused histopathological changes in the thyroid and pituitary similar to those described above in animals with adrenal-regeneration hypertension treated with amphenone.

Adrenal

The adrenal cortex was markedly widened, an increase in the width of the fasciculata and to a lesser extent of the reticularis zone, being responsible. The cells of all zones were filled with lipid, with the exception of a narrow lipid free zone between the glomerulosa and the fasciculata. Lipid accumulation in cortical cells was generally even and the nuclei were central, however, in the outer half of the fasciculata occasional cells showed a picture of fatty metaplasia

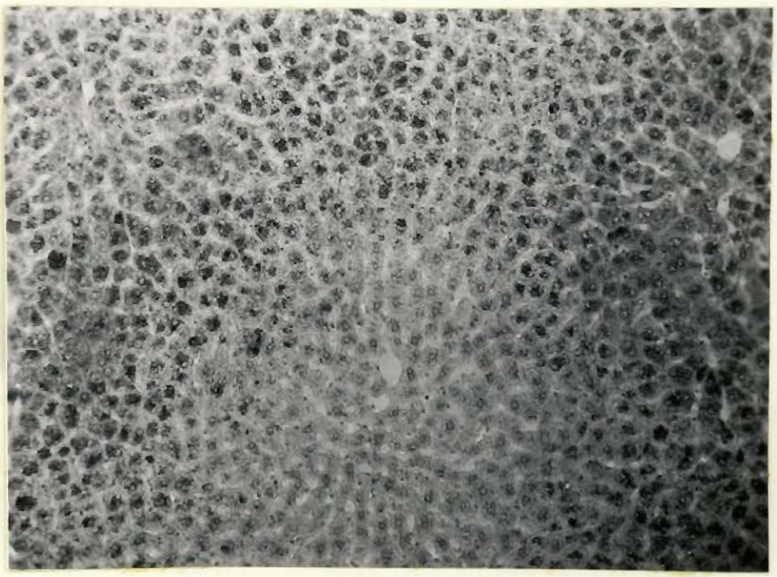

Fig. 28 - Liver of intact rat treated with amphenone for 7 weeks. The lipid infiltration appears as darkly stained material. Sudan III and Haematoxylin X 125.

with the nucleus pushed to the side. These changes may be observed in a comparison of Figures 26 and 27.

Liver

The architecture of this organ was well maintained, but with dilatation of the central veins and centrolobular congestion. In the periphery of the liver lobules the liver cells showed fatty infiltration (Fig. 28). The nuclear staining was well preserved, and many cells contained mitotic figures or two nuclei. The other tissues in these animals were normal.

Group VI. (DCA Hypertension treated with Amphenone)

The treatment of DCA hypertensive rats with amphenone led to changes in the liver and thyroid gland, similar to those seen in the other groups treated with this drug. The cardiovascular-renal lesions in this group were severe and did not appear to have been altered by treatment with amphenone.

DISCUSSION

It is clear from the results of the three groups of animals with adrenal-reganeration hypertension which were treated with amphenone, that this agent exerted a marked effect on the blood pressure in this form of hypertension. Amphenone was capable under these circumstances of reversing an established hypertension, or when therapy was begun soon after adrenal enucleation, it prevented the development of hypertension. The same dose of amphenone had no effect on the blood pressure of normal animals nor did it effect the blood pressure in DCA hypertension. Thus amphenone was unlike antihypertensive agents such as Rauwolfia, Veratrum derivatives, or ganglionic blocking agents in its mechanism of action, since these latter therapeutic agents have been shown to exert a depressor effect in DCA hypertension in the rat (171). The possibility must be recognized that the depressor effect of amphenone could be related to an increased excretion of sodium at the kidney or a decrease in consumption of saline, but again, since both adrenal-regeneration and DCA hypertension in the rat, as mentioned earlier, are dependent on excessive intake of sodium the difference between the effects of amphenone in these two forms of hypertension would be difficult to ex-

plain on this basis. It is evident particularly in Group IV (adrenal-regeneration hypertension treated with amphenone at 2 weeks) that amphenone treatment did not interfere with regeneration of the adrenal gland on the basis of size. The weight of the adrenal in these animals was equal to that of the controls. However, these glands had undergone a fatty metaplasia similar to that seen in the adrenal glands of normal animals treated with this drug. It is logical therefore to infer that the same depressant effects on adrenal function are exerted in regenerating adrenal glands as in the normal. It is believed that these depressant effects of amphenone on adrenal cortical function are primarily responsible for the effect of the drug on adrenal-regeneration hypertension. We do not know whether adrenal-regeneration hypertension is related to increased, abnormal, normal or decreased adrenal cortical secretion. However, these results do suggest that whatever the state of the function of the adrenal cortex depression of this gland is not compatable with the maintenance of the hypertensive state.

The absence of renal and cardiac hypertrophy or cardiovascular and renal lesions in the rats with enucleated adrenals treated with amphenone was striking. Since no renal scars were seen in any of these rats, it must be assumed that the time interval prior to amphenone did not allow these

lesions to develop and therefore reversal of such lesions did not occur. On the other hand, since none of these animals had such lesions it is evident that treatment with amphenone prevented their development. Masson et al (197) in recent studies have demonstrated the close association between blood pressure levels and the presence and character of cardiovascular and renal lesions in renal hypertension. Thus it is probable that the absence of these lesions in the amphenone treated enucleated rats is simply a reflection of the effects of this drug on blood pressure. It is well known that there is a close correlation between heart weight and blood pressure in experimental hypertension in the rat. It is considered likely that the lower heart weight in the rats treated with amphenone is also secondary to the effects of this drug on the blood pressure.

Amphenone as mentioned earlier has antithyroid activity and an atypical estrogenic activity. It was possible that the effects of this drug on adrenal regeneration hypertension were related to these properties. The experiments in the next section were undertaken to investigate these possibilities.

PART III

EFFECT OF PROPYLTHIOURACIL AND ESTROGEN ON ADRENAL-REGENERATION HYPERTENSION

It has been shown in the experiments of the previous section that amphenone would either prevent or reverse the hypertension which occurs with adrenal regeneration in sensitized rats. This compound is known to have effects on endocrine organs other than the adrenal, and it appeared important to determine what role, if any, these actions might play in aleviating the hypertension. Amphenone is known particularly to have antithyroid properties (285, 288, 289) and also weak atypical estrogenic activity (285).

It is well established that important functional interrelationships exist between the thyroid gland and adrenal cortex. In animals rendered hyperthyroid the adrenal cortex enlarges, and it shrinks in size in animals made hypothyroid by thyroidectomy or antithyroid drugs. Studies in this field have been summarized by Deane and Greep (306). Recently Masson et al showed that treatment of adrenal-regeneration hypertensive rats with thyroxine resulted in higher blood pressures and increased the severity of the vascular lesions (307). Similarly Skelton et al (308) have demonstrated a preventive effect of thyroparathyroidectomy

on adrenal-regeneration hypertension. Thus both hyper and hypothyroidism have been shown to influence adrenal-regeneration hypertension and theoretically the protective action of amphenone could be attributed to its depressant effect on the thyroid gland.

The possible role of the estrogenic effect of amphenone in adrenal-regeneration hypertension is less clear, however it is well known that estrogens influence the adrenal cortex. Estradiol causes a variable hypertrophy of the adrenal gland (309) and a rapid loss of lipids from the cortex (310). Vogt (311) has demonstrated that hexoestrol decreases the steroidogenesis of corticosterone the principal glucocorticoid in the rat. Thus, in theory at least, the estrogenic activity of amphenone could by depressing the adrenal gland play some part in the action of this compound on blood pressure.

The following experiments which were designed to throw some light on these possibilities were run concurrently with those on amphenone. However, for sake of clarity they are presented separately.

EXPERIMENTAL DESIGN

Group I - Unilateral nephrectomy (10 animals). The right kidney was removed and 1% saline was

substituted for the drinking water.

Group II - Adrenal-regeneration hypertension treated with propylthiouracil (10 animals). The right kidney and right adrenal gland were removed and the left adrenal was enucleated. The drinking water was replaced with 1% saline. Five weeks after this operation treatment was started with propylthiouracil (PTU) 40 mg/kg orally each day in aqueous solution.

Group III - Adrenal-regeneration hypertension treated with estrogen (10 animals). The operative procedure in this group was the same as that in Group 2. One week after operation treatment with estrogen was begun. Premarin (R) a mixture of water-soluble estrogen conjugates was given orally. The daily dose was equivalent in estrogenic potency to 1 mg/kg of sodium estrone sulfate.

RESULTS

1. Blood Pressure

The animals of group I which were unilaterally nephrectomized and given 1% saline to drink failed to develop hypertension. As shown in Figure 29 only a moderate rise in blood pressure was seen after this treatment.

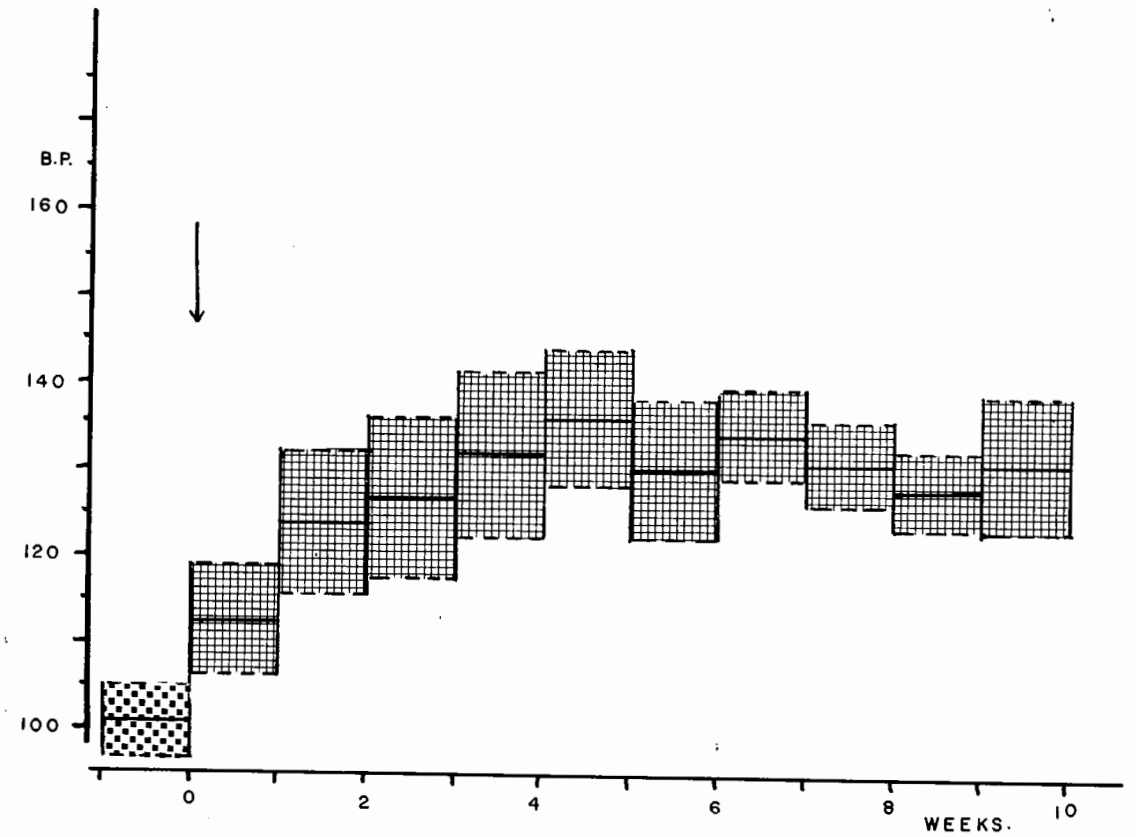

Fig. 29 - Moderate rise in blood pressure after unilateral nephrectomy and saline administration. Arrow indicates the time of operation.

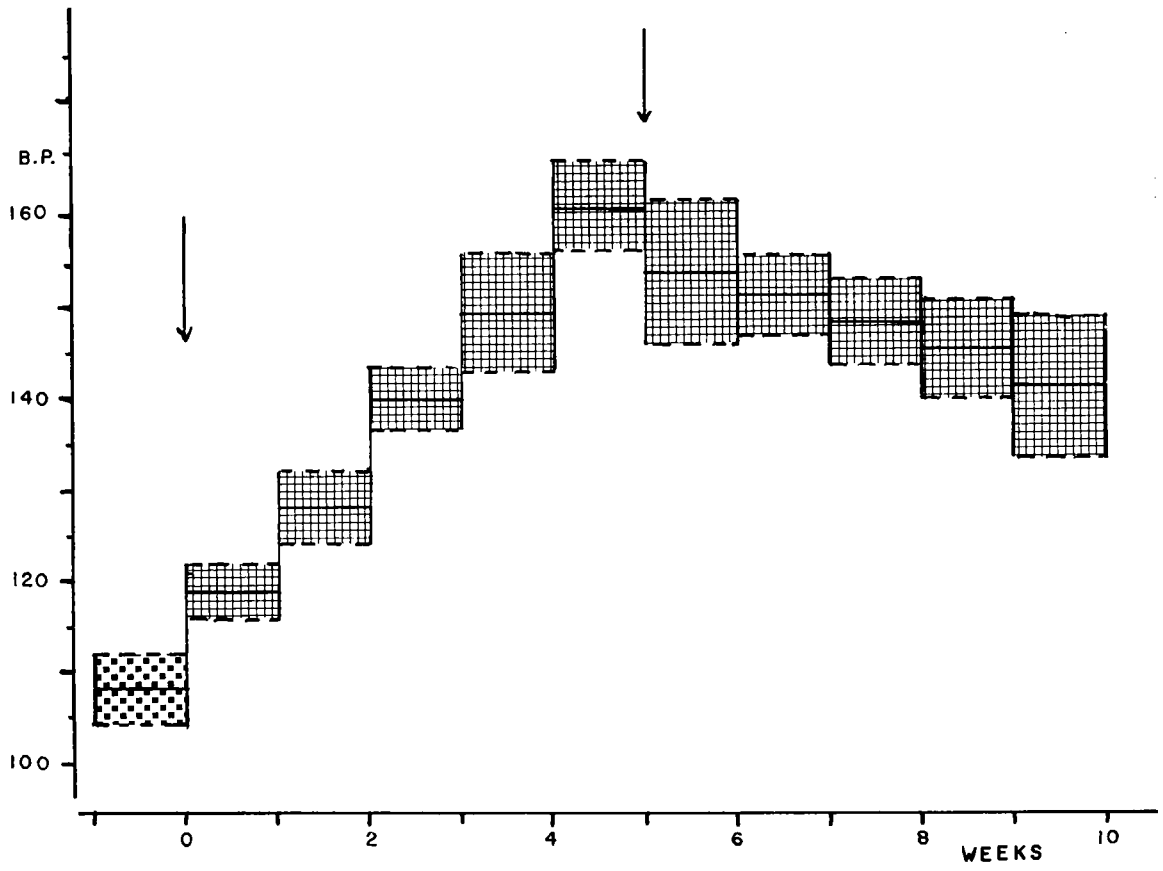

Fig. 30 - Depressant effect of PTU treatment on the blood pressure of adrenal-regeneration hypertensive rats. Animals were prepared at left arrow and PTU treatment was started as indicated by the arrow on the right.

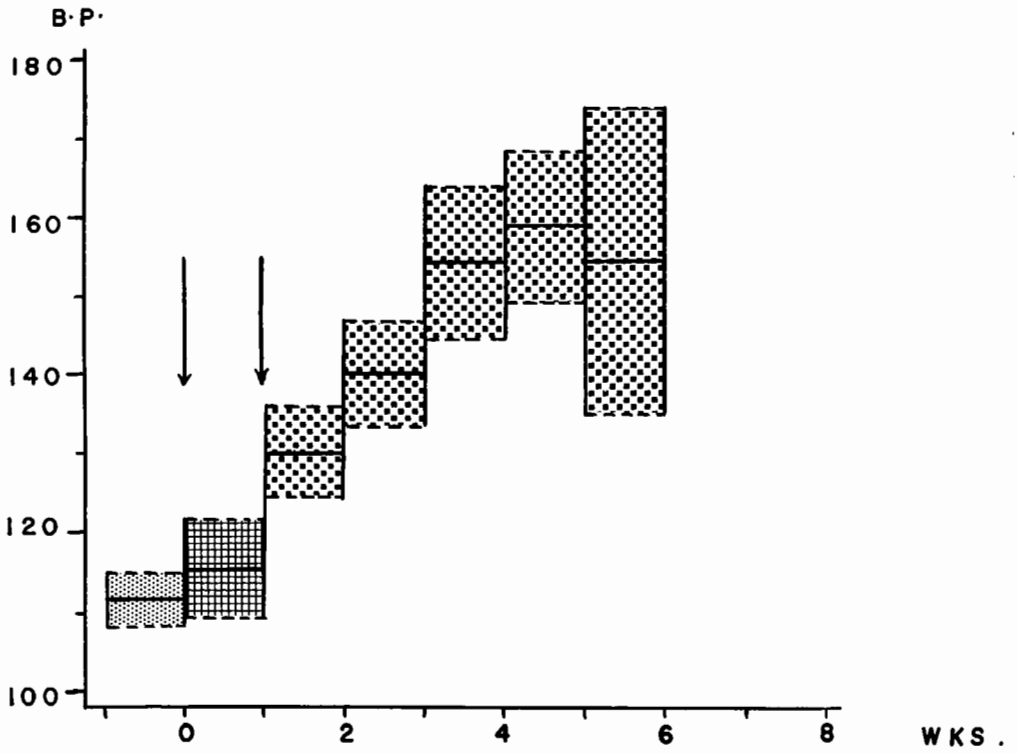

Fig. 31 - Failure of daily treatment with estrogen to influence the development of adrenal-regeneration hypertension. The rats were operated at the left arrow and Premarin therapy was started at the arrow on the right.

The systolic blood pressures of the animals of Group II rose in the usual manner to hypertensive levels during the 5 week post-operative period. When the mean blood pressure was 160 mm Hg treatment with PTU was started. During the succeeding 5 weeks of PTU therapy (Fig. 30), the blood pressure progressively declined to a level comparable to that of the Group I controls.

As shown in Figure 31 estrogen therapy commenced soon after operation failed to influence the development of the ensuing hypertension.

2. Body and Tissue Weights

The body and tissue weights of the animals from the three groups are contained in Table VI. The animals of the group treated with PTU, and the estrogen treated group, failed to gain weight in a normal manner. This is a well known effect of either estrogen therapy or PTU therapy when administered in high doses. The weights of the heart, kidney, spleen and thymus of the PTU treated group were even lower than those of the nephrectomized controls. Similarly the weights of the thymus, thyroid, pituitary and liver of the estrogen treated rats were significantly decreased. However these differences were not significant when the respective values were related to body weight. When compared on this basis

TABLE VI BODY AND TISSUE WEIGHTS

	Group I Unilateral Nephrectomy	Group II Adrenal-Regeneration Hypertension + P T U	Group III Adrenal-Regeneration Hypertension+Estrogen
Thymus (mg)	392 ± 38	218 ± 15	222.5 ± 16
Thyroid	23.7 ± 1.5	61.6 ± 4	13.8 ± 0.5
Adrenal (s)	48.6 ± 4	21.4 ± 1.3	24.5 ± 4.0
Pituitary	11.0 ± .5	16.4 ± .8	8.8 ± 1.9
Spleen	629 ± 30	444 ± 31	400 ± 0.09
Heart (g)	.999 ± .045	.792 ± .031	968 ± 85
Liver	14.1 ± .8	10.8 ± .6	8.8 ± 0.5
Brain	1.81 ± .04	1.74 ± .03	1.58 ± 0.03
Kidney	2.18 ± .1	1.64 ± .06	2.21 ± 0.06
Body Weight	331 ± 16	266 ± 7	1.78 ± 12.0

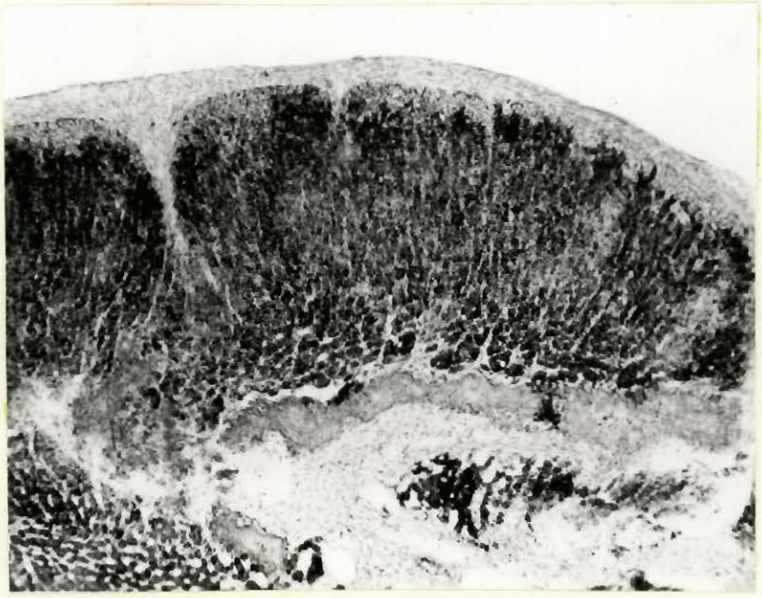

Fig. 32 - Regenerated adrenal cortex from animal of Group II after treatment with PTU. Atrophy of fasciculata zone is obvious when compared with Fig. 33. Sudan IV. Hematoxylin X 125.

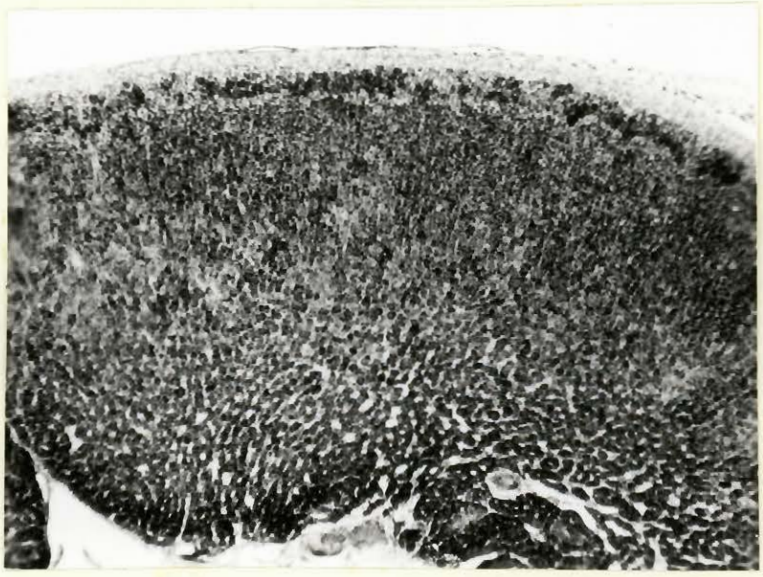

Fig. 33 - Regenerated adrenal cortex from a rat with adrenal-regeneration hypertension, zonation is distinct with a moderate amount of lipid accumulation. Sudan IV - hematoxylin X 125

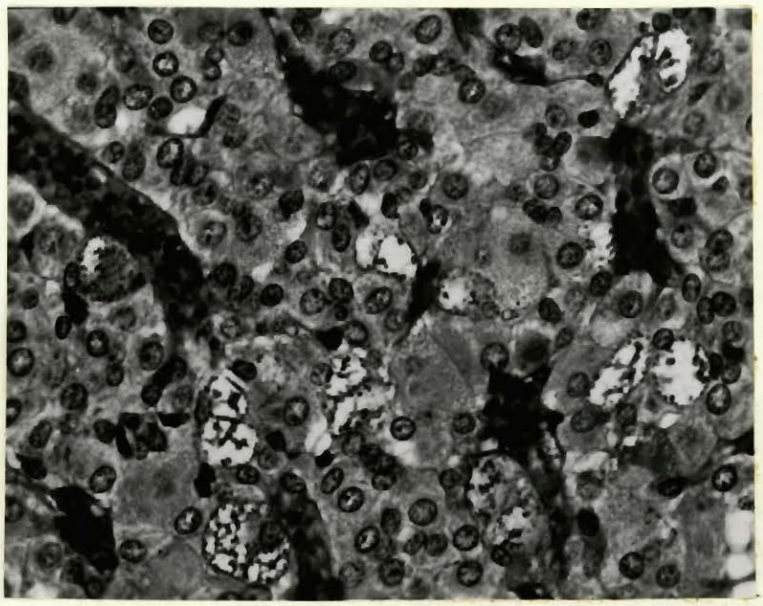

Fig. 34 - Pituitary changes in animal from Group II treated with PTU. Marked hyperemia, large, vacuolar basophils and degranulation of eosinophils. One basophil at left, below a capillary, contains rough hyaline granules. Should be compared with Figure 10. Rona-Morvay stain X 310.

the heart and kidney weights of the estrogen treated rats were significantly greater. This was anticipated in view of the unrelieved hypertension in this group. The thyroid and pituitary weights of the rats treated with PTU were significantly larger than those of the other two groups.

The weights of the sex glands of the rats were not routinely recorded in these studies. An exception was the group treated with estrogen, where it was important to know whether the animals had been under an adequate level of therapy. The weights of these organs in g/100 g body weight in the estrogen treated group and the control group were: testes 1.08 $\pm$ 0.02 and 1.2 $\pm$ .03, seminal vesicles .09 $\pm$ .01 and .12 $\pm$ .005, prostate .027 $\pm$ .009 and 0.15 $\pm$ .005 respectively. These differences were significant at the 95% level of confidence indicating that a significant atrophy of these organs was present in the group treated with estrogen.

3. Gross and Microscopic Pathological Changes.

Group I (Unilateral Nephrectomy). Only one animal in this group showed any evidence of pathological lesions. This rat had a mild degree of chronic focal pyelonephritis without glomerular or vascular involvement. Terminal blood pressure of this animal was 159 mm of mercury.

Group II (Adrenal-Regeneration Hypertension Treated with PTU). No evidence of either renal or vascular lesions was found in animals of this group. The regenerated adrenal gland (Fig. 32) was small and contained large masses of fibrous connective tissue and calcified areas. Zonation of the adrenal cortex was distinct, though marked differences were apparent compared to the untreated regenerated cortex (Fig. 33). The glomerulosa was normal in width and composed of darkly stained cells containing little lipid. The fasciculata was quite narrow, and this difference in size appeared to account for the overall decrease in size of the gland. The cells of this zone were well outlined but cytoplasm and nucleus were reduced in size. The reticularis appeared relatively broad with deep penetrations of cells into the central connective tissue mass. The thyroid gland was composed of dilated acini surrounded by tall columnar cells. The colloid content of these acini was diminished with frequent resorption vacuoles at the periphery. Papillary infolding was prominent. In the pituitary the changes resembled those seen after thyroidectomy, the number of both eosinophil and chromophobe cells was reduced. Eosinophil cells were degranulated. Basophils appeared to make up about one-half of cells of the pituitary. These cells were swollen and stained only faintly with aniline

blue. In more than half of these basophils (Fig. 34) the cytoplasm showed a honeycomb structure containing numerous vacuoles. Some basophils were ballooned out so that outlines were no longer visible. In a small percentage large distinct hyaline globules were found.

Group III (Adrenal-Regeneration Hypertension Treated with Estrogen). These animals had histologic changes in the prostate and seminal vesicles indicative of a functional atrophy and compatible with the tissue weight changes which were mentioned above. They also had widespread renal and cardiovascular changes which could not be differentiated in incidence or severity from those seen in earlier control groups with adrenal-regeneration hypertension.

DISCUSSION

The failure of treatment with estrogens to influence the development of adrenal-regeneration hypertension is somewhat surprising since Skelton (308), in an identical experiment, prevented the development of hypertension using the synthetic estrogen diethylstilboestrol. There are several possibilities which may explain the differences between the results of the two studies. Skelton used a synthetic estrogen, whereas in our experiments a mixture of natural estrogens was administered. Nevertheless in both experiments,

as judged by the weights of the secondary sex glands, the animals were under an estrogenic effect. A second possibility lies in the difference between the estrogenic activity of the two hormone preparations. Both were administered in the same absolute dose, 1 mg/kg stilboestrol and 1 mg/kg expressed as estrone, but since the former is a more powerful estrogen the animals treated with stilboestrol would be under a greater estrogenic effect. A third possibility would be that these results represent a difference between natural and synthetic estrogens in their effect on the rat. Although Vogt (311) has shown that hexoestrol depresses adrenal corticosterone synthesis, no one has as yet reported similar studies on estrone or other natural estrogens.

It is clear from these results that PTU treatment lowers the blood pressure in adrenal-regeneration hypertension. This effect is probably analagous to that reported by Skelton (308) after thyroparathyroidectomy. This finding is also compatible with the increased severity of the hypertension and cardiovascular-renal lesions reported by Masson after the administration of thyroid hormone to rats developing adrenal-regeneration hypertension (307). It would be of interest to know whether the effect shown on blood pressure is accompanied by a favourable influence

on the development of cardiovascular and renal lesions. However, as described earlier, the incidence of these lesions in control animals is considerably less than 100%, and although no such lesions were found in the animals treated with PTU, no definite conclusions may be drawn from groups of this size.

Although the atrophy of the adrenal gland produced by thyroidectomy or antithyroid drugs is well known, it would be presumptive to attribute the antihypertensive effect of PTU to a depressant action on the adrenal gland. Antithyroid drugs have been shown to lower the blood pressure of renal hypertensive rats (312) and rats with metacorticoid hypertension (173). In either case an effect on the adrenal may be important, however Salgado has also shown that PTU inhibited the development of hypertension in adrenalectomized rats given sodium chloride and desoxycorticosterone (314). Thus it is probable that an extra-adrenal action of PTU plays a role in its action on hypertension.

It is evident that with the exception of weights of the pituitary and thyroid glands, there is a general reduction in tissue weights in the PTU treated group. The generally lower weight of animals treated with antithyroid drugs is well known (315, 316) and probably plays a large

part in causing the decrease in tissue weights. Although the regenerated adrenal of PTU treated animals was not significantly lower in weight than that of the controls when expressed in terms of body weight, definite histological differences were seen when adrenals from these groups were compared. The shrinking of the fasciculata zone which has been described has also been reported by Deane and Greep following thiouracil therapy (306).

When the actions of PTU and amphenone on the adrenal cortex are compared, it is evident that the two drugs have almost opposite effects. Amphenone caused enlargement of the fasciculata and reticularis zones with increase in size of the cells and intense lipid accumulation. PTU on the other hand caused shrinking of the fasciculata zone and the cells of this zone were shrunken with scanty cytoplasm and no lipid accumulation. Unfortunately, although the effects of PTU on adrenal histology are well known, the action of this drug on the secretory function of the adrenal gland of the rat has not been reported.

The effect of PTU on adrenal-regeneration hypertension reported herein, the preventive effect of thyroparathyroidectomy disclosed by Skelton (308), and Masson's demonstration that thyroxine aggravates adrenal-regeneration

hypertension (307), point to an important role for the thyroid gland in the development and maintenance of this form of hypertension. However it cannot be concluded from these results that the effect of amphenone in adrenal-regeneration hypertension can be attributed entirely to its antithyroid properties. It was shown in Part II of this study that amphenone does not depress the blood pressure in DCA hypertension in adrenalectomized rats, while Salgado (314) has shown a marked effect for PTU under these conditions. The possibility remains that amphenone acts both directly on the adrenal gland and on the thyroid gland in adrenal-regeneration hypertension.

PART IV

ALDOSTERONE SECRETION IN ADRENAL-REGENERATION HYPERTENSION

The experiments reported above have confirmed the development of hypertension and cardiovascular-renal lesions during the process of adrenal regeneration in suitably sensitized rats. They have also shown that amphenone, which is known to affect adrenal function, will also influence adrenal-regeneration hypertension but not DCA hypertension. Although these studies add to the evidence pointing to a primary role of the adrenal gland in this syndrome, they do not implicate any specific adrenal factors which might play an etiologic role.

Most of the experimental evidence which has been reviewed relates the hypertensive effect of adrenal hormones, particularly DCA, to sodium retaining ability. Since aldosterone is believed by some to play a primary role in essential hypertension (43, 44), and since this sodium retaining hormone can produce hypertension in the rat (180, 181), it was natural to consider this agent as a possible causative factor in adrenal-regeneration hypertension. This possibility was proposed by Skelton (242) soon after he first

described adrenal-regeneration hypertension. The following experiments were therefore designed to study the aldosterone secretion in adrenal-regeneration hypertension, and compare it with suitable control groups.

EXPERIMENTAL DESIGN

Group I - Adrenal-Regeneration Hypertension - Females (14 animals). These animals were unilaterally nephrectomized and adrenalectomized on the right side, and the left adrenal was enucleated. The drinking water was replaced with 1% saline.

Group II - Adrenal-Regeneration Hypertension - Males (20 animals). The operative techniques were the same as those in Group I. Drinking water was replaced with 1% saline.

Group III - Adrenal Hypertrophy Controls (13 animals). These rats were unilaterally nephrectomized and adrenalectomized on the right side and were given 1% saline to drink.

Group IV - Adrenal-Regeneration without saline (23 animals). These rats were unilaterally nephrectomized and adrenalectomized on the

right side and the left adrenal was enucleated. The drinking water was left unchanged.

The blood pressure of these animals was measured prior to operation, and any rats with a blood pressure greater than 110 mm of mercury were discarded. Blood pressures were again measured at the end of the 4 week experimental period, before anaesthetizing the animals for collection of adrenal vein blood as described under methods. Samples of adrenal effluent blood were collected over a period of one hour from each animal. These samples were refrigerated at 36°F after collection, and sent within 48 hours to Dr. C.J. P. Giroud of the Montreal Children's Hospital for extraction and determination of the aldosterone content by biological assay.

RESULTS

The aldosterone secretion rates of the various groups are given in Table VI. The secretion rate of aldosterone was 0.119 mg/kg/adrenal/hour in the female group with adrenal-regeneration hypertension and 0.095 in the corresponding male group. The secretion rate in the two control groups was slightly higher, 0.124 mcg/kg/adrenal/hour in Group 3 with adrenal hypertrophy, and 0.146 in Group 4

in which the adrenals were enucleated but which were given only tap water to drink.

It is clear that there is no indication of hypersecretion of aldosterone in adrenal-regeneration hypertension. Actually the secretion is somewhat less in the two groups with elevated blood pressure than in either of the control groups. The adrenal glands of the female rats are significantly greater in size than those of the male animals, and the elevation of blood pressure in this group was slightly greater, however there was no real difference between the aldosterone secretion of the two sexes. The highest aldosterone secretion was found in the group which received tap water to drink during the period of adrenal regeneration.

DISCUSSION

The results of this study indicate that any theory of the etiology of adrenal-regeneration hypertension which is based only on a hypersecretion of aldosterone is clearly untenable. Masson et al (175) showed that in vitro the aldosterone production of adrenal glands from rats with adrenal-regeneration hypertension was less than that of the glands from normotensive controls. In these experiments it

TABLE VI SECRETION OF ALDOSTERONE IN THE ADRENAL VEIN

BLOOD OF RATS WITH ADRENAL - REGENERATION HYPERTENSION

	Group	Sex	Number of Animals	Mean Body Weight g	Final Blood Pressure mm Hg	Adrenal Weight mg	Collection Volume cc/kg/adr./hr.	Aldosterone mcg/kg/adr. hr.
I	Rt.nephrectomy and adrenalectomy, left adrenal enucleation	F	14	192	166	40	11.5	0.119
II	" "	M	20	284	150.2	26.3	9.5	0.095
III	Rt.nephrectomy and adrenalectomy	M	13	289	131	37.3	13.1	0.124
IV	Rt.nephrectomy and adrenalectomy, left adrenal enucleation	M	23	300	138	30.4	11.3	0.146

has been shown that in vivo the aldosterone secretion of rats with adrenal-regeneration hypertension is probably lower and certainly not greater than that from normotensive animals with regenerated adrenals or those with adrenal hypertrophy. Subsequent to these experiments a personal communication was received from Miss C. Laplante and Dr. C.J.P Giroud. Determination of corticosterone content of the blood samples used in this experiment showed that the secretion of this steroid in either male or female rats with adrenal-regeneration hypertension was only slightly lower than that in the two control groups (328). Since corticosterone is the principal glucocorticoid secreted by the rat adrenal it appears unlikely that rats with adrenal-regeneration hypertension have a hypersecretion of glucocorticoids. The possibility still exists of course that an imbalance of adrenocortical hormones is the cause of adrenal-regeneration hypertension. If a deficiency of some opposing factor was present, the low secretion of aldosterone in absolute terms might act in the animal like a relative hypersecretion.

There is one facet of this intriguing question which is difficult to resolve in the light of our present limited knowledge. If hypersecretion of aldosterone was indeed the

basic cause of this hypertension why would an increased sodium intake be essential to its development since increased sodium intake is known to act as a depressant to aldosterone production (317, 318).

PART V

COMPARISON OF HYPERTENSION INDUCED BY CORTICOSTERONE WITH ADRENAL-REGENERATION HYPERTENSION

It has been shown in the preceding section that aldosterone secretion was less in rats with adrenal-regeneration hypertension than in normal controls. These data are in agreement with Masson's finding of lower than normal in vitro production of aldosterone by adrenal tissue of rats in this form of hypertension (175). These results served to focus attention on the possible role of other corticoids in this form of hypertension.

In 1954 Selye (319) reported that corticosterone, the principal glucocorticoid secreted by the rat adrenal, did not produce renal hyalinosis or hypertension when administered to the rat at a dose of 1 mg per day. More recently Gross (191) reported that 5 mg/day of corticosterone caused the development of hypertension and cardiovascular-renal lesions in this species.

Theoretically at least, since corticosterone is capable of causing hypertension and associated morphological changes, it is possible that hypersecretion of this glucocorticoid could be the cause of adrenal-regeneration hypertension. In

this study hypertension was produced in adrenalectomized rats using the technique and dosage employed by Gross (191). The changes in blood pressure and morphological lesions were compared with those in earlier groups having adrenal-regeneration hypertension. This was done with the view that if the latter syndrome is due to excessive corticosterone secretion by the regenerating adrenal gland basic similarities in the two types of hypertension might exist.

EXPERIMENTAL DESIGN

Corticosterone hypertension was produced in 40 bilaterally adrenalectomized rats by giving them 5 mg of corticosterone subcutaneously each day in crystalline suspension. The animals were unilaterally nephrectomized, and the drinking water was replaced with 1% saline.

RESULTS

Only 15 out of the 40 animals were able to tolerate this high dose of corticosterone. Treatment of the remainder was stopped after 4 weeks because of the development of multiple subcutaneous abscesses and septicaemia. Most of these animals survived after treatment was stopped but did not become hypertensive. The results hereafter are limited

to those of the 15 animals which survived treatment with corticosterone for 12 weeks.

1. Blood Pressure

The hypertensive response of the adrenalectomized, unilaterally nephrectomized rat to corticosterone and saline administration is shown in Figure 35. In each of the earlier studies of adrenal-regeneration hypertension the blood pressure rose to hypertensive levels in a 4-5 week period, and almost invariably stayed at a plateau thereafter. In corticosterone hypertension the rise in blood pressure was more gradual and an increase was seen each week of corticosterone administration. At least 6-7 weeks were required for the blood pressure to reach clearly hypertensive levels.

2. Body and Tissue Weights

The animals treated with corticosterone were cachexic at the end of the experiment with a mean weight of 257 g. Normal or adrenal-regeneration hypertension animals of a similar age would weigh well over 300 g. The corticosterone treated animals showed depression in the weight of the thyroid, thymus and spleen when the weights were expressed in terms of body weight and compared with those of animals with adrenal-regeneration hypertension in earlier experiments.

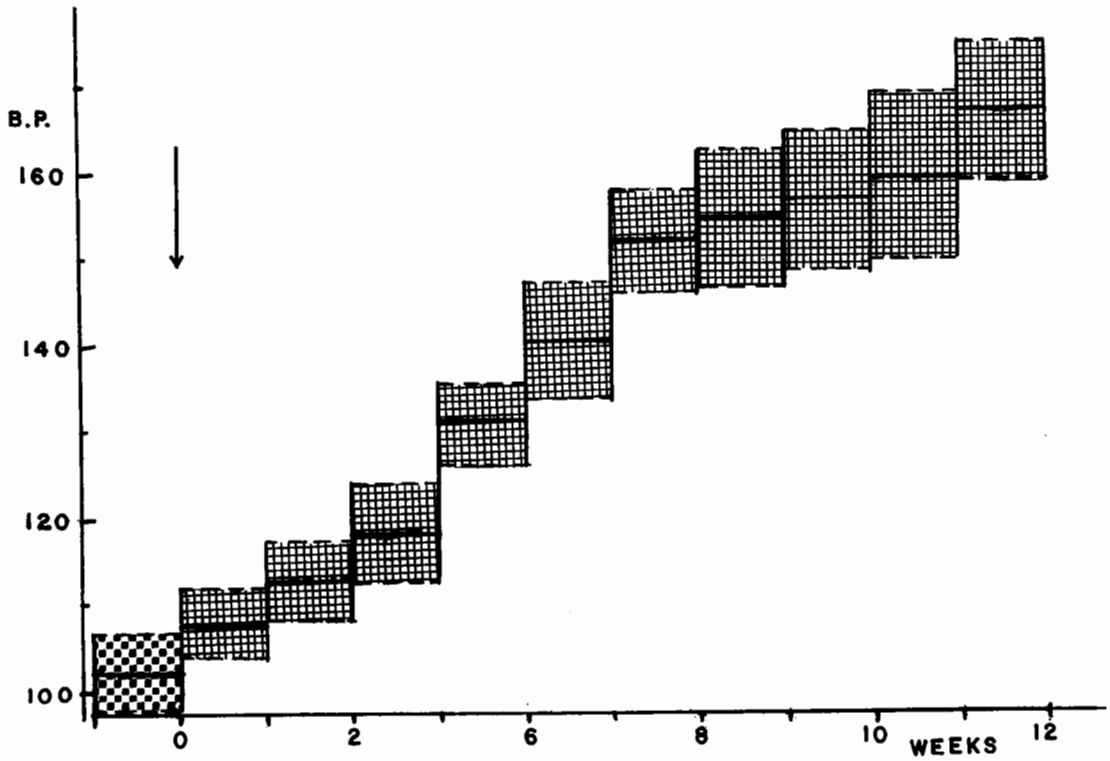

Fig. 35 - Hypertensive response following corticosterone treatment 5 mg per day to the adrenalectomized rat.

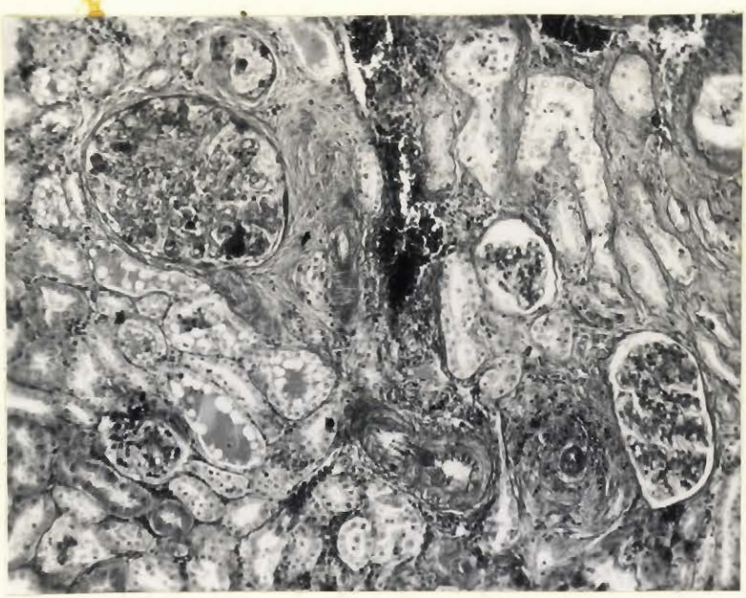

Fig. 36 - Nephrosclerosis and vascular lesions in kidney of rat with adrenal-regeneration hypertension. Casons Trichrome X 125.

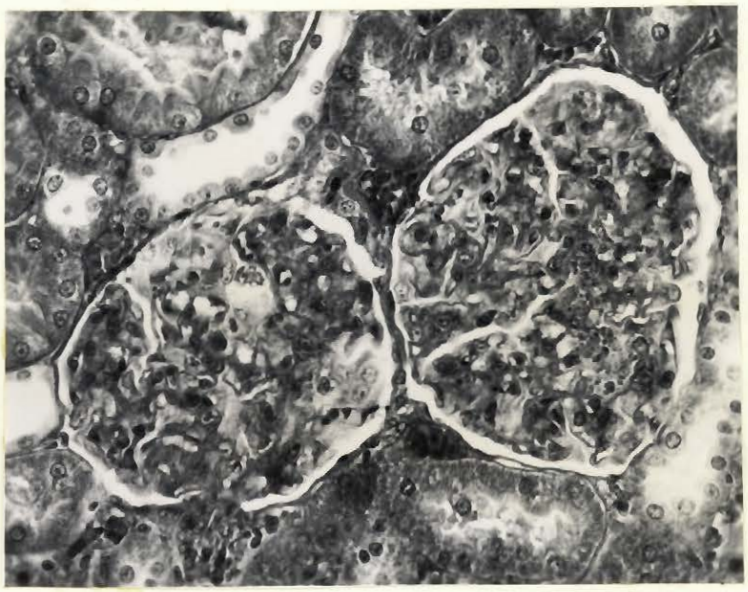

Fig. 37 - Mild glomerulohyalinosis and capsular thickening in kidney of rat treated with corticosterone Cason's Trichrome X 310.

These tissue weight changes are the expected effects of glucocorticoid treatment in high dosage, but it is significant that they are not found in adrenal-regeneration hypertension.

3. Gross and Microscopic Pathological Changes.

The most pronounced difference between hypertension induced by corticosterone and adrenal-regeneration hypertension lay in the microscopic appearance of the tissues. Whereas in adrenal-regeneration hypertension there was nephrosclerosis and widespread vascular lesions (Fig. 36), the animals with corticosterone hypertension had only slight glomerulohyalinosis, capsular thickening in the kidney (Fig. 37) and no vascular lesions. There were also marked differences in the islet tissue of the pancreas. The animals treated with corticosterone had enlarged Islets of Langerhans with beta cell hyperplasia. The beta cells which were moderately degranulated occupied most of the islets (Fig. 38) with alpha cells remaining in only a narrow rim around the periphery. The islets from animals with adrenal-regeneration hypertension were normal in size and cellular distribution with well preserved granule content (Fig. 39).

The pituitaries from animals with adrenal-regeneration hypertension as already described appeared to have a relative

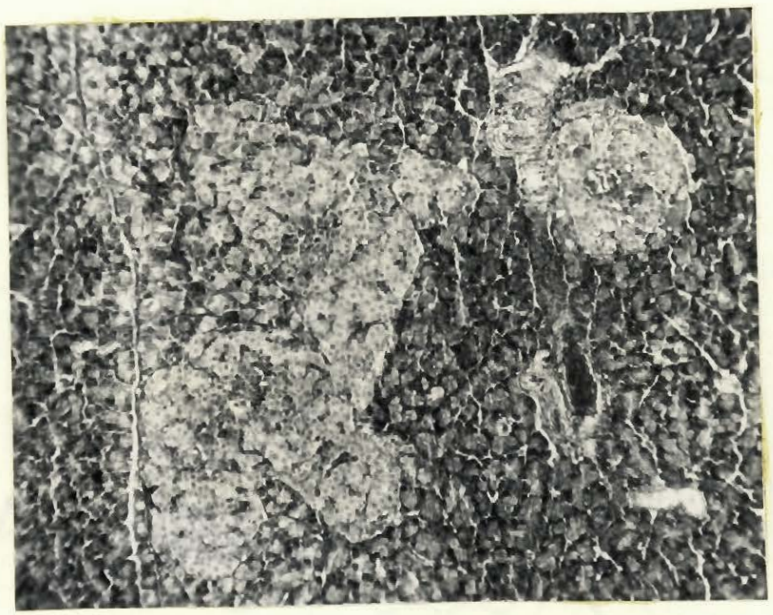

Fig. 38 - Enlarged Islet of Langerhans with beta cell hyperplasia in a rat treated with corticosterone. Rona-Morvay X 125.

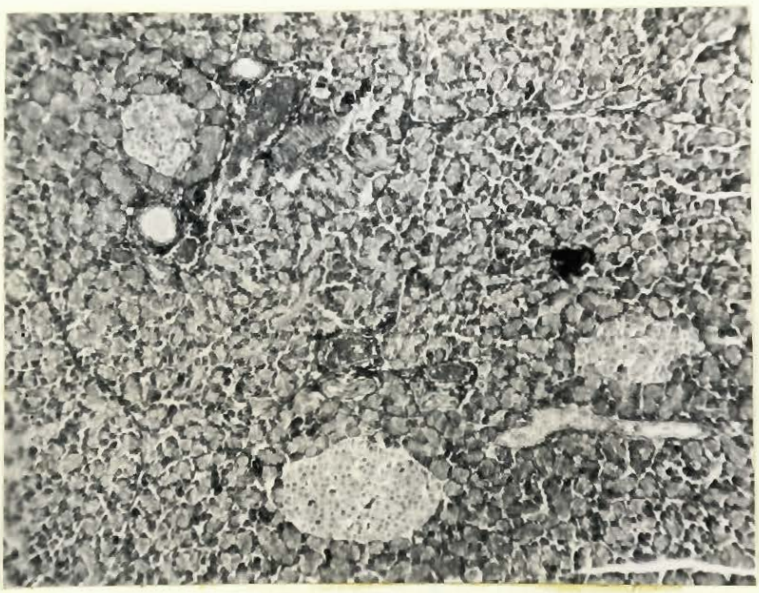

Fig. 39 - Normal Islets of Langerhans from a rat with adrenal-regeneration hypertension. Rona-Morvay X 125.

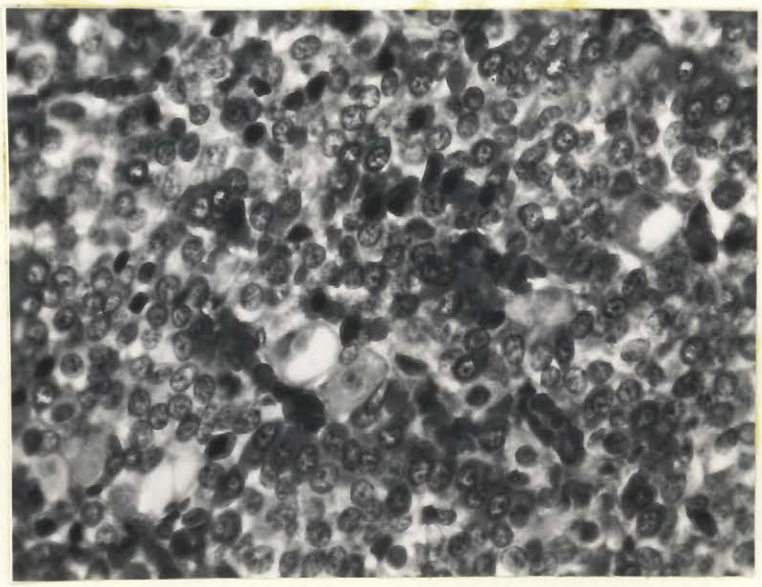

Fig. 40 - Pituitary from a rat with corticosterone hypertension. Basophils are decreased in numbers compared with eosinophils and chromophobes. Large colloid inclusions may be seen. Rona-Morvay X 310.

increase in the number of basophils. The pituitaries from rats treated with corticosterone had few basophils as shown in Fig. 40, and these with poorly stained cytoplasm. Some of these basophils showed cytoplasmic colloid inclusion bodies.

DISCUSSION

It would appear from the measurements of rate of increase in blood pressure and observations of tissue weights, and differences in histological appearance of the kidneys, blood vessels, pancreas and pituitary that adrenal-regeneration hypertension and hypertension induced by this amount of corticosterone are quite dissimilar. There seems to be no basis on which one could postulate that these two forms of hypertension have a common pathogenetic mechanism. The possibility still remains that following enucleation of the adrenal, unilateral nephrectomy and a high salt intake, the rat is more sensitive to the pressor effects of corticosterone, and that hypertension and cardiovascular lesions might be produced without any other manifestations of glucocorticoid excess. This possibility seems remote.

GENERAL DISCUSSION

It is proposed to correlate in the following discussion the known facts concerning adrenal-regeneration hypertension as mentioned in the review of the literature with the findings of the studies reported herein. On the basis of this knowledge it is possible to put forth several theories which might explain the etiology of this form of experimental hypertension.

Skelton (242) showed in 1955 that, when one adrenal of a unilaterally nephrectomized unilaterally adrenalectomized rat was enucleated, and the sodium chloride intake of the animal was increased, hypertension developed as regeneration of the enucleated adrenal took place. This finding has been confirmed in this study. Adrenal-regeneration hypertension in many respects resembles that produced in the rat by the injection of DCA. Like the latter it is dependent on an increased intake of sodium chloride (245), and there is a striking similarity in the cardiovascular and renal lesions in the two forms of hypertension. In one respect there is a difference between DCA and adrenal-regeneration hypertension. This difference lies in the severity of the hypertension, cardiovascular and renal lesions

in the two forms of the disease. The blood pressure levels as shown in Part II of this study are higher in DCA hypertension in the rat, and the histopathological lesions are both more severe and more uniform in this disease. However, these differences, it is believed, are quantitative and the basic similarities in the two forms of hypertension may point to common mechanisms of etiology.

There are a number of factors which may be regarded as essential to the development of adrenal-regeneration hypertension. Among these is the necessity of an increased salt intake which was first pointed out by Skelton (245). The failure of the animals of Group IV, Part IV, of this study (which were on a water intake) to develop hypertension confirms Skelton's observations. The role of sodium in experimental hypertension as discussed in the literature review is so important that adrenal enucleation and regeneration may possibly be considered as a process which sensitizes the rat to the hypertensive effects of this ion. Skelton found that removal of one kidney was necessary for the development of adrenal-regeneration hypertension (245). Another similarity to DCA hypertension is evident in this respect as unilateral nephrectomy also sensitizes the rat to the hypertensive effects of DCA (153).

There are a number of factors which may be regarded as essential to the development of adrenal-regeneration hypertension. Among these is the necessity of an increased salt intake which was first pointed out by Skelton (245). The failure of the animals of Group IV, Part IV, of this study (which were on a water intake) to develop hypertension confirms Skelton's observations. The role of sodium in experimental hypertension as discussed in the literature review is so important that adrenal enucleation and regeneration may possibly be considered as a process which sensitizes the rat to the hypertensive effects of this ion. Skelton found that removal of one kidney was necessary for the development of adrenal-regeneration hypertension (245). Another similarity to DCA hypertension is evident in this respect as unilateral nephrectomy also sensitizes the rat to the hypertensive effects of DCA (153).

A number of studies which have been performed in adrenal-regeneration hypertension point to a primary though as yet obscure role for the regenerating adrenal cortex in this syndrome. In Part I of this study it was shown that if one adrenal of the rat was enucleated, and the second left intact, no regeneration of the enucleated gland took place and hypertension did not develop. When at the end

of 5 weeks the intact adrenal was removed, regeneration occurred, and hypertension rapidly developed. Thus it is evident that the enucleation per se does not cause the development of hypertension, but that regeneration of the gland must take place. Skelton's demonstration (245) that removal of the hypophysis which prevents adrenal regeneration also prevents the development of adrenal-regeneration hypertension points as well to the essential nature of the regeneration process.

It is interesting in this respect to compare the effects of adrenal-enucleation and regeneration with unilateral adrenalectomy and resultant hypertrophy of the remaining gland. Such a comparison may be made between Groups II and IV of Part I.

Adrenal hypertrophy in Group IV was not capable of providing the stimulus for the development of hypertension. Group II on the other hand, which had regeneration of an enucleated gland, became hypertensive. The studies of Part IV and the recent communication from Laplante and Giroud (328) indicate that the regenerating adrenal gland has almost normal secretion rates of aldosterone and corticosterone 30 days after enucleation.

Similar secretion rates of aldosterone and corticosterone were also found in the rats with adrenal hypertrophy (Part IV). If it is assumed that the adrenal cortical function in these two states does not differ in other respects such as the presence or absence of other active adrenal cortical substances, then aside from the medulla in the hypertrophied adrenal the other obvious difference between the two processes was the period of adrenal deficiency present after enucleation of the gland. It is unlikely that the failure of the rats with adrenal hypertrophy to become hypertensive is due simply to a protective effect provided by the adrenal medulla. Such a role would not be in keeping with the pressor properties of the hormones of the adrenal medulla, also if this were the case, hypertension would have occurred in the bilaterally adrenalectomized rats of Group III, Part I, which were sensitized by a high salt intake and unilateral nephrectomy. Thus the period of adrenal deficiency, which will be discussed later, may be of primary importance in the etiology of adrenal-regeneration hypertension.

For purposes of discussion it is possible to divide the sequence of events in adrenal-regeneration hypertension into three periods in terms of adrenal function. First, the period of adrenal deficiency which is present for 5 - 7 days

after enucleation of the gland (237), second, a period of 4 - 5 weeks during which histological regeneration of the adrenal takes place (229), presumably an increasing secretion of aldosterone and corticosterone develops and the animals become hypertensive, and third the period thereafter when the blood pressure remains on a plateau at hypertensive levels.

It appears from the comparison made above of adrenal regeneration and adrenal hypertrophy in sensitized animals that the period of adrenal deficiency is an essential component in the development of adrenal-regeneration hypertension. Several quite recent studies support this observation. Skelton (190) administered 1 mg of corticosterone to rats immediately after enucleation of the adrenal gland, contralateral adrenalectomy and sensitization by unilateral nephrectomy and increasing the intake of sodium chloride. This treatment prevented the development of hypertension. In a similar experiment Grollman (189) prevented the development of adrenal-regeneration hypertension by administering 1 mg each of corticosterone and DCA to the rat for the first 10 days after enucleation of the adrenal. In either of these experiments the administration of corticoids pre-

vented the occurrence of a period of adrenal deficiency, however it might also have influenced the functional regeneration of the gland, so that these experiments do not provide conclusive evidence that a period of adrenal deficiency is essential to the development of adrenal-regeneration hypertension.

Most of the studies on adrenal-regeneration hypertension have been directed toward exploration of the nature of the secretion of the regenerating adrenal glands and the study of factors which affect it. Masson (175) showed that in vitro the aldosterone production of adrenal glands from rats with adrenal-regeneration hypertension was less than that of the glands from normotensive controls. The studies reported herein demonstrate that aldosterone secretion by regenerated adrenal glands is slightly lower than that of normal adrenals. Also recent work by Laplante and Giroud indicate that corticosterone secretion by regenerated adrenals is within the normal range (328). Thus all the present evidence suggests that adrenal function in respect to aldosterone and corticosterone is normal or only slightly below normal when adrenal-regeneration hypertension is fully developed.

Although the secretion of adrenal cortical steroids in

adrenal-regeneration hypertension is apparently in the normal range, a number of studies amply demonstrate that this level of adrenal cortical function is essential to the development and maintenance of the hypertension, and the production of cardiovascular and renal lesions. Skelton (249) showed that re-enucleation or removal of the regenerated adrenal gland after hypertension had been established caused the blood pressure to return to normal levels. When the adrenal was completely removed the fall in blood pressure was permanent, while after re-enucleation the fall was transient, and hypertension returned as the adrenal regenerated again. Transplantation of the regenerating adrenal to the spleen (325) or mesentery (326) prevented the development of hypertension, presumably because from these sites the adrenal steroids enter the portal circulation and are destroyed in the liver. On the other hand transplanting the regenerating adrenal to the renal capsule with drainage through the systemic circulation does not prevent hypertension (326).

It would be interesting to know in rats with adrenals transplanted to the spleen whether passage through the liver leads to an alteration in the effectiveness of steroids in their action on other tissues, eg. the kidney in such animals, since factors apparently essential to the development of adrenal-regeneration hypertension are destroyed by hepatic drainage.

The studies reported herein also demonstrate the importance of the secretions of the adrenal gland in the second and third stages of adrenal-regeneration hypertension. The administration of the adrenal depressant drug amphenone to rats which had an established hypertension caused a return of the blood pressure to normal levels. When amphenone treatment was started soon after enucleation of the adrenal glands the usual rise in blood pressure was prevented.

Propylthiouracil exerts a favourable influence on adrenal-regeneration hypertension as shown in Part III of this study. Masson has demonstrated (247) that thyroxine caused a further increase in the blood pressure and aggravated the cardiovascular and renal lesions. These experiments do not provide evidence which can be interpreted in terms of adrenal function, however the results are in keeping with the direct relationship which is believed to exist between thyroid and adrenal function.

Additional evidence on the essential role of the adrenal steroids in adrenal-regeneration hypertension is contained in a communication from Kagawa and Sturtevant (167). These workers reported recently that 3-(3-oxo-17β hydroxy-4 androsten-17-yl) propionic acid γ-lactone (SC-5233) blocked the effects of aldosterone and DCA on salt excretion (155).

This blocking action was attributed to competition between SC-5233 and the natural steroids for receptor sites in the renal tubule. Kagawa and Sturtevant (167) found that the administration of 40 mg/kg/day of SC-5233 to rats immediately after adrenal enucleation prevented the development of hypertension and cardiac hypertrophy. Other measurements of body weight, saline intake, and adrenal weight were unaffected. It appears therefore that the action of steroids from the regenerating adrenal cortex at the renal level are necessary for the development of adrenal-regeneration hypertension.

As mentioned earlier the evidence available suggests that adrenal function in adrenal-regeneration hypertension is normal in terms of the secretion rate of aldosterone and corticosterone. However, in spite of these normal secretory rates evidence exists which indicates that these animals are not completely normal in respect to water and electrolyte metabolism. Komrad and Wyman (237), and later Jones and Spalding (238), showed that after adrenal enucleation (without the sensitization of unilateral nephrectomy and increased salt intake) the rat was unable to excrete a water load in the normal manner, and that the animals were deficient in this respect as long as three months after operation. These observations appeared important enough to warrant confirmation in sensitized enucleated rats with

adrenal-regeneration hypertension. Accordingly a group of hypertensive animals were prepared in the usual manner, and water intoxication tests performed by the method of Komrad and Wyman (237). Thirty days after enucleation when the animals were hypertensive, there was a marked decrease in their ability to excrete the water load with a consequent delay in the diuresis. At 80 days however, no difference was seen between the hypertensive group and the controls in the handling of the water load. Recently del Greco (320) found that rats with adrenal-regeneration hypertension 4 - 5 weeks after enucleation of the adrenal gland did not respond in a normal manner to an osmotic diuresis induced by mannitol. Thus it appears that, although rats with regenerated adrenals apparently have normal secretory rates of corticosterone and aldosterone, and can be maintained satisfactorily without extra sodium, under the stress of a water load a full functional capacity is not present. This may indicate the lack of a functional reserve in these animals, however, although no studies on this subject are known, a similar deficiency may exist in unilaterally adrenalectomized rats so that the significance of these observations is not clear.

From the facts outlined in the foregoing discussion,

the following hypothesis are presented as possible explanations of adrenal-regeneration hypertension.

1. That the regenerating adrenal gland secretes some as yet unknown steroid or steroids which are directly responsible for the increase in blood pressure, cardiovascular and renal lesions.

It must be admitted that there is no experimental evidence to indicate the existence of such a steroid. However, because of the difficulties of determining the presence of an unknown steroid by available methods, and until an alternative theory of the etiology is definitely proven, this possibility cannot be ignored.

2. That the regenerating adrenal gland is deficient in the secretion of some unknown steroid whose effect would balance the hypertensive action of aldosterone or corticosterone. Thus according to this theory, the secretion of aldosterone by the regenerating adrenal cortex is the primary cause of the hypertension, but it is the absence of some unknown factor which allows its development. Although no direct evidence exists to support this concept, it is compatible with the available facts concerning adrenal-regeneration hypertension. The normal secretory rate of aldosterone and corticosterone according to this theory and in

the absence of the balancing steroid could be considered as a relative hypersecretion, and the factors which interfere with this relative hypersecretion would act in a manner which would tend to restore the balance and thus reduce the blood pressure.

The theory that the adrenal secretes a steroid which balances the sodium retaining hormones is not new. Sayers (30) advanced this theory on the basis of the failure of rats to develop hypertension following the administration of adrenal cortical extract, and increased sensitivity in terms of blood pressure of adrenalectomized rats or humans to DCA (30,95,96,97). Freidman has shown that the administration of adrenal cortical extract would inhibit the development of DCA hypertension and cardiac enlargement in the rat (47). Thus there is some evidence to support the existence of an antihypertensive steroid. Recently Wettstein et al (321) reported that 16 hydroxy allopregnanolone isolated from normal beef adrenals would cause sodium excretion in the rat. This group has also shown that this compound has antihypertensive properties in rats with renal hypertension (48). This steroid with its sodium excreting and antihypertensive effects might be expected to act in a manner opposite to that of aldosterone, and thus could theo-

retically be the balancing factor of adrenal-regeneration hypertension.

However, one must bear in mind that, although both aldosterone and DCA have sodium retaining properties and can produce hypertension in the rat, there is no proof that these two properties are related to each other in all steroids. By the same reasoning therefore, it is possible that an antihypertensive steroid may exist in the normal adrenal which does not effect sodium metabolism at the renal level.

3. The final theory is that in adrenal-regeneration hypertension a hypersensitivity to pressor adrenal hormones exists as a result of the period of adrenal deficiency, which is present in these animals for 5 - 7 days immediately after enucleation of the adrenal gland. Like each of the foregoing postulates, there is a lack of direct evidence to support this theory. However it is like the others compatible with the available facts concerning adrenal-regeneration hypertension.

If a hypersensitivity to the pressor effects of adrenal hormones is produced by a short period of adrenal deficiency, it explains the failure of rats with adrenal hypertrophy (Part I) which had no period of complete absence of adrenal steroids to develop hypertension. Although the

secretion of aldosterone (Part IV) is almost the same in rats with adrenal hypertrophy compared to those with regenerated adrenals, the hypersensitivity in the latter animals would explain the development of the hypertension.

It should be possible to test this theory experimentally. If hypersensitivity to adrenal hormones is produced by a period of deficiency, then the administration of maintenance doses of aldosterone and corticosterone to sensitized rats 7 days after bilateral adrenalectomy should result in hypertension.

If this should be the case then the question arises as to whether it is simply a hypersensitivity as a result of a period of adrenal deficiency which is responsible for the development of adrenal-regeneration hypertension, or the fact that the animals are unilaterally nephrectomized and on a high salt intake during this period. It would not appear difficult to design appropriate experiments to resolve these questions.

It would certainly be premature to attempt to relate the results of these studies and these concepts of the etiology of adrenal-regeneration hypertension to hypertensive cardiovascular disease in man. However it would appear worth-

while to draw attention to recent conclusions regarding this disease. The concept of essential hypertension as being of primarily renal origin is no longer widely held, but no other generally acceptable explanation of the etiology of this disease has been put forth. The possibility exists that elucidation of the etiology of adrenal-regeneration hypertension may provide information of direct value in the study of the clinical disease.

SUMMARY

1. The development of hypertension and cardiovascular-renal lesions during adrenal regeneration in unilaterally nephrectomized rats on a high salt intake has been confirmed.

2. It was shown that this hypertension did not develop in rats which were unilaterally nephrectomized on a high salt intake when the adrenals were left intact, when both adrenals were removed or when one adrenal was removed completely and the other left intact. Thus adrenal hypertrophy was shown to be inadequate to produce this hypertension.

3. By delaying the removal of one adrenal after enucleation of the opposite gland, it was shown that regeneration of cortical tissue does not occur unless adrenal insufficiency exists. Similarly hypertension did not occur until the stimulus for regeneration was provided by removal of the intact adrenal gland.

4. The characteristic lesions of adrenal-regeneration hypertension reported earlier were demonstrated in histological studies. However, unlike earlier studies no periarteritis nodosa was found, and the incidence and severity of the cardiovascular and renal lesions was also less.

5. Alterations in pituitary histology, particularly in the basophils, were demonstrated for the first time in

adrenal-regeneration hypertension. The pituitary changes were found to be unlike those seen after corticosterone or propylthiouracil administration.

6. Evidence of the specificity of the role of the regenerating cortical tissue in adrenal-regeneration hypertension was gained by the demonstration that the adrenal depressant drug amphenone would prevent the development of this hypertension, or reverse the blood pressure increase to normal after it had developed.

7. In parallel studies it was shown that amphenone did not affect the blood pressure of normal rats or adrenalectomized rats with hypertension produced by desoxycorticosterone. These results indicated that the effect of this drug on adrenal-regeneration hypertension was mediated by a depressant action on the adrenal gland, and not by a direct effect on the blood vessels or vasomotor regulating mechanisms.

8. It was found by treating rats having adrenal-regeneration hypertension with estrogen and propylthiouracil that the effect of amphenone on the blood pressure could not be attributed to its estrogenic activity, and it appeared unlikely that its effect was mediated by the thyroid gland.

9. Adrenal effluent blood was collected from several groups of rats by cannulation of the adrenal vein. Measure-

ment of the aldosterone content of these samples by bioassay showed that no hypersecretion of this steroid existed in adrenal-regeneration hypertension. The secretion rates were slightly lower than those in the controls.

10. No significant difference was found between the severity of hypertension or the aldosterone secretion of male and female rats with adrenal-regeneration hypertension.

11. It was shown earlier, and confirmed in this study, that unilaterally nephrectomized rats with unilateral adrenal enucleation and contralateral adrenalectomy did not become hypertensive if their salt intake was limited to that found in their normal diet, in spite of the fact that regeneration of the adrenal had occurred.

12. Hypertension was produced in bilaterally adrenalectomized unilaterally nephrectomized rats on a high salt intake by the administration of high doses of corticosterone. The development of this form of hypertension, and the lesions produced were compared to those in adrenal-regeneration hypertension. The characteristic changes in the pituitary and pancreas produced by corticosterone, which are absent in adrenal-regeneration hypertension, and the differences in cardiovascular and renal lesions in these forms of hypertension support the belief that they have different etiologic mechanisms.

13. Three hypotheses have been presented which might serve as the basis for further studies on the etiology of adrenal-regeneration hypertension.

CONTRIBUTION TO KNOWLEDGE

1. It has been shown in this study for the first time that adrenal hypertrophy, which occurred after unilateral adrenalectomy of sensitized rats, was not an adequate stimulus for the development of hypertension.

2. It has also been shown that delayed regeneration of an enucleated adrenal gland in sensitized rats resulted in the development of hypertension, cardiovascular, and renal lesions in these animals.

3. In this study alterations in pituitary histology have been described in adrenal-regeneration hypertension. These changes have not been described elsewhere.

4. It has been shown for the first time that the adrenal depressant drug amphenone reversed or prevented the development of adrenal-regeneration hypertension.

5. By treating rats with adrenal-regeneration hypertension with propylthiouracil, it was shown that antithyroid drugs had effects in adrenal-regeneration hypertension similar to those of thyroidectomy.

6. By direct cannulation of the renal vein, collection of adrenal effluent blood, and measurement of the aldoster-

one content, it was shown for the first time that hyper-secretion of this steroid does not exist in adrenal-regeneration hypertension, and that the secretory rate of the regenerated adrenal is within the range of that of a single normal adrenal.

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