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The Relationship of Dopamine to Blood Pressure in Experimental Renal Hypertension

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THE RELATIONSHIP OF DOPAMINE TO BLOOD PRESSURE IN EXPERIMENTAL RENAL HYPERTENSION

BY

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ABSTRACT

The possible relationship among urinary dopamine levels, circulating dopamine levels, kidney dopamine levels and arterial blood pressure was studied in hypertensive rats and dogs. The role of the kidney in the formation of dopamine from dihydroxyphenylalanine (DOPA) was also investigated in cats.

Hypertension was produced by right renal nephrectomy followed in two weeks by contralateral renal artery compression.

The blood pressure of both the rats and dogs showed a sharp increase the first week following the second operation and remained elevated for the duration of the study. Individual pressures as high as 205 mm Hg were encountered.

Dopamine was extracted from urine by absorption onto alumina, converted to its trihydroxyindole derivative and measured fluorimetrically. Tissue and blood samples were for the most part homogenized in trichloroacetic acid, then passed through alumina columns. Dopamine was separated from DOPA by passing trichloroacetic acid extracts through columns of sodium Dowex 50W-X8 to extract dopamine, then through alumina columns to extract DOPA.

Urinary dopamine levels were reported as micrograms per liter (μg/l), micrograms per 24 hours (μg/24h) and micrograms per killogram per 24 hours (μg/kg/24h). In comparisons with a control group, the dopamine levels (μg/l) were significantly lower in the hypertensive group on several occasions, however dopamine levels expressed as μg/24h or μg/kg/24h differed only on two occasions; once prior to any operational procedures and then the first week following renal artery compression. A diuresis was

observed in the hypertensive animals which could account for the decrease in urinary dopamine concentration (μg/l) which was obtained. Over the duration of the rat study, mean dopamine levels (μg/l) remained relatively constant and varied between 168 and 418 μg/l, paired mean dopamine levels (μg/24h) showed a tendency to rise and varied between 4.61 and 10.80 μg/24h and mean dopamine levels (μg/kg/24h) showed a slight tendency to decrease, ranging from 11.51 to 18.53 μg/kg/24h.

Correlation and regression analyses for the dependency of arterial blood pressure on urinary dopamine levels (μg/l, μg/24h and μg/kg/24h) did not detect any statistically significant relationships. No significant relationship was established between dopamine levels (μg/kg/24h) and weight (gm).

Dopamine could not be detected in the blood of hypertensive rats or dogs, or in the ischemic kidneys of hypertensive rats.

The infusion of DOPA into cats with and without functional renal tissue produced similar tissue dopamine levels in the liver, heart and spleen, However, renal arterio-venous differences showed that some DOPA was decarboxylated to dopamine in the kidney.

No evidence was obtained in the study which implicated dopamine in the hypertensive process and no relationship between arterial blood pressure and urinary or circulating dopamine was established.

DOCTOR OF PHILOSOPHY THESIS

OF

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I. INTRODUCTION

Although much has been learned within the past several decades regarding the synthesis, distribution, physiological disposition and the role of the catecholamines in regards to nerve physiology, little progress has been made in the possible role of these hormones in organic disease. Except in a few isolated cases such as pheochromocytoma, attempts at demonstrating a causal relationship to various organic disease states have resulted in failure. Because of the prominent pressor effects of the catecholamines on the cardiovascular system, there have been repeated attempts to show a relationship to hypertensive disease. Most of these studies have concerned themselves with the actions of norepinephrine and epinephrine and are inconclusive in their findings. For the most part, the catecholamine dopamine (3-hydroxytyramine) has been ignored even though it is known to possess rather powerful pressor effects. For this reason, DeFanti and DeFeo undertook a study of the possible role of this substance in the etiology of experimental renal hypertension in albino rats. Their findings showed an increased urinary excretion of dopamine which correlated with the severity of hypertension.

The purpose of this problem was to extend their work in the hopes of further elucidating the possible role of dopamine in the etiology of renal hypertension.

Early in the study it was discovered that Dowex 50W-X8 resin extracted a substance with native fluorescence from rat urine and that the blank utilized in the original study, that of Carlsson and Waldeck, quenched this native fluorescence. Therefore, a good portion of this study was directed towards the development of a reliable method for the

assay of urinary dopamine. Also because of this finding, certain aspects of the original study had to be repeated. In addition to this, an attempt was made to demonstrate the presence of dopamine in the blood of hypertensive rats and dogs, in the kidneys of hypertensive rats, and also to determine the extent of involvement of the kidney in the increased tissue levels of dopamine known to follow the intravenous infusion of dihydroxyphenylalanine (DOPA).

II. REVIEW OF LITERATURE

Since the first successful production of sustained experimental hypertension by Goldblatt in 1934, numerous methods have been reported in the literature for the production of experimental hypertension. Most successful methods either directly or indirectly involve renal function; and as a consequence, etiological studies have centered their attention on the function of the kidneys and other systems known to exert an influence upon kidney function (i.e., the nervous and endocrine systems).

DeFanti (1961) has extensively reviewed the literature in this area, and the interested reader is referred to his work for developments in the field prior to 1961. The present review will center its attention on more recent advances, making reference to the earlier works only where necessary to establish a background for the present discussion.

There is by no means complete agreement on what initiates hypertension or maintains it. Most investigators, however, feel that renal hypertension is initially a consequence of one of the following: (1) the release of a substance from the kidney which in itself is not pressor, but which in turn causes the formation of an extra-renal pressor substance (i.e., the renin-hypertensin or angiotensin system), (2) retention of a pressor substance within the body due to impaired renal elimination or failure of the kidney to destroy pressor substances (i.e. renoprival hypertension) or (3) the formation and secretion of an active pressor substance from within the ischemic renal tissue itself. There is evidence to support all of these theories, and yet the evidence cannot be considered conclusive for any.

The renin-angiotensin theory probably receives the greatest support. Figure 1 depicts the proposed mechanism:

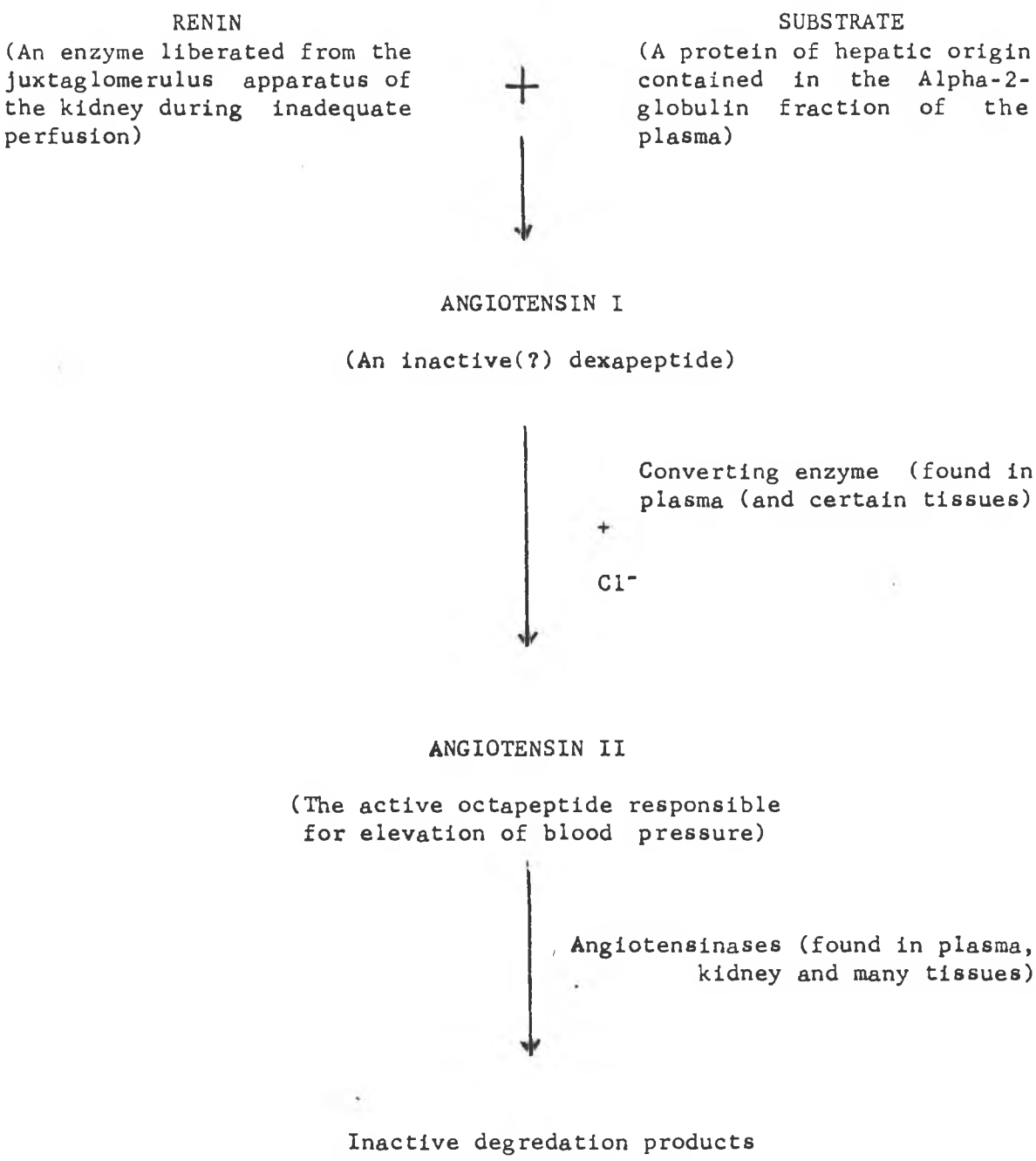

Fig. 1. Pathway for the formation and destruction of Angiotensin II. (Formulated after Skeggs et al., 1967)

According to this theory, whenever the kidney is endangered by inadequate perfusion pressure, it releases the enzyme renin. It in turn causes the formation of angiotensin II, which elevates the blood pressure by a vaso-constrictive action on the blood vessels—thus restoring adequate perfusion pressure.

Research in this area was stimulated by the findings of Laragh et al. (1960) and Biron et al. (1961), who demonstrated that angiotensin II stimulates the release of aldosterone from the adrenal cortex, thus directly implicating the adrenals in the renin-angiotensin theory of hypertension. A tie-in with the adrenals had been suspected for some time since Goldblatt (1951) found that the adrenals were necessary for the development of hypertension following renal ischemia. Helmer et al. (1951) presented evidence to suggest that the adrenal steroids are necessary for maintenance of renin substrate in the plasma.

If hypertension is caused by this mechanism, one would expect to find not only elevated levels of angiotensin II but aldosterone as well. The evidence, however, is conflicting. Kahn et al. (1952) reported only slightly elevated angiotensin levels in benign essential hypertensive patients, but levels twenty times the norm in malignant hypertensive patients. Skeggs et al. (1958) obtained similar results. On the other hand, Haynes et al. (1947) and Taquini et al. (1958) found no evidence of elevated angiotensin levels. More recent work is no less confusing. Genest et al. (1964) failed to demonstrate consistently elevated angiotensin levels in dogs with experimental malignant hypertension, while Scornik et al. (1961) failed to demonstrate abnormal levels in the human form of this disease. Mulrow et al. (1964) also failed to detect appreciable angiotensin II levels in renal hypertensive patients. However,

Morris et al. (1964) consistently found angiotensin II to be elevated in renal hypertensive patients. It is quite possible that methodology has contributed to the confusion.

A similar situation exists for aldosterone. While Genest et al. (1958) found the mean urinary aldosterone level to be significantly elevated in a group of patients suffering from essential, renal, and malignant hypertension, 57% of the individual patients were within the normal range. Laragh (1960a; 1960b) found consistently elevated aldosterone levels in malignant hypertension, but could find no abnormality in benign essential hypertension. In a subsequent study (Laragh et al., 1966), the aldosterone level in uncomplicated essential hypertension was either normal or low.

An interesting observation was made by Ames et al. (1965) when they discovered that prolonged infusions of angiotensin in human subjects, on a normal sodium diet, resulted in an increase in vascular sensitivity to angiotensin. Over the period of the infusion (up to 11 days), less and less angiotensin was required to maintain a consistent pressor response. In addition, the secretion of aldosterone was found to decrease to near normal levels as the dose of angiotensin was decreased. These findings were attributed to sodium retention and a resulting increase in vascular sensitivity since a group of subjects on a low-salt diet did not develop increased sensitivity. On the basis of these findings, Laragh (1967) has proposed a possible mechanism for the development of hypertension in which neither angiotensin or aldosterone levels remain permanently elevated. Supposedly, the process is initiated by a primary renal disturbance, causing the formation of excessive angiotensin levels—which in turn increase the secretion of aldosterone from the adrenal cortex. Sodium

retention and hypertension develop and act in concert to suppress further formation of angiotensin. Eventually a point is reached where the angiotensin blood level drops below the concentration necessary to stimulate aldosterone production, and the levels of the steroid return to normal. However, the increased vascular sensitivity to angiotensin, as a result of elevated intravascular stores of sodium, maintains the hypertensive state despite the return of angiotensin levels to normal values.

The above data indicate that renin, angiotensin, and aldosterone most likely play a role in certain phases or types of hypertensive disease. Yet there are discrepancies which have not been adequately explained: the rapid return of blood pressure to normal when the circulation to a clipped kidney is restored and the contralateral kidney removed, but in addition, the maintenance of hypertension when the clipped kidney is removed and the contralateral kidney left in place (Tobian et al., 1959). An investigation into this phenomenon by Singer et al. (1963) found the secretion of aldosterone to be elevated in hypertensive rats in which one renal artery was clipped and the other kidney unaltered but not in rats with one renal artery clipped and the contralateral kidney removed. It is also difficult to understand the failure of severe salt restriction to lower blood pressure in renal hypertensive rats (Redleaf and Tobian, 1958) or the failure of diets low in salt content to lower blood pressure in more than two-thirds of all hypertensive patients (Corcoran, et al., 1951).

Evidence in support of the renoprival theory of hypertension is limited. Grollman et al. (1949) were the first to demonstrate the development of hypertension in animals following removal of both kidneys. Muirhead et al. (1956) suggested that normal kidneys secrete a substance with antihypertensive properties and that hypertension results when the

kidneys fail to secrete this substance. Later it was demonstrated that peritoneal implantation of renal medullary tissue prevents the development of renoprival hypertension (Muirhead et al., 1959), and in 1966 a lipid displaying antihypertensive properties was isolated from the medullary tissue (Muirhead et al., 1966). It was proposed that functional renal tissue is necessary for maintenance of the normotensive state. Mendlowitz et al. (1961) state that it is not as yet known if there is a human counterpart for this type of experimental hypertension.

The catecholamines, in particular, norepinephrine (NE) and epinephrine (E), have long been suspected of playing a role in the etiology of hypertension primarily because of their prominent actions on the cardiovascular system. However, as Pert (1966) points out, there is hardly any evidence of credit which implicates increased circulating catecholamines in ordinary cases of hypertension with the noteable exception of pheochromocytoma. Therefore, if E or NE is to be implicated, some change must be demonstrated in either their synthesis, degradation, storage, release, or sensitivity of the vascular wall to locally released NE.

The synthetic pathway for the formation of the catecholamines has now been well established with the isolation and characterization of tyrosine hydroxylase by Nagatsu et al. (1964). Although this enzyme was the first to be involved in the synthesis of the catecholamines, it was the last to be isolated and characterized. The major pathway for the synthesis of NE and E is shown in Figure 2.

Tyrosine hydroxylase seems to be localized in subcellular particles which sediment at 15,000 to 20,000 x g (i.e., the mitochondrial fraction). It requires a tetrahydropteridine cofactor, Fe^{++} and oxygen (Udenfriend, 1966). L-Dopa decarboxylase (aromatic L-amino acid decarboxylase) activity

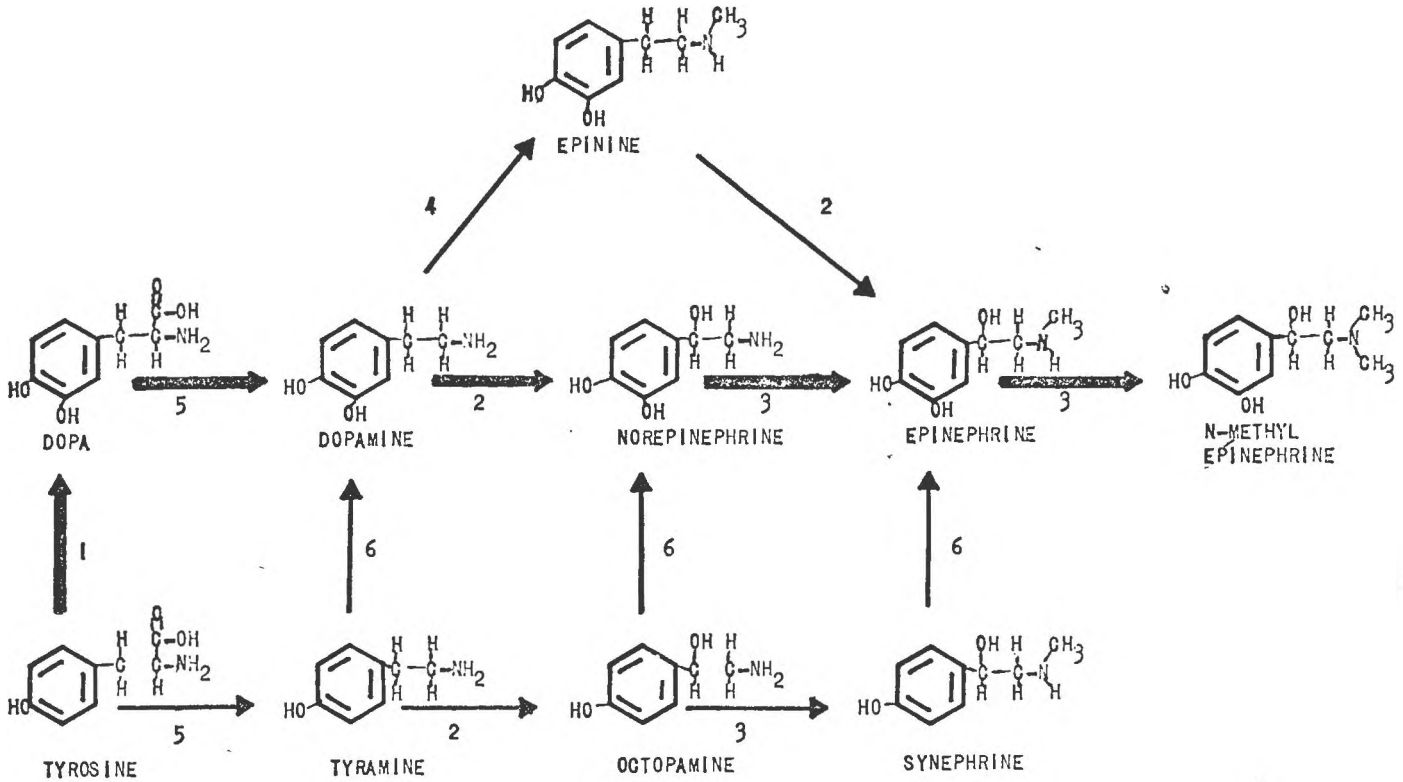

Fig. 2. Main and alternate pathways in the formation of catecholamines: 1) tyrosine hydroxylase, 2) dopamine β-oxidase, 3) phenylethanolamine-N-methyl transferase, 4) nonspecific N-methyl transferase in lung, 5) aromatic amino acid decarboxylase, 6) catechol-forming enzyme (After Axelrod, 1966).

remains in the supernatant fraction following high-speed centrifugation and is not, therefore, particle bound (Lovenberg et al., 1962). This enzyme requires pyridoxal phosphate as a cofactor (Green, 1945). Dopamine-β-oxidase activity has been linked to the chromaffin granules of the adrenal medulla (Levin et al., 1960; Oka et al., 1967) and the granulated vesicles of sympathetic nerve endings (Potter et al., 1963). It requires oxygen, ascorbic acid, and a dicarboxylic acid as cofactors (Levin et al., 1960). Phenylethanolamine-N-methyl transferase is for the most part confined to the adrenal medulla in mammals and is found in the supernatant of cell homogenates. It requires S-adenosylmethionine as a methyl donor (Axelrod, 1962a). Tyrosine is readily available in the circulation in levels of 10-15 mg/l (Spector et al., 1963). It is therefore presumed that tyrosine is taken up into sympathetic nerve endings and other sites of catecholamine synthesis by an active transport system similar to that acting in the brain (Chrivos et al., 1960). Within the cell, tyrosine passes from the cytoplasm to the mitochondria where it is converted to 3,4-dihydroxyphenylalanine (DOPA) which returns to the cytoplasm where it is decarboxylated to dopamine (DM). Finally, DM enters the granulated vesicles where it is converted to NE. In the adrenal medulla some of the formed NE passes back into the cytoplasm where it is converted to E. The E then re-enters the granulated vesicles for storage (Axelrod, 1966).

The available evidence supports tyrosine as the immediate precursor from which the catecholamines are synthesized. In addition to being readily available in the circulation (*hoc. sit*), adequate levels are maintained even in starvation states (Melmon et al., 1964). Other possible precursors such as DOPA or DM are not readily available in the circulation (Anton et al., 1964).

Factors controlling the rates of catecholamine synthesis are not yet fully understood. Udenfriend (1966) presents rather convincing evidence that tyrosine hydroxylase activity represents the rate-limiting step in NE synthesis: Of the three possible precursors (i.e. tyrosine, DOPA and DM), saturation could be achieved only with tyrosine. The V_{max} for tyrosine hydroxylase is two or three orders of magnitude lower than the values for DOPA decarboxylase or dopamine-β-oxidase, its activity being expressed in millimicromoles of tyrosine oxidized per gram of tissue per hour compared with micromoles of DOPA or DM converted per gram of tissue per hour. Thus, tyrosine hydroxylase becomes limiting because the amount of enzyme is limiting. Studies with inhibitors add further support. α-Methyl-p-tyrosine, α-methylphenylalanine, 3-iodotyrosine, 3-iodo-α-methyltyrosine and Hassle 22/54, all inhibitors of tyrosine hydroxylase, lower tissue levels of NE when administered to animals. However, disulfiram, which inhibits dopamine-β-oxidase, will also lower tissue levels (Musacchio et al., 1964). In contrast, benzyloxyamine and certain benzylhydrazine analogs, also inhibitors of dopamine-β-oxidase, will not lower NE levels appreciably <u>in vivo</u> (Nikodijevic et al., 1963). There is therefore a possibility that under certain circumstances dopamine-β-oxidase could be rate-limiting. DOPA decarboxylase cannot be considered rate-limiting since it apparently cannot be inhibited sufficiently to interfere with catecholamine synthesis (Hess et al., 1961).

Factors other than enzyme activity could conceivably influence the rate of catecholamine biosynthesis. Availability of substrate, enzyme cofactors, and integrity of the cellular compartments may play a role. In regard to this latter point, it has been shown that reserpine depresses NE synthesis in sympathetic nerves possibly by causing damage to the

granulated vesicles (Dahlstrom et al., 1965). The activity of the sympathetic nervous system itself may play a role. NE synthesis from tyrosine is depressed in the denervated submaxillary gland (Musacchio and Weise, 1965). However, available evidence, though sparse, suggests that sympathetic tone is normal in hypertension (Pickering, 1936; Pert, 1966).

Since an increase in synthesis of the catecholamines would necessarily be reflected by an increase in their degradation products, it is necessary to have an understanding of the basic mechanisms of catecholamine metabolism. A great deal of advancement has been made in this area within recent years. Basically two enzymes are involved in the metabolic degradation of the catecholamines: Monoamine oxidase (MAO) and catechol-O-methyl transferase (COMT).

MAO is widely distributed in the tissues, being localized in the mitochondria (Blaschko et al., 1957). Especially large quantities occur in sympathetic nerve endings (Snyder et al., 1965). It appears that its role in catecholamine metabolism is limited to disposing of excess stored material within the sympathetic nerve endings (Koplin, 1964). MAO has no apparent role in the physiological inactivation of NE or E since near total inhibition of the enzyme has no effect on the physiological responses to either injected or nerve-released catecholamines (Burn et al., 1954; Griesemer et al., 1953). On the contrary, MAO plays an important role in regulating the tissue levels of the catecholamines. Marked inhibition of MAO leads to a rise in the tissue levels of NE and other endogenous amines, especially serotonin, DM and tyramine which serve as better substrates for MAO than NE and E (Koplin, 1964; Shore et al., 1957). In a latter publication, Shore (1966) points out the importance of MAO in regulating the uptake and storage of biogenic monoamines in sympathetic innervated tissues.

Catechol-O-methyl transferase (COMT) is found in almost all tissues and is highly concentrated in the liver and kidneys (Axelrod and Tomchick, 1958). COMT activity remains in the cytoplasm following high speed centrifugation and its function is considered to be extra-neuronal since tissue levels of NE are unaffected by the activity of the enzyme (Crout et al., 1961). Axelrod (1966) presents extensive evidence to support the view that COMT acts primarily upon circulating amines and amines released from sympathetic nerve endings. In addition, he presents additional evidence to implicate the enzyme, phenylethanolamine N-methyl transferase (PEMT) in the metabolism of NE. The major, and some minor, pathways for the catabolism of NE and E are shown in Figure 3.

The three major excretory products of NE and E are 3-methoxy-4-hydroxymandelic acid (vanilylmandelic acid; VMA), normetanephrine (NMN) and metanephrine (MN) (Armstrong et al., 1957; Axelrod and Tomchick, 1958). The major portion of VMA in the urine probably represents NE produced and deaminated within the nerve ending before it has a chance to produce a physiological effect (Kopin, 1964). Therefore, NMN for the most part represents NE which has been physiologically discharged from the sympathetic nerves.

There have been surprisingly few well-controlled studies regarding E and NE levels in ordinary cases of hypertension. Euler et al. (1954) found some evidence of increased excretion of free and conjugated NE in a limited number of hypertensive patients. Romano et al. (1966) reported urinary VMA levels to be approximately twice as high in hypertensive subjects as compared to control values. Stott and Robinson (1967) found NMN excretion higher in hypertensives. However, the majority of investigators have failed to find any consistent elevation in tissue or urinary levels

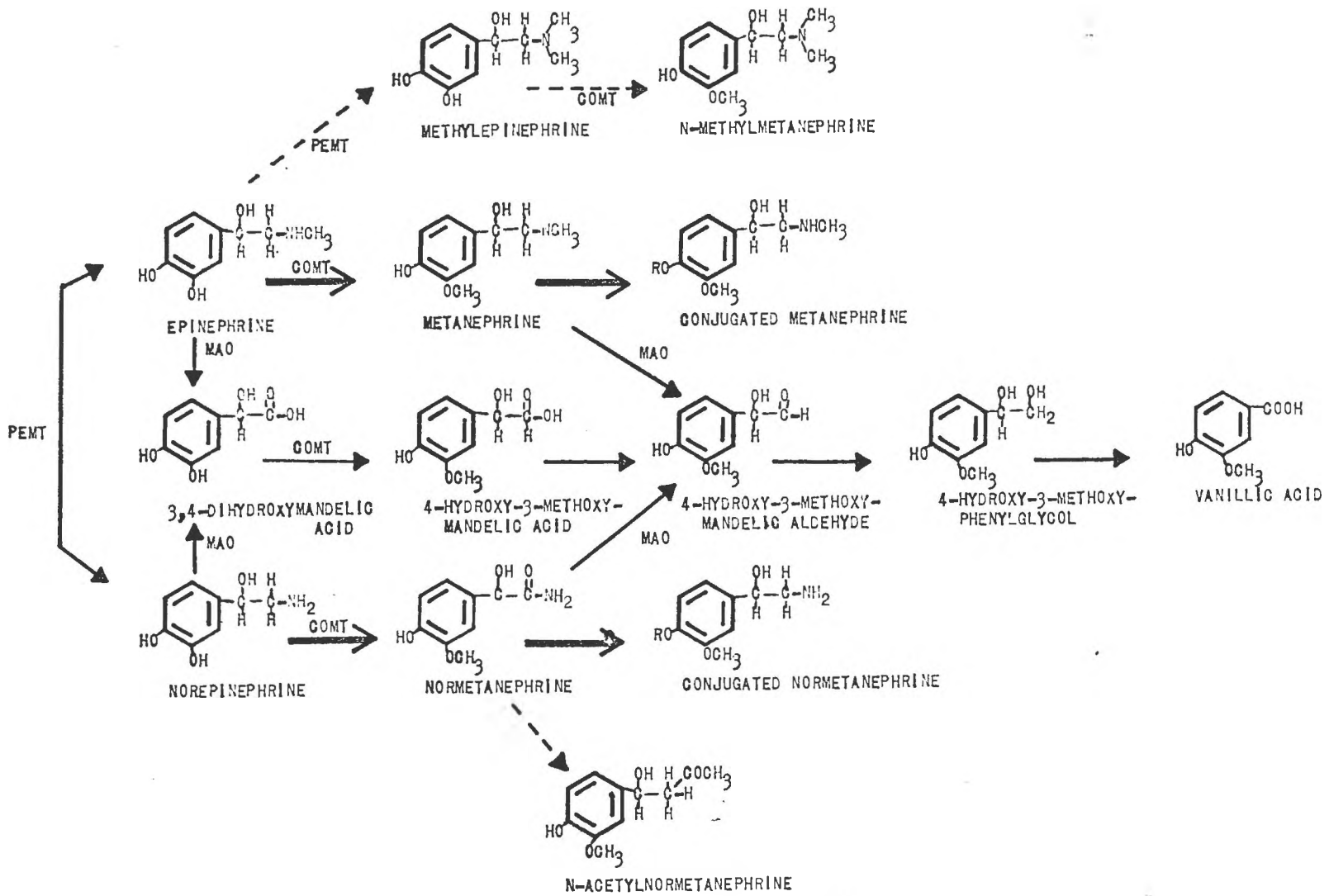

Fig. 3. Metabolism of norepinephrine and epinephrine. Heavy arrows indicate main pathway for tissue metabolism, solid arrows indicate main pathway for intraneuronal metabolism, dashed arrows indicate minor pathways of metabolism. Catechol-O-methyl transferase (COMT), monoamine oxidase (MAO), phenylethanolamine N-methyl transferase (PEMT). (After Axelrod, 1966).

of E, NE or their metabolites in hypertensive disease(Brunjes, 1964; Gitlow et al., 1963; Gitlow et al., 1962; Petrasek et al., 1966; Sjoerdsma, 1961; Raab, 1961; Wegmann et al., 1962).

It is quite possible tha NE could participate in the hypertensive process at the local tissue level without giving rise to increased plasma levels or excretion rates. If both the release and re-uptake of NE from its storage sites were increased, then increased sympathetic activity would be possible without any outward signs of NE involvement. Studies into the possibility of an increased local turnover have been rather limited. Gitlow et al. (1964), using tritiated NE, found a trend for decreased metabolic clearance in advanced hypertensive patients. Mendlowitz et al. (1959) proposed a hypothetical deficiency of COMT in the vascular wall. However, Sjoerdsma et al. (1961) could show no deficiency of COMT or MAO activity in hypertension. Reciently DeChamplain et al. (1967) demonstrated decreased accumulation of tritiated NE in certain tissues of DOCA (desoxycorticosterone acetate) hypertensive rats. A highly significant inverse relationship was shown between blood pressure and both endogenous and labeled NE levels in the heart. They also demonstrated an increase in NE metabolites in the hypertensive animals. A defect in amine storage was offered as a possible explanation.

It is also possible for NE to participate in the hypertensive process at normal tissue levels if the vascular wall becomes over-responsive. There are a growing number of reports which suggest such a possibility (Raab, 1961; Doyle and Fraser, 1961; McCubbin and Page, 1963; Baum and Shropshire, 1967). Pert (1966) sheds doubt on the theory of increased vascular sensitivity and advocates caution in the interpretation of experimental results. Additional studies are necessary in this area before the importance of these findings can be established.

Recent evidence suggests that angiotensin is a highly potent releaser of adrenal catecholamines (Feldberg and Lewis, 1964; Ibid. 1965). The implications are that the catecholamines may be involved in the renin-angiotensin theory of hypertension. Other investigators, however, have not been able to confirm these findings. Vincent et al. (1965) could show no effect of angiotensin administration on VMA excretion in labile hyperrensive subjects, and Healy (1967) also could not show any effect on either VMA or urinay catecholamine excretion rates.

One would have to conclude that no convincing evidence currently exists to directly implicate NE or E in the hypertensive process, but one would equally have to conclude that there is no convincing evidence which would completely exonerate them.

The immediate precursor to NE, dopamine (DM), has received very little attention as a possible parricipant in the hypertensive process. And yet, DM possesses all the attributes of a vasoactive substance. It is pressor in the cat (Holtz et al., 1963), predominantly so in the dog (Goldberg and Sjoerdsma, 1959; Maxwell et al., 1960; Holmes and Fowler, 1962; McDonald and Goldberg, 1963), in man (Horwitz et al., 1962; Allwood and Ginsburg, 1964), and in the rat (Holtz et al., 1942). Most of these investigators attributed the pressor response to an increase in cardiac output since peripheral resistance was found to either decrease or remain relatively unaffected. Small doses of DM, its administration following drugs which block the alpha receptors, and usual doses in the rabbit or guinea pig result in a depressor response which is unaffected by prior administration of beta adrenergic blockers (McDonald and Goldberg, 1963; Vanov, 1963; Holtz et al., 1963; and Fble, 1964). These findings would suggest that the pressor effect of DM is at least partially due to action

on the alpha receptors while the depressor response results from an action other than stimulation of beta receptors. Prior administration of reserpine does not affect the response to DM but completely prevents the usual pressor response to tyramine, a potent releaser of NE (McNay and Goldberg, 1966). In addition, Stone et al. (1963) showed that DM does not deplete tissue catecholamines. These findings, coupled with those of Horwitz et al. (1960) who showed that MAO inhibition greatly augmented the pressor response of DM but had little effect on the pressor response to NE, would suggest that DM itself is pressor and does not act by releasing NE or by enzymatic conversion to another catecholamine. Horwitz et al. (1962) have estimated the pressor potency of DM to be 1/25 to 1/50 that of NE in man.

Dopamine is widely distributed in the tissues. Schumann (1959) reported DM to comprise 2% of the total catecholamines in the adrenal medulla, 50% in sympathetic nerves and ganglia, 90% in the liver, 98% in the lungs and 30 to 50% of the total catecholamines in the spleen, pancreas parotid gland, and brain. Euler and Lishajko (1957) reported similar findings for lung and spleen. Other investigators have not been able to confirm the presence of large quantities of DM in mammalian tissues. Wegmann (1963) could detect the presence of DM only in the spleen and kidney of the dog. Anton and Sayre (1964) generally found DM tissue levels to be low, except in the brain for which their values were in general agreement with those of Carlsson (1959). These same authors were unable to detect the presence of either DM or DOPA in the plasma of humans, dogs, or rabbits,

As the immediate precursor to NE, DM is taken up from the cytoplasm of sympathetic nerves by granulated vesicles in the nerve endings

(Kirshner, 1962; Musacchio et al., 1964) and oxidized to NE (Levin et al., 1960; Potter and Axelrod, 1963). Recently an enzyme has been found in rabbit lung which will N-methylate DM to form epinine (Axelrod, 1962b). Dopamine-beta-oxidase will hydroxylate epinine to E (Bridgers and Kaufman, 1962), thus establishing a minor pathway for the synthesis of E. The major metabolic pathway for the degredation of DM leads to the formation of homovanillic acid (HVA) Hornykiewicz, 1966). There is evidence, at least in the brain, that DM is first attacked by MAO leading to the formation of 3,4-dihydroxyphenylacetic acid which is subsequently O-methylated by COMT to produce HVA (Anden et al., 1963; Carlsson and Hillarp, 1962). Carlsson and Waldeck (1964) have reported the normal occurrence of small amounts of 3-methoxytyramine in the brain and a considerable increase following administration of MAO inhibitors. This would indicate that small amounts of DM may be O-methylated prior to oxidative deamination. It seems logical that MAO should be the first enzyme involved in the catabolism of DM in light of what is known about the tissue distribution of DM and MAO. Both the enzyme and its substrate are concentrated within sympathetic nerve endings (hoc.sit.), and there is no good evidence to suggest that dopamine is normally released as a consequence of nerve stimulation. The major pathways for the synthesis and catabolism of DM are presented in Figure 4.

Dopamine and its metabolites are known to increase in the urine in neuroblastoma (Kaser, 1966), melanoma (Duchon and Gregora, 1962), familial dysautonomia (Smith et al., 1963) and malignant pheochromocytoma (Robinson et al., 1964); but the possible role of DM and its metabolites in hypertension have been given only passing interest. Bischoff and Torres (1962) report the urinary excretion of DM to be within the normal range in a

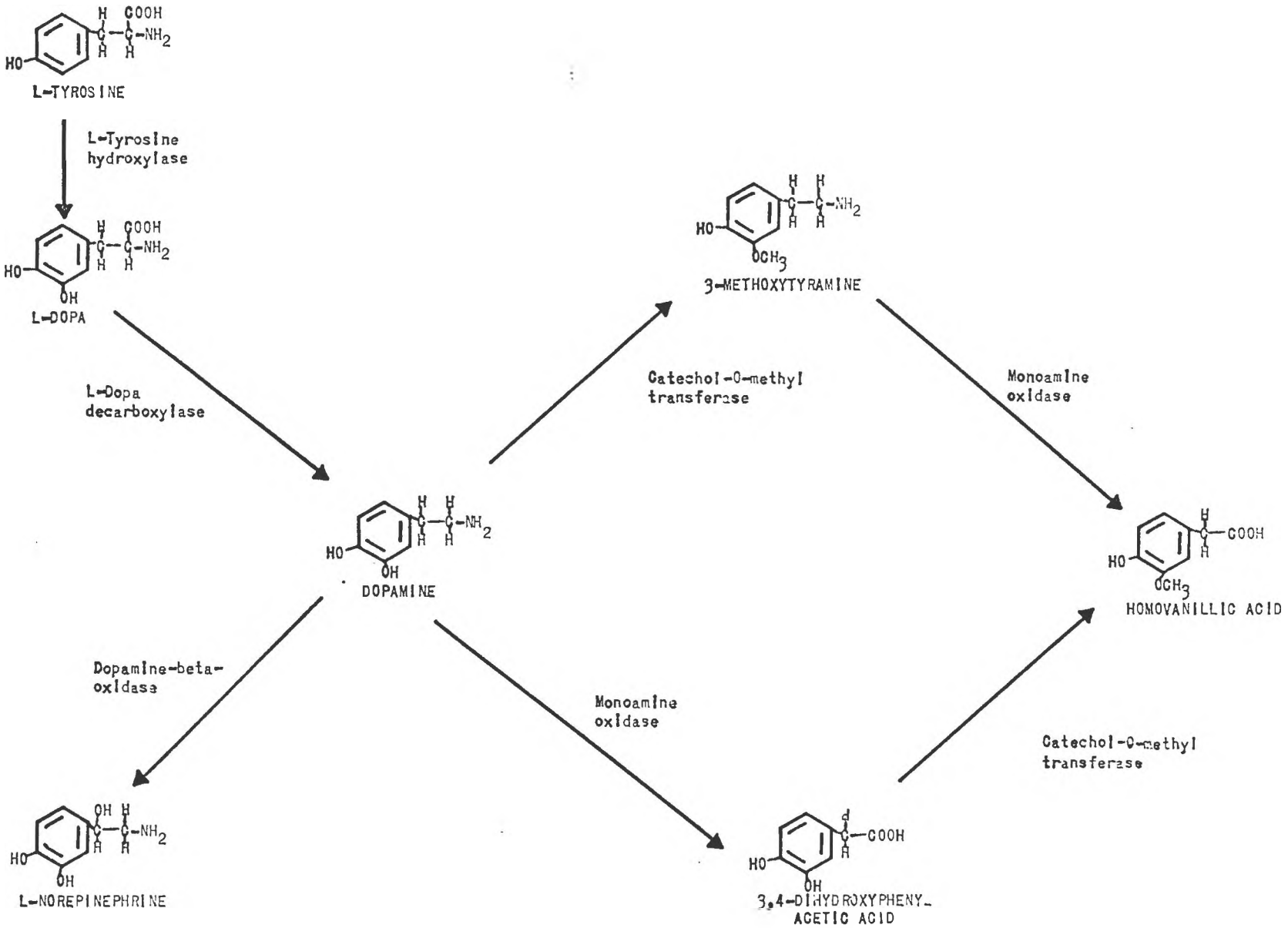

Fig. 4. Main pathway of biosynthesis and catabolism of dopamine in the mammalian organism. L-dopa: L-dihydroxyphenylalanine; MAO: monoamine oxidase; COMT: catechol-O-methyltransferase. (After Hornykiewicz, 1966).

very limited number of hypertensive patients, and Petrasek et al. (1966) reported HVA excretion to be normal in a larger number of patients. The only study dealing directly with the possible role of DM in hypertension appears to be that of DeFanti and DeFeo (1963). These investigators found the urinary excretion of DM elevated in renal hypertensive rats and found a positive correlation between the blood pressure in these animals and the logarithm DM concentration.

The findings of DeFanti and DeFeo (1963), the established pressor activity of DM in man and animal, and the lack of controlled studies into the possible involvement of DM in renal hypertension suggest the need for further studies in this area.

III. INVESTIGATION

A. OBJECTIVES

The objectives of this investigation are as follows:

1. To develop a reliable method for the assay of dopamine in rat urine.

2. To determine if the [illegible] urinary excretion of dopamine in rats and dogs rendered hypertensive by renal ischemia.

3. To determine if the arterial blood pressure is significantly dependent upon the increase in dopamine production.

4. To determine the role of the kidney in the elevation of tissue dopamine levels which follow the administration of dihydroxyphenylalanine.

B. MATERIALS AND METHODS

1. Production of Experimental Hypertension

Male albino rats of the Sprague Dawley strain weighing 90 to 100 g were used for this investigation. Experimental renal hypertension was induced by a two-stage operation under ether anesthesia according to the method of Goldblatt et al. (1934) as modified by Dury (1938). The first consisted of removal of the right kidney through a dorsal incision; and the second, compression of the left renal artery. A period of two weeks separated the two operations to allow time for the animals to recover and to carry out the procedures listed below. Compression of the left renal artery was carried out in the following manner: The artery was located through a ventral midline incision in the lower abdomen and carefully separated from the renal vein and surrounding connective tissue. A wire stylus obtained from either a 19-, 20- or 21-gauge needle was laid alongside the artery, the choice of size being based upon an attempt to produce a

reduction in renal blood flow of at least 50%. A silk ligature was then double-knotted around both the artery and stylus, temporarily cutting off renal blood flow. The stylus was then carefully removed, leaving the ligature in place. The muscle layer was sutured and the skin incision closed with wound clamps. The rats were divided into three groups of 16 each and housed 8 to a cage.

A group of sham-operated rats served as controls and was run concurrently with the experimental groups. These animals were also subjected to two operations: the first, removal of the right kidney; the second, manipulation of the left renal artery. The indirect systolic blood pressure of each animal was obtained initially at weekly intervals and then at two-week intervals. An Infraton[1] unit connected to a sensitive oscilloscope (1 mV/cm deflection) was used to obtain a pulse wave from the tail. A special plexiglass holder was devised for the pressure-sensitive microphone which, with a screw, permitted adjustment of the pressure applied to the microphone in order to obtain the maximum pulse wave. A rat tail cuff attached to an aneroid manometer was placed proximal to the microphone on the rat's tail. The cuff was inflated to a pressure well above the anticipated systolic pressure. Systolic blood pressure was recorded as that point, to the nearest 5 mm Hg, where the pulse wave returned as the pressure was slowly released from the cuff. As temperature variation was found to create difficulties in obtaining an adequate pulse wave, all determinations were carried out in a constant temperature room at 25° C.

2. Collection of Urine

Following the blood pressure determinations, rats within the same

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1. Beckman Instruments Inc., Spinco Division, Palo Alto, California.

group having the same or nearly the same blood pressures were paired and placed in stainless steel metabolism cages to collect 24-hour urine samples. A sufficient quantity (4-5 drops) of concentrated sulfuric acid was added to the collection vessel to insure an acid milieu in order to prevent breakdown of urinary catecholamines. The total urinary output for 24 hours was recorded in ml and the samples frozen at -40°C for subsequent determination of dopamine content.

3. Collection of Blood Samples

At the end of the experiment each of the surviving rats was sacrificed by decapitation and the blood collected. A small amount of heparin was added to each tube to prevent coagulation. The samples were then centrifuged to separate the plasma, which was removed and frozen for subsequent dopamine assay.

4. Comparison of Kidney Dopamine Content

An additional group of hypertensive rats was prepared for the purpose of determining any changes in the renal tissue content of dopamine which might be associated with the hypertensive state. Under ether anesthesia the right kidneys were removed, blotted to remove excess blood, weighed, and then frozen in liquid nitrogen and stored at -40°C to await assay for dopamine content. Eight weeks following the second operation, the surviving rats were sacrificed by decapitation, and the left kidneys removed and treated as above. The blood from these animals was pooled to form one large sample which was subsequently assayed for dopamine content. The blood pressure of these animals was recorded only twice: just prior to the initial operation while under ether anesthesia and just prior to sacrifice, again under ether anesthesia.

5. Hypertensive Dogs

One female mongrel dog and one male mongrel dog were also rendered hypertensive by a two-stage operation. In the first procedure the right kidney was removed under pentobarbital anesthesia, 30 mg/kg, through an incision made in the dorsal surface just below the rib cage. A double silk ligature was tied around the renal artery, vein and ureter near the renal pelvis. A cut was made proximal to these ties, and the kidney was then removed. Care was exercised to prevent damage to the adrenal gland. The muscle incision was closed with chromic gut and the skin with dermal silk. After a two-week recovery period, the second stage of the operation was carried out, again under pentobarbital anesthesia. An incision was made in the linea alba from the xyphoid process to include the umbilical scar. The left renal artery was freed from the renal vein and stripped clean of fascia. A silk ligature was placed underneath the artery; and a metal stylus, prepared from a series of common finishing nails, was selected with the intent of reducing the lumen of the artery approximately 50% and was laid alongside the artery. A double-knotted tie was then completed around both the artery and the stylus, momentarily cutting off renal blood flow. The stylus was then removed, restoring renal blood flow but leaving a constriction in the artery. The loose end of the ligature was anchored to the dorsal muscle surface to prevent slippage. The incision was then closed in the usual manner. Two dogs were utilized as controls. One male underwent surgical removal of the right kidney and simultaneous manipulation of the left renal artery to serve as a sham-operated control; the other, a male, served as an unoperative control. All surgery was carried out under aseptic technique, and post-operative antibiotics were utilized to minimize the chances of infection.

Weekly blood pressure recordings were obtained from the tail of the dogs using the Infraton unit and finger-occluding cuff attached to an aneroid manometer. Twenty ml blood samples were collected at weekly intervals using EDTA-charged Evacuettes[2]. The dogs were also placed in metabolism cages each week for collection of 24-hour urine samples. Some difficulty was encountered in getting the dogs to void while within the confines of the metabolism cage, and 5 mg of Urecholine Cl[3] subcutaneously was occasionally employed to force emptying the bladder. Sulfuric acid was again utilized to provide an acid environment during the collection period. The total urinary output for 24 hours was recorded and an aliquot taken and frozen along with the blood samples at -40°C for subsequent dopamine assay.

6. Cat Infusion Studies

A separate experiment was undertaken in cats to determine the role of the kidney in the elevation of tissue dopamine levels known to occur following infusion of d,l-dihydroxyphenylalanine (Wegmann, 1963). Two female cats and two male cats were sacrificed as controls to determine endogenous levels of dopamine. They were first anesthetized with sodium pentobarbital 35 mg/kg intraperitoneally, then cardiac standstill was produced by the intravenous administration of potassium chloride. The heart, left kidney, spleen and one lobe of the liver were removed, blotted dry and their weights recorded. The organs were immediately placed in 10% trichloroacetic acid and homogenized in a Virtis "45"[4] homogenizer. The mixture was

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2. A product of Becton, Dickinson & Co., Rutherford, New Jersey

3. Merck, Sharpe & Dohme's Trade Name for Bethanechol Cl.

4. The Virtis Co. Inc., Division of Cenco Instruments, Gardiner, New York

transferred to centrifuge tubes and centrifuged at 12,000 RPM for 20 minutes in a Lourdes Model AB centrifuge to separate the clear supernatant. The volume of the supernatant was adjusted to 50 ml with 10% trichloroacetic acid and a 25 ml aliquot utilized for the determination of DOPA and dopamine.

In four other cats, d,l-DOPA[5] 50 mg/kg was infused into the femoral vein over a 20-minute period. Sodium pentobarbital 35 mg/kg was used as the anesthetic. An arterial catheter was inserted into the abdominal artery via the femoral artery to a point just below the origin of the left renal artery for the purpose of obtaining 5 ml blood samples midway through the infusion period and one hour after initiation of the infusion. A catheter was likewise inserted through the left ovarian vein or the left spermatic vein into the left renal vein to obtain corresponding venous blood samples. The right renal artery and vein were completely ligated in these experiments. One hour after starting the infusion, the cats were sacrificed by an iv injection of potassium chloride and the heart, left kidney, spleen and a lobe of the liver removed and treated as described above. The blood samples were immediately treated by the addition of 10 ml of 10% trichloroacetic acid.

In four additional cats 50 mg/kg of d,l-DOPA was infused as above except that both renal arteries and veins were tightly ligated to remove the kidneys from the general circulation. Blood samples were not taken in these animals since they would not be representative of a renal A-V difference. The heart, spleen and a lobe of the liver were removed as previously described and assayed for their dopamine and DOPA content.

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5. d,l-dihydroxyphenylalanine was obtained from Nutritional Biochemicals Corp., Cleveland 28, Ohio.

By a comparison of the results from these studies, it was hoped that the contribution of the kidney to tissue levels of dopamine in the heart, liver and spleen could be determined.

7. Determination of Dopamine and DOPA

With certain modifications, the trihydroxyindole method of Carlsson and Waldeck (1958) was used for the fluorimetric estimation of dopamine and DOPA. The blank as employed by these investigators left out sodium sulfite, which is included as an ingredient in one of the reagents used to develop the fluorophore of dopamine and DOPA. The omission of this substance allows a faint iodine color to remain in the blank which acts as a quenching agent resulting in erroneously low blank values. In the present procedure sodium sulfite was added out of the normal sequence, following the addition of all other reagents. Thus, the blank utilized in the present procedure contains all of the chemicals found in a standard assay solution. Additional modifications of the original method included: adjustment of the strength of certain of the reagents, alterations of reaction times and heating the solution prior to UV irradiation. The necessary reagents and a description of the development procedure which was used, are as follows:

0.1 M Phosphate buffer pH6.5 Dissolve 5.34 g Na_2HPO_4, 2 H_2O in 500 ml of water. Dissolve 9.59 g KH_2PO_4 (Sorensen) in 500 ml water. Add the former solution to the latter, utilizing a pH meter to adjust the pH to 6.5.

Iodine Solution Dissolve 0.252 g iodine and 5.0 g potassium iodide in 100 ml water.

4.5 N Sodium Hydroxide Solution

Alkaline Sulfite Solution Dissolve 5.04 g Na_2SO_3, 7 H_2O in 10 ml water and dilute with 5 N sodium hydroxide to 100 ml.

6 N Acetic Acid Solution Dilute 34.7 ml glacial acetic acid with water to 100 ml.

Sodium Sulfite Solution Dissolve 5.04 g Na_2SO_3, 7 H_2O in 10 ml water.

Glass distilled water was used throughout.

A three ml aliquot of a column eluate (see sections 8, 9, 10, 11) was transferred to a 15 ml volumetric centrifuge tube and the pH adjusted to 6.5 by the addition of one ml of 0.3 M potassium carbonate in the case of a 0.2 N acetic acid eluate or by the dropwise addition of 5.0 M potassium carbonate to a 2.0 N hydrochloric acid eluate followed in either case by the addition of one ml phosphate buffer pH 6.5. One-tenth of a ml of iodine solution was added to the tube, the contents mixed and allowed to react for three minutes. Then one ml of alkaline sulfite reagent was added, the contents mixed and this reaction allowed to proceed for four minutes. Finally, the pH of the solution was adjusted to approximately 5.4 by the addition of one ml of 6 N acetic acid and the final volume adjusted to 10 ml by the addition of distilled water. The tube was then transferred to a boiling water bath for five minutes, cooled and centrifuged. Two to 3 ml of the resultant solution was transferred to a fused quartz cell and irradiated under a short wave (254 mμ mercury lamp for 10 minutes.[6] The fluorescence of the solution was read in an Aminco-Bowman Spectrophotofluorometer[7] at an activating wavelength of 325 mμ and a fluorescent wavelength of 380 mμ (uncorrected instrument values).

A blank was determined for the assay by utilizing an additional 3 ml column aliquot and treating it as outlined above except that 4.5 N NaOH was substituted for the alkaline sulfite reagent and 0.1 ml of sodium

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6. Chromato-Vue, Black Light Eastern Corp., New York, New York.

7. Aminco-Bowman Spectrophotofluorometer, American Instrument Co., Inc., Silver Springs, Maryland. Specifications for the test: Xenon Lamp, Slit Arrangement Number 5, Photomultiplier Tube iP28.

sulfite solution was added to 9.9 ml of the resultant solution just prior to centrifugation. The procedure allowed the handling of up to 18 samples at one time.

Estimation of dopamine or DOPA content in the developed solutions was made by dividing the corrected fluorescent intensities of the unknowns by a value calculated to represent the fluorescent intensity of a solution of dopamine or DOPA containing 0.1 μg/ml. This standard value was determined each time a series of unknowns was assayed to guard against deterioration of the reagents and day-to-day variation in fluorescent intensity and was obtained in the following manner: Acidic solutions of dopamine or DOPA to give final concentrations of 0.05, 0.1, 0.2 and 0.3 μg/ml and a blank were subjected to the procedures outlined above. These concentrations were all within the range of linearity reported by Carlsson and Waldeck (1958), confirmed by DeFanti (1961) and supported by the present work. Correction for fluorescence contributed by the reagents was made by subtracting the fluorescent intensity of the blank from the fluorescent intensities of the standard solutions. These corrected values were then adjusted and averaged to obtain a single value representing the fluorescent intensity of a 0.1 μg/ml solution of dopamine or DOPA. The following formulas summarize the mechanics:

$$I_s = \sum \left(F_s - R.B. \times \frac{0.1}{C}\right) / n$$

where I_s = fluorescent intensity of a 0.1 μg/ml solution of dopamine or DOPA

F_s = uncorrected fluorescent intensity of the developed solutions at concentration C

R.B. = fluorescent intensity of the reagent blank

C = final concentrations of dopamine or DOPA in μg/ml

n = the number of determinations

and finally:

$$C_u = (F_u - B_u) \ / I_s$$

where C_u = the concentration of dopamine or DOPA (μg/ml) in the developed solution of the unknown

F_u = the uncorrected fluorescence intensity of the developed solution of the unknown

B_u = the fluorescent intensity of corresponding blank of the unknown

I_s = the fluorescent intensity of the standard as determined above

Since the final volume of the developed solutions was 10 ml, the total dopamine or DOPA content of a 3 ml column aliquot was equivalent to 10 times the concentration in μg/ml (10 C_u) and the total in a 10 ml eluate to 100 $C_u/3$. The total amount of dopamine or DOPA in a sample was then calculated by the use of the following formula:

$$D_t = \frac{V_1}{V_2} \cdot \frac{100\ C_u}{3}$$

where D_t = the total quantity (μg) of dopamine or DOPA in a sample

V_1 = the volume (ml) of the sample extracted for dopamine or DOPA content

V_2 = the total volume (ml) of the sample

C_u = the concentration of dopamine or DOPA (μg/ml) in the developed solution of the unknown

8. Extraction of Catecholamines from Urine

Following modification of the blank as outlined above, it was discovered that the sodium form of Dowex 50W-X8 was extracting a substance from rat urine which displayed native fluorescence interfering with the estimation of dopamine. It was therefore necessary to develop a different method for the extraction of catecholamines from urine other than that

employed in the original study (DeFanti, 1961; Crawford and Law, 1958; Bertler et al., 1958). Various methods of absorption on alumina at pH 8.4 were tried (Weil-Malherbe and Bone, 1952; v. Euler and Lishajko, 1953; Weil-Malherbe, 1961; Weil-Malherbe, 1964; Small, 1963) and found to avoid the problems encountered with Dowex. The final extraction method as outlined below is an adaptation of these various methods incorporating what was found by trial and error to give the best urinary recoveries of dopamine.

Alumina for chromatographic analysis supplied by British Drug Houses, LTD[8] was found to possess the most desirable characteristics and was used in this investigation. Prior to use, the alumina was suspended in 500 ml of 2 N hydrochloric acid and heated to 80°C for 20 minutes with constant stirring, filtered onto a glass-sintered filter and washed with an additional 500 ml of 2 N hydrochloric acid. The alumina was then resuspended in 500 ml of glass-distilled water, stirred, allowed to settle for approximately 15 seconds and the water decanted to remove the alumina fines. This was repeated a total of 10 times, and then the alumina was dried at 105°C overnight. Alumina columns were prepared by suspending 0.7 g of the acid-washed alumina in 10 ml of 0.1 M ammonium acetate buffer pH 8.0 and adjusting the pH to 8.4 by the addition of 1 N sodium hydroxide (the quantity of 1 N sodium hydroxide required was determined for each lot of alumina and was usually about 0.3 ml). The suspension was agitated for one minute and then poured into a column previously filled with glass-distilled water and plugged with a small pledget of cotton. The columns were constructed of 7 mm I.D. pyrex glass tubing drawn to a 4 mm O.D. tip

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8. *Exclusive United States Distributors: Gallard-Schlesinger Manufacturing Corp., 580 Mineola Avenue, Carle Place, Long Island, New York 11514

to which polyethylene tubing was attached. The flow rate through the columns was regulated to 2 to 3 ml per minute, when necessary, by adjustable clamps on the tubing. Nalgene[R] funnels served as the reservoirs. The formed columns were washed with 5 ml of glass-distilled water and clamped until addition of the urine samples. Dog or rat urine aliquots were first centrifuged for 20 minutes in a clinical centrifuge and then 10 ml (minimum 5 ml) transferred to large pyrex test tubes. Two drops of concentrated hydrochloric acid and 2.0 ml of 0.2 M ethylenediamine tetraacetic acid (disodium salt) were added to each tube. The tubes were stoppered with corks equipped with 21 g needles as relief valves and heated in a boiling water bath for 20 minutes to hydrolyze any dopamine present in conjugated form. The tubes were cooled and their contents transferred to plastic beakers using three 5 ml rinses of distilled water. The pH of each was then adjusted to 8.4 with 1 N sodium hydroxide using a model 28 Radiometer automatic titrator[9] and immediately added to an alumina column. One ml of 1% sodium metabisulfite was added to each sample during the pH adjustment to guard against oxidation of the catecholamines before absorption onto the alumina. After passage of the urine samples, the columns were washed with 5 ml of 1 M sodium acetate followed by 5 ml of distilled water. Elution was performed by the addition of two separate 5 ml portions of 0.2 N acetic acid. All additions were made just as the meniscus of the previous solution entered the alumina bed. Three ml aliquots of the combined 10 ml eluates from the columns were then assayed for dopamine content as previously described. If this was not carried out within one-half hour, the eluates had to be frozen to prevent breakdown of their catecholamine content.

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9. Radiometer Copenhagen, 811 Sharon Drive, Westlake, Ohio.

Internal standards were run at various intervals throughout the experimental period by the addition of known amounts of dopamine to the urine prior to processing. The recovery values for these standards are recorded in Table 2, the average recovery being 82.43 ± 11.73 % which is somewhat lower than the average recovery of 88.74 ± 5.93 % obtained for known concentrations of dopamine in 0.2 N acetic acid (see Table 1).

9. Extraction of Catecholamines from Rat Plasma

Three methods were used to prepare the blood plasmas of the rats sacrificed at the termination of the experimental period for extraction of their catecholamine content. In one-third of the samples, the plasma proteins were precipitated with perchloric acid and centrifuged; in another third, the samples were hydrolyzed in 1.0 N perchloric acid at 100° C for 10 minutes and centrifuged (Haggendal, 1963); the remaining samples were passed through a column of Sephadex G-25[10] which separated the plasma into catecholamine and protein containing portions (Marshall, 1963). The catecholamines were then extracted from the samples for fluorimetric assay using columns of the sodium form of Dowex 50W-X8 as subsequently described in section 11.

10. Extraction of Catecholamines from Whole Blood and Tissues

The tissues and whole blood samples from the other portions of this study were all extracted by homogenization in 10% trichloroacetic acid. These include the rat kidneys and pooled blood sample from section 4, the blood samples obtained from the dog experiments (section 5) and the tissues and blood samples obtained from the cat infusion studies (section 6). Where only the dopamine content was under investigation,

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10. A product of Pharmacia Fine Chemicals, Inc., 800 Centennial Ave., Piscataway, New Market, New Jersey 08854

TABLE 1

Percent recovery of dopamine from 0.2 N acetic acid

Dopamine Added (μg)	Dopamine Found (μg)	Percent Recovery
2.00	1.80	90.00
2.00	1.93	96.50
2.00	2.13	106.50
2.00	1.67	83.50
2.00	1.77	88.50
2.00	1.77	88.50
4.00	3.57	89.25
4.00	3.57	89.25
4.00	3.57	89.25
4.00	3.67	91.75
4.00	3.43	85.75
4.00	3.43	85.75
4.00	3.57	89.25
6.00	4.90	81.67
6.00	4.90	81.67
6.00	5.47	91.17
6.00	4.77	79.50
6.00	5.47	91.17
6.00	5.23	87.17

Mean recovery $\pm$ standard deviation = 88.74 $\pm$ 5.93 %

TABLE 2

Percent recovery of dopamine added to urine samples

Endogenous Dopamine (μg)	Dopamine Added (μg)	Total Dopamine Calculated(μg)	Found(μg)	Percent Recovery
1.50	2.00	3.50	4.00	114.28
1.00	2.00	3.00	2.1	70.00
1.60	2.00	3.60	2.93	81.39
1.30	2.00	3.30	2.33	70.61
2.73	2.00	4.73	4.03	85.20
1.27	2.00	3.27	3.07	93.88
1.70	2.00	3.70	2.77	74.86
2.07	2.00	4.07	3.33	81.82
0.73	2.00	2.73	2.53	92.67
1.10	2.00	3.10	2.10	67.74
0.73	2.00	2.73	2.50	91.57
0.83	3.00	3.83	3.57	93.21
1.03	3.00	4.03	3.73	92.55
0.87	3.00	3.87	2.73	70.54
1.27	3.00	4.27	3.50	81.97
1.57	3.00	4.57	3.67	80.31
1.07	4.00	5.07	3.90	76.92
1.23	4.00	5.23	3.93	75.14
0.93	4.00	4.93	3.53	71.60

Mean recovery $\pm$ standard deviation = 82.43 $\pm$ 11.73 %

the trichloroacetic acid extracts were passed through columns of alumina as described for urine. The samples from the cat infusion studies, however, were passed through a two-column procedure as described in the following section.

11. Separation of Dopamine and DOPA

A two-column procedure was used to separate dopamine from DOPA in the samples obtained following the infusion of d,l-DOPA in cats. The first column was prepared using the sodium form of Dowex 50W-X8 which has been shown by Bertler et al. (1958) to selectively bind dopamine while allowing DOPA to pass on through the column. The sodium form of the resin was prepared by cycling the resin first through 2 N hydrochloric acid followed by several washes with glass-distilled water, then through several changes of 5 N sodium hydroxide and finally washed with glass-distilled water until the supernatant was neutral. The resin was then filtered by suction onto a glass-sintered filter and stored in moist form in a tightly stoppered bottle. The columns were prepared by suspending 500 mg of the prepared resin in distilled water and pouring the mixture into columns constructed from the discarded barrels of 1 ml tuberculin syringes which had been previously filled with distilled water and plugged with small pledgets of cotton. After the columns had formed, 10 ml of 1.0 N sodium acetate-acetic acid buffer pH 6.0 was passed through each column followed by 5 ml of distilled water. The columns were then ready for use. The samples were prepared for addition to the columns by selecting suitable aliquots of the trichloroacetic acid extracts, adjusting the pH to 6.5 with 5 M potassium carbonate, adding 2 ml of 0.2 M ethylenediamine tetraacetic acid (disodium salt) and 1 ml of 1% sodium metabisulfite to each sample. As a sample passed through a

column (flow rate = 0.25 ml/minute), the effluent (E-1) was collected in a small plastic beaker resting on a magnetic stirrer which contained 1 ml of 2 N hydrochloric acid and a bar to permit continual agitation of the effluent. This was found necessary to prevent destruction of DOPA in the effluent. The column was then washed with two 10-ml portions of distilled water which was combined with the effluent (E-1). The dopamine was then eluted from the column using two separate 5 ml portions of 2 N hydrochloric acid. A 3 ml aliquot of this was assayed for dopamine content as previously described. The volume of the combined effluent and washings (E-1) was adjusted to 50 ml with distilled water, and a 5 ml aliquot taken for extraction and assay of the DOPA content using alumina columns at pH 8.4 and the fluorimetric procedure as previously described for the urine samples. The method as described achieved an excellent separation of DOPA and dopamine with a 94.62 $\pm$ 4.30 % recovery of dopamine and a 78.67 $\pm$ 4.6 % recovery of DOPA as determined from known mixtures of the two substances (see Table 3).

TABLE 3

Percent recovery of DOPA[a] and dopamine from mixtures in 10% trichloroacetic acid

Dopamine			DOPA		
Added(μg)	Found(μg)	Percent Recovered	Added(μg)	Found(μg)	Percent Recovered
4	3.83	95.75	4	3.05	76.25
4	4.17	104.25	4	3.14	78.50
4	3.63	90.75	4	3.25	81.25
4	3.95	98.75	6	4.13	68.83
4	3.65	91.25	6	4.61	76.83
4	3.57	89.25	6	4.87	81.17
4	3.69	92.25	20[b]	16.87	84.35
4	3.91	97.75	20[b]	16.30	81.50
4	3.77	94.25	20[b]	15.48	77.40
0	0	0	20[b]	15.65	78.25
0	0	0	20[b]	16.70	83.50
0	0	0	20[b]	15.25	76.25
4	3.71	92.75	0	0	0
4	3.89	97.25	0	0	0
4	3.65	91.25	0	0	0

Mean recovery + standard deviation: Dopamine = 94.62 $\pm$ 4.30 % DOPA = 78.67 $\pm$ 4.16 %

[a] dihydroxyphenylalanine

[b] an aliquot one-fifth the volume of the combined washings and effluent from the dowex column was assayed for DOPA content.

C. RESULTS

All of the results of the various phases of this study, which include the values from the control and experimental rat study on the relationship of urinary dopamine levels to renal hypertension and the statistical evaluation of these results; the results from the assay of the plasma obtained upon sacrifice of the hypertensive rats surviving the twenty-week study; the results from the assay of the left ischemic kidneys from a group of hypertensive rats; the urinary and blood dopamine levels from the study in dogs; and the results obtained when d,l-DOPA was infused into cats with and without functional kidney tissue, are presented in this section.

1. Data for the Control Group of Rats in the Urinary Dopamine Study are presented in TABLES 4 - 14. Each entry in these tables represents the results obtained from a pooled sample comprised of two animals.

TABLE 4

Control Group (week one). Relationship of urinary dopamine levels (paired samples) to arterial blood pressure (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopamine µg/l	Dopamine µg/24h	Dopamine µg/kg/24h
130	420	4.20	12.28
140	178	2.31	7.86
130	453	6.34	18.43
135	320	4.96	14.21
123	250	4.87	13.16
120	380	3.42	9.83
115	440	5.28	15.00
125	600	6.30	17.40
113	540	3.78	11.32
Mean ± standard deviation			
125.7 + 8.97	397.9 + 133.6	4.61 + 1.37	13.28 + 3.41

[a] control values prior to any operative procedures

TABLE 5

Control Group (week two). Relationship of urinary dopamine levels (paired samples) to arterial blood pressure (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopamine μg/l	Dopamine μg/24h	Dopamine μg/kg/24h
115	163	5.22	11.25
128	237	4.38	11.46
120	597	6.87	15.10
110	440	8.58	20.57
120	400	7.60	16.89
118	514	4.11	9.34
120	437	10.49	24.11
100	323	5.81	13.83
116	353	7.94	17.30
Mean ± standard deviation			
115.7 ± 15.3	384.9 ± 134	6.78 ± 2.10	15.54 ± 4.75

[a] control values prior to any operative procedures

TABLE 6

Control Group (week four). Relationship of urinary dopamine levels (paired samples) to arterial blood pressure (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopamine μg/l	Dopamine μg/24h	Dopamine μg/kg/24h
120	290	9.57	20.19
125	186	6.42	13.78
123	347	6.25	13.21
120	253	3.41	7.41
110	267	5.87	12.36
108	254	6.22	13.40
130	373	10.44	21.79
105	120	4.02	8.20
115	343	7.20	15.25
Mean ± standard deviation			
117.5 ± 8.59	270.3 ± 80.9	6.60 ± 2.28	13.95 ± 4.76

[a] one week following right renal nephrectomy

TABLE 7

Control Group (week six). Relationship of urinary dopamine levels (paired samples) to arterial blood pressure (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopamine μg/l	Dopamine μg/24h	Dopamine μg/kg/24h
115	213	7.24	14.51
130	233	5.83	11.75
113	340	7.82	15.83
115	320	3.84	7.22
120	350	8.75	17.71
123	177	4.51	8.18
115	113	2.77	5.53
120	303	9.09	17.58
110	414	7.45	13.45
Mean ± standard deviation			
117.9 ± 6.05	274 ± 95.8	6.37 ± 2.24	12.42 ± 4.53

[a] one week following left renal artery manipulation

TABLE 8

Control Group (week eight). Relationship of urinary dopamine levels (paired samples) to arterial blood pressure (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopamine μg/l	Dopamine μg/24h	Dopamine μg/kg/24h
118	276	6.21	11.52
110	260	7.14	12.53
105	296	9.03	15.28
123	331	8.11	15.24
125	251	6.78	11.87
120	318	5.72	9.48
110	298	8.34	14.30
120	402	7.63	13.43
110	338	7.43	12.70
Mean $\pm$ standard deviation			
115.7 $\pm$ 7.02	307.8 $\pm$ 46.4	7.38 $\pm$ 1.04	12.91 $\pm$ 1.89

[a] three weeks following left renal artery manipulation

TABLE 9

Control Group (week ten). Relationship of urinary dopamine levels (paired samples) to arterial blood pressure (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopamine μg/l	Dopamine μg/24h	Dopamine μg/kg/24h
117	175	5.15	9.35
130	314	10.37	17.51
115	262	4.72	7.89
120	385	7.13	13.06
110	318	8.76	14.29
110	196	5.89	9.88
120	304	8.21	14.11
113	466	6.76	11.76
125	372	9.11	15.16
Mean $\pm$ standard deviation			
117.8 $\pm$ 6.75	310.2 $\pm$ 91.8	7.34 $\pm$ 1.91	12.56 $\pm$ 3.10

[a] five weeks following left renal artery manipulation

TABLE 10

Control Group (week twelve). Relationship of urinary dopamine levels (paired samples) to arterial blood pressure (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopamine μg/l	Dopamine μg/24h	Dopamine μg/kg/24h
125	257	8.22	14.12
130	332	9.13	14.54
123	458	6.41	9.97
118	324	7.95	13.68
110	445	10.24	15.68
115	210	5.79	9.12
125	336	6.21	10.02
110	394	7.89	13.30
125	389	10.88	17.24
Mean ± standard deviation			
120.1 ± 7.19	349.4 ± 81.9	8.08 ± 1.8	13.07 ± 2.79

[a] seven weeks following left renal artery manipulation

TABLE 11

Control Group (week fourteen). Relationship of urinary dopamine levels (paired samples) to arterial blood pressure (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopamine μg/l	Dopamine μg/24h	Dopamine μg/kg/24h
135	435	6.31	9.55
123	321	8.82	13.12
125	166	4.97	7.15
118	371	10.21	14.77
115	617	15.13	21.19
110	378	6.99	9.55
105	374	7.11	10.20
130	336	8.23	11.38
125	327	9.17	14.06
Mean $\pm$ standard deviation			
120.7 $\pm$ 9.58	369.4 $\pm$ 118.5	8.55 $\pm$ 2.94	12.33 $\pm$ 4.12

[a] nine weeks following left renal artery manipulation

TABLE 12

Control Group (week sixteen). Relationship of urinary dopamine levels (paired samples) to arterial blood pressure (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopamine μg/l	Dopamine μg/24h	Dopamine μg/kg/24h
95	397	9.13	13.50
130	399	10.78	15.69
117	410	6.97	9.79
135	319	9.41	13.09
110	316	6.48	8.91
115	403	13.29	17.64
125	368	11.03	15.27
110	263	7.24	9.88
130	323	5.98	8.64
Mean ± standard deviation			
118.5 ± 12.7	355.3 ± 51.8	8.92 ± 2.46	12.49 ± 3.31

[a] eleven weeks following left renal artery manipulation

TABLE 13

Control Group (week eighteen). Relationship of urinary dopamine levels (paired samples) to arterial blood pressure (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopamine μg/l	Dopamine μg/24h	Dopamine μg/kg/24h
125	400	9.21	12.68
120	417	11.47	15.29
130	322	10.29	13.48
135	366	6.77	9.04
110	560	14.01	17.57
123	704	10.21	13.41
120	470	8.23	10.46
115	229	6.31	8.62
130	300	9.01	11.36
Mean $\pm$ standard deviation			
123.1 $\pm$ 7.89	418.7 $\pm$ 144.3	9.50 $\pm$ 2.37	12.43 $\pm$ 2.91

[a] thirteen weeks following left renal artery manipulation

TABLE 14

Control Group (week twenty). Relationship of urinary dopamine levels (paired samples) to arterial blood pressure (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopamine μg/l	Dopamine μg/24h	Dopamine μg/kg/24h
135	412	5.97	7.67
125	413	13.23	16.51
123	595	16.37	19.81
117	266	6.53	8.17
130	623	11.21	13.29
115	314	7.23	9.07
130	321	9.15	10.92
120	213	6.71	8.48
125	305	8.23	9.66
Mean ± standard deviation			
124.4 ± 6.53	384.7 ± 142.1	9.40 ± 3.54	11.51 ± 4.21

[a] fifteen weeks following left renal artery manipulation

2. Data for the Experimental Group of Rats in the Urinary Dopamine Study are presented in TABLES 15 - 25. Each entry in these tables represents the results obtained from a pooled sample comprised of two animals.

TABLE 15

Experimental Group (week one). Relationship of urinary dopamine levels (paired samples) to arterial blood pressure (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopamine µg/l	Dopamine µg/24h	Dopamine µg/kg/24h
115	294	8.82	22.16
120	270	4.86	15.43
120	282	4.94	14.57
113	365	8.58	25.61
140	487	6.82	16.24
130	200	5.50	21.74
115	274	8.50	25.52
120	229	5.50	18.09
135	295	8.40	21.82
135	337	6.40	16.33
125	320	7.04	18.57
120	307	5.53	15.36
120	652	8.48	20.82
110	-	-	-
115	350	5.60	15.38
118	250	7.50	21.55
108	337	5.90	16.34
123	347	9.72	26.41
105	383	7.66	13.30
110	473	8.04	15.00
115	300	10.5	21.52
120	313	8.45	15.01
105	373	6.71	11.39
103	460	8.51	15.70
110	400	10.00	19.01
118	493	10.85	20.43
110	363	10.16	18.47
Mean ± standard deviation			
117.7 ± 9.37	352.± 97.8	7.29 ± 2.21	18.53 ± 3.97

[a] control values prior to any operative procedures

LE 16

erimental G eek two) tionship of urinary dopamine levels
aired sample rterial pressure (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopa μg/	Dopamine μg/24h	Dopamine μg/kg/24h
10	424	9.54	17.73
	466	7.92	14.45
20	199	4.29	8.46
110	438	6.35	12.40
125	624	12.17	21.02
115	325	7.48	14.38
120	382	8.59	16.61
120	390	7.02	13.98
120	345	7.60	15.17
120	410	8.61	16.40
110	387	6.97	12.38
95	-	-	-
133	327	7.52	12.99
110	457	10.28	18.97
123	413	8.26	14.59
110	337	6.74	12.76
110	347	7.46	13.23
115	-	-	-
115	320	5.04	11.56
110	390	4.68	9.79
115	317	3.80	8.08
120	240	4.92	11.55
125	347	4.51	9.84
120	277	4.43	9.42
125	353	5.12	12.86
115	477	5.96	13.42
115	200	3.60	7.83
Mean ± standard deviation			
117.0 ± 7.91	367.7 ± 91.5	6.75 ± 2.16	13.19 ± 3.34

[a] control values prior to any operative procedures

TABLE 17

Experimental Group (week four). Relationship of urinary dopamine levels (paired samples) to arterial blood pressure (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopamine μg/l	Dopamine μg/24h	Dopamine μg/kg/24h
105	187	2.99	5.67
110	246	4.67	8.28
120	287	4.02	7.96
123	263	7.10	12.33
108	323	6.94	13.17
110	257	3.98	7.71
103	157	3.69	6.38
118	190	3.04	6.31
115	383	8.81	17.41
115	243	7.65	15.84
120	157	4.95	10.51
135	210	6.09	11.69
105	180	5.94	11.81
130	267	8.54	17.18
108	163	4.73	10.35
113	233	6.29	12.14
120	210	7.56	15.52
123	303	11.82	23.17
115	167	5.51	11.43
110	163	7.01	14.13
123	206	6.18	13.26
135	146	5.62	12.95
103	303	8.79	19.15
120	200	4.80	9.66
113	214	6.85	15.46
128	207	8.69	18.65
120	207	8.07	17.54
Mean ± standard deviation			
116.6 ± 9.03	224.9 ± 58.5	6.31 ± 2.07	12.80 ± 4.39

[a] one week following right renal nephrectomy

TABLE 19

Experimental Group (week eight). Relationship of urinary dopamine levels (paired samples) to arterial blood pressure (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopamine μg/l	Dopamine μg/24h	Dopamine μg/kg/24h
155	186	6.14	11.41
150	274	9.32	17.10
163	214	7.49	12.98
163	254	7.24	12.64
135	160	5.76	10.59
173	90	5.76	9.23
205	166	10.37	14.65
150	103	6.39	9.86
173	170	7.39	16.42
168	180	8.46	13.22
190	100	6.15	10.57
183	146	6.64	11.37
175	153	7.65	12.77
145	555	7.22	12.73
180	142	6.74	11.74
Mean ± standard deviation			
167.2 ± 18.5	192.9 ± 112.8	7.25 ± 1.31	12.48 ± 2.24

[a] three weeks following left renal artery constriction

TABLE 20

Experimental Group (week ten). Relationship of urinary dopamine levels (paired samples) to arterial blood pressure (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopamine μg/l	Dopamine μg/24h	Dopamine μg/kg/24h
163	185	8.68	12.43
173	207	10.35	17.49
205	235	8.21	13.22
173	345	8.46	16.24
168	168	10.91	18.77
175	166	7.47	12.40
163	136	6.48	10.57
200	213	4.91	6.86
120	291	9.47	15.62
180	148	7.75	12.95
135	190	6.75	13.47
190	266	7.84	13.17
Mean ± standard deviation			
170.4 ± 24.3	212.5 ± 62.2	8.11 ± 1.67	13.60 ± 3.18

[a] five weeks following left renal artery constriction

TABLE 21

Experimental Group (week twelve). Relationship of urinary dopamine levels (paired samples) to arterial blood pressure (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopamine μg/l	Dopamine μg/24h	Dopamine μg/kg/24h
163	223	12.69	18.65
163	114	7.10	10.51
173	181	8.50	13.18
155	129	3.81	6.67
205	171	9.39	14.65
173	145	6.20	9.42
150	221	8.61	13.26
205	103	4.96	7.83
180	103	5.81	9.23
150	172	10.66	14.23
135	292	6.71	10.54
Mean ± standard deviation			
168.3 ± 22.1	168.5 ± 59.0	7.68 ± 2.60	11.65 ± 3.50

[a] seven weeks following left renal artery constriction

TABLE 22

Experimental Group (week fourteen). Relationship of urinary dopamine levels (paired samples) to arterial blood pressure (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopamine µg/l	Dopamine µg/24h	Dopamine µg/kg/24h
173	206	10.84	15.17
173	252	15.61	22.16
150	373	17.91	25.61
150	137	5.35	7.61
175	403	9.27	13.26
135	144	9.68	14.23
180	489	11.24	13.98
163	214	8.02	13.50
205	89	6.19	8.97
Mean ± standard deviation			
167.1 ± 20.5	256.3 ± 136.2	10.46 ± 4.11	14.94 ± 5.72

[a] nine weeks following left renal artery constriction

TABLE 23

Experimental Group (week sixteen). Relationship of urinary dopamine levels (paired samples) to arterial blood pressure (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopamine μg/l	Dopamine μg/24h	Dopamine μg/kg/24h
170	287	10.62	12.21
180	207	11.80	15.55
190	307	7.06	7.83
190	283	6.51	8.75
163	200	14.00	21.12
200	130	7.15	8.97
135	140	11.90	15.16
180	429	9.86	12.80
Mean ± standard deviation			
176.0 ± 20.3	247.9 ± 98.96	9.86 ± 2.73	12.80 ± 4.45

[a] eleven weeks following left renal artery constriction

TABLE 24

Experimental Group (week eighteen). Relationship of urinary dopamine levels (paired samples) to arterial blood pressure (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopamine μg/l	Dopamine μg/24h	Dopamine μg/kg/24h
168	143	9.15	11.10
180	223	9.81	10.51
175	357	12.49	14.13
200	254	22.10	27.35
200	163	7.33	9.07
120	160	7.44	9.23
155	143	6.15	8.93
175	326	9.62	13.14
Mean $\pm$ standard deviation			
171.6 $\pm$ 25.79	221.1 $\pm$ 84.4	10.51 $\pm$ 5.07	12.93 $\pm$ 6.13

[a] thirteen weeks following left renal artery constriction

TABLE 25

Experimental Group (week twenty). Relationship of urinary dopamine levels (paired samples) to arterial blood (paired means) in male rats.[a]

Blood Pressure mm Hg	Dopamine μg/l	Dopamine μg/24h	Dopamine μg/kg/24h
173	204	12.67	13.69
150	224	10.66	12.26
163	163	8.81	9.86
135	371	13.72	16.29
173	391	11.33	15.64
173	178	11.55	13.07
200	142	6.87	7.83
Mean ± standard deviation			
166.7 ± 20.6	239.0 ± 100.73	10.80 ± 2.32	12.63 ± 3.01

[a] fifteen weeks following left renal artery constriction

3. A Statistical Evaluation of the Results Obtained from the Study on the Relationship of Urinary Dopamine Levels in Arterial Blood Pressure is presented in TABLES 26 and 27.

TABLE 26

Comparisons of the means of the control and experimental groups using "Student's" t distribution.[a] The calculated /T/ is followed by the tabulated t (in parentheses) at the 0.05 confidence level and corresponding degrees of freedom.[b]

Week	mm Hg	μg/l	μg/24h	μg/kg/24h
1	2.240(2.032)*	1.102(2.035)	3.404(2.035)**	3.536(2.035)**
2	0.332(2.032)	0.426(2.037)	0.036(2.037)	1.614(2.037)
4[c]	0.262(2.032)	1.825(2.032)	0.355(2.032)	0.667(2.032)
6[d]	6.181(2.060)***	0.062(2.060)	3.269(2.060)**	2.967(2.060)**
8	7.952(2.074)***	2.884(2.074)**	0.253(2.074)	0.482(2.074)
10	6.275(2.093)***	2.911(2.093)**	0.985(2.093)	0.750(2.093)
12	6.247(2.101)***	5.734(2.101)***	0.392(2.101)	1.212(2.101)
14	6.148(2.120)***	1.877(2.120)	1.134(2.120)	1.109(2.120)
16	7.089(2.131)***	2.846(2.131)*	0.747(2.131)	0.164(2.131)
18	5.388(2.131)***	1.047(2.131)	0.537(2.131)	0.219(2.131)
20	5.863(2.145)***	2.287(2.145)*	0.903(2.145)	0.594(2.145)

[a] /T/ calculated according to Snedecor, 1956

$$/T/ = (\bar{x}_1 - \bar{x}_2) \sqrt{\frac{n_1 n_2 (n_1 + n_2 - 2)}{(n_1 + n_2)\sum x^2}}$$

[b] t = /T/ t rejects Ho: $_1 - _2 = 0$ indicating a significant difference between the control and experimental means
* signifies a difference at the 0.05 level or less
** signifies a difference at the 0.01 level or less
*** signifies a difference at the 0.001 level or less

[c] week following right renal nephrectomy in both experimental and control groups

[d] week following left renal artery constriction in the experimental group; left renal artery manipulation in the control group

TABLE 27

Regression coefficients and correlation coefficients with confidence intervals and tests of the hypotheses for the rat data of the control and experimental groups from week 6 through week 20.

Relationship Investigated	Regression Coefficient[a] b	Correlation Coefficient[a] r	t_{cal}[b]	$t_{05}(N-2)$
	CONTROL GROUP			
Blood Pressure on Dopamine μg/l	0.005(-0.013 to 0.023)	0.062(-0.149 to 0.268)	0.522	1.994
Blood Pressure on Dopamine μg/24h	0.253(-0.535 to 1.041)	0.076(-0.303 to 0.156)	0.639	1.994
Blood Pressure on Dopamine μg/kg/24h	-0.082(-0.672 to 0.508)	-0.033(-0.262 to 0.199)	0.276	1.994
Dopamine μg/kg/24h on Weight in Grams	-0.002(-0.10 to 0.006)	-0.072(-0.299 to 0.160)	0.602	1.994
	EXPERIMENTAL GROUP			
Blood Pressure on Dopamine μg/l	-0.040(-0.086 to 0.006)	-0.181(-0.376 to 0.029)	1.705	1.987
Blood Pressure on Dopamine μg/24h	-0.868(-2.330 to 0.594)	-0.126(-0.326 to 0.085)	1.179	1.987
Blood Pressure on Dopamine μg/kg/24h	-0.943(-1.919 to 0.033)	-0.203(-0.391 to 0.011)	1.922	1.987
Dopamine μg/kg/24h on Weight in Grams	-0.006(-0.014 to -0.002)	-0.164(-0.358 to 0.049)	1.544	1.987

[a] All calculation concerned with the regression coefficients, correlation coefficients and tests of hypotheses were carried out according to Snedecor, 1956.

[b] Since t_{cal} does not exceed $t_{05}(N-2)$ in any instance the hypotheses: Ho:$\beta = 0$ and Ho:$\rho = 0$ are not rejected.

4. Analysis of the Plasma from Hypertensive Rats Sacrificed at the End of the Twentieth Week.

Assay of the individual plasmas obtained from the 14 hypertensive rats which survived the 20-week study failed to demonstrate the presence of dopamine in any of the samples regardless of the manner in which the plasma was treated prior to its subsequent passage through Dowex^{R} resin.

5. The Results Obtained from the Assay of the Ischemic Left Kidneys of Hypertensive Rats for Dopamine Content are presented in TABLE 28.

TABLE 28

Influence of renal hypertension of eight weeks duration on kidney dopamine content in male rats.[a]

Initial Blood Pressure[b] mm Hg	Terminal Blood Pressure mm Hg	Dopamine Content[c] Left Kidney (μg/gm)
85	120	0
110	160	0
95	150	0
100	120	0
90	140	0
80	140	0
95	110	0
95	105	0
105	125	0
100	125	0
100	130	0
115	145	0
Dopamine content of the pooled blood from the above animals (80 ml) = 0		

[a] The rats were subjected to a simultaneous right renal nephrectomy and left renal artery compression.

[b] Both initial and terminal blood pressure was determined under ether anesthesia.

[c] The blank values slightly exceeded the standard determinations in all assays.

6. The Data for the Study of Urinary and Blood Dopamine Levels in Control and Renal Hypertensive Dogs are presented in TABLE 29.

TABLE 29

Relationship of urinary dopamine and blood dopamine levels to arterial blood pressure in control and renal hypertensive dogs

Subject	Week of Experiment 1	2	4[a]	6[b]	7	8	9	10
Male:Control								
Blood Pressure(mmHg)	150	160	160	155	160	160	165	160
Urinary Dopamine(μg/24h)	1342	497	1220	406	105	318	346	-
Blood Dopamine(μg/20 ml)	-	0.13	0.07	0.13	0.17	0.07	0.17	0.20
Male:Unilateral Nephrectomy								
Blood Pressure(mmHg)	160	170	165	155	160	170	150	165
Urinary Dopamine(μg/24h)	518	246	215	199	373	-	414	105
Blood Dopamine(μg/20ml)	-	0.10	0.13	0.17	-	0.07	0.13	0.23
Female:Hypertensive								
Blood Pressure(mmHg)	140	150	150	-	200	210	195	205
Urinary Dopamine(μg/24h)	271	231	333	-	278	320	209	366
Blood Dopamine(μg/20ml)	-	0.07	0.17	0.10	0.10	0.17	0.13	0.13
Male:Hypertensive								
Blood Pressure(mmHg)	175	160	170	-	180	200	210	215
Urinary Dopamine(μg/24h)	247	290	311	-	317	343	352	308
Blood Dopamine(μg/20ml)	-	0.10	0.10	0.13	0.07	0.07	0.17	0.20

[a] first week following right renal nephrectomy in latter three subjects

[b] first week following left renal artery constriction in the hypertensive subjects

7. The Data Obtained from the Intravenous Infusion of d,l-DOPA in Cats are presented in TABLES 30 - 32.

TABLE 30

Levels of dopamine and DOPA in various tissues of the untreated cat

Cat No.	Heart (μg/gm)	Liver (μg/gm)	Spleen (μg/gm)	Kidney (μg/gm)
		DOPAMINE		
1	0.18	0.16	0.31	0.07
2	0.18	0.00	0.00	0.00
3	0.07	0.01	0.005	0.02
4	0.009	0.02	0.00	0.009
Average	0.11	0.05	0.08	0.02
		DOPA		
1	0.18	0.12	0.23	0.27
2	0.00	0.00	0.00	0.00
3	0.00	0.008	0.03	0.02
4	0.01	0.01	0.03	0.05
Average	0.05	0.03	0.07	0.08

TABLE 31

Tissue levels of dopamine and DOPA in various tissues of the cat following intravenous infusion of 50 mg/kg d,l-DOPA in cats with left kidney function.

Cat No.	Heart (μg/gm)	Liver (μg/gm)	Spleen (μg/gm)	Kidney (μg/gm)	Renal Blood (μg/l) Arterial-1 (10 minute samples)	Venous-1	Arterial-2 (one hour samples)	Venous-2
				DOPAMINE				
1	1.91	2.6	3.30	7.22	74	106	74	60
2	0.44	3.96	2.62	24.78	16	134	30	180
3	-	1.07	0.83	4.78	30	-	30	30
4	0.40	0.56	0.24[a]	4.40	-	106	30	30
Average	0.92	2.05	2.25	10.29	40	115	41	75
				DOPA				
1	23.62	8.40	13.75	111.55	22,296	16,560	4,966	3,980
2	25.38	11.20	23.90	98.76	15,960	27,600	5,300	4,846
3	28.60	18.70	48.24	59.02	2,984	9,142	1,320	1,306
4	43.04	13.06	53.34	104.42	3,370	1,044	2,926	850
Average	30.16	12.84	34.81	93.44	11,152	13,586	3,628	2,745

[a] Some of this sample was lost during handling and the value is not included in the average.

TABLE 32

Tissue levels of dopamine and DOPA in various tissues of the cat following intravenous infusion of 50 mg/kg d,l-DOPA in cats without kidney function

Cat No.	Heart (μg/gm)	Liver (μg/gm)	Spleen (μg/gm)
		DOPAMINE	
1	1.54	1.22	2.08
2	0.98	4.48	2.10
3	0.80	2.43	3.60
4	0.70	2.75	2.11
Average	1.00	2.72	2.47
		DOPA	
1	35.43	10.65	20.87
2	31.02	18.84	16.48
3	32.24	12.58	15.32
4	22.42	14.10	18.10
Average	30.28	14.04	17.69

IV. DISCUSSION

The data from the major portion of the study, dealing with the relationship of urinary dopamine levels to arterial hypertension in renal hypertensive rats are presented in Tables 4 through 25. It is immediately apparent (Figure 5) that the method used to induce hypertension (Goldblatt et al., 1934; Dury, 1938) was successful. The mean blood pressure values of the control group varied within a rather narrow range of from 115.7 mm Hg (Table 5) to 125.7 mm Hg (Table 4), while those of the experimental group ranged from a low of 116.6 mm Hg (Table 17) prior to left renal artery constriction to a maximum of 176 mm Hg, 11 weeks following constriction (Table 23).

The mean blood pressure of the control group was highest the first week and differed significantly ($.01 < p < .05$) from the experimental group for the same week. However, the mean blood pressure of the control group dropped the second week and then showed a tendency to rise slowly throughout the duration of the 20-week study, never exceeding the first week's average. Individual variation within the control group, ignoring time as a possible factor, ranged from 95 mm Hg to 140 mm Hg.

The mean of the experimental group showed a sharp increase in blood pressure one week following left renal artery constriction (Table 18), and the group means remained significantly elevated above those of the controls for the duration of the study ($p < .001$). Although the mean blood pressure continued to rise subsequent to renal artery constriction, the magnitude of the rise was offset by deaths which occurred within this group as a consequence of the severity of hypertension and its resultant complications. While 36 rats survived the first week subsequent to renal-artery

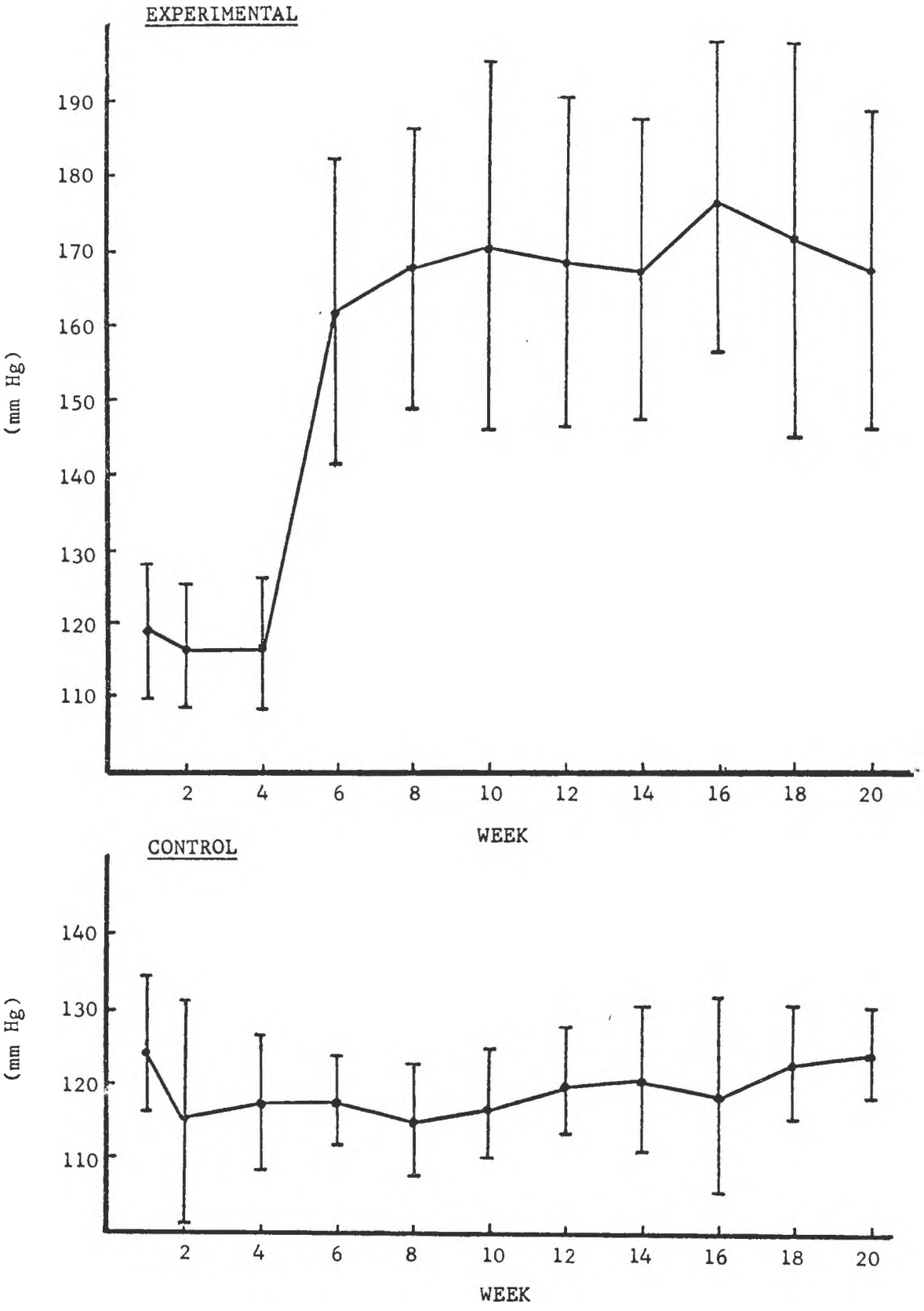

Fig. 5. Mean blood pressures and standard deviations (mm Hg) for the experimental and control groups for week 1 through 20.

constriction, only 14 animals survived to the conclusion of the 20-week study. Individual variation within the experimental group following renal-artery constriction, ranged from 120 mm Hg to 205 mm Hg, indicating that not all of the animals developed arterial hypertension.

Urinary dopamine levels, expressed as micrograms per liter, remained relatively constant for the control group; the group means varied between 270 and 418 μg/l (Figure 6). Individual variation ranged from 113 to 623 μg/l. Dopamine concentration decreased in the control group the fourth week of the experiment (one week following right nephrectomy) and then progressively rose to reach a mean level, comparable to those obtained initially, about the twelfth week of the experiment.

The group means of the experimental group varied between 168 and 367 μg/l, while individual variation ranged between 89 and 652 μg/l. A similar decrease in dopamine concentration was seen in this group following right-renal nephrectomy (Figure 6). However, in the case of the experimental group rather than a tendency to rise, a tendency was noted for the dopamine concentration to decrease further. Comparisons between the group means of the control and experimental animals showed the urinary dopamine concentration to be significantly lower in the experimental animals for the eighth ($.01 > p > .001$), tenth ($.01 > p > .001$), twelfth ($p < .001$), sixteenth ($p < .05$) and twentieth ($p < .05$) weeks of the study (Table 26).

The regression and correlation coefficients for the dependency of blood pressure on dopamine concentration (μg/l) from the sixth through the twentieth week of the study are shown in Table 27. The regression and correlation coefficients of the control group are $b=0.005$ and $r=0.062$ respectively, while those of the experimental group are $b= -0.040$ and $r= -0.181$ respectively. However, none of these values are statistically

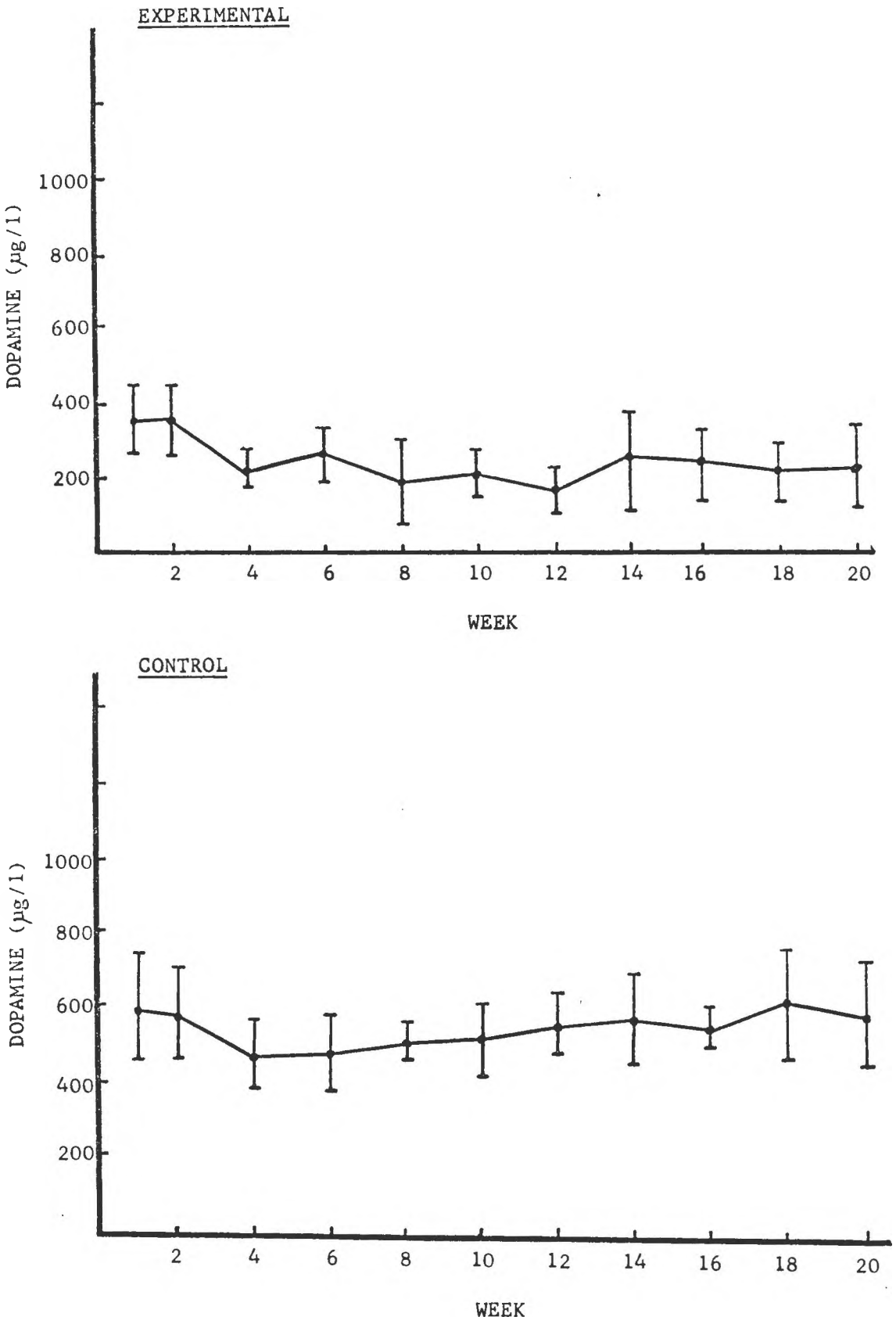

Fig. 6. Mean urinary dopamine levels and standard deviations (µg/l) for the experimental and control groups for week 1 through 20.

significant and the hypothesis Ho: $\beta = 0$ and Ho: $\rho = 0$ cannot be rejected; this implies that no relationship exists between dopamine concentration and arterial blood pressure.

DeFanti (1961) reported finding a highly significant positive regression coefficient for dependency of blood pressure on logarithm dopamine concentration in hypertensive rats, for dopamine concentrations ranging from 500 to 1000 µg/l. The above results are not in agreement with these findings. Possible explanations for the discrepancy lie in the different methods of statistical analysis and assay methods used in the two studies. DeFanti worked with six groups of experimental animals comprised of from one to six animals each which survived left renal artery compression. The mean values for blood pressure and dopamine concentration from these six groups were averaged to obtain pooled means and standard deviations of dopamine concentration and blood pressure for each week of the study. Apparently, the contribution of each group to the pooled mean was weighed equally despite variation in the number of animals comprising each group. These pooled means, from the first through the twelfth week following left renal artery compression, were then utilized in the regression analysis. In the present study, the values from 88 paired assays were utilized for the analysis.

Based upon the regression analysis statistics, DeFanti concluded that, "72% of the variations occurring in blood pressure could be explained on the basis of the linear regression of blood pressure on dopamine." No direct comparisons were made between the dopamine levels of the control group and the experimental group. Dopamine levels were reported for the control group for the first, second, third, fourth, fifth, sixth, and eighth weeks following right nephrectomy. Non-statistical comparison of

the dopamine levels of the control group with those of the experimental groups for these same weeks showed the dopamine levels in the control group to exceed those of the experimental group in 17 out of a possible 33 instances. In addition, the pooled means of the hypertensive animals were below that of the pooled control mean (obtained prior to left renal artery constriction) from these same animals for the first five weeks following left renal artery compression. However, during this five-week period the blood pressure had risen from a control value of 122+4.3 mm Hg to 173 +11.2 mm Hg. During this major rise in blood pressure, the dopamine levels remained below the pre-operational control value. Under these circumstances it is difficult to accept the dependancy of blood pressure on urinary dopamine levels.

Probably a more important contribution to the differences seen in the two studies was made by differences in methodology rather than differences in statistical evaluation of the data. While DeFanti used Dowex Sodium 50W-X8 to extract dopamine from the urine, the present study used alumina. In addition, changes were made in the assay procedure itself, particularly in the blank. These changes were necessitated by the discovery, early in the present study, that Dowex resin was extracting a substance with native fluorescence from the urine of hypertensive rats and that the blank (Carlsson and Waldeck, 1958) by quenching this fluorescence was resulting in erroneously high urinary dopamine levels. By using alumina and changing the order of addition of reagents in the blank, this difficulty was circumvented in the present study. The explanation seems compatible with the results; for while alumina extracted 82.43 +11.73% of variable amounts of dopamine added to urine (Table 2) compared with 74.52 +10.99% reported by DeFanti, the range of dopamine values was lower

in the present study (89-652 μg/1) than reported by DeFanti (97-1569 μg/1). Other investigators have encountered difficulties with the dopamine assay, and various modifications can be found in the literature (Drujan et al., 1959; McGeer and McGeer, 1962; Brodie et al., 1962; Carlsson and Lindqvist, 1962; Anton and Sayre, 1964). The strong cation exchange resins (e.g. Dowex 50W-X8) have been reported to give off fluorescent material (Haggendal, 1962; Ibid., 1966); and absorption onto alumina at pH 8.6 seems superior and more specific, particularly for urine (Anton and Sayre, 1962; Ibid., 1964; Haggendal, 1966).

(Tables 4 through 25 also present urinary dopamine levels as total micrograms excreted per 24 hours (μg/24 hr) and micrograms per killogram per 24 hours (μg/kg/24 hr). Since a change in the volume of urine produced in a 24-hour period could have a significant influence on dopamine levels when reported as concentration, it was thought necessary to compare the total micrograms excreted per 24 hours between the control and experimental groups. The results of the two groups differed significantly only for the first and sixth weeks of the experiment ($.001 < p < .01$). The discrepancy for the first week could possibly be attributed to variable adaptation to experimental procedure. The difference seen the sixth week (first week following left renal artery constriction) probably is a result of temporary alteration of renal function or a rebound phenomenon consequent to renal impairment. The excretion of dopamine (μg/24 hr) tended to increase (Figure 7) in both groups over the duration of the study, indicating a possible relationship with time (e.g. age). This possibility was not statistically evaluated, but is in agreement with other studies (Burn, 1953). The relationship between blood pressure and dopamine (μg/24 hr), however, was investigated for both groups. No relationship

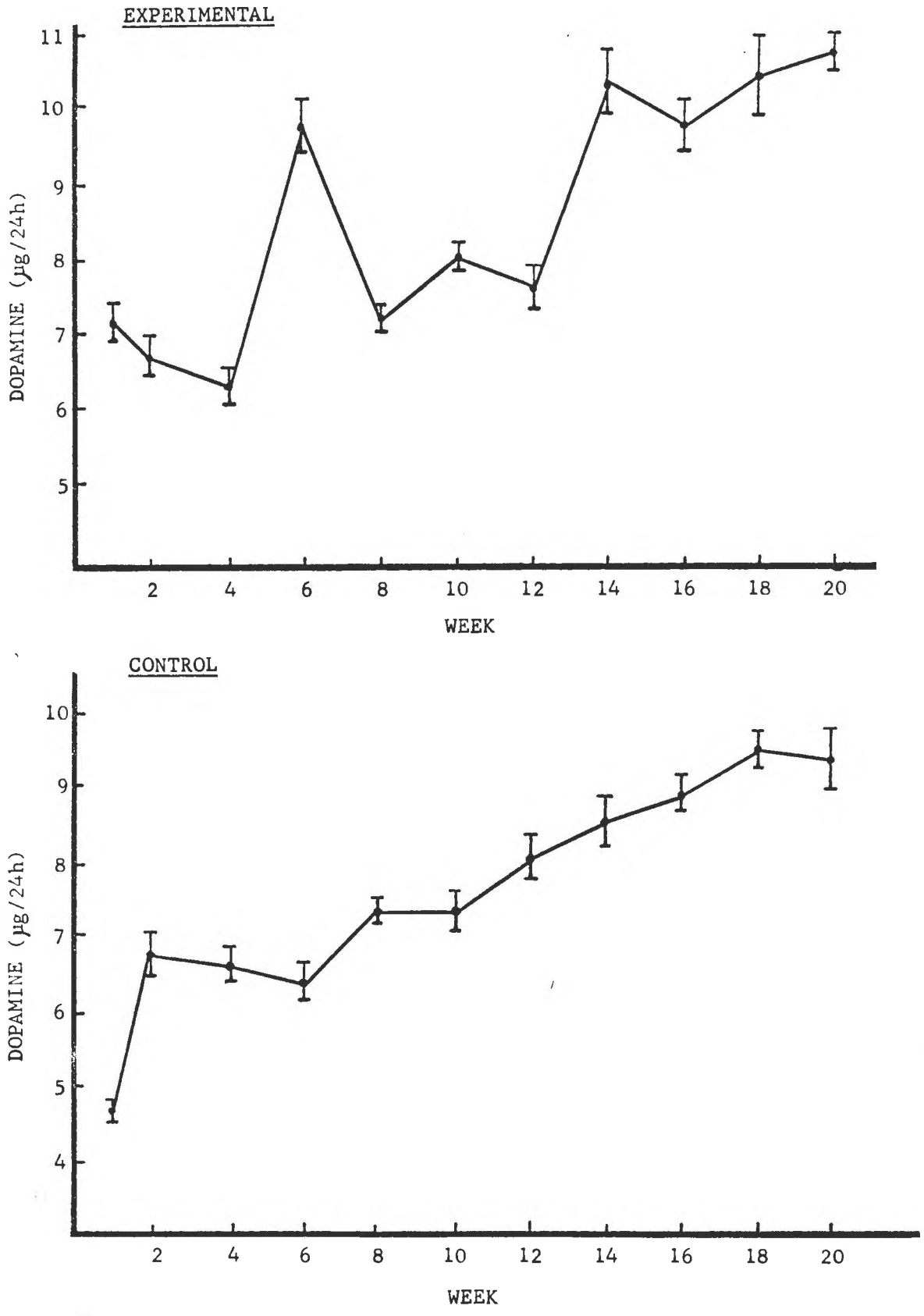

Fig. 7. Mean urinary dopamine levels and standard deviations (µg/24h) for the experimental and control groups for week 1 through 20.

was found to exist for either of the groups when the data from the sixth week on, independent of time, was subjected to a determination of the regression and correlation coefficients. The values are shown in Table 27. The regression coefficient (b) for the control group was 0.253 and for the experimental group -0.868. The correlation coefficient (r) was 0.076 for the control group and-0.126 for the experimental group. Although these values took different directions, it is important to note that they were not statistically significant.

Urinary dopamine values expressed as micrograms per kilogram per 24 hours were reported because DeFanti and DeFeo (1963) reported finding an increased excretion of dopamine (μg/kg/24 hr) when plotted against weight (gm) for hypertensive rats, contrasted with a decrease in normotensive rats. Leduc (1961) also reported a decrease in urinary dopamine levels (μg/kg/24 hr) in normotensive rats as body weight increased. In the present study a slight tendency for dopamine levels (μg/kg/24 hr) to decrease with increased weight was noted for both the experimental and control groups; however, neither trend proved statistically significant (Table 27). Also subjection of the data on dependency of blood pressure to dopamine levels (μg/kg/24 hr) to a determination of the regression and correlation coefficients resulted in values of low magnitude which were not statistically significant. Comparisons between the two groups (Table 26; Figure 7) disclosed a significant difference between the two groups for only the first and sixth weeks, resulting in a situation similar to that encountered when dopamine levels (μg/24 hr) were compared.

In light of the above findings, it appears that the quantity of dopamine excreted in the urine, expressed either as total micrograms per 24 hours or as micrograms per killogram per 24 hours, follows a similar

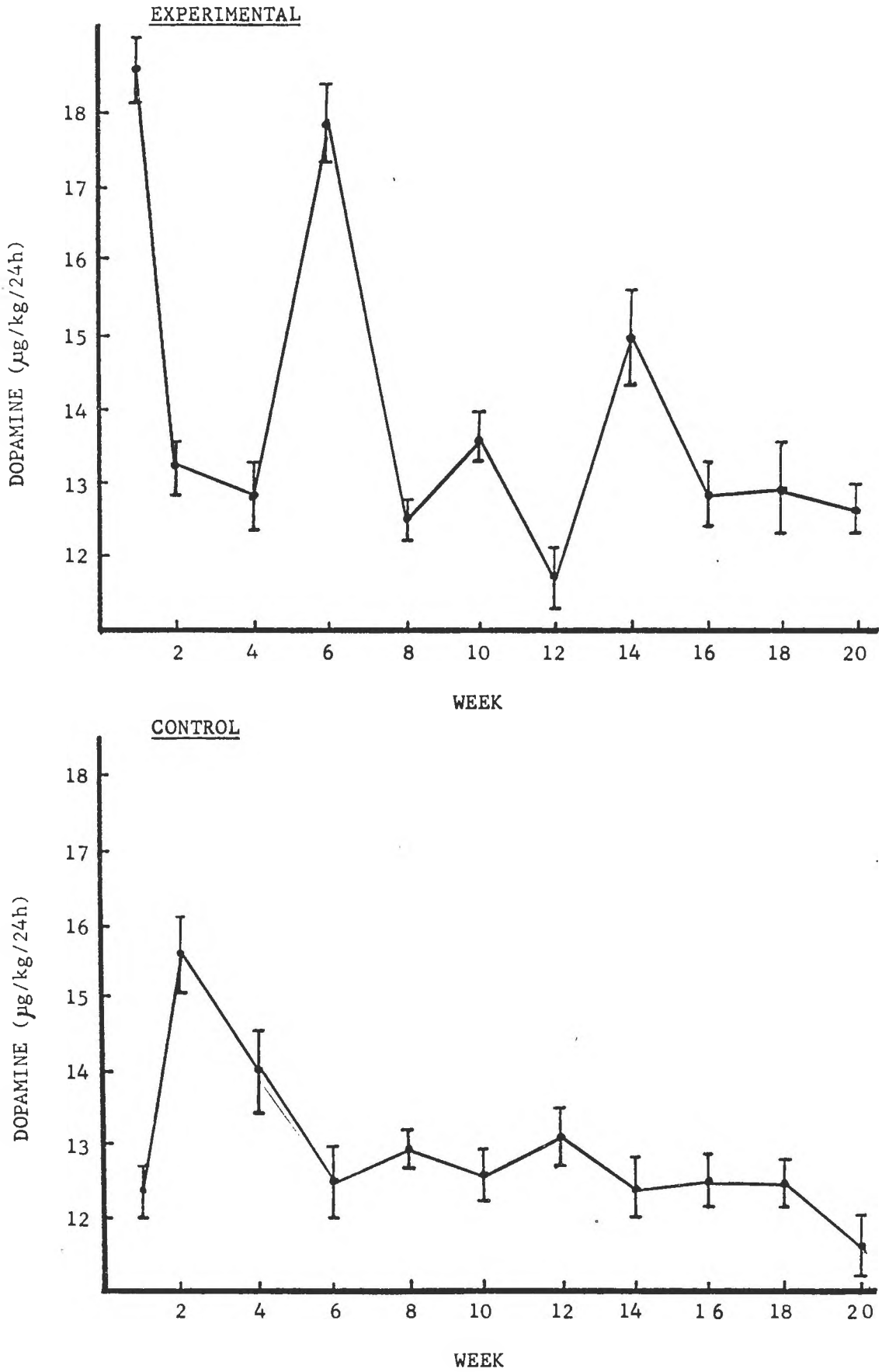

Fig. 8. Mean urinary dopamine levels and standard deviations (µg/kg/24h) for the experimental and control groups for week 1 through 20.

pattern in normotensive and hypertensive rats. It also appears that the total quantity of dopamine excreted per 24 hours tends to increase with time (e.g., age), while the dopamine levels reported as micrograms per killogram per 24 hours tend to remain relatively constant or display a slightly downward trend as body weight increases. Since dopamine levels expressed as micrograms per liter were significantly lower on several occasions in the hypertensive groups of animals, an explanation is necessary. The explanation would seem to be an increase in urinary volume which occurred in the experimental group subsequent to left renal artery constriction. An increase in urinary output is commonly encountered in renal hypertension as the kidneys lose their power to concentrate the urine. If this dilution factor is taken into account, as it is when dopamine levels are reported as micrograms excreted per 24 hours or micrograms excreted per killogram per 24 hours, then no consistent significant differences are seen to exist between the normotensive and hypertensive animals. The only possible conclusion is that urinary dopamine levels bear no relationship to the increase in blood pressure as a result of renal ischemia in rats.

The urinary dopamine levels (μg/24 hr) for the study carried out in dogs are presented in Table 29. These values are quite variable; however, there are no obvious differences between the values for the hypertensive dogs, the control dog or the unilaterally nephrectomized control dog.

The infusion of d,l-DOPA in cats with and without functional renal tissue (Tables 30, 31 and 32) was carried out to determine the role of the kidneys in the increased tissue levels of dopamine reported to occur following DOPA infusion (Wegmann, 1963). The tissue levels of dopamine and DOPA found in the untreated control cats are presented in Table 30.

The dopamine levels were low in all the tissues examined; the mean levels were highest in the heart (0.11 μg/gm), next highest in the spleen (0.08 μg/gm), next in the liver (0.05 μg/gm), and lowest in the kidney (0.02 μg/gm). Mean DOPA levels were very low (0.08 μg/g in all tissues). Wegmann (1963) reported detectable levels of dopamine in the spleen and kidney of untreated dogs but not in heart and liver. Anton and Sayre (1964) reported finding very low levels of dopamine in the tissues of the dog, the highest value being found in the heart (0.05 μg/gm). These same investigators reported the highest level of dopamine in the cat spleen (0.12 μg/gm) and similar levels in the heart, liver, and kidney (0.07 μg/gm). Both investigators failed to detect DOPA in any of these tissues. Following the infusion of d,l-DOPA in cats with left renal function, there was an increase in the dopamine content of all the tissues examined. The pattern of increase was the same as that obtained by Wegmann, the dopamine level being highest in the kidney (10.29 μg/gm) followed in order by the spleen (2.25 μg/gm), liver (2.05 μg/gm), and heart (0.92 μg/gm). The DOPA level was highest in the kidney (93.44 μg/gm), followed in order by the spleen (34.81 μg/gm), heart (30.16 μg/gm), and liver (12.84 μg/gm); again the pattern was similar to that reported by Wegmann. Blood samples taken during the experiment showed that some synthesis of dopamine was taking place in the kidney. An arterial blood sample taken midway through the infusion period from the abdominal aorta near the origin of the left renal artery showed a dopamine content of 40 μg/l, while a venous blood sample obtained from the left renal vein via the ovarian vein for the same time showed a dopamine content of 115 μg/l. Blood samples obtained in a similar manner one hour after initiation of the infusion (40 minutes after conclusion of the infusion) showed an arterial dopamine level of 41 μg/l and

a venous level of 75 μg/l. As might be expected, the blood levels of DOPA were very high. The values presented in the same order as above were arterial: 11,152 μg/l; venous, 13,586 μg/l; arterial, 3.628; venous, 2.745 μg/l. The high value obtained for the first venous sample probably represents a slight contamination of the sample with blood from the abdominal vein through which DOPA was being infused. Similar tissue levels of dopamine were obtained in the heart (1.00 μg/gm), liver (2.72 μg/gm), and spleen (2.47 μg/gm) when d,l-DOPA was infused into cats without kidney function. This finding would imply that the kidney contributes very little to the increased dopamine levels seen in these tissues. Dopamine synthesis either takes place in situ within the heart, liver, and spleen or as suggested by Wegmann, possibly the liver contributes to the tissue levels in the other two organs. Circumstantial evidence might support this. The liver is known to possess a high degree of DOPA decarboxylase activity (Blaschko, 1939) and yet the dopamine level in this organ following DOPA infusion is surprisingly low. However, the DOPA content of the liver is also relatively low following DOPA infusion. This could indicate a rapid turnover of DOPA in the liver with subsequent release of dopamine into the circulation.

None of the results from this investigation implicate dopamine in the renal hypertensive process. No relationship was established between urinary dopamine levels and arterial blood pressure in hypertensive rats or dogs. In addition, dopamine could not be demonstrated in the blood of hypertensive rats or dogs or in the renal tissue of hypertensive rats.

Doubt was also cast upon the role of the kidney in contributing to an increase in tissue dopamine levels following the infusion of d,l-DOPA. Evidence was presented that the kidney does decarboxylate DOPA, but

evidence was also presented that this had little influence on dopamine levels in heart, liver and spleen.

A question raised by the investigation is the nature of the native fluorescence found present in Dowex 50W-X8 extracts of hypertensive rat urines. High blanks were also encountered in the assay of the ischemic kidneys. Is it possible that the substances are one and the same and may be involved in the hypertensive process? Further investigation along this line might prove profitable.

V. SUMMARY AND CONCLUSIONS

Arterial hypertension was induced in male rats of the Wistar strain and in mongrel dogs by right renal nephrectomy and left renal artery constriction for the purpose of studying the relationship of urinary, blood, and kidney dopamine levels to the resulting increase in blood pressure. In the rats a significant increase in blood pressure was obtained the first week following left renal artery constriction, and the pressure remained significantly elevated above that of a sham-operated control group for the duration of the study. The highest mean blood pressure for the hypertensive group (176 mm Hg) was obtained on the eleventh week following renal artery constriction. Individual blood pressures ranged as high as 205 mm Hg. Similar results were obtained in the hypertensive dogs.

A new method was developed for the fluorimetric assay of urinary dopamine by modification of existing methods. Alumina was used to extract dopamine, and this eliminated certain difficulties which were initially encountered when Dowex sodium 50W-X8 was used. Recovery of known quantities of dopamine added to urine samples averaged 82.43 +11.73%. Dopamine was extracted from blood and tissues principally by homogenization in 10% trichloroacetic acid followed by passage through alumina. A method was also devised to separate DOPA from dopamine contained in the same tissue extract. This was accomplished by first passing a trichloroacetic acid extract through sodium Dowex to extract dopamine, then through alumina to extract DOPA. Excellent separation and recoveries were obtained.

Comparisons of urinary dopamine levels were carried out between the control and hypertensive groups of rats. Dopamine concentration (μg/l)

was significantly lower on several occasions in the hypertensive group. However, dopamine levels reported as micrograms per 24 hours or micrograms per kilogram per 24 hours differed significantly on only two occasions--once during the pre-operational control period and again on the first week following left renal artery constriction. A diuresis was observed in the hypertensive rats, which could explain the apparent decrease in dopamine concentration (μg/l). Dopamine levels (μg/24 hr) showed a tendency to increase in both the control and experimental groups during the period of the study while dopamine levels (μg/k/24 hr) remained relatively constant and displayed only a slight tendency to decrease. Regression and correlation coefficients were determined in both groups of rats for the following relationships: blood pressure on urinary dopamine (μg/l), blood pressure on urinary dopamine (μg/24 hr), blood pressure on urinary dopamine (μg/kg/24 hr), and dopamine (μg/kg/24 hr) on weight (gm). No statistically significant relationships were discovered. Dopamine could not be detected in the blood or kidneys of hypertensive rats or in the blood of hypertensive dogs.

A relationship between dopamine and the hypertensive process was not established in the present study, and it is doubtful that dopamine plays a role in the elevation of blood pressure which takes place following right nephrectomy and left renal artery constriction in the rat or dog.

d,l-DOPA was infused into cats with and without functional renal tissue to determine the contribution of the kidneys to tissue levels of dopamine. Tissue dopamine levels were elevated to a similar degree in liver, spleen, and heart in both instances; however, renal arterio-venous differences indicated that some decarboxylation of DOPA to dopamine did take place in the kidney. The similar tissue levels were probably a

result of saturation of uptake sites in the tissues, and it was suggested that the liver may have contributed to the tissue levels in the heart and spleen of the totally nephrectomized cats.

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VITA

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He entered the University of Pittsburgh, College of Pharmacy in 1953 and graduated with a Bachelor of Science in Pharmacy in 1957. He continued his education at the same institution where he served as a graduate assistant in pharmacognosy and pharmacology and received the degree of Master of Science in Pharmaceutical Sciences with a major in pharmacology in 1961. He accepted the position of instructor of pharmacology at the University of Rhode Island, College of Pharmacy that same year and entered the graduate school to continue work toward the doctorate. In 1965 he was promoted to the rank of assistant professor.

He is a member of the following societies and professional organizations, Rho Chi, national honor society, Kappa Psi Pharmaceutical Fraternity, Sigma Xi, honorary science society and the American Pharmaceutical Association. He is licensed to practice pharmacy in three states.

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