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INCREASED RESISTANCE AND IMPAIRED VASODILATION
IN THE NORMOTENSIVE HINDQUARTERS OF
RATS WITH COARCTATION HYPERTENSION

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INCREASED RESISTANCE AND IMPAIRED VASODILATION IN THE NORMOTENSIVE HINDQUARTERS OF RATS WITH COARCTATION HYPERTENSION

By

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ABSTRACT

INCREASED RESISTANCE AND IMPAIRED VASODILATION IN THE NORMOTENSIVE HINDQUARTERS OF RATS WITH COARCTATION HYPERTENSION

By

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The role of pressure in structural vascular changes in coarctation hypertension was examined in isolated, innervated, pump-perfused hindlimbs of rats with 4 weeks of abdominal aortic coarctation hypertension (Group A); normotensive control rats (Group B); and normotensive abdominal aortic coarcted rats with hindquarters atrophy (Group C). Hindquarters intravascular pressures were always normotensive in Group A. In the hindlimbs of Group A compared to either groups B or C, pressure-flow curves were displaced toward the pressure axis ($p < 0.01$) while resting resistance, resistance after maximal vasodilation, and plasma renin concentrations were increased ($p < 0.05$). Thus, in normotensive beds of rats with coarctation hypertension: 1) Resistance is elevated; 2) Structural vascular changes indicated by impaired vasodilation may contribute to this elevation; 3) The changes are not attributable to hindquarters atrophy but may be related to plasma renin; 4) Elevated resistance and impaired vasodilation were not caused by high intravascular pressures.

DEDICATION

To my wife Pamela whose constant patience and support was always felt and greatly appreciated. My greatest debt of thanks belongs to you.

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Introduction

In chronic arterial hypertension the systemic arterioles have thickened walls and narrowed lumens. The wall thickening has been attributed to medial hypertrophy and an increased wall water content.

Both have been assumed to result from increased intravascular pressure. Animals with coarctation hypertension provide an interesting model for examining the role of pressure in the development of such vascular changes.

In this type of hypertensive animal the arterial regions above the coarctation exhibit an increased intravascular pressure. However, arterial pressure in the regions below the coarctation are usually normal or near normal. Therefore, any vascular changes seen in these normotensive vascular beds could not be attributed to high intravascular pressures.

In an earlier investigation, we observed that increased vascular wall water content occurs in the normotensive vascular beds of rats with coarctation hypertension. Therefore, we believe the assumption that vascular wall thickening in hypertension results from increased intravascular pressure is in question.

The purpose of the present investigation was to determine if resistance changes accompany this vascular wall edema, by examining the isolated, innervated blood-pump perfused hindlimb of rats with coarctation of the

abdominal aorta above the origin of both renal arteries. Consequently, the experiments were designed to measure resistence at rest and over a range of flows, in a normotensive vascular bed of rats with coarctation hypertension.

The pressure-flow studies were intended to measure the effects of passive distention in the hindlimb vasculature. Also, resistance was to be measured in the acutely denervated hindlimb during maximum active vasodilation induced by sodium nitroprusside.

Measurements of both passive and active vasodilation were intended to give the investigator some insight into the actual structure of the vessel walls in the hindlimb. An elevation in the resistance measurements, (especially after maximal vasodilation) found in the normotensive hindlimb of rats with coarctation hypertension would be evidence that increased vascular resistance and structural alterations in the vessels in hypertension are <u>not</u> a secondary adaptation to an elevated intravascular pressure, but, instead, might somehow be related to the cause of the hypertension itself.

Literature Review

One of the major features of chronic arterial hypertension is an increase in peripheral vascular resistance, which, in turn, is responsible for the maintenance of the elevated blood pressure. Since blood viscosity, the sympathetic nerves, a variety of humoral stimuli, myogenic influences and the structure of arterial vessels can all influence vascular resistance, any or all of these factors then could, theoretically, be implicated in the etiology of hypertension.

Pickering, in his early text on high blood pressure, reviewed previous investigations which showed that a generalized wall thickening of the arterioles occurs in chronic arterial hypertension. (40) Although recent histoligical studies by Bevan et al. support these results (3, 4), very often it is hard to detect true differences in the wall-to-lumen ratio between two vessels. One can not tell, for example, if he is observing a vessel with a truly thickened wall, or, a thin walled vessel that had contracted to a thicker state at the time of tissue fixation. Therefore, some other method is needed for examining the structure of the arterial vessels in hypertension.

Smirk first suggested, and then Folkow and his co-workers investigated the hemodynamic consequences of vascular wall thickening; an area of which, by itself,

little was known at the time of his initial studies. Folkow postulated that different types of wall thickening could exist in hypertension. He also felt that the structural characteristics of the resistance vessels which in turn are affected by wall thickness, are best displayed when all smooth muscle activity in the vasculature is abolished; that is, when the vasculature has been brought to the state of maximal relaxation. (46, 10)

Folkow hypothesized that, depending on the type of vessel thickening, three values for vascular resistances could be expected during a state of maximal vasodilation. Firstly, the vascular wall thickening could expand the vessel wall primarily in an outward direction so that, at maximal dilation, the lumen (and the resultant resistance to flow) of the hypertensive vessel would be the same as a normotensive vessel under the same conditions. Secondly, the wall thickening could progress in a primarily inward direction thus encroaching on the lumen of the hypertensive vessel even at maximal vasodilation. This, then, would result in an increase in resistance during maximal vasodilation. Finally, the vascular wall thickening could proceed in such a manner as to expand both the vessel wall and the lumen in an outward direction. In this latter case, the hypertensive vessel would show a decreased resistance at maximum vasodilation.

More importantly, the thickened vessel wall, in and of itself, would affect vascular resistance in a much

different manner than a "normal" thin-walled vessel, as soon as the smooth muscle of these vessels begins to contract. Folkow reasoned that the thicker vessel wall would have more tissue mass situated inside the line of force of smooth muscle contraction. Consequently, in a thickened vessel, more tissue mass would be forced into the lumen at any given level of smooth muscle shortening compared to a vessel with a normal wall. It follows also, then, that the resultant increase in resistance from the shortening of the muscle in the thickened wall would be considerably greater than that for a normal vessel. This exaggerated increase in vascular resistance for a given level of smooth muscle shortening has been termed "structural hyperresponsiveness" and would occur totally apart from any variation in vascular smooth muscle sensitivity. (10, 12, 14, 16, 45)

Moreover, Sivertsson has shown that this phenomenon will be observed in any comparison of a thick walled vessel with a thinner walled vessel by his study of A-V shunts in cats. (45) The forelimb vasculature of the cat contains two structurally different types of arterial circuits. One circuit is primarily involved with regulation of nutritional blood flow to the forelimb tissue, while the other "shunt" circuit is involved with the thermoregulation of the limb. The resistance vessels of this latter circuit have thicker walls compared to the resistance vessels involved in regulation of the nutritional flow. Sivertsson observed a vascular

hyperresponsiveness to sympathetic nerve stimulation and intra-arterial norepinephrine in the thick walled vessels of the shunt circuit compared to the relatively thin walled vessels of the nutritional circuit. Consequently, vascular hyperresponsiveness would be expected in hypertension if, indeed, the vessel walls were thickened.

As a first step in investigating these theories in hypertension, Folkow and his co-workers examined the forearm circulation of humans with established, benign, essential hypertension. They found that resistance was still increased in the forearms of patients with essential hypertension, even during metabolically induced maximal vasodilation. (10) Also, in a separate study, Conway had shown that the diastolic pressure of hypertensive rabbits was still elevated, compared to normotensive controls during maximal vasodilation with nitroglycerine. (6)

These findings at maximal vasodilation, suggested, but did not absolutely prove that a primarily inwardly directed vascular wall thickening occurs in the vasculature of hypertensives, i.e., there was some structurally based component to the increased resistance seen in hypertension. This structural component would raise the baseline from which all vascular changes would take place, and thus, as theorized by Folkow, cause an exaggerated increase in resistance at any given level of smooth muscle shortening. (In addition, Sivertsson and Folkow have stated that an increased resistance at complete vascular smooth muscle

relaxation, apart from any thickening of the vessel wall, will also raise the baseline from which all vascular changes take place and, therefore, also cause an exaggerated (though not as great as if the vessel wall was thickened too) increase in resistance at any given level of smooth muscle contraction. (12, 45))

The finding of evidence suggestive of vascular wall thickening in hypertension is very important because of its implication to the development and especially the maintenance of the elevated arterial pressure in hypertension. Theoretically, such a structural vascular change could elevate peripheral resistance and arterial blood pressure even though all other factors (such as neurogenic or humoral based smooth muscle vasoactivity) remained unchanged.

Therefore, structural changes in the vessel wall could contribute greatly or even account for all the increase in resistance seen in chronic arterial hypertension. Because of this possibility, considerable attention has been given to finding evidence for structural vascular changes in chronic arterial hypertension (3, 5, 6, 10, 11, 12, 14, 15, 16, 17, 24, 25, 26, 28, 30, 33, 34, 35, 36, 38, 40, 45, 46, 49, 50, 51, 52, 53).

Folkow and his associates have conducted subsequent studies in experimental hypertension in this regard. These studies support the theory that vascular wall thickening occurs in chronic arterial hypertension. The experimental model of hypertension employed by Folkow and his associates

consisted of rats with a genetic predisposition toward hypertension. These rats naturally become hypertensive early in their development without any experimental intervention by the investigator. Thus, these animals are often referred to as spontaneously hypertensive rats. (11, 12, 14, 15, 16, 27, 28, 36, 38, 39, 44, 53)

In these spontaneously hypertensive rats perfused with an artificial medium, Folkow's group has found that vascular resistance of the hindlimb is increased at maximal vasodilation, and, in addition that the slopes of norepinephrine dose-response curves for these rats are significantly increased compared to normotensive controls, even though the thresholds to norepinephrine remained the same. (12, 16) In other words, this vascular bed showed the type of vascular hyper-reactivity that would be expected from vessels with thickened walls. This same vascular bed also showed an increased maximal response to vasoconstrictor agents. (An increased maximal vasoconstrictor response and slope of the dose-response curve has also been demonstrated by Folkow in the renal vascular beds of spontaneously hypertensive rats. (14)) An increased maximal vasoconstrictor response is indicative of an increase in the amount of contractile tissue mass in the vessel. This increased maximal vasoconstrictor response, along with the increased resistance at maximal dilation and increased slope of the dose response curve, <u>together</u> then, can according to Folkow, only be explained

on the basis of a primarily medial hypertrophy of the smooth muscle in the resistance vessels of the hindlimbs of these rats. (12, 16) In addition, Folkow states that the hemodynamic consequence of the proposed structural change (calculated from a model based on the data concerning the "hypertrophied" vessel) can largely alone account for the raised resistance at rest in the SHR without necessitating any increased smooth muscle activity. (12)

Other investigators have now provided a variety of data that is supportive of vascular wall thickening in hypertension. Sivertsson, in 1968, not only reproduced the attenuated vasodilation in the forearm of humans with essential hypertension, but also reported a significant increase in forearm vascular response to norepinephrine. (45) As in Folkow's study with spontaneously hypertensive rats, no increased sensitivity to norepinephrine was demonstrated.

Also, Lais and Brody have reported significant vascular hyperresponsiveness in spontaneously hypertensive rats that they felt was at least, in part, due to vascular wall thickening. (27, 28)

Phelan reported a significantly increased resistance in the pump perfused hindlimbs of chronic two-kidney Goldblatt hypertensive rats at maximal vasodilation, (38) as did Overbeck in the pump perfused forelimb of dogs with chronic one-kidney perinephritic hypertension. (34) In Overbeck's study, passive distention of the vasculature, caused by increasing the perfusion pressure, could not reduce resistance in the hypertensive animals' forelimb

to a level attained in normotensive dogs at a distending pressure half as great as that in the hypertensive animals' limb.

This impaired passive distention has also been demonstrated in the renal and general systemic circuit of spontaneously hypertensive rats by Folkow. (11, 14) Importantly, impaired passive distention is another characteristic of vessels with thickened walls.(11, 14, 35, 50)

Medial hypertrophy is not the only structural vascular change seen in hypertension. Tobian and Binion, in 1952, reported that the renal arteries and psoas muscle of hypertensive patients contained significantly elevated amounts of water and sodium compared to normotensive controls. Such tissue "waterlogging", could substantially elevate peripheral resistance by increasing the wall-to-lumen ratio of the resistance vessels.

Since structural vascular changes do apparently occur in chronic arterial hypertension, and have profound hemodynamic implications, a great deal of attention has been given to the etiology of these changes. Specifically, a large number of studies have centered on the role that pressure plays in such changes. These studies are generally directed at examining the vascular effects of artifically produced hypotension, or at the comparison of vascular beds of patients, or animals, with aortic coarctation hypertension.

Aortic coarctation provides a unique tool for

examining the effects of pressure on vascular characteristics in that the vascular beds above and below the coarctation are exposed to two quite different lateral pressures. Arterial pressures above the coarctation are elevated, usually into the hypertensive range, while those below the coarctation are usually normal or near normal. (1, 3, 4, 5, 17, 23, 33, 36, 41, 43, 51, 52)

Bevan and his associates measured the wall thickness and internal circumference of arterial strips taken from rabbits with two weeks of coarctation of the abdominal aorta above the renal arteries. They found a significant positive correlation between the wall thickness of arterial strips taken from the hypertensive regions, above the coarctation, and the carotid (hypertensive) arterial blood pressure of the animal. They did not find any correlation between carotid arterial blood pressure and the wall thickness of strips taken from the normotensive regions below the coarctation. They did find, however, an occasional correlation between the wall thickness of strips from the normotensive regions and the femoral arterial blood pressure of the rabbit. There was no apparent correlation between the internal <u>circumference</u> of any arterial strips and any blood pressure. (3)

In addition, Bevan reported that the maximum norepinephrine contractile response of the "hypertensive" arterial strips was positively correlated with the carotid arterial blood pressure, while no such correlation was

found for the "normotensive" arterial strips. Again, there was an occasional positive correlation for these strips and the femoral arterial pressure. Furthermore, in a later study, Bevan found this same relationship concerning contractile response, using stimulation of the sympathetic nerves instead of norepinephrine as the stimulus. (5)

Of significant importance in these findings is that the hyperresponsiveness of the hypertensive strips was shown to be due to the increased wall mass of the vessel and not due to an increased sensitivity to norepinephrine, since the norepinephrine ED_{50} was not altered by changes in arterial pressure.

In patients with thoracic coarctation hypertension, Samanek and his associates have recently studied the hemodynamics of two vascular regions, above and below, the site of coarctation, an average of 11.5 years after successful surgical correction of the coarctation. (43) Even after this extended period of time, these investigators found that resting blood flows were significantly reduced in the upper extremities (forearm) compared to the vascular regions of the calf muscle, while blood flow in this latter region did not differ significantly from normotensive controls. These differences between upper and lower extremities remained significantly different during maximal reactive hyperemia and during maximal vasodilation with amyl nitrate.

Subsequent calculations revealed that the reduced

blood flow in the upper extremities during all three manuevers was attributable to an elevated resistance in the upper extremity, which, according to Samanek, was probably due to the structure of the vessels, as evidenced by the increased resistance at maximum vasodilation.

In studies on regional hypotension, Sivertsson (45) found that the vascular bed of the hindlimb with 3 to 5 weeks partial occlusion of the supplying artery in the cat exhibited a significantly decreased responsiveness compared to normal hindlimbs. This vascular hyporesponsiveness was revealed as a decrease in the vascular response of the occluded bed to graded sympathetic nerve stimulation and norepinephrine infusions. Threshold responses to norepinephrine were the same for both experimental groups.

Similar results were later reported by Martin and Conrad in the hindlimb of the dog. (30) Six weeks after ligation of the supplying artery these investigators found that the resting resistance of the limb was significantly lower than that of acutely occluded controls. This lower resistance could not be attributed to alteration of the blood gas tension of carbon dioxide or oxygen. However, histomeric analysis revealed that the vessel walls of the hypotensive vasculature were significantly thinner than those from the control animals.

Folkow and his co-workers produced hypotension in the hindlimbs of young spontaneously hypertensive and

normotensive control rats by ligating the aorta below the renal arteries. In contrast to finds in the unligated spontaneously hypertensive or normotensive control rats, the hindquarters vasculature, pump-perfused with an artificial medium, of the ligated animals exhibited a decreased resistance at maximal dilation, a decrease in the slope of the norepinephrine dose-response curve, and also a decreased maximal response to vasoconstrictors. Since the threshold to norepinephrine was not shown to be changed in the ligated animals, these results, together, were explained on the basis of a thinning of the vessel walls.

What is of further interest, is that these same variables, when measured in the unligated normotensive rats, yielded values that fell in between those of spontaneously hypertensive rats and those of the ligated animals. Thus, three separate sets of resistance measurements were obtained, each seemingly directly related to the level of pressure in the hindquarters.

In another study, Folkow was able to reverse evidence for vascular structural changes in young spontaneously hypertensive rats by treating them for twelve weeks with the hypotensive drugs guanethidine and hydralazine. (15) In these treated animals, the slope of the norepinephrine dose-response curve was decreased as was the maximal vasoconstrictor response, while there was no alteration in the threshold to norepinephrine.

Weiss and Hallback recently studied the time course of regional hypotension in the hindlimbs of adult spontaneously hypertensive rats, pump-perfused with an artificial medium, by ligating the abdominal aorta below the renal arteries. (53) After only three days of ligation the hindlimb vasculature already began to show signs of regression of the hypertensive vascular thickening. By three weeks the vessels had completely reversed their thickened state by exhibiting a widened lumina and a decrease in the wall mass. However, even after 17 weeks of hypotension these hindlimbs of adult rats would not adapt to the level seen in similarly treated young spontaneously hypertensive rats. (The implication of this finding is discussed in the Discussion Section of this thesis.)

Studies on vessel water and salt content in coarctation hypertension have also been conducted. Hollander and his co-workers, in their study on thoracic coarctation hypertension in dogs, reported that the sodium, chloride, and water content of the hypertensive portion of the thoracic aorta was increased compared to the same portion in normotensive dogs, while the water and electrolyte content of the normotensive portion of the aorta was virtually identical to a portion from the same area in control dogs. (26) In addition, Hollander reported an increase in the amount of ion-binding mucopolysaccharides in the coarcted dog in the hypertensive portion of the

aorta only. He then reasoned that the increased ion and water content of the hypertensive aorta was due to the increased amount of ion-binding mucopolysaccharides whose rate of synthesis was, in turn, positively influenced by an increased intravascular pressure.

Villamil later reported similar results to Hollander's both in a preliminary investigation on experimental thoracic coarctation in dogs, (51) and also in a more complete study on the same experimental model with Matloff. (52) Tissue samples from the aorta proximal to the coarctation, taken four weeks after coarctation, showed significant increases in magnesium, calcium, water and intracellular sodium, compared to the same portion of aorta in normotensive controls, or to the distal segments of the aorta of the coarcted animal. Carotid artery samples, taken four weeks after coarctation showed significant increases in total and non-inulin sodium as well as increases in calcium. Importantly the ionic composition of the femoral artery which was not exposed to an elevated pressure, was not affected by the coarctation.

The studies just mentioned on ionic composition, as well as the hemodynamic and histomeric studies on regional hypotension and coarctation hypertension, all seem to suggest that pressure is involved in, and may even be responsible for bringing about the structural vascular changes seen in hypertension. Because of these results, the investigators believe that the structural vascular

changes in hypertension are merely a secondary adaptive alteration to the increase in intravascular pressure. (3, 4, 5, 10, 11, 12, 14, 16, 40, 45, 50, 53)

This, of course, by no means precludes the involvement of other factors, such as neural and humoral influences, in the pathogenesis and maintenance of the structural changes, their involvement being direct, or indirect. Indeed, there is a body of evidence that seems to suggest that an increase in intravascular pressure is not necessary for bringing about certain vascular alterations in hypertension. This becomes partially evident when one examines a recent study by Hansen and Bohr. (25)

These investigators produced a hypotensive vascular bed by partially occluding the external iliac artery in only one hindlimb of groups of spontaneously hypertensive rats, rats with deoxycorticosterone acetate (DOCA) hypertension, and normotensive control rats. They then compared the contractility (maximum contractile force) and threshold response (to a variety of agonists) of helical strips from the "low" and "high" pressure femoral arteries. To complete the analysis these strips were also compared with respective strips taken from the normotensive control rats.

In general, it was found that <u>both</u> the "high" and "low" pressure strips of either group of hypertensive rats exhibited a significantly increased sensitivity to some type of agonist, when compared to "high" and "low" pressure strips of normotensive rats. Furthermore, it was shown that there was no difference in the sensitivity of "high" and "low" pressure strips in any group of rats.

Therefore, lowering the transmural pressure of a previously hypertensive limb could not reverse the changes in sensitivity seen in hypertension; and also, protecting a vascular bed from an induced hypertension could not prevent this same altered sensitivity from occuring.

It was also found that the contractility of both types of strips was significantly reduced in both the spontaneously hypertensive and DOCA hypertensive rats compared to the appropriate normotensive controls. What is of further interest, is that, in all three groups of rats, the "low" pressure strips exhibited a significantly reduced contractility. Hansen and Bohr, therefore, concluded that functional changes in the sensitivity of a vessel are not dependent on the level of a transmural pressure. Extrapolating this to the development of hypertension in general, these investigators have postulated that structural changes in hypertension are secondary to an increase in intravascular pressure, while changes in sensitivity are not.

From a hemodynamic study done in 1963, Nolla-Panandes published a report on experimental coarctation in the rat. (33) In rats with aorta coarcted above the origin of both renal arteries, forelimb arterial pressure was elevated, while hindlimb arterial pressure was normal compared to animals clipped below the renal arteries or to sham operated controls. These latter two groups had normal fore and hindlimb pressures. However, when the hindlimbs of

these rats were pump perfused with an artificial medium the following results were obtained.

Even though the hindlimbs were not exposed to an elevated intravascular pressure, perfusion pressures generated in the hindlimbs, at a constant flow, in rats with coarctation hypertension were significantly elevated compared to the other control groups. In addition, the mean pressure rise in response to a single dose of norepinephrine was significantly greater in the rats with coarctation hypertension compared to the control animals. There were no significant differences between the sham controls and the rats coarcted below the renal arteries. Also, for rats coarcted above the renal arteries only, Nolla-Panandes found a significantly positive regression of hindlimb norepinephrine responses on the initial hindlimb perfusion pressure.

According to the author, the findings in the rats coarcted above the renal arteries could not be attributed to differences in the size of the animal, or to effects of coarctation on the development of the hindlimb vasculature, as body and limb weights were similar in all the groups of rats. Also, he reported that the vasculatures showed no residual muscular tone. He concluded that the changes seen obviously could not be attributed to an elevated intravascular pressure and instead might in some way be related to the mechanism that produced the hypertension!

In a more recent study Pamnani and Overbeck examined the electrolyte and water content of rats coarcted about the aorta in the same manner as the models used by Nolla-Panandes. (36) Blood pressure recordings revealed that the rats coarcted above the renal arteries had elevated carotid arterial pressures compared to the sham operated normotensive controls, while there was no difference between the femoral arterial pressures for these two groups, as these pressures remained normotensive.

In contrast to the findings of Hollander in thoracic coarctation in the dog, Pamnani and Overbeck found that the water, sodium and potassium content was significantly elevated in the abdominal aorta and veins, as well as the thoracic aorta of rats coarcted above the renal arteries, compared to controls. In addition, there were no significant differences in ionic or water content between the hypertensive and normotensive portions of the aorta, nor was there any correlation between the magnitude of the vascular changes in the aorta, or veins, and the level of the carotid arterial pressure. These results, then, suggest that altered levels of vascular ions and water in hypertension, are not necessarily directly caused by an elevated intravascular pressure.

Although these last three studies do not totally exclude the possibility that an increased intravascular pressure plays a role in determining vascular wall structure, and function in hypertension, it would now seem

that increased intravascular pressure is not the sole determinant of vessel wall structure and function.

As an additional determinant, the role of the kidney, especially through its renin-angiotensin system, has also been implicated in the pathogenisis of hypertension. This implication is based on the fact that the renin-angiotensin system is known to be able to effect changes in blood pressure through the vasoconstrictive nature of angiotensin II, and through the system's effect on sodium reabsorption. (51, 18, 40) This system is stimulated by a decrease in the perfusion pressure in the afferent arteriole of the kidney. Such a situation could arise from a decreased blood flow to the kidney.

It has been seen that coarctation hypertension has been used in examining the effects of pressure on blood vessels, and that, this type of hypertension involves a constriction of the aorta above the renal arteries. Since the constriction could affect blood flow to the kidneys, one could postulate the renal system as an additional factor besides pressure in the development of the hypertension, in this experimental model.

There has been general evidence since the 1930's that renal factors might be involved in the development of hypertension due to coarctation of the aorta. This was first demonstrated by Goldblatt who found that he could produce hypertension in dogs by coarcting the aorta above the renal arteries but not below. (21)

Similar results have since been obtained by Habib et al in dogs (23) and, as previously stated, by Nolla-Panandes in rats. (33) Both of these investigators have provided additional evidence in support of a renal factor in coarctation hypertension.

Habib has found a generalized increase in blood pressure in coarctation of the thoracic aorta, not just an increase in arterial pressure above the coarctation. Also, he has found that transplanting a kidney to a vascular region above the coarctation will substantially (but not totally) reduce arterial pressure above and below the level of coarctation.

Besides showing that coarcting the abdominal aorta would only produce hypertension if instituted above the renal arteries, Nolla-Panandes analyzed the time course of the blood pressure changes as a further indication of renal involvement. He observed, that immediately after coarcting the abdominal aorta above both renal arteries in rats, the fore and hindlimb blood pressures drop. After this point in time, both pressures rise within a day in a parallel manner until the forelimb pressure is hypertensive and the hindlimb pressure is normotensive again at the end of two weeks. Nolla-Panandes stated that this type of time course in the development of the hypertension could not be totally explained by a gradual change in the severity of the coarctation itself (i.e. such as a gradual sclerotic

narrowing of the coarctation). If this alone was the only development in the days following the coarctation, then as the aorta narrowed, pressure below the constriction would decrease as pressure above the constriction increased.

More direct evidence for renal involvement in coarctation hypertension has been provided by Ribeiro and Krakoff. (41) In a preliminary study these investigators found an elevated plasma renin activity in human coarctation hypertension but not in essential hypertension. Neither group of patients had any type of renovascular disease. Additionally, it was found that 30 minute infusions of saralasin, a specific angiotensin antagonist, would substantially reduce the blood pressure in the patients with coarctation of the aorta, while patients with essential hypertension were not affected by the same infusion.

Bagby and her associates have found a significant decrease in renal plasma flow and the glomerular filtration rate in salt-restricted dogs with thoracic aortic coarctation hypertension, compared to salt-restricted control dogs. The coarcted dogs also showed a significantly increased plasma renin activity and reactivity. (1)

Timmis and Gordon have also implicated the renal pressor system in the pathogenesis of coarctation hypertension and have suggested that this system is stimulated by

the coarctation's effect of dampening the pulsatile nature of the renal blood flow. (48)

Furthermore, the renin-angiotensin system may be involved in the development of the structural cardiovascular changes that have been postulated to be largely responsible for the maintenance of the elevated arterial pressure in hypertension. Sen and her co-workers have reported an increase in ventricular weight and plasma renin activity in young spontaneously "hypertensive" rats before the animal actually becomes hypertensive. (44) The ventricular weight and plasma renin activity were positively correlated in these rats. In addition, antihypertensive drug therapy using methyldopa or hydralizine was able to reduce blood pressure in the hypertensive rats. However, ventricular weight was reduced by methyldopa only. Interestingly, methyldopa reduced plasma renin activity in the rats while hydralizine increased the plasma renin activity.

Although these findings suggest a possible hypertrophic effect of the renin-angiotensin system on cardiac muscle tissue, it is not known whether or not this system can affect vascular smooth muscle in a similar manner.

In this regard, Villamil (51) has shown that chronic exposure of arteries to angiotensin II significantly increases their total sodium, magnesium, and calcium content. Exposure to angiotensin also increased the artery's permeability to sodium and decreased the inulin space of the artery.

Importantly, the increased permeability seems to be specifically related to the angiotensin.

In contrast to the findings that the renin-angiotensin system is involved in hypertension, Markiewicz et al reported decreased plasma renin activities in humans with coarctation hypertension and, in addition, could not demonstrate a correlation between the plasma renin activity and the level of arterial blood pressure. (29) However, there was no renal underperfusion present in these patients at the time of the study.

Also, in Bevan's study on abdominal coarctation in rabbits, the investigators stated that their experimental model had, not to their knowledge been associated with an elevated plasma renin activity (3), and this study did not reveal any arterial alterations below the site of the coarctation.

A humoral factor such as the renin-angiotensin system, would largely be expected to effect the entire vascular system of the animal. Whether or not such a system was brought into play during a certain type of hypertension might explain why there is conflicting data in animal models primarily designed to study the effects of different intravascular pressures (i.e. coarctation hypertension).

Aside from humoral factors, neurogenic factors have been another area of investigation in the pathogenesis of hypertension. Bevan et al have reported significant

increases in the norepinephrine content, release, and neuronal uptake of the terminal adrenergic nerve plexus in the "hypertensive" ear artery of rabbits with coarctation of the abdominal aorta. They have also reported an increase in the vessels' permeability to norepinephrine. (4, 5) These changes were not seen in the "normotensive" vasculature or the veins. However, extraneuronal uptake of the norepinephrine as well as the activity of norepinephrine degradating enzymes were also shown to be increased in the ear artery.

Coupled with the fact that, compared to controls, the media of these arteries were thickened, these last two factors concerned with norepinephrine disposition would tend to decrease the effects of sympathetic nerve activity, thus, according to Bevan, negating the effects of the increased activity in the terminal adrenergic nerve plexus.

Lais and Brody have reported a selective increase in the sensitivity to norepinephrine in the pump perfused hindlimbs of spontaneously hypertensive rats. (28) They correspondingly concluded that altered sensitivity and vascular wall thickening were involved in the vascular hyperresponsiveness seen in these rats. It should be noted that, in contrast to Folkow's perfusion studies, this investigation used blood as the perfusate; or to use the vernacular of the investigators, the experiments were performed in a vascular bed exposed to a "full

humoral complement". Therefore, Lais and Brody stated that the altered sensitivity may in some way be related to circulating hormones. In this respect, Goldberg has shown that angiotensin II, can release norepinephrine from the nerve terminals of veins. (20).

Turning to other neurogenic influences, although the baroreceptors appear to be "reset" in hypertension, (17, 27) it has been believed that actual sympathetic discharge is nevertheless normal. (17, 40, 45) Recent evidence by Lais and Brody would appear to support this belief. (27)

In summary, then, the increased peripheral resistance seen in chronic hypertension appears to result, at least in part, from the structural characteristics of the resistance vessels, although there is evidence that the smooth muscle cells may be hypersensitive to certain vasoconstrictors.

The studies suggest that the smooth muscle lining these vessels is hypertrophied and that the vessel walls contain elevated amounts of salt and water and fibrous tissue. These factors tend to encroach on the lumens of the vessels thus elevating their resistance to flow, even at maximal vasodilation.

A large body of evidence has suggested that these structural vascular changes are a secondary adaptive development to an increased intravascular pressure. However, other studies, while not excluding pressure as a

possible influence on these changes, seem to suggest that other factors may be involved.

A likely candidate as an additional factor in the development of the structural changes may be the renin-angiotensin system.

Hypothesis

One of the characteristics of chronic arterial hypertension is an increase in total peripheral vascular resistance. This increased resistance is, to a large extent, based on an underlying thickening of the walls of the resistance vessels which encroach upon their lumens, even at maximal vasodilation, thus raising the baseline from which all resistance changes take place. It has been believed that this vascular wall thickening is a secondary adaptation of the resistance vessels to a primary increase in blood pressure. To test this hypothesis, resistance measurements were made in the normotensive vascular beds of rats with coarctation hypertension. Any evidence for increased resistance and/or vascular wall thickening in these normotensive vascular beds of a hypertensive animal could not be a secondary adaptation to high intravascular pressure, but instead, then, may be related to the etiology of the hypertension itself.

Methodology

Pre-experimental

Male, outbred, Sprague-Dawley rats, weighing between 150 and 200 grams were used in the study. Under sterile conditions and ether anesthesia, a midline/abdominal longitudinal incision was made in each animal.

The rats were then randomly divided into four groups. In one group, hereafter to be called Group A, a small, circular silver clip, approximately 1 to 2 mm wide and 0.813 mm in interior diameter, was placed around the abdominal aorta above the origin of both renal arteries to produce coarctation hypertension.

In a second group, hereafter to be called Group B, a silver clip, with an internal diameter of 1.48 mm or 1.70 mm (See Appendix A) was placed around the abdominal aorta above the origin of both renal arteries. This group of rats served as sham operated normotensive controls, for the clip was too large to cause significant constriction of the abdominal aorta.

A third group of rats, hereafter called Group C, were designed as controls for the hindlimb atrophy that might occur in Group A. In this group a constricting silver clip, with an internal diameter of 0.610 mm was placed around the abdominal aorta below the origin of both renal arteries.

This type of aortic coarctation reduces intravascular

pressure and flow in the vascular regions below the clip, but does not produce hypertension in the vascular regions above the clip.

Finally, two-kidney Goldblatt hypertension was created in a fourth group of rats, hereafter called Group D, by constricting the renal artery of the left kidney with a silver clip with an interior diameter of 0.406, 0.419, or 0.434 mm (See Appendix A) and leaving the other kidney intact. The purpose of this final group of rats was to ensure that elevated hind-limb resistance could be detected in the perfusion experiments described below.

After clipping, the incision was closed. The animal's weight in grams was recorded, after which it was placed in a plastic cage, three or four rats per cage, and allowed to recover from the effects of the anesthesia.

The rats were then kept in a temperature controlled room (approximately 24° C) in the Laboratory Animal Care Service at Michigan State University for a post-operative/pre-experimental period of not less than four, and no greater than five weeks. During this period the animals were maintained on a diet of standard rat chow and tap water. No drugs were administered at any time during this four week period and with few exceptions, all the animals remained healthy. The mortality rate was less than 10% for all the groups of rats.

Blood pressure and body weights were recorded during the second, third, and fourth weeks after clipping. Blood

pressures were taken in rats lightly anesthetized with ether by tail plethysmography, as described by Phelan. (37) Two readings of caudal arterial systolic pressure were measured and recorded. These pressures were used to represent arterial pressures in the regions below the site of coarctation.

Experimental Methodology

The experiments in this study were designed to measure hemodynamics in the isolated innervated, blood pump perfused hindlimb of the four groups of rats just described. Experiments on the rats were always performed at least four weeks, and always less than five weeks, after a set of rats were clipped.

For an experiment on a particular day, three rats clipped on the same day of the same group were selected so that their body weights would be as close to 350 grams as possible. The majority of rats attained this weight sometime during the fourth week after they were clipped.

Once selected, the rats were moved to the room where all the experiments were performed during the study. Here the rats were weighed to the nearest gram and the weights were recorded.

Of the three rats selected, one rat was to be perfused, another was to be used as a blood donor for the first, and the third rat was used as a backup for either the perfused rat or the blood donor. On days when two

experiments were to be performed, the same blood donor was used for both rats.

To prepare a rat for an experiment it was first placed in an anesthesia chamber containing a few drops of ether. Once fully anesthetized (no whisker twitch) the rat was removed from the chamber and layed supine on a small surgical table where its limbs were extended and taped down. Anesthesia was continued using a nose cone with an ether soaked cotton plug.

A small midline neck incision was then made separating the lobes of the thyroid gland, and the sternothyroid muscle thus exposing the animal's trachea which was then isolated.

Next a 2 cm inguinal incision was made in the animal's right groin to expose the right femoral artery and vein. This vein was then cannulated in a proximal direction and secured using a small L-shaped glass cannula connected to a piece of rubber uterine tubing.

The ether nose cone was then removed, and 70 mg/Kg in 0.7 cc of normal saline of alpha-chlorolose was given to the animal I.V. through the femoral vein cannula as in initial anesthesia. (Note: the initial dose of anesthesia was not sufficient for the purposes of the experiments if less than 50 mg/Kg was given to the rat. Insufficient anesthesia was considered due cause to invalidate data gathered on such an animal. See Appendix A)

With the rat anesthetized, a small incision was then

made in the trachea allowing the insertion of a short (≈ 1.5 cm) piece of polyethylene tubing (0.066 inches of I.D.; 0.095 inches O.D.). This "tracheal tube" was then secured with the sutures already present in the area and allowed one to aspirate any tracheal mucous and other fluids out of the lungs. The right carotid artery was then isolated and packed with gelfoam (Thrombin in plastic foam; Upjohn Co.) and 00 black silk suture was carefully slipped underneath it, to aid in its retraction.

Procedures were then directed to isolating the rats left hindlimb which was to be used in the perfusion experiments for this study. Briefly, this hindlimb was isolated from the body by severing (with cautery) and ligating skin and muscle connections and by dislocating the hip from the pelvis with a heavy ligature. The femoral artery, vein, and nerve were left undisturbed as was the sciatic nerve and its medial branch.

Thirty minutes after the last surgical procedure, heparin was administered to the animal (400 USP units, Wolins Industries).

During this thirty minute interval, allowed for clotting, the skin incision was closed, with the exception of approximately 1 1/2 cm directly over the femoral vein, artery and nerve. This area was gently packed with normal saline soaked cotton to keep structures moist, and a small amount of Gelfoam for hemostasis. The limb was gently extended and secured by connecting a hemostat to one toe,

then securing the hemostat to the table with tape.

Also during this thirty minute interval the donor rat was then anesthetized with ether, laparotomized, and aortic blood was drawn into a 12 cc heparinized syringe. This blood was quickly transferred to a heparinized beaker and then swirled by hand to insure complete mixing with the heparin (and thus avoid the formation of microclots). The beaker was then covered with Parafilm to prevent evaporation and reduce the chance of contamination. An autopsy was then performed on the donor rat. If anything proved wrong with this rat such as a lung infection or renal infarction, its blood was discarded and a new donor was used.

The total amount of time used to prepare the experimental animal for perfusion including the 30 minutes waiting period before administering the heparin was monitored for each rat. If this time exceeded 2 hours and 30 minutes in duration, the animal would not remain sufficiently anesthetized during the full length of the perfusion experiment and thus any data from animals of this type were discarded. By staying under the 2 hour, 30 minute level, it was assured that all animals studied would be equally sedated during the entire length of the experiment. (See Appendix A)

At this time the pump used for this study was filled with normal saline and allowed to run at maximum flow. This pump was a modification of a pump previously described (9); this pump provides a nonpulsatile flow, is pressure

independant to at least 350 mm Hg, and produces negligible hemolysis. For this study, side branches of the pump's inflow and outflow tubing could be connected to Statham P23Gb pressure transducers. These pressures could then be measured on a Hewlett Packard 7702B dual channel recorder-amplifier and a H.P. Moseley 7100B strip chart recorder. The pump was enclosed in a plexiglas cover, used to keep the pumping mechanism at a temperature of 37° C. The entire pump was on a platform that could be rotated 360 degrees.

Before the perfusion of each animal, heparinized cannulae were inserted in a proximal direction into the right femoral and carotid arteries and secured with sutures and by means of adjustable clamps on the side of the surgical table. This table was then raised to the level of the Statham strain gauges and by means of a Silastic, normal saline filled, tube connected to one of the strain gauges, mean carotid and femoral arterial pressures could be directly recorded alternately. These pressures were used to represent arterial pressures in the regions above and below the clip, respectively. At least two mean carotid and mean femoral arterial pressures were recorded for each experiment. If each pair of carotid or femoral pressures did not agree within 5 mm Hg of each other, another carotid and femoral pressure was obtained until two successive pairs of pressures agreed in the prescribed manner. The cannulae were then clamped and left in place.

With this segment of the experiment completed, the

Silastic tubing was disconnected and the pump inflow and outflow tubing was connected to the strain gauges via their side tubing. All the pump's tubing was flushed with normal saline and the entire pump was run at maximum flow while being rotated to 90^{o} and 180^{o} to remove any and all trapped air.

At this time, supplemental anesthesia was given to the rat as an IV injection of pentobarbital (J.T. Baker Industries) 16 mg/Kg body weight and alpha-chloralose (25 mg/Kg) (See Appendix A).

With the supplemental anesthesia given to the rat, a heparinized cannula was inserted into the left femoral artery (the lone artery to the isolated limb) in a downstream (distal) direction and secured in a nonobstructive position with sutures and a side clamp connected to the surgical table.

Before the limb could be perfused the pump was primed with the heparinized donor blood at 1cc per minute. (cc used interchangably with ml throughout paper; flow was actually measured in ml's) When the pump was completely filled with blood the pump inflow tubing was connected to the carotid artery cannula, being sure that no air got into the system during the connection of the pump tubing to the cannula.

With this connection made, the clamp on the carotid artery cannula was removed and the pump was started at 1 cc per minute. Tube and cannula resistance produced an immediate drop in the inflow pressure of about 60 mm Hg. If a much greater drop in pressure occurred at this time, it was taken to mean that the carotid artery was in some manner

obstructed. The pump was stopped, and the cannula repositioned.

If no occlusion was indicated, the pump outflow tubing was connected to the left femoral artery via the cannula and the isolated limb was then perfused at 1 cc per minute for 15 minutes to establish a steady state. Blood leaving the isolated left hindlimb was allowed to freely return to the animals body via the left femoral vein. Only the right carotid artery was used to supply blood to the pump during the experiment. The right femoral arterial and venous cannulae were not included in the pump circuit nor were they used for any other measurements during the perfusion of the animals left hindlimb.

Pump outflow (perfusion) pressure and pump inflow pressure were both recorded continuously on the strip chart recorder. Pump flow was adjusted by a digital pump rate control unit connected to the pump motor.

After the 15 minute steady state period was completed, pump flow was then adjusted so that steady state perfusion pressure (minus the pressure drop across the tubing and cannula (see below)) was similar to the rat's femoral arterial pressure. The pressure and pump flow was recorded and designated "resting limb pressure" and "resting limb blood flow." "Resting limb resistance" was calculated using these values.

Once resting flow and resistance were obtained, the effects of passive distension of the hindlimb arteries was

studied. To accomplish this, pump flow was first adjusted to 0.125 cc/minute and allowed to remain there for at least 5, but no longer than 9 minutes. In this manner enough time was allotted for achievement of a steady state at this flow. Flow was then adjusted, in order, to 0.25, 0.5, 1.0, 1.5, and 2.0 cc/minute. As in the case of the flow at 0.125 cc/minute, a steady state was first obtained at each flow before moving on to the next flow, providing steady state was attained again before the ninth minute. (See Appendix A)

After this set of flows was complete, the pump was stopped momentarily in order to monitor the carotid blood pressure of the animal as a rather rough indicator of the general condition of the animal. Also, the drop in limb perfusion pressure, once the pump was stopped, was watched to see if it dropped below 5-7 mm Hg. If this was the case, it was considered evidence for complete isolation of the hindlimb. Then the same set of flows were repeated and generated pressures again recorded.

After completion of the two sets of flows, flow was returned to 1.0 cc/minute. Once a steady state was achieved, (or in 9 minutes) first the femoral then the sciatic nerves were cut. A drop in perfusion pressure after cutting the femoral and sciatic nerves was considered evidence that they were viable. Ten minutes after severing the femoral nerve, the steady state perfusion pressure was recorded. The resistance calculated was thus termed <u>resistance after acute denervation</u>.

With the limb denervated, and the pump still at 1 cc/minute, a supramaximal dose of the vasodilator sodium nitroprusside (0.6 mg/Kg in 1 cc normal saline/Kg) was injected rapidly into pump tubing upstream to the pump. This agent is believed to act directly on the vascular smooth muscle to produce relaxation of the smooth muscle. (22) Maximal vasodilation of the hindlimb vasculature was tested by successively doubling the dose of the nitroprusside until no further decrease in hindlimb perfusion pressure could be detected. Perfusion pressure 4 minutes after the final injection was recorded; calculated resistance was designated "resistance after maximal sodium nitroprusside vasodilation."

With the pump still running at 1 cc/minute the cannula in the perfused limb was cut free from the femoral artery ending the experiment. Two heparinized capillary tubes were quickly filled with the free-flowing blood. The rest of the animal's blood was pumped into a beaker. At this time, in all rats, the pressure gradient across the outflow tubing and cannula was measured at each flow rate used during the experiment (Figure 1). This value was subtracted from the respective perfusion pressure recorded and the net value was used to calculate all limb resistances. Also, for these calculations femoral venous pressure in the isolated hindlimb was taken to be constant during each portion of the experiment. The capillary tubes were used to determine the blood hematocrit of the rat while the beaker of blood

was used for determining plasma creatinine, sodium, potassium, calcium, and magnesium.

The rat was autopsied, and the animal's clip type was verified. The animal's kidneys and heart were excised and weighed. Both hindlimbs were removed and weighed. These limbs were then dried in an oven at about 86^{o} C and weighed not less than one week later.

Resistance was expressed as net perfusion pressure/ cc minute $gram^{-1}$ perfused limb wet and dry weight and also in terms of the opposite limb wet and dry weight (for discussion, see Appendix B).

Sixteen additional rats of groups A and B were prepared after the end of the study. Samples were taken from these rats for plasma renin concentrations 4 1/2 weeks after clipping, by decapitating the animal and collecting the first two seconds of blood flow from the severed aorta. Blood was prepared by technicians from Dr. M. Bailie's laboratory at Michigan State University. The animals were then autopsied and the hearts were weighed. Body weights were also recorded at the 4 1/2 week mark.

The renin activity was determined by radioimmunoassay procedures and expressed as ng angiotensin I/ml/hour. Plasma creatinines were analyzed with an autoanalyzer and expressed as mg percent while plasma Na^{+} and K^{+} levels (m Eq/L) were analyzed on the Beckman Flame Photometer. Calcium and magnesium levels (m Eq/L) were analyzed on the Perkin-Elmer Atomic Absorbtion Spectrometer. Calculated

resistances were analyzed with the one-way analysis of variance and the means were compared with the Student-Newman-Kuels test. Other experimental variables were analyzed among groups in the same manner, while comparisons within groups were done with the Student paired t-test. In addition to the analysis of variance, the pressure-flow and resulting resistance-flow curves were analyzed with profile analysis. (31)

As a final note to the reader, it must be mentioned that the experimental protocol used in this study was as much a result of solutions to problems that occured during the study as it was a result of planned protocol. These problems in many cases dictated the "hows" and "whys" of our experimental protocol. These problems were fairly numerous and, therefore, it was felt that to mention them at each point in the methodology would only serve to make the methodology disjointed and thus hard for the reader to follow. Therefore, two appendices have been included in this paper in an effort to put in a concise form how our experimental protocol came to be. It is hoped that this will give the reader a better understanding of why certain methods had to be used in this study, as well as serve as an aid to problem solving for future students in this field.

Figure 1. Pressure drop across pump outflow tubing and cannula

mm Hg = pressure in millimeters of mercury
cc/minute=flow in cubic centimeters per minute

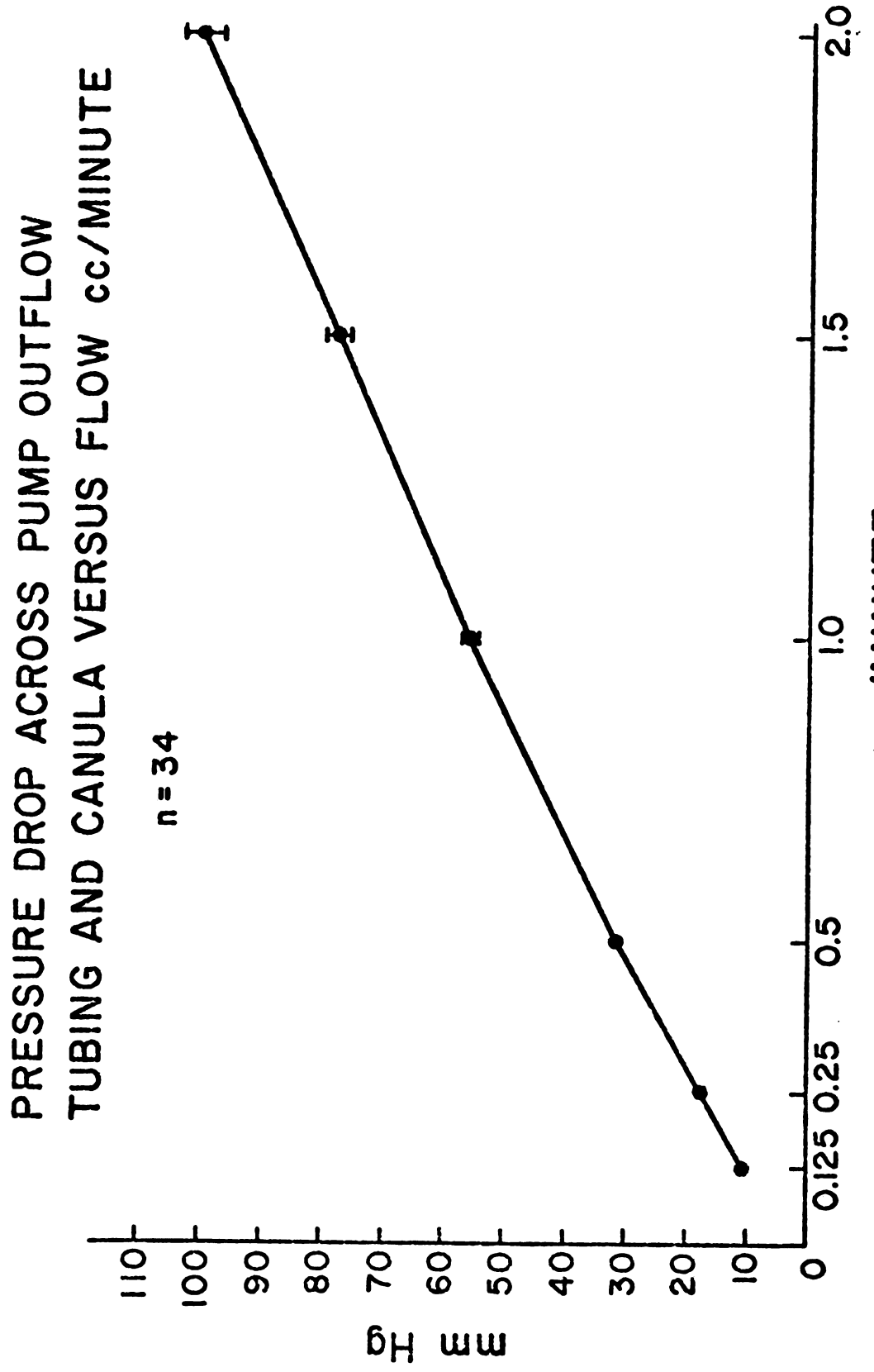
PRESSURE DROP ACROSS PUMP OUTFLOW
TUBING AND CANULA VERSUS FLOW cc/MINUTE
n=34
mm Hg
110
100
90
80
70
60
50
40
30
20
10
0
0.125
0.25
0.5
1.0
1.5
2.0
cc/MINUTE

Figure 1

Results

I. General Parameters and Blood Pressures

Body weights, blood pressures, hematocrits, plasma creatinines and electrolytes, etc., are listed in Table 1. As can be seen the groups of animals did not differ significantly in their body weight, nor was there any difference in their plasma electrolytes. Similar hematocrits that were also within normal ranges suggest that no significant bleeding occurred during the experimental procedure in any group of rats.

Although kidney mass was slightly reduced in Group A rats compared to Group B (significant for the left kidney only, $p < 0.05$), plasma creatinines were similar in all four groups of rats.

Heart weight expressed in terms of body weight was significantly increased ($p < 0.01$) in hypertensive rats of Groups A and D compared to sham controls (by 44 and 21% respectively). There was also a slight ($\simeq$ 1%) increase in heart weight/body weight in Group C rats compared to sham controls ($p < 0.05$).

One of the most important features of the above data is the fact that tail systolic pressures remained normotensive and similar in Groups A and B throughout the four week pre-experiment period. Note also that there were no significant week-to-week variations of the pressures within groups. This similarity was further verified by the

direct measurements of blood pressure taken just prior to the start of the experiments. These direct measurements show that the mean femoral arterial pressures of these two groups did not differ significantly and remained normotensive, although mean carotid blood pressure was significantly higher in Group A rats compared to Group B ($p < 0.01$). Of additional interest is the fact that, the mean femoral blood pressure of Group C was significantly hypotensive ($p < 0.01$), although the mean carotid blood pressures for Groups B and C were normotensive and virtually identical. Also, as expected, Group D rats were hypertensive both in the carotid and femoral arteries ($p < 0.01$ for both vs. the respective sham control), as well as during the 4 weeks prior to the experiments.

For clarity purposes the following figures list only the statistical comparisons that are most important for the particular item being depicted. A statistical summary of all intergroup comparisons for the data in table 1 (and also for all subsequent data) is given in Appendix C.

II. Limb Weight

Opposite limb wet weight, expressed in terms of body weight, was slightly but significantly reduced in rats of Groups A and C (6.0 and 5.3 percent respectively, $p < 0.01$), indicating that slight limb atrophy had occured in these two groups of rats (Figure 2). Consequently, all flow and

TABLE 1

GENERAL PARAMETERS

All values represent means ± standard errors of the means. Group A - rats with coarctation hypertension; Group B - normotensive sham operated control rats; Group C - rats with coarctation of the abdominal aorta below the origin of both renal arteries; Group D - rats with two-kidney Goldblatt hypertension. wt = weight; mg% = milligrams percent; mEq/L = milliequivelents per liter; (* significant difference, $p < 0.05$; ** significant difference $p < 0.01$; *** significant difference $p < 0.001$ all compared to Group B).

( ) = n, number of experimental units

✓✓ $p < 0.01$ within group
✓ $p < 0.05$ within group

TABLE 1

	Group A	Group B	Group C	Group D
Body weight grams	352.8 ± 4.86 (16)	353.1 ± 2.93 (21)	358.9 ± 3.72 (15)	358.7 ± 10.13
Systolic Caudal Arterial Pressures mm Hg				
2nd week	109.2 ± 4.74 (7)	110.9 ± 1.56 (10)	49.8 ± 5.93 (3)	1.73 ± 3.12 (4)
3rd week	108.5 ± 4.57 (10)	109.6 ± 2.13 (13)	56.0 ± 4.90 (6)	182.2 ± 7.60 (6)
4th week	112.8 ± 2.68 (12)	112.7 ± 2.18 (13)	56.7 ± 4.82 (16)	173.3 ± 11.74 (6)
3 week average	110.5 ± 2.19 (29)	111.1 ± 1.17 (36)	55.1 ± 2.99 (15) *	176.6 ± 5.12 (16) *
Carotid Arterial Pressure mm Hg	152.8 ± 5.96 (13) **	110.3 ± 2.46 (20)	110.2 ± 3.84 (13)	149.1 ± 8.88 (9) **
Femoral Arterial Pressure mm Hg	112.3 ± 5.52 (13)	104.9 ± 2.49 (20)	75.4 ± 3.61 (13) **	146.7 ± 9.2 (9) **
Heart Weight/Body Weight	0.0048 ± 0.00015 (16) **	0.0033 ± 0.00006 (21)	0.0034 ± 0.00007 (15) **	0.0040 ± 0.00014 (9) **
Left Kidney Wt./Body Wt.	0.00326 ± 0.00011 (16) *	0.00374 ± 0.00010 (21)	0.00370 ± 0.00010 (15)	0.00273 ± 0.00028 (19) **
Rt. Kidney Wt./Body Wt.	0.00340 ± 0.00014 (16)	0.00370 ± 0.00012 (21)	0.00370 ± 0.00012 (15)	0.00439 ± 0.00027 (9) **
Creatinine mg%	1.14 ± 0.11 (12)	0.91 ± 0.12	1.24 ± 0.17 (8)	1.25 ± 0.20 (5)
Sodium meq/L	130.3 ± 1.88 (11)	134.9 ± 2.05 (11)	131.6 ± 2.52 (8)	134.2 ± 2.35 (6)
Potassium meq/L	4.26 ± 0.20 (11)	4.46 ± 0.15 (11)	4.39 ± 0.22 (7)	4.19 ± 0.23 (4)
Calcium meq/L	3.28 ± 0.15 (11)	3.28 ± 0.09 (11)	3.37 ± 0.12 (7)	3.25 ± 0.09 (4)
Magnesium meq/L	2.55 ± 0.11 (10)	2.72 ± 0.14 (11)	2.45 ± 0.8 (7)	2.42 ± 0.06 (4)
Hematocrits	44.2 ± 0.77 (13)	44.4 ± 0.70 (14)	43.5 ± 0.81 (12)	44.4 ± 0.87 (7)
Plasma Renin Concentrations ng/ml/hr Angiotensin I	21.86 ± 2 **	12.10 ± 0.75		
Heart Wt./Body Wt. for rats used for Plasma Renin Concentrations	0.0047 ± 0.00015 ***	0.0033 ± 0.00006		
Perfused limb H_2O content gm	14.10 ± 0.18 (14)	14.22 ± 0.19 (20)	13.90 ± 0.20 (15)	14.83 ± 0.42 (9)
Opposite limb H_2O content gm	13.10 ± 0.21 (14) * /	13.89 ± 0.15 (20)	13.46 ± 0.22 (15) //	14.10 ± 0.39 (9) /

resistance data were expressed in terms of limb weight as detailed in Appendix B.

III. Resting Resistance and Flow

Resting resistance and resting flow, both expressed in terms of opposite limb wet weight, are depicted in Figures 3 and 4 respectively. As expected resting resistance in the hindlimb vascular bed was elevated in rats with two-kidney Goldblatt hypertension (67%; $p < 0.01$). Hindlimb resistance was also elevated in Group A rats that were coarcted above the renal arteries; i.e., rats that had hypertensive forequarters and normotensive hindquarters. This elevation in resistance in Group A rats is statistically significant when compared with either Group B or C ($p < 0.01$; 58% and 119% elevation, respectively).

Resting hindlimb resistance, expressed in terms of opposite limb wet weight was reduced by almost 29% in rats of Group C compared to sham controls, ($p < 0.01$) using the two-tailed Students t-test.

Resting flows per gram opposite limb wet weight were reduced by 29% in Group A rats compared to sham controls using the Student-Newman-Kuels test ($p < 0.05$). Resting flows were significantly reduced in Groups C and D compared to sham controls ($p < 0.05$, 17 and 22 percent, respectively) when the differences between the means were tested with the Student's two-tailed t-test. (Note: comparisons of flow

Figure 2. Opposite Limb Wet Weight/Body Weight

Coarc Ab = Group A = rats with abdominal aortic coarctation above the origin of both renal arteries

Sham = Group B = normotensive sham operated control rats

Coarc Bl = Group C = rats with abdominal aortic coarctation below the origin of both renal arteries

Renal = Group D = rats with two kidney Goldblatt hypertension

gm = gram; p = probability that the differences between means of the groups occured by chance

n = number of experimental units

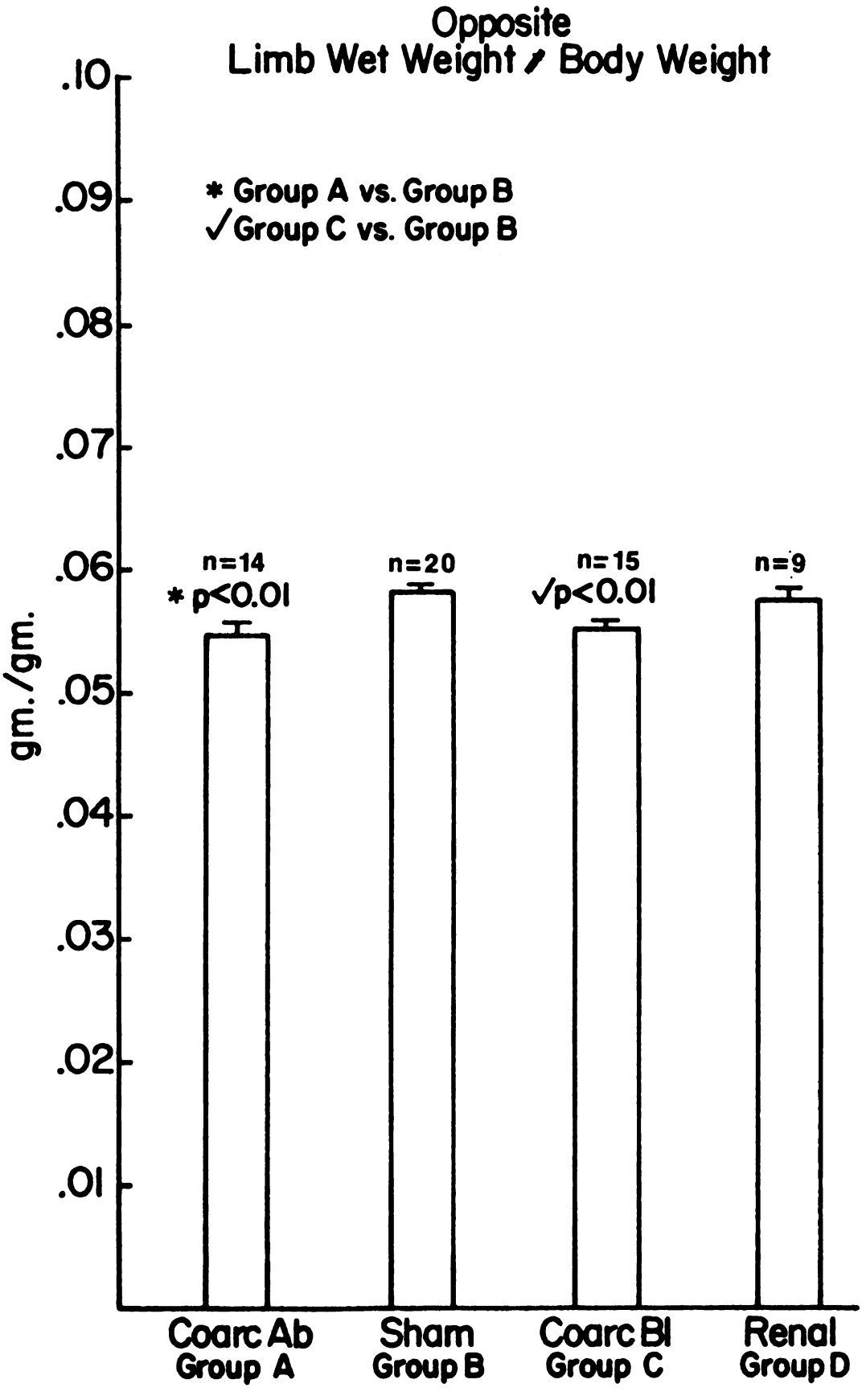
Opposite
Limb Wet Weight / Body Weight
* Group A vs. Group B
✓ Group C vs. Group B
gm./gm.
.10
.09
.08
.07
.06
.05
.04
.03
.02
.01
n=14
* p<0.01
n=20
n=15
✓p<0.01
n=9
Coarc Ab
Group A
Sham
Group B
Coarc Bl
Group C
Renal
Group D

Figure 2

Figure 3. Resting Resistance x Limb Wet Weight

Coarc Ab = Group A = rats with abdominal aortic coarctation above the origin of both renal arteries

Sham = Group B = normotensive sham operated control rats

Coarc Bl = Group C = rats with abdominal aortic coarctation below the origin of both renal arteries

Renal = Group D = rats with two kidney Goldblatt hypertension

p = probability that the differences between means of the groups occured by chance

mm Hg = pressures in millimeters of mercury

ml min^{-1} gm^{-1} = milliliters per minute per gram

n = number of experimental units

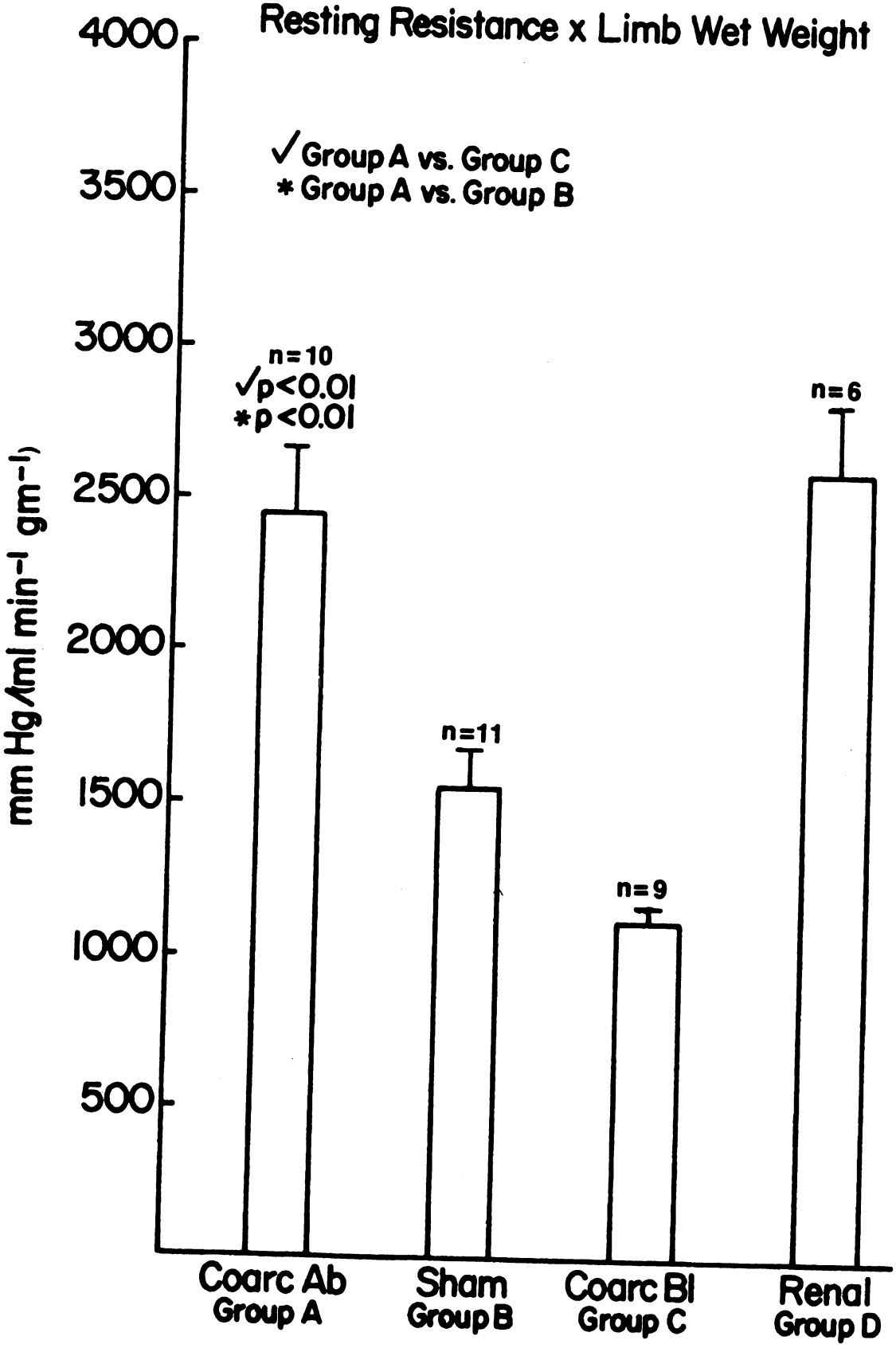
Resting Resistance x Limb Wet Weight
✓Group A vs. Group C
* Group A vs. Group B
4000
3500
3000
2500
2000
1500
1000
500
mm Hg/ml min⁻¹ gm⁻¹
n=10
✓p<0.01
*p<0.01
n=11
n=9
n=6
Coarc Ab
Group A
Sham
Group B
Coarc Bl
Group C
Renal
Group D

Figure 3

had to be made to Group B regardless if one considers this value "normal" or not. Variations in animal strain, type and level of anesthesia, amount of skin present relative to muscle, and whether or not measurements are made with artificial media or blood, all make any comparisons of this study's animals resting flow to those of another study tenuous at best. For those interested, this resting flow in Group B of 69 cc/minute/Kg falls between separate resting values for muscle and skin in man (18).)

IV. Effects of Passive Distention

In an effort to produce passive distention in the hindlimb vasculature of the rats, pump flow was adjusted sequentially from 0.125 cc/minute to 2.0 cc/minute thereby increasing perfusion pressure. From the pressure generated by each flow, pressure-flow, resistance-flow, and resistance-pressure curves were constructed which are depicted in Figures 5, 6, and 7 respectively. These curves are depicted with data not normalized by limb wet weight, although all statistical analyses and comparisons are made with the opposite limb wet weight normalization.

In an attempt to analyze the curves as a whole, a profile analysis (31) was performed on the pressure-flow and resistance-flow data. The nature of this analysis did not allow one to use data expressed in terms of any limb weight. Profile analysis revealed that the pressure-flow and resistance-flow curves of Group A were significantly elevated compared to Groups B or C ($p < 0.0001$). This

Figure 4. Resting Flow/gram Limb Wet Weight

Coarc Ab = Group A = rats with abdominal aortic coarctation above the origin of both renal arteries

Sham = Group B = normotensive sham operated control rats

Coarc Bl = Group C = rats with abdominal aortic coarctation below the origin of both renal arteries

Renal = Group D = rats with two kidney Golblatt hypertension

p = probability that the differences between means of the groups occured by chance

$ml/mingm^{-1}$ = milliliters per minute per gram

n = number of experimental units

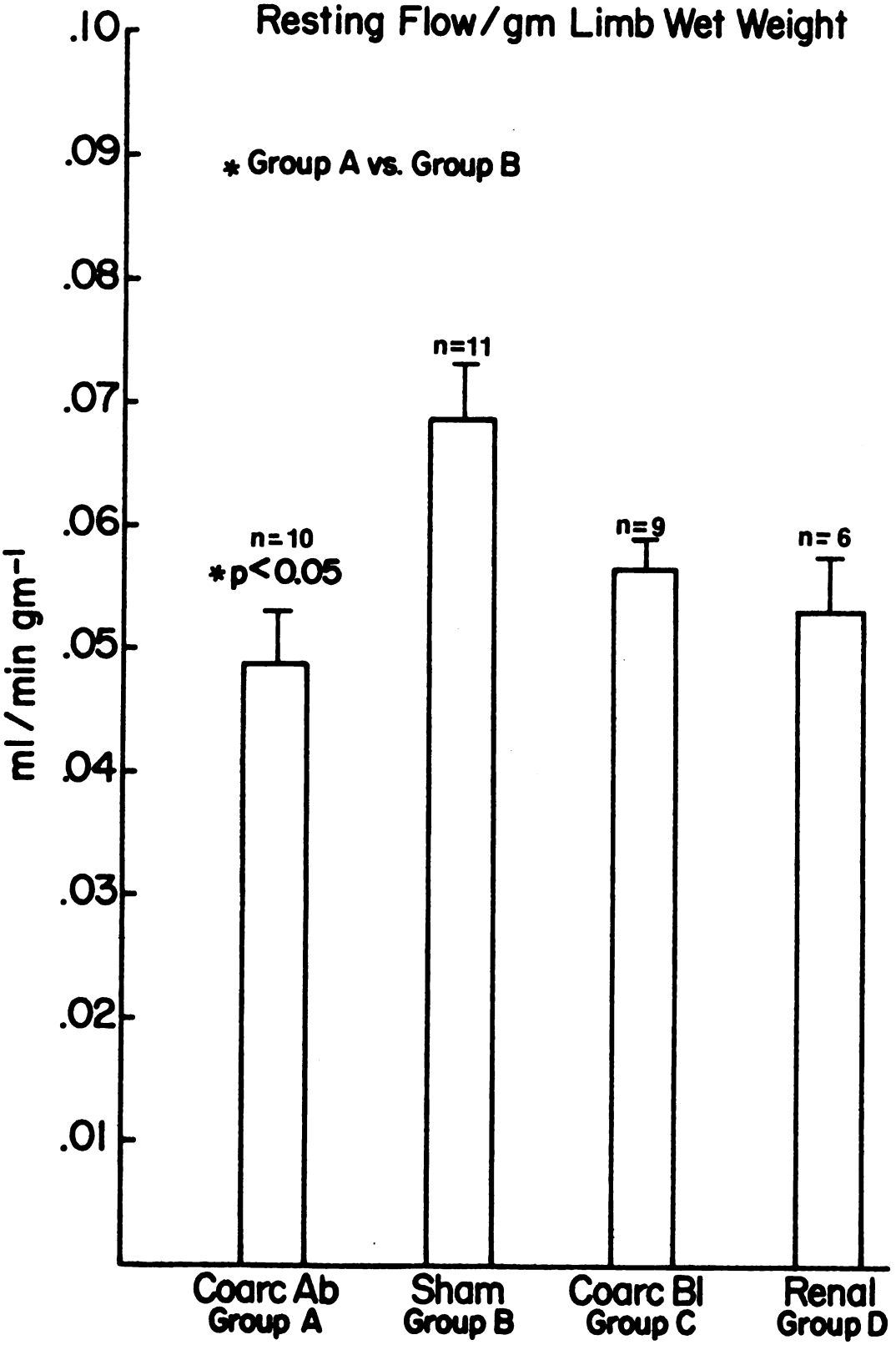
Resting Flow/gm Limb Wet Weight
* Group A vs. Group B
ml/min gm⁻¹
.10
.09
.08
.07
.06
.05
.04
.03
.02
.01
n=10
*p<0.05
n=11
n=9
n=6
Coarc Ab
Group A
Sham
Group B
Coarc Bl
Group C
Renal
Group D

Figure 4

analysis also revealed, however, that the curves deviated significantly from parallelism thus rendering any comparisons between the relative levels of the curves inconclusive. Consequently, to see if resistance was increased or decreased in any group over the range of flows studied, the data had to be analyzed one point at a time, with the one-way analysis of variance and the S-N-K test. Their 66 individual comparisons are tabulated in Appendix C.

To summarize significantly higher pressures and resistance were seen at all flows in rats of Group A compared to Groups B or C ($p < 0.01$). (The lone exception to this pattern was the comparison between Groups A and B at 2.0 cc/minute. It seems that this inconsistency in the data is due to an increase in the total amount of variability at 2.0 cc/minute compared to the previous two flows. This insignificance disappears when the data at this flow is not expressed in terms of opposite limb wet weight.)

Also, rats of Group D exhibited significantly higher pressures and resistances compared to Group B at all flows while at flows $\geq$ 1.0 cc/minute Group C exhibited significantly lower pressure and resistances compared to Group B.

The results in Group A and D suggest an impairment of passive distention in the hindlimb vessels of these groups relative to Group B. These phenomena can be better seen in Figure 7 which shows, for example, that a distending pressure of 185 mm Hg in Group A could not passively

Figure 5. Average Pressure versus Flow

Coarc Ab = Group A = rats with abdominal aortic coarctation above the origin of both renal arteries

Sham = Group B = normotensive sham operated control rats

Coarc Bl = Group C = rats with abdominal aortic coarctation below the origin of both renal arteries

p = probability that the differences between means of the groups occured by chance

Goldblatt hyper = rats with two kidney Goldblatt hypertension

mm Hg = pressure in millimeters of mercury

cc/min = flow in cubic centimeters per minute

n = number of experimental units

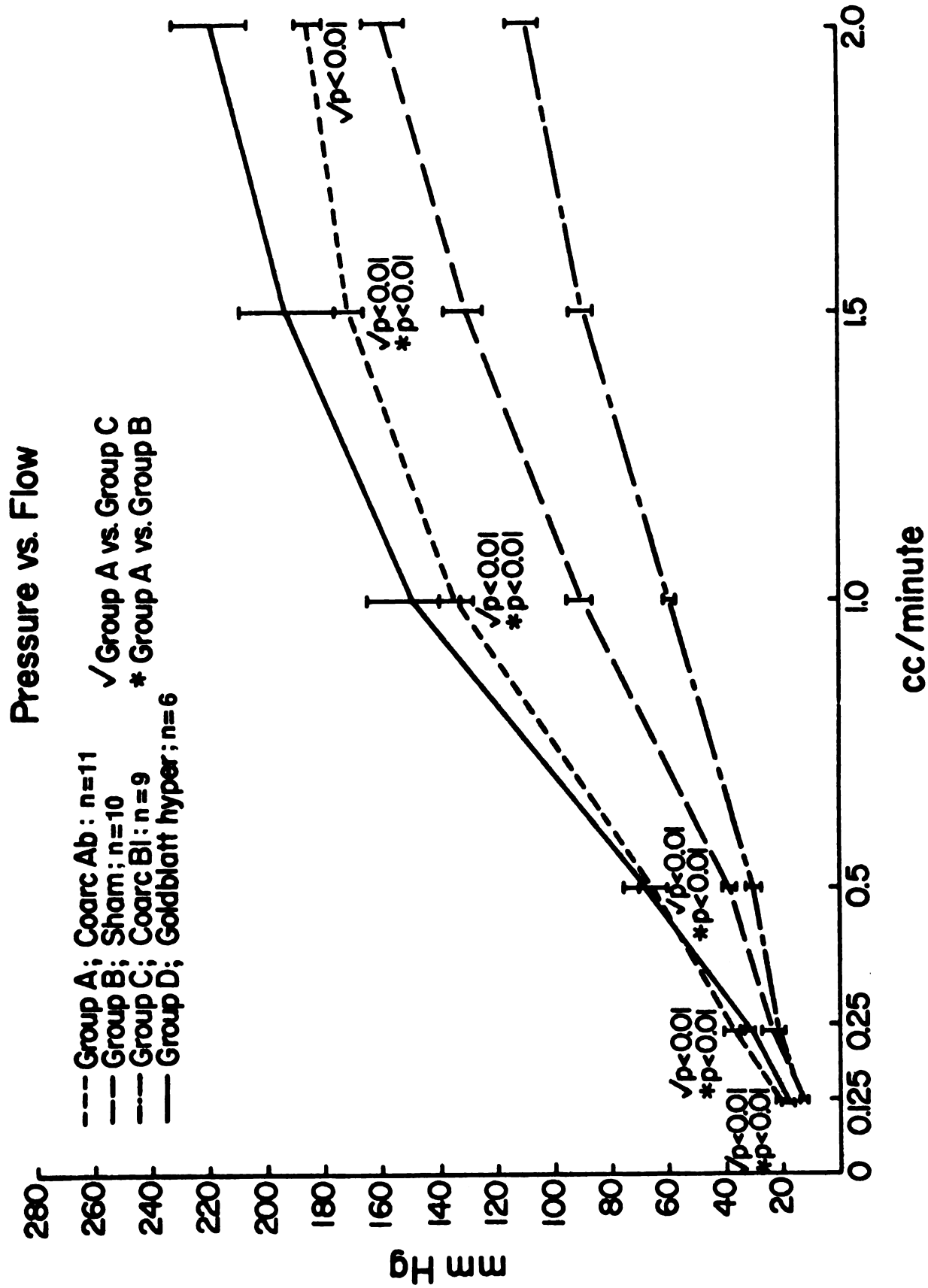
Pressure vs. Flow
Group A; Coarc Ab : n=11
Group B; Sham; n=10
Group C; Coarc Bl : n=9
Group D; Goldblatt hyper; n=6
√ Group A vs. Group C
* Group A vs. Group B
mm Hg
cc/minute
280
260
240
220
200
180
160
140
120
100
80
60
40
20
0
0.125
0.25
0.5
1.0
1.5
2.0
√p<0.01
*p<0.01

Figure 5

Figure 6. Average Resistance versus Flow

Coarc Ab = Group A = rats with abdominal aortic coarctation above the origin of both renal arteries

Sham = Group B = normotensive sham operated control rats

Coarc Bl = Group C = rats with abdominal aortic coarctation below the origin of both renal arteries

p = probability that the differences between means of group occured by chance

Goldblatt hyper = rats with two kidney Goldblatt hypertension

mm Hg/ml min^{-1} = resistance

n = number of experimental units

cc/minute = cubic centimeters per minute

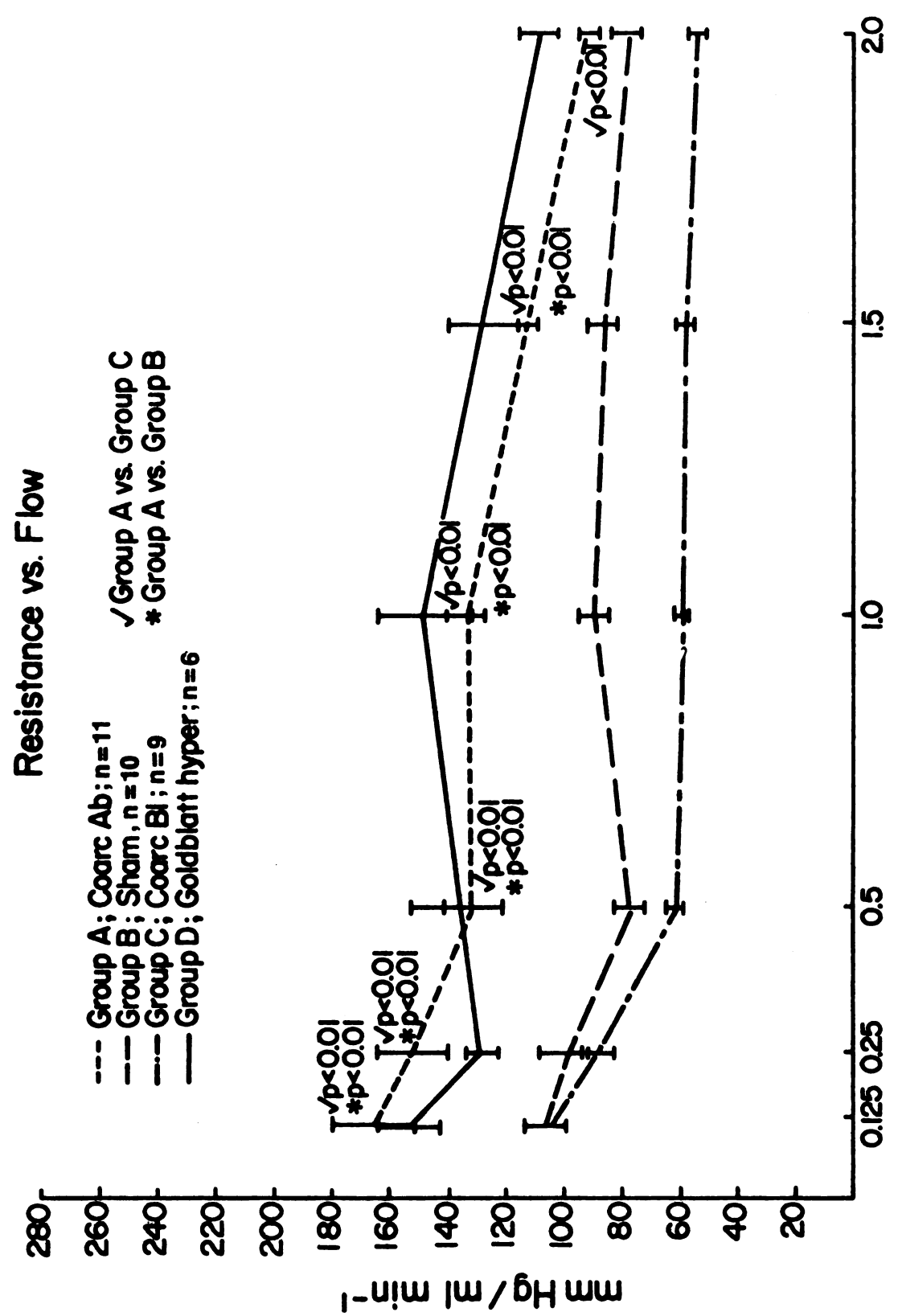
Resistance vs. Flow
--- Group A; Coarc Ab; n=11
— — Group B; Sham, n=10
— · — Group C; Coarc Bl; n=9
—— Group D; Goldblatt hyper; n=6
√ Group A vs. Group C
* Group A vs. Group B
mm Hg / ml min⁻¹
cc/ minute
0.125
0.25
0.5
1.0
1.5
2.0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
√p<0.01
*p<0.01

Figure 6

Figure 7. Average Resistance versus Average Pressure

Coarc Ab= Group A = rats with abdominal aortic coarctation above the origin of both renal arteries

Sham = Group B = normotensive sham operated control rats

Coarc Bl = Group C = rats with abdominal aortic coarctation below the origin of both renal arteries

Renal = Group D = rats with two kidney Goldblatt hypertension

mm Hg/ml min^{-1} gm^{-1} = resistance expressed as pressure in milliliters of mercury divided by flow expressed as milliliters per minute per gram opposite limb wet weight

n = number of experimental units

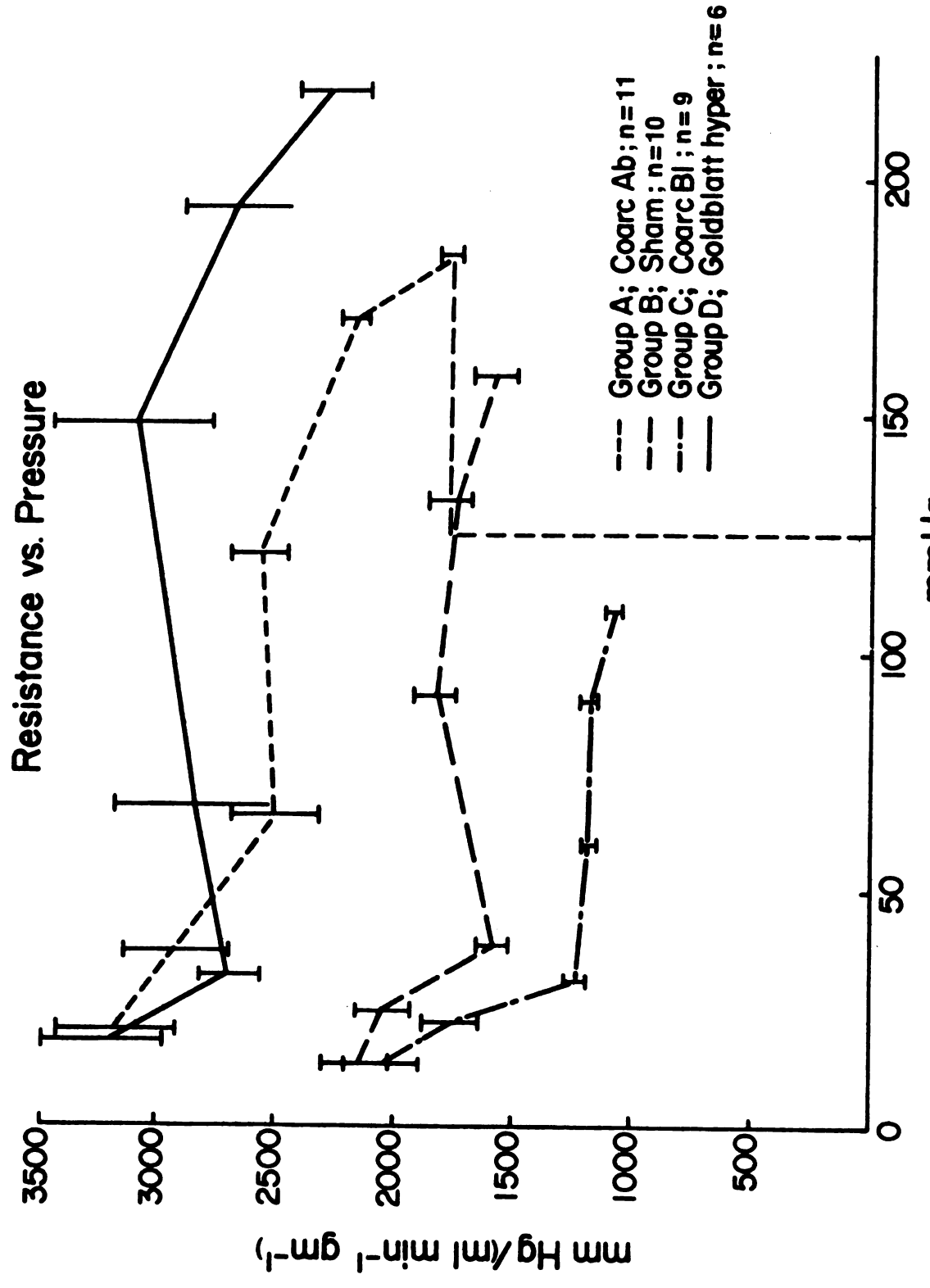
Resistance vs. Pressure
mm Hg/(ml min⁻¹ gm⁻¹)
3500
3000
2500
2000
1500
1000
500
0
50
100
150
200
mmHg
Group A; Coarc Ab; n=11
Group B; Sham; n=10
Group C; Coarc Bl; n=9
Group D; Goldblatt hyper; n=6

Figure 7

reduce resistance to a level attained in Group B at a level of only 125 mm Hg.

V. Acute Devervation

After acute denervation, resistance at a flow of 1.0 cc/minute (not normalized for limb weight) remained elevated by 17% in Group A compared to Group B rats. With the opposite limb wet weight normalization Group A rats exhibited a significantly higher resistance compared to Group C only (52%, $p < 0.01$). Groups C and D also exhibited significant differences after acute denervation compared to sham controls ($p < 0.01$) being reduced 28% and increased 58% respectively. These results are shown graphically in Figure 8.

VI. Resistance after Infusion of Sodium Nitroprusside

Residual resistance in the denervated hindlimb of the four groups of rats after injection of supramaximal doses of the vasodilator sodium nitroprusside are presented in Figure 9. All groups differ significantly from all other groups ($p < 0.01$; $p < 0.05$ Group A versus Group B). Of interest is the fact that resistance remains elevated in rats of Group A compared to the sham-control group, presumably after all smooth muscle activity had been abolished.

Figure 8. Resistance after Acute Devervation x Limb Wet Weight; Flow = 1 cc/minute

Coarc Ab = Group A = rats with abdominal aortic coarctation above the origin of both renal arteries

Sham = Group B = normotensive sham operated control rats

Coarc Bl = Group C = rats with abdominal aortic coarctation below the origin of both renal arteries

Renal = Group D = rats with two kidney Goldblatt hypertension

mm Hg/ml min^{-1} gm^{-1} = resistance expressed as pressure divided by flow, expressed as milliliters per minute per gram opposite limb wet weight

n = number of experimental units

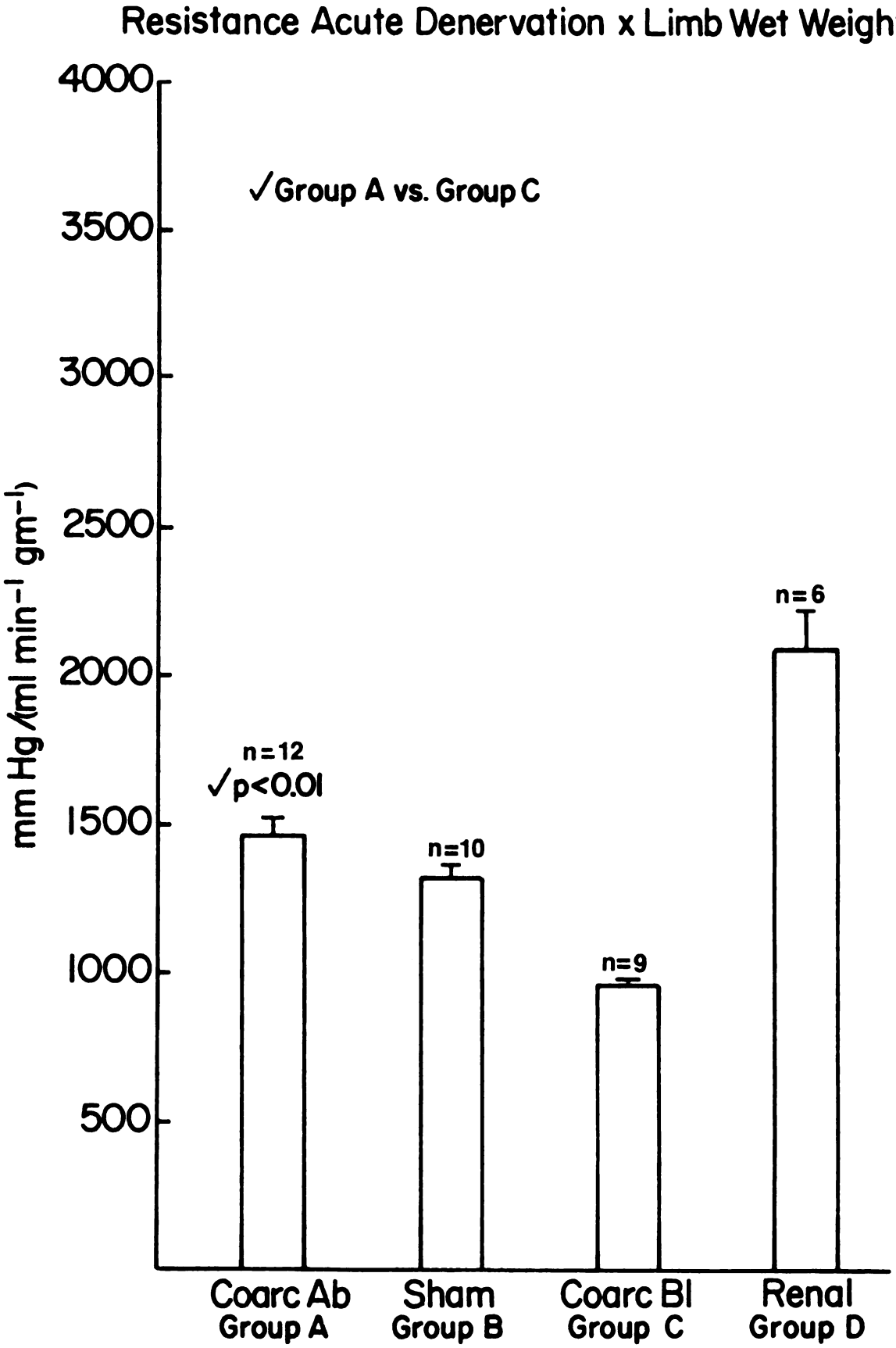
Resistance Acute Denervation x Limb Wet Weight
4000
3500
3000
2500
2000
1500
1000
500
mm Hg/ml min⁻¹ gm⁻¹
✓Group A vs. Group C
n=12
✓p<0.01
n=10
n=9
n=6
Coarc Ab
Group A
Sham
Group B
Coarc Bl
Group C
Renal
Group D

Figure 8

Figure 9. Resistance Times Opposite Limb Wet Weight at 1.0 cc/minute after Supramaximal Injection of Sodium Nitroprusside

Coarc Ab = Group A = rats with abdominal aortic coarctation above the origin of both renal arteries

Sham = Group B = normotensive sham operated control rats

Coarc Bl = Group C = rats with abdominal aortic coarctation below the origin of both renal arteries

Renal = Group D = rats with two kidney Goldblatt hypertension

mm Hg/ml min^{-1} gm^{-1} = resistance expressed as pressure divided by flow, expressed as milliliters per minute per gram opposite limb wet weight

NaNP = Sodium nitroprusside

n = number of experimental units

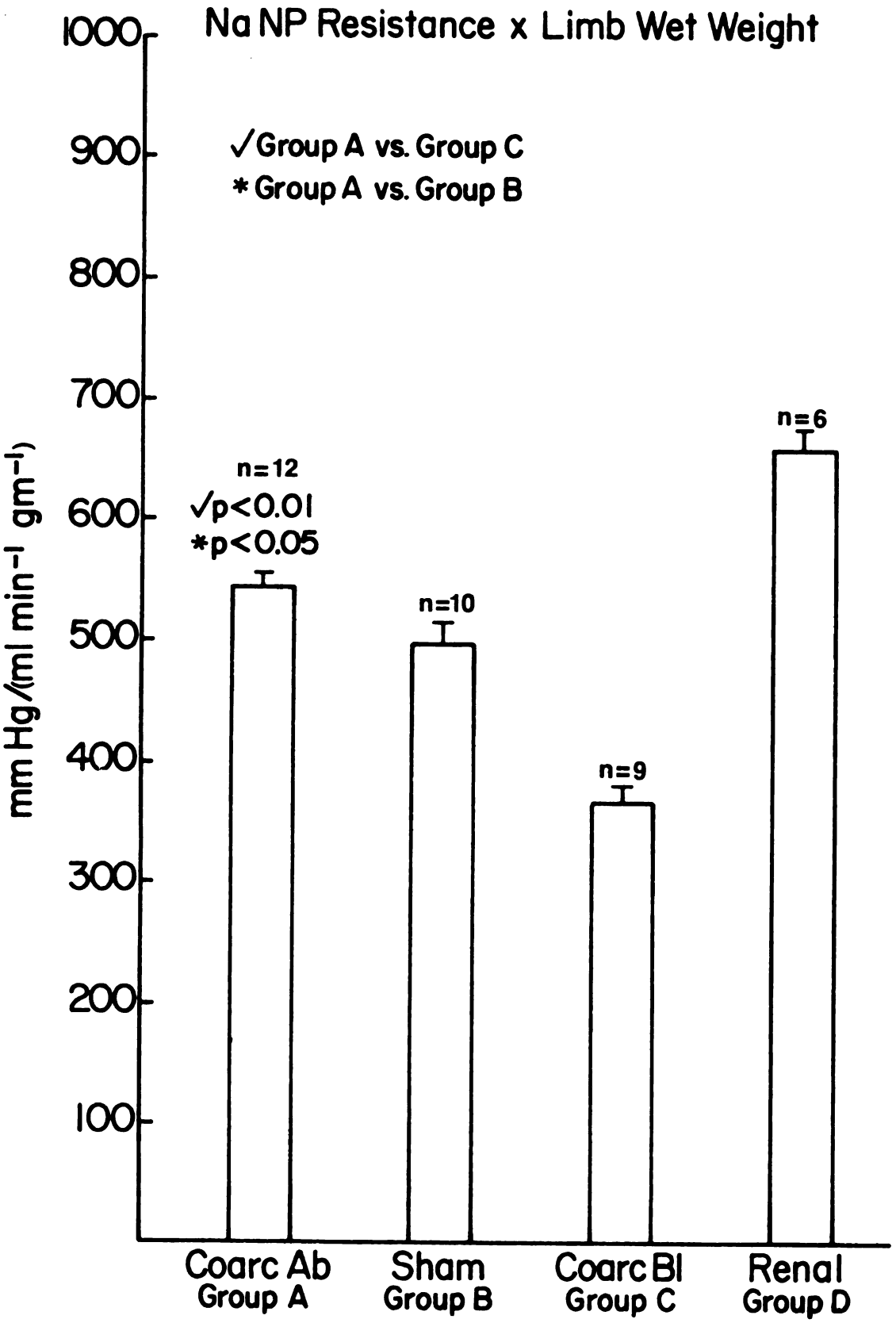
Na NP Resistance x Limb Wet Weight
✓Group A vs. Group C
* Group A vs. Group B
mm Hg/(ml min⁻¹ gm⁻¹)
1000
900
800
700
600
500
400
300
200
100
n=12
✓p<0.01
*p<0.05
n=10
n=9
n=6
Coarc Ab
Group A
Sham
Group B
Coarc Bl
Group C
Renal
Group D

Figure 9

VII. Plasma Renin Concentration

The results of the measurements of plasma renin concentration in rats of Group A and B are presented in Figure 10. Plasma renin concentration was found to be increased by 80% in Group A rats relative to Group B ($p < 0.001$) when compared with the Student's two-tailed t-test. These data are listed in Appendix C.

Figure 10. Plasma Renin Concentration

ng/ml/hour angiotensin I = nanograms per milliliter per hour of angiotensin I

Coarc Ab = rats coarcted above the renal arteries around the abdominal aorta (coarctation hypertensive)

Sham = normotensive sham operated control rats

n = number of experimental units

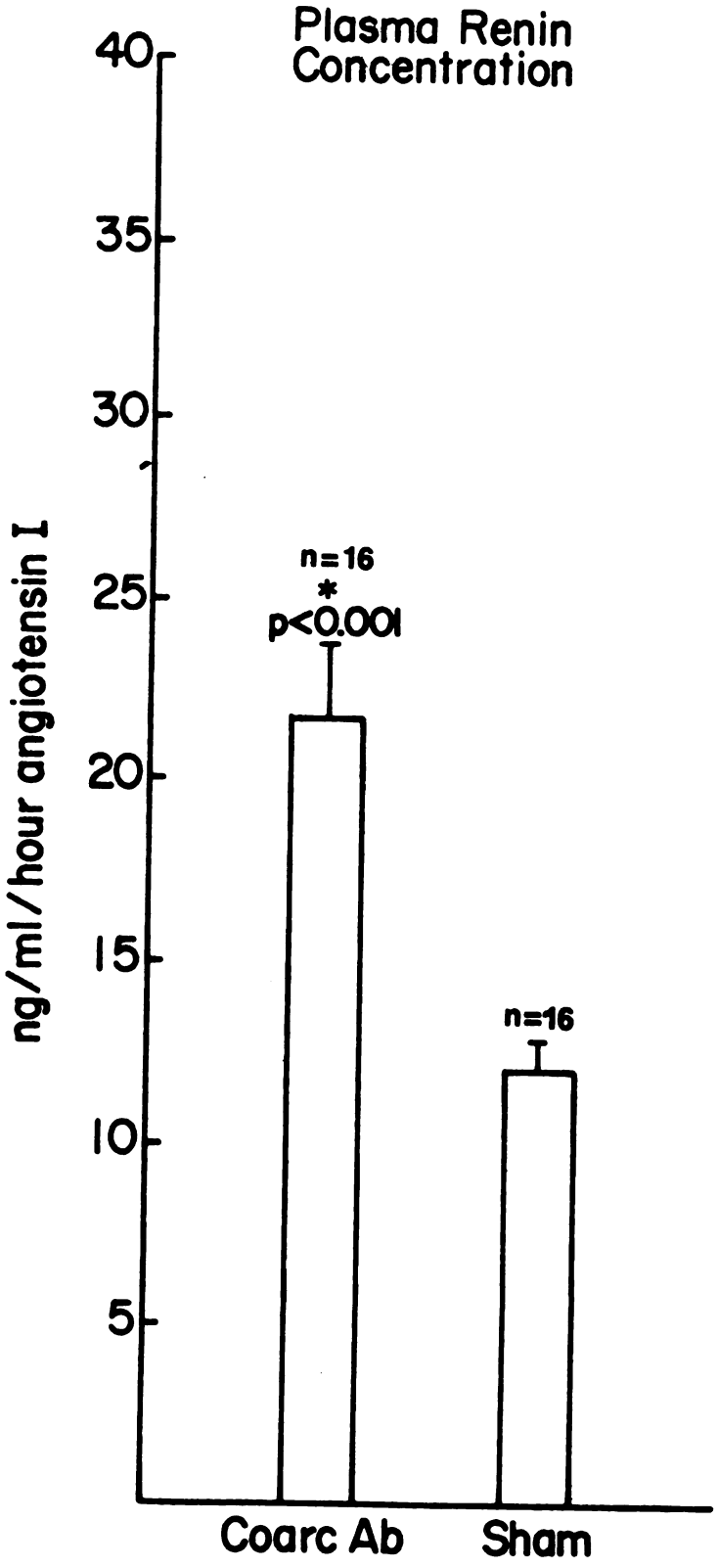
Plasma Renin
Concentration
ng/ml/hour angiotensin I
40
35
30
25
20
15
10
5
n=16
*
p<0.001
n=16
Coarc Ab
Sham

Figure 10

Discussion

To test the hypothesis that the vascular wall thickening in hypertension results from increased intravascular pressure, hindlimb pressure-flow relationships were measured in rats with experimental aortic coarctation hypertension.

As is the case in most animals with this type of hypertension, the hindlimb vascular bed of the rats with coarctation hypertension in this study was not exposed to increased intravascular pressures. On the day of the perfusion experiments, and in the prior four weeks, arterial pressure in the regions distal to the coarctation did not differ significantly from the corresponding area in the sham operated normotensive control animals, these vascular regions remaining normotensive.

It was not possible, in this study, to measure pressure weekly in the arterial region above the level of the coarctation. However, direct readings of mean carotid arterial pressure, on the day of each experiment, showed a significantly elevated, hypertensive pressure in rats coarcted above the renal arteries. Also, the presence of a cardiac hypertrophy in these animals (as well as those with two kidney Goldblatt hypertension) would suggest that a hypertensive condition was present before the conduction of the experiments as well. Cardiac hypertrophy is a common finding in hypertension and is approximately proportional to the level of the hypertension. (17, 33, 40)

Concerning the hemodynamic portion of the present study there are three major findings from the data in the normotensive hindlimb vasculature of Group A compared to the data from Group B. These are:

1) Resistance is significantly elevated, both at rest and over a range of flows while resting flow is reduced.

2) Pressure-flow relationships suggest impaired passive dilation of the resistance vessels.

3) Residual vascular resistance after maximal vasodilation is significantly increased.

These findings are intriguing because, in the majority of previous studies by other investigators, vascular beds of hypertensive animals that are not exposed to an elevated intravascular pressure do not exhibit an increased resistance at maximal vasodilation, or at any level of smooth muscle contraction, and do not display evidence for the vascular wall thickening found in hypertensive vascular beds. (3, 5, 10, 11, 12, 13, 14, 15, 16, 24, 26, 28, 37, 38, 40, 45, 49, 53) In addition, early studies on coarctation hypertension state that resting flow and presumably resistance are normal in the vascular regions distal to the coarctation. (17)

The increased hindlimb vascular resistance in Group A, as manifested by the pressure-flow relationships (Figures 5 and 6) and by resistance values at rest (Figure 3), could theoretically be attributed to neural, humoral, or structural

factors or a combination of these factors.

With regards to humoral factors it was found in the present study that the plasma renin concentration of Group A rats was elevated by 81% compared to rats of Group B. However, basal tone (resistance after acute denervation/ resistance after NaNP) in these two groups was almost identical. Therefore, it is unlikely that elevated levels of plasma angiotensin, or any other circulating vasconstrictors directly contributed to the elevated resistance via their effect on smooth muscle contraction. (45)

The present study was not designed to investigate the role of the sympathetic nerves in hypertension. However, since hindlimb resistance in the hypertensive animals coarcted above the renal arteries was still significantly increased after the presumed abolishment of all smooth muscle activity,it can be concluded that a portion of the increased resistance was not due to the immediate effects of neural factors (but neural factors operating chronically could have changed the vessel structurally).

Plots of Resistance versus Pressure (Figure 7) suggest that passive dilation of the vasculature is impaired in Group A compared to Group B. Impaired passive dilation of the arteries is a characteristic of hypertension and has been used by previous investigators to argue for the existance of a vascular wall thickening and/or an increase in the wall-to-lumen ratio of the resistance vessels. (11, 14, 17, 34)

Totally aside from any implication of vessel wall

structure, the existence of a hypertensive characteristic (impaired passive dilation) in the hindlimb vasculature of Group A is significant in that this vasculature was not hypertensive during the study.

One might argue that passive distention is not even occuring in the Groups during the pressure-flow studies because resistance increases or stays constant between the 3rd and 4th flows (0.5 & 1.0 cc/minute) (2nd and 3rd for Group D, 0.25 & 0.5 cc/minute) rather than decreases. (Figures 6 & 7) This then would suggest that some auto-regulatory phenomenon is being displayed by the pressure-flow studies.

Therefore, it can not be denied that smooth muscle contraction could have influenced vascular resistance during the flow sequences. However, one can not rule out passive distention as a major determinant of vascular resistance during the flow sequences. It should be noted that even though resistance increases for example in Groups A & B between the third and fourth flows, resistance in both groups at the fourth flow (1.0 cc/minute) is still below that at the initial flow (0.125 cc/minute) for both groups. Were it not for a considerable distending force at the fourth flow resistance would be even higher than at the initial flow of 0.125 cc/minute due to both metabolic and myogenic factors. Besides, resistance again continued to decrease in both groups at flows higher than 1.0 cc/minute.

According to the law of LaPlace ($T = P (r/w)$) the

internal radius of the vessels in all groups would have to decrease, and thus resistance increase, beyond the initial values (at a flow of 0.125 cc/minute) in order to completely negate the distending force of the increase in pressure at each successive flow. (13) Since, relative to the initial value, this never occurs in any group it may be said that the vessels were for the most part being distended over the flow range studied. It may then be postulated that distention of the vasculature was impaired in Group A, since resistance remained elevated over these flows in Group A compared to Group B.

Whether or not this impaired distention is due to an underlying increase in vessel wall thickness (thus increasing the wall-to-lumen ratio) in Group A relative to Group B cannot be determined for certain from the pressure-flow study.

Profile analysis revealed that the level of the pressure-flow curves for Group A was greater than the curves for Group B, but that the curves were not parallel to each other. Although this would be the case if the hindlimb vessels of Group A had structurally based greater wall-to-lumen ratios than Group B, one cannot tell for certain if this is the case in the present study, since smooth muscle activity was not abolished in any of the groups during the pressure-flow experiments. In explanation, one

cannot tell if the implied increase in wall-to-lumen ratio was due to an underlying thickened vessel wall or, instead, due to an increased amount of vascular smooth muscle contraction or both. (13)

Nevertheless, it can at least be said that the hindlimb arteries of the intact animals with coarctation hypertension are less distensible than those for normotensive controls.

One might suggest that more conclusive data may have been obtained if the pressure-flow studies were done during complete vascular smooth muscle relaxation. However, such a study has its own set of experimental problems.

Firstly, if the vessels are completely relaxed in the intact animal, blood pressure is markedly reduced so that the life support of the animal is severely compromised. Thus, the hindlimbs (if that is to be the vascular region studied) have to be completely separated (nerves and vessels severed) from the animal and the animal killed if a pressure-flow study is to be performed. (11) Also, in this type of study with the hindlimbs separated from the animal's body an oxygenated plasma substitute has to be used as a perfusate. (11, 12, 14, 15, 16) Thus, data on an intact animal (full neural and humoral factors present) cannot be obtained.

Secondly, with the vessels completely relaxed the formation of edema is considerably facilitated in the

hindlimbs aside from what might be normally expected from high perfusion pressures and flows. In fact, Folkow, in this type of pressure-flow experiment stated that intravascular pressures had to be kept between 10 and 40 mm Hg in order to avoid edema formation in the hindlimb. (11) This is hardly within the normal physiological pressure range for the animal. As can be seen, whatever extrapolation is made between data gathered in this manner (no vascular tone, low pressures, artificial media, etc.) and the intact animal under normal physiological conditions is tenuous at best. There apparently seems to be no "good" way to conduct pressure-flow studies on the hindlimb vasculature.

In the present study, in the hindlimbs of Group A compared to either Groups B or C, resistance at maximal active vasodilation was significantly increased. It is unlikely that this difference is due to an increase in extravascular pressure through tissue edema because similar water contents were found in the perfused limbs of all groups. (Table 1)

This impaired active vasodilation suggests that some structural component is involved in the general elevated hindlimb resistance seen in the rats with coarctation hypertension. Concerning structural factors, the changes in resistance in the hindlimbs of Group A cannot be explained on the basis of a decreased number of vessels due to the slight hindquarters atrophy that occurred after

clipping, because there was similar limb atrophy in the normotensive rats with aortas clipped below the renal arteries (Group C); and, in these latter rats hindlimb resistance was reduced, passive vasodilation was enhanced, and residual resistance after maximal vasodilation was reduced, compared to the sham operated control rats. In other words, aortic coartation alone (Group C) would tend to cause changes in the resistance of the hindlimb vessels in the opposite direction from those that were observed in the rats with coarctation hypertension. Indeed, the findings in the control group of rats coarcted below the renal arteries add increased significance to the observations made in the rats with coarctation hypertension. Consequently, from this logic it is not surprising that resistance at maximal vasodilation in Group A is increased by "only" 10% compared to Group B.

One may wonder why resistance measurements in Group A & B differ by 10% at maximal vasodilation yet differ by 57% at rest. These anomalies are depicted in Figure 11 which depicts theoretical radii and internal circumferences for all the groups at rest and maximal vasodilation. These values are derived from Poiseuille's equation which states that resistance = $\frac{3Ln}{\pi r^4 N}$. (13) If comparisons are made within each group (which will be the case below), $\frac{8Ln}{\pi N}$ can be an arbitrary constant. For purposes of the model, the internal vessel radius of Group B at maximal

Figure 11. Theoretical vessel radii and circumferences at maximal vasodilation and at rest in the rat hindlimb

Group A = rats with coarctation hypertension

Group B = sham operated normotensive control rats

Group C = rats coarcted about the aorta below both renal arteries

Group D = rats with two-kidney Goldblatt hypertension

Δ% Maximal Vasodilation to rest = percent difference in circumference from maximal vasodilation to rest

THEORETICAL VESSEL INTERNAL RADII AND CIRCUMFERENCES AT MAXIMAL VASODILATION AND AT REST IN THE RAT HINDLIMB EXPRESSED IN ARBITRARY UNITS

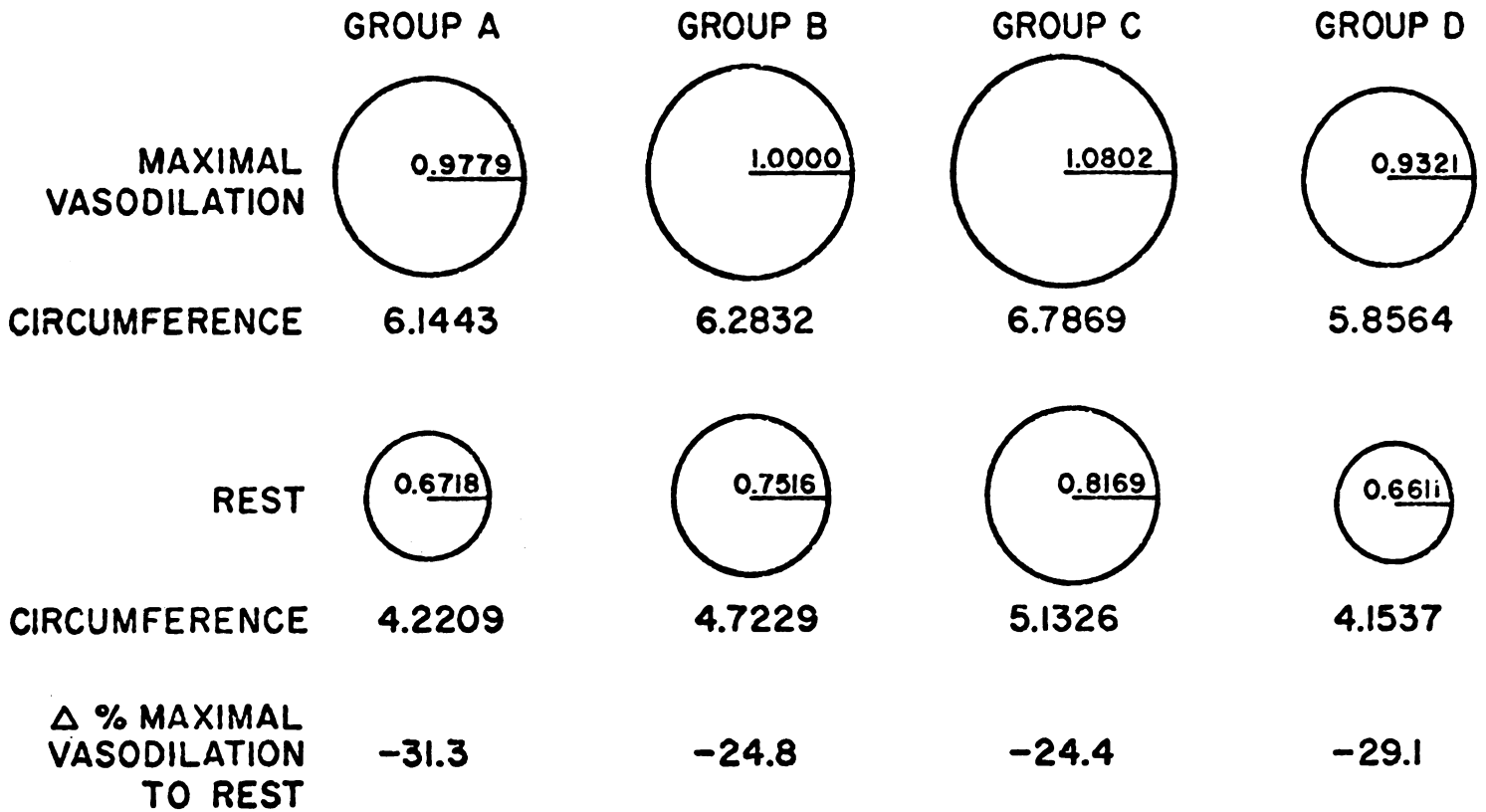

Figure 11

dilation is set at 1.0. This, then sets the constant of proportionality, $\frac{8Ln}{\pi N}$, relating resistance to the internal radius of the vessel at 498.4. Using the conservative case by which one sets the wall-to-lumen ratio of each vessel equal at maximal vasodilation, one can calculate the percent of vessel contraction necessary to elevate flow resistance in each group from the level of maximal vasodilation to the level at rest.

As can be seen from Figure 11 a contraction of roughly 24% is required to elevate resistance from maximal dilation to resting values in Groups B & C. However, a contraction of 29% is required in Group D, and a still higher contraction of slightly over 31% is required in Group A, to obtain resting resistance values for these two groups.

These last two values pose an interesting, though purely speculative, problem in that the maximum amount of vessel contraction believed to be obtainable in a vessel with normal wall structure is about 27 and not more than 30 percent. (12, 13) Consequently, from this point of view, the increase in resistance in Groups B and C from maximal vasodilation to rest could be accounted for solely on the basis of an increase in the amount of vessel contraction. However, since the amount of contraction in the vessel, by definition, cannot exceed the maximum amount attainable, then resistance at rest in Group D and especially Group A cannot be explained simply by a certain

amount of vessel contraction. With this the case, then the only way Group A could attain its resistance value at rest would be for it to have an increased amount of vessel wall mass displaced into the vessel lumen for each given level of vascular contraction compared to Group B. In other words, Group A would have to have an increase in vessel wall thickness relative to Group B.

Of course, this statement is not proof positive for vessel wall thickening and depends on the fact that the resistance vessels cannot constrict more than 27 to 30 percent. However, coupled with the fact that the increase in resistance at maximal vasodilation in Group A relative to the normotensive sham controls (Group B) cannot be simply explained by tissue edema or limb atrophy, the possibility that this increased resistance at maximal vasodilation in Group A reflects an underlying vessel wall thickening now seems somewhat more plausible.

Whatever the case may truly be, of prime importance related to finding elevated resistance measurements in the hindlimb vasculature of rats with coarctation hypertension (Group A) is that this vasculature was <u>not</u> exposed to an elevated intravascular pressure. Therefore, high intravascular pressure cannot be postulated as being responsible for the resistance changes seen in these rats.

The findings of impaired vasodilation in the hindlimbs of these rats would especially appear to contradict

the hypothesis that structural changes in vascular walls of hypertensive animals (which can account for almost all the elevated resistance) are the result of increased intravascular pressure. (3, 5, 10, 11, 12, 15, 16, 26, 30, 45, 51, 52, 53)

There are a number of reasons why this study's results apparently contradict this hypothesis.

First are the interpretation of data and types of experimental models used by some investigators to advance the opinion that structural changes in hypertension result from increased intravascular pressure.

In separate studies, Folkow, Sivertsson, Weiss, and Martin have shown that hypotension can reduce resistance by causing an atrophy of the arterial vessel walls in the hypotensive region. (15, 16, 45, 53, 30) These investigators then used a "reverse" logic to postulate that an increased pressure would, consequently, lead to a thickening of the vessel wall, as is the case in hypertension. Indeed, using the results, in the present study, in animals with coarctation of the abdominal aorta below the renal arteries, and those with two-kidney Goldblatt hypertension, one might come to the same conclusion. In explanation, the hindquarters of the rats with Goldblatt hypertension were exposed to an elevated intravascular pressure. These hindlimbs exhibited a significantly increased resistance at maximal vasodilation compared to the groups of rats with normotensive hindlimbs. (A & B) Conversely, the

hindquarters of rats coarcted below the renal arteries were exposed to a decreased intravascular pressure and these hindlimbs showed a decreased resistance at maximal vasodilation.

Concerning this latter point, however, finding that hypotension can cause vessel wall thinning does not necessarily prove the opposite situation can be true.

It is possible that other factors may come into play in a hypotensive vascular bed besides the reduced intraluminal pressure. As suggested by Weiss and Hallback (53), and Folkow (16) the underperfusion of the vascular region exposed to hypotension may decrease the "nutritional" supply to the vessels, thus resulting in an atrophy of the vessel wall. Therefore, the influence of blood pressure on vascular changes may not be strictly a pressure phenomenon when low levels of pressure are present.

Also, it is important to note that, unlike the present study, Folkow's studies never examined a normotensive vascular bed of a hypertensive animal. Instead, Folkow examined the "protected" (hypotensive) vascular bed of spontaneously hypertensive rats. Data from these beds, compared to normotensive control rats, provided evidence, through reverse logic, that structural changes in the resistance vessels result from increased intravascular pressure.

However, the appropriate control for a "protected" vascular bed in a hypertensive animal may, instead of a normotensive bed, be a similarly "protected" bed in a normotensive animal. Using this type of comparison, Weiss

and Hallback's data in "protected" beds of adult spontaneously hypertensive rats provide evidence that vessel wall thickness remains greater in the hypertensives. Since decreased pressure could not totally reverse the structural change, this result then suggests that other factors besides pressure may be involved in the vascular structural changes.

It would also seem, then, that a better test for the hypothesis that, structural changes are secondary to changes in pressure alone, might be provided by examining a normotensive, rather than a sub-normotensive vascular bed of a hypertensive animal. This type of model was used in the present study and in the studies of Nolla-Panandes (33) and, Pamnani and Overbeck (36), and these studies have provided evidence for elevated resistance and structural vascular changes in the normotensive vascular beds of rats with coarctation hypertension. Therefore, it seems that some factor may be present in this type of experimental model that is not present in other models used to investigate the role of pressure in the development of structural vascular changes and an elevated resistance.

It has already been noted that the rats with coarctation hypertension in this study (Group A) had elevated levels of plasma renin compared to normotensive sham controls (Group B), but that it was unlikely that this factor directly caused the elevated resistance seen in Group A. However, it is possible that the elevated renin

levels may have affected vascular resistance through effects on vascular wall structure. Sen and her associates have correlated plasma renin activity in spontaneously hypertensive rats with ventricular hypertrophy. (44) Also, exposure to elevated levels of renin or angiotensin may have altered the ionic composition of the arterial walls in rats with coarctation hypertension, as had been demonstrated by Villamil. (51)

Using the findings of Villamil (51) and, Pamnani and Overbeck, (36) one could form a hypothesis concerning why increased hindlimb resistance was found in this study's rats with coarctation hypertension.

In short, Villamil found that chronic exposure to angiotensin increases the salt content and sodium permeability of arterial strips. Pamnani and Overbeck found increases in the sodium, potassium and water content of arteries taken from the normotensive vascular beds of rats with coarctation hypertension.

In this same experimental model, the present study has shown that plasma renin concentrations are increased as is hindlimb vascular resistance at maximal vasodilation.

Therefore, increased plasma renin concentrations in the present study's rats with coarctation hypertension could have increased the amount of salt and water content in the rats' hindlimbs (as in Pamnani's and Overbeck's study) thus causing a thickening of the vessel walls. This in turn would have resulted in an increased hindlimb resistance

even at maximal vasodilation, which indeed, was truly the case in the present study's rats with coarctation hypertension.

With respect to other investigators' work in coarctation hypertension, the involvement of the renin-angiotensin system in coarctation hypertension has both been supported and denied. Whether or not, and to what extent, this system is brought into play in coarctation hypertension may explain some of the differences in the results of studies conducted by different investigators. In this regard, most studies that have shown that no vascular alterations occur in the vascular regions distal to the coarctation have been on thoracic aortic coarctation. It is possible that the differing results may be due to different sites of coarctation (thoracic or abdominal) and the possible resulting difference in renin-angiotensin involvement.

Aortograms in dogs with thoracic coarctation hypertension have shown that this type of coarctation hypertension is associated with an extensive amount of collateral circulation (23). It is possible that this collateral circulation could reduce the degree of involvement of the renal pressor system in thoracic coarctation through its effect on renal perfusion (1). Recall that, in the present study coarctation hypertension was produced by coarcting the abdominal aorta just above the origin of the renal arteries, which

obviously brought the renin-angiotensin system into play. (Figure 10)

It is true, however, that in another study of abdominal coarctation hypertension Bevan et al. (3) did not find elevated levels of salt and water in either the hypertensive or the normotensive arterial regions. Additionally, Bevan et al. found that the vessel wall thickness of only the arteries from hypertensive regions could be correlated with the level of hypertension.

Bevan's study implies that high intravascular pressure may be responsible for increasing the wall thickness of the arteries. This implication, and his results on water and ions, differ from the implications of the present study and results reported by Pamnani et al. (36), respectively, both of whom, like Bevans, used animals with abdominal coarctation as the experimental model. This apparent dicotomy between data on similar experimental models may be attributable to a large variety of factors. Firstly, the model of hypertension studied by Bevan et al. apparently does not have elevated levels of plasma renin in contrast to the present study. Also, it appears that Bevan et al. did not produce as severe a coarctation as was produced by the present study, which then may have reduced the involvement of the renin-angiotensin system. The pressure gradient between the average carotid and femoral arterial pressures was only 26 mm Hg in the rabbits (127 mm Hg-101 mm Hg), compared to 40 mm Hg in the rats of the present study (152 mm Hg-112 mm Hg).

Also, the duration of the coarctation was only two weeks in the rabbits studied by Bevan, compared to four weeks in the rats used by Pamnani et al. and the present

investigator.

Finally, and most importantly, Bevan et al. studied the arterial vessels in vitro and did not make direct comparisons between hypertensive and control animals.

In summary, certainly, the results of the present study do not exclude the possibility that elevated intraluminal pressure may alter vessel wall structure. Indeed, hindlimb resistance after maximal vasodilation was significantly higher in rats with Goldblatt hypertension (which were exposed to higher mean arterial pressures) than the rats with coarctation hypertension. The plasma renin concentrations of the rats with Goldblatt hypertension were not measured, however. (See Appendix A) Therefore, the possibility remains that a greater degree of vascular alteration was brought about by elevated levels of plasma renin and not by elevated levels of intravascular pressure in Group D. Two kidney Goldblatt hypertension has been previously associated with elevated plasma renin levels (40), but this cannot be demonstrated in the present study. Consequently, the results of the present study (Group A versus B, and, Group D versus A) suggest that, although elevated intravascular pressure may cause structural changes in the resistance vessels, it is not the only factor that can cause these changes and therefore is not necessary for bringing about the structural vascular changes seen in hypertension. In addition the results of Group A versus B further suggest that structural vascular changes (indicated by impaired maximal vasodilation) and

elevated vascular resistance are not necessarily a secondary vascular adaptation to a primary increase in intravascular pressure but instead may be related to the cause of hypertension itself.

Additional avenues of investigation should be pursued to determine if the resistance changes seen in the rats with coarctation hypertension were actually due to an increase in the wall-to-lumen ratio of the hindlimb vessels, for example, by conducting norepinephrine dose-response experiments from a level of maximum dilation to maximum constriction, as prescribed by Folkow and his coworkers (12). Also, pressure-flow studies, done on the vasculature during complete smooth muscle relaxation, although difficult, would help in this regard.

Similar studies could also be done on rats with coarctation hypertension where the effects of neural and renal humoral factors are abolished to determine what role these components may play in the vascular alterations. This might be accomplished by severing the sympathetic nerve supply to the hindlimb vessels in the coarcted rats, or by creating chemical sympathectomy (and adrenal demedullation) before doing the hemodynamic studies. It would also be interesting to block angiotensin II in these rats (e.g. with Saralysin).

In conclusion, then, the results of the present study state:

1) Normotensive vascular beds of rats with coarctation

hypertension display an elevated resistance.

2) Structural vascular changes, indicated by impaired vasodilation, may contribute to this elevated resistance.

3) These changes are not attributable to hindquarters atrophy, but may be related to elevated levels of circulating renin; and,

4) In contrast to previous theories, the elevated resistance and impaired vasodilation were not caused by high intravascular pressures or increased limb blood flows.

APPENDICES

The following appendices
are designed to serve as
a guide to the development
of experimental protocol and
as a reference for statistical
analyses.

APPENDIX A

PROBLEM SOLVING AND THE DEVELOPMENT OF EXPERIMENTAL PROTOCOL

When dealing with the perfusion of a small vascular bed, such as the rat hindlimb, the possibility of embolizing the bed becomes quite high. With such a vascular bed, even the smallest amounts of air or foreign material, amounts that can easily pass through the perfusion circuit virtually unnoticed, can significantly occlude the perfusion circuit at the level of the small blood vessels.

During early attempts at conducting experiments for this study, the rats' hindlimb frequently became embolized, presumably by air, as soon as the perfusion started. Since this condition prevented all portions of the experiment from being completed, the occurrence of embolization was a serious problem.

As stated previously, as a general means of preventing this problem, the transparent pump tubing leading to the hindlimb was carefully watched for the first eleven minutes of every perfusion so that potential emboli could be removed. However, watching the tubing did nothing to prevent embolization immediately as the perfusion started.

Since immediate, as well as early, embolization could be caused by small blood clots, and general particulate matter, as well as air, changes were made in the experimental preparation so that the occurrence of these potential emboli might be eliminated.

Consequently, as opposed to early experimental attempts, all beakers of normal saline used for flushing canuli, etc., were then covered with parafilm to prevent their exposure to particulate matter. To further prevent blood clots from forming, heparin was to be placed inside the syringe and beaker used for collection of donor blood, instead of just the beaker, as had been the case during the early experiments. Also, whenever an unsuccessful attempt at cannulating a femoral or carotid artery occurred, the resultant air space in the cannula was to be flushed out with heparin instead of saline. To aid in flushing out trapped air in the cannula after their insertion into an artery, the rubber part of the cannula was to be trimmed back so that a needle, attached to a syringe of saline, could be inserted all the way to the bottom of the cannula. In this manner one could insure that air could be removed from the entire length of the cannula.

By taking special care during the pre-perfusion preparations, and by implementing these changes, the problem of embolization was greatly, if not entirely, eliminated.

During the period of time that the embolization problem was being worked on, a Sigmamotor finger pump was used

for perfusing the rat's hindlimb. This pump possessed its own unique problem in that it produced pulse pressures in excess of 150 mm Hg (at a flow of 1.0 cc/min) when connected to a non-embolized limb. Since this pulse pressure was well outside the physiological norm for the rat, steps had to be taken to try to reduce the pulsations to a more acceptable level.

As a first attempt at solving this problem, a saline filled "Windkessel" was connected to the downstream pump tubing. This significantly reduced the pulsations during the initial part of the perfusion. However, the gradual increase in hindlimb resistance due to reactive hyperemia caused a large back-up of blood into the "Windkessel". This then produced two undesirable effects. Firstly, this significantly reduced the air space in the Windkessel thereby increasing the pulse pressure. Secondly, this caused a displacement of blood from the animal's circulation which in turn further increased resistance by activating the baroreceptors. This then established a positive feedback loop which prevented a steady state from ever being established in the preparation. The use of duel Windkessels could not prevent this situation from occurring.

As an alternative to the Windkessel concept, attempts were made to reduce the pulse pressure by reducing the stroke output of the pump and alternatively by increasing the pump downstream capacitance. Increasing the pump's

capacitance by increasing the volume available in the pump circuit proved unsatisfactory. Although this alteration significantly reduced the pulsations it displaced too much blood from the rat's circulation. Consequently, many types of pump tubing of varying diameters were tested to see if the large pulse pressure could be reduced by reducing the stroke output of the pump. This too proved unsatisfactory because pump tubing of a small enough diameter to adequately reduce the stroke output could not deliver flows to the hindlimb in amounts greater than 0.75 cc/min. Conversely, pump tubing of a diameter large enough to deliver higher flows did so at unacceptable pulse pressures. After much searching for tubing of an optimum diameter it was found that the "best" tubing still produced pulse pressures in excess of 100 mm Hg at the upper levels of flows to be used in the experiments. Since such pulsations were still abnormally high for the rat hindlimb the Sigma motor pump had to be abandoned altogether.

A duel chamber hydrolic pump was then employed as the blood pump for the perfusion studies. This pump was designed such that pulse pressures were never higher than 8 mm Hg. However, this pump had a drawing pressure of 60 mm Hg at 1.0 cc/min.

In the preparation prior to the use of this pump, blood was drawn through the pumping mechanism from the rats right femoral artery. In rats clipped below the renal arteries,

mean arterial pressure in this region was approximately 75 mm Hg. Consequently, whenever flow was increased above 1.0 cc/min., a negative pressure was induced in the inflow circuit of the pump. This cannot only damage the pump but also facilitate the passage of air into the pump tubing. Therefore, the surgical procedure on the rats had to be altered so that the carotid artery could be isolated and used to supply blood to the pump. By incorporating the isolation of the carotid artery into the pre-perfusion preparations, it was then possible to measure arterial pressures above and below the site of the coarctation.

In a previous study by Pamnani et al. (36), on the same type of rats, these same pressures were measured with the rats under light ether anesthesia. To simulate this condition using chlorolose and nembutol anesthesia, the previously used anesthesia combination had to be altered. This altered combination had to leave the rat "light" enough for blood pressure measurements yet deep enough to keep the animal completely calm for the remainder of the experiment. Before it was decided that both carotid and femoral arterial pressures were to be measured, the rat was given its total dosage of both anesthetics early in the surgical preparation. This dosage regimen kept the animal too deep for the purposes of measuring the arterial pressures. After experimenting with various sequences of dosage administration, the best dosage sequence was the one that has been

previously mentioned in the methodology.

The hydraulic pump used for the perfusion studies contained about 1 cc of fluid space outside of the rats circulation. Early in the study this space was filled with normal saline which was then pumped into the animal at the start of each experiment. The resulting displacement of 1 cc of blood from the rat's circulation helped reduce arterial pressure to the extent that negative pressures were occasionally incurred in normotensive rats. For this reason it was decided to prime the pump with blood taken from an appropriate donor. Therefore allowances had to be made for yet another addition to the pre-experimental protocol. Since the rats used for the perfusion at this time were from a highly inbred colony transfusion reactions could be discounted. Unfortunately at this time the colony of rats contracted pneumonia. This problem initially caused the elimination of a number of experiments due to the discovery of the disease upon autopsy of the animal. However, this problem became so severe that rats had to be obtained from outside the colony to continue the study.

Due to financial considerations the new rats had to be obtained from an outbred colony. Consequently, these rats had to be tested for transfusion reactions before they could be employed in the experiments.

Five pairs of rats were randomly selected and paired from the outbred colony. Using the standard testing

procedures with washed cells and serum from each rat, compatibility tests were then commenced on all rats. The tests showed no signs of hemolysis or agglutination either macroscopically or microscopically for any pair of rats. Therefore, the rats of the outbred colony were deemed compatible. During subsequent experiments, no signs of transfusion reactions were ever noted.

This new strain of rats, however, was associated with some quite unexpected problems. Because this strain was outbred, the rats were much heartier than inbred rats. Consequently, the new, outbred strain grew to be an average of 100 grams larger than the rats used by Pamnani (36), over the same four week pre-experimental period. The clip sizes for the normotensive sham controls then became relatively too small for the new larger breed of rats. Consequently, this "control" group started to develop coarctation hypertension.

New clips were then constructed to be approximately the same percentage larger in internal diameter as the new rats were in general body size. The first set of new clips (1.48 mm I.D.) maintained the carotid-femoral arterial pressure gradient at less than 6 mm Hg. One other set of clips, with an overly large interior diameter (1.70 mm) were then employed to see if this gradient could further be reduced. There was virtually no difference seen in the gradient between animals with either sham clip, but as an

exercise in caution the larger clip was used for the sham controls for the remainder of the study.

A more serious problem with the new colony of rats was revealed during the surgical isolation of the hindlimb of the rats. Either for reasons of a variation in the hindlimb vasculature or for lack of a good clotting capacity, the outbred rats bled greatly with little or no clotting when the muscles were teased apart, as had been the prescribed method for isolating the hindlimb. This bleeding never stopped during the experiment because heparin had to be used in the blood to prevent it from clotting in portions of the pump. Consequently, the rats were invariably in some stage of shock during an experiment, and, therefore, the results of such experiments could not be considered valid.

As a first measure to combat this problem the time between the completion of the hindlimb isolation and administering heparin to the rat was extended 15 minutes above the normal 30 minute period. However, waiting an extra 15 minutes let the animals become so light, that it was impossible to get readings of the carotid and femoral arterial pressures. More importantly this maneuver did little to stop the bleeding from the hindlimb.

Subsequently, additional ligatures and gelfoam were employed to stop the bleeding but these measures proved to be ineffectual. Finally, it was decided upon to use cautery to isolate the limb. By sealing the ends of severed

muscles and small blood vessels this method proved highly effective in preventing any bleeding from occurring during the experiment.

What experiments were completed, before cautery was used to isolate the hindlimb, seemed to reveal that there were no differences in the resistances of rats with coarctation hypertension compared to the normotensive sham controls. This could have simply been related to the hemorrhaging of the hindlimb but there was no way that could be determined for certain. Therefore, to determine if the results in the rats with coarctation hypertension were artificial or truly valid, a fourth group of rats were to be included in the study. This group consisted of rats with two-kidney Goldblatt hypertension and were to be used to determine if the experimenter could detect resistance changes in hypertension.

Three different renal clip sizes were used before the perfect size was determined. The smallest clip size totally occluded the kidney, which then caused either malignant hypertension or no hypertension at all (presumably since the damaged kidney could no longer produce renin). Conversely, the largest clip apparently did not cause a severe enough reduction of renal flow, as the majority of the rats prepared in this manner did not develop a substantial hypertension. Nevertheless, regardless of the clip used, any resultant hypertensive rat was used to comprise

the fourth group of experimental animals.

Through all the aforementioned problems and their solutions a series of criteria were devised to serve as a set of guidelines for rejecting any data obtained from completed experiments. These criteria are as follows:

1) excessive bleeding and/or hematocrit below 40%;
2) lung infection of any type;
3) infarcted kidney(s) in any group except renal hypertensives;
4) creatinine values outside two standard deviations of the mean;
5) carotid arterial-femoral arterial pressure gradient above 10.0 mm Hg in sham operated animals;
6) carotid arterial pressure below 130 mm Hg in rats coarcted above the renal arteries;
7) carotid arterial pressure sham rats or rats coarcted below above 130 mm Hg in the renal arteries under conditions of normal sedation;
8) surgery lasting longer than 2 hrs 30 mins (surgery defined as time animal is placed in ether until the start of perfusion; includes 30 minutes wait for heparin, taking carotid and femoral arterial pressure twice, and pump set up);
9) animal getting less than 0.5 cc of chloralose (normal dosage was 0.7 cc chloralose);

10) animal significantly embolized, i.e., NaNP resistance greater than 2 standard deviations of the mean, or sharp increase (> 30 mm Hg rise in 12 seconds or less) in perfusion pressure without a return to pre-increase levels, $\pm$ 5 mm Hg, after corrective measures were taken;

11) differences between flow sets of greater than 30% where there is evidence for embolization before differences;

12) acute denervation and/or NaNP resistance segments of experiment are to be eliminated if the animal constantly moves and tugs against cannula during entire measurement period;

13) no decrease in perfusion pressure after acute denervation thus signifying a non-vital nerve supply to the hindlimb;

14) experimental values beyond $\pm$ 3 standard deviations of the mean

APPENDIX B

REASONS FOR LIMB WEIGHT NORMALIZATION

When perfusing any extremity (such as the rat's hind limb) at a constant flow, one of the determinants of vascular resistance can be the size of the extremity; the logic being, that limbs of different sizes contain different numbers of vessels arranged in parallel. Differences in limb size can add to the amount of variability already present in the resistance measurements in each experimental group, thus making it all the more difficult to pick up true difference in the values of each group. Consequently, it is common practice to express resistance measurements in terms of the amount of tissue mass (weight) in which the resistance was determined. Of course, if these weights were the same in all the experimental groups studied, there would be no need for such a "normalization" of the data.

In this study a dicotomy existed in the data on the weights of the perfused hindlimbs. The limb weights, expressed in terms of body weight, of the rats with coarctation hypertension were not different from those of sham controls. However, both these limb weight/body weight ratios were significantly greater than either of the ratios for rats coarcted below the renal arteries or for the rats with two-kidney Goldblatt hypertension. Therefore the question arose as to in what manner the data should be expressed.

Presumably there should be some form of inverse relationship between the size of the limb and the resistance to flow in the limb; all other factors being constant. Consequently a linear regression was done of resistance during maximal vasodilation versus the limb weight of each group of rats.

In all groups, whether the perfused limb wet weight or dry weight was used, or the opposite limb's wet or dry weight was used, or whether a linear or exponential curve was being fitted to the data, there was absolutely no significant regression or correlation of any type present in the data.

However, negative results do not necessarily mean that no relationship truly exists. Therefore, other means had to be used to determine whether or not a limb weight normalization was to be used on the data.

In a subsequent analysis, it was found that the perfused limbs of all the groups of rats contained more water, presumably from edema due to the perfusion, than did the opposite non-perfused limb. Therefore, the perfused limb wet weights of the rats may have been different from the true limb weights of the rats (before perfusion). Therefore, these weights could not be used to normalize the resistance data.

It was also found that the dry limb weight data, on either limb, had higher coefficients of variability than the other limb weights, so much so that resistance data

corrected with dry limb weight actually contained more variability than the resistance data alone. Consequently, dry limb weights could not be used to normalize the data.

This left only the opposite limb wet weights as the closest estimate to the true limb weight of the animal. These weights expressed in terms of body weight, were significantly less in either coarcted group of rats compared to sham controls. In addition, resistance data expressed in terms of these weights had the lowest amount of variability compared to the same data expressed with or without the other limb weights.

In spite of the results of the regression analysis, since the opposite limb weight/body weight ratios signified some significant atrophy in the hindlimbs of coarcted rats, it was felt that all the resistance data should be expressed in terms of some type of limb weight. Since the opposite limb wet weights were shown to best reduce the variability in the data, they were chosen for the limb weight normalization of the data.

APPENDIX C

DATA AND RESULTS OF STATISTICAL ANALYSES

TABLE 2

AVERAGE SYSTOLIC CAUDAL ARTERIAL PRESSURE

First Measurement

Group	A	B	C	D
n	7	10	3	4
Mean	109.21	110.95	49.83	173.38
S.D.	12.55	4.93	10.28	6.24
VAR	157.49	24.30	105.58	38.90
SEM	4.74	1.56	5.93	3.12

S-N-K TEST

Comp	Range	Diff.	LSR 0.05	Sig 0.05
D vs C	4	123.6	18.46	*
D vs A	3	64.2	13.69	*
B vs C	3	61.1	14.38	*
D vs B	2	62.4	10.66	*
B vs A	2	1.74	8.88	No
A vs C	2	59.4	12.43	*

TABLE 3

AVERAGE SYSTOLIC CAUDAL ARTERIAL PRESSURE

Second Measurement

GROUP	A	B	C	D
n	10	13	6	6
Mean	108.5	109.62	56	182.17
S.D.	14.44	7.69	13.16	18.61
VAR	208.56	59.13	144.25	346.27
SEM	4.57	2.13	4.90	7.60

S-N-K TEST

Comp	Range	Diff	LSR 0.05	Sig 0.05
D vs C	4	126.2	19.99	*
D vs A	3	73.7	16.21	*
B vs C	3	53.6	15.50	*
D vs B	2	72.6	13.43	*
B vs A	2	1.1	10.94	No
A vs C	2	52.5	13.43	*

TABLE 4

AVERAGE SYSTOLIC CAUDAL ARTERIAL PRESSURE

Third Measurement

Group	A	B	C	D
n	12	13	6	6
Mean	112.83	112.69	56.75	173.25
S.D.	9.27	7.86	11.81	28.76
VAR	85.97	61.73	139.58	827.38
SEM	2.68	2.18	4.82	11.74

S-N-K TEST

Comp	Range	Diff	LSR 0.05	Sig 0.05
D vs C	4	116.5	22.0	*
D vs B	3	60.6	17.04	*
A vs C	3	56.1	17.26	*
D vs A	2	60.4	14.31	*
A vs B	2	00.14	11.46	No
B vs C	2	55.9	14.13	*

TABLE 5

AVERAGE SYSTOLIC CAUDAL ARTERIAL PRESSURE

Total Measurement

Group	A	B	C	D
n	29	36	15	16
Mean	110.47	111.10	55.07	176.63
S.D.	11.78	7.03	11.58	20.46
VAR	138.73	49.45	134.21	418.65
SEM	2.19	1.17	2.99	5.12

S-N-K Test

Comp	Range	Diff	LSR 0.05	Sig 0.05
D vs C	4	121.6	11.54	*
D vs A	3	66.2	9.10	*
B vs C	3	56.0	8.98	*
D vs B	2	65.5	7.32	*
B vs A	2	0.63	6.08	No
A vs C	2	55.4	7.75	*

TABLE 6

WEEK-TO-WEEK COMPARISONS OF AVERAGE SYSTOLIC CAUDAL ARTERIAL PRESSURE

	1st vs. 2nd	2nd vs 3rd	3rd vs 1st
Group A	Not sig	Not sig	Not sig
	$p > 0.8$	$p > 0.4$	$p > 0.4$
Group B	Not Sig	Not Sig	Not Sig
	$p > 0.6$	$p > 0.3$	$p > 0.5$
Group C	Not Sig	Not Sig	Not Sig
	$p > 0.4$	$p > 0.9$	$p > 0.4$
Group D	Not Sig	Not Sig	Not sig
	$p > 0.3$	$p > 0.5$	$p > 0.9$

TABLE 7

DIRECT MEAN CAROTID ARTERIAL PRESSURE

Group	A	B	C	D
n	13	20	13	9
Mean	152.75	110.29	110.19	149.11
S.D.	21.50	10.98	13.84	26.63
VAR	462.31	120.66	191.66	709.31
SEM	5.96	2.46	3.84	8.88

S-N-K TEST

Comp	Range	Diff	LSR 0.01	Sig 0.01	LSR 0.05	Sig 0.05
A vs C	4	42.55	23.16	*	-	-
A vs B	3	42.46	21.57	*	-	-
D vs C	3	38.92	22.07	*	-	-
A vs D	2	3.64	20.92	No	15.69	No
D vs B	2	38.82	19.36	*	-	-
B vs C	2	0.10	17.19	No	12.89	No

TABLE 8

DIRECT MEAN FEMORAL ARTERIAL PRESSURE

Group	A	B	C	D
n	13	20	13	9
Mean	112.28	104.88	75.37	146.67
S.D.	19.9	11.14	11.32	27.48
VAR	395.99	124.20	128.07	775.02
SEM	5.52	2.49	3.61	9.16

S-N-K TEST

Comp	Range	Diff	LSR0.01	Sig 0.01	LSR 0.05	Sig 0.05
D vs C	4	71.3	24.27	*	-	-
D vs B	3	41.79	20.91	*	-	-
A vs C	3	36.91	20.44	*	-	-
D vs A	2	34.39	19.82	*	-	-
A vs B	2	7.4	16.29	No	12.22	No
B vs C	2	36.91	16.29	*	-	-

TABLE 9

RESTING RESISTANCE X OPPOSITE LIMB WET WEIGHT

Group	A	B	C	D
n	10	11	9	6
Mean	2447.2	1561.18	1119.33	2609.5
S.D.	693.17	395.25	122.78	564.98
VAR	480,481	156,221	15075.0	319,200
SEM	219.20	119.17	40.93	230.65

S-N-K TEST

Comp	Range	Diff	LSR 0.01	Sig 0.01	LSR 0.05	Sig 0.05
D vs C	4	1490.17	868.0	*	-	-
A vs C	3	1327.87	704.2	*	-	-
D vs B	3	1648.32	688.9	*	-	-
D vs A	2	162.30	689.9	No	512.96	No
A vs B	2	886.02	583.7	*	-	-
B vs C	2	441.85	600.5	*(t-test)	446.47	No

TABLE 10

RESTING FLOW (cc/Minute)/GRAM OPPOSITE LIMB
WET WEIGHT

Group	A	B	C	D
n	10	11	9	6
Mean	0.04896	0.06895	0.05713	0.05343
S.D.	0.01355	0.01470	0.00580	0.01097
VAR	0.00018	0.00022	0.00003	0.00012
SEM	0.00429	0.00443	0.00193	0.00447

S-N-K TEST

Comp	Range	Diff	LSR 0.05	Sig 0.05
B vs A	4	0.01999	0.01730	*
B vs D	3	0.01552	0.01822	No
C vs A	3	0.00817	—	No
B vs C	2	0.01182	0.01337	* (t-test)
C vs D	2	0.00370	—	No
D vs A	2	0.00447	—	No

TABLE 11

RESISTANCE AFTER ACUTE DENERVATION X
OPPOSITE LIMB WET WEIGHT FLOW = 1.0 CC/MINUTE

Group	A	B	C	D
n	12	10	9	6
Mean	1457.75	1326.3	956.44	2094.33
S.D.	230.21	131.42	74.86	330.91
VAR	52997.5	17271.8	5603.3	109499.5
SEM	66.46	41.56	24.95	135.09

S-N-K TEST

Comp	Range	Diff	LSR 0.01	Sig 0.01	LSR 0.05	Sig 0.05
D vs C	4	1137.89	357	*	-	-
D vs B	3	768.03	326	*	-	-
A vs C	3	501.31	278	*	-	-
D vs A	2	636.58	275	*	-	-
A vs B	2	131.45	235	No	175.06	No
B vs C	2	369.86	253	*	-	-

TABLE 12

RESISTANCE AFTER SODIUM NITROPRUSSIDE X
OPPOSITE LIMB WET WEIGHT

Flow = 1.0 cc/Minute

Group	A	B	C	D
n	12	10	9	6
Mean	545.0	498.4	366.11	660.33
S.D.	41.34	57.27	44.97	44.68
VAR	1709.27	3280.27	2022.61	1996.67
SEM	11.93	18.11	14.99	18.24

S-N-K TEST

Comp	Range	Diff	LSR0.01	Sig 0.01	LSR 0.05	Sig 0.05
D vs C	4	294.22	84.42	*	-	-
D vs B	3	161.93	76.99	*	-	-
A vs C	3	178.89	65.74	*	-	-
D vs A	2	115.33	65.00	*	-	-
A vs B	2	46.6	55.67	No	41.12	*
B vs C	2	132.29	59.73	*	-	-

TABLE 13
AVERAGE PRESSURE x OPPOSITE LIMB WET WEIGHT
Flow = 0.125 cc/Minute

Group	A	B	C	D
n	11	10	9	6
Mean	397.27	270.30	258.67	400.83
S.D.	102.94	57.08	56.14	70.78
VAR	10597	3259	3151	5010
SEM	31.04	18.05	18.71	28.90

S-N-K TEST

Comp	Range	Diff	LSR0.01	Sig 0.01	LSR 0.05	Sig 0.05
D vs C	4	142.16	135.59	*	-	-
D vs B	3	130.53	123.60	*	-	-
A vs C	3	138.60	107.62	*	-	-
D vs A	2	3.56	105.95	No	78.76	No
A vs B	2	126.97	91.20	*	-	-
B vs C	2	11.67	95.91	No	71.30	No

TABLE 14

AVERAGE RESISTANCE X OPPOSITE LIMB WET WEIGHT

Flow = 0.125 cc/Minute

Group	A	B	C	D
n	11	10	9	6
Mean	3179	2161	2068	3211
S.D.	823.73	455.00	450.60	571.38
VAR	678538	207031	203036	326480
SEM	248.37	143.89	150.20	233.27

S-N-K TEST

Comp	Range	Diff	LSR0.01	Sig 0.01	LSR 0.05	Sig 0.05
D vs C	4	1142.4	1086	*	-	-
D vs B	3	1049.6	990	*	-	-
A vs C	3	1110.9	852	*	-	-
D vs A	2	31.5	848	No	630.8	No
A vs B	2	1018.1	730	*	-	-
B vs C	2	92.8	768	No	571.1	No

TABLE 15

AVERAGE PRESSURE X OPPOSITE LIMB WET WEIGHT
Flow - 0.25 cc/Minute

Group	A	B	C	D
n	11	10	9	6
Mean	730.00	511.75	445.60	673.30
S.D.	185.1	94.12	92.20	75.63
VAR	34261	8859	8501	5720
SEM	55.81	29.76	30.73	30.88

S-N-K TEST

Comp	Range	Diff	LSR0.01	Sig 0.01	LSR 0.05	Sig 0.05
A vs C	4	284.4	194.0	*	-	-
A vs B	3	218.25	175.6	*	-	-
D vs C	3	227.7	212	*	-	-
A vs D	2	56.7	178	No	132	No
D vs B	2	161.3	181	No	134	*
B vs C	2	66.4	161	No	120	No

TABLE 16

AVERAGE RESISTANCE X OPPOSITE LIMB WET WEIGHT
Flow - 0.25 cc/Minute

Group	A	B	C	D
n	11	10	9	6
Mean	2918.9	2053	1771	2693
S.D.	740.9	370.5	350.8	302.7
VAR	549007	137302	123092	91599
SEM	223.4	117.17	116.9	123.6

S-N-K TEST

Comp	Range	Diff	LSR0.01	Sig 0.01	LSR 0.05	Sig 0.05
A vs C	4	1147.9	767.17	*	-	-
A vs B	3	865.9	694.3	*	-	-
D vs C	3	922	837	*	-	-
A vs D	2	225.9	703	No	523	No
D vs B	2	640	715	No	532	*
B vs C	2	282	636	No	473	No

TABLE 17

AVERAGE PRESSURE X OPPOSITE LIMB WET WEIGHT

Flow = 0.5cc/Minute

Group	A	B	C	D
n	11	10	9	6
Mean	1256.9	794	617.6	1426
S.D.	307.08	94.52	71.55	399.57
VAR	94298	8933	5119	159659
SEM	92.59	29.87	23.85	163.13

S-N-K TEST

Comp	Range	Diff	LSR0.01	Sig 0.01	LSR 0.05	Sig 0.05
D vs C	4	808.4	430	*	-	-
D vs B	3	632	392	*	-	-
A vs C	3	639.4	341	*	-	-
D vs A	2	169.1	336	No	250	No
A vs B	2	462.9	289	*	-	-
B vs C	2	176.4	304	No	226	No

TABLE 18

AVERAGE RESISTANCE X OPPOSITE LIMB WET WEIGHT

Flow - 0.5 cc/Minute

Group	A	B	C	D
n	11	10	9	6
Mean	2513.18	1587.9	1235	2853
S.D.	614.13	189.27	143.32	798.7
VAR	377156	35822	20541	637904
SEM	185.17	59.85	47.77	326

S-N-K TEST

Comp	Range	Diff	LSR0.01	Sig 0.01	LSR 0.05	Sig 0.05
D vs C	4	1618	859	*	-	-
D vs B	3	1265	783	*	-	-
A vs C	3	1278	682	*	-	-
D vs A	2	339.8	671	No	499	No
A vs B	2	925.3	578	*	-	-
B vs C	2	352.9	608	No	452	No

TABLE 19

AVERAGE PRESSURE AND RESISTANCE X OPPOSITE LIMB
WET WEIGHT Flow - 1.0 cc/Minute

Group	A	B	C	D
n	11	10	9	6
Mean	2580	1840.8	1183.3	3111.7
S.D.	395.7	281.07	71.2	833.4
VAR	156572	79003	5070	694566
SEM	119.3	88.88	23.73	340.2

S-N-K TEST

Comp	Range	Diff	LSR0.01	Sig 0.01	LSR 0.05	Sig 0.05
D vs C	4	1928.4	757	*	-	-
D vs B	3	1270.9	691	*	-	-
A vs C	3	1396.7	601	*	-	-
D vs A	2	531.7	591.7	No	440	*
A vs B	2	739.2	509.4	*	-	-
B vs C	2	657.5	536	*	-	-

TABLE 20

AVERAGE PRESSURE X OPPOSITE LIMB WET WEIGHT

Flow - 1.5 cc/Minute

Group	A	B	C	D
n	11	10	9	6
Mean	3275	2657.9	1770.1	4033
S.D.	310.1	457	177.1	839
VAR	96169	208818	31369	703884
SEM	93.5	144.5	59.04	342.5

S-N-K TEST

Comp	Range	Diff	LSR0.01	Sig 0.01	LSR 0.05	Sig 0.05
D vs C	4	2263	806	*	_	_
D vs B	3	1375.1	735	*	_	_
A vs C	3	1504.9	646	*	_	_
D vs A	2	758	630	*	_	_
A vs B	2	617.1	542	*	_	_
B vs C	2	887.8	570	*	_	_

TABLE 21

AVERAGE RESISTANCE X OPPOSITE LIMB WET WEIGHT

Flow = 1.5cc/Minute

Group	A	B	C	D
n	11	10	9	6
Mean	2183	1773.9	1190.6	2688.7
S.D.	206.6	307.1	115.3	559.2
VAR	42682	94318	13290	312755
SEM	62.29	97.1	38.43	228.3

S-N-K TEST

Comp	Range	Diff	LSR0.01	Sig 0.01	LSR 0.05	Sig 0.05
D vs C	4	1498.1	540	*	-	-
D vs B	3	914.8	493	*	-	-
A vs C	3	992.4	429	*	-	-
D vs A	2	505.7	422	*	-	-
A vs B	2	409.1	363.4	*	-	-
B vs C	2	583.3	382	*	-	-

TABLE 22

AVERAGE PRESSURE X OPPOSITE LIMB WET WEIGHT

Flow - 2.0 cc/Minute

Group	A	B	C	D
n	11	10	9	6
Mean	3547	3197.8	2164	4566
S.D.	348.4	525.1	270.2	725
VAR	121374	275737	72996	526266
SEM	105.0	166.1	90.06	296

S-N-K TEST

Comp	Range	Diff	LSR0.01	Sig 0.01	LSR 0.05	Sig 0.05
D vs C	4	2402	828	*	-	-
D vs B	3	1368	754	*	-	-
A vs C	3	1383	657	*	-	-
D vs A	2	1019	646	*	-	-
A vs B	2	340.2	557	No	414	No; sig w/o limb weight
B vs C	2	1033.8	585	*	-	-

TABLE 23

AVERAGE RESISTANCE X OPPOSITE LIMB WET WEIGHT

Flow = 2.0 cc/Minute

Group	A	B	C	D
n	11	10	9	6
Mean	1773.5	1599.2	1082	2283
S.D.	174.1	262.6	135.1	363
VAR	30313	68938	18244	131639
SEM	52.49	82.03	45.02	148

S-N-K TEST

Comp	Range	Diff	LSR0.01	Sig 0.01	LSR 0.05	Sig 0.05
D vs C	4	1201	414	*	-	-
D vs B	3	683.8	377	*	-	-
A vs C	3	691.5	328	*	-	-
D vs A	2	509.5	323	*	-	-
A vs B	2	174.3	278	No	207	No;sig w/o limb weight
B vs C	2	517	293	*	-	-

TABLE 24

BODY WEIGHTS

(grams)

Group	A	B	C	D
n	16	21	15	9
Mean	352.8	353.1	358.9	358.7
S.D.	19.42	13.41	14.41	30.40
VAR	377.3	179.7	297.6	821.3
SEM	4.86	2.93	3.72	10.13

No significant differences among groups

TABLE 26

HEART WEIGHT/BODY WEIGHT

Group	A	B	C	D
n	16	21	15	9
Mean	0.00481	0.00333	0.00337	0.00403
S.D.	0.00059	0.00027	0.00025	0.00041
VAR	3.52×10^{-7}	7.31×10^{-8}	6.35×10^{-8}	1.65×10^{-7}
SEM	0.00015	0.00006	0.00007	0.00014

S-N-K TEST

Comp	Range	Diff	LSR0.01	Sig 0.01
A vs B	4	0.0148	0.00043	*
A vs C	3	0.0144	0.00043	*
D vs B	3	0.0070	0.00048	*
A vs D	2	0.0078	0.00044	*
D vs C	2	0.0068	0.00044	*
C vs B	2	0.0004	0.00036	*

TABLE 27

LEFT KIDNEY WEIGHT/BODY WEIGHT

Group	A	B	C	D
n	16	21	15	9
Mean	0.00326	0.00374	0.00370	0.00273
S.D.	0.0042	0.00045	0.00039	0.00083
VAR	1.77×10^{-7}	2.00×10^{-7}	1.51×10^{-7}	6.8×10^{-7}
SEM	0.00011	0.00010	0.000010	0.00028

S-N-K TEST

Comp	Range	Diff	LSR0.01	Sig 0.01	LSR 0.05	Sig 0.05
B vs D	4	0.00101	0.00065	*	-	-
B vs A	3	0.00048	0.00050	No	0.00040	*
C vs D	3	0.00097	0.00064	*	-	-
B vs C	2	0.00004	0.00045	No	0.00035	No
C vs A	2	0.00044	0.00048	No	0.00037	*
A vs D	2	0.00053	0.00055	No	0.00042	*

TABLE 28

RIGHT KIDNEY WEIGHT/BODY WEIGHT

Group	A	B	C	D
n	16	21	15	9
Mean	0.0034	0.0037	0.0037	0.0044
S.D.	0.00055	0.00056	0.00046	0.00081
VAR	3.07×10^{-7}	3.16×10^{-7}	2.14×10^{-7}	6.56×10^{-7}
SEM	0.00014	0.00012	0.00012	0.00027

S-N-K TEST

Comp	Range	Diff	LSR0.01	Sig 0.01	LSR 0.05	Sig 0.05
D vs A	3	0.00099	0.00073	*	-	-
D vs B	2	0.00069	0.00062	*	-	-
D vs C	2	0.00069	0.00065	*	-	-
B vs A	2	0.00030	0.00051	No	0.00038	No
C vs A	2	0.00030	0.00056	No	0.00042	No

TABLE 29

PLASMA CREATININE mg%

GROUP	A	B	C	D
n	12	11	8	5
Σ	13.70	10.00	9.90	6.25
Mean	1.14	0.91	1.24	1.25
S.D.	0.366	0.408	0.486	0.456
VAR	0.134	0.166	0.236	0.208
SEM	0.106	0.123	0.172	0.204

No significant differences among groups

TABLE 30

PLASMA SODIUM mEq/L

GROUP	A	B	C	D
n	11	11	8	6
Σ	1433.5	1484.5	1053.0	805
Mean	130.3	134.9	131.6	134.2
S.D.	6.23	6.81	7.13	6.99
VAR	38.91	46.42	50.77	48.87
SEM	1.88	2.05	2.52	2.85

No significant differences among groups

TABLE 31

PLASMA POTASSIUM mEq/L

GROUP	A	B	C	D
n	11	11	8	6
Σ	46.85	49.10	35.10	25.15
Mean	4.26	4.46	4.39	4.19
S.D.	0.65	0.50	0.62	0.57
VAR	0.43	0.25	0.39	0.32
SEM	0.20	0.15	0.22	0.23

No significant differences among groups

TABLE 32

PLASMA CALCIUM mEq/L

GROUP	A	B	C	D
n	10	11	7	4
Σ	32.775	36.025	23.6	13.0
Mean	3.28	3.28	3.37	3.25
S.D.	0.480	0.297	0.309	0.175
VAR	0.230	0.088	0.100	0.03
SEM	0.152	0.090	0.117	0.090

No significant differences among groups

TABLE 33

PLASMA MAGNESIUM mEq/L

Group	A	B	C	D
n	10	11	7	4
Σ	25.15	29.935	17.14	9.665
Mean	2.552	2.721	2.449	2.416
S.D.	0.341	0.462	0.204	0.124
VAR	0.116	0.213	0.042	0.015
SEM	0.108	0.139	0.077	0.062

No significant differences among groups

TABLE 34

HEMATOCRITS

GROUP	A	B	C	D
n	13	14	12	7
Σ	574.25	622	521.75	310.75
Mean	44.17	44.43	43.48	44.39
S.D.	2.76	2.61	2.81	2.30
SEM	0.766	0.697	0.811	0.869
VAR	7.629	6.802	7.892	5.289

No significant differences among groups

TABLE 35

PLASMA RENIN CONCENTRATION

(ng/ml)/hr ANGIOTENSION I

GROUP	A	B
Mean	21.9	12.1
S.D.	7.209	2.708
VAR	51.977	7.331
SEM	2.000	0.751
n	13	13

Significant difference 2-tailed t-test $p < 0.001$

TABLE 36

HEART WEIGHT/BODY WEIGHT FOR RATS
USED FOR PLASMA RENIN CONCENTRATIONS

	Group A	Group B
n	13	15
Mean	0.00468	0.00330
S.D.	0.00054	0.00025
VAR	2.91×10^{-7}	6.15×10^{-8}
SEM	0.000149	0.000064

Means are statistically significantly different with students two-tailed t-test, $p < 0.001$.

APPENDIX C KEY

GROUP A - rats with coarctation of the abdominal aorta above the origin of both renal arteries (coarctation hypertension)

GROUP B - normotensive sham operated control rats

GROUP C - rats with coarctation of the abdominal aorta below the origin of both renal arteries

GROUP D - rats with two-kidney Goldblatt hypertension

n = number of rats in each group

mean = average group value for the parameter listed

S.D. = standard deviation of the group

Var. = group variance

SEM. = standard error of the mean of the group

S-N-K test = Student-Newman-Kuels test

comp = intergroup comparison being made

range = number of group means the comparison encompasses

Diff = difference between the means of the two groups being compared

LSR 0.01 - (Least Significant Range) smallest difference needed between the means of the two groups for the difference to be statistically significant, $p < 0.01$

LSR 0.05 - least significant range, $p < 0.05$

Sig 0.01 - significant difference at $p < 0.01$

Sig 0.05 - significant difference at $p < 0.05$

* - a statistically significant difference exists

No - no statistically significant difference exists

\- - no need for statistical comparison

t test - test used was students two-tailed t-test

mEq/L - milliequivalents per liter

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