# BLOOD PRESSURE CHANGES AND RENIN ACTIVITY ASSOCIATED WITH RENAL ARTERIOVENOUS FISTULAE IN THE DOG

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## ABSTRACT

## BLOOD PRESSURE CHANGES AND RENIN ACTIVITY ASSOCIATED WITH RENAL ARTERIOVENOUS FISTULAE IN THE DOG

By

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In humans, renal arteriovenous (a-v) fistulae have been associated with diastolic hypertension in 48% of demonstrated antemortem cases, (Maldonado and Sheps, 1966). The hemodynamic changes usually associated with a-v fistulae allow for a small decrease in diastolic blood pressure. The question then arises why diastolic hypertension occurs with a-v fistulae at this particular anatomical site. Renovascular anomalies have long been known to cause hypertension. There is much evidence that this renovascular hypertension, to some degree, is mediated through the renin-angiotensin-aldosterone (RAA) system. Any large non-renal a-v fistula might also cause increased RAA activity, but not hypertension. This study was undertaken to determine whether 1) a communication between renal artery and vein could be successfully constructed, 2) whether this would produce diastolic hypertension in the dog, 3) whether there would be a rise in renin activity and if so, would there be a quantitatively greater rise than found with non-renal a-v fistulae.

Surgical a-v fistulae between renal artery and vein were successfully constructed in ten dogs who were then followed for

a four week period to observe any change in blood pressure or renin activity from the control period. Diastolic pressure rose an average 10 mm Hg from 95 mm Hg to 105 mm Hg. A greater rise was seen in the first two post-operative weeks with a subsequent lowering to near control values. Renin activity, measured by a rat blood pressure assay method, did not change significantly. The diastolic pressure rise was a small one, and by no means is it certain that the rise represented clinical hypertension.

Eight dogs had fistulae constructed between the renal artery and splenic veins. This condition does not raise renal venous pressure as in the previous series, but does decrease the perfusion pressure and flow to the kidney. There was no rise in blood pressure or renin activity.

Four dogs had fistulae constructed between the femoral artery and vein. There was a slight but insignificant drop in diastolic blood pressure and no change in renin activity. A sizable drop in perfusion pressure and flow in the distal arterial segment was demonstrated as well as a large increase in distal segment venous pressure. The pressure drop from arterial to venous circuit was greatly reduced. It is assumed that similar hemodynamic changes occur at the site of any a-v fistula.

Surgical construction of a-v fistulae proved to be feasible. A small increase in diastolic pressure was seen only with renal a-v fistulae, however renin activity did not increase nor was it elevated in the other two series of fistulae. Ischemia, necrosis, and loss of renal function were noted. This was more pronounced in the series where venous pressure was elevated. The effect of an elevated renal venous pressure is implicated. When the renal vein pressure increase was prevented there was no rise in diastolic blood pressure.

## REFERENCES

Maldonado, J. E. and S. G. Sheps. Renal arteriovenous fistula. Postgrad. Med. 40: 263, 1966.

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# INTRODUCTION

Since Goldblatt's classic experiment in 1934, alterations in renal hemodynamics have been implicated in renal hypertension. Among the known alterations which produce renal hypertension are renal arteriovenous (a-v) fistulae. Although a rare condition, and one which is seldom diagnosed antemortem, it is associated with a high incidence of hypertension. Indeed, Maldonado and Sheps reviewing the world literature in 1966 found an incidence of hypertension of 48% when renal a-v fistula was suspected and subsequently demonstrated. The cause-effect relation in the etiology of this particular kind of renal hypertension has not been established. Many investigators have felt the renin-angiotensin-aldosterone (RAA) system is involved. Much evidence has accumulated over the years to suggest an intrinsic involvement of this hormonal system in other types of renal hypertension, although all the mechanics have not been established and are the subject of no small controversy. In renal a-v fistulae there are more complex hemodynamic changes than usually seen in other renovascular hypertensive situations. These hemodynamic changes in themselves may in some way account for elevation of blood pressure secondary to renal changes. The hypertension may be mediated either through the RAA system or occur independently. In one sense this might be considered a paradoxical hypertension inasmuch as a-v fistulae usually cause a mild drop in diastolic pressure. In the presence of an a-v fistula there is a drop in peripheral resistance and a short circuiting of blood from the arterial to the venous compartment. The decrease in resistance is accompanied by a drop in diastolic blood pressure. This is sensed by the baroreceptors which reflexly stimulate the heart to increase rate and stroke force. Venous return increases because of the aforementioned short circuiting plus the action of the reflexly mediated venoconstriction through sympathico-adrenal discharge. The net result is an increased cardiac output that is reflected in an increased systolic blood pressure and a return of diastolic pressure to near normal although a mild diastolic hypotension often remains. The pulse pressure is usually increased because of an increased stroke volume in the presence of a decreased total peripheral resistance. In the presence of large shunts or weak hearts, congestive heart failure of the high output variety can occur. It should be pointed out that congestive cardiac failure can occur with any a-v fistula, renal or otherwise. This appears to be related to the size of the shunt, and obviously in the face of hypertension would be accelerated. When cardiac failure occurs, aldosterone levels are elevated. It is also possible to demonstrate aldosterone elevations in experimental a-v fistulae before frank failure has occurred. If it can be presumed that angiotensin is the major, if not the only, stimulant to increased secretion of aldosterone in the presence of a-v fistulae, the question arises as to the fundamental differences between non-renal and renal a-v fistulae. Perhaps in the former angiotensin only stimulates aldosterone secretion whereas in the latter it may also have a hypertension-producing effect. Quantitative differences are one of several considerations, and this possibility initially prompted this study. The experimental canine model required successful surgical creation of a renal a-v shunt followed by demonstrable hypertension, and although early pilot studies suggested that dogs may react to renal a-v fistulae with elevations in blood pressure, as in humans, a later series of animals has cast considerable coubt on this premise. However, on the basis of the preliminary promising results, the decision was made to study renin activity levels in dogs with renal a-v fistulae as well as other hemodynamic parameters in an attempt to fix a cause for this unusual hypertension.

## LITERATURE REVIEW

*Cardiovascular changes associated with arteriovenous fistulae.*

Since William Hunter first described an arteriovenous fistula in 1757, numerous clinical case reports have appeared in world medical literature. However, it remained until the early 1920's before the pathophysiology was explored clinically and experimentally. Emile Holman has been a prolific investigator of this anomaly, and in 1923 he reported on his findings from 21 cases at the Johns Hopkins Hospital. He noted the characteristic blood pressure variations, viz., an elevated systolic and decreased diastolic pressure with resulting increased pulse pressure - all of which changed toward normal after surgical repair. He also noted the presence of cardiomegaly and the dilatation of the artery proximal to the shunt. He was also aware of an increase in cardiac output and attempted to explain this phenomenon. Lewis and Drury in 1923 (cited by Cohen *et al.*, 1948) had reported that there was no increase in cardiac output. Holman disputed this. He stated there is a necessary rise in venous pressure as an initiating event. This, when later investigated, proved not to be the case. Still, according to Holman's observations there is an increase in cardiac output. He noted that "the volume of blood which flows through the heart per unit time is obviously greatly increased when short circuiting occurs". He postulated that this causes increased myocardial work and hypertrophy. Later physiologic observations shed more accurate light on the actual mechanisms of increased cardiac output and subsequent cardiomegaly. Holman also noted an increase in blood volume based on the observation of low red cell counts which approached normal following surgical repair. His explanation was a Starling effect with the lowered blood pressure causing net reabsorption from the tissue fluids. This was not an unattractive explanation in 1923 when methods for body fluid measurements were not developed nor was there knowledge of aldosterone and its role in producing hypervolemia under circumstances of cardiac compromise. Holman speculated on the nature of the dilatation proximal to the fistula. He correctly observed that the final limiting factor determining the quantity of blood that will pass through a fistula is the resistance of the fistula itself. He suggested that the artery would dilate until its cross sectional area equals that of the arteriovenous communication. He stated that the dilatation will not occur if the arterial cross section is initially larger than the area of the shunt.

In one of the first experimental arteriovenous fistulae, Holman reported that on opening the shunt there was an immediate rise in heart rate from 148 to 224 while systolic pressure dropped from 204 to 176 and diastolic pressure dropped from 124 to 80. When the fistula was then quickly compressed, pressure returned to 208/110.

Reid (1925) reported observations on humans and dogs with experimental femoral arteriovenous fistulae. He noted the condition is a powerful stimulus to the development of collateral circulation. He observed that the artery proximal to the shunt becomes dilated and thin walled with degenerative changes while proximal veins dilate with thickening of the wall. He commented on "grave cardiac disturbances" to the point of sudden death. Reid also noted that circulation in an affected limb distal to the shunt is impaired, but there is an increase in the capillary bed.

Reid (1920) had previously reported on local findings in experimental femoral and carotid fistulae. The presence of a thrill and a bruit were noted. He also commented on the proximal dilatation of the artery and also observed a distal narrowing which takes considerable time (up to 3 years) to develop. Cardiomegaly was incidentally noted, but the author admitted he does not know how this is functionally related to the a-v fistula.

Matas (1925) delivered a comprehensive lecture on the clinical findings associated with a-v fistulae. Although he presented little physiologic explanation, he did note that the degree of cardiac decompensation and the systemic findings depend on the size of the fistula, the caliber of the involved vessels, the proximity to the heart, the duration, and the volume of the arterial stream.

Holman (1924), following his report on the Johns Hopkins series reported on a series of experimental fistulae to verify and attempt to explain the findings in human patients. He was successful in creating a surgical fistula between the aorta and vena cava. He remarked on the striking thrill and bruit and noted both disappeared when the proximal vein was occluded. Holman's explanation for this is inconclusive. As previously mentioned, on opening the shunt, heart rate increases and blood pressure falls. However, after a period of time the systolic pressure returns to normal although diastolic pressure remains low. The heart rate also slows. Holman suggested that these chronic changes occurred as a result of increase in blood volume. He measured blood volume (method of Hooper) in four dogs with femoral a-v fistulae and found an increase after the shunt had been open long enough to reverse the immediate changes. Holman also cites the increase in blood volume to explain the changes which occur on closing the shunt, namely, a marked slowing of heart rate and increase in both systolic and diastolic blood pressure. Following closure, blood volume returns to normal as do heart rate and blood pressure.

Venous pressure was elevated in the proximal segment near the fistula, but was normal in other peripheral veins if the heart was functioning well. In this same series of experiments, Holman reported dilation and hypertrophy of the heart and dilatation of the artery and vein proximal to the fistula.

Ellis and Weiss (1929) reported on their findings in two patients with an a-v fistula. One had the classic findings of congestive heart failure. The authors were able to manually compress the fistulae and noted a drop in cardiac output with a decrease in heart rate and rise in blood pressure. They speculated that there was an accumulation of blood in the venous segment followed by increased total blood volume. Local findings were also mentioned, specifically regional arteriolar dilatation, capillary pulsations and an increased skin temperature.

Cohen _et al_. (1948) because of a conflict in the literature on changes in cardiac output in patients with arteriovenous fistulae felt further investigation was needed. Their observations were made on 12 male patients with fistulae resulting from wounds less than 2 years old. Cardiac output was determined by catheterization and blood flow in the forearm was determined by venous occlusion plethysmography. These authors found an increase in cardiac output and the increase had some relation to the size of the fistula. Heart rate was also moderately increased. When the fistulae were closed by manual compression, there was a marked decrease in cardiac output along with a decreased pulse and an increase in systolic blood pressure. Flow through forearms was normal, but increased on compression of the fistulae. When the nerves to the forearm were blocked with procaine, the increase in flow on compression was reduced suggesting dilatation as a cause of increased flow rather than the rise in mean blood pressure.

Van Loo et al. (1949) examined the systemic effects of 2 cm long femoral a-v shunts in 20 dogs. They, too, felt the problem needed re-exploration because of some conflicts in the early literature. Similar to other reports they noted a rise in cardiac output and heart rate on opening the fistula. There was also an immediate drop in systolic, diastolic and mean blood pressure. Central venous pressure was unchanged.

Elkin and Warren (1947) published the first of several articles concerning their findings in the cardiovascular system in wounded veterans with a-v fistulae at the Ashford Hospital. In this first article, the authors reviewed local diagnostic and systemic signs. In a small group of patients, cardiac output was measured by critically damped low frequency ballistocardiography. More than half the patients had higher cardiac outputs than those found after surgical correction. There was generally a small depression of diastolic blood pressure with a wide pulse pressure. A small increase in blood volume was also noted.

Warren et al. (1951) with a larger group of patients at the same facility again reported on cardiac output in patients with traumatic a-v fistulae using ballistocardiography. Predictably, most showed an increased cardiac output. The authors suggest a decrease in cardiac output may indicate a failing heart, i.e., the so called high output heart failure. In this series of 47 patients there was no evidence of frank congestive heart failure.

The blood volume in 41 of these patients was measured using T1824. Twenty-Three of these patients (56%) following surgical correction showed a decrease in blood volume from preo-operative values of 200 ml or less, a value the authors felt was not significant. However, 18 patients (44%) showed a decrease of 200 to 1000 ml following surgery. In general, the patients with increased blood volume were those with functionally large fistulae.

Nickerson *et al.* (1951) in work also done at the Ashford Hospital examined venous pressures in six dogs with experimental femoral a-v fistulae. They found insignificant (0.5 mm Hg) pressure rises in the right atrium and peripheral veins on opening the shunt. However, venous pressure in the distal segment near the fistula is notably increased. Pressure in the artery proximal to the fistula falls on opening the fistula.

Epstein *et al.* (1953) observed the effects of a-v fistulae in a variety of sites on renal hemodynamics in 17 Korean war casualties. Clearances of inulin and PAH (creatinine in some cases) were found to be normal. These did not change on closing the fistulae. However, upon closure diastolic blood pressure rose an average of 15 mm Hg. Sodium excretion also increased following closure of the fistula. Although an explanation for the increase in sodium excretion was not forthcoming, in light of present knowledge of aldosterone this finding is predictable.

Frank *et al.* (1955) measured several parameters in dogs with acute artificial shunts through tubing. They found an increased stroke volume and cardiac output in all experiments. When the a-v fistula accomodated less than 20% of the cardiac output, the cardiac output increased by the full amount of fistula flow. In larger shunts, cardiac output did not increase to the full amount of fistula flow which can only mean a decrease in systemic capillary flow. The authors suggest that the factor which limits the degree of increase in cardiac output in the presence of large fistulae is inadequency of venous return, and the limit to the rate of venous return seems to be the volume of blood available. Consequently, the increase in blood volume seen in chronic a-v fistulae may be compensatory and not an indication of congestive heart failure. The authors feel that in small shunts, the increased venous blood probably comes from blood reservoirs. In large ones, these are exhausted and there is a shift from the periphery.

Muenster et al. (1959) reported on six patients, two of whom were in congestive failure. The physiologic findings were the same as previously reported but the additional parameter of arteriovenous oxygen difference was measured. The A-$VO_2$ difference was markedly reduced in all cases. Central venous pressure was elevated in the two cases with clinical failure. The authors made the point that heart failure may be produced in the apparently healthy heart by a peripheral circulatory load.

Hilton et al. (1955) studied the effects of an acute arteriovenous fistula on renal function. Filtration rate was normal or minimally reduced. However, renal blood flow always decreased, producing an increase in filtration fraction. This suggests a postglomerular constriction and perhaps minimal afferent constriction. Since mean arterial pressure did not fall, there must have been increased renal vascular resistance.

Dart *et al.* (1966) studied hemodynamic effects of femoral venous occlusion, before and after a-v shunting. Cardiac output was measured by placing an electromagnetic flowmeter probe on the ascending aorta. The femoral shunt was constructed using a rapidly polymerizing glue, and some were constructed with sutures. Prior to opening the fistula, venous occlusion caused an average 7% drop in cardiac output. Femoral artery flow decreased by 24%. There were no changes in blood pressure. When the shunt was opened, cardiac output increased 31%. Femoral artery flow increased 92% while mean blood pressure dropped 16 mm Hg. Proximal venous pressure was unchanged, (a finding at variance with other investigators) while distal venous pressure increased to 1/2 - 2/3 of arterial pressure. When the proximal venous segment was occluded, cardiac output dropped 13% while femoral artery flow decreased 84%. Mean femoral artery pressure rose 18% and proximal and distal venous pressures increased to near the femoral artery pressure. This was one of the few studies where flow was measured about the fistula.

Holman (1952) examined pressure relations at the site of an a-v fistula and noted some findings concerning flow. He stated "the effects of a fistula depend in large measure upon its size, but even more significantly upon the relationship of its size to the caliber of the vessels in which the fistula lies". He noted that fistulae of uniform size will pour a greater volume of blood through them, the nearer the heart they lie. This paper deals with experimental femoral and iliac a-v fistulae and observations on flow and size of vessels after one of the three segments, viz., distal artery, proximal vein and distal vein, is ligated. He noted that banding the proximal artery to prevent dilatation increased flow through the shunt via the distal artery and collateral bed. Formation of new collaterals always occurred after a-v fistulae were formed. Dye injections indicated a reversal of flow in the distal arterial segment through the shunt. This depends on the size of the fistula. Retrograde flow occurs only in large shunts when they are larger than the caliber of the artery. Pressure relations about the fistula in the above circumstance favored retrograde flow in the distal artery. Much of the flow available for this retrograde movement is assumed to come from collateral circulation, and, indeed, in the case of older shunts, where such collateral vessels had time to develop, the retrograde flow was greater. In a few experiments, the occurrence of a negative pressure in the proximal vein was noted. This was related to the high velocity of flow and was explained on the basis of the Venturi principle.

## Renal Hypertension

The complicity of the kidney in hypertension has been a consideration of both clinician and physiologist since 1898 when Tigerstedt and Bergman demonstrated the presence of an extractable renal pressor substance which they named "renin". However, it wasn't until 1934 when Goldblatt with his classic experiments on reduction of renal blood flow with subsequent hypertension in the canine model that investigators began to grasp the potential importance of this excretory organ as an explanation for the etiology and pathophysiology of at least one form of blood pressure elevation. Work carried on simultaneously in Indianapolis by Page and Helmer (1940) and Braun-Menendez _et al_., in

Buenos Aires (1940) indicated that the active principle responsible for vascular pressor activity was not renin *per se*, but another compound named "angiotonin" by the former investigators and "hypertensin" by the latter. By international agreement, the compound is now known as "angiotensin". Work subsequently done in these two laboratories, as well as in others, demonstrated conclusively that renin, the substance from the kidney, acts as a proteolytic enzyme which catalyzes the formation of a decapeptide known as angiotensin *I* from a substrate usually referred to as angiotensinogen. This substrate appears to originate in the liver and migrates electrophoretically as an $\alpha_2$ globulin. Angiotensin *I*, once liberated, is further reduced in the presence of a converting enzyme to angiotensin *II* - the active pressor substance. Page and Bumpus (1961) as well as Braun-Menendez *et al.* (1946) have written excellent reviews of the properties, chemical nature, synthesis, fate and activity of the various components of this system beginning with renin and ending with angiotensin *II*.

The control of renin release has been recently reviewed by Vander (1967). The review suggests that reduction in perfusion pressure such as occurs in the Goldblatt experiment or in similar situations such as arteriovenous fistula can increase the renal output of renin.

Increase in renin output has been the basis of numerous studies to determine if this event will cause transient or sustained hypertension. With the advent of methods for quantitative determination of plasma renin levels, such studies became possible. One thing clearly has evolved from these studies, namely that there is no consistent evidence of an elevated circulating renin level under conditions where hypertension of the renovascular (Goldblatt) type is suspected or known. Brown *et al.* studied several cases of surgically proved renal artery stenosis by assaying plasma renin concentration. They found many elevated, many normal and a very few below normal.

Fasciolo *et al.* (1964) studied plasma renin in dogs with unilateral clamped renal arteries. In six dogs they observed a rise in blood pressure with a subsequent return to normal. There was no rise in renin activity. When the contralateral kidney was later removed, blood pressure again rose, but, there was still no rise in renin. The authors concluded that canine renal hypertension is not caused by elevated circulating renin.

Laragh (1962) reviewed the considerable evidence that angiotensin *II* is a direct stimulant to the adrenal cortical zona glomerulosa to release the salt-retaining hormone aldosterone. Such an interaction inspired considerable research into the possible role of aldosterone in renal or other types of hypertension.

Davis *et al.* (1964) created experimental aortic-caval a-v fistulae below the kidneys in 16 dogs. The dogs all developed clinical signs of congestive heart failure along with the previously described systemic signs of a-v fistula. They found a marked hyper-secretion of aldosterone. This prompted the production of secondary aldosteronism in a second group of dogs by constriction of the thoracic inferior vena cava. Plasma renin levels were found to be elevated in all cases. This is indirect evidence that arteriovenous fistulae cause elevated renin levels concurrent with a drop in mean blood pressure.

Davis *et al.* (1962) clamped both renal arteries in 15 dogs. Eight of these developed a chronic benign hypertension while the other seven exhibited a malignant hypertension. The plasma renin content in the former group was two times normal while that in the latter group was ten times normal. This later group also demonstrated a striking aldosteronism. This paper presents evidence in conflict with studies showing no rise in renin levels in the presence of classic Goldblatt hypertension.

Malrow (1964) presented his findings in 16 patients with renal vascular lesions and hypertension. He was unable to demonstrate any significant rise in plasma renin activity.

Cohen et al. (1965) studied nine cases of renovascular hypertension. They stressed the importance of sampling blood while the patient is in the upright position. Six of their nine cases had markedly elevated values (931-3554 ng%) for renin activity.

These are a few of the many studies on plasma renin activity in suspected or proved clinical or experimental renal hypertension. The disparity of results and conclusions is provocative of skepticism and chagrin. Hypotheses are rampant and speculation concerning the actual role of renin in renovascular hypertension should yield a host of additional studies. A common criticism of the studies reported to date is concerned with methodology for assay of renin activity. Standarization has been loose at best, but with improved techniques, hopefully, this pitfall will be eliminated.

## Measurement of Renin Activity and Angiotensin

A chemical means to accurately assess circulating levels of renin and angiotensin *II* has been the subject of considerable research in the past 70 years. Braun-Menendez (1946) reviews much of the early efforts advanced toward this goal. Until recently, there was no accurate assay for angiotensin *II*. A measure of renin activity has been available for a number of years. The actual measurement of renin has never been accomplished since this enzyme has never been isolated in pure form. Renin activity implies the generation of angiotensin *II* by incubation and is measured by bioassay using a pressor response in an assay animal as the index of activity. Variations on three methods have been most widely accepted and used in the past decade. Helmer and Judson (1963) described a method of dialysing heparinized blood - a method which removed other pressor agents but unfortunately did not completely inactivate angiotensinases. The end product is assayed in a 48 hour nephrectomized pithed cat. Numerous modifications in various laboratories have improved the validity of this assay.

Boucher et al. introduced the use of Dowex. This is a resin which removes small peptides while excluding other pressor agents and protects the angiotensin from proteolytic enzymes. EDTA was added to the plasma as an anticoagulant and, serendipitously was found to inhibit angiotensinase. The plasma is usually incubated at 37°C for exactly three hours. The angiotensin is eluted with diethlyamine, lypophylized, reconstituted in alcohol and assayed in the nephrectomized, pentolinium-ized rat. Again, improvements in various laboratories since introduction of this method have yielded a better assay. This method has been quite commonly used.

Gunnells (1967) has introduced a bioassay similar to Boucher's with modifications and improvements. Details are described in the Methods Section elsewhere in this dissertation.

The introduction of the radioimmunoassay has now made it possible to assay directly for angiotensin - a long awaited event. Vallotton and Page (1967) published a method requiring an extraction procedure from plasma whereas Grocke (1969) has simplified the technique by assaying from plasma directly. When the procedure is functioning well in the numerous laboratories where renin and angiotensin are being studied, it can be expected that much more consistency in results from similar investigations will be forthcoming.

## Renal Arteriovenous Fistula: A Special Case

Renal arteriovenous fistulae are uncommon pathologic events, but they are of interest because of the high incidence of associated hypertension in humans. In 1966, Maldonado and Sheps reviewed the world literature and found only 86 reported cases of this anomaly since the first case was described by Varela in 1928. In reviewing the reported cases, these authors found the incidence of diastolic hypertension (pressure more than 90 mm Hg) to be 48%. Fistulae following biopsy were not included since most were characterized by hypertension before the biopsy. Forty-six cases were considered. From the 68 cases reported where etiology could be determined, a classification evolved, (Table 1). Maldonado, Sheps, Bernart, DeWeerd and Harrison (1964) reported a case where radioisotope renography and split renal function studies demonstrated ischemia distal to the fistula. Pathologic examination of the kidney in this case showed areas of incomplete infarction in which juxtoglomerular cell counts were high. Tobian (1966) states that this finding is characteristic of Goldblatt hypertension, and a Goldblatt mechanism has been suggested as the cause of hypertension in some cases of renal a-v fistulae.

Three additional cases of renal arteriovenous fistula have been reported since the above review. Papadopoulous _et al._ (1967) reports on a 24 year old male with diastolic hypertension which was successfully treated surgically. Sharif _et al._ (1967) reported a case with borderline hypertension (160/100) in a 48 year old female. Following surgery, blood pressure dropped to 130/90. These authors feel the hypertension results from an ischemic kidney, i.e., a Goldblatt hypertension. Ditchek _et al._ (1969) diagnosed and surgically corrected a fistula in a 50 year old female who was not hypertensive.

TABLE 1

CAUSES OF RENAL A-V FISTULAE

| Category | Number | Per Cent of 68 |
|---|---|---|
| Congenital & Idiopathic | 23 | 34 |
| Acquired | | |
| hypernephronma | 10 | 15 |
| trauma | | |
| penetrating | 9 | 13 |
| nonpenetrating | 2 | 3 |
| nephrolithotomy | 2 | 3 |
| Partial nephrectomy | 2 | 3 |
| Percutaneous biospy | 16 | 24 |
| inflammation | 3 | 4 |
| atherosclerosis | 1 | 1 |
| TOTAL | 68 | 100 |

## Experimental Renal Arteriovenous Fistula

Lasher and Glen in 1939 reported their findings from a series of 12 dogs in which they had surgically created renal a-v fistulae. The authors did not statistically analyze their data, but there was no apparent rise in blood pressure until a contralateral renal a-v fistula was created. Following this second procedure pressure rose only slightly and the dogs soon died of uremia. The authors point out that one major difference between the Goldblatt experiment and their own is that in the Goldblatt studies there is no rise in venous pressure whereas in their own, there is. They suggest that the elevated venous pressure may in part be responsible for the altered physiology but propose no mechanism.

In 1969, Secrest reported the results of his investigation of the effects of a renal a-v fistula on renal function and blood pressure. They found that after operation, there was a slight consistent widening of pulse pressure but no elevation of diastolic pressure. There was no rise in renin activity. One dog with a femoral a-v fistula was used as a control with no post-operative change in blood pressure or renin activity.

## MATERIALS AND METHODS

1. Animals.

A. Conditioning.

Fifty-five to eighty lb dogs of either sex were selected for ease of handling. They were vaccinated against canine distemper, leptospirosis and hepatitis; wormed with standard veterinary antihelminthics; examined for general good health and checked for anemia. The dogs were observed for a two week period while on standard dry dog chow and weight changes were noted. Daily the animals were brought to a quiet laboratory where they were trained to lie quietly on a table for blood pressure determination. The pressure was obtained by femoral arterial puncture with a 21 gauge needle attached to a Statham P23G pressure transducer and was recorded on a Gilson ink-writing recorder (Model CH-CPBB, Gilson Medical Electronics, Middleton, Wisconsin). Every dog had at least two pressures in the acceptable range before being operated. Dogs whose resting pressure would not fall below 160/110 mm Hg were not used in the study.

B. Pre-operative care and observations.

Blood pressure was checked on the day of surgery. A pre-anesthetic 10 ml venous blood sample was drawn for renin activity determination. The dogs were induced with sodium thyamylal (Parke Davis - Detroit, Michigan) 1:250, 1 ml/5 lbs body weight. They were intubated and maintained on methoxyflurane (Pitman Moore) gas anesthesia with an oxygen flow of 350 ml/min. Respiratory assistance was not required. They were closely shaved and the skin in the operative area was scrubbed with Septisol (Vestal Laboratories - St. Louis, Mo.) followed by alcohol rinsing. Sterile procedure was followed throughout with standard draping.

C. Operative procedure: Renal a-v fistula.

A surgical incision was made along the left subcostal area from the lateral border of the paraspinous muscles to a point halfway to the venteromedial line. A muscle-splitting grid incision was then made down to the peritoneum. The kidney was identified and freed from surrounding fascia. It was then retracted ventrally through the incision to expose the renal vessels. The kidney was wrapped in a warm sponge moistened with saline while the vessels were coated with warm parrafin oil. In some cases renal venous samples were taken for renin activity determination. The vessels were stripped of all fat and fascia for a 2 cm distance where possible. Carefully noting the time, the artery and vein were approximated and clamped proximally and distally with small serrafine clamps. A 1 cm longitudinal incision was then made in both artery and vein so the lengths coincided. A stay suture of 5-0 silk was placed midway in the superior lip of each incision to allow for retraction and visibility of the lumena. The ends of each incision were then approximated, artery to vein, and tied with 6-0 mersilene suture. While retracting on the stay sutures the bottom lips were sewn together intraluminally with a continuous stitch. Upon reaching the opposite end of the incisions, the running stitch was tied to the previously-placed approximating suture. The superior lips of the incisions were then sewn, stay sutures were removed and top-running stitch tied at the end where bottom suturing began (Figures 1 and 2).

Figure 1. Suture arrangement in the ostia which will form the fistula.

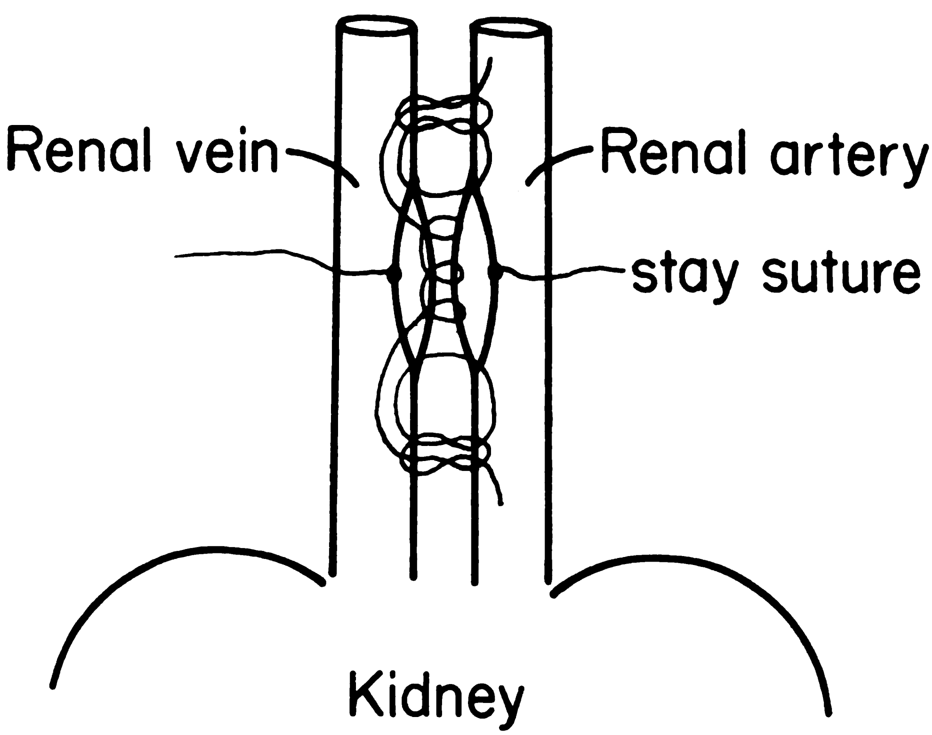
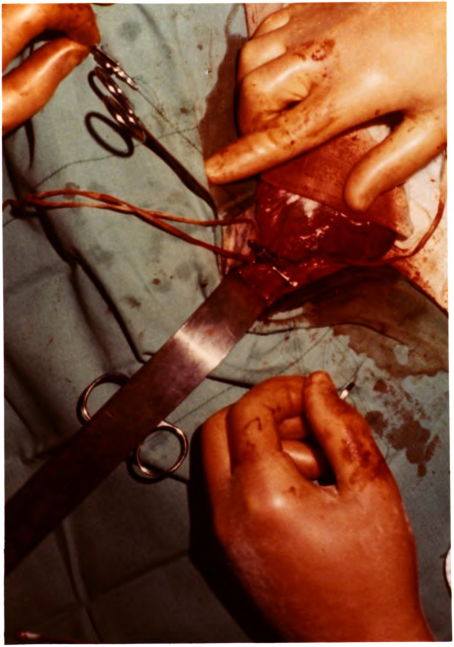

Renal vein
Renal artery
stay suture
Kidney

Figure 2. Photograph of a renal artery and vein with ostia being sewn together.



Gelfoam (Upjohn Co., Kalamazoo, Michigan) was placed beneath and atop the sutured vessels and clamps were removed. Bleeding greater than 10-20 ml rarely occurred and was controlled in all cases. A palpable thrill and audible bruit were immediately apparent and testified to the patency of the shunt. Duration of vessel occlusion for surgery ranged from 25 - 40 min. After 10 minutes the kidney was replaced in the retroperitoneal fossa and wound layers were closed with 4-0 cotton. By completion of suturing, animals were showing signs of recovery from anesthesia. Two ml of Penstrep (Merck and Co. Inc., Rahway, N.J.) were administered i.m. and the animal returned to his cage.

D. Operative procedure: Renal artery - splenic vein fistula controls.

The procedure was carried out as in C to the point of stripping the renal artery. The renal vein was left intact. The animal's spleen was delivered onto the field and major vessels were identified. Considerable time was required to free-up and strip a suitable length (usually 4-5 cm) of vein. This was then approximated along side the renal artery and anastomosis was carried out as in C. Because of greater technical difficulty, clamping times ranged from 40 - 60 minutes. The procedure was completed as in C above.

E. Operative procedure: Femoral a-v fistulae controls.

An incision was made through the femoral triangle to expose the femoral artery and vein. A 1 cm anastomosis was then carried out in the manner described in C taking care to allow sufficient distance on both the proximal and distal limbs for electromagnetic flowmeter probe (Model 300, Carolina Medical Electronics, Inc., Winston Salem, N.C.) placement. Clamping time ranged from 20-25 minutes.

Following hemostasis, flows were determined in the proximal and distal limbs of both artery and vein. (This had been attempted in some of the renal shunts, but the anatomy precluded obtaining satisfactory results). Pressures were then obtained in all four limbs with a 23 g needle attached to a Statham P23G pressure transducer and recorded on a Gilson ink-writer recorder. Where low pressures were anticipated, a Statham P23A low pressure transducer was used. The incision was closed with 4-0 cotton. From this point, procedure was as in C above.

F. Post-operative care and observation.

There was no operative morbidity or wound infection. All dogs were ambulatory and were observed to urinate on the first post-operative day. Two dogs developed nonrelated illnesses (probably viral) and were dropped from the study. Each dog's pressure was checked once or twice weekly for a total of eight times. Venous blood samples were drawn at the first sign of elevated blood pressure, and all dogs were sampled terminally whether hypertensive or not. Careful auscultation for flank bruit was done at time of pressure determination as an index of shunt patency.

G. Terminal and postmortem observations.

After 4 - 5 weeks of pressure determinations, the dogs were anesthetized and surgical wounds reopened. Kidneys and vessels were inspected. The dogs were euthanized and the kidneys and vessels retained for gross and histopathological examination.

11. Renin Activity Determination.

A. Preparation of plasma (Vallotton and Page).

Ten ml of whole venous blood were withdrawn into a plastic tube containing 0.9 ml of 3.8% citrate solution and immediately chilled to $4^{o}C$. The blood was then centrifuged at $4^{o}C$. for ten minutes at 2500 rpm. The plasma was immediately withdrawn and frozen until preparation for assay. The plasma was just thawed to a point about $4^{o}C$. and maintained in an ice bath. The pH was adjusted to 5.5 with 1 N HCl. Following this, 3 drops of a 5% solution of diisopropylflurophosphate (DFP) in isopropyl alcohol were added to eliminate the activity of angiotensinase. The samples were then incubated for exactly 3 hours at $37^{o}C$. and again placed in an ice bath to stop all reaction. Two ml of the plasma were mixed with 2 ml of a 10% suspension of Dowex 50 W-X2 resin (Dow Chemical - Midland, Michigan) in .2M ammonium acetate at pH 6.0. The resin was prepared for use by cycling first through water, then .6N HCL, another water wash followed by 4N NaOH, then rewashing and finally suspending in ammonium acetate at pH 6.0. This resin is designed to pick up small polypeptides such as angiotensin *II*. The mixture was placed in a vitamin assay tube (Column) with a filter disc punched from standard filter paper. The resin was allowed to drain, then washed with 6 ml of .2N ammonium acetate followed by 6 ml distilled water. These washings were discarded. The peptide (angiotensin *II*) was then eluted from the resin with 1 ml of diethylamine (Sigma Chemical, St. Louis, Mo.) followed by 1 ml of .2N $NH_4OH$. The eluant was then lyophylized and saved until time of assay whereupon it was reconstituted with 1 ml of 1M tris buffer pH 7.5 to which had been added 100 mg of lysozyme (Sigma Chemical, St. Louis, Mo.) for each 100 ml of buffer.

B. Bioassay - Method of J. Gunnells (1967).

Male Sprague-Dawley rats weighing 150-200 gm were bilaterally nephrectomized under ether anaesthesia 18-22 hours prior to assay. They were permitted neither food nor water during this interval. At the time of bioassay, the rats were anesthetized with sodium pentobarbital, 30 mg/kg injected intraperitoneally. Anaesthesia was maintained at a constant level by subcutaneous injection of sodium pentobarbital, 50 mg/ml through a polyethylene catheter at intervals of six minutes. The agent was delivered from a microsyringe at a constant volume of 2 ul. Ganglionic blockade was achieved by injection of 15-60 ul of pentolinium tartrate (Wyeth, Philadelphia, Pa.) (10 ug/ml). The trachea was cannulated with PE 240 tubing. A carotid artery was cannulated with PE 50 polyethylene tubing for determination of blood pressure. This was connected to a Sanborn low pressure transducer (Sanborn, Boston, Mass.) and the tracing monitored on a Sanborn recorder (Series 1700H.P., Sanborn, Boston, Mass.). The opposite jugular vein and a femoral vein were isolated. These were cannulated with PE 10 tubing, one being used for known quantity standards of angiotensin *II*, the other for the unknown plasma samples. All tubes were filled with saline containing heparin (National Biochemicals, Cleveland, Ohio) (10 units/ml). The commercial valine-5 angiotensin *II* (Ciba, Summit, N.J.) was prepared daily in a dose of 0.1 ng/ul from a stock solution containing 10 ug/ml in saline prepared weekly. This was introduced into the assay animal in increments of 2 ul up to 10 ul to establish a standard curve. The unknown plasma was injected in aliquots of 20 ul up to 100 ul depending on the degree of response. The recorder was adjusted so that 1 mm Hg rise in pressure equaled at least a 4 mm rise of the recording sylus. A rise of 6 mm to an injection of 2 ul of standard was required to accept the animal for assay. The unknown samples were bracketed with known standards in doses to cause pressure to be recorded just above and below that observed for the unknown. The animal's blood pressure was maintained at 60-80 mm Hg with repeated doses of pentolinium tartrate.

## RESULTS

Group 1: Renal Artery to Renal Vein Anastomosis.

Diastolic Blood Pressure. The presence of an arteriovenous fistula between the renal artery and vein resulted in a significant post-operative rise in diastolic blood pressure, but not into a range generally considered to be hypertensive. Ten dogs whose blood pressures were checked twice weekly for four weeks showed an average rise of 10 mm Hg. ($\bar{X}_1$ = 95.0, $\bar{X}_2$ = 105.6, S.E. = 1.45, N = 10, ($P < .001$).* Furthermore, much of the rise occurred in the first two weeks after opening the shunt with a subsequent return to near control values in most cases (Table 2). When a shunt sealed off or a thrombus occurred, such a return to normal might be predicted. The disappearance of the bruit in the flank region is the index that such an event may have occurred. This situation was noted in animal 19B where the fistula spontaneously closed. A thrombus was present in animals 22B and 30B; and, in the latter case, it interferred with the drainage of the left spermatic vein sufficiently to cause a large varicocoele. Although statistically highly significant, the average rise in blood pressure from a clinical standpoint was not impressive.

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*$\bar{X}_1$ = Mean for controls, $\bar{X}_2$ = Mean for experimentals, S.E. = Standard error of the mean difference, N = Sample size.

Renal Pathology. Prior to sacrificing the animals, cannulation of the left ureter yielded insufficient urine flow to perform renal function studies.

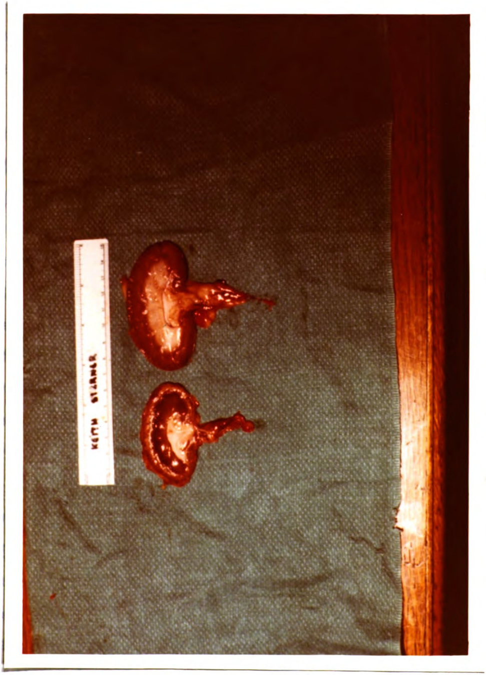
Postmortem examination revealed normal kidneys in 3 cases while the rest were shrunken or contracted to a fraction of the size of the contralateral kidney (Figure 3). Histopathologic changes (Table 3) varied from minimal to complete coagulation necrosis. Where there was sufficient intact anatomy for assessment, tabular hyaline deposits and connective tissue infiltration were common features. The complete pathology reports are in Appendix A.

TABLE 2

DIASTOLIC BLOOD PRESSURE MEASUREMENTS IN DOGS WITH RENAL A-V FISTULAE

| Dog # | Pre-Op. | 1st Post-Op. | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | Post-Op. Average |
|---|---|---|---|---|---|---|---|---|---|---|
| 19B | 105 | 130 | 120 | 155 | 105 | 110 | 115 | 115 | 120 | 121 |
| 22B | 90 | 95 | 110 | 125 | 95 | 90 | 105 | 85 | 95 | 100 |
| 25B | 105 | 130 | 125 | 135 | 130 | 115 | 110 | 105 | | 121 |
| 27B | 100 | 115 | 115 | 115 | 110 | 100 | 110 | 100 | 120 | 111 |
| 28B | 90 | 95 | 100 | 115 | 115 | 95 | 100 | 90 | 85 | 99 |
| 30B | 85 | 130 | 105 | 85 | 90 | 85 | 100 | 95 | 100 | 99 |
| 32B | 95 | 95 | 120 | 95 | 105 | 100 | 100 | 90 | 100 | 101 |
| 35B | 100 | 105 | 95 | 90 | 100 | 105 | 110 | 95 | 100 | 101 |
| 40B | 80 | 85 | 95 | 100 | 95 | 95 | 100 | 100 | 100 | 96 |
| 2C | 100 | 110 | 120 | 115 | 110 | 110 | 85 | 100 | 105 | 107 |
| Avg. | 95.0 | 109 | 111 | 113 | 106 | 101 | 104 | 98 | 103 | 105.6 |

Figure 3. Photograph comparing normal dog kidney to necrotic kidney resulting from renal a-v fistula.


KEITH STARNER

Renin Activity Levels. The control values of renin activity for all peripheral venous samples were pooled and compared with the pooled post-operative values in each of the three treatment groups, renal a-v, splenic renal a-v, femoral a-v. Over the period of sampling following surgical creation of the shunt, there was no significant rise in renin activity. ($\overline{X}_1$ = 5.70, N = 7, $\overline{X}_2$ = 5.73, N = 16, S.E. = .413, values taken from Table 4 and converted to natural logarithms). Values in nanograms percent are recorded in Table 4.

Group *II.* Renal Artery to Splenic Vein Anastomosis.

Diastolic Blood Pressure. When an a-v fistula was created between the renal artery and splenic vein, no significant rise in diastolic blood pressure could be found in the post-operative period. ($\overline{X}_1$ = 104, $\overline{X}_2$ = 107, S.E. = 3.22, N = 8). Values are presented in Table 5.

Renal and Splenic Pathology. Histopathologic features are recorded in Table 6. In several cases, urine output on the shunted side was apparent, but quantity was too small for accurate renal function studies. Generally, the kidneys, in gross examination, appeared to be healthier than in the renal a-v shunt series. The spleens all showed signs of congestion secondary to the elevation in venous pressure. Complete pathology reports are in Appendix A.

Renin Activity Levels. The statistical analysis of data on renin activity was handled as in the preceeding section. Details are in Appendix B. Again, there was no significant rise in post-operative renin activity. ($\overline{X}_1$ = 5.70, N = 7, $\overline{X}_2$ = 6.10, N = 4, S.E. = .441 converted to natural logarithms). Values in Ng percent are recorded in Table 7.

TABLE 3

SUMMARY OF RENAL PATHOLOGIC FEATURES IN DOGS WITH RENAL A-V FISTULAE

+ = present, - = absent, 0 = not reported

| Dog | Gross | Necrosis | Tubular Hyaline | Connective Tissue or Scarring | Capsular Thickening | Microvascular Proliferation |
|---|---|---|---|---|---|---|
| 19B | Normal | None | + | - | - | - |
| 22B | Contracted, indurated, thrombus in renal v. | Coagulation | + | + | + | + |
| 25B | Contracted | Coagulation | + | + | - | - |
| 26B | Contracted | Coagulation complete | 0 | + | + | 0 |
| 28B | Contracted | Coagulation complete | + | + | + | 0 |
| 30B | Contracted, thrombi in hilar vessels | Coagulation complete | 0 | 0 | 0 | 0 |
| 32B | Normal | None | + | - | - | - |
| 2C | Normal | None | + | - | - | - |

TABLE 4

PRE- AND POST-OPERATIVE RENIN ACTIVITY VALUES (ng Percent*) IN RENAL A-V FISTULAE

| Dog # | Control | Post-Operative |
|---|---|---|
| 19B | 1820 | 1800, 450, 850, 300, 250 |
| 22B | 310 | 95, 120, 190 |
| 23B | 50 | |
| 25B | 234 | 125, 350, 300 |
| 26B | 200 | 190 |
| 28B | | 1050 |
| 30B | 200 | |
| 32B | | 400, 375, 150 |
| | $\bar{X}_1 = 469$ | $\bar{X}_2 = 437$ |

*Nanograms per 100 ml plasma

TABLE 5

BLOOD PRESSURE MEASUREMENT IN DOGS WITH RENAL SPLENIC A-V FISTULAE

| Dog # | Pre-Op | 1st Post-Op | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | Avg. |
|---|---|---|---|---|---|---|---|---|---|---|
| 10B | 115 | 125 | 115 | 130 | 115 | 105 | 115 | | | 117 |
| 6B | 105 | 100 | 120 | 120 | 110 | 110 | | | | 112 |
| 13B | 110 | 115 | 120 | 120 | 100 | 110 | 95 | | | 110 |
| 12B | 100 | 120 | 95 | 105 | 100 | 120 | 100 | 125 | 100 | 108 |
| 15B | 95 | 110 | 140 | 110 | 110 | 100 | 100 | 105 | 100 | 105 |
| 16B | 110 | 130 | 115 | 85 | 95 | 115 | 100 | 100 | 100 | 109 |
| 17B | 110 | 105 | 95 | 100 | 95 | 95 | 90 | 105 | 90 | 95 |
| 18B | 85 | 95 | 100 | 100 | 95 | 100 | 105 | 105 | 100 | 100 |
| $\bar{X}_1$ | 104 | | | | | | | | $\bar{X}_2$ | 107 |

TABLE 6

RENAL AND SPLENIC PATHOLOGIC FEATURES IN DOGS
WITH RENAL SPLENIC A-V FISTULAE

| Dog | Renal Gross | Splenic Congestion | Necrosis | Tubular Hyaline | Connective Tissue | Capsular Thickening |
|---|---|---|---|---|---|---|
| 6B | Contracted | + | + | 0 | + | + |
| 10B | None | + | - | - | - | - |
| 12B | Normal | + | - | + | - | - |
| 13B | Contracted | + | moderate coagulation | + | + | 0 |
| 15B | Contracted | + | focal coagulation | + | 0 | + |
| 16B | Normal | + | minimal coagulation | 0 | + | + |
| 17B | Normal | + | - | - | - | - |
| 18B | Contracted | + | widespread coagulation | 0 | - | + |

TABLE 7

RENIN ACTIVITY AS ng PERCENT IN DOGS
WITH RENAL-SPLENIC A-V FISTULAE

| Dog # | Pre-Operative | Post-Operative |
|---|---|---|
| 13B | 805 | |
| 12B | | 500 |
| 15B | | 480 |
| 16B | | 370 |
| 18B | | 550 |
| | $\bar{X}_1 = 469$ | $\bar{X}_2 = 475$ |

Group III. Femoral Artery to Femoral Vein Anastamosis.

Diastolic Blood Pressure. Unlike the preceding two series where post-operative diastolic pressure rose, in one case significantly, the other slightly, but not significantly, in this series of four animals, three had decreased diastolic pressure. However, overall there was no significant change ($\bar{X}_1$ = 97.5, $\bar{X}_2$ = 93.5, S.E. = 2.34). Values are recorded in Table 8.

Renin Activity Levels. In four pooled post-operative venous blood samples from the same dog, the renin activity did not differ significantly from the pooled controls of all groups ($\bar{X}_1$ = 5.70, N - 7, $\bar{X}_2$ = 5.85, N = 4, S.E. = .61). Values are in Table 9.

TABLE 8

DIASTOLIC BLOOD PRESSURE MEASUREMENTS IN DOGS WITH FEMORAL A-V FISTULAE

| Dog # | Pre-Op | 1st Post-Op | 2nd | 3rd | 4th | 5th | 6th | 7th | 8th | Avg. |
|---|---|---|---|---|---|---|---|---|---|---|
| 34B | 95 | 110 | 100 | 95 | 95 | 100 | 95 | 95 | 100 | 98 |
| 36B | 95 | 85 | 90 | 80 | 75 | 80 | 85 | 90 | 90 | 84 |
| 37B | 100 | 100 | 90 | 100 | 100 | 90 | 110 | 100 | 95 | 98 |
| 38B | 100 | 90 | 90 | 85 | 100 | 85 | 105 | 95 | | 93 |
| $\bar{X}_1$ | 97.5 | | | | | | | | $\bar{X}_2$ | 93.5 |

## Flow and Pressure Changes at the Site of the Femoral A-V Fistula

Upon opening the a-v fistula, both blood pressures and blood flows were measured in all four segments influenced by the shunt (Fig. 4). Since the caliber of the renal vessels is close to that of the femoral vessels, similar values should be obtained for renal vessels (See Discussion). The values for blood pressure are presented in Table 10 and those for flow in Table 11.

TABLE 9

RENIN ACTIVITY LEVELS IN ONE DOG WITH A FEMORAL A-V FISTULA EXPRESSED ng PERCENT

| Dog # | Pre-Operative | Post-Operative |
|---|---|---|
| 36B | - <br> $\bar{X}_1 = 469$ | 625, 1000, 140, 170 <br> $\bar{X}_2 = 484$ |

TABLE 10

BLOOD PRESSURE VALUES FOLLOWING THE OPENING OF A FEMORAL A-V FISTULA IN mm Hg

| Dog # | Systemic Blood Pressure | Prox Art. BP | Distal Art. BP | Prox. Vein BP | Distal Vein BP |
|---|---|---|---|---|---|
| 34B | 185/125 | 145/95 | 95/60 | 3-4 | 60/30 |
| 36B | 105/80 | 110/75 | 75/60 | 4-5 | 30 |
| 37B | 140/110 | 135/95 | 80/60 | 4 | 60/40 |
| 38B | 180/115 | 140/100 | 80/65 | 3-4 | 65 |

Figure 4. A typical a-v anastomosis showing direction of blood flow into three segments.

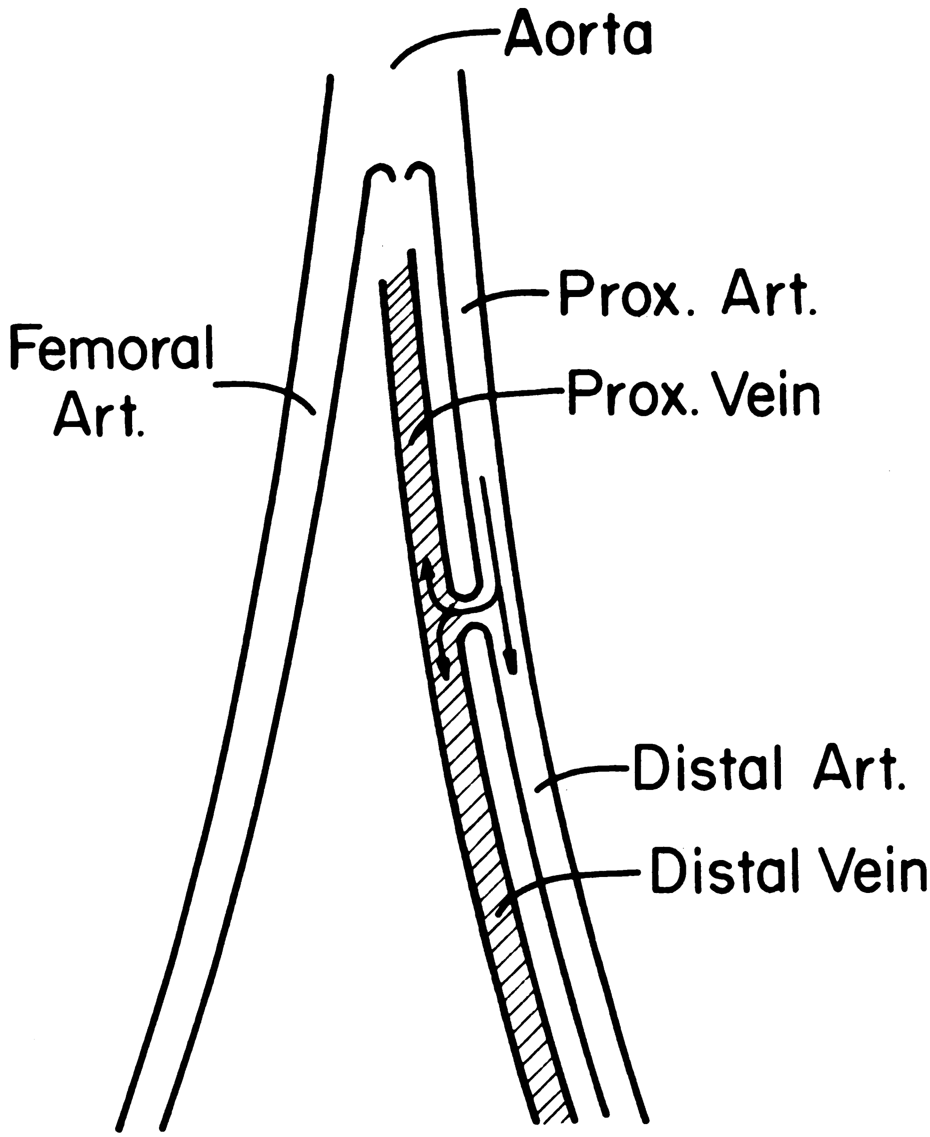

Aorta
Prox. Art.
Femoral Art.
Prox. Vein
Distal Art.
Distal Vein

TABLE 11

BLOOD FLOW VALUES FOLLOWING THE OPENING OF A FEMORAL A-V FISTULA IN ml/min

| Dog # | Pre Shunt Arterial | Post Shunt Prox Art. | Distal Art. | Prox. Vein | Distal Vein |
|---|---|---|---|---|---|
| 34B | 130 | 840 | 25-30 | 820 | 150 (R) |
| 36B | 80 | 980 | 40 | 940 | 50 |
| 37B | 50 | 1000 | 70 | 900 | 50 |
| 38B | 90 | 900 | 35 | 850 | 40 |

(R) indicates retrograde flow

## DISCUSSION

Renovascular anomalies or pathology have for some time been known to be associated with hypertension in humans. Although the number of reported cases is small, it is scarcely surprising that renal hypertension results from about half the cases of renal a-v fistula. The exact mechanism of renovascular hypertension has not been absolutely established, nor is it certain that the diastolic hypertension associated with renal a-v fistula results in part or entirely from identical circumstances found in cases of renovascular hypertension. Reduced renal perfusion pressure and/or flow as an initiating factor seems to be a common feature. The role of the renin-angiotensin-aldosterone (RAA) system in response to decreased pressure and flow, as the direct cause of the blood pressure elevation, has been the source of much research and speculation. It has been suggested (Davis and Urquhart, 1964) that the RAA system is more active in any large a-v fistula, but, where renal vessels are not involved as in femoral a-v fistulae, clearly hypertension is not a feature. Renal a-v fistulae, then, become a special situation where, when they are large enough, one might expect an increase in RAA activity, systemic findings common to large a-v fistulae and, perhaps paradoxically, diastolic hypertension.

Many of the hypothetically predictable findings in renal a-v fistulae could not be demonstrated in this study. In the case of renal a-v fistulae, there was a clear cut, significant, but small, rise in diastolic pressure. Diastolic pressures, rather than systolic or mean pressures, are studied here because the hypertension associated with renal a-v fistulae in humans is diastolic in nature. Also, the fistula may cause a rise in systolic pressure as a reflection of increased cardiac output and not represent clinical systolic hypertension. The pressure rise was highest in the first few days after the shunt was open, but consistently dropped toward control values by the end of the test period. In a few cases where the shunt lost patency, an explanation for the pressure drop was obvious so long as it was certain that flow through the renal vessels was not interrupted. We were certain of this in only one dog. Notwithstanding a statistical rise in diastolic pressure in this group of ten dogs, clinical hypertension never resulted. For purposes of discussion, a steady increase of 25-30 mm Hg diastolic pressure should be in evidence to assume clinical hypertension. No animal's pressure rose to this extent. Still, it remains to be explained how a renal a-v shunt causes the mild, presumably factitious, rise in diastolic pressure which was noted. A drop in renal perfusion pressure and flow will also occur when blood is shunted from the renal artery to the splenic vein, yet no rise in systemic pressure of any magnitude was observed in this situation. The marked congestion with secondary anatomical changes observed in every spleen where a renal-splenic a-v shunt was present makes it quite apparent that an elevation in distal segment venous pressure can be very punishing to the organ. Along this line it was also noted that when the renal venous pressure was elevated as in the renal a-v fistula, damage to the kidney was generally much more pronounced than when arterial blood was merely shunted away from the kidney. Whether or not this phenomenon of venous pressure elevation can explain the different blood pressure response is a point of mystery and speculation.

As has been pointed out elsewhere in this dissertation, renin may somehow be involved in renal hypertension. From this study, it appears there is no significant rise in circulating levels of this enzyme where either the renal a-v or renal-splenic a-v fistulae were present. It's not possible, therefore, to postulate elevated plasma renin either secondary to reduced renal perfusion pressure, reduced flow or elevated venous pressure as the cause of the rise in diastolic pressure observed in renal a-v shunts. The possibility exists, certainly, that the observed pressure rise is a spurious one, i.e., not actually related to the fistula. However, it would be very difficult to explain on what other basis it occurred since the rise did not occur in the other two series of dogs who were handled in almost identical fastion. The operation *per se* was the only difference.

There is no reason to believe that a decrease in perfusion pressure to the kidney was more pronounced in the renal a-v shunt as compared to the renal-splenic a-v shunt. The nature of the anatomical region around the kidney made it difficult to control bleeding, while studying pressure and flow relations about the site of the fistula. Consequently, in order to gain an indirect idea of flow changes, studies were done in animals with a femoral a-v fistula. Pre-operative pressures in the femoral and renal vessels are nearly identical, but flow is much higher to the kidney. This is to be expected since the resistance in this organ is much lower than in the hindlimb. The critical factor is the caliber of the vessels which again is nearly identical. The caliber determines the amount of blood flow through fistulae of the same size and resistance. These studies on femoral a-v fistulae leave no doubt that perfusion pressure and flow to the distal arterial limb, and ultimately the organ, are very significantly reduced. In the kidney, the reduction is sufficient to cause ischemic changes. One would also predict from reading Vander's (1967) review that a rise in renin output would occur. The fact that renin activity levels did not increase was therefore unexpected. Some explanations for the failure to show an increase can be hypothesized. The extensive necrosis of the kidney noted on histopathologic examination raises the question whether there remained enough viable tissue to react to the stimuli for increased production of renin. Also, if renin secretion is an active process, the degree of ischemia may not have permitted delivery of sufficient oxygen to increase renin output. The latter point is more attractive in the cases where only minimal ischemic changes were noted, (Braverman 1970).

Another consideration has to do with disruption of the lymphatic vessels leading from the kidney hilus. The surgery required to construct the shunt almost certainly produced such disruption. The lymphatics are important conveyors of renin from the kidney to the peripheral blood (Baillie 1970). In the event the lymphatics are draining into the abdominal cavity, peripheral values for renin may be falsely low. This becomes especially important in the presence of elevated venous pressure which augments lymphatic drainage secondary to increased tissue pressure.

Finally, errors in methodology and statistical analysis are not completely ruled out. Removal from the study of one animal with unusually high values, both control and experimental, might alter the conclusions concerning the statistical hypothesis. However, such a practice, if possible, should be avoided. Removal of the high values alone did not alter the significance of the conclusion in the renal a-v series.

In the absence of an elevated renin, it becomes very difficult to suggest a mechanism for the small rise in diastolic pressure found in the dogs with renal a-v fistulae. From this study, it is strongly suggested that the rise in renal venous pressure is at least implicated, even if not the major responsible factor. No explanation of the mechanism involved in elevated renal venous pressure without renin changes is suggested.

Actually, one must wonder if this canine model as described is acting similarly to the hypertension-producing renal a-v fistulae in humans. The scarcity of such cases precludes any early accumulation of data concerning renin activity in these patients. Even if renin activity is ultimately found to be normal, the uncertain duration of the shunt prior to diagnosis in most cases would cloud the issue of possible elevations of renin activity at onset of hypertension. The whole question of using canine models to study human hypertension is raised. Quite clearly, here is one example that proved unrewarding in gaining information about its human counterpart.

Finally, the hemodynamic findings about the femoral a-v fistula are of interest. Again, there was no rise in renin activity, (At least in the one animal where it was measured). A small peripheral shunt such as this did not drop diastolic pressure significantly. Therefore an elevated renin activity would not be expected. The pressure flow changes are not surprising. The fact that there is no elevation of venous pressure in the proximal venous segment after shunting the large quantities of blood is testimony to the capacity of the venous system. Holman (1956) using india ink, observed retrograde flow in the distal venous segment. With a flowmeter, we observed the same phenomenon in one dog. How this occurs is difficult to explain in light of the backward resistance of the organ. Eddy currents interfering with the activity of the flow probe is a possibility. The high pressures in the distal venous segment were predictable. The low arterial-venous pressure differential must certainly alter microcirculatory phenomena and it would seem that survival of the organ would depend on rapid development of collateral circulation as observed by early workers in this field.

## SUMMARY AND CONCLUSIONS

Ten dogs with surgically created renal arteriovenous fistulae were followed for a four week post-operative period to determine if hypertension would develop or renin activity levels would rise. While there was no clear cut hypertension, average diastolic blood pressure increased 10 mm Hg. There was no significant rise in renin activity. Eight dogs were similarly prepared, only the shunt went from renal artery to splenic vein, thus obviating any rise in renal venous pressure. This series of dogs had no significant rise in blood pressure or renin activity suggesting that the elevation of renal venous pressure in the former series may in some way explain the small rise in blood pressure. Renin activity does not seem to be a factor. Four dogs with femoral a-v fistulae were studied as controls. Renin activity did not change in the one animal where measured. Blood pressure and blood flow values were determined in the four arterial and venous segments about the fistula. These data indicated that perfusion pressure and flow in the distal arterial segment were both reduced and pressure in the distal vein segment was elevated. Such a set of circumstances will cause ischemia in the organ distal to the shunt and in the case of the kidney causes sufficient ischemia to produce necrosis and loss of function. In what manner, if any, this may influence a rise in blood pressure is not clear.

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# APPENDICES

# APPENDIX A

# PATHOLOGICAL CHANGES

## CASE NUMBER 6B

### HISTOPATHOLOGY REPORT:

The spleen was highly congested and there was some increase of reticulum cells in the red pulp. The most prominent change in the kidney was atrophy of the tubules. There was some increase in connective tissue, particularly in the madullary region. Due to the over-all tubular atrophy, the glomeruli appeared especially numerous and prominent. These glomeruli were, in many instances, hypercellular and the Bowman's capsule was somewhat thickened. A few tubules were dilated and there was some evidence of tubular regeneration. There appeared to be an increase in collateral circulation, and the kidney capsule was somewhat thickened. In the sections examined I could not find evidence of thrombosis, but the larger vessel did appear to have thicker walls than would be anticipated.

### CONCLUSIONS:

Renal ischemia resulting from experimental surgery. Splenic congestion.

## CASE NUMBER 10B

HISTOPATHOLOGY REPORT:

Evidently the surgical procedure had not appreciably interfered with renal circulation because the kidney was essentially normal in appearance. There also was not the marked increase in collateral circulation in the region of the hilus of the kidney that was observed in the previous specimens. The splenic changes however were severe. In the indurated areas not only was there marked congestion but there was fibroblastic and endothelial proliferation. In these areas there were regions in which the blood had undergone hemolysis. In other splenic sections the most noticeable feature was congestion with a replacement of the lymphocytic tissue.

CONCLUSIONS:

Marked splenic congestion with some granulation tissue. Kidney essentially normal.

## CASE NUMBER 12B

### HISTOPATHOLOGY REPORT:

A major portion of the renal tissue was relatively normal. As contrasted with previous sections there were no areas of coagulation necrosis. In some areas there was an atrophy of the tubules and evidence of regeneration. Some tubules were dilated and the epithelium was flattened. There were hysline casts in many of the tubules. The capsule was relatively normal. The major microscopic changes were in the splenic tissue. The capsule was thickened considerably and there were many new capillaries and some fibroblasts in the capsular region. The splenic nodules were still present but appeared to be less active and smaller than normal. The major portion of the spleen was composed of red pulp and in this area there was an increase in reticuloendothelial cells and some erythrophagocytosis. The degree of congestion was considerably less than the other sections from previous dogs and was in marked contrast to case number 083079 which will be reported later. The only area in which congestion was prominent was just under and in the thickened capsule.

### CONCLUSIONS:

Mild renal degenerative changes, thickening and congestion of the splenic capsule and reticuloendothelial cell proliferation in the splenic red pulp.

## CASE NUMBER 13B

### HISTOPATHOLOGY REPORT:

The renal tissue from this dog presented a varied appearance. There were areas of coagulation necrosis particularly in the medulla but also in some cortical regions. In the cortex in the areas in which coagulation necrosis had taken place there was considerable increase in connective tissue. Away from the necrotic areas two changes were particularly evident. In certain locations there was a marked atrophy and disappearance of the tubules. There did appear to be some evidence of regeneration. In other areas the most prominent change was a dilatation of the tubules with a flattening of the epithelium. There were many hyalin casts. It must be pointed out that some areas of the kidney were relatively normal and overall the glomeruli were relatively normal. When the splenic tissue was cut for sectioning it was noticed that there was a layer of whitish tissue adhered to the spleen. On section this tissue was composed of connective tissue and fat. Much of the fat had undergone necrosis. In the region where the capsule would normally be there was marked fibroblastic proliferation. Under the capsule there was severe congestion. There were some intact splenic nodules but few normal appearing lymphocytes, especially around the periphery of the splenic nodules. Most of the cells appeared to be reticular cells. There was of course considerable blood pigments and erythrophagocytosis. There was also considerable erythropoiesis. The major portion of the tissue, however, was blood. Examination of the bladder epithelium disclosed a few eosinophilic bodies which could be distemper inclusion bodies. However, these were not numerous nor were they sufficiently characteristic to warrant an unequivocal diagnosis of canine distemper. I have submitted the bladder and renal tissue for special stains for inclusion bodies. If these special stains confirm the presence of inclusion bodies, I will report in a follow-up letter. In the future if canine distemper is suspected, it would be advisable not only to submit the bladder but also to submit the lung, traches and brain.

### CONCLUSIONS:

Renal necrosis and degeneration due to ischemia, and splenic congestion.

## CASE NUMBER 15B

### HISTOPATHOLOGY REPORT:

There was a moderate thickening of the capsule though it was to a lesser degree than in some of the previous sections. There were focal areas of coagulation necrosis which were especially severe in the subcapsular region of the cortex. Necrosis was complete, involving the glomeruli as well as the rubules. There were a few foci of calcium deposits in these areas. Areas of the kidney which were not necrotic also had considerable changes. The major change consisted in a constriction and disappearance of tubules with some evidence of regeneration. There were also many dilated tubules. Casts were numerous. The glomeruli appeared enlarged. There was a thickening of Bowman's capsule but an increase in Bowman's space. In the medullary region some of the tubules were dilated and others constricted. There was also some connective tissue hyalinization in the medulla. In the spleen there was a localized thickening of the capsule. This thickening overlayed the normal capsule and consisted mainly of fibrocytes and fibroblasts and endothelial proliferation. Could these areas have been traumatized during surgery? There was congestion, not extensive, but especially noticeable in the subcapsular region. The germinal centers were hyperplastic and there was also reticuloendothelial hyperplasia in the red pulp.

### CONCLUSIONS:

Focal coagulation necrosis in the kidney and moderate congestion and hyperplasia of the spleen.

## CASE NUMBER 16B

### HISTOPATHOLOGY REPORT:

The "normal kidney" had several small cysts in the cortical region and one was seen in the medulla. Many tubules were dilated. There was no evidence of necrosis or inflammation. The left kidney presented a highly variable appearance. The areas of necrosis and degeneration involved not more than 20% of the renal tissue. Many of these areas were focalized. In one section there was a well demarcated area in the cortex which had undergone coagulation necrosis of many of the tubules, some of which were attempting regeneration. Most of the glomeruli in these regions were still viable. The capsule over the necrotic area was markedly thickened and necrosis of the connective tissue was evident. In one small area there was a focus of granulomatous inflammation with many giant cells. There was one area in the medulla that had undergone coagulation necrosis and this area was also well demarcated. In the spleen there was congestion in the capsule but this was not as severe as in previous spleens submitted. The major change in the splenic architecture appeared to be an increase in the amount of red pulp. Much of this increase was due to proliferation of reticuloendotheilai cells in addition to the mild congestion. There was some phagocytosis of pigments. There was also some increase in connective tissue.

### CONCLUSIONS:

Focal areas of necrosis and degeneration in the left kidney. Cysts in the right kidney. Splenic congestion and RE cell proliferation.

## CASE NUMBER 17B

### HISTOPATHOLOGY REPORT:

The surgical procedure had evidently not appreciably interfered with renal circulation because the kidney was essentially normal in appearance. The only change in the kidneys was some postmortem change. The splenic changes consisted of considerable congestion particularly under the capsule. There was an increase in hemosiderin and also some erythrophagocytosis. There was also some erythropoiesis. In some areas the capsule was markedly thickened and the connective tissue which had proliferated in this area was undergoing some hyaline degeneration.

### CONCLUSIONS:

Splenic congestion. Kidney essentially normal.

## CASE NUMBER 18B

### HISTOPATHOLOGY REPORT:

There was a widespread coagulation necrosis in the renal tissue but the pattern was different than in dog 15B. In 18B there also was some coagulation necrosis in the cortical region but it was not involving the glomeruli to the extent that it did in 15B. The particularly prominent feature was the nearly complete coagulation necrosis in the medullary region. In the cortex, tubules were constricted for the most part with only a few being dilated. Most of the glomeruli were viable. There was thickening of Bowman's capsule and congestion of the glomerular tuft. The capsule was thickened and there did appear to be an increase in vasculature in this region. In the spleen there was also an area of granulation tissue overlying the capsule in some areas. Congestion was somewhat more severe than in 15B. Hyperplasia of germinal centers was not evident but there was more congestion in the red pulp away from the subcapsular regions as compared to 15B.

### CONCLUSIONS:

Coagulation necrosis of the kidney due to renal ischemia.
Splenic congestion.

## CASE NUMBER 19B

HISTOPATHOLOGY REPORT:

Compared to other kidneys submitted the renal tissue is relatively normal. There were no areas of coagulation necrosis. The most noticeable pathologic feature is an increase in hyaline casts. Examination of the region where the surgery was performed revealed the presence of a mild inflammatory reaction where the suture material had been used. In the section examined there was a marked build up between the inner elastic membrane of an artery and the intimai endothelium. Most of the cells appeared to be fibroblasts. There was also considerable degeneration in the wall of the artery. In the adventitia there was an increased number of capillaries which were congested. The tissue seen, especially in the intimal region of the artery, could have contributed to the sealing off of the shunt. Evidently, the blood supply of the kidney had not been appreciably interfered with because of the viability and relatively normal appearance.

CONCLUSIONS:

Relatively normal kidney. Vasculitis and medial degeneration in region of surgical procedure.

## CASE NUMBER 22B

HISTOPATHOLOGY REPORT:

There was extensive and nearly complete coagulation necrosis in the medullary region of this kidney and the cortex was nearly as seriously affected. The only viable tissue was a few glomeruli. A few remnants of tubules and some regeneration of tubules had occurred at the outer most area of the cortex. In this area also there were some tubules that were filled with hyalin-like material. Connective tissue appeared to be more abundant in the cortical region than in some of the previous sections. The capsule was thickened and in the outer most portion of the capsule there was considerable capillary and small vessel proliferation. There did appear to be a well organized thrombus in tissue which most likely was the renal vein. There was some suture material in the area but very little inflammatory reaction. The thrombus had nearly completely obliterated the lumen of the vessel. There was only limited recanalization.

CONCLUSIONS:

Coagulation necrosis of the kidney due to renal ischemis.
Thrombus of the renal vein.

## CASE NUMBER 25B

### HISTOPATHOLOGY REPORT:

There was no normal-appearing renal tissue. The changes ranged from complete coagulation necrosis, which was especially prominent in the medullary region, to areas in certain areas of the cortex in which the glomeruli were viable and the tubules in the area distended and filled with a pinkish hyaline material. The tubules in other areas were noticeably constricted and there was some evidence of regeneration. The interstitial areas contained more connective tissue than some of the other sections have shown. In certain areas the interstitial tissue appeared edematous and many vacuoles were seen. The vessels near the site of the operation had undergone some changes. There was a mild granulomatous inflammatory reaction near the suture material and also areas of necrosis. In the adventitia of the vessels there was also some inflammatory reaction with lymhocytes being the most prominent cell. There appeared to be an increase in the number of blood vessels surrounding the large vessel which has been operated on.

### CONCLUSIONS:

Coagulation necrosis of the kidney due to renal ischemia. Arteritis. Necrosis near the suture material. Increase in collateral circulation.

## CASE NUMBER 26B

### HISTOPATHOLOGY REPORT:

There was very little viable tissue that was remaining in this kidney. A few glomeruli were still viable and some of the tubules had begun to undergo regeneration. In many areas, however, the glomeruli, as well as the tubules, were necrotic. Considerable connective tissue replacement had taken place. As was seen in some of the earlier cases, there was a marked increase in collateral circulation particularly in the capsule and also in the region near the hilus of the kidney.

### CONCLUSIONS:

Coagulation necrosis due to renal ischemia.

## CASE NUMBER 28B

### HISTOPATHOLOGY REPORT:

Necrosis is probably more complete in this kidney than in any other of the series submitted. The medullary region was completely necrotic and in the cortex there were only a few viable glomeruli and no normal-appearing tubules. The tubules that were present were either filled with hyalin casts with the epithelium flattened or they were markedly constricted. There was only a minimal amount of regeneration. There was some connective tissue proliferation in the interstitial region in the cortex but very little, if any, in the medullary region. The capsule was considerably thickened but the vascularity had not been increased. In the perirenal areas there was an inflammatory reaction with lymphocytes and plasma cells being the most prominent cell type. The right "normal" kidney had a cortex which appeared unaffected but there was considerable dilation of tubules beginning at the corticomedullary junction and extending down into the medulla.

### CONCLUSIONS:

Coagulation necrosis of the kidney due to renal ischemia.

## CASE NUMBER 30B

### HISTOPATHOLOGY REPORT:

The renal parenchymal tissue had nearly all undergone coagulation necrosis. There were only a few viable glomerular cells and virtually no tubular cells that were still viable. The main difference between this case and the other ones is that there was considerable evidence of thrombosis in the vessels in the hilus of the kidney. The thrombi were well organized but there was only limited recanalization. Sections taken through the vasculature near the site of the operation revealed well organized thrombi in the large vessels. Sections through the spermatic cord revealed marked congestion and edema in addition to the dilatation of the veins. Also there was a marked dilatation of the lymphatic vessels. The major change in the testis was severe congestion and hemorrhage which had crowded out many of the seminiferous tubules and caused considerable necrosis. The seminiferous tubules away from the areas of hemorrhage had undergone considerable vacuolar degeneration.

### CONCLUSIONS:

Coagulation necrosis of the kidney due to renal ischemia. Marked thrombosis of the renal vessels. Congestion and hemorrhage in the testis and spermatic cord.

## CASE NUMBER 32B

### HISTOPATHOLOGY REPORT:

The renal parenchymal tissue, as compared to most of the others in this series, was in fairly good condition. The most noticeable change involved the tubules. There was marked dilatation and the epithelial cells were flattened. There also appeared to be some increase in the space between the glomerulus and the parietal portion of Bowman's capsule. There were also a few hyaline casts and focalized areas where tubules had probably undergone severe degeneration and were regenerating. In these areas the tubules were much smaller than normal.

### CONCLUSIONS:

Tubular dilatation and epithelial degenerative changes. Overall the kidney is in much better shape than most of the kidneys submitted from this series.

## CASE NUMBER 2C

### HISTOPATHOLOGY REPORT:

Compared to the kidney from dog 1C, the changes in this dog were relatively mild. However, many of the tubules were dilated and in certain areas hyalin casts were more prominent. There were also some areas of focal interstitial nephritis with lymphocytes and some connective tissue being present. Some tubules had also undergone atrophy. Coagulative necrosis was not a feature in this kidney. Sections taken from the area in which the operation was performed revealed only minor changes. In one area there was some connective tissue in the region of the intima of an artery. This was localized and apparently the vessel was patent.

### CONCLUSIONS:

Tubuler dilatation, hyalin casts and some tubular atrophy. Focal areas of interstitial nephritis. Vessels apparently patent.

# APPENDIX B

# STATISTICAL METHODS

Since each dog could be studied prior to surgery, ideally he should serve as his own control. In such a case, a paired student T test is best used for analysis of data. This was done in analysing the blood pressure data. The animal's immediate unanesthetized pre-operative diastolic blood pressure was used as one paired value while the average of all that animals post-operative diastolic blood pressure values was used for the other paired value--the difference being $d_j$. $\bar{d}$ represents the mean difference, and also equals $\bar{X}_1 - \bar{X}_2$. $\bar{X}_1$ = mean for controls and $\bar{X}_2$ = mean for experimentals. The test statistic (t) $= \frac{\bar{d}}{S_{\bar{d}}}$. $S_{\bar{d}}$ equals the standard error of the mean paired difference and is calculated from

$$S_{\bar{d}} = \sqrt{\frac{SS_d}{n - 1}/n}$$

where $SS_d = \sum_{j=1}^{n} d_j^2 - \frac{(\sum^{n} d_j)^2}{n}$

The hypothesis being tested is:

$$H_0: \mu_d = 0, \quad H_A: \mu_d \neq 0$$

The critical value t ($\alpha$, n-1 pairs) is found in a standard "t table" in any text book of statistics or scientific tables. The probability of type 1 error ($\alpha$) for this study was a minimum of .01 ($P < .01$).

In the case of analysis of the renin activity data, missing samples precluded use of a paired t test. Consequently a pooled t test was used. Control values for peripheral venous renin activity from all animals were compared to the post-operative pooled peripheral venous renin activity values for each treatment group (renal A-V fistula, renal-splenic A-V fistula, and femoral A-V fistula). Statistically equal variances are a requisite for use of this test. The hypothesis that $S_1^2 = S_2^2$ was tested with an F test with the following test statistic:

$$F = \frac{S^2\ (\text{large})}{S^2\ (\text{small})} = \frac{SS\ (\text{large})/N-1}{SS\ (\text{small})/N-1}$$

The critical value is F (.05, N-1, N-1) where $\alpha = .1$. However the critical value for a two sided F test is calculated for $\alpha/2$.

If the hypothesis for equal variance is not rejected, then one proceeds with the pooled t test where the test statistic (t) = $\frac{\bar{X}_1 - \bar{X}_2}{S_{\bar{X}_1 - \bar{X}_2}}$ WHERE

$$S_{\bar{X}_1 - \bar{X}_2} = S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}$$

and S is $\sqrt{S^2}$ which is calculated from:

$$S^2 = \frac{SS_{X_1} + SS_{X_2}}{N_1 + N_2 - 2}$$

The critical value t $(\alpha, n_1 + N_2 - 2)$ is again taken from a standard t table.

In the event the hypothesis $S_1^2 = S_2^2$ is rejected (as was the case in the renal-splenic A-V fistulae) an approximate t test is used where the test statistic (t') =

$$\frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}}$$

It should be pointed out that it is also necessary to calculate an approximate value for degrees of freedom, but this was not necessary in this series since there was no significance at any $\alpha$ for any degree of freedom in the sample range.

Because of the presence of an "outlier" in both the control and renal A-V fistula populations, the variance is predictably large. In an attempt to reduce the variance, all values were converted to natural logarithme with a resulting standard error that was only 7% of the average of the two means whereas before the transformation it was close to 50%. Although the values in question clearly belonged in another population, they were kept in the study. Their presence (nor their absence) did not interfere with the significance of the hypothesis test, and with the transformation to natural logarithms the variances were acceptable.

# APPENDIX C

# PRELIMINARY STUDY ON THE VALIDITY OF THE BIOASSAY

Prior to the use on this project of the rat pressor bioassay technique described in the Method's section, samples from another series of experiments where it was expected that values would rise were tested. The consistent rise in experimental values over controls suggests the bioassay is a valid method. Values are presented in Table 1.

TABLE 1

RENIN ACTIVITY LEVELS IN ng %

| Control | | Experimental | |
|---|---|---|---|
| 1-1 | 700 | 1-2 | 2500 |
| 2-1 | 1500 | 2-2 | 2500 |
| 3-1 | 2750 | 3-2 | over 5000 |
| 4-1 | 2000 | 4-2 | 2750 |
| 6-1 | 2000 | 6-2 | 4000 |
| 7-1 | 2000 | 7-2 | 2750 |
| 8-1 | 2500 | 8-2 | 2000 |
| 10-1 | 3000 | 10-2 | 4500 |
| Avg. | 2375 | Avg. | 3250 + |