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IMMATURITY OF RENAL FUNCTION IN NEWBORN PIGS: FACTORS AFFECTING RENAL HEMODYNAMICS, SODIUM EXCRETION AND PLASMA RENIN ACTIVITY

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# IMMATURITY OF RENAL FUNCTION IN NEWBORN PIGS: FACTORS AFFECTING RENAL HEMODYNAMICS, SODIUM EXCRETION AND PLASMA RENIN ACTIVITY

By

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ABSTRACT

IMMATURITY OF RENAL FUNCTION IN NEWBORN PIGS: FACTORS AFFECTING RENAL HEMODYNAMICS, SODIUM EXCRETION AND PLASMA RENIN ACTIVITY

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Renal blood flow of neonatal animals is low compared to adults. Also, following infusion of a saline load, the newborn animal does not increase renal sodium excretion as rapidly as older animals. Young piglets have a low total renal blood flow (RBF) and low outer to inner cortical blood flow ratio (O/I ratio). This immaturity in renal hemodynamics is due in part to a high renal vascular resistance. As the kidney matures, RBF and the O/I ratio increase concomitantly with a decrease in renal vascular resistance. In the present experiments, factors which may contribute to the postnatal changes in renal blood flow and renal sodium excretion were investigated in piglets between 1 and 50 days of age.

The high renal resistance during the perinatal period may result from undifferentiated renal vascular spaces in the outer cortex. Kidneys of anesthetized developing piglets were perfused with silastic rubber at low pressure (25-50 mmHg) and physiologic pressure (2 day = 75 mmHg; 50 day = 125 mmHg). Following low pressure perfusion, the filling of the renal vasculature in the outer cortex of young piglets

was less than that in older animals. This reduced outer cortical filling appeared to be located within the postglomerular vessels. After perfusion at physiologic pressure, kidneys of both 2 and 50 day old pigs were filled equally suggesting that the high neonatal renal vascular resistance was not due to the absence of morphological vascular differentiation.

The plasma renin activity (PRA) and angiotensin II (AII) concentrations are elevated in newborn animals. PRA of young piglets also was found to be greater than older animals. The present data indicate that this high PRA did not result from an insensitivity to negative feedback by AII or from the stimulation of renin secretion by prostaglandins. The circulating half-life of renin in 1-5 day old pigs was not different from 45-50 day animals suggesting that the high PRA does not result entirely from immature renin catabolism. Therefore, it was suggested that neonatal hyperreninemia might result from a low plasma volume at birth.

The effect of AII and prostaglandins on renal function was investigated in conscious piglets during infusion of the competitive angiotensin II antagonist, $Sar^1$-$Ala^8$ angiotensin II (saralasin) and the prostaglandin synthetase inhibitor, indomethacin. Following drug infusion the intravascular volume was expanded with isotonic saline (2% of the body weight). Saralasin did not affect RBF or O/I ratio suggesting that AII does not increase vascular resistance in the outer cortex of young pigs. Saralasin infusion, however, reduced the natriuresis after volume expansion of young pigs but did not affect this natriuretic response in older animals. Thus, in 1-5 day old pigs circulating AII may be a factor in the control of sodium excretion. Indomethacin did not affect neonatal

renal hemodynamics but decreased RBF and increased O/I ratio in conscious 45-50 day old animals. Also, inhibition of prostaglandin synthetase did not affect sodium excretion after volume expansion in pigs between 1 and 50 days of age. Therefore, prostaglandins do not appear to contribute to basal RBF or alter renal sodium excretion in neonates.

Neonatal sodium excretion was investigated by volume expansion (8% of the body weight) of conscious pigs with saline (VE) and saline plus bovine serum albumin (BSA). VE did not alter RBF in piglets between 1 and 50 days of age. Sodium excretion, however, increased to a similar degree in all animals suggesting that immature neonatal renal hemodynamics does not prevent the immediate natriuretic response to VE. Additionally, the natriuresis following VE was not due to changes in the intrarenal distribution of blood flow. BSA infusion decreased hematocrit but prevented the natriuresis in both 1-5 and 45-50 day animals suggesting that passive renal tubular handling of sodium is similar between newborn and more mature pigs.

Dedicated To

B.J., Debbie and C.D.

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# INTRODUCTION

This thesis describes some of the factors which may contribute to the immaturity of renal blood flow and renal sodium excretion in young pigs. To adequately examine the physiological control of the neonatal kidney, adult renal function first will be reviewed. The intention here is not to provide a complete review of renal functional development but rather describe the specific aspects of kidney function in adult animals which in the present investigation were examined in the developing pig. First, renal blood flow and the extrinsic factors which affect renal blood flow in adult animals will be described. The effects of intrarenal blood flow distribution, plasma oncotic pressure and hormonal factors on renal sodium excretion in adult kidneys also will be examined. The discussion will then focus on a review of renal blood flow and the renin-angiotensin system in young animals. Finally, renal sodium excretion after saline infusion into neonates will be discussed. It is important that the reader continually compare and contrast the neonatal renal function with the same renal function in older animals.

## 1. Renal Blood Flow in the Adult Kidney

### a. Renal Vascular Patterns in Adult Kidneys

The renal artery originates as a branch of the descending aorta and divides near the hilum of the kidney into a dorsal and ventral branch. Each branch divides again into several interlobar

arteries which project within the kidney toward the junction of the renal cortex and medulla. Some small interlobular arteries may branch from these interlobar vessels and proceed directly toward the cortical surface. The arcuate arteries, however, are the major branches of the interlobar arteries, and these vessels traverse the corticomedullary junction parallel to the surface of the cortex. Most of the interlobular arteries branch from the arcuates and project perpendicularly to the surface of the kidney. As the interlobular arteries pass through the cortex, they give off afferent arterioles which terminate in the glomerular capillaries (Stein, 1976).

The intrarenal microvasculature is unique in that the glomerular capillaries are positioned between two arterioles: the afferent arteriole entering the glomerulus and the efferent arteriole which leaves this capillary network. The efferent arteriole is responsible in part for the regulation of glomerular capillary pressure as well as glomerular blood flow (Barger and Herd, 1971). The renal efferent arterioles are divided into 3 groups by their anatomical position within the cortex: the subcapsular cortical arterioles, the midcortical arterioles, and the juxtamedullary cortical arterioles.

The subcapsular cortical efferent arterioles of rat kidneys leave the glomeruli and proceed toward the cortical surface where they branch into the subcapsular peritubular capillaries (Evan and Dail, 1977). These efferent arterioles have a small diameter when they leave the glomerulus but may widen as much as 3-fold before reaching the peritubular capillaries. Not all subcapsular efferent arterioles proceed directly to the cortical surface, but some arterioles may branch relatively close to the glomerulus. Many of these branches,

however, eventually project to the surface of the kidney (Evan and Dail, 1977). Uniquely, these efferent arterioles near the cortical surface are exclusively associated with the tubules of the nephron from which they originated (Beeuwkes and Bonventre, 1975; Weinstein and Szymewicz, 1978). In the midcortical region, nearly all of the efferent arterioles have identical patterns of branching (Evan and Dail, 1977). These efferent arterioles divide immediately after leaving the glomerulus into a widely anastomosing capillary network (Beeuwkes, 1971). Unlike the efferent arterioles and peritubular capillaries of the subcapsular region, these arterioles are associated with many different tubules throughout the cortex (Beeuwkes and Bonventre, 1975). Juxtamedullary efferent arterioles are large and usually equal in diameter to the afferent arteriole. These arterioles descend into the medulla and form the capillary network of the vasa rectae. In some juxtamedullary vessels, a small lateral branch may emerge from the descending arteriole and form a capillary network in this region similar to the midcortical peritubular capillaries (Evan and Dail, 1977).

Anatomically, the venous circulation parallels the arterial system in most species. Outer cortical venous blood empties into superficial cortical veins which anastomose into the arcuate veins. Inner cortical blood drains directly into the arcuate veins, and these vessels then coalesce to form interlobar veins. Venous blood leaves the kidney by way of the renal vein and reenters the systemic circulation at the ascending vena cava (Stein, 1976).

b. Extrinsic Factors in the Regulation of Renal Blood Flow

There are two major extrinsic factors which are involved in the regulation of renal blood flow in the adult: the sympathetic

nervous system and vasoactive hormones. The renal nerves originate primarily from the celiac plexus and upper splanchnic nerves and enter the kidney at the hilus with the renal artery. Both cholinergic and adrenergic nerve fibers innervate the renal parenchyma (McKenna and Angelakos, 1968; Gosling, 1969), and most of these fibers proceed to the cortex of the kidney. The renal cortical fibers form neural junctions with the afferent arterioles, efferent arterioles and renal tubules (Barajas, 1978), whereas those fibers which enter the renal medulla innervate the vasa recta (Gosling, 1969).

Despite the abundant renal innervation, tonic renal nerve activity does not appear to contribute to the maintenance of renal vascular resistance. Berne (1952) has shown that renal denervation does not alter renal resistance in the normal conscious dog. Although alpha, beta and cholinergic receptors are present in the kidney (Vander, 1964), blockade of these receptors does not significantly alter renal blood flow (Stein et al., 1973a). Renal denervation also does not appear to produce renal hyperemia. This effect was demonstrated in unanesthetized dogs in which the clearance of para-aminohippuric acid (PAH), glomerular filtration rate, renal plasma flow or electrolyte excretion were not altered by unilateral renal denervation (Maluf, 1943; Berne, 1952). Similarly, in normal humans anesthesia of the 5th thoracic segment of the spinal cord did not affect the clearance of diodrast (Smith et al., 1939). Thus, the data demonstrate that the renal circulation is not influenced by tonic activity in sympathetic pathways.

Although tonic sympathetic activity does not affect renal resistance in the normal kidney, the renal nerves may have an active

role in the regulation of renal blood flow during altered states of sympathetic activation such as low systemic blood pressure. Direct renal nerve stimulation decreased total renal blood flow (Houck, 1951; Pomerantz et al., 1968; Stein et al., 1973a) but this decrease was not associated with any change in the intrarenal distribution of blood flow (Stein et al., 1973a). Infusion of epinephrine and norepinephrine into the kidney caused a dose dependent vasoconstriction of the renal vasculature, and this decrease in renal blood flow again did not alter the distribution of blood flow in the renal cortex (Rector et al., 1972). In contrast to these data, Gotshall and Itskovitz (1977) reported that in the isolated perfused dog kidney, renal nerve stimulation not only decreased renal blood flow but also resulted in a redistribution of blood flow to the outer cortex. These authors cited two possible reasons for the differences observed between their data and previously reported results. First, the experiments of Gotshall and Itskovitz were performed in isolated perfused dog kidneys, and it is possible that a different response occurs in the isolated organ system than would occur in an in situ kidney. Secondly, the level of renal nerve stimulation as well as dose of norepinephrine used in the isolated perfused kidney was less than that utilized in the in situ experiments. Renal nerve stimulation and norepinephrine infusion (0.25-0.5 μg/min) in the isolated perfused kidney reduced renal blood flow by 28% and 23%, respectively. In the anesthetized dog, renal nerve stimulation reduced renal blood flow by 40% (Stein et al., 1973a) and norepinephrine (4 μg/min) decreased renal blood flow by 49%. Thus, the changes in intrarenal distribution of blood flow after renal nerve stimulation

or norepinephrine infusion may be a function of the magnitude of decrease in renal blood flow.

Severe exercise in animals also has been utilized to demonstrate neurogenic control of renal blood flow. In normal dogs, severe exercise increased cardiac output and blood pressure but did not affect renal blood flow suggesting renal resistance increased (Henrick et al., 1939; Millard et al., 1972). This response was not altered by denervation or by alpha-adrenergic blockade (Millard et al., 1972), thus the increase in renal resistance was attributed to the intrinsic ability of the kidney to autoregulate. In contrast, steady state exercise and severe exercise decreased total renal blood flow in adult miniature swine by 26% and 70%, respectively, without changing the intrarenal distribution of blood flow (Sanders et al., 1976). Mean aortic blood pressure and cardiac output were significantly increased in these exercise states. Previously, it appeared that in dogs the renal sympathetic nerves primarily decrease renal blood flow in pathogenic states, and renal autoregulation maintained flow during physiologic stress. In miniature swine, however, physiologic changes may result in renal sympathetic nerve stimulation and decreases in total renal blood flow.

Vasoactive hormones circulating within the systemic plasma or synthesized and released intrarenally also may act to regulate renal blood flow and the intrarenal distribution of blood flow. The enzyme renin is synthesized and stored in the juxtaglomerular cells of the afferent arterioles. Renin is released into the blood and renal interstitial space and acts on its protein substrate angiotensinogen releasing the decapeptide angiotensin I. Angiotensin I is converted to

the octapeptide angiotensin II by the action of a dipeptidylcarboxypeptidase, angiotensin I converting enzyme. Angiotensin II is a potent stimulus of vascular smooth muscle contraction and also stimulates the release of aldosterone from the adrenal cortex.

Angiotensin II has been investigated as a factor regulating renal blood flow by the intrarenal infusion of the hormone and by the use of the competitive angiotensin II antagonist, $Sar^1$-$Ala^8$-angiotensin II (saralasin). Angiotensin II infusion into the kidney induced a prompt decrease in renal blood flow, whereas intrarenal infusion of angiotensin I had little effect upon renal resistance (Osborn et al., 1974). Similar results were observed in animals with elevated plasma renin concentration and presumably increased angiotensin II concentration. Renal artery constriction of one kidney increased circulating plasma renin concentration, and saralasin infusion into the contralateral kidney of these animals increased renal blood flow (Satoh and Zimmerman, 1975; Zimmerman, 1973). Furthermore, the magnitude of this vasodilation was directly correlated with the arterial plasma renin concentration (Satoh and Zimmerman, 1975). Saralasin infusion also increased renal blood flow in animals whose plasma renin concentration was increased by sodium depletion (Freeman et al., 1973; Mimran et al., 1974), thoracic vena caval constriction (Freeman et al., 1973; Slick et al., 1975) and high output heart failure (Freeman et al., 1975b). Thus, following experimental stimulation of the renin-angiotensin system, angiotensin II inhibition with saralasin increases renal blood flow and decreases renal resistance. In contrast to these data, saralasin infusion into dogs or rats with normal plasma renin activities, did not change renal blood flow or renal resistance (Davis, 1975;

Freeman et al., 1973; Ishikawa and Hollenberg, 1975). Therefore, angiotensin II in the normal animal does not appear to reduce renal blood flow tonically. However, in chronic states of hyperreninemia, circulating angiotensin II may increase renal resistance and decrease renal blood flow.

In addition to the observed effects of angiotensin II on total renal blood flow, circulating angiotensin II also may affect the intrarenal distribution of blood flow. In anesthetized dogs, Rector et al. (1972) demonstrated that angiotensin II infusion resulted in a uniform decrease in renal cortical blood flow. In the isolated perfused dog kidney, however, infusion of angiotensin II consistently decreased blood flow to the outer cortex but had little effect on inner cortical blood flow (Itskovitz and McGiff, 1974). Itskovitz and McGiff (1974) hypothesized that the variable response in inner cortical blood flow after angiotensin II may have been due to an interaction between infused angiotensin and renal prostaglandin synthesis. The primary prostaglandin synthesized in the renal medulla is prostaglandin $E_2$ ($PGE_2$) (Daniels et al., 1967; Lee et al., 1967), and this hormone is a potent vasodilator (Chang et al., 1975). Furthermore, angiotensin II has been shown to stimulate the release of PGE into the renal venous effluent of anesthetized dogs (McGiff et al., 1970; Aiken and Vane, 1973). From these observations, Itskovitz and McGiff postulated that the variability in inner cortical blood flow after angiotensin II may have resulted from concomitant $PGE_2$ release, and any effect of angiotensin II to reduce the inner cortical blood flow was offset by vasodilation from the prostaglandin. The effect of angiotensin II infusion on renal blood flow distribution was investigated further by infusion

of angiotensin II after inhibition of renal prostaglandin synthesis with indomethacin. Following indomethacin infusion, angiotensin II significantly decreased renal blood flow in both the inner and outer cortex of the isolated perfused dog kidney. This result was similar to the effect of angiotensin II on renal blood flow distribution in situ (Rector et al., 1972). This potential interaction between angiotensin II and $PGE_2$ in the inner cortex was identified more completely in experiments utilizing the angiotensin I converting enzyme inhibitor, SQ20,881 (Bailie and Barbour, 1975). In these experiments, the renal cortex was divided into 4 zones with zone 1 the outermost cortical zone and zone 4 the juxtamedullary cortex. Inhibition of angiotensin I conversion with SQ20,881 increased renal blood flow in cortical zones 2, 3 and 4 with the largest increase occurring in zone 3. Renal blood flow in the outer cortical zone 1 was unchanged after SQ20,881 infusion. Thus, inhibition of angiotensin II synthesis with SQ20,881, selectively increased inner cortical blood flow. This increase in inner cortical blood flow may have resulted from removal of angiotensin II vasoconstriction, and in the presence of $PGE_2$, vasodilation of the inner cortical blood vessels occurred. It must be noted, however, that since angiotensin converting enzyme is the same enzyme which catabolizes bradykinin, converting enzyme blockade in these experiments could not distinguish between vasodilation from an increased concentration of bradykinin or a decreased concentration of angiotensin II.

Prostaglandin $E_2$ also has been considered to be a significant extrinsic factor in the regulation of renal blood flow. The effects of $PGE_2$ on renal blood flow have been investigated by direct intrarenal

infusion and by use of several prostaglandin synthetase inhibitors including indomethacin, sodium meclofenamate and aspirin. As previously mentioned, $PGE_2$ is synthesized primarily within the renal medulla (Daniels et al., 1967; Lee et al., 1967). Although $PGE_2$ synthesis has been demonstrated in the renal cortex, the enzyme responsible for $PGE_2$ catabolism, prostaglandin 15-hydroxydehydrogenase, is present in high concentrations in this region of the kidney (Larsson and Anggard, 1973). Therefore, $PGE_2$ synthesized and released in the renal cortex is rapidly degraded.

The direct intrarenal infusion of $PGE_2$ (Itskovitz et al., 1974; Itskovitz and McGiff, 1974; Chang et al., 1975; Itskovitz and Campbell, 1976) or its biosynthetic precursor, arachidonic acid (Splawinski et al., 1972; Larsson and Anggard, 1974) increased renal blood flow. This increase in total renal blood flow was associated with a larger increase in blood flow to the inner cortex than the outer cortex (Itskovitz et al., 1974). It was originally reported by Lonigro et al. (1973) that inhibition of prostaglandin synthesis by indomethacin or sodium meclofenamate in anesthetized dogs decreased renal blood flow and this reduction was correlated with a decrease in renal venous $PGE_2$ concentration from 0.32 ng/ml to 0.06 ng/ml. Several investigators also have shown that indomethacin or meclofenamate decreased renal blood flow as well as increased the ratio of outer cortical to inner cortical blood flow (Swain et al., 1975; Zins, 1975; Zimmerman, 1978; Noordewier et al., 1978). In addition, indomethacin (Kirschenbaum et al., 1974; Noordewier et al., 1978) and meclofenamate (Kirschenbaum et al., 1974) decreased the absolute blood flow in both the outer and inner cortical regions, however, the outer to inner cortical blood flow

ratio (O/I ratio) was increased. This increase in O/I ratio resulted from a greater decrease in blood flow to the inner cortex than the outer cortex. Therefore, the data are in agreement with the concept that the vasodilator lipid $PGE_2$, synthesized and released from the renal medulla, may increase inner cortical blood flow. This hormone probably acts only as an intrarenal hormone since $PGE_2$ is significantly catabolized in the renal cortex (Larsson and Anggard, 1973) and the pulmonary circulation (McGiff et al., 1969).

Although prostaglandin synthetase inhibition altered renal hemodynamics in dogs stressed by anesthesia, indomethacin did not affect total renal blood flow (Kirschenbaum and Stein, 1976; Swain et al., 1975) or the distribution of renal blood flow (Zins, 1975) in conscious animals. In contrast, meclofenamate decreased renal blood flow in conscious dogs (Swain et al., 1975). These observations concerning prostaglandin synthetase inhibition and renal blood flow in conscious and anesthetized dogs are difficult to interpret, and it may be possible that specific effects of the synthetase inhibitors on the renal vasculature produce different results. Blasingham and Nasjelletti (1978) have offered the alternate explanation that this discrepancy between conscious and anesthetized animals is due to the relative state of sodium balance of the experimental animal. In their experiments, sodium meclofenamate did not change renal hemodynamics in sodium replete anesthetized dogs whereas in sodium deplete animals prostaglandin inhibition decreased renal blood flow and increased renal resistance.

The recently discovered prostaglandin, prostacyclin or $PGI_2$ also may be active in the hormonal regulation of renal blood flow. Blood vessels dissected from the cortex of bovine kidneys were shown to synthesize $PGI_2$ (Terragno et al., 1978a). In the intact dog, infusion of $PGI_2$ produced a rapid reduction in systemic blood pressure (Bunting et al., 1976) and this vasodepressor response has been verified by the ability of $PGI_2$ to relax arterial strips in vitro (Needleman et al., 1978). Bolger et al. (1978) reported that $PGI_2$ dramatically alters renal hemodynamics in dogs. After the intrarenal infusion of $PGI_2$, total renal blood flow increased from 4.1 ml/min/g kidney weight to 6.1 ml/min/g. Both outer cortical blood flow and inner cortical blood flow also were increased, but the outer to inner cortical blood flow ratio was decreased. Therefore, inner cortical blood flow increased to a greater extent than outer cortical flow. Thus, the effect of $PGI_2$ on renal hemodynamics is similar to that of $PGE_2$ in that both hormones increase total renal blood flow and increase inner cortical blood flow more than outer cortical flow. These renal hemodynamic effects of prostacyclin may be particularly important since unlike $PGE_2$, prostacyclin does not appear to be significantly degraded upon passage through the lung (Waldman et al., 1978). Therefore, synthesis and release of $PGI_2$ into the systemic circulation by other tissues may affect renal hemodynamics.

It is important to note that significant interactions may exist between angiotensin II, $PGE_2$, $PGI_2$ and the sympathetic nervous system. Angiotensin II stimulates the release of PGE from the kidney (McGiff et al., 1970; Aiken and Vane, 1973) and vasoconstriction by angiotensin II may be opposed by $PGE_2$-induced vasodilation. Thus,

angiotensin II infusion into isolated dog kidneys did not change inner cortical blood flow. However, in the presence of prostaglandin synthetase blockade, angiotensin II decreased blood flow to the inner cortex (Itskovitz and McGiff, 1974). This interaction between angiotensin and renal prostaglandins also was reported by Aiken and Vane (1973). In these experiments, angiotensin II infusion decreased renal blood flow to a greater extent in animals pretreated with the prostaglandin synthetase inhibitors indomethacin or meclofenamate than angiotensin II infused into untreated dogs. It was suggested that inhibition of prostaglandin synthesis increased the sensitivity of the renal vasculature to vasoconstriction. Furthermore, bradykinin (McGiff et al., 1972b), norepinephrine, epinephrine (Needleman et al., 1974; McGiff et al., 1972a) and renal nerve stimulation (Dunham and Zimmerman, 1970; Davis and Horton, 1972) have been shown to increase the release of prostaglandin E-like material from the kidney. McGiff et al. (1972a) demonstrated that renal blood flow decreased following norepinephrine infusion. However, in spite of continued drug infusion, partial to complete recovery of renal blood flow was observed within 1 to 3 minutes. These authors were able to directly correlate this recovery of renal blood flow with the renal venous PGE concentrations. Therefore, the renal vasoconstrictor effect of norepinephrine may be opposed by concomitant prostaglandin release and renal vasodilation. These results substantiate the possibility that complex interrelationships may exist between the various extrinsic factors which are known to alter renal hemodynamics when their release is stimulated or when they are infused alone. Thus, these interactions must be considered in

experiments in which these factors are being tested as possible mediators of renal blood flow.

2. Regulation of Sodium Excretion by Adult Kidneys

a. Intrarenal Blood Flow Distribution and Sodium Excretion

The regulation of sodium excretion by the kidney is a necessary requirement for the control of extracellular fluid volume. Intrarenal hemodynamics may affect the tubular sodium handling. The blood which enters the kidney does not perfuse the renal cortex in a uniform manner and this heterogeneous distribution of blood flow has been correlated with renal sodium excretion (Barger, 1966). Micropuncture experiments have revealed at least two types of populations of nephrons: the superficial cortical units with short loops of Henle and the juxtamedullary nephrons with loops extending deep into the medulla of the kidney. It has been proposed that the long juxtamedullary nephrons demonstrate more efficient sodium reabsorption than the short outer cortical nephrons and Barger (1966) categorized these units into superficial "salt losing" nephrons and juxtamedullary "salt retaining" units. The correlation which exists between nephron distribution and the renal arteries supplying blood flow to their respective glomeruli suggests a potential relationship between the intrarenal distribution of blood flow and nephron function.

Micropuncture experiments have shown that the function of nephrons between different cortical layers is not uniform. Horster and Thurau (1968) reported that in sodium loaded rats single nephron glomerular filtration rate in superficial cortical glomeruli averaged 38.1 nl/min while the single nephron glomerular filtration rate of

juxtamedullary nephrons was 16.5 nl/min. In sodium depleted rats, the superficial glomerular filtration rate was depressed to 23.5 nl/min and the juxtamedullary glomerular filtration rate increased to 58.2 nl/min. In anesthetized rats fed a normal diet, the superficial cortical glomeruli had filtration rates averaging 30.5 nl/min and those of nephrons originating from juxtamedullary glomeruli were 59.7 nl/min (Stumpe et al., 1969). Thus, in rats on a low sodium diet, 41% of the total glomerular filtrate was formed in the juxtamedullary nephrons, whereas rats fed a high sodium diet had only 11% of the glomerular filtrate formed by this same population of nephrons (Horster and Thurau, 1968; Stumpe et al., 1969). These data suggest that external factors which alter the intrarenal distribution of blood flow may change sodium excretion by shifting the intrarenal distribution of glomerular filtration to different nephron populations.

Alterations in urine volume and composition have been observed with different rates of blood flow through the juxtamedullary cortex and outer medulla (Birtch et al., 1967; Barger, 1966). These experiments demonstrated that blood flow through the deeper portions of the kidney was increased in sodium retaining states, but in sodium losing states, blood flow was decreased to these regions. Intrarenal distribution of blood flow also is altered during saline diuresis in anesthetized dogs. Jones and Herd (1970) showed by Krypton-85 autoradiography that the renal cortex and medulla had nearly equal concentrations of radioactivity in control experiments. During saline diuresis, however, a decreased entry and exit of Krypton-85 was seen in the juxtamedullary portions of the cortex suggesting that saline diuresis was associated with a shift in blood flow toward the outer cortex.

Recently, the hypothesis that a direct correlation exists between shifting renal blood flow distribution and changes in sodium excretion has been challenged. The changes in glomerular filtration of superficial cortical and juxtamedullary nephrons in rats receiving differing amounts of dietary sodium reported by Horster and Thurau (1968) only inferred that these effects were due to the shifting of renal blood flow. In contrast, Hsu and Kurtz (1976) were unable to show a change in renal blood flow distribution of rats on a low, high or normal sodium diet. By measuring the renal blood flow distribution with radioactive microspheres, Blantz et al. (1971) reported that during saline diuresis of dogs, total renal blood flow was increased throughout the cortex. The fractional cortical blood flow, however, was decreased in outer cortical zone 1 and increased in the juxtamedullary zone 4. This result has been verified by Stein et al. (1973b), and these authors suggested that the validity of these data in comparison to the measurement of blood flow distribution by the inert gas technique used in earlier experiments, rests in the definition of "outer cortex". Measurement of outer cortical blood flow with inert gases represents the majority of outer cortical nephrons and not the superficial cortex alone. On the other hand, using microspheres it is possible to separate the outer cortex of adult kidneys into 4 zones with relative ease and accuracy (Katz et al., 1971; McNay and Abe, 1970). These measurements of renal blood flow distribution during saline diuresis with microspheres are supported by the observation that vasodilation with acetylcholine (Stein et al., 1971; Itskovitz and Campbell, 1976), and histamine (Itskovitz and Campbell, 1976) increased renal sodium excretion but shifted renal blood flow away from the outer

cortex and toward the juxtamedullary nephrons. Thus, the previously held concept of "salt losing" and "salt retaining" nephrons does not appear to be valid and the relationships between shifts in renal blood flow and changes in sodium excretion are probably coincidental rather than directly related.

b. Oncotic Pressure and Sodium Excretion

Sodium excretion by the kidney may be regulated by the renal tubules and the intrarenal forces which participate in maintaining the proper sodium balance of animals include both active and passive transport processes. Reabsorption of sodium by active transport has been demonstrated throughout the nephron (Schultz, 1976). However, several investigators also have shown that a direct correlation exists between peritubular capillary oncotic pressure and passive proximal tubular sodium reabsorption (Lewy and Windhager, 1968; Brenner et al., 1969; Spitzer and Windhager, 1970; Daugherty et al., 1972; Brenner and Troy, 1971; Ott et al., 1975). Two physical forces contribute to the passive regulation of peritubular sodium reabsorption. First, a decrease in perfusion pressure between the glomerulus and the peritubular capillaries establishes a low peritubular capillary hydrostatic pressure. Secondly, formation of an ultrafiltrate of plasma increases the oncotic pressure of blood passing into the peritubular capillaries. Thus, in the peritubular capillaries there exists a low hydrostatic pressure and high oncotic pressure, both of which facilitate passive reabsorption of fluid from the tubule into the peritubular capillary. Therefore, changes in either peritubular capillary hydrostatic pressure or peritubular capillary oncotic pressure may act either to enhance or to suppress delivery of sodium out of the proximal tubule.

Infusion of saline into animals increases sodium excretion and this natriuresis may result in part from decreased proximal tubular sodium reabsorption (Dirks et al., 1965; Cortney et al., 1965; Rector et al., 1967). Micropuncture experiments have shown that saline loading in dogs (Dirks et al., 1965) and rats (Cortney et al., 1965) decreased the fractional reabsorption of sodium and water in the proximal tubule without increasing glomerular filtration rate. Although changes in glomerular filtration rate also may contribute to the natriuresis of saline loading (Stein et al., 1973c), these observations demonstrate that the proximal tubule is capable of directly decreasing fractional sodium reabsorption. A reduction of peritubular capillary oncotic pressure has been proposed as a possible mechanism for the decrease in fractional sodium reabsorption by the proximal tubule observed after volume expansion (Lewy and Windhager, 1968). The large fluid load may result in dilution of the plasma thereby decreasing the peritubular capillary oncotic pressure. The effect of hemodilution on tubular sodium reabsorption has been investigated by micropuncture perfusion of the peritubular capillaries with solutions of different oncotic pressure. Brenner and Troy (1971) have shown that peritubular capillary perfusion with solutions containing bovine serum albumin decreased the sodium concentration in the proximal tubular fluid. Similarly, in rats whose fractional sodium reabsorption was initially reduced by intravenous saline infusion, microperfusion of the peritubular capillaries with hyperoncotic Ringer's solution reversed this effect by increasing proximal tubular sodium reabsorption (Brenner et al., 1971). The effect of peritubular capillary oncotic pressure on proximal tubular fluid reabsorption also was investigated during aortic

constriction (Brenner and Troy, 1971). Suprarenal aortic constriction in rats decreased single nephron glomerular filtration rate and also decreased postglomerular arteriole plasma protein concentration. This fall in peritubular capillary protein concentration was associated with a concomitant decrease in proximal tubular fluid reabsorption. Peritubular capillary microperfusion with hyperoncotic albumin solutions during aortic constriction again reversed this effect increasing proximal tubular fluid reabsorption 25% above the control values despite glomerular filtration rate remaining below the initial control measurements. These results suggest that the decrease in peritubular capillary oncotic pressure which occurs after saline loading of the extracellular fluid or after suprarenal aortic constriction may suppress sodium reabsorption in the proximal tubule. Thus, Brenner and coworkers concluded that peritubular capillary protein concentration is a critical determinant of proximal tubular fluid reabsorption.

This concept that peritubular capillary oncotic pressure regulates proximal tubule sodium reabsorption has been questioned by other investigators. Holzgreve and Schrier (1975b) reported that during microperfusion of peritubular capillaries with normal saline, intravenous saline infusion decreased tubular fluid reabsorption from 3.72±0.61 nl/min/mm nephron to 3.38±1.54 nl/min/mm nephron. Reabsorption rate of tubular fluid into adjacent peritubular capillaries perfused with saline plus bovine serum albumin during control periods was 3.64±0.92 nl/min/mm nephron. After volume expansion, the fluid reabsorption in these tubules similarly decreased to 3.02±1.47 nl/min/mm nephron. In another experiment, these same authors demonstrated that proximal tubular fluid reabsorption was similarly reduced by

aortic constriction in tubules whose peritubular capillaries were perfused by saline or saline plus bovine serum albumin. These results have been supported by Conger and coworkers (1976) who demonstrated that peritubular capillary perfusion with either concentrated rat plasma or protein free rat plasma did not alter sodium reabsorption in the surrounding proximal tubules. In these experiments, peritubular capillary oncotic pressure was increased up to 40 mmHg without producing detectable changes in sodium reabsorption.

The discrepancies in results between Conger et al., Holzgreve and Schrier and results obtained in similar experiments by Brenner and coworkers may be due to differences in experimental procedures. First, the experiments of Holzgreve and Schrier were carefully conducted so that perfusates containing saline and saline plus albumin did not differ in osmolality. Osmolality was shown to be a critical factor in peritubular capillary microperfusion experiments (Holzgreve and Schrier, 1975a). A difference in osmolality of 10 mOsm/kg between the tubular fluid and peritubular capillary perfusate resulted in a change in fluid flux across the tubule of 1.03 nl/min/mm nephron. It is not clear from the earlier experiments by Brenner and coworkers whether peritubular capillary perfusate osmolality was similar to the osmolality of circulating rat plasma. The reports by Brenner and coworkers do not mention whether peritubular capillaries in control experiments were microperfused with a control medium but instead appeared to be perfused by normal blood flow. Thus, differences between the osmolality of circulating rat plasma and the microperfusate solutions could have accounted for the changes in tubular fluid reabsorption in these experiments. This possibility is supported by the experiments of Conger et al.

(1976). Rat plasma or concentrated rat plasma used as the peritubular capillary perfusate solution did not affect tubular fluid reabsorption. Since osmolality may be a critical factor governing proximal tubule fluid reabsorption, the plasma perfusate is much more physiological than the bovine albumin or dextran solutions used in previous experiments. Thus, recent experiments appear to demonstrate that during volume expansion, decreased peritubular capillary oncotic pressure does not account for increased fractional sodium excretion by the proximal tubules but rather changes in peritubular capillary osmolality may significantly alter proximal tubular fluid reabsorption.

This conclusion, however, still must be reconciled with the data which show that systemic infusion of hyperoncotic albumin solutions suppresses the natriuresis observed after saline infusion alone (Petersdorf and Welt, 1953; Schrier et al., 1968; Daugharty et al., 1968; Earley et al., 1966; Martino and Earley, 1967; Levy and Levinsky, 1971). Regulation of sodium reabsorption in more distal nephron segments may be an alternative explanation to this problem. Several investigators have shown that changes in proximal tubule sodium reabsorption do not necessarily reflect similar changes in sodium excretion (Howards et al., 1968; Knox et al., 1968; Burke et al., 1971; Knox et al., 1973) suggesting that more distal nephron sites also are critically involved in the regulation of sodium balance. Knox et al. (1973) demonstrated that saline volume expansion of dogs increased sodium delivery from the proximal tubule as well as increased sodium excretion. Subsequent albumin infusion, however, reduced the sodium delivery from the proximal tubule back to control values but the previously established natriuresis remained elevated. These data suggest

that the natriuresis observed after saline infusion must result at least in part from decreased sodium reabsorption beyond the proximal tubule. Stein et al. (1973c) have attempted to identify the nephron segments responsible for increasing sodium excretion after volume expansion. These authors reported that Ringer's volume expansion decreased proximal tubule sodium reabsorption to a greater degree than did volume expansion with Ringer's plus albumin. Despite this difference in proximal tubular sodium reabsorption between Ringer's infusion and Ringer's plus albumin, both fractional and absolute sodium delivery to the early and late distal nephron were similar. Collecting duct fractional reabsorption, however, decreased from 96% to 31% during Ringer's infusion but decreased to only 80% after albumin infusion. Thus, it was concluded that inhibition of sodium excretion after albumin infusion must have resulted from increased sodium reabsorption in the collecting duct. Although these data offer an alternative explanation for the discrepancies of previously discussed experiments, further investigation is necessary to isolate the tubular sites involved in the reduction of natriuresis by infusion of hyperoncotic albumin solutions.

c. Renal Hormonal Factors and Sodium Excretion

In addition to plasma oncotic pressure affecting sodium reabsorption by the renal tubules, the E prostaglandins also have been implicated in the regulation of sodium excretion by the kidney. Infusion of prostaglandins of the E series into anesthetized dogs has been shown to increase sodium excretion, but this natriuresis was associated with an increase in renal blood flow (Vander 1968; Gross and Bartter, 1973; Strandhoy et al., 1974; Johnston et al., 1968). The experiments

of Johnston et al. (1968) demonstrated that the intrarenal infusion of prostaglandin $E_1$ at 0.01 μg/min, 1.0 μg/min and 2.0 μg/min increased renal plasma flow and absolute urinary sodium excretion in a dose dependent manner. In similar experiments, Gross and Bartter (1973) reported that intrarenal $PGE_1$ (2 μg/min) infusion increased sodium excretion from 42.3 μEq/min to 102.8 μEq/min. Renal plasma flow concomitantly increased from 96.3 ml/min to 160.2 ml/min but glomerular filtration rate and systemic arterial blood pressure were unchanged. These data have been supported by the observation that administration of the prostaglandin synthetase inhibitor indomethacin (10 mg/kg) to rats significantly decreased both renal plasma flow and sodium excretion (Dusing et al., 1976). This effect of indomethacin on renal plasma flow and sodium excretion has been verified by similar results with prostaglandin inhibition after acetylsalicylic acid administration to anesthetized dogs (Berg and Bergan, 1976). Elevation of plasma acetylsalicylic acid concentrations to 20-80 μg/ml, 80-200 μg/ml and 200-400 μg/ml decreased sodium excretion and renal blood flow in a dose related fashion. Prostaglandin inhibition with meclofenamate also decreased sodium excretion and renal blood flow in sodium replete anesthetized dogs (Blasingham and Nasjletti, 1978). Thus, since PGE infusion increased sodium excretion and prostaglandin synthetase inhibition decreased sodium excretion, $PGE_1$, $PGE_2$ or both may function as an intrarenal "natriuretic hormone".

Many of the previous experiments examining prostaglandins and renal function have been conducted utilizing several different prostaglandins. Lee et al. (1967) identified $PGE_2$, $PGF_{2\alpha}$ and $PGA_2$ in the

rabbit renal medulla, however, $PGE_2$ was present in the highest concentrations. Recently, Larsson and Anggard (1976) measured $PGE_2$, $PGF_{2\alpha}$, and $PGA_2$ in rabbit kidneys by gas chromatography mass spectroscopy. $PGE_2$ was the most abundant renal prostaglandin and measured 0.19±0.04 μg/g tissue in the renal cortex and 4.36±1.04 μg/g tissue in the medulla. Prostaglandins of the E and F series are metabolized by the enzyme 15-hydroxyprostaglandin dehydrogenase. Consequently, the low $PGE_2$ concentrations in the renal cortex in comparison to the renal medulla are due to the presence of 10-fold more 15-hydroxyprostaglandin dehydrogenase in the cortical portions of the kidney (Larsson and Anggard, 1973). Therefore, $PGE_2$ currently is considered to be the most abundant renal prostaglandin and this hormone may be the most significant prostaglandin with respect to physiological renal function.

The mechanism by which $PGE_2$ enhances sodium excretion is difficult to determine since $PGE_2$ infusion and prostaglandin synthetase inhibition both result in dramatic hemodynamic changes. Therefore, the natriuretic effect of $PGE_2$ may be secondary to renal vasodilation. Indirect measurements have implied that fractional sodium reabsorption in the proximal tubule is reduced after intrarenal $PGE_1$ infusion resulting in increased sodium excretion (Johnston et al., 1968; Martinez-Maldonado et al., 1972; Shimzu et al., 1969). In these experiments, $PGE_1$ infusion increased renal blood flow, sodium excretion and free water clearance. The increase in free water clearance after $PGE_1$ was interpreted as indirect evidence for increased solute delivery to the distal nephron. Although other filtered ions could not be excluded from causing the increased free water clearance, sodium excretion was increased in conjunction with free water clearance suggesting that

sodium reabsorption in the proximal tubule or possibly the loop of Henle may have been reduced. Distal nephron sodium reabsorption did not compensate for this larger sodium load resulting in enhanced sodium excretion. Micropuncture experiments in rats demonstrated that $PGE_2$ did not affect proximal tubular sodium reabsorption or single nephron glomerular filtration rate but did increase fractional sodium excretion (Fulgraff and Meiforth, 1971). These data were supported by Strandhoy et al. (1974) and these authors reported different effects of $PGE_1$ and $PGE_2$ on proximal tubule sodium reabsorption in dogs. Both $PGE_1$ and $PGE_2$ infusion increased fractional sodium excretion but only $PGE_1$ infusion was shown to decrease proximal tubule sodium reabsorption. The increase in renal plasma flow after $PGE_2$ infusion increased peritubular capillary and interstitial space hydrostatic pressure but did not change peritubular capillary oncotic pressure. These hemodynamic changes, however, were not correlated with a change in proximal tubule sodium reabsorption. Thus, as in the rat, the infusion of $PGE_2$ in the dog was postulated to have a direct tubular effect, inhibiting sodium reabsorption in the loop of Henle, distal nephron or the collecting duct.

To demonstrate a direct tubular effect of $PGE_2$ on sodium reabsorption, hemodynamic changes which result from $PGE_2$ infusion must be eliminated. Tannenbaum et al. (1975) infused the prostaglandin precursor, sodium arachidonate into dogs at low doses to dissociate renal hemodynamic changes from possible direct tubular effects of renal prostaglandins on sodium excretion. Intrarenal arachidonate infusion at 1 and 3 μg/min only slightly increased renal blood flow but sodium

excretion was nearly doubled. The small increase in renal blood flow after arachidonic acid was not statistically significant. These effects of sodium arachidonate were abolished by the simultaneous infusion of the prostaglandin synthetase inhibitor 5,8,11,14-eicosatetraynoic acid (20:4), suggesting that increased prostaglandin synthesis after arachidonate resulted in a natriuresis which was relatively free from hemodynamic changes. Recently, Blasingham and Nasjletti (1978) published preliminary evidence that in sodium replete dogs, prostaglandin synthetase inhibition with meclofenamate did not change glomerular filtration rate or renal blood flow but sodium excretion was significantly decreased from 57 μEq/min to 28 μEq/min. These data suggest that renal prostaglandins may directly enhance sodium excretion without significantly changing renal hemodynamics in the sodium replete dog. These results were supported by the observation that inrarenal injection of sodium chloride in dogs increased prostaglandin E concentration of the renal venous effluent (Terrashima et al., 1976). Thus, high plasma sodium concentration can stimulate PGE release from the kidney, and this prostaglandin may directly increase sodium excretion.

Some investigators have been unable to demonstrate a natiuretic effect of renal prostaglandins when renal blood flow is held constant. Kirschenbaum and Stein (1976) have shown in conscious dogs undergoing water diuresis that prostaglandin synthetase inhibition with sodium meclofenamate or RO 20-5720 increased sodium excretion without changing renal plasma flow. These data support an antinatriuretic function of renal prostaglandins. Furthermore, Bohan and Wesson (1976) have shown that pretreatment of rats with indomethacin did not change the natriuresis observed after saline loading of rats. Experiments in

*in vitro* also have suggested that prostaglandins may directly increase renal sodium reabsorption. The toad bladder previously has been used as an *in vitro* model of the collecting duct and $PGE_1$ stimulated sodium transport in the toad bladder (Lipson and Sharp, 1971). Frog skin also has been utilized as a similar model and both $PGE_1$ (Hall and O'Regan, 1974; Gerencser, 1978) and $PGE_2$ (Haylor and Lote, 1976) were shown to increase the permeability of frog skin to sodium. These results indicate that renal prostaglandins may directly increase tubular sodium reabsorption. Thus, intrarenal synthesis and release of $PGE_2$ may function as an intrarenal "antinatriuretic hormone".

Recently, however, investigators have failed to demonstrate a direct tubular effect of renal prostaglandins on sodium transport in isolated tubules. Addition of $PGE_1$ and $PGE_2$ to the medium of tubule suspensions from the renal cortex or medulla had no effect on sodium uptake (Dunn, 1975). Fine and Trizna (1977) used the isolated perfused tubule technique to examine the effect of $PGE_2$ on tubular sodium transport. $PGE_2$ did not change sodium flux in rabbit thick ascending limbs or medullary collecting ducts when added to the perfusion solution or surrounding bath medium. Stokes and Kokko (1977) have even demonstrated a natriuretic effect of $PGE_2$ in isolated cortical and outer medullary collecting tubules of rabbits. Sodium reabsorption of the cortical collecting tubule was decreased when bathed with medium containing $PGE_2$ in concentrations of 0.1 μM, 1 μM and 10 μM. Similar effects were observed in outer medullary collecting tubules at $PGE_2$ concentrations of 10 μM and 100 μM. Therefore, an antinatriuretic effect of $PGE_1$ and $PGE_2$ has not been demonstrated in the isolated

perfused tubule, and in fact, $PGE_2$ may actually increase sodium excretion by the collecting duct.

The discrepancies between the data which demonstrate that renal prostaglandins increase sodium excretion and the results suggesting renal prostaglandins are antinatriuretic are difficult to explain. It is possible that inrarenal prostaglandins may influence sodium excretion by two mechanisms. First, $PGE_1$ and $PGE_2$ increased renal blood flow, and this vasodilation was associated with an increase in sodium excretion. Administration of prostaglandin synthetase inhibitors in some experimental situations decreased renal blood flow, and this decrease was correlated with a reduction in sodium excretion. Thus, renal prostaglandins may increase sodium excretion by vasodilation of the renal vasculature. With the recent discovery of prostacyclin ($PGI_2$), this intrarenal hormone also may influence sodium excretion by this mechanism since Bolger et al. (1978) reported that $PGI_2$ infusion into dogs increased renal blood flow as well as sodium excretion. Secondly, renal prostaglandins may have direct tubular effects on sodium reabsorption. This mechanism of prostaglandin regulation of sodium excretion is controversial. Clearly, any direct effect of the renal prostaglandins on sodium transport occurs at a distal nephron site probably within the collecting duct. Recent evidence has indicated that if $PGE_2$ alters tubular sodium reabsorption then the effect of this hormone is to inhibit tubular sodium transport. Further investigation, however, is necessary to identify the specific tubular effects of renal prostaglandins on sodium reabsorption.

Angiotensin II has been proposed as another humoral factor mediating renal sodium excretion. Angiotensin stimulates aldosterone

release from the adrenal cortex and aldosterone increases sodium reabsorption in the distal nephron. Regardless of this well established effect of aldosterone on sodium transport, angiotensin II has been shown to directly alter sodium excretion. Several investigators have demonstrated that angiotensin II infusion increased sodium excretion without changing glomerular filtration rate (Hughes-Jones et al., 1949; Louis and Doyle, 1965; Porush et al., 1967; Healy and Elliott, 1970; Akinkugbe et al., 1966). In these experiments, renal plasma flow was not increased suggesting that angiotensin II may have a direct effect on sodium reabsorption. Langford (1964), demonstrated that angiotensin II infusion into chicken kidneys directly inhibited sodium reabsorption. The chicken kidney has a unique renal portal vascular system in addition to the normal arterial perfusion of the glomerular capillaries. In the portal system, the renal peritubular capillaries are directly perfused by the portal vessels bypassing the glomerular capillaries. By infusing angiotensin II into the venous portal vasculature, the direct tubular effects of the hormone circulating through the peritubular capillaries may be investigated. In these chickens, angiotensin II infused into the right renal portal vessels at 0.25-2 μg/kg/min increased sodium excretion without affecting glomerular filtration rate. Langford concluded that peritubular capillary perfusion with angiotensin II directly reduced renal tubular sodium reabsorption in the chicken kidney.

The experiments demonstrating a natriuretic effect of angiotensin II prompted a search for the tubular site at which angiotensin may directly affect sodium reabsorption. Vander (1963) using the stop-flow technique in anesthetized rats demonstrated that distal tubular

sodium concentration was increased after angiotensin II infusion. Since glomerular filtration rate was unaffected by angiotensin infusion, the data suggested that distal tubular sodium reabsorption was decreased by a direct effect of angiotensin II. Micropuncture experiments also have identified specific tubular actions of angiotensin II. In rats, infusion of angiotensin II ($4.6 \times 10^{-5}$ ng/min) into peritubular capillaries increased capillary hydrostatic pressure from 17 to 23.3 cm of water (Steven and Thorpe, 1977). This increase in peritubular capillary hydrostatic pressure was associated with a reduction in proximal tubular fluid reabsorption rate from 32.3 to 24.9 nl/min. Thus, peritubular capillary perfusion with angiotensin II decreased tubular fluid reabsorption in adjacent proximal tubules. These data do not directly correlate angiotensin II with decreased proximal tubule sodium reabsorption but rather only infer that sodium was retained in the tubular fluid of the proximal tubule. This inference would seem to be valid, however, since Healy et al. (1969) have demonstrated that angiotensin II does not alter active sodium transport in isolated tubule suspensions. Therefore, any effect angiotensin II might have on sodium reabsorption would result from passive forces, and inhibition of tubular fluid reabsorption also would reduce tubular sodium reabsorption.

The effect of angiotensin II on peritubular capillary hydrostatic pressure and tubular fluid reabsorption is not merely an artifact of the infusion of pharmacological amounts of angiotensin II. Infusion of the competitive angiotensin II inhibitor, $Sar^1$-$Ala^8$-angiotensin II (saralasin) into peritubular capillaries decreased the hydrostatic pressure in both the capillaries and the surrounding

proximal tubules (Steven and Thorpe, 1977). These data suggest that endogenous angiotensin II may suppress tubular sodium reabsorption by increasing peritubular capillary hydrostatic pressure. Lowitz et al. (1969) have used the micropuncture technique to investigate the effects of angiotensin II on distal tubular sodium reabsorption in rats. In these experiments, angiotensin II infusion (1.0-1.5 μg/kg/min i.v.) increased urine flow rate and sodium excretion without affecting systemic blood pressure or glomerular filtration rate. Sodium concentration of tubular fluid recollected from distal tubules before angiotensin infusion averaged 37.5 mEq/l whereas the distal tubule sodium concentration after angiotensin infusion was 51.9 mEq/l. This increase in distal tubule sodium concentration could have resulted from three factors: 1) Increased tubular water reabsorption; 2) increased delivery of sodium from the proximal tubule; or 3) decreased distal tubule sodium reabsorption. The ratio of tubular fluid to plasma inulin concentration was slightly reduced following angiotensin infusion suggesting that the increase in tubular sodium concentration may have been partially due to an increase in water reabsorption. The effect of angiotensin II on proximal and distal tubule sodium reabsorption was investigated by peritubular capillary perfusion with a solution containing 2.5 μg/ml angiotensin. In contrast to the experiments of Steven and Thorpe (1977), Lowitz et al. (1969) were unable to demonstrate any change in proximal tubular sodium reabsorption following peritubular capillary perfusion with angiotensin II. Distal tubule sodium reabsorption, however, was significantly increased. These authors suggested that circulating angiotensin II in the peritubular capillaries does not change delivery of sodium from the proximal tubule

but may reduce renal sodium reabsorption by a direct tubular effect on the distal nephron. At present, no clear explanation is available for the apparent controversy concerning the effect of angiotensin II on sodium reabsorption in the proximal tubule. However, the micropuncture data do confirm that angiotensin II enhances sodium excretion and this natriuresis may result from decreased sodium reabsorption in the distal nephron.

Recently, some investigators have been unable to show a natriuretic effect of angiotensin II and have reported that angiotensin may be antinatriuretic. In dogs, the intrarenal infusion of angiotensin II (1 μg/kg/min) significantly decreased sodium excretion (Waugh, 1972; Fagard et al., 1978). In these experiments, antinatriuresis following angiotensin II infusion was associated with either a decrease in renal blood flow (Waugh, 1972) or a decrease in both renal blood flow and glomerular filtration rate (Fagard et al., 1978). The effect of endogenous angiotensin II on sodium excretion also has been investigated by intrarenal angiotensin II blockade in dogs previously placed on a low sodium diet. Trippodo et al. (1977) reported that intrarenal infusion of saralasin increased sodium excretion and renal blood flow but did not alter glomerular filtration rate. Lohmeier et al. (1977) demonstrated an increase in renal blood flow and sodium excretion in sodium depleted dogs after saralasin infusion but in these experiments glomerular filtration rate also was increased. These data indicate that intrarenal blockade of angiotensin II in the sodium depleted dog increased both renal blood flow and sodium excretion. Therefore, sodium reabsorption may have been increased by endogenous angiotensin II.

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The conflict between an antinatriuretic effect of angiotensin II and the proposal that angiotensin II directly increases sodium excretion may be explained by changes in renal hemodynamics after angiotensin infusion. Experiments which demonstrated a natriuresis after angiotensin II infusion were associated with no change in renal plasma flow or glomerular filtration rate. Thus, angiotensin II may increase sodium excretion by directly altering peritubular capillary fluid reabsorption. Conversely, in recent experiments angiotensin II infusion decreased sodium excretion but also decreased renal blood flow or glomerular filtration rate. Therefore, the antinatriuretic effect of angiotensin II may be secondary to a decreased renal perfusion pressure, decreased filtered sodium load or both. Angiotensin II induced changes in renal sodium excretion also may be dependent upon the dose of angiotensin infused into the kidney. Malvin and Vander (1967) reported that infusion of 10-100 ng/kg/min angiotensin II decreased glomerular filtration rate and also decreased sodium excretion. Larger doses of angiotensin II (100-1000 ng/kg/min) also decreased glomerular filtration rate but increased sodium excretion suggesting angiotensin II directly decreased sodium reabsorption. Similarly, Lameijer et al. (1966) demonstrated that intravenous infusion of 3 ng/kg/min angiotensin II decreased sodium excretion whereas 60 ng/kg/min enhanced sodium excretion. In these experiments, neither dose of angiotensin II affected glomerular filtration rate or renal plasma flow. Thus, the effect of angiotensin II at low doses decreased renal sodium excretion whereas larger doses resulted in a natriuresis. Therefore, in addition to the indirect effects of angiotensin II on sodium excretion by stimulation of aldosterone release, circulating

angiotensin II also may increase sodium excretion by directly reducing tubular sodium reabsorption.

3. Renal Blood Flow in the Developing Kidney

a. Renal Vascular Development

The development of the renal vasculature begins early in fetal life. The renal artery originates from a series of nonsegmental splanchnic arteries extending laterally from the aorta (McCrory, 1972). Vascularization of the renal cortex and medulla in rabbit embryos was described at 21 days gestation (Lewis, 1958). These experiments demonstrated that the renal cortex was occupied by a dense plexus of vessels among the mesenchyme which resembled a sinusoidal vascular system (Lewis, 1958). At 23 days gestation of the rabbit embryo, blood flow through the glomeruli was low and all renal blood flow was shunted around the glomerular capillaries to the sinusoids. The sinusoidal system became capilliform at 26 days gestation in the rabbit and interlobular arteries were developing as arcuate branches. A high resistance in the glomerular capillaries was still evident at this age, and the primary pathway of blood flow remained toward the sinusoidal system. Lewis (1958) has described that at least in the rabbit, direct arterial connections between the arteries and peritubular plexus remain intact until 12 days after birth. Furthermore, these interconnections between interlobular arteries and peritubular capillaries were of low resistance so that an effective vascular shunt bypassing the glomeruli existed in the young rabbit.

The embryological development of renal cortical efferent arterioles also has been described in the sheep and human fetus.

Davies (1950) reported that in the mesonephros of sheep, efferent vessels branch from the glomeruli and join a plexus of sinusoidal veins around the tubules. In fetal human kidneys, efferent arterioles appear to develop only after the glomeruli and peritubular capillaries are present. These vessels begin as solid cords of cells and often do not develop a lumen until the glomerular capillary already is filled with blood (Edwards, 1951). Although the evidence describing renal vascular development is limited, the available data suggest that the renal vasculature is not mature at birth, and significant postnatal changes in renal hemodynamics must occur.

b. Regulation of Renal Blood Flow in Young Animals

The postnatal maturation of renal blood flow has been described in several species of animals including humans (Ljundquist, 1963), sheep (Alexander and Nixon, 1962), rats (Aperia and Herin, 1975) and piglets (Gruskin et al., 1970). Fetal renal blood flow in near-term lambs was reported as 0.96 ml/min/g kidney weight (Dunne et al., 1972) which was significantly lower than renal blood flow in lambs 31-48 days of age (Aperia and Herin, 1976). In newborn dogs, Aschinberg et al. (1975) reported that mean renal blood flow increased from 0.39 ml/min/g kidney weight at 7 days of age to 2.06 ml/min/g kidney weight at 42 days. Jose et al. (1971) also demonstrated in puppies that renal blood flow was 1.2 ml/min/g at 42 days and increased to adult values of 3.5 ml/min/g by 98-112 days of age. Renal blood flow measurements in 17 day old rats have been reported as 0.25 ml/min/g kidney (Aperia and Herin, 1975). This flow increased to 5 ml/min/g by 60 days of age. Similarly, newborn piglets have low renal blood flow. Gruskin et al. (1970) demonstrated that in the first 24 hours of life, piglet renal

blood flow averaged 43 ml/min/$m^2$. This perfusion rate increased to 760 ml/min/$m^2$ by 45 days of age. Adult renal plasma flow values in swine have been determined by the clearance of PAH as approximately 3.85 ml/min/g (Nielsen et al., 1966). These data document that renal blood flow in the newborn kidney is low and increases as both the kidney and the animal mature.

The low renal blood flow in the undeveloped kidney may be the result of high renal vascular resistance, low cardiac output, low mean arterial blood pressure or a combination of the three. In piglets between the ages of 1 and 45 days, renal blood flow increased by a factor of 18 while renal resistance decreased by 86%. Cardiac output, however, only increased by a factor of 7 over this same age range suggesting that the increase in renal blood flow and fall in renal resistance in piglets is only partially due to the increase in cardiac output (Gruskin et al., 1970). These data have been substantiated in the human neonate in which the kidney receives approximately 5-6% of the cardiac output in comparison to the adult kidney which receives nearly 15-25% (Bolomey et al., 1949). Kleinman and Lubbe (1972) have determined the relationships between blood pressure, renal vascular resistance, renal plasma flow and glomerular filtration rate (GFR) in newborn dogs. Renal plasma flow increased from 0.7 ml/min/g at 1 day of age to 1.8 ml/min/g at 30 days of age. Mean arterial blood pressure increased from 40 mmHg to 80 mmHg over this age range, and glomerular filtration rate increased from 0.16 ml/min/g kidney at birth to 0.35 ml/min/g at 30 days. In these experiments, increases in both GFR and renal plasma flow were directly related to the increase in mean arterial blood pressure. Since mean renal vascular resistance in the newborn

puppy was not different from that of the adult, the age dependent increase in renal plasma flow in the newborn puppy resulted from increased arterial blood pressure rather than decreased renal resistance.

Nephrogenesis begins in the juxtamedullary cortex and maturation proceeds outward to the superficial cortex (Potter, 1972; Speller and Moffat, 1977). Spitzer and Brandis (1974) noted in the first 2 weeks of postnatal development in the guinea pig, that the total glomerular filtration rate increased by an average of 0.96 nl/min per nephron, while single nephron filtration rate of the superficial cortical glomeruli increased only 0.17 nl/min. Clearly, a more marked increase in single nephron filtration rate occurred in the juxtamedullary nephrons. In contrast, the increase in single nephron glomerular filtration rate during the third and fourth week of postnatal development could be attributed almost entirely to the outer cortex. Horster and Valtin (1971) demonstrated that in puppies between 21 and 69 days of age a 7-fold increase in single nephron glomerular filtration rate occurred in outer cortical glomeruli, while whole kidney filtration rate increased 4.5-fold.

It may be postulated from these observations that the uneven distribution of glomerular filtration rate at birth results from a similar heterogeneity of renal blood flow. The distribution of renal blood flow in young animals has been investigated primarily in the canine puppy. Olbing et al. (1973) showed that between 5 and 36 hours after birth of dogs, inner cortical glomeruli were perfused at a rate 5 times that of the outer cortex. At 6 weeks of age, however, flow to the outer cortical glomeruli had increased to a value greater than the juxtamedullary areas. Aschinberg et al. (1975) showed in the puppy

that during the first week of life, the renal cortex was homogeneously perfused at a rate of 0.88 ml/min/g kidney which represented only 35% of the total renal blood flow. By 2 weeks of age, 75% of the total renal blood flow perfused the cortex. However, of this cortical blood flow only 15% perfused the outer cortex, and 60% was received by inner cortical nephrons. Between 2 and 10 weeks of age the percentage of total renal blood flow to the cortex only increased by another 10% but this flow was redistributed, 65% to the outer cortex and 20% to the inner cortex. Jose et al. (1971) utilizing the Xenon-133 washout technique and autoradiography in dogs, also showed that blood flow to the outer cortex increased by 16% from 6 weeks to 16 weeks of age. These data indicate that renal blood flow is immature in the newborn puppy, and that as the kidney matures not only does total renal blood flow increase but also the ratio of outer to inner cortical blood flow increases.

Kleinman and Reuter (1973) determined in the newborn dog that the increase in outer cortical to inner cortical flow ratio between 1 and 14 days of age was primarily due to the rise in outer cortical blood flow. Furthermore, these authors reported a direct correlation between the increase in outer cortex:inner cortex flow ratio and mean systemic blood pressure. These authors concluded, however, that although the transient rise in blood pressure with age may be a factor in increasing outer cortical blood flow, the maturation and development of outer cortical glomeruli as well as other factors (possibly hormonal) must also contribute to the large increase in total renal blood flow and outer cortical flow which occur after birth.

It has been postulated that the high renal resistance and low outer cortical blood flow in the newborn is due to a mature and active sympathetic nervous system (Jose et al., 1972). Gootman et al. (1972) have demonstrated that in the newborn piglet the central vasomotor regulatory centers are functional at birth although not fully mature. Reddy et al. (1972) also have reported that low frequency sciatic nerve stimulation in newborn pigs decreased renal blood flow, whereas high frequency stimulation increased renal flow. Stimulation of peripheral regulatory systems in newborn pigs has been accomplished by hypercapnia and hemorrhage. In anesthetized piglets 1-22 days of age, severe hypercapnic acidosis and hemorrhage increased renal blood flow (Reddy et al., 1972; Gootman et al., 1971).

Specific affects of epinephrine and norepinephrine have been shown in canine puppies. Jose et al. (1974) reported that epinephrine (10-19 ng/g kidney weight/min i.v.) decreased renal cortical blood flow in the canine puppy while not affecting cortical flow in the adult. Furthermore, 21-31 ng/g/min reduced renal blood flow by 92% in the puppy and only 27% in the adult. In the puppy, cortical blood flow was redistributed to the inner cortex by epinephrine. This evidence indicates that the neonatal dog kidney has an increased sensitivity to circulating catecholamines and these hormones may in part maintain the low renal blood flow in the puppy. Jose et al. (1972) also reported that in 8-10 week old puppies the intrarenal infusion of the alpha-adrenergic antagonist phenoxybenzamine significantly increased blood flow to the outer cortex. This result suggests that sympathetic nerve activity or circulating catecholamines in the newborn dog may

decrease total renal blood flow as well as reduce blood flow to the outer cortex.

4. Renin-Angiotensin System in Developing Animals

The existence of a functional renin-angiotensin system in newborn animals has been determined in several species (Pohlova and Jelinek, 1974; Broughton-Pipkin et al., 1974; Granger et al., 1971; Alward et al., 1978) including humans (Kotchen et al., 1972). The systemic plasma renin concentration in neonatal dogs (12 to 48 hours after birth) has been reported to be 25.6 ng/ml compared to 1 ng/ml in adult dogs (Granger et al., 1971). Broughton-Pipkin et al. (1974) reported that the plasma renin concentration of fetal lambs averaged 9 ng AI/ml and increased to 22 ng AI/ml by 10 days of age postpartum. Rat plasma renin activity was already 9.2 ng AII/ml/hr in 1 day old animals and decreased to 3.37 ng AII/ml/hr by 80 days of age (Pohlova and Jelinek, 1974). Kotchen et al. (1972) reported that less than 7 hours after birth plasma renin activity in infants averaged 2.4 ng AI/ml/hr and increased to 8.8 ng AI/ml/hr within 24 hours. By 3-6 days of age plasma renin activity was 11.6 ng/ml/hr and then decreased to 2.3 ng AI/ml/hr at 3-6 weeks of age. The values at 6 hours, 24 hours and 3-6 days were all significantly greater than adult control values of 0.7 ng AI/ml/hr. Clearly, the plasma renin activities of these animals were high at birth and decreased with age. This altered state of hyperreninemia may have important effects on neonatal renal function.

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The physiological mechanisms which lead to the high plasma renin activity in neonates are unclear. Potentially, a high plasma renin substrate concentration could increase the activity of the renin enzyme

in the circulating plasma. Plasma renin activity is determined by incubation of a plasma sample containing endogenous renin and renin substrate. Incubation is carried out in the presence of both converting enzyme and angiotensinase inhibitors so that the generated angiotensin I is an estimate of the plasma renin activity. If renin substrate is present in sufficient concentrations so that the enzymatic reaction between renin and renin substrate demonstrates zero order kinetics, then the absolute renin concentration is estimated. However, in some species the endogenous renin substrate concentration may not be sufficient to establish zero-order reaction kinetics (Wallace et al., 1979). Therefore, the determination of plasma renin activity may be dependent on both the concentration of renin and the concentration of renin substrate. If the endogenous renin substrate concentration in newborn animals does limit the reaction between renin and renin substrate, then the high plasma renin activity in young animals may result from a similar elevation of plasma renin substrate concentration. Thus, changes in plasma renin activity after birth may be a function of renin substrate concentration rather than the plasma renin concentration.

Plasma renin substrate concentration has been determined in developing rats and humans. Pohlova and Jelinek (1974) reported that plasma renin substrate concentration in rats 1 day of age averaged 365.5±29.2 ng AII/ml. This value was not significantly different than the plasma renin substrate concentration at 80 days of age (328.9±22.9 ng AII/ml). Over this same age range, rat plasma renin concentration decreased from 9.82±1.87 ng AII/ml/hr to 3.37±0.24 ng AII/ml/hr.

Kotchen et al. (1972) demonstrated that plasma renin substrate concentration of infants less than 24 hours old averaged 2,484 ng AI/ml. At 3-6 days and 3-6 weeks of age, plasma substrate was unchanged averaging 2,538 ng AI/ml and 2,652 ng AI/ml, respectively. In these same infants, plasma renin activity was 8.8 ng AI/ml at birth which increased to 11.6 ng AI/ml at 6 days of age and then declined to 2.3 ng AI/ml by 3-6 weeks. These data show that although plasma renin activity increased after birth and then declined toward adult levels, renin substrate concentration remained constant. Therefore, the changes observed in plasma renin activity appear to result from alterations in plasma renin concentration.

Immature renal function in young animals may affect the rate of renin secretion and consequently the systemic plasma renin concentration. Early reports suggested that granulated juxtaglomerular cells did not appear until 14 days postpartum in the rat, but a progressive rise in total juxtaglomerular index occurred from the 14th through the 60th day of life (Dauda and Endes, 1963; Alexander and Grimason, 1967). In mice, juxtaglomerular granulation was not observed until the second to fourth week of life (Friedberg, 1965). An absence of juxtaglomerular granulation in 1 day old piglets also has been reported (Bing and Kazimierczak, 1964). Recently however, electron photomicrographs of fetal rat kidneys (18th day gestation) (Bruhl et al., 1974) and fetal lamb kidneys (Smith et al., 1974) have identified specific secretory granules in the epitheloid cells of the juxtaglomerular apparatus of these species. Schmidt et al. (1972) have isolated juxtaglomerular granules by differential centrifugation of rat kidney homogenates taken from animals 5-6 hours of age. These homogenates contained

pressor activities 7.4 times higher than adult rat homogenates. Electron photomicrographs also demonstrated the presence of granulated epitheloid cells in the juxtaglomerular apparatus of these rats. Eguchi et al. (1974) have measured a granular cell index in the juxtaglomerular cells of fetal rats, and this index increased within 1 day after birth. These recent data show the existence of specific juxtaglomerular granules in the newborn rat kidney suggesting that in young rats, the secretion of renin occurs from stored granules.

The increased plasma renin activity in young animals when compared with adults could be due to enhanced activation of renin release. Before discussing the possible developmental changes in renin secretion, a brief synposis of the control of renin release in adult animals is necessary. Three basic mechanisms have been proposed which regulate the release of renin from the kidney. First, the sympathetic nervous system directly innervates the juxtaglomerular apparatus. Renal nerve stimulation may increase renin release by beta-adrenergic receptor activation. Secondly, the macula densa region in the early distal tubule senses changes in tubular sodium or chloride load or changes in sodium (chloride) transport. Increases in distal tubular sodium (chloride) load or macula densa cell sodium (chloride) transport decrease renin release. The third mechanism located in the afferent arteriole is a vascular baroreceptor. This vascular receptor responds to changes in renal perfusion pressure or afferent arteriole wall tension. Thus, decreases in renal perfusion pressure increase renin release (Davis and Freeman, 1976).

The vascular baroreceptor may stimulate renin release in young animals. Trimper and Lumbers (1972) have presented indirect evidence

for the existence of a functional baroreceptor in the neonate. The diuretic, furosemide, has been shown to stimulate renin release at both the vascular and macula densa receptors (Corsini et al., 1975). Furosemide administration to fetal lambs (110-115 days of gestation) significantly increased plasma renin activity (Trimper and Lumbers, 1972). Although these experiments do not isolate the exact mechanism of renin release following furosemide administration in fetal lambs, it is evident that the intrarenal release of renin in the newborn may be sensitive to changes in sodium (chloride) transport at the macula densa, decreased renal perfusion pressure or both. Broughton-Pipkin et al. (1974) indirectly have investigated the vascular baroreceptor in fetal and newborn lambs. Removal of 25% of the circulating blood volume increased plasma renin activity in both age groups. In these experiments, hemorrhage reduced systemic blood pressure between 27% and 39% of control. Smith et al. (1974) have demonstrated similar results after removal of 8-10% of the plasma volume from fetal lambs between 120 and 145 days of gestation. Although renal perfusion pressure likely was decreased after hemorrhage, these data do not distinguish between baroreceptor stimulation of renin release and activation of the sympathetic nervous system. In fetal lambs, Smith et al. (1974) have investigated the vascular baroreceptor more directly by suprarenal aortic constriction. Reduction of renal perfusion pressure by 8-10 mmHg significantly increased the plasma renin activity of lambs, 120-130 days of gestation. This result indicates that the vascular baroreceptor may be functional in these animals. The possibility does exist, however, that aortic constriction decreased the filtered sodium or chloride load, and renin secretion was stimulated by the macula densa receptor.

Arterial pressure and renal blood flow are low in the newborn, consequently renal perfusion pressure also is low. According to the baroreceptor hypothesis, a low renal perfusion pressure increases renin secretion (Blaine et al., 1970). Therefore, as the animal matures and perfusion pressure increases, renin secretion should concomitantly decrease.

Stimulation of the sympathetic nervous system and subsequent intrarenal beta receptor activation also may increase renin release from neonatal kidneys. The presence of a functional sympathetic nervous system in young piglets has been documented (Gootman et al., 1972). As previously mentioned, Jose et al. (1974) reported that the renal arterioles of puppies may have an increased sensitivity to epinephrine. Although tonic renal efferent nerve activity has not been measured in neonatal animals, these observations suggest that activation of the sympathetic nervous system possibly by the low systemic blood pressure, may increase renin release and subsequently increase plasma renin concentration.

The regulation of renin release in young animals by the tubular macula densa receptor has not been investigated. However, several observations of renal function in neonates suggest that renin release is stimulated by this tubular receptor. As previously mentioned, furosemide administration to fetal lambs increased plasma renin activity (Trimper and Lumbers, 1972). Furosemide may stimulate renin release at the macula densa in young animals by inhibition of sodium or chloride transport (Vander and Carlson, 1969). At birth, glomerular filtration rate is low and increases with age (Horster and Valtin, 1971; Spitzer and Brandis, 1974), but plasma sodium and chloride

concentrations are not significantly different from those of older animals (Pownall, 1970). Therefore, the filtered sodium or chloride load by neonatal kidneys should be less than older animals. Consequently, the sodium (chloride) load to the macula densa also may be lower in young animals thereby increasing renin release. Horster and Larsson (1976) have shown that fluid reabsorption in immature rabbit proximal tubules is increased. The fluid reabsorption in these young kidneys may enhance proximal tubular sodium or chloride reabsorption and again increase renin release because the sodium (chloride) load presented to the macula densa would be lower than in adult animals. These potential mechanisms by which the macula densa may contribute to the high plasma renin activity in newborns remain speculative. Further investigation is necessary before firm conclusions can be made about macula densa influences on tonic renin release in young animals.

Other factors which may contribute to the high plasma renin activity in newborns include renin derived from the maternal plasma, hormonal influences on renin secretion and renin catabolism. Maternal hyperreninemia occurs during pregnancy in humans (Brown et al., 1963), sheep (Smith et al., 1974) and dogs (Hodari et al., 1967). Renin, however, is a large protein (molecular weight approximately 40,000 daltons) and it does not seem likely that this large molecule would cross the feto-placental barrier. Maternal renin, therefore, would not be expected as a source of the elevated plasma renin activity in the neonate.

In adult animals, angiotensin II, prostaglandin $E_2$ ($PGE_2$) and prostacyclin ($PGI_2$) have been shown to alter renin release. Angiotensin II inhibition of renin secretion is well established in adult

animals (Shade et al., 1973; Naftilan and Oparil, 1978). Preliminary evidence presented by Jose et al. (1973) suggested that angiotensin II blockade in puppies with saralasin increased plasma renin activity. Therefore, negative feedback by angiotensin II on the juxtaglomerular cells may be functional in young dogs. Both $PGE_2$ (Osborn et al., 1978) and prostacyclin (Whorton et al., 1977a) increased renin release in adult animals and this effect may be due to direct stimulation of the juxtaglomerular cells. Terragno et al. (1978b) have demonstrated that the renal vasculature in fetal pigs is capable of synthesizing more $PGE_2$ and $PGI_2$ than adult animals. In young animals, however, the possibility that renin secretion is stimulated by $PGE_2$ or $PGI_2$ has not been investigated.

Solomon et al. (1977) recently suggested that the rate of renin disappearance from the plasma of infant rats was lower than adults. These data suggest that decreased renin catabolism may account for the high neonatal plasma renin activity. The half-life of renin in the infants, however, was determined by nephrectomizing individual littermate animals and killing each animal at different times for the determination of their plasma renin activity. Only one renin disappearance curve obtained from young rats was reported. Therefore, the effect of renin catabolism on plasma renin activity of young animals is not clearly established at this time.

From this discussion it is evident that the known mechanisms which regulate renin release in adult animals also may be present in the newborn. Although renin secretion could be a factor in elevating neonatal plasma renin activity, the absolute cause of this hyperreninemia has not been identified. The high plasma renin concentration and

consequently circulating angiotensin II may be a factor suppressing both total renal blood flow and outer cortical blood flow in the neonate. Consequently, the altered renal hemodynamics may lead to the immaturity of other renal functions such as glomerular filtration rate and renal sodium excretion.

5. Sodium Excretion by Young Animals After Saline Infusion

Numerous experiments have shown that young animals have a limited ability to increase renal sodium excretion following intravascular volume expansion by saline infusion (Kleinman and Reuter, 1974; Aperia et al., 1975b; McCance and Widdowson, 1957; Aperia et al., 1974b; Bengele and Solomon, 1974). The mechanisms limiting sodium excretion by newborns may involve; 1) a low glomerular filtration rate which would reduce the filtered sodium load, 2) low total renal blood flow accompanied by a shifted distribution of blood flow toward the inner cortex, 3) an inability of the neonatal kidney to decrease sodium reabsorption after volume expansion and 4) larger expansion of the extracellular fluid volume than intravascular volume after saline infusion.

Aperia et al. (1975b) have shown that glomerular filtration rate of infant humans increased exponentially with age whereas fractional sodium excretion increased linearly. This observation correlated with the experiments of Kleinman and Reuter (1974) in which saline infusion into both puppies and adult dogs increased glomerular filtration rate and therefore the filtered sodium load to a similar degree. Puppies however, excreted only 2.4% of the filtered sodium while adults were able to excrete 5.6%. Similar results have been reported in developing sheep (Aperia et al., 1975a). Thus, the data substantiate the lack of

a correlation between glomerular filtration rate and the ability of the young to increase sodium excretion after saline infusion.

The effect of changes in renal blood flow distribution on renal sodium excretion in adults was previously discussed. In young animals, the low total renal blood flow and low outer cortical flow originally were proposed to reduce sodium excretion. The limited response of lambs, 5-28 days of age, to enhance sodium excretion after volume expansion was correlated with an increase in blood flow to the inner cortex. This shift in blood flow following saline infusion was not observed in older animals (Aperia et al., 1975a). A conclusive interpretation of these data, however, was difficult due to the large variability in the ratio of inner cortical to outer cortical blood flow after saline infusion. Kleinman and Reuter (1974) investigated this question in puppies, and although saline expansion shifted blood flow toward the outer cortex, there was no correlation between changes in intrarenal blood flow distribution and sodium excretion. Thus, data concerning renal blood flow distribution and sodium excretion from newborn animals appears to coincide with those data from adult dogs suggesting that shifts in the distribution of intrarenal blood flow and the regulation of sodium excretion are not directly related.

Sodium excretion after intravascular volume expansion of newborns also may be altered by the inability of neonates to inhibit sodium reabsorption. Horster and Larsson (1976) have determined the hydraulic conductance of isolated perfused proximal tubules dissected from developing rabbits. Tubules from young rabbits showed significantly higher hydraulic conductivity than tubules from older animals. In addition, when tubules from rabbits 2-6 days of age were bathed in a

hyperoncotic medium, fluid reabsorption during perfusion increased by 40%. During perfusion of more mature tubules (30-38 days of age) in the same medium, fluid reabsorption only increased 19%. Following ultrastructural examination of both young and mature tubules, these authors suggested that age related differences in the development of intercellular connections may exist within the proximal tubule. On the basis of their conductance data, a paracellular shunt for both electrolytes and water was proposed in immature tubules. This "leaky" proximal tubule would then be more susceptible to the peritubular capillary oncotic pressure which may regulate sodium reabsorption in this nephron segment. Furthermore, if the vascular resistance of the outer cortex in the neonate is elevated, then the peritubular capillary hydrostatic pressure in the outer cortex would be low. Indeed, peritubular capillary hydrostatic pressure in the puppy was reported as 3 mmHg at 10 days of age which increased to 11 mmHg by 60 days after birth (Horster and Valtin, 1971). In developing dogs (Horster and Valtin, 1971) and rats (Ichikawa et al., 1978), proximal tubule hydrostatic pressure did not change. Therefore, in the young animal, a relatively high proximal tubular hydrostatic pressure is opposed by a low peritubular capillary hydrostatic pressure. This combination of hydrostatic forces favors tubular fluid reabsorption, and the newborn may not adequately increase sodium excretion after saline infusion due to an inability to decrease tubular sodium reabsorption.

The proposal that young animals fail to reduce tubular sodium reabsorption after volume expansion also has been investigated in vivo. In young rabbits, Horster and Larsson (1976) proposed that immature proximal tubules may reduce sodium excretion but Kleinman (1975) has

suggested that in puppies distal tubular sodium reabsorption is enhanced. In these experiments, distal tubule blockade of sodium transport with ethacrynic acid and chlorothiazide in animals undergoing saline diuresis decreased fractional sodium reabsorption from 98% to 51%. Distal blockade in hydropenic puppies decreased fractional sodium reabsorption to 70%, and when these animals were then volume expanded, sodium reabsorption fell even further to 49%. This latter fall in sodium reabsorption by 21% must have been due to inhibition of proximal tubular sodium reabsorption. Thus, it was concluded that the low sodium excretion after saline loading of puppies must have resulted from augmented distal reabsorption. Recently, these experiments have been confirmed utilizing the diuretic amiloride to provide distal tubular blockade of sodium reabsorption (Banks and Kleinman, 1978). These results indicated that at least in the puppy the reduced response to increase sodium excretion after a salt challenge may result from increased distal tubular sodium reabsorption.

Specific effects of prostaglandin $E_2$ and angiotensin II on sodium excretion by adult animals were previously discussed. Tubular sodium reabsorption in young animals also may be altered by these hormones. In adult animals, angiotensin II may directly increase sodium reabsorption in the proximal tubule (Steven and Thorpe, 1977), distal tubule (Vander, 1963; Lowitz et al., 1969) or both. Since the plasma angiotensin II concentration of young animals is elevated, the increased reabsorptive capacity of neonatal proximal or distal tubules may result from excess angiotensin II. Similarly, specific effects of prostaglandin $E_2$ on renal tubular sodium reabsorption have been described.

Terragno et al. (1978b) reported that renal medullary slices from fetal pigs synthesized more $PGE_2$, $PGI_2$ and $PGF_{2\alpha}$ than adult kidneys and suggested that the lower synthetic activity of adults was due to the presence of an endogenous prostaglandin synthetase inhibitor. Since this inhibitor did not seem to be present in fetal kidneys, the prostaglandins, $PGE_2$ and $PGI_2$ were suggested as important modulators of renal function in the fetus. Therefore, it is possible that the prostaglandins present in the neonatal kidney may significantly depress renal sodium excretion by increasing sodium reabsorption. Presently, however, the effect of angiotensin II or prostaglandins on the regulation of neonatal renal function has not been investigated.

After administration of a large saline load, the increase in vascular volume of neonates may be relatively small in comparison to changes in extracellular fluid volume. A low plasma protein concentration at birth has been documented (Pownall, 1970) which may result in a low oncotic pressure of the circulating plasma. The infused saline load could possibly leave the vascular space increasing the extracellular fluid and not become available for renal excretion. This hypothesis is supported by preliminary evidence that in puppies after addition of bovine serum albumin (10 g/dl) to a saline expansion fluid, plasma volume was significantly increased and sodium excretion was concomitantly enhanced (Arant, 1978). Clearly, further investigation is necessary to validate the specific effects of plasma oncotic pressure in the regulation of neonatal sodium excretion.

## RATIONALE

The morphological and functional development of neonatal kidneys begins within the inner cortex and proceeds toward the outer cortex (Ljundquist, 1963; Horster and Valtin, 1971; Spitzer and Brandis, 1974). Since the renal outer cortex is immature in young animals, significant differences may exist in renal function of infants when compared with adults. Piglets were chosen as the animal model in the present experiments primarily for two reasons. First, the anatomical size of newborn pigs allows for surgical procedures to be performed without producing extensive trauma. Secondly, the degree of maturity at birth of piglets and infant humans are comparable (Glauser, 1966).

Renal hemodynamics in young animals are characterized by a low total renal blood flow and low outer to inner cortical blood flow ratio. As the kidney matures, total renal blood flow increases and this increase in renal blood flow is accompanied by a proportionately greater increase in blood flow to the outer cortex than the inner cortex (Aschinberg et al., 1975; Kleinman and Reuter, 1973; Aperia et al., 1977). The immaturity of neonatal renal blood flow results in part from a high renal resistance (Gruskin et al., 1970). Since cardiac output and perfusion pressure also are low at birth, the maturational changes in renal hemodynamics may be partially due to the rising blood pressure (Kleinman and Lubbe, 1972).

The renal resistance component, however, may result from two factors. First, if the renal vascular spaces are not yet morphologically developed in the young animal, then these vessels might present a large resistance to kidney perfusion. In the present experiments, the renal vascular development of pigs 1-5, 18-22 and 45-50 days of age, was investigated. In piglets of each age group, perfusion of the renal vasculature with silastic rubber, Microfil, formed renal vascular casts which allowed observation of the structural development of the renal vasculature and localization of a possible site for increased renal resistance at younger ages. Secondly, the high plasma renin activity and circulating angiotensin II concentrations in the neonate, may constrict the renal vasculature resulting in the altered renal hemodynamics observed during the perinatal period. As the animal matures, plasma renin activity and plasma angiotensin II concentration decreases, which in turn may decrease renal vascular resistance in older animals. Therefore, in piglets 1-5, 18-22 and 45-50 days of age, renal blood flow and the intrarenal distribution of blood flow were determined both before and after competitive angiotensin II receptor blockade with $Sar^{1}$-$Ala^{8}$-angiotensin II (saralasin).

Because of the observed differences in plasma renin activity between younger and more mature animals, it was of interest to investigate possible mechanisms which may cause neonatal hyperreninemia. In adults, circulating angiotensin II directly inhibits further renin release from the juxtaglomerular apparatus (Naftilan and Oparil, 1978; Shade *et al.*, 1973) and since angiotensin II also is elevated in the young, these kidneys may have a decreased sensitivity to this negative feedback loop. To identify the negative feedback of angiotensin II on

the juxtaglomerular apparatus of developing piglets, plasma renin activity was determined before and after infusion of the competitive angiotensin antagonist, saralasin, into piglets 1-5, 18-22 and 45-50 days of age. Prostacyclin and $PGE_2$ also have been suggested as direct mediators of renin release in adult kidneys (Whorton et al., 1977a; Osborn et al., 1978; Dew and Michelakis, 1974). Furthermore, prostaglandin synthetase blockade with indomethacin decreased plasma renin activity in different species (Romero et al., 1976; Bailie et al., 1976; Speckart et al., 1977). Terragno et al. (1978b) have suggested that the concentration of $PGE_2$ and $PGI_2$ are elevated in the newborn, thus, renal $PGE_2$ and $PGI_2$ or both may mediate the high plasma renin activity observed following birth. This possibility was evaluated in piglets between the ages of 1 and 50 days by the determination of plasma renin activity before and after administration of indomethacin. In contrast to mechanisms which alter renin release from the kidney, the catabolism of renin also may affect the circulating concentration of the enzyme. The liver is the primary site of renin catabolism (Haecox et al., 1967). However, hepatic metabolic function is immature at birth (Greengard, 1974) suggesting that decreased renin catabolism by newborns could be responsible for the elevated plasma renin activity. To evaluate the possibility that reduced neonatal liver metabolism elevates plasma renin activity, the rate of disappearance of renin from the circulating plasma of nephrectomized piglets 1-5 and 45-50 days of age was determined.

In conjunction with the immaturity of renal hemodynamics in newborns, it has been observed that these young animals do not excrete

a saline load as rapidly as adults (Aperia et al., 1975b; Kleinman and Reuter, 1974; McCance and Widdowson, 1957). This inability to enhance sodium excretion may result from the low renal blood flow, low outer to inner cortical blood flow ratio or both. Although glomerular filtration rate also is low in neonates, a correlation between increasing glomerular filtration and increasing sodium excretion with age does not appear to exist (Aperia et al., 1975a). The regulation of plasma sodium concentration and plasma volume after saline loading by adult animals appears to result from inhibition of tubular sodium reabsorption thereby increasing sodium excretion. Experiments by Kleinman (1975) have shown that puppies decreased proximal tubular sodium reabsorption after volume expansion, but the inability of these animals to rapidly excrete the infused sodium load resulted from increased sodium reabsorption in the distal tubule. Arant (1978) also has presented evidence suggesting that a low plasma oncotic pressure in newborns may reduce sodium excretion by allowing the infused saline load to substantially increase the extravascular space rather than the vascular space. To more clearly identify the regulation of sodium excretion in neonates, renal function was assessed in conscious piglets 1-5 and 45-50 days of age both before and after volume expansion with saline and saline plus bovine serum albumin.

The limited renal response by young animals to increase sodium excretion after saline infusion also may be due to humoral factors present within the kidney or in the systemic circulation. In adult animals, $PGE_2$ and $PGI_2$ infusion increased renal blood flow and renal sodium excretion (Vander, 1968; Gross and Bartter, 1973; Bolger et al.,

1978). In addition, $PGE_2$ may directly affect tubular sodium transport (Tannenbaum et al., 1975; Stokes and Kokko, 1977). $PGE_2$ and $PGI_2$ were recently postulated as mediators of renal function in fetal pigs (Terragno et al., 1978b). It was demonstrated that fetal pig renal vascular strips synthesized greater amounts of $PGE_2$ and 6-keto $PGF_{1\alpha}$, the stable metabolic product of $PGI_2$, than adults. Therefore, renal prostaglandins may significantly alter sodium excretion in newborn animals. Another humoral factor which may regulate tubular sodium excretion is angiotensin II. Renal vasoconstriction by angiotensin II could decrease glomerular filtration rate and suppress renal sodium excretion. Angiotensin II also has been shown to directly alter renal tubular sodium reabsorption (Hughes-Jones et al., 1945; Langford, 1964; Vander, 1963). Therefore, the high circulating concentrations of angiotensin II in the newborn may affect renal sodium excretion following a salt challenge. In the present experiments, the effect of these humoral factors on renal sodium excretion was investigated by angiotensin II inhibition with saralasin and prostaglandin synthetase blockade with indomethacin in piglets 1-5, 18-22 and 45-50 days of age both before and after intravascular volume expansion.

## METHODS

1. Renal Vascular Patterns During Development of Piglets

The renal vasculature was defined in kidneys from anesthetized piglets 2, 20 and 50 days of age using the silastic rubber Microfil (MV-112, Canton Biomedical Products). Experiments were conducted on at least 2 animals at each age. Piglets were anesthetized with sodium pentobarbital (35 mg/kg i.p.), a tracheostomy performed and artifically ventilated with a positive pressure respirator (Harvard Apparatus). A femoral artery was cannulated with polyethylene tubing and the animal given 6 mg of sodium heparin. The right and left kidneys were exposed through a midline abdominal incision and two loose ligatures were positioned around the abdominal aorta, one anterior to the right renal artery and the other posterior to the left renal artery. The aorta was cannulated with polyethylene tubing from below the kidneys and the tip of the cannula placed between the ligatures. Upon ligation of the anterior ligature, infusion of a solution containing 140 mEq/liter sodium chloride and 3.2 mEq/liter potassium chloride warmed to 37°C was begun with a peristaltic pump. The ascending vena cava was clamped below the left renal vein and severed through an incision in the thoracic cavity for subsequent drainage and evacuation of perfusate.

Experiments were conducted utilizing 2 perfusion pressure protocols. Protocol A: Kidneys from piglets in each age group (2, 20 and

50 days) were perfused at low pressure (25-50 mmHg). In one experiment, a 2 day piglet was pretreated with acetylcholine (0.229 mg/kg/min i.v.) in order to dilate the renal vasculature and subsequently perfused at low perfusion pressure. Acetylcholine was initially infused at 0.440 mg/kg/min, a dose which decreased systemic blood pressure by 15 mmHg. The infusion rate was then decreased to 0.229 mg/kg/min and blood pressure returned to control. Protocol B: Kidneys from piglets 2 and 50 days of age were perfused at their physiologic systemic arterial blood pressure of approximately 75 and 125 mmHg, respectively. The kidneys in all experiments were cleared at these pressures with the warm saline until they were blanched. Infusion of a 2% glutaraldehyde solution at 37°C adjusted to pH 7.3 with 0.1 M sodium phosphate buffer was then begun. A period of 10-15 minutes was required for adequate fixation of the kidney tissue. During kidney fixation, perfusion pressures were maintained at the appropriate level by altering the rate of infusion. Microfil then was infused into the fixed renal vasculature through the same abdominal cannula. The Microfil medium consisted of Microfil compound and 5% by volume of dibutylene dilaurate (MV curing agent). To ensure complete vascular filling, the Microfil perfusion was maintained until the silicone perfusate was visualized on the kidney surface and within the renal veins. The renal arteries and veins then were clamped and the system left undisturbed _in situ_ to allow the silicone rubber to polymerize (30 minutes). The kidneys were removed and stored at 4°C overnight. Subsequently, kidney slices approximately 5 mm in diameter were cut perpendicular to the long axis of the kidney. These slices were serially dehydrated at 24 hour intervals with 25, 50, 75, 95 and 100% ethanol and the tissue cleared in

methyl salicylate for at least 24 hours. The 5 mm kidney sections were thin sliced (approximately 300 μ) manually, and the thin slices were suspended in methyl salicylate for examination by light microscopy.

2. General Surgical Procedures for Unanesthetized Piglet Experiments

Piglets between 1 and 50 days of age were anesthetized initially with a nitrous oxide-oxygen mixture (ratio 3:1) and ketamine HCl (Parke-Davis Co.). Animals 1-5 and 18-22 days of age received 10 mg/kg ketamine (i.m.), and piglets 45-50 days of age were administered 20 mg/kg (i.m.). Polyethylene catheters were placed in the descending aorta via a femoral artery, in the femoral vein and in the left external jugular vein. A polyethylene catheter was placed in the bladder via a small midline abdominal incision. A catheter also was placed in the left ventricle of the heart via the left carotid artery. Placement of this latter catheter was verified by the decrease in diastolic pressure at the time of catheter entry into the left ventricle and also by inspection at the end of each experiment. The incisions were closed using 4-0 silk suture. Throughout each experiment, body temperature was maintained at 38°C using a Thermistemp Temperature Controller (Yellow Springs Inst. Co.) and heat lamp. Each animal was placed in a comfort sling and at least 1 hour was allowed for recovery from anesthesia.

3. Verification of the Use of Radioactive Microspheres for the Determination of Total Renal Blood Flow and the Intrarenal Distribution of Blood Flow in Developing Piglets

Total renal blood flow and the intrarenal distribution of blood flow were determined by the radioactive microsphere technique previously

described for use in adult dogs (Warren and Ledingham, 1975; McNay and Abe, 1970; Katz et al., 1971) and puppies (Kleinman and Reuter, 1973). To determine if microsphere injection had any deleterious effects on cardiac or renal function of young piglets, microspheres (15±5 μ) were injected into piglets ranging in age from 1-23 days. Experiments were conducted on conscious animals surgically prepared as previously described. In 4 piglets 12-23 days old, approximately 100,000 microspheres (15±5 μ) labelled with either $Sr^{85}$ or $Ce^{141}$ were each consecutively injected into the left ventricle of the heart. The effect of microsphere injection on renal function also was determined in 2 additional piglets, 5 and 6 days of age. An infusion of $H^3$-methoxyinluin (2 μCi/ml at 0.03 ml/min/kg i.v.) was began 1 hour prior to the start of each experiment. The renal clearance of inulin was determined from the collection of urine during 2 control periods of 20 minutes duration each. An arterial blood sample (1 ml) was drawn at the midpoint of each collection period for the determination of plasma inulin concentration. Glomerular filtration rate was estimated from the inulin clearance. Microspheres (approximately 100,000) labelled with either $Sr^{85}$ or $Cr^{51}$ were randomly injected on 2 successive occasions, and each injection was followed by 2 additional 20 minute inulin clearance periods. Kidney sections from these experiments were fixed with 10% acetate buffered formalin. Thin section slices (30 μ) were made using a microtome (American Optical 820) and stained with hematoxylin and eosin for subsequent histologic examination.

The following techniques were used for microsphere injection, the determination of renal blood flow and the determination of intrarenal

distribution of blood flow. Immediately prior to injection, microspheres were suspended in 0.4 ml of 20% dextran solution and separated by sonification. Following microsphere injection, the catheter was flushed with 3.0 ml saline over 30 seconds. The stopcock was thoroughly rinsed to prevent contamination of later injections with differently labelled microspheres. A reference blood sample was withdrawn from the descending aorta at a rate of 1.75 ml/min for 1.5 minutes beginning just before the start of each microsphere injection. At the end of the experiment, kidneys were excised, decapsulated and weighed. To determine the outer to inner cortical blood flow ratio, the kidney was laid flat and 3 slices (3-5 mm wide) were removed by sectioning the kidney perpendicular to the long axis. One slice was removed about 8 mm from each pole of the kidney and one slice was removed from the middle of the kidney. Each slice then was laid flat and 4 sections were removed from around the circumference of the slice (2 sections from the dorsal side of the kidney slice and 2 from the ventral portion of the kidney slice). These 4 sections were quickly frozen in dry ice and acetone and sliced equally between outer and inner cortical tissue. The outer and inner cortical pieces then were pooled into their respective groups and weighed. Radioactivity in each group of 4 tissue slices, the reference blood sample and the remainder of each kidney was determined by gamma scintillation spectrometry (Searle Analytical Inc.). Total renal blood flow was calculated from the following equation:

$$\text{RBF (ml/min)} = \frac{\text{(Total kidney counts)(1.75 ml/min)}}{\text{Reference blood counts}}$$

Outer and inner cortical slice counts were normalized for tissue weight and the intrarenal distribution of blood flow was calculated as the ratio of outer to inner slice counts (O/I ratio). Values of O/I ratio and renal blood flow normalized for renal mass were calculated for each kidney and expressed as the mean of the two kidneys. Absolute renal blood flow also was determined for each kidney and expressed as the sum total of the blood flow to both kidneys.

4. Effect of Saralasin and Indomethacin on Renal Function in Developing Piglets

Experiments were performed on conscious piglets 1-5, 18-22 and 45-50 days of age and were surgically prepared as previously described. Inulin (6 g/dl) was infused intravenously at 0.03 ml/min/kg into 18-22 and 45-50 day old animals for the determination of glomerular filtration rate. Piglets 1-5 days of age received $H^3$-methoxy inulin (2 μCi/ml) at the same infusion rate. Inulin clearance, urine flow rate, sodium excretion and potassium excretion were determined from urine collection periods of 20 minutes duration in 1-5 day and 18-22 day old animals, while 10 minute periods were used in 45-50 day old pigs. Arterial blood (1 ml) was obtained at the midpoint of each urine collection period for the determination of plasma inulin, sodium and potassium concentration and plasma renin activity. Blood was placed in a chilled tube containing 10 μl of 0.4 molar ethylenediaminetetraacetic acid (EDTA) and was immediately centrifuged at 4°C (International Equipment Co.). The plasma then was removed and placed on ice and the packed cells were resuspended in a 3 g/dl bovine serum albumin (BSA) solution and returned to the animal to minimize blood loss.

Initially, two control clearance periods were obtained in each experiment. Angiotensin II inhibition then was achieved in six animals of each age group by the administration of saralasin (5-10 μg/kg/min). Angiotensin II blockade also was produced in three additional piglets 1-5 days of age, by treatment with the angiotensin I converting enzyme inhibitor SQ20,881 (100 μg/kg as a bolus followed by an infusion of 13 μg/min). In each experiment, the dose of saralasin or SQ20,881 was sufficient to completely block the rise in systemic blood pressure observed after injection of at least 2 μg angiotensin II or angiotensin I, respectively. Prostaglandin synthetase blockade was produced in seven 1-5 day piglets, six 18-22 and six 45-50 day animals by administration of indomethacin (3.0 mg/kg as a bolus followed by an infusion of 2.0 mg/kg/hr). Control experiments were conducted in seven, six and seven 1-5, 18-22 and 45-50 day pigs, respectively, replacing the saralasin or indomethacin with an infusion of saline at 0.03 ml/min/kg. Drug concentrations were calculated such that all compounds were infused at the same rate (0.03 ml/min/kg). At least 30 minutes were allowed for drug equilibration. Microspheres (15±5 μ) labelled with either $Sr^{85}$, $Ce^{141}$ or $Cr^{51}$, then were randomly injected into the left ventricle of the heart for the determination of renal blood flow and the intrarenal distribution of blood flow followed by two additional inulin clearance periods. While drug infusion was continued, all animals were infused intravenously with a solution containing 140 mEq/liter NaCl and 3.2 mEq/liter KCl in a volume equal to 2% of the body weight over 30 minutes. The infusion rate of saline was adjusted at the end of the 30 minute infusion period to equal the urine flow

rate. Microspheres were again injected and two post-saline infusion clearance periods collected.

5. Regulation of Plasma Renin in Developing Piglets

The effects of volume expansion, saralasin, indomethacin and the combination of saralasin and indomethacin with volume expansion on plasma renin were determined in unanesthetized piglets between 1 and 50 days of age. Plasma renin was determined on all arterial samples collected in the previous experiment (Section 4) before and after drug treatment as well as before and after volume expansion.

Anesthetized piglets were used to estimate neonatal renin metabolism. Piglets 1-5 and 45-50 days of age were anesthetized with sodium pentobar-- bital (35 mg/kg i.v.), a tracheostomy performed and artificially ventilated with a positive pressure respirator (Harvard Apparatus). A femoral artery and femoral vein were cannulated with polyethylene tubing. Both kidneys were exposed by a midline incision and a loose ligature was placed around the renal artery and renal vein tfo each kidney. Renin release then was stimulated by ligating both ureters and injecting 25-50 µg of isoproterenol intravenously. Isoproterenol induced a transient fall in systemic blood pressure of approximately 30 mmHg. Plasma renin concentration was allowed to increase for 15 minutes at which time the renal artery and vein of both kidneys were ligated and the kidneys removed. The time of ligation of the second kidney was designated time zero. An arterial blood sample (1.0 ml) was immediately collected and placed in a chilled tube containing 10 µl of 0.4 M EDTA. Blood was centrifuged at 4°C (International Equipment Co.) and the plasma was removed and placed on ice. The packed cells were resuspended

in a 3 g/dl BSA solution and returned to the animal to minimize blood loss. Subsequent blood samples were collected at 5, 10, 15, 30, 60 and 90 minutes post-nephrectomy.

6. Effect of Intravascular Volume Expansion with Isocontic Bovine Serum Albumin Solution (BSA) on Renal Function of Developing Piglets

Piglets 1-5 and 45-50 days of age were surgically prepared as previously described. $H^3$-Methoxyinulin (2 μCi/ml) and inulin (6 g/dl) were infused intravenously (0.03 ml/min/kg) to 1-5 and 45-50 day old pigs, respectively, for at least 1 hour prior to the start of each experiment. Inulin clearance, urine flow rate, sodium excretion and potassium excretion were determined from urine collection periods of 20 minutes duration in 1-5 day old piglets and periods of 10 minutes duration in 45-50 day animals. Arterial blood was obtained at the midpoint of each urine collection period for the determination of plasma inulin concentration and plasma renin activity. The blood was placed in a chilled tube containing 10 μl of 0.4 M EDTA and was immediately centrifuged at 4°C. The plasma was removed and placed on ice and the packed cells were resuspended in a 3 g/dl bovine serum albumin solution and returned to the animal to minimize blood loss. Microspheres labelled with either $Sr^{85}$ or $Ce^{141}$ were injected initially into the left ventricle of the heart for the determination of control renal blood flow and the intrarenal distribution of blood flow. Microsphere injection was followed by two control clearance periods. The intravascular volume of animals then was expanded with either a sodium-potassium chloride solution (140 mEq/l $Na^+$ and 3.2 mEq/l $K^+$) or with an identical solution containing bovine serum albumin (4 g/dl). Animals of each age group were infused with saline or saline plus BSA equal to

2% of the body weight (low saline load). In other experiments, animals of each age group were infused with saline or saline plus BSA in a volume equal to 8% of the body weight (high saline load). The total volume of saline was administered over a 30 minute period. All solutions were warmed and maintained at 37°C throughout the experiment. At the end of the initial 30 minute loading period, the infusion of each solution was adjusted to equal the urine flow rate. Microspheres were again injected and two post-volume expansion clearance periods collected.

7. Analytical and Statistical Procedures

Systemic blood pressure was measured in all experiments using a pressure transducer (Statham P23AA) and a direct writing oscillograph (Grass polygraph). Urine and plasma inulin concentration from experiments in 18-22 and 45-50 day pigs were determined by the method of Walser et al. (1955). $H^3$-Methoxy inulin concentration in urine and plasma from 1-5 day piglets was determined by liquid scintillation spectrometry (Beckman Instruments). The clearance of inulin from the plasma was used to estimate glomerular filtration rate and calculated in milliliters per minute as the product of urine flow rate and urine inulin concentration divided by the plasma inulin concentration. Sodium and potassium concentration of both urine and plasma were determined by flame photometry (Instrumentation Laboratories, Inc.). The percentage of filtered sodium or potassium excreted was calculated from the following equation:

$$F_{Na^+ \text{ or } K^+}(\%) = \frac{(Na^+ \text{ or } K^+)u \cdot (\dot{V}) \cdot 100}{(GFR) \cdot (Na^+ \text{ or } K^+)p \cdot (0.95)}$$

$(Na^+ \text{ or } K^+)u$ = Urinary sodium or potassium concentration

$\dot{V}$ = Urine flow rate

GFR = Glomerular filtration rate

$(Na^+ \text{ or } K^+)p$ = Plasma sodium or potassium concentration

(0.95) = Gibbs-Donnan equilibrium constant for positive singly charged ions

Hematocrit of arterial blood samples was obtained by the micromethod. Renal plasma flow was calculated as the product of one minus the hematocrit and the absolute renal blood flow. Filtration fraction then was calculated as the quotient of glomerular filtration rate and renal plasma flow. Renal resistance was determined by dividing the mean systemic blood pressure by the total renal blood flow. Total body surface area (SA) was calculated from the following formula:

$SA = 0.097\ (BW^{\cdot 693})$ where BW = body weight in kilograms (Brody, 1945).

Plasma renin activity was determined by radioimmunoassay for angiotensin I and expressed as ng/ml/hr (Haber et al., 1969). Plasma renin concentration was determined in renin disappearance experiments by incubation of plasma samples in the presence of hog renin substrate prepared by the method of Skeggs et al. (1963).

The effects of saralasin and indomethacin treatment on renal function (Section 4) were compared to control animals in each age group by a one-way analysis of variance. The effect of intravascular volume expansion during drug treatment within animals of each age group was analyzed by a two-way analysis of variance. These mean differences were tested by the least significance difference. The effect of

saralasin, indomethacin and volume expansion on plasma renin activity is expressed as the percent change from control. Values were compared to 0% change and analyzed by the Student's $t$ procedure (Sokal and Rohlf, 1969). The effects of intravascular volume expansion with saline and saline plus BSA on renal function (Section 6) were compared to control values in each age group by a paired t analysis. The rate of disappearance of renin from the systemic circulation (Section 5) was plotted as the log of renin concentration versus time. Lines representing the decrease in renin with time were calculated by linear regression (least squares method) and the renin half-life was determined as the quotient of 0.693 and the slope of the regression line (Fingl and Woodbury, 1975). The slopes, y-intercepts and renin half-times of 1-5 and 45-50 day old pigs were analyzed by a one-way analysis of variance and mean differences were compared by the least significant difference test. Comparisons of normal renal function between 1-5, 18-22 and 45-50 day pigs were made by one-way analysis of variance and mean differences were tested by the Student-Neuman-Keuls procedure. The 0.05 level of probability was considered significant for all data.

# RESULTS

## 1. Patterns of Renal Vascular Development in Piglets

### Protocol A: Low Perfusion Pressure (25-50 mmHg)

The renal vasculature of piglets 2, 20 and 50 days of age was perfused with saline, glutaraldehyde and the silastic rubber Microfil at 25-50 mmHg to determine the pattern of renal vascular development. The perfusion pressure of 25-50 mmHg was chosen because it was less than the normal perfusion pressure at any age. In 2 day old piglets, afferent arterioles and glomerular capillaries exhibited relatively low resistance to vascular filling at this low perfusion pressure as indicated by the filling with silastic rubber (Figure 1). Renal efferent arterioles and peritubular capillaries, however, were poorly perfused particularly within the inner cortex (Figure 1). Although some peritubular capillary filling was demonstrated in the outer cortical areas (Figure 1), under high magnification (100X) efferent arterioles could not be visualized in the inner cortex at this age after low perfusion pressure fixation. Also, the size of the 2-day piglet interlobar arteries projecting toward the cortical surface appeared to be smaller than interlobar arteries in older animals (Figure 1). The vasculature of piglets 20 days of age demonstrated adequate afferent arteriolar and glomerular capillary filling (Figure 2). In this age animal, inner cortical peritubular capillary filling was increased, but the outer cortex remained poorly perfused beyond the glomerular capillaries (Figure 2).

Figure 1. Renal vascular cast of a 2 day old piglet following perfusion with Microfil at low pressure (25-50 mmHg). The subcapsular outer cortex is shown in the upper left corner and the vasa rectae of the outer medulla are shown in the lower right corner. Light photomicrograph (25X).

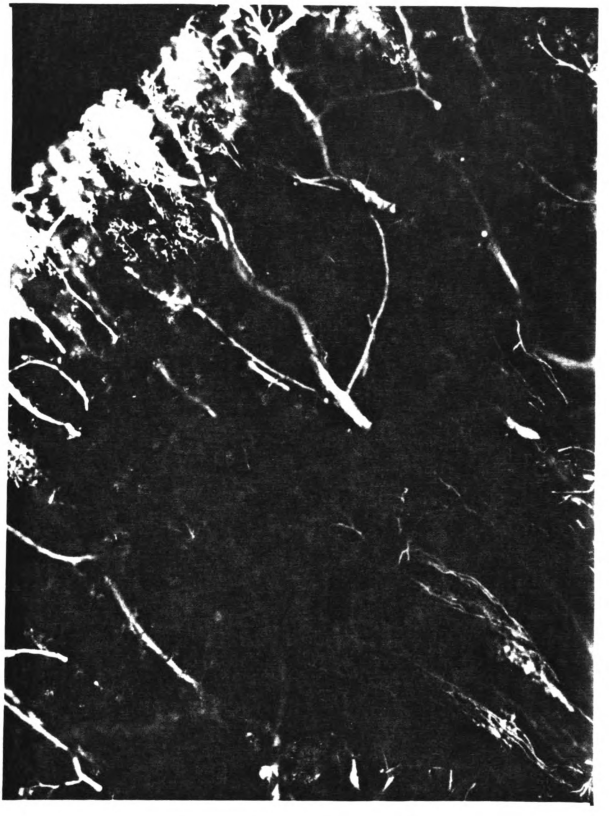

Figure 1

Figure 2. Renal vascular cast of a 20 day old pig following perfusion with Microfil at low pressure (25-50 mmHg). The outer cortex is shown at the top of the figure and the inner cortex is shown at the bottom of the figure. Light photomicrograph (30X).

Figure 2

By 50 days of age, low pressure renal vascular perfusion not only filled afferent arterioles and glomerular capillaries but also filled the peritubular capillaries throughout the cortex (Figure 3). In these kidneys the interlobar arteries (bottom of Figure 3 with arcuate and interlobular arteries branching to the cortex) appeared relatively large in comparison to the younger animals.

Protocol B: Physiologic Perfusion Pressure (2 day: 75 mmHg, 50 day: 125 mmHg)

The renal vascular filling of 2 day old piglet kidneys perfused at 75 mmHg is shown in Figure 4. In contrast to filling observed at low perfusion pressure (Figure 1), both afferent arterioles and peritubular capillaries throughout the renal cortex were visible at the higher perfusion pressure. In 50 day old piglets, physiologic perfusion pressure revealed vascular filling similar to that observed in 2 day old piglets (Figure 5). Furthermore, the postglomerular capillary filling at this physiologic perfusion pressure in 50 day old animals was similar to the vascular filling observed in 50 day pigs at low perfusion pressure (Figure 3).

To further assess the differences between renal vascular filling of 2 day old piglets at low perfusion pressure and high perfusion pressure, acetylcholine (0.229 mg/kg/min i.v.) was infused prior to renal vascular fixation. In this experiment, renal vascular filling of the 2 day piglet was accentuated (Figure 6). This increased filling was observed in the postglomerular vessels of both the outer and inner cortex, but when compared to untreated 2 day kidneys (Figure 1) the most striking effect appeared in the inner cortical peritubular capillaries (Figure 6).

Figure 3. Renal vascular cast of a 50 day old pig following perfusion with Microfil at low pressure (25-50 mmHg). The outer cortex is shown at the top of the figure and the inner cortex is at the bottom of the figure. Light photomicrograph (25X).

Figure 3

Figure 4. Renal vascular cast of a 2 day old piglet following perfusion with Microfil at physiologic pressure (75 mmHg). The outer cortex is shown at the top of the figure and the vasa rectae of the outer medulla are at the bottom of the figure. Light photomicrograph (20X).

Figure 4

Figure 4. Renal vascular cast of a 2 day old piglet following perfusion with Microfil at physiologic pressure (75 mmHg). The outer cortex is shown at the top of the figure and the vasa rectae of the outer medulla are at the bottom of the figure. Light photomicrograph (20X).

Figure 4

Figure 5. Renal vascular cast of a 50 day old pig following perfusion with Microfil at physiologic pressure (125 mmHg). The outer cortex is shown at the top of the figure and the inner cortex is at the bottom of the figure. Light photomicrograph (20X).

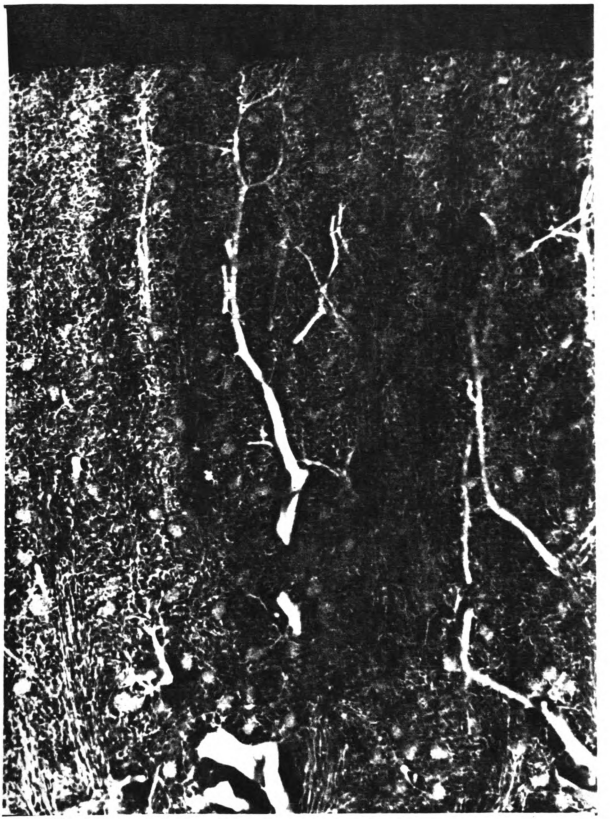

Figure 5

Figure 6. Renal vascular cast of 2 day old piglet pretreated with acetylcholine (0.229 mg/kg/min i.v.) and perfused with Microfil at low pressure (25-50 mmHg). The outer cortex is shown at the top of the figure and the vasa rectae of the medulla are present at the bottom of the figure. Light photomicrograph (20X).

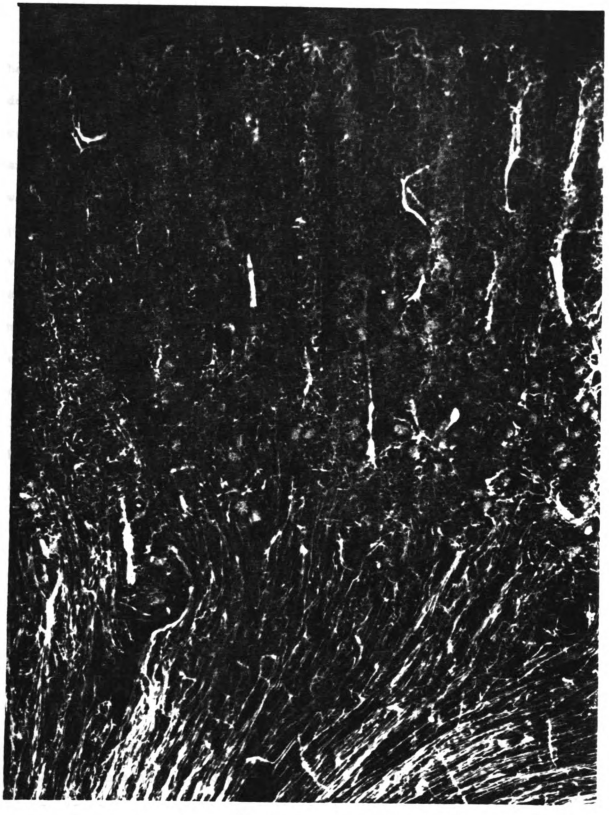

Figure 6

## 2. Verification of the Use of Radioactive Microspheres for the Determination of Renal Blood Flow and the Intrarenal Distribution of Blood Flow in Developing Piglets

The effect of microsphere injection into the left ventricle of the heart on systemic blood pressure, heart rate and renal hemodynamics was determined in 4 piglets. Injection of 0.4 ml of 20 g/dl dextran solution containing approximately 200,000 microspheres decreased blood pressure and increased heart rate in experiment #1 (Table 1). Heart rate also was elevated after microsphere injection in experiment #2, but blood pressure was unchanged (Table 1). It was determined that these cardiovascular changes were induced by rapid saline clearing of the catheter after the microspheres were injected. Subsequent injections in experiments #3 and #4 by the technique described in Methods did not affect either blood pressure or heart rate (Table 1). In these experiments, approximately 200,000 microspheres injected on 2 successive occasions increased renal blood flow in experiment #1 but did not affect renal blood flow or the intrarenal distribution of blood flow in subsequent experiments (Table 1). In 2 additional piglets, 5 and 6 days of age, the effect of microsphere injection on renal function was tested. Injection of a total of approximately 200,000 microspheres (100,000 injected twice) did not decrease urine flow rate, glomerular filtration rate, fractional sodium excretion or fractional potassium excretion in either experiment (Table 2). Histologic examination of kidney sections from these piglets showed microspheres to be lodged either within the glomerular capillaries or within the afferent arterioles (Figure 7). Low power examination of these kidneys demonstrated a small number of glomeruli containing microspheres (Figure 8). In Figure 8, approximately 40 glomeruli are shown, but only 2 microspheres were identified

TABLE 1

Effect of Microsphere Injection on Systemic Blood Pressure, Heart Rate and Renal Hemodynamics in Young Piglets

| | Experiment | Control | During Sphere Injection 1 | Post-Sphere Injection 1 | During Sphere Injection 2 | Post-Sphere Injection 2 |
|---|---|---|---|---|---|---|
| Heart Rate | 1 | 112 | 204 | 161 | 141 | 171 |
| | 2 | 148 | 171 | 207 | 159 | 185 |
| | 3 | 72 | 71 | 69 | 71 | 69 |
| | 4 | 62 | 60 | 60 | 62 | 65 |
| Blood Pressure | 1 | 76 | 40 | 76 | 41 | 70 |
| | 2 | 86 | 75 | 80 | 83 | 85 |
| | 3 | 93 | 96 | 95 | 92 | 95 |
| | 4 | 85 | 86 | 87 | 92 | 87 |
| RBF | 1 | | | 17.3 | | 32.3 |
| | 2 | | | 40.7 | | 37.9 |
| | 3 | | | 122.2 | | 118.5 |
| | 4 | | | ----- | | ----- |
| O/I Ratio | 1 | | | 1.095 | | .934 |
| | 2 | | | .923 | | 1.142 |
| | 3 | | | 1.793 | | 1.712 |
| | 4 | | | 1.102 | | 1.186 |

Heart Rate = beats/min; Blood Pressure = mmHg; RBF = Renal Blood Flow (ml/min); O/I ratio = Ratio of outer to inner cortical blood flow.

TABLE 1

Effect of Microsphere Injection on Systemic Blood Pressure, Heart Rate and Renal Hemodynamics in Young Piglets

| | Experiment | Control | During Sphere Injection 1 | Post-Sphere Injection 1 | During Sphere Injection 2 | Post-Sphere Injection 2 |
|---|---|---|---|---|---|---|
| Heart Rate | 1 | 112 | 204 | 161 | 141 | 171 |
| | 2 | 148 | 171 | 207 | 159 | 185 |
| | 3 | 72 | 71 | 69 | 71 | 69 |
| | 4 | 62 | 60 | 60 | 62 | 65 |
| Blood Pressure | 1 | 76 | 40 | 76 | 41 | 70 |
| | 2 | 86 | 75 | 80 | 83 | 85 |
| | 3 | 93 | 96 | 95 | 92 | 95 |
| | 4 | 85 | 86 | 87 | 92 | 87 |
| RBF | 1 | | | 17.3 | | 32.3 |
| | 2 | | | 40.7 | | 37.9 |
| | 3 | | | 122.2 | | 118.5 |
| | 4 | | | ----- | | ----- |
| O/I Ratio | 1 | | | 1.095 | | .934 |
| | 2 | | | .923 | | 1.142 |
| | 3 | | | 1.793 | | 1.712 |
| | 4 | | | 1.102 | | 1.186 |

Heart Rate = beats/min; Blood Pressure = mmHg; RBF = Renal Blood Flow (ml/min); O/I ratio = Ratio of outer to inner cortical blood flow.

TABLE 2

Effect of Microsphere Injection on Renal Function in Newborn Piglets

| Experiment | Control | | | | Post-Sphere #1 (100,000 microspheres) | | | | Post-Sphere #2 (100,000 microspheres) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | V | GFR | $F_{Na}+$ | $F_K+$ | V | GFR | $F_{Na}+$ | $F_K+$ | V | GFR | $F_{Na}+$ | $F_K+$ |
| 1 | .106 | 6.90 | .18 | 45.6 | .126 | 8.84 | .13 | 51.2 | .146 | 7.11 | .18 | 58.6 |
| 2 | .117 | 5.83 | .16 | 40.0 | .129 | 7.17 | .18 | 48.8 | .281 | 9.19 | 1.18 | 39.5 |

V = Urine flow rate (ml/min); GFR = Glomerular Filtration Rate (ml/min); $F_{Na}+$ = Fractional sodium excretion (%); $F_K+$ = Fractional potassium excretion (%).

Figure 7. Light photomicrograph (100X) of radiolabelled microsphere (15±5 μ) lodged at the junction of the afferent arteriole and glomerular capillaries. The photograph was obtained from a renal cortical slice (30 μ) of a 5 day old piglet after injection of approximately 200,000 microspheres into the left ventricle of the heart.

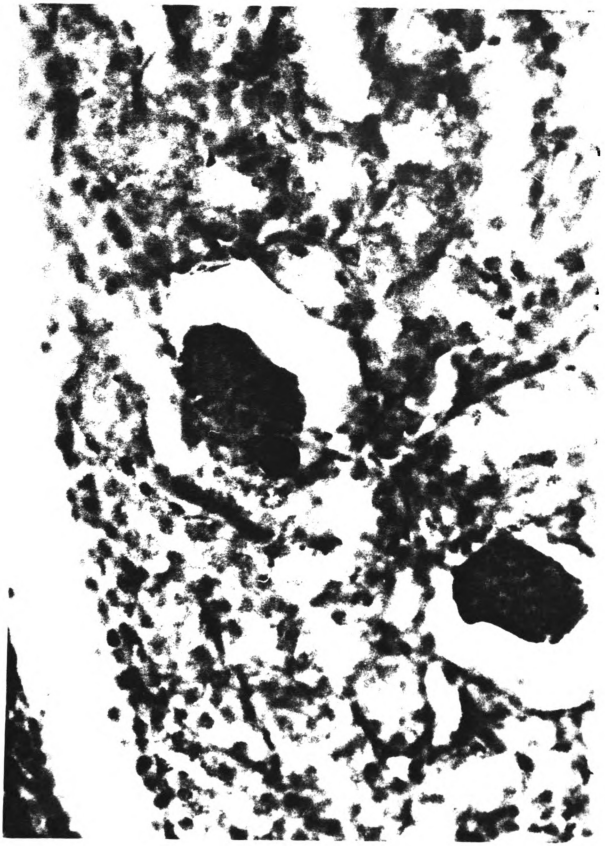

Figure 7

Figure 8. Light photomicrograph (40X) of the renal cortex from a 5 day old piglet following injection of 200,000 microspheres (15±5 μ) into the left ventricle of the heart. Microspheres lodged within the afferent arterioles are designated by the arrows. The outer cortex is at the top of the figure.

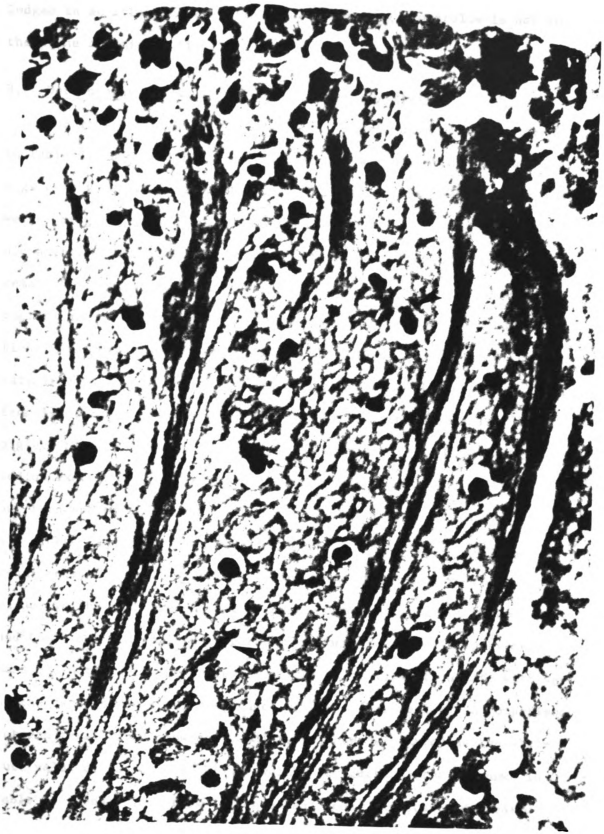

Figure 8

in this section. The microsphere near the bottom of the figure is lodged in an afferent arteriole whose connecting glomerulus is not in the plane of this section.

3. Development of Renal Function in Conscious Piglets

The growth rate of piglets between 1 and 50 days of age is shown in Table 3. Body weight, kidney weight and surface area increased in pigs over this age range (Table 3). The ratio of kidney weight to body weight progressively decreased between 1-5 day piglets and 45-50 day old animals (Table 3). Kidney weight to surface area ratio, however, remained unchanged between 1 and 50 days of age (Table 3). Developing piglets also demonstrated several age dependent changes in renal function. Systemic blood pressure, renal blood flow, glomerular filtration rate (Figure 9), O/I ratio and filtration fraction (Table 4) increased from 1-5 through 45-50 days of age. Renal resistance decreased in piglets between the ages of 1 and 50 days (Figure 9). Fractional sodium excretion also decreased between birth and 22 days of age, but then sodium excretion increased slightly in older animals (Table 4). These changes were calculated without correction for increases in renal mass with age. When renal blood flow was normalized for increased kidney weight with age, no significant change was apparent between 1-5 and 45-50 day old piglets (Figure 10). Conversely, dividing by renal mass did not affect the increase in glomerular filtration rate with age or the decrease in renal resistance with age (Figure 10). A comparison between renal blood flow and renal resistance calculated as the absolute value or factored by kidney weight and surface area is shown in Table 5. Renal blood flow increased and renal resistance decreased

TABLE 3

Developmental Changes in Kidney Weight, Body Weight and Surface Area of Pigs

| | Body Weight (kg) | Surface Area ($m^2$) | Kidney Weight (g) | KW/BW | KW/SA |
|---|---|---|---|---|---|
| 1-5 Day | 2.00 | 0.150 | 17.13 | 8.61 | 114.2 |
| ± SEM | 0.08 | 0.004 | 0.71 | 0.27 | 3.5 |
| n | 26 | 26 | 25 | 25 | 25 |
| 18-22 Day | 4.66[a] | 0.258[a] | 30.72[a] | 6.65[a] | 119.3 |
| ± SEM | 0.12 | 0.005 | 1.09 | 0.27 | 4.5 |
| n | 18 | 18 | 18 | 18 | 18 |
| 45-50 Day | 10.32[a,b] | 0.423[a,b] | 51.82[a,b] | 5.10[a,b] | 122.5 |
| ± SEM | 0.36 | 0.010 | 1.51 | 0.13 | 2.6 |
| n | 28 | 28 | 28 | 28 | 28 |

n = Number of observations; SEM = standard error of the mean; KW/BW = kg/g; KW/SA = $g/m^2$.

[a]Significantly different from 1-5 day piglets.

[b]Significantly different from 18-22 day piglets.

Figure 9. **Arterial blood pressure (BP), renal blood flow (RBF),** glomerular filtration rate (GFR) and renal resistance (RR) in piglets 1-5, 18-22 and 45-50 days of age. Absolute values are shown without factoring for kidney weight. Each bar indicates the mean ± standard error of the mean. ( ) = number of observations. * = Significantly different from 1-5 day piglets ($p<0.05$). § = Significantly different from 18-22 day piglets ($p<0.05$).

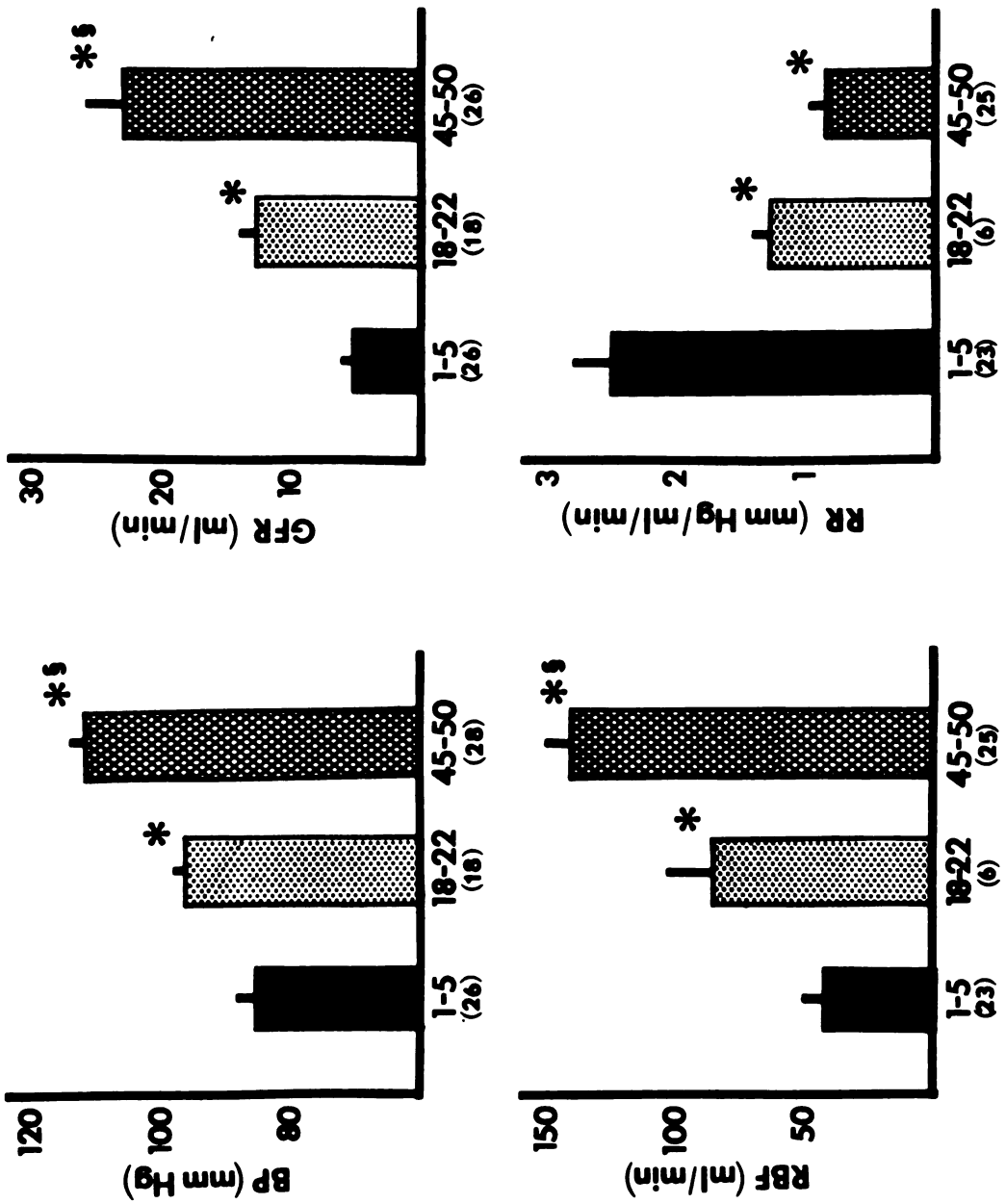
GFR (ml/min)
30
20
10
1-5 (26)
18-22 (18)
45-50 (26)
RR (mm Hg/ml/min)
3
2
1
1-5 (23)
18-22 (6)
45-50 (25)
BP (mm Hg)
120
100
80
1-5 (26)
18-22 (18)
45-50 (28)
RBF (ml/min)
150
100
50
1-5 (23)
18-22 (6)
45-50 (25)

Figure 9

TABLE 4

Fractional Sodium Excretion, O/I Ratio and Filtration Fraction in Developing Piglets

| | $F_{Na}+$ (%) | O/I Ratio | Filtration Fraction |
|---|---|---|---|
| 1-5 Day | 0.27 | 1.196 | 0.178 |
| ± SEM | 0.04 | 0.057 | 0.018 |
| n | 26 | 23 | 23 |
| 18-22 Day | 0.09[a] | 1.349 | 0.245 |
| ± SEM | 0.01 | 0.105 | 0.027 |
| n | 17 | 6 | 6 |
| 45-50 Day | 0.20 | 1.478[a] | 0.254[a] |
| ± SEM | 0.05 | 0.055 | 0.031 |
| n | 26 | 27 | 22 |

SEM = Standard error of the mean; O/I = Outer to inner cortical blood flow ratio; n = Number of observations.

[a]Significantly different from 1-5 day piglets ($p<0.05$).

Figure 10. Renal blood flow (RBF), renal resistance (RR) and glomerular filtration rate (GFR) in piglets 1-5, 18-22 and 45-50 days of age. Values shown are calculated per gram kidney weight. Each bar indicates the mean ± the standard error of the mean. ( ) = Number of observations. * = Significantly different from 1-5 day piglets ($p<0.05$).

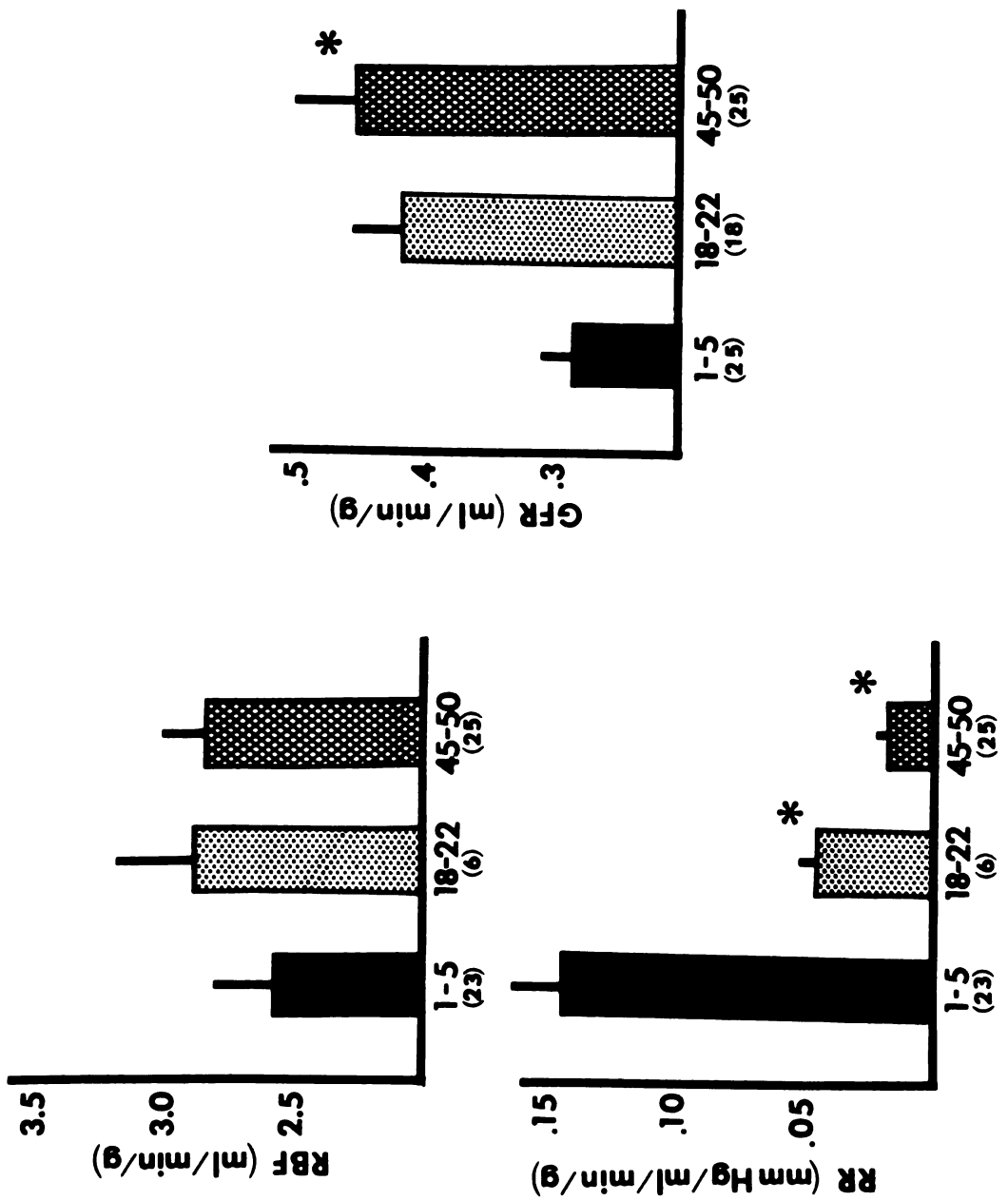
GFR (ml/min/g)
.5
.4
.3
1-5 (25)
18-22 (18)
45-50 (25)
*
RBF (ml/min/g)
3.5
3.0
2.5
1-5 (23)
18-22 (6)
45-50 (25)
RR (mm Hg/ml/min/g)
.15
.10
.05
1-5 (23)
18-22 (6)
45-50 (25)
*
*

Figure 10

TABLE 5

Renal Blood Flow and Renal Resistance in Developing Pigs

| | | 1-5 Day (23) | 18-22 Day (6) | 45-50 Day (25) |
|---|---|---|---|---|
| Renal Blood Flow | ml/min | 44.3±4.5 | 84.0±12.9[a] | 143.5±8.8[a,b] |
| | ml/min/g | 2.527±0.227 | 2.867±0.289 | 2.831±0.163 |
| | $ml/min/m^2$ | 292.5±27.1 | 328.7±46.6 | 339.4±18.3 |
| Renal Resistance | mmHg/ml/min | 2.30±0.23 | 1.25±0.16[a] | 0.85±0.05[a] |
| | mmHg/ml/min/g | 0.145±0.019 | 0.045±0.006 | 0.017±0.002[a] |
| | $mmHg/ml/min/m^2$ | 15.81±1.82 | 4.95±0.63[a] | 2.07±0.16[a] |

[a] Significantly different when compared to 1-5 day pigs ($p<0.05$).

[b] Significantly different when compared to 18-22 day pigs ($p<0.05$).

Values expressed as mean ± standard error of mean. ( ) = Number of observations.

between 1 and 50 days of age when calculated as absolute flow and resistance (Table 5, Figure 9). Renal resistance also decreased with age when calculated per gram kidney weight and per square meter body surface area (Table 5). However, when renal blood flow was factored by either kidney weight or body surface area, the age-related increase in blood flow was not apparent (Table 5).

4. Effect of Saralasin and Indomethacin on Renal Function in Unanesthetized, Developing Piglets

In all animals, initial values of urine flow rate, fractional sodium excretion ($F_{Na}+$), fractional potassium excretion ($F_{K}+$), glomerular filtration rate, blood pressure and hematocrit prior to drug treatment were determined. In each age group, pretreatment values were compared between piglets which would subsequently be administered saralasin, indomethacin or saline. There were no significant differences observed in any of these parameters between animals of each age group tested (Table 6).

Renal blood flow and the intrarenal distribution of blood flow (O/I ratio) were determined in control, saralasin and indomethacin treated piglets between 1 and 50 days of age. In 1-5 day old piglets, neither saralasin nor indomethacin altered renal blood flow, O/I ratio (Figure 11), renal resistance or filtration fraction (Figure 12). In the 45-50 day age group, again saralasin did not alter renal hemodynamics or filtration fraction (Figures 11 and 12). However, in this older age group, renal blood flow after indomethacin treatment averaged 2.112±0.221 ml/min/g which was significantly lower than control renal blood flow of 3.446±0.446 ml/min/g at this age (Figure

TABLE 6

Urine Flow Rate (V), Glomerular Filtration Rate (GFR), Fractional Sodium Excretion ($F_{Na}+$), Fractional Potassium Excretion ($F_K+$), Blood Pressure (BP) and Hematocrit (Hct) Prior to Drug Treatment[a]

| | Group | V (ml/min) | GFR (ml/min) | $F_{Na}+$ (%) | $F_K+$ (%) | BP (mmHg) | Hct (%) |
|---|---|---|---|---|---|---|---|
| 1-5 Day | Control (7) | 0.074±0.014 | 4.62±0.60 | 0.23±0.05 | 16.37±5.89 | 82±2 | 28.5±1.8 |
| | Saralasin (7) | 0.042±0.007 | 3.34±0.74 | 0.29±0.09 | 14.54±6.71 | 79±4 | 28.7±1.8 |
| | Indomethacin (6) | 0.058±0.016 | 4.04±0.50 | 0.27±0.07 | 15.39±5.13 | 81±3 | 26.3±0.7 |
| 18-22 Day | Control (6) | 0.095±0.012 | 14.18±1.23 | 0.05±0.01 | 4.93±1.54 | 95±5 | 28.6±3.4 |
| | Saralasin (6) | 0.111±0.014 | 11.83±1.22 | 0.07±0.01 | 10.54±2.54 | 98±2 | 24.8±3.8 |
| | Indomethacin (6) | 0.109±0.027 | 11.55±2.24 | 0.13±0.03 | 9.11±2.57 | 97±2 | 23.7±4.1 |
| 45-50 Day | Control (7) | 0.131±0.019 | 25.82±7.18 | 0.10±0.05 | 17.77±9.58 | 114±6 | 30.8±1.8 |
| | Saralasin (6) | 0.182±0.039 | 37.92±9.31 | 0.16±0.06 | 6.71±2.11 | 112±6 | 27.7±0.9 |
| | Indomethacin (6) | 0.182±0.028 | 32.16±3.40 | 0.11±0.03 | 13.92±3.38 | 118±5 | 30.4±1.2 |

[a]Values are mean ± 1 standard error of the mean. () = Number of observations.

Figure 11. Effect of saralasin and indomethacin on renal blood flow (RBF) and outer to inner cortical blood flow ratio (O/I ratio) in piglets 1-5, 18-22 and 45-50 days of age. C = control, S = saralasin, I = indomethacin. Each bar indicates the mean ± the standard error of the mean. * = Significantly different from control experiments ($p<0.05$).

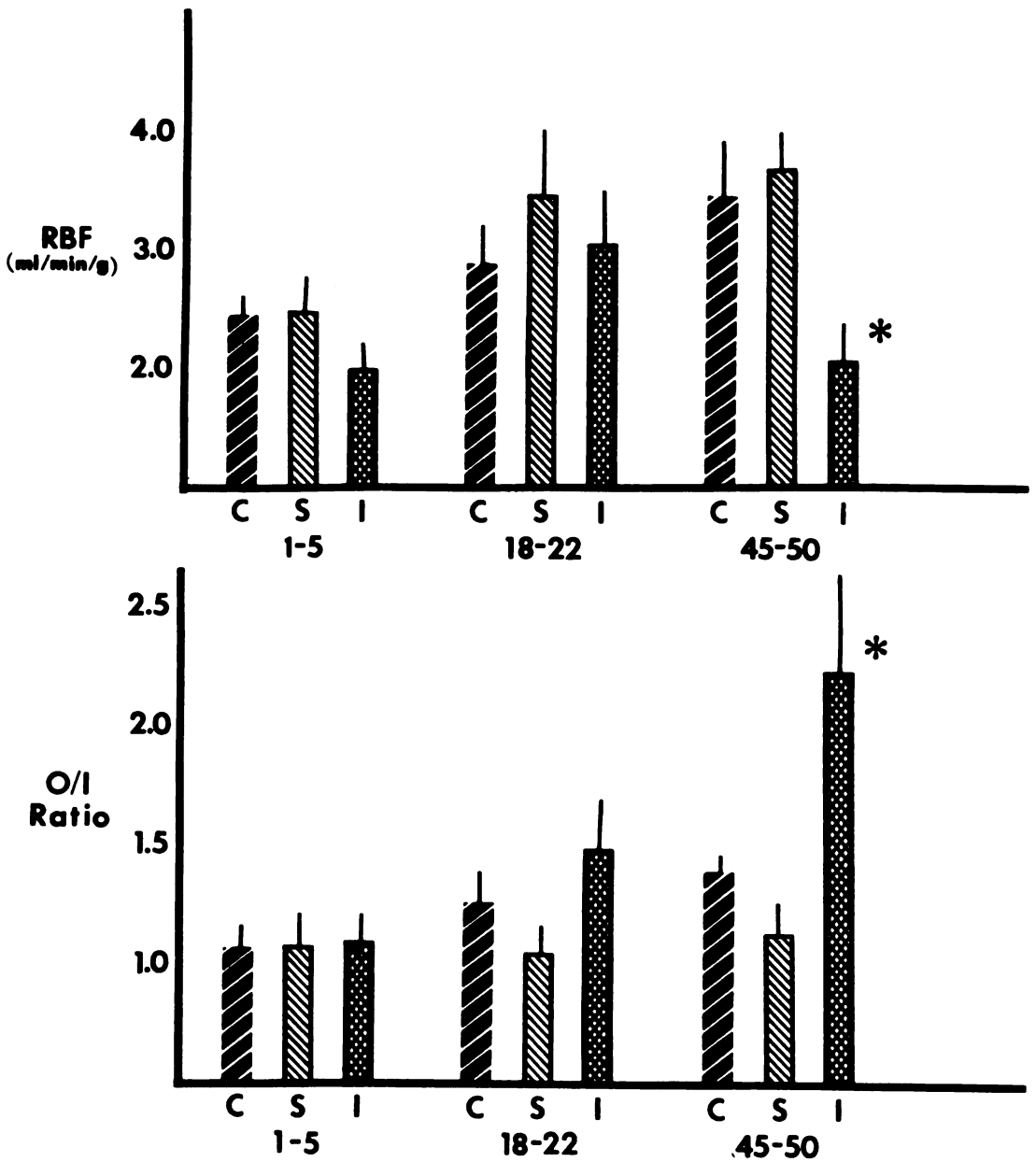
RBF
(ml/min/g)
4.0
3.0
2.0
C S I C S I C S I
1-5
18-22
45-50
*
O/I
Ratio
2.5
2.0
1.5
1.0
C S I C S I C S I
1-5
18-22
45-50
*

Figure 11

Figure 12. Effect of saralasin and indomethacin on filtration fraction (FF) and renal resistance (RR) in piglets 1-5, 18-22 and 45-50 days of age. C = control, S = saralasin, I = indomethacin. Each bar indicates the mean ± the standard error of the mean. * = Significantly different from control experiments ($p<0.05$).

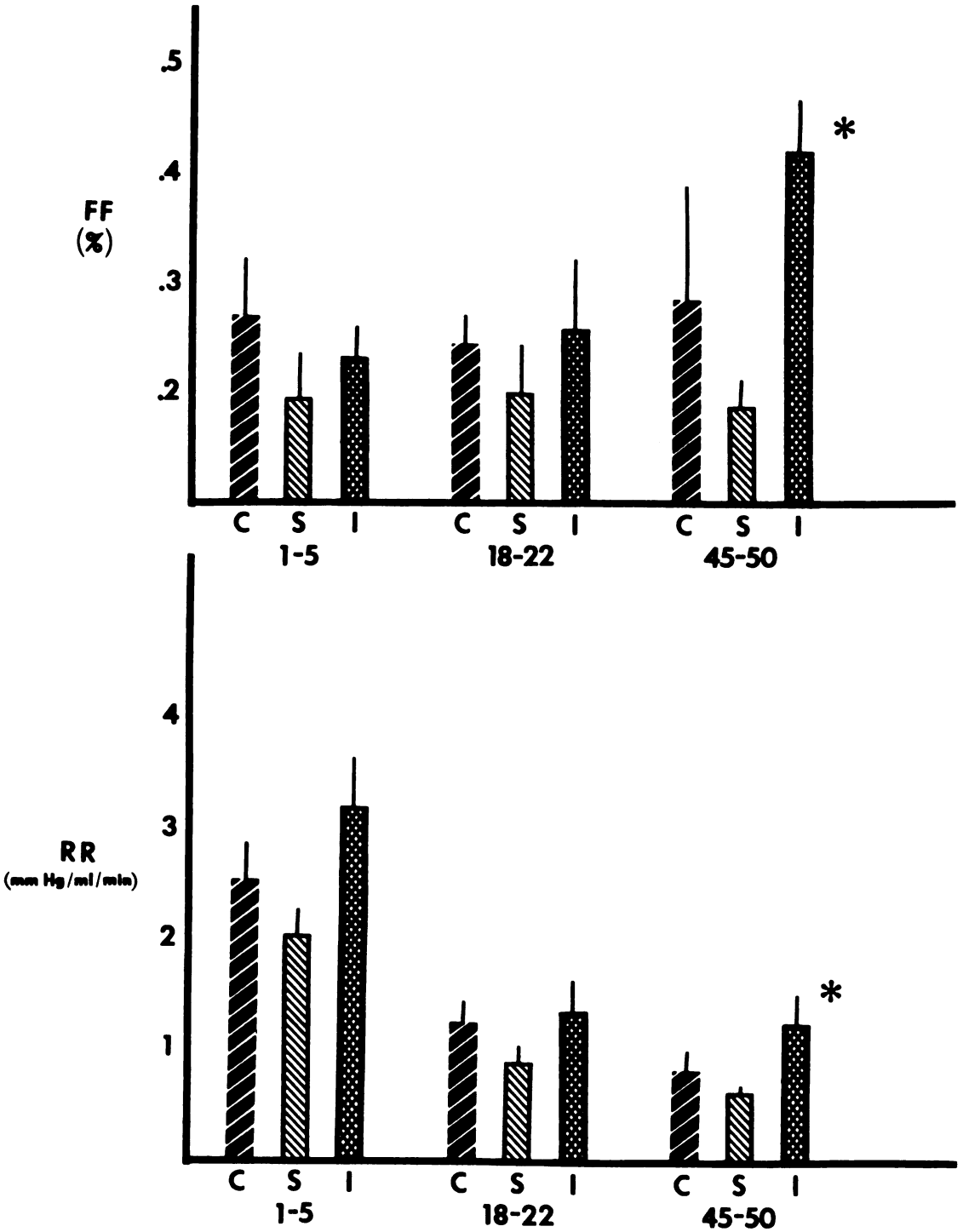
.5
.4
.3
.2
FF
(%)
*
C S I C S I C S I
1-5
18-22
45-50
4
3
2
1
RR
(mm Hg/ml/min)
*
C S I C S I C S I
1-5
18-22
45-50

Figure 12

11). Indomethacin also significantly increased O/I ratio of 45-50 day pigs when compared to control experiments (Figure 11). These changes in renal blood flow following indomethacin were associated with concomitant increases in filtration fraction and renal resistance (Figure 12).

The effect of prostaglandin synthetase inhibition on renal blood flow and O/I ratio of unanesthetized 45-50 day old pigs was verified in three experiments. Renal blood flow and O/I ratio were measured by microspheres injected prior to and after indomethacin administration. Indomethacin decreased renal blood flow and increased O/I ratio and renal resistance in all three animals tested (Table 7).

The effect of angiotensin II blockade with saralasin and renal prostaglandin synthetase inhibition with indomethacin on the excretion of an acute sodium load also was examined in these developing piglets. Intravascular volume expansion alone did not alter renal blood flow, O/I ratio, renal resistance or filtration fraction in piglets 1 to 50 days of age (Table 8). Despite the inability of volume expansion to change renal hemodynamics at any of these ages, saline infusion increased fractional sodium excretion in 1-5, 18-22 and 45-50 day pigs (Figure 13). In each age group, volume expansion also slightly increased urine flow rate and fractional potassium excretion but glomerular filtration rate was unchanged (Table 8).

Saralasin administration alone did not change fractional sodium excretion from control values in 1-5 day old animals. In these young piglets however, angiotensin II inhibition blocked the volume expansion induced natriuresis previously observed in control experiments (Figure 13). At 18-22 and 45-50 days of age, this inhibition of the natriuresis by saralasin was not present, and volume expansion resulted in a

TABLE 7

Effect of Indomethacin on Renal Hemodynamics in Unanesthetized 45-50 Day Old Pigs

| Expt. | RBF | | O/I | | RR | |
|---|---|---|---|---|---|---|
| | C | I | C | I | C | I |
| 1 | 5.391 | 3.263 | 1.403 | 1.691 | 0.514 | 0.747 |
| 2 | 4.246 | 2.594 | 1.695 | 2.521 | 0.439 | 0.761 |
| 3 | 2.699 | 2.243 | 1.577 | 2.154 | 0.725 | 0.934 |
| Mean | 4.112 | 2.700 | 1.558 | 2.122 | 0.559 | 0.814 |
| ± SEM | 0.780 | 0.299 | 0.085 | 0.240 | 0.086 | 0.060 |

RBF = Renal Blood Flow (ml/min/g); O/I = Outer to Inner cortical blood flow ratio; RR = Renal Resistance (mmHg/ml/min); C = control; I = Indomethacin; SEM = standard error of the mean.

TABLE 8

Effect of Volume Expansion on Renal Function of Control, Saralasin and Indomethacin Treated Piglets

| | | Control | Volume Expansion | Saralasin | Volume Expansion | Indomethacin | Volume Expansion |
|---|---|---|---|---|---|---|---|
| 1-5 | RBF (5) | 2.40±0.13 | 2.40±0.49 | 2.42±0.30 | 2.07±0.19 | 1.98±0.18 | 1.94±0.22 |
| | O/I (6) | 1.05±0.09 | 1.16±0.07 | 1.06±0.15 | 1.07±0.11 | 1.06±0.14 | 1.12±0.10 |
| | RR (5) | 2.55±0.32 | 2.24±0.41 | 2.00±0.27 | 2.35±0.31 | 3.18±0.47 | 3.41±8.77 |
| | FF (5) | 0.27±0.05 | 0.27±0.05[a] | 0.19±0.04 | 0.28±0.04[a] | 0.23±0.03 | 0.28±0.09 |
| | V (7) | 0.08±0.01 | 0.14±0.03[a] | 0.05±0.01 | 0.08±0.02[a] | 0.04±0.01 | 0.12±0.05 |
| | GFR (7) | 5.21±0.65 | 6.03±0.99 | 5.10±0.92 | 6.57±0.67 | 4.85±0.69 | 5.11±0.72 |
| | $F_K+$ (7) | 18.09±6.71 | 28.29±9.21 | 15.83±3.68 | 22.19±4.18 | 8.96±2.67 | 22.18±7.53 |
| 18-22 | RBF (6) | 2.87±0.29 | 3.08±0.42 | 3.43±0.51 | 3.49±0.43 | 3.03±0.42 | 3.32±0.58 |
| | O/I (6) | 1.25±0.11 | 1.36±0.16 | 1.04±0.11 | 0.96±0.08 | 1.46±0.20 | 1.33±0.11 |
| | RR (6) | 1.25±0.16 | 1.18±0.16 | 0.88±0.13 | 0.80±0.07 | 1.35±0.29 | 1.26±0.21 |
| | FF (6) | 0.24±0.03 | 0.24±0.04 | 0.20±0.04 | 0.19±0.04 | 0.26±0.06 | 0.21±0.04 |
| | V (6) | 0.12±0.01 | 0.29±0.09[a] | 0.18±0.05 | 0.31±0.07[a] | 0.12±0.02 | 0.80±0.36[a] |
| | GFR (6) | 13.48±0.82 | 13.91±1.61 | 13.63±2.77 | 13.61±2.25 | 16.12±3.11 | 15.37±1.84 |
| | $F_K+$ (6) | 7.30±1.94 | 27.48±5.85[a] | 12.98±3.36 | 33.71±6.58[a] | 6.20±1.94 | 24.95±6.36[a] |
| 45-50 | RBF (6) | 3.45±0.47 | 3.71±0.52 | 3.64±0.30 | 3.82±0.29 | 2.11±0.22 | 2.86±0.44 |
| | O/I (6) | 1.37±0.05 | 1.42±0.08 | 1.10±0.13 | 1.46±0.10 | 2.12±0.38 | 1.89±0.36 |
| | RR (6) | 0.84±0.16 | 0.76±0.12 | 0.62±0.07 | 0.60±0.06 | 1.27±0.23 | 1.00±0.19 |
| | FF (6) | 0.28±0.10 | 0.21±0.05 | 0.18±0.03 | 0.14±0.03 | 0.41±0.05 | 0.34±0.05 |
| | V (7) | 0.34±0.13 | 0.53±0.21 | 0.24±0.07 | 0.32±0.01 | 0.42±0.29 | 1.55±0.72 |
| | GFR (7) | 26.58±7.41 | 24.09±6.36 | 30.10±4.22 | 24.33±5.69 | 31.96±3.90 | 32.96±2.46 |
| | $F_K+$ (7) | 19.18±8.93 | 27.33±8.57[a] | 13.15±3.33 | 35.50±15.97 | 11.44±3.85 | 20.04±7.05 |

[a] $p<0.05$; () = Number of observations; RBF = Renal Blood Flow (ml/min/g); O/I = Outer to Inner Cortical Blood Flow Ratio; RR = Renal Resistance (mmHg/ml/min); FF = Filtration Fraction; V = Urine Flow Rate (ml/min); GFR = Glomerular Filtration Rate (ml/min); $F_K+$ = Fractional Potassium Excretion (%).

Figure 13. Effect of intravascular volume expansion (VE) on fractional sodium excretion ($F_{Na}+$) of control (C), saralasin (SAR) and indomethacin (INDO) treated piglets 1-5, 18-22 and 45-50 days of age. Each bar indicates the mean ± the standard error of the mean. * = Significantly different from treatment periods ($p<0.05$).

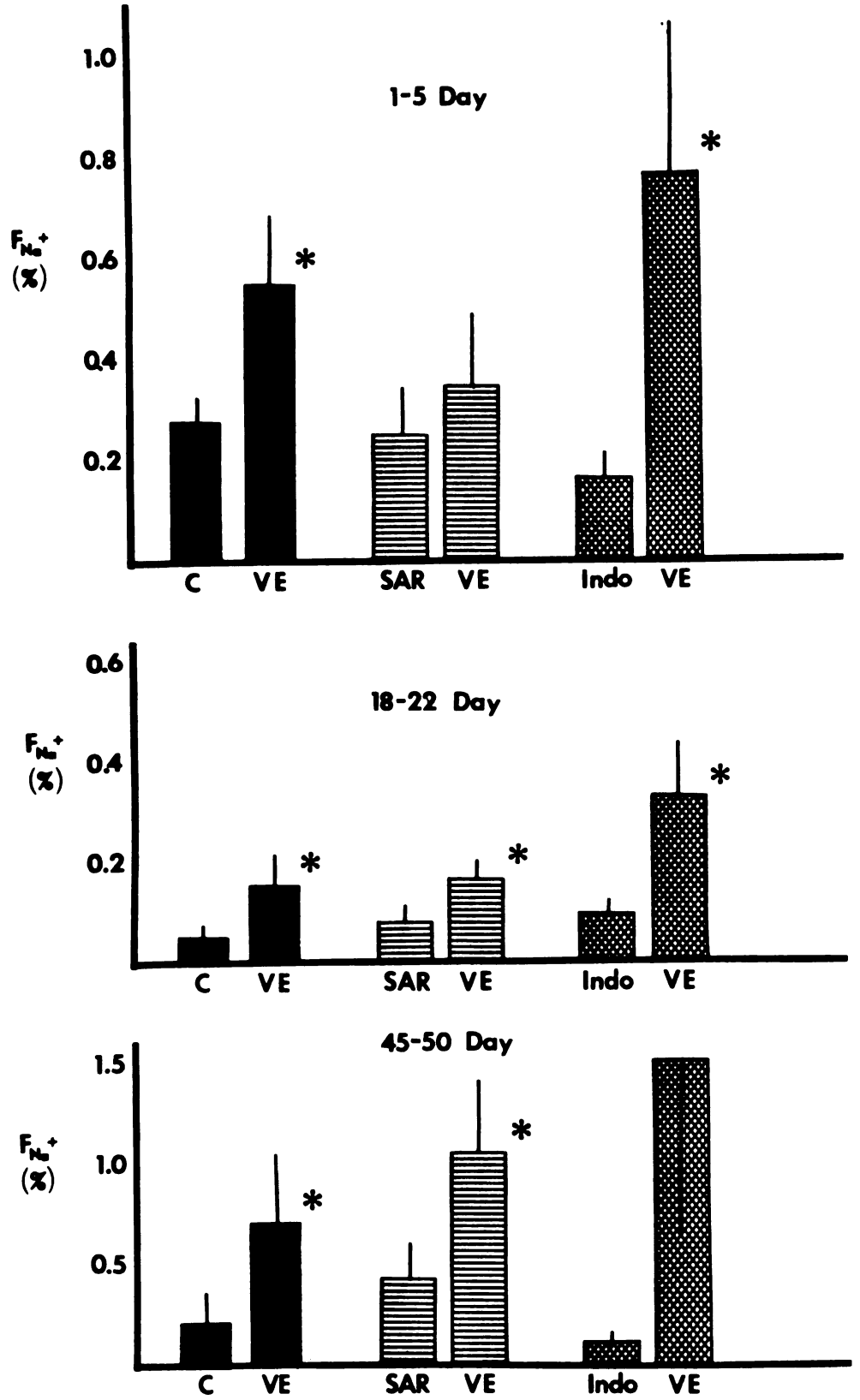
1-5 Day
1.0
0.8
0.6
0.4
0.2
$F_{Na^+}$ (%)
*
C
VE
SAR
VE
Indo
VE
18-22 Day
0.6
0.4
0.2
45-50 Day
1.5
1.0
0.5

Figure 13

significant increase in fractional sodium excretion during saralasin infusion (Figure 13). In all saralasin treated pigs, volume expansion did not affect renal blood flow, O/I ratio, renal resistance or filtration fraction (Table 8).

Since in 1-5 day piglets saralasin inhibited the volume expansion induced increase in fractional sodium excretion, the effect of angiotensin II inhibition on renal sodium excretion was further tested using the antiotensin I converting enzyme inhibitior SQ20,881. Three piglets 1-5 days of age were treated with SQ20,881. After angiotensin I converting enzyme blockade, volume expansion did not change fractional sodium excretion in 2 of the 3 piglets tested (Table 9). Intravascular volume expansion after angiotensin II blockade of piglets in all three age groups slightly increased urine flow rate and fractional potassium excretion but did not change glomerular filtration rate (Tables 8 and 9).

Treatment of piglets in each age group with indomethacin did not alter fractional sodium excretion from control values. Volume expansion of indomethacin treated 1-5 and 18-22 day old animals significantly increased fractional sodium excretion similar to those increases observed after volume expansion of control piglets (Figure 13). In pigs 45-50 days of age receiving indomethacin, saline loading increased fractional sodium excretion in 4 out of 6 animals. Due to the large variability in the magnitude of this response, the increase was not statistically significant (Figure 13). Volume expansion in all of the indomethacin treated pigs produced small increases in urine flow rate and fractional potassium excretion but did not affect glomerular filtration rate (Table 8). In addition, volume expansion after indomethacin

TABLE 9

Effect of SQ20,881 on Urine Flow Rate and Fractional Sodium Excretion Before and After Volume Expansion in 1-5 Day Piglets

| Experiment | V | | | $F_{Na^+}$ | | |
|---|---|---|---|---|---|---|
| | C | SQ | VE | C | SQ | VE |
| 1 | 0.051 | 0.051 | 0.068 | 0.26 | 0.10 | 0.50 |
| 2 | 0.052 | 0.035 | 0.046 | 0.19 | 0.17 | 0.35 |
| 3 | 0.051 | 0.050 | 0.075 | 0.67 | 0.37 | 0.41 |
| Mean | 0.051 | 0.045 | 0.063 | 0.37 | 0.21 | 0.42 |
| ± SEM | 0.0003 | 0.005 | 0.009 | 0.15 | 0.08 | 0.04 |

V = Urine Flow Rate (ml/min); $F_{Na^+}$ = Fractional Sodium Excretion (%); C = Control; SQ = SQ20,881; VE = Volume Expansion; SEM = standard error of the mean.

did not change renal hemodynamics in pigs of any age group (Table 8).

5. Regulation of Plasma Renin in Developing Piglets

The plasma renin activities of untreated piglets 1-5, 18-22 and 45-50 day of age are shown in Figure 14. Plasma renin activity of 1-5 day old animals averaged 13.38±1.68 ng/ml which was not significantly different from piglets 18-22 days of age (17.32±2.73 ng/ml). By 45-50 days after birth, the plasma renin activity had decreased to 9.32±0.72 ng/ml. This value was significantly lower than the plasma renin activities measured in 18-22 day old piglets (Figure 14).

The effect of saralasin, indomethacin and intravascular volume expansion on circulating plasma renin activity was examined in developing piglets. In control experiments of each age group, plasma renin activity remained constant and also did not change following saline infusion in a volume equalling 2% of the body weight (Figure 15). Angiotensin II receptor blockade with saralasin, markedly increased plasma renin activity in 1-5 and 18-22 day old animals (Figure 15). Subsequent volume expansion, however, decreased plasma renin activity in both age groups (Figure 15). In 45-50 day old pigs, saralasin alone did not affect plasma renin activity (Figure 15). Volume expansion of older piglets in the presence of saralasin significantly suppressed plasma renin activity (Figure 15). Prostaglandin synthetase blockade with indomethacin did not affect plasma renin activity in 1-5 or 45-50 day piglets but slightly decreased the plasma renin activity in 18-22 day animals (Figure 15). Although mild volume expansion alone did not change PRA, volume expansion in the presence of indomethacin, signifi-

Figure 14. Plasma renin activity (PRA) in piglets 1-5, 18-22 and 45-50 days of age. Individual points are the plasma renin activities for each animal. Squares indicate the mean plasma renin activity for each age group. * = Significantly different from 18-22 day piglets ($p<0.05$).

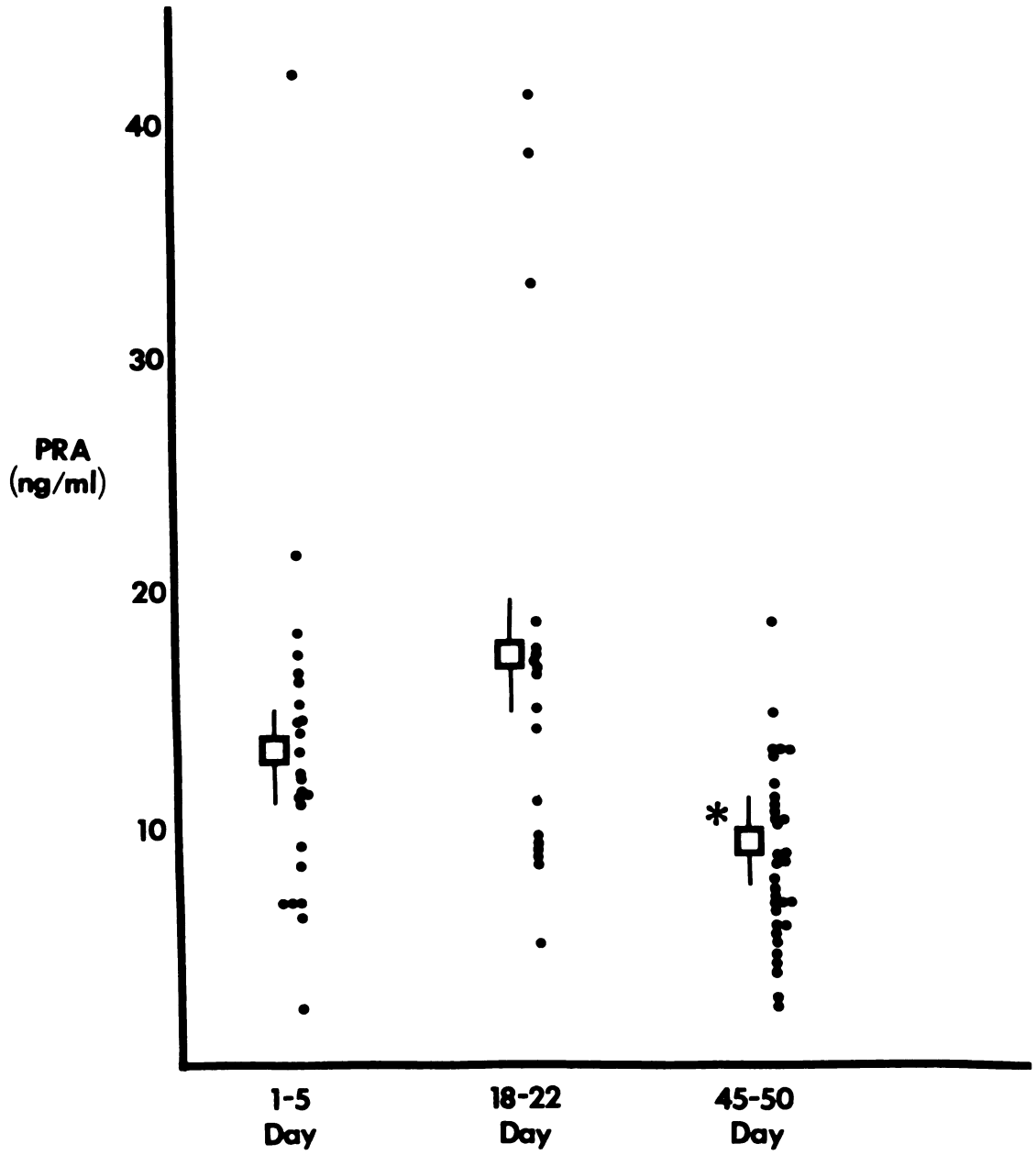


Figure 14

Figure 15. Change in plasma renin of developing piglets following volume expansion (VE), saralasin (SAR) and indomethacin (INDO). Con = control values. * = Significantly different from zero ($p<0.05$).

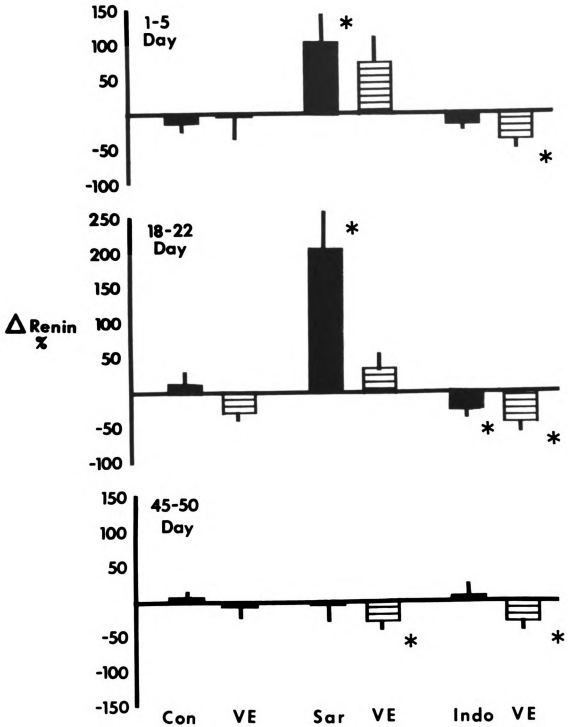
150
100
50
-50
-100
1-5
Day
*
*
250
200
150
100
50
-50
-100
18-22
Day
Δ Renin
%
*
*
*
150
100
50
-50
-100
-150
45-50
Day
*
*
Con
VE
Sar
VE
Indo
VE

Figure 15

cantly decreased the plasma renin activity of 1-5, 18-22 and 45-50 day piglets (Figure 15).

The disappearance of renin from the systemic circulation also was investigated in piglets 1-5 and 45-50 days of age. The calculated half-life of renin in 1-5 day piglets was 17.08±3.04 minutes which was not significantly different from the renin half-life of 12.92±0.95 minutes determined for 45-50 day pigs (Table 10). Similarly, the mean slopes of the regression lines for 1-5 and 45-50 day piglets were not different (Table 10). The regression lines for each individual experiment of 1-5 and 45-50 day old pigs are shown in Figures 16 and 17, respectively. Regression lines for renin disappearance in 1-5 day old piglets were more variable than the older animals. In 5 of the 7 experiments within this age group, the regression lines are nearly parallel (Figure 16), whereas 2 experiments produced steep slopes and correspond to the experiments with renin half times of 7.79 and 7.14 minutes (Table 10). Regression analysis of 45-50 day old piglets, however, exhibit less variation resulting in nearly parallel lines of renin disappearance from the plasma (Figure 17). Calculated correlation coefficients of variation for all lines were greater than 0.70.

6. Excretion of a Sodium Load in Developing Piglets: Effect of BSA

Experiment A: Effect of Low Intravascular Volume Expansion (2% of BW) and Low Intravascular Volume Expansion + Bovine Serum Albumin (BSA) on Renal Function in Developing Piglets

Mild saline infusion (2% of body weight) did not affect renal blood flow in piglets 1-5 or 45-50 days of age (Figure 18). After addition of BSA (4 g/dl) to the saline infusion solution, volume

TABLE 10

Catabolism of Renin in Piglets 1-5 and 45-50 Days of Age

| | Experiment | Slope | Y-Intercept | $T_{\frac{1}{2}}$ |
|---|---|---|---|---|
| 1-5 Day Piglets | 1 | -0.032 | 32.46 | 21.66 |
| | 2 | -0.055 | 24.36 | 12.60 |
| | 3 | -0.029 | 53.79 | 23.90 |
| | 4 | -0.037 | 80.32 | 18.73 |
| | 5 | -0.089 | 55.65 | 7.79 |
| | 6 | -0.097 | 96.83 | 7.14 |
| | 7 | -0.025 | 82.93 | 27.72 |
| | Mean | -0.052 | 60.91 | 17.08 |
| | ± SEM | 0.011 | 10.21 | 3.04 |
| 45-50 Day Piglets | 1 | -0.070 | 36.16 | 9.90 |
| | 2 | -0.055 | 83.93 | 12.60 |
| | 3 | -0.043 | 43.25 | 16.17 |
| | 4 | -0.063 | 32.59 | 11.00 |
| | 5 | -0.053 | 27.50 | 13.08 |
| | 6 | -0.047 | 32.75 | 14.75 |
| | Mean | -0.055 | 42.70 | 12.92 |
| | ± SEM | 0.004 | 8.52 | 0.95 |

SEM = Standard error of the mean.

Figure 16. Disappearance of renin from the circulating plasma of 1-5 day old piglets after bilateral nephrectomy. Regression lines of renin disappearance in 7 animals are shown. PRC = plasma renin concentration.

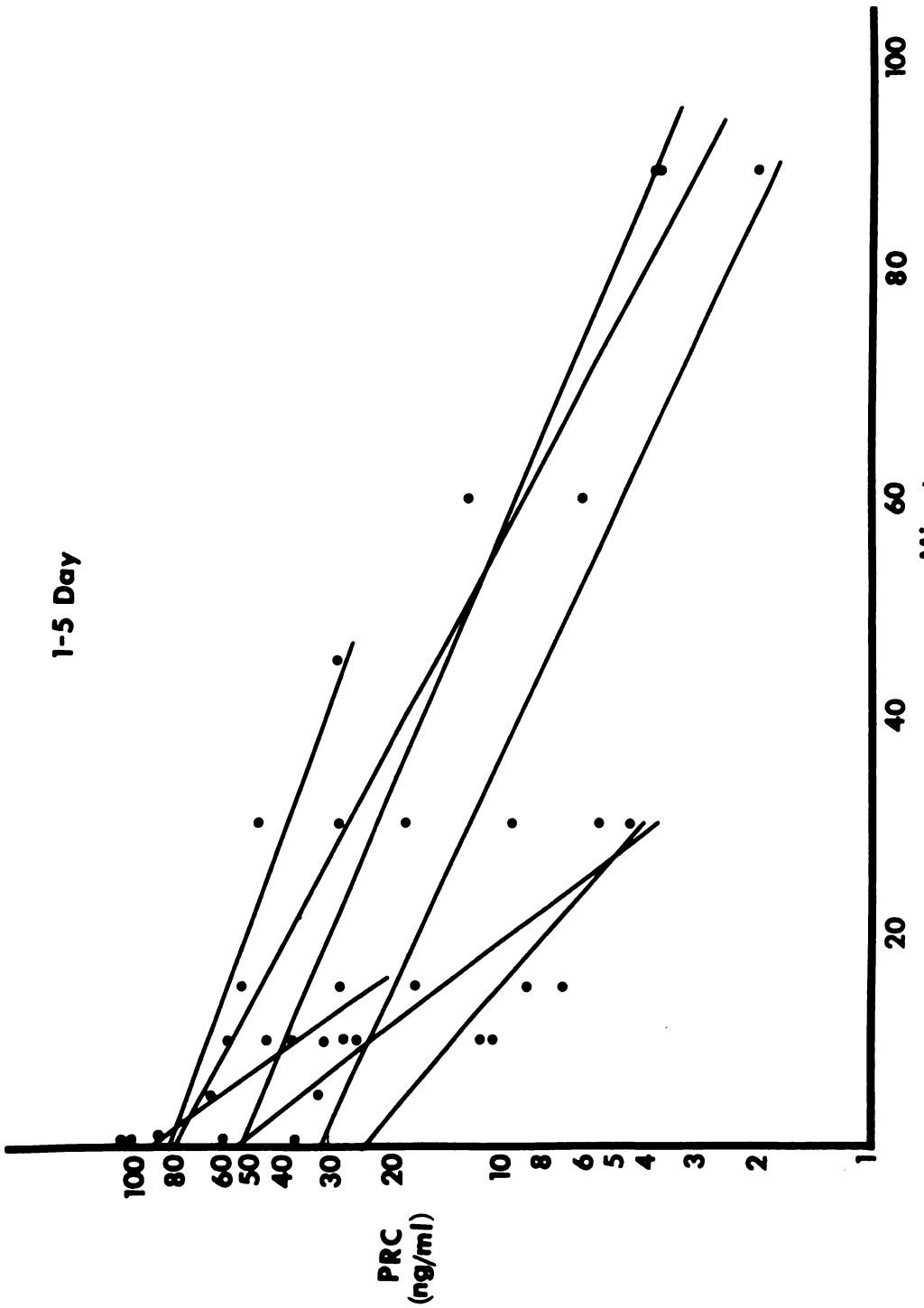
1-5 Day
PRC
(ng/ml)
100
80
60
50
40
30
20
10
8
6
5
4
3
2
1
20
40
60
80
100
Minutes

Figure 16

Figure 17. Disappearance of renin from the circulating plasma of 45-50 day old pigs after bilateral nephrectomy. Regression lines of renin disappearance in 5 pigs are shown. PRC = plasma renin concentration.

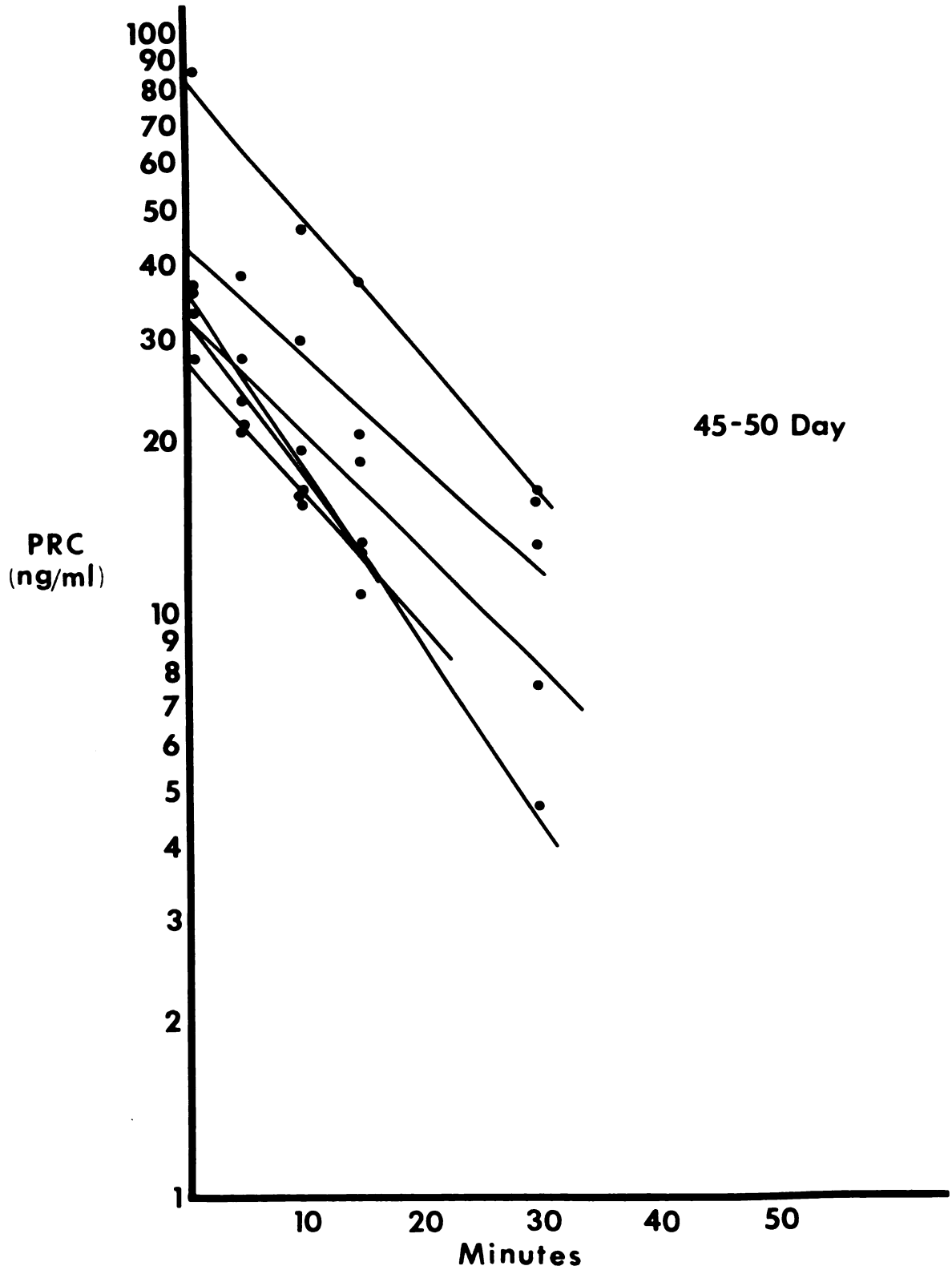
100
90
80
70
60
50
40
30
20
10
9
8
7
6
5
4
3
2
1
PRC
(ng/ml)
45-50 Day
10
20
30
40
50
Minutes

Figure 17

Figure 18. Effect of low (2% of body weight) intravascular volume expansion with saline (VE) and saline plus bovine serum albumin (VE + BSA) on renal blood flow (RBF) and outer cortical to inner cortical blood flow ratio (O/I ratio) in 1-5 and 45-50 day old piglets. Con = control periods. O/I ratio of individual experiments are shown by the solid lines. Squares and dotted lines indicate the mean values of the O/I ratio before and after volume expansion. Each bar indicates the mean ± the standard error of the mean. * = Significantly different from control periods ($p<0.05$).

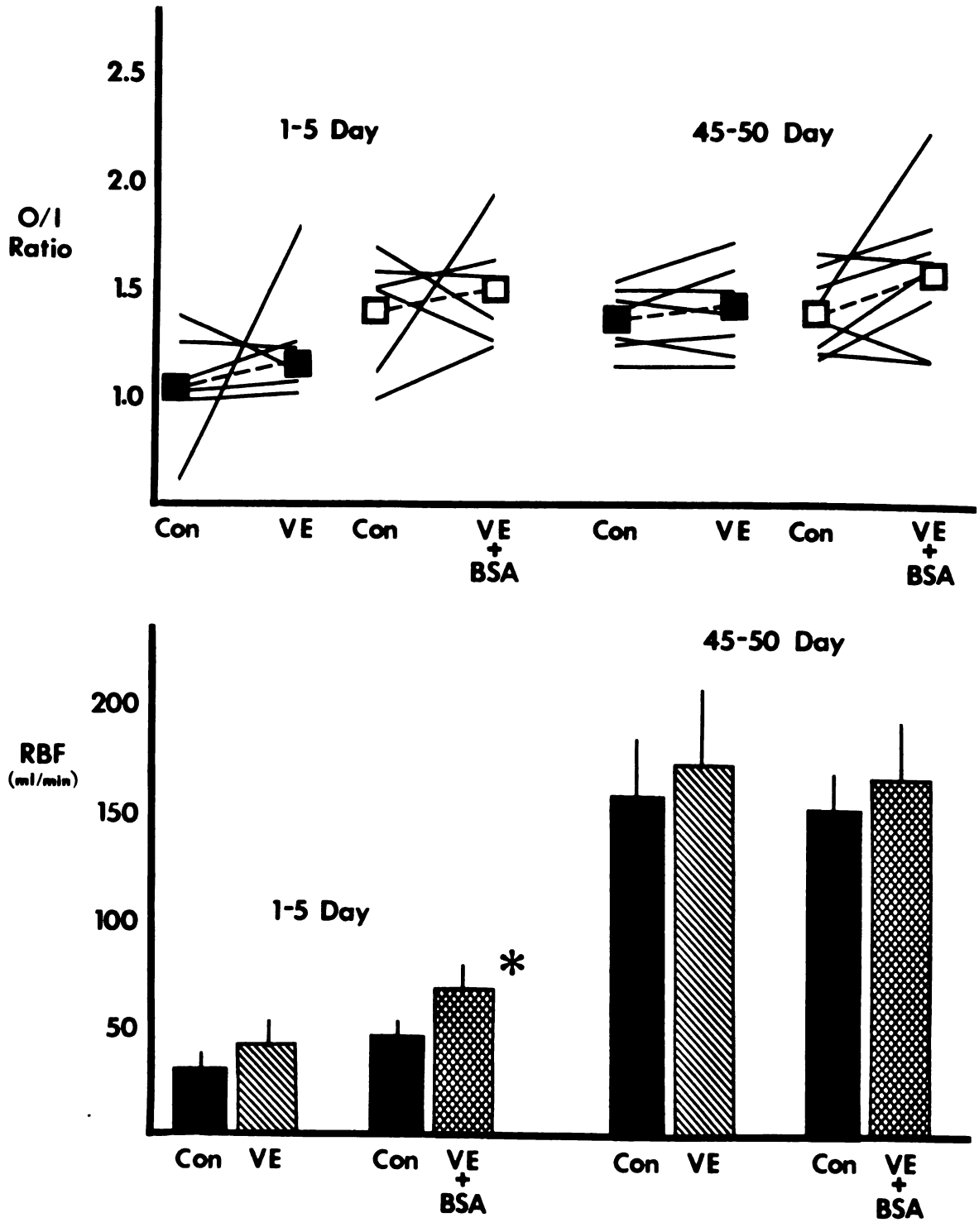
2.5
2.0
1.5
1.0
O/I
Ratio
1-5 Day
45-50 Day
Con
VE
Con
VE + BSA
Con
VE
Con
VE + BSA
200
150
100
50
RBF
(ml/min)
45-50 Day
1-5 Day
*
Con
VE
Con
VE + BSA
Con
VE
Con
VE + BSA

Figure 18

expansion increased renal blood flow in 1-5 day animals but again did not affect 45-50 day piglets (Figure 18). Low volume expansion with saline or saline plus BSA did not alter the intrarenal distribution of blood flow in piglets of either age group (Figure 18).

In piglets 1-5 days of age, low saline infusion increased fractional sodium excretion in 6 out of 7 animals tested, and volume expansion with BSA did not change this response (Figure 19). Similar effects were observed in 45-50 day old animals in which fractional sodium excretion increased equally after infusion of saline and saline plus BSA (Figure 19). Low volume expansion with or without BSA did not change blood pressure, filtration fraction or glomerular filtration rate of 1-5 or 45-50 day pigs (Table 11). Saline infusion alone tended to decrease hematocrit in both age groups (Table 11). BSA saline infusion significantly decreased hematocrit in 45-50 day pigs, whereas hematocrit in 1-5 day animals only fell slightly (Table 11). Intravascular volume expansion of piglets 1-50 days of age with both saline and saline plus BSA also slightly increased urine flow rate and fractional potassium excretion (Table 11).

Experiment B: Effect of High Intravascular Volume Expansion (8% of BW) and High Intravascular Volume Expansion + Bovine Serum Albumin (BSA) on Renal Function in Developing Piglets

Intravascular volume expansion of 1-5 day piglets did not significantly affect hematocrit, whereas saline infusion into 45-50 day animals decreased hematocrit (Table 12). Infusion of saline plus BSA, however, decreased hematocrit in both age groups tested (Table 12). Despite these apparent changes in vascular volume, infusion of either saline or saline plus BSA had little effect on systemic blood pressure,

Figure 19. Effect of low (2% of body weight) intravascular volume expansion with saline (VE) and saline plus bovine serum albumin (VE + BSA) on fractional sodium excretion ($F_{Na}+$) by 1-5 and 45-50 day old piglets. Solid lines indicate the change in sodium excretion after volume expansion in individual experiments. Squares and dotted lines show the mean values of fractional sodium excretion before and after volume expansion. * = Significantly different from control (Con) periods ($p<0.05$).

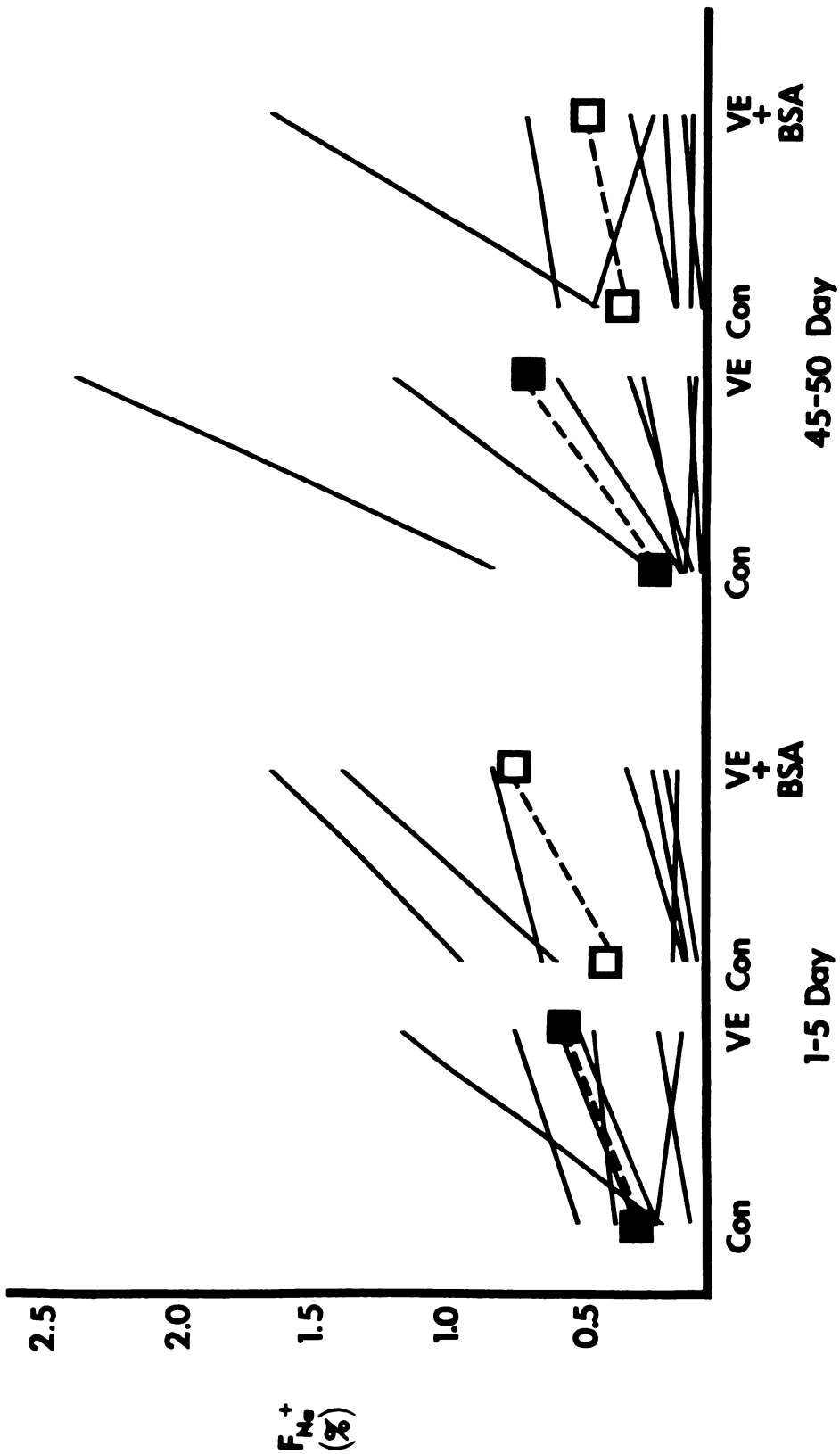
2.5
2.0
1.5
1.0
0.5
$F^{+}_{Na}$ (%)
Con
VE
Con
VE + BSA
1-5 Day
Con
VE
Con
VE + BSA
45-50 Day

Figure 19

TABLE 11

Effect of Low Intravascular Volume Expansion (2% BW) and Low Intravascular Volume Expansion + BSA on Renal Function in Developing Piglets

| | | | BP | Hct | V | FF | GFR | $F_K+$ |
|---|---|---|---|---|---|---|---|---|
| 1-5 Day | Saline (7) | Control | 83 | 28.2 | 0.078 | 0.269 | 5.21 | 18.09 |
| | | ± SEM | 1 | 1.8 | 0.014 | 0.046 | 0.65 | 6.71 |
| | | Volume Expansion | 80 | 26.7 | 0.138[a] | 0.273 | 6.03 | 28.29 |
| | | ± SEM | 2 | 1.8 | 0.034 | 0.051 | 0.99 | 9.21 |
| | Saline + BSA (7) | Control | 81 | 25.6 | 0.060 | 0.156 | 5.03 | 5.72 |
| | | ± SEM | 4 | 1.4 | 0.007 | 0.019 | 0.57 | 2.35 |
| | | BSA | 84 | 24.4 | 0.107 | 0.133 | 5.92 | 15.02[a] |
| | | ± SEM | 3 | 0.9 | 0.023 | 0.019 | 0.31 | 7.03 |
| 45-50 Day | Saline (7) | Control | 112 | 32.5 | 0.338 | 0.282 | 26.58 | 19.18 |
| | | ± SEM | 5 | 1.8 | 0.127 | 0.102 | 7.41 | 8.93 |
| | | Volume Expansion | 111 | 30.8 | 0.532 | 0.214 | 24.09 | 27.33[a] |
| | | ± SEM | 4 | 1.6 | 0.207 | 0.053 | 6.36 | 8.57 |
| | Saline + BSA (8) | Control | 111 | 33.3 | 0.144 | 0.190 | 24.06 | 8.68 |
| | | ± SEM | 4 | 1.4 | 0.017 | 0.034 | 4.37 | 2.03 |
| | | BSA | 108 | 28.7[a] | 0.224 | 0.154 | 26.51 | 15.93 |
| | | ± SEM | 4 | 1.1 | 0.043 | 0.021 | 5.28 | 4.05 |

BP = Blood Pressure (mmHg); Hct = Hematocrit (%); V = Urine flow rate (ml/min); FF = Filtration fraction; GFR = Glomerular filtration rate (ml/min); $F_K+$ = Fractional potassium excretion (%); SEM = Standard error of the mean; [a] $p<0.05$.

TABLE 12

Effect of High Intravascular Expansion (8% BW) and High Intravascular Volume Expansion + BSA on Renal Function in Developing Piglets

| | | | BP | PRA | Hct | Plasma $Na^+$ | Plasma $K^+$ | V |
|---|---|---|---|---|---|---|---|---|
| 1-5 Day | Saline (6) | Control | 87 | 9.72 | 27.3 | 150.6 | 4.32 | 0.076 |
| | | ± SEM | 4 | 2.24 | 1.5 | 1.0 | 0.13 | 0.013 |
| | | Volume Expansion | 93[a] | 8.05 | 26.2 | 152.2 | 4.54 | 0.390 |
| | | ± SEM | 3 | 3.03 | 1.2 | 1.3 | 0.07 | 0.137 |
| | Saline + BSA (6) | Control | 90 | 18.48 | 32.8 | 143.4 | 4.07 | 0.060 |
| | | ± SEM | 2 | 5.31 | 1.3 | 5.3 | 0.23 | 0.014 |
| | | BSA | 91 | 11.06[a] | 25.4[a] | 143.9 | 4.22 | 0.231 |
| | | ± SEM | 3 | 4.02 | 1.1 | 6.5 | 0.20 | 0.079 |
| 45-50 Day | Saline (6) | Control | 108 | 8.22 | 33.4 | 144.9 | 3.77 | 0.117 |
| | | ± SEM | 2 | 1.83 | 0.5 | 4.0 | 0.13 | 0.021 |
| | | Volume Expansion | 110 | 5.57[a] | 30.4[a] | 144.9 | 4.14[a] | 1.061[a] |
| | | ± SEM | 3 | 1.67 | 1.2 | 4.3 | 0.08 | 0.235 |
| | Saline + BSA (8) | Control | 115 | 9.53 | 33.4 | 139.7 | 3.66 | 0.159 |
| | | ± SEM | 2 | 1.17 | 0.9 | 2.9 | 0.19 | 0.024 |
| | | BSA | 109 | 7.66 | 23.7[a] | 138.7 | 3.78 | 0.413 |
| | | ± SEM | 3 | 1.29 | 0.5 | 3.1 | 0.18 | 0.129 |

BP = Blood Pressure (mmHg); PRA = Plasma renin activity (ng/ml); Hct = Hematocrit (%); Plasma sodium and potassium concentration (mEq/l); V = Urine flow rate (ml/min).
() = Number of observations; SEM = Standard error of the mean. [a] $p<0.05$.

plasma sodium or plasma potassium concentration (Table 12). Although high intravascular volume expansion with saline increased systemic blood pressure of 1-5 day old piglets, saline infusion did not change the blood pressure of pigs 45-50 days of age (Table 12). After addition of BSA to the saline solution, volume expansion did not affect blood pressure in either age group (Table 12). In 1-5 day piglets, urine flow rate was increased to a similar degree after volume expansion with saline or infusion of saline plus BSA (Table 12). Saline infusion into 45-50 day pigs increased urine flow rate, whereas unlike younger animals, BSA saline infusion reduced this diuresis (Table 12). Plasma renin activity of 1-5 day piglets was not changed after saline infusion alone, but high volume expansion with BSA decreased the plasma renin activity in this age group. In contrast, saline infusion decreased plasma renin activity in piglets 45-50 days of age, but this decrease in plasma renin activity was not observed after volume expansion with BSA (Table 12).

In 1-5 day piglets, volume expansion with saline alone did not affect renal blood flow (Figure 20) or the intrarenal distribution of blood flow (Figure 21). Volume expansion with BSA added to the saline solution concomitantly increased total renal blood flow (Figure 20) and O/I ratio (Figure 21). In 45-50 day pigs, saline infusion also did not change renal blood flow (Figure 20), but unlike younger animals, the ratio of outer to inner cortical blood flow was increased (Figure 21). Volume expansion of 45-50 day pigs with BSA increased renal blood flow similar to 1-5 day animals (Figure 20) but did not change the cortical distribution of blood flow (Figure 20).

Figure 20. Effect of high (8% of body weight) intravascular volume expansion with saline (VE) and saline plus bovine serum albumin (VE + BSA) on renal blood flow (RBF) in 1-5 and 45-50 day old piglets. Con = control periods. Each bar indicates mean ± standard error of mean. * = Significantly different from control periods ($p<0.05$).

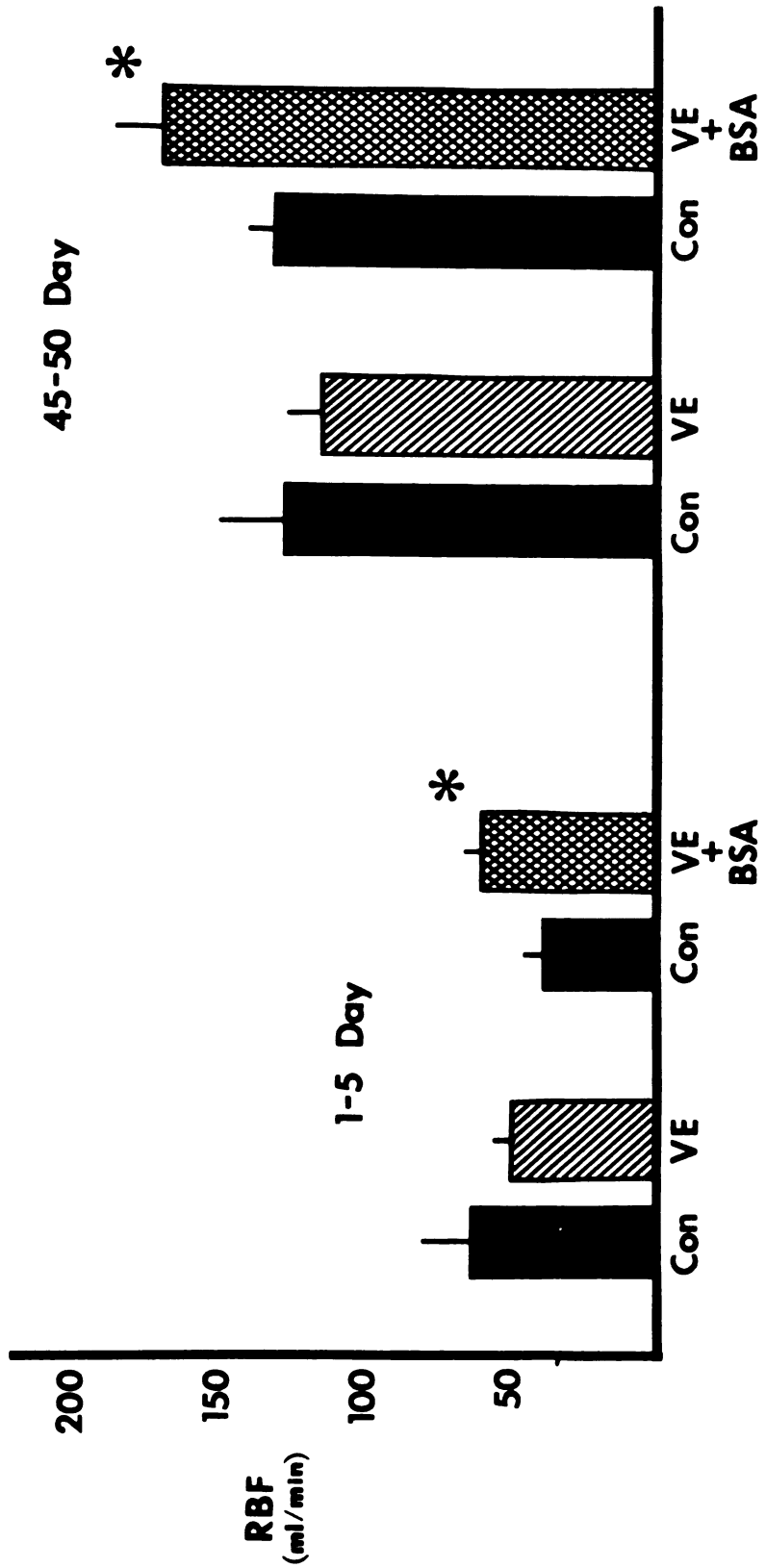


Figure 20

Figure 21. Effect of high (8% of body weight) intravascular volume expansion with saline (VE) and saline plus bovine serum albumin (VE + BSA) on the outer to inner cortical blood flow ratio (O/I ratio) in 1-5 and 45-50 day old piglets. Con = control periods. Ratios of the individual experiments are shown by the solid lines. Squares and dotted lines indicate the mean values of the O/I ratio before and after volume expansion. * = Significantly different from control periods ($p<0.05$).

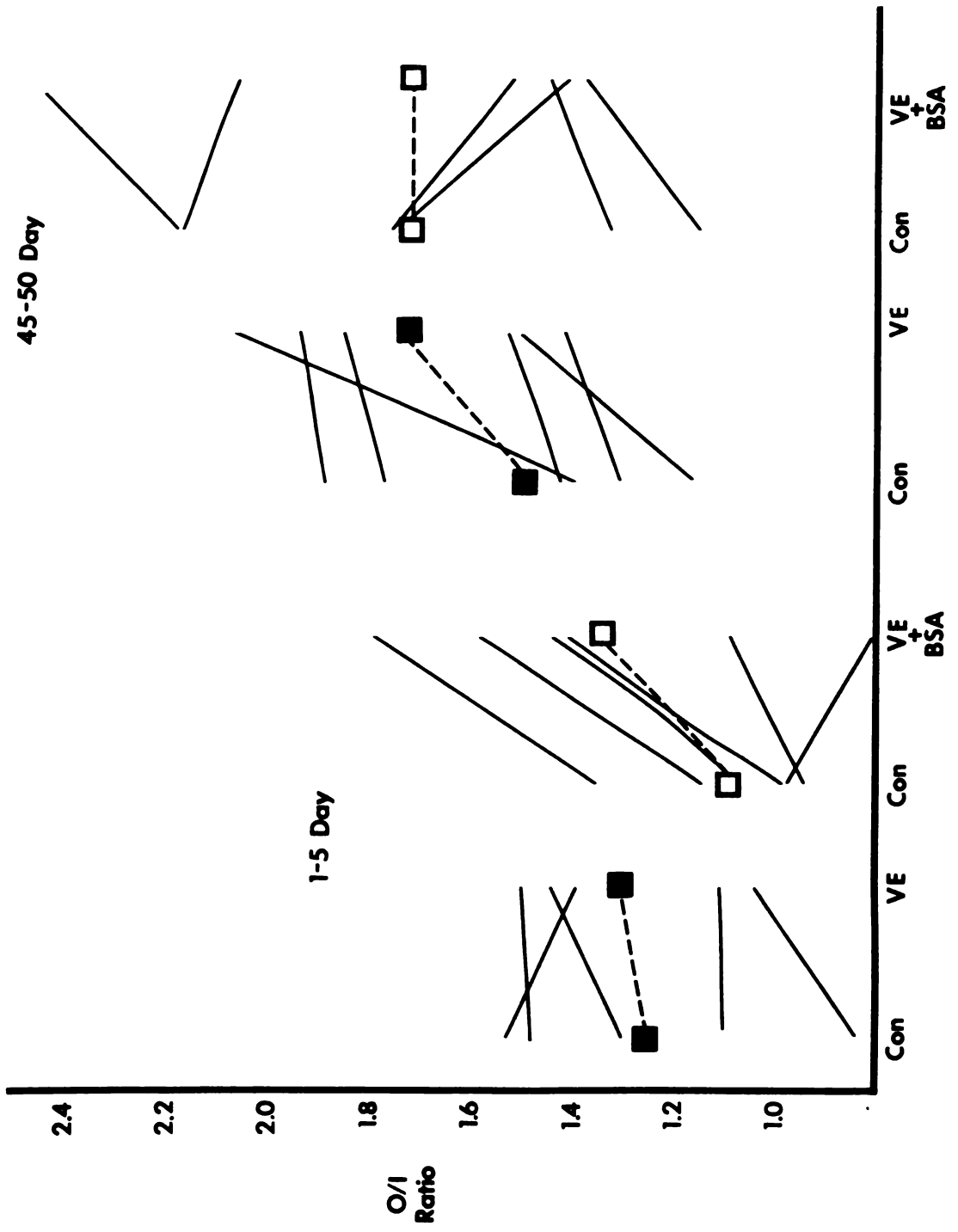
45-50 Day
1-5 Day
O/I Ratio
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
Con
VE
Con
VE + BSA
Con
VE
Con
VE + BSA

Figure 21

After volume expansion of 1-5 day piglets with saline, the small decrease in renal blood flow (Figure 20) and slight increase in glomerular filtration rate (Figure 22) resulted in a significant increase in filtration fraction (Figure 22). After BSA saline infusion into 1-5 day piglets, the increase in renal blood flow (Figure 20) was accompanied by a significant increase in glomerular filtration rate (Figure 22). Since the magnitude of these increases were relatively equal, fitration fraction remained unchanged (Figure 22). Saline infusion into 45-50 day old animals did not alter glomerular filtration rate or filtration fraction (Figure 22), and although BSA saline increased renal blood flow (Figure 20), filtration fraction and glomerular filtration rate remained unchanged in this age group (Figure 22). Fractional sodium excretion and fractional potassium excretion also were determined in developing pigs after high intravascular volume expansion with saline and saline plus BSA. After saline infusion alone, fractional sodium excretion was increased to a similar degree in both 1-5 day piglets and in 45-50 day animals (Figure 23). Infusion of saline plus BSA, however, blocked this natriuresis in both age groups tested (Figure 23). Fractional potassium excretion was significantly increased following intravascular volume expansion of both 1-5 and 45-50 day pigs. In contrast to the effect of BSA saline infusion on fractional sodium excretion, volume expansion with BSA increased fractional potassium excretion in both 1-5 and 45-50 day pigs similar to those animals receiving only saline (Figure 23).

Figure 22. Effect of high (8% of body weight) intravascular volume expansion with saline (VE) and saline plus bovine serum albumin (VE + BSA) on filtration fraction and glomerular filtration rate (GFR) in 1-5 and 45-50 day old piglets. Con = control periods. Each bar indicates mean ± standard error of mean. * = Significantly different from control periods ($p<0.05$).

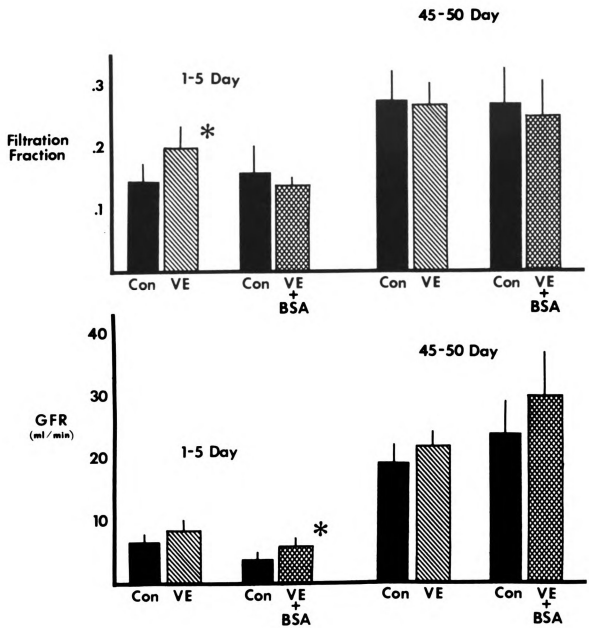
45-50 Day
1-5 Day
Filtration Fraction
.3
.2
.1
*
Con
VE
Con
VE + BSA
Con
VE
Con
VE + BSA
40
30
20
10
GFR (ml/min)
45-50 Day
1-5 Day
*
Con
VE
Con
VE + BSA
Con
VE
Con
VE + BSA

Figure 22

Figure 23. Effect of high (8% of body weight) intravascular volume expansion with saline (VE) and saline plus bovine serum albumin (VE + BSA) on fractional sodium excretion ($F_{Na}+$) and fractional potassium excretion ($F_{K}+$) by 1-5 and 45-50 day old piglets. Con = control periods. Fractional excretions of individual experiments are shown by the solid lines. Squares and dotted lines indicate the mean values of fractional excretion before and after volume expansion. * = Significantly different from control periods ($p<0.05$).

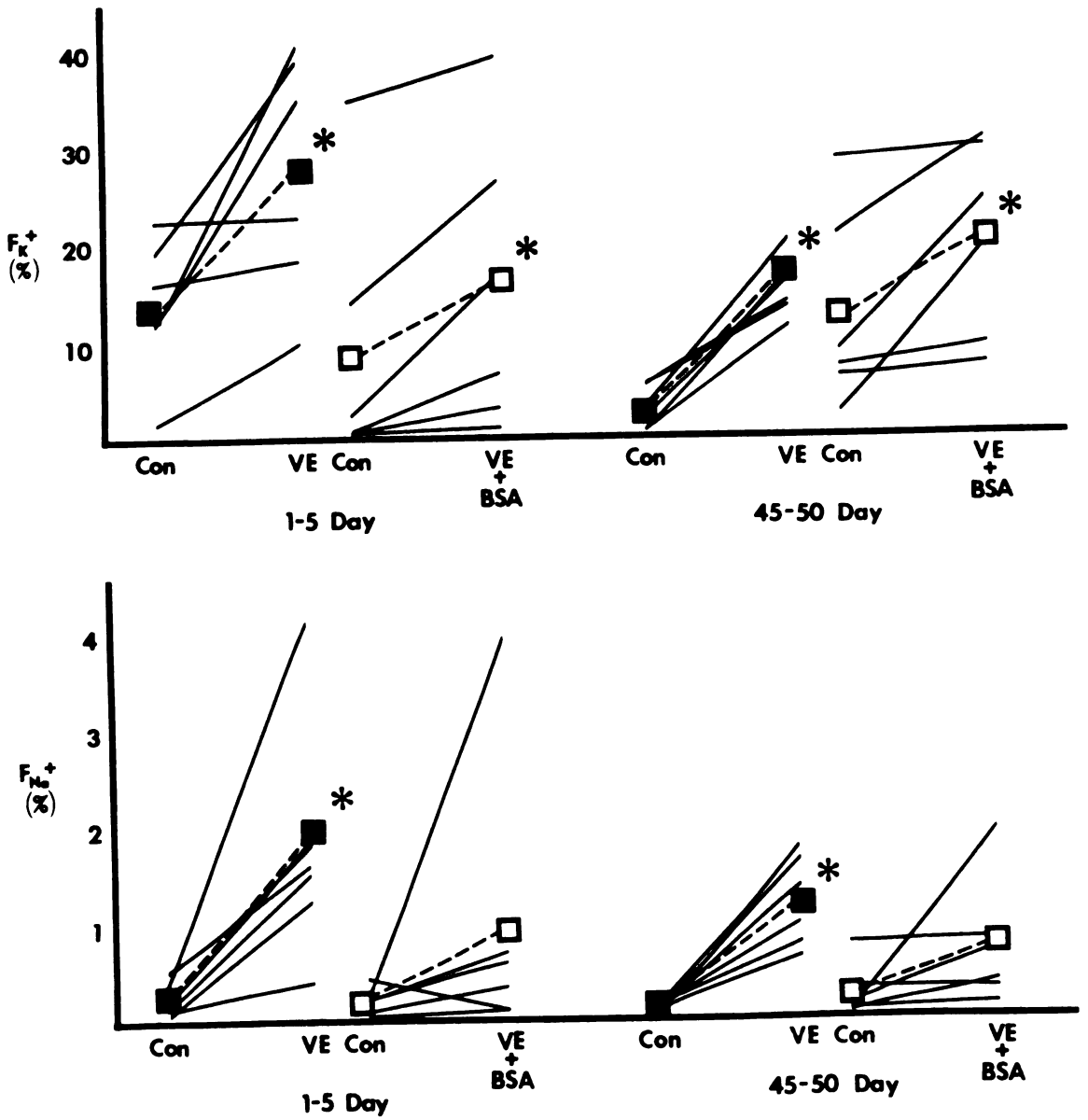
40
30
20
10
$F_K^+$ (%)
*
*
*
*
Con
VE Con
VE + BSA
1-5 Day
Con
VE Con
VE + BSA
45-50 Day
4
3
2
1
$F_{Na}^+$ (%)
*
*
*
*
Con
VE Con
VE + BSA
1-5 Day
Con
VE Con
VE + BSA
45-50 Day

Figure 23

# DISCUSSION

## 1. Verification of the Use of Radioactive Microspheres for the Determination of Renal Blood Flow and Intrarenal Distribution of Blood Flow in Developing Piglets

The use of radioactive microspheres for the measurement of both total renal blood flow and the distribution of renal blood flow has been evaluated and verified in adult (McNay and Abe, 1970; Stein et al., 1973b; Katz et al., 1971) and newborn dogs (Kleinman and Reuter, 1973). Since microspheres 15±5 microns in diameter are totally extracted by adult dog kidneys (Katz et al., 1971), and this size microsphere does not interfere with renal function in puppies (Kleinman and Reuter, 1973), it was assumed that microspheres 15±5 microns would be suitable for use in experiments on young piglets. To confirm this assumption, 7 experiments testing microsphere injection on cardiac and renal function were performed in conscious piglets. The injection of microspheres (approximately 200,000 on 2 successive occasions) suspended in dextran (20 g/dl) and withdrawal of blood from the descending aorta (1.75 ml/min for 1.5 minutes) had little effect upon cardiac or renal function of young piglets (Tables 1 and 2). The data also demonstrate that in untreated conscious piglets, renal blood flow and outer to inner cortical blood flow ratio may be measured with microspheres at least twice in succession with a high degree of precision (Table 1).

The technique used in determining the O/I ratio included sectioning both the right and left kidneys near each pole and near the hilus.

Each section was then sliced around the circumference of the kidney to obtain 4 samples of outer and inner cortical tissue. This technique produced 12 outer and 12 inner cortical samples per kidney which were pooled into groups of 4 for radioactive counting. The O/I ratios determined by this technique were similar to the O/I ratios previously reported in conscious piglets by Alward et al. (1978). The precision of the determination of renal cortical blood flow distribution by this technique may result from the elimination of intrarenal variation in calculated O/I ratios by selecting a large sample size throughout the kidney and pooling these samples for radioactive counting.

Histologic examination of these kidneys revealed that microspheres 15±5 microns were of sufficient diameter to flow freely into the afferent arteriole and lodge either within the afferent arteriole or at the junction of the afferent arteriole and the glomerulus (Figure 7). Furthermore, histological observation of the renal cortex demonstrated that a very low percentage of the glomerular capillary beds were obstructed by injected microspheres (Figure 8). Since microspheres prevented blood flow to only a small number of glomeruli, it was not surprising that microsphere injection had little effect on overall renal function (Table 2). Thus, it appears that radioactive microspheres can be effectively used for the quantification of both total renal blood flow and the intrarenal distribution of blood flow in unanesthetized developing piglets without significantly altering cardiac or renal function.

2. Renal Functional Development of Conscious Piglets

Piglets between 1 and 50 days of age demonstrated significant increases in body weight, kidney weight and surface area (Table 3). Comparing the increase in kidney weight to the changes in body weight and surface area, however, demonstrated that the kidney weight to body weight ratio decreased with advancing age, but the ratio of kidney weight to surface area was unchanged between 1 and 50 days after birth (Table 3). The decrease in kidney weight to body weight ratio with age resulted from a more rapid rate of increase in body weight than kidney weight during maturation. In contrast, surface area and kidney weight increased at similar rates up to 50 days of age.

The development of renal function has been described in several species (Horster and Valtin, 1971; Dlouha, 1976; Spitzer and Brandis, 1974; McCance and Widdowson, 1956) including infants (Edelmann and Spitzer, 1969; Aperia et al., 1975b). In rats, glomerular filtration rate calculated as a function of kidney weight increased from birth through 20 days of age (Dlouha, 1976; Horster and Lewy, 1970). Glomerular filtration rate also increased with age in neonatal lambs when calculated either with or without normalizing for renal mass (Aperia et al., 1974a), but in fetal lambs glomerular filtration rate only increased with maturation when expressed as the absolute value (Robillard et al., 1975). The present data demonstrate that in piglets, glomerular filtration rate also was low at birth and increased with age (Figure 9). This age related increase in glomerular filtration rate was evident when calculated as absolute flow (Figure 9) or when calculated as a function of kidney weight (Figure 10). Several factors may contribute to the low glomerular filtration rate at birth. Kleinman

and Lubbe (1972) reported that in puppies a significant correlation existed between increasing blood pressure and increasing glomerular filtration rate with age. These authors concluded that blood pressure maturation alone could not be totally responsible for the large increase in glomerular filtration rate which occurred between birth and adulthood. Spitzer and Edelmann (1971) have estimated from urinary stop-flow measurements in guinea pigs that glomerular capillary pressure increased from 18.8 mmHg at birth to 38.0 mmHg at 7 weeks of age. Over this same age range, effective filtration pressure increased despite the observation that factors opposing filtration, proximal tubular hydrostatic pressure and plasma oncotic pressure, also were increasing. These authors concluded that the increase in effective filtration pressure with age, primarily was due to the rise in glomerular capillary pressure. Recently, glomerular capillary pressure of young rats (35-45 days of age) was directly measured by micropuncture and found to be no different than adults. Single nephron glomerular filtration rates, in young rats, however, were significantly lower than the single nephron glomerular filtration rates of older animals (Ichikawa, 1978). These micropuncture experiments suggest that at least in rats, increasing renal perfusion pressure is not responsible for the rise in glomerular filtration rate with age.

Since many renal tubules are morphologically immature at birth (Potter, 1972), an elevated intratubular pressure was originally suggested as a factor producing the low postnatal rate of glomerular filtration (Edelmann and Spitzer, 1969). Spitzer and Edelmann (1971) later reported that proximal intratubular hydrostatic pressure is low

in young guinea pigs and increased to adult values by 50 days of age. Similarly, Horster and Valtin (1971) showed that in dogs between 20 and 80 days of age proximal intratubular pressure was constant but nephron filtration rate increased 8-fold over this age range. These data demonstrated that intratubular hydrostatic pressure cannot be responsible for the low glomerular filtration rate of infants.

Another factor which may contribute to the low rate of glomerular filtration in neonates is a low coefficient of filtration ($K_f$). The coefficient of filtration ($K_f$) is a relative measure of the permeability of the glomerular membrane and the total surface area available for filtration. Preliminary evidence by Ichikawa et al. (1978) have shown that the $K_f$ of young rats was lower than adult values. This result is supported by the observation that filtration fraction in rats also is low at birth and increases with age (Horster and Lewy, 1971). In these experiments, the increase in filtration fraction was due to an increase in glomerular filtration rate without appreciable changes in renal plasma flow between birth and 20 days of age. Therefore, the increase in glomerular filtration rate and consequently filtration fraction may have resulted from increased glomerular permeability or possibly from an increase in glomerular capillary surface area. In puppies, however, filtration fraction did not change with age suggesting that a low $K_f$ is not responsible for reducing glomerular filtration rate in the newborn dog (Kleinman and Lubbe, 1972).

In the present experiments, systemic blood pressure significantly increased between 1 and 50 days of age (Figure 9). This rise in perfusion pressure may be a contributing factor to the maturation of glomerular filtration rate in pigs similar to the direct relationship

between blood pressure and glomerular filtration rate in developing dogs (Kleinman and Lubbe, 1972). Unlike the puppy, however, developing piglets demonstrated an increase in filtration fraction between 1 and 50 days of age as well (Table 4). This increase in filtration fraction occurred despite an increased renal blood flow and glomerular filtration rate with age (Figure 9). Thus, the rate of increase in glomerular filtration must have exceeded the rate of increase in renal blood flow. This increase in filtration fraction suggests that an increase in the coefficient of filtration ($K_f$) also may contribute to the rise in glomerular filtration rate of developing pigs. Factors which may contribute to an increase in $K_f$ include postnatal glomerulogenesis thereby increasing glomerular capillary surface area, the postnatal rise in renal perfusion pressure (Figure 9) and increased glomerular capillary permeability. Further investigation, however, is necessary to more clearly identify which of these factors may be specifically responsible for the maturational changes in glomerular filtration rate of young piglets.

Several investigators have observed that renal blood flow is low at birth and increases with age (Kleinman and Lubbe, 1972; Aperia et al., 1977; Horster and Valtin, 1971; Calcagno and Jose, 1972; Aschinberg et al., 1975; Gruskin et al., 1970). Gruskin et al. (1970) have investigated the maturation of renal hemodynamics in conscious piglets. These authors reported that renal blood flow increased from 0.043 $l/min/m^2$ body surface area in 1 day old piglets to 0.76 $l/min/m^2$ by 45 days of age. This rise in renal blood flow with age was associated with an increase in systemic blood pressure from 64 mmHg to 108 mmHg

and a decrease in renal resistance from 1605 mmHg/l/min/$m^2$ to 138 mmHg/l/min/$m^2$ over this same age range. The development of renal hemodynamics in conscious piglets also was demonstrated in the present experiments (Figures 9 and 10). Total renal blood flow (Figure 9) increased and renal vascular resistance decreased between the ages of 1 and 50 days (Figure 9). Renal blood flow and renal resistance, however, are presented as the absolute value without factoring for kidney weight or body surface area. In contrast to the data reported by Gruskin et al., renal blood flow of 1-5 day piglets calculated per gram kidney weight or per square meter body surface area was not significantly different from 45-50 day pigs (Figure 10 and Table 5). Renal resistance factored by kidney weight or body surface area, however, decreased with age similar to renal resistance calculated as mmHg/ml/min (Figure 9 and Table 5). It is not surprising that renal blood flow or renal resistance factored by kidney weight and surface area demonstrated similar age related changes since the kidney weight and surface area appear to increase with age at a similar rate (Table 3). The difference in maturation of absolute renal blood flow and renal blood flow per square meter or per gram kidney, however, were not expected, particularly since Gruskin et al. (1970) reported that in piglets renal blood flow per square meter increased between 1 and 49 days of age. In the present experiments, the fact that renal blood flow per gram kidney or per square meter did not significantly change with age suggests that renal blood flow and kidney weight or body surface area are increasing at a similar rate. Indeed, between 1 and 50 days of age renal blood flow increased 69% and kidney weight increased by 67%.

The differences in maturational changes of renal blood flow per square meter between the present data and those of Gruskin et al. (1970) may be due to the techniques used in the determination of renal blood flow. Gruskin reported that renal blood flow in conscious 1-5 day old piglets averaged between 0.04 and 0.08 $l/min/m^2$ whereas the present data show renal blood flow of 1-5 day piglets to equal 0.29 $l/min/m^2$ (Table 5). In the previous experiments, renal blood flow was determined as the product of cardiac output and the fraction of cardiac output perfusing the kidney. In contrast to the method of Gruskin et al., renal blood flow was determined in the present experiments by withdrawal of blood from the descending aorta simultaneously with microsphere injection. Renal blood flow then was calculated from the ratio of radioactive counts obtained at the known withdrawal rate and the radioactive counts which entered the kidney. Therefore, it is possible that the discrepancy between renal blood flow reported by Gruskin et al. and renal blood flow in the present data resulted from these differences in technique. The renal blood flow determined in the present experiments is in very close agreement with the renal blood flow of developing pigs reported by Bailie et al. (1979). In these experiments, blood flow was measured directly with a noncannulating electromagnetic flowmeter. These authors reported that renal blood flow per gram kidney weight in 3 day old pigs averaged 2.0 ml/min/g and was approximately 2.7 ml/min/g at 53 days of age. This change in renal blood flow with age was not grossly different from the present data (Table 5). Thus, it appears that the microsphere method used for the determination of renal blood flow in the present experiments has

been substantiated by more direct techniques. In addition, the observation that absolute increases in renal blood flow of developing piglets may be masked by factoring by kidney weight has been confirmed.

Developmental changes in renal hemodynamics have been investigated in other species as well, and it appears that maturational changes in renal blood flow calculated per gram kidney weight may be species specific. Maturation of renal plasma flow in rats was reported by Horster and Lewy (1970). Renal plasma flow was measured between 2 and 20 days of age using the clearance of paraminohippuric acid (PAH) divided by the renal extraction of PAH. Over this age range, rat renal plasma flow did not change when calculated per gram kidney weight despite maturational increases in PAH clearance, inulin clearance and PAH extraction. Similarly, renal blood flow of lambs normalized by kidney weight did not change between 135 days gestation and 9 days after birth (Aperia et al., 1977). In contrast to these data are several reports that renal blood flow calculated per gram kidney weight in developing dogs increased between birth and 80 days of age (Horster and Valtin, 1971; Aschinberg et al., 1975; Calcagno and Jose, 1972; Kleinman and Lubbe, 1972). It appears that at least in the puppy renal hemodynamics are quite immature at birth and renal blood flow increases at a faster rate than does kidney weight. In lambs, rats and piglets, however, renal blood flow and kidney weight mature at similar rates. This result does not suggest that the increase in absolute blood flow with age in these species is due entirely to renal growth. In piglets, perfusion pressure increased (Figure 9) and renal resistance per gram kidney (Figure 10) decreased between 1 and 50 days of age. Therefore,

the increase in renal blood flow of developing piglets must have resulted from factors other than merely growth of the renal vasculature.

Horster and Valtin (1971) have proposed an explanation for the relationship between renal function and tissue mass. These authors suggested that postnatal renal maturation may be divided into two phases. Early renal development may be characterized by disproportionate maturation of renal function and tissue mass. During the latter part of the postnatal period, a constant relation between renal function and tissue mass is achieved. Using this concept of renal development, it is possible that in some species early renal functional maturation may exceed tissue growth, but in other species, this early maturational phase can be very short. The latter may in fact be the case for the maturation of renal blood flow in young piglets and possibly lambs and rats as well. In the piglet at least, the early phase of renal maturation where increases in renal blood flow were independent of renal growth was not demonstrated after birth. This early phase of development, therefore, may occur in this species during fetal development, and only the later phase of renal blood flow maturation in which blood flow increases in proportion to renal mass is observed during postnatal maturation.

3. Regulation of Plasma Renin Activity in Young Piglets

Plasma renin activity as well as circulating angiotensin II are elevated in the newborn of several animal species and this plasma renin activity decreases with advancing age (Pohlova and Jelinek, 1974; Kotchen et al., 1972; Granger et al., 1971; Broughton-Pipkin et al.,

1974; Broughton-Pipkin et al., 1971; Broughton-Pipkin and Symonds, 1977). The present data show that the developmental changes in plasma renin activity of conscious piglets were not as dramatic as those changes previously reported in other species. Animals 18-22 days of age demonstrated significantly greater plasma renin activities than 45-50 day old pigs, but the plasma renin activity of 1-5 day pigs was not significantly different than either older age group (Figure 14). Bailie et al. (1979), however, have shown that the plasma renin concentration of anesthetized piglets is higher in 3-5 day animals than at 42-53 days of age. This discrepancy between the maturational changes in plasma renin activity of anesthetized piglets (Bailie et al., 1979) and the present data may be due to the large variability in plasma renin activities of the conscious animals. Although these animals rested quietly and appeared to be fully awake, an acute stress of anesthesia had been administered. Therefore, this acute stress could produce variability in the plasma renin activity at all ages preventing significant differences between the 1-5 and 45-50 day age groups.

The mechanisms which have been considered to contribute to the high neonatal plasma renin activity include: 1) elevated renin secretion by young animals, 2) decreased degradation of the enzyme, or 3) a low circulating plasma volume at birth which would increase the concentration of renin in the plasma. The newborn of different species are capable of altering their plasma renin activity in response to a variety of stimuli including suprarenal aortic constriction (Smith et al., 1974), hemorrhage (Broughton-Pipkin et al., 1974), furosemide (Siegel and Fisher, 1977), isoproterenol and propranolol (Bailie et

al., 1979). These observations suggest that these stimuli change renin release in young animals by activation of the sympathetic nervous system, baroreceptor mechanism or macula densa receptor. Thus, it appears that the tubular, vascular and sympathetic mechanisms which regulate renin release in adult animals are intact in the newborn as well.

The regulation of renin secretion by the distal tubular macula densa receptor was investigated in the present experiments by the determination of plasma renin activity before and after volume expansion in developing piglets. Horster and Larsson (1976) have demonstrated that proximal tubules of young rabbits reabsorb more fluid than do proximal tubules from older animals. These immature proximal tubules in young animals may enhance proximal tubular sodium reabsorption thereby decreasing the sodium load to the macula densa receptor. This low sodium load then might be a stimulus for the hypersecretion of renin in young animals resulting in a high plasma renin activity. The data from the present experiments demonstrate that mild intravascular volume expansion (2% of the body weight over 30 minutes) with a sodium-potassium chloride solution did not change the plasma renin activity of developing piglets between 1 and 50 days of age (Figure 15). These results indicate that hypersecretion of renin stimulated by the tubular macula densa mechanism probably does not occur in young piglets since the same saline load was ineffective in altering the plasma renin activity of both young and more mature animals. It is possible that this saline load was not of sufficient magnitude to suppress renin secretion at all. This does not seem likely, however, since

intravascular volume expansion at this rate did increase fractional sodium excretion (Figure 13), fractional potassium excretion and urine flow rate (Table 8) in piglets of each age group. Furthermore, in animals 1-5 and 18-22 days of age, plasma renin activity initially was elevated by saralasin treatment, and low level volume expansion significantly decreased plasma renin (Figure 15). Thus, the macula densa mechanism in immature piglet kidneys does appear to be functional.

Renal humoral factors including angiotensin II and renal prostaglandins also may be involved in elevating neonatal renin secretion. Angiotensin II has been shown to inhibit renin release directly by a negative feedback mechanism acting on the juxtaglomerular cells of the afferent arteriole (Shade et al., 1973; Naftilan and Oparil, 1978). If the juxtaglomerular apparatus of newborn animals were insensitive to the negative feedback of angiotensin II, then neonatal renin secretion would be elevated. The present experiments show that angiotensin II inhibition with saralasin increased plasma renin activity in 1-5 and 18-22 day piglets but had no effect on plasma renin at 45-50 days of age (Figure 15). These results are similar to those of Solomon et al. (1976) who reported that saralasin significantly increased plasma renin activity of 2 week old rats. The effect of saralasin administration on plasma renin activity in adult animals has been shown to correlate with the concentration of renin in the plasma at the time of angiotensin blockade. Thus, saralasin did not alter basal renin secretion in adult animals with normal plasma renin activities (Davis, 1975; Freeman et al., 1973; Satoh and Zimmerman, 1975; Ishikawa and Hollenberg, 1975) but increased renin release in animals with various states of hyperreninemia

(Satoh and Zimmerman, 1975; Slick et al., 1975; Mimran et al., 1974; Freeman et al., 1973). Therefore, young piglets whose plasma renin activity was elevated, responded to angiotensin II inhibition by increasing plasma renin activity similar to adult animals with hyperreninemia. Similarly, in 45-50 day pigs in which plasma renin activity was reduced, saralasin administration did not alter the plasma renin activity. Thus, the absence of a negative feedback system by angiotensin II on renin release must not be responsible for elevating the plasma renin activity of neonatal piglets.

The renal prostaglandins are another humoral factor which may be responsible for elevating plasma renin activity in young animals. Prostaglandin $E_2$ ($PGE_2$) and prostacyclin ($PGI_2$) have been shown to increase renin release by a direct action upon the juxtaglomerular cells (Whorton et al., 1978a; Dew and Michelakis, 1974; Osborn et al., 1978). Terragno et al., (1978b) recently demonstrated that renal blood vessels dissected from fetal pigs synthesize more $PGE_2$ and $PGI_2$ than do renal blood vessels from adults. If these renal prostaglandins are present in high concentrations within the neonatal kidney, then renin secretion may be activated in these young animals by $PGE_2$, $PGI_2$ or both. The present data document that in 1-5 or 45-50 day pigs prostaglandin synthetase inhibition with indomethacin did not change plasma renin activity, and indomethacin only slightly suppressed renin activity in 18-22 day animals (Figure 15). Thus, no age related effect of indomethacin on plasma renin activity was demonstrated in these pigs, and the increased plasma renin activity of young piglets does not appear to result from the stimulation of renin secretion by prostaglandins.

The observation that indomethacin did not affect plasma renin activity of 45-50 day pigs (Figure 15) was not expected since indomethacin has been shown to decrease plasma renin activity in adult animals of different species (Romero et al., 1976; Speckart et al., 1977; Bailie et al., 1976). Therefore, the data also suggest that renal prostaglandins may not be a major factor contributing to the basal rate of renin release in older pigs. Although mild saline infusion alone did not alter plasma renin activity at any age (Figure 15), volume expansion of indomethacin treated piglets between 1 and 50 days of age significantly decreased plasma renin activity (Figure 15). Therefore, since concomitant suppression of the macula densa receptor and renal prostaglandin synthesis decreased plasma renin activity, renal prostaglandins and the macula densa receptor may modulate juxtaglomerular cell secretion of renin. This potential modulation of renin secretion, however, is only of minor importance in the regulation of plasma renin activity since neither indomethacin nor saline infusion alone reduced plasma renin activity.

In summary, the elevated plasma renin activity in young animals may result from activation of one or a combination of the several stimuli known to regulate the release of renin from the kidney. The present data suggest that renal prostaglandins do not change the plasma renin activity of developing piglets. In addition, inhibition of the tubular macula densa receptor did not affect the plasma renin activity of piglets 1 to 50 days of age. Furthermore, the data suggest that an insensitivity to angiotensin II negative feedback is not responsible for increasing the plasma renin activity of young piglets since angiotensin II inhibition with saralasin increased the plasma renin activity

of pigs between 1 and 22 days of age. Recently, Bailie et al. (1979) reported that in pigs between the ages of 3 and 53 days, plasma renin concentration decreased from 4.4 ng/ml to 0.7 ng/ml, but there was no significant change in renin secretion rate from these kidneys. This result in conjunction with the data presented from the present experiments suggests that at least in the pig elevated rates of renin secretion are not responsible for the increased plasma renin activity of young piglets.

The plasma renin activity of neonates, however, may be affected by the relative rate of renin catabolism. Renin is significantly catabolized in both the intact (Schneider et al., 1968) and isolated (Tapia et al., 1972) canine liver, but since metabolic liver function is immature at birth (Greengard, 1974), renin catabolism may be depressed in young animals resulting in an elevated plasma renin activity. The present experiments also investigated the role of renin catabolism in developing piglets as a potential factor contributing to the altered state of renin activity in young animals. The rate of disappearance of renin ($T_{\frac{1}{2}}$) from arterial plasma of 1-5 day piglets was not different from 45-50 animals (Table 7). This result is confirmed by the relatively parallel nature of the regression lines from piglets 1-5 and 45-50 days of age (Figures 16 and 17). Solomon et al. (1977) have reported that the $T_{\frac{1}{2}}$ of renin in rats less than 2 weeks of age was significantly greater than the renin half-life in 4-6 week old animals. The renin disappearance curve from these young rats, however, was constructed from blood samples obtained at various times after nephrectomy of different individual litter mate animals. The present data document a

large inter-animal variability (range of renin $T_{\frac{1}{2}}$ from 7 to 27 minutes) which may be due to varying rates of liver development in individual animals. Thus, it does not appear that in young piglets a decreased rate of renin disappearance from the circulating plasma is entirely responsible for elevating the neonatal plasma renin activity.

The total circulating plasma volume has been determined in developing piglets (Talbot and Swenson, 1970) as well as infants (Cropp, 1971). Talbot and Swenson (1970) reported that plasma volume of piglets averaged 89.9 ml at birth and increased to 569 ml by 42 days of age. Since renin secretion rate in piglets does not change with age (Bailie et al., 1979), and renin catabolism in this same species does not account for the fall in plasma renin activity, it is possible that the higher plasma renin activity in young animals may be the result of a low plasma volume at birth. If renin secretion remained constant during development, then as the circulating plasma volume increased with age the plasma renin concentration would decrease. This rising plasma volume, however, cannot account for the entire decrease in plasma renin activity. Plasma renin activity in some species may depend upon both the concentration of renin and the concentration of renin substrate (Wallace et al., 1979). Therefore, unless renin substrate synthesis also increases with the rising plasma volume, substrate concentration will fall proportionately, and plasma renin activity would be the same in both newborn and older animals. In infant humans, plasma renin activity decreased between 1 and 6 weeks of age, however, plasma renin substrate concentration was unchanged during this period (Kotchen et al., 1972). Similarly, plasma renin substrate

concentration was shown to be constant in developing rats (Pohlova and Jelinek, 1974). These results indicate that renin substrate secretion may increase during development. Therefore, if the elevated plasma renin activity in newborns is due at least in part to a contracted plasma volume, then renin substrate synthesis and secretion must increase with age presumably in conjunction with advancing liver development. Currently, sufficient data are not available regarding the development of hepatic renin substrate synthesis, and further investigation is necessary to totally evaluate this hypothesis.

4. Renal Blood Flow in Developing Piglets

The low renal blood flow (Figure 9) demonstrated in young piglets as well as other animals (Ljundquist, 1963; Alexander and Nixon, 1962; Aperia and Herin, 1975; Aschinberg et al., 1975) may result from the high neonatal renal vascular resistance (Figures 9 and 10). In addition, the outer to inner cortical blood flow ratio (O/I ratio) of piglets was low at birth and increased with age (Table 4). Thus, this high renal vascular resistance appears to be localized within the outer cortex, and the increase in renal blood flow with age is due to a selective increase in outer cortical blood flow (Aschinberg et al., 1975). Possible factors which may contribute to this elevated renal vascular resistance include renal humoral factors such as angiotensin II and the lack of morphological development of the renal vasculature in the outer cortex. To evaluate the possibility that this elevated resistance arises from undeveloped renal vascular spaces, renal vascular patterns of pig kidneys 2, 20 and 50 days of age were investigated

with the silastic rubber, Microfil. Patency of the renal vasculature after fixing the kidneys with glutaraldehyde perfusion in situ was verified by histologic examination of a rat kidney fixed by this technique (Figure 24). In Figure 24, an afferent arteriole is shown projecting from the bottom of the figure into the capillaries of a glomerulus. Bowman's space is evident, and the visceral simple squamous epithelia surrounding the glomerulus is clearly identified. Proximal tubules, distal tubules and the afferent arterioles have wide lumens and none of these structures appear to be collapsed. Therefore, following glutaraldehyde fixation both renal vascular and tubular structures are patent and subsequent vascular filling with microfil represents a reproducible cast of the renal vasculature.

Kidneys from piglets 2, 20 and 50 days of age when perfused at low pressure demonstrated an age related increase in peritubular capillary filling (Figures 1, 2 and 3). This increase in peritubular capillary filling originated within the inner cortex at 20 days of age and progressed to the outer cortex by 50 days of age. In addition, pretreatment of a 2 day old piglet with acetylcholine significantly increased peritubular capillary filling following low pressure perfusion of the renal vasculature (Figure 6). These experiments visually substantiate that the renal vascular resistance of young piglets is high at birth and that this resistance decreases as the kidney matures. Afferent arterioles and glomerular capillaries appeared to be equally filled by low perfusion pressure at all ages, and the increased vascular filling of older pigs occurred within the postglomerular vessels. This observation suggests that the high renal vascular resistance of young

Figure 24. Light photomicrograph (40X) of the cortex from a rat kidney following glutaraldehyde perfusion *in situ*. AA = afferent arteriole; C = peritubular capillaries; G = glomerulus; BC = Bowman's capsule; PT = proximal tubule; DT = distal tubule.

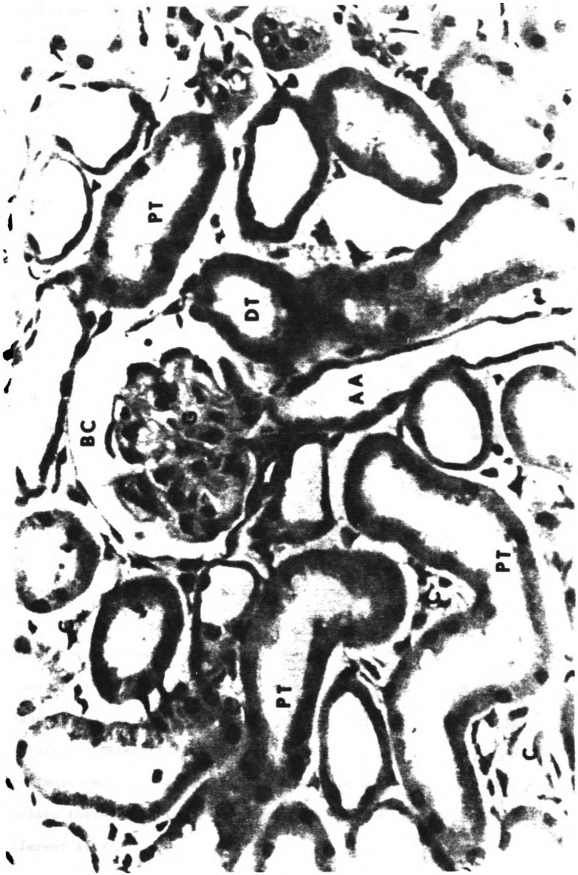


Figure 24

piglets may be localized within the efferent arteriole. Furthermore, the postglomerular resistance appeared to decrease in a centrifugal pattern beginning in the inner cortex and progressing toward the outer cortex with kidney maturation. Thus, the low outer to inner cortical blood flow ratio of 1-5 day old piglets (Table 3) may have resulted from a high postglomerular vascular resistance located in the vessels of the outer cortex. The resistance in the outer cortical vessels then decreased with age, and the increase in piglet O/I ratio between 1 and 50 days of age correlates with the increased outer cortical vascular filling after low pressure perfusion with Microfil over this same age range.

Although maturational changes in renal resistance were demonstrated following renal vascular perfusion at low pressure, kidney perfusion at physiologic pressures did not reveal any significant differences in renal vascular resistance between 2 and 50 day old pigs (Figures 4 and 5). This result was not expected since calculated renal resistances of 1-5 day old piglets are significantly greater than the renal resistance of 45-50 day animals (Figure 9). The use of renal vascular perfusion with Microfil for the localization of renal resistance is an imprecise technique. Despite the fact that differences in piglet renal resistance appear to exist between 1-5 and 45-50 day animals, it is possible that these differences cannot be detected at higher perfusion pressures by the formation of vascular casts with microfil. Therefore, future investigation of renal vascular development must rely upon more sophisticated techniques to conclusively isolate the site of renal resistance in young animals within the efferent arteriole.

Renal vascular development has been previously investigated in sheep (Davies, 1950) and rabbit kidneys (Lewis, 1958). These experiments were conducted in fetal kidneys and described a vascular plexus in the renal cortex connected to the afferent arteriole which resembled a sinusoidal system. The sinusoids in the fetal sheep kidney developed at approximately the same time as the glomerular circulation was established (Davies, 1950). This sinusoidal plexus in the rabbit persisted until 12 days after birth (Lewis, 1958). It was suggested that immature renal function, in particular the low glomerular filtration rate, in young rabbits partially was the result of the shunting of blood flow around the glomerular capillaries directly to the sinusoidal vessels (Lewis, 1958). To facilitate the shunting of blood flow away from the glomerular capillaries to the sinusoidal system, there must exist a lower resistance in the sinusoids than the glomerular capillaries. In the young piglet, these low resistance sinusoids were not identified by renal vascular perfusion with Microfil (Figure 6). In fact, these experiments demonstrated a high vascular resistance in the post-glomerular cortical vessels suggesting that at least in the neonatal pig the presence of low resistance sinusoids do not exist and cannot be responsible for reducing glomerular filtration rate or overall renal function.

Presently, investigations concerning renal anatomical development have dealt primarily with glomerulogenesis and renal tubular maturation and very little data are available which describe the development of the renal vasculature in neonates. In human kidneys, the efferent arteriole develops only after afferent arterioles and glomerular capillaries are present. Thus, the efferent arteriole is the last noncapillary vessel to mature in the kidney (Edwards, 1951). Therefore,

it is possible that low total renal blood flow, low outer cortical blood flow and high renal resistance in neonates may be due to undifferentiated vascular spaces in the kidney. The resistance to renal blood flow in 2 day old piglets, however, does not appear to result from undifferentiated blood vessels in the renal cortex since both perfusion of immature kidneys at 75 mmHg (Figure 4) and low pressure perfusion after vasodilation with acetylcholine (Figure 6) substantially enhanced the filling in the postglomerular capillaries. Thus, the low renal blood flow and high renal resistance at these young ages must result from some factor other than the absence of vascular morphological differentiation in the outer cortex of the kidney.

The possible humoral agent which has been considered to increase renal vascular resistance in the immature kidney is circulating angiotensin II. Plasma renin activity and circulating angiotensin II are elevated at birth, and since angiotensin II is a potent stimulus for vascular smooth muscle contraction, this hormone could increase renal resistance in the outer cortex and reduce renal blood flow in young animals. The effect of angiotensin II on renal hemodynamics of developing piglets was tested in these experiments using the competitive angiotensin receptor antagonist $Sar^1$-$Ala^8$-angiotensin II (saralasin). Angiotensin II blockade did not change renal blood flow, the intrarenal distribution of blood flow (Figure 11) or renal resistance (Figure 12) in piglets 1-5, 18-22, or 45-50 days of age. These data demonstrate no apparent age dependent relationship between competitive angiotensin II blockade with saralasin and renal hemodynamics suggesting that angiotensin II is not a factor increasing the renal resistance of the

neonatal pig. Although saralasin did not alter renal hemodynamics in developing pigs, it is difficult to derive specific conclusions about the effect of angiotensin II upon renal resistance since the possibility remains that the competitive antagonist may not have reached the proper receptor sites. In each experiment, saralasin completely blocked the increase in systemic blood pressure produced by 0.5, 1.0 and 2.0 μg angiotensin II injected intravenously. However, since the renal parenchyma has been shown to contain considerable angiotensinase activity (Itskovitz and Miller, 1966), significant angiotensin II antagonist metabolism could have occurred within the kidney despite evidence that systemic vascular angiotensin II receptors were effectively inhibited.

The present data also demonstrate that prostaglandin synthetase inhibition with indomethacin in 1-5 and 18-22 day old conscious piglets did not change total renal blood flow, the distribution of renal blood flow (Figure 11) or renal resistance (Figure 12). The inability of indomethacin to change basal renal hemodynamics in young piglets where renal resistance already is elevated is not surprising since the renal prostaglandins $PGE_2$ and $PGI_2$ dilate the renal vasculature (Itskovitz and McGiff, 1974; Bolger et al., 1978). Terragno et al. (1978a) demonstrated that blood vessels dissected from fetal calf kidneys released 10-fold more 6-keto $PGF_{1\alpha}$, the stable metabolic product of $PGI_2$, than adult vessels. This result was substantiated in pigs, and it was suggested that the increased prostaglandin biosynthetic capacity of fetal pig kidneys was due to their inability to produce an endogenous prostaglandin synthetase inhibitor (Terragno et al., 1978b). These investigators concluded that synthesis of prostaglandins may have

a regulatory function in fetal pig kidneys. However, the failure of indomethacin administration to piglets 1-5 days of age to alter renal hemodynamics (Figures 11 and 12) suggests that renal prostaglandins are not responsible for establishing basal renal blood flow in young pigs.

Since Terragno et al. (1978b) have shown enhanced prostaglandin synthetase activity of fetal pig renal arteries in vitro, it is possible that the dose of indomethacin employed in these experiments was not sufficient to provide complete enzyme blockade in vivo. Effective inhibition of renal prostaglandin synthetase by the dose of indomethacin administered to 15 day piglets in these experiments, was verified by arachidonic acid injection both before and after indomethacin. Arachidonate (1 mg and 2 mg i.v.) decreased renal blood flow as measured by a noncannulating electromagnetic flowmeter (Figure 25). Indomethacin (3.0 mg/kg followed by 2.0 mg/kg/hr) blocked the decrease in renal blood flow observed at both the 1 mg and 2 mg doses (Figure 25) confirming that this dose of indomethacin effectively inhibited the renal prostaglandin synthetase in 15 day piglets.

Other mechanisms contributing to the low renal blood flow in young animals may involve a combination of low systemic blood pressure and cardiac output and increased renal efferent sympathetic nerve activity. Indeed, systemic blood pressure (Figure 9) and cardiac output (Gruskin et al., 1970) of newborns are less than adult values. In puppies, the rise in renal plasma flow with age correlated directly with increasing systemic blood pressure (Kleinman and Lubbe, 1972), however, a similar correlation between cardiac output and renal blood flow did not exist in piglets (Gruskin et al., 1970). The low systemic blood pressure in

Figure 25. The effect of intravenous arachidonic acid (AA) injection on renal blood flow (RBF) and arterial blood pressure (BP) before and after indomethacin.

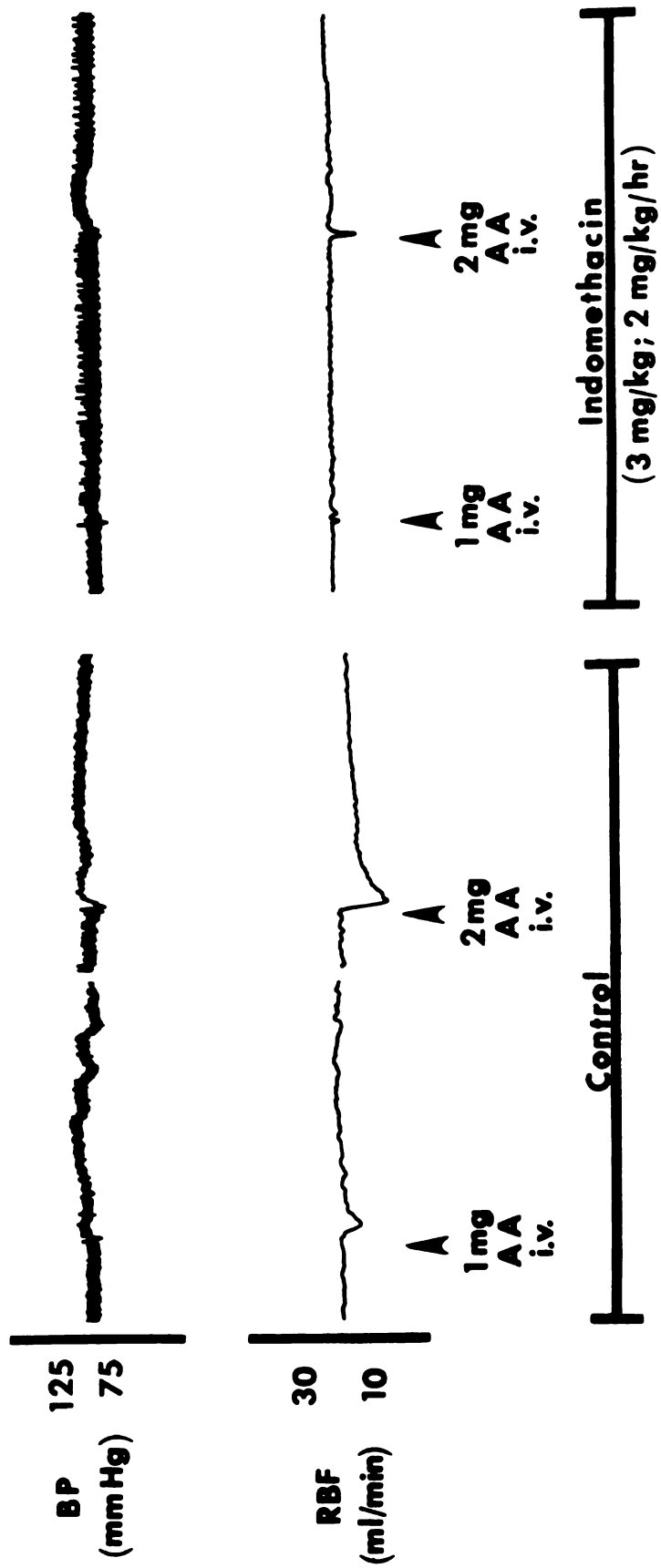
BP
(mm Hg)
125
75
RBF
(ml/min)
30
10
1mg
AA
i.v.
2mg
AA
i.v.
Control
1mg
AA
i.v.
2mg
AA
i.v.
Indomethacin
(3 mg/kg; 2 mg/kg/hr)

Figure 25

newborns may activate the sympathetic nervous system which could be responsible for suppressing renal blood flow. Gootman et al. (1972) have shown that the sympathetic nervous system is developed in young piglets and responds to various physiologic stimuli (Gootman et al., 1971). Furthermore, the renal vasculature of neonatal puppies was shown to have an increased sensitivity to norepinephrine (Jose et al., 1974). The possibility that sympathetic nerve activity alters neonatal renal hemodynamics was supported by evidence that α-adrenergic receptor inhibition in these animals shifted blood flow to the outer cortex (Jose et al., 1972). Clearly, more than one factor may be involved in suppressing the renal blood flow of young animals. The present experiments document increased postglomerular capillary resistance of the outer cortex in newborn pig kidneys, but this resistance did not appear to result from undifferentiated renal vascular spaces or increased circulating angiotensin II. Certainly, further investigation of other proposed factors leading to the altered renal hemodynamics of newborns is necessary.

Although prostaglandin synthetase inhibition did not affect renal blood flow in young piglets, indomethacin administration to conscious 45-50 day animals decreased renal blood flow and increased O/I ratio (Figure 11 and Table 7) and renal resistance (Figure 11). This decrease in renal blood flow was accompanied by no change in glomerular filtration rate resulting in an increase in filtration fraction (Figure 12). These data indicate that indomethacin, presumably through inhibition of prostaglandin synthesis, increased the renal vascular resistance of older pigs within the efferent glomerular arteriole, thereby enhancing filtration fraction. It is well established that indomethacin

decreases renal blood flow (Lonigro et al., 1973; Noordewier et al., 1978; Zimmerman, 1978) and increases O/I ratio in anesthetized dogs (Noordewier et al., 1978; Itskovitz and McGiff, 1974), but the effect of prostaglandin synthetase inhibition on renal hemodynamics in conscious animals is unclear. Swain et al. (1975) reported that prostaglandin synthetase inhibition with sodium meclofenamate reduced renal blood flow in conscious dogs, but indomethacin did not alter renal hemodynamics. Recently, Zimmerman (1978) demonstrated that meclofenamate had no effect on renal resistance in normotensive, conscious dogs which is in agreement with previous reports that indomethacin does not affect renal blood flow (Kirschenbaum and Stein, 1976) or the intrarenal distribution of blood flow (Zins, 1975) in unanesthetized animals. In the unanesthetized pig, indomethacin decreased total renal blood flow and increased the ratio of outer to inner cortical blood flow. Noordewier et al. (1978) reported that in anesthetized dogs indomethacin decreased renal blood flow to outer cortical zone 1 by 29%, whereas the blood flow to inner cortical zone 4 decreased by 55%. Since the renal medulla is the primary site of renal prostaglandin synthesis (Crowshaw and Szlyk, 1970; Bohman and Larsson, 1975), the increase in O/I ratio is due to a greater decrease in inner cortical blood flow rather than a shift in blood flow from the inner to the outer cortex.

It must be mentioned that although these pigs are fully awake at the time of experimentation, they have undergone an acute stress of anesthesia and critical comparisons between these animals and trained, conscious dogs may not be valid. The differences observed in renal

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It must be mentioned that although these pigs are fully awake at the time of experimentation, they have undergone an acute stress of anesthesia and critical comparisons between these animals and trained, conscious dogs may not be valid. The differences observed in renal

hemodynamics between conscious and anesthetized animals may result from increased sympathetic nerve activity following stress or anesthesia. Catecholamine infusion increased renal PGE release from isolated perfused kidneys (Needleman et al., 1974; McGiff et al., 1972a). Thus, catecholamine release by renal efferent nerve activity may increase prostaglandin synthesis above basal levels present in the normal animal. Administration of indomethacin during this altered state of medullary prostaglandin synthesis then may result in decreased renal blood flow with an increase in fractional blood flow to the outer cortex. Thus, the acute stress subjected to the piglets in the present experiments may have been sufficient to alter sympathetic nerve activity, and therefore, the changes in renal hemodynamics observed after indomethacin treatment were similar to the effects of indomethacin in anesthetized dogs.

5. Excretion of a Saline Load by Developing Piglets

It has been demonstrated previously that in addition to the low renal blood flow and high renal resistance at birth, the newborn of several species do not increase sodium excretion as rapidly as adult animals following saline infusion (Kleinman and Reuter, 1974; Aperia et al., 1975a; McCance and Widdowson, 1957; Aperia et al., 1974b; Bengele and Solomon, 1974). Four factors may be involved which limit sodium excretion by the neonatal kidney after saline infusion: 1) Since both glomerular filtration rate and renal blood flow are lower in young animals than adults (Figure 9), the infused sodium load may never become available to the renal tubules for excretion. 2) The renal tubule may not reduce passive sodium reabsorption after infusion of an

acute salt load. 3) After saline infusion into young animals, the sodium load may be distributed disproportionately to the extracellular fluid space rather than remaining within the systemic circulation. 4) Renal humoral factors which include angiotensin II and renal prostaglandins may directly affect neonatal tubular sodium transport.

The interrelationships between renal sodium excretion after volume expansion and renal hemodynamics initially were investigated utilizing both a low (2% of body weight) and high (8% of body weight) saline load. Mild saline loading (2% of body weight) tended to decrease hematocrit in both 1-5 and 45-50 day piglets (Table 11), however, this saline load did not affect renal hemodynamics of either age group (Figure 18). Fractional sodium excretion (Figure 19), urine flow rate and fractional potassium excretion (Table 11) were increased equally after volume expansion of each age group suggesting that young piglets are capable of excreting a mild sodium load as well as older animals which have a higher total renal blood flow and O/I ratio.

Since the low saline load induced only a mild natriuresis and diuresis, the relationship between renal hemodynamics and sodium excretion in developing piglets was investigated further using a large saline load (8% of body weight). Volume expansion of 1-5 and 45-50 day pigs increased fractional sodium excretion (Figure 23) without affecting renal blood flow (Figure 20). Saline infusion did not affect the intrarenal distribution of blood flow in young piglets, but the O/I ratio was increased in 45-50 day pigs (Figure 21). It was originally reported by Barger (1966) that sodium excretion in adult dogs was associated with a concomitant shift in blood flow to the outer cortex,

and it was suggested that the shorter outer cortical nephrons reabsorbed less sodium than the long juxtamedullary nephrons thereby enhancing renal sodium excretion. Subsequent experiments, however, have been unable to demonstrate a significant correlation between changes in cortical blood flow distribution and sodium excretion (Stein et al., 1973b; Blantz et al., 1971). The present data substantiate these observations. Volume expansion shifted renal blood flow toward the outer cortex in 45-50 day pigs but did not change intrarenal hemodynamics in younger pigs. Sodium excretion, however, was equally increased after infusion of the high saline load. Therefore, the increase in sodium excretion and the shift in blood flow to the outer cortex after saline infusion into 45-50 day pigs are probably not directly related.

Aperia et al. (1975a) demonstrated that lambs 5-28 days of age did not change the intrarenal distribution of blood flow after volume expansion, but 48-57 day animals increased outer cortical blood flow after a similar saline load. Kleinman and Reuter (1974) reported an increase in outer cortical blood flow following intravascular volume expansion in puppies with saline. However, both lambs and puppies failed to increase sodium excretion as rapidly as older animals. In both of these species of young animals, there was no significant correlation between changes in intrarenal blood flow distribution and sodium excretion suggesting that altered renal hemodynamics in the neonatal period were not responsible for their inability to increase sodium excretion after volume expansion. Intravascular volume expansion at both 2% and 8% of the body weight of 1-5 and 45-50 day piglets equally increased fractional sodium excretion and this increase was not

associated with any change in intrarenal hemodynamics in 1-5 day animals (Figures 21 and 23). Thus, these data suggest that the low renal blood flow and low outer to inner cortical blood flow ratio do not limit the ability of young piglets to enhance sodium excretion following intravascular volume expansion.

Conscious piglets 1-5 days of age increased fractional sodium excretion after high intravascular volume expansion to the same degree as 45-50 day old animals (Figure 23). This result was similar to the changes in sodium excretion of developing pigs after infusion of a low saline load (Figure 19). It is possible that the similar natriuresis of 1-5 day pigs after saline infusion and the sodium excretion of 45-50 day pigs following volume expansion is due to the fact that the maturity of renal regulation of sodium excretion is not different in these two age groups. Thus, the renal sodium excretion mechanisms of pigs are either quite mature at birth, or these mechanisms remain immature at 45-50 days of age. Maturational changes in renal function (Figure 9 and Table 4) as well as fractional sodium excretion (Table 4) over this age range, however, were documented in these piglets. Fractional sodium excretion significantly decreased between 1-5 and 18-22 day old animals. Sodium excretion did tend to increase between 18-22 and 45-50 day animals but this increase most likely was due to the weaning of piglets at about 40 days of age, and the conversion of their diets from sow's milk to a mineral, supplemented chow. Therefore, it appears that tubular regulation of sodium excretion did change in these pigs between 1 and 22 days of age. The explanation that the ability of the renal tubule in 45-50 day pigs to decrease sodium reabsorption remains

immature does not seem likely since fractional sodium excretion, renal blood flow, glomerular filtration rate, outer to inner cortical blood flow ratio and filtration fraction all showed distinct maturational changes with age (Figure 9 and Table 4).

The similar natriuresis after saline infusion of a volume equal to 2% of the body weight in 1-5 day piglets when compared to 45-50 day animals was probably due to the small saline load. However, after saline infusion in a volume equal to 8% of the body weight, the natriuresis of 1-5 day animals again, was similar to the 45-50 day age group (Figure 23). This result does not necessarily imply that the mechanisms regulating renal sodium excretion following saline infusion in piglets are mature at birth. The present experiments investigated the increase in fractional sodium excretion which occurred within 40 minutes after saline infusion, and the data indicated that the immediate natriuretic response to volume expansion of 1-5 day piglets was similar to 45-50 day old animals. However, the ability of young piglets to excrete a saline load also must be evaluated for an extended time period. It is possible that young piglets would require a longer period of time to totally excrete an infused sodium load than older animals.

An alternative explanation may be that the filtered sodium load of 1-5 day piglets was increased after volume expansion. Infusion of the large saline load into piglets of either age group did not change renal blood flow (Figure 20) or glomerular filtration rate (Figure 22) significantly. In 1-5 day piglets, however, the small decrease in renal blood flow (Figure 20) and slight increase in glomerular

filtration rate (Figure 22) resulted in a significant increase in filtration fraction (Figure 22) after volume expansion with saline. In addition, plasma sodium concentration of 1-5 day pigs increased slightly from 150.6 mEq/l to 152.2 mEq/l after saline infusion. Therefore, the natriuresis of 1-5 day piglets after volume expansion, which was similar to 45-50 day animals, may have resulted from a combination of these changes which increased the filtered sodium load. In older animals, neither filtration fraction nor plasma sodium concentration increased after volume expansion suggesting that other factors may be responsible for the natriuresis in this age group.

The natriuresis which occurs after volume expansion of normal adult animals with saline alone is produced by a decrease in tubular sodium reabsorption (Dirks et al., 1965; Cortney et al., 1965; Rector et al., 1967). In addition, several investigators have shown that plasma oncotic pressure may alter tubular sodium transport (Petersdorf and Welt, 1953; Schrier et al., 1968; Daugharty et al., 1968; Earley et al., 1966; Martino and Earley, 1967). Micropuncture experiments have demonstrated that a direct correlation existed between proximal tubular sodium reabsorption and peritubular capillary oncotic pressure (Lewy and Windhager, 1968; Spitzer and Windhager, 1970; Brenner et al., 1969; Daugharty et al., 1972; Brenner and Troy, 1971). Thus, as peritubular capillary oncotic pressure increased, proximal tubular sodium reabsorption also increased. These data do not mean that the distal tubule is incapable of altering sodium excretion. Knox et al. (1973) have reported that the natriuresis which occurs after saline infusion must result at least in part from decreased sodium reabsorption beyond

the proximal tubule. Thus, alterations in proximal tubule sodium reabsorption may not necessarily produce a "quantitatively" similar change in sodium excretion (Howards et al., 1968; Knox et al., 1968; Burke et al., 1971; Knox et al., 1973) suggesting that sodium excretion also is regulated in the distal tubule or collecting duct.

Alterations in sodium reabsorption by the proximal or distal tubule may affect the ability of newborn animals to enhance sodium excretion after volume expansion. Horster and Larsson (1976) reported that in isolated proximal tubules of neonatal rabbits hydraulic hydrostatic conductance was 7-fold greater than in adult proximal tubules. Similarly, when these proximal tubules were perfused in the presence of normal or hyperoncotic serum, the fluid reabsorption from neonatal tubules increased more than fluid reabsorption from mature tubules. These authors concluded that based on both this hydrostatic conductance data and ultrastructural observation of the proximal tubule cells, the immature tubule may be "leaky". This "leaky" proximal tubule, therefore, may result in enhanced proximal tubular sodium reabsorption. Experiments conducted in puppies, however, have not isolated the enhanced reabsorptive capacity to the proximal tubule but rather suggest that it is enhanced distal tubular sodium reabsorption after saline infusion which may prevent adequate sodium excretion by neonates. This conclusion was based on the observation that after saline infusion in puppies, distal tubule blockade of sodium reabsorption with chlorothiazide and ethacrynic acid increased fractional sodium excretion equal to that of mature animals (Kleinman, 1975).

Renal sodium reabsorption of piglets 1-5 and 45-50 days of age was investigated in the present experiments by volume expansion at 2% and 8% of their body weights with saline plus bovine serum albumin (BSA). BSA infusion in a volume equal to 2% of the body weight of both 1-5 and 45-50 day pigs increased fractional sodium excretion similar to the natriuresis after saline infusion alone (Figure 19). This result suggests that at least in the young pig, the sodium reabsorption of the renal tubules after a low sodium load is equal to that of more mature animals.

Careful examination of the effect of volume expansion with saline and saline plus BSA on plasma renin activity may identify the portion of the nephron where sodium reabsorption is being altered by volume expansion in these piglets. In 45-50 day animals, large volume saline infusion (8% of body weight) significantly decreased plasma renin activity (Table 12) and increased fractional sodium excretion (Figure 23). After volume expansion, the decrease in plasma renin activity suggests that the sodium load delivered to the macula densa region of the distal nephron was increased. The increased delivery of sodium to the distal nephron could not have resulted from an increased filtered sodium load since filtration fraction, glomerular filtration rate (Figure 22) and plasma sodium concentration (Table 12) were not affected by saline infusion. Therefore, the increased sodium load delivered to the distal nephron may have resulted from decreased reabsorption of sodium in the proximal tubule. These data suggest that the natriuresis after saline infusion into 45-50 day pigs was due in part to decreased fractional proximal tubular sodium reabsorption. In addition, volume

expansion (8% of the body weight) of 45-50 day pigs with saline plus BSA prevented the increase in sodium excretion previously observed after saline infusion alone (Figure 23). Again, saline plus BSA infusion did not alter filtration fraction, glomerular filtration rate (Figure 22) or plasma sodium concentration (Table 12) indicating that the filtered sodium load was unchanged following BSA volume expansion. Furthermore, in these experiments, plasma renin activity was not altered by infusion of saline plus BSA (Table 12) suggesting that the sodium load delivered to the macula densa also was unchanged after volume expansion. Since the filtered sodium load was not affected by infusion of saline plus BSA, the inhibition of sodium excretion by bovine serum albumin may have resulted from increased sodium reabsorption in the proximal tubule. This increase in proximal tubular sodium reabsorption may be due to the rise in peritubular capillary oncotic pressure following albumin infusion.

The nephron segment which may be responsible for natriuresis following volume expansion of 1-5 day pigs also may be identified by examination of the plasma renin activity. After saline infusion alone (8% of body weight), sodium excretion was significantly increased (Figure 23), but plasma renin activity was unchanged (Table 12). Although filtration fraction did increase in 15 day piglets after volume expansion with saline (Figure 22), glomerular filtration rate (Figure 22) and plasma sodium concentration (Table 12) were unchanged. These data indicate that the sodium load leaving the proximal tubule was similar during control periods and after volume expansion. Therefore, unlike 45-50 day pigs, the natriuresis which occurs after volume

expansion of 1-5 day pigs did not result from decreased proximal tubule sodium reabsorption. Instead, the data suggest that the increased fractional sodium excretion following saline infusion resulted from a reduction of fractional sodium reabsorption in the more distal portions of the nephron. These results also suggest that the proximal tubule of newborn piglets may be functionally similar to the proximal tubule of young rabbits. Horster and Larsson (1976) documented that young rabbit proximal tubules reabsorb more fluid than do proximal tubules from older animals. The present data suggest that proximal tubules of young piglet kidneys also are immature. After volume expansion, the proximal tubules of 1-5 day piglets did not effectively decrease their fractional sodium reabsorption whereas by 45-50 days of age saline infusion appeared to decrease fractional sodium reabsorption in the proximal tubule. Sodium excretion after volume expansion of young pigs, however, was similar to the sodium excretion of older animals. Thus, in contrast to the puppy in which distal tubular function may be immature (Kleinman, 1975), the neonatal piglet compensated for the immature proximal tubule by reducing sodium reabsorption in the more distal portions of the nephron.

The nephron segment where inhibition of sodium excretion occurs after saline plus BSA infusion into 1-5 day piglets is not identified as easily. Plasma renin activity decreased following BSA saline loading (Table 12). Plasma sodium concentration (Table 12) was not changed by BSA volume expansion but glomerular filtration rate was increased significantly (Figure 22). These data indicate that both the filtered sodium load and the delivery of sodium to the distal nephron

were increased after BSA volume expansion. The natriuresis observed after saline infusion alone, however, was blocked by addition of BSA to the infusion solution (Figure 23). Therefore, this inhibition of sodium excretion may have resulted from increased sodium reabsorption in the distal tubule or collecting duct. This conclusion, however, is complicated by the observation that the absolute value of glomerular filtration rates before and after BSA volume expansion were less than the glomerular filtration rates of piglets receiving only saline (Figure 22). Therefore, despite the increase in glomerular filtration rate following volume expansion with BSA, the total filtered sodium load of these piglets was less than the filtered sodium load of animals volume expanded with saline. Thus, the reduction in sodium excretion after BSA saline infusion could have resulted from a lower filtered sodium load.

The hormones, angiotensin II and prostaglandin $E_2$, have been postulated as factors which may affect renal sodium excretion in adult animals either by directly altering tubular sodium reabsorption or by changing renal hemodynamics. Circulating angiotensin II as well as intrarenal angiotensin synthesis may affect neonatal renal sodium excretion after saline infusion. Preliminary reports have shown that angiotensin II inhibition with saralasin decreased both glomerular filtration rate and PAH clearance in puppies (Jose et al., 1973) and fetal lambs (Moore et al., 1974). These authors suggested that angiotensin II may regulate tubular function in the neonatal kidney. Therefore, it was of interest to investigate the effect of angiotensin II inhibition with saralasin ($Sar^1$-$Ala^8$-angiotensin II) on renal sodium

excretion of developing piglets before and after saline infusion (2% of the body weight). The dose of saralasin infused (5-10 μg/min) was adjusted to provide complete blockade of the blood pressure response following intravenous injection of 0.5, 1.0 and 2.0 μg of angiotensin II. Angiotensin II has been measured in the renal lymph of dogs (Bailie et al., 1971) confirming that the intrarenal synthesis of angiotensin II occurs within the renal interstitium. Although this dose of saralasin produced sufficient systemic angiotensin II blockade the possibility remains that intrarenal angiotensin II receptors may be only partially inhibited.

Intravascular volume expansion of piglets 1-5, 18-22 and 45-50 days of age increased fractional sodium excretion (Figure 14). In 1-5 day piglets, angiotensin II inhibition with saralasin, however, blocked this natriuresis induced by saline infusion (Figure 13) without changing renal hemodynamics, filtration fraction or glomerular filtration rate (Table 8). This antinatriuretic effect of saralasin was not observed in piglets 18-22 or 45-50 days of age (Figure 15) and again saralsin did not affect renal hemodynamics of these older animals (Table 8). These data indicate that since inhibition of angiotensin II reduced the natriuresis observed after saline infusion into 1-5 day piglets, angiotensin II may function as a natriuretic hormone in young piglets.

The analogue $Sar^{1}$-$Ala^{8}$ angiotensin II (saralasin) may exhibit agonistic properties of angiotensin II. Some investigators have suggested that angiotensin II may decrease sodium excretion (Waugh, 1972; Fagard et al., 1978) and this antinatriuretic effect of angiotensin II was shown to occur after infusion of low doses of the hormone (Malvin

and Vander, 1967; Lameijer et al., 1966). Therefore, manifestation of these agonistic properties by saralasin could prevent the natriuretic response in young piglets. In 3 piglets 1-5 days of age, angiotensin II synthesis was blocked by the angiotensin I converting enzyme inhibition SQ20,881 (100 µg/kg as a bolus followed by an infusion of 13 µg/min) both before and after volume expansion. In these experiments, this dose of SQ20,881 prevented the increase in arterial blood pressure observed after injection of 2 µg of angiotensin I. In each animal tested, volume expansion after AII blockade produced a small diuresis but reduced the natriuresis which was observed previously in untreated piglets (Table 9). Thus, in piglets pretreated with saralasin or SQ20,881 the observed inhibition of volume expansion induced natriuresis resulted from angiotensin II blockade rather than from an agonistic effect of the angiotensin analogue.

A direct intrarenal role of angiotensin II regulating sodium excretion has been examined by several investigators (Hughes-Jones et al., 1949; Louis and Doyle, 1965; Porush et al., 1967; Langford, 1964; Trippodo et al., 1977; Lohmeier et al., 1977). Angiotensin II infusion decreased sodium excretion in dogs (Waugh, 1972; Fagard et al., 1978), and this antinatriuretic effect may be a function of the animal's initial state of sodium balance (Trippodo et al., 1977). A direct effect of angiotensin on renal tubular sodium reabsorption, however, is difficult to interpret from these data since angiotensin II infusion also decreased both renal blood flow and glomerular filtration rate. Micropuncture experiments have shown that angiotensin II infused into the peritubular capillaries may directly inhibit distal tubular sodium

reabsorption (Lowitz et al., 1969). The present data suggest that systemically circulating angiotensin II may be natriuretic in young piglets. Since circulating angiotensin concentrations in young animals are elevated (Broughton-Pipkin et al., 1971; Broughton-Pipkin et al., 1974; Broughton-Pipkin and Symonds, 1977), plasma angiotensin in young piglets may directly increase sodium excretion, and therefore, angiotensin II inhibition with saralasin would diminish the natriuresis following saline volume expansion. In older pigs where plasma renin concentration is reduced (Bailie et al., 1979) and presumably circulating angiotensin is lower, inhibition of angiotensin II would have little effect upon volume expansion induced sodium excretion. Indeed, the fractional sodium excretion of 1-5 day piglets was significantly greater than the sodium excretion of older animals (Table 4). Thus, circulating angiotensin II in newborn piglets may actually decrease sodium reabsorption and increase sodium excretion. In addition, Lowitz et al. (1969) have suggested that angiotensin II in the peritubular capillaries may directly decrease sodium reabsorption in the distal nephron. The natriuresis after volume expansion (8% of the body weight) of 1-5 day piglets may have resulted from decreased distal tubular sodium reabsorption as previously discussed. Therefore, the present data suggest that in newborn piglets the natriuresis after saline infusion may be due to tonic inhibition of sodium reabsorption by circulating angiotensin II.

Prostacyclin ($PGI_2$) and $PGE_2$ are synthesized in the renal vasculature and medulla, respectively, and these hormones have been shown to alter renal sodium excretion either by increasing total renal blood

flow (Johnston et al., 1968; Martinez-Maldonado et al., 1972; Shimzu et al., 1969) or by directly affecting tubular sodium reabsorption (Tannenbaum et al., 1975; Kirschenbaum and Stein, 1976; Lipson and Sharp, 1971; Haylor and Lote, 1976). In anesthetized dogs, the intrarenal infusion of $PGE_2$ (Johnston et al., 1968; Tannenbaum et al., 1975) and $PGI_2$ (Bolger et al., 1978) resulted in a prompt increase in fractional sodium excretion, but this natriuresis may have been due to increased renal blood flow after prostaglandin administration. Kirschenbaum and Stein (1976), however, demonstrated that in conscious dogs two structurally dissimilar inhibitors of prostaglandin synthetase increased sodium excretion in the absence of renal hemodynamic changes suggesting that renal prostaglandins may be antinatriuretic. These data are supported by the observation that $PGE_1$ stimulates and indomethacin inhibits sodium transport in isolated toad bladder (Lipson and Sharp, 1971) and frog skin (Hall and O'Regan 1974; Gerencser, 1978) preparations.

Since renal prostaglandins have been implicated as mediators of salt excretion in adult animals, these hormones also may function to alter the ability of the neonatal kidney to increase sodium excretion after saline infusion. Terragno et al. (1978b) have demonstrated that fetal blood vessels synthesize $PGE_2$ and $PGI_2$ at a faster rate than adult vessels. These investigators concluded that synthesis of prostaglandins may have a regulatory function in fetal pig kidneys. The present experiments invesigated the interrelationships between prostaglandin synthetase inhibition and renal blood flow before and after intravascular volume expansion (2% of the body weight) of developing

pigs. In 1-5 and 18-22 day old conscious piglets, total renal blood flow, the distribution of renal blood flow (Figure 11), renal resistance and glomerular filtration rate were unaffected by prostaglandin synthetase inhibition with indomethacin both before (Figures 11 and 12) and after (Table 8) mild volume expansion. In addition, indomethacin administration to these piglets did not alter the natriuresis observed after saline infusion (Figure 13). Although renal prostaglandin synthesis in young animals may have an as yet, undetermined role in renal function, it does not appear that these hormones function in the normal regulation of sodium excretion following intravascular volume expansion of conscious piglets. In indomethacin treated 45-50 day old pigs, volume expansion increased fractional sodium excretion in 4 out of 6 experiments (Figure 13). This response was highly variable and the significance of prostaglandin synthetase inhibition in the regulation of sodium excretion after saline infusion in these older pigs would be speculative.

Another factor which may affect neonatal sodium excretion after volume expansion is the diffusion of infused saline from the vascular spaces into the extravascular compartment. Plasma protein concentration is low at birth and increases with age (Pownall, 1970), and this low oncotic pressure may prevent an increase in vascular volume after saline infusion. Arant (1978) reported that in puppies, addition of 10% BSA to the saline infusion solution significantly increased plasma volume as well as fractional sodium excretion above control values observed after saline expansion alone. In the present experiments, saline infusion (8% of the body weight) into 1-5 day piglets did not

affect hematocrit (Table 12) whereas intravsacular volume expansion of 45-50 day animals significantly reduced hematocrit (Table 12). Sodium excretion of both 1-5 and 45-50 day pigs increased equally after saline infusion (Figure 23). These results indicate that although volume expansion may have increased the vascular volume of 45-50 day pigs more than the vascular volume of 1-5 day animals, the difference in hemodilution between these two age groups did not affect renal sodium excretion. BSA saline infusion (8% of body weight) increased vascular volume of both 1-5 and 45-50 day pigs as shown by the dramatic decrease in hematocrit (Table 12), but in contrast to the data of Arant (1978), BSA infusion prevented the natriuresis of volume expansion in both age groups (Figure 23). Presently, reasons for the discrepancies between the present data and those of Arant are not entirely clear. It is possible that since the natriuresis after saline infusion of 1-5 and 45-50 day pigs was not different, comparisons between piglets and puppies may not be valid. Further investigation of the problem concerning vascular volume changes following saline infusion in other species is necessary before definite conclusions can be made.

## SUMMARY

The purpose of this investigation was three-fold: 1) To identify factors which may be involved in the control of hemodynamics in neonatal kidneys. 2) To provide evidence for an interrelationship between neonatal renal hemodynamics and the inability of young animals to excrete an acute saline load. 3) To investigate factors which may be involved in the control of plasma renin activity in young animals. The results of the present experiments have provided evidence for the following conclusions.

1. In the newborn piglet, low resistance sinusoids were not identified using renal vascular perfusion with Microfil. Thus, the immature renal function in young piglets does not result from the shunting of blood flow around the glomerular capillaries to these sinusoids.

2. Incomplete vascular filling was observed after low pressure perfusion in the outer cortex of young piglet kidneys verifying the high vascular resistance in this region of immature kidneys. In more mature animals, peritubular capillary filling increased suggesting that the high vascular resistance in newborn kidneys may be localized within the postglomerular vessels.

3. Vascular filling following perfusion at physiologic pressure and after vasodilation with acetylcholine was complete indicating that the low renal blood flow and high renal resistance in newborn piglets

must result from some factor other than the absence of morphological differentiation in the outer cortex.

4. No apparent age dependent relationship between competitive angiotensin II antagonism with saralasin and renal hemodynamics was demonstrated. Therefore, angiotensin II does not appear to be a factor increasing renal resistance in the neonatal pig.

5. The failure of indomethacin administration to young piglets to alter renal hemodynamics suggests that renal prostaglandins are not responsible for establishing basal renal blood flow in these young animals.

6. Following intravascular volume expansion (8% of the body weight) of developing pigs, no relationship between total renal blood flow, the intrarenal distribution of blood flow and fractional sodium excretion was demonstrated. Therefore, the increase in sodium excretion and changes in renal hemodynamics which occur after volume expansion of piglets are not directly related.

7. Since fractional sodium excretion of newborn and older pigs following both low and high saline loading was increased equally, the low renal blood flow and low outer to inner cortical blood flow ratio does not limit the magnitude of the rise in fractional sodium excretion of young piglets following intravascular volume expansion.

8. Following volume expansion, the similar natriuresis of developing pigs demonstrates that the immediate natriuretic response of newborn pigs to saline infusion is relatively mature.

9. Although volume expansion may have increased the vascular volume of 45-50 day pigs more than that in 1-5 day animals, the

difference in hemodilution between these age groups did not affect fractional sodium excretion. Therefore, possible diffusion of the infused saline load out of the vascular space does not reduce the immediate natriuretic response of young piglets to volume expansion.

10. Following volume expansion of newborn piglets treated with saralasin, an antinatriuretic effect of angiotensin II antagonism was demonstrated. In young piglets, the natriuresis after saline infusion may be due in part to inhibition of sodium reabsorption by circulating angiotensin II.

11. Prostaglandin synthetase inhibition with indomethacin did not alter the natriuretic effect of volume expansion in developing piglets indicating that renal prostaglandins are not involved in the regulation of sodium excretion following saline infusion in young pigs.

12. Since infusion of the same saline load into both newborn and more mature pigs was ineffective in altering the plasma renin activity, renin secretion stimulated by the macula densa receptor is not responsible for the elevated plasma renin activity at young ages.

13. Plasma renin activity of young piglets was increased following competitive angiotensin II receptor antagonism with saralasin suggesting that renin release at younger ages is not enhanced by the absence of angiotensin II negative feedback on the juxtaglomerular cells.

14. No age related effect of prostaglandin synthetase inhibition with indomethacin on plasma renin activity was demonstrated in pigs between 1 and 50 days of age. Thus, the increased plasma renin activity

of young piglets does not appear to result from renin secretion stimulated by prostaglandins.

15. The circulating half-life of renin in newborn pigs was not significantly different from that in more mature animals. Therefore, in young piglets immature hepatic function and a decreased rate of renin catabolism is not entirely responsible for elevating neonatal plasma renin activity.

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