AN ABSTRACT OF THE THESIS OF

Nancy D. Binder for the Doctor of Philosophy Degree in Physiology

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The kidneys play an essential role in the long term regulation of blood pressure in the postnatal mammal through their regulation of extracellular fluid volume. In the prenatal animal the kidney is not known to regulate extracellular fluid volume since excreted ions and water remain a part of the conceptus. These experiments were therefore conducted to test the hypothesis that the fetal kidney is unnecessary for blood pressure regulation.

Bilateral nephrectomies were performed and chronic indwelling vascular, amniotic fluid, and allantoic fluid catheters were inserted into fetal lambs. Their arterial blood pressures were measured 4 - 23 days after the operation. The fetuses continued to grow after bilateral nephrectomy and had arterial blood pressures which were within the range found in normal chronically catheterized fetal lambs.

Volumes of distribution and placental permeability surface area products for $^{22}Na^{+}$ and $^{36}Cl^{-}$ were also measured in intact and anephric fetuses. The anephric lambs had volumes of distribution and permeability surface area products for $^{22}Na^{+}$ that were two times greater than those for controls and volumes of distribution and permeability surface area products for $^{36}Cl^{-}$ that were 1.5 times greater than those for controls. There were also differences in maternal and fetal plasma concentrations of Na^{+} and Cl^{-} after nephrectomy. It was speculated that these concentration differences reflect differences in extracellular fluid regulation between anephric fetuses and normal fetuses.

The roles of the autonomic nervous system, the renin angiotensin system and vasopressin secretion in the regulation of fetal arterial blood pressure were discussed. It was concluded that the fetal kidneys are unnecessary for arterial blood pressure control before birth and the mechanism of arterial blood pressure regulation is still unknown.

ARTERIAL BLOOD PRESSURE IN THE ANEPHRIC SHEEP FETUS

by

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APPROVED:

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(Chairman, Graduate Council)

To Lois and Augie

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

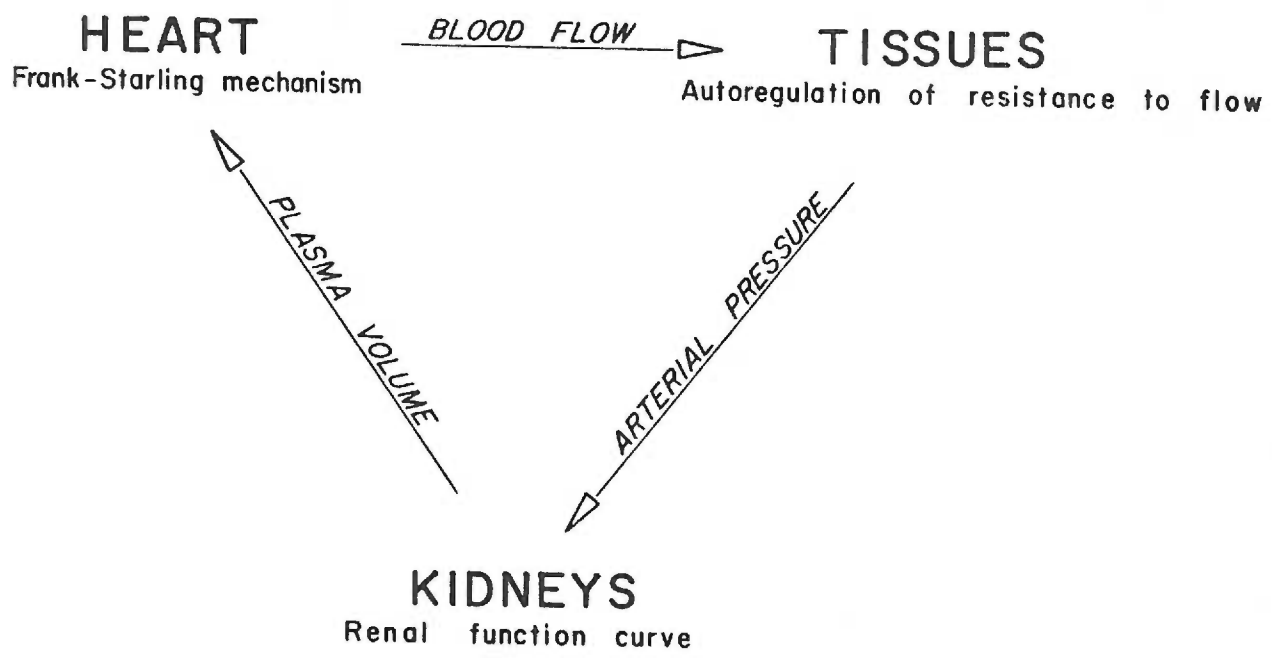
INTRODUCTION

Arterial blood pressure is the driving force which supports the flow of blood to the various tissues of the body at a rate proportional to the metabolic need of the tissue. As a starting point for understanding the mechanisms which may be involved in the regulation of arterial blood pressure of the fetal lamb, it would be useful to look at some of the mechanisms that are involved in blood pressure regulation in the postnatal mammal.

Guyton and his colleagues have presented a detailed analysis of the complex interrelationships of many physiologic mechanisms that are known to affect arterial blood pressure (Guyton, Coleman, Cowley, Manning, Norman, and Ferguson, 1974; Coleman, Cowley, and Guyton, 1975). The important conclusions of their analysis are that the short term control of arterial blood pressure is a function which is primarily vested in the nervous system, but that the long term regulation of arterial blood pressure is governed through the mechanisms which regulate the fluid balance of the body. According to their analysis the kidney is the principal organ for long term blood pressure regulation because it provides an "integral" control mechanism whereby the volume of fluid circulating in the body is adjusted in relation to the arterial blood pressure (Guyton, Coleman, Cowley, Sheel, Manning, and Norman, 1972).

According to their analysis, the intrinsic relationships shown in Figure 1 are adequate to provide a negative feedback system to regulate arterial blood pressure. The kidneys excrete water and solutes at a rate which balances the net difference between fluid intake and nonrenal

Figure 1. Diagram of the basic components of the negative feedback system for the long term regulation of arterial blood pressure. The plasma volume is a fraction of the total extracellular fluid of the body and changes directly with changes in extracellular fluid. The volume of fluid in the vasculature determines the filling pressure of the heart. The heart translates end ventricular diastolic volume into cardiac output by means of the Frank Starling mechanism. The blood vessels to the tissues change their resistances in accordance with the metabolic needs of the tissues. Arterial blood pressure is the product of the cardiac output and total peripheral resistance. The rate of fluid excretion by the kidney is determined by the arterial blood pressure and thus the amount of extracellular fluid in the body is changed.


HEART
Frank-Starling mechanism
BLOOD FLOW
TISSUES
Autoregulation of resistance to flow
PLASMA VOLUME
ARTERIAL PRESSURE
KIDNEYS
Renal function curve

losses so that in the steady state the volume of fluid in the body is constant. The body fluid is proportioned between intracellular, interstitial, and intravascular compartments by the dynamics of fluid exchange between the compartments. The portion of fluid that is within the vasculature functions with the capacitance of the systemic vasculature to determine the filling pressure of the heart. The Frank-Starling mechanism of the heart adjusts the cardiac output so that it is roughly proportional to ventricular end diastolic pressure. The tissues dilate or constrict to keep their flow of blood appropriate for their metabolic needs, and the individual resistances of the tissues determine the total peripheral resistance. The rate of fluid excretion by the kidney is determined by arterial blood pressure. If the blood pressure is below a required level the kidney retains fluid which would otherwise be excreted. This acts to increase venous pressure and cardiac output and thus restores arterial blood pressure to the normal level. Conversely, if arterial pressure is too high, the kidneys increase fluid excretion until blood pressure is lowered to its normal level.

There are many physiologic mechanisms which are involved in the control of arterial blood pressure. The basic difference between short term mechanisms of blood pressure regulation, such as the baroreceptor system, and the long term mechanism is in the nature of the feedback effector of the system. The short term mechanisms of blood pressure regulation are all "proportional control" systems. The correction effected by such a system is approximately proportional to the "error" sensed by the system. A measure of the effectiveness of a proportional control device is its "open loop gain" which is the ratio of the applied

correction and the remaining difference between corrected pressure and ideal pressure. The word remaining needs to be emphasized because if no error remained a proportional control device would no longer effect a correction. It is therefore impossible for a proportional control system to completely correct an alteration in blood pressure; due to the finite gain of the system an "error" needs to be sensed in order for the system to operate. Examples of proportional control systems for arterial blood pressure control include the baroreceptor mechanism and feedback systems which cause the release of vasopressin and the generation of angiotensin to provide vasoconstriction in the face of a reduction in arterial blood pressure.

One of the important conclusions of Guyton's systems analysis is that a proportional control system cannot govern the long term regulation of blood pressure. Long term control must be subserved by an integral control mechanism because it is the only type of mechanism which is capable of completely correcting an altered blood pressure. The effect of such a system is equal to the time integral of all its activities since it began to operate. The gain of this type of system is variable and will approach infinity as the blood pressure is restored to normal. The regulation of body fluid and thus of blood volume by the kidney does represent an integral control mechanism. A change effected by this system, for instance an increase in circulating blood volume, will persist even after the kidneys have returned the fluid volume of the body to normal and net excretion is again exactly equal to intake at an arterial blood pressure which is recognized as normal by the kidney.

Although proportional control devices are ineffective for long

term regulation of arterial blood pressure they can provide life saving "buffer" responses that prevent excesses in blood pressure during the time it takes for the integral control system to cause complete correction. The initial action of a proportional control system in response to an alteration in blood pressure prevents the integral control system from acting as rapidly as it would in the absence of the proportional control system because it causes a reduction in sensory input to the integral control device. This is a comparatively small price in view of the great advantage that the proportional control system provides for keeping acute fluctuations in arterial blood pressure within narrow limits. The separation of systems which regulate blood pressure into purely proportional control systems or purely integral control systems is not possible in a practical sense. For instance, the renin-angiotensin system can act as a proportional control device by causing vasoconstriction in response to an acute decrease in blood pressure, but it also acts through a stimulation of aldosterone to cause the kidney to retain a greater amount of sodium and thus a greater amount of fluid.

If the fetus is to make a successful adaptation to life outside the uterus it is important that these control mechanisms be developed by the time of birth, but there is no a priori reason to assume that the process of long term regulation of arterial blood pressure by the fetus is governed through the same mechanisms that operate after birth. There is extensive documentation that the kidneys of the sheep fetus are present and elaborating urine by at least the 60th day of gestation (Alexander, Nixon, Widdas, and Wohlzogen, 1958; Alexander and Nixon, 1964; Hervey and Slater, 1968; Buddingh, Parker, Ishizai, and Taylor

1971; Gresham, Rankin, Makowski, Meschia, and Battaglia, 1971; Mellor and Slater, 1972; Rankin, Gresham, Battaglia, Makowski, and Meschia, 1972; Robillard, Sessions, Kennedy, Hamel-Robillard, and Smith, 1977). It has also been shown that the sheep fetus responds appropriately to volume expansion effected by the intravenous infusion of 50 ml of normal saline by increasing glomerular filtration and sodium excretion (Hurley, Kirkpatrick, Pitlick, Friedman, and Mendoza, 1977). However, any urine which is elaborated by the kidney of the fetal lamb passes into the amniotic and allantoic fluids which are actually special extracellular fluid compartments of the fetus. A net gain or loss of water or solute from the conceptus as a whole can occur only through exchange with the maternal fluids across the placenta. Examination of the principle routes of extracellular fluid exchange of the fetal lamb (Figure 2) points to the placenta as the organ through which the fluid balance of the fetus is regulated. If there is a relationship between the regulation of body fluid and the regulation of arterial blood pressure in the fetus which is similar to the relationship which exists in the postnatal mammal, then the placenta must replace the kidney as a link in the feedback loop for long term regulation of arterial blood pressure in the fetus (Figure 3).

The umbilical vessels apparently do not possess nervous innervation and they are remarkably unresponsive to direct effects of vasoconstrictive substances (Assali, Holm, and Sehgal, 1962; Dawes, 1968; Goodwin, Mahon, and Reid, 1968; Roach, 1973; Berman, Goodlin, Heymann, and Rudolph, 1978; Rankin and Phernetton, 1978). Since the resistance of the umbilical circulation does not appear to be under the control of the sheep fetus,

Figure 2. Principle sites for water and solute exchange with the fetal plasma and maternal and fetal extracellular fluids. Fetal and maternal plasmas interchange freely with their respective interstitial fluids and the interstitial fluid of the placenta. Unidirectional arrows from fetal plasma to amniotic and allantoic fluids represent contributions to these fluids by the excretion of urine by the fetal kidneys. These fluids probably exchange with each other and with the interstitial fluid of the fetus. The separation of maternal and fetal extracellular fluids is indicated by a broken line because the principle site of exchange between mother and fetus is in the shared interstitial fluid of the placenta.

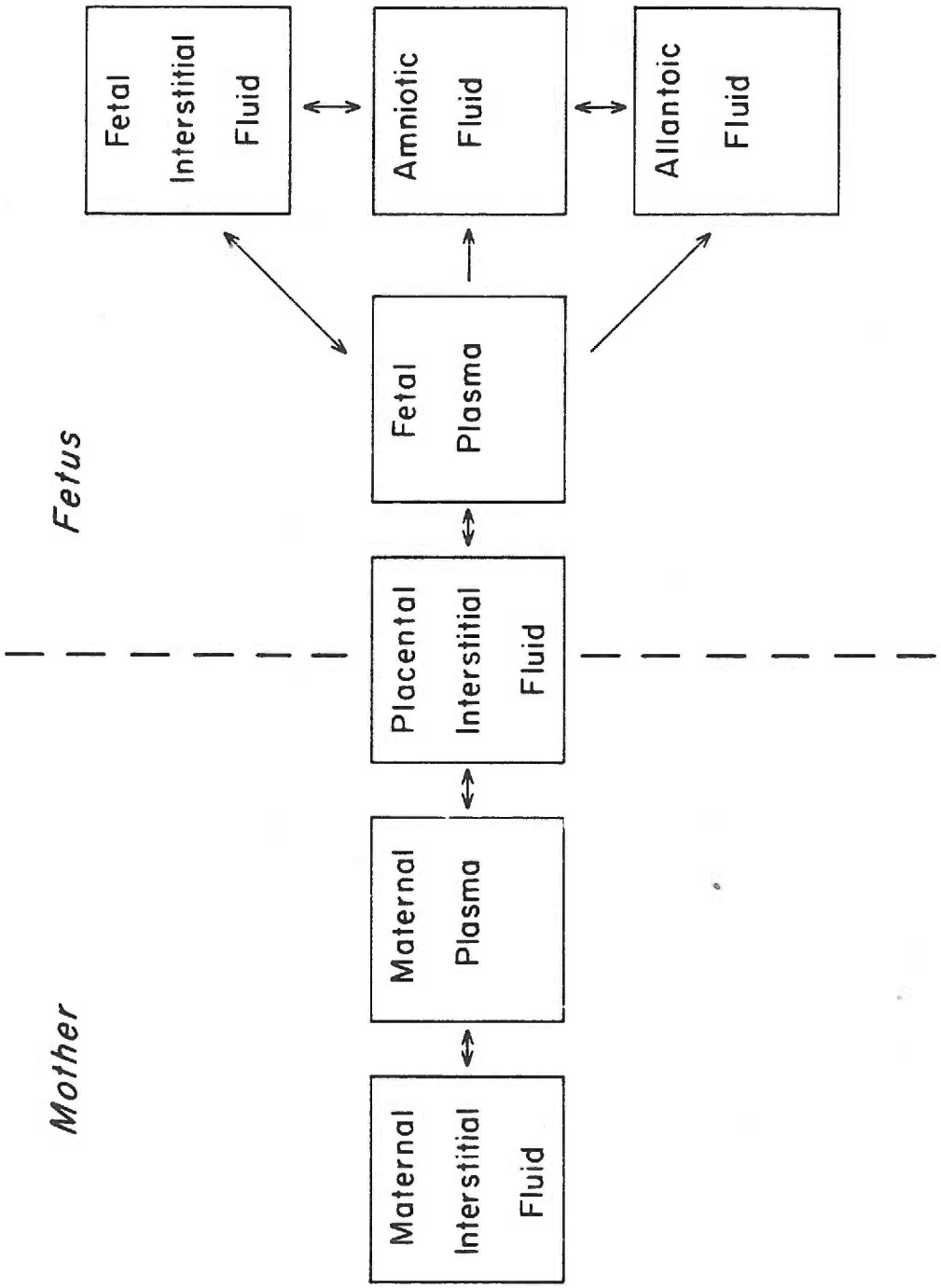
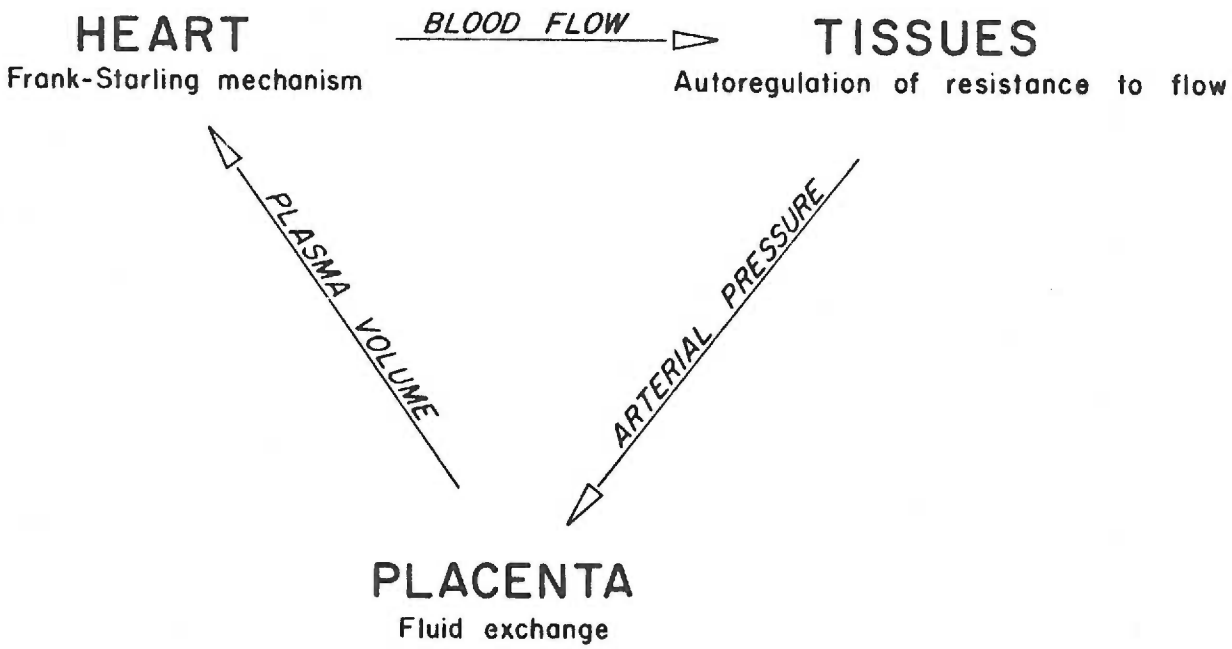

Fetal Interstitial Fluid
Amniotic Fluid
Allantoic Fluid
Fetal Plasma
Fetus
Placental Interstitial Fluid
Maternal Plasma
Maternal Interstitial Fluid
Mother

Figure 3. Diagram of a possible negative feedback system for the long term regulation of fetal arterial blood pressure. The mechanism involves the same components as the system shown in Figure 1 for the postnatal mammal except that the regulation of extracellular fluid volume takes place via placental exchange of water and solutes rather than by renal excretion. In order for such a mechanism to regulate blood pressure there must be some relationship between fetal arterial blood pressure and net fluid exchange in the capillaries of the placenta.


HEART
Frank-Starling mechanism
BLOOD FLOW
TISSUES
Autoregulation of resistance to flow
PLASMA VOLUME
ARTERIAL PRESSURE
PLACENTA
Fluid exchange

the flow through the umbilical circulation is proportional to the fetal arterial blood pressure. If it is assumed that pre- and postcapillary resistances remain constant just as total resistance in the umbilical circulation remains constant, then an increase or decrease in arterial blood pressure of the fetus would be reflected by a proportional change in the pressure of the blood within the fetal capillaries of the placenta. Since water exchange across the placenta must occur under the influence of hydrostatic and osmotic forces, a change in the hydrostatic pressures in the fetal capillaries of the placenta would act to change the balance of forces operating to move water across the placenta. Thus a decrease in arterial blood pressure of the fetus would tend to increase its rate of acquisition of water and an increase of fetal arterial blood pressure would tend to decrease its rate of water acquisition. Thus it appears that the placenta is in a position to provide for an interdependent regulation of fluid volume and arterial blood pressure of the fetus (Faber, 1971). Although a balance of hydrostatic and osmotic forces between maternal and fetal capillaries of the placenta is adequate to explain regulation of fetal fluid volume and long term maintainence of constant fetal arterial blood pressure, it is unable to provide an explanation for an increase in the fluid volume of the fetus with growth, particularly in the face of the increase in arterial blood pressure that occurs during growth of the sheep fetus (Barcroft, 1946; Dawes, 1968; Boddy, Dawes, Fisher, Pinter, and Robinson, 1974; Robillard, Matson, Sessions, and Smith, 1979). Any theory which attempts to explain the long term regulation of arterial blood pressure of the fetus must account for these changes.

The purpose of the research described in this thesis was to investigate the role of the kidneys in the long term regulation of arterial blood pressure in the sheep fetus. It was predicted that anephric sheep fetuses would maintain normal blood pressure and would sustain a normal increase in blood pressure with advancing gestation. It was also predicted that the anephric fetus would continue to acquire water and solutes necessary for growth. This is in contrast to the prediction by Mott (1978) that the anephric sheep fetus would have a lower than normal arterial blood pressure because of the loss of the kidneys as the major source of renin. It is also in contrast with the report by Dutton, Mott, and Valdes-Cruz (1978) that six sheep fetuses developed higher than normal arterial blood pressures within two to nine days following bilateral nephrectomy *in utero*.

In addition to measuring blood pressure in bilaterally nephrectomized and intact sheep fetuses we measured plasma and blood volumes in anephric sheep fetuses to look for an altered relationship between blood volume and arterial blood pressure in case the blood pressures of anephric fetuses were found to be different from those of intact fetuses.

We also wished to compare water and solute acquisition by intact and nephrectomized sheep fetuses. This was done by measuring transplacental gradients for solutes from the concentrations of solutes found in materal and fetal plasmas. We measured placental diffusion permeabilities for $^{22}Na^{+}$ and $^{36}Cl^{-}$ because Armentrout, Katz, Thornburg and Faber (1977) and Conrad and Faber (1977) have shown that water exchange across the placenta of the sheep does not depend in a simple way on the hydrostatic and osmotic pressures in the maternal and fetal capillaries

in the sheep placenta. They have shown that the major constraint on the acquisition of water by the sheep fetus is the rate at which it can acquire electrolytes, particularly Na^+ and Cl^-. The diffusional permeabilities for these ions will be reported as "permeability surface area products" (PS), a parameter which is the product of the permeability constant (P) equal to the ratio of the flux of a solute per unit area of membrane and the concentration difference of the solute across the membrane (Kotyk and Janáček, 1975), and the total surface area of the membrane available for diffusion (S). These terms were incorporated into a single coefficient because the area available for exchange between the maternal and fetal capillaries cannot readily be measured.

MATERIALS AND METHODS

Animals

Pregnant ewes of mixed western breed were purchased from a local farmer, who grazed them on pasture. After purchase they were transferred to the animal care facilities where they were housed in pens and given ad libitum access to alfalfa pellets and water. Their diet was supplemented with corn, oats, and alfalfa hay.

The approximate duration of pregnancy was estimated from the size and degree of ossification of the fetal skeleton on a radiograph of the ewe's abdomen. Surgery was performed when the gestation was estimated to exceed 90 days. Before this age fetal sheep are small (less than approximately 0.5 kg) and fragile and surgical procedures are difficult to perform. Our intention was to study anephric fetuses for as long as possible before the expected delivery at approximately 147 days of gestation. Therefore, an attempt was made to select ewes carrying younger fetuses for these studies.

Surgical Protocols

The ewes were deprived of food but not water 24 hours prior to surgery. Anesthesia was induced with 4% halothane in a mixture of two-thirds nitrous oxide and one-third oxygen administered via a specially designed face mask. The ewe was placed on the surgery table in the supine position after the induction of anesthesia. She was then intubated with a cuffed endotracheal tube. The cuff was inflated and the tube was secured by taping it to her muzzle. Anesthesia was maintained with the same gas mixture while the halothane concentration

was varied as needed for complete anesthesia of the ewe and fetus during surgery.

After the endotracheal airway had been established and the ewe had been stabilized on maintainence concentrations of anesthetic gases, her feet were taped to the table. Her abdomen was shorn with clippers and shaved thoroughly with a straight razor. Her abdomen was then thoroughly scrubbed with Betadine surgical soap, an iodophor, and rinsed.

The surgeons scrubbed for a minimum of 10 minutes with a brush and an iodine containing soap after donning face masks and hair covers. One surgeon donned sterile gloves and scrubbed the abdomen of the ewe with Betadine for a second time. The surgical field was then rinsed with 95% ethanol, dried with sterile gauze, and sprayed with a 1% solution of iodine dissolved in 95% ethanol. A sterile drape was then placed over the ewe's lower extremities. The surgeon who performed these procedures scrubbed for an additional three minutes before gowning. The surgeons then donned sterile disposible paper gowns and surgical gloves. The ewe, Mayo stand, and instrument table were draped in a sterile manner with disposible paper and plastic drapes.

All instruments and catheters used in surgery had been previously autoclaved for 20 minutes at a temperature of 250^{o}F and a pressure of 15 PSI. These strict sterile precautions are necessary because the sheep fetus does not have circulating immunoglobulins and thus is extremely susceptible to infection (Marault, 1970). Although the fetus is capable of mounting an immune response in some circumstances, it is usually not sufficient to avert death within 12 hours if there is a break in sterile technique.

A midline abdominal incision was made through the ewe's skin from the umbilicus to the udder. The subcutaneous tissues were bluntly dissected and significant bleeders were ligated with 00 silk suture. An incision was made in the fascia and peritoneum with scissors and was extended as far caudad as possible to prevent subsequent compression of uterine or umbilical vessels against the edge of the wound. The uterine horn containing the fetus was brought out through the incision. A purse string suture of no. 3 silk was placed in the myometrium around an area where placental cotyledons could be avoided. The myometrium was incised with a scalpel in a direction parallel to maternal vessels in order to minimize bleeding. The allantoic and amniotic membranes were opened with a hemostat and the part of the fetus that was to be operated was delivered through the opening. The purse string suture was tightened around the fetus to prevent spillage of amniotic fluid after it was ascertained that blood flow in the umbilical cord would not be compromised by cord compression.

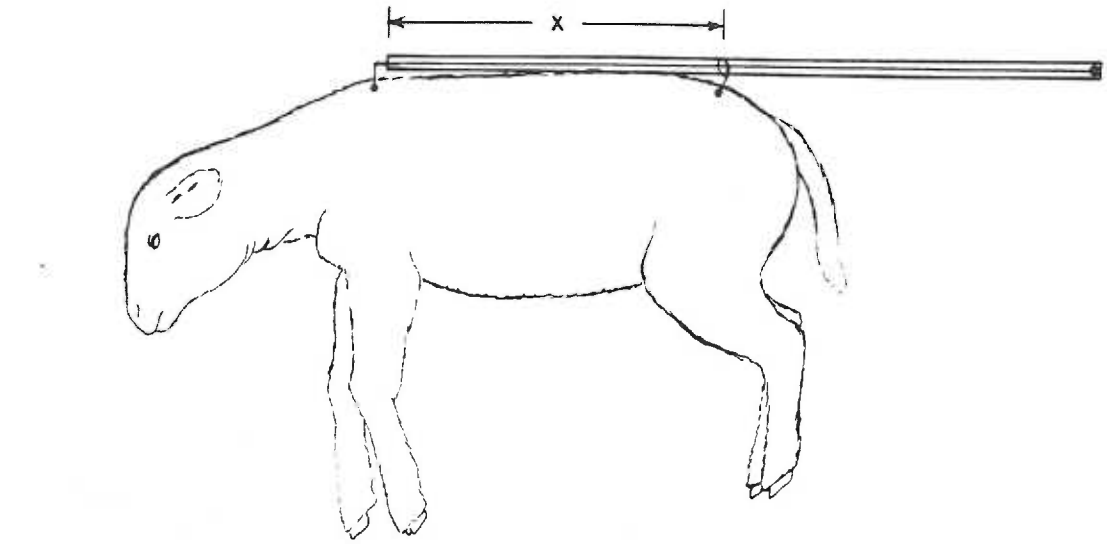
In surgeries on experimental fetuses one hindlimb was delivered first. An incision was made through the skin overlying the groin and the femoral artery and vein were isolated by blunt dissection. These vessels were separately ligated with 00 silk suture. A small incision was made in each vessel proximal to the ligature with small scissors and a polyvinyl catheter (1.0 mm ID, 1.3 mm OD) filled with heparinized saline was inserted into the vessel for approximately 10 - 15 cm. The catheters were secured in two places with 00 silk suture. The skin was closed with a continuous suture. The catheters were then anchored with an additional suture placed through the skin. A free loop of catheter

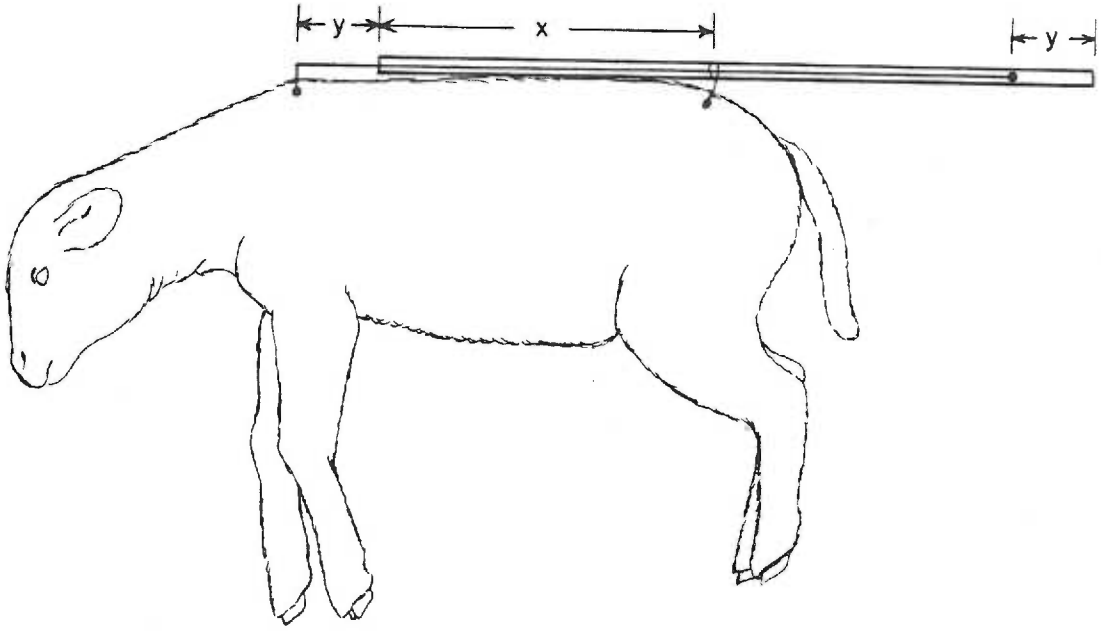
was left to allow for fetal growth. After this a silastic catheter (3 mm ID) with several side holes was anchored to the skin. This catheter was used later for sampling amniotic fluid and measuring intrauterine pressure.

The purse string suture was loosened for delivery of the hindquarters of the fetus and then it was carefully drawn around the fetus caudad to the umbilical cord. Nephrectomies were performed through bilateral paravertebral incisions in the lumbar region. The subcutaneous tissues were bluntly dissected to gain access to the retroperitoneal space without entering the peritoneal cavity. The kidney was gently pressed through the incision and the perirenal connective tissue was gently wiped towards the hilum with gauze. The dissection was done carefully to avoid damage to the adrenal glands.

A length gauge of the type described by Mellor and Matheson (1979) was placed on 10 of the experimental fetuses. This simple device consists of a monofilament nylon line placed in a polyethylene catheter which is closed at one end. The line is sutured to the skin overlying the proximal end of the spine and the catheter is anchored to the skin overlying the sacrum in such a way that the line is free to slip inside the catheter. As the fetus increases in length the line is pulled out of the catheter and an increase in distance between the closed end of the catheter and the end of the line is observed. This is illustrated in Figure 4. For placement of the gauge the fetus was removed from the uterus until his shoulders appeared at the uterine incision. One surgeon suspended the fetus by his feet while holding the edge of the uterus away from the umbilical cord. The other surgeon tunneled the

Figure 4. Illustration of how linear growth of the sheep fetus can be measured while it is in utero. The length gauge consists of a monofilament line whose end is attached to the skin overlying the spinous process of the C-7 vertebra inside a polyethylene catheter which is anchored to the skin overlying the sacrum of the fetus. When the length of the fetus, x, increases after placement of the gauge by an amount, y, a change in the distance between a knot on the distal end of the monofilament line and the closed end of the catheter equal to y is observed.


x


y
x
y

catheter under the skin from the sacrum to the lower neck using a metal rod as a guide. The monofilament line was sutured over the prominence of the spinous process of the seventh cervical vertebra (C-7). The distance between C-7 and the sacrum was measured with a suture cut to the same length. All but the hindquarters of the fetus was quickly returned to the uterus and the purse string suture was again tightened while the length gauge catheter was anchored to the skin overlying the sacrum. It usually took between 1-2 minutes to place the gauge, and the heart rate of the fetus usually did not decrease below 100 beats per minute. No attempt was made to place the proximal end of the length gauge on the crown of the fetus because it was believed that the stress of removing the fetus completely from the uterus would have been too great.

The fetus was then returned to the uterus. In some cases a liter of saline was poured into the opening to facilitate relaxation of the uterus so that the fetus could be returned without tearing the uterus or the extrafetal membranes. The catheters were brought out through the uterine incision. A short length of catheter was left in the uterus to provide for slack. The membranes were gathered around the catheters and tied with a 00 silk suture to form a seal. The purse string suture in the uterine wall was then tightened to close the incision around the exiting catheters.

Another purse string suture was placed through the myometrium at the tip of the uterine horn. The uterus and allantoic membrane of the fetus were opened in the manner previously described and a 3 mm ID catheter was inserted into the allantoic cavity. The membranes and

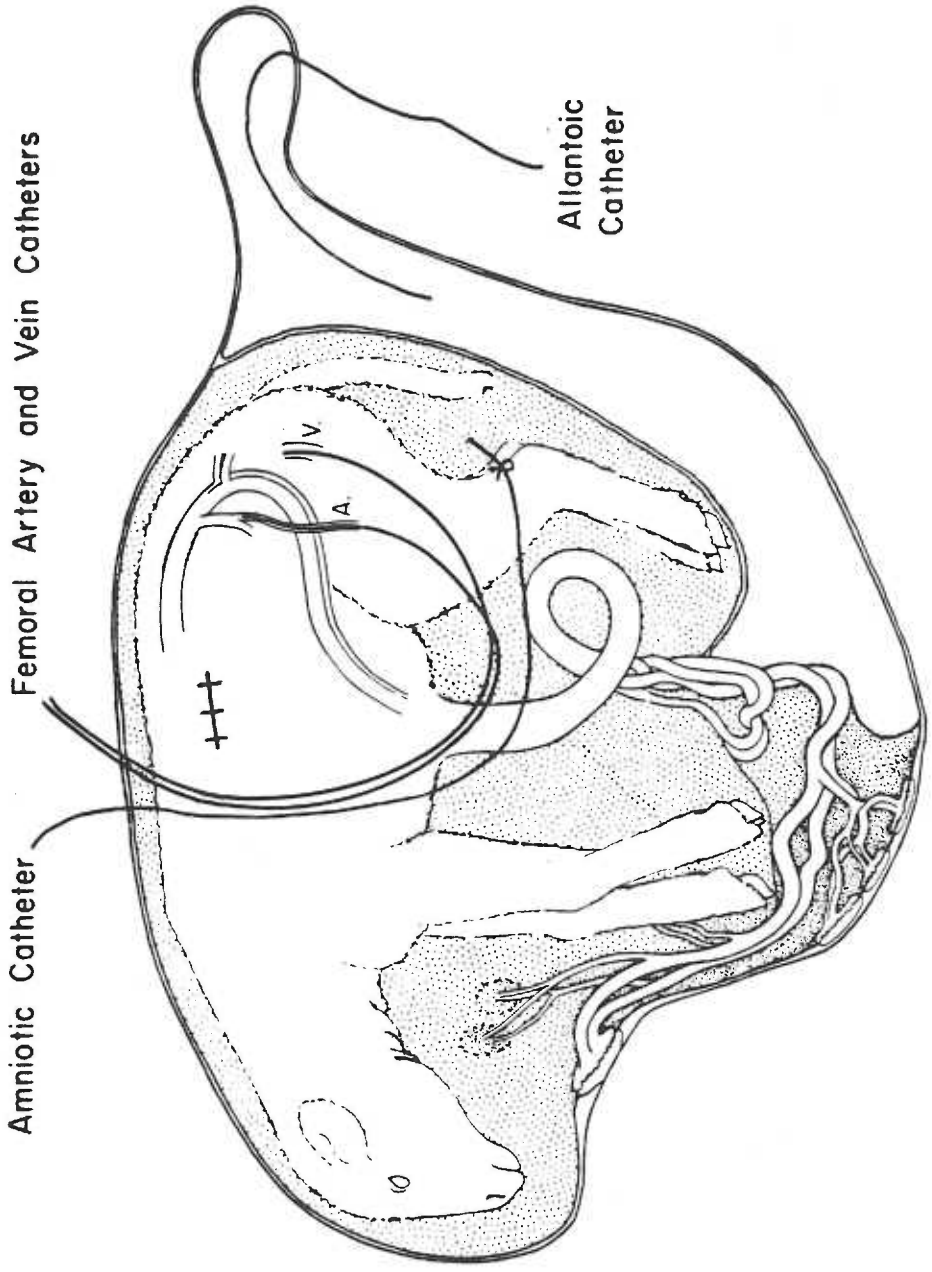
uterus were closed as described above.

One million units of penicillin-G dissolved in 6 ml heparinized saline was then injected via the amniotic catheter followed by a 6 ml heparinized saline flush. No additional antibiotics were given to the fetus after surgery, but occasionally intramuscular penicillin-G was given to the ewe as treatment for wound infection. The catheters were tied with different numbers of knots to permit their later identification.

After the uterus had been repaired and replaced, a hole was made through the abdominal fascia of the ewe and the catheters passed through it. Her peritoneum and fascia were closed with interrupted no. 3 silk sutures. In most cases the catheters were then passed through a subcutaneous tunnel and exteriorized through a small incision made on the flank of the ewe. The catheters were placed in a nylon pouch which was sutured to the skin above the point of exit of the catheters. In the other cases the catheters were tunneled subcutaneously to the ewe's flank with a guide string. The catheters were left under the skin and the guide string was tied into an interrupted suture used to close the small flank incision. The risk of infection spreading along the catheter tract was greatly reduced by leaving the catheters in a subcutaneous position until the day preceding an experiment. The abdominal skin of the ewe was then closed with metal wound slips. A summary of the surgical preparation is shown in Figure 5.

At the conclusion of surgery the ewe was allowed to breathe room air. She was removed from the surgery table and extubated as soon as she showed signs of recovering from the anesthesia. She was then returned to the pen with the other sheep and allowed immediate access

Figure 5. A diagram summarizing the surgical preparation of an anephric sheep fetus. The site of incision for removal of the kidney is indicated by a line with cross hatches. The sites for placement of femoral artery, femoral vein, and amniotic and allantoic fluid compartment catheters are also shown.


Amniotic Catheter
Femoral Artery and Vein Catheters
Allantoic Catheter
V
A

to food and water.

Controls

The fetuses which served as controls in these experiments were also subjected to similar intrauterine surgeries where chronic indwelling catheters were placed, but during which the fetal kidneys were untouched. The locations of the vascular catheters differed in some cases. Some of the control fetuses had electromagnetic flow probes placed on the pulmonary artery or a catheter placed in the trachea. A few fetuses had an inflatable occluder placed around the umbilical cord. The degree of trauma imposed by these surgeries was considered to be equivalent to that of fetal nephrectomy.

Twins

Mellor (1969a) describes up to a 65% incidence of anastomoses of chorionic vessels smaller than 0.8 mm diameter in twin pregnancies, but states that a low incidence of erythrocyte mosaicism suggests that the mixing of blood from the two fetuses is usually minimal. He also states that the occurrence of freemartinism, although its incidence in sheep is low, implies that hormones can pass from one fetus to the other. The occurrence of a significant communication between the circulations of sheep fetuses has been demonstrated by the rapid passage of Evans blue dye from one twin to the other (Valdes Cruz, Taylor, Mott & Carver, 1977). Since it would not be possible to detect anastomosis between the circulations of twins at the time of surgery and because it would be impossible to rule out that a nephrectomized fetus did not receive hormones from an intact twin, all fetuses in a multiple pregnancy were nephrectomized.

Catheter Maintenance

During the week following surgery the intravascular catheters of the fetus were opened and flushed every other day with 1.5 ml of a sterile bacteriostatic saline solution containing 250 units sodium heparin per ml. Strict sterile precautions were observed at all times, and a 1% solution of iodine in 95% ethanol was used on the catheters and instruments as a bactericidal agent. In subsequent weeks the catheters were flushed in a similar manner biweekly. The dates and amounts of fluid injected on each occasion were recorded. The difference in length between the nylon line and the closed end of the length gauge was recorded daily, except in some cases in which the measurement for a single day was omitted.

Experimental Protocols

On the day of the experiment two ewes, to keep each other quiet, were placed in stanchions and wheeled to the laboratory. They were placed with their heads together and food and water was provided between them. Waste was collected in plastic tubs to prevent contamination of the laboratory. The ewes remained in the stanchions for the duration of the experiments (up to 24 hours) and were returned to the pen afterwards.

Experiments were performed on sheep carrying anephric fetuses only after the third postoperative day. Not all procedures described in this section were attempted for all fetuses. The number of successful experiments will be given in the results sections.

Procedures for Blood Sampling

Fetal blood samples were obtained from the indwelling arterial catheter under the strict sterile precautions previously described for catheter flushing. Before a sample was taken 3 ml of fluid, a volume equal to at least three times the catheter dead space, was withdrawn into a sterile syringe. After sampling this "dead space," fluid was returned to the fetus and the catheter was flushed with 2 ml sterile heparinized saline solution. Blood losses were not replaced with homologous blood or with protein solutions to avoid transfer of hormones to the fetus. The volumes of fluid withdrawn from and injected into a fetus during the course of an experiment were recorded. The net loss or gain of fluid was usually less than 10 ml.

The first blood sample drawn was for the determination of plasma renin activity (PRA). This sample was drawn first because it has been reported (Broughton Pipkin, Lumbers, and Mott, 1974a; Smith, Lupu, Barajas, Bauer, and Bashore, 1974) that hemorrhage is a potent stimulus for the release of renin by the sheep fetus. A blood loss of 4 ml has been found to significantly increase PRA in the sheep fetus. For these determinations a 1 ml sample of blood was collected in a sterile syringe, transferred to a test tube containing 1 mg EDTA and placed on ice immediately. It was centrifuged at $4^{o}C$ within one hour. The plasma was removed, frozen immediately, and stored at $-20^{o}C$. PRA and renin substrate concentrations were determined by radioimmunoassay as described later.

A second 1 ml sample was removed under anaerobic conditions into a syringe containing heparin in its dead space. The sample was analyzed immediately for arterial blood pH, P_{CO_2}, and P_{O_2} on a Radiometer micro

blood gas analyzer (Radiometer, Copenhagen, Denmark) which had been calibrated at 39°C. Three capillary tubes were filled with blood from this sample and spun in an Autocrit centrifuge (Clay Adams, Parsippany, New Jersey). The hematocrit was taken as the mean of the three determinations.

A 6 ml sample was then removed under anaerobic conditions into a syringe containing a known amount of heparin solution in its dead space. A similar 6 ml sample was obtained by puncture of the jugular vein of the ewe within a few minutes of taking the fetal sample. The syringes were immediately covered with mercury filled caps and centrifuged. The separated plasma was used for determinations of osmolality, chemical composition, and to provide blanks for spectrophotometry (See below).

An Advanced Osmometer Model 3W (Advanced Instruments, Needham Heights, Massachusetts) was used for the measurement of maternal and fetal plasma osmolality by freezing point depression. Calibrations were performed with 256 mOsm and 319 mOsm standards. The measured osmolality of a sample was corrected for the contribution made by heparin (Woods, Thornburg, and Faber, 1978).

Maternal and fetal plasma concentrations of Na^+, K^+, Cl^-, Ca^{2+}, Mg^{2+}, PO_4^{3-}, glucose, urea, creatinine, uric acid, lactate, total protein, and albumin were measured in coded samples by the Department of Clinical Pathology with a Technicon SMAC AutoAnalyzer system.

When possible, samples of amniotic and allantoic fluid were obtained for the measurement of osmolality and chemical concentration as described above. An amount equal to three times the catheter dead space volume was withdrawn and discarded prior to sampling.

Measurement of Fetal Blood Pressure

The fetal arterial, venous, and amniotic fluid catheters were connected to Gould-Statham P23 ID pressure transducers (Gould Statham, Oxnard, California) with silastic tubing in series with two three-way stopcocks. The tubing was flushed with 1% iodine in 95% ethanol prior to filling with heparinized saline. The transducers were calibrated against two reservoirs of water whose hydrostatic pressure difference was equal to 40 mm Hg. Pressures were recorded for one hour on a Beckman R612 Dynograph recorder (Beckman, Schiller, New Jersey). The intrauterine pressure recorded from the amniotic fluid catheter was subtracted from the recordings from the arterial and venous catheters to give fetal intravascular pressures. Fetal heart rate was determined from the number of systolic pressure peaks in a given time interval.

Fetal Plasma and Blood Volumes

A volume of 2-3 ml of Evans blue dye in saline solution (approximately 0.75 mg/ml) was injected via the fetal venous catheter. The catheter was immediately flushed with 6 ml sterile heparinized saline. The amount of solution injected was obtained by weighing the syringe before and after injection. Three ml samples were taken from the arterial catheter of the fetus 15 minutes and 30 minutes after the time of injection. The sampling syringes contained heparin in their dead space.

To insure sterility, the Evans blue stock solution was boiled before each experiment. Because of the possibility that boiling changed the concentration of stock solution, a new standard was made each time the solution was boiled. To make the standard a weighed volume (2-3 ml)

of stock solution was placed in a volumetric flask and diluted to one liter with distilled water.

The concentrations of the standard and samples were determined colorimetrically from their absorbances at 620 nm on a Coleman Spectrophotometer Model 6/20 (Coleman Instruments, Maywood, Illinois). Fetal plasma obtained before the injection of dye was used as a blank to eliminate the nonspecific absorbance of other compounds present in the plasma (Gregersen, 1938). Samples showing hemolysis were rejected.

The concentrations found in the samples at 15 minutes and 30 minutes were plotted semilogarithmically and extrapolated to time zero to obtain the concentration that would have been present in the fetal circulation if mixing had occurred instantaneously. The period of mixing has been found to be complete by six minutes in the sheep fetus (Barcroft, 1939), so that change in concentration of the dye between 15 and 30 minutes represents its loss from the vascular compartment. The plasma volume was obtained by dividing the amount injected by the extrapolated concentration at time zero (Pitts, 1974).

Blood volume (BV) was calculated from the plasma volume (PV) and hematocrit (hcrt) from the following formula:

$$BV = PV/(1 - hcrt/100) \qquad (1)$$

Diffusion Permeabilities and Volumes of Distribution of $^{22}Na^{+}$ and $^{36}Cl^{-}$

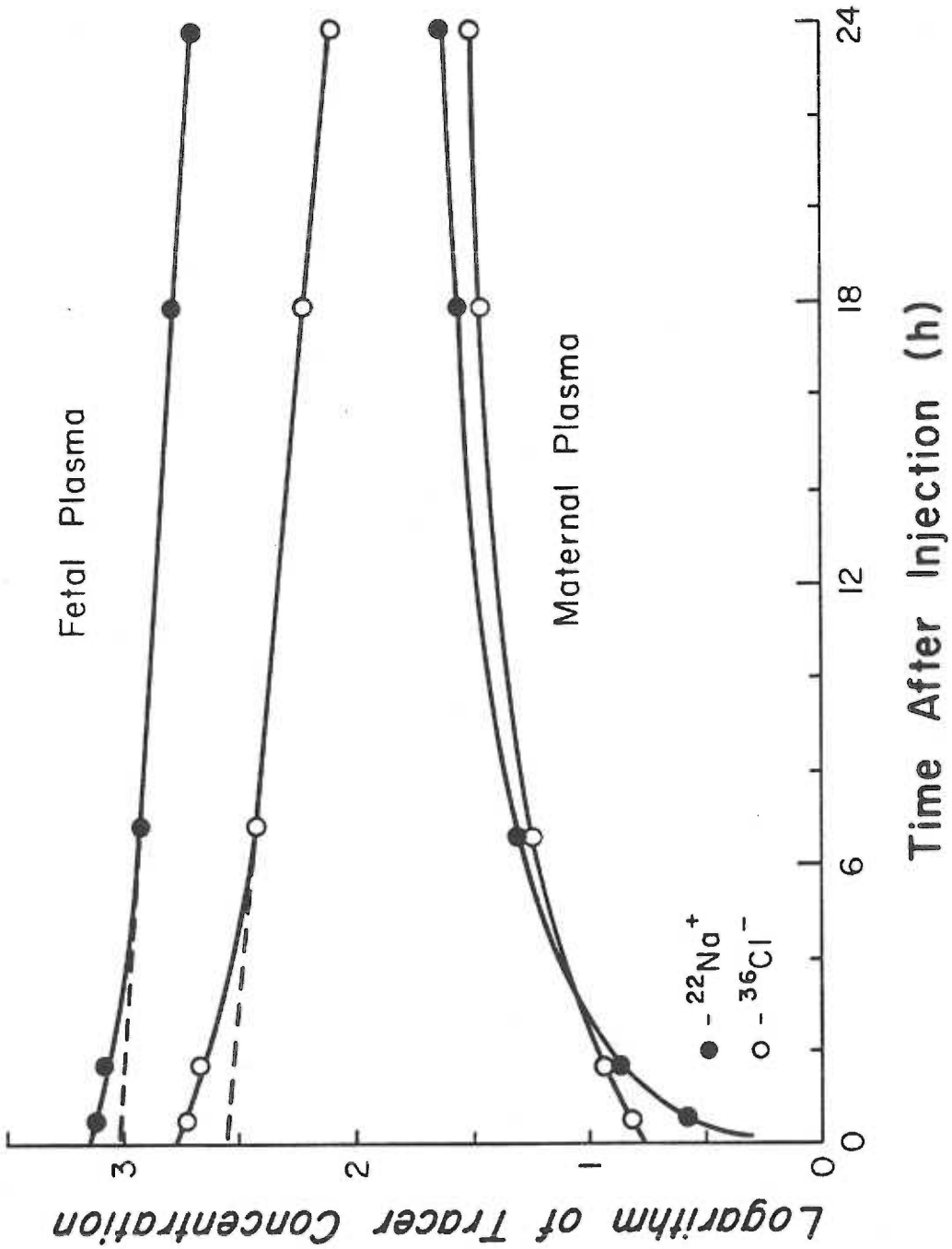
Approximately 10 μCi $^{22}Na^{+}$ and $^{36}Cl^{-}$ in saline solution (New England Nuclear, Boston, Massachusetts) were injected via the fetal venous catheter at the time of Evan blue dye injection. Radioisotope concentrations were determined in fetal arterial blood samples taken 15 minutes,

6 hours, 12 hours, and 20 hours after the time of injection.

The blood samples were centrifuged and the supernatant plasmas were removed. The activity (counts per minute per millimeter plasma) of $^{22}Na^{+}$ in a 0.5 ml aliquot of plasma was determined by gamma spectrometry on a Packard Model 3002 scintillation spectrometer (Packard Instruments, Downers Grove, Illinois). The plasma proteins in samples containing $^{36}Cl^{-}$ were precipitated by the addition of 0.5 ml 15% (w/v) trichloroacetic acid to 0.5 ml plasma. The solution was thoroughly mixed and centrifuged. 0.5 ml of the supernatant was dissolved in a fluor (Aquasol ^{R}, New England Nuclear) and was counted by beta spectrometry in a Packard Tricarb Model 3200 liquid scintillation spectrometer. Reference standards in sheep plasma were counted by both methods. In samples containing both $^{22}Na^{+}$ and $^{36}Cl^{-}$ the activity of each isotope was determined by the channels ratio method (Rudolph and Heymann, 1967). The activities were corrected for background counts and for dilution by the heparin solution in the dead space of the sampling syringe.

Fetal plasma concentrations declined logarithmically with time between 6 and 20 hours after injection (Figure 6). The non-linear part of the curve during the first six hours was attributed to the equilibration of the tracer in fetal plasma with fetal intracellular, extracellular, and extrafetal fluids. The linear part of the semilogarithmic curve was extrapolated to the time of injection to obtain the concentration that would have been present in fetal plasma if equilibration had occurred instantaneously at the time of injection. The amount of isotope injected was divided by the extrapolated concentration to obtain the volume of distribution of the isotope in the conceptus.

Figure 6. Relative concentrations of $^{22}Na^{+}$ (closed circles) and $^{36}Cl^{-}$ (open circles) in maternal and fetal plasma as a function of time after injection of 10-20 μCi isotope into the femoral vein of the fetus. Concentrations are in arbitrary units on a logarithmic scale. Note the linear decline in fetal plasma concentrations between 6 and 24 hours and linear extrapolation to time of injection.


Logarithm of Tracer Concentration
Time After Injection (h)
Fetal Plasma
Maternal Plasma
● - $^{22}Na^{+}$
○ - $^{36}Cl^{-}$
0
1
2
3
0
6
12
18
24

Placental permeability surface area products (PS) were calculated as the ratio of the amount of radioactivity lost by the fetus between 6 and 20 hours and the time integral of the concentration difference of the isotope between fetal and maternal plasmas ($\int_1^2 (C^f - C^m)\, dt$). The amount lost by the fetus was calculated as the product of the volume of distribution (V) and the difference in concentration of the isotope in fetal plasma at 6 hours (C^f_1) and 20 hours (C^f_2). The time integral of the concentration difference between mother and fetus was determined from the area between the maternal and fetal concentration curves between 6 and 20 hours (Figure 7). Hence:

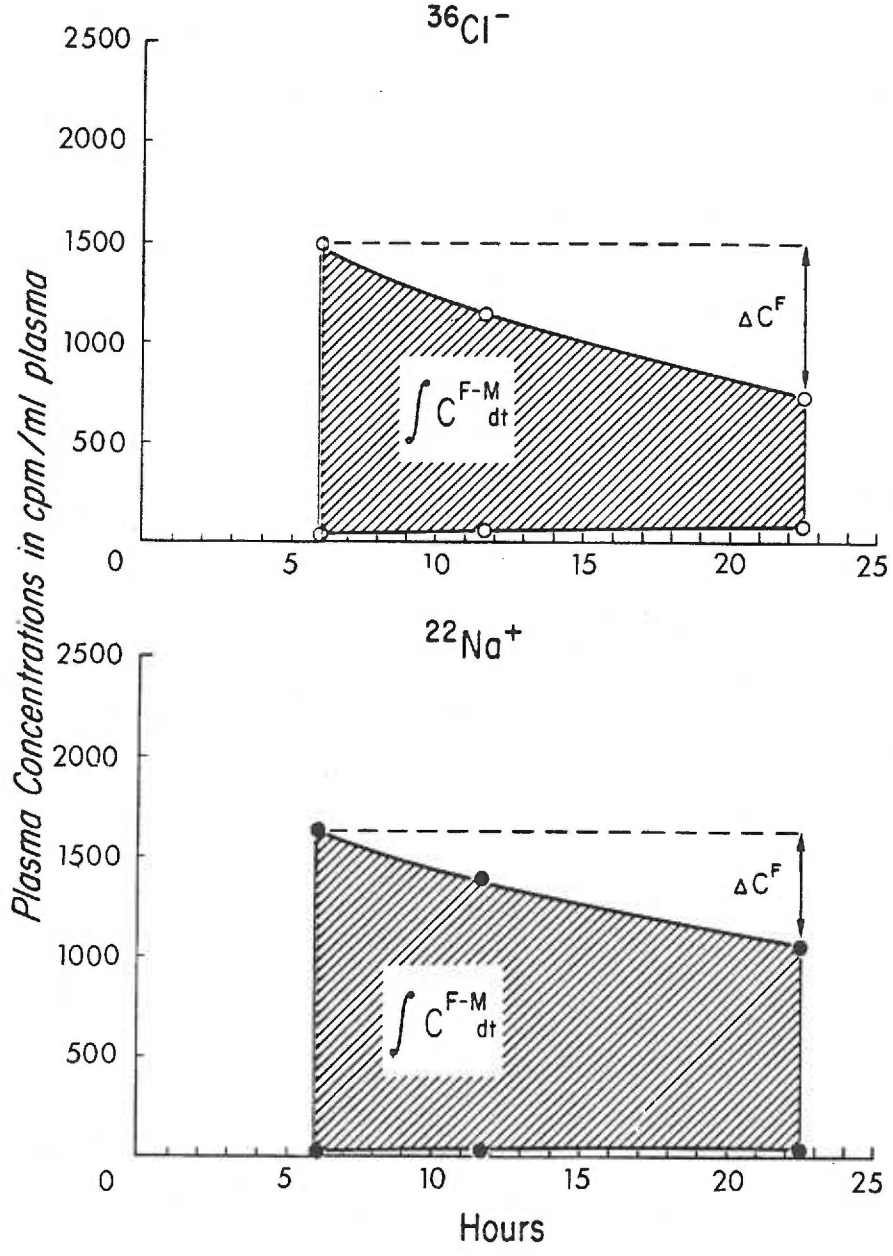
$$PS = V\ (C^f_1 - C^f_2)\ /\ \int_1^2 (C^f - C^m)dt,\ \mu l/sec \cdot kg \qquad (2)$$

Plasma Renin Activity

Plasma renin activities were determined by radioimmunoassay of angiotensin I by Dr. D. M. Potter of the Veteran's Administration Medical Center, Portland, Oregon, according to modifications of previously described methods (Cohen, Grim, Klough, Guyer, Kem, and Lucas, 1971; Potter, McDonald, Metcalfe, and Porter, 1977). PRA measures the rate of production of angiotensin I by renin in plasma acting upon endogenous renin substrate during a controlled period of time.

100 µl aliquots of the plasma samples obtained in these experiments were incubated at 37°C after the addition of 3.5 mmol diisopropyl-fluorophosphate (Sigma, St. Louis, Missouri) to inhibit the action of angiotensinases present in plasma. The pH of the samples was adjusted to 6.2 with 0.38 molar citric acid before incubation because the maximum rate of production of angiotensin I was found to occur between pH 6.0

Figure 7. Linear plots of concentrations of $^{36}Cl^-$ (upper figure) and $^{22}Na^+$ (lower figure) in maternal and fetal plasmas as functions of time after injection of 10-20 μCi of isotope into the femoral vein of the fetus. The differences in fetal plasma concentrations of isotopes between 6 and 22 hours ($C^f_1 - C^f_2$) are indicated by vertical arrows. The time integrals of the concentration differences of isotope between fetal and maternal plasmas $[\int_1^2 (C^f - C^m)\, dt\ ]$ is equal to the shaded area between fetal and maternal concentration curves.


$^{36}Cl^-$
2500
2000
1500
1000
500
0
5
10
15
20
25
ΔC^F
$\int C^{F-M} dt$
$^{22}Na^+$
2500
2000
1500
1000
500
0
5
10
15
20
25
ΔC^F
$\int C^{F-M} dt$
Plasma Concentrations in cpm/ml plasma
Hours

and 6.4 in pooled maternal and fetal sheep plasmas. The optimum incubation period was defined as the time during which generation of angiotensin was linear with respect to time. Samples obtained from ewes and intact fetuses were incubated for 15 minutes and samples from anephric fetuses were incubated for one hour.

The radioimmunoassay mixture was prepared at an Automatic Pipette Station (Micromedic Systems, Horsham, Pennsylvania). The reagents were ^{125}I-angiotensin I (New England Nuclear, Boston, Massachusetts), rabbit antibody to synthetic angiotensin I (University of Michigan, Ann Arbor, Michigan), and 0.1 molar Tris buffer with 0.01% (w/v) egg lysozyme (Sigma). 100 µl radiolabeled angiotensin I and 100 µl of antibody were added to 5, 10, or 20 µl of plasma. The volume of plasma used depended on the expected plasma renin activity. The volume of Tris buffer with egg lysozyme used was adjusted so that the final volume of the reaction mixture was 1 ml. All samples were assayed in duplicate. The reaction mixture was incubated in polyethylene tubes for 18 hours at 4^{o}C.

Separation of antibody bound and free angiotensin I was achieved by the addition of 1 ml dextran coated charcoal (Norit A; Matheson, Coleman, and Bell, Norwood, Ohio) to the reaction mixture. The samples were thoroughly mixed and centrifuged, the supernantant was removed, and the activity of free ^{125}I-angiotensin I on the charcoal pellet was determined by gamma spectrometry (Nuclear Chicago, Chicago, Illinois).

The standard curve was prepared with angiotensin I (Schwarz-Mann, Orangeburg, New York) which has been standardized against Medical

Research Council Research Standard A, code 71/328. The reliability of an assay was checked against aliquots of standard angiotensin I, pooled dog plasma, and hog renin in renin substrate enriched plasma (0.02, 0.01, and 0.005 Goldblatt units) prepared in duplicate in the same manner as samples at the beginning and end of the assay. Maximum and minimum binding of ^{125}I-angiotensin I and antibody were determined with labeled angiotensin I in Tris buffer with egg lysozyme in the presence and absence of antibody. All samples from a single set of experiments were assayed in one group. Some samples were reassayed if the amount of angiotensin I could not be quantitated because it was much greater or less than expected and therefore outside the reliable range of the assay. Intraassay variability was <13% and interassay variability was <17%.

Termination of Experiments

Ewes carrying anephric fetuses were allowed to continue their pregnancy until the fetus was born, aborted, or died in utero. Anephric fetuses that were born alive were killed within a few hours of birth before they showed evidence of renal failure. Ewes carrying control fetuses were usually killed with intravenous barbituate at the end of an experiment. Fetuses were weighed and the distance between the seventh cervical vertabra (C-7) and the sacrum was measured. An autopsy was performed to inspect for edema, to identify grossly normal adrenal glands in experimental fetuses, to check the placement of catheters, and to determine cause of death. Death of experimental fetuses was usually associated with infection along the maternal subcutaneous catheter tract.

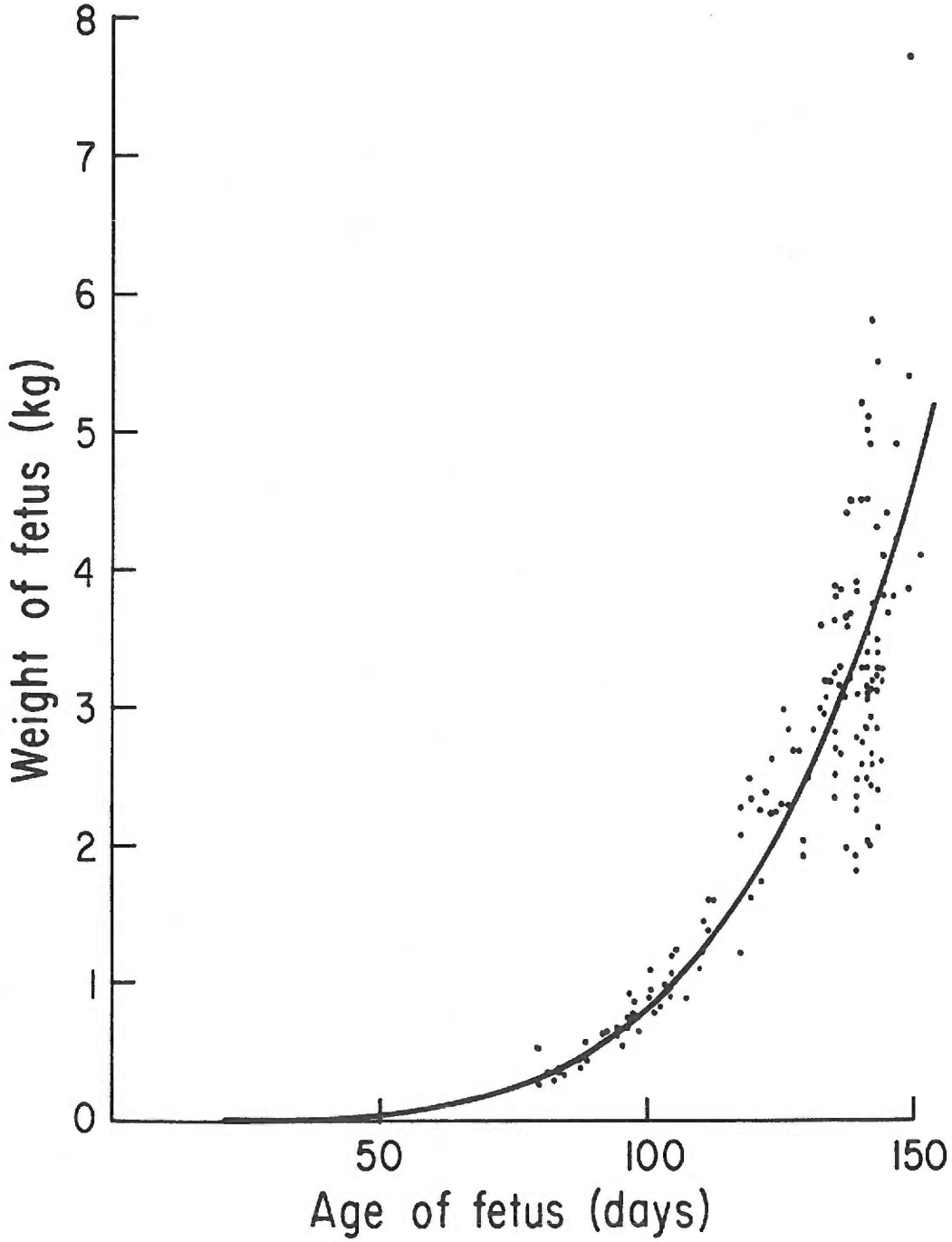
RESULTS

Growth

During the last part of gestation, from 90 days to term at approximately 147 days, the fetal lamb grows almost an order of magnitude from approximately 0.5 to 5 kg (Figure 8). In order to study the effects of bilateral nephrectomy on arterial blood pressure of the fetus apart from the changes that are associated with growth of the fetus, it was necessary to look for a reliable method of estimating the weight of the fetus *in utero* so that experiments performed on the same anephric fetus over a period of weeks could be interpreted. Since other investigators have reported that bilaterally nephrectomized sheep fetus do not grow as well as intact twin controls (Thorburn, 1974) or as well as controls of known gestational age (Oakes, Cabalum, Lowe, Buster, Gluckman, Kaplan, Grumbach, and Nathanielz, 1980), we could not make *a priori* assumptions about the growth of anephric fetuses in these experiments. We could not directly compare the weights of our fetuses to published growth curves of fetal weight as a function of gestational age because the breeding dates of the sheep used in these experiments were unknown. Other factors that would make comparison to published growth curves difficult are the effects of genetic differences (Stephenson, 1958) and the effects of differences in nutrient intake by pregnant ewes (Wallace, 1948; Rattray, Garrett, East, and Hinman, 1974; Alexander, 1974; Mellor and Matheson, 1979) on the growth of the fetal lamb.

The weight of the sheep fetus has been found to correlate closely with its plasma volume or with its blood volume (Creasy, Drost, Green,

Figure 8. The relationship between weight and gestational age of fetal sheep studied by Barcroft (1946). The equation for the curve was derived from a linear regression of the logarithm of fetal weight versus the logarithm of gestational age. Fetal weight (y) in kilograms is a function of gestational age (x) in days according to the formula, $y = 4.7 \times ^{4.1}$. Note the large range for birth weight at approximately 147 days of gestation.


Weight of fetus (kg)
0
1
2
3
4
5
6
7
8
50
100
150
Age of fetus (days)

and Morris, 1970; Broughton Pipkin and Kirkpatrick, 1972; Caton, Wilcox, Abrams, and Barron, 1974). Barcroft and Kennedy (1938) used Evans blue dye to measure the plasma volumes of sheep fetuses and found that blood volume was equal to approximately 15% of fetal weight. We used the relationship 156.1 ml of blood, determined from measured plasma volume, per kilogram fetal weight (Creasy, et al., 1970) to estimate the weight of our fetuses from their calculated intravascular volumes. We measured the plasma volume of six anephric sheep fetuses within 24 hours of their death or delivery and found that there was a reliable correlation between the weight of the fetus calculated from Creasy's formula and the actual weight of the fetus measured at autopsy ($r = 0.97$, $p<0.001$) (Figure 9). In 17 cases we measured the plasma volume of anephric fetuses 2 to 16 days before autopsy. Two measurements were made on eight fetuses and three measurements were made on one fetus. In fetuses where successive measurements were made, the plasma volume always increased with time. To see if this increase in plasma volume could be used to provide a reliable estimate of fetal growth, the weight of the fetus estimated from its intravascular volume was plotted against the weight of the fetus at autopsy corrected for growth between experiment and autopsy (Figure 10) with the assumption that the mean rate of growth was the same as that shown by the sheep studied by Barcroft (Figure 8). There was a reliable correlation ($r = 0.94$, $p<0.001$) between these two estimates of fetal weight (Figure 10).

Additional information about the rate of growth of anephric fetuses in this study was obtained from changes in the length of the fetus observed with length gauges and from the differences in measured distance

Figure 9. The relationship between fetal weight estimated from fetal blood volume calculated from plasma volume measured with Evans blue dye and fetal arterial blood hematocrit and the actual weight of the fetus measured at autopsy for 6 fetal lambs who had plasma volumes measured within 24 hours of autopsy. The formula used to estimate fetal weight was 156.1 ml blood per kilogram fetal weight (Creasy, et al, 1970). The diagonal line is the line of identity for the two values. There was a reliable correlation between estimated and actual fetal weight ($r = 0.97$, $p < 0.001$). The slope of the regression line was 0.89.

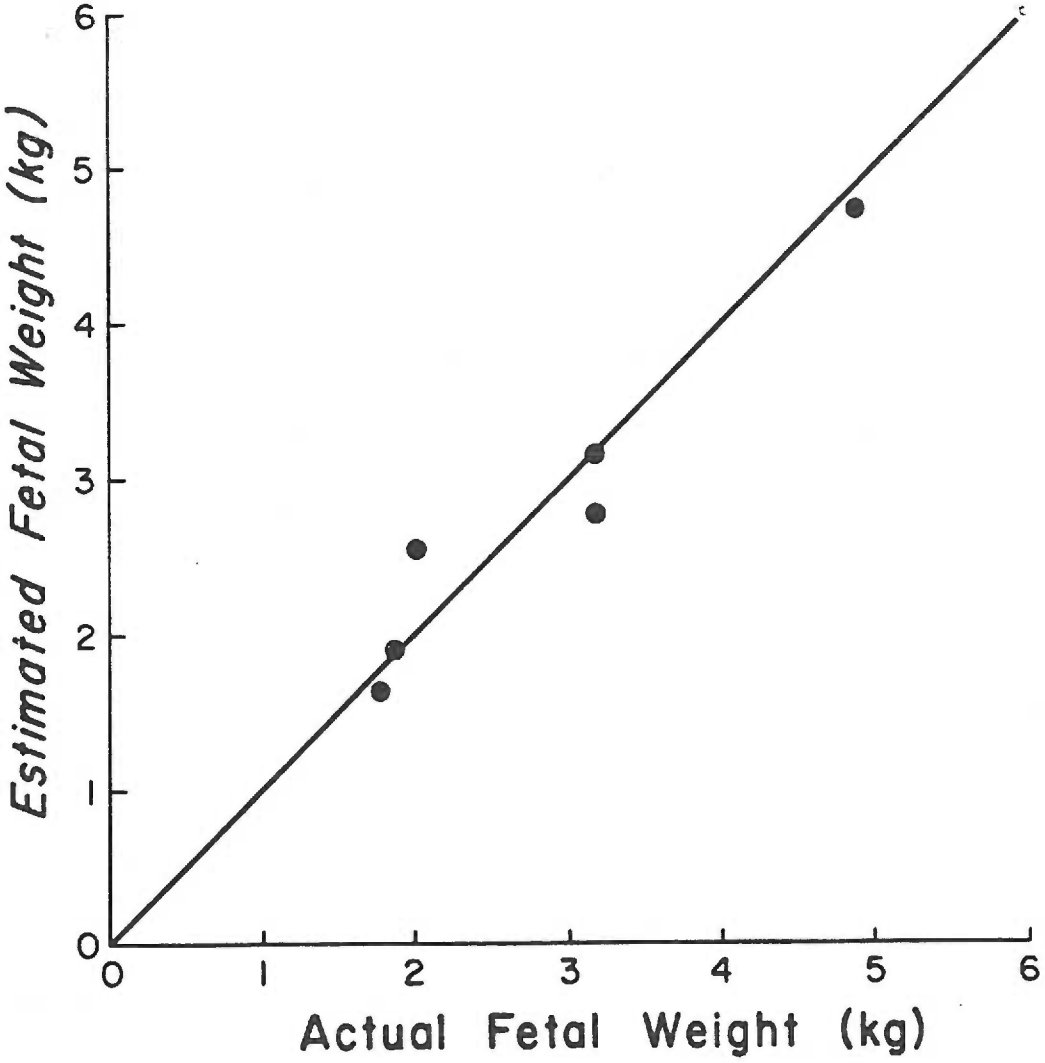
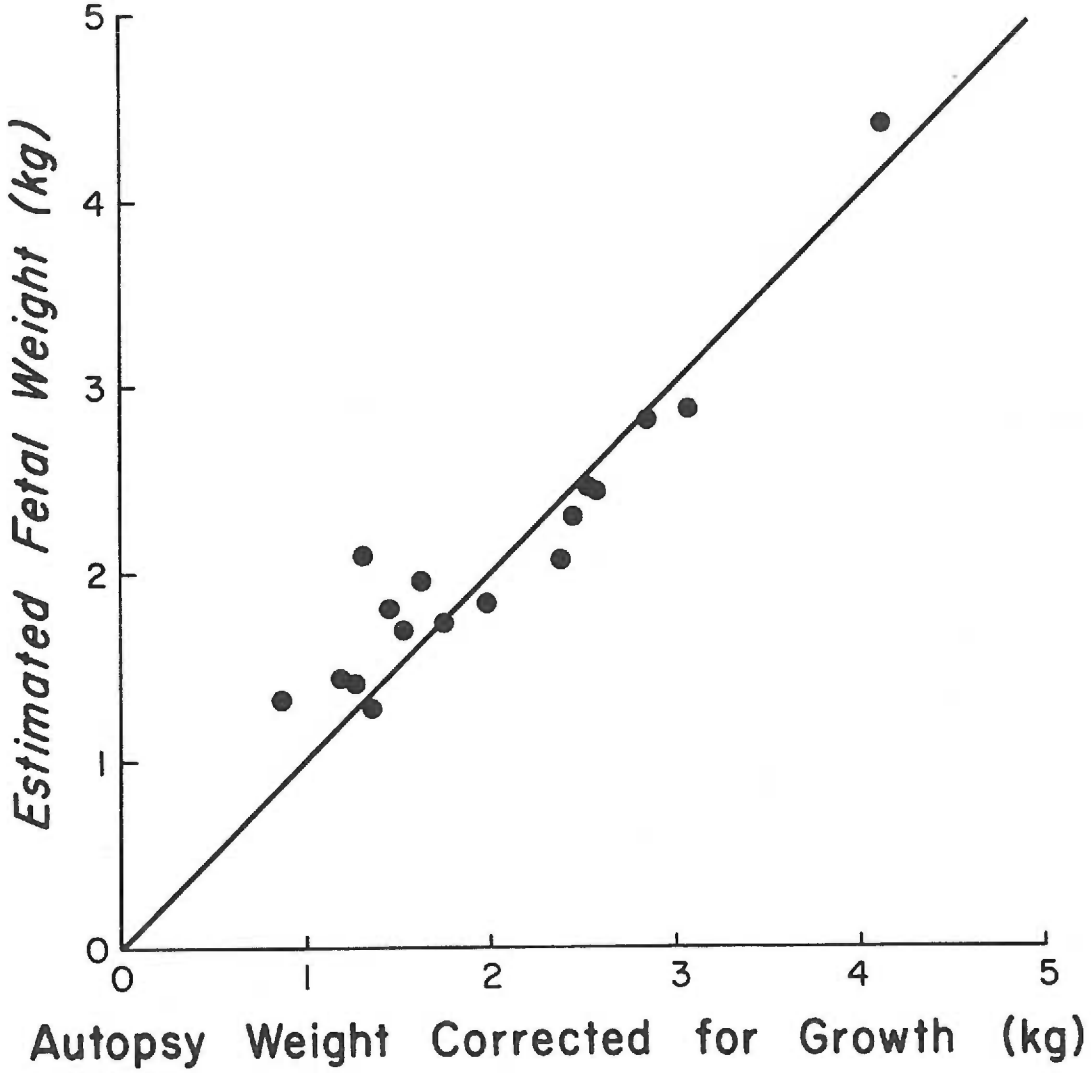

Estimated Fetal Weight (kg)
Actual Fetal Weight (kg)
0
1
2
3
4
5
6

Figure 10. The relationship between two estimates of fetal weight for 17 fetuses who had plasma volumes measured with Evans blue dye more than 24 hours before autopsy. One estimate of fetal weight was obtained from the relationship between blood volume and fetal weight (156.ml per kilogram fetus) reported by Creasy et al. (1970). The other estimate was obtained by correcting the weight of the fetus measured at autopsy for expected growth between the time of plasma volume measurement and autopsy based on the assumption that anephric fetuses in this study grew at the same rate as the fetuses shown in Figure 8. The diagonal line is the line of identity for the two values. There was a reliable correlation between the two estimates of fetal weight ($r=0.94$, $p < 0.001$). The slope of the line was 0.85.


Estimated Fetal Weight (kg)
0
1
2
3
4
5
0
1
2
3
4
5
Autopsy Weight Corrected for Growth (kg)

between C-7 and sacrum found at autopsy and during surgery. The mean (± SEM) changes in fetal length with time observed in 10 fetuses with length gauges was 4.2 ± 0.1 mm/day. The apparent mean (± SEM) rate of increase in fetal length, calculated from differences in the measured distance between C-7 and sacrum at autopsy and surgery, was 4.3 ± 0.1 mm/day in six anephric fetuses without length gauges. These results are within the range of reported rates of increase of fetal crown-rump length as a function of gestational age of the fetus during the last third of gestation (Table 1). Since the distance between C-7 and the sacrum is a fraction of the crown-rump length, we would expect that the rate of increase in the measurement of distance between C-7 and sacrum would be less than the rate of increase in crown-rump length in our fetuses.

It appears that the anephric sheep fetuses in this study continued to grow after bilateral nephrectomy and that their rate of growth is within the range reported by other investigators. In particular, the growth of anephric fetuses in this study as measured by increases in weight calculated from measured blood volumes and by increases in length is comparable to the rate of growth of fetuses studied by Barcroft (1946).

Arterial Blood Pressures

Arterial blood pressures were measured in 51 control fetuses studied 3 to 9 days (mean 5) after operation and in 30 anephric fetuses 3 to 24 (mean 7) days after operation. Studies were not performed before the third postoperative day to allow time for cardiac output and arterial pressure to stabilize after surgery (Rudolph and Heymann, 1973; Faber, unpublished observations). Blood pressures measured in fetuses

Table 1. Changes in Length of Sheep Fetuses During the Last Third of Gestation.

Number of Fetuses	ΔLength (mm/day)	Period of Gestation (days)	Breed	Investigator	Ref.
56	3.6	90-140*	Merino	Malan & Curson	1
186	4.2	99-140*	Not noted	Barcroft	2
10	4.2	>90[+]	Mixed Western	Binder, et al.	3
6	4.3	>90[‡]	Mixed Western	Binder, et al.	3
49	4.4	80-125*	Merino	Cloete	1
--	4.4	80-125*	various	Evans & Sack	4
53	4.5	60-145*	Welsh	Huggett & Widdas	5
100	5.6	80-125*	New Zealand Romney	Stephenson	1
28	4.6-6.6	100-140[o]	Scottish Blackface	Mellor & Matheson	6

*Crown-rump length measured at autopsy on fetuses of known gestational age.

[+]C-7-sacrum length measured with length gauges.

[‡]Calculated from changes in measured C-7 sacrum length.

[o]Crown-rump length measured with length gauges.

1. Stephenson, 1959
2. Barcroft, 1946
3. This Study
4. Evans and Sack, 1973
5. Huggett and Widdas, 1950
6. Mellor and Matheson, 1979

with an arterial blood pH less than 7.30, or in fetuses who were born or who died within 24 hours of recording were not included in the results. All control fetuses were killed and weighed within 24 hours of the experiment. For experimental fetuses studied more than 24 hours prior to autopsy, the weight at autopsy was corrected for growth between experiment and autopsy assuming that growth could be predicted from the equation for the curve shown in Figure 8. Anephric fetuses were autopsied 0 - 20 (mean 6) days after experiment.

Control data from fetuses used in these experiments are reported in Table 2. Anephric and intact control fetuses did not differ with respect to arterial P_{O_2}, P_{CO_2}, pH, or hematocrit. Although the range of weights was similar for both groups (1.12 - 5.11 kg for control fetuses versus 1.16 - 4.48 kg for anephric fetuses), the mean weight of experimental fetuses was 1 kg less than the mean weight of the controls. This difference was attributed to the deliberate selection of younger fetuses for the experimental group. The mean heart rate of the experimental fetuses was greater than that of the controls. This difference in mean heart rate between the two groups was attributed to a difference in mean age between the groups as demonstrated by weight differences since fetal heart rate has been shown to decrease with advancing gestational age (Barcroft, 1946; Boddy, Dawes, Fisher, Pinter, and Robinson, 1974; Robillard, Matson, Sessions, and Smith, 1979). Linear regression analysis of the relationship between heart rate and weight for both groups revealed that the two samples were probably drawn from the same population ($t = 1.23$ for the difference of slopes). The equation for the relationship between fetal heart rate (HR) and fetal weight (Wt) in kilograms was

Table 2. Fetal Control Data (means ± S.D.) Recorded at the Time of Blood Pressure Measurement.

	Fetal Weight (kg)	Arterial Blood P_{O_2} (mm Hg)	Arterial Blood P_{CO_2} (mm Hg)	pH	hcrt (%)	HR (beats/min)
Control Fetuses	3.49±0.90 (51)	17.4±4.1 (32)	51.2±6.4 (30)	7.37±0.04 (41)	35±7 (41)	167±23 (44)
Anephric Fetuses	2.50±1.06* (30)	17.8±3.5 (23)	51.4±3.7 (26)	7.36±0.03 (27)	30±5 (16)	190±23 (28)
Difference of means, p	<0.001	NS	NS	NS	NS	<0.001

*Calculated from fetal weight at autopsy and the rate of growth predicted by Figure 8

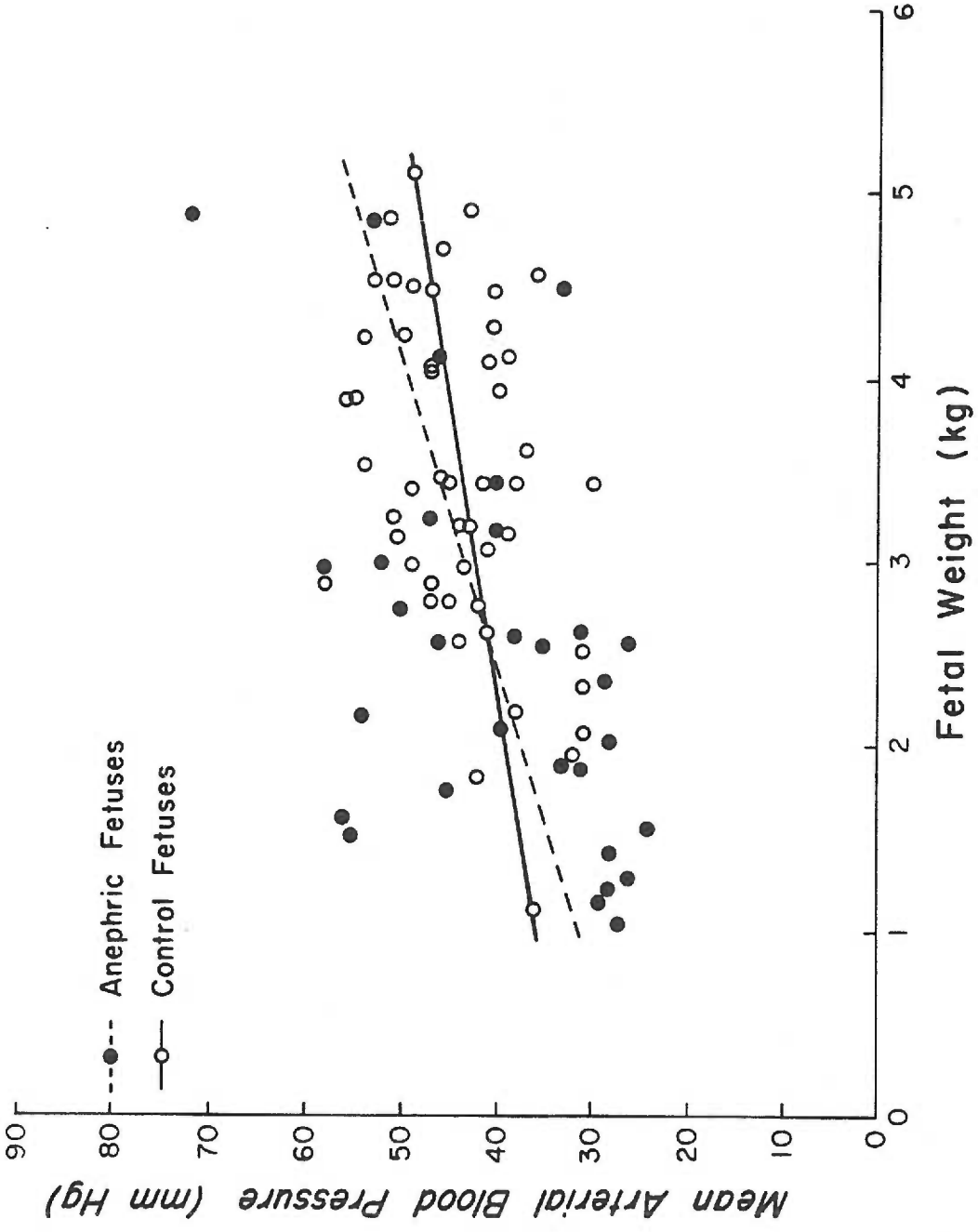
HR = 207 - 10 Wt , r = -0.45, $p<0.001$ for the combined groups.

Figure 11 shows the relationship between mean arterial blood pressure and fetal weight for control and anephric fetuses. Arterial blood pressure increased with fetal weight for both control and anephric fetuses. The linear regression lines for the two groups were not significantly different from each other (t = 1.57). Arterial blood pressures measured in the group of anephric fetuses showed more variability than those of controls. Part of this difference could be due to uncertainty in determination of the actual weight of anephric fetuses at the time of experiment, but the difference in variability may also reflect a true difference between the groups. If we have overestimated the rate of growth of anephric fetuses, then the true weight of the fetus at the time of blood pressure measurement would have been greater than the estimated weight. This type of error would tend to bias our data towards showing a higher arterial blood pressure in experimental versus control fetuses. In spite of this, our data fail to support the previous report of increased arterial blood pressure in bilaterally nephrectomized fetal lambs (Dutton, et al., 1978).

Plasma Renin Activity

Plasma renin activities were measured in samples obtained from 8 pregnant ewes, 10 intact control fetuses, and 24 anephric fetuses studied 3 to 21 days after operation. No maternal sample came from a ewe carrying an anephric fetus. These results are shown on the first line of Table 3. Values for plasma renin activity were higher in normal fetuses than in pregnant ewes, which has also been shown by

Figure 11. Mean arterial blood pressure as a function of fetal weight for intact control (open circles, broken line) and anephric (closed circles, solid line) sheep fetuses. Fetuses were weighed at autopsy and the weights of anephric fetuses were corrected for growth occurring between blood pressure measurement and autopsy based on the rate of growth predicted by the curve in Figure 8. The slopes of the regression lines were significantly different from zero for both control ($p < 0.005$) and anephric ($p < 0.005$) but the slopes of the regression lines were not significantly different from each other ($t = 1.57$).


Mean Arterial Blood Pressure (mm Hg)
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
6
Fetal Weight (kg)
Anephric Fetuses
Control Fetuses

other investigators (Table 3). The values for plasma renin activity in samples obtained from bilaterally nephrectomized fetuses in this study were very low; the amount of angiotensin I generated during incubation of these samples was below the limits of detection by radioimmunoassay under the conditions employed in this study. This occurred even though samples from anephric fetuses were incubated four times longer than those from control fetuses, and even though the volume of plasma used in the radioimmunoassay was 20 µl for samples from anephric fetuses versus 5 µl for samples from control fetuses. The low value for plasma renin activity for anephric fetuses is also consistent with the results of other investigators (Table 3).

Our values for plasma renin activity for pregnant ewes and intact fetuses appear to be higher than mean values reported by some other groups, but the mean value of plasma renin activity for intact fetuses is within the range reported by Fleishman, Oakes, Epstein, Catt, and Chez, (1975). They measured plasma renin activity in 15 sheep fetuses at three day intervals beginning on the fourth postoperative day. Their daily means ranged from 6.6 to 15.5 ng/ml/hr. The higher values for ewes sampled in this study may reflect the effects of stress on the secretion of renin (Reid, Morris, and Ganong, 1978) because sampling was performed on our ewes within 15 minutes of moving them from the pen to the laboratory. Differences in assay techniques could explain the differences in results, but the use of angiotensin I which has been standardized against the Medical Research Council standard should allow comparison of these results to those obtained by other groups.

Table 3. Plasma Renin Activity Measured as the Generation of Angiotensin I (ng/ml/hr) in Pregnant Ewes and Chronically Catheterized Intact and Bilaterally Nephrectomized Sheep Fetuses. Results Are Means ±S.E.M. Number of Measurements Are Given in Parentheses.

Days p.o.	Ewes	Intact Fetuses	Anephric Fetuses	Ref.
>3	7.1±3.4 (8)	13.4±1.7 (16)	<2.0 (27)	1
1-4			0.1 (6)	2
1-4	2.9±0.6 (14)	8.3±1.5 (18)	0.62 (2)	3
5-19	1.5±0.2 (40)	10.7±1.1 (55)		3
>2	2.70±0.22 (58)	9.71±2.39 (28)		4
6	2.9±1.3 (3)	9.0±1.3 (3)		5
>4	2.2-4.0* (15)	6.6-15.5* (15)		6

*Range of daily means

1. This study
2. Oakes, Fleishman, Catt, and Chez, 1977
3. Broughton Pipkin, Lumbers, and Mott, 1974a
4. Broughton Pipkin and O'Brien, 1978
5. Smith, Lupu, Barajas, Bauer, and Bashore, 1974
6. Fleishman, Oakes, Epstein, Catt, and Chez, 1975

Volumes of Distribution and Diffusion Permeabilites for $^{22}Na^{+}$ and $^{36}Cl^{-}$

Volumes of distribution (VD) and permeability surface area products (PS) for placental diffusion were measured in 7 control fetuses and in 16 anephric fetuses for $^{22}Na^{+}$ and in 8 control and 10 anephric fetuses for $^{36}Cl^{-}$. Tracer studies were repeated in one anephric fetus after an interval of 2 weeks. Both tracers were injected into 7 of the control fetuses and into 10 of the anephric fetuses. Control fetuses were studied 1 - 9 days after operation and anephric fetuses were studied 2 - 21 days postoperatively. The control data for fetuses in these experiments are given in Table 4. The weights of anephric fetuses were calculated as previously described. Anephric fetuses were significantly smaller than control fetuses. As discussed previously, this difference was attributed to the selection of younger fetuses to undergo bilateral nephrectomy. The groups had similar mean arterial blood P_{O_2} and P_{CO_2}, but the mean arterial pH of control fetuses was slightly but statisically significantly lower than that of anephric fetuses. This difference was attributed to the shorter time interval between surgery and experiment in the control group. There was no significant difference between results from fetuses studied shortly after operation versus results from fetuses studied several days after operation.

The relationship between volume of distribution for $^{22}Na^{+}$ and $^{36}Cl^{-}$ and fetal weight is shown in Figure 12 for control fetuses and in Figure 13 for anephric fetuses. In both groups the volume of distribution for each of the ions appears to increase linearly with increasing fetal weight. This would be the logical result if the proportion of the ion in the fetus remained constant. When the volumes of distribution

Table 4. Fetal Control Data (means ± S.D.) Recorded at the Time of Injection of Radioisotopes for the Measurement of Volume of Distribution and Placental Permeabilities. Numbers of Measurements Are Given in Parentheses.

Tracer Study	Experimental Group	Fetal Weight (hg)	Arterial Blood P_{O_2} (mm Hg)	Arterial Blood P_{CO_2} (mm Hg)	Arterial Blood pH
Na^+	Control	3.07±0.70**[1] (7)	17.2±3.7 (7)	53.3±7.0 (7)	7.30±0.09* (7)
	Anephric	2.08±0.91** (17)	17.7±3.8 (14)	50.6±3.0 (14)	7.37±0.03* (14)
Cl^-	Control	3.04±0.66‡[1] (8)	18.4±4.8 (8)	52.6±6.8 (8)	7.31±0.09* (8)
	Anephric	1.76±0.61‡ (10)	17.9±4.2 (7)	50.2±2.7 (7)	7.39±0.02*

[1]Calculated from fetal weight at autopy and the rate of growth predicted by Figure 8.

Difference of means, experimental versus control:

*$p<0.05$
**$p<0.01$
‡$p<0.001$

Figure 12. Linear regressions for volumes of distribution (VD) in liters (l) of $^{22}Na^{+}$ (open circles, solid line) and $^{36}Cl^{-}$ (open triangles, broken line) as a function of fetal weight (kg) for intact control fetuses. The slopes of the regression lines were not significantly different from zero.

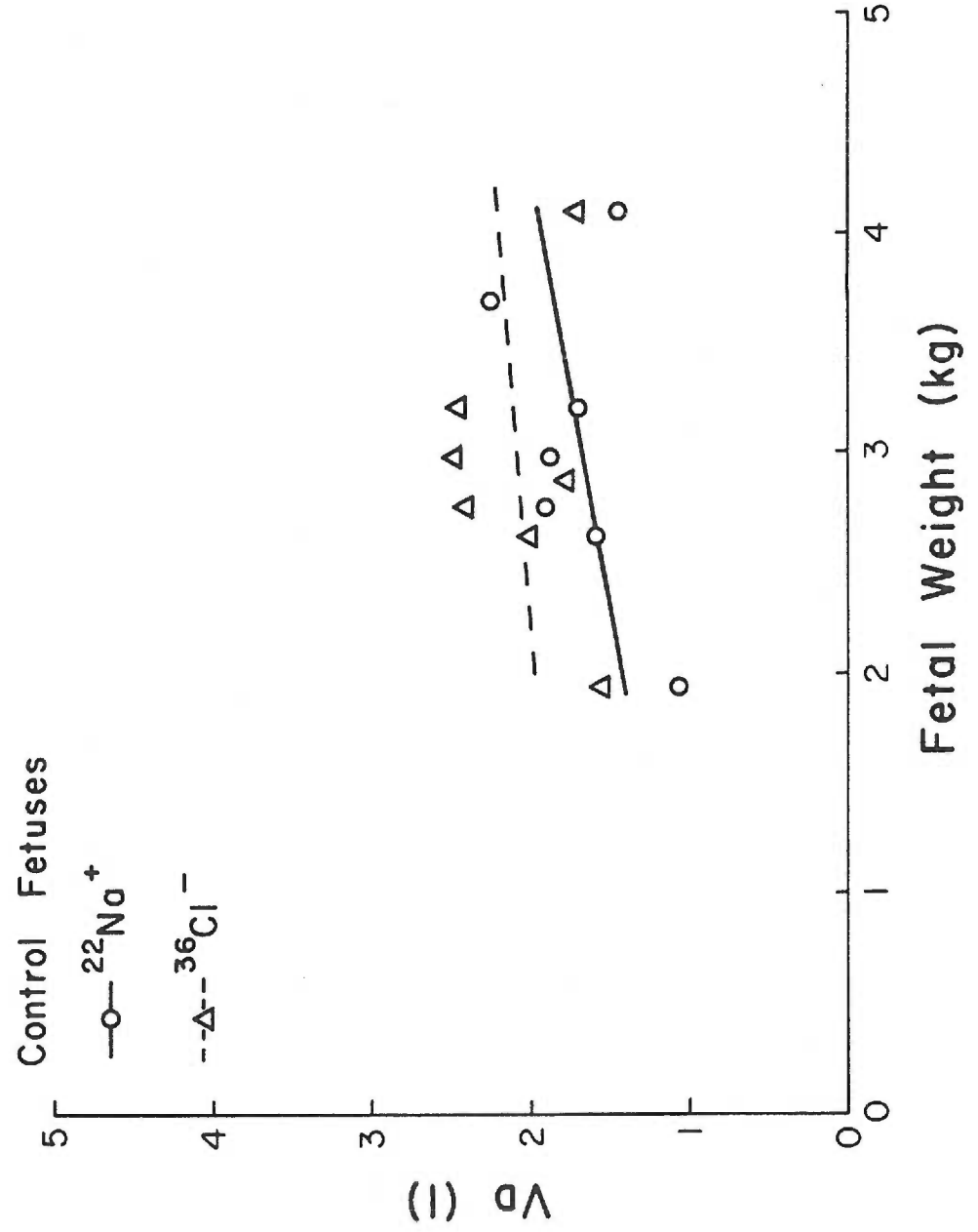
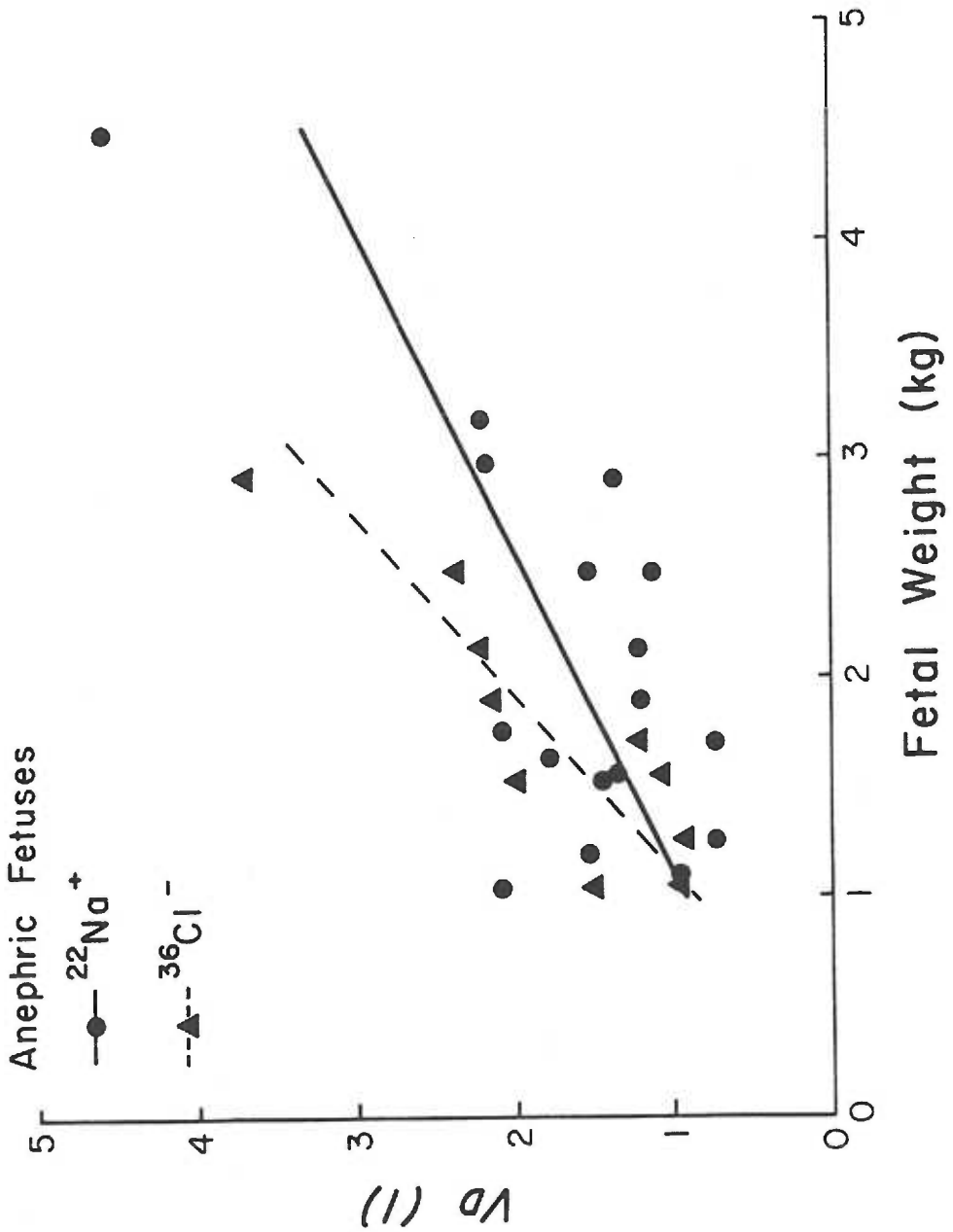

Control Fetuses
$^{22}Na^{+}$
$^{36}Cl^{-}$
Vd (l)
Fetal Weight (kg)
0
1
2
3
4
5

Figure 13. Linear regressions for volumes of distribution (VD) in liters (l) as a function of fetal weight for $^{22}Na^{+}$ (closed circles, solid line) and $^{36}Cl^{-}$ (closed triangles, broken line) in anephric sheep fetuses. The slopes of the regression lines were significantly different from zero for both $^{22}Na^{+}$ ($p < 0.005$) and $^{36}Cl^{-}$ ($p < 0.005$). The autopsy weights of the fetuses were corrected for growth as described in the legend for Figure 11.


Anephric Fetuses
$^{22}Na^{+}$
$^{36}Cl^{-}$
Vb (l)
Fetal Weight (kg)
0
1
2
3
4
5

were expressed per kilogram fetal weight there was no reliable correlation between volume of distribution per kilogram fetal weight and the weight of the fetus for either $^{22}Na^{+}$ or $^{36}Cl^{-}$ in either the control or the experimental group. Therefore, volumes of distribution were normalized per kilogram fetal weight to permit comparison of results from fetuses of different sizes. For control fetuses the volume of distribution for $^{36}Cl^{-}$ was greater than the volume of distribution for $^{22}Na^{+}$ ($p < 0.005$, by paired t test). The opposite was true for anephric fetuses where the volume of distribution for $^{36}Cl^{-}$ was less than the volume of distribution for $^{22}Na^{+}$ ($p < 0.05$, paired t test).

Placental permeability surface area products are shown as a function of fetal weight in Figure 14 for control fetuses and in Figure 15 for anephric fetuses. These figures show that diffusion permeabilities for both ions increase linearly with fetal weight for both control and anephric fetuses. Permeability surface area products for both ions expressed per kilogram fetal weight showed no significant correlation with fetal weight in either group. Therefore, permeability surface area products for $^{22}Na^{+}$ and $^{36}Cl^{-}$ were also normalized per kilogram fetal weight. Permeability surface area products were greater for $^{36}Cl^{-}$ than for $^{22}Na^{+}$ in both the control ($p < 0.005$, paired t test) and anephric ($p < 0.005$, paired t test) fetuses.

A comparison for volumes of distribution expressed per kilogram fetal weight in control versus anephric fetuses is shown in Figure 16 for $^{22}Na^{+}$ and in Figure 17 for $^{36}Cl^{-}$. Volumes of distribution per kilogram fetal weight were significantly higher in anephric fetuses than in control fetuses for both $^{22}Na^{+}$ and $^{36}Cl^{-}$. There was more

Figure 14. Linear regressions for placental permeability surface area products (PS) in µl/sec as a function of fetal weight in kg for $^{22}Na^{+}$ (open circles, solid line) and $^{36}Cl^{-}$ (open triangles, broken line) in control fetuses. The slopes of the regression lines were significantly different from zero for both $^{22}Na^{+}$ ($p < 0.05$) and $^{36}Cl^{-}$ ($p < 0.05$).

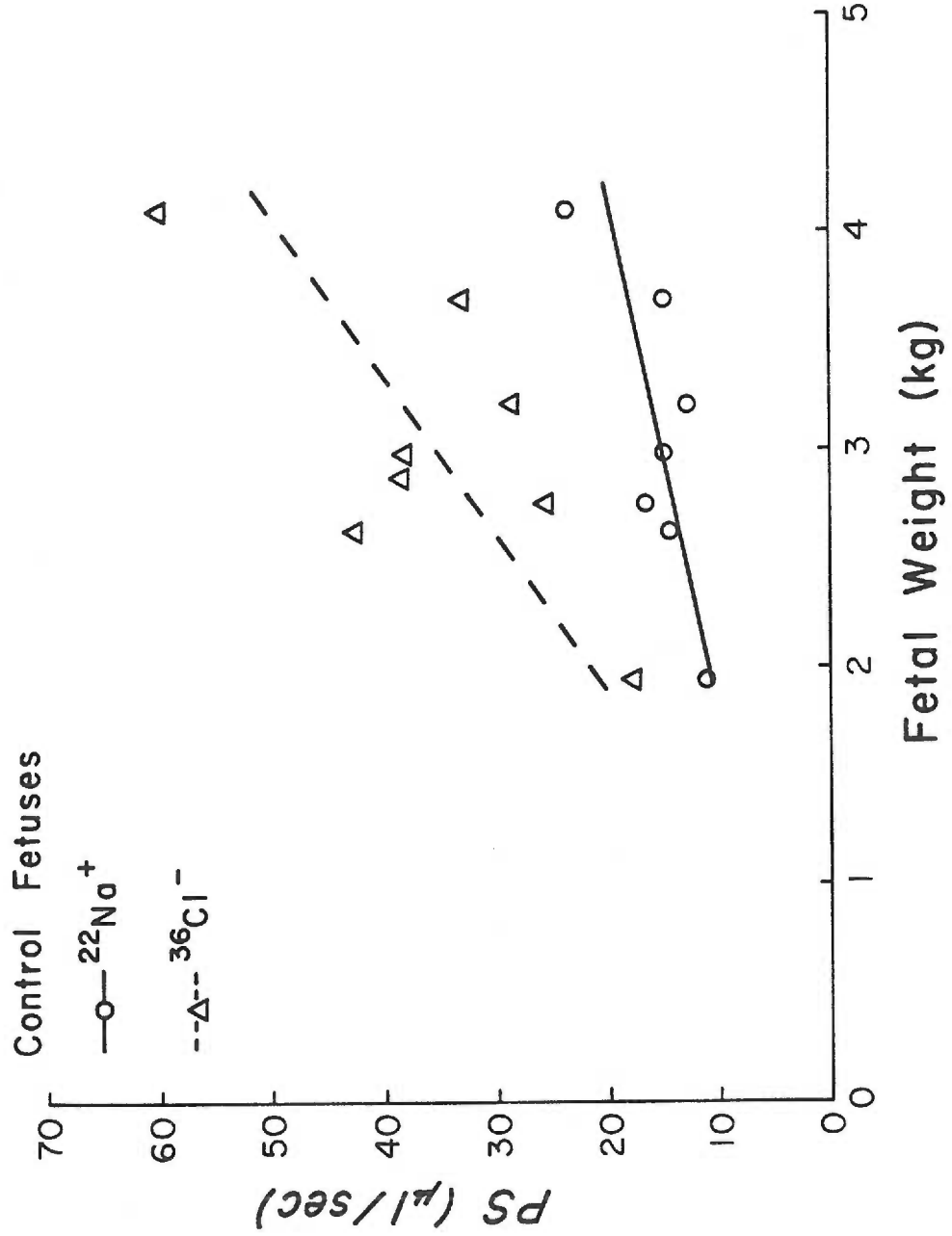
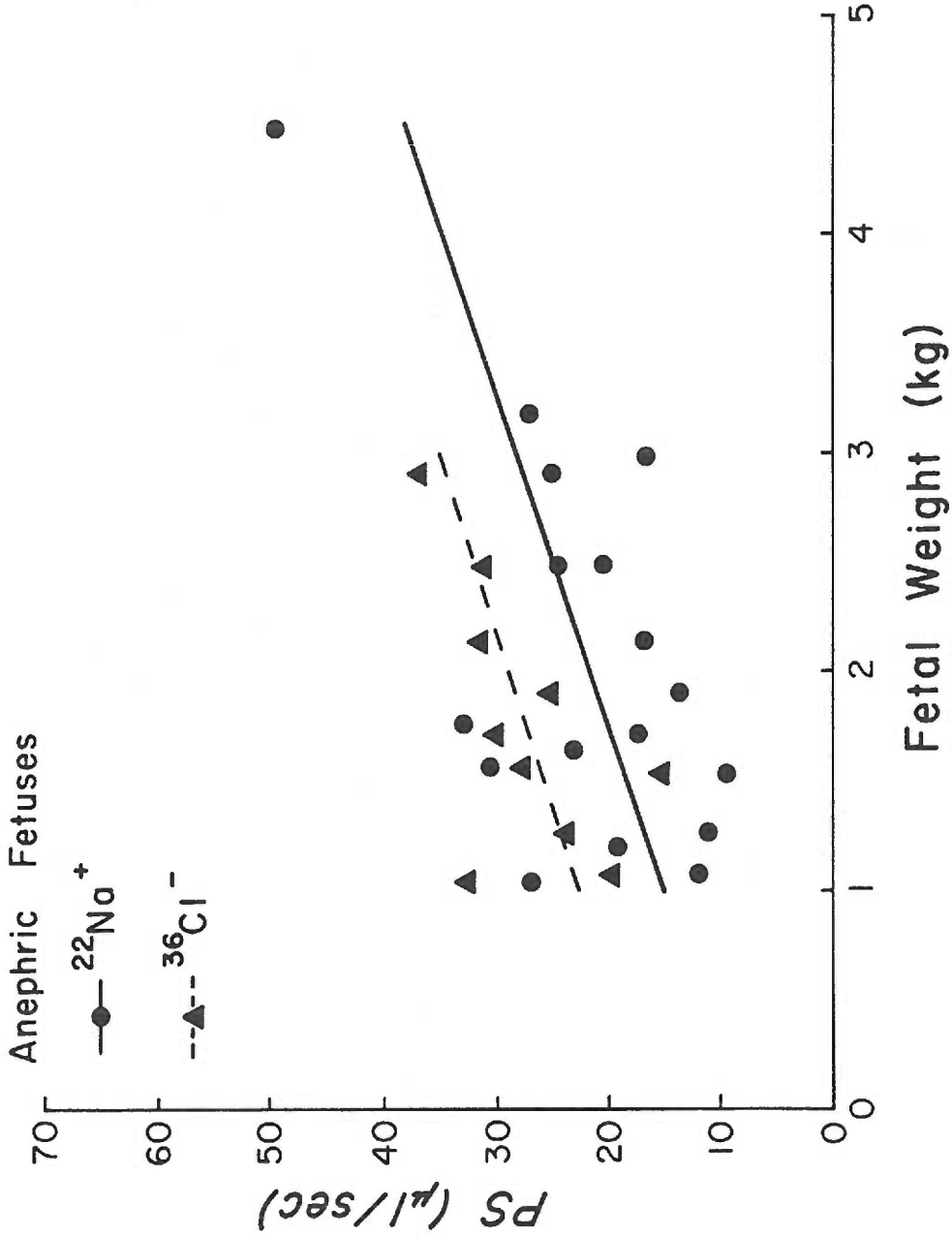

Control Fetuses
—o— $^{22}Na^{+}$
--Δ-- $^{36}Cl^{-}$
PS (μl/sec)
70
60
50
40
30
20
10
0
0
1
2
3
4
5
Fetal Weight (kg)

Figure 15. Linear regressions for placental permeability surface area products (PS) in µl/sec as a function of fetal weight in kg for $^{22}Na^{+}$ (closed circles, solid line) and $^{36}Cl^{-}$ (closed triangles, broken line) in anephric sheep fetuses. The slope of the regression line for $^{22}Na^{+}$ was significantly different from zero ($p < 0.01$), but the slope of the regression line for $^{36}Cl^{-}$ was not ($p < 0.075$).


Anephric Fetuses
$^{22}Na^+$
$^{36}Cl^-$
PS (μl/sec)
70
60
50
40
30
20
10
0
0
1
2
3
4
5
Fetal Weight (kg)

variability in the results from anephric fetuses than in results from control fetuses.

Placental permeability surface area products per kilogram fetal weight in control and anephric fetuses are compared in Figure 18 for $^{22}Na^{+}$ and in Figure 19 for $^{36}Cl^{-}$. For both ions the placental diffusion permeabilities per kilogram fetal weight were higher in anephric fetuses than in control fetuses. There was more variability in results from anephric fetuses versus results from control fetuses.

There was a significant ($p < 0.01$) positive correlation between volume of distribution per kilogram for $^{22}Na^{+}$ and volume of distribution per kilogram for $^{36}Cl^{-}$ measured in fetuses injected with both isotopes (Figure 20). There was also a significant ($p < 0.01$) positive correlation between permeability surface area product per kilogram for $^{22}Na^{+}$ and permeability surface area product per kilogram for $^{36}Cl^{-}$ in the same fetuses (Figure 21).

A summary of mean volumes of distribution and permeability surface area products expressed per kilogram fetal weight for $^{22}Na^{+}$ and $^{36}Cl^{-}$ in control and anephric fetuses is given in Table 5. Mean values of volumes of distribution per kilogram and placental diffusion permeabilities for $^{22}Na^{+}$ were approximately 2 times higher in anephric fetuses than in control fetuses. The means of these values for $^{36}Cl^{-}$ were approximately 1.5 times higher in anephric fetuses than in control fetuses.

Figure 16. A comparison of volumes of distribution for $^{22}Na^{+}$ expressed per kilogram fetal weight (VD/kg) in L/kg in control (open circles) and anephric (closed circles) sheep fetuses. The mean values for both groups are indicated by horizontal lines. The weights of anephric fetuses were calculated as described in the legend for Figure 11.

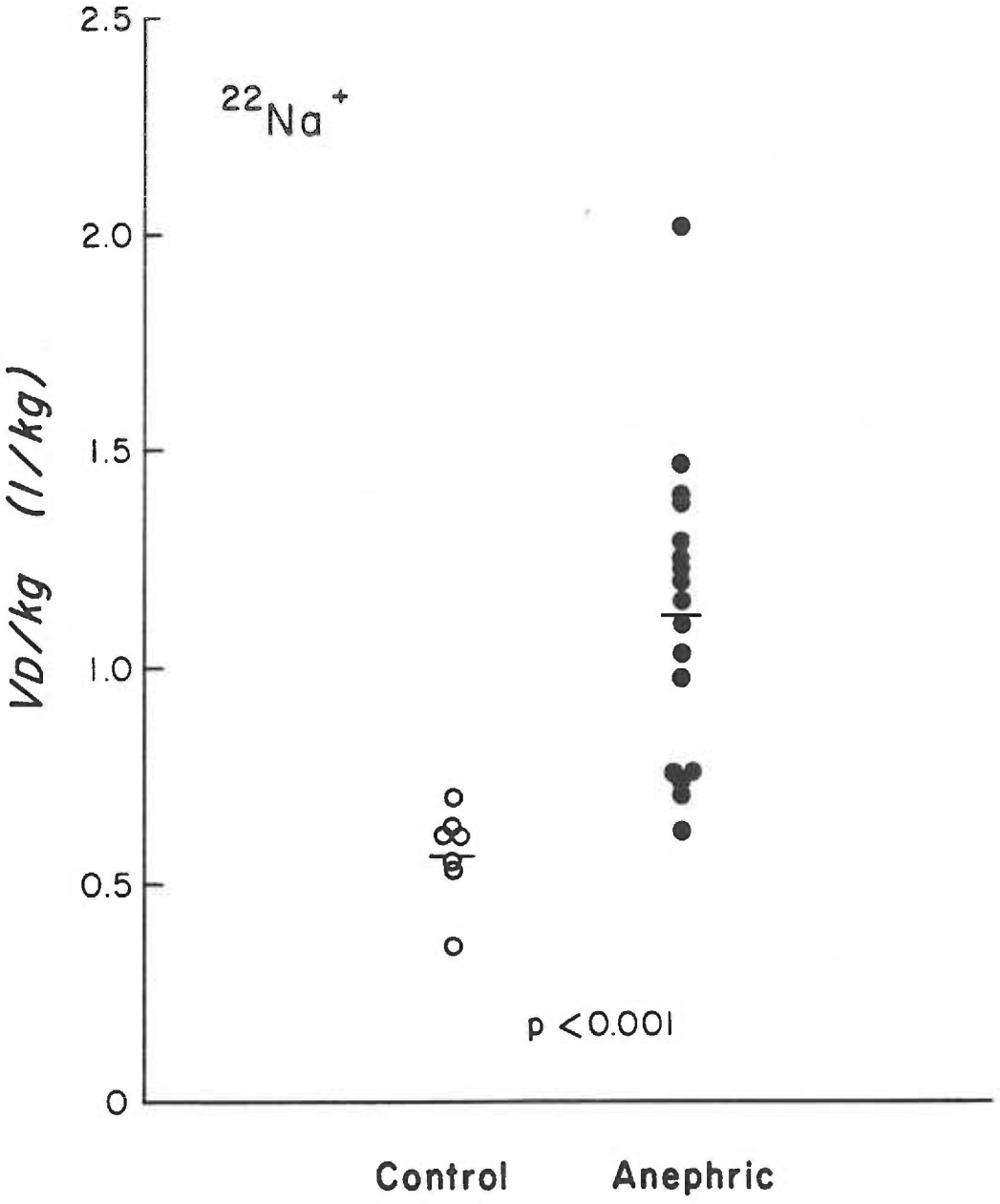

2.5
2.0
1.5
1.0
0.5
0
$^{22}Na^{+}$
VD/kg (l/kg)
p <0.001
Control
Anephric

Figure 17. A comparison of volumes of distribution for $^{36}Cl^-$ expressed per kilogram fetal weight (VD/kg) in L/kg in control (open circles) and anephric (closed circles) sheep fetuses. The mean values for both groups are indicated by horizontal lines. The weights of anephric fetuses were calculated as described in the legend for Figure 11.

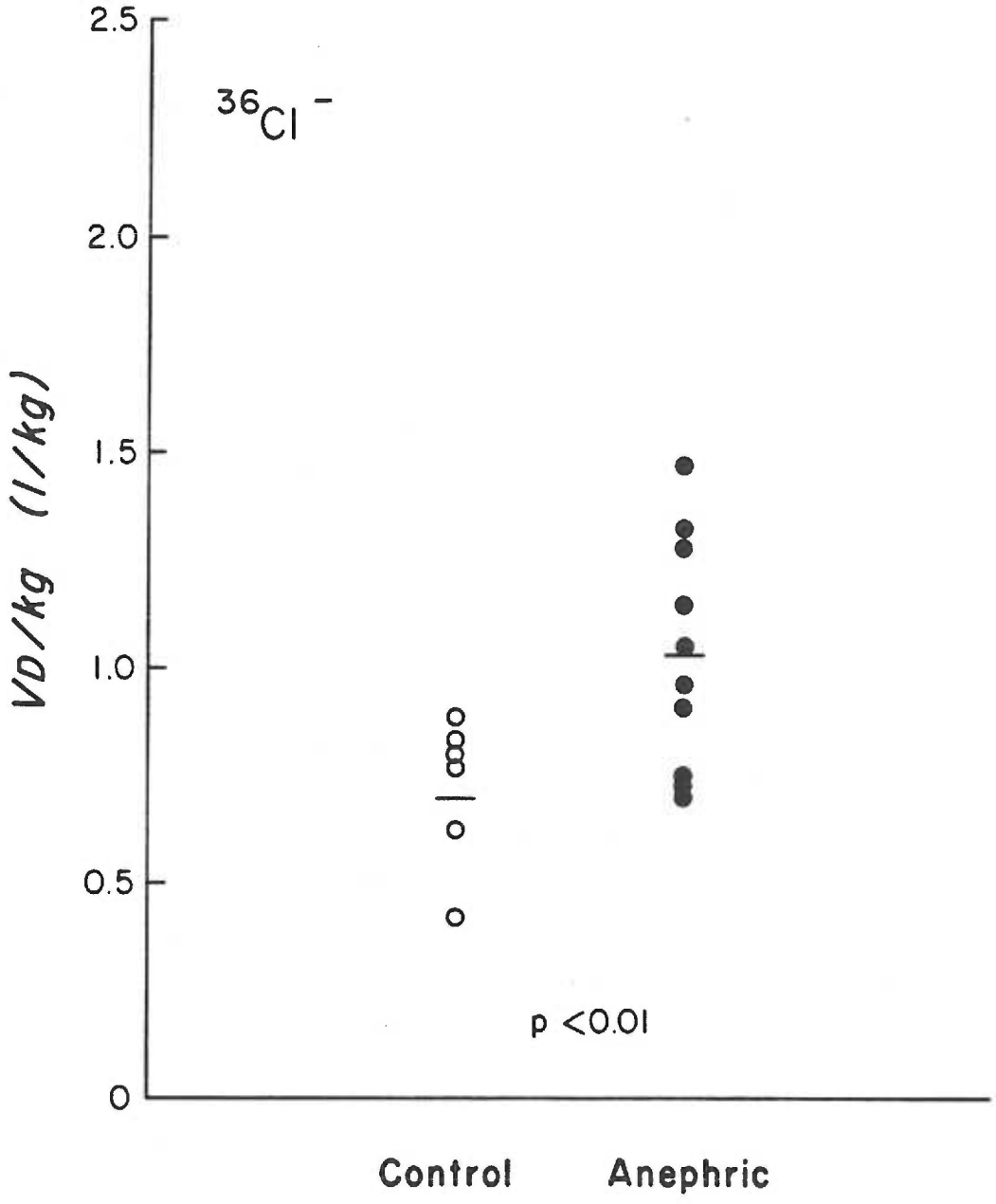

2.5
2.0
1.5
1.0
0.5
0
$^{36}Cl^-$
V_D/kg (l/kg)
p <0.01
Control
Anephric

Figure 18. A comparison of permeability surface area products for $^{22}Na^{+}$ expressed per kilogram fetal weight (PS/kg) in µl/sec/kg for control (open circles) and anephric (closed circles) sheep fetuses. The mean values for both groups are indicated by horizontal lines. The weights of anephric fetuses were calculated as described in the legend for Figure 11.

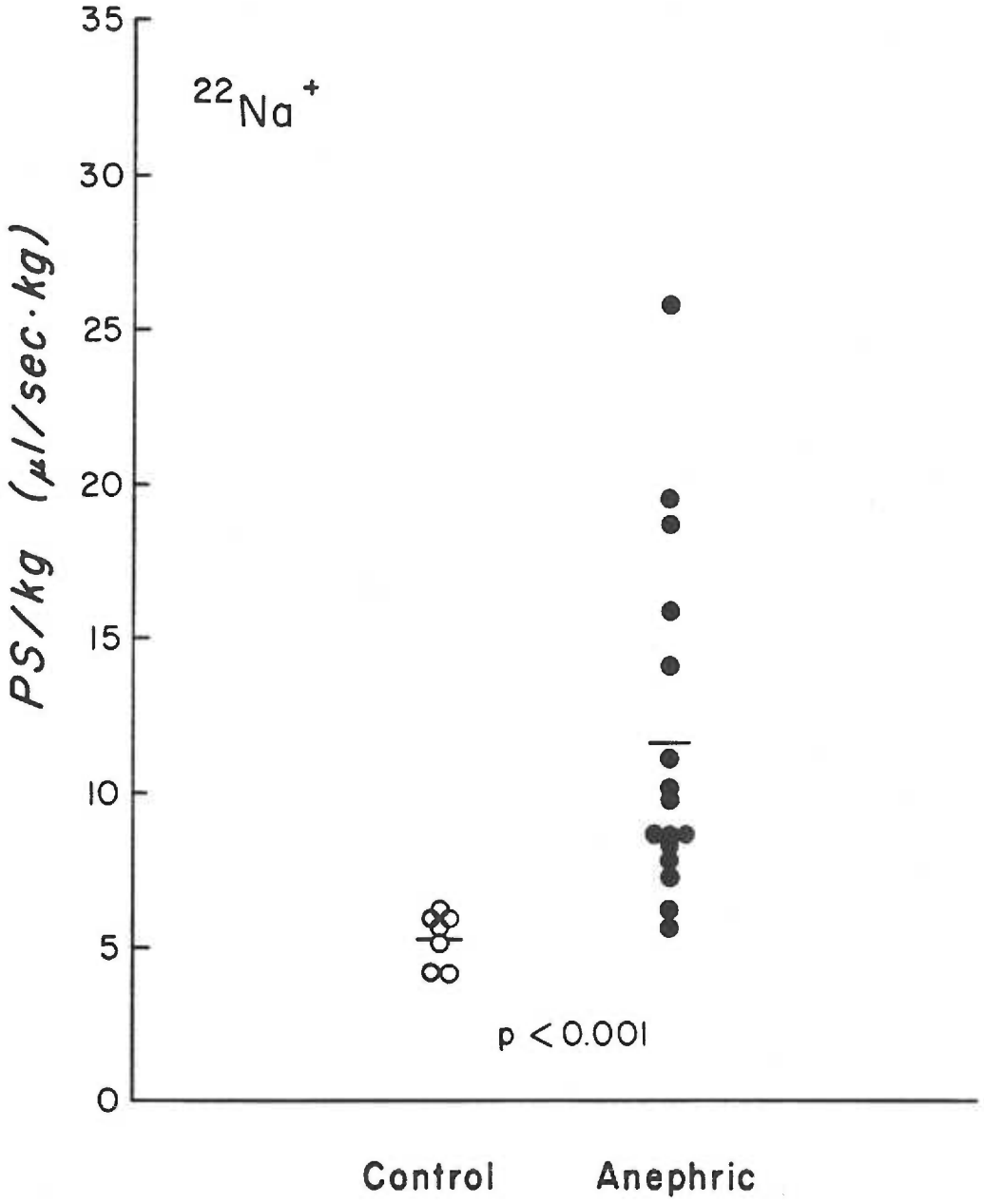

35
30
25
20
15
10
5
0
$^{22}Na^+$
PS/kg (μl/sec·kg)
p < 0.001
Control
Anephric

Figure 19. A comparison of permeability surface area products for $^{36}Cl^-$ expressed per kilogram fetal weight (PS/kg) in µl/sec/kg for control (open circles) and anephric (closed circles) sheep fetuses. The mean values for both groups are indicated by horizontal lines. The weights of anephric fetuses were calculated as described in the legend for Figure 11.

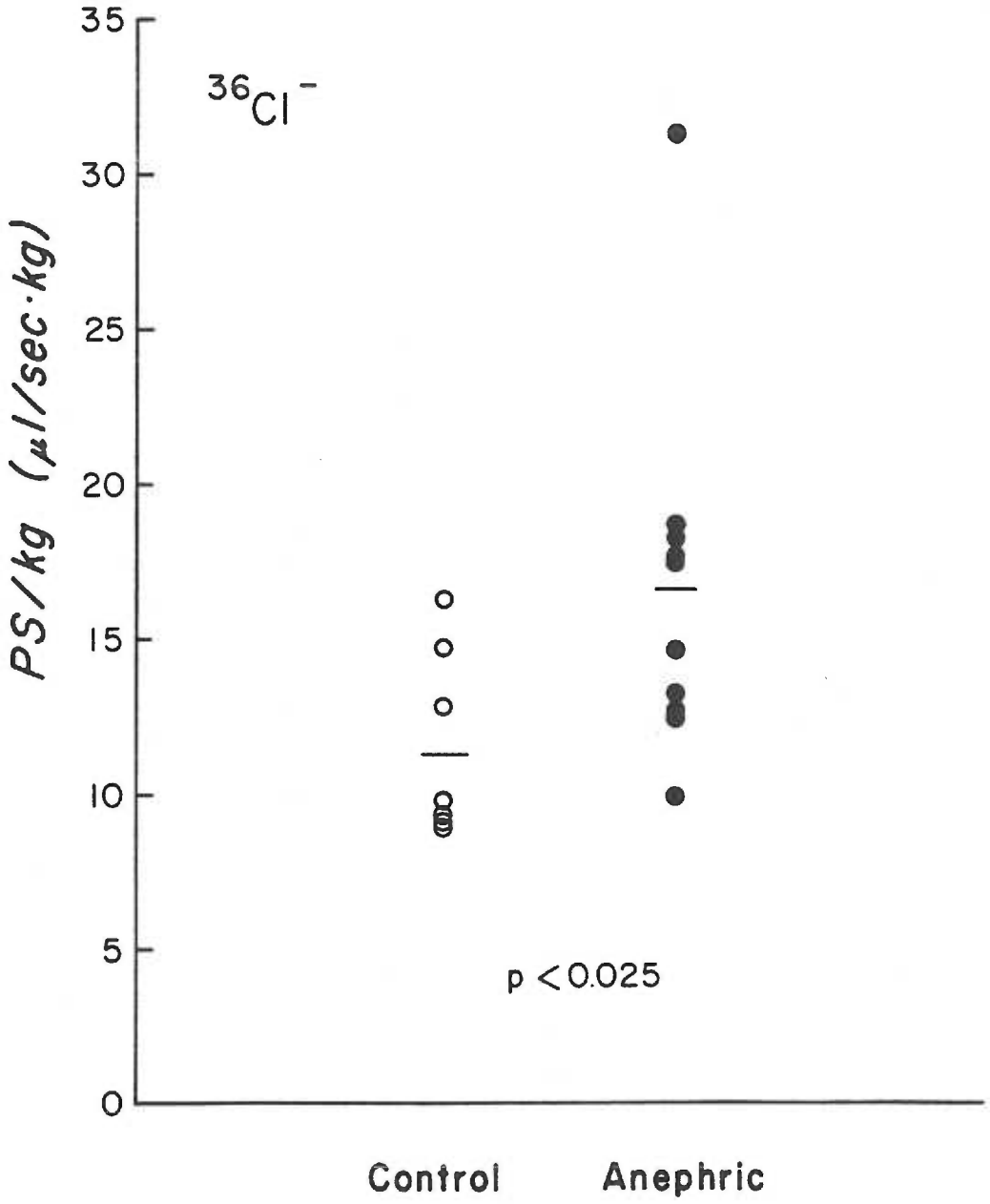

35
30
25
20
15
10
5
0
$^{36}Cl^-$
PS/kg (µl/sec·kg)
p < 0.025
Control
Anephric

Figure 20. The relationship between volume of distribution per kilogram fetal weight of $^{22}Na^{+}$ ($VD_{Na}+/kg$) and $^{36}Cl^{-}$ ($VD_{Cl}-/kg$) expressed in L/kg in fetuses who were injected with both isotopes. Control fetuses are represented by open circles and anephric fetuses are represented by closed circles. There was a reliable correlation between $VD_{Na}+/kg$ and $VD_{Cl}-/kg$ ($r = 0.85$, $p < 0.001$). The autopsy weights of anephric fetuses were corrected for growth as previously described.

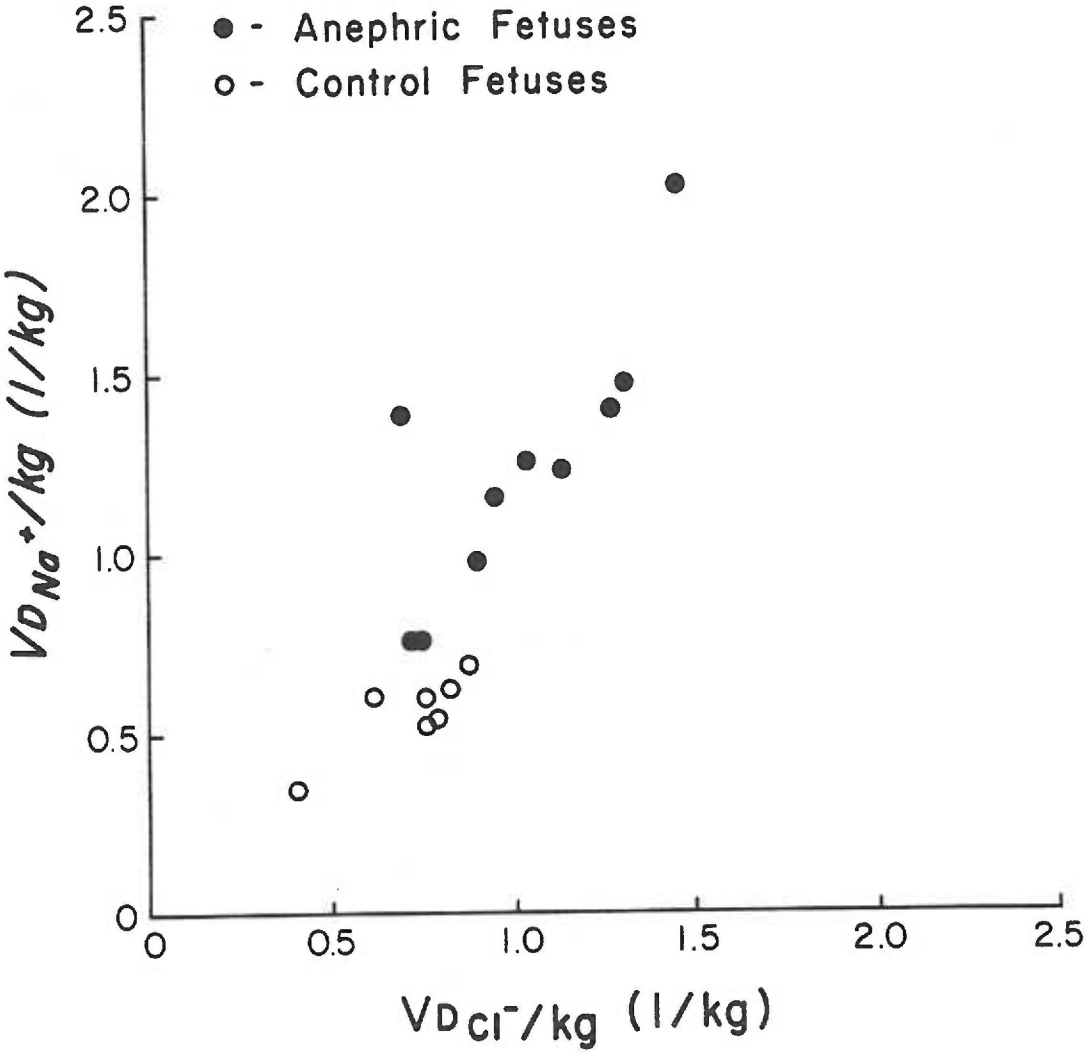

● - Anephric Fetuses
o - Control Fetuses
2.5
2.0
1.5
1.0
0.5
0
0
0.5
1.0
1.5
2.0
2.5
VD Na+/kg (l/kg)
VD Cl-/kg (l/kg)

Figure 21. The relationship between permeability surface area product per kilogram fetal weight for $^{22}Na^{+}$ ($PS_{Na}+/kg$) and $^{36}Cl^{-}$ ($PS_{Cl}-/kg$) expressed in µl/sec/kg in fetuses who were injected with both isotopes. Control fetuses are represented by open circles and anephric fetuses are represented by closed circles. There was a reliable correlation between $VD_{Na}+/kg$ and $VD_{Cl}-/kg$ ($r=0.85$, $p < 0.001$). The autopsy weights of anephric fetuses were corrected for growth as previously described.

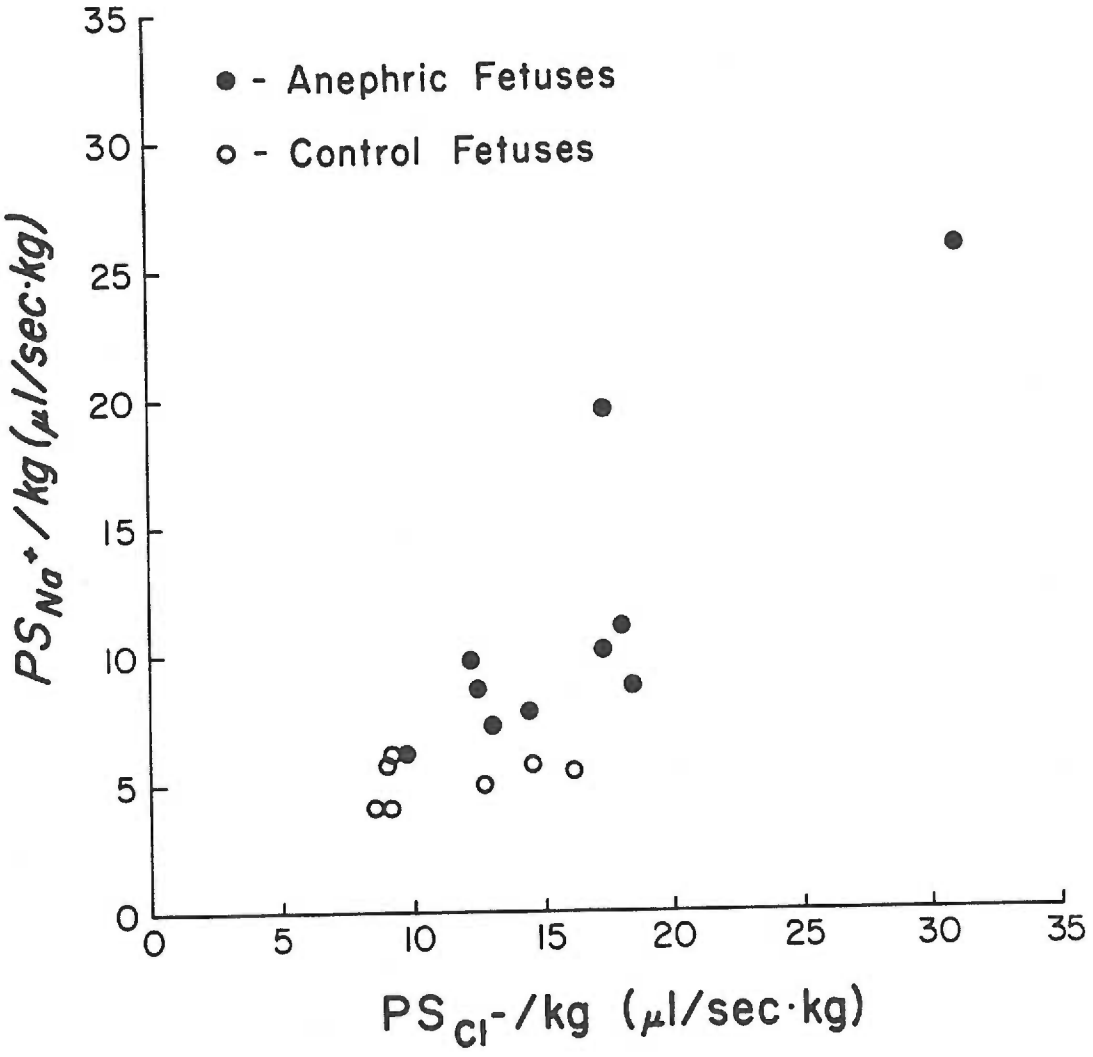

● - Anephric Fetuses
○ - Control Fetuses
PS_{Na^+}/kg (μl/sec·kg)
PS_{Cl^-}/kg (μl/sec·kg)
0
5
10
15
20
25
30
35

Table 5. Mean (±SEM) Volumes of Distribution (VD) and Placental Permeability Surface Area Products (PS) for Anephric and Intact Control Fetuses.

	VD_{Na^+}/kg (ml/kg)	VD_{Cl^-}/kg (ml/kg)	PS_{Na^+}/kg (µl/sec./kg)	PS_{Cl^-}/kg (µl/sec./kg)
Anephric*	1122±88 (17)	1030±85 (10)	11.54±1.33 (17)	16.56±1.88 (10)
Control**	568±41 (7)	710±53 (8)	5.19±0.32 (7)	11.23±1.04 (8)
Difference	544±95	320±100	6.35±1.37	5.33±2.48
p	<0.001	<0.01	<0.001	<0.025

*Based on weights calculated from the weight of the fetus at autopsy and the rate of growth predicted by Figure 8.
**Based on autopsy weights of fetuses.

Solute Concentrations in Maternal and Fetal Plasma

The concentrations of various solutes were measured in plasma samples taken from 18 pregnant ewes and their 23 anephric fetuses on the day when arterial blood pressures were measured in the fetuses. Mean concentrations of electrolytes in maternal and fetal plasma are shown in Table 6. Mean concentrations of nonelectrolytes in maternal and fetal plasma are shown in Table 7. Maternal plasma concentrations of Na^+, Cl^-, glucose, total protein, and albumin exceeded fetal plasma concentrations of these substances. Fetal concentrations of Mg^{++}, Ca^{++}, PO_4^{3-}, urea, creatinine, uric acid, and lactate exceeded maternal concentrations of these solutes. The osmolality of fetal plasma was slightly less than the osmolality of maternal plasma.

The concentrations of solutes in the plasma of pregnant ewes and their intact fetuses are shown in Table 8 for electrolytes and in Table 9 for nonelectrolytes. The data presented in these tables has been taken from published and unpublished results of Armentrout, et al. (1977). They obtained blood samples from catheterized sheep using the same techniques reported in the methods section of this thesis. Their plasma samples were analyzed in the same laboratory by the same methods used to analyze plasma samples from anephric fetuses and their mothers.

Interesting differences in maternal and fetal plasma concentrations of Na^+ and Cl^- and in transplacental concentration differences for these ions were noted between ewes with control fetuses and ewes with anephric fetuses. Dutton and Mott (1979) have described differences in concentrations of Na^+ and K^+ in plasma samples from anephric fetal lambs and their mothers compared to normal fetuses and ewes. These differences

Table 6. Mean (± SEM) Concentrations of Electrolytes (mM) and Osmolalities (mOsm) of Plasma Samples from Anephric Sheep Fetuses and Their Mothers. Differences in Concentration Between Maternal and Fetal Plasmas ($C_m - C_f$) Have Been Calculated from Paired Data; a Positive Value Indicates That Maternal Concentration Is Higher Than Fetal Concentration. The Probability That a Difference in Concentration Exists Has Been Calculated from Student's t Test on Paired Data. The Numbers of Measurements Are Shown in Parentheses.

Constituent	Maternal Plasma*	Fetal Plasma**	$(C_m - C_f)$	P
Na^+	150.1 ± 0.6 (18)	148.3 ± 0.9 (23)	+1.81 ± 0.87 (22)	<0.05
K^+	4.42 ± 0.14 (18)	4.41 ± 0.17 (23)	+0.09 ± 0.15 (22)	NS
Mg^{++}	0.88 ± 0.04 (10)	1.21 ± 0.06 (13)	-0.34 ± 0.06 (13)	<0.001
Ca^{++}	2.29 ± 0.04 (18)	2.77 ± 0.08 (23)	-0.47 ± 0.08 (22)	<0.001
Cl^-	113.5 ± 0.8 (18)	96.2 ± 1.4 (23)	+17.1 ± 1.5 (22)	<0.001
PO_4^{3-}	0.37 ± 0.03 (18)	0.83 ± 0.04 (22)	-0.53 ± 0.03 (21)	<0.001
Osmolality	295.7 ± 1.6 (10)	290.1 ± 1.5 (13)	+4.17 ± 1.26 (10)	<0.01

* Sample taken from jugular vein of ewe

**Sample taken from femoral artery of fetus

Table 7. Mean (± SEM) Concentrations of Nonelectrolytes (mg/dl) in Plasma Samples From Anephric Sheep Fetuses and Their Mothers. Differences in Concentration Between Maternal and Fetal Plasma (C_m - C_f) Have Been Calculated from Paired Data; a Positive Value Indicates That Maternal Concentration is Higher Than Fetal Concentration. The Probability That a Difference in Concentration Exists Has Been Calculated from Student's t Test on Paired Data. The Numbers of Measurements are Shown in Parentheses.

Constituent	Maternal Plasma*	Fetal Plasma**	(C_m - C_f)	P
Glucose	71.4 ± 4.5 (18)	12.9 ± 1.8 (23)	+54.0 ± 3.3 (22)	0.001
Urea	17.0 ± 5.5 (18)	21.3 ± 1.45 (23)	-5.09 ± 1.02 (22)	0.001
Creatinine	0.8 ± 0.5 (18)	2.5 ± 1.5 (23)	-1.71 ± 0.15 (22)	0.001
Uric Acid	0.21 ± 0.03 (11)	0.52 ± 0.08 (15)	-1.31 ± 0.08 (15)	0.01
Lactate	1.1 ± 0.2 (10)	3.3 ± 0.5 (13)	-2.3 ± 0.5 (13)	0.001
Total Protein	7.18 ± 0.16 (18)	3.39 ± 0.08 (23)	+3.79 ± 0.16 (22)	0.001
Albumin	3.04 ± 0.06 (18)	1.84 ± 0.27 (23)	+1.02 ± 0.08 (22)	0.001

* Sample taken from jugular vein of ewe

**Sample taken from femoral artery of fetus

Table 8. Mean (± SEM) Concentrations of Electrolytes (mM) and Osmolalities (mOsm) of Plasma Samples from Intact Sheep Fetuses and Their Mothers. Differences in Concentration Between Maternal and Fetal Plasmas ($C_m - C_f$) Have Been Calculated from Paired Data; a Positive Value Indicates That Maternal Concentration Is Higher Than Fetal Concentration. The Probability That a Difference in Concentration Exists Has Been Calculated from Student's t Test on Paired Data. The Numbers of Measurements Are Shown in Parentheses.

Constituent	Maternal Plasma*	Fetal Plasma**	$(C_m - C_f)$	P
Na^+	145.3 ± 1.2 (9)	143.7 ± 1.9 (9)	+2.29 ± 0.64	0.01
K^+	4.49 ± 0.10 (9)	3.91 ± 0.14 (9)	+0.55 ± 0.14	0.01
Mg^{++}	0.85 ± 0.08 (7)	0.81 ± 0.06 (7)	+0.04 ± 0.05	NS
Ca^{++}	2.12 ± 0.07 (9)	2.16 ± 0.08 (9)	-0.79 ± 0.06	0.001
Cl^-	107.7 ± 1.7 (9)	101.3 ± 2.4 (9)	+5.47 ± 1.37	0.01
PO_4^{3-}	0.48 ± 0.03 (6)	0.73 ± 0.05 (6)	-0.27 ± 0.3	0.01

* Sample taken from uterine artery of ewe

**Sample taken from umbilical artery of fetus

Table 9. Mean (± SEM) Concentrations of Nonelectrolytes (mg/dl) in Plasma Samples From Intact Sheep Fetuses and Their Mothers. Differences in Concentration Between Maternal and Fetal Plasma ($C_m - C_f$) Have Been Calculated from Paired Data; a Positive Value Indicates That Maternal Concentration is Higher Than Fetal Concentration. The Probability That a Difference in Concentration Exists Has Been Calculated from Student's t Test on Paired Data. The Numbers of Measurements Are Shown in Parentheses.

Constituent	Maternal Plasma*	Fetal Plasma**	$(C_m - C_f)$	P
Glucose	59.7 ± 4.9 (8)	17.4 ± 4.9 (8)	+42.2 ± 0.83	<0.001
Urea	16.4 ± 2.0 (9)	19.0 ± 2.1 (9)	-2.56 ± 0.29	<0.001
Creatinine	0.9 ± 0.1 (6)	1.5 ± 0.1 (6)	-0.62 ± 0.15	<0.01
Uric Acid	0.30 ± 0.15 (6)	0.58 ± 0.13 (5)	-0.42 ± 0.17	<0.05
Total Protein	6.60 ± 0.25 (6)	3.43 ± 0.22 (6)	+3.32 ± 0.25	<0.01
Albumin	2.78 ± 0.20 (6)	1.84 ± 0.27 (5)	+0.90 ± 0.13	<0.001
Osmolality	301.1 ± 2.9 (8)	300.0 ± 2.3 (8)	+2.2 ± 0.7	<0.01

* Sample taken from uterine artery of ewe

**Sample taken from umbilical artery of fetus

Table 10. A comparison of Maternal and Fetal Plasma Concentrations of Na^+, K^+, and Cl^- Between Ewes Carrying Normal Fetuses and Ewes Carrying Anephric Fetuses. A Similar Comparison of the Plasma Concentrations of These Ions Is Shown for Pregnant Ewes Carrying Normal Fetuses at 70 and 140 Days of Gestation. C_m Refers to Maternal Plasma Concentration, C_f to Fetal Plasma Concentration, and $(C_m - C_f)$ to the Difference in Concentration Between Maternal and Fetal Plasmas. Values Are Expressed as Means.

	Na^+			K^+			Cl^-			
	C_m (mM)	C_f (mM)	(C_m-C_f) (mM)	C_m (mM)	C_f (mM)	(C_m-C_f) (mM)	C_m (mM)	C_f (mM)	(C_m-C_f) (mM)	Ref.
Control Fetuses	145.3	143.7	2.29	4.49	3.91	0.55	107.7	101.3	5.47	1
Anephric Fetuses	150.1	148.3	1.81	4.42	4.41	0.09	113.5	96.2	17.1	2
p	<0.01	<0.05	NS	NS	<0.05	<0.05	<0.001	<0.001	<0.001	
Control Fetuses	144	141		3.9	4.3					3
Anephric Fetuses	153	151		4.3	5.0					3
p	<0.001	<0.001		<0.001	<0.001					
Normal Fetuses, 70 days	147.0	142.6		4.0	3.8		104.9	108.2		4
Normal Fetuses, 140 days	157.0	155.1		4.4	5.8		110.7	109.2		4
p	<0.001	<0.001		<0.01	<0.001		<0.001	NS		

1. Armentrout, Faber, and Thornburg (1977)
2. This Study
3. Dutton and Mott (1979)
4. Kaiser and Cummings, (1958)

are summarized in Table 10. Maternal and fetal plasma concentrations of Na^+, K^+, and Cl^- in pregnant ewes and their normal fetuses at 70 and 140 days of gestation are also shown in Table 10 to illustrate that there are gestational trends for the concentrations of these ions in maternal and fetal sheep plasmas. The differences in plasma concentrations of Na^+ and Cl^- between anephric fetuses and their mothers and intact fetuses and their mothers that were observed in this study probably are real. The concentrations of Na^+ in maternal and fetal plasmas normally increase with advancing gestation, but the anephric fetuses in this study are smaller, and therefore younger, than the intact fetuses in the study reported by Armentrout, et al. (1977). Therefore, it would be expected that the concentrations of Na^+ in maternal and fetal plasmas reported in this study would be less than those reported by Armentrout, et al. (1977). The mean concentration of Cl^- in plasma from anephric fetuses is considerably less than fetal plasma concentrations of Cl^- for normal fetuses shown in Table 10. Therefore, changes in maternal and fetal plasma concentrations of these ions during gestation are unlikely to account for the differences between anephric and intact fetuses.

Solute Concentrations in Amniotic and Allantoic Fluids

The concentrations of solutes in amniotic and allantoic fluids were measured at the time of operation for 15 fetuses. 4 amniotic fluid samples and 2 allantoic fluid samples were obtained from anephric fetuses 1 week after operation. It was not possible to obtain samples of extrafetal fluids from other anephric fetuses. At the time of autopsy anephric fetuses were found to have almost no free fluid in

their gestational sacs; usually a viscous gel was found. The presence of only small amounts of extrafetal fluids is similar to the oligohydramnios of human infants with renal agenesis (Potter, 1946 and 1965) or to the presence of only small amounts of extrafetal fluids in the amniotic and allantoic sacs of fetal sheep after drainage of fetal urine for several days (Gresham, et al., 1972).

Concentrations of electrolytes in amniotic and allantoic fluids of sheep fetuses before and after fetal nephrectomy are shown in Table 11, and the concentrations of nonelectrolytes in these fluids are shown in Table 12. The concentrations of solutes in these fluids at the time of operation are within the ranges reported by other investigators (Rapoport and Mann, 1970; Mellor 1972). There have been no previous reports of solute concentrations in the extrafetal fluids of anephric fetal lambs. It is interesting to note that the amniotic fluid of anephric fetuses tends toward equilibrium with fetal plasma after bilateral fetal nephrectomy, but that this is not the case for allantoic fluid.

Table 11. Mean (± SEM) Concentration of Electrolytes (mM) and Osmolalities (mOsm) of Amniotic and Allantoic Fluids of Fetal Sheep Sampled Before and After Bilateral Nephrectomy of the Fetuses. Samples Were Taken at the Time of Operation and One Week Later. The Numbers of Measurements Are Shown in Parentheses.

Constituent	Amniotic Fluid		Allantoic Fluid	
	Before	After	Before	After
Na^+	107.4 ± 3.50 (15)	140.0 ± 5.37 (4)	52.33 ± 4.84 (15)	34.0 (3)
K^+	8.26 ± 0.72 (14)	6.9 ± 1.5 (4)	65.90 ± 10.47 (14)	16.6 (2)
Mg^{++}	0.08 ± 0.01 (15)	0.15 ± 0.05 (4)	1.13 ± 0.13 (15)	2.13 (2)
Ca^{++}	0.11 ± 0.03 (15)	0.15 ± 0.01 (4)	0.25 ± 0.06 (15)	0.79 (2)
Cl^-	98.3 ± 3.04 (15)	114.0 ± 4.97 (4)	11.8 ± 3.39 (15)	-0- (2)
PO_4^{3-}	0.02 ± 0.003 (15)	0.06 ± 0.016 (4)	0.11 ± 0.026 (15)	0.33 (2)
Osmolality	278.25 ± 1.99 (15)	286.73 ± 1.91 (4)	283.49 ± 1.87 (15)	281.95 (2)

Table 12. Mean (± SEM) Concentrations of Nonelectrolytes (mg/dl) of Amniotic and Allantoic Fluids of Fetal Sheep Sampled Before and After Bilateral Nephrectomy of the Fetuses. Samples Were Taken at the Time of Operation and One Week Later. The Numbers of Measurements Are Shown in Parentheses.

Constituent	Amniotic Fluid		Allantoic Fluid	
	Before	After	Before	After
Glucose	7.60 ± 1.62 (15)	5.25 ± 2.17 (4)	11.27 ± 1.93 (15)	2.50 (2)
Lactate	1.04 ± 0.38 (15)	2 (4)	0.76 ± 0.19 (15)	0.55 (2)
Urea	43.2 ± 5.01 (15)	19.0 ± 0.82 (4)	40.4 ± 2.65 (15)	31 (2)
Creatinine	5.0 ± 0.55 (15)	3.3 ± 1.16 (4)	21.3 ± 1.92 (15)	10 (2)
Uric Acid	0.57 ± 0.10 (13)	1.13 ± 0.41 (4)	2.64 ± 0.38 (13)	7.8 (2)
Total Protein	0.11 ± 0.02 (15)	0.30 ± 0.07 (4)	0.91 ± 0.07 (15)	2.45 (4)
Albumin	0.01 ± 0.01 (15)	0.18 ± 0.05 (4)	0.09 ± 0.02 (15)	1.35 (2)

DISCUSSION

The results of these experiments indicate that anephric sheep fetuses are able to maintain arterial blood pressures which are within the range of pressures found in normal sheep fetuses. These results support the hypothesis presented in the introduction that the kidneys of the fetus are not necessary for blood pressure control in the fetus because they are not in a position to regulate arterial blood pressure through changes in the extracellular volume of the fetus. The results also indicate that hormones elaborated by the fetal kidneys, such as renin, are not crucial for the regulation of arterial blood pressure by the fetus.

In the introduction it was stated that long term arterial blood pressure regulation by the postnatal mammal is mediated through the regulation of extracellular fluid volume and thus of blood volume. It was also stated that the placenta is probably the most important organ for regulation of extracellular fluid volume of the fetus. Thus it was implied that long term regulation of fetal arterial blood pressure was mediated by mechanisms governing water and solute exchange between maternal and fetal capillaries in the placenta. The fact that sheep fetuses maintain normal blood pressure after bilateral nephrectomy is supporting evidence for the hypothesis that the placenta is the site for extracellular fluid volume and arterial blood pressure regulation in the fetus. However, this hypothesized mechanism for long term blood pressure regulation by the fetus should not be accepted without examining the dynamics of water and solute exchange across the placental barrier.

Barcroft (1946) was one of the first to comment on the importance

of water acquisition by the fetus. Since approximately 70% of the fetal lamb is water, as it grows at the rate of approximately 40 g/kg/day, it needs to accumulate 28 g of water per kilogram per day or 1.56 moles of water per kilogram per day. By comparison, oxygen uptake by the fetus is approximately 10 ml/kg/minute (Clapp, 1978) or about 0.64 moles O_2 per kilogram per day. Fetal plasma is normally hypotonic to maternal plasma (Meschia, Battalia, and Barron, 1957; Armentrout, et al.). Never the less the net movement of water must be from the mother to the fetus, in order to support an ever growing fetus with an ever expanding volume of water. This movement of water must occur as the result of the action of hydrostatic and osmotic forces, so it is logical to assume that a change in the hydrostatic pressures in the fetal capillaries would act to change the balance of forces tending to move water from the mother to the fetus.

The osmotic reflection coefficients for Na^+ and Cl^- are approximately 0.8 for the placental exchange barrier of the sheep (Thornburg, Binder, and Faber, 1979a) and Na^+ and Cl^- are the most abundant solutes present in sheep plasma. Thus there cannot be a significant movement of water across the placenta of the sheep under the influence of hydrostatic pressures without the creation of a large opposing osmotic pressure gradient due to changes in the transplacental concentration gradients for Na^+ and Cl^-. (It can be predicted from van't Hoff's law, $\pi = \Delta cRT$, that a 1 mM concentration difference of a completely impermeant solute would create an osmotic pressure of approximately 19 mm Hg across the placental exchange barrier at 39°C). Thus it appears that the major factor which limits the rate of water transfer across the placenta

of the sheep is the rate at which electrolytes, particularly Na^+ and Cl^- are transferred (Conrad and Faber, 1977).

If the passive transfer of various inert solutes limits the rate at which the fetus can acquire water, then it would be necessary for placental diffusion permeabilities for these solutes to increase as the size of the fetus increased simply because a larger fetus would require a larger influx of water and solutes to maintain growth. The size of the placenta does not increase after the 95th day of gestation in the sheep (Barcroft, 1946), but the permeability of the sheep placenta to urea (Meschia, Breathnach, Cotter, Hellegers, and Barron, 1963; Kulhanek, Meschia, Makowski, and Battaglia, 1974), Na^+ (Boyd, Stacey, Ward, and Weedon, 1978; Weedon, Stacey, Ward, and Boyd, 1978), and Na^+ and Cl^- (Thornburg, et al., 1979a) does increase linearly with increasing fetal weight. Thus it appears that some mechanism exists to adjust the functional permeability of the sheep placenta to provide for changing requirements for water and solute acquisition by the fetal lamb. In view of the importance of electrolyte acquisition for the acquisition of water by the fetus, it would be of interest to know if the observed changes in Na^+ and Cl^- concentrations in maternal and fetal plasmas and of placental permeability surface area products for $^{22}Na^+$ and $^{36}Cl^-$ offer information about the regulation of extracellular fluid in the fetal lamb.

Validity of Measurements of Volumes of Distribution and Placental Diffusion Permeabilities for $^{22}Na^+$ and $^{36}Cl^-$

Volumes of distribution for $^{22}Na^+$ and $^{36}Cl^-$ were calculated from the ratio of a known amount of isotope injected into the fetus and extra-

polated concentration of the isotope in fetal plasma at the time of injection. Extrapolation of the linear part of the semilogarithmic curve of fetal plasma concentration as a function of time after injection (Figure 6) is based on the assumption that the rate of loss of isotope from the fetus to the mother during the distribution phase occurring during the first six hours after injection is proportional to a fetal plasma concentration predicted from extrapolation of the log linear portion of the fetal plasma concentration curve during the elimination phase after six hours. However, an exponential disappearance rate suggests that clearance of the tracer from fetal plasma depends upon the concentration in fetal plasma. Therefore, actual loss of tracer to the mother during the distribution phase would be expected to be somewhat greater than assumed loss of tracer to the mother (Lawson, 1962). This is because actual fetal concentrations were higher than the extrapolated concentrations during the first six hours after injection (Figure 6). Although it is unlikely that this error would be more severe in anephric fetuses than in normal fetuses, if the neglected loss of tracer from fetal plasma was greater for nephrectomized fetuses than for normal fetuses, it would be possible for the calculated volume of distribution of isotope in the conceptus to be greater for anephric fetuses than for normal fetuses. Since our calculation of placental permeability surface area product (equation 2) utilizes the volume of distribution of the isotope to calculate the amount of isotope present in the conceptus at a given time, the assumption of a value for the volume of distribution of the isotope in the conceptus which is greater than the true value for volume of distribution would lead to the

calculation of a permeability surface area product which would also be greater than the true value.

During the course of the experiments it became apparent that calculated volumes of distribution and permeability surface area products for $^{22}Na^{+}$ and $^{36}Cl^{-}$ were higher in anephric fetuses than in control fetuses. Therefore, we decided to kill one of the anephric fetuses immediately after taking maternal and fetal blood samples 20 hours after injection of $^{22}Na^{+}$ into the fetus. The fetus, membranes, and extrafetal fluids were dissolved in NaOH to determine the total amount of radioisotope in the conceptus. The actual volume of distribution of the isotope in the conceptus was taken as the ratio of the total radioisotope content of the conceptus at the time of autopsy and the concentration of the isotope in the terminal fetal plasma sample. This directly determined volume of distribution was 2,583 ml versus 2,242 ml calculated from the ratio of the amount of isotope injected and the extrapolated concentration at the time of injection. This represents an error of 15% in our calculated volume of distribution. The mean directly measured volume of distribution of $^{22}Na^{+}$ in five control fetuses was 10% greater than the mean calculated volume of distribution (Thornburg, et al., 1979a). Thus it appears that the mean volume of distribution of $^{22}Na^{+}$ expressed per kilogram fetal weight for anephric fetuses is indeed twice as high as the mean for control fetuses.

The calculated permeability surface area products for diffusion of $^{22}Na^{+}$ and $^{36}Cl^{-}$ across the placenta of the sheep are subject to errors made in the calculated volumes of distribution and to errors made in the measurements of the concentrations of radioisotopes in maternal

and fetal plasma. The percentage errors made in calculations of permeability surface area products for anephric fetuses should be approximately the same as the percentage errors made in the calculations of these values for control fetuses. Therefore, the differences in permeability surface area products for $^{22}Na^{+}$ and $^{36}Cl^{-}$ between control and anephric fetuses are probably real.

The movement of ions across membranes are governed by forces generated by concentration differences and electrical potential differences across the membrane and by the bulk flow of solution through the membrane (Ussing, 1949). The measurement of a permeability coefficient takes into account only the flux of an ion under the influence of a concentration gradient (Ussing, 1952). In cases where the permeability of a substance is estimated by means of a radioactive tracer the flow of tracer may be influenced by the flow of the abundant species (Ussing, 1952; Essig and Kaplan, 1979) in which case the tracer permeability will differ by a small amount from the permeability coefficient for the abundant species of ion, but generally these errors are within limits of measurement for the permeability coefficient (Curran, Taylor, and Solomon, 1967; Kotyk and Janáček, 1974). In the sheep placenta the movement of Na^{+} and Cl^{-} is governed principally by diffusion, since the ultrafiltration coefficients for Na^{+} and Cl^{-} are approximately 0.8 (Thornburg, et al., 1979a). The effect of bulk flow of the isotopically abundant species from the mother to the fetus would be expected to decrease the permeability coefficient determined from measurement of tracer flux from the fetus to the mother, as in this study. However, this effect appears to be negigible because of the equality (within the limits of measurement)

of bidirectional Na^+ fluxes across the sheep placenta measured with $^{22}Na^+$ and $^{24}Na^+$ (Weedon, Stacey, Ward, and Boyd, 1979). An electrical potential difference of approximately 50 mV has been measured between the pregnant ewe and fetal lamb (Widdas, 1961; Mellor, 1970; Weedon, et al., 1979), but the transplacental part of the total electrical potential difference is only on the order of 1 mV (Thornburg, Binder, and Faber, 1979b). It can be calculated from the Nernst equation that an electrical potential difference of this magnitude would exert a force equivalent to that associated with a ratio of ion concentrations in maternal and fetal plasmas of approximately 1.05/1, whereas the concentration ratios of isotopes in fetal and maternal plasmas were on the order of 10/1 to 100/1 between 6 and 20 hours after the injection of isotope into the fetus. The effects of bulk flow of isotopically abundant species and a small transplacental electrical potential difference were approximately the same for both control and anephric fetuses and in any case these effects are probably well within the limits of the accuracy of the measurement.

The fact that we have observed differences in permeability surface area products for $^{22}Na^+$ and $^{36}Cl^-$ between anephric and control fetuses indicates that there probably are differences between these two groups of fetuses in the membrane characteristics of the placental exchange barrier that govern the transfer of water and solutes between mother and fetus. However, we cannot make a statement about the fluxes of these ions or the movement of water across the placenta based on data obtained in this study. The total flux of an ion is equal to the sum of the diffusional flux, the flux occurring with bulk flow of solution

through the membrane, and the flux occuring under the influence of a difference in electrical potential across the membrane. We can calculate values for the diffusional flux on an ion from the calculated permeability surface area product and transplacental concentration difference, but we cannot calculate the other fluxes without knowing the flow of water, the ultrafiltration reflection coefficient for the ion, and without knowing the transplacental difference in electrical potential. It would be hazardous to assume that these are the same for anephric fetuses and normal fetuses. We cannot calculate the transplacental flow of water without knowing the hydraulic conductivity of the membrane and without knowing the hydrostatic and osmotic pressure differences (Andreoli and Schaffer, 1976).

Therefore, the fact that we found changes in placental permeability surface area products for $^{22}Na^{+}$ and $^{36}Cl^{-}$ and changes in transplacental concentration gradients for these ions after bilateral fetal nephrectomy indicates that there probably has been a change in the characteristics of the placental membrane between the ewe and the anephric fetus, and that there probably is some difference in the way in which anephric fetuses acquire solutes and water. However, the overall acquisition of water by anephric fetuses appears to be normal as evidenced by a normal rate of growth and a normal intravascular volume. Differences between normal and anephric fetuses in volumes of distribution and placental permeability surface area products for $^{22}Na^{+}$ and $^{36}Cl^{-}$ were completely unexpected when the experiments were designed, and the relevance of these differences to an understanding of blood pressure control in normal and anephric fetuses is not immediately apparent.

Other Mechansisms For Blood Pressure Regulation in the Fetal Lamb

Less is known about the regulation of fetal arterial blood pressure than is known about regulation of blood pressure of the postnatal mammal. Most investigators who have considered the question of fetal blood pressure regulation have examined only responses made by the fetus to acute alterations in blood pressure. As discussed in the introduction, these immediate responses represent the effects of "proportional" control systems. These mechanisms are not considered capable of regulating arterial blood pressure over the long term in postnatal mammals and there is no reason to assume that these mechanisms could serve as long term regulators of arterial blood pressure in the fetus before birth. Even so, an understanding of proportional control mechanisms for blood pressure control in the fetus may contribute to an understanding of overall regulation of arterial blood pressure in the fetus.

The most frequently investigated mechanisms for control of arterial blood pressure of the fetus are the autonomic nervous system, the renin-angiotensin system, and the secretion of vasopressin. These will be discussed below with regard to the possible roles of these systems in the overall regulation of fetal arterial blood pressure.

The Role of the Autonomic Nervous System in Regulation of Fetal Arterial Blood Pressure

The ability of the autonomic nervous system to influence the blood pressure of the fetus depends upon the growth of nerves, their ability to synthesize and release active neurotransmitters, the development of receptor sites, the maturation of the reflex center and the responsiveness

of effector organs. The processes involved in the development of the various facets of autonomic nervous system control of fetal cardiovascular functions are not known in detail. Results obtained by different investigators depend not only upon the gestational ages of fetuses but also upon the use of "acute" and "chronic" preparations. Fetal responsiveness to a pharmacologic agent used to test the autonomic nervous system depends not only upon the dosage of the drug employed but also upon the site of injection or infusion of the drug. No attempt will be made to discuss differences which depend on these factors. Instead this discussion will focus on general findings which may be applicable to the physiologic role of the autonomic nervous system in blood pressure regulation by the fetus.

Barcroft and Barron (Barcroft, 1946) showed that stimulation of the distal end of a severed vagus nerve was able to produce slowing of the heart by the 77th day of gestation, and that stimulation of the proximal end of the cut vagus was able to produce cardiac slowing by the 88th day. Dawes (1968) found that vagal stimulation produced barely perceptible slowing of the heart at 60 days and that the magnitude of cardiac slowing increased with increasing age of the fetus. Assali, Brinkman, Woods, Dandavino, and Nuwayhid (1977, 1978) also found that vagal stimulation produced bradycardia and that systemic arterial pressure and blood flows in the major vessels were relatively unaffected by the bradycardia because of an increase in stroke volume. They also found that bilateral cervical vagotomy did not produce a significant change in either the resting heart rate or the systemic pressure of the fetus (Assali, et al., 1978). Vagal stimulation is capable of producing a decrease in myocardial contractility

which can be seen as a decrease in stroke volume when heart rate is held constant by right atrial pacing during vagal stimulation (Rudolph and Heymann, 1978).

Intravenous administration of acetylcholine produces a slowing of heart rate that is similar to what is seen during vagal stimulation (Vappavouri, Shinebourne, Williams, Heymann, and Rudolph, 1973; Assali, et al., 1977, 1978). Large doses of acetylcholine produced a decrease in arterial blood pressure associated with bradycardia (Assali, Bekey, Morrison, 1968; Vappavouri, et al., 1973; Assali, et al, 1978; Berman, Goodlin, Heymann, and Rudolph, 1978). Intravenous administration of atropine can abolish the slowing of heart rate seen with vagal stimulation (Rudolph and Heymann, 1978) or with acetylcholine (Assali, et al., 1978). Atropine infusion will produce an increase in the resting heart rate of the sheep fetus even in fetuses of less than 100 days (Vapaavouri, et al., 1973; Rudolph and Heymann, 1978). The magnitude of these increases in heart rate increases with the gestational age of the fetus (Assali, et al., 1977 and 1978).

Barcroft (1946) also noted that infusion of epinephrine into the sheep fetus resulted in an increase in arterial blood pressure followed by bradycardia. If the vagus had been sectioned then infusion of epinephrine resulted in tachycardia. Similar results have been obtained by Assali, et al. (1968, 1977, 1978); Berman, et al. (1978), and Harris and van Petten (1979). Most investigators have found that the fetus responds to an infusion of norepinephrine in a similar fashion although the magnitude of the increase in arterial pressure is greater for norepinephrine than for epinephrine (Harris and van Petten, 1979). The

fetus has a decreased responsiveness to norepinephrine infusion when compared to the effects seen in the neonatal lamb or adult (Assali, Holm, and Shegal, 1962; Assali, et al., 1968, 1977, 1978; Berman, et al., 1978; Chez, Eherenkranz, Oakes, Walker, Hamilton, Brennan, and McLaughlin, 1978). The fetus is also capable of responding to stress with the release of both norepinephrine and epinephrine from the adrenal medulla (Comline, Silver, and Silver, 1965).

Alpha adrenergic stimulation with methoxamine leads to an increase in fetal arterial blood pressure and a decrease in heart rate (Barrett, Heymann, and Rudolph, 1972; Chez, et al., 1978; Rudolph and Heymann, 1978). Phenoxybenzamine infusion blocks the effects of methoxamine (Barrett, et al., 1972). When phenoxybenzmine is infused alone it generally results in a decreased arterial blood pressure (Rankin and Phernetton, 1978) which may be followed by a later rise in heart rate (Assali, et al., 1977, 1978). Chez, et al. (1978) found that phenoxybenzamine had little effect on fetal arterial blood pressure but that it caused a slight increase in heart rate. Vapaavouri, et al. (1973) found that hypotension produced by phenoxybenzamine could persist for one or two days. Phentolamine produces a blood pressure drop similar in magnitude to that produced by phenoxybenzamine (Vapaavouri, et al., 1973; Rudolph and Heymann, 1978) but the duration of the effect is not as long.

Isoproteronol infusion can lead to an increase in fetal heart rate by the 60th day of gestation (Barrett, et al., 1972; Rudolph and Heymann, 1978). The tachycardia produced by isoproteronol generally results in little change in arterial blood pressure (Assali, et al., 1977, 1978;

Chez, et al., 1978). Administration of propranolol results in a decrease in fetal heart rate (Rudolph and Heymann, 1978) especially after the 120th day of gestation (Vapaavouri, et al., 1973). Assali, et al. (1977, 1978) reported that propranolol produces a greater percentage change in fetal heart rate than atropine. Propranolol administration does not appear to change arterial pressure of the fetus (Chez, et al., 1978).

Ganglionic blockade with hexamethonium has been shown to decrease both heart rate and blood pressure (Dawes, 1968). Ganglionic blockade with trimethapen generally does not produce a significant effect in fetuses of approximately 100 days, but a decrease in blood pressure following trimethapen has been noted in near term fetuses (Assali, et al., 1978).

Baroreceptor reflexes have been elicited in sheep fetuses in response to a number of stimuli. In general the magnitude of the response elicited increases with increasing age of the fetus. The earliest demonstration of baroreceptor reflexes in the fetal lamb was by Barcroft and Barron (Barcroft, 1946). They observed that the slowing of the heart in response to a pressor dose of epinephrine could be abolished by sectioning or blocking the vagal nerves. Shinebourne, Williams, Heymann, and Rudolph (1972) produced slowing of the fetal heart after inflating a balloon in the lower aorta to increase the arterial pressure in the carotid arteries and upper aorta. They found that slowing of the heart could be abolished by cutting the sinus nerves and by stripping the aortic arch. Dawes (1968) has implicated baroreceptor mechanisms in the acceleration of fetal heart rate after bilateral

occlusion of the carotid arteries. Faber, Green, and Thornburg (1974) found that the open loop feedback gain for the response of baroreceptors to a decrease in arterial blood pressure following fetal hemorrhage was not significantly different from zero and that there is no significant difference in responses by fetuses who had received hexamethonium versus those who had not received the ganglionic blocking agent. Thus it appears that baroreceptors do not exert much control even over fluctuations in blood pressure of less than a few minutes duration in the fetal lamb.

Changes in fetal heart rate and blood pressure following hypoxia and acidemia have been attributed to chemoreceptor reflexes. Dawes (1969) has shown that increased blood pressure, tachycardia, and vasoconstriction of the hindlimbs of the fetus following hypoxia can be blocked by section of the vagal nerves. Goodlin and Rudolph (Rudolph and Heymann, 1978) have found that stimulation of either carotid or aortic chemoreceptors with sodium cyanide can produce bradycardia and changes in fetal arterial blood pressure. However, Longo, Wyatt, Hewitt and Gilbert (1978) have concluded that the fact that fetal hypoxia produces no significant change in fetal cardiac output and only a moderate increase in flow to the brain and myocardium indicates that the chemoreceptors are poorly developed in the fetus and that they function with very low gain amplification.

The examples given above indicate that the sheep fetus responds to classic autonomic agents and manipulations, depending on gestational age, in a way that is qualitatively similar to adult sheep. However, the fact that normal blood pressure is maintained even in the relatively immature fetus where autonomic responses are diminished indicates that the autonomic nervous system plays at most a minor role in the maintainence

of normal blood pressure by the sheep fetus, and in any case the effects of the autonomic nervous system are subject to the limitations that apply to all proportional control devices.

The Renin Angiotensin System and Fetal Arterial Blood Pressure

Angiotensin II acts directly to cause vasoconstriction of the systemic vasculature of the sheep fetus (Assali, et al., 1962; Rudolph, 1977; Berman, et al., 1978). Basal levels of renin, as measured by plasma renin activity, appear to be high in fetal lambs as compared to adult sheep (Table 3), but fetal plasma concentrations of angiotensin II as measured by radioimmunoassay do not appear to be higher than those of adult sheep (Broughton Pipkin, et al., 1974a). The renin angiotensin system can be stimulated by hemorrhage (Broughton Pipkin, et al., 1974a; Smith, et al., 1974), aortic constriction (Smith, et al., 1974), bilateral carotid occlusion (Broughton Pipkin, Kirkpatrick, Lumbers, and Mott, 1974) and by the infusion of furosemide into the fetus (Trimper and Lumbers, 1972; Fleishman, et al., 1975; Siegel and Fisher, 1980), but not by the infusion of epinephrine into the fetus (Broughton Pipkin, et al., 1974a). Although the sheep fetus responds to furosemide infusion with an increase in plasma renin activity to approximately five times basal activity, the plasma aldosterone concentration of the fetal lamb does not increase following furosemide infusion (Siegel and Fisher, 1980). The sheep fetus responds to (Sar^1) (Ala^8) angiotensin II, an agent which blocks vasoconstriction induced by angiotensin II, with a reduction in blood pressure (Iwamoto and Rudolph, 1977) or an equivocal reduction in blood pressure (Broughton Pipkin and O'Brien, 1977 and 1978) and to (Sar^1) (Ile^8) with no significant reduction in blood

pressure (Rankin and Phernetton, 1978). Thus it appears that the renin-angiotensin system of the fetal lamb can respond appropriately to various stimuli and that release of angiotensin II may cause vasoconstriction in the fetal lamb, but the role of the renin-angiotensin system in the maintainence of the normal blood pressure of the fetal lamb is not known.

The experiments reported in this thesis confirm previous reports that bilateral nephrectomy removes the major source of circulating renin in the fetal lamb (Pipkin, et al., 1974a and 1974b; Oakes, et al., 1977; Mott, 1978). This loss of circulating renin would not be expected to affect long term regulation of fetal arterial blood pressure unless renin was involved in regulating the fluid balance of the fetus as it is in the adult. The fetuses in these experiments maintained arterial blood pressures which were within normal limits. This result indicates that renin provided by the fetal kidneys is not essential for the long term regulation of arterial blood pressure in the fetal lamb.

Vasopressin and Fetal Arterial Blood Pressure

Arginine vasopressin has been demonstrated in the neurohypophysis and the plasma of the sheep fetus during the last half of gestation (Vizsolyi and Perks, 1969; Perks and Vizsolyi, 1973; Alexander, Bashore, Britton, and Forsling, 1974; Weitzman, Fisher, Robillard, Erenberg, Kennedy, and Smith, 1978; Rurak, 1978; Czernichow, 1979). Even though fetal plasma is slightly hypotonic to maternal plasma, levels of vasopressin in the plasmas of maternal and fetal sheep are similar after the 100th day of gestation (Weitzman, et al., 1978). There is a log linear relationship betwen plasma osmolality and basal arginine

vasopressin concentration in fetal lambs (Weitzman, et al., 1978) which is similar to the relationship between plasma osmolality and vasopressin concentration in adult sheep (Weitzman and Fisher, 1977). The similarity of concentrations in maternal and fetal plasmas is not due to transplacental passage of the hormone (Leake, Weitzman, Effros, Siegel, and Fisher, 1979), but rather to the similarity of maternal and fetal osmolalities. Fetal vasopressin concentrations increase after infusion of hypertonic saline into the fetus or its mother (Leake, et al., 1979) Hemorrhage was a potent stimulus for the release of arginine vasopressin in the fetal sheep (Alexander, Britton, Firsling, Nixon, and Ratcliffe, 1974; Robillard, Weitzman, Fisher, and Smith, 1979) with concentrations of arginine vasopressin increasing approximately 40 times over basal levels. This response appeared to be more closely related to a decrease in blood volume than a decrease in blood pressure. Two hours after correction of the fetal blood volume vasopressin concentrations were normal (Robillard, et al., 1979). Rurak (1978) demonstrated that hypoxemia is also a potent stimulus for the release of vasopressin from the neurohypophysis of the sheep fetus, and that the secretion of vasopressin in response to a hypoxic stimulus was less in fetuses with severed cerival vagal trunks. Rurak (1978) found that hypoxia produced an increase in blood pressure and a decrease in heart rate in sheep fetuses; the magnitude of the increase in heart rate was closely correlated with the concentration of vasopressin in the fetal plasma. He also found that vasopressin infused at rates which yielded plasma concentrations similar to those seen in fetuses during hypoxia resulted in similar increases in fetal arterial blood pressure and similar decreases in fetal heart rate.

The hypertensive effects of vasopressin were relatively greater in fetuses than in ewes (Rurak, 1978). Iwamoto, Rudolph, Keil, and Hyemann (1979) demonstrated similar effects after infusion of vasopressin into sheep fetuses. Furosemide when given as an intravenous infusion to newborn lambs increased plasma renin activity and the concentration of arginine vasopressin; administration of (Sar^1) (Ala^8) angiotensin II before furosemide infusion blunted the increase in vasopressin concentration (Seigel, Weitzman, and Fisher, 1978). Robillard, et al., (1979) speculated that vasopressin could act on the sheep placenta to increase isoosmotic water fluxes, from mother to fetus after fetal hemorrhage, but pointed out that vasopressin would also have to stimulate Na^+ fluxes at the same time. Thornburg, et al., (1979a) have speculated that vasopressin does increase the diffusional permeability of the placenta for Na^+. In view of the effects of vasopressin on a variety of epithelia (Andreoli and Schaffer, 1976) to change fluxes of water and Na^+ across the membrane barriers, it is interesting to speculate about a possible role for vasopressin in the regulation of fetal arterial blood pressure, especially in light of the apparent large increases in vasopressin secretion after decreases in fetal blood volume and the vasoconstrictor properties of physiologic amounts of vasopressin in the sheep fetus.

SUMMARY AND CONCLUSIONS

It was found that fetal lambs continued to grow after bilateral nephrectomy *in utero* and that they had arterial blood pressures within the range of blood pressures of intact control fetal lambs. It was also found that the plasma renin activities of anephric fetuses were substantially less than the plasma renin activities of intact fetuses.

Anephric fetal lambs had differences in volumes of distribution and placental permeability surface area products for $^{22}Na^{+}$ and $^{36}Cl^{-}$ as compared to intact control fetuses. There were also differences in maternal and fetal plasma concentrations of Na^{+} and Cl^{-} in ewes with anephric lambs compared to those with intact fetuses. These differences may reflect changes in extracellular fluid regulation by fetal lambs after nephrectomy.

The kidneys are essential for long term regulation of arterial blood pressure by the postnatal animal, but the results of these experiments indicate that the kidneys and hormones of renal origin are not required for blood pressure regulation by the sheep fetus before birth. However, the mechanism for long term regulation of arterial blood pressure in the fetus remains intriguingly elusive.

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