12-1984

The Role of Vasopressin in the Fetal Renal Response to Hypertonicity

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THE ROLE OF VASOPRESSIN IN THE FETAL RENAL RESPONSE TO HYPERTONICITY

by

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A Dissertation in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in Physiology

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December 1984

Each person whose signature appears below certifies that this dissertation in his/her opinion is adequate, in scope and quality, as a dissertation for the degree Doctor of Philosophy.

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Acknowledgments

The author wishes to express her appreciation to Drs. Robert A. Brace and Cecilia Y. Cheung for their guidance and support in these investigations.

I would also like to thank Debi Brittingham, Howard Wieland, Ruth McLarty, Kirk Turner, Susan Moore, and Donald Monroe for their technical assistance.

I am grateful to Dr. Maurice Manning for his kind gift of the vasopressin antagonist, and to Mr. Percy Herbert for the use of his chloridometer.

I especially want to thank my fellow graduate students Kirk Turner, Denise Cook, Ruth McLarty, and Peter Gold for their friendship and moral support throughout the time I spent in the research and writing of this dissertation.

Finally, I want to express my gratitude to the members of the Division of Perinatal Biology whose grant money supported this work.

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I. INTRODUCTION

In the adult, the kidneys and the thirst mechanism provide the primary means for regulation of the volume and osmolality of body fluids. Adjustment of urine volume and osmolality makes it possible to maintain a relatively constant internal environment despite wide variations in intake. Adult animals are able to concentrate their urine to osmolalities several times that of plasma in response to antidiuretic hormone (arginine vasopressin - "AVP") secreted by the posterior pituitary under conditions of stress, including hemorrhage, hypoxia, and hypertonicity.

In general, renal function in the fetus is characterized by a low GFR, high urine flow rate, and low urine osmolality relative to that of the adult. The human fetus produces urine at the rate of about 25 ml/hr at 40 weeks gestation (51,54), and this urine is considerably hypotonic to plasma (31). In acute preparations, urine flow averaged 5.0 ml/kg/hr in fetal monkeys and urine was either hypo- or isotonic to plasma (8). Due to the stress involved in acute studies, these values probably represent lower than normal flows and greater than normal osmolalities.

A number of laboratories have measured renal function in fetal sheep under control conditions and during a variety of perturbations. The results are summarized in Table 1, and references are included in the table. In the unstressed

Table 1. Measurements of Renal Function in Chronically Catheterized Fetal Sheep

Reference	G.A. (days)	Urine flow (ml/min) ± SE	Urine osmolality (mOsm/kg) ± SE	GFR (ml/min) ± SE	Perturbation
Gresham et al '72 (17)	117-131	0.09-0.31 /kg 0.14	65-160	1.05±0.05 /kg (in)	C
Robillard & Weitzman '80 (37)	106-142	0.045-0.61 /kg 0.26±0.04 0.04-0.90 0.25±0.05	103-489 197±23 243-512 321±16 *	0.47-1.51 /kg 1.08±0.113 (io) 0.65-2.35 1.36±0.13 *	C AVP inf 683 uU/min/kg for 2 hr
Wintour et al '82 (53)	86-120 121-term		154±45 118±35		
Gomez et al '84 (15)	102-120	0.72±0.12 0.15±0.04 *	125±9 325±22 *	2.48±0.38 (io) NS	C 31% hemorrhage
	132-144	0.86±0.16 0.15±0.03 *	150±26 413±13 *	4.00±0.47 NS	C 33% hemorrhage
Robillard et al '81 (38)	106-119 131-141	0.41±0.04 0.81±0.14	124±6 171±29	1.73±0.1 (io) 3.94±0.4	C
Schroder et al '84 (44)	110-135	0.174 /kg incr by 37% * 0.06 *	160±13 NS 295±21 *	1.22±0.12 /kg NS (cr) NS	C +50 ml blood -109 ml blood
Gomez & Robillard '84 (16)	103-119	0.66±0.15 0.15±0.04 *	111±10 325±22 *	2.57±0.41 2.13±1.0	C 31% hemorrhage
	132-144	1.07±0.16 0.15±0.04 *	142±29 414±13 *	4.78±0.47 3.21±0.65	C 33% hemorrhage

Table 1 continued.

Reference	G.A. (days)	Urine flow (ml/min)	Urine osmolality (mOsm/kg)	GFR (ml/min)	Perturbation
Rankin et al '72 (35)	122-134			1.05±0.052 /kg	C
Hurley et al '77 (19)	110-145	0.55±0.06		1.87±0.11 (in)	C
		NS		2.59±0.42 *	+50 ml saline to F over 30 min
		NS		NS	+ 1 L saline to M over 60 min
Lingwood et al '78 (25)	90-109	0.3±.083	159±45		
	130-term	"	99±36		
	96-141	variable changes	250-480 *		AVP inf 140 pmol/ hr for 4 hr
	126-140	1/3-1/4 of control	360-480 *		AVP inf 35 pmol/ hr for 2 hr
Daniel et al '84 (11)	117-136	0.27±.045 /kg	168±31		C
		0.17±.046 /kg *	325±31 *		Hypoxia, decrease of P_{O2} by 50%

G.A.=gestational age, * = significantly different from control, (in)=measured as inulin clearance, (io)=measured as iothalamate clearance, (cr)=measured as creatinine clearance, C=control values, NS=no significant difference from control, F=fetus, M=mother, incr=increased, inf=infusion

fetal lamb, urine flow has been found to average from 0.1 to 0.3 ml/min/kg, and on this per-weight basis remains relatively constant over the latter third of gestation. Urine osmolality under normal conditions usually ranges between 100 and 170 mOsm/kg and there is no clear age-related change over this part of gestation. Glomerular filtration rate (GFR) in the normal fetus averages around 1 ml/min/kg, and has been estimated using ^{14}C-inulin, ^{125}I-iothalamate, and endogenous creatinine clearances. Creatinine clearance tends to overestimate fetal GFR because some creatinine is secreted by the tubules (17).

When a chronically catheterized sheep fetus is hemorrhaged, no change is seen in GFR, but urine flow falls to 15-35% of control values and urine osmolality increases to equal or slightly exceed that of plasma. In contrast, during volume loading GFR and fetal urine flow may or may not increase, and no change is seen in urine osmolality. Hypoxia appears to be less of a stimulus for reduction in urine flow than is hemorrhage: when fetal P_{O2} is reduced by 50%, urine flow falls only to 63% of control. Urine osmolality becomes slightly hypertonic to plasma but may not reach values as high as those seen during hemorrhage.

Following infusion of AVP into the fetal lamb, variable changes are seen in fetal urine flow, probably as a result of interactions between the vasopressor and antidiuretic

actions of the hormone. GFR may increase. In all studies, at a variety of different doses, urine osmolality increased during AVP infusion, reaching values as high as 512 mOsm/kg.

AVP levels have also been measured in fetal and maternal sheep plasma by a number of different investigators. The results of these studies are summarized in Table 2. Two different sets of units have been used throughout the literature for expressing plasma AVP concentrations: pg/ml and uU/ml. The conversion factor is 2.5-2.8 pg/uU, depending on the purity of the hormone. As can be seen in Table 2, unstressed fetal and maternal plasma AVP levels are similar in the sheep, and the average values reported range from 0.5 to 3 uU/ml, or about 1 to 7 pg/ml. AVP is released by the fetal pituitary in response to hypertonicity, hypoxia, hemorrhage, and hypotension (10) so that fetal plasma levels during these perturbations are increased five- to over a hundred-fold, depending on the study. It also appears that the fetus may show an even greater AVP response to a given stimulus than does the mother.

Thus it is known that the fetal lamb normally puts out large volumes of urine that is markedly hypotonic to plasma, even though fetal AVP levels are similar to those in the adult animal. Fetal plasma AVP levels rise several- to many-fold in response to hypoxia and hemorrhage, while urine osmolality rises and urine output falls. However, under neither

Table 2. Measurements of AVP Levels in Chronically Catheterized Pregnant Sheep and Their Fetuses

Reference	G.A. (days)	Control Plasma AVP (pg/ml)	Peak Response Plasma AVP (pg/ml)	Perturbation
Wintour et al '82 (53)	<120	5.6±2.1 (F)	-	-
	121-term	5.5±2.1 (F)	-	-
Leake et al '79 (23)	112-139	1.8±0.5 (F) *	21.0±6.0 *	Hypertonic saline to M, increased osm by 20 mOsm/kg
		1.8±0.5 (M) *	6.5±4.5 *	
Siegel et al '80 (46)	102-114	2.55±0.90 (F) *	10.7±4.1 *	Hypertonic saline to F, increased osm by 20 mOsm/kg
	121-130	2.78±0.60 *	29.3±7.5 *	Hypertonic saline to F, increased osm by 20 mOsm/kg
Robillard et al '81 (38)	106-119	1.33±0.5 (F) *	25.3±5.5 *	Hypoxia, P_{O2} decreased from 22 to 12 mmHg
	131-141	1.63±0.3 *	53.8±9.3 *	Hypoxia, P_{O2} decreased from 22 to 13 mmHg
Iwamoto et al '79 (20)	123-140	4.83±1.17 (F)	-	-
		3.16±0.70 (M)	-	-
Daniel et al '84 (11)	117-136	1.33±0.53 (F)	46.4±4.71	Hypoxia, P_{O2} decreased by 50%
Drummond et al '80 (14)	96-124	1.4±0.3	31.7±14.7	Hemorrhage 20% of F blood volume at rate of 2 %/min

Table 2 continued.

Reference	G.A. (days)	Control Plasma AVP (pg/ml)	Peak Response Plasma AVP (pg/ml)	Perturbation
DeVane et al '82 (12)	130-140	3.5-5.0 (M) *	4.3-10.0 *	Acute asphyxia with pentobarbital
		4.0-5.5 (F) *	85-963 *	
		7.0±0.8 (M) *	NS	Hypoxia, P_{O2} decreased from 19.9 to 13.8 mmHg
		7.3±1.1 (F) *	NS	
		6.5±0.4 (F) *	25.5±6.5 *	Hypoxia, P_{O2} decreased from 20.5 to 12.9 mmHg
Weitzman et al '78 (52)	101-120	4.8±1.2 (F) *		
	121-141	1.9±0.5 (F) *		
		2.1±0.4 (M) *		
Rurak '79 (41)	124-142	<8 (bioassay) *	170±73 *	Hemorrhage 16-70% of F blood volume at 0.7-1.9 %/min
			438±105 *	Post-hemorrhage
Robillard & Weitzman '80 (37)	106-140	3.40±0.65 *	62.5±5.33 * (mean during infusion)	AVP infusion, 0.683±0.040 mU/min/kg

G.A.=gestational age, osm=plasma osmolality, NS=no significant difference from control, F=fetus, M=mother, *=converted from author's units of uU/ml using a conversion factor of 2.5 pg/uU

of these circumstances has the fetal urine osmolality been found to reach the levels which can be achieved by the adult kidney under similar conditions.

In the adult animal several factors must be present in order for a highly concentrated urine to be produced in response to an osmotic or other stimulus:

1) The osmoreceptors (or volume receptors, etc.) in the hypothalamus must be functional in detecting the stimulus and in signaling the release of AVP by the posterior pituitary.

2) The neurosecretory cells of the hypothalamus must be able to synthesize AVP, and stores of the hormone must be available for release by the posterior pituitary.

3) A cortico-papillary intrarenal solute gradient must be present.

4) The kidney must possess functional AVP receptors which when stimulated will bring about an increase in the permeability of the collecting tubules to water.

As mentioned above, studies have shown that both osmoreceptors (46,52) and volume receptors (39) are present and functional in the near-term sheep fetus, and that plasma AVP levels reached during hypertonic, hypovolemic, and hypoxic stresses are at least as large as those reached in the adult (15,23,48). Therefore it does not appear that the inability of the fetal kidney to concentrate urine is due to inadequacies in factors 1) or 2) above.

On the other hand, it has been shown that the intrarenal solute gradient is indeed normally very small in the fetus (47), and this could account for the lack of concentrating

ability. However, it is not known whether this small intrarenal gradient is due to an inherent inability of the fetal kidney to create a gradient, or to washout of a potential solute gradient by the large diuresis normally present in the fetus. In the adult rat, the normal intrarenal gradient virtually disappears within 5-6 hours after the onset of sustained diuresis (4). The original gradient is re-established after 4-5 hours of AVP infusion in these animals (18). Thus it is possible that the normal production of large volumes of hypotonic urine prevents the fetal kidney from generating an intrarenal solute gradient. Although the renal effects of infusion of AVP at sub-pressor doses have been studied in the fetal sheep (25,37), the maximum length of these infusions was only 2 hours. According to the adult data, this length of time is insufficient for establishment of an intrarenal gradient after washout caused by diuresis. Thus the possibility remains that the fetal kidney might be capable of developing an intrarenal solute gradient if AVP were infused for a longer period of time.

The fourth factor which is known to be necessary for elaboration of a concentrated urine is the presence of functional AVP receptors in the renal medulla. It is possible that the renal AVP receptors are relatively low in either affinity or number or both in the fetus, so that the circulating AVP, although present in adequate concentrations, is

relatively ineffective. It is also possible that coupling between the AVP receptor and activation of adenylate cyclase is not as tight in fetal kidney as it is in the adult. I am aware of only three developmental studies of either renal AVP receptor binding or adenylate cyclase activation. One study suggested that postnatally the appearance of specific vasopressin binding precedes the onset of adenylate cyclase responsiveness in the rat (34), and another study provided further evidence that the enzyme is hyporesponsive to vasopressin in newborn rats and rabbits (43). However, a third group of investigators found no age-related differences in responsiveness in either newborn rabbits or dogs (26). The reasons for these discrepancies are not clear. In any case, to my knowledge no such studies have been done on fetal tissue. Although there is in vivo evidence for maturation of renal AVP receptors over gestation (32,36,53), receptor binding characteristics have not been studied in the fetus. Thus, a comparative study of the renal AVP receptor binding characteristics and adenylate cyclase activation over gestation and postnatally will be an important step in elucidating the reasons for the lack of urine concentrating ability in the fetus, and in gaining a better overall understanding of the fetal kidney and other factors controlling fetal fluid balance.

Adult animals are able to concentrate their urine in re-

sponse to solute loading (hypertonicity), thus excreting the excess solute while conserving water (3). Renal responses to induced pure hypertonicity have not been studied in the fetus. After hypertonic NaCl injection into the sheep fetus, plasma osmolality increases but returns to normal within two hours (55). Fetal AVP levels also increase (46). When hypertonic NaCl is injected into the mother, the fetus becomes hypertonic and amniotic fluid osmolality gradually increases (56), suggesting that fetal urine osmolality may have also increased. It is not known to what extent the fetal kidney is involved in the regulation of fetal osmolality. Because of the normal fluctuations in maternal and fetal osmolalities due to eating and drinking, as well as the clinical interest in fluid administration to newborns and pregnant women, it is important to gain a better understanding of the fetal kidney and its role in the regulation of fetal osmolality and fluid volumes.

II. OBJECTIVES

The objectives of these studies were to measure the changes in fetal renal function in response to a step increase in plasma osmolality of 10-15 mOsm/kg with minimum changes in body fluid volumes, and to determine the role of AVP in this response. The latter goal was accomplished by administering an AVP antagonist to a second group of fetuses prior to inducing hypertonicity. In addition, a third group of fetuses received an AVP infusion before and during the hypertonic period so that the responses at high and sustained plasma AVP levels could be compared to normal responses. At the end of some experiments the fetal intrarenal osmolality gradient was to be measured.

The objectives of the *in vitro* studies were to determine the binding characteristics of the renal AVP receptors in the fetus, and to compare them at several stages of gestation as well as in the newborn and adult.

The questions which I attempted to answer are the following:

1) How does the fetal kidney respond to prolonged hypertonicity?

2) What is the role of AVP in this response?

3) What changes, if any, occur in the fetal intrarenal solute gradient during prolonged hypertonicity or AVP infusion?

4) Under conditions of hypertonicity and/or high AVP levels for a prolonged period, to what extent can the fetal kidney concentrate urine?

5) Are the affinity and capacity of renal AVP receptors different in the fetus and the adult?

The specific hypotheses to be tested were:

1) The inability of the fetal kidney to concentrate urine relative to that of the adult is due to either a) the relatively low number and/or affinity of renal AVP receptors, or b) the small intrarenal solute gradient.

2) Under conditions of prolonged hyperosmolality or AVP infusion, the fetal kidney may be able to increase the intrarenal solute gradient, and consequently, the osmolality of the urine.

III. METHODS

A. Whole Animal Studies

1. Animal Preparation: Thirteen pregnant ewes at approximately 125 days of gestation were used for these studies. Food was withheld for 24-48 hours prior to surgery. Animals were anesthetized with 500 mg sodium thiopental (Abbott) and maintained with 0.5-1.5% Halothane in a 2:3 mixture of nitrous oxide and oxygen via an endotracheal tube. Ewes received 1000 ml of 5% dextrose in lactated Ringer's intravenously during surgery.

The ewe was placed in a supine position, and catheters were put in a maternal tibial artery and femoral vein. The uterus was exposed through a midline abdominal incision and a pursestring suture was made in an area free of cotyledons. A fetal hindlimb was withdrawn through a small incision in the uterus, and the pursestring drawn around the leg to prevent leakage of amniotic fluid. Catheters were placed in the tibial artery and saphenous vein, and advanced into the descending aorta and inferior vena cava respectively. An amniotic fluid catheter was sewn to the fetal skin, and the hindlimb replaced. In a similar manner, the tibial artery and saphenous vein in the opposite fetal hindlimb were catheterized, and two additional amniotic fluid catheters were sutured to the skin. The first hindlimb was then re-exposed, and the caudal 1/4 of the fetal body trunk exteriorized by

pulling gently on the limbs. A midline incision approximately 1.5 cm in length was made in the fetal abdomen just anterior to the pubic symphysis. The fetal bladder was exposed and a pursestring suture was made in the bladder wall. A 15-gauge catheter with two polyvinyl rings glued approximately 2 cm from the tip was used. Several holes were cut in the wall of the catheter within 1.5 cm of the tip, and the catheter was inserted 2 cm into the fetal bladder via a puncture wound. The pursestring was drawn up tightly between the two rings and tied. The fetal body wall and skin were closed separately, and the fetus replaced in the uterus. The membranes were tied closed, and the uterine incision closed by inverting the cut edges and tightening the pursestring. Catheters were brought out through the midline incision in the body wall and tunneled subcutaneously to a nylon pouch sewn to the ewe's left flank.

Another midline incision was made through the udder, and the maternal bladder was exposed. Using a 15-gauge catheter with two sets of polyvinyl rings glued 7 and 8.5 cm from the tip, the maternal bladder was catheterized in a manner similar to that used for the fetal bladder. The pursestring was drawn up either between or just above the rings nearest the tip, and the catheter was then further secured to the bladder by three sets of ties knotted between the more distal set of rings. This catheter was also tunneled subcu-

taneously to the nylon pouch.

2. Post-operative Procedures: Fetal and maternal blood samples were taken daily during the post-operative period (usually around 8:00 am) for measurement of blood gases, osmolalities, hematocrits, and plasma protein concentrations. Amniotic fluid and fetal and maternal urine samples were taken daily for determination of osmolalities and sodium and potassium concentrations.

Animals were maintained on daily antibiotics (ewes: 400,000 units procaine penicillin G and dihydrostreptomycin sulfate intramuscularly (Combiotic, Pfizer), and fetuses: 500 mg ampicillin sodium (Polycillin-N, Bristol Laboratories) into the amniotic fluid) throughout the post-operative period. Catheters in blood vessels were flushed daily with sodium heparin (600-1000 units/ml) to keep them patent.

Experiments were begun on the sixth post-operative day, except in one case where the experiment was done on the fifth post-operative day. In cases where more than one protocol was done on an animal, a two-day recovery period was allowed between experiments. Ewes were denied food and water during the experiment. The animals were brought into the recording laboratory at least two hours prior to the beginning of the control period, and remained standing in their carts throughout the experiment.

3. Experimental Measurements: During the control and experimental periods, mean pressures in a fetal artery and vein, and amniotic fluid, as well as fetal heart rate were recorded continuously on a Beckman R612 polygraph and simultaneously on disks with an on-line computer (Texas Instruments 990/10). Fetal arterial and venous pressures were corrected for amniotic fluid pressure. Fetal and maternal bladder catheters were drained into continuously weighed vials, and mean urine outputs were calculated over two-minute intervals from the change in weight of the vials, assuming a specific gravity of unity.

Fetal and maternal arterial blood samples were taken at the beginning and end of the experiment for determination of blood gases (Radiometer ABL2, Copenhagen, Denmark), which were measured at 37°C and corrected to body temperature (39°C in the mother and 39.5°C in the fetus). All blood samples were collected into plastic syringes containing a small amount of dry heparin; we verified that this amount did not change the osmolality of 0.5-1.0 ml samples detectably. Sample syringes were capped immediately after samples were taken. Amniotic fluid and urine were collected into non-heparinized syringes. A dead-space volume four times the volume of the catheter was withdrawn before each sample and replaced immediately after sampling. The total volume of fetal blood removed due to sampling did not exceed 25 ml over an eight-

hour period.

Whole blood samples were analyzed for osmolality (Advanced DigiMatic Osmometer, model 3DII, Advanced Instruments, Inc., Needham Heights, MA), and plasma sodium and potassium concentrations (Instrumentation Laboratory System 502, Instrumentation Laboratory, Inc., Lexington, MA). Hematocrits were run in triplicate; the average standard deviation of this triplicate reading was 0.1 hematocrit units (0.1%). Plasma proteins were determined with a hand-held refractometer (AO TS meter, American Optical Corp., Keene, NH). The remainder of the blood was centrifuged and the plasma frozen for later determination of plasma chloride concentrations (Buchler-Cotlove Chloridometer, Buchler Instruments, Fort Lee, NJ), and plasma urea concentrations (Urea Nitrogen Test Kit #640, Sigma Chemical Co., St. Louis, MO). At specified timepoints, 1.7 ml maternal and fetal blood samples were also taken and put immediately into iced tubes containing 170 ul of 0.3 M EDTA (pH 7.4), centrifuged at 4°C, and the plasma frozen at -80°C for later AVP assay (Immuno Nuclear Corporation, Stillwater, Minnesota).

Urine collecting vials were emptied 3 minutes prior to the blood sampling timepoints, and urine samples were then taken out of the vials 3 minutes after sampling blood, so that urine values represent 6-minute averages around the blood sample points. Maternal and fetal urine samples were

analyzed for osmolality, sodium, and potassium, and frozen for later determination of chloride and urea. All fetal urine not used for chemical measurements was injected into the amniotic space after the amniotic fluid sample was taken. Only osmolality was measured on the amniotic fluid samples.

4. Estimation of Fetal Glomerular Filtration Rate (GFR): Fetal GFR was estimated by the renal clearance of ^{14}C-inulin (New England Nuclear, Boston, MA),(35). 10 uCi of ^{14}C-inulin in 1 ml saline was injected into a fetal venous catheter about one hour prior to the beginning of the control period. 100 ul aliquots of fetal urine and plasma samples were counted for ^{14}C in 9 ml PCS fluor (Amersham Corp., Arlington Hts., IL) in a Packard Liquid Scintillation Counter. Counting efficiency was 93.4%. Fetal GFR was calculated as (U_{in}/P_{in}) x UF, where U_{in} = the urine concentration of inulin, P_{in} = the plasma concentration of inulin, and UF = the average urine flow for the time period over which the urine sample was collected.

5. Measurement of Blood Volume: Prior to the beginning of the control period, fetal blood volume was determined by a standard indicator dilution method using ^{99}Tc labeled autologous fetal red cells. Samples were counted for ^{99}Tc in a Packard Auto-Gamma Scintillation Spectrometer, and fetal blood volume was determined by extrapolating cpm/ml to time zero. Since ^{99}Tc has a short half-life (6 hours), counts

were corrected for decay occurring during the time the tubes were being counted.

Transient changes in fetal blood volume were calculated using the equation

$$BV = \frac{FRCM \times BV_o \times HCT_o}{HCT \times (.43 + .57 \times OSM/OSM_o)}$$

where

$$FRCM = \frac{RCV_o - \sum [\, V_s \times HCT \times (.43 + .57 \times OSM/OSM_o)]}{RCV_o}$$

BV = blood volume at times of individual samples

FRCM = fraction of original red cell mass remaining in vasculature

HCT = hematocrit of individual samples

OSM = osmolality of individual samples

RCV_o = initial total red cell volume = $HCT_o \times BV_o$

V_s = volume of individual samples

.57 = fraction of red cell volume which is osmotically active (24)

and the subscript o represents initial conditions. This equation has the advantage that red cells lost due to sampling are taken into account in calculating blood volume changes from hematocrit and osmolality data. No corrections were made for the volumes of injectates or infusates.

6. Experimental Protocols: Three different protocols were used, involving hypertonic injections into normal, AVP-infused, and antagonist-infused fetuses.

a. Hypertonic injection into normal fetuses:

During a one-hour control period, fetal and maternal arterial blood, urine, and amniotic fluid were sampled at 30-minute intervals. After the control period, 15 ml of sterile 9% NaCl was injected as a bolus into the fetal vein catheter, and 2 ml/kg body weight of 9% NaCl was infused as fast as possible (within two minutes) into the maternal vein. Immediately thereafter an infusion of 0.45-0.89 ml/min of 9% NaCl was begun into the maternal vein and continued throughout the experiment. Fetal and maternal arterial blood, fetal and maternal urine, and amniotic fluid were sampled at 5,15, 30,60,120,180, and 240 minutes following the injection. Fetal blood was sampled at -30,-5,5,15,60,120, and 240 minutes for measurement of plasma AVP.

The ewe was sacrificed immediately following the experiment, and the fetus was weighed. The fetal kidneys were removed and weighed, and rapidly frozen at -80°C for later dissection and determination of the intrarenal osmolality gradient.

b. Hypertonic injection into AVP-infused fetuses:

In six animals, an infusion of arginine vasopressin (1.2 ng/min in .025 ml/min saline) into a fetal vein was begun after a one hour control period and continued for six hours. Two hours after beginning the AVP infusion (reference time = 0), the hypertonic injections into mother and fetus were

performed as described above. Fetal and maternal arterial blood, fetal and maternal urine, and amniotic fluid were sampled at 30-minute intervals during the control and AVP infusion periods, and at 5,15,30,60, and 240 minutes after hypertonic injection during AVP infusion. Fetal and maternal blood was sampled for measurement of plasma AVP levels at 30 and 5 minutes before beginning the AVP infusion (time = -150 and -125), at 30 and 115 minutes after beginning the AVP infusion (time = -90 and -5), and at 15 and 180 minutes after the hypertonic injections (time = 15 and 180).

c. Hypertonic injection into AVP-blocked fetuses:

In five animals, after a one-hour control period, an AVP antagonist was given as a bolus (25 ug in 1 ml saline) followed by an infusion (0.57 ug/min in .025 ml/min saline) over six hours. Because the antagonist was expected to cross-react with AVP in the radioimmunoassay, blood samples for measurement of AVP were taken only during the control period (time = -150 and -125 minutes). In all other respects sampling times and hypertonic injection protocols were identical to those described for AVP-infused fetuses.

7. Rationale: The rationale behind choosing these three protocols was as follows. Hypertonic injections into normal animals were done first to determine the normal responses of the fetal kidney to hypertonicity. AVP levels were measured in these experiments in order to give insight into whether

changes in plasma AVP might be responsible for the changes in renal function. In a second group of fetuses, AVP infusions were done to determine the effects of AVP on the fetal kidney. The dose of AVP chosen was intended to have maximal antidiuretic and minimal vasopressor effects in the fetus. Changes which were seen both after hypertonic injection into normal fetuses and during AVP infusion alone were probably AVP-mediated responses. Hypertonic injections during AVP infusion were also done to determine the role of AVP in the response of the fetal kidney to hypertonicity; normal responses to hypertonic injection which were still seen during AVP infusion were probably not due to AVP, but to some other factor such as the hypertonicity itself. The AVP blocker alone was given to determine the role of AVP in maintenance of fetal renal function under normal conditions; any changes seen during infusion of the antagonist would suggest that AVP was normally involved in controlling these variables. Hypertonic injection during antagonist infusion was done to gain further insight into the role of AVP in the normal response of the fetal kidney to hypertonicity; with AVP blocked, any changes seen in fetal renal function were probably due to some mechanism other than AVP. If these same changes were seen during hypertonic injection into normal fetuses, it would suggest that they were also not AVP-mediated in the normal animal. Because the question

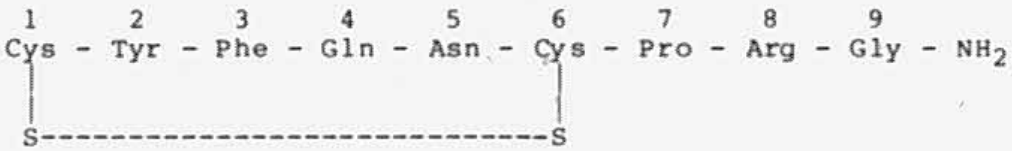
of the role of AVP in the fetal renal response to hypertonicity was approached from several angles, thus providing cross-checks on the conclusions drawn, the likelihood of coming to incorrect conclusions was minimized.

Vascular pressures were measured in all fetuses to determine if pressure changes might be responsible for non-AVP-mediated responses - the existence of pressure changes would only suggest but not prove that pressures might have been important in causing the renal responses. Maternal renal function was measured simultaneously in all animals to provide an index of normal responses of the adult kidney to similar conditions. Although the injection protocols were slightly different for fetus and mother (both received a bolus injection while only the ewe received a subsequent continuous infusion), the plasma levels of osmolality, Na^+, and Cl^- seen by the fetal and maternal kidneys were similar. The continuous infusion into the ewe was necessary to keep both maternal and fetal plasma levels elevated. Bolus injections were given to both mother and fetus in order to increase maternal and fetal plasma osmolalities simultaneously and therefore minimize transplacental fluid movement.

These experiments tested only fetal and maternal renal responses to hypertonic NaCl and the role of AVP in the fetal response. They did not test responses to some other hypertonic agent such as mannitol, nor did they determine the role

of AVP in the maternal response. NaCl was chosen as the hypertonic agent because it is an endogenous substance, because Na^+ and Cl^- are the major anions of plasma and the major contributors to plasma osmolality, and because Na^+ and Cl^- are likely to be elevated in plasma under naturally-occurring conditions of hypertonicity and dehydration.

8. AVP Antagonist: Recently a number of synthetic AVP analogs have become available which show varying degrees of agonistic and antagonistic activity to the pressor, antidiuretic, and behavioral effects of the naturally-occurring hormone (29,42). The structure of the parent compound (naturally-occurring AVP) is shown below.



Four changes in the molecule have been shown to enhance antidiuretic activity (29):

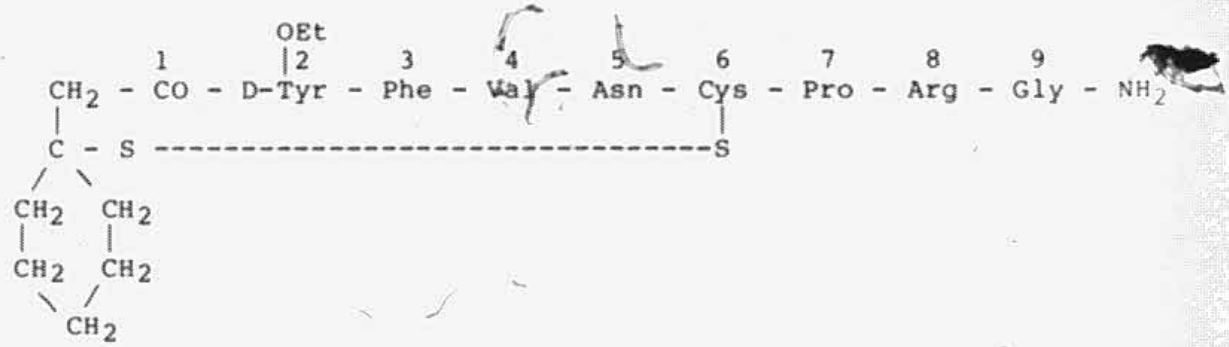
1) deamination at position #1
2) substitution of phenylalanine at position #2
3) substitution of a lipophilic amino acid at position #4
4) substitution of D-arginine at position #8.

On the other hand, in order to achieve antidiuretic antagonism a different set of structural modifications is required (28):

1) O-alkylation of tyrosine at position #2 with ethyl, methyl, isopropyl, or N-propyl groups, the ethyl group being most effective

2) substitution of a β,β-cyclopentamethylene group at position #1
3) substitution of a valine at position #4.

I chose to use [1(β-mercapto-β,β-cyclopentamethylene proprionic acid),2-(O-ethyl)D-tyrosine,4-valine] arginine vasopressin (shown below) because it has high anti-antidiuretic potency and shows minimal pressor activity (30). It has an "effective dose" (that dose which reduces the response seen from 2X units of agonist to the response with 1X units of agonist) of 1.1 ± 0.2 nmol/kg for anti-antidiuretic activity, and of 0.45 ± 0.11 nmol/kg for anti-vasopressor activity (30).



[1(β-mercapto-β,β-cyclopentamethylene proprionic acid), 2-(O-ethyl)D-tyrosine,4-valine] arginine vasopressin

9. Data Analysis: The data are expressed as the mean ± one standard deviation or one standard error as indicated. Transient changes are presented graphically as means ± SE at each sampling timepoint. Peak changes were also calculated as mean change from control or pre-injection value

± SE, and level of significance was evaluated using a paired t-test or analysis of variance.

B. Receptor Binding Studies

1. Membrane Fraction Preparation: A kidney tissue plasma membrane fraction was prepared according to a modification of the procedure of Rajerison, et al (34). Pregnant sheep of approximately 130 days gestation carrying twin fetuses were anesthetized with 30 ml pentobarbital sodium (Nembutal, Abbott Laboratories, North Chicago, IL), and the uterus exposed through a midline incision. The fetuses were delivered and the umbilical cords tied and cut. Fetal and maternal kidneys were quickly excised and immersed in ice cold buffered isotonic solution (5 mM Tris-HCl, 3 mM $MgCl_2$, 1 mM EDTA, and 250 mM sucrose, pH 7.4). On ice, the cortical and medullary regions regions were dissected out and weighed. The tissues were homogenized in approximately 40 ml isotonic solution, made up to 70 ml with the same solution, and filtered through glass wool. The homogenates were centrifuged twice at 100g for 15 minutes at 4°C, and the pellets discarded. The supernatants were centrifuged for 20 minutes at 1500g, and the resulting pellets dispersed in 70 ml hypotonic solution (5 mM Tris-HCl, 3 mM $MgCl_2$, and 1 mM EDTA, pH 7.4). The tubes were kept at room temperature for 10 minutes and then centrifuged at 1500g for 20 minutes. The pellets were dispersed in approximately 6 ml of hypotonic solution. In

preliminary studies it was found that the percent of total AVP binding which was specific (i.e. that displaced by a 100-fold excess of unlabeled hormone) was at most 30% after storing the preparation at -80°C. This percentage was doubled if the binding experiments were performed the same day as the preparation was made. Therefore in all subsequent studies binding experiments were performed immediately after preparing the membrane fraction, and only the data from these studies are included in the Results.

The protein concentration of the membrane preparations was determined by the method of Bradford (6).

2. AVP Binding Experiments: AVP receptor binding experiments were performed by incubating membrane protein (approximately 100 ug per tube) with varying concentrations of tritiated AVP in a final volume of 250 ul in the presence or absence of a 100-fold excess of unlabeled hormone. The incubation was done in a 30°C shaking water bath for 30 minutes. After incubation, 750 ul of ice cold buffer was added to each tube and it was vortexed. The contents of the tubes were aspirated with Pasteur pipettes, vacuum filtered on pre-soaked cellulose acetate filters (EH, 0.5 u pore size, Millipore Corporation, Bedford, MA), washed with 12 ml ice cold buffer, and placed in scintillation vials containing 9 ml scintillation cocktail (Econofluor, New England Nuclear, Boston, MA) and 300 ul Protosol (New England Nuclear). The vials were

counted for ^3H the following day in a Packard Liquid Scintillation Counter. Counting efficiency averaged 57%. Preliminary studies were performed to determine these optimum conditions for filtering and counting. Total binding was defined as the radioactivity bound in the absence of unlabeled hormone, non-specific binding as that bound in the presence of a 100-fold excess of unlabeled hormone, and specific binding as the difference between total and non-specific binding.

3. Data Analysis: Binding of ^3H-AVP to renal medullary tissue was expressed as cpm bound per 100 ug membrane protein. Scatchard analysis of the data was performed by plotting the ratio of bound to free hormone vs bound hormone. The best-fit lines were determined by the method of symmetry (5), and statistical significance evaluated by the same method. K_D, a measure of the affinity of the binding sites, was calculated as -1/slope, and B_{max}, a measure of the number of binding sites present, was taken as the x-intercept of the best-fit line through the points.

IV. RESULTS

A. Whole Animal Studies

1. Post-operative Data: The results of analyses of fetal and maternal blood and urine, and amniotic fluid for the post-operative period in each animal are shown in Figures 1-8. Fetal and maternal plasma osmolalities fell slightly but not significantly during the first six post-operative days. Fetal hematocrit also fell significantly for the first two to three days (Figure 2), but maternal hematocrit did not change. Fetal and maternal plasma protein concentrations also did not change during the post-operative period (Figure 3). Maternal blood pH was low the first day after surgery but did not change significantly after the second post-operative day (Figure 4). Fetal blood pH was 7.322 $\pm$ 0.005 on the first post-operative day and was not significantly different from this on any subsequent day but tended to parallel that of the mother. Figure 5 shows the relationship between fetal and maternal blood pH values during the post-operative period.

The mean osmolality of fetal urine was 161 mOsm/kg on the first post-operative day and did not change significantly during the next five days (Figure 6). However, urine osmolality in two animals increased to 309 and 340 mOsm/kg on the sixth day. Fetal urine Na^+ increased significantly during the post-operative period (Figure 7), however fetal urine K^+

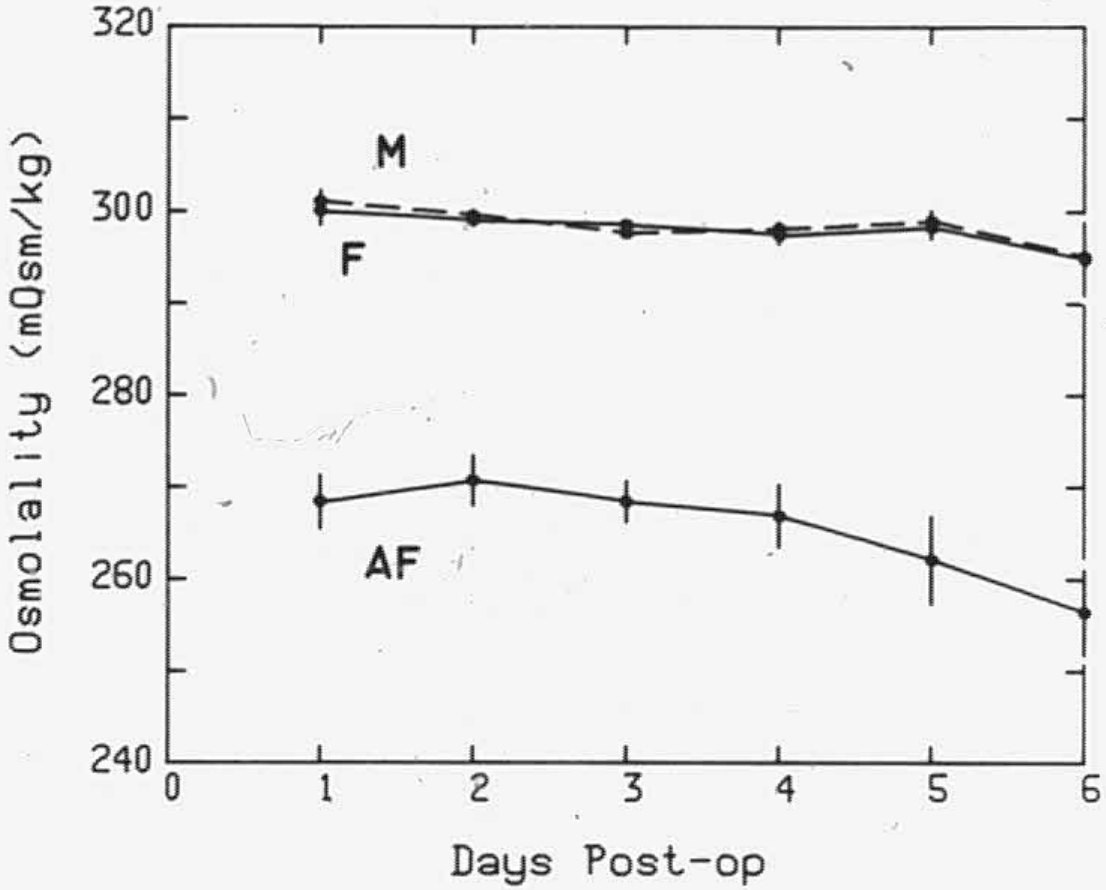


Figure 1. Post-operative changes in fetal (F) and maternal (M) plasma, and amniotic fluid (AF) osmolalities. Mean ± SE, n = 13.

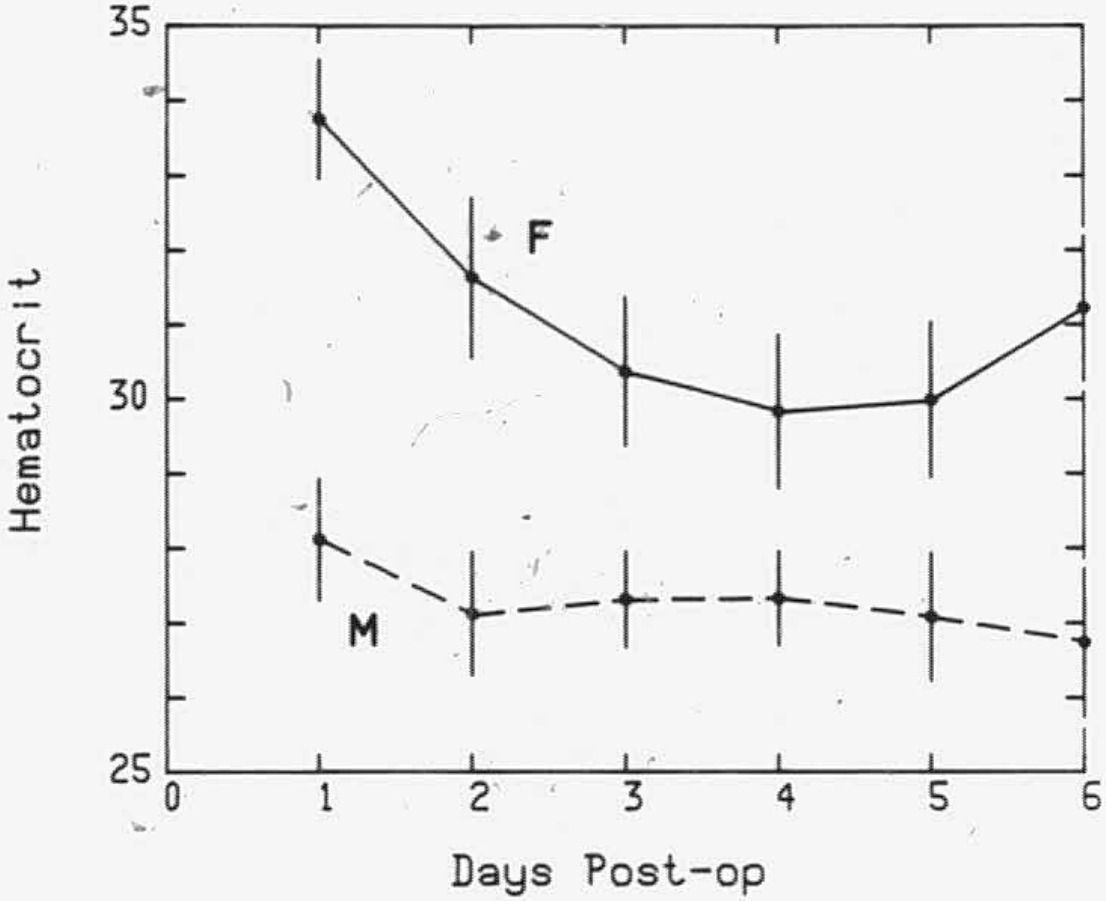


Figure 2. Post-operative changes in fetal (F) and maternal (M) hematocrits. Mean ± SE, n = 13.

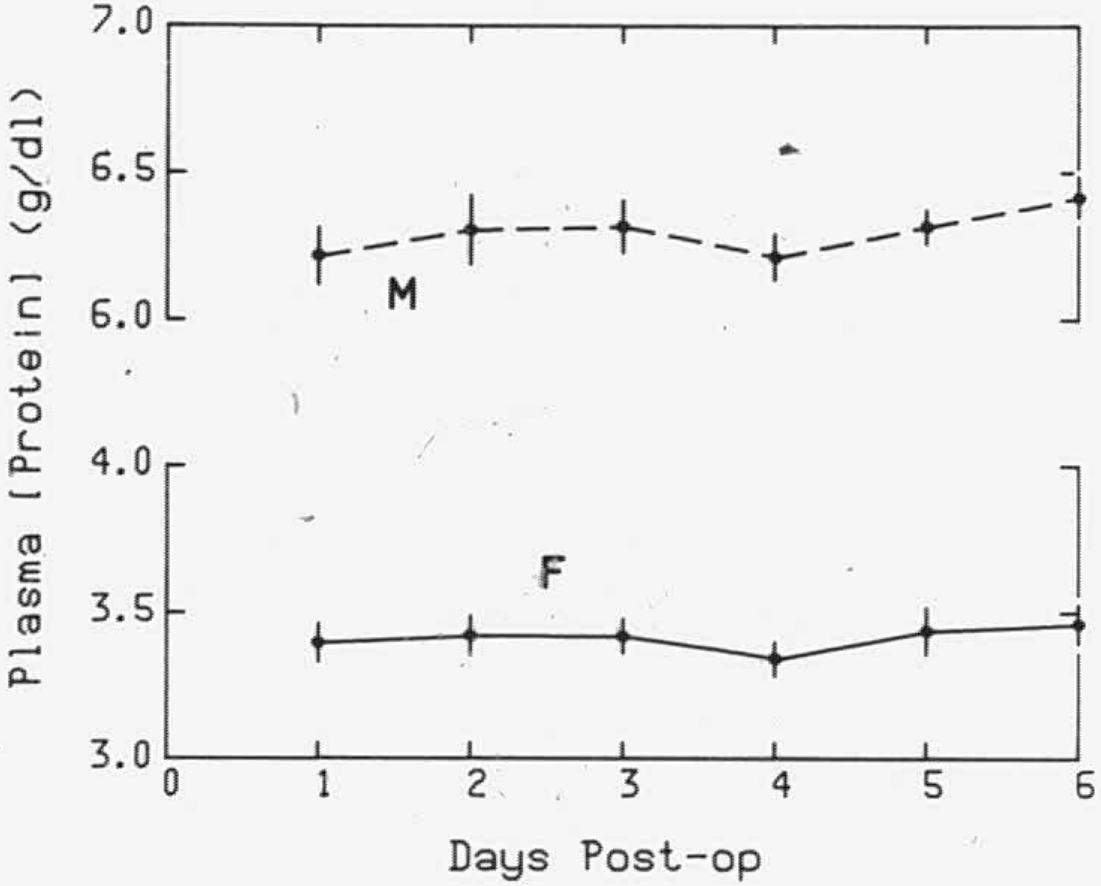


Figure 3. Post-operative changes in fetal (F) and maternal (M) plasma protein concentrations. Mean ± SE, n = 13.

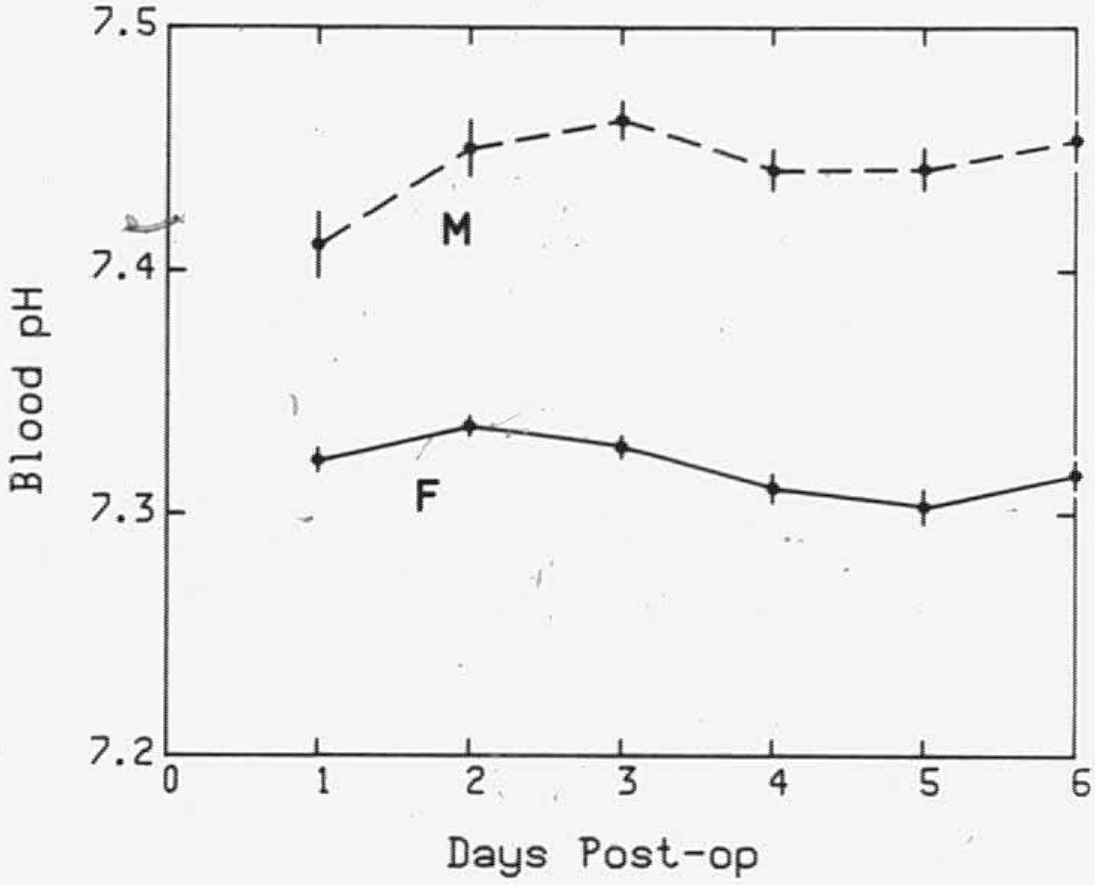


Figure 4. Post-operative changes in fetal (F) and maternal (M) blood pH. Mean ± SE, n = 13.

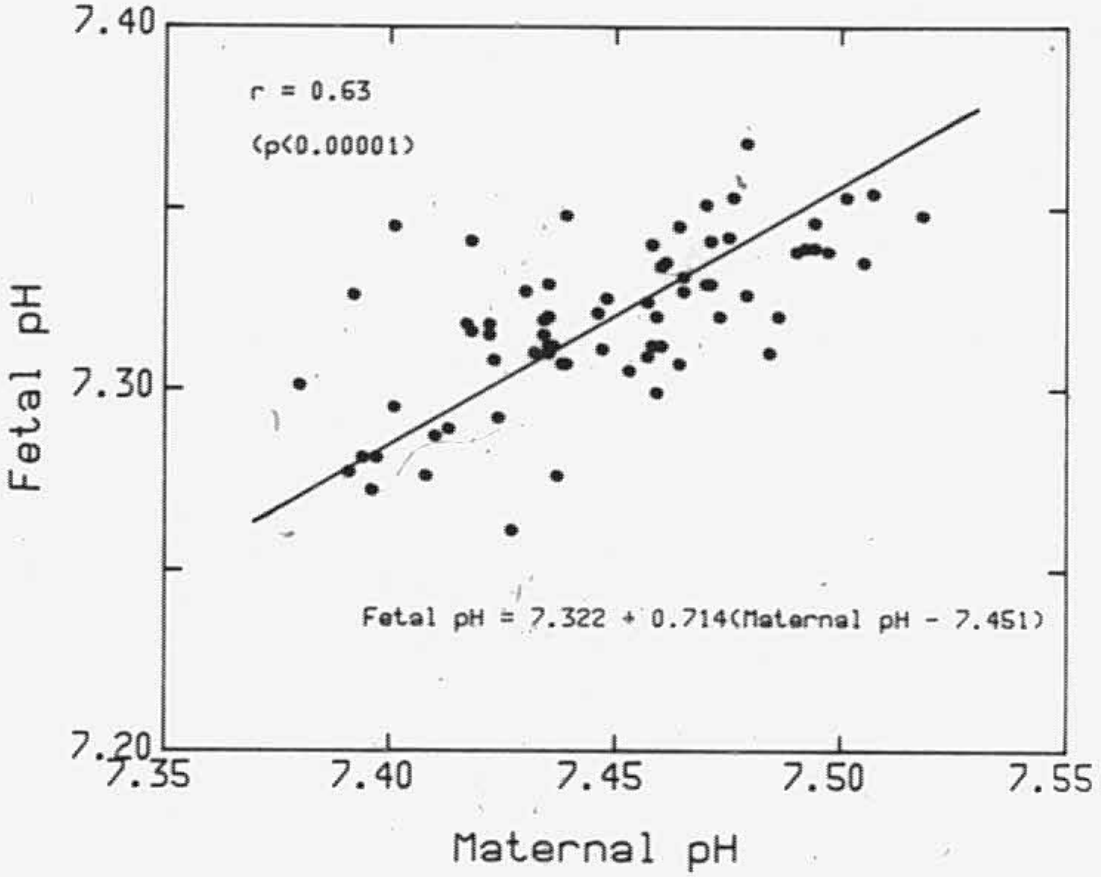


Figure 5. Relationship between fetal and maternal blood pH values in the first six days after surgery.

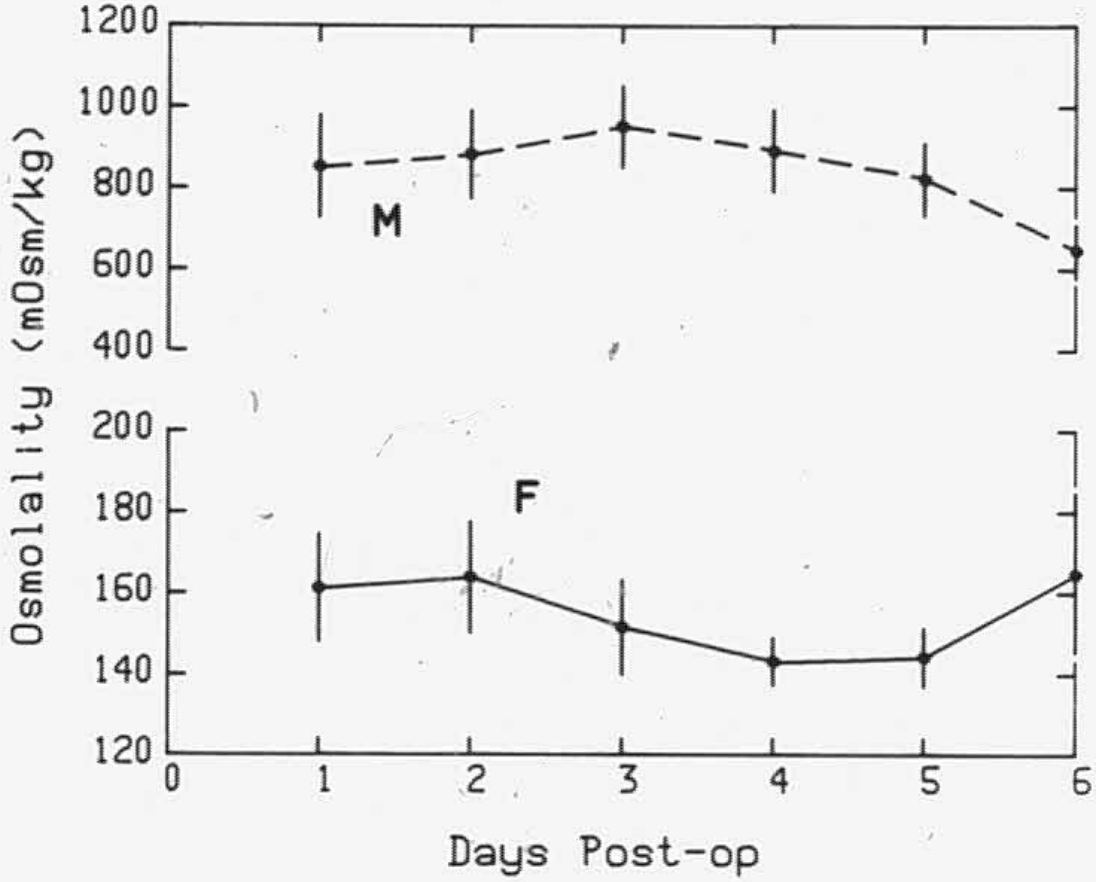


Figure 6. Post-operative changes in fetal (F) and maternal (M) urine osmolalities. Mean ± SE, n = 13.

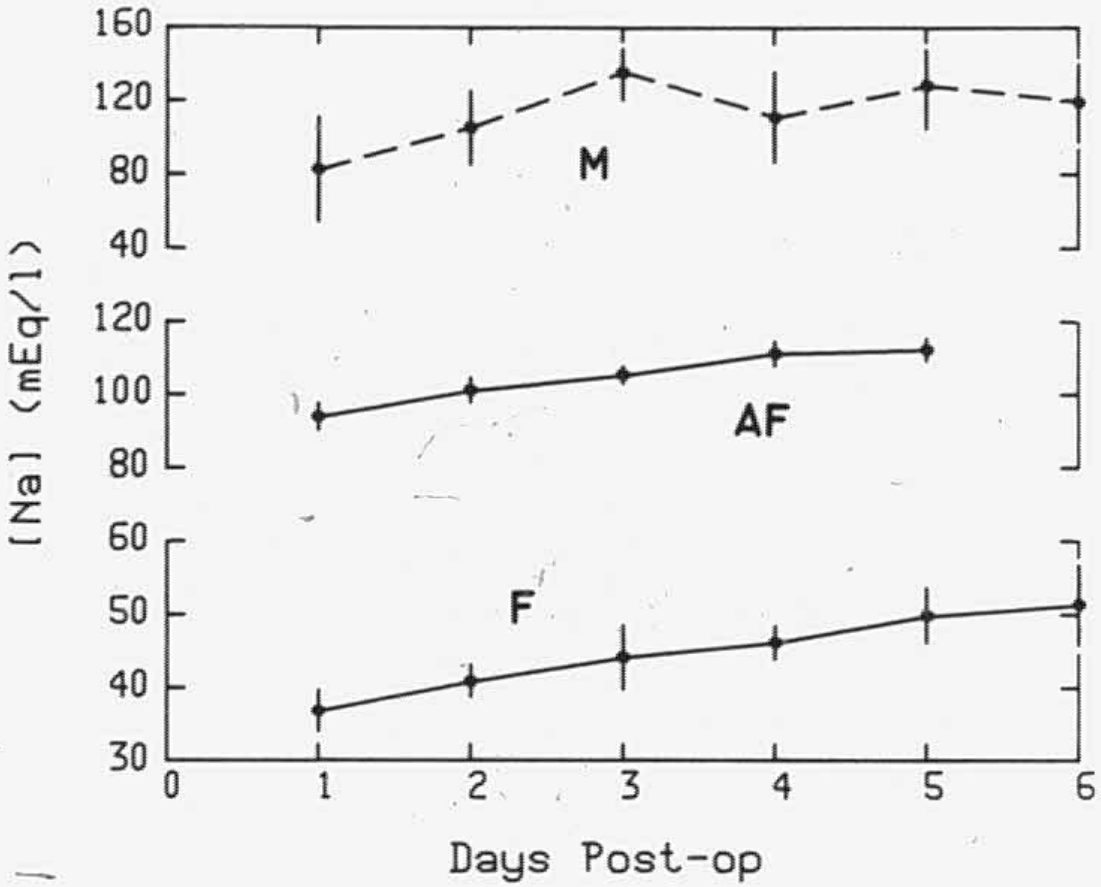


Figure 7. Post-operative changes in fetal (F) and maternal (M) urine and amniotic fluid (AF) Na+ concentrations. Mean ± SE, n = 13.

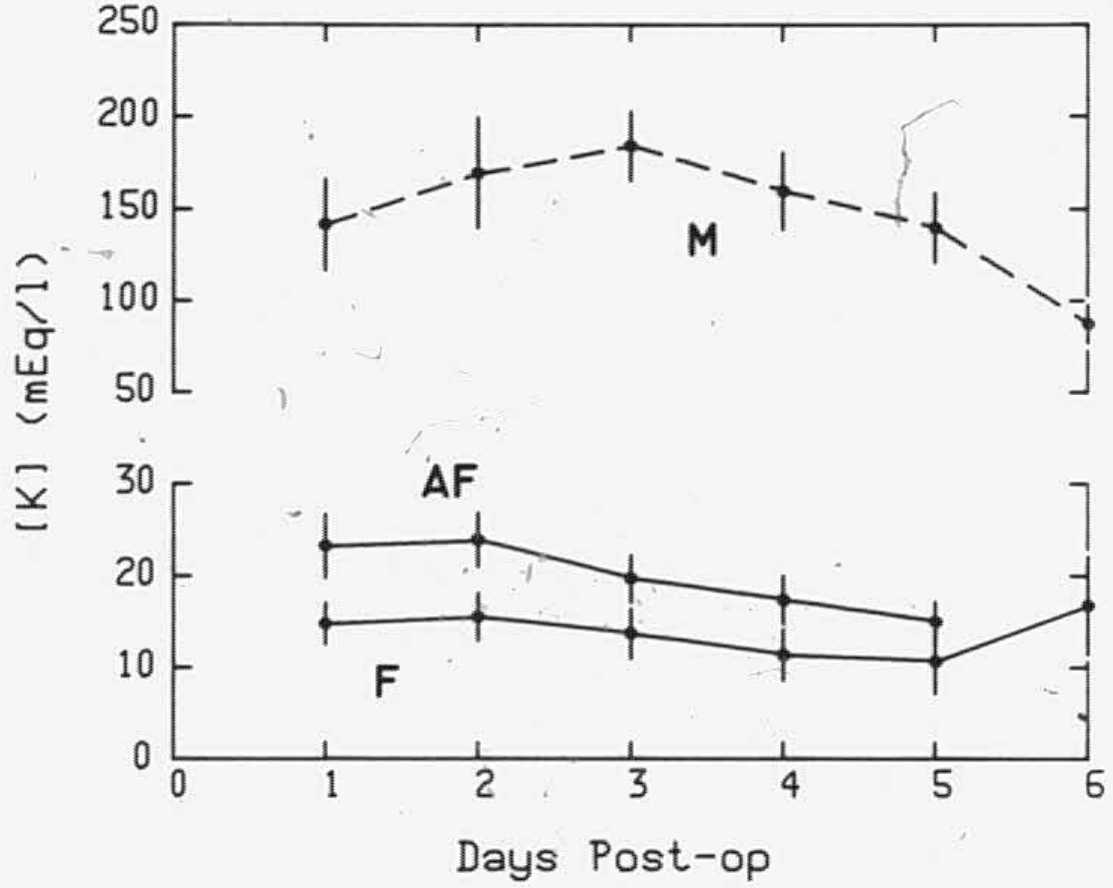


Figure 8. Post-operative changes in fetal (F) and maternal (M) urine and amniotic fluid (AF) K+ concentrations. Mean ± SE, n = 13.

did not change (Figure 8). Maternal urine osmolality and Na^+ showed no significant changes after surgery (Figures 6 and 7), but the decrease in maternal urine K^+ was significant (Figure 8). Amniotic fluid osmolality fell significantly during the first six days after surgery (Figure 1), while amniotic fluid Na^+ increased significantly (Figure 7). Amniotic fluid K^+ also tended to fall somewhat after surgery (Figure 8).

2. Control Values: The mean values of fetal and maternal fluid constituents, cardiovascular variables, and renal function parameters during the control periods are given in Table 3.

3. Hypertonic Injections into Normal Fetuses:

a. Effects on plasma, urine, and amniotic fluid constituents

The effects of hypertonicity on the constituents of fetal and maternal plasma and amniotic fluid are shown in Figures 9-11. In response to hypertonic injection, fetal and maternal plasma osmolalities rose initially by an average of 13 $\pm$ 1 and 15 $\pm$ 1 mOsm/kg respectively, and were still elevated by 10 $\pm$ 2 and 12 $\pm$ 2 mOsm/kg four hours following the injections (Figure 9). Amniotic fluid osmolality rose gradually by an average of 11 $\pm$ 3 mOsm/kg over the four hours post-injection ($p<.01$). Changes in fetal plasma Na^+ and Cl^- levels paralleled the changes in osmolality, peaking at 6.9 $\pm$ 0.4 mEq/l plasma water and 6.4 $\pm$ 0.8 mEq/l plasma above

Table 3. Control values, mean ± SE.

	HI (n=8)	AVP + HI (n=6)	ANT + HI (n=5)
Fetal arterial pressure (mmHg)	38.1 ± 1.0	41.9 ± 1.8	41.6 ± 1.3
Fetal venous pressure (mmHg)	2.6 ± 0.2	4.0 ± 0.7	3.9 ± 0.1
Amniotic fluid pressure (mmHg)	12.3 ± 0.9	11.9 ± 1.4	11.8 ± 1.4
Fetal heart rate (bpm)	169 ± 5	147 ± 8	156 ± 4
Fetal urine flow (ml/min)	1.10 ± 0.23	0.68 ± 0.21	0.95 ± 0.08
Maternal urine flow (ml/min)	1.29 ± 0.40	2.83 ± 0.04	3.66 ± 1.59
Fetal GFR (ml/min)	3.79 ± 0.42	4.36 ± 0.32	4.77 ± 0.48
Fetal blood volume (ml)	433 ± 20	472 ± 22	441 ± 18
Fetal weight (gm)	3731 ± 123	3638 ± 307	3675 ± 359
Maternal weight (kg)	45.8 ± 2.4	49.1 ± 0.9	49.3 ± 1.0
Gestational age (days)	131.6 ± 0.4	133.3 ± 0.8	132.4 ± 1.4
Fetal pH	7.305 ± 0.014	7.298 ± 0.012	7.316 ± 0.011
Fetal P_{O2} (mmHg)	——	19.9 ± 0.7	21.6 ± 0.9
Fetal P_{CO2} (mmHg)	54.5 ± 1.3	52.0 ± 1.4	50.9 ± 0.8
Maternal pH	7.460 ± 0.011	7.425 ± 0.008	7.446 ± 0.015
Maternal P_{O2} (mmHg)	87.7 ± 3.8	93.8 ± 2.0	92.6 ± 4.3
Maternal P_{CO2} (mmHg)	38.7 ± 0.9	36.4 ± 1.0	36.3 ± 1.9
Fetal plasma Na^+ (mEq/l)	149.6 ± 0.6	147.6 ± 1.0	148.3 ± 0.4
Fetal plasma K^+ (mEq/l)	4.1 ± 0.2	4.5 ± 0.2	4.4 ± 0.1
Fetal plasma osmolality (mOsm/kg)	293.1 ± 1.2	296.2 ± 1.0	295.5 ± 0.9
Fetal plasma urea (mmol/l)	6.6 ± 0.6	6.6 ± 0.6	6.2 ± 1.0
Fetal plasma Cl^- (mEq/l)	105.5 ± 1.1	105.5 ± 2.0	104.5 ± 1.6

Maternal plasma Na^+ (mEq/l)	153.8 ± 0.8	152.3 ± 1.1	152.7 ± 0.6
Maternal plasma K^+ (mEq/l)	4.4 ± 0.1	4.4 ± 0.2	4.4 ± 0.2
Maternal plasma osmolality (mOsm/kg)	293.9 ± 1.3	296.3 ± 0.9	296.7 ± 0.9
Maternal plasma urea (mmol/l)	6.3 ± 0.6	6.0 ± 0.5	5.3 ± 0.7
Maternal plasma Cl^- (mEq/l)	105.3 ± 1.3	107.3 ± 1.2	106.8 ± 1.6
Fetal urine Na^+ (mEq/l)	56.9 ± 8.6	46.1 ± 7.4	55.2 ± 20.1
Fetal urine K^+ (mEq/l)	16.7 ± 7.9	38.2 ± 14.2	27.7 ± 11.0
Fetal urine osmolality (mOsm/kg)	188 ± 31	226 ± 42	164 ± 29
Fetal urine urea (mmol/l)	26.7 ± 7.3	49.4 ± 12.0	19.8 ± 5.2
Fetal urine Cl^- (mEq/l)	41.7 ± 7.2	32.7 ± 10.1	25.2 ± 7.1
Maternal urine Na^+ (mEq/l)	97.5 ± 30.6	145.5 ± 25.2	176.5 ± 32.3
Maternal urine K^+ (mEq/l)	122.1 ± 21.0	108.8 ± 18.7	100.4 ± 20.6
Maternal urine osmolality (mOsm/kg)	762 ± 53	732 ± 90	703 ± 153
Maternal urine urea (mmol/l)	218 ± 19	171 ± 25	148 ± 42
Maternal urine Cl^- (mEq/l)	101 ± 22	147 ± 23	158 ± 32
Amniotic fluid osmolality (mOsm/kg)	259 ± 7	273 ± 6	259 ± 5
Fetal plasma AVP (pg/ml)	8.0 ± 1.8	7.8 ± 0.5	5.6 ± 1.0
Maternal plasma AVP (pg/ml)	2.5 ± 0.7	6.3 ± 0.4	3.6 ± 0.5
Fetal C_{Na} (ml/min)	0.37 ± 0.07	0.21 ± 0.08	0.43 ± 0.18
Fetal C_K (ml/min)	1.9 ± 0.4	3.4 ± 0.9	6.3 ± 2.9
Fetal C_{osm} (ml/min)	0.53 ± 0.07	0.39 ± 0.09	0.62 ± 0.17
Fetal C_{urea} (ml/min)	2.97 ± 0.39	3.63 ± 0.71	3.49 ± 1.32
Fetal C_{Cl} (ml/min)	0.41 ± 0.11	0.17 ± 0.06	0.26 ± 0.08

Maternal C_{Na} (ml/min)	0.96 ± 0.46	2.44 ± 0.25	3.11 ± 0.97
Maternal C_K (ml/min)	32.8 ± 13.2	61.0 ± 11.0	54.7 ± 12.2
Maternal C_{osm} (ml/min)	3.0 ± 0.8	6.3 ± 0.4	5.6 ± 1.3
Maternal C_{urea} (ml/min)	43.4 ± 11.5	78.4 ± 11.1	60.8 ± 13.1
Maternal C_{Cl}	1.12 ± 0.44	3.29 ± 0.29	3.70 ± 1.02

HI = animals used for hypertonic injection studies, AVP + HI = animals used for AVP infusion followed by hypertonic injection, ANT + HI = animals used for antagonist infusion followed by hypertonic injection

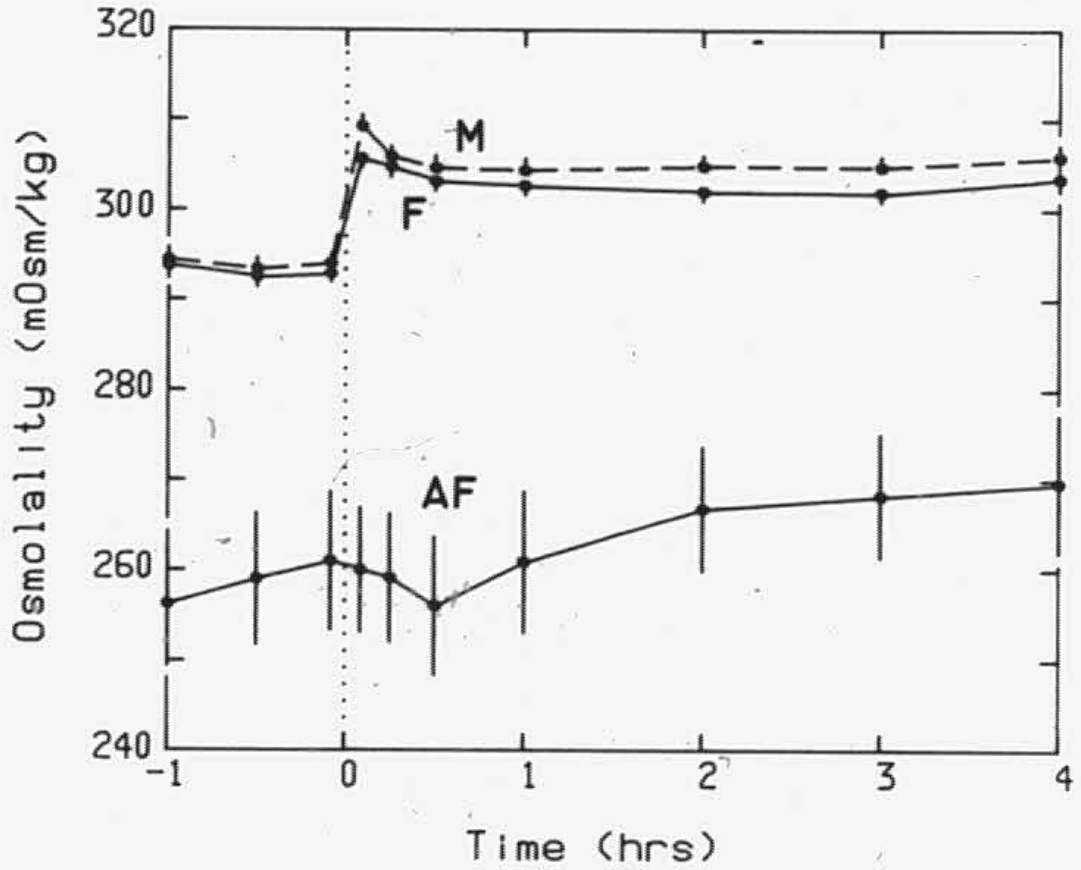


Figure 9. Time course of fetal (F) and maternal (M) plasma and amniotic fluid (AF) osmolalities following hypertonic injection at time zero. Mean $\pm$ SE, n = 8.

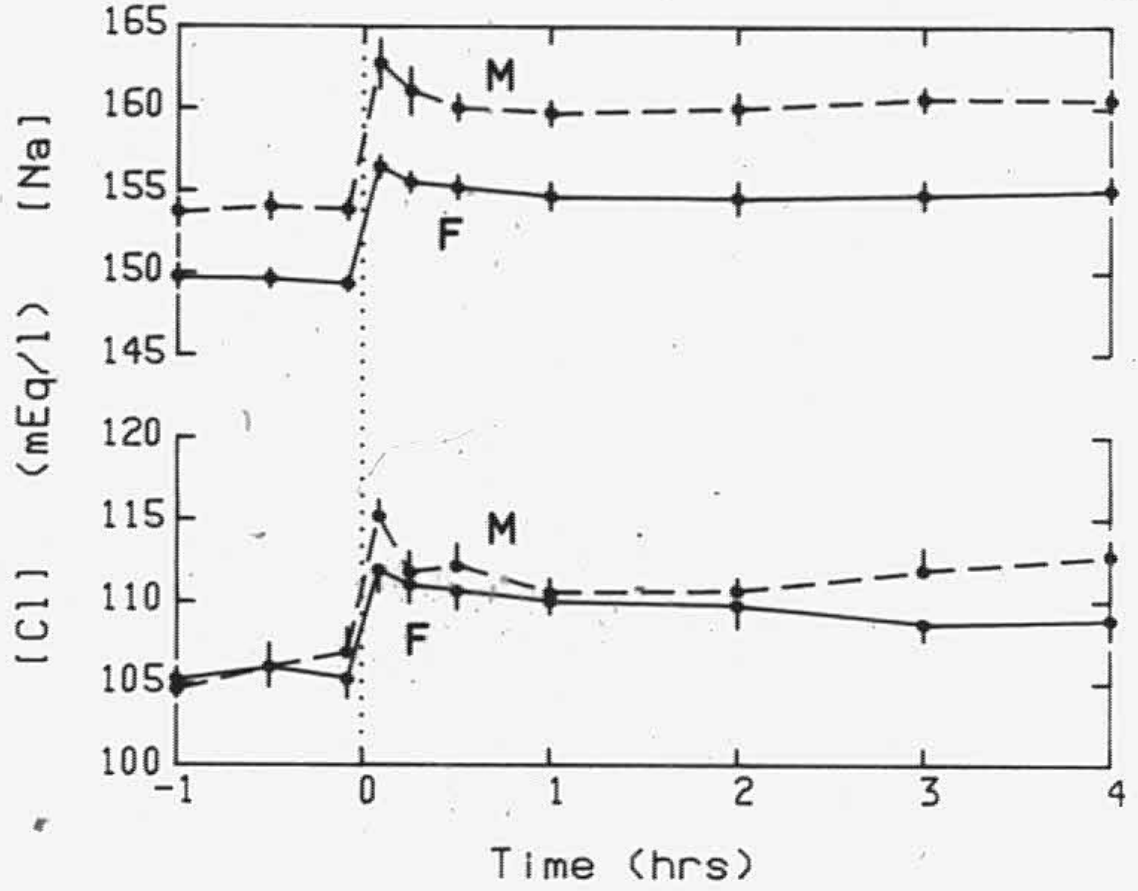


Figure 10. Time course of fetal (F) and maternal (M) plasma Na+ and Cl- concentrations following hypertonic injection at time zero. Mean ± SE, n = 8.

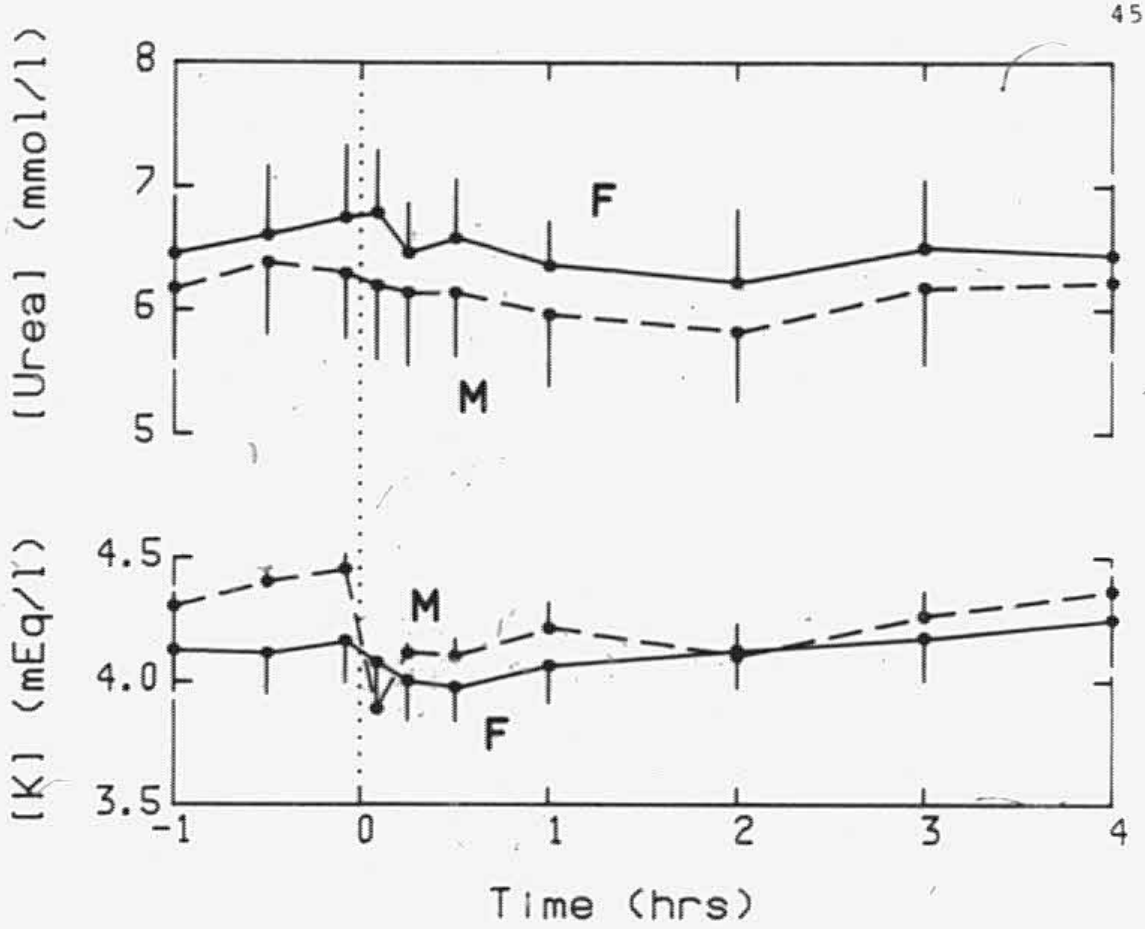


Figure 11. Time course of fetal (F) and maternal (M) plasma urea and K+ concentrations following hypertonic injection at time zero. Mean ± SE, n = 8.

control respectively (Figure 10). Maternal plasma Na^+ and Cl^- showed similar changes, peaking at 8.9 ± 1.2 mEq/l plasma water and 9.3 ± 0.7 mEq/l plasma above the control levels respectively. Fetal plasma K^+ showed no change (Figure 11), while maternal plasma K^+ fell significantly by 0.5 ± 0.1 after hypertonic injection ($p<.001$) and gradually returned to normal. Fetal and maternal plasma urea concentrations showed no significant changes.

The effects of hypertonic injections on fetal and maternal urine constituents are shown in Figures 12-16. Fetal urine osmolality increased to a value 89 ± 24 mOsm/kg above control by one hour post-injection ($p<.01$) and remained at that level thereafter. Maternal urine osmolality fell slightly but significantly to 63 ± 19 mOsm/kg below the control value by 5 minutes ($p<.02$), and then increased to 254 ± 59 mOsm/kg above control by four hours post-injection ($p<.01$). Fetal urine Na^+ increased to 27 ± 4 mEq/l above control by 15 minutes after hypertonic injection ($p<.001$), and remained relatively constant thereafter (Figure 13). In contrast, maternal urine Na^+ increased rapidly for the first 30 minutes following injection and then more slowly, but was still increasing after four hours. At this time, maternal urine Na^+ had increased about 3 1/2-fold, or by 235 ± 23 mEq/l over control values ($p<.001$). The responses of urine Cl^- concentrations in mother and fetus showed similar pat-

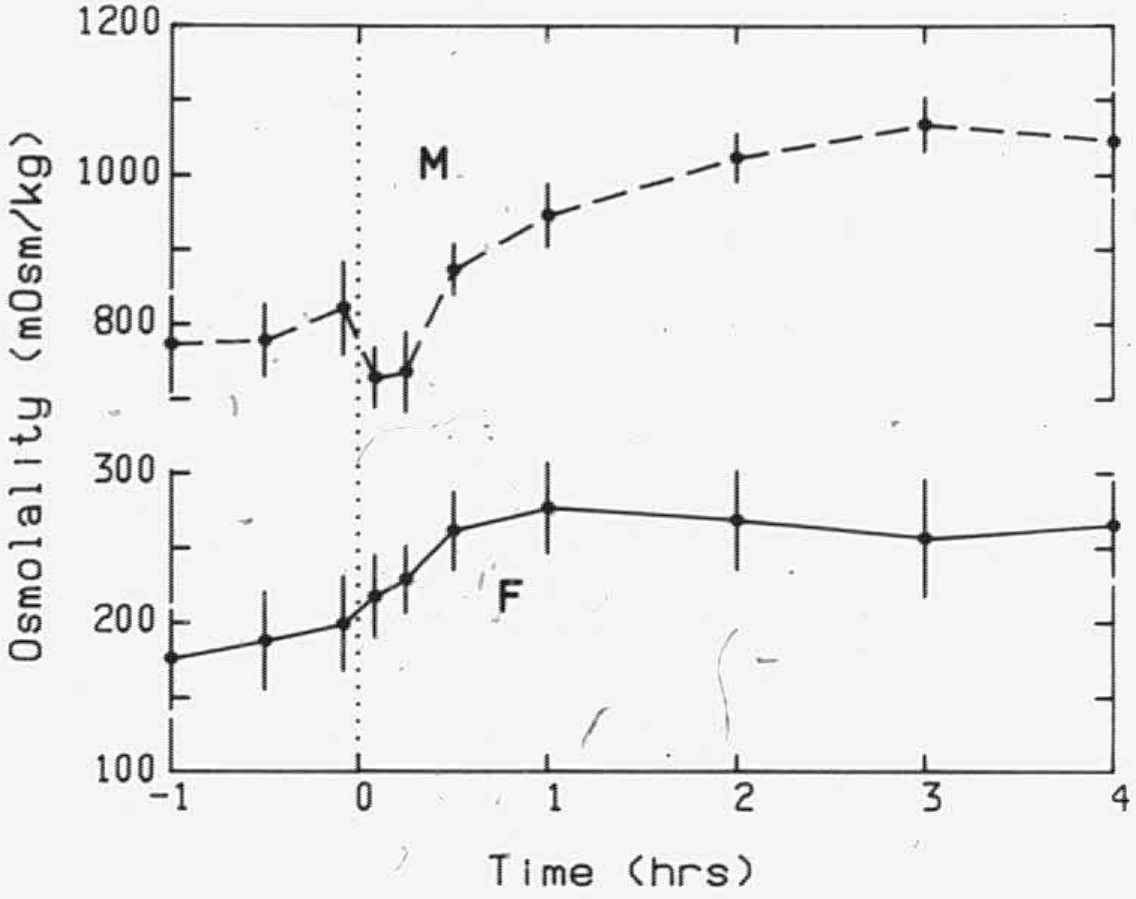


Figure 12. Time course of fetal (F) and maternal (M) urine osmolalities following hypertonic injection at time zero. Mean ± SE, n = 8.

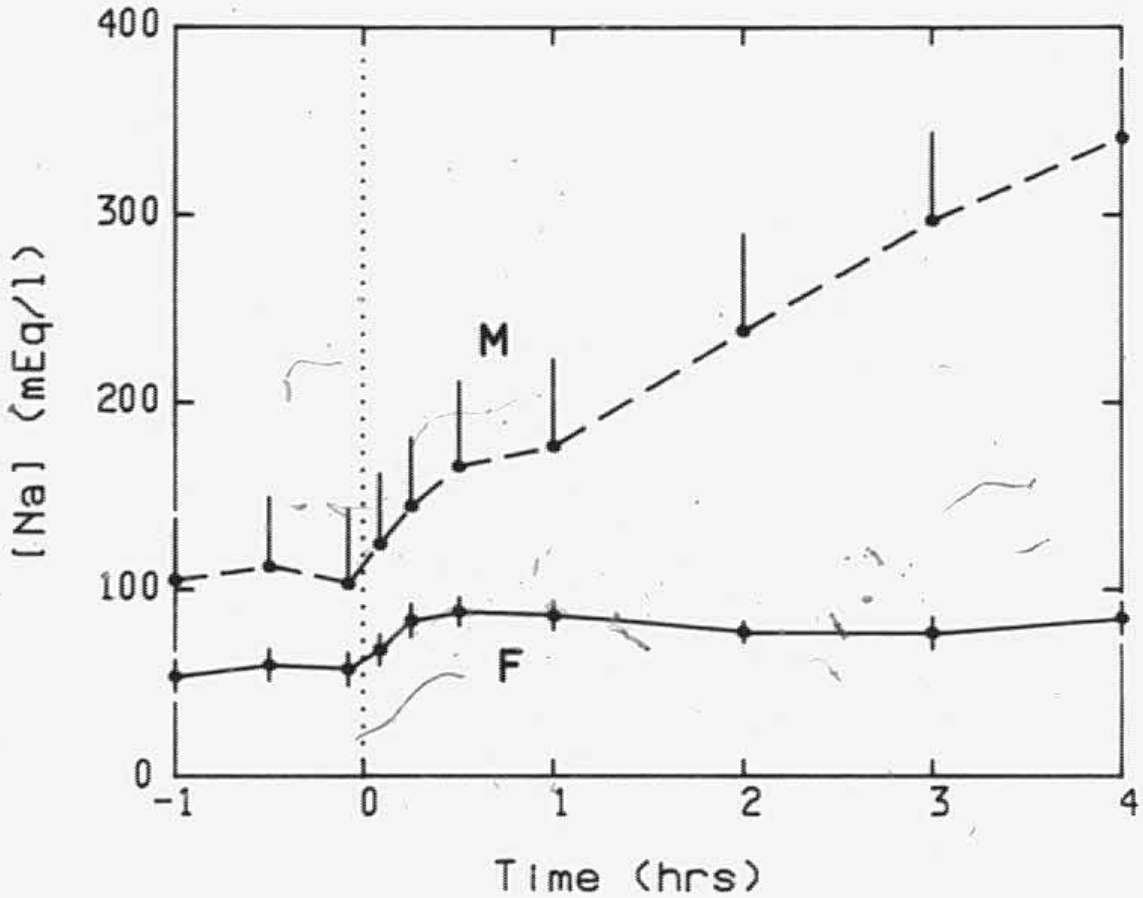


Figure 13. Time course of fetal (F) and maternal (M) urine Na+ concentrations following hypertonic injection at time zero. Mean ± SE, n = 8.

terns to those of Na^+ (Figure 14). Fetal urine Cl^- was increased by 31 ± 4 mEq/l at 30 minutes post-injection ($p<.001$), and then tended to fall somewhat, but remained above control levels for the entire four hours. Maternal urine Cl^- increased rapidly to 152 ± 16 mEq/l above control by 30 minutes ($p<.001$), and then continued to increase more gradually to 227 ± 16 mEq/l above control by four hours post-injection. Fetal urine K^+ showed no significant change during the experiment (Figure 15), while maternal urine K^+ fell to 18 ± 12 mEq/l below control by 5 minutes after injection, returned to the control level by 30 minutes, and then gradually fell to 46 ± 11 mEq/l below control over the next 3 1/2 hours ($p<.01$). The concentration of urea in fetal urine fell slightly but not significantly at 15 minutes after hypertonic injection and then tended to increase above control for the rest of the experimental period (Figure 16). Maternal urine urea, on the other hand, had fallen dramatically by 79.6 ± 14.8 mmol/l at 15 minutes after hypertonic injection ($p<.01$) and then increased somewhat, but did not return to the control level by four hours post-injection.

b. Effects on plasma AVP levels

The effects of hypertonic injections on fetal plasma AVP levels are shown in Figure 17. Fetal AVP was increased by 2.9 ± 1.1 pg/ml over the immediate pre-injection value five minutes after hypertonic injection and then fell to normal

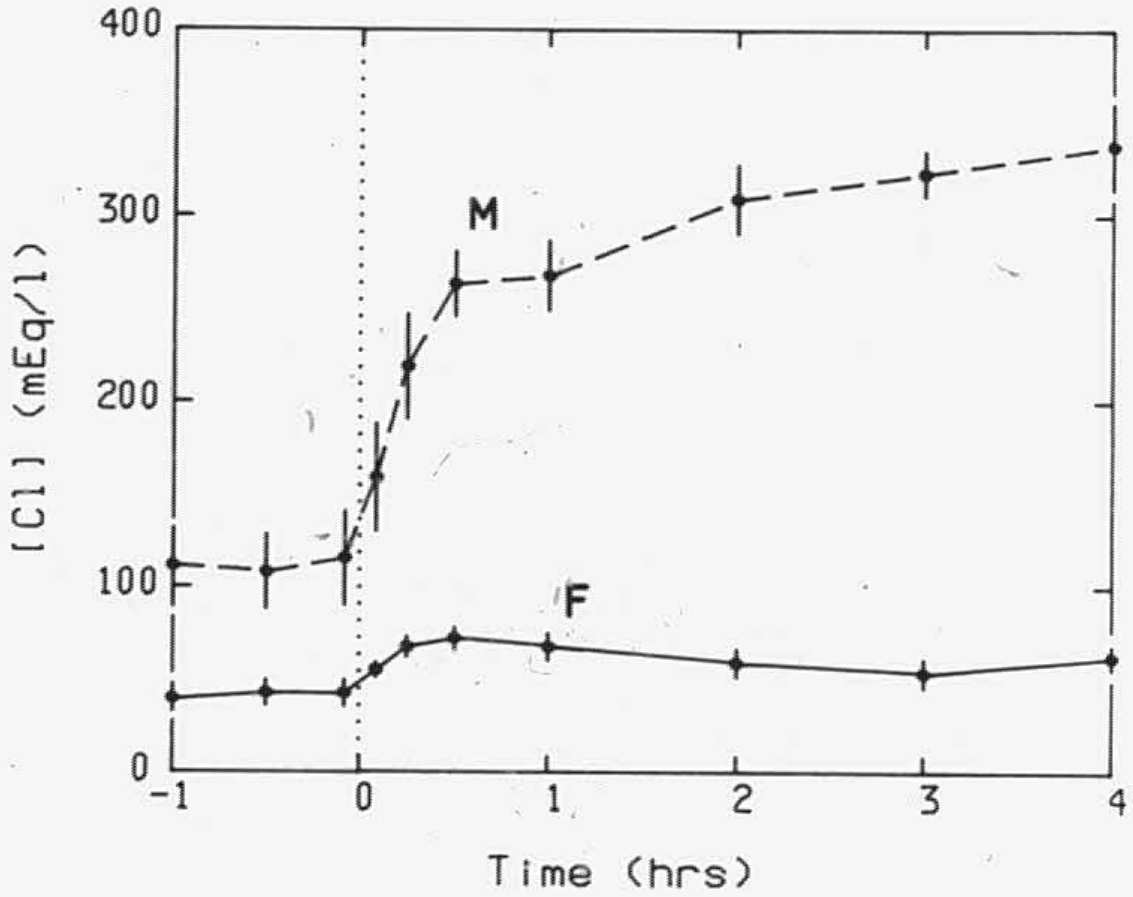


Figure 14. Time course of fetal (F) and maternal (M) urine Cl- concentrations following hypertonic injection at time zero. Mean ± SE, n = 8.

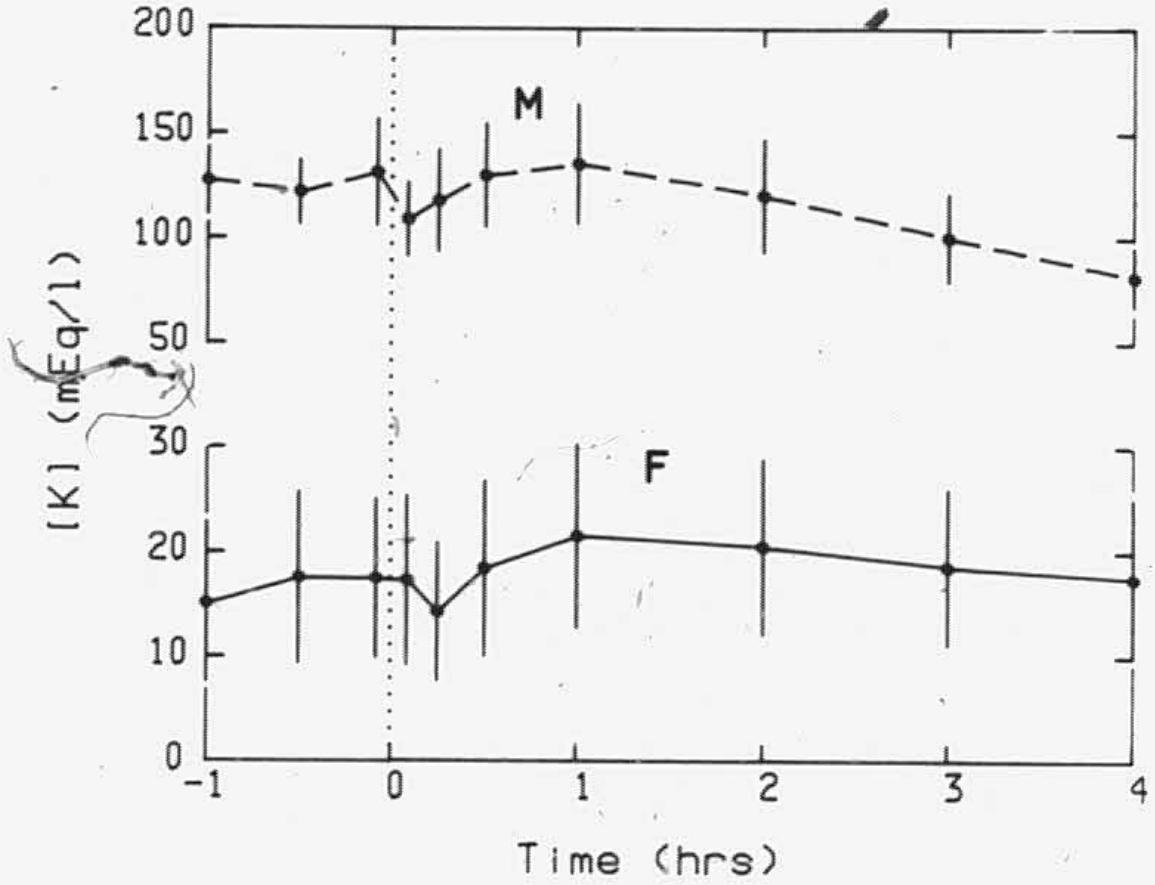


Figure 15. Time course of fetal (F) and maternal (M) urine K+ concentrations following hypertonic injection at time zero. Mean ± SE, n = 8.

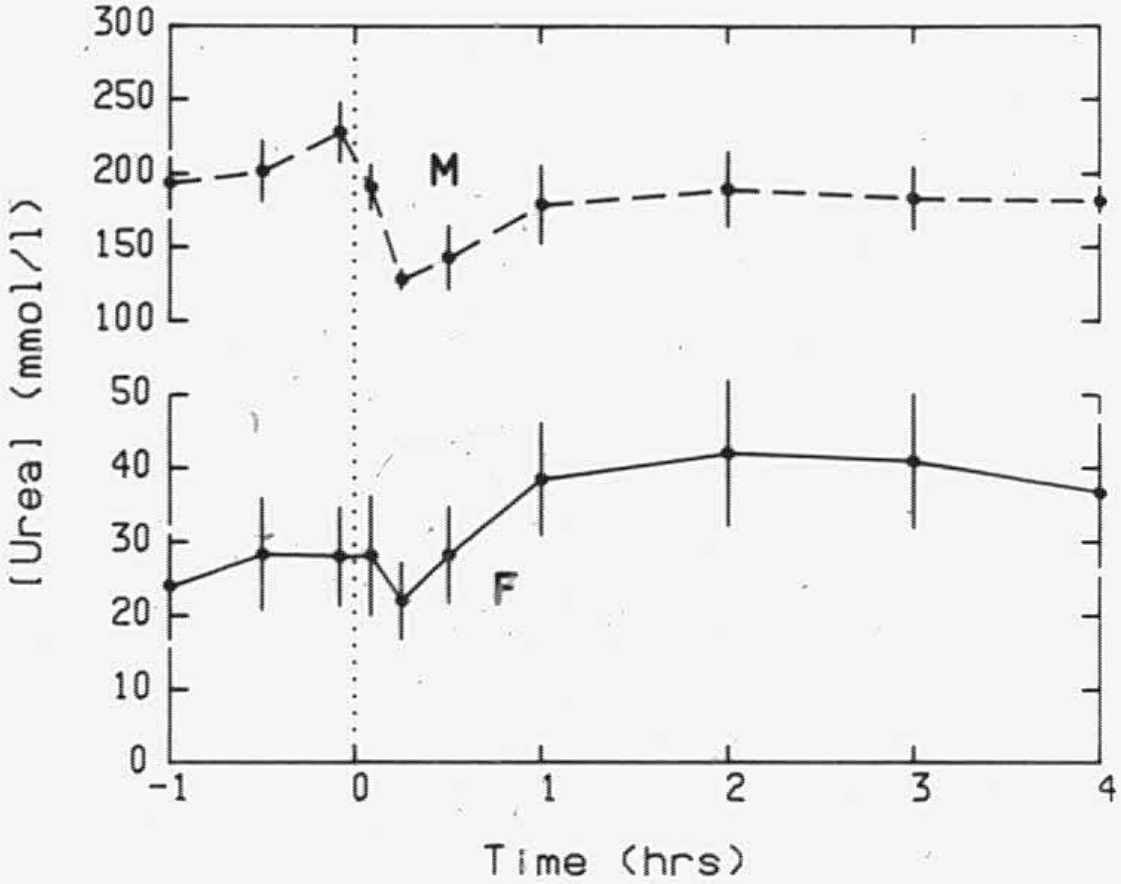


Figure 16. Time course of fetal (F) and maternal (M) urine urea concentrations following hypertonic injection at time zero. Mean ± SE, n = 8.

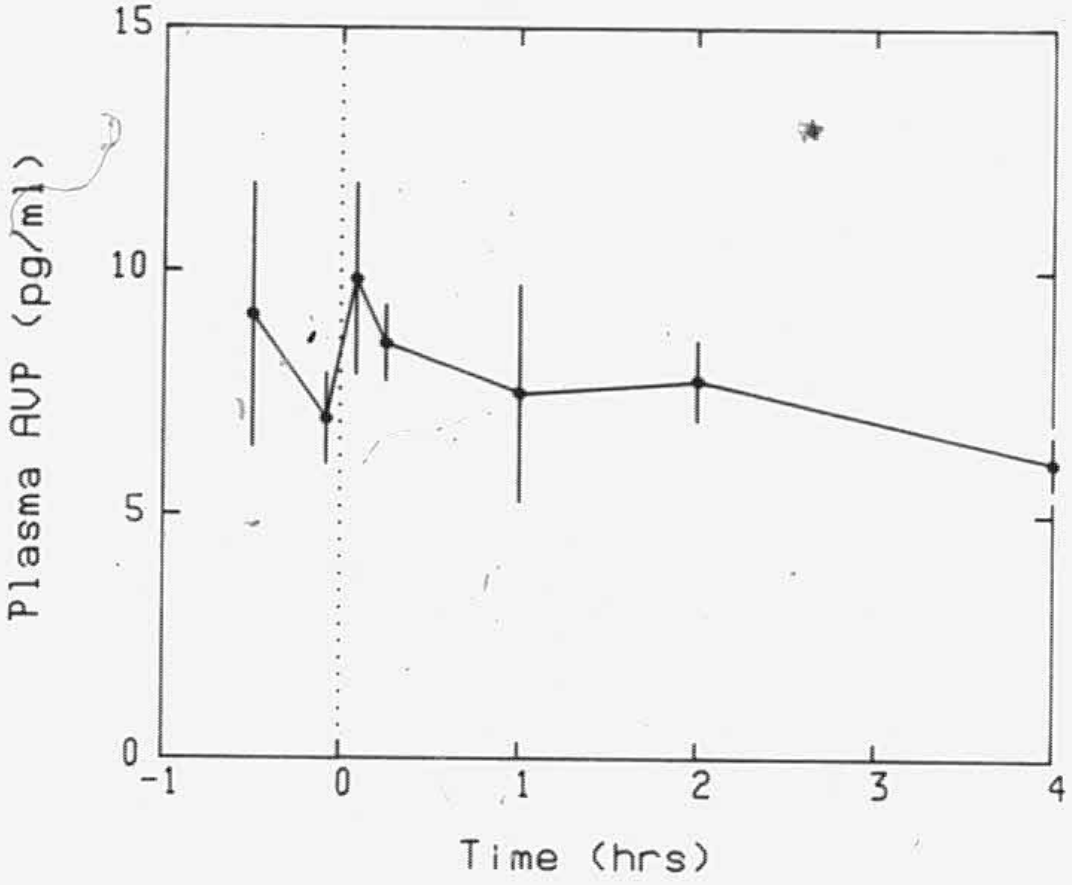


Figure 17. Time course of fetal plasma AVP levels in response to hypertonic injection at time zero. Mean ± SE, n = 3.

during the remainder of the experimental period.

c. Effects on continuously-measured variables

The effects of hypertonic injections on fetal cardiovascular variables are shown in Figures 18-20. Fetal arterial pressure was increased slightly but not significantly during the first two minutes following hypertonic injection, and then was decreased significantly by 1-3 mmHg for at least the next two hours. Fetal venous pressure increased by a peak of 2.3 $\pm$ 0.5 mmHg two minutes after injection ($p<.01$) but returned to normal within 5-10 minutes and did not change significantly thereafter. Fetal heart rate increased immediately in response to the injection, to a peak of 29 $\pm$ 4 bpm above control at 10 minutes ($p<.001$). Fetal heart rate remained elevated for most of the next four hours. Amniotic fluid pressure (not shown) showed no change during the experiment.

The responses of fetal urine output to hypertonic injection are shown in Figure 21. Fetal urine flow increased immediately following injection, to a peak of 0.8 $\pm$ 0.2 ml/min above control values at 5 minutes ($p<.02$), and remained elevated for at least 10 minutes. After one hour, fetal urine flow was decreased by an average of 0.3 $\pm$ 0.15 ml/min for the next three hours.

Due to catheter problems, reliable maternal urine flow rates were obtained in only three animals in this group.

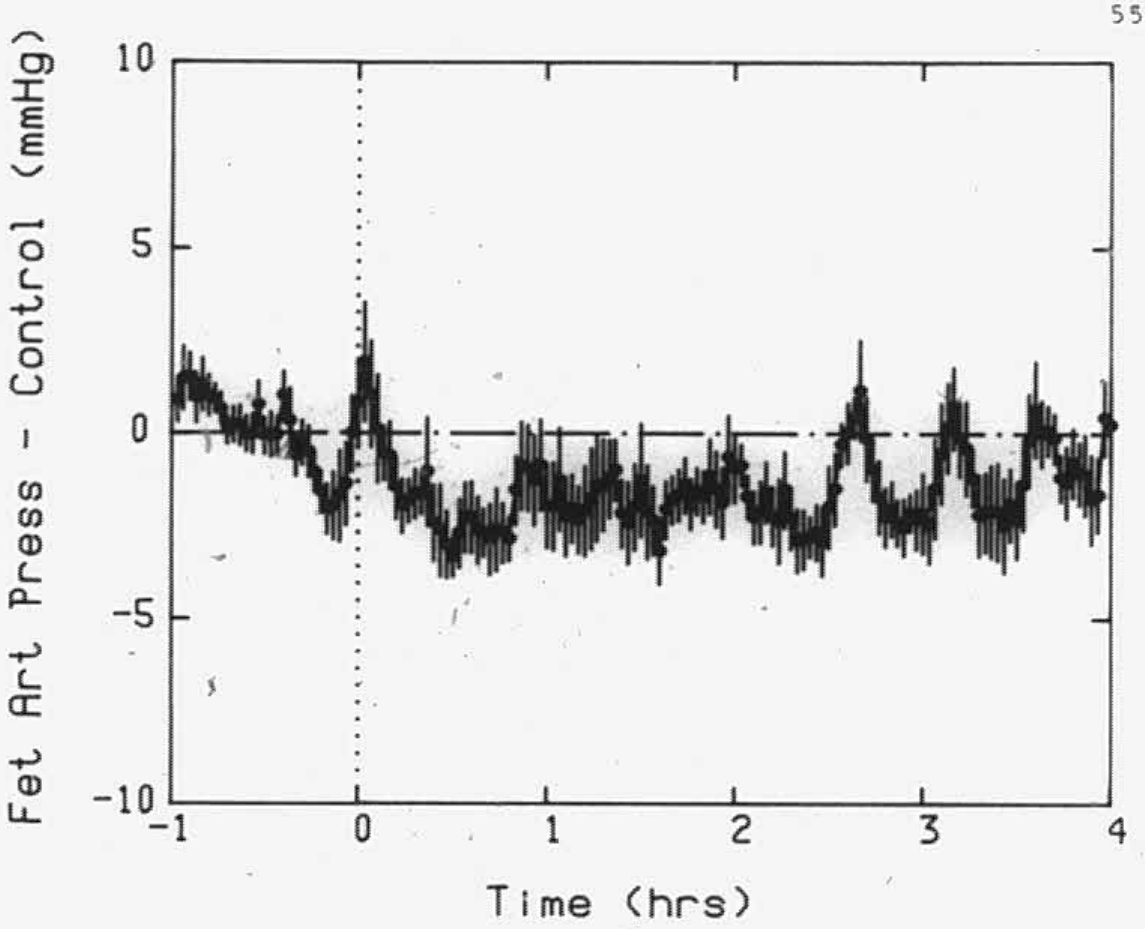


Figure 18. Time course of fetal arterial pressure changes in response to hypertonic injection at time zero. Control = 38.1 ± 1.04 mmHg. Mean change from control ± SE, n = 8.

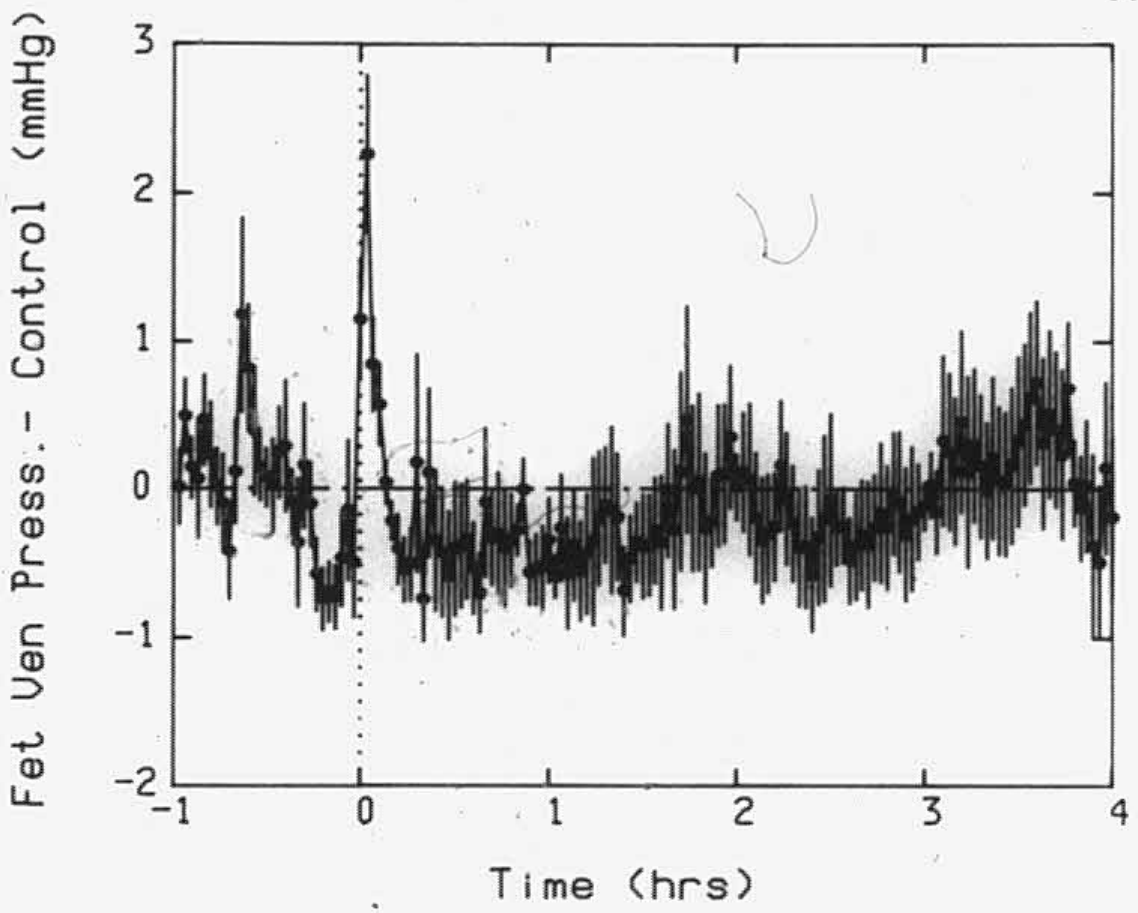


Figure 19. Time course of fetal venous pressure changes in response to hypertonic injection at time zero. Control = 2.58 ± 0.23 mmHg. Mean change from control ± SE, n = 8.

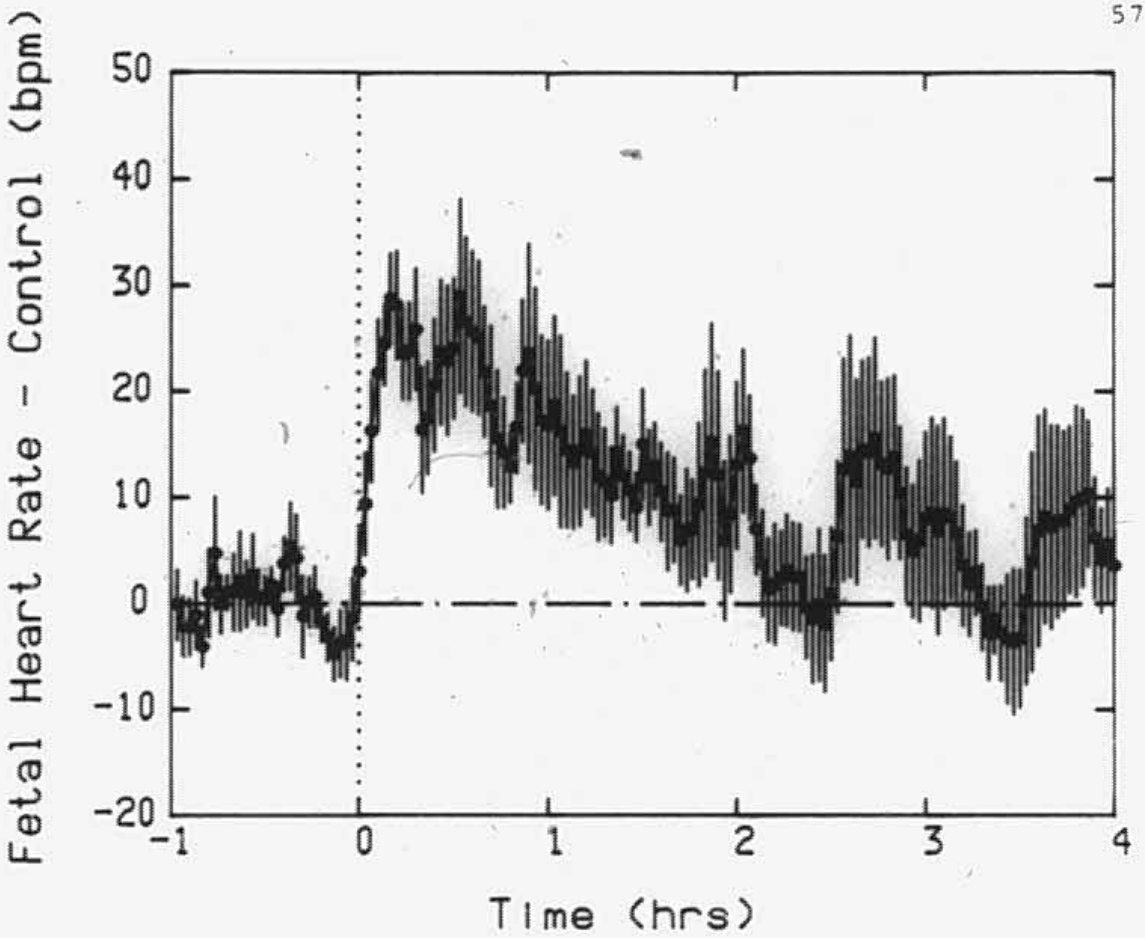


Figure 20. Time course of fetal heart rate changes in response to hypertonic injection at time zero. Control = 169 ± 5 bpm. Mean change from control ± SE, n = 8.

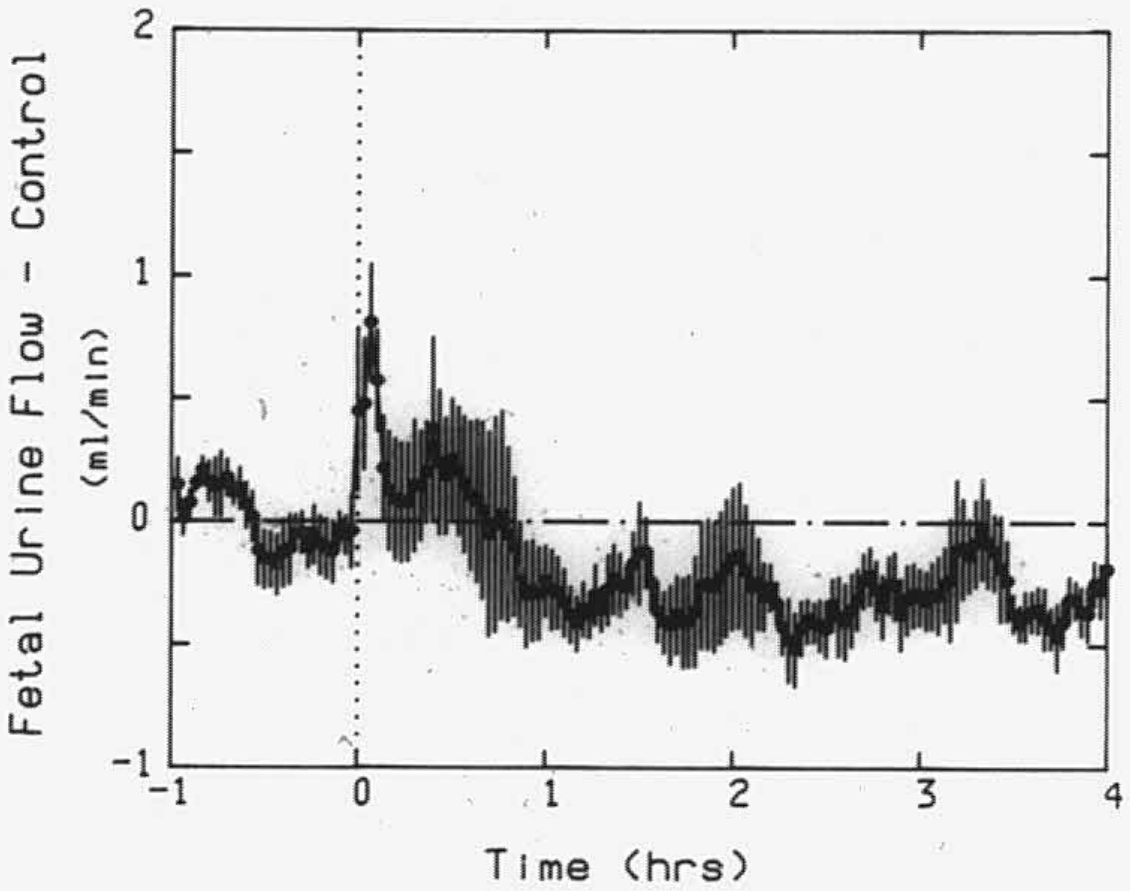


Figure 21. Time course of fetal urine flow changes in response to hypertonic injection at time zero. Control = 1.1 ± 0.2 ml/min. Mean change from control ± SE, n = 8.

The results are not included here, but were not different from maternal responses in the fetal antagonist and AVP infusion studies (Figure 45).

d. Effects on fetal GFR

The effects of hypertonic injection on fetal plasma and urine inulin concentrations and calculated fetal GFR are shown in Figures 22-23. Approximately 55% of the labeled inulin present in the fetal plasma at the beginning of the experiment was lost over the next five hours. Fetal GFR was increased to 6.5 $\pm$ 1.2 ml/min five minutes after hypertonic injection but then returned to the control value and did not change for the duration of the experiment.

e. Effect on renal clearance of chemical constituents

The effects of hypertonic injections on the renal clearance of plasma constituents are shown in Figures 24-28. Fetal C_{Na}, C_{Cl}, and C_{osm} were all approximately doubled for the first hour after hypertonic injection and were not different from control thereafter. Fetal C_K and C_{urea}, on the other hand, were increased only at the 5 minute sampling point, by an average of 103% and 69% respectively. Maternal clearance values are not shown, but the changes seen were not different from those in the mothers of AVP- and antagonist-infused fetuses (Figures 52-56).

f. Effect on free water clearance (C_{water})

The effects of hypertonic injection on C_{water} in the

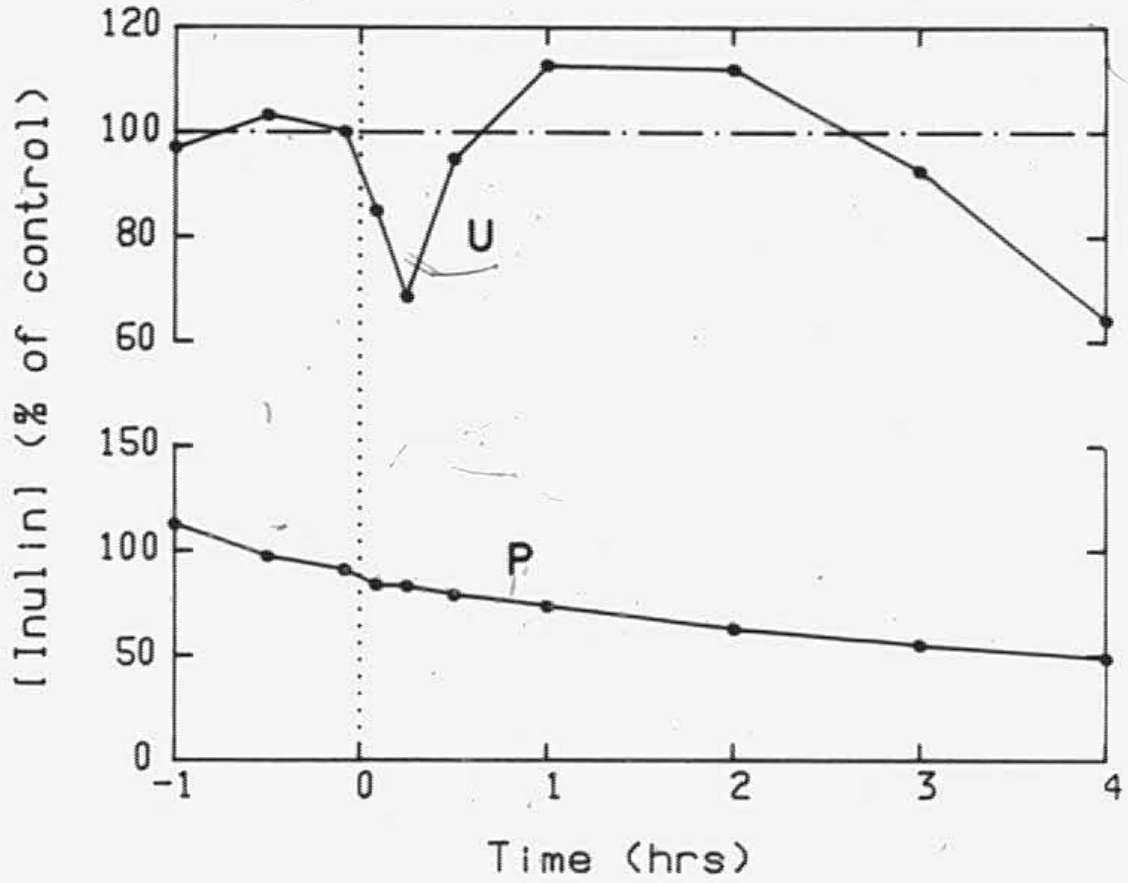


Figure 22. Time course of fetal urine (U) and plasma (P) inulin concentrations in response to hypertonic injection at time zero. Mean, n = 7.

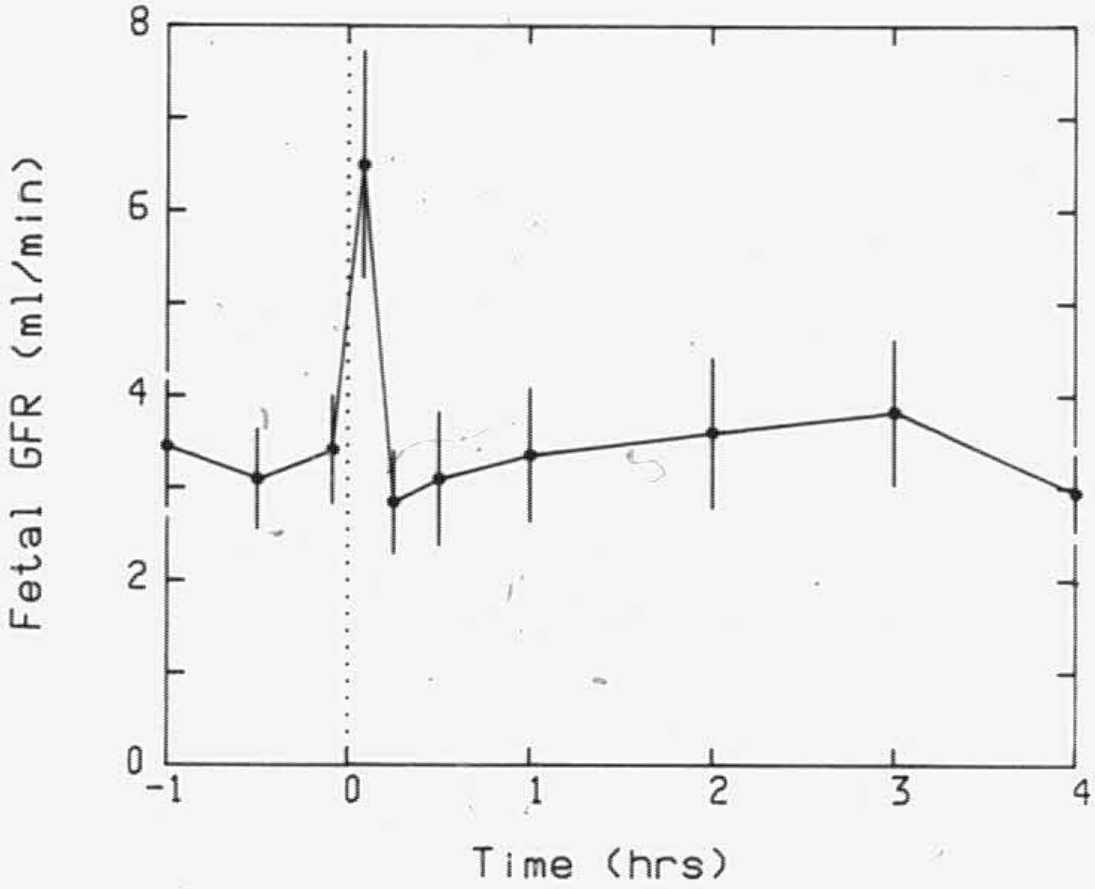


Figure 23. Time course of fetal GFR in response to hypertonic injection at time zero. Mean ± SE, n = 7.

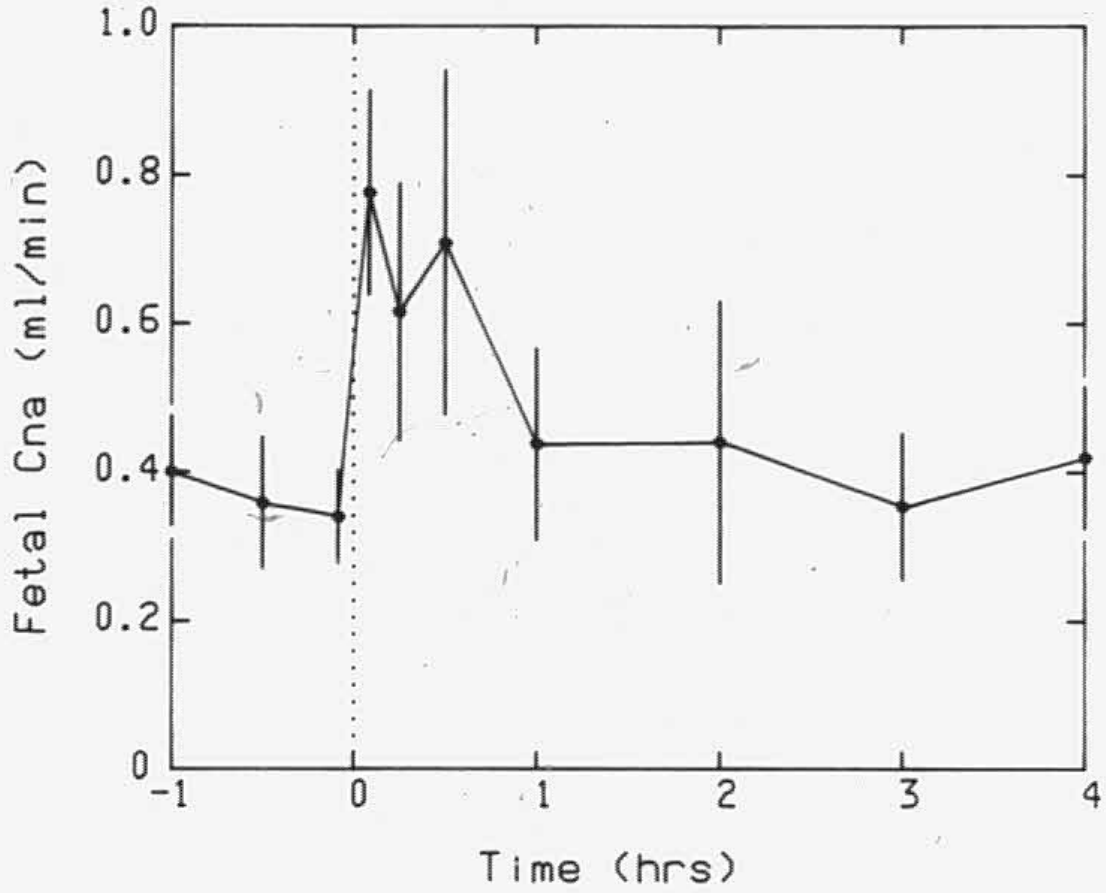


Figure 24. Time course of fetal Na+ clearance in response to hypertonic injection at time zero. Mean ± SE, n = 8.

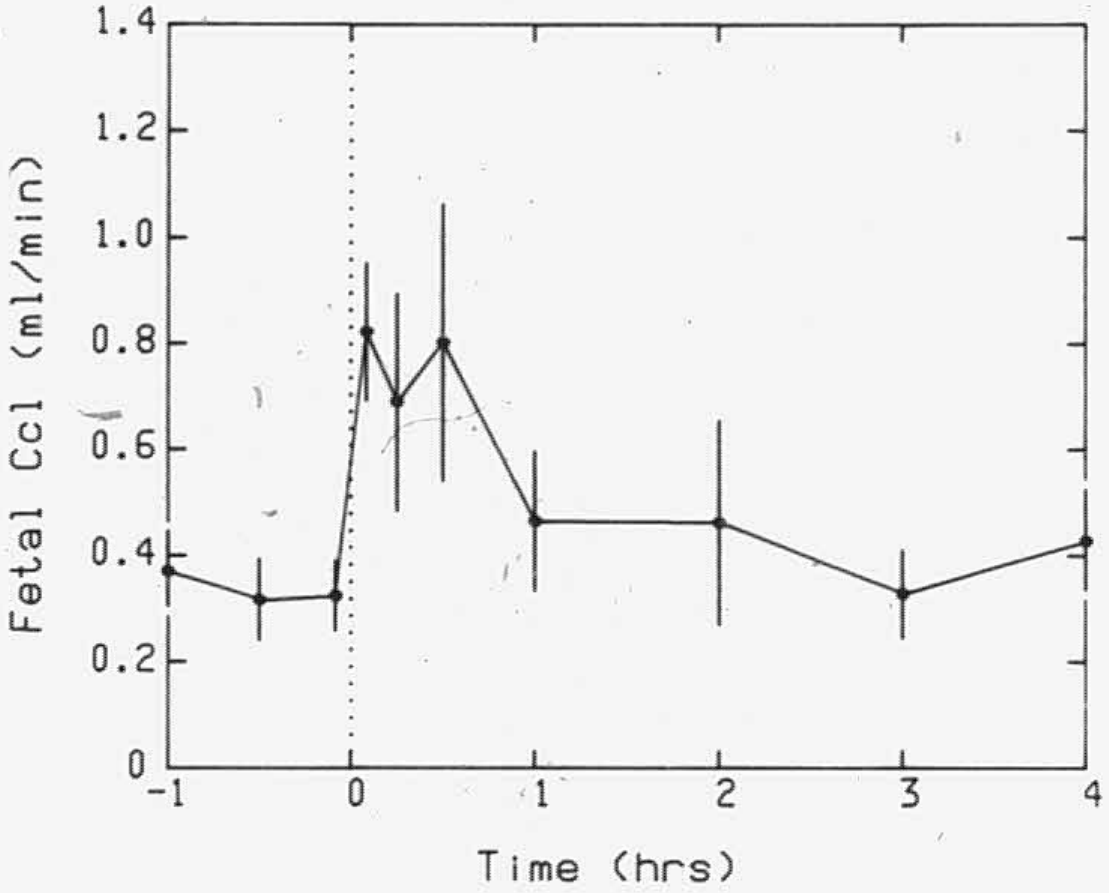


Figure 25. Time course of fetal Cl- clearance in response to hypertonic injection at time zero. Mean ± SE, n = 8.

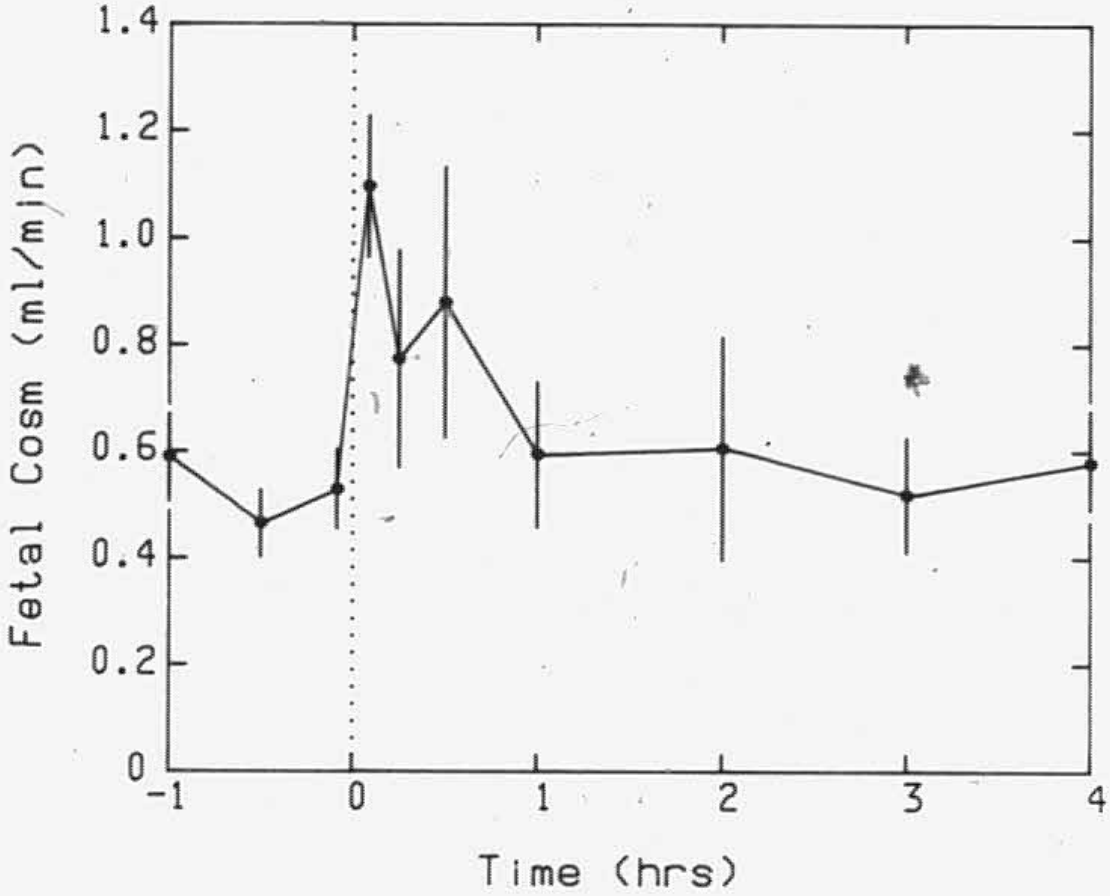


Figure 26. Time course of fetal osmolar clearance in response to hypertonic injection at time zero. Mean ± SE, n = 8.

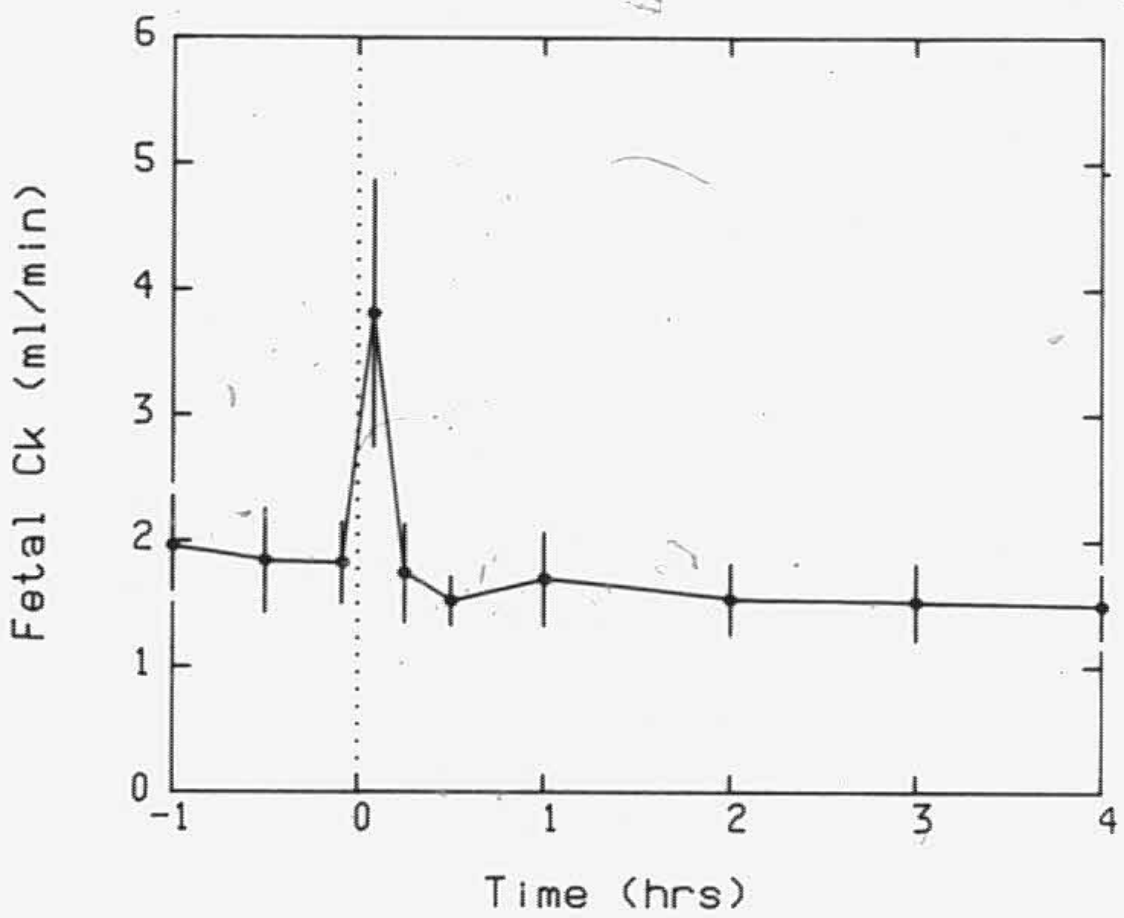


Figure 27. Time course of fetal K+ clearance in response to hypertonic injection at time zero. Mean ± SE, n = 8.

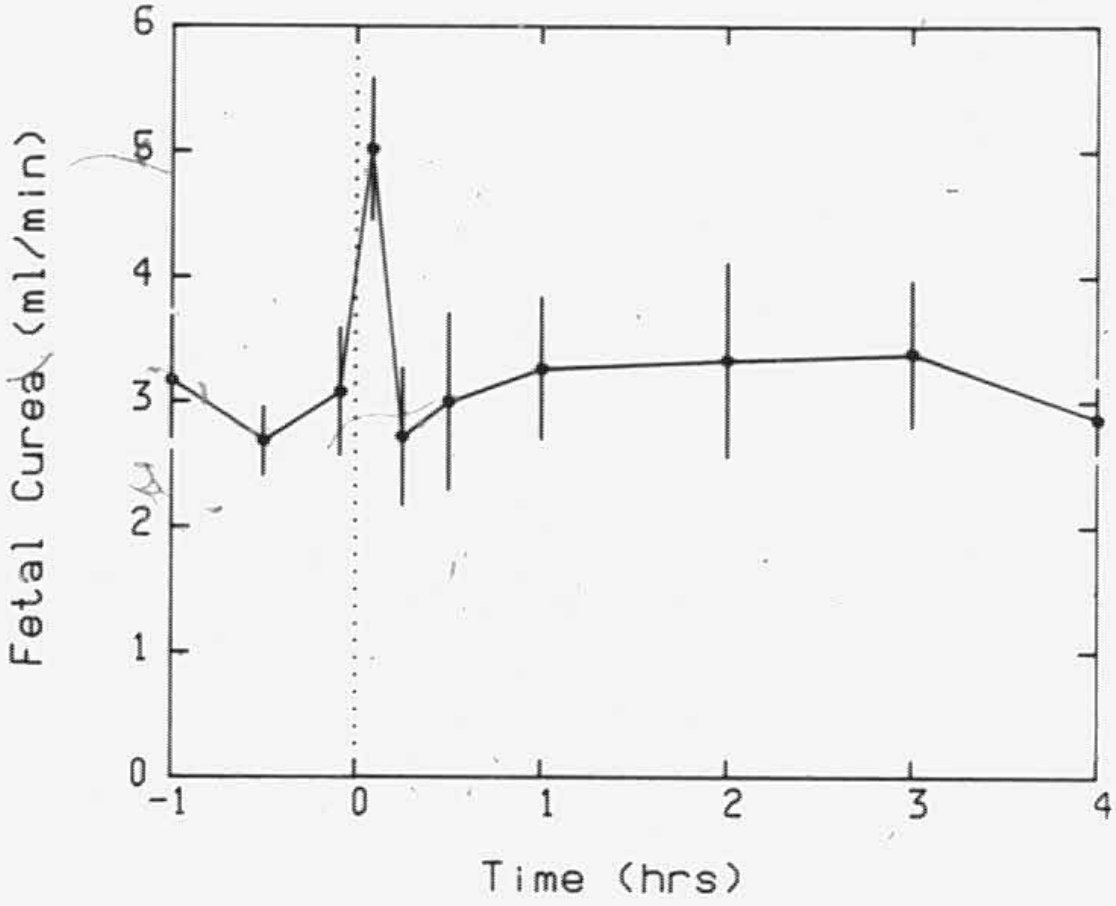


Figure 28. Time course of fetal urea clearance in response to hypertonic injection at time zero. Mean ± SE; n = 8.

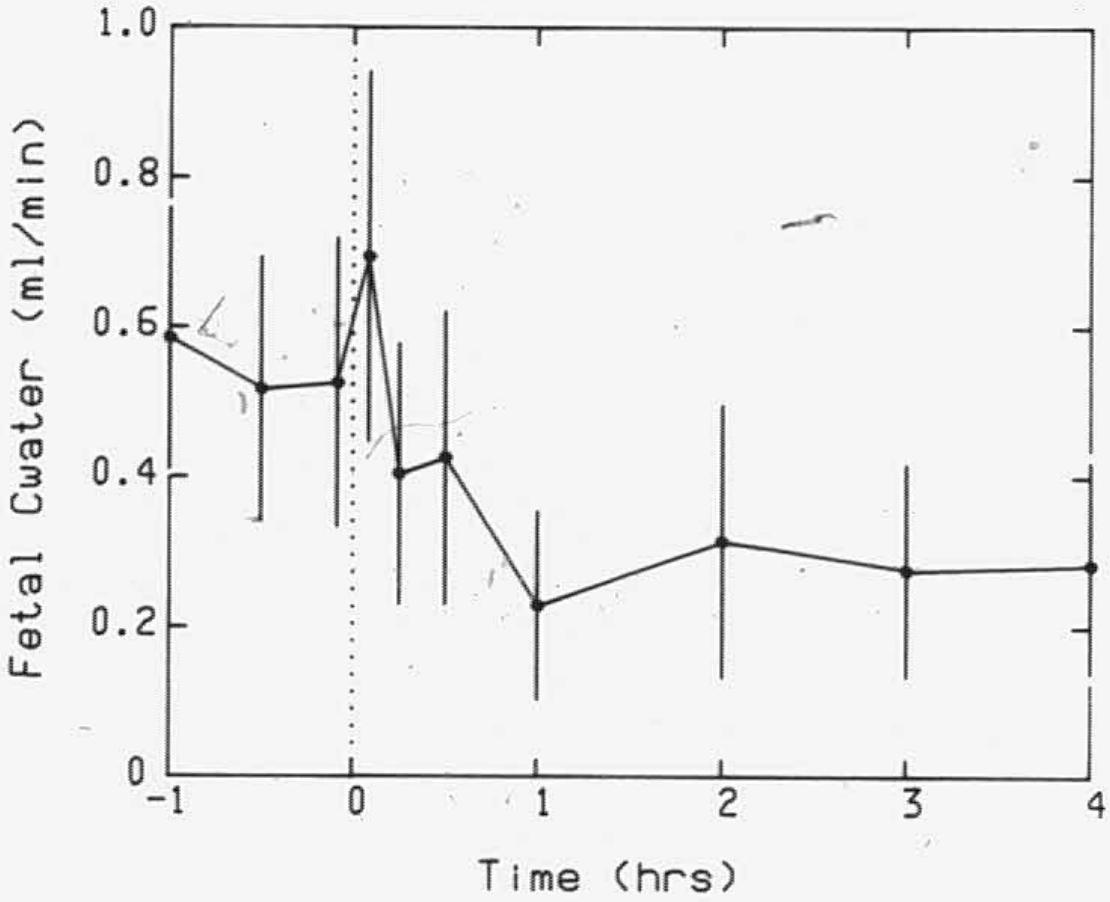


Figure 29. Time course of fetal free water clearance in response to hypertonic injection at time zero. Mean ± SE, n = 8.

fetus are shown in Figure 29. Fetal C_{water} had fallen from 0.54 ± 0.18 to 0.23 ± 0.13 ml/min at one hour post-injection and remained constant thereafter. Despite this fall, fetal C_{water} did not become negative during the experiment (i.e. free water was not reabsorbed).

g. Effect on fetal blood volume

As shown in Figure 30, hypertonic injection into mother and fetus, followed by hypertonic infusion into the ewe, had no significant effect on fetal blood volume.

4. Hypertonic Injections into AVP-Infused Fetuses:

a. Effects on plasma, urine, and amniotic fluid constituents

The effects of AVP infusion followed by hypertonic injection on fetal and maternal fluids are shown in Figures 31-39. Fetal plasma osmolality fell significantly during the first hour of AVP infusion, to a value 3 ± 0.6 mOsm/kg below control after one hour of infusion ($p<.01$). Fetal plasma osmolality remained below control values until the hypertonic NaCl was injected. Maternal plasma osmolality fell gradually during the period of AVP infusion into the fetus, to 2 ± 0.8 mOsm/kg below control after two hours ($p<.05$). After hypertonic injection, fetal and maternal plasma osmolalities rose to 12 ± 1 and 15 ± 1 mOsm/kg above control and were still elevated by 10 ± 2 and 14 ± 2 mOsm/kg at the end of the experiment. Amniotic fluid osmolality rose slightly but not significantly during fetal AVP infusion, and continued to rise

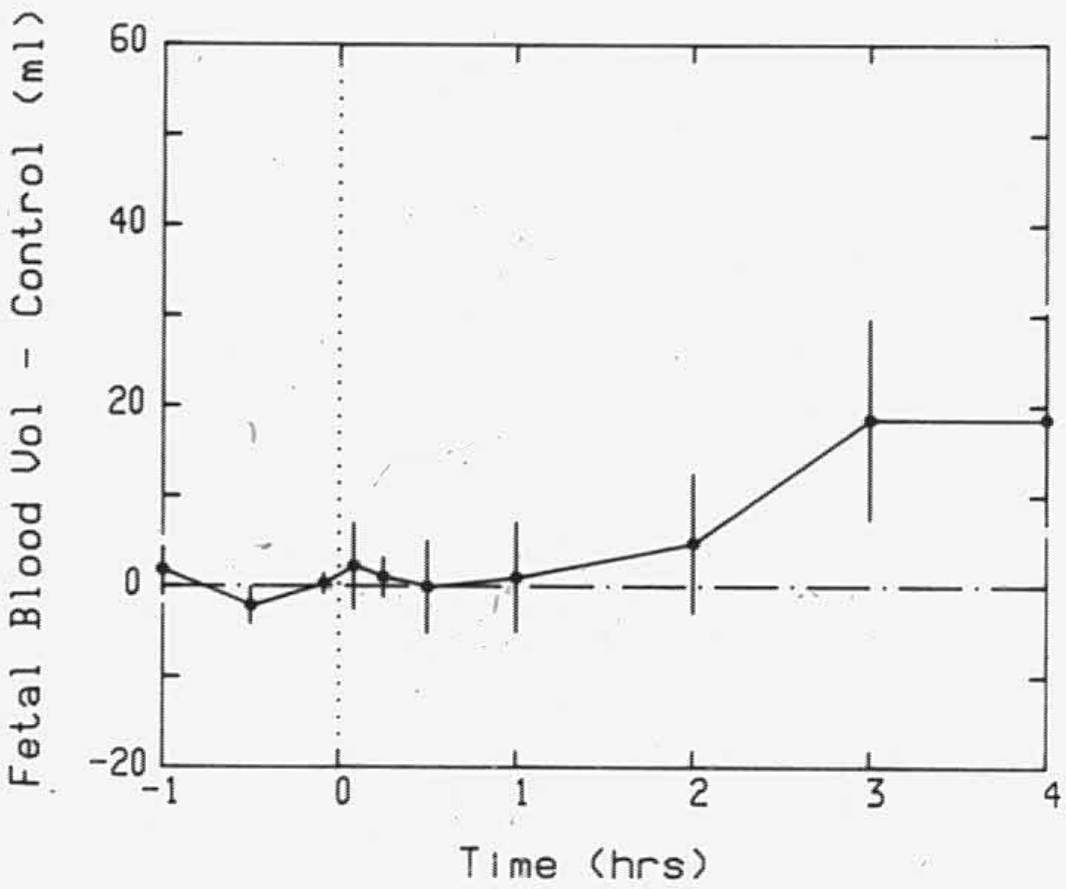


Figure 30. Time course of fetal blood volume changes in response to hypertonic injection at time zero. Control = 433 ± 20 ml. Mean change from control ± SE, n = 8.

gradually after hypertonic injection, reaching a value 14 ± 2 mOsm/kg above control by the end of the experiment ($p<.01$).

Fetal plasma Na^+ had fallen by 1.2 ± 0.3 mEq/l plasma water after two hours of fetal AVP infusion ($p<.01$), while maternal plasma Na^+ did not change significantly (Figure 32). Fetal and maternal plasma Cl^- also did not change. Following hypertonic injection, fetal and maternal plasma Na^+ rose to 6.3 ± 1.0 and 8.0 ± 1.2 mEq/l plasma water above control respectively and were still elevated by 4.8 ± 1.0 and 6.8 ± 0.8 mEq/l plasma water after four hours. Fetal and maternal plasma Cl^- rose by 9.4 ± 1.8 and 11.9 ± 2.6 mEq/l plasma, and were 9.1 ± 3.9 and 10.9 ± 1.6 mEq/l plasma above control at the end of the experiment.

Fetal plasma urea did not change significantly during the experiment (Figure 33). Fetal plasma K^+ did not change during AVP infusion, but was reduced by an average of 0.17 ± 0.03 mEq/l plasma water for the first thirty minutes after hypertonic injection ($p<.01$) and then returned to normal (Figure 34). Maternal plasma urea increased slightly during the control and fetal AVP infusion periods but did not change significantly after hypertonic injection (Figure 33). Maternal plasma K^+, on the other hand, remained constant until the hypertonic injection, when it fell by 0.44 ± 0.17 mEq/l plasma water ($p<.05$). Maternal plasma K^+ was not different from control values by the end of the experiment. Fetal

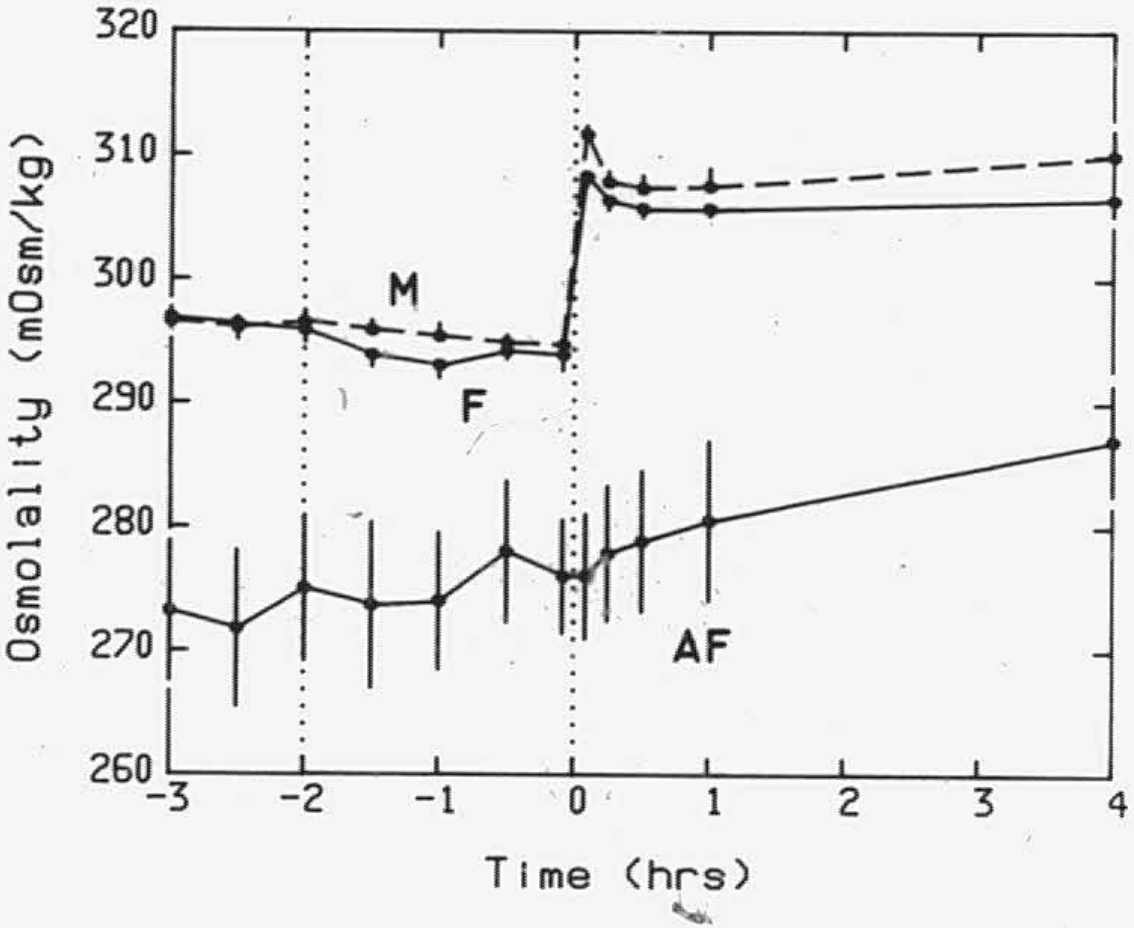


Figure 31. Time course of fetal (F) and maternal (M) plasma and amniotic fluid (AF) osmolalities. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 6.

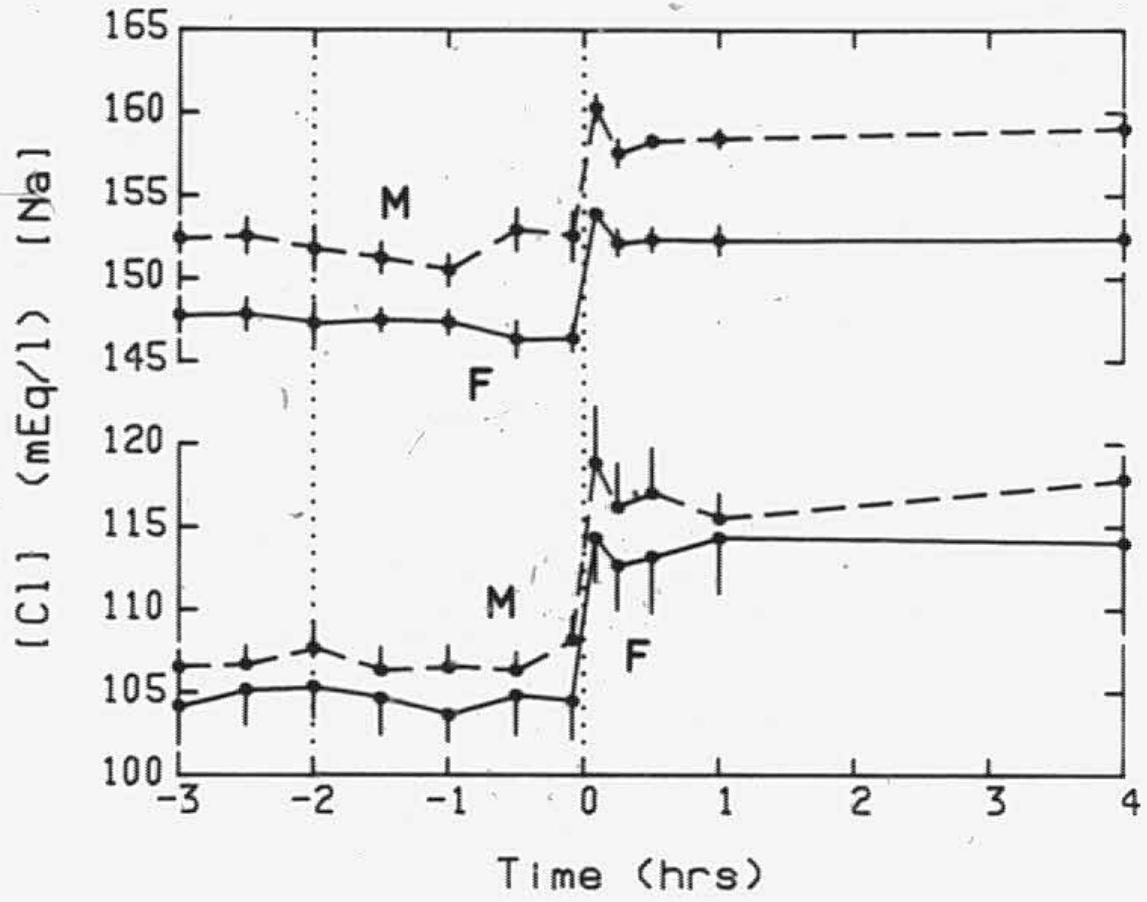


Figure 32. Time course of fetal (F) and maternal (M) plasma Na+ and Cl- concentrations. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 6.

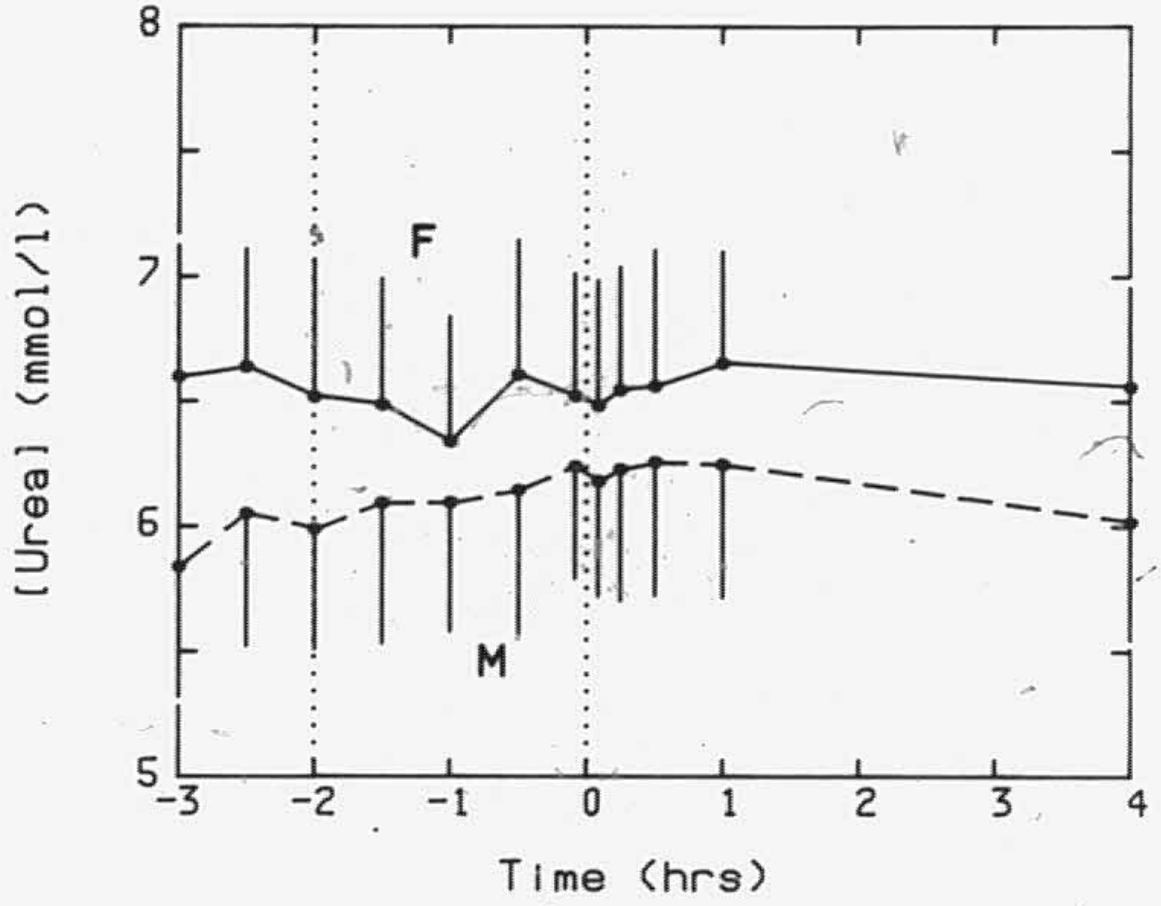


Figure 33. Time course of fetal (F) and maternal (M) plasma urea concentrations. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 6.

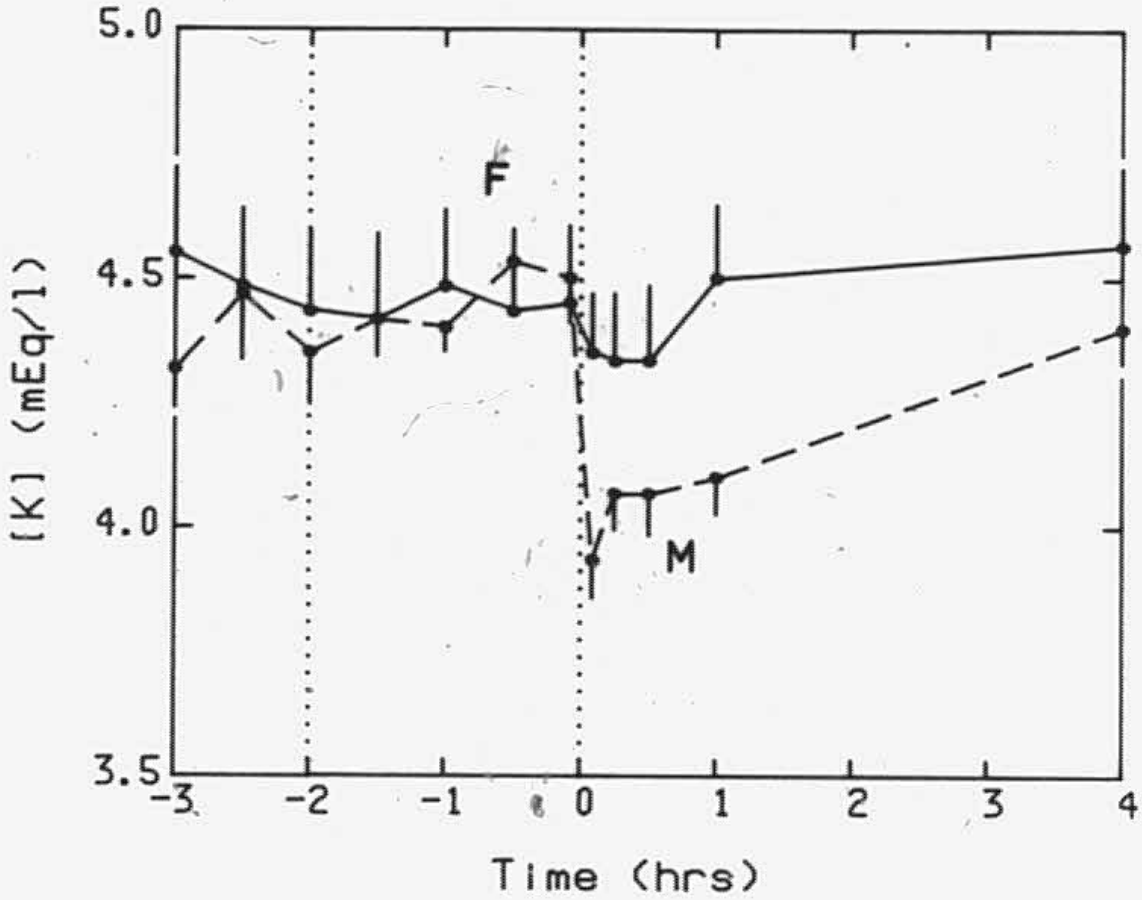


Figure 34. Time course of fetal (F) and maternal (M) plasma K+ concentrations. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 6.

urine osmolality increased in response to AVP infusion (Figure 35), reaching a value 123 ± 39 mOsm/kg above control one hour after the onset of infusion ($p<.05$). Throughout the remainder of the AVP infusion, as well as after hypertonic injection, fetal urine osmolality remained essentially constant. During the control period and the first hour of AVP infusion, maternal urine osmolality increased significantly from 683 ± 91 to 919 ± 90 mOsm/kg. Maternal urine osmolality tended to level off during the second hour of fetal AVP infusion. In response to hypertonic injection maternal urine osmolality fell initially to 657 ± 51 mOsm/kg, returned to pre-injection values within fifteen minutes, and then continued to increase.

Fetal urine Na^+ and Cl^- increased during the first hour of AVP infusion by 21 ± 11 and 15 ± 4 mEq/l respectively, and then plateaued (Figures 36 and 37). After hypertonic injection fetal urine Na^+ and Cl^- increased further to 62 ± 13 and 62 ± 11 mEq/l above the control level respectively at fifteen minutes post-injection ($p<.01$), and remained at these levels for the duration of the experiment. Maternal urine Na^+ did not change significantly during the period of fetal AVP infusion, while maternal urine Cl^- increased to 69 ± 9 mEq/l above control after two hours ($p<.001$). In response to hypertonic injection, maternal urine Na^+ and Cl^- increased rapidly at first and then more slowly, reaching

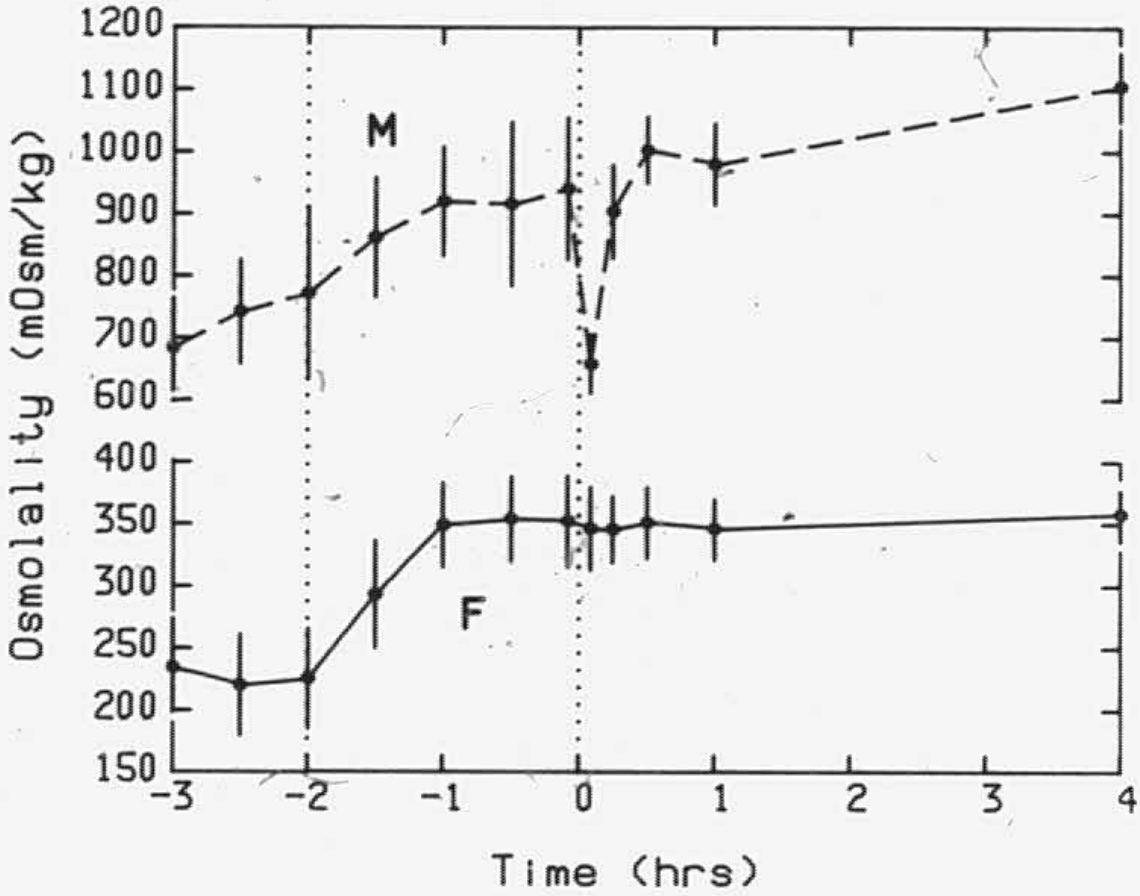


Figure 35. Time course of fetal (F) and maternal (M) urine osmolalities. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 6.

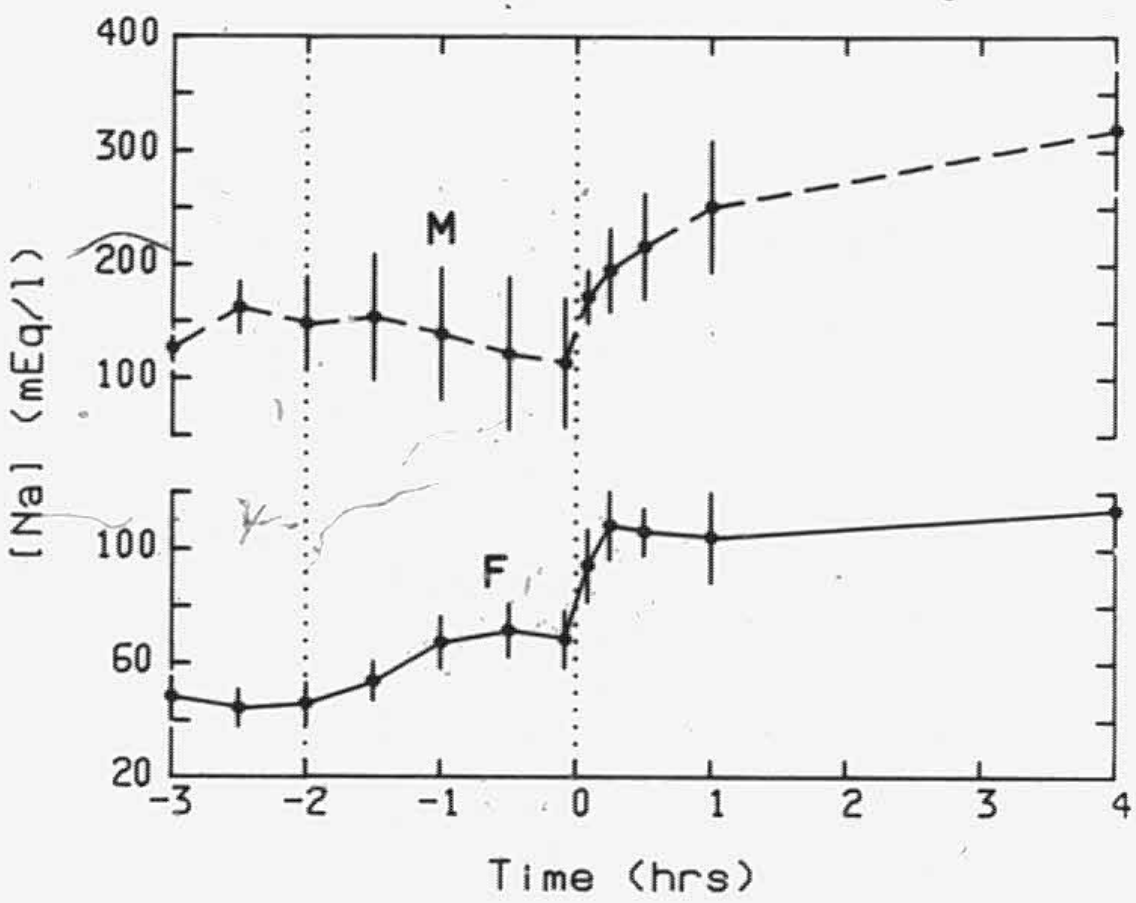


Figure 36. Time course of fetal (F) and maternal (M) urine Na+ concentrations. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean $\pm$ SE, n = 6.

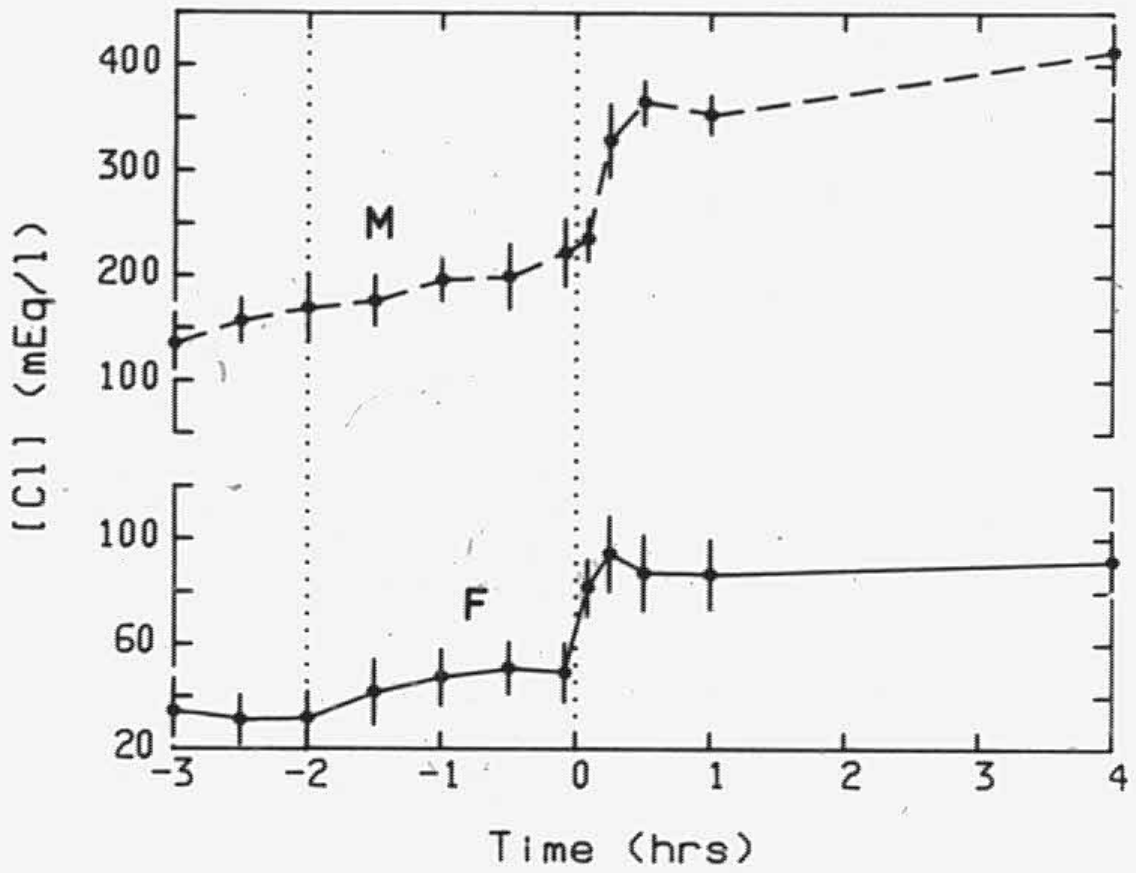


Figure 37. Time course of fetal (F) and maternal (M) urine Cl- concentrations. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 6.

173 ± 45 ($p<.02$) and 259 ± 16 mEq/l ($p<.001$) above control values after four hours.

Fetal urine urea and K^+ increased during the first hour of AVP infusion, to 34.1 ± 12.5 mmol/l and 16 ± 5 mEq/l above control respectively ($p<.05$), and then remained relatively constant during the second hour of infusion (Figures 38 and 39). After hypertonic injection, fetal urine urea and K^+ fell to 24.2 ± 5.6 mmol/l ($p<.01$) and 22 ± 7 mEq/l ($p<.05$) below pre-injection values respectively at fifteen minutes post-injection and remained essentially constant thereafter. Maternal urine urea increased during the control period and the first 1 to 1 1/2 hours of fetal AVP infusion, from 139 ± 21 to 283 ± 54 mmol/l after 2 1/2 hours, and then plateaued (Figure 38). Maternal urine K^+ showed a similar pattern, increasing from 84 ± 24 to 163 ± 26 mEq/l after two hours and then tending to level off (Figure 39). After hypertonic injection, maternal urine urea fell by 119 ± 47 mmol/l from the immediate pre-injection value and then increased but did not again reach pre-injection levels. Maternal urine K^+ also fell in response to hypertonic injection, by 67 ± 22 mEq/l from the immediate pre-injection level ($p<.05$), returned to pre-injection values within fifteen minutes, and had fallen to 75 ± 27 mEq/l below the pre-injection level after four hours ($p<.05$).

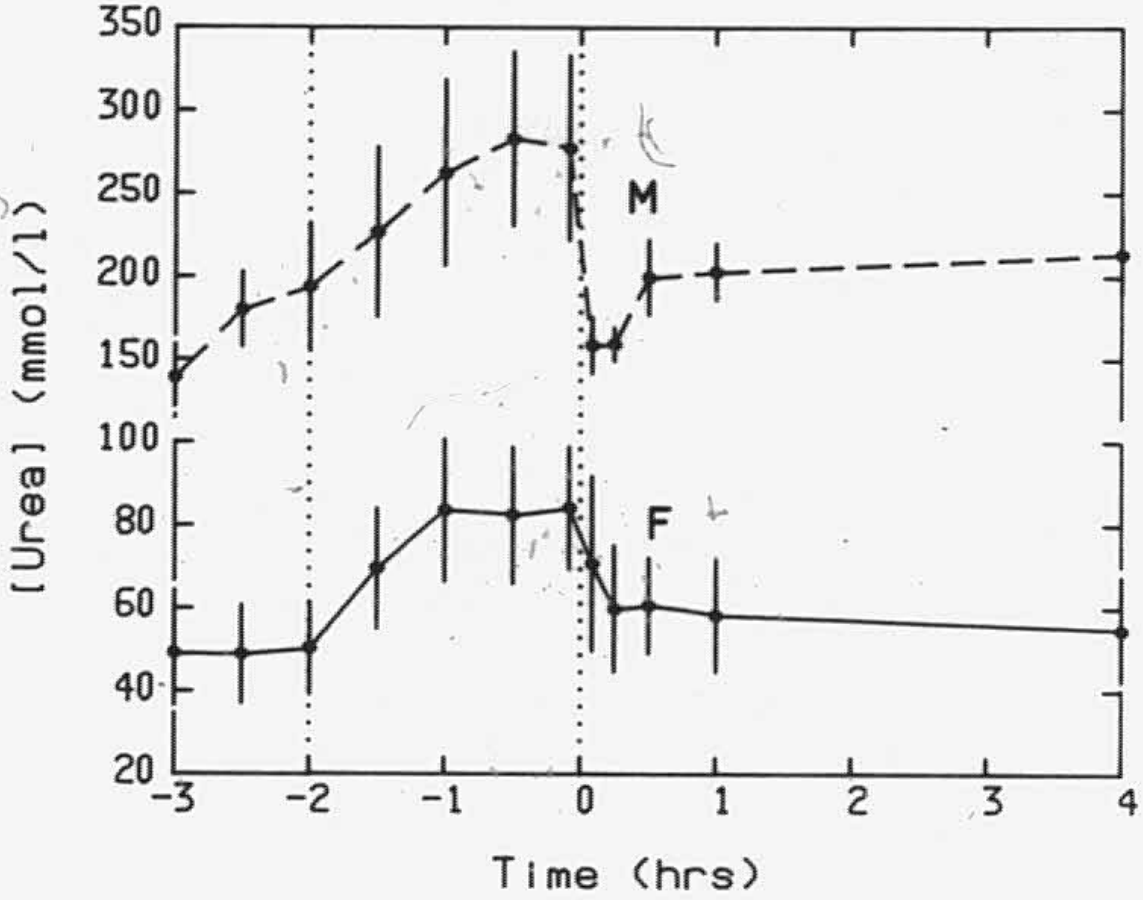


Figure 38. Time course of fetal (F) and maternal (M) urine urea concentrations. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero.

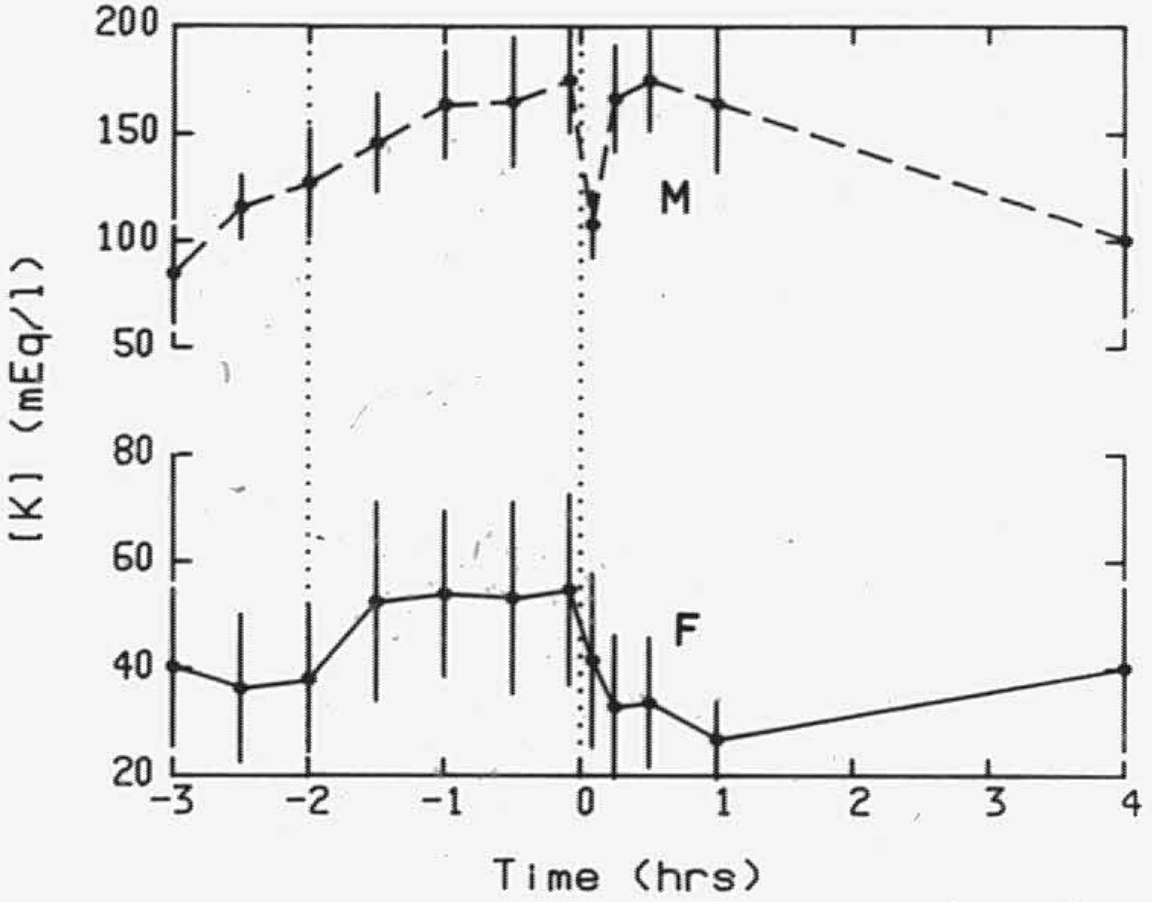


Figure 39. Time course of fetal (F) and maternal (M) urine K+ concentrations. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 6.

b. Effects on plasma AVP levels

The effects on plasma AVP levels of fetal AVP infusion followed by hypertonic injection are shown in Figure 40. Fetal AVP had increased by 2.6 ± 1.0 pg/ml 30 minutes after the onset of the infusion and continued to increase. After hypertonic injection fetal AVP increased further by 2.0 ± 1.6 pg/ml. Maternal plasma AVP (not shown) did not change significantly during fetal AVP infusion, and responses to hypertonic injection were small (1-2 pg/ml) and variable so that the changes were not significant.

c. Effects on continuously-measured variables

The effects of AVP infusion and hypertonic injection on fetal cardiovascular variables are shown in Figures 41-43. Fetal arterial pressure was elevated by an average of 2 mmHg during AVP infusion, was increased transiently by 3.9 ± 1.9 mmHg over control values in response to hypertonic injection, returned to control values for approximately two hours, and then tended to be elevated by approximately 2 mHg for the remainder of the experiment. Fetal venous pressure tended to show a similar pattern although the changes were not significant because pressures were so variable. Fetal heart rate tended to be decreased during AVP infusion, although this change was not statistically significant. Following hypertonic injection, fetal heart rate was increased by an average of 21 ± 8 bpm over values during the infusion period

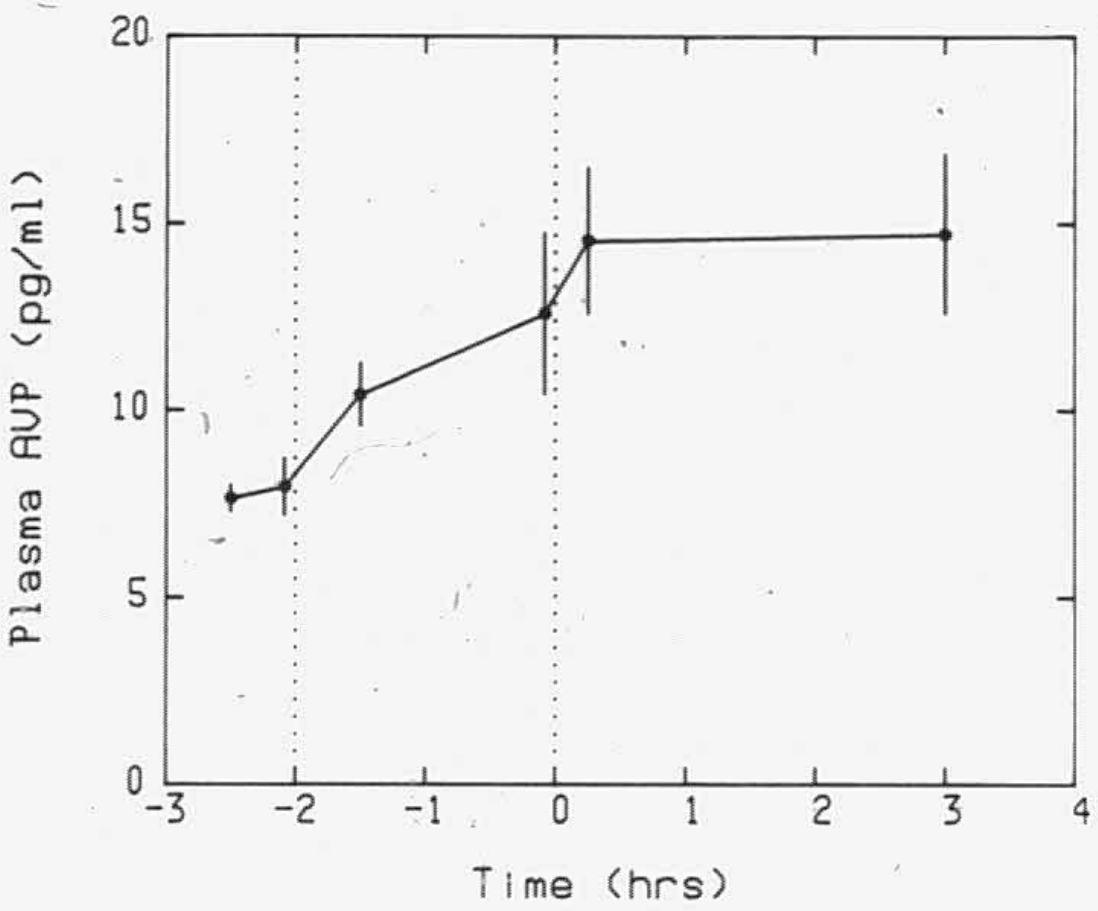


Figure 40. Time course of fetal plasma AVP levels. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean $\pm$ SE, n = 6.

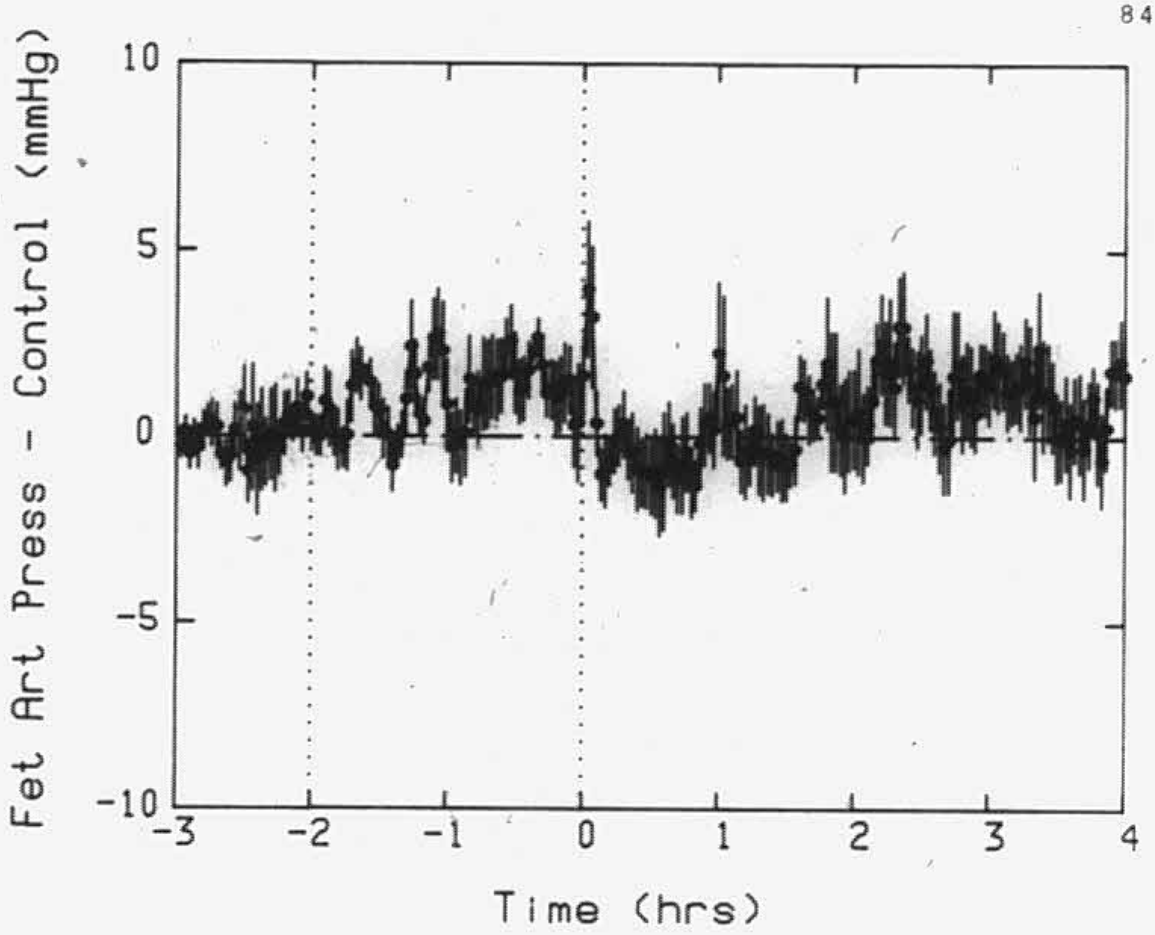


Figure 41. Time course of fetal arterial pressure changes. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Control = 41.9 ± 1.8 mmHg. Mean change from control ± SE, n = 6.

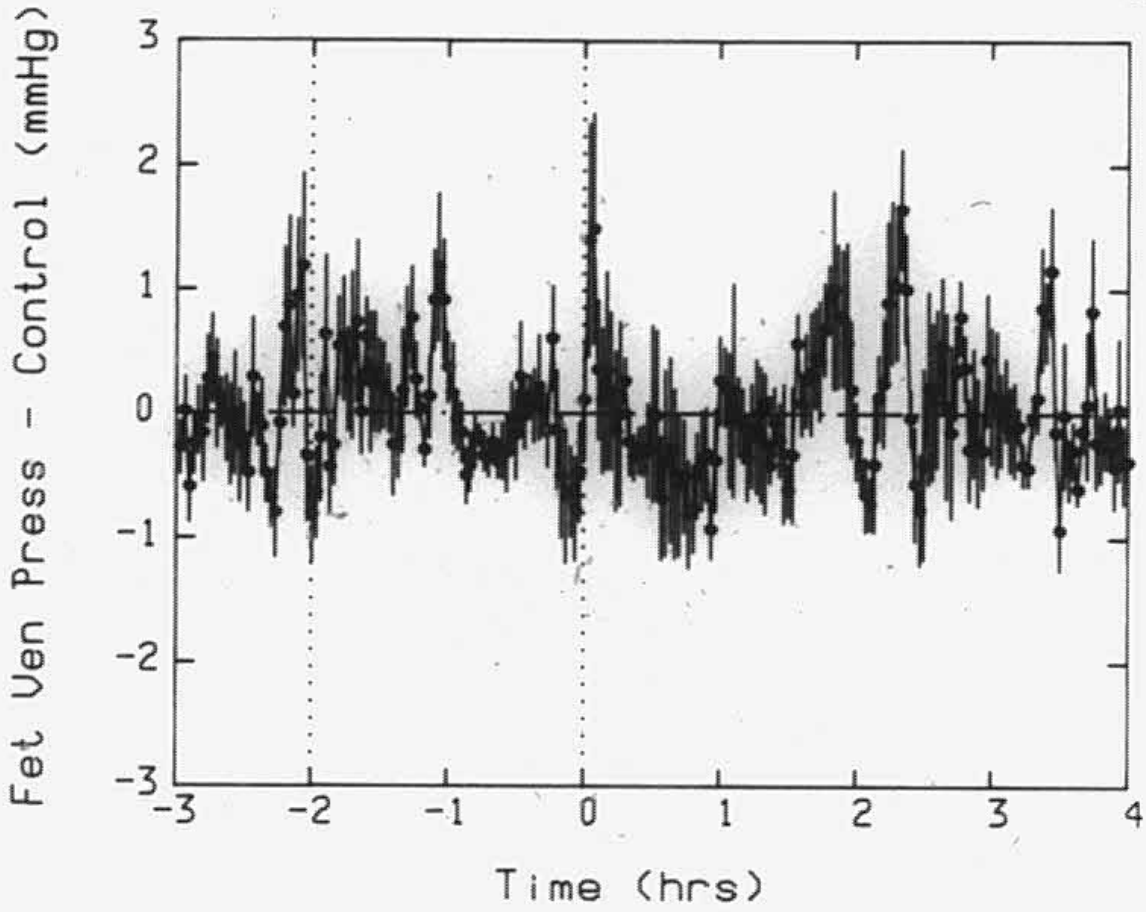


Figure 42. Time course of fetal venous pressure changes. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Control = 4.00 $\pm$ 0.67 mmHg. Mean change from control $\pm$ SE, n = 6.

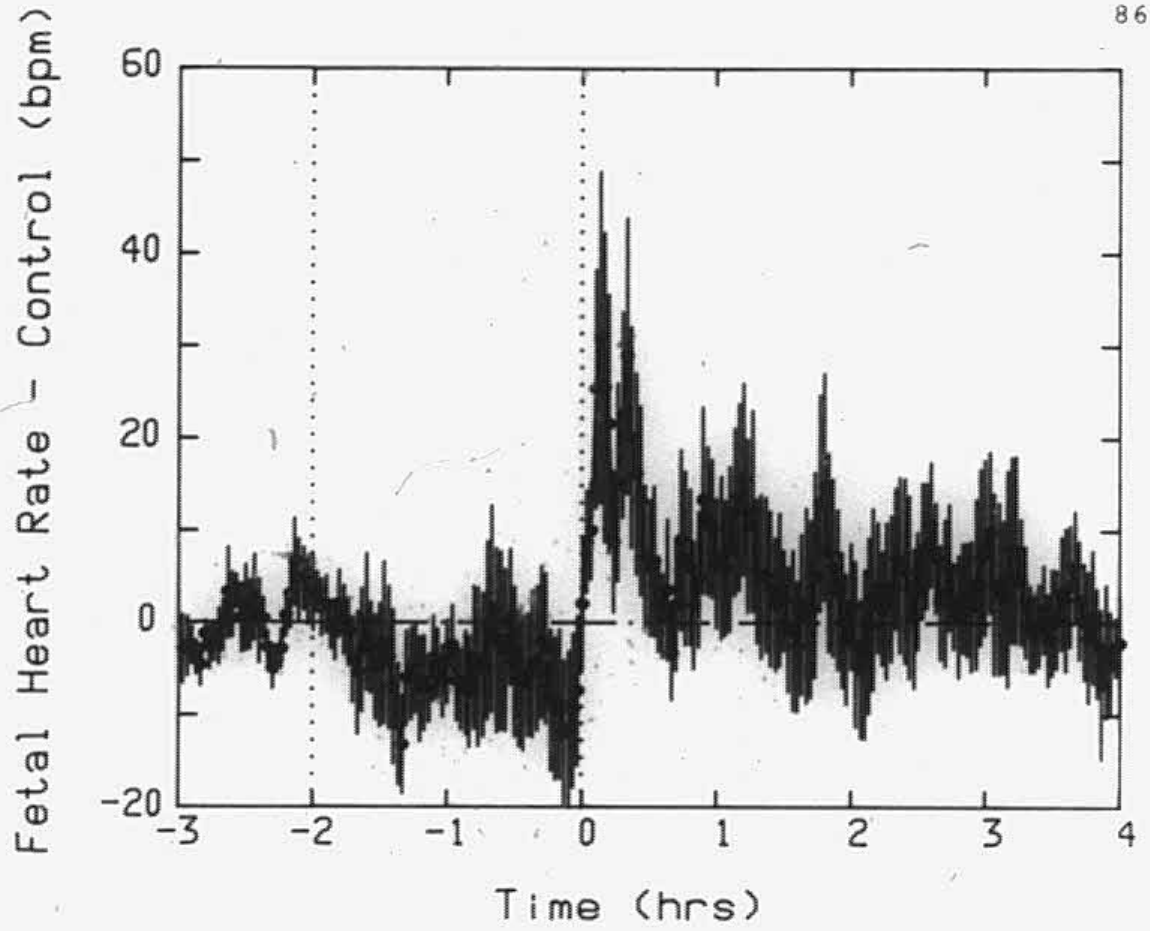


Figure 43. Time course of fetal heart rate changes. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Control = 147 ± 8 bpm. Mean change from control ± SE, n = 6.

($p<.05$) for the first half hour and tended to remain elevated for the duration of the experiment.

The responses of fetal urine output to AVP infusion and hypertonic injection are shown in Figure 44. Fetal urine flow had fallen by 0.21 ± 0.07 ml/min within five minutes after the onset of AVP infusion ($p<.05$), and averaged 0.25 ml/min below control for the next two hours. After hypertonic injection, fetal urine flow increased transiently to a peak of 1.10 ± 0.41 ml/min above control values ($p<.05$) but returned to the control level within five to ten minutes and did not change significantly during the rest of the experiment.

The responses of maternal urine output were not different in the AVP-infusion and antagonist-infusion experiments, and so these two sets of data were treated as one group. The effects of hypertonic injection on maternal urine flow in these animals are shown in Figure 45. Maternal urine flow fell gradually during the control and fetal infusion periods, from an initial value of 3.9 ± 0.9 to 1.4 ± 0.2 ml/min three hours later. Following hypertonic injection, maternal urine flow rose sharply by a peak of 4.2 ± 0.8 ml/min over the immediate pre-injection value ($p<.001$), fell to 1.0 ± 0.3 ml/min above the pre-injection value within 15-20 minutes, and remained at this level for the rest of the experiment.

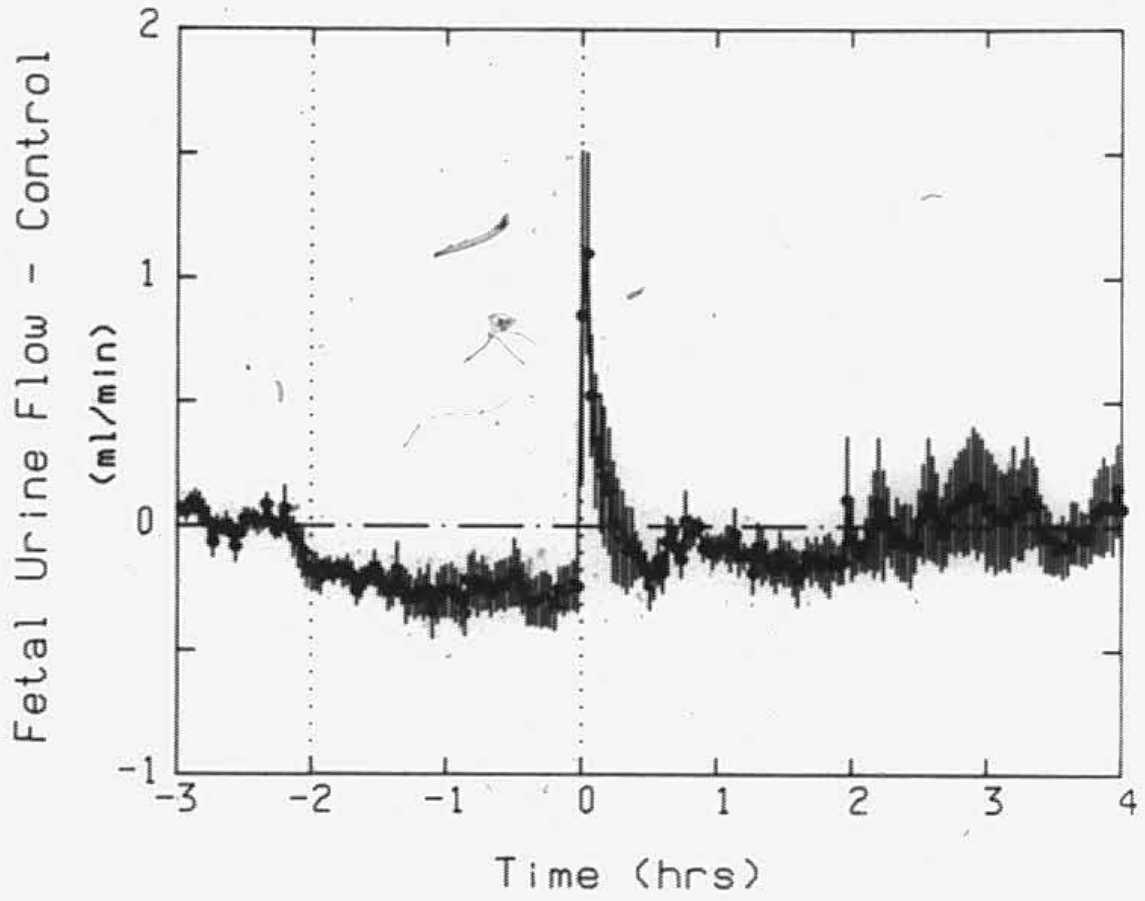


Figure 44. Time course of fetal urine flow. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Control = 0.7 $\pm$ 0.2 ml/min.. Mean change from control $\pm$ SE, n = 6.

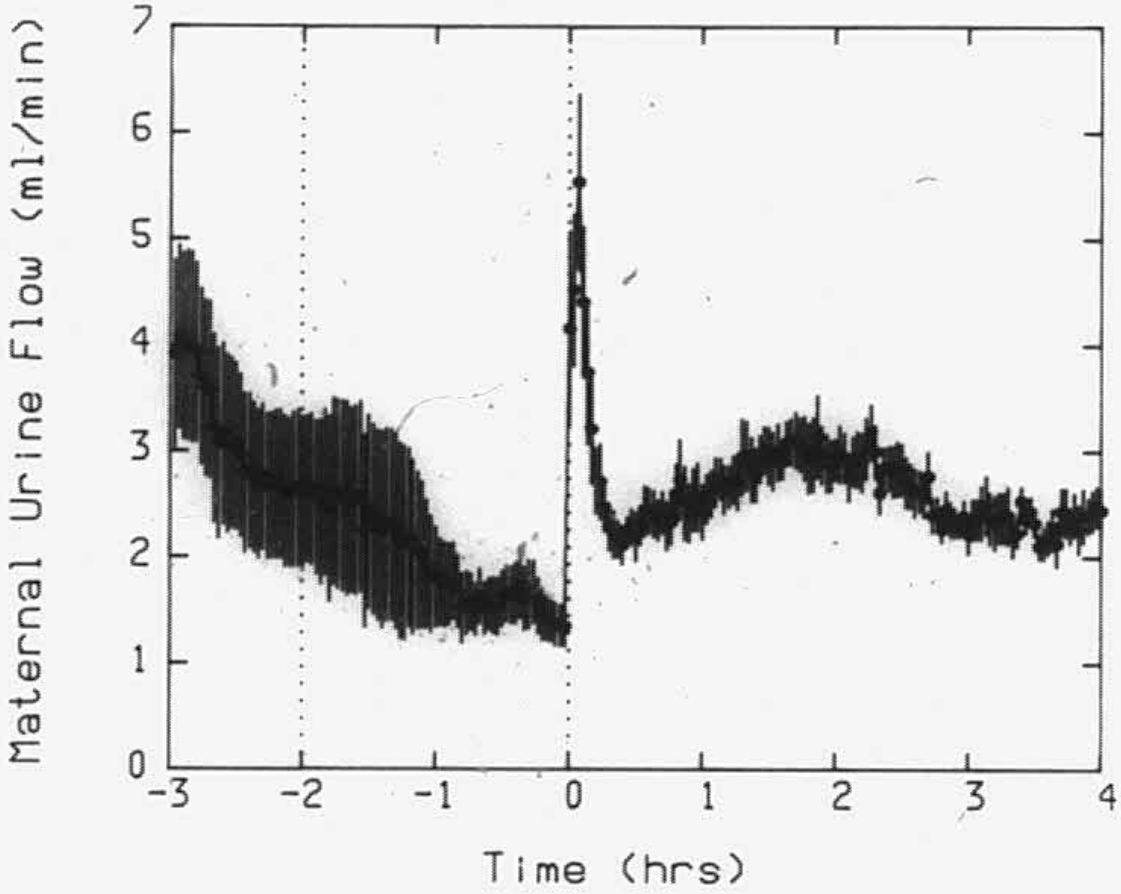


Figure 45. Time course of maternal urine flow. Fetal AVP or antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 10.

d. Effects on fetal GFR

The effects of AVP infusion followed by hypertonic injection on fetal GFR are shown in Figure 46. Fetal GFR fell from 5.2 ± 0.5 to 3.6 ± 0.4 ml/min during the control period and then increased slightly to 4.4 ± 0.4 ml/min during the AVP infusion. Fetal GFR was increased to 9.0 ± 1.3 ml/min five minutes after hypertonic injection but had returned to normal ten minutes later and remained constant thereafter.

e. Effects on renal clearance of chemical constituents

The effects on fetal renal clearance values of AVP infusion followed by hypertonic injection are shown in Figures 47-51. Fetal C_{Na}, C_{Cl}, C_{osm}, C_K, and C_{urea} were essentially unchanged during the control and AVP-infusion periods. After hypertonic injection, fetal C_{Na}, C_{Cl}, and C_{osm} rose transiently to 0.71 ± 0.27, 0.76 ± 0.24, and 1.08 ± 0.25 ml/min respectively. These three variables fell toward control values until 30 minutes post-injection and then rose gradually to 0.60 ± 0.27, 0.59 ± 0.24, and 0.78 ± 0.27 ml/min respectively by the end of the experiment. Fetal C_K and C_{urea} increased transiently in response to hypertonic injection, to 6.9 ± 1.8 and 8.5 ± 1.6 ml/min respectively, but returned to control values by 15 minutes post-injection and did not change thereafter.

The responses of maternal renal clearance values during fetal AVP infusion were not different from those during

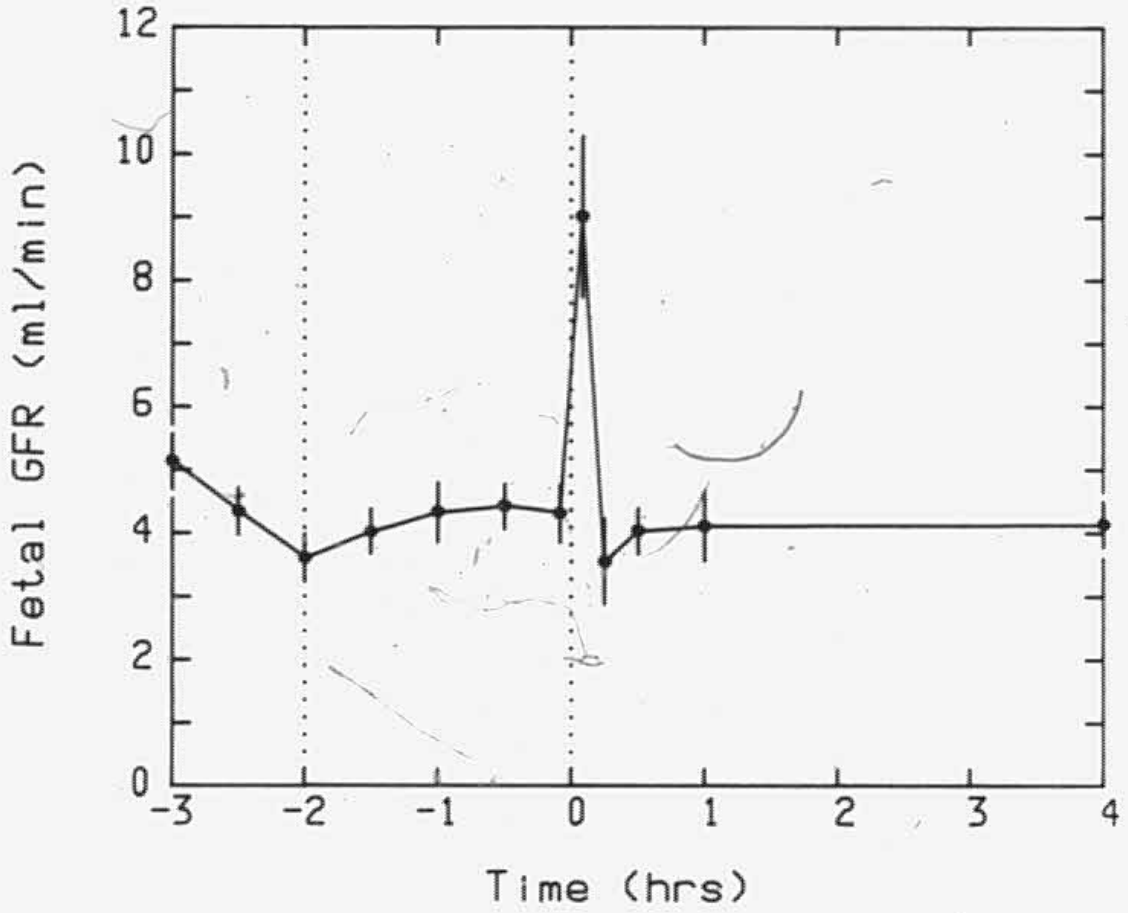


Figure 46. Time course of fetal GFR. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean $\pm$ SE, n = 6.

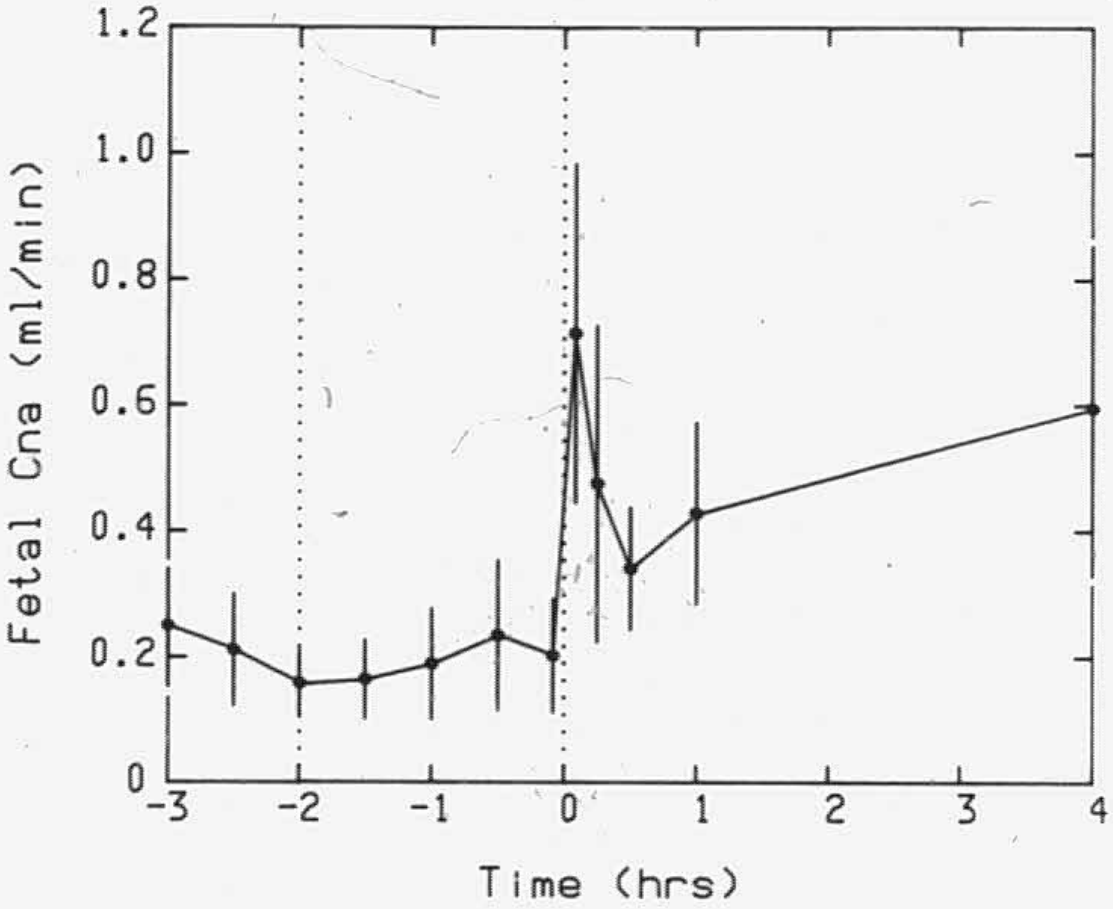


Figure 47. Time course of fetal Na+ clearance. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 6.

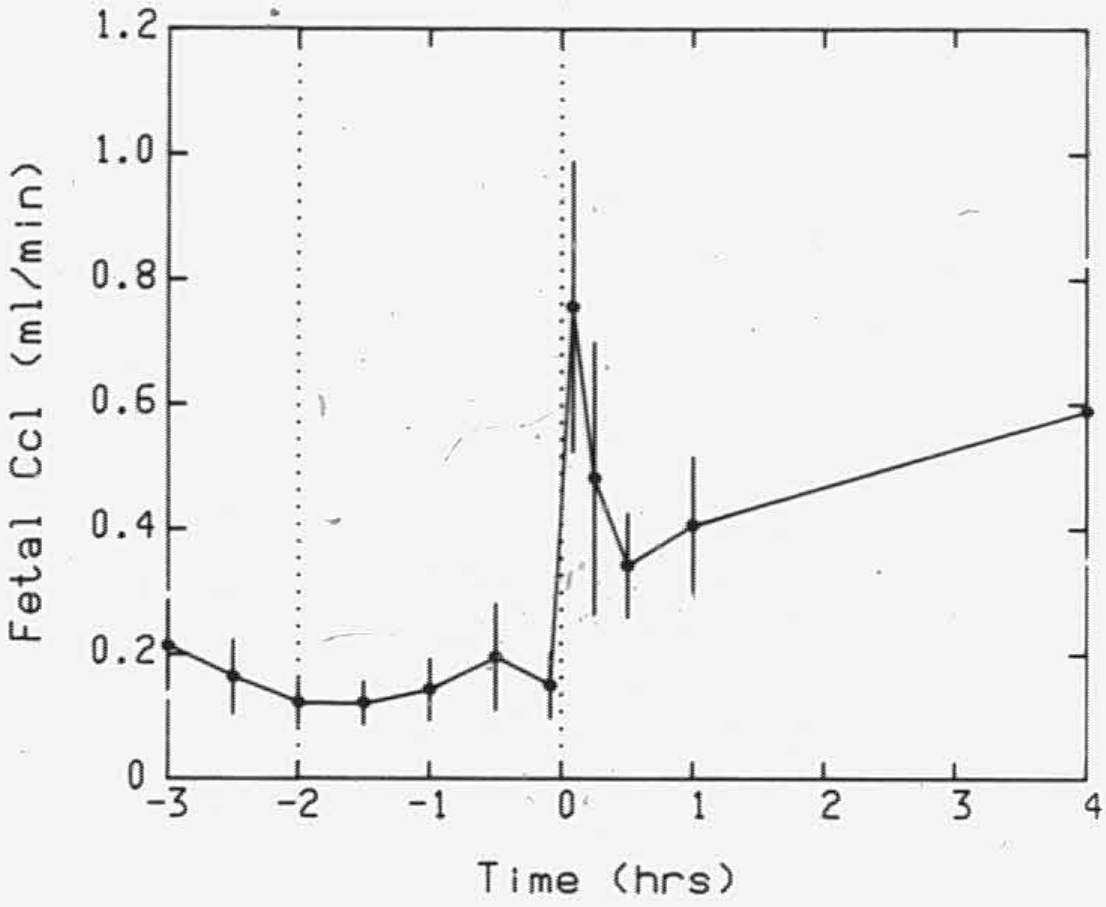


Figure 48. Time course of fetal Cl- clearance. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 6.

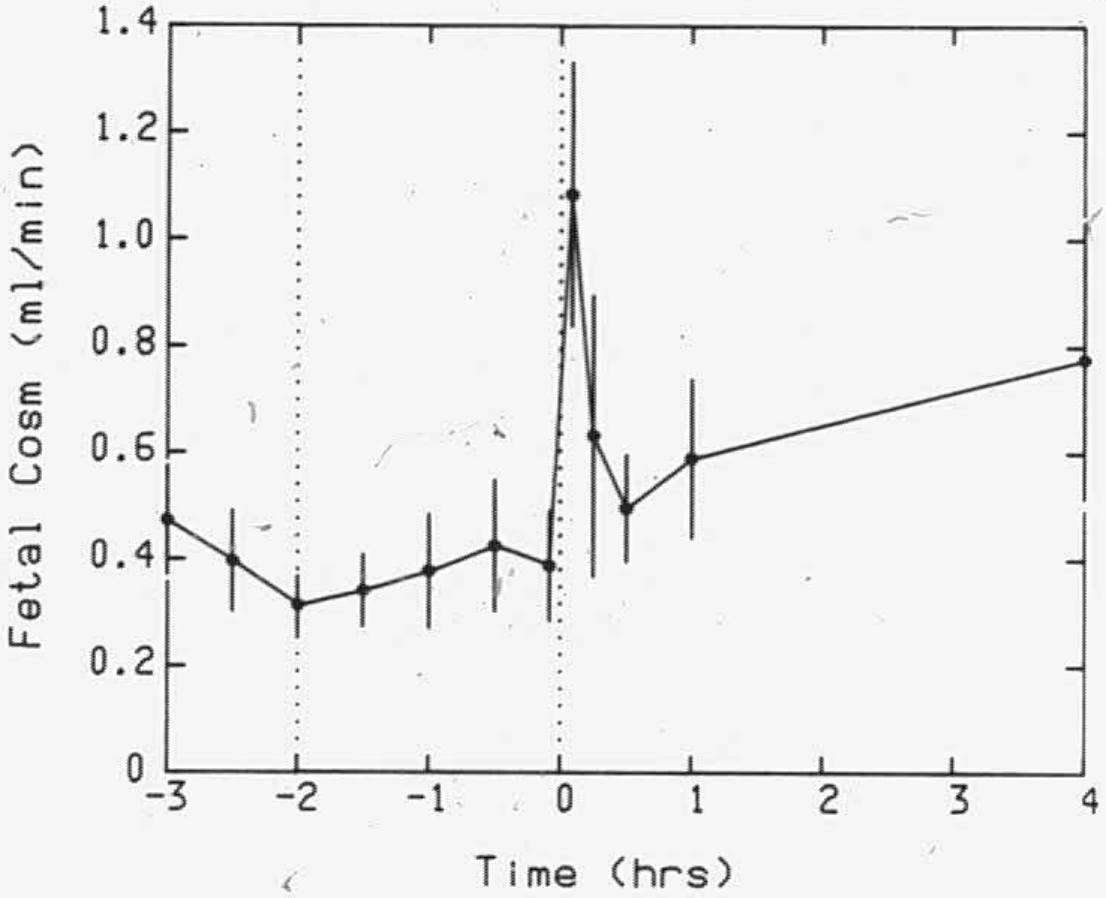


Figure 49. Time course of fetal osmolar clearance. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean $\pm$ SE, n = 6.

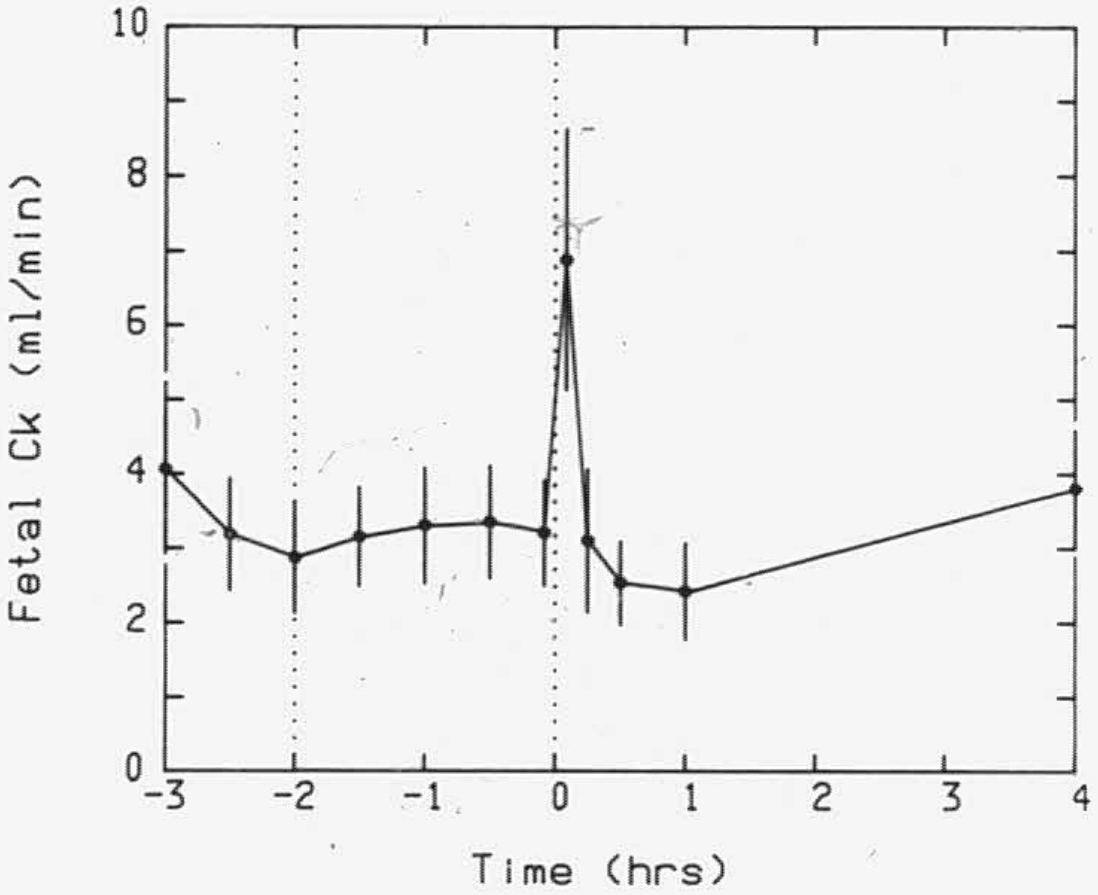


Figure 50. Time course of fetal K+ clearance. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 6.

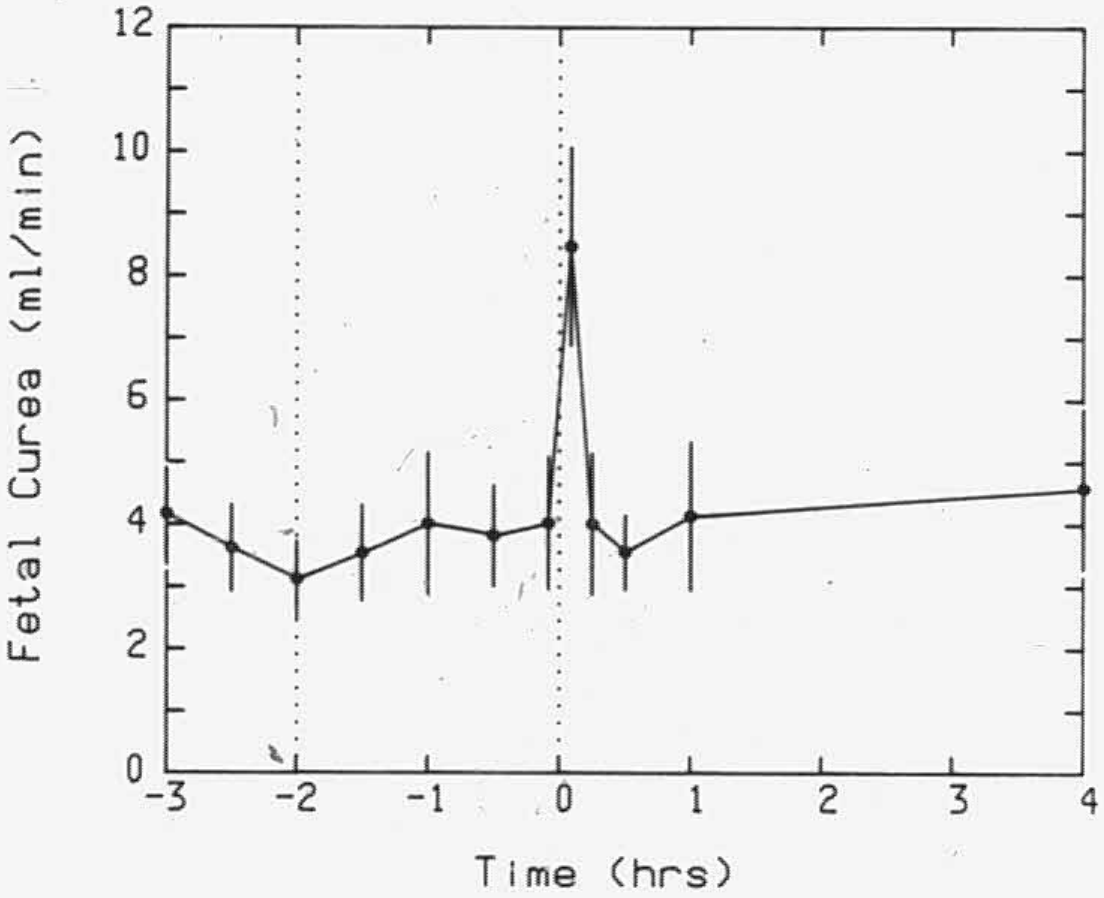


Figure 51. Time course of fetal urea clearance. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 6.

antagonist infusion, so these data were pooled. The results are shown in Figures 52-56. Maternal C_{Na}, C_{Cl}, and C_{osm} fell gradually during the control and fetal infusion periods. Following hypertonic injection, maternal C_{Na} rose initially to 5.5 $\pm$ 0.9 ml/min, fell to 1.4 $\pm$ 0.3 ml/min by 30 minutes post-injection, and then increased to 5.5 $\pm$ 0.7 ml/min by the end of the experiment. C_{Cl} followed a similar pattern, peaking at 10.0 $\pm$ 1.4 ml/min, falling to 6.9 $\pm$ 0.4 ml/min at 30 minutes, and increasing to 8.4 $\pm$ 0.8 ml/min by four hours post-injection. Likewise, maternal C_{osm} increased to 10.5 $\pm$ 1.6 ml/min in response to hypertonic injection, fell to 7.0 $\pm$ 0.4 ml/min in 30 minutes, and then increased to 8.6 $\pm$ 0.8 ml/min by the end of the experiment.

Maternal C_K fell slightly during the control and fetal infusion periods, increased to 110 $\pm$ 16 ml/min immediately following hypertonic injection, and gradually fell to control values by the end of the experiment. Maternal C_{urea} increased transiently to 117 $\pm$ 16 ml/min in response to hypertonic injection, returned to control values by 15 minutes post-injection, and then rose slightly during the rest of the experiment.

f. Effect on free water clearance

The effects of AVP infusion and hypertonic injection on fetal renal C_{water} are shown in Figure 57. Fetal C_{water} was positive during the control period, fell to 0.03 $\pm$ 0.08

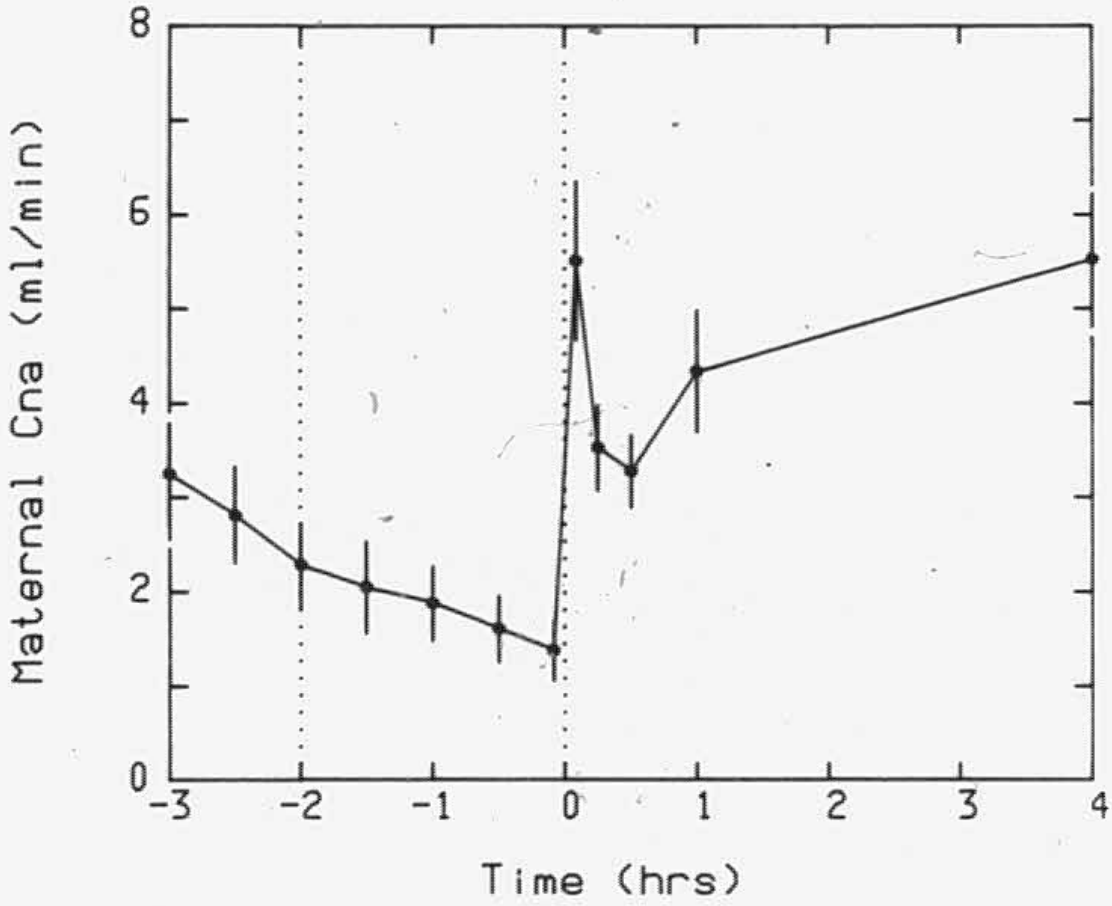


Figure 52. Time course of maternal Na+ clearance. Fetal AVP or antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 10.

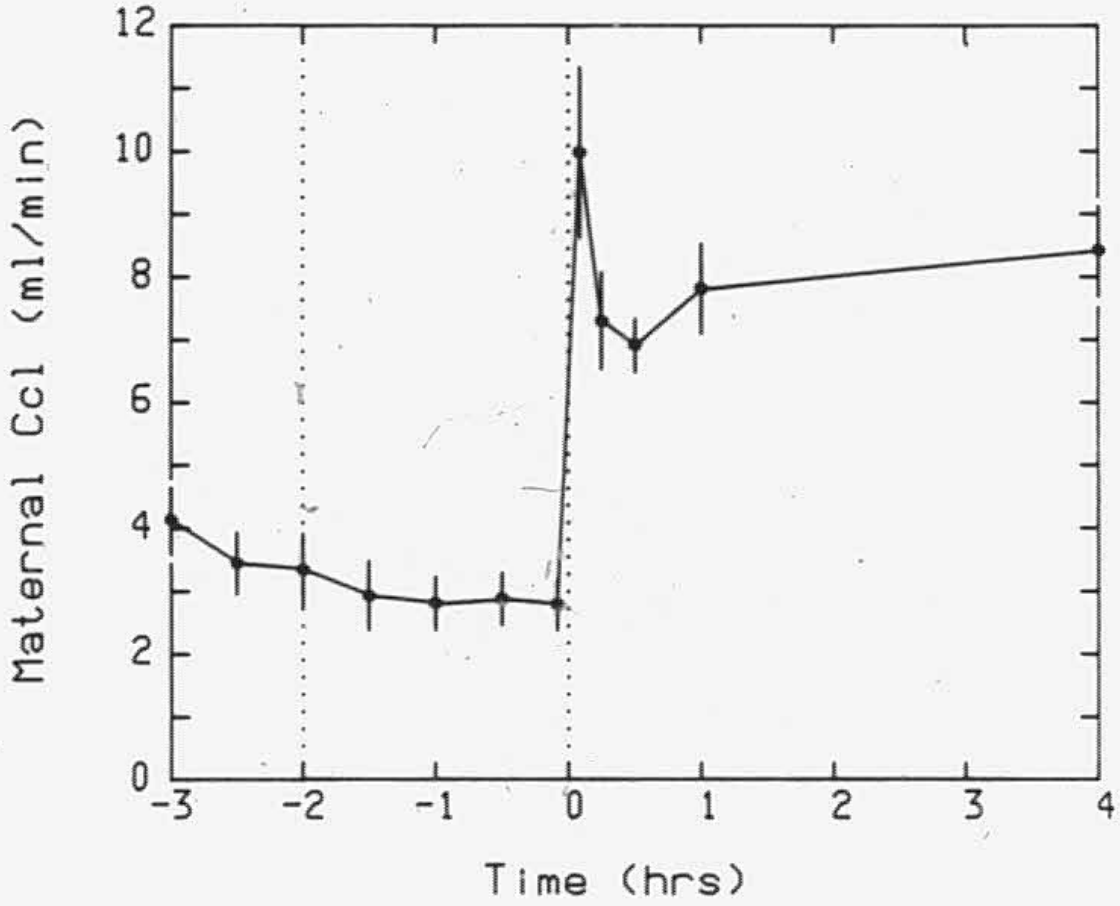


Figure 53. Time course of maternal Cl- clearance. Fetal AVP or antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 10.

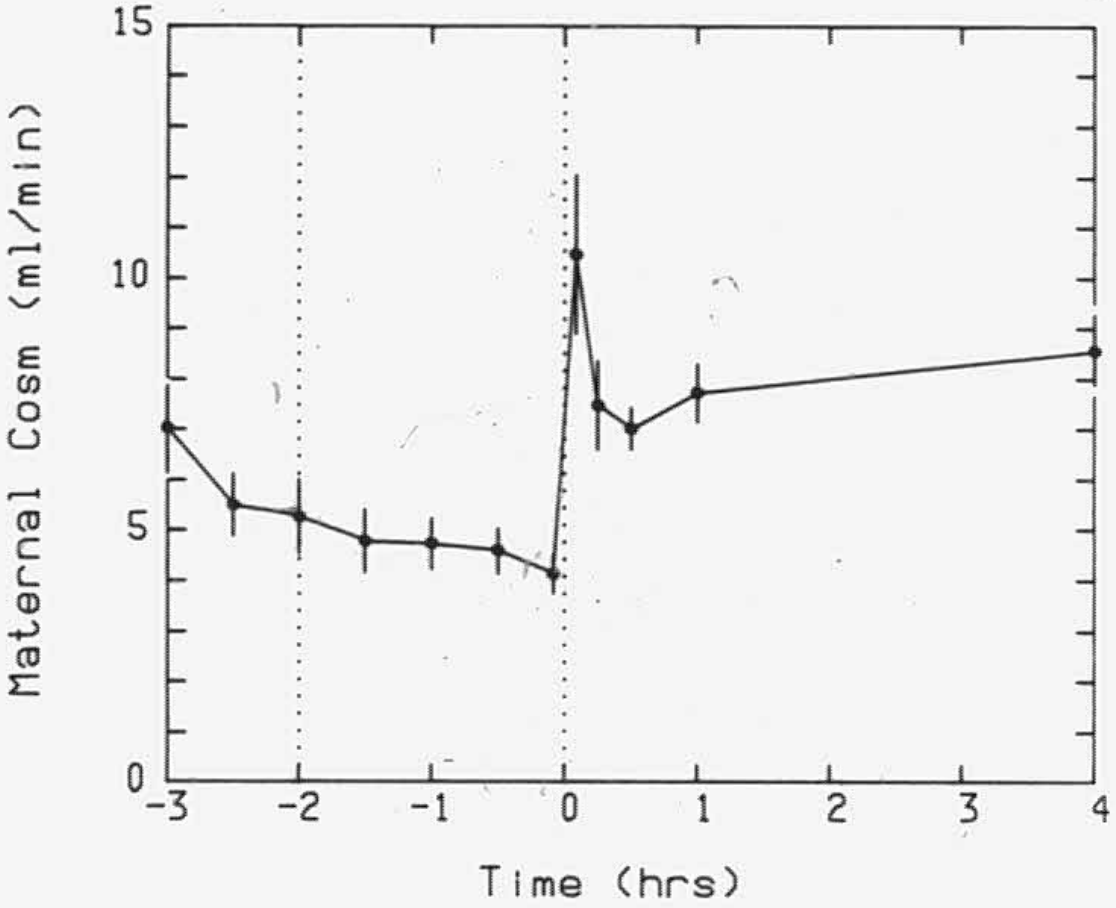


Figure 54. Time course of maternal osmolar clearance. Fetal AVP or antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 10.

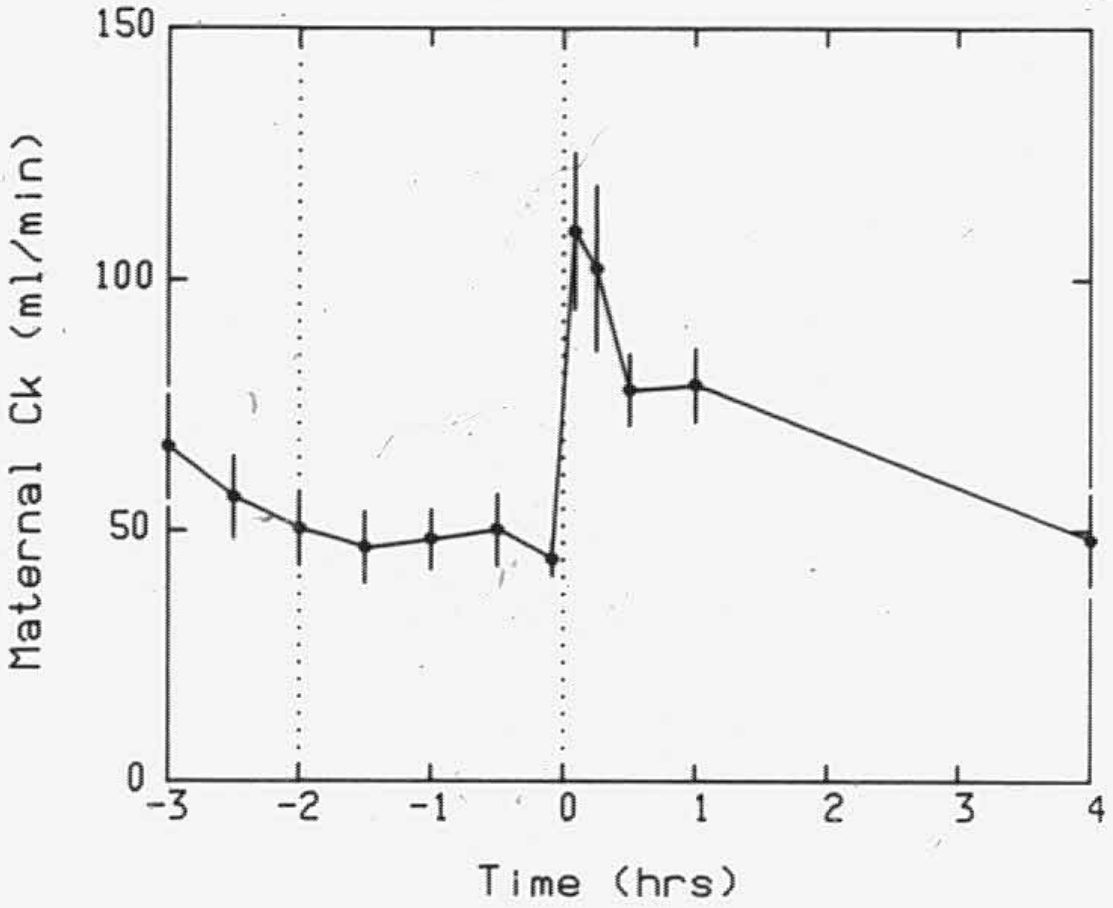


Figure 55. Time course of maternal K+ clearance. Fetal AVP or antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 10.

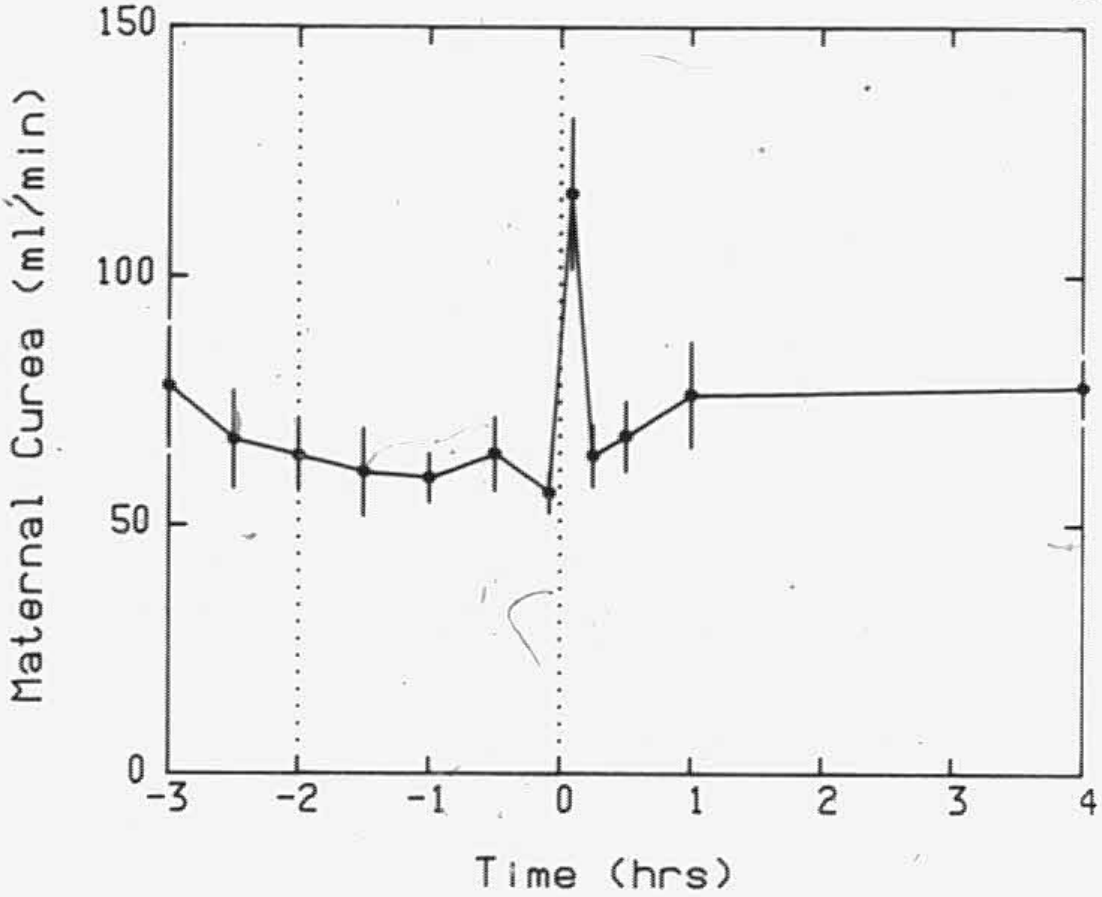


Figure 56. Time course of maternal urea clearance. Fetal AVP or antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 10.

ml/min during the first hour of AVP infusion, and remained constant for the rest of the experiment.

The responses of maternal C_{water} during fetal AVP and antagonist infusions were not different, so these data were pooled. The results are shown in Figure 58. Maternal C_{water} was constant and negative during the control and infusion periods, fell to -5.5 $\pm$ 1.1 ml/min after hypertonic injection, and remained at this level thereafter.

g. Effect on fetal blood volume

As shown in Figure 59, fetal blood volume fell slightly but significantly during AVP infusion, to 8 $\pm$ 2 ml below control 1 1/2 hours after the onset of infusion ($p<.01$). After hypertonic injection fetal blood volume was increased for at least 30 minutes, by a peak of 10 $\pm$ 4 ml at 15 minutes. Beyond one hour post-injection fetal blood volume was not different from the control value.

5. Hypertonic Injections into Antagonist-Infused Fetuses:

a. Effects on plasma, urine, and amniotic fluid constituents

The responses of fetal and maternal plasma and amniotic fluid constituents to antagonist infusion and hypertonic injection are shown in Figures 60-62. Fetal and maternal plasma osmolalities fell slightly but not significantly during the antagonist infusion, to 1.5 $\pm$ 0.7 and 2.5 $\pm$ 1.0 mOsm/kg below control respectively two hours after the onset of infusion. Following hypertonic injection, fetal and maternal

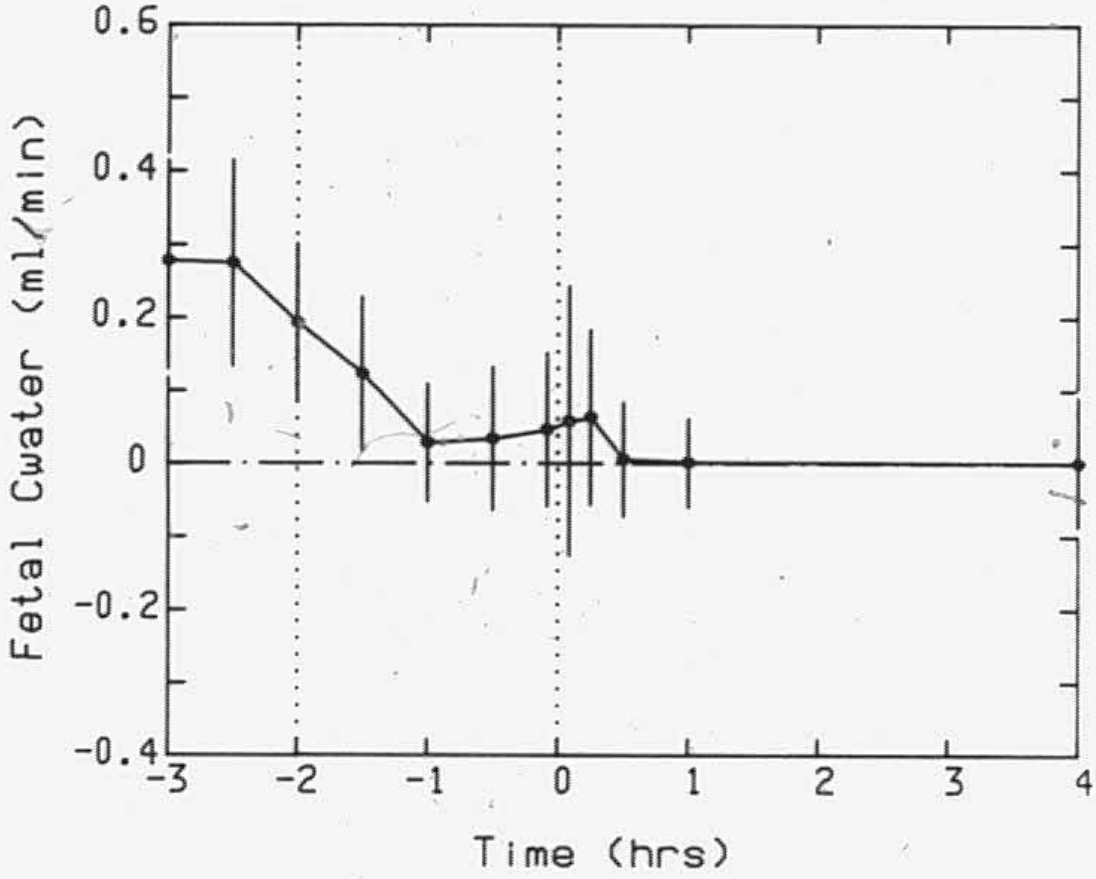


Figure 57. Time course of fetal free water clearance. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 6.

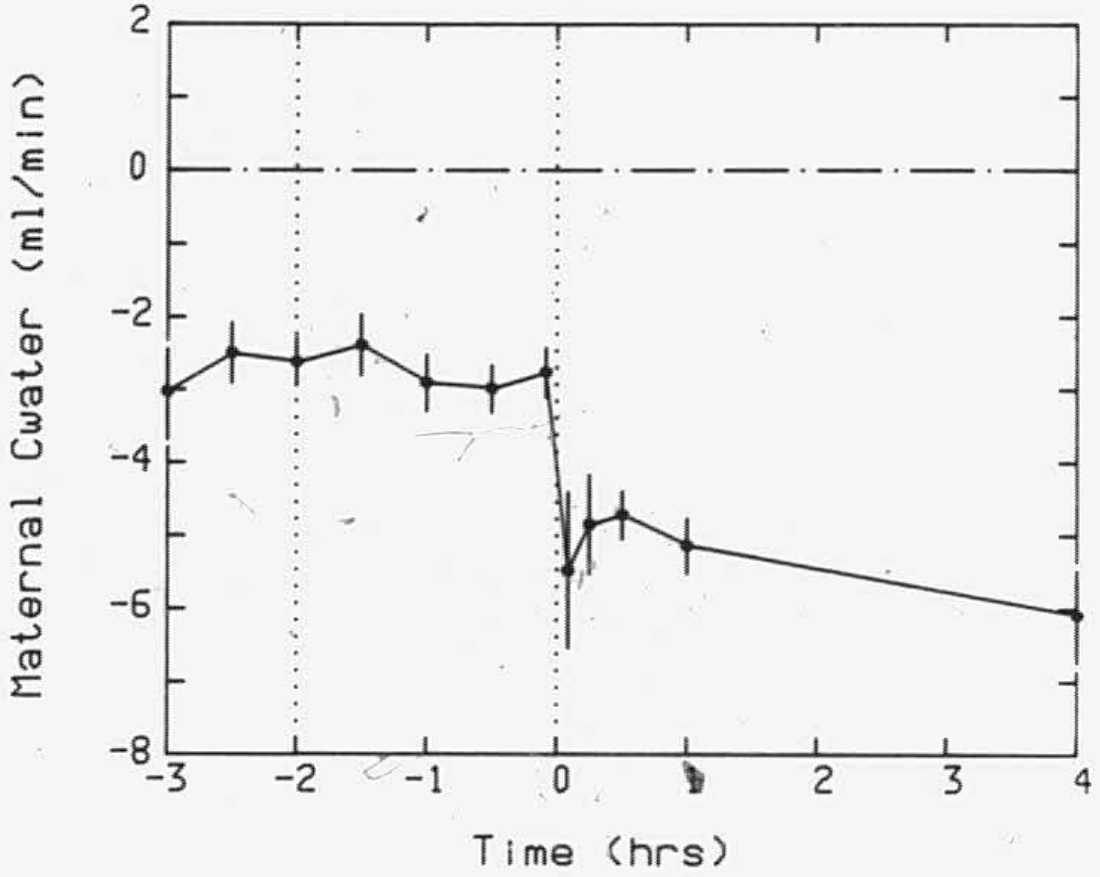


Figure 58. Time course of maternal free water clearance. Fetal AVP or antagonist injection was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 10.

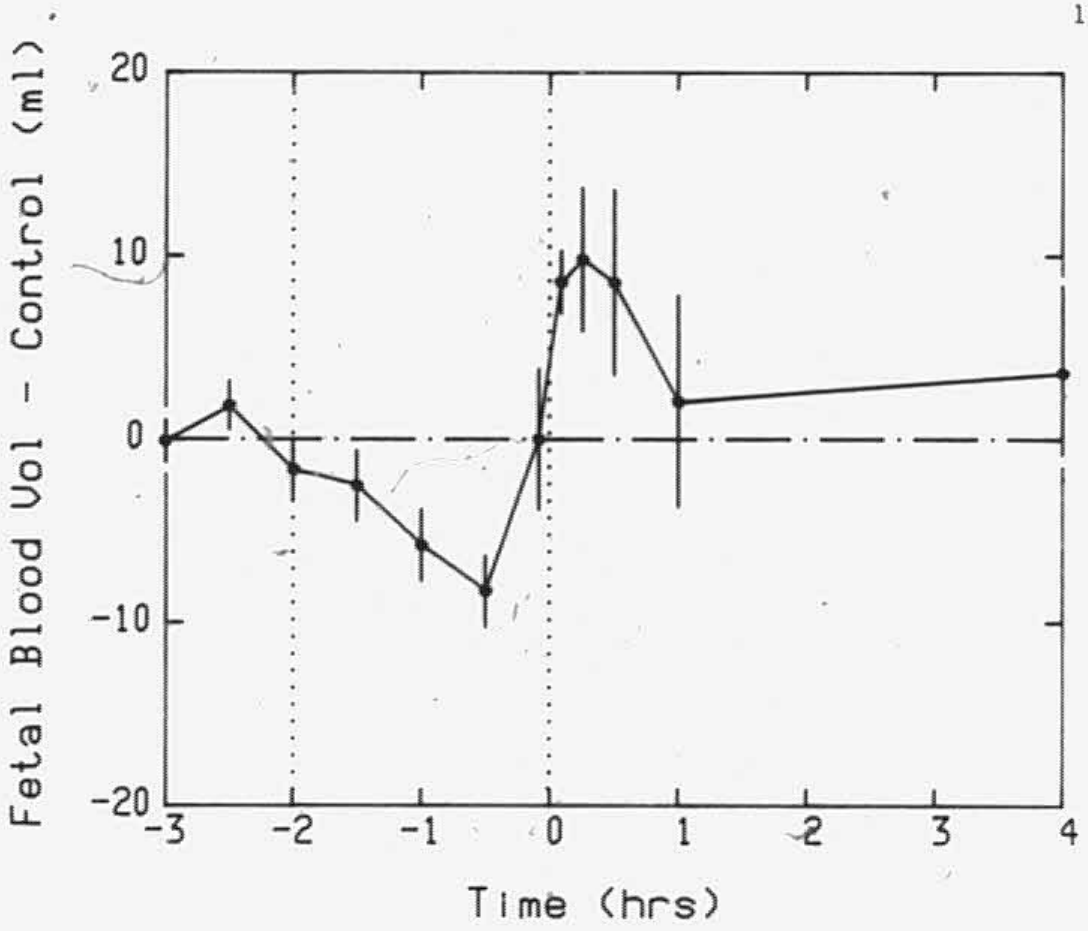


Figure 59. Time course of fetal blood volume changes. Fetal AVP infusion was begun at -2 hrs and hypertonic injection was done at time zero. Control = 472 ± 22 ml. Mean change from control ± SE, n = 6.

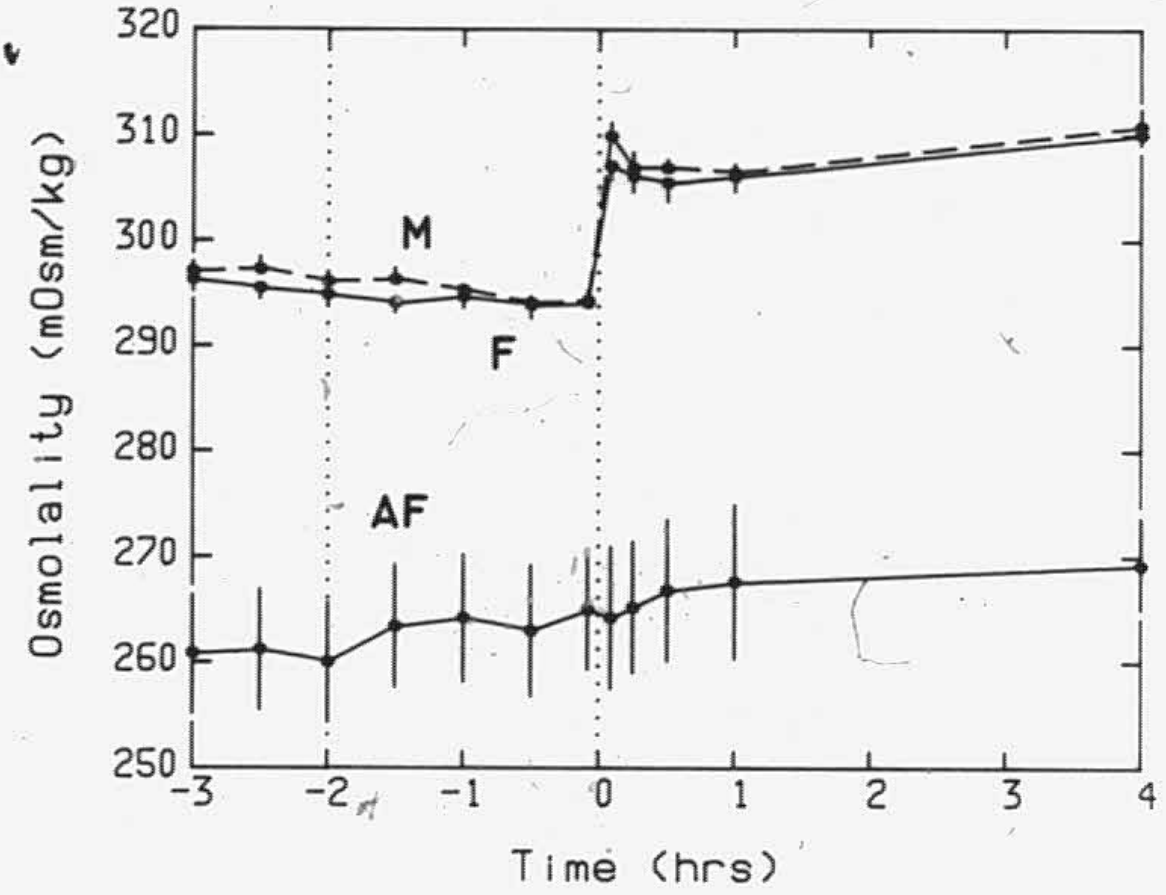


Figure 60. Time course of fetal (F) and maternal (M) plasma and amniotic fluid (AF) osmolalities. Fetal antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 5.

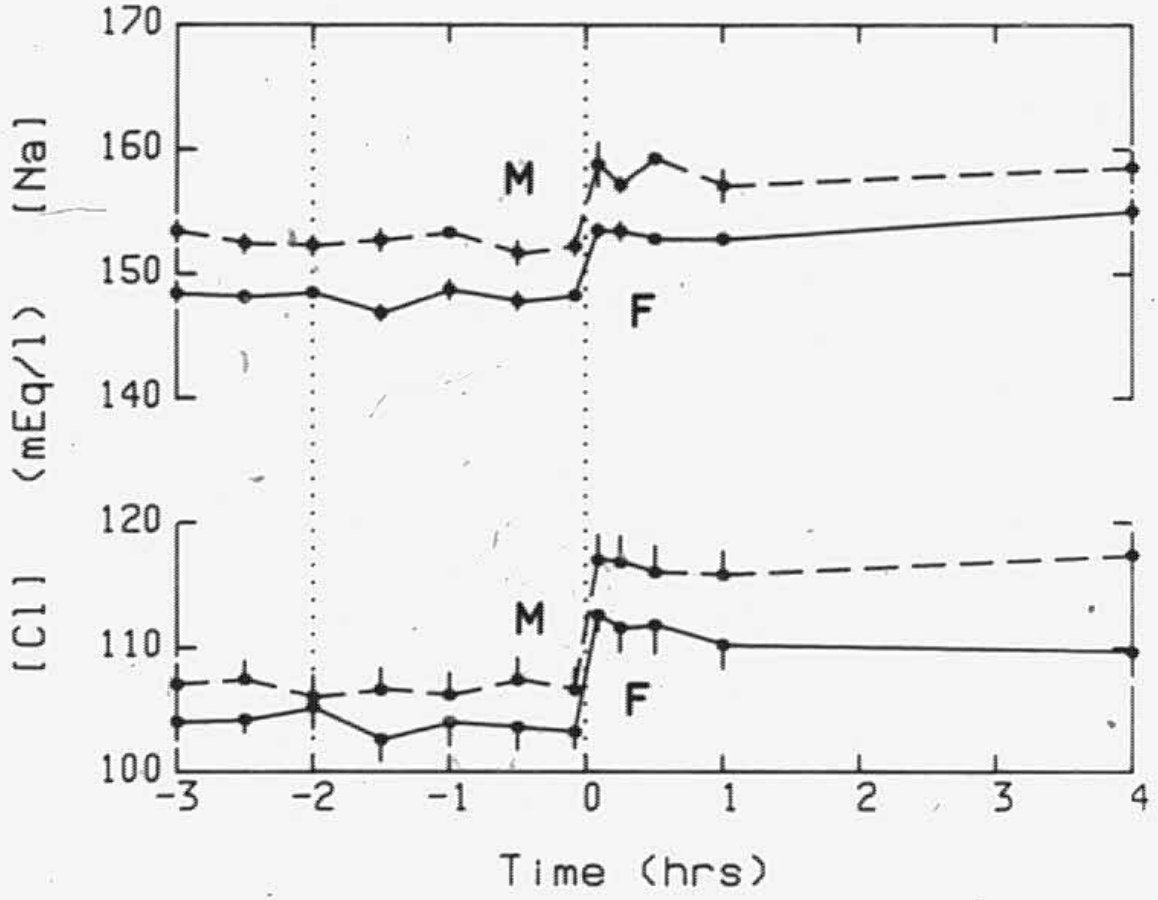


Figure 61. Time course of fetal (F) and maternal (M) plasma Na+ and Cl- concentrations. Fetal antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 5.

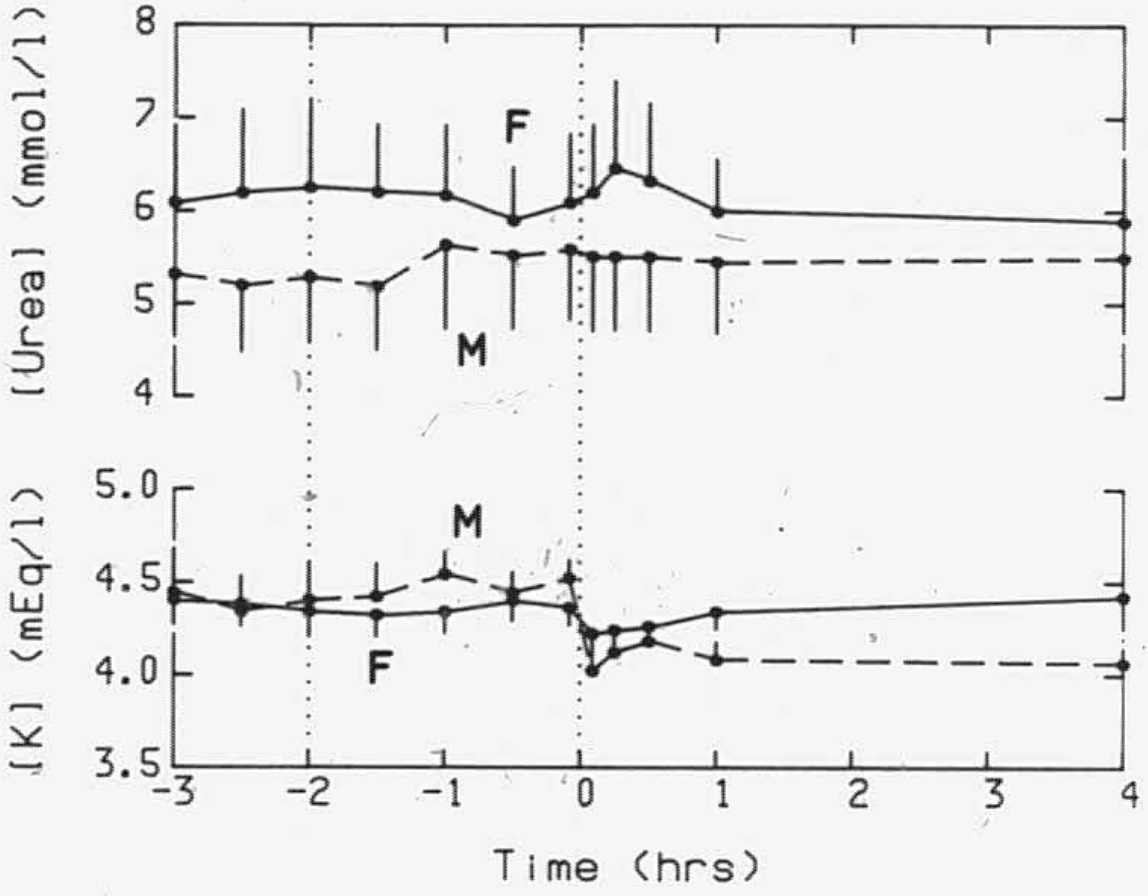


Figure 62. Time course of fetal (F) and maternal (M) plasma urea and K+ concentrations. Fetal antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 5.

plasma osmolalities rose to 12 ± 1 and 13 ± 1 mOsm/kg above control respectively, fell slightly, and then increased again to 15 ± 1 and 14 ± 2 mOsm/kg above control respectively by the end of the experiment. Amniotic fluid osmolality did not change significantly during the antagonist infusion and the increase in amniotic fluid osmolality after hypertonic injection was small (4 ± 1 mOsm/kg). Fetal and maternal plasma Na^+ concentrations did not change significantly during the antagonist infusion but were increased by 5.1 ± 0.5 and 6.1 ± 1.8 mEq/l plasma water after hypertonic injection and remained at these levels thereafter (Figure 61). Fetal plasma Cl^- fell slightly but significantly by 1.9 ± 0.5 mEq/l plasma 30 minutes after the onset of antagonist infusion ($p<.02$) but was not different from control over the next 1 1/2 hours. Fetal and maternal plasma Cl^- was increased by 8.1 ± 1.2 and 10.2 ± 1.2 mEq/l plasma following hypertonic injection and remained at these levels for the duration of the experiment.

Fetal plasma K^+ did not change during the antagonist infusion, fell by 0.19 ± 0.05 mEq/l plasma water immediately following hypertonic injection ($p<.02$), and had returned to control values one hour later (Figure 62). Maternal plasma K^+ tended to fall after hypertonic injection, but this decrease did not reach statistical significance. Fetal and maternal plasma urea concentrations did not change significantly during the experiment.

The responses of fetal and maternal urine constituents are shown in Figures 63-67. The osmolality of fetal urine did not change significantly during the antagonist infusion and after hypertonic injection (Figure 63). Maternal urine osmolality increased from 648 ± 155 to 1018 ± 154 mOsm/kg during the control and fetal antagonist infusion periods, fell to 671 ± 93 mOsm/kg immediately following hypertonic injection ($p<.02$), and then increased again, rapidly at first and then more slowly, to 1044 ± 88 mOsm/kg by the end of the experiment. Fetal urine Na^+ and Cl^- did not change significantly during the antagonist infusion, but rose significantly by 24 ± 7 ($p<.05$) and 27 ± 5 ($p<.05$) mEq/l respectively over the immediate pre-injection value in response to hypertonic injection and remained elevated for the rest of the experiment (Figures 64 and 65). Maternal urine Na^+ increased following hypertonic injection, by 195 ± 26 mEq/l after four hours ($p<.01$). Maternal urine Cl^- increased gradually from 143 ± 31 to 238 ± 46 mEq/l during the control and fetal infusion periods. After hypertonic injection, maternal urine Cl^- increased rapidly at first and then more slowly, reaching 389 ± 22 mEq/l by the end of the experiment ($p<.05$).

Fetal urine K^+ did not change during antagonist infusion, but fell after hypertonic injection to 12 ± 9 mEq/l below control by one hour post-injection and remained low for the duration of the experiment (Figure 66). Likewise,

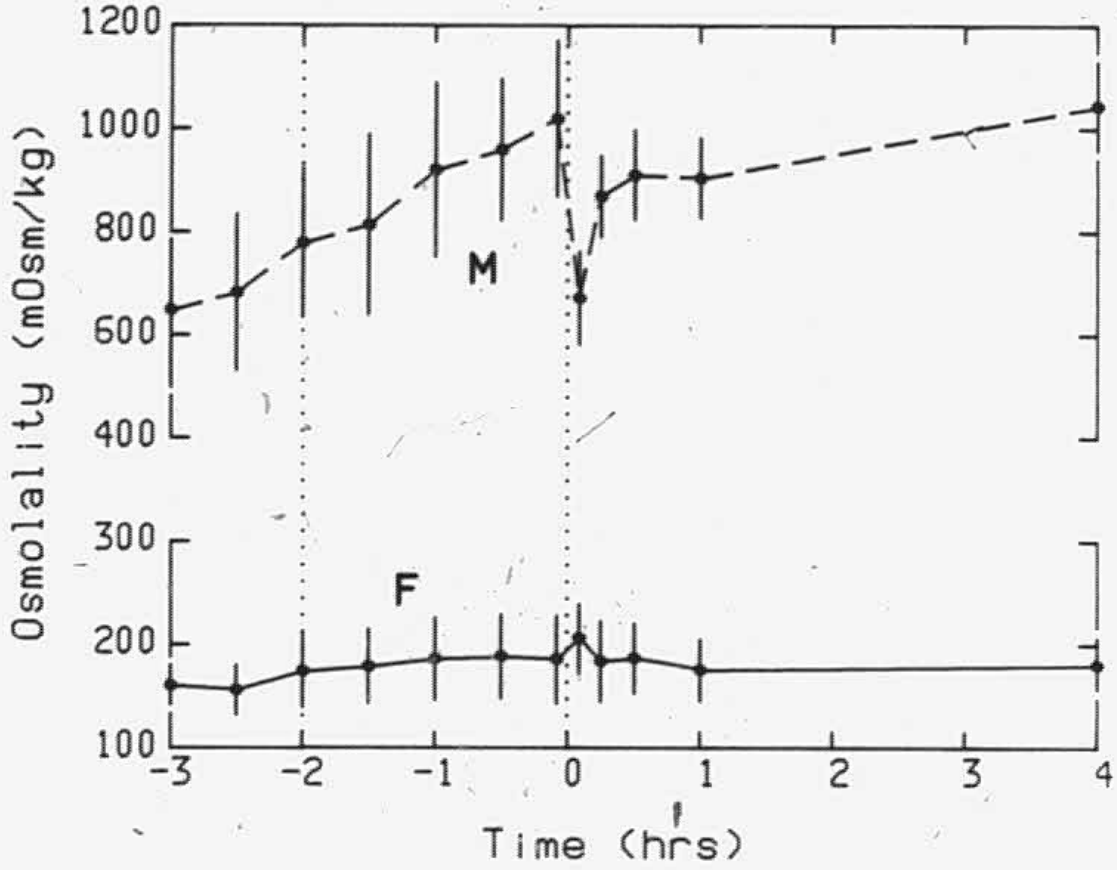


Figure 63. Time course of fetal (F) and maternal (M) urine osmolalities. Fetal antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 5.

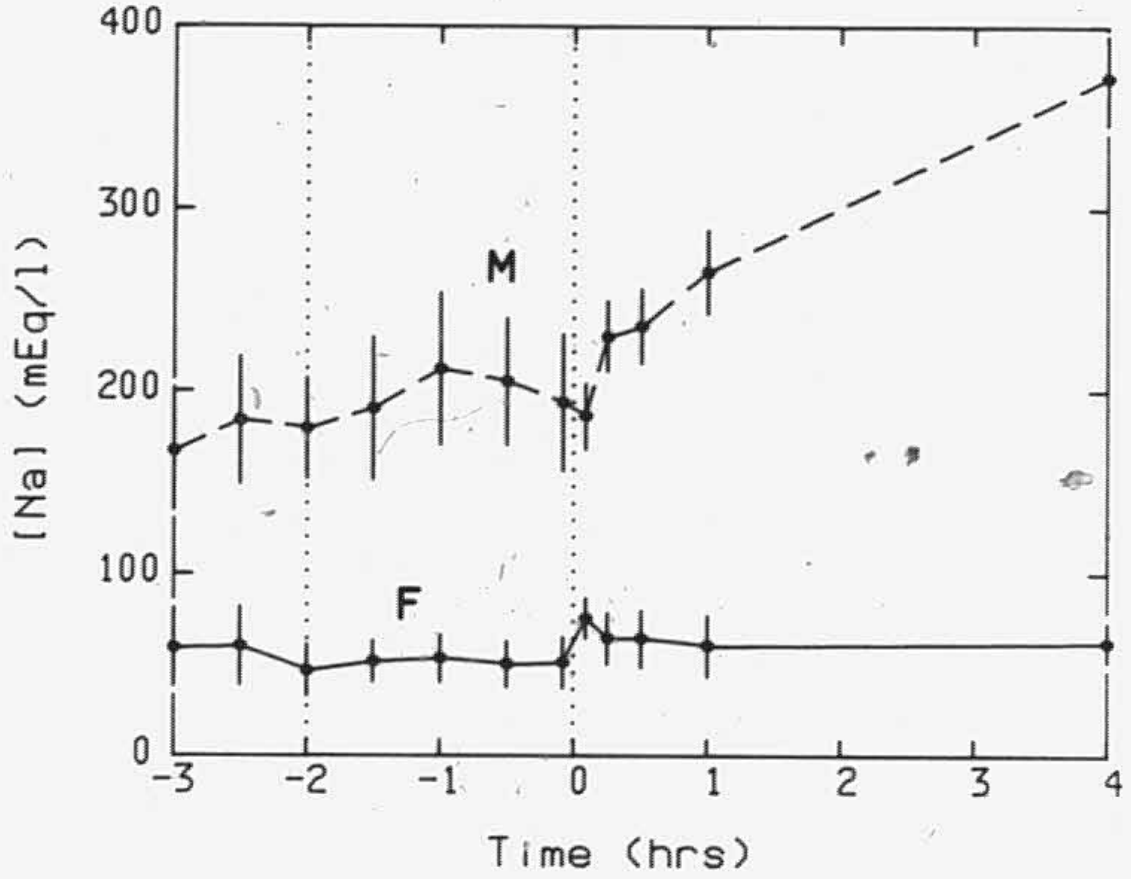


Figure 64. Time course of fetal (F) and maternal (M) urine Na+ concentrations. Fetal antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean $\pm$ SE, n = 5.

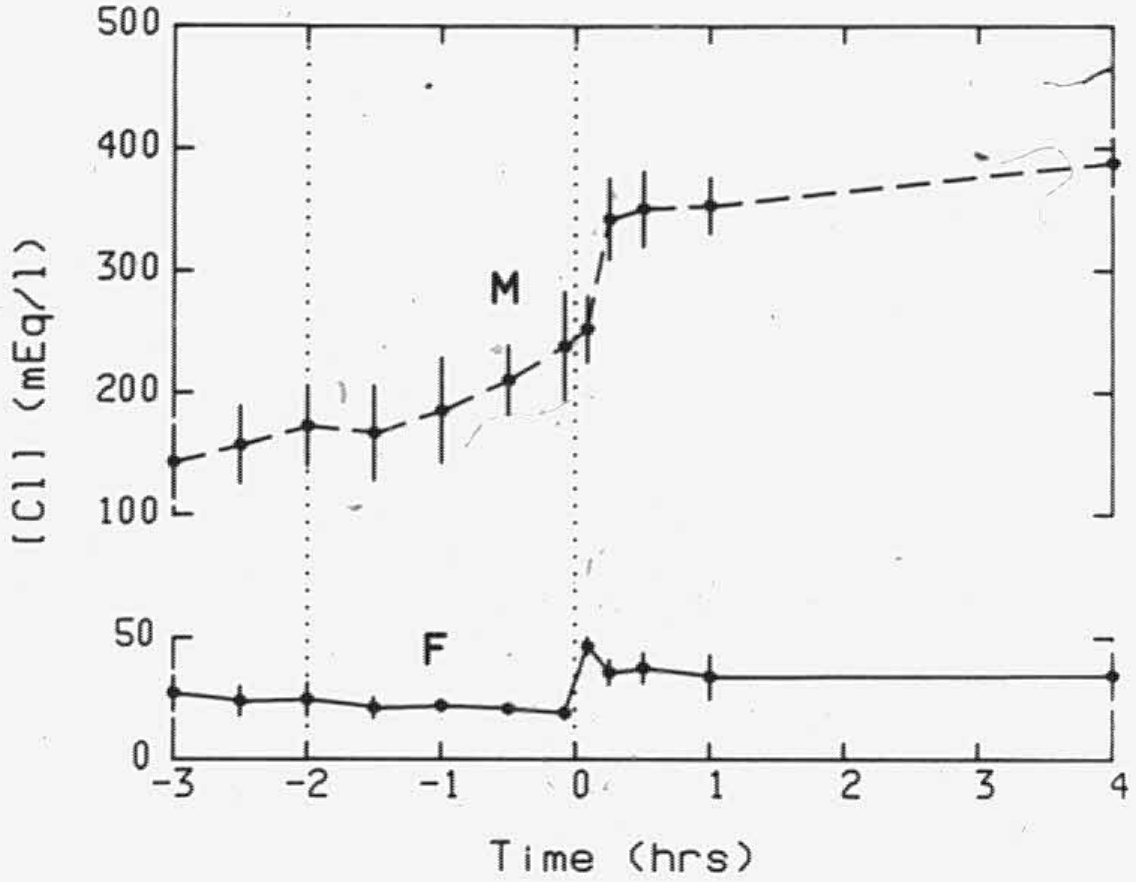


Figure 65. Time course of fetal (F) and maternal (M) urine Cl- concentrations. Fetal antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 5.

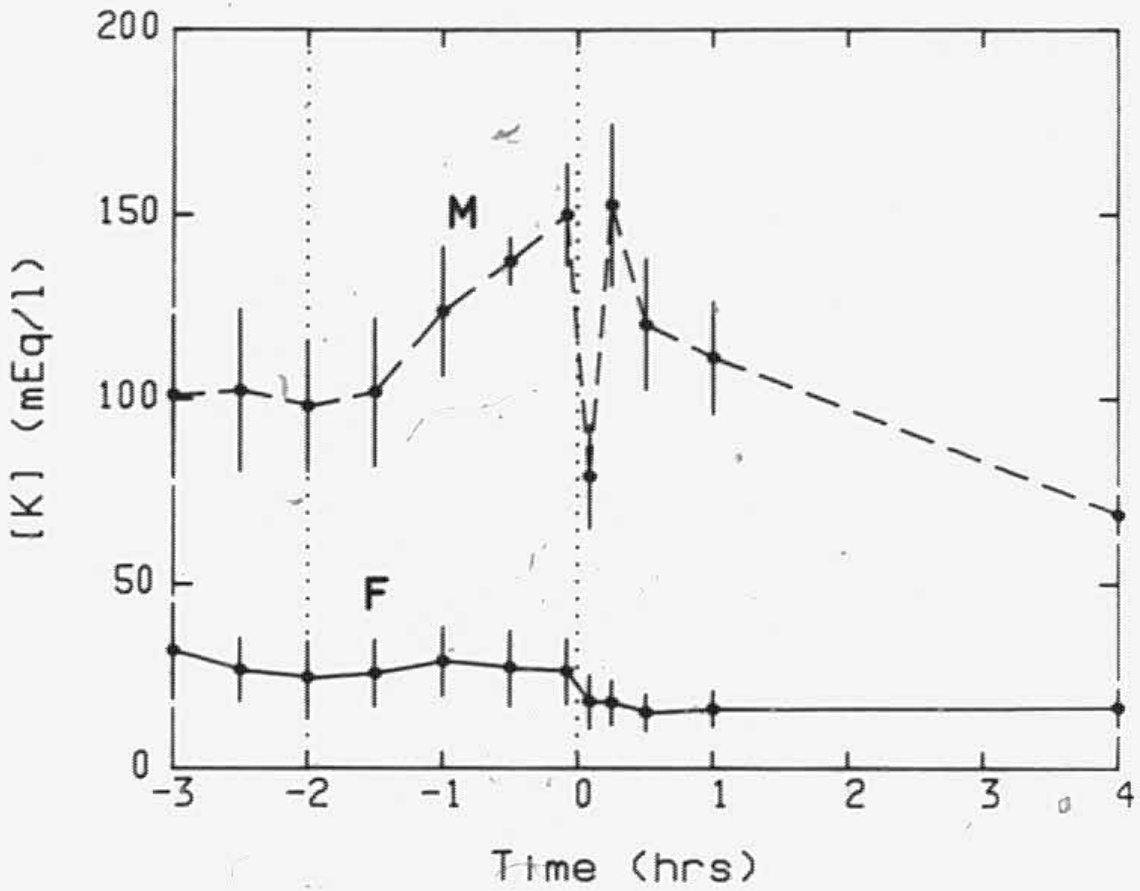


Figure 66. Time course of fetal (F) and maternal (M) urine K+ concentrations. Fetal antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 5.

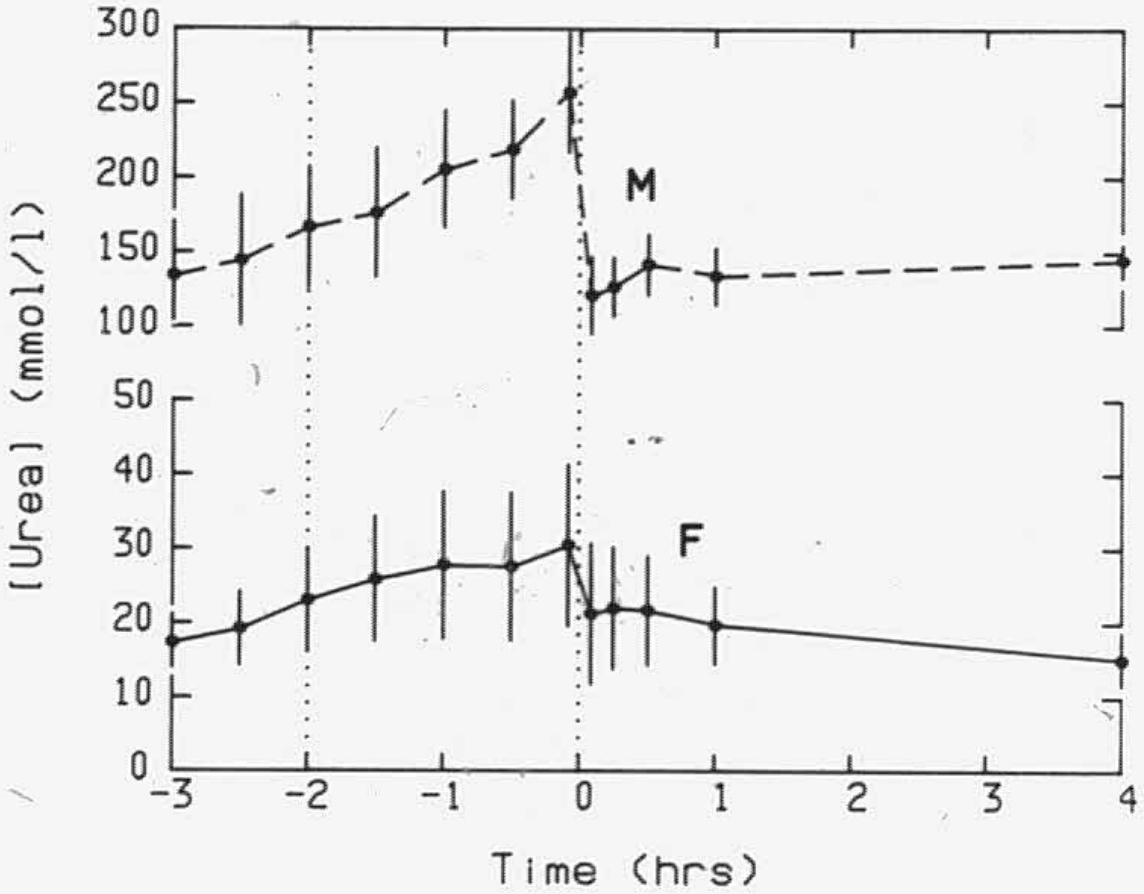


Figure 67. Time course of fetal (F) and maternal (M) urine urea concentrations. Fetal antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 5.

fetal urine urea was unchanged during the antagonist infusion, but fell by 9.2 ± 4.8 mmol/l from the immediate pre-injection value in response to hypertonic injection and remained decreased for the rest of the experiment (Figure 67). Maternal urine urea increased from 134 ± 40 to 257 ± 41 mmol/l during the control and fetal infusion periods, fell to 120 ± 27 mmol/l in response to hypertonic injection ($p<.01$), and remained low for the rest of the experiment. Maternal urine K^+ did not change during the control period but increased by 50 ± 9 mEq/l during the fetal infusion period. Following hypertonic injection, maternal urine K^+ fell transiently to 71 ± 14 mEq/l below the immediate pre-injection value ($p<.01$), returned to the pre-injection level by 15 minutes, and then gradually decreased to 81 ± 17 mEq/l below pre-injection by the end of the experiment ($p<.01$).

b. Effects on continuously-measured variables

The effects of antagonist infusion and hypertonic injection on fetal cardiovascular variables are shown in Figures 68-70. Mean fetal arterial pressure was reduced by an average of 1.4 mmHg during antagonist infusion, although this change was not statistically significant. After hypertonic injection fetal arterial pressure fell significantly below the control value, by 3-4 mmHg for the first hour and by 2-3 mmHg for the remainder of the experiment. Fetal venous pressure tended to fall slightly during the antagonist in-

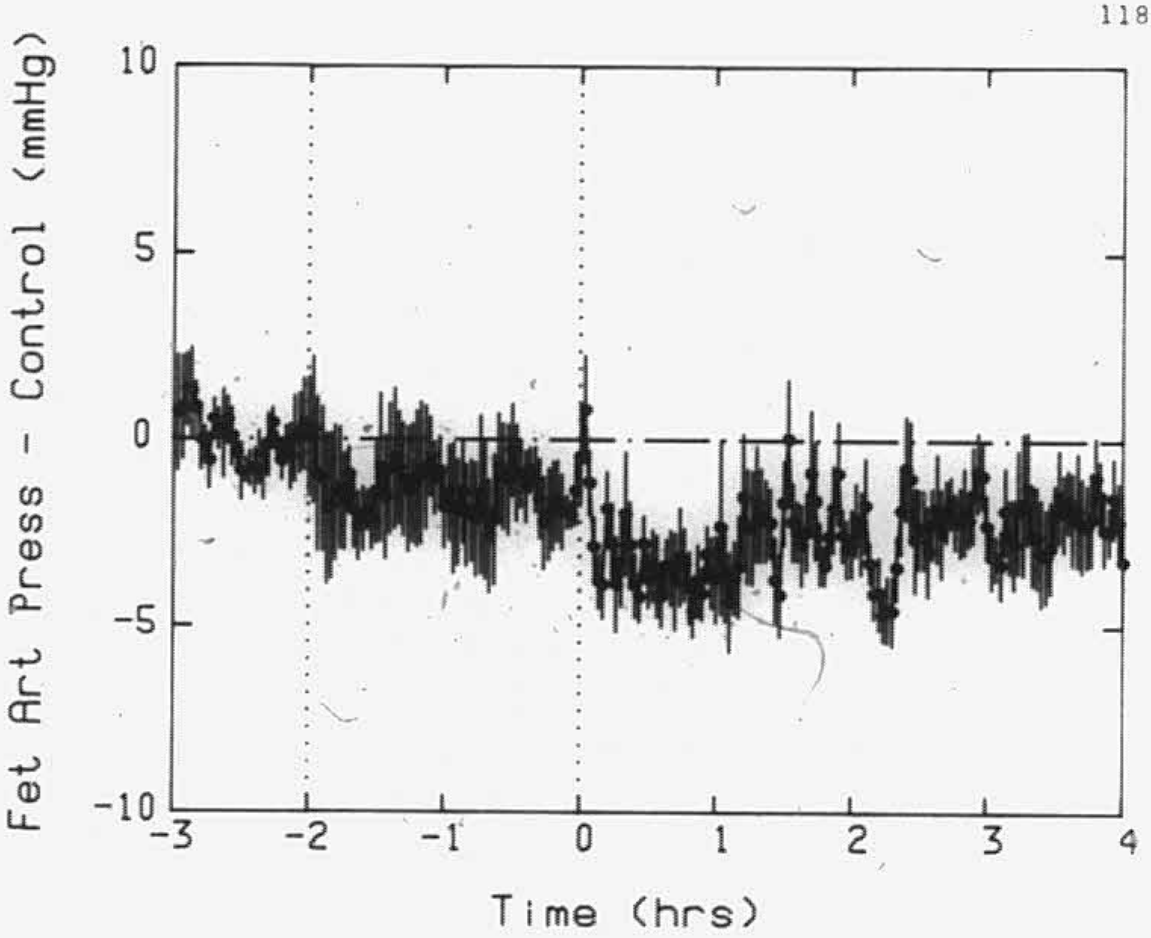


Figure 68. Time course of fetal arterial pressure changes. Fetal antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Control = 41.6 ± 1.3 mmHg. Mean change from control ± SE, n = 5.

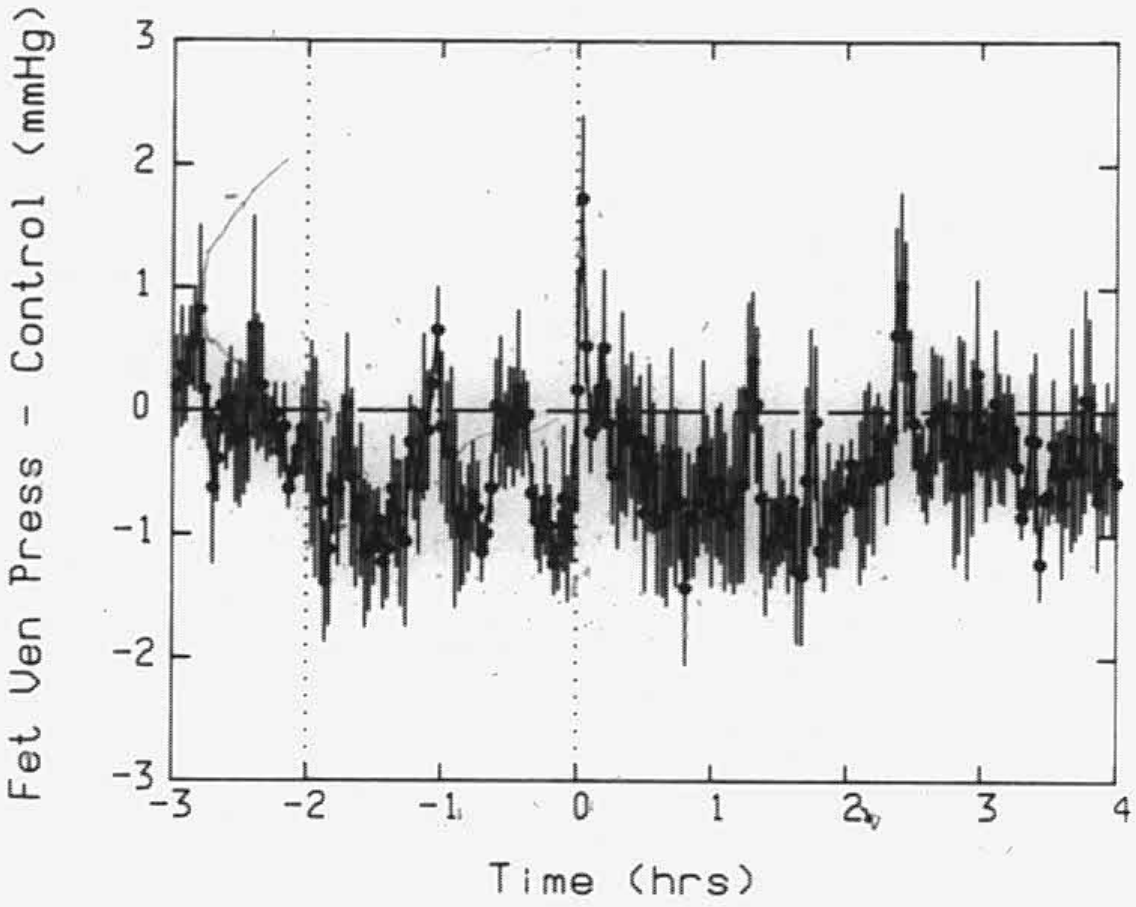


Figure 69. Time course of fetal venous pressure changes. Fetal antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Control = 3.92 ± 0.08 mmHg. Mean change from control ± SE, n = 5.

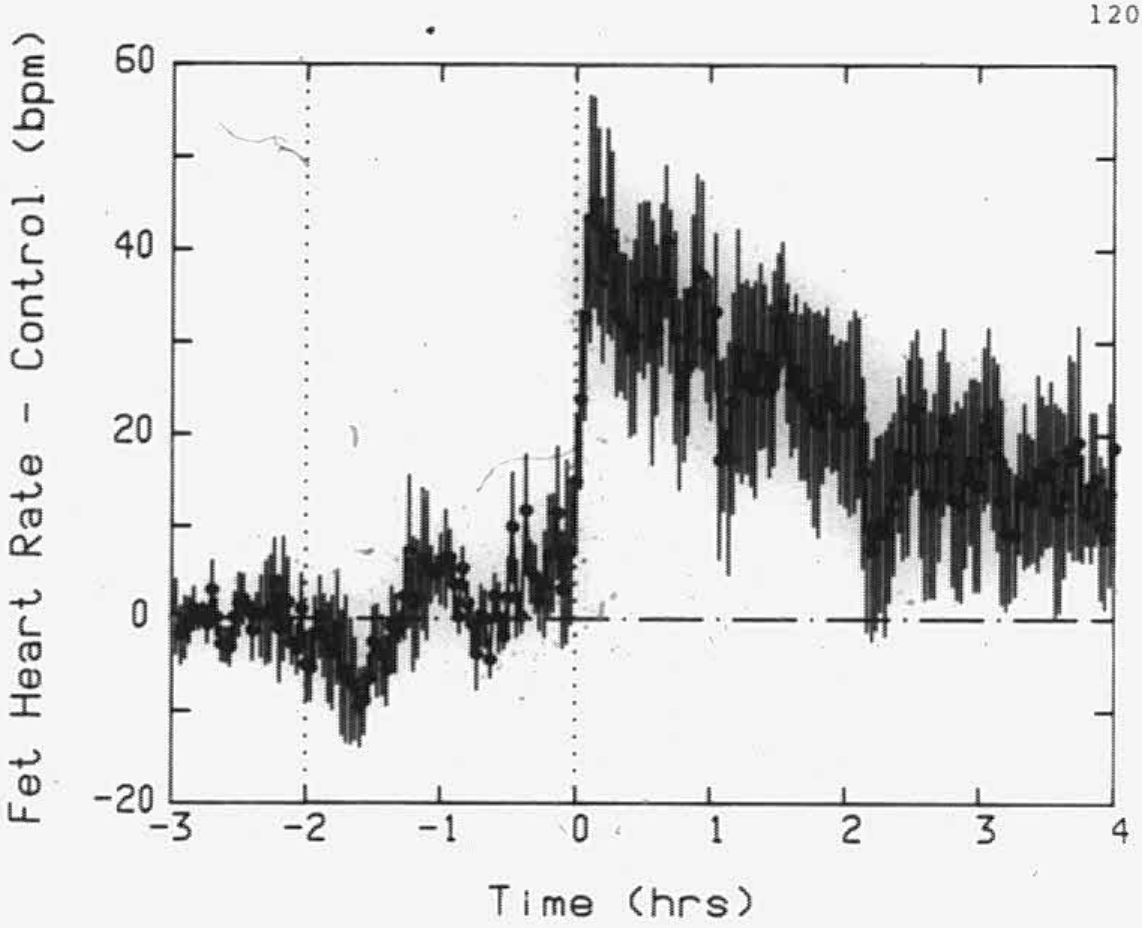


Figure 70. Time course of fetal heart rate changes. Fetal antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Control = 156 ± 4 bpm. Mean change from control ± SE, n = 5.

infusion, but was so variable that this decrease was not statistically significant. Fetal venous pressure increased transiently to 1.7 ± 0.7 mmHg above the mean control value in response to hypertonic injection and then returned to pre-injection values. The variability of fetal heart rate was increased during antagonist infusion, but the mean fetal heart rate was not significantly different from control during this period. Following hypertonic injection fetal heart rate was increased by a peak of 45 ± 12 bpm at eight minutes post-injection ($p<.02$) and fell gradually after this but was still elevated at the end of the experiment.

The responses of fetal urine output to antagonist infusion and hypertonic injection are shown in Figure 71. Fetal urine flow fell significantly during the control period, from 1.4 ± 0.3 to 0.8 ± 0.1 ml/min. During antagonist infusion fetal urine flow was not significantly different from the immediate pre-injection value. After hypertonic injection fetal urine flow increased transiently by a peak of 1.4 ± 0.3 ml/min over the average during the infusion period ($p<.01$), fell to 0.3 ± 0.1 ml/ min above the steady-state value within ten minutes, and remained at this level for the duration of the experiment.

c. Effects on fetal GFR

The effects of antagonist infusion followed by hypertonic injection on fetal GFR are shown in Figure 72. Fetal

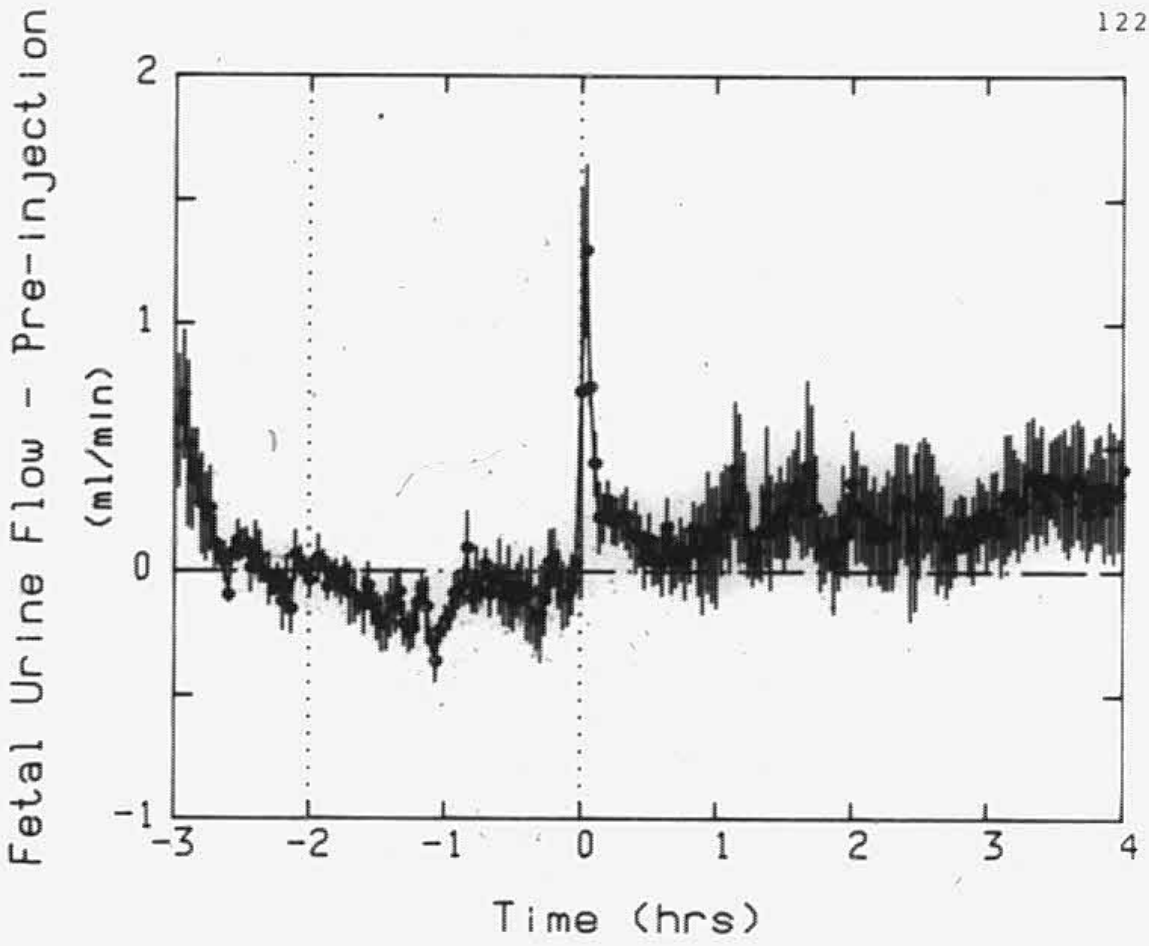


Figure 71. Time course of fetal urine flow. Fetal antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Control = 1.0 $\pm$ 0.1 ml/min. Mean change from control $\pm$ SE, n = 5.

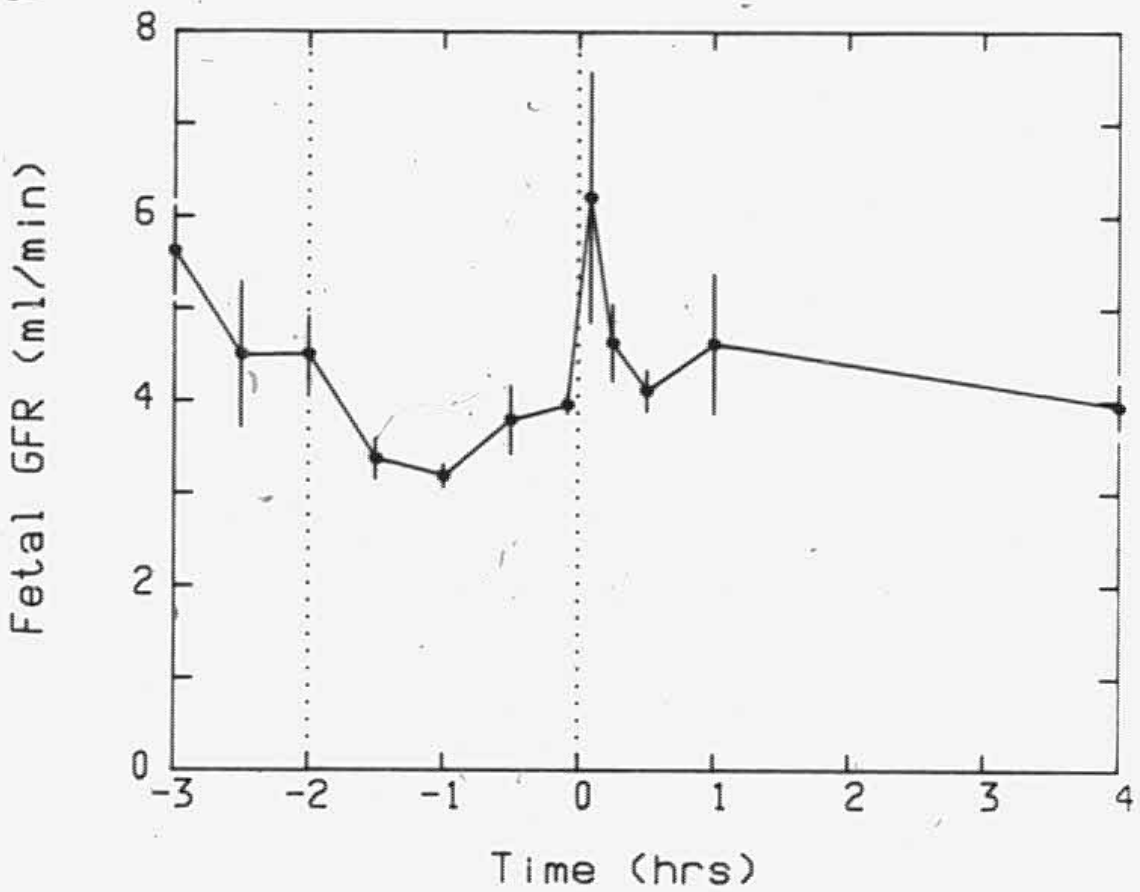


Figure 72. Time course of fetal GFR. Fetal antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 5.

GFR was decreased during the antagonist infusion, to a minimum of 3.2 ± 0.1 ml/min after one hour. After hypertonic injection fetal GFR increased transiently to 6.2 ± 1.4 ml/min, returned to the control value within 15 minutes, and remained essentially constant thereafter.

d. Effects on renal clearance of chemical constituents

The effects of antagonist infusion followed by hypertonic injection on fetal renal clearance values are shown in Figures 73-77. All fetal clearance values were elevated at the beginning of the control period but were in a steady state during the second half hour of this period. No significant changes were seen in any of these clearances during the antagonist infusion, although C_{urea} and C_{osm} tended to be reduced during this period. Following hypertonic injection, fetal C_{osm} was increased to a peak of 0.99 ± 0.11 ml/min after five minutes, fell to 0.57 ± 0.08 ml/min by 15 minutes, and remained elevated at this level for the rest of the experiment. C_{Na} and C_{Cl} showed a similar pattern, peaking at 0.74 ± 0.11 and 0.64 ± 0.11 ml/min respectively. Fetal C_K and C_{urea} increased transiently in only three out of five animals, so that there were no statistically significant changes in these two variables after hypertonic injection.

e. Effects on free water clearance

The effects of antagonist infusion followed by hyper-

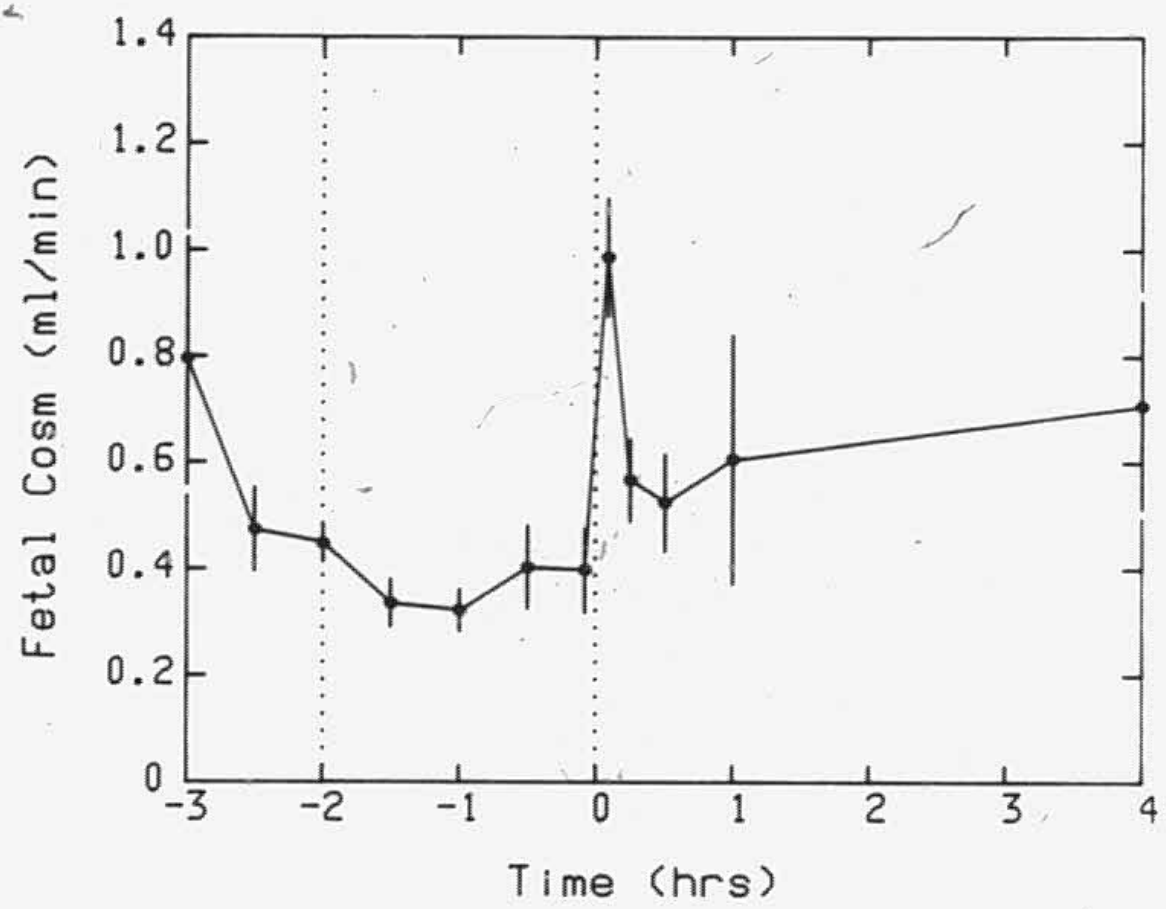


Figure 73. Time course of fetal osmolar clearance. Fetal antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 5.

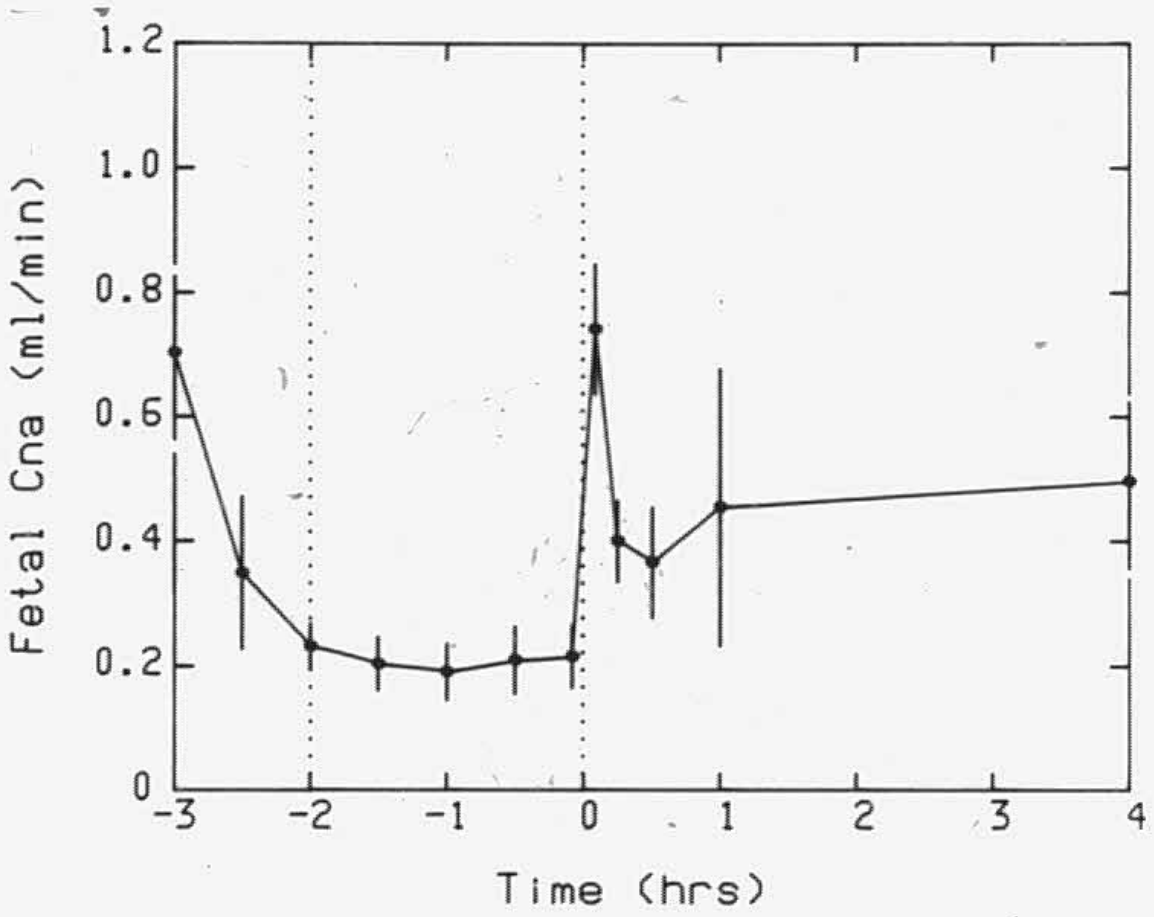


Figure 74. Time course of fetal Na+ clearance. Fetal antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 5.

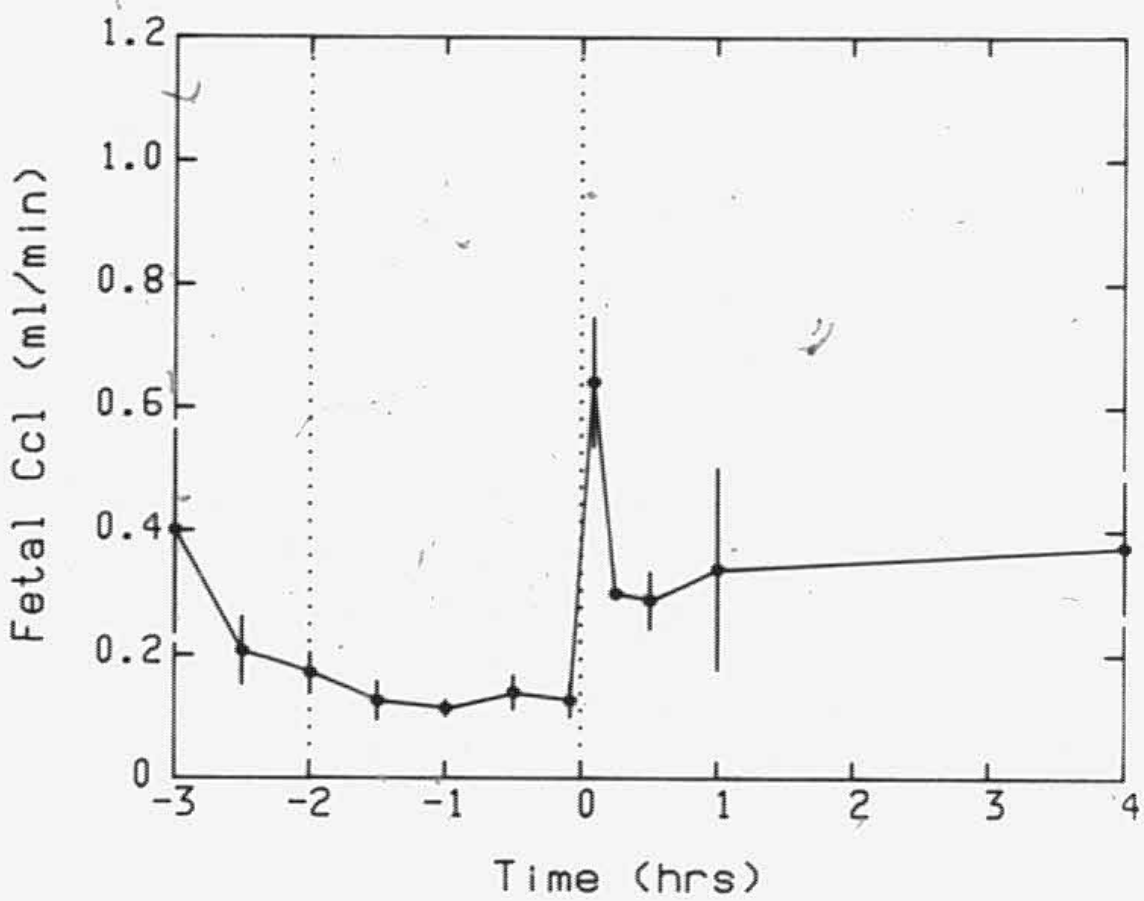


Figure 75. Time course of fetal Cl- clearance. Fetal antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 5.

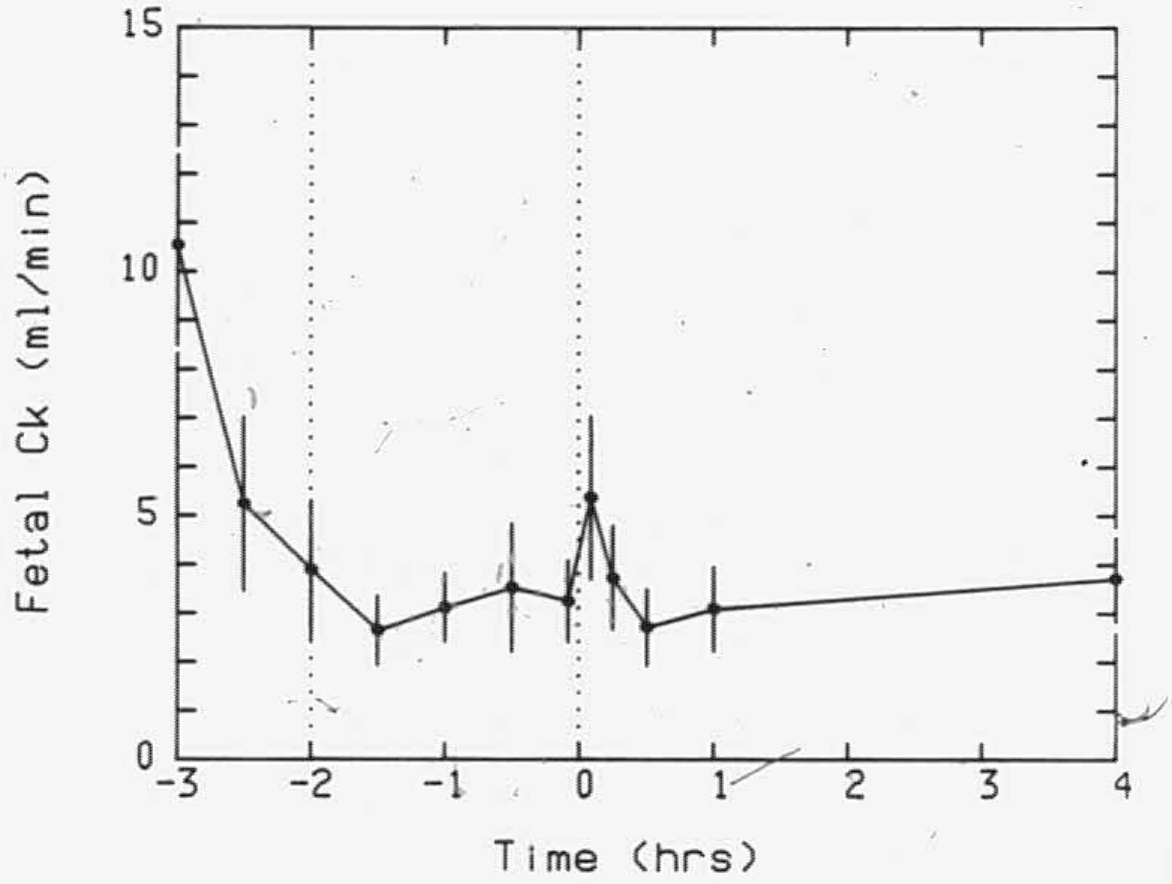


Figure 76. Time course of fetal K+ clearance. Fetal antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 5.

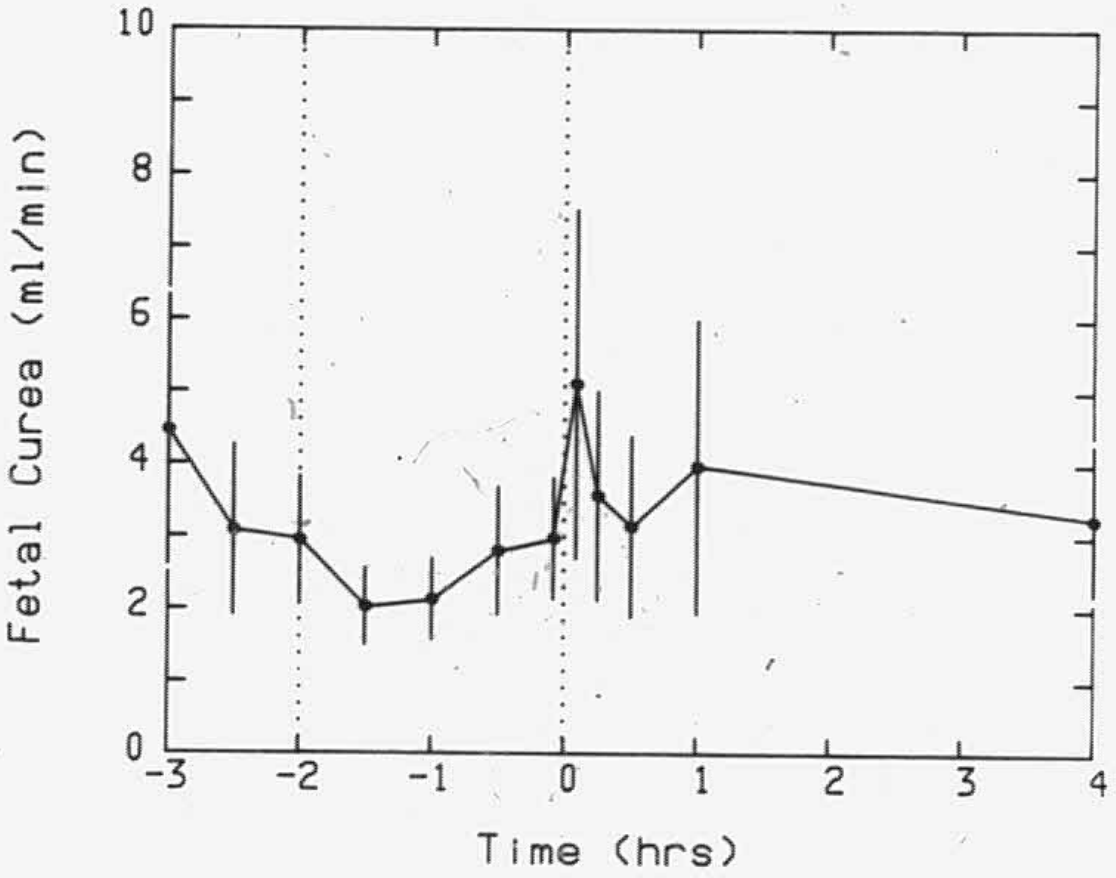


Figure 77. Time course of fetal urea clearance. Fetal antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 5.

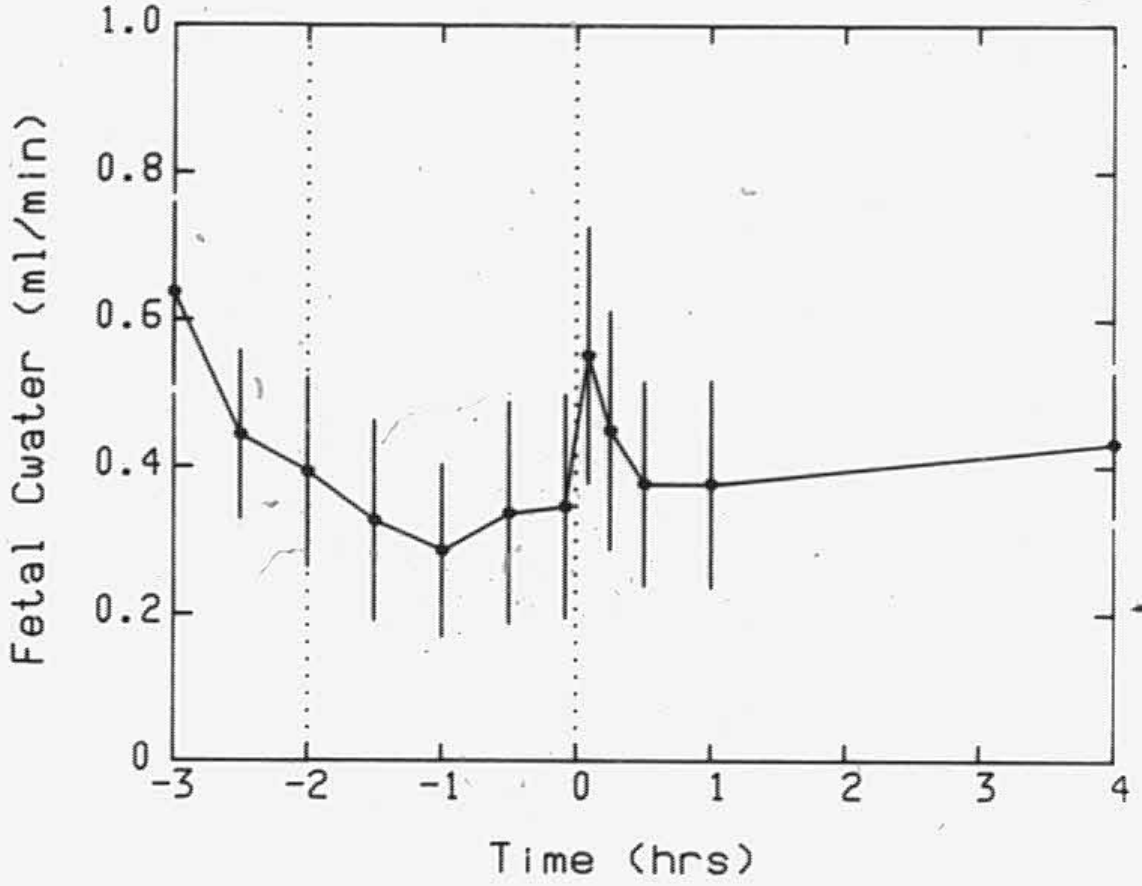


Figure 78. Time course of fetal free water clearance. Fetal antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean ± SE, n = 5.

tonic injection on fetal C_{water} are shown in Figure 78. Fetal C_{water} remained positive throughout the experiment. The responses of C_{water} to antagonist infusion and hypertonic injection varied from animal to animal, so that there were no statistically significant changes in this variable during the experiment.

f. Effect on fetal blood volume

As shown in Figure 79, no statistically significant changes were seen in fetal blood volume during antagonist infusion. Following hypertonic injection, fetal blood volume increased by a peak of 14 $\pm$ 7 ml and remained elevated for at least 30 minutes.

B. Receptor Binding Studies

The binding of ^{3}H-AVP to fetal and maternal renal medullary tissue is depicted in Figures 80 and 81. Total, nonspecific, and specific binding per 100 ug protein were all less in fetal than in maternal tissue preparations at any given final ^{3}H-AVP concentration. (Note difference in scales.) Specific binding as a percent of total binding was also less in fetal than in maternal tissue.

Scatchard plots of these data are shown in Figure 82. For fetal tissue, K_D was 0.44 nM and B_{max} was 15.2 fmol/mg protein. For maternal tissue, K_D was 0.80 nM and B_{max} was 67.2 fmol/mg protein. The slopes of the two lines; and therefore the K_D's, were not significantly different.

However, B_{max} in maternal tissue was over four times that in fetal tissue, suggesting that maternal tissue has several-fold more binding sites per unit membrane protein than does fetal kidney tissue.

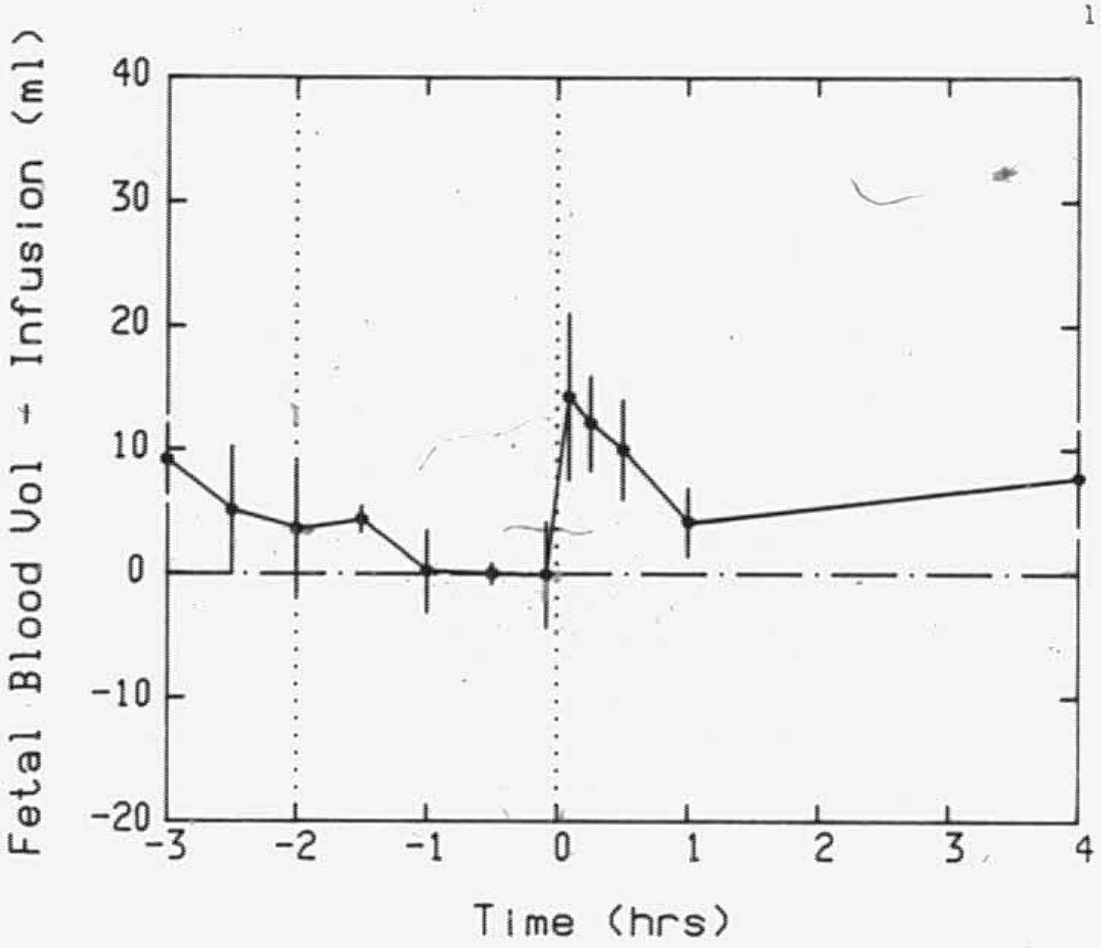


Figure 79. Time course of fetal blood volume changes. Fetal antagonist infusion was begun at -2 hrs and hypertonic injection was done at time zero. Mean change from mean value during last hour of antagonist infusion ± SE, n = 4.

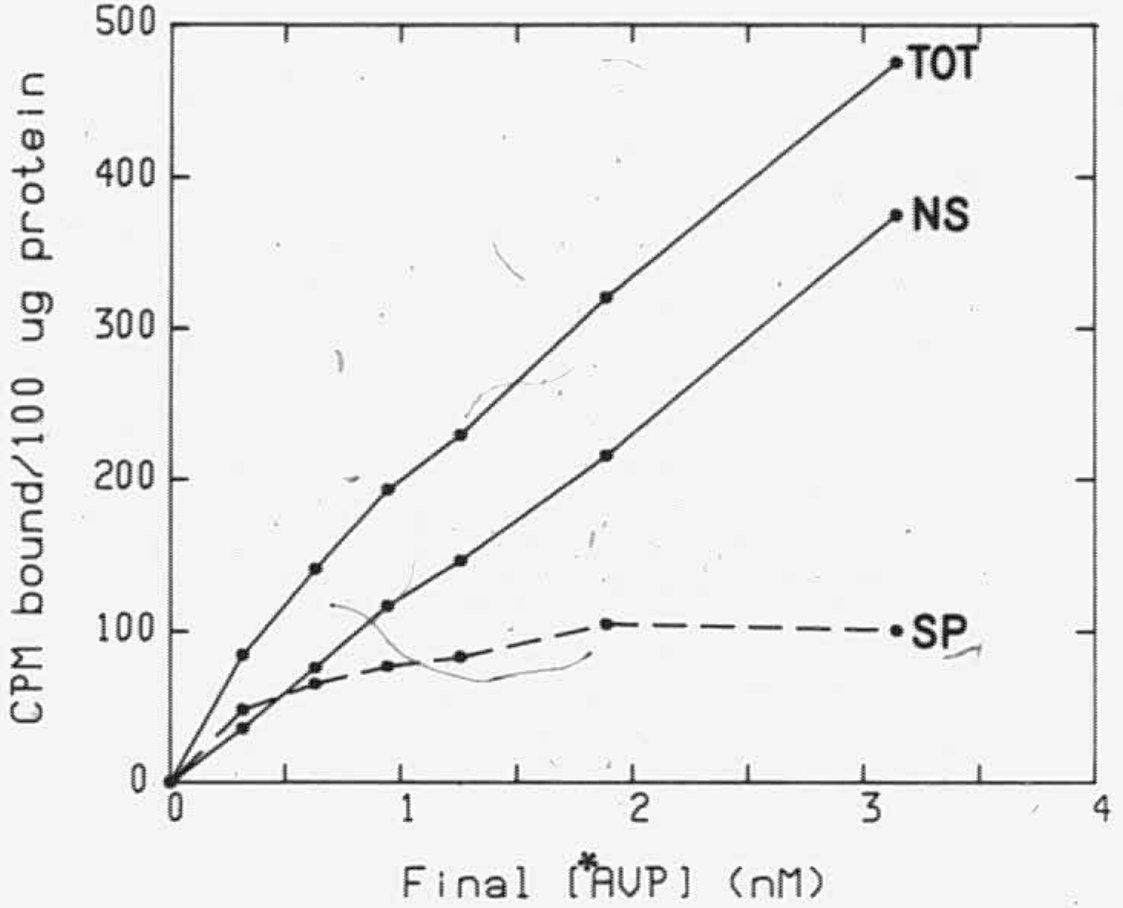


Figure 80. Binding of labeled AVP to fetal renal medullary tissue. Mean of two experiments. TOT = total binding, NS = non-specific binding, SP = specific binding.

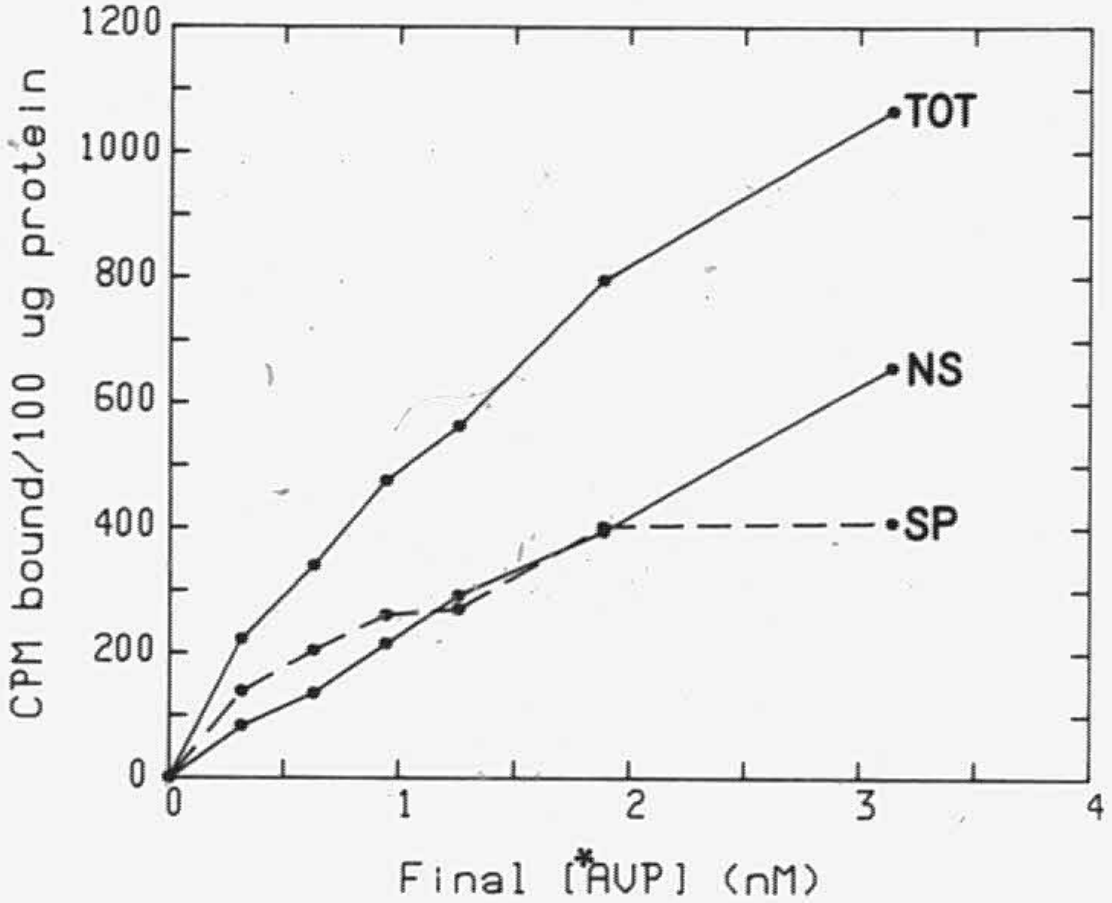


Figure 81. Binding of labeled AVP to maternal renal medullary tissue. Mean of two experiments. TOT = total binding, NS = non-specific binding, SP = specific binding.

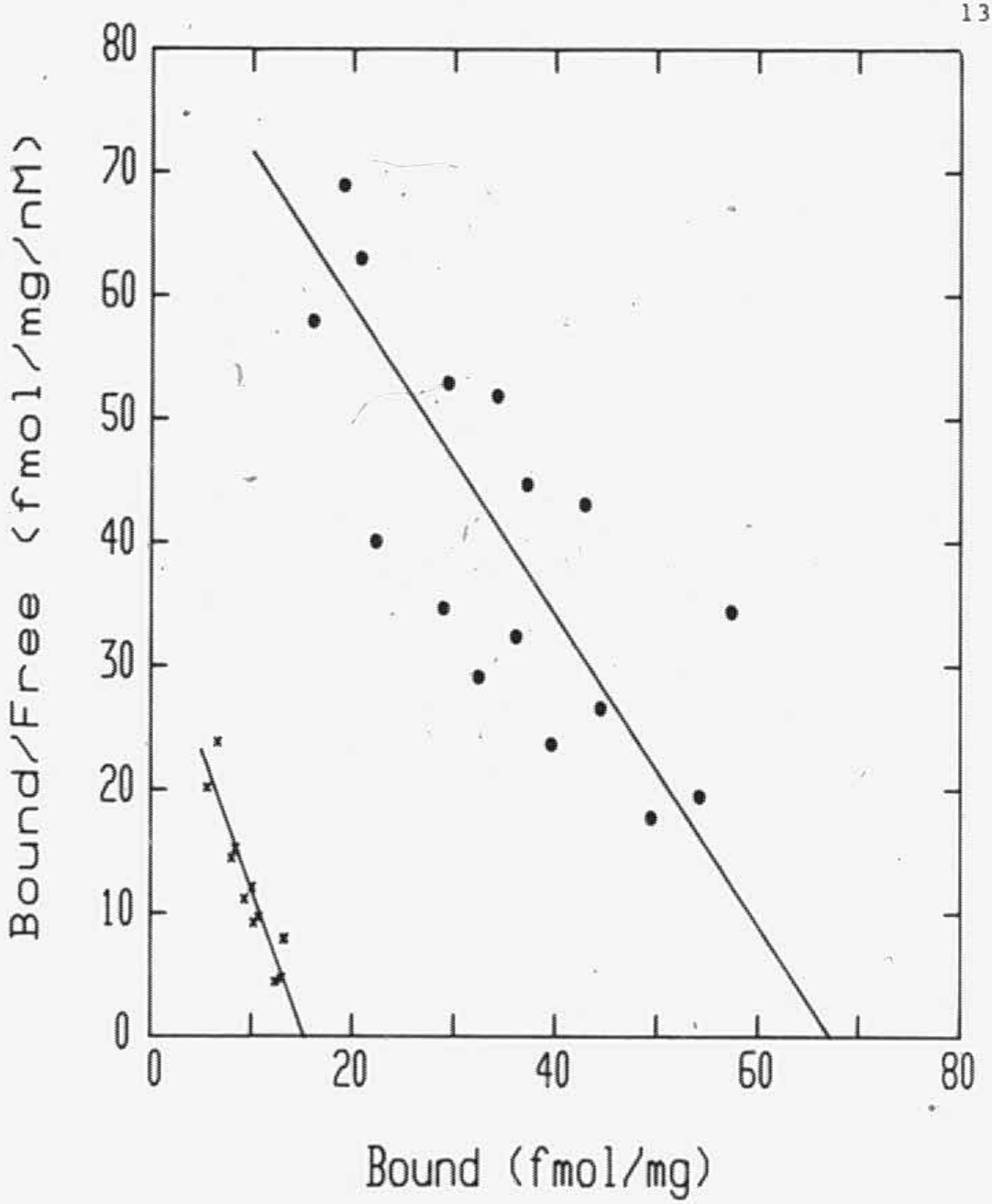


Figure 82. Scatchard plots of AVP binding to fetal (x) and maternal (●) renal medullary tissue. For fetal tissue K_D = 0.44 nM and B_{max} = 15.2 fmol/mg protein. For maternal tissue K_D = 0.80 nM and B_{max} = 67.2 fmol/mg protein.

V. DISCUSSION

A. AVP and the Renal Response to Hypertonicity

The fetal kidney responded to prolonged hyperosmolality with increases in urine osmolality, Na^+, and Cl^-, a transient increase in urine flow followed by a decrease to below the control level, transient increases in renal clearance values and GFR, and a decrease in free water clearance. With the exception of C_{osm}, C_{Na}, and C_{Cl}, the transient changes appeared to be due to a transient increase in arterial pressure following hypertonic injection. C_{osm}, C_{Na}, and C_{Cl} were elevated for up to one hour, despite the return to normal arterial pressure within a few minutes, because the urine concentrations of these solutes increased before urine flow decreased. Beyond one hour, however, only C_{water} and urine osmolality, Na^+ and Cl^- were different from control values, suggesting that the long-term response of the fetal kidney to hyperosmolality is merely to conserve water. In contrast, the maternal kidney was able to increase C_{osm}, C_{Na}, and C_{Cl} long-term both by increasing urine flow over the pre-injection value and by continuing to increase urine concentrations of these solutes.

To my knowledge only one previous study has looked at the effects of hyperosmolality on fetal renal function (27). This study is not directly comparable to the present work because it involved hypertonic mannitol infusions into the

ewe and not the fetus, which probably resulted in fluid movement out of the fetus across the placenta, a reduction in fetal blood volume, and possibly a decrease in fetal arterial pressure (not reported). In the present study, on the other hand, hypertonic NaCl was injected into both mother and fetus in order to minimize transplacental fluid movement, and fetal blood volume remained constant (Figure 30). However, Lumbers and Stevens (27) found that fetal urine flow decreased and urine osmolality increased after hypertonic infusion, with no change in GFR or osmolar clearance. Considering the fact that these investigators collected data during a series of experimental periods of at least 30 minutes duration, and that transient responses were therefore undetectable, these data are not inconsistent with the present study.

With the effects of AVP blocked, the fetal kidney responded to prolonged hyperosmolality with increases in urine Na^+ and Cl^- but not osmolality, a transient increase in urine flow followed by a decrease to values which averaged above the pre-injection level, transient increases in GFR, C_K, C_{urea}, and C_{water}, and prolonged increases in C_{osm}, C_{Na}, and C_{Cl}. These prolonged increases over control values of C_{osm}, C_{Na}, and C_{Cl} appear to be due solely to the prolonged elevation of urine flow relative to that seen in normal fetuses. Thus, not surprisingly, it appears that the decrease in fetal

urine flow and increase in urine osmolality after hypertonic injection are due to the actions of AVP on the kidney. This is consistent with the finding that AVP infusion alone causes a similar decrease in fetal urine flow and increase in urine osmolality.

In AVP-infused fetuses, the kidney was apparently already maximally stimulated by AVP prior to hypertonic injection, as urine osmolality did not increase further with hypertonicity. This also lends support to the idea that it is AVP and not some other mechanism which is primarily responsible for the fetal renal responses to hypertonicity.

Apart from AVP-mediated responses, the major effect of hypertonic NaCl injection on the fetal kidney appears to be to increase urine concentrations of these electrolytes. In normal, AVP-infused, and antagonist-infused fetuses, urine concentrations of Na^+ and Cl^- increased following hypertonic injection, and the magnitudes of the increases were not different in the three groups. In normal fetuses these increases paralleled an increase in urine osmolality, so that the relative contributions of Na^+ and Cl^- to total urine osmolality remained constant (Figure 83). In both AVP- and antagonist-infused fetuses, in which urine osmolality did not change after hypertonic injection, urine Na^+ and Cl^- increased while K^+, urea, and the unidentified solutes showed proportionate decreases (Figure 83). In the maternal ewe, urine

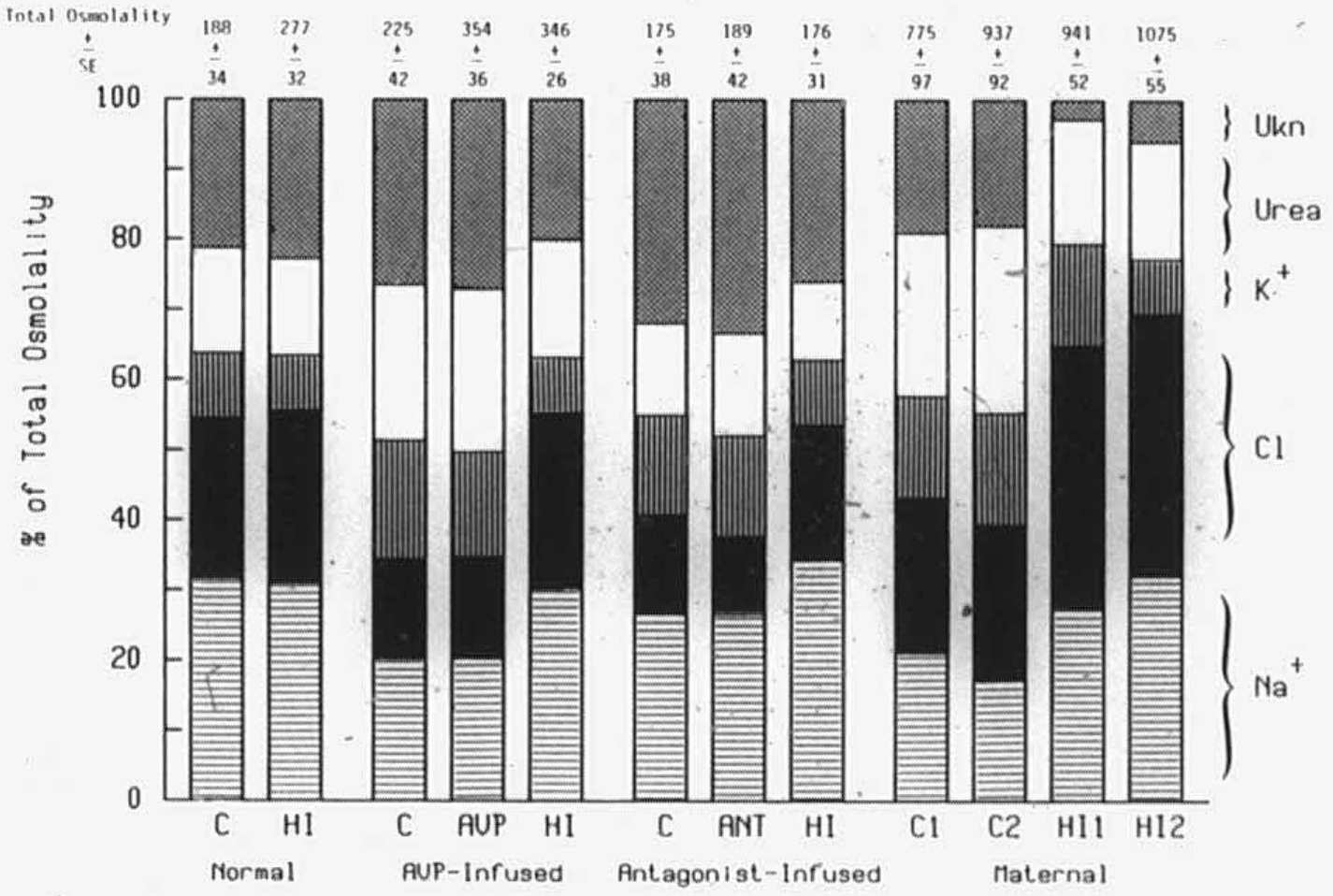


Figure 83. Relative contributions of urine constituents to total urine osmolality during control and experimental periods in normal, AVP-infused, and antagonist-infused fetuses and the maternal ewes. C = control, AVP = during AVP infusion, ANT = during antagonist infusion, HI = after hypertonic injection.

osmolality as well as the relative contributions of Na^+ and Cl^- increased after hypertonic injection.

B. Maternal Responses and Comparison With Those of the Fetus

As mentioned in the Results, maternal responses to hypertonicity in the mothers of AVP-infused fetuses were not different from those in the mothers of antagonist-infused fetuses, and so the results were pooled. Moreover, these responses were not different from those in ewes carrying fetuses that received no agonist or antagonist infusion. This is not surprising, because it is known that AVP does not cross the placenta in the sheep (23), and by virtue of its size and structural similarity to the naturally-occurring hormone, it is highly unlikely that the antagonist would cross the placenta either. Thus an infusion of these substances into the fetus would not be expected to affect the ewe.

One problem I encountered in the maternal studies is that of increasing urine osmolality, Na^+, Cl^-, K^+, and urea, and decreasing urine flow during the control period. While this was unfortunate, it was not unexpected, because the ewe was denied access to water during the experiment and therefore was unable to replenish the fluid lost in urine and feces as well as insensible losses. On the other hand, in preliminary studies I found that plasma osmolalities in both mother and fetus were unstable when the ewe was allowed free

access to water, and certainly she could not be allowed to drink after hypertonic injection if plasma osmolality was to be maintained constant and elevated. Therefore, changes during the control period were judged to be the lesser of two evils. At any rate, in many cases maternal urine values were stable during the last hour before hypertonic injection.

Maternal renal function was followed in these studies primarily to provide a measure of adult responses with which to compare those of the fetus. With several exceptions, fetal responses to hypertonic injection were qualitatively if not quantitatively similar to those of the mother.

One difference between fetal and maternal renal responses to hypertonicity was the transient fall in maternal urine osmolality immediately after injection, which had no counterpart in the fetus. This decrease in the osmolality of the urine induced by hypertonic injection is well-documented in the literature (3,7,13,33). It is presumably due to the rapid flow of fluid through the kidney tubules during this osmotic diuresis, with relatively little time for modification of the fluid, and to addition of fluid which is nearly isotonic with plasma to the tubular fluid as it flows through the kidney, thus causing the osmolality of the urine to tend toward plasma osmolality. However, in adult humans, when the osmolality of the urine was originally hypotonic, injection of hypertonic mannitol caused urine osmolality to in-

crease (7,13). This is consistent with the present findings in the fetus, where the initial urine osmolality was below that of the plasma.

Another contrast between fetal and maternal responses to hyperosmolality is that the concentrations of Na^+ and Cl^- in the maternal urine continue to increase for the duration of the experiment, while those in fetal urine quickly reach a plateau. Related to this is the prolonged increase in the urea concentration of the fetal urine after hypertonic injection, when maternal urine urea is decreased. It appears that the maternal kidney is better able to adjust the components of the urine to rid the body of the solute which is in excess (in this case NaCl). The fetus, on the other hand, appears to have an obligatory proportion of urine solutes devoted to urea, or conversely, a maximum level for Na^+ and Cl^- in the urine.

C. Effect of AVP on Fetal Renal Function

AVP appears to be unimportant in the control of urine osmolality in unstressed fetuses, as suggested by the lack of response to antagonist infusion (Figure 63). This is not surprising, considering the normally low urine osmolality in the fetus. This study confirms the results of other studies (25,37) that fetal urine osmolality does increase in response to exogenous AVP but that a maximum is reached at urine values only slightly hypertonic to plasma, even despite the

prolonged infusion times in the present study. I found that fetal urine flow was decreased by 0.25 ml/min or about 37% of control during AVP infusion. In comparison, Lingwood, et al found a somewhat larger reduction in urine flow at AVP doses approximately half those of the present study, and variable responses in urine flow at doses twice those of this study (25). At even higher doses, Robillard and Weitzman found variable responses resulting in no change in mean fetal urine flow (37). AVP was significantly pressor at the higher doses in both studies. Therefore, it appears that AVP causes fetal urine flow to fall when given at low doses, but that at higher doses a pressor effect comes into play which counteracts the antidiuretic effects of AVP on urine flow.

In contrast to the study by Robillard and Weitzman (37), in which GFR increased during AVP infusion, in the present study GFR did not change significantly. Again, however, the dose of AVP given in that study was several fold higher and had a much greater pressor effect than in the present work, so the differences in the response of GFR are not unexpected.

D. Effect of AVP and Antagonist on Fetal Vascular Pressures and Heart Rate

The infusion of AVP at the dose used in this study was mildly pressor (Figure 41), raising fetal arterial pressure by about 2 mmHg. In comparison, Lingwood, et al (25) saw no change in arterial pressure at doses about half of those

used in the present study, and an average increase in arterial pressure of 18 mmHg at doses about twice those of the present study. At even higher doses, Robillard and Weitzman reported an average increase of 8 mmHg in fetal arterial blood pressure (37), and at yet higher doses Iwamoto et al found an increase of 9 mmHg (20). Thus, the dose of AVP used in the present study appears to be near the threshold for a pressor response.

AVP infusion also tended to depress fetal heart rate in the present study, as well as depressing the normal response of fetal heart rate to hypertonic injection. However, this decrease in fetal heart rate was small (5-10 bpm) and did not reach statistical significance. In contrast, at doses approximately ten-fold higher, Iwamoto et al found that fetal heart rate was decreased by 30 bpm (20). Again, the doses of AVP used in the present study appear to be near the threshold for a negative chronotropic response.

Although the AVP antagonist used in this study was intended to block the antidiuretic actions of the hormone, it appeared to affect the pressor effects of the hormone as well. Fetal arterial pressure tended to be decreased during the antagonist infusion by 1-2 mmHg. This probably accounts for the simultaneous decrease in GFR, but apparently had no other effects on the kidney. No change was seen in fetal heart rate. To my knowledge, only one other study has looked

at the effects of a vasopressin antagonist in the fetus (21). These investigators used a different antagonist, $d(CH_2)_5Tyr(Me)AVP$, which is designed to block the pressor effects of AVP. They found that injection of the antagonist had no effect on resting fetal blood pressure or heart rate, however hemorrhage produced a greater fall in fetal arterial pressure in the presence of the blocker than in its absence. They concluded that AVP is not important in maintenance of fetal arterial pressure in the resting state, but that AVP plays a role in recovery of fetal blood pressure after hemorrhage. No details are given as to the effective dose of the antagonist used, but since these synthetic molecules invariably have mixed actions, it is possible that the effective dose for an antivasopressor effect of the antagonist used in the present study was similar to or less than that in the study of Kelly et al (21). Thus, although my intent was to block the antidiuretic effects of AVP, the antagonist I used may also have been an even better blocker of pressor effects than was that used by Kelly et al. At any rate, the results of the present study suggest that AVP may play a small role in the maintenance of normal arterial blood pressure in the fetus under resting conditions.

E. AVP and Control of Fetal Blood Volume

Fetal blood volume fell by a small but significant amount during the infusion of AVP. While no change was seen

in fetal blood volume during infusion of comparable doses of AVP in another study from our laboratory (49), the infusion lasted only 30 minutes in that study, and 15 ml of saline were infused as the vehicle. Since fetal blood volume decreased by a maximum of only 8 ml in the present study, and the decrease was not yet significant at 30 minutes post-injection, these data are not inconsistent. In the present study the reduction in blood volume is probably not due to the simultaneous slight elevation in arterial pressure tending to force fluid out of the vasculature into the interstitium, since venous pressure, and therefore probably capillary pressure, did not change. It has been suggested that AVP may change the permeability characteristics of the placenta (9,22,50), and this might account for the decrease which was seen in fetal blood volume if fluid may have moved from fetus to mother across the placenta.

Fetal blood volume appeared to be controlled during hypertonic injection in normal fetuses, while in both AVP-infused and AVP-blocked fetuses blood volume increased after hypertonic injection, i.e. fetal blood volume did not appear to be as carefully controlled in these animals. The reason for the similar responses in AVP-infused and AVP-blocked fetuses is unclear, since one would tend to expect opposite responses in the two cases.

F. Recovery of Fetal Renal Function Following Surgery

In 1972, Gresham, et al (17) measured urine output, urine osmolality, GFR, and a number of other indices of renal function in fetal sheep daily for several weeks following surgery. These investigators concluded that it takes three to six days for recovery of normal renal function post-operatively in the fetal lamb, and the conclusions of that study have become dogma within the field. Consequently, investigators wishing to study aspects of renal function in the fetal lamb have felt obligated to allow their animals to recover for six days before beginning experiments.

The present study does not support the results of Gresham, et al. In my animals fetal urine osmolality was considerably hypotonic to plasma the day after surgery and did not change significantly between the first and sixth post-operative days. The exception to this is that in two animals fetal urine osmolality jumped to 309 and 340 mOsm/kg on the sixth day. Blood gases were normal in these animals and there were no other indications of fetal distress. It is possible that the increase in urine osmolality in these fetuses was indicative of impending delivery, as urine osmolality is known to rise just prior to birth (53). Changes in urine and amniotic fluid Na^+ and K^+ were relatively minor, and trends continued through the sixth day without leveling off. Although fetal hematocrit was elevated immediately

after surgery, values on the third postoperative day were not different from those on the sixth day. Maternal blood pH was low the first day after surgery but did not change significantly after the second day. Fetal pH the first day after surgery was not different from that on any subsequent day, although fetal pH tended to follow variations in maternal pH, and the two variables were significantly correlated (Figure 5). Thus, it appears that normal blood and urine values in both fetus and mother were obtained on the third and all subsequent postoperative days, suggesting that it is unnecessary to wait more than three days after fetal surgery before performing renal function studies in the sheep.

G. Control Values

In general, the mean control values (Table 3) were not different among the three groups of animals. The values reported for blood gases and cardiovascular variables are consistent with average values for healthy fetuses in our laboratory (55). Values for plasma and urine constituents, renal clearances, and GFR are in good agreement with those reported in the literature (2,17,36). Fetal urine flow is somewhat higher than that reported by Gresham, et al (17) but is consistent with more recently published values (7,37).

H. Changes in Amniotic Fluid Osmolality

It is known that the osmolality of amniotic fluid increases after maternal plasma is made hypertonic (40,56),

and some investigators have interpreted these findings to reflect osmotic transfer of water from the amniotic fluid to the mother across the fetal membranes and uterine wall (40). While this is one possible explanation, fetal urine may also be responsible for the observed increase in amniotic fluid osmolality. Hypertonic injections into the mother are known to cause hyperosmolality, hypovolemia, and elevated AVP levels in the fetus (23,57). Since AVP causes an increase in urine osmolality (25,37,present study) and fetal urine is known to be a major source of amniotic fluid (45), it is not unreasonable to expect that fetal urine might be important in increasing amniotic fluid osmolality.

In the present study, amniotic fluid osmolality increased slightly during AVP infusion, and by about 11 mOsm/kg after hypertonic injection in both normal and AVP-infused fetuses. In these experiments fetal urine osmolality also increased. However, after hypertonic injection in antagonist-infused fetuses, when fetal urine osmolality was prevented from increasing, the increase in amniotic fluid osmolality was much smaller (4 mOsm/kg). This suggests that a major part of the increase in amniotic fluid osmolality following maternal hypertonic injection is due to the increased osmolality of fetal urine, and that water movement across the membranes and uterine wall plays a relatively minor role in increasing amniotic fluid osmolality under

these conditions.

I. Binding of AVP to Renal Medullary Tissue

Given the relative lack of urine concentrating ability of the fetal kidney despite the presence of circulating AVP levels which are at least as high as those in the adult, one might expect that the sensitivity and/or the capacity of the AVP receptors in the fetal renal medulla is reduced. As shown in Figure 82, B_{max} is over 4-fold higher in maternal tissue than in fetal tissue, while the slopes of the lines ($-1/K_D$) are not significantly different. Thus, it appears that the sensitivities of the AVP receptors are not different, but that the fetal kidney simply has fewer receptors through which AVP can act. It cannot be determined from the present data, however, whether or not the coupling between AVP receptors and adenylate cyclase activation is different in fetal and adult tissue.

J. Problems Encountered in Carrying Out the Studies

The primary problem encountered in these studies was a relatively small number of observations in three areas, resulting from several factors. One area was that of the AVP assay. Although I checked into the proper procedures for collection of samples before beginning the experiments, it was not until half of the experiments had been completed that I found that heparin present in the samples interfered with the assay. Thus the samples already stored had to be

discarded.

A second area in which relatively small numbers of observations were made involved infusion of the AVP antagonist. While it would have been ideal to be able to carry out eight to ten experiments using this blocker, I was able to secure sufficient antagonist for only five experiments. Considering the biological variation routinely present in these whole animal experiments, it is difficult to demonstrate statistical significance of small changes with an "n" of only five.

The third part of the studies in which fewer-than-ideal numbers of experiments were run is the receptor binding assays. This problem resulted from two factors. The first is that the tissue used for these studies was secured from animals which were being sacrificed for other studies, so that often the tissue became available at times which conflicted with my whole animal work. Thus I was able to take advantage of only a fraction of the tissue which became available. The second reason for the low number of experiments in this part of the study is simply a time factor. Several months were spent in developing the assay, optimizing conditions for binding, etc., and once these aspects of the techniques were perfected, relatively little time was left for carrying out the final experiments. However, I hope to continue these studies in the future because it appears that differences do exist between AVP receptors in fetal and

adult renal tissue.

Aside from these difficulties, the major technical problem which I encountered in these studies was in the measurement of the intrarenal osmolality gradient. In preliminary studies, it appeared that it would be possible to estimate tissue osmolality simply by homogenizing a weighed piece of tissue in a known amount of distilled water and measuring osmolality in a freezing point depression osmometer. Later I encountered difficulties with this method because of the small amount and fibrous nature of the tissue. Since the experimental design also required sacrificing each animal after only one experiment, and measurement of the intrarenal gradient was judged not to be a vital part of the study, I elected to forego intrarenal gradient measurements in favor of the option of performing more than one experiment per animal. However, there is no indirect evidence of an increase in the intrarenal gradient in these studies, i.e. fetal urine osmolality plateaued after AVP infusion and/or hypertonic injection rather than gradually continuing to increase as might be expected if the intrarenal gradient were increasing over time.

VI. CONCLUSIONS

In order to study the responses of the fetal kidney to hypertonicity and to determine the role of AVP in this response, hypertonic NaCl was injected simultaneously into pregnant ewes and their fetuses, and maternal and fetal renal function were measured over the next four hours. These experiments were performed in normal, AVP-infused, and AVP-blocked fetuses. In vitro studies were also performed to compare AVP receptor binding characteristics in fetal and maternal renal medullary tissue. The major findings of the study were:

1) The fetal kidney responds to hyperosmolality with transient increases in urine flow, GFR, and renal clearance values, prolonged increases in urine osmolality, Na^+, and Cl^-, and prolonged decreases in urine flow and free water clearance.

2) The transient changes in fetal renal function after hypertonic injection appear to be due to the effects of transient increases in arterial pressure, while the prolonged changes appear to be due to the effects of elevated AVP levels.

3) The exception to the above statement is that urine Na^+ and Cl^- concentrations increased by the same amounts in all fetuses after hypertonic injection, independent of the effects of AVP. In AVP- and antagonist-infused fetuses, where urine osmolality did not increase after hypertonic injection, as well as in the maternal ewe, the relative contributions of Na^+ and Cl^- to total urine osmolality were increased after hypertonic injection, while they remained constant in normal fetuses.

4) In spite of the elevation in fetal urine osmolality and decrease in urine flow in response to hypertonicity, the fetal kidney was not able to concentrate urine to the same extent as that of the mother, as evidenced by the smaller rise in urine osmolality and the smaller fall in free

water clearance in the fetus. Moreover, there was no evidence of an increase in the fetal intrarenal solute gradient during AVP infusion and/or hypertonic injection.

5) Injection of the AVP antagonist $d(CH_2)_5$D-tyr(Et)VAVP had no effect on fetal plasma and urine levels of Na^+, Cl^-, K^+, or urea, nor on osmolality, and also did not affect fetal urine flow. However, mean fetal blood pressure tended to be reduced by about 2 mmHg during antagonist infusion. The antagonist also prevented the normal prolonged increase in urine osmolality and decrease in urine flow in response to hypertonic injection. It appears that AVP is unimportant in maintenance of normal fetal renal function in the resting state, but may be involved in the normal maintenance of fetal blood pressure.

6) Fetal renal function does not appear to change substantially in the first six days after surgery.

7) The increase in amniotic fluid osmolality after maternal plasma is made hypertonic appears to be due primarily to the increase in fetal urine osmolality rather than to water movement across the membranes and uterine wall.

8) The capacity of AVP receptors in fetal renal medullary tissue is less than one fourth of that of maternal tissue, while the affinities of the receptors in the two types of tissues are not significantly different. Thus it appears that the relatively weak response of the fetal kidney to circulating AVP may be due to a sparsity of AVP receptor sites.

VII. RECOMMENDATIONS

During the course of these studies several ideas came to mind as to directions which might be taken next in this area. As mentioned earlier, a logical way to proceed with the receptor binding studies would be first to complete several more experiments identical to those presented here so that a more rigorous statistical analysis can be performed. Then it would be advisable to characterize the receptors further by performing temperature- and pH-dependency as well as competition studies. It would also be important to determine the relationship between AVP receptor binding and activation of adenylate cyclase in the tissues. Finally, it would be valuable to do these studies at several stages of gestation, in the newborn animal at perhaps one week and two months of age, as well as in the adult non-pregnant animal, in placental tissue, and perhaps in another species also. Now that the techniques are worked out, this would be a relatively simple and potentially rewarding area to pursue.

Another direction one might want to take as a next step is to study the effects of AVP on placental permeability characteristics, including the reflection coefficients and permeability surface area products for osmotically active particles, as well as the filtration coefficient of the placenta. As mentioned earlier, it has been suggested that AVP may change these parameters, but it is not at all clear

at this point what these changes might be. A variety of different approaches might be taken to this problem, including receptor binding and other in vitro studies, placental perfusion, whole animal work, and computer modeling. In any case, this area promises to be an exciting frontier to explore.

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LOMA LINDA UNIVERSITY

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THE ROLE OF VASOPRESSIN IN THE FETAL RENAL RESPONSE TO HYPERTONICITY

by

Lori L. Woods

A Dissertation in Partial Fulfillment of the
Requirements for the Degree Doctor of Philosophy
in Physiology

December 1984

Abstract

THE ROLE OF VASOPRESSIN
IN THE FETAL RENAL RESPONSE TO HYPERTONICITY

by

Lori L. Woods

In order to study the effects of hyperosmolality on fetal renal function and to gain insight into the role of AVP in the fetal urine concentrating mechanism, two types of studies were performed. In whole animal studies, 9% NaCl was injected intravenously into 8 chronically catheterized pregnant ewes of 130-135 days gestation and their fetuses, followed by a continuous infusion of 9% NaCl into the ewes. Fetal and maternal plasma osmolalities rose initially by 5% and remained elevated for the next four hours. Fetal arterial and venous pressures increased transiently. Fetal urine flow increased transiently by 73%, remained elevated for at least 10 minutes, and averaged 27% below control beyond one hour. Fetal GFR, C_K, and C_{urea} were increased transiently; fetal C_{Na}, C_{Cl}, and C_{osm} were approximately doubled for up to one hour. Fetal urine osmolality, $[Na^+]$, and $[Cl^-]$ increased by 47%, 47%, and 74% respectively and remained at these levels beyond about 30 minutes. Fetal C_{water} had fallen by 43% after one hour, but did not become negative, and remained constant thereafter. Fetal plasma

AVP rose initially by 42% and then fell towards normal.

In six fetuses AVP was infused for two hours before and four hours after hypertonic injection, causing an average rise of 48% in plasma AVP levels. During AVP infusion fetal arterial pressure was increased by 5%. Fetal urine flow averaged 37% below control; fetal urine osmolality became slightly hypertonic by one hour and then plateaued; fetal GFR increased by 22%. Fetal C_{water} fell to zero. After hypertonic injection fetal urine osmolality did not change, but the relative contributions of Na^+ and Cl^- to total osmolality increased. Fetal C_{Na}, C_{Cl}, and C_{osm} were elevated for the duration of the experiment. Fetal AVP levels increased an additional 27%. In five animals the AVP antagonist $d(CH_2)_5$D-tyr(Et)VAVP was infused into the fetus for two hours before and four hours after hypertonic injection. Fetal arterial pressure was reduced an average of 3% and GFR fell by 33% but no other changes were seen in response to the antagonist. After hypertonic injection fetal arterial pressure fell to 7-10% below control. Fetal urine flow showed a transient increase of 175% and a prolonged increase of 38%. Fetal urine osmolality did not change but fetal urine $[Na^+]$ and $[Cl^-]$ rose by 47% and 142%. C_{osm}, C_{Na}, and C_{Cl} showed transient increases of 170-500% and prolonged increases of 50-200%.

Binding of ^{3}H-AVP to fetal and maternal renal medullary

tissue preparations was also studied. Scatchard plots of the data yielded average K_D values of 0.44 and 0.80 nM, and intercepts of 15.2 and 67.2 fmol/mg protein for fetal and maternal tissue respectively. The K_D's were not significantly different.

It was concluded that the transient changes in fetal renal function after hypertonic injection appear to be due to transient increases in arterial pressure, while, except for the increases in urine $[Na^+]$ and $[Cl^-]$, the prolonged changes appear to be due to the effects of elevated AVP levels. The weak response of the fetal kidney to hypertonicity relative to that of the adult appears to be due at least in part to a relatively low number of AVP receptor sites in fetal medullary tissue.