

An Evaluation of Fetal Renal Function in a Chronic Sheep Preparation

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ABSTRACT Fetal renal function in the sheep was investigated in a chronic preparation which permitted repeated evaluations of urine flow and osmolality as well as renal clearances in animals which were unanesthetized and remote from acute surgical stress. Measurements of fetal blood pressure, pH, osmolality, fetal growth in utero, and final outcome did not indicate an adverse effect of the experimental procedure on the fetus.

Fetal urine flow and osmolality were highly variable during the early postoperative period. They did not stabilize until 3–6 days after surgery, when urine osmolality became markedly hypotonic (range 65–160 mOsm/kg H₂O) and urine flow rose to approximately 0.14 ml/min·kg. Fluctuations in urine flow and osmolality in the early postoperative period were the result of tubular reabsorption of water rather than a change in the glomerular filtration rate.

The inulin-¹⁴C clearance, used as a measure of the glomerular filtration rate, was 1.05 ± 0.05 ml/min·kg (mean \pm SEM) for all animals studied. Urea, fructose, sodium, and chloride were partially reabsorbed by the fetal kidney, while creatinine was secreted.

Continuous drainage of fetal urine for 18 days in one animal demonstrated that the fetus was able to excrete large amounts of water, sodium, and fructose without apparent detrimental effects.

INTRODUCTION

Knowledge of renal function in prenatal life is meager because of the technical difficulties of obtaining reliable information from the fetus. In the early part of this century, the bulk of the information was derived from

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the analysis of fetal and newborn urine (2, 3). Subsequent studies of human fetal renal function have been limited to histologic examination of kidneys from the abortus (4), radiographic evidence of dye excretion (5), and evaluation of bladder urine at the time of delivery (6).

Recently, investigators have begun to measure urine flow and renal clearances in fetuses of such experimental animals as sheep and goats. These measurements have been limited to the time period during surgery or to a few hours after the operation. In the sheep fetus older than 115 days gestational age, estimates of glomerular filtration rate (GFR)¹ have ranged from 0.2 to 1.4 ml/min·kg body weight and urine osmolalities from 60 to 600 mOsm/kg H₂O (7–11). It is not clear whether these wide ranges observed represent normal variability between animals or are, somehow, the product of abnormal experimental conditions.

The major deficiencies of acute experimentation on the mammalian fetus include the effects of operative and anesthetic stress and the limited time available before the preparation deteriorates. In order to circumvent such problems, we have developed a chronic preparation in which the urine flow and renal clearances of the fetal lamb may be studied in the same fetus over a period of several days.

METHODS

Experimental design. A schematic representation of the surgical preparation is presented in Fig. 1. Catheters were inserted into a maternal femoral artery and a fetal pedal artery and vein for blood sampling and infusion. Additional catheters were placed into the fetal bladder by way of the urachus and into either the amniotic or allantoic sac. This experimental model permits either continuous urine

¹ Abbreviation used in this paper: GFR, glomerular filtration rate.

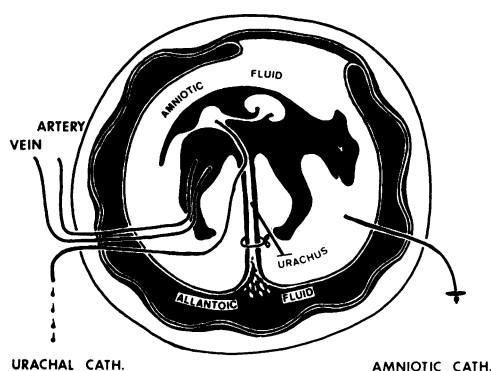


FIGURE 1 Surgical preparation. Anatomical characteristics of the sheep fetal-maternal unit and catheter deployment for this study are presented in a schematic fashion. (Basic drawing modified from Alexander and Nixon [30]).

drainage to the outside for extended periods of time or return of urine into the amniotic or allantoic sac by joining the urachal and sac catheters. Accurately timed urine specimens were collected into a fraction collector which was positioned below the level of the fetus in order to drain the fetal bladder continuously and decrease the possibility of retrograde urinary tract infection. Urine was collected in 25–50 min fractions with thymol added to all specimens as a preservative, except to those obtained during clearance studies. The latter samples were analyzed immediately.

Surgery. Nine ewes of Dorset or mixed western breed were studied. The gestational ages of animals M, 1, and 2 were 117, 122, and 130 days respectively at the day of surgery. The ages of the other fetuses are given in Table II. Hay and grain were withheld for 48 hr before surgery, although water was permitted ad lib. Penicillin (600,000 U aqueous procaine) and streptomycin (0.5 g) were given intramuscularly before surgery and on the first 3 days post-operatively.

Pentobarbital (5 mg/kg) was administered through a jugular vein catheter for sedation, and surgery was performed under spinal anesthesia (6 mg Pontocaine, in hypertonic glucose).

After a sterile abdominal preparation, a midline incision was made in the lower abdomen. The pregnant uterine horn was delivered onto the abdomen and wrapped in a Silver Swaddler (12) to minimize drying and radiant heat loss. A small segment of umbilical cord near its fetal insertion was exposed through a transverse incision of the uterine wall and fetal membranes. After a longitudinal incision in the cord, the urachus was identified as a small tubular structure surrounded by a distinct vascular plexus. The urachus was ligated distally and a polyvinyl catheter (1.2 mm o.d.) was threaded through the proximal stump into the urinary bladder—a distance of approximately 6 cm. The catheter was secured with a silk suture ligature and tissue adhesive (isobutyl-2-cyanoacrylate monomer, Ethicon, Somerville, N. J.). The fetal membranes were closed with a 3–0 silk purse-string suture, and the myometrium was repaired with a continuous locking 3–0 silk suture.

A second incision was made near the tip of the pregnant horn in order to expose the fetal hindlimb. Polyvinyl catheters were advanced approximately 16 cm into a pedal artery and vein. Through the same incision a polyvinyl catheter was placed either into the amniotic (animals 5, 6, 7,

9, 10) or allantoic sac (animals M, 1, 2, 4). The uterus and fetal membranes were closed as previously described. A final catheter was then placed into a maternal femoral artery. All catheters were exteriorized through a subcutaneous tunnel and placed into a cloth pouch on the animal's flank. The catheters were irrigated daily with 0.5 ml of heparinized saline (1,000 U/ml). The ewe was usually standing and eating within 6 hr after the operation. Postoperatively, the sheep was confined in a specially constructed cart for the duration of the experiment. This allowed mild exercise without jeopardizing the exteriorized catheters.

Analytical methods. Urine and plasma osmolalities were measured by freezing-point depression of a 0.25 ml sample (Osmette osmometer, Precision Systems, Inc, Waltham, Mass.). Samples of a standard solution were reproducible within $\pm 0.5\%$. Fetal and maternal blood samples for osmolality were obtained at approximately weekly intervals. They were collected, centrifuged, and stored as previously described (13). Fructose concentrations were determined by the resorcinol method of Yaphe and Arsenaault (14), which was adapted for automated analysis. Urea concentrations were measured with a Technicon AutoAnalyzer (Technicon Corporation, Ardsley, N. Y.) by the carbamide-diacetyl reaction (15).

A microtechnique for obtaining true endogenous creatinine concentrations was used, based upon the method described by Owen, Iggo, Scandrett, and Stewart (16). Values obtained from the microtechnique agreed within $\pm 1\%$ of the macromethod. Sodium and potassium concentrations were measured by flame photometry (17) and chloride concentration by potentiometric titration (18). Blood for pH determination was collected anaerobically in heparinized glass syringes and stored in ice. The pH was measured at 39.5°C within 5 min of sampling by means of a capillary glass electrode (Radiometer Co., Copenhagen, Denmark). Radioactivity of inulin- ^{14}C in blood and urine was counted by a liquid scintillation method. The precautions suggested by Gennari, Cortell, and Schwartz (19) were followed in order to avoid loss of ^{14}C activity in the scintillation vial.

Physiologic techniques. In sheep 1, 2, and 4 inulin- ^{14}C clearances were determined on the basis of two sets of plasma concentrations and urinary excretion rates measured near the end of a 150 min constant infusion period. This method was then discontinued in favor of a simpler technique capable of yielding more information. In sheep 5, 6, 7, 9, and 10, inulin clearances were calculated by injecting approximately $10\ \mu\text{Ci}$ inulin- ^{14}C (inulin-carboxyl- ^{14}C , New England Nuclear Corp., Boston, Mass.) into the fetal pedal vein catheter and determining the concentrations of fetal arterial plasma and the renal excretion rates of ^{14}C in five to eight separate time periods over a total of 10 hr.² The fetal arterial blood pressure was obtained by measuring the pressure difference between the pedal arterial and the amniotic or allantoic catheters. One fetus (fetus M) was used to observe urinary output and osmolality with as minimum disturbance of the normal physiology of the preparation as possible. Hence this fetus did not receive any infusions and its urine was collected intermittently.

Data analysis. The intrauterine fetal weight at a given day was estimated from the weight measured at the time of delivery, by means of the following equation:

$$\log \text{intrauterine weight (g)} = \log \text{birth weight (g)} - 0.0153 \times \Delta t \quad (1)$$

² A detailed description and analysis of this technique is in preparation.

where Δt represents the time difference, in days, between the day of delivery and the day of estimate. Equation 1 was derived from data on the growth of fetal sheep which are available in the literature (20–23). 73 fetal lambs of known gestational ages from 110 days to term were included in the construction of an intrauterine growth curve described by the equation:

$$\log \text{ fetal weight (g)} = 1.4208 + 0.0153 \times \text{gestational age (days)} \quad (2)$$

The factor 0.0153 in equation 1 represents the slope of the curve. By calculating the 95% confidence limits of the slope (0.0137–0.0169), it can be determined with the same degree of confidence that the probable error in estimating intrauterine weight is $\pm 1.8\%$ when Δt is 5 days and 3.6% when Δt is 10 days.

The statistical significance of the ratios of the various clearances to the clearance of inulin- ^{14}C was estimated by the application of Fieller's theorem (24). This statistical analysis permits the determination of confidence limits for a ratio of two dependent variables which are assumed to be normally distributed. The confidence limits are not symmetrical about the mean, since a ratio has skewed distribution.

RESULTS

Fetal outcome. Of the nine fetuses that were studied, three were delivered vaginally (ewes M, 5, 9) and six by cesarean section. All fetuses maintained normal arterial pH, plasma osmolality, and blood pressure throughout the experiment (see Table II). They were alive at time of delivery, appeared healthy, and were of appropriate weight for gestational age. Although fetal urine was collected continuously for 18 days in fetus 4, clearances were measured only on the 4th and 8th day, because the fetal arterial catheter ceased to function. Animals 2, 5, and 9 were not included in Fig. 2 because the urachal catheter became occluded 3 days after surgery in animals 2 and 5, and the fetus 9 delivered shortly after the first clearance study at the end of the 3rd postoperative day.

Fetal urine flow and osmolality. Fig. 2 presents the results obtained in six of the nine animals studied. In each case the first urine sample was collected during surgery. The specimen was markedly hypotonic when it was obtained within 1 hr of the start of the surgical procedure (animals 4, 6, 7). When sampling was delayed (animals M, 1, 10), the urine osmolality was greater. After surgery, the tonicity of the fetal urine was elevated for several hours and then fell below 200 mOsm/kg H_2O . A rebound was observed on the 2nd or 3rd day, so that a stable, low osmolality was attained only 3–6 days after surgery. The rebound was noted in all fetuses regardless of whether the fetus had urine returned to the allantoic sac intermittently (M), returned to the sac continuously (fetus 1), or drained from the fetus continuously (fetuses 4, 6, 7, 10). Performance of renal clearance studies had no consistent

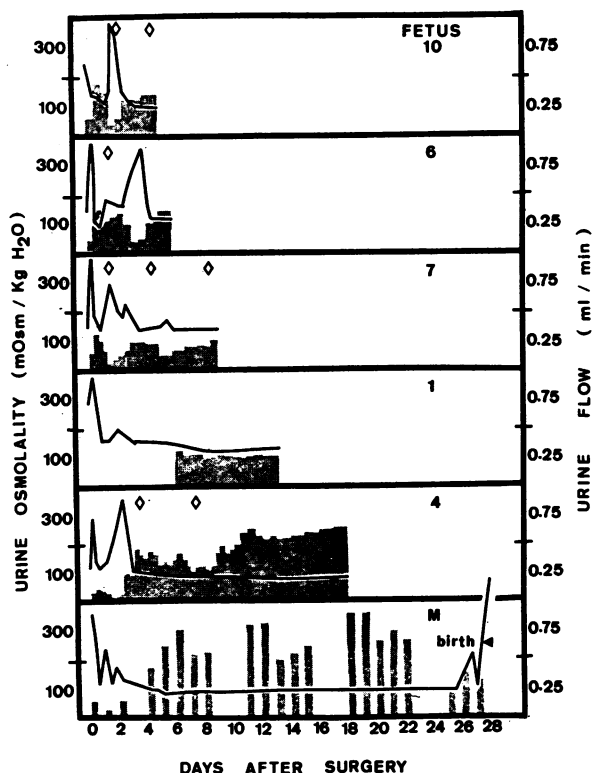


FIGURE 2 Fetal urine flow and osmolality. Osmolalities are represented by the solid line, while urine flows and sampling intervals are shown by the bar graphs. The diamonds indicate the time of the clearance studies presented in Table II.

effect on urine flow or tonicity. The marked oscillations in urine flow and osmolality during the early postoperative period produced a range of urine tonicity from 90 to 400 mOsm/kg H_2O . Urine flow was inversely related to osmolality and became less than 0.03 ml/min·kg during the hypertonic rebound. When stability was achieved, the flow rose to a mean of 0.14 ml/min·kg (range 0.09–0.31). In the stable period, there were fairly large interindividual differences in urine osmolality and flow (range 65–160 mOsm/kg H_2O and 0.09–0.31 ml/min·kg), but the intraindividual differences were much less, as Fig. 2 demonstrates. Approximately 36 hr before labor and delivery there was a temporary increase in urine osmolality in fetus M. During labor the urine osmolality rose to 340 mOsm/kg H_2O and was 480 mOsm/kg H_2O in the first *postpartum* sample.

Fetal urine composition. The results of analysis of the composition of hypotonic and hypertonic fetal urine are presented in Fig. 3. Approximately 90% of the total solute could be accounted for by fructose, urea, creatinine, and the electrolytes: sodium, potassium, calcium, chloride, and phosphate. Sodium was the major

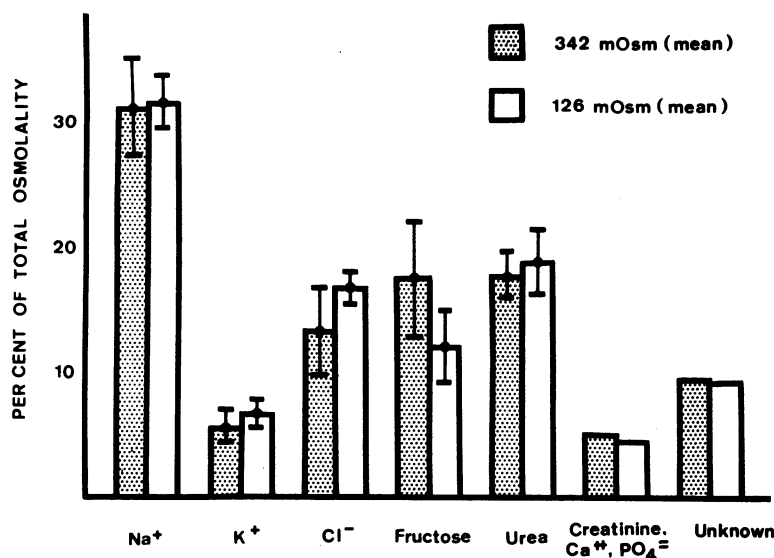


FIGURE 3 Fetal urine composition. Relative contributions of various urine solutes to total osmolality during periods of maximal and minimal concentration are represented as per cent of total \pm SEM.

contributor to the total solute concentration. The contribution of urea was relatively small and comparable to that of fructose and chloride. In contrast to fructose, glucose was present in trace amounts only (approximately 1 mg/100 ml). As shown by Fig. 3, the proportions of various urine solutes were quite similar during periods of maximal (mean 342 mOsm/kg H₂O) and minimal (mean 126 mOsm/kg H₂O) concentrations. No variability is given for calcium, phosphate, and creatinine because these substances were measured once in pooled samples of portions. The rates of sodium excretion during maximal and minimal urine concentration were not significantly different (5.2 ± 1.7 and 4.3 ± 0.90 μ Eq/min per kg, $P > 0.05$).

Fetal renal clearances. Eight fetuses received a total of 14 injections of radioactive inulin. The mean GFR's

calculated on the basis of these 14 observations are presented in Fig. 4. Statistical analysis of the diagram shown in Fig. 4 demonstrated no systematic change of the GFR per kg of fetus in the days after surgery ($P > 0.2$, test for independence of Y on X). The mean of the 14 observations was 1.05 ± 0.05 SEM. All but one of these observations were within $\pm 20\%$ of the mean. Since in 9 of the 14 observations the GFR was measured repeatedly over a 10 hr period, we were able to determine that random errors of measurement and fluctuations of the measured variable within a given observation period contributed but a small fraction of the total variance (see Table I). Simultaneous renal clearances of inulin-¹⁴C, fructose, urea, and creatinine were determined in six of the animals. The results are presented in Table II. The mean ratios of the clearances of fructose, urea, and creatinine to inulin are presented in Table III. This table demonstrates that the creatinine/inulin ratio was significantly higher than one and that the other ratios were significantly less than one.

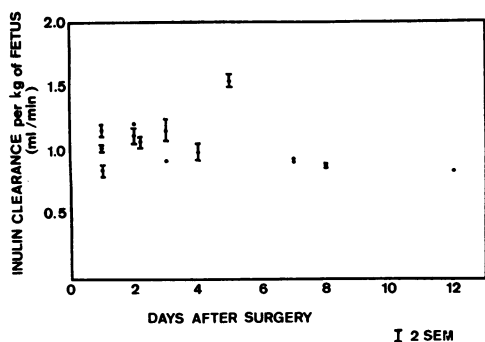


FIGURE 4 The inulin-¹⁴C clearances per kilogram of fetus are plotted against the days after surgery. For nine of the observations, the range of variability is indicated.

TABLE I
Analysis of Variance of Inulin-¹⁴C Clearance Measurements

Source of variation	Sum of squares	Degrees of freedom	Mean squares	F ratio
Between observations	1.7600	8	0.22000	69.4
Within observations	0.1235	39	0.00317	$F_{0.99}(8,39) = 3.00$
Total	1.8835	47		

TABLE II
Summary of Fetal Renal Clearances

Animal	Fetal age	Clearance						Arterial pH	Mean arterial pressure	Estimated fetal wt	Birth wt	Days after surgery
		Inulin	Fructose	Urea	Creatinine	Sodium	Chloride					
	days			ml/min per kg					mm Hg	kg		
4	122	0.94	0.44	0.32	—	0.0189	0.00527	—	41.2	3.00	4.90	3
	126	0.94	0.46	0.44	—	0.0137	0.004	7.320	39.9	3.45	—	7
5	129	0.86	0.55	0.67	1.67	0.180	0.0139	—	—	3.00	3.84	1
	131	1.17	0.69	0.82	1.73	—	—	—	—	—	—	3
6	132	1.17	0.77	0.73	1.88	—	—	7.310	44.6	2.30	2.55	2
7	125	1.04	0.48	0.40	1.13	0.0250	0.0134	7.315	43.7	2.07	2.95	2
	128	1.00	0.74	0.56	1.54	0.0548	0.0358	7.326	42.4	2.30	—	5
	132	0.89	0.75	0.69	1.67	—	—	7.340	41.8	2.65	—	8
9	134	1.13	0.48	0.51	—	—	—	7.345	42.8	2.88	3.20	3
10	128	1.08	0.50	0.54	1.13	0.0521	0.0154	7.333	46.0	2.11	2.70	2
	131	1.54	0.60	0.76	1.88	—	—	7.318	44.1	2.34	—	5
Mean \pm SEM		1.07 \pm 0.06	0.59 \pm 0.03	0.59 \pm 0.04	1.58 \pm 0.10							

Effects of continuous fetal urine drainage. The total volume of urine excreted by the unstressed fetus is much larger than the combined volumes of the allantoic and amniotic fluids at term. Thus, under normal circumstances there is a circulation of water and solutes from the fetus into the amniotic and allantoic sacs and back to the maternal and fetal organisms. Diversion of the fetal urinary output to the outside for a prolonged period of time disrupts this circulation and therefore affords the opportunity of testing its function. This was the case in animal 4, where the fetal urinary output was drained continuously to the outside for 18 consecutive days. The data presented in Table IV demonstrate that this experimental procedure caused the fetus to lose large amounts of water, sodium, and fructose. The sodium lost was approximately equal to, and the water lost approximately five times greater than, the amounts needed by the fetus for normal growth and development in the 18 day period. Despite the magnitude of these losses, the composition of fetal plasma remained constant throughout the experiment (see Table IV), and

an apparently normal lamb weighing 4900 g was delivered by cesarean section on the 148th day of gestation. At the time of the section, it was noted that the uterine wall conformed closely to the fetal body. This observation suggested the presence of abnormally low volumes of allantoic and amniotic fluids. Consequently the uterine cavity was opened carefully in order to avoid loss of fluids and to permit measurements of amniotic and allantoic volumes. The allantoic cavity was reduced to a virtual space with no detectable fluid, while the amniotic sac contained only 50 ml.

Fetus 1, which had its urine drained continuously for 7 days, presented a similar picture at the time of cesarean section and had a total combined volume of amniotic and allantoic fluid of 130 ml.

DISCUSSION

The primary aim of this investigation was to provide information about renal function in a chronic fetal preparation from the time of surgery, through the recovery period, to the re-establishment of a steady state. This

TABLE III
Statistical Analysis of Clearance Ratios

Mean = $\frac{\sum \text{Clearance X}}{\sum \text{Clearance Inulin-}^{14}\text{C}}$			
Ratio	Fructose/Inulin	Urea/Inulin	Creatinine/Inulin
Mean	0.55	0.55	1.48
95% Confidence limits	0.46–0.65	0.46–0.64	1.31–1.65

TABLE IV
Results of Continuous Urine Drainage for 18 Days (Animal 4)

	Total urinary excretion	Plasma concentration	
		4th day	18th day
Urine	9,250 ml		
Na ⁺	165 mEq	144.4 mEq/liter	146.8 mEq/liter
K ⁺	33 mEq	3.8 mEq/liter	4.0 mEq/liter
Cl ⁻	83 mEq	109.5 mEq/liter	109.9 mEq/liter
Fructose	33,318 mg	92.0 mg/100 ml	87.0 mg/100 ml
Urea	9,644 mg	21.4 mg/100 ml	35.3 mg/100 ml
Osmolality		293 mOsm/kg H ₂ O	296 mOsm/kg H ₂ O

study clearly demonstrates that in the steady state, fetal renal function has the following main characteristics: (a) Production of very large amounts of hypotonic urine. The urine production rate per kg body weight and the tonicity are comparable with those of an adult animal with water diuresis. (b) Sodium excretion rate of approximately 6 mEq/day per kg of body weight. (c) GFR/kg body weight approximately 30–50% that reported for a newborn lamb and for an adult animal (8). This statement is based on the assumption that the clearance of inulin-¹⁴C is a measure of GFR in the fetus. Previous estimates of GFR in acute experiments on exteriorized fetal lambs had given the results summarized in Table V. The use of test substances with markedly different clearances, such as creatinine, inulin, and urea, accounts for some of the discrepancies in the results. In addition, it is possible that the use of acute preparations for these measurements contributed to the wide range of variability.

Our data demonstrate that acute stress could reduce the fetal urinary flow severalfold and produce a hypertonic urine with respect to plasma osmolality. Recovery of fetal renal function from the stress of surgery was

slow (3–6 days) and characterized by large fluctuations of urine flow and osmolality. In this period of instability, the inulin-¹⁴C clearances and Na excretion rates were approximately the same as in the steady state. Thus it would appear that stress was affecting primarily the tubular reabsorption of water.

The possibility that diversion of the fetal urinary flow to the outside might adversely affect the fetus and/or alter the flow rate and osmolality of fetal urine was considered. This was thought possible since under normal circumstances the substances excreted by the fetal kidneys are still available to the fetus through swallowing of amniotic fluid and reabsorption across the fetal membranes. Surprisingly, continuous drainage of fetal urine for as long as 18 days had no demonstrable adverse effect on fetal growth and plasma composition, despite considerable losses of water, electrolytes, and fructose (see Table IV). The urine flow rate and osmolality were comparable in continuous and discontinuous drainage. This experimental finding proves that the fetus is able to acquire from its environment amounts of water, sodium, chloride, and carbohydrates which are in great excess of the quantities needed for metabolism and

TABLE V
Comparison of GFR in Fetal Sheep of >115 Days of Gestation

Method	GFR (mean)	Coefficient of variation	Reference
	<i>ml/min · kg</i>		
Mean of creatinine, urea, and fructose clearances	0.6	53	Alexander, Nixon, Widdas, and Wohlzogen (7)
Sodium- ¹³¹ I iothalamate	0.75	27	Smith and Schwartz (25)
Exogenous creatinine	1.41	—	Alexander and Nixon (9)
Inulin	0.75	33	Smith, Adams, Bordon, and Hilburn (10)
Inulin- ¹⁴ C	1.05	18	Present study

growth. The only clear effect of continuous fetal urine drainage on the system appeared to be a marked reduction in the volumes of allantoic and amniotic fluids.

After 18 days of urinary drainage, fetus 4 had excreted more than 33 g of fructose (see Table IV), while plasma concentrations changed very little. This suggests that the blood levels were maintained by an active regulation of the synthesis of this carbohydrate and not by simply reabsorbing the large urinary losses from the liquors. The metabolic implications remain obscure, since there is no conclusive evidence that the sheep fetus utilizes the fructose which is available.

In order to consider the physiologic meaning of these observations, it is necessary to realize that in the last stages of pregnancy each fetal organ must be adapted to the conditions of intrauterine life as well as being prepared to change its mode of operation with birth, when the fetus is exposed to a new environment. Thus, in fetal life a small GFR by adult standards may have the advantage of reducing the amount of Na presented to the tubules for reabsorption, in turn reducing renal O₂ consumption. A low renal O₂ consumption would have the additional advantage of requiring a relatively low renal blood flow. The recent finding (26, 27) that renal blood flow in fetal life is considerably less than after birth agrees with this hypothesis. Despite the low GFR, the normal fetus maintains a high rate of urine flow by the simple mechanism of reduced tubular reabsorption of water. This high urine flow serves to maintain a large volume of allantoic and amniotic fluids, as the observations on renal agenesis in human fetuses (28) and the present experiments indicate. It is clear that these peculiarities of renal function are advantageous only in fetal life and would have harmful consequences if they persisted beyond birth, hence the necessity of physiologic mechanisms that induce a change in the modality of renal function at or near birth. Since the changes in urine flow and osmolality induced by surgical stress and by natural birth are similar (see Fig. 2, animal M), it is possible that the stress associated with birth is one of the events that trigger the transition between fetal and neonatal renal function.

The marked variability of osmolality and urine flows observed by us during surgery and in the immediate postoperative period is comparable with that observed by others in acute preparations. Obviously, such variability is an artifact of the experimental procedure and should not be interpreted as representative of normal intrauterine conditions. It is of some interest that according to the data of Alexander, Nixon, Widdas, and Wohlzogen (7) small excretory rates of hypertonic urine are found more commonly in acute preparations of sheep fetuses close to term (> 120 days gestational age). In the light of the present study, this phenomenon

may represent a more pronounced response of the older fetus to stress. Previously, a comparison of the hemoglobin concentrations in blood of acute and chronic fetal preparations had led also to the hypothesis that the effects of acute stress are more evident in older fetuses (29). At any rate, this study of fetal renal function provides one more example of the fact that a correct description of "normal" organ function in the fetus cannot be obtained by studies carried out in acute preparations subject to operative stress.

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