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Measurement of Blood Flow and Oxygen Consumption in the Pelvic Limb of Fetal Sheep (42695)

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Abstract. In order to determine blood flow and oxygen consumption in the pelvic limb of fetal sheep, we applied the Fick principle of measurement of oxygen consumption in seven paired experiments in seven fetal sheep under normal conditions and after treatment with pancuronium bromide. Catheterization procedures, which minimized interference with the study limb circulation, avoided changes of catheter tip position during fetal movements, and prevented collateral circulation to and from tissues not located in the pelvic limb, were utilized. Blood flow through the external iliac artery was measured by means of a transit time ultrasonic method. Six sample sets for oxygen content were drawn from the external iliac artery and vein during 45-min control period and repeated after neuromuscular blockade. Normal oxygen consumption under these experimental conditions was determined to be 20.7 ± 1.9 (mean ± SEM) μmole · min^{-1} · 100 g^{-1}. Neuromuscular blockade caused oxygen consumption to decrease significantly ($P < 0.01$) by 12% to 18.1 ± 2.1 μmole · min^{-1} · 100 g^{-1} and decreased the average coefficient of variation from 15 to 8%. The data demonstrate that spontaneous skeletal muscle activity accounts for a significant amount of oxygen consumption, the level of which can vary widely over brief periods of time. These results suggest that such tissues with significant spontaneous changes in metabolic activity require repeated blood flow measurements with simultaneous determination of substrate arteriovenous differences to best describe metabolism under normal conditions.

Fetal limb metabolism is of interest because the limb is representative of nonvisceral tissues (the "carcass"). Such tissues account for approximately 70% of the body weight in late gestation fetal lambs and exhibit a much more variable growth rate than neural tissues. Although measurements of metabolic substrate arteriovenous differences across the circulation of the fetal limb have yielded useful information (1–5), development of a more complete understanding of fetal limb metabolism has been hampered by the inability to simultaneously measure substrate arteriovenous differences and blood flow to the fetal limb under well-defined experimental conditions. Furthermore, the results of different studies may not be comparable because the venous catheter placement for fetal hindlimb studies may have altered limb circulation and variably included effluent from the genitourinary and lower gastrointestinal tracts. The Fick principle has been applied in studies of newborn (6) and adult (7) animals under experimental conditions which assume metabolic steady state and constant perfusion of the limb tissues. Whether steady-state conditions prevail *in utero* is open to question given the intermittent, spontaneous muscle activity observed in the normal fetus (8).

The purpose of the present study was to measure blood flow and oxygen consumption in the pelvic limb of fetal sheep under normal conditions and conditions of neuromuscular blockade. Blood flow was measured by means of a transit time ultrasonic flow transducer. Microspheres were used to verify the ultrasonic flow measurement and to clearly define the anatomical area where perfusion and metabolism were under investigation.

Materials and Methods. *Surgery and animal care.* Seven mixed-breed (Rambouillet-Columbia) ewes in the last month of pregnancy were fasted for 48 hr before surgery. Under intravenous pentobarbital sedation and 1% tetracaine hydrochloride spinal anesthesia (10–12 mg), polyvinyl catheters (1.38 mm o.d.) were placed in a fetal hindlimb pedal artery with the tip positioned in the external iliac artery and in a fetal forelimb brachial vein. The hindlimb containing the

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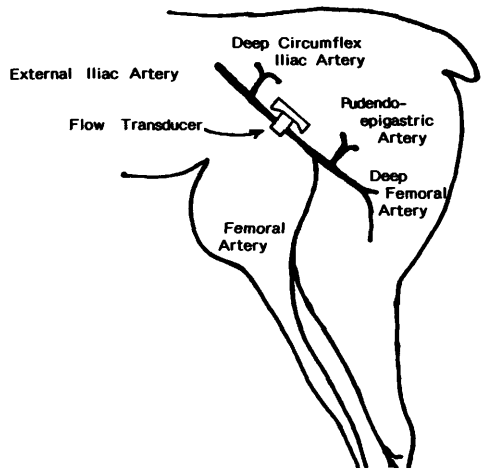

FIG. 1. Diagram of the arterial circulation and flow transducer placement in the pelvic limb of the ovine fetus. See text for details of catheter placement.

arterial catheter was designated the "non-study" limb. Smaller polyvinyl catheters (1.00 mm o.d.) were inserted in the contralateral deep circumflex iliac artery with the tip at the origin of this artery as it branches from the external iliac artery for local injection and in the pudendoepigastric venous trunk with the tip advanced 1 cm into the external iliac vein. The hindlimb drained by this iliac vein was designated the "study" limb (Fig. 1). The deep circumflex iliac vein and the pudendoepigastric arterial trunk were ligated in the study limb, thereby minimizing collateral circulation to and from midline structures such as genitalia, tail, or distal gastrointestinal tract which otherwise contaminate the external iliac circulation serving the skin, muscle, and bone of the hindlimb. These catheterization procedures have been described previously (5). A catheter was also placed in the amniotic fluid cavity for blood pressure reference and instillation of antibiotic. Pancuronium bromide (Organon Inc., West Orange, NJ) was injected during surgery via the brachial vein catheter (0.2 mg/kg estimated fetal weight) and the fetus observed for loss of skeletal muscle tone.

A 3-mm transit time ultrasonic blood flow transducer (Transonic Systems, Inc., Ithaca, NY) was positioned around the external iliac artery of the study limb for continuous blood flow measurement. The theoretical basis for the transit time method has been previously reported (9). These transducers were precalibrated *in vitro* for linearity of response from zero to 150 ml/min; zero flow corrections were obtained *in situ* at autopsy.

The animals recovered promptly from surgery and were standing and feeding in their individual pens within 6 hr. Ampicillin (500 mg) and streptomycin (1 g) were given intramuscularly to the ewe on the day of surgery. Ampicillin (500 mg) was injected into the amniotic fluid on the day of surgery and daily for the first 3 postoperative days. The catheters were flushed daily with a heparinized bacteriostatic saline solution (35 U heparin/ml). The ewes were given water and fed *ad libitum* with their standard alfalfa pellet diet.

Study design. The animals were allowed 7 days of recovery from operative stress prior to study. For the purpose of the present investigation, the animals were studied once between 127 and 133 days of gestation (mean 129 days). No experiments were performed during the recovery period. However, between this study and the time of autopsy a second 3-hr study was performed on the effect of hypoxia on blood flow to the pelvic limb (10). On each study day a normal control period was established over a 45-min period during which six sets of blood samples for hemoglobin concentration and oxygen saturation (0.5 ml into heparin-coated capillaries) were drawn simultaneously from the external iliac artery and vein catheters. Blood flow was measured continuously during each sample set. Sample sets required 2 min to draw; blood flow for the sample set was determined as the average of the mean blood flow noted every 0.2 min during the sampling interval.

Pancuronium bromide was then injected via the brachial vein catheter (0.2 mg/kg estimated fetal weight). Following a 30-min equilibration period the six sample sets for hemoglobin concentration and oxygen saturation and blood flow measurements were then repeated during a 45-min study period.

Prior to autopsy, which occurred 14 days after surgery, 15-μm-diameter microspheres labeled with ^{46}Sc (3M Company, St. Paul, MN) were injected systemically over 1 min

via the brachial vein catheter in order to measure blood flow to both the study and the nonstudy hindlimbs. During the injection, a reference blood flow sample (11) was drawn at a constant rate (4.0 ml·min^{-1}) from the nonstudy limb external iliac artery catheter; blood flow to the study limb was measured simultaneously using the transit time ultrasonic blood flow transducer. In three animals microspheres with a different label were slowly injected locally via the deep circumflex iliac artery catheter in order to determine which tissues were perfused by the external iliac artery of the study limb. Each animal was then killed by injection of an euthanasia solution, at which time the condition and placement of the catheters and the blood flow transducer were verified and the fetus and hindlimb tissue weights were measured. Both the study and the nonstudy pelvic limbs were separated into skin, muscle, and bone, weighed, and then analyzed for microspheres (Tracor 3-Channel Analytic Gamma Counter, Model 1185, Nuclear-Chicago Corp., Chicago, IL).

Measurements and calculations. Amniotic pressure and fetal arterial blood pressure were measured with a strain gauge transducer (Gould-Statham, Cleveland, OH). Fetal heart rate was counted from the arterial pulse frequency.

Blood hemoglobin concentration expressed as oxygen capacity (Hb; mmole·liter^{-1}) and oxyhemoglobin saturation (SO_2; %) were measured in duplicate by an automatic, direct reading photometer (OSM-2, Radiometer, Copenhagen). Blood oxygen content [(O_2); mM] was calculated as: $(O_2) = Hb \times SO_2/100$, since the contribution of physically dissolved oxygen to oxygen content was negligible.

Oxygen consumption by the pelvic limb ($\dot{V}_{O_2}$; μmole·min^{-1}) was calculated by application of the Fick principle: $\dot{V}_{O_2} = F[(O_2)_a - (O_2)_v]$ where F(ml·min^{-1}) is the mean blood flow determined during the sampling interval as outlined above, $(O_2)_a$ is the oxygen content in the external iliac artery, and $(O_2)_v$ is the oxygen content in the external iliac vein of the study limb.

Statistics. The mean, variance, standard deviation, and coefficient of variation (CV = 100% SD/mean) of each independent variable were determined separately for each animal during the control period and the study period using the six sample sets obtained during each period. Statistical significance ($P < 0.05$) was determined by means of the Student t test with paired analysis between the control and study periods. The mean variability was compared using a paired t test after log transformation of variance as suggested by Scheffe (12). Where appropriate, means and standard errors of the mean were determined for the group of animals studied.

Results. A total of seven paired experiments were obtained across the study limb of seven fetal lambs, the mean ± SEM gestational age for which was 129 ± 1 days. Heart rate, mean blood pressure, and hemoglobin concentration were not significantly different between the control and study periods: 169 ± 5 versus 177 ± 4 beats·min^{-1} ($P > 0.2$), 45 ± 2 versus 45 ± 2 mm Hg ($P > 0.2$), and 7.10 ± 0.24 versus 7.06 ± 0.23 mm·l^{-1} ($P > 0.3$), respectively.

Table I presents the fetal gestational age, weight, and the study and nonstudy pelvic limb weights at autopsy, and the external iliac artery blood flow per 100 g of tissue determined by the microsphere method on the day of autopsy. Because the day of autopsy occurred an average of 7 days following the day of study, Table I also presents an estimate of the individual fetal pelvic limb weights as of the day of study calculated by means of a standard mathematical formulation of the fetal sheep growth curve observed in our laboratory (13). The estimated weight at day of study was used to calculate limb blood flow per 100 g of tissue for the purpose of comparison with data in the literature.

Pelvic limb tissues. Serial sections of limb tissues locally injected with microspheres demonstrated that the skin perfused by the external iliac artery was approximately delineated by a line perpendicular from the spine to the iliac crest and from the iliac crest over the inguinal canal to the midline. It did not include skin covering genitalia, rectum, or tail. Muscle included those tissues originating in the femoral region and inserting in the abdominal and pelvic areas. Bone included the femur but not the iliosacral structures because there was less than 20% of the counts per gram of tissue in these pelvic

TABLE I. INDIVIDUAL ANIMAL AGE, WEIGHT, STUDY LIMB, AND NONSTUDY LIMB WEIGHTS AT AUTOPSY, STUDY LIMB FLOW ON DAY OF AUTOPSY, AND ESTIMATED STUDY LIMB WEIGHT ON DAY OF STUDY

	Day of autopsy					Day of experiment
Animal no.	Age at autopsy	Fetal weight	Nonstudy limb weight	Study limb weight	Study limb blood flow	Estimated study limb weight[a]
1	129	2840	276	298	23.5	260
2	138	3800	374	390	16.9	328
3	136	3240	325	329	14.8	249
4	132	3000	288	310	9.5	237
5	142	3420	335	340	12.1	259
6	136	3110	305	309	8.0	258
7	139	2970	—	280	24.3	216
Mean	136	3235[b]	317	329[b]	16.9[c]	258
± SEM	±2	±139	±14	±14	±2.4	±13

Note. Age is in days; weights are in grams; blood flow is ml · min^{-1} · 100 g tissue^{-1}.

[a] Study limb weight on the day of study for each individual animal was estimated as the product of the estimated fetal weight (13) on day of study times the limb weight to fetal weight ratio determined at autopsy for each individual animal.

[b] Because the nonstudy limb weight was not obtained in animal No. 7, the fetal weight and study limb weights for animal No. 7 are not included in the mean values.

[c] For animal No. 6, flow transducer noted at autopsy to be compressing vessel; blood flow measurement in animal No. 6 is not included in the mean study limb blood flow.

bones compared to the leg bones. The study pelvic limb defined in this manner for all seven animals had a mean weight of 323 ± 13 g, or approximately 10% of the fetal weight, which is consistent with an earlier estimate of fetal hindlimb weight (14). As indicated in Table I, the nonstudy pelvic limb, in which the external iliac artery catheter had been placed, was slightly, but significantly, smaller than the study limb, 317 ± 14 g versus 329 ± 14 g ($P < 0.01$).

In three animals in which the measurement was compared, there was no difference between blood flow per gram of tissue to the study versus nonstudy pelvic limb as measured by microspheres: 18.4 ± 2.6 ml · min^{-1} · 100 g^{-1} versus 18.6 ± 3.4 ml · min^{-1} ($P > 0.3$), respectively. The study limb tissues were separated into bone, skin and muscle, the distribution of which by weight and by blood flow as measured by systemic microsphere injection are presented in Table II.

Blood flow measurement. The value for blood flow to the study limb measured by means of the transit time ultrasonic blood flow transducer correlated well with ($r = 0.991$) and was not different from (paired t test, $P > 0.5$) the value for blood flow measured simultaneously by standard microsphere technique, as illustrated in Fig. 2. The calculated line of regression fits the equation, $y = 0.91\ (x) + 2.6$, where y is the transit time blood flow (ml · min^{-1}), and x is the microsphere blood flow (ml · min^{-1}).

Individual and mean data for external iliac artery blood flow, oxygen saturation and content, and oxygen consumption before and after neuromuscular blockade are presented in Table III. Blood flow decreased significantly by 12% after administering pancuronium bromide. The average coefficient of variation within each sample set was 7% before and 6% after neuromuscular blockade. Blood flow variance was not signifi-

TABLE II. PELVIC LIMB TISSUE DISTRIBUTION BY WEIGHT AND BY BLOOD FLOW IN SEVEN FETAL SHEEP

Tissue	Weight	Blood flow
Bone	95 ± 4	17.0 ± 1.2
Skin	69 ± 4	13.5 ± 1.2
Muscle	159 ± 6	19.5 ± 1.7

Note. Values are means ± SEM. Toe tissue included as bone tissue. Weight is in gram; blood flow is in ml · min^{-1}.

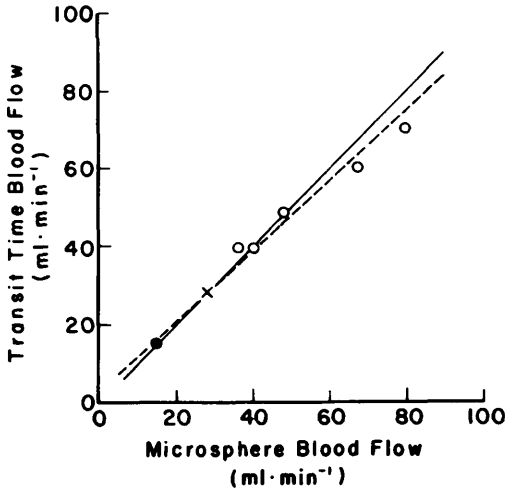

FIG. 2. Comparison of microsphere blood flow measurement to transit time ultrasonic blood flow measurement in the external iliac artery of fetal sheep. The solid line (—) is the line of identity. The interrupted line (- - -) is the calculated line of regression which fits the equation: $y = 0.91\ (x) + 2.6$, where y = transit time ultrasonic blood flow and x = microsphere blood flow. $r = 0.991$. Symbols: (X) transducer noted at autopsy to be compressing vessel; (●) fetus made hypoxic in order to obtain a decrease in blood flow; (○) normal values.

cantly different ($P > 0.10$), suggesting that spontaneous fetal movements did not make a major contribution to blood flow variability (Table III).

Oxygen Content. There was a significant increase in oxygen saturation and content in the eternal iliac artery and vein after neuromuscular blockade (Table III). Arterial content increased by 17% and venous content by 23%. There was a decrease in the mean coefficient of variation within each sample set, for arterial oxygen content from 6 to 2%, and for venous oxygen content from 8 to 2%. Oxygen saturation and content variances were significantly less during neuromuscular blockade (Table III).

Oxygen consumption. Neuromuscular blockade significantly decreased oxygen consumption by 2.6 $\mu m \cdot min^{-1} \cdot 100\ g^{-1}$, or 12%, in the study limb (Table III). Although the study limbs were of comparable weight (Table I) and the fetuses of similar gestational age (Table III), there was interanimal variability with respect to pelvic limb oxygen consumption per 100 g of tissue both before and after neuromuscular blockade, as indicated in Table III. With respect to intraanimal variability, the mean variance of oxygen consumption values observed within sample sets for each fetus was larger during the normal, control periods than after pancuronium was administered. The decrease in variance observed after neuromuscular blockade was significant ($P < 0.005$), as indicated in Table III.

Discussion. Studies of the fetal hindlimb have depended upon measurements of either substrate arteriovenous differences (1, 2, 15) or metabolic quotients (3–5) for a description of the metabolic question of interest. Interpretation and comparison of the metabolic data so obtained are problematic since the placement of the venous sampling catheter in most studies includes blood from the pudendoepigastric trunk and blood from the deep circumflex iliac vein with its cranial and caudal branches (Fig. 1). Catheter placement in the inferior vena cava below the renal veins also likely includes inferior mesenteric effluent. Oxygen content data from the control period in the present study are similar to those observed in a study in which the external iliac circulation was comparably modified (5). The present study is the first to apply the Fick principle to the pelvic limb of fetal sheep for the purpose of determining oxygen consumption. This has been accomplished by defining anatomically the tissues served by the circulation under study, by continuous measurement of blood flow to those tissues, and by measuring oxygen content in the appropriate venous effluent.

The observation that the blood flow per gram of tissue to the study limb was not different from the blood flow to the nonstudy limb indicates that the placement of the transit time ultrasonic flow transducer did not alter blood flow to the study limb, although this comparison was not made in the one animal (No. 6) where compression of external iliac artery was noted at autopsy. Agreement between blood flow measurements by the microsphere method and the transit time method (Fig. 2) indicates that the accurate continuous measurement of pelvic limb fetal blood flow is feasible. It is interesting that a 4% difference in pelvic limb weights was noted (Table I), suggesting that

TABLE III. INDIVIDUAL ANIMAL MEAN AND STANDARD DEVIATION OF SIX DETERMINATIONS FOR BLOOD FLOW, ARTERIAL AND VENOUS OXYGEN SATURATION AND CONTENT, AND OXYGEN CONSUMPTION IN THE PELVIC LIMB OF FETAL SHEEP: CONTROL (C) VERSUS NEUROMUSCULAR BLOCKADE (P)

Animal no.	Age at study	F		$(SO_2)_a$		$(SO_2)_v$	
		C	P	C	P	C	P
1	125	24.1	21.7	50.4	63.4	40.0	52.7
		±1.8	±2.2	±4.8	±0.9	±4.4	±1.9
2	132	23.9	20.9	58.3	63.6	42.8	46.7
		±1.0	±1.5	±2.6	±1.9	±3.5	±1.0
3	127	26.4	23.7	53.6	61.6	43.4	50.0
		±1.6	±0.6	±2.5	±0.7	±3.2	±0.8
4	124	17.4	12.6	59.9	68.1	45.9	54.3
		±0.9	±0.3	±2.1	±0.8	±3.0	±0.9
5	132	29.3	24.7	41.6	55.8	29.1	42.4
		±2.6	±2.5	±4.5	±1.7	±2.8	±1.3
6	130	13.4	12.1	56.8	63.4	39.1	46.8
		±0.5	±0.4	±2.3	±1.2	±1.8	±0.6
7	130	22.1	21.5	58.0	68.5	44.6	56.7
		±3.2	±1.7	±4.6	±1.4	±6.2	±1.6
Mean[a]		23.9	20.9	53.6	63.5	41.0	50.5
±SEM		±1.6	±1.8	±2.8	±1.9	±2.5	±2.1
P value[b]		<0.01		<0.001		<0.005	
P value[c]		ns; >0.10		<0.001		≪0.001	

Animal no.	Age at study	$(O_2)_a$		$(O_2)_v$		$\dot{V}_{O_2}$	
		C	P	C	P	C	P
1	125	3.96	4.85	3.15	4.11	19.3	15.8
		±0.38	±0.13	±0.32	±0.11	±4.1	±2.6
2	132	3.93	4.33	2.91	3.23	24.3	22.8
		±0.15	±0.17	±0.24	±0.09	±3.6	±1.8
3	127	4.19	4.82	3.43	3.95	19.9	20.7
		±0.18	±0.06	±0.23	±0.08	±3.1	±1.0
4	124	3.83	4.34	2.97	3.51	14.8	10.5
		±0.10	±0.05	±0.15	±0.06	±2.9	±0.3
5	132	3.10	4.14	2.18	3.16	27.9	24.1
		±0.31	±0.13	±0.20	±0.09	±3.2	±1.4
6	130	3.98	4.46	2.77	3.32	16.2	13.7
		±0.15	±0.09	±0.15	±0.05	±1.9	±0.5
7	130	3.72	4.32	2.89	3.62	17.9	15.0
		±0.28	±0.08	±0.36	±0.10	±2.8	±1.7
Mean[a]		3.79	4.47	2.92	3.60	20.7	18.1
±SEM		±0.15	±0.12	±0.17	±0.16	±1.9	±2.1
P value[b]		<0.001		<0.005		<0.025	
P value[c]		<0.005		≪0.001		<0.005	

Note. Age at study is in days. F is blood flow through the external iliac artery, ml · min^{-1} · 100 g^{-1}. $(SO_2)_a$ and $(SO_2)_v$ are the oxygen saturations, %, and $(O_2)_a$ and $(O_2)_v$ are the oxygen contents, mM, measured in the external iliac artery and vein, respectively. $\dot{V}_{O_2}$ is the oxygen consumption across the external iliac circulation, micromol · min^{-1} · 100 g^{-1}. C is the Control Period and P is the Pancuronium-treated Period.

[a] Flow transducer noted at autopsy to be compressing vessel for animal No. 6; results from this animal are not included in the calculation of the mean values.

[b] P is the probability by paired t test that the mean control value is different from the mean study value for each independent variable.

[c] P is the probability by paired t test that the variance observed during the Control Period is different from the variance observed during the Pancuronium-Treated Period for each independent variable (see text for explanation).

the arterial catheterization of the nonstudy limb had a small adverse effect on its growth. More importantly, comparison with data in the literature (14) showed no evidence that the ligation of the pudendoepigastric and deep circumflex iliac arteries and veins, as described, caused a decrease in the growth of the study limb.

Blood flow to the study limb did not increase significantly from the day of study to the day of autopsy. On the assumption that the fetus grew normally, this represents a decrease in flow per gram of tissue (Table I versus Table III), a trend noted by Dawes *et al.* (14). It seems unlikely that growth was adversely affected by the brief, 3-hr hypoxia study performed prior to autopsy. The normal blood flow per 100 g tissue observed in the present study (Table III) was approximately four times greater than the blood flow measured under acute experimental conditions (14), emphasizing the physiologic changes induced by stress and anesthesia, and is in agreement with other more recent measurements of total carcass blood flow by the microsphere method (16, 17).

The present study demonstrates that with neuromuscular blockade there was a significant decrease in pelvic limb blood flow and oxygen uptake and a marked decrease in the variance of the oxygen uptake measurement. Blood flow and oxygen uptake decreased to 88% of control, and the oxygen uptake variance decreased to 23% of control. These changes are likely to reflect a decrease in both magnitude and variability of oxygen demands by fetal skeletal muscle. Given the large and random variability of normal fetal limb oxygen demands, it is clear that precise studies of fetal limb metabolism require repeated within-animal measurements of blood flow and arteriovenous concentration differences of substrates. It is interesting to note also that neuromuscular blockade did not reduce appreciably the differences in limb oxygen uptake among fetuses, thus indicating that there is another major, yet unidentified, source of variation in addition to muscular activity.

Previous investigations have demonstrated that fetal injection of the neuromuscular blocking agents pancuronium (18) and gallamine (19) is associated with a significant increase of fetal blood PO_2 and oxygen content. In agreement with these observations we have shown that fetal blood oxygen saturation and content are markedly elevated in the 30- to 75-minute period following the injection of pancuronium (Table III). In addition, our study demonstrates that pancuronium decreased significantly the variance of blood saturation and content to approximately 20% of control. These observations have suggested the hypothesis that the decrease in fetal oxygen demands induced by neuromuscular blockade decreases oxygen extraction across the uterine and umbilical circulations, thus increasing the PO_2 of both fetal arterial and umbilical venous blood (19). The evidence bearing on the validity of this hypothesis is inconclusive. In the study of fetal oxygen uptake before and after gallamine injection, umbilical arterial and venous PO_2's increased even in those fetuses that did not show a decrease in umbilical oxygen saturation and uptake (19). Furthermore, in the present study, pancuronium decreased limb oxygen uptake by 12% only. If this change is representative of the overall change in carcass oxygen uptake, which is estimated to represent normally 40% of fetal oxygen demands (20), the effect of pancuronium on umbilical oxygen uptake should be relatively small.

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