Changes in cerebral blood flow, cerebral metabolites, and breathing movements in the sheep fetus following asphyxia produced by occlusion of the umbilical cord

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Yan EB, Baburamani AA, Walker AM, Walker DW. Changes in cerebral blood flow, cerebral metabolites, and breathing movements in the sheep fetus following asphyxia produced by occlusion of the umbilical cord. Am J Physiol Regul Integr Comp Physiol 297: R60–R69, 2009. First published April 29, 2009; doi:10.1152/ajpregu.00047.2009.—Severe global fetal asphyxia, if caused by a brief occlusion of the umbilical cord, results in prolonged cerebral hypoperfusion in fetal sheep. In this study, we sought evidence to support the hypothesis that cerebral hypoperfusion is a consequence of suppressed cerebral metabolism. In the 24 h following complete occlusion of the umbilical cord for 10 min, sagittal sinus blood flow velocity was significantly decreased for up to 12 h. Capillary blood flow, measured using microspheres, decreased at 1 and 5 h after cord occlusion in many brain regions, including cortical gray and white matter. Microdialysis probes implanted in the cerebral cortex revealed an increase in extracellular glucose concentrations in gray matter for 7–8 h postasphyxia, while lactate increased only briefly, suggesting decreased cerebral glucose utilization over this time. Although these data, as well as the concurrent suppression of breathing movements and electrocortical activity, support the concept of hypometabolic hypoperfusion, the significant increase of pyruvate and glycerol concentrations in dialysate fluid obtained from the cerebral cortex at 3–8 h after cord occlusion suggests an eventual loss of membrane integrity. The prolonged increase of breathing movements for many hours suggests loss of the pontine/thalamic control that produces the distinct pattern of fetal breathing movements.

cerebral blood flow; asphyxia; cerebral metabolism; fetal breathing movements; microdialysis

IN BOTH ADULT STROKE AND PERINATAL hypoxia-ischemia, brain damage results not only from the initial acute hypoxic event, but also during the recovery phase, when tissue perfusion and oxygenation are reestablished. Factors contributing to this “secondary” wave of damage include prolonged vascular patency, cerebral edema, breakdown of the blood-brain barrier, recruitment of phagocytes, induction of inflammatory processes, and free radical formation (27–29).

In pregnancy, clinical evidence suggests that tangling of the umbilical cord, placental abruption, and birth asphyxia are well-recognized conditions contributing to fetal asphyxia, which can result in severe neurologic disorders in children after birth (39). Regardless of whether the period of greatest vulnerability for the brain is antepartum or intrapartum, the precise causal mechanisms of developmental brain injury await more sophisticated means of detection and definition.

In two previous studies in fetal sheep, we showed that the highly toxic hydroxyl radical (OH·) increased in cortical gray and white matter in two phases following severe fetal asphyxia in late gestation - the first lasting for 1–2 h and beginning during the asphyxial episode caused by occluding the umbilical cord for 10 min, and a second increase of similar magnitude occurring at 8–10 h after the asphyxia (33, 44). Although it is known that the OH· may affect vascular conductance and cellular metabolism during pathological conditions (32, 45), it is also known that the highly reactive oxygen and nitrogen free radicals alter structure and function of many cellular constituents, including proteins, DNA, RNA, and lipids (6, 33, 40).

In this study, our aim was to determine the consequence of brief, global asphyxia on the fetal brain in relation to changes of total and regional cerebral blood flows, cerebral metabolism, and fetal behavior. The hypothesis tested was that cellular injury of the brain accompanies global fetal asphyxia, and because this is not readily repaired, a number of behavioral sequelae emerge, indicating the loss of CNS function. Although it has been argued that fetal cerebral metabolism is suppressed by acute hypoxic or asphyxic events—and this in itself may be an adaptive response to the hypoperfusion and hypo-oxygenation of the brain (20)—we hypothesized that nevertheless it would be possible to show that breakdown of cell structure and loss of normal fetal activity occurred after the asphyxic insult.

MATERIALS AND METHODS

We induced fetal asphyxia by occluding the umbilical cord for 10 min and used the microdialysis probe to measure real-time changes in brain extracellular fluid of glycerol and intracellular metabolites that would indicate the loss of cellular integrity. Regional cerebral blood flow was measured using microspheres, complemented by sagittal sinus blood flow velocity measured continuously over 24 h using a piezoelectric ultrasonic probe as a measure of global cortical flow. We also measured electrocortical activity and breathing movements as indices of cerebral function, since these have well-characterized patterns of activity (10, 11), and changes in them might indicate timing of the onset of cellular metabolic stress in the brain.

Animal surgery. Pregnant sheep carrying a singleton fetus were used in this study. The use of these animals and all procedures had received prior approval from the School of Biomedical Science Animal Ethics Committee of Monash University. At 124–126 days gestational age (term is 146 days), surgery was performed under general anesthesia, induced by injecting the ewe with 1 g thiopentone...
sodium in water (50 mg/ml iv; Pentothal, Boehringer Ingelheim, Australia), and then maintaining anesthesia with 1.5–2% isoflurane (Isoflo, Abbott Australasia, Kurnell, Australia) given via an endotracheal tube. Under aseptic conditions the fetal head and forelimbs were exposed through a uterine incision. A polyvinyl catheter (0.86 mm internal diameter) was inserted into a brachial artery to obtain blood samples and measure arterial pressure, and a saline-filled catheter directed caudally was inserted into the trachea to measure fetal breathing movements. A flexible microdialysis probe (polycarbonate membrane, 20-kDa cutoff; CMA Microdialysis, Solna, Sweden) was inserted into the gray matter (8-mm depth) of cerebral cortex using external coordinates of 5 mm forward of the coronal suture and 10 mm lateral to the sagittal suture (44). The probe was inserted through a 1-mm hole drilled in the skull, and held in place using cyanoacrylate glue. A piece of parietal bone (44). The probe was inserted through a 1-mm hole drilled in the skull, and held in place using cyanoacrylate glue. A piece of parietal bone was removed over the sagittal suture to expose the dura, and a piezoelectric ultrasonic crystal probe (−1 mm × 1 mm, 20 MHz; Iowa Doppler Products, Iowa City, IA) was placed directly above the sagittal sinus to measure blood flow velocity (35). A pair of fine wire electrodes (type AS633, Cooner Wire, Chatsworth, USA) was placed bilaterally on the dura over the parasagittal parietal cortex to record the electrocorticogram (ECOG). The dialysis probes, ultrasonic crystal, and ECOG electrodes were all covered with dental cement to ensure they were immobilized, and the skin incision was repaired. The fetus was then further withdrawn from the uterus to allow an inflatable Silastic cuff (16HD; In Vivo Medical, Sacramento, CA) to be placed around the umbilical cord which, on inflation, would cause complete cessation of blood flow in the cord.

A second group of fetuses (n = 4) was also prepared, as described above, except that microdialysis probes were not inserted, and a catheter was inserted into one femoral vein for administration of 15-μm colored microspheres (E-Z Trac; IMT Laboratories, Irvine, CA) and into the second brachial artery for reference blood sampling. These fetuses were also subjected to cord occlusion for 10 min, as described below.

Animal experiments. Experiments were performed 4–5 days after surgery. During this time (and from the time immediately after recovery from surgery), the ewes were held in a mobile cage with a floor area of 1.2 m × 0.63 m and could sit and stand comfortably and move from side-to-side but were not able to turn around. Fetal blood pressure and amniotic pressure were measured using solid state pressure transducers (DTXPlus, B-D Medical Systems) attached to the side of the cage, and fetal arterial pressure was calculated by electronic subtraction of amniotic pressure. Fetal heart rate was calculated online from the blood pressure pulse. ECOG was recorded using a high common mode rejection amplifier, and the signal recorded after low-pass filtering (30 Hz). Sagittal sinus blood flow velocity was measured using a directional pulsed Doppler flowmeter (545C-5, Bioengineering; The University of Iowa, Iowa City, IA), with the range (depth) of the reflected signal adjusted if necessary to ensure that the peak velocity was always being recorded. All analog signals were converted into digital data (100 data points per second) continuously from the 3rd postoperative day and stored on the hard disk of a computer using chart software (PowerLab, ADInstruments, Castle Hill, NSW, Australia).

Perfusion of the microdialysis probe was started 24 h before the designated time for umbilical cord occlusion (UCO). The probe was perfused at a rate of 2 μl/min with modified artificial cerebrospinal fluid (aCSF; NaH2PO4 12.5 mM, Na2HPO4 37.5 mM, NaCl 90 mM, KCl 4 mM, pH 7.4). The dialysate was collected over 30-min epochs beginning 4 h before and continuing for 24 h after the designated time for the 10 min UCO, or a sham maneuver. A refrigerated fraction collector was attached to the side of the cage, to which the outflow tube was connected, allowing automated collection over the 28-h period of the experiment. At 4- to 6-h intervals, the chilled dialysate samples (60 μl each tube) were removed from the fraction collector and stored at −70°C until analysis. Fetal asphyxia was induced by inflating the Silastic cuff for 10 min with 2–3 ml sterile water (n = 5); for the sham occlusion group, the cuff was not inflated (n = 5). Fetal blood samples (0.5 ml) were obtained at −2 and −1 h, −5 min, +5 min, +9 min, and at 0.5, 1, 2, 6, 12, and 24 h relative to the start of the occlusion. At 24 h after the actual or sham UCO, the ewe and fetus were humanely euthanized by intravenous injection of pentobarbital sodium (Lethabarb; Virbac, Milperra, NSW, Australia) to the ewe. The fetal brain was removed and immersion-fixed in 4% paraformaldehyde for determining the location of the probe tip.

Blood samples were used immediately to measure Po2, Pco2, O2 saturation, pH, and hematocrit using a Radiometer ABL5 analyzer (Radiometer Medical A/S, Brønshøj, Denmark). Glucose, lactate, pyruvate, and glycerol were measured in the microdialysis samples using an ISU/S autoanalyzer (CMA Microdialysis). Quality controls were analyzed after every 10 samples; the intra-assay coefficient for each analyte was <5%, and the interassay coefficients were <10%. Metabolite values reported in results have been corrected for recovery at the flow rate of 2 μl/min, which is 13%, 23%, and 26% for glucose, lactate, and pyruvate, respectively, as provided by the manufacturer (CMA Microdialysis).

In the second group of fetuses, cerebral capillary blood flow was determined using permanently colored microspheres (22). A reference blood sample was withdrawn at a constant rate (0.8 ml/min) from the brachial artery catheter starting 1 min before and continuing until 3 min after the injection of each set of microspheres. Thirty seconds after the start of brachial blood sampling ~5×10⁶ microspheres (E-Z Trac, IMT Laboratories, Irvine, CA) suspended in 1 ml 0.05% Tween 80-saline solution was injected into the inferior vena cava via the femoral vein catheter over 10 s. Microspheres of different colors (blue, green, red, black, coral) were used to determine cerebral blood flow at −1, +1, +5, +10 and +24 h, respectively; microspheres (orange) were also injected at 7 min after starting the UCO. Animals were euthanized 24 h after the real or sham UCO. The fetal brain was quickly removed and divided into cortical and noncortical regions; for this study, the areas of interest were the outer layer of the frontal and occipital cortex (primarily gray matter), midbrain, pons, and medulla. Each region was weighed and placed in hemolysis reagent (0.2% ethanol, 0.02% sodium azide), and the tissues were then digested in 2 M sodium hydroxide for 72 h at 70°C. The microspheres were extracted from the solubilized tissue by centrifugation at 2000 g for 30 min at room temperature. The pellet containing microspheres was washed (0.2% Tween 80, 0.005% sodium azide, 0.025% sodium dodecyl benzene sulfonate) several times before manual counting was performed using a hemocytometer (Neubauer Improved Bright-line Hemocytometer, Blaubrand, Germany) at ×100 magnification. Ten aliquots from each brain region were counted and summed to minimize counting errors.

| Table 1. Fetal blood gas parameters and pH before, during, and after UCO or sham UCO |
|---------------------------------|---------|---------|---------|
|                                | PreUCO | −1 h    | UCO, 9 min | PostUCO, +1 h |
| Po2, mmHg                       |         |         |          |               |
| Sham                            | 21.03±0.43 | 22.38±0.75 | 22.20±0.72 |
| UCO                             | 24.77±1.99 | 10.13±1.34† | 27.11±1.47 |
| Pco2, mmHg                      |         |         |          |               |
| Sham                            | 47.18±1.26 | 44.80±1.75 | 46.38±1.5  |
| UCO                             | 46.14±1.49 | 102.05±4.88† | 45.21±1.02 |
| pH                              |         |         |          |               |
| Sham                            | 7.362±0.011 | 7.373±0.006 | 7.363±0.007 |
| UCO                             | 7.364±0.009 | 6.978±0.020† | 7.311±0.012 |

Data are shown as means ± SE; n = 5 fetuses per group. UCO, umbilical cord occlusion. *Significant differences (P < 0.05) between UCO and sham groups at the time point indicated. †Significant differences (P < 0.05) between preUCO and time points indicated within the treatment group.
sampling and pipetting errors. Regional cerebral blood flow \(Q_r\) (ml/min/100 g wet tissue weight), was calculated using the following equation:

\[
Q_r = \frac{N^{\text{sample}}}{N^{\text{ref}}} \times \frac{Q^{\text{ref}}}{N^{\text{ref}}},
\]

where \(N^{\text{sample}}\) = number of microspheres per 100 g of wet tissue sample; \(Q^{\text{ref}}\) = withdrawal rate of the reference blood sample (ml/min); and \(N^{\text{ref}}\) = total number of microspheres per milliliter of the reference blood sample. The intra-run coefficient of variation for counting microspheres was <5%, and the inter-run coefficient was <10%.

Data analysis. All data are presented as means ± SE. A mean value of sagittal sinus blood flow velocity was obtained for successive 10 min epochs across the entire recording period of each experiment (i.e., -4 to +24 h), and the data for each fetus were then expressed as a percentage of the mean amplitude calculated for the 2-h period immediately prior to the time of UCO (or sham) to account for different signal levels between fetuses. The ECoG record was divided into epochs of high and low voltage activity (7, 10) using the mean amplitude for each type of activity obtained over at least 4 h of control (i.e., pre-UCO) recording, and the results are expressed as the percentage of the time that each activity occurred during consecutive 1-h periods of the experiment. Very low amplitude ECoG activity (hereafter referred to as sub-low voltage) occurred after UCO in most fetuses and was defined as an amplitude sustained for at least 1 min that was <25% of the amplitude of normal low-voltage ECoG activity. Seizure-like activity was quantified by an algorithm that identified “spiking” transients in the ECoG, based on the criterion that the amplitude of the spike was 50% greater than the average ECoG amplitude for 1 min prior to occurrence of the appearance of these transients. The number of these spikes was counted for each 1-h period throughout the 24-h experiment for both the UCO and sham fetuses.

Statistics. Two-way repeated-measures ANOVA with treatment and time as the factors was used to analyze the differences of the blood gas parameters, pH, blood pressure, cerebral metabolites, sagittal sinus blood flow velocity, and ECoG activities between control and treatment groups. Where the ANOVA indicated significant \((P < 0.05)\) differences between the treatment and time, a Student’s \(t\)-test was applied post hoc to identify the significant differences between the treatments at each sampling time. The change of cerebral capillary blood flow relative to the control, pre-UCO blood flow measured at -1 h, was evaluated by a 1-way repeated-measures ANOVA, with a post hoc Dunnet’s test for significance.

RESULTS

Systemic blood gases and cardiovascular data. As expected, the 10 min of UCO produced profound changes in fetal arterial blood, including hypoxia (PO2 10.13 ± 1.34 mmHg), hypercapnia (Pco2 102.03 ± 4.88 mmHg), and acidemia (pH 6.978 ± 0.020) compared with the preocclusion values (Table 1). However, by 1 h after the occlusion, the arterial PO2 (27.11 ± 1.47 mmHg), Pco2 (45.21 ± 1.02 mmHg), and pH (7.311 ±

### Table 2. Fetal mean arterial pressure and heart rate before, during, and after UCO or sham UCO

<table>
<thead>
<tr>
<th></th>
<th>PreUCO (-15 min)</th>
<th>UCO, 2 min</th>
<th>UCO, 9 min</th>
<th>PostUCO, +2 min</th>
<th>PostUCO, +15 min</th>
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</thead>
<tbody>
<tr>
<td><strong>MAP, mmHg</strong></td>
<td></td>
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<tr>
<td>Sham</td>
<td>35.5±2.1</td>
<td>35.7±1.9</td>
<td>34.5±1.4</td>
<td>35.3±1.6</td>
<td>36.7±1.8</td>
</tr>
<tr>
<td>UCO</td>
<td>36.7±2.2</td>
<td>68.6±6.0†</td>
<td>27.1±3.4†</td>
<td>49.4±5.7*</td>
<td>41.5±3.1</td>
</tr>
<tr>
<td><strong>HR, beats/min</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sham</td>
<td>154.8±2.6</td>
<td>150.9±2.8</td>
<td>149.6±3.3</td>
<td>151.4±3.8</td>
<td>154.8±7.0</td>
</tr>
<tr>
<td>UCO</td>
<td>173.6±10.6</td>
<td>112.0±12.0†</td>
<td>110.0±14.5†</td>
<td>245.4±34.0*</td>
<td>212.5±12.9†</td>
</tr>
</tbody>
</table>

Data are shown as means ± SE; \(n = 5\) fetuses per group. *Significant differences \((P < 0.05)\) between UCO and sham groups at the time point indicated. †Significant differences \((P < 0.05)\) between preUCO and time points indicated within the treatment group. MAP, mean arterial pressure; HR, heart rate.
0.012) had recovered to the normal range for fetuses of this gestational age (Table 1). Mean arterial pressure (MAP) reached a maximum value of 68.6 ± 6.0 mmHg at 2 min after the cord was completely occluded, and then it decreased to below normal values by the end of the 10-min period of occlusion (27.1 ± 3.4 mmHg) (Table 2). Deflation of the occluder cuff resulted immediately in fetal hypertension, but the arterial pressure then subsided so that by 2–3 h after the occlusion, the MAP was not different between the UCO and sham groups. Bradycardia was observed as soon as the cord was occluded, with heart rate reaching a minimum of 65.1 ± 0.3 beats/min at 4 min after the onset of occlusion, and it remained significantly below the preocclusion heart rate of 147.2 ± 9.1 beats/min for the entire period of occlusion. Tachycardia was observed immediately after release of the occlusion (245.4 ± 34.0 beats/min at 2 min). As for MAP, the heart rate also returned to the preocclusion levels by 1–2 h after the end of the occlusion. No significant variations of MAP and heart rate occurred in the sham occlusion group over the 24-h experimental period (Table 2).

Cerebral blood flow. Immediately after occluding the umbilical cord, sagittal vein flow velocity showed a complex set of changes—an initial decrease, followed by an increase to reach a peak value of 218.9 ± 42.6% above the control value at 6 min after initiating UCO, before decreasing rapidly to a level below (62.7 ± 10.8%) that of the control (pre-UCO) velocity after 9 min had elapsed. Release of the cuff after 10 min of cord occlusion resulted in an increase of sagittal vein flow velocity, before the flow velocity decreased significantly to below control values, as noted above (Fig. 1). An analysis of the distribution of microspheres indicated that UCO for 10 min significantly decreased capillary blood flow in frontal and occipital cortical gray matter, midbrain, pons, and medulla at 1, 5, and 10 h after UCO (Fig. 2), the perfusion of these regions returning to the pre-UCO levels by 24 h after the UCO. The changes of regional cerebral perfusion were to some extent matched by changes of sagittal sinus flow velocity, which between ~1 and 10 h after UCO was only ~70% of the pre-UCO blood flow velocity (Fig. 1).

Fig. 2. Capillary blood flow as measured by microspheres; data are shown as means ± SE (n = 4 fetuses per group) of the change of blood flow (ml per min per 100 grams wet tissue) from basal values at −1 h before umbilical cord occlusion. Blood flow in gray matter of the frontal cortex (A) and occipital cortex (B), midbrain (C), pons (D), and medulla (E). *Significant differences (P < 0.05) between −1 h and the time point indicated.
**Cortical glycerol concentration.** Extracellular glycerol increased in two phases in cortical gray matter after UCO (Fig. 3A). There was a 3.5-fold increase that occurred during the collection of dialysate that began at the time of onset of UCO, with the values falling back to control values by 2–3 h after UCO. Then, there was a second, more gradual, and sustained trend increase of glycerol at +7 to +8.5 h after onset of the UCO (P = 0.09), after which the extracellular glycerol concentrations values decreased, and from 10 to 24 h, the concentrations were not different from the pre-UCO values, or from the time-matched values obtained from the sham-occluded fetuses.

**Metabolic data.** The glucose and lactate concentrations in the efflux from the microdialysis probes inserted into cortical gray matter were 0.29 ± 0.09 and 0.73 ± 0.15 mM, respectively, for samples obtained prior to UCO; for all fetuses, these concentrations were lower than the corresponding values in plasma (glucose, 1.02 ± 0.09 mM; lactate, 1.80 ± 0.29 mM). UCO for 10 min resulted in increases of cerebral extracellular concentrations of glucose, lactate, and pyruvate (Fig. 3, B–D). Glucose concentrations had increased by the time the first complete dialysate sample had been collected after starting the UCO (i.e., at 30 min after UCO), and it remained significantly higher than pre-UCO values until ~5 h after the UCO (Fig. 3B). Extracellular lactate was significantly increased only for the first sample collected after onset of the UCO, and it was not different thereafter from the pre-UCO values or compared with the time-matched samples obtained from the sham-occluded fetuses (Fig. 3C). Changes in the concentrations of pyruvate (Fig. 3D) were similar to those of extracellular glucose with a significant increase observed between 1 and 5 h after UCO.

**Electrocortical activity and fetal breathing movements.** Concurrently with these metabolic changes in the brain, fetal ECoG activity and breathing movements were altered for many hours following UCO. Before occlusion of the umbilical cord, the ECoG showed episodes of high- and low-voltage activity of approximately equal duration, as expected for fetal sheep at this gestational age (10). Cord occlusion resulted in a significant decrease of the high-voltage activity to effectively zero incidence for up to 4 h after the occlusion, and the incidence of high-voltage activity remained below the preocclusion values and less than that for the sham-occluded group, for much of the time up to the end of the experiment (Fig. 4, A and B). The incidence of low-voltage activity was also decreased following the occlusion (Fig. 4, A and C) and was replaced by activity that was either very low in amplitude (<25% of the normal low-voltage amplitude for each fetus), or effectively isoelectric; i.e., equal to amplifier noise (Fig. 4, A and D). High-amplitude spiking activity in the ECoG record occurred in 4 of the 5 fetuses after the 10 min of UCO. This seizurelike activity appeared 15–30 min after the UCO, reached a maximum incidence at 1–3 h after the occlusion, and then returned to the very low levels typical of normal fetal ECoG activity by 7 h after the occlusion (Fig. 4, A and E). Episodes of high- and low-voltage activity had returned by 15 h after the occlusion in most fetuses, but the coherent pattern of high- and low-voltage episodes present before the occlusion was usually not regained before the end of the 24-h experiment.

Before the UCO, a normal pattern of low-amplitude (3–5 mmHg), episodic fetal breathing movements was observed, with these movements being present for 49.5 ± 8.0% of the time. As expected from previous studies (26), movements occurred mainly during periods of low-voltage ECoG activity (Fig. 5A). This type of fetal breathing was rapidly abolished by UCO, which induced isolated, breathing movements (gases), which were short in duration but high in amplitude, generating 10- to 20-mmHg decreases of pressure in the fluid-filled
Fig. 4. Representative ECoG activity (A) of a fetus in respect to umbilical cord occlusion (UCO). Incidences of high-voltage ECoG activity (B); low voltage activity (C); and sub-low voltage ECoG activity (D) are shown for sham (dashed lines) and cord occluded (solid lines) fetuses for 2-h epochs from −4 to +24 h with respect to the umbilical cord occlusion at time 0. The area between the two dotted lines in the second record (0 h to +1 h) indicates the period of 10-min UCO. Note the reemergence of episodes of high- and low-amplitude ECoG activity (+12 h to +15 h), with the beginning of continuously breathing movements as shown in Fig. 5A. The number of “spike” (E) transients per hour in the ECoG record for sham (dashed lines) and cord occluded (solid lines) fetuses is shown. Data are shown as means ± SE; n = 4 fetuses per group. *Significant differences (P < 0.05) between groups.
trachea (Fig. 5C). Normal episodic breathing movements gradually returned between 3 and 8 h after the UCO, but then the incidence increased until the breathing movements became continuous (i.e., 100% incidence) by 19–20 h after UCO (Fig. 5B). At this time, breathing movements continued through the reemerging episodes of high-voltage ECoG activity (Fig. 6). The incidence of breathing movements had decreased somewhat after 20 h post-UCO, but the incidence was still
Fig. 6. Concurrent fetal ECoG activity (A) and breathing movements (B) at 21–22 h after UCO in a fetus at 134 days gestation. Expanded section below each main trace shows the ECoG and breathing activities over 1.5 min.

DISCUSSION

This study has shown that brief, global fetal asphyxia has significant effects on total and regional cerebral blood flows, cerebral metabolism, and fetal behavior. The findings support the hypothesis that cellular injury caused by the asphyxia leads to a number of behavioral sequelae indicative of compromised CNS function.

The global fetal asphyxia that arises from complete, but reversible occlusion of the umbilical cord has previously been shown to induce gray and white matter damage, and hippocampal and cerebellar neuronal cell death when the brain is examined 1–3 days after the occlusion (9, 14, 16, 30, 31, 43). This brief (10 min) interruption to umbilical blood flow had relatively transient effects on fetal systemic physiological parameters, as shown by the almost complete recovery from the hypoxia, acidosis, and cardiovascular perturbations by 1–2 h after the occlusion. However, while the MAP and acid-base status had stabilized soon after the occlusion, blood flow velocity in the sagittal sinus vein continued to fall, reaching levels ~70% of the preocclusion velocity for up to 10 h, before returning to the preocclusion level. This post-UCO decrease of flow velocity in the sagittal sinus—the major site of drainage of blood from the cerebral hemispheres—was to some extent matched by decreases in regional cerebral perfusion as measured by microspheres, and could arise directly from cerebral vasoconstriction, or indirectly from increased intracranial pressure as a result of cerebral edema, a response that has been noted in other studies (4, 5, 12, 36). On the other hand, Jensen et al. (20) have proposed that posthypoxic hypoperfusion in the fetal brain may reflect suppressed cerebral metabolism, a response that may protect the immature brain from metabolite-related brain damage (20).

Some of the microdialysis data are consistent with suppression of cerebral metabolism, such as increased extracellular glucose (indicating decreased in cellular utilization) and, except for a transient increase, the low levels of lactate (indicating the maintenance of aerobic metabolism). A decrease in cerebral metabolism following UCO is also consistent with a study of cerebral heat production in which it was calculated that O₂ consumption and glucose uptake were diminished to ~10% and 86%, respectively, after 10 min of cord occlusion in fetal sheep (18). However, despite these changes, it is evident that cellular integrity was compromised, as shown by the release of intracellular pyruvate into extracellular fluid, and the associated increase of glycerol. Extracellular glycerol increased shortly after initiating the cord occlusion and lasted for ~1 h before concentrations returned to basal levels. A second increase of glycerol, although not as great in magnitude as the first, started ~7 h after the occlusion event and lasted for a further 1.5 h. Energy failure and intracellular Ca²⁺ overload are the two major contributors to membrane phospholipid degradation following brain ischemia (37), and extracellular glycerol has been used as a marker of membrane phospholipid degradation and cell damage. Energy failure induced by hypoxia/ischemia results in the influx of calcium into the cells, activation of phospholipases, decomposition of cell membranes, and liberation of glycerol into extracellular fluid (17). This sequence of events can result from the overproduction of reactive oxygen species, in particular, hydroxyl radical, which we have shown elsewhere to be increased by umbilical cord occlusion (33, 44). Animal study has shown an immediate and sustained increase in brain extracellular glycerol following fluid percussion brain injury, with the increase in glycerol concentration suggested to be due to free radical productions after the mechanical trauma, as it is decreased by free radical scavenger treatment (32). In the present study, the glycerol increase may have resulted from a quasi-mechanical insult in that large changes of blood pressure and cerebral blood flow might be associated with vascular damage and subsequent intracerebral hemorrhage. We have shown that hydroxyl radical increases in the fetal brain in two phases, similar to the change of glycerol reported here (33, 44). We now suggest that the second, slower increase of glycerol is a result of the increase of reactive oxygen species and that it signifies “loss of membrane integrity,” as shown by the appearance of pyruvate in the extracellular (i.e., microdialysate) fluid.

In these experiments, as in another study in which the umbilical cord was occluded (22), plasma glucose concentrations were increased. Hypoxia-induced increases in adrenergic activity and plasma catecholamines increase plasma glucose concentrations in fetal sheep (21) and lead to increased plasma glucose concentrations through glycogenolysis (38). Some of the increase of brain extracellular glucose observed in this study may be derived from this systemic hyperglycemia. However, increased expression of the 55-kDa microvascular iso-
form of GLUT1 has been shown in the hypoxic immature rat brain (41), and if this were to occur postasphyxia in fetal sheep, greater glucose transport might be the result. The increase of extracellular glucose is also consistent with decreased glucose utilization, and the hypometabolic state of the CNS, proposed to be a major consequence of global fetal asphyxia in fetal sheep (20), and also noted as a result of prolonged fetal hypoxemia in the fetal llama (13).

An additional finding of this study was the large increase in amplitude and incidence of fetal breathing movements from about 8 h after the brief episode of UCO. Breathing movements in the late-gestation sheep fetus occur as discontinuous episodes associated with rapid eye movement (REM) and low-voltage electrocortical activity. These movements are readily suppressed by hypoxemia (7) due to descending inhibition arising from the thalamic parafascicular nuclear complex (26) and possibly also from the pontine parabrachial/subcoruleus nuclei (8). Thus, the significant reduction of fetal breathing movements during and for a time after the asphyxial episode was not unexpected, although a hypometabolic state within the brain stem (as the marked fall of blood flow to these regions indicates; see Fig. 2), may also contribute to decreased activity within the respiratory networks for several hours following cord occlusion. However, the eventual increase of both the amplitude and incidence of breathing movements and the continuance of breathing throughout the reemerging episodes of high-voltage ECoG, suggests that a profound change had occurred either within the region of the brain stem concerned with the generation of respiratory rhythm. Continuous fetal breathing activity has been produced by total midcollicular transection of the fetal brain (11) or by placement of punctate lesions in the rostral pons or thalamus (15, 26). Continuous fetal breathing after cord occlusion in sheep has been noted by others, but the experimental context was different. Baier et al. (2) and Adamson et al. (1) were able to induce continuous breathing when the cord was occluded and air or 100% O2 was delivered to the fetal lungs through an endotracheal tube. However, the period of observation was short (30 min), and these experiments were directed at establishing whether the placenta released substances that inhibited fetal respiration, rather than to examine the effects of brief asphyxia on the brain (1, 2).

More extended observations over days were made by Kawagoe et al. (23) and Watson et al. (42) who used repeated, short (90 s) episodes of cord occlusion. These authors observed that the incidence of normal breathing movements were decreased while “deep inspiratory efforts” [gasps] were increased, and they interpreted these changes as an adaptation to the frequent periods of hypoxemia (23, 42). The experiment that comes closest to ours, in terms of the time of cord occlusion (10 min), is that of Hunter et al. (18), who made observations of cerebral blood flow for 1 h after the occlusion, but made no observations of fetal breathing movements (19).

The impact of the brief, but profound, asphyxia on the fetal brain may result in the loss of activity in the supra-pontine networks that produce the normal cyclic ECoG activity and the descending control of brain stem respiratory activity. Alternatively, as treatment with prostaglandin synthesis inhibitors (24, 25), or 5-hydroxytryptophan (34) also produce continuous fetal breathing activity, we cannot rule out that changes to metabolism and neurotransmitter release within the medulla, pons, or diencephalon following cord occlusion that might also increase fetal breathing activity. It is worthy of note that the recovery of sagittal vein blood flow occurred at approximately the time when the mean amplitude of the ECoG was increasing, when distinct episodes of high and low amplitude reemerged in ECoG activity, and when extracellular glucose, pyruvate, and glycerol concentrations were falling back to their normal ranges.

**Perspectives and Significance**

Our results do not contradict the concept of a linkage between cerebral hypoperfusion and hypometabolism following a single brief episode of fetal asphyxia but do show that cell membrane function is impaired in the hours following the insult. This impairment does not seem to occur in association with anaerobic glycolysis, thus contrasting this clinically relevant model of in utero fetal stress with the Rice-Vannucci model of hypoxia-ischemia in the immature rodent brain where a period of significant anaerobic glycolysis occurs in association with cell death and expansion of the cerebral infarct. Should widespread impairment of cellular function occur throughout the cortex, it might explain the alteration of fetal breathing movements in relation to the recovering ECoG activity. It has been known since Barcroft’s pioneering studies that development of the fetal sheep brain includes increasing inhibitory control of hindbrain activity by the developing rostral centers (3), a fact confirmed by brain transection studies (11). The changes in fetal activity that we have observed following global fetal asphyxia may not be readily seen in the human fetus due to the relatively short (15–30 min) periods of observation usually made by obstetric ultrasonographers who are concerned with diagnosing the presence or absence of movement, not its prolongation.

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**REFERENCES**


