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THE EFFECTS OF OXYGEN DEPRIVATION

ON BRAIN METABOLITES IN VARIOUS

GESTATIONAL AGE SHEEP FETUSES

By

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B.A., Colorado College, 1978

Presented in partial fulfillment of the
requirements for the degree of

Master of Science

UNIVERSITY OF MONTANA

1981

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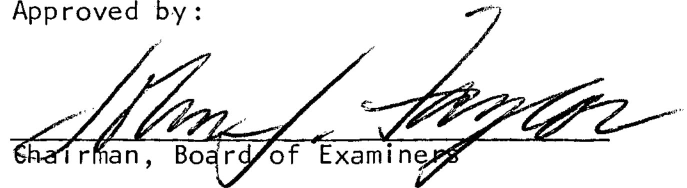

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The Effects of Oxygen Deprivation on Brain Metabolites in Various Gestational Age Sheep Fetuses

Director: J. J. Taylor

Fetuses are more resistant than adults to brain injury following oxygen deprivation. Although a metabolic basis for this resistance has been postulated, the mechanism for this resistance of the fetal brain has not been resolved. Previous biochemical and pathological studies in adult monkeys and goats established that the accumulation of lactic acid above a threshold concentration of 17-20 μmoles/g in brain tissue as a result of oxygen deprivation, led to injury. The purpose of this study was to determine if the metabolic basis for fetal brain tolerance to anoxia during early gestation was its inability to accumulate lactic acid above threshold concentrations. This hypothesis was tested by examining the brain metabolite concentrations in sheep fetuses exposed to circulatory arrest at 50, 80, 110, and 140 days of gestation. These brain metabolite concentrations were compared to brain metabolites from normally oxygenated fetuses of the same gestational age. Cortical gray and white matter, and brain stem were sampled and fluorometric enzyme assays were performed to determine lactate, glycogen, glucose, fructose, ATP, ADP, AMP, and phosphocreatine concentrations. The results of this study indicated that lactic acid accumulated to threshold levels only in the brain stem of 110 and 140 day sheep fetuses. These biochemical findings correlated very well with pathology studies which showed that the brain stem nuclei of late gestational age fetuses were the only structures damaged following anoxia. Lactic acid did not accumulate to supra-threshold levels because carbohydrate substrates, in particular glycogen, were not present in high enough concentrations until late in gestation. Although ATP, PCr, and energy charge values decreased significantly during anoxia, there was no correlation between energy charge, ATP, or PCr values and brain pathology. Thus, the conclusion was made that early gestational age fetuses were more resistant to brain injury following oxygen deprivation because lactic acid did not accumulate in vulnerable brain structures above threshold levels.

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INTRODUCTION

A variety of circumstances leading to severe oxygen deprivation in the human fetus ultimately result in brain injury (1,4,12). In order to examine possible mechanisms underlying the development of brain injury, animal models are used. Results from these animal experiments have led to various explanations for the mechanism of brain injury following oxygen deprivation (37, for reviews see 24-26,29,44). Most theories suggest that the lack of oxygen is the only determinant of brain injury although experimental evidence to the contrary exists (26,31). In 1976 Myers and Yamaguchi proposed that, during oxygen deprivation, lactic acid accumulation above a threshold concentration, leads to brain edema and tissue necrosis (30). A number of pathological and biochemical studies using different models of oxygen deprivation in Dr. Myers', and more recently, in four other laboratories support this theory (9-11, 23, 35, 36,42,48,51). The present thesis will test this and other pathogenic mechanisms in fetal brains and will examine the correlation between lactic acid accumulation and brain injury in the sheep fetus exposed to oxygen deprivation. Furthermore, this study will attempt to find a basis for the extended tolerance fo the fetal brain to lack of oxygen.

I. Statement of the Problem

This study will examine the effect of anoxia on several brain metabolites in various brain regions of sheep fetuses of different

gestational ages. Previous brain pathology studies in fetuses subjected to varying lengths of anoxia indicate that the fetal brain is more tolerant of anoxic exposure than the adult brain (5, 7, 11). Studies in adult animals show that lactic acid accumulation correlates well with the development of brain pathology following oxygen deprivation. As a result of this earlier work, biochemical studies are needed to test the hypothesis that the fetal brain is more tolerant to anoxia because the fetal brain accumulates less lactic acid during oxygen deprivation than the adult.

The maximum concentration to which lactic acid accumulates during anoxia, as a result of circulatory arrest, depends primarily upon the levels of carbohydrate substrates in the tissue prior to anoxia. Lower glycogen, glucose and fructose concentrations may be found in the fetus compared to adults in the normally oxygenated state. In addition, specific structures of the brain may contain varied amounts of each carbohydrate and change as a function of gestational age. Thus, substrate availability determines the degree of lactic acid accumulation during anoxic exposure and if the hypothesis is correct, defines the vulnerability of a particular structure to injury with varying gestational ages.

Several investigators have proposed that brain injury occurs because of a deficient energy availability. This study will determine the concentrations of adenosine-5'-triphosphate (ATP), phosphocreatine (PCr) and energy charge and will attempt to correlate a decreased energy availability with brain injury. Although ATP, PCr and energy charge have

not been shown to be correlated with pathology in adults, the fetus has not been examined (25).

The results of the present investigation will provide information on critical biochemical changes in brain caused by oxygen deprivation. The correlation between these biochemical investigations and brain pathology, will provide a better understanding of the remarkable tolerance of the fetal brain to injury from oxygen deprivation.

II. Background

A. The Influence of Magnitude and Duration of Oxygen Deprivation on Patterns of Brain Injury.

Early studies concerned with the effects of oxygen deprivation on brain tissue focused on patterns of injury produced by exposure to different magnitudes and durations of oxygen deprivation (39). In these studies the magnitude of oxygen deprivation ranged from anoxia to various levels of hypoxia. Anoxia, a state of total oxygen deprivation, has been used as a model for both adult and neonatal human brain injuries resulting from oxygen deprivation. Newborn rhesus monkeys exposed to total asphyxia, produced by clamping the umbilical cord and preventing breathing, exhibited a pattern of injury restricted to brain stem nuclei (24). In these animals the inferior colliculus was the primary brain stem structure injured. Frequent injury was also present in the superior olivary nucleus, descending nucleus of the fifth cranial nerve, lateral

and spinal vestibular nuclei, gracile and cuneate nuclei and the posterior and lateral ventral nuclei of the thalamus (24). Food deprived, juvenile and adult monkeys subjected to circulatory arrest also exhibited injury in these brain stem structures, in addition to the IIIrd, IVth and VIth cranial nerve nuclei (22). However, this pattern of restricted injury to brain stem nuclei without involvement of the cerebral hemispheres has rarely been reported in humans exposed to oxygen deprivation. These results suggested that the circumstances producing brain injury in the human were different from this experimental model of a total and sudden lack of oxygen (24,30,31).

Hypoxia, another model commonly used, is a state of oxygen deprivation in which there is a decreased oxygen content in arterial blood. In the fetus arterial blood contents are normally 10-12 volumes percent. A reduction not exceeding 4 volumes percent did not produce neurological damage or significant changes in cardiovascular performance, while 3-4 volumes percent or lower produced cardiovascular changes but not neurological damage (25). Brain injury began to occur in fetuses of food deprived animals when the fetus was exposed to an arterial blood oxygen content equal to or less than 0.5 volumes percent for a duration of 25-30 minutes (25). Fetuses exposed to less than 0.5 volumes percent oxygen (arterial blood) often developed severe cardiovascular collapse from which the animals could not be resuscitated if the exposure lasted longer than 20 minutes (24). Those fetuses subjected to less than 1.5 volumes percent oxygen developed brain edema and necrosis of the cerebral hemispheres. This pattern of pathology is similar to the one observed in severely

asphyxiated human newborns, who subsequently develop cerebral palsy (20, 24). This hypoxic exposure proved to be a valid model for brain injury due to asphyxia in the perinatal period.

B. Theories of Brain Injury Following Oxygen Deprivation.

Several theories have been proposed to explain the pattern of brain injury resulting from exposure to oxygen deprivation. In 1922 Vogt proposed that the selective vulnerability of the brain to injury from oxygen deprivation was the result of different patterns of metabolic or chemical organization in various brain structures (ref. in 26). In contrast to this chemical basis for tissue injury, Spielmeyer proposed that brain injury resulted from changes in vascular perfusion during or after oxygen deprivation (ref. in 26). An offshoot of the vascular theory of brain injury was the border zone hypothesis. Current supporters of the border zone hypothesis propose that the most susceptible areas of the brain to injury lie furthest from the major cerebral arteries and are the first to suffer reductions in perfusion when a decrease in blood flow occurs (31). Another hypothesis, termed the "no-reflow phenomenon", was put forth by Ames and coworkers (2). In this theory they propose that brain cell injury occurs as a result of swelling in perivascular glial and endothelial cells which impairs recirculation after oxygen deprivation.

C. Other Variables Which Determine the Pattern of Brain Injury Following Oxygen Deprivation.

Further studies from Myers and coworker defined a factor, unrelated to those proposed by earlier investigators, that was found to be a crucial determinant of brain pathologic outcome from anoxic exposures. These studies revealed that the magnitude and duration of oxygen deprivation were not the only variables involved in determining the development of brain injury. Evidence for this new variable came from studies in which monkeys were food deprived overnight and then received glucose injections prior to 10 minutes of anoxia (31). Surprisingly, these animals died hours after exposure with major cerebral necrosis and edema with herniation of the cerebellar tonsils and vermis, as well as a flattening of cerebral convolutions. In contrast, monkeys that received saline infusions and also were exposed to 10 minutes of anoxia developed injury restricted to the nuclei in the brain stem with no involvement of the cerebral hemispheres (23,31). Thus, the animal's serum glucose concentration and, by inference, the carbohydrate state, which was changed by food deprivation or glucose infusions, was proposed to be the important variable determining the pattern of brain injury following identical anoxic exposure (23-26).

Biochemical studies with saline- and glucose-infused rhesus monkeys exposed to 10 minutes of anoxia indicated that the only significant difference between the two groups was that glucose-infused monkeys developed lactic acid levels greater than 30 μmoles/g while saline-infused

animals accumulated lactic acid to 10-12 μmoles/g (25,30). The highly vulnerable brain stem nuclei in food deprived monkeys and goats exposed to circulatory arrest also accumulated the highest lactic acid concentrations in the brain (29, 49). Brainstem nuclei also contain more glycogen stores than other brain structures (28,49). Thus, there was a direct correlation between the pattern of brain pathology and the accumulation of lactic acid to high concentrations in the various brain regions.

A similar relationship between lactic acid accumulation and brain injury was found in monkeys exposed to 10 and 25 minutes of hypoxia (3.5 volumes % O2) (25). Ten minutes of exposure to severe hypoxia did not lead to the development of brain injury and cortical lactic acid concentrations only reached 10-12 μmoles/g. However, monkeys exposed to 25 minutes of hypoxia accumulated lactic acid concentrations of 24 umoles/g in the brain and they developed brain injury. Based upon these results and those from the anoxia studies, Myers concluded that the accumulation of lactic acid to concentrations greater than 17-20 μmoles/g was the threshold value for brain injury (25, 26).

Additional biochemical studies were performed to determine whether the concentration or activity of other metabolites and enzymes, respectively, correlated with the development of brain injury. Investigators have long argued that a depletion of ATP or a severe decrease in energy charge caused brain injury (8, 32). Although ATP levels were markedly reduced, they were not significantly different between glucose- and saline-infused animals exposed to circulatory arrest, even though the animals with high serum glucose concentrations developed brain edema and tissue necrosis

while all animals with low serum glucose levels survived and showed minimal or no neurologic damage (25). No significant correlation was found between brain injury and certain enzyme activities, including sodium-potassium ATPase (Na+K+ATPase), acid phosphatase and alkaline phosphatase (24, 25, 30).

Lactic acid accumulation in brain tissue during oxygen deprivation can be understood on the basis of brain carbohydrate metabolism. An increase in the production of lactic acid from pyruvate, rather than formation of acetyl coenzyme A (acetyl CoA), occurs because of the increase in the reduced nicotinamide (NADH) to oxidized nicotinamide (NAD) ratio in an oxygen deprived cell. The increase in the NADH/NAD ratio and the reduction in the concentrations of acetyl CoA slow tricarboxylic acid (TCA) cycle flux and result in lower levels of ATP and higher concentrations of adenine-5'-dinucleotide (ADP) and adenine-5'-mononucleotide (AMP) in the cell (16). Enhancement of ADP and AMP concentrations stimulate phosphofructokinase (PFK) activity, an enzyme which serves as a primary regulatory enzyme in the glycolytic pathway (18). This increase in activity of glycolysis and in addition, glycogenolysis results in a rapid depletion of tissue stores of glycogen and glucose and leads to a 5-10-fold increase in lactic acid concentrations in brain tissue (18). Thus, tissue stores of glycogen and glucose would determine the level to which lactic acid accumulated during a prescribed interval of oxygen deprivation (19,48).

D. Studies of Oxygen Deprivation and Metabolism in the Fetus

In certain respects, fetuses and neonates do not resemble their adult counterparts with respect to brain injury induced by oxygen deprivation. For example, fetal and neonatal brain tissue is more resistant to injury from oxygen deprivation (15). Furthermore, fetal metabolism differs in many respects from the adult (13). Many investigators utilize these metabolic differences to explain the higher susceptibility of the adult to brain injury, from oxygen deprivation, compared to the fetus. However, definitive experimental proof is not available to support these theories. The theory that lactic acid accumulation above threshold levels results in brain injury has not been tested with respect to fetuses. Therefore, biochemical studies are needed to test whether the greater tolerance of the fetal brain to injury following oxygen deprivation is due to decreased lactic acid production.

Several different studies have demonstrated that fetuses and newborns were more resistant than adults to oxygen deprivation. In these studies young animals were consistantly more tolerant, on the basis of survival, behavioral deficits, physiologic reflexes and gross brain pathology (7,15,25). Thus, the theories proposed to explain the resistance of the fetal and neonatal brain to injury, following oxygen deprivation, must consider these earlier observations.

Various animal and oxygen deprivation models were used to study the relationship of state of development to brain injury resulting from oxygen deprivation. Fetal and neonatal models, including monkeys, dogs, cats,

rabbits, mice, guinea pigs and rats were compared to each other and to the corresponding adults (5,7,13,15,25,44,46). Monkey fetuses were asphyxiated by clamping the umbilical cord, and fetal hypoxia was produced by respiring the mother with a decreased oxygen concentration (25). Term rat fetuses, as well as neonatal rats, were decapitated or placed in a 100 percent nitrogen gas environment (7,45,46). The brains of neonatal puppies were exposed to anoxia either by occlusion of the ascending aorta (5) or by abrupt inflation of a cervical pressure cuff following laminectomy (15). Hypoxia was achieved by respiring animals with a decreased oxygen gas (25, 44). In these models it was demonstrated that fetuses were more resistant to brain injury than neonates (7), while both fetuses and neonates were more resistant than adults (5,7,25,46).

Several parameters were used to define resistance in the studies mentioned above. In 1941 Kabat examined three different parameters which revealed that puppies were more resistant than adults. He demonstrated that after anoxia puppies recovered normal behavior faster (revival time); survived longer periods of anoxia, without permanent changes in behavior (survival time); and regained corneal and respiratory reflexes more quickly (recovery time) (15). Similar results with survival and behavior were found with rats and dogs (5, 7).

Studies from Dr. Myers' laboratory also revealed differences between newborn and young adult monkeys (25,26). Newborn monkeys of food deprived mothers and food deprived, young adults monkeys exposed to 12.5 minutes of anoxia developed lesions in brain stem nuclei. However, in the newborn the inferior colliculus was more susceptible to injury, while in the adult

the IIIrd, IVth and VIth cranial nerve nuclei were more readily injured (25). Another investigation revealed that at least 25 minutes of 0.8-1.5 volumes percent oxygen was needed for fetuses to develop brain edema and injury to the cerebral hemispheres (25). In contrast young adult brains exhibited a similar outcome after 25 minutes of respiring air with a much higher oxygen content (3.5 volumes percent).

Different theories concerned with the increased resistance of the fetal brain to injury following oxygen deprivation have focused on differences between fetal and adult carbohydrate metabolism. These differences in carbohydrate metabolism include:

1) pathways available to fetuses and adults;
2) substrate concentrations in fetuses and adults; and
3) rate of metabolism in both groups.

The hypothesis of Dr. Myers and coworkers is that these differences in carbohydrate metabolism may result in lower levels of lactic acid and also, a lower rate of lactic acid accumulation in fetal brain tissue as compared to the adult. These 2 factors result in the extended tolerance of the fetal brain to anoxia.

Adults and fetuses of the same species do not always have the same pathways available for carbohydrate metabolism. Radioactive tracer studies using chronically cannulated sheep fetuses revealed that glucose was broken down to lactate or carbon dioxide plus water and that gluconeogenesis did not occur (50). Both catabolism of glucose and synthesis of glucose from metabolites, such as lactate, occurred in adult sheep (14,50). The enzymes and hormones necessary to stimulate

gluconeogenesis were present in the fetus. However, the enzymes were either inactive or inadequately stimulated by the hormones (40,50). Fetal serum glucose came from the transport of glucose across the placenta and glycogen stores in the fetus (14,40,50). Glycogen was synthesized and broken down in sheep fetuses and adults (50).

The radioactive tracer studies also demonstrated that the placenta converted maternal glucose to fructose (50). This conversion resulted in a favorable concentration gradient for glucose transport across the placenta (41). Subsequent radioactive tracer studies and analysis of loss of serum fructose in the urine suggested that sheep fetuses did not metabolize fructose (41, 50). However, other studies found that the amniotic membranes in the fetus utilize fructose and that stressful situations resulted in the use of serum fructose by the fetus (3, 6). Investigators found insignificant fructose levels in adult sheep which implied fructose did not make a major contribution to the carbohydrate pool (50).

In addition to pathways, serum glucose, fructose, and lactate concentrations have been examined in sheep fetuses, neonates and adults. In these studies the investigators concluded that serum lactate levels were higher in fetuses (2.19±0.6mM) than in suckling lambs (1.78±0.46mM)(50). However, serum glucose levels were lower in sheep fetuses (0.59±0.15mM) than in suckling lambs (3.61±0.58mM) or in adults (2.67±0.31mM)(50). Serum fructose values also were higher in fetuses (5.09±0.89mM) than in newborns and adult sheep, where they were

undetectable (14, 50). Subsequent studies showed that brain glucose and lactate levels were lower than serum values, but they followed the serum concentrations (21).

Investigators also found that carbohydrate metabolism proceeded at a different rate in fetuses compared to adults. Chronical y cannulated sheep fetuses utilized glucose faster (10.5 mg/min/kg) than did adults (1.4 mg/min/kg) (14). It has been suggested that the increased rate of glucose consumption in the fetus resulted from a greater dependence on glucose metabolism; adults depended more on noncarbohydrate substrates. However, 30-day old rats consuming a high carbohydrate diet showed an increased rate of glucose metabolism compared to 2-day old newborns (30-day=12.13 μmole glucose/min/100 g body wt., 2-day=6.62 μmole glucose/minute/100 g body wt.) (47).

Brain tissue of decapitated fetal rats also metabolized glucose to lactate at a slower rate (0.27mM/min) than 7-day old postnatal rats (0.58mM/min) (7). Decapitated fetal rats also metabolized brain glycogen at a slower rate (0.01mM/min) than similarly treated postnatal rats (0.17mM/min)(7). The maximal rate of lactate accumulation in brain tissue occurred faster in 7 day old postnatal rats (1.9mM/min) compared to fetuses (0.9mM/min)(7). Based on ATP, phosphocreatine and ADP measurements, the study found adult rat brains used ene gy at about a 10 times faster rate (26.6 mmole-P/kg/min) than did 7 day old (2.58 mmole-P/kg/min), fetal (1.57 mmole-P/kg/min) and 1 day old (1.33 mmole-P/kg/min) rats (7).

The results from these metabolic studies form the basis for the theories explaining the increased tolerance of the fetal brain to oxygen deprivation compared to adults. It is the hypothesis of this thesis that a decreased rate of glycolysis and/or a lower carbohydrate reserve is present in the fetal brain during anoxia. The higher rate of glucose utilization in decapitated postnatal rat brain (0.70 mmoles/kg/min) compared to fetal rat brain (0.08 mmoles/kg/min) supports the idea of decreased glycolytic rates in fetuses compared to older animals (7). Carbohydrate reserves also may be lower in fetuses as shown by sheep serum glucose levels (fetus= 0.59 ± 0.5mM, mother= 2.67 ± 0.31mM) (50). These lower serum glucose levels indicate lower levels of glucose will be found in the fetal brain compared to adults (2[illegible]) During anoxia, these two factors could lead to lower lactic acid levels, thus preventing injury to the fetal brains (29).

Other investigators have proposed that the fetal brain has a lower rate of energy consumption than do adult brains, and, therefore during anoxia the rate of energy reserve depletion would allow the brain of the fetus to survive longer than the adult brain (7,13,46). Himwich proposed that during oxygen deprivation, the fetus was able to obtain an adequate amount of energy from glycolysis to sustain neurological function, while in the adult these energy requirements could not be met by this pathway (13). This theory was based on experiments in which fetuses of different species survived asphyxia for longer periods than did adults (13). However, metabolic studies were not done to prove that different energy utilization rates existed for fetuses, neonates and adults.

Duffy et al. focused on this theory when they reported that cerebral energy utilization rates during anoxia were higher for adult rats (26.8 mmole-P/kg/min) than for term fetuses (1.57 mmole-P/kg/min) (7). However, the brains of the term fetuses exhibited a higher energy utilization rate than 1 day old rats (1.33 mmoles-P/kg/min), yet fetuses survived anoxia for twice as long as these neonates (Survival was based on the presence of the gasping reflex which previously has been defined as a neurological function that indicated survival of brain tissue.) (7). These investigators concluded other factors, in addition to energy requirements, were involved in fetal brain tolerance to oxygen deprivation. Subsequent studies revealed that the availability of readily mobilizable glycogen plus the decreased energy requirements of the neonate increased the ability of the neonatal rat brain to survive asphyxia (46). However, fetal studies were not performed to determine whether glycogen was more easily mobilized by fetal, 2 day old or 10 day old postnatal rat brains. Thus, insufficient evidence exists to support the theory that the resistance of fetal brains to the cessation of the gasping reflex, resulting from oxygen deprivation, is caused by a decreased energy utilization rate. Furthermore, these results suggest a correlation between survival and the development of brain injury which may not be a valid extrapolation since a brain may survive anoxia, despite the presence of brain pathology (injury) (24, 25).

The experiments in this thesis will examine the change of several metabolite concentrations (i.e. lactic acid, glucose, glycogen) during anoxia and compare the results with brain injury (gross and histologic

brain damage). Alterations in ATP and PCr concentrations and energy charge will also be examined to determine whether the energy deficit correlates with the occurrence of fetal brain injury.

MATERIALS AND METHODS

I. Animal Preparation

Pregnant ewes of Dorset or mixed stock are used for this study. Ewes carrying fetuses of approximately 50, 80, 110 or 140 days gestation are food deprived for 48 hours prior to the experiment. Then, the pregnant ewe is anesthetized with pentobarbital (35 mg/kg). In addition, individual 3.8-6.0 mg/kg doses of pentobarbital are given to maintain anesthesia as needed, at approximately 20 minute intervals. Following induction of anesthesia, the ewe is intubated and mechanically ventilated with a Harvard respirator. Body temperature is maintained at $39 \pm 2^{\circ}C$ with a warm water mattress. Adequate oxygenation of the fetus is ensured by ventilating the mother with 100% oxygen. The femoral artery of the mother is then catheterized and arterial blood is sampled for determinations of pH, pO2, pCO2, base excess (BE), hematocrit, glucose and lactate.

The period between the conclusion of surgical manipulations and the initiation of the cesarean section is referred to as the "control period". During this period blood gases, pH, and base excess are measured using a Corning blood gas analyzer. The pH, pO2 and pCO2 are measured directly by electrodes within the blood gas analyzer. Base excess is a valued derived from the plasma concentrations of hemoglobin, bicarbonate (a value calculated from the pCO2) and pH. The base excess is defined as the amount of acid or base needed to titrate 1 liter of blood back to a pH of 7.40. Hematocrit measurements also are made and converted to hemoglobin values to be used to determine base excess. Hematocrit is the

packed red blood cell volume which is measured by centrifuging a capillary pipet filled with blood and determining the red cell volume from a Critocap chart (Lancer Co.). At the time of sampling arterial blood for blood gases and pH determinations, a 0.3 ml sample is taken and centrifuged immediately. The resulting plasma is then assayed for lactate and glucose as described in the "Assay" section. Prior to the initiation of the cesarean section, the respirator rate is adjusted to achieve the following control values in arterial blood: pO_2= 80 mmHg, pCO_2=30-40 mmHg, pH=7.40-7.50, BE=0$\pm$2 mmoles/l.

The experiment is initiated at 1 hour following induction of anesthesia, if the blood gases and pH values of the mother s arterial blood are within the limits indicated above. Following the cesarean section the fetus, which s still attached to the mother via the umbilical cord, is removed from the uterus and samples of blood are taken from the intact umbilical artery and vein for blood gases, pH, and plasma lactate, glucose and fructose.

1. Cardiac arrest. Immediately following blood sampling, the heart of the fetus is extirpated. The umbilical cord is then clamped and cut and the fetus is placed back in the abdomen of the mother to maintain its body temperature. These fetuses are referred to as "arrested fetuses". To determine the maximum concentration of lactic acid in brain tissue, the fetus is left in the abdomen for 1-2 hours depending upon the gestational age. The fetal brain is then frozen by pouring liquid nitrogen over the fetal head while the body of the fetus is cooled in dry ice.

2. Controls - in situ freezing. In control animals, the brain is frozen in situ as previously described for rats and cats (33, 52). The bottom of a styrofoam cup is cut out and the cup is attached to the head of the fetus with silicone grease (Dow-Corning). Liquid nitrogen is poured into the cup and refilled as required. This method permits continued circulation to the various brain regions until the time of freezing, thus preventing anoxic alterations in metabolite concentrations. After the brain is frozen, the entire animal is then frozen in dry ice or alternatively the animal is decapitated and the head is frozen.

II. Tissue Sampling

Brain and other tissue specimens are stored in the -80°C freezer until they can be sectioned and sampled. All sectioning and sampling is performed in a -20°C room to prevent alterations of metabolite concentrations. Coronal sections of the brain are cut using a band saw. Small samples (mg) are taken from different brain regions which are exposed by the sectioning process. The structures sampled in each age group are listed in Table 1. These brain regions are clearly defined by their location and their morphology. These tissue samples are weighed on a Roller-Smith precision balance and placed in Tenbroeck homoogenizers containing 100 μl of 0.1N-HCl in methanol. The homogenizers are placed in a dry ice-ethanol bath at -40°C and the samples are dispersed with a glass rod. Next, the homogenizer is placed in ice at 0°C and 1.0 ml of 0.02N-HCl is added. Samples are then homogenized by hand and a 200 μl

TABLE 1

Structures Sampled from Sheep Fetus Brain at Each Gestational Age

Structure	50 Day	80 Day	110 Day	140 Day
Cortex	Hemispheral Wall(Mid.) Hemispheral Wall(Post)	Cortex (gray matter)	Precentral Gyrus Superior Parietal Occipital Superior Temporal Gyrus	Precentral Gyrus Superior Parietal Occipital Superior Temporal Gyrus
White Matter		Occipital Anterior	Centrum Semiovale	Centrum Semiovale
Brain stem	Medulla Inferior Colliculus	Tegmentum Inferior Colliculus	Inferior Colliculus Tegmentum Vestibular Nucleus Inferior Olive Gracile Nucleus Cuneate Nucleus Sub Gelatinosa	Inferior Colliculus Tegmentum Vestibular Nucleus Inferior Olive Gracile Nucleus Cuneate Nucleus Sub Gelatinosa

aliquot is removed from the brain homogenates for glycogen assays. The aliquots of brain and homogenates of liver, heart and muscle are heated at 100°C for 10 minutes and stored at -80°C until assayed. To precipitate protein, 112.5 µl of 3M perchloric acid is added to the brain tissue homogenates and the homogenate is then centrifuged at 12,500 g for 20 minutes. The perchlorate extract is neutralized to pH 6 to 7 by the addition of 2.5M-$KHCO_3$. The tubes are left on ice for 30 minutes in order to precipitate potassium perchlorate. Next, the tubes are centrifuged for 10 minutes at low speed and the supernatant is removed and stored at -80°C until assayed.

III. Assays

1) Introduction

All metabolite concentrations are determined by enzymatic assays employing either fluorometric or spectrophotometric methods (17). Fluorescence techniques are used to measure nmole quantities of a metabolite. In this study, a Farrand Optical fluorometer will be used. This instrument measures the fluorescence emitted by NADH or NADPH at 450 nm following the excitement of these compounds at 355 nm. Tissue metabolite concentrations are calculated on the basis of standards which have been standardized by spectrophotometric methods and run along with the tissue extracts. Along with standards, calculations take the mg of sample/µl of supernatant into account, such that the fluorescence of a sample is expressed as umoles of metabolite/gram of tissue.

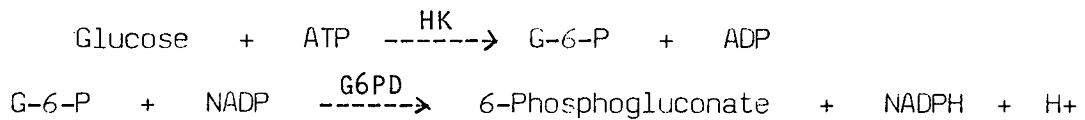
Spectrophotometric assays are employed when μmole or mmole quantities of a metabolite are present. For all assays except serum glucose determinations (see Serum Assays) the absorbance of NADH or NADPH is measured at 340 nm. In all reactions the concentration of NADH or NADPH is stoichiometrically related to the particular metabolite being assayed. Only one reading of the sample tube plus a blank reagent tube is required for the spectrophotometric assays. The concentration of the metabolite is calculated from the absorbance and the molar extinction coefficient of 6270 for NADH or NADPH at 340 nm.

2. Fluorometric Assays

a. General

All of the assays except one utilize the reagents in Table 2. The reactions are run in a total volume of 1.0 ml at the final concentrations indicated. Tris, in these reactions, is employed as a buffer while the dithiothreitol (DTT) stabilizes the thiol groups of enzymes in the mixture. The magnesium chloride (MgCl2) is required as a cofactor for the hexokinase (HK) (EC 2.7.1.1) reactions. Both glucose and ATP are substrates for HK and therefore, the reduced nicotinamide dinucleotide phosphate (NADPH) produced as a result of coupling the HK reaction with glucose-6-phosphate dehydrogenase (G6PD) (EC 1.1.1.49) reflects the original quantitity of the particular metabolite which was present in the tissue. The specific reactions catalyzed by HK and G6PD are:

Figure 1



b. Brain Tissue Metabolite Assays

(1) Glycogen and Glucose

Glycogen and glucose are assayed on unneutralized homogenates of tissue samples as described by Passonneau and Lauderdale (34). Initially, glycogen is converted to glucose using α-1,4-α-1,6-amyloglucosidase (AG). The reagents employed to determine glucose concentrations are listed in Table 2. The assay is carried out as follows: aliquots (50 μl) of brain tissue homogenates are added to tubes containing 100 ul of 100 mM sodium acetate (NaAc), pH 4.7. AG (0.0015 U), which hydrolyzes the α-1,4-α-1,6 bonds of glycogen to yield free glucose, is added to those tubes in which glycogen will be assayed. Parallel tubes are similarly assayed without AG to determine the concentration of free glucose present in the homogenates. The NaAc buffers the incubation mixture at a pH optimum for AG of 4.7. Following addition of AG the tubes are incubated for 30 minutes at room temperature, and the reaction is stopped by adding 1.0 ml of the tris reaction mixture (Table 2) without the HK and G6PD to all the tubes. This reaction mixture elevates the pH to 7-8 which inhibits AG and is an optimum pH for HK and G6PD. Following addition of the tris reaction mixture, the blank fluorescence of all tubes is read. Then HK and G6PD are added to all the tubes to start the

TABLE 2

Metabolite Measured	Tris-HCl µmoles-pH8.1	MgCl2 µmoles	DTT µmoles	NADP µmoles	HK units	G6PD units	Other Additions
Glucose	50	1.0	0.5	0.05	0.15	0.03	ATP-0.3 µmoles NaAc -10mM (pH 4.7)
ATP and PCr	50	1.0	0.5	0.05	0.15	0.03	ADP-0.1 µmoles AMP-0.1 µmoles Creatine kinase-1.0 unit Glucose-0.1mM

Note: For explanation of reagent abbreviations, see text.
All enzymes diluted with bovine serum albumin (20 µl/ml)and 20mM Tris.

reaction and the tubes are incubated at room temperature for 30 minutes (for reaction see Fig. 1). The fluorescence is then read. The concentration of glycogen is determined by subtracting the tubes assayed without AG (glucose) from the parallel tubes to which AG is added. These later tubes contain the sum of glucose units from glycogen plus the free glucose in the tissue.

(2) Fructose

The tubes without AG in the glycogen-glucose assay are assayed for fructose after the fluorescence resulting from the presence of glucose has been determined. Phosphoglucose isomerase (PGI) (EC 5.3.1.9; 3.5 U) is added to these tubes and the change in fluorescence is read after 30 minutes. The HK present in the reaction mixture converts the fructose to fructose-6-phosphate (F6P). PGI converts the F6P to glucose-6-phosphate (G6P). The G6P and NADP then can be converted to 6-phosphogluconate and NADPH, respectively (See Fig. 1). Thus, the fructose present in the brain tissue is stoichiometric with the NADPH fluorescence measured by the fluorometer.

(3) ATP and Phosphocreatine

The ATP and PCr tissue assay also utilizes the reaction mixture described in Table 2. ATP and PCr are assayed on the same tissue samples. Aliquots (100 μl) of the neutralized perchlorate extracts are added to 1.0 ml of the reaction mixture listed in Table 2. Following the reading of sample blank fluorescence, the reaction is started with HK-G6PD (for reaction see Fig. 1) and the fluorescence (which is a measure of the ATP present) is read after 30 minutes. Next, ADP, AMP

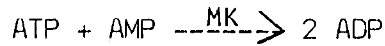
and creatine kinase are added to the reaction mixture. Creatine kinase breaks down PCr to creatine and catalyzes the phosphorylation of ADP to ATP. The fluorescence is then read after 30 minutes. This third fluorescence reading is a measure of the amount of PCr present.

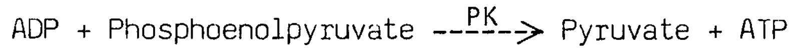
Falsely high PCr values result if AMP is not included in the PCr assay to inhibit adenlyate kinase. This enzyme is present in the tissue extracts and is acid stable (i.e. not precipitated by HClO4)(17).

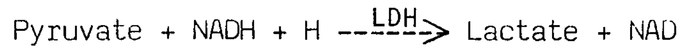
(3) ADP and AMP

ADP and AMP are assayed on the same tissue sample. Aliquots (200 μl) of each extract are added to 800 μl of reaction mixture. The reaction mixture for each tube consists of 50 mM imidazole, pH 7.0, 0.01 mM NADH, 2.0 mM MgCl2, 75 mM potassium chloride (KCl) and 0.02 mM phosphoenolpyruvate. After the sample and lactate dehydrogenase (LDH) (0.4 U) are added to the tubes, the blank fluorescence is read. Pyruvate kinase (0.8 U) (PK) is then added and the decrease in fluorescence for ADP is determined after 15 minutes. The AMP concentration is determined by adding myokinase (0.4 U) (MK) plus 0.005 mM ATP to the tubes. Following a second 15 minute incubation the fluorescence is measured. The sequence of reactions involved in this assay are listed in Figure 2.

Figure 2







(4) Lactic Acid

Lactic acid is assayed in a reaction mixture containing 200 mM hydrazine, pH 9.7, 60 mM NAD, and 1 mM ethylenediamine tetraacetate (EDTA). The reaction is started by adding LDH (7.8 U) which converts lactate present in the brain tissue extract and NAD to pyruvate and NADH, respectively. This reaction is pulled toward pyruvate formation by hydrazine which reacts with pyruvate to form pyruvate hydrazone. The EDTA binds manganese (Mn++) whose presence in the sample inhibits LDH. The reaction mixture is incubated for 30 minutes and then the fluorescence from the NADH is measured. The NADH concentration is stoichiometric with the lactate concentration.

3. Spectrophotometric Assays

a. Serum Assays

(1) Lactic Acid

This assay uses the same reagents described in the tissue assay. However, a ten-fold higher NAD concentration is required. Absorbance is read at 340 nm.

(2) Glucose

Glucose is assayed by a colorometric method described in Sigma Technical Bulletin No. 510. The reagent purchased from Sigma Chemical Co. consists of glucose oxidase and peroxidase. Glucose oxidase catalyzes the formation of gluconic acid and hydrogen peroxide from the glucose present in serum. The peroxide then reacts with colorless o-dianisidine to form o-dianisidine (brown). Reagent blanks, as well as standards are required for this assay. The absorbance of o-dianisidine (brown) is read at 450 nm and the serum glucose concentration is calculated from the values of the standards.

(3) Fructose

This assay employs the same reagents and the same concentrations described in the glucose fluorometric assay (see Table 2) with 2 exceptions. In the tris reaction mixture a 10-fold higher concentration of NADP is used. PGI also is added as described in the fructose fluorometric assay.

D. Calculations

1. Energy charge is calculated from the concentrations of AMP, ADP and ATP obtained from brain tissue assays. The formula used to calculate energy charge is:

$$EC = \frac{[ATP] + 1/2[ADP]}{[ATP] + [ADP] + [AMP]}$$

2. The metabolite concentrations for each group were compared to each other with an unpaired Student's t-test. Metabolite concentrations were significantly different if p 0.05. The equation used for this t-test is:

$$t = \frac{x - y}{\frac{(n_x S_x - n_y S_y)}{(n_x + n_y + 2)} \left(\frac{1}{n_x} + \frac{1}{n_y} \right)}$$

Note: n = number of samples in each group.

a,b = group letter.

x,y = mean for groups a and b, respectively.

S = standard deviation.

RESULTS

I. Arterial and Venous Blood Gas, pH and Metabolite Concentrations.

Blood gases, pH and serum metabolite concentrations were determined in the ewes and fetuses to provide knowledge of their oxygenation and acid-base status prior to exposure. One hour following the induction of anesthesia (i.e. end of the "control period"), ewes of control or arrested fetuses did not exhibit significantly different pH, blood gas or serum metabolite values with respect to gestational age. Thus, these data were pooled to determine if control pH, blood gas and serum metabolite values in ewes of control fetuses were different than the values observed in the ewes of arrested fetuses (Table 3). The arterial blood gas and pH values present in the ewes of control and arrested fetuses were within the ranges specified in the Materials and Methods. Lactate, glucose and fructose concentrations, in ewes of control and arrested fetuses, were not significantly different during the control period (Table 3). The arterial and venous blood were not significantly different between control and arrested fetuses with 2 exceptions (Tables 4, 5). First, the mean arterial pCO2 during the control period was significantly lower (p 0.05) in the 80 day fetuses subsequently exposed to anoxia compared to the control fetuses (Table 4). Arterial serum fructose levels also were significantly lower (p 0.05), during the control period, in 50 and 110 day arrested fetuses compared to the corresponding control fetuses (Table 4).

TABLE 3

Ewe Arterial Blood Gas, pH and Metabolite Concentrations During Control Period

Sample	Control	Arrest
pH	7.45±0.01 (18)	7.47±0.01 (16)
pO2 (mmHg)	344±21 (18)	354±29 (16)
pCO2 (mmHg)	35±1 (18)	33±1 (15)
HCO3 (mmole/l)	23±1 (16)	23±1 (14)
Base Excess (mmole/l)	2±1 (18)	1±1 (16)
O2 Saturation (vol %)	100±1 (14)	100±1 (13)
O2 Content (mmoles/l)	15±1 (13)	14±0 (13)
Hematocrit (vol %)	29±1 (17)	29±1 (15)
Glucose mM	4.25±0.18 (18)	4.20±0.15 (16)
Fructose mM	0.27±0.09 (16)	0.20±0.05 (15)
Lactate mM	2.95±0.25 (18)	3.12±0.24 (16)

*Values expressed as mean ± SEM.
( ) = Number of samples.

TABLE 4

Arterial Blood Gas, pH and Metabolite Concentrations During Control Period for Sheep Fetuses of Various Gestational Ages*

	50 Day		80 Day	
Sample	Control	Arrest	Control	Arrest
pH	7.46 (1)	7.35 (2)	7.40±0.02 (4)	7.37±0.01 (3)
PO2 (mmHg)	21 (1)	17 (2)	20±2 (4)	17±2 (3)
pCO2 (mmHg)	39 (1)	49 (2)	46±2 (3)	29±2 (3)
HCO3 (mmole/l)	27 (1)	27 (2)	27±0 (3)	27 (2)
Base Excess (mmole/l)	4 (1)	3 (2)	3±1 (4)	1±2 (4)
O2 Saturation (vol %)	33 (1)	19 (1)	33±2 (3)	31±7 (3)
O2 Content (mmole/l)	4 (1)	3 (2)	6±0.5 (2)	8±1 (3)
Hematocrit (vol %)	25 (1)	31 (2)	33±0.3 (3)	35 (2)
Glucose mM	1.73±0.30 (3)	1.35±0.39 (3)	1.48±0.06 (3)	1.73±0.13 (4)
Fructose mM	4.80±0.20 (3)	6.08±0.31 (3)	7.05±0.88 (4)	9.47±0.23 (3)
Lactate mM	2.20±0.15 (3)	2.50±0.19 (3)	1.46±0.02 (3)	1.36±0.14 (4)

TABLE 4 (con't.)

	110 Day		140 Day	
Sample	Control	Arrest	Control	Arrest
pH	7.33±0.01 (4)	7.38±0.02 (3)	7.33±0.01 (3)	7.33±0.00 (4)
PO2 (mmHg)	28±3 (5)	23±0 (4)	24±1 (4)	23±3 (4)
pCO2 (mmHg)	53±1 (4)	49±1 (3)	53±1 (4)	54±2 (3)
HCO3 (mmole/l)	27±0 (4)	25±1 (4)	27±1 (5)	27±1 (4)
Base Excess (mmole/l)	1±0 (5)	2±1 (3)	1±1 (4)	1±1 (4)
O2 Saturation (vol %)	38±5 (5)	28±2 (4)	26±1 (3)	35±3 (3)
O2 Content (mmole/l)	6±1 (4)	5±0 (3)	6±0 (4)	7±1 (3)
Hematocrit (vol %)	38±1 (4)	39±1 (4)	49±1 (4)	45±0 (3)
Glucose mM	1.85±0.14 (5)	1.61±0.20 (4)	0.88±0.05 (3)	1.10±0.10 (3)
Fructose mM	9.97±1.11 (3)	7.80±0.90 (4)	3.70±0.10 (3)	2.60±0.30 (3)
Lactate mM	2.03±0.14 (4)	1.97±0.27 (4)	2.73±0.11 (4)	2.54±0.14 (3)

*Values expressed as mean ± SEM.
( ) = Number of samples.

TABLE 5

Venous Blood Gas, pH and Metabolite Concentrations During Control Period for Sheep Fetuses of Various Gestational Ages*

	50 Day		80 Day	
Sample	Control	Arrest	Control	Arrest
pH			7.41±0.02 (4)	7.41±0.01 (3)
PO2 (mmHg)			37±2 (3)	47±4 (3)
pCO2 (mmHg)			41±1 (3)	40±4 (3)
HCO3 (mmole/l)			26±1 (3)	27 (2)
Base Excess (mmole/l)			3±1 (4)	4±1 (3)
O2 Saturation (vol %)			74±7 (4)	78±7 (3)
O2 Content (mmole/l)			10±1 (3)	14±2 (3)
Hematocrit (vol %)			32±1 (3)	35 (2)
Glucose mM			2.13±0.20 (5)	1.96±0.06 (3)
Fructose mM			9.04±1.22 (5)	9.77±0.15 (3)
Lactate mM			1.59±0.15 (5)	1.47±0.13 (4)

TABLE 5 (con't)

	110 Day		140 Day	
Sample	Control	Arrest	Control	Arrest
pH	7.37±0.03 (4)	7.39±0.03 (4)	7.34±0.01 (3)	7.36±0.01 (4)
PO2 (mmHg)	38±1 (3)	43±3 (4)	35±2 (4)	32±4 (3)
pCO2 (mmHg)	47±1 (3)	41±2 (4)	50±1 (4)	48±1 (3)
HCO3 (mmoles/l)	25±1 (5)	23±1 (4)	26±1 (5)	26±1 (3)
Base Excess (mmoles/l)	2±0 (4)	2±0 (3)	1±1 (5)	1±1 (4)
O2 Saturation (vol %)	60±1 (4)	74±3 (4)	47±2 (3)	58±10 (4)
O2 Content (mmoles/l)	12±1 (4)	13±0 (4)	12±1 (4)	14±1 (3)
Hematocrit (vol %)	39±0 (3)	39±1 (4)	49±1 (4)	45±0 (3)
Glucose (mM)	2.15±0.12 (4)	1.61±0.19 (4)	1.27±0.10 (3)	1.10±0.10 (3)
Fructose (mM)	10.43±0.93 (4)	9.10±0.30 (3)	3.89±0.11 (4)	3.00±0.30 (3)
Lactate (mM)	2.24±0.11 (4)	2.06±0.30 (4)	2.76±0.05 (3)	2.86±0.02 (3)

*Values expressed as mean ± SEM.
( ) = Number of samples.

Serum concentrations of lactate, glucose and fructose changed significantly with gestational age ($p<0.05$). Arterial lactate concentrations decreased significantly from 50 to 80 days in control and arrested fetuses during the control period (Table 4, Fig. 3;$p<0.05$). Arterial and venous lactate levels increased significantly from 80 to 140 days in control and arrested fetuses (Table 4, 5, Fig. 3;$p<0.05$). In contrast to lactate, arterial glucose levels peaked at 80 days of gestation and decreased significantly by 140 days of gestation in both control and arrested fetuses, during the control period (Table 4, Fig. 4;$p<0.05$). In venous blood, the glucose levels also decreased significantly between 80 and 140 days of gestation in the control period (Table 5, $p<0.05$). Although fructose levels were higher in serum, they tended to parallel the changes in glucose with respect to gestational age. Arterial fructose concentrations during the control period in control and arrested fetuses peaked between 80 and 110 days of gestation and then decreased significantly by 140 days (Table 4, Fig. 4;$p<0.05$). There was no significant difference between 80 and 110 day fructose levels in arrested or control fetuses during the control period (Table 4, Fig. 5). Furthermore, fructose levels decreased significantly between 110 and 140 days for both groups of fetuses during the control period. Similar results were found in the venous samples from these fetuses (Table 5).

II. Brain Metabolites in Control and Arrested Fetuses

Carbohydrate and energy metabolites from several brain regions were determined for control and arrested fetuses at 50, 80, 110 and 140 days of

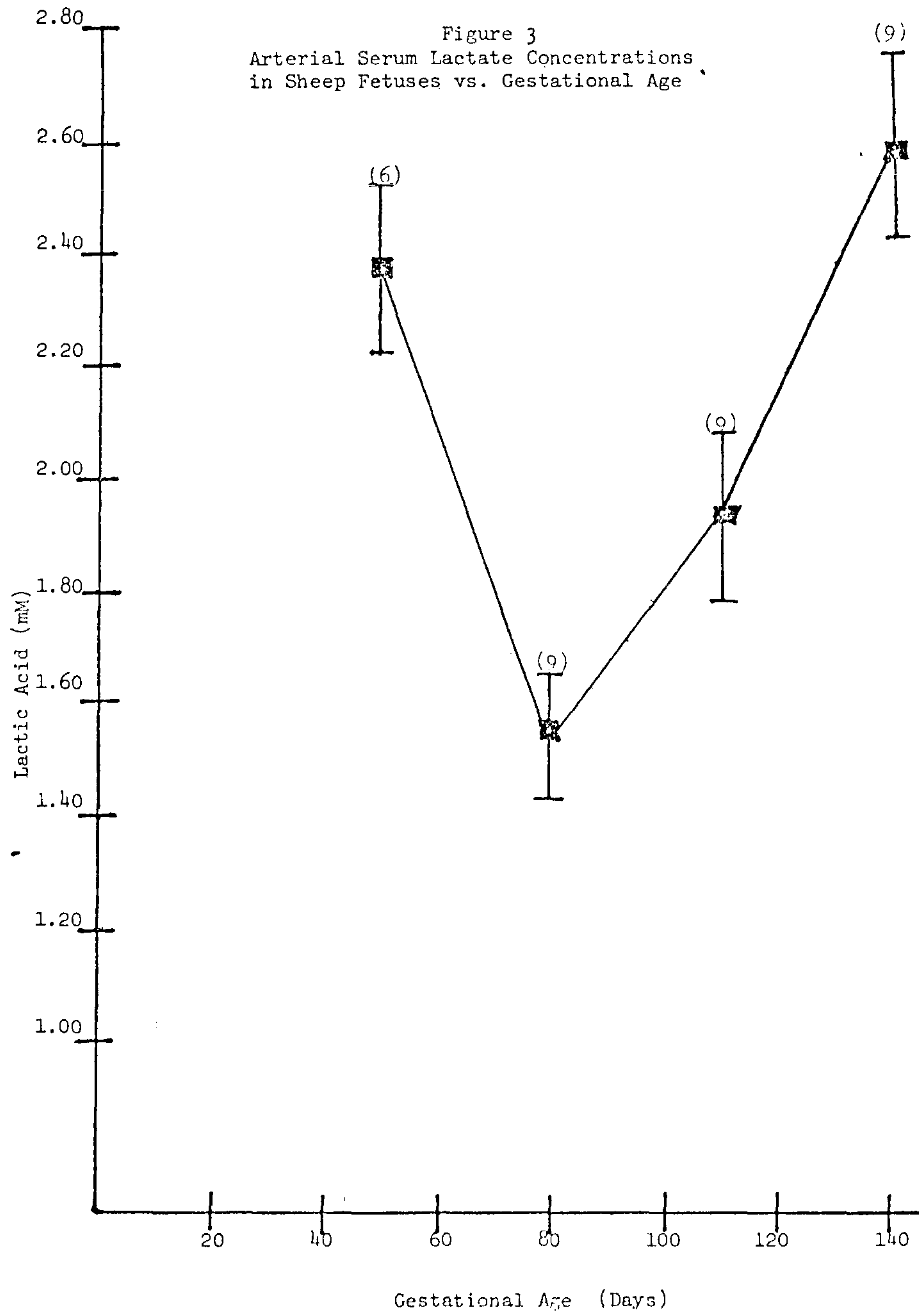


Figure 3
Arterial Serum Lactate Concentrations in Sheep Fetuses vs. Gestational Age

Figure 4
Arterial Serum Glucose Concentrations
in Sheep Fetuses vs. Gestational Age

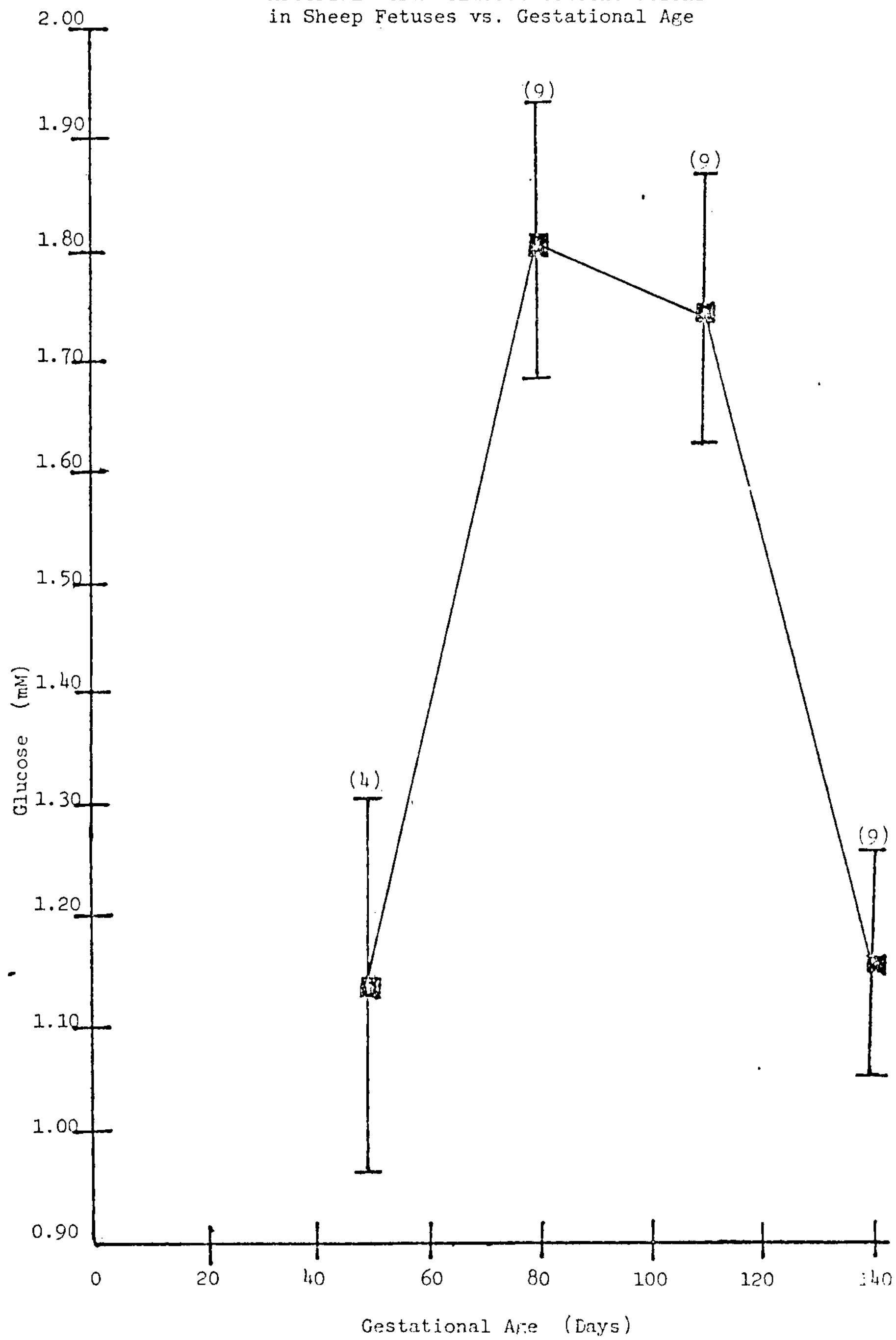
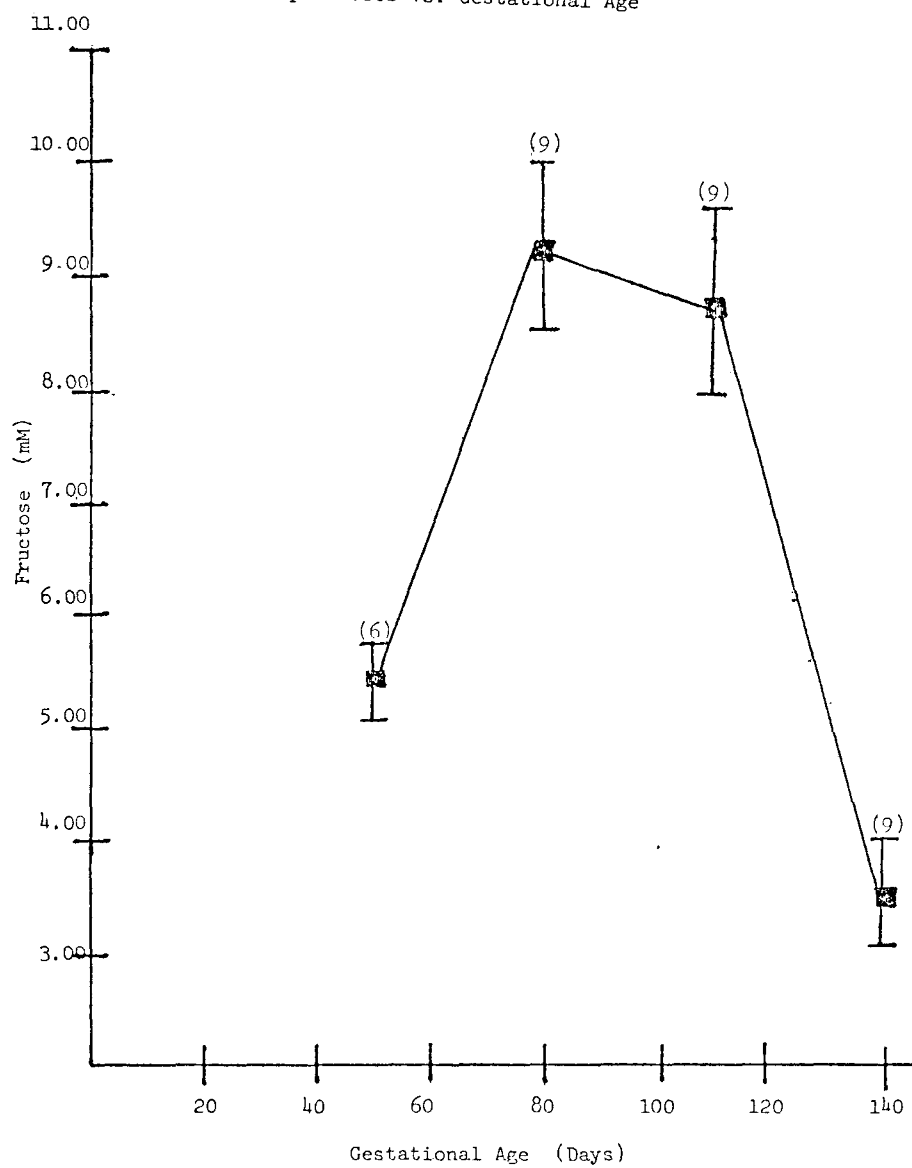


Figure 5 .
Arterial Serum Fructose Concentrations
in Sheep Fetuses vs. Gestational Age



gestation. The metabolite concentrations of cortical gray and white matter and brain stem were compared because previous pathologic studies with monkey and sheep fetuses, of various gestational ages exposed to anoxia, showed injury to brain stem nuclei but not cortex or white matter. Monkey fetuses of less than 80 days gestation rarely exhibited any brain injury following 30 minutes of anoxia (24).

The lactate concentrations of all brain structures of control fetuses decreased with gestational age. The concentrations of lactate were similar in the cortex and white matter at all gestational ages examined. Brainstem lactate levels in control fetuses were similar to cortex levels at 50 and 80 days and significantly higher than cortex at 110 and 140 days of gestation (Table 6).

Following circulatory arrest the lactate concentrations were higher compared to control lactate concentrations, in all brain regions at all gestational ages ($p<0.05$, Fig. 6). The concentration to which lactate accumulated also increased with gestational age in cortex and brain stem of arrested fetuses. However, only in the brain stem of arrested 110 and 140 day fetuses did lactate accumulate to the threshold levels previously determined to cause injury (17-20 μmoles/g) (Table 6). The lactate levels present in the brain stem of the arrested 80, 110 and 140 day fetuses were significantly higher than those concentrations in the corresponding cortex ($p<0.05$, Fig. 6). White matter lactate concentrations in arrested fetuses did not change with gestational age (Table 6). Furthermore, lactate concentrations in the cerebral white matter of arrested fetuses were significantly higher at 110 days ($p<0.05$) than cortical gray matter

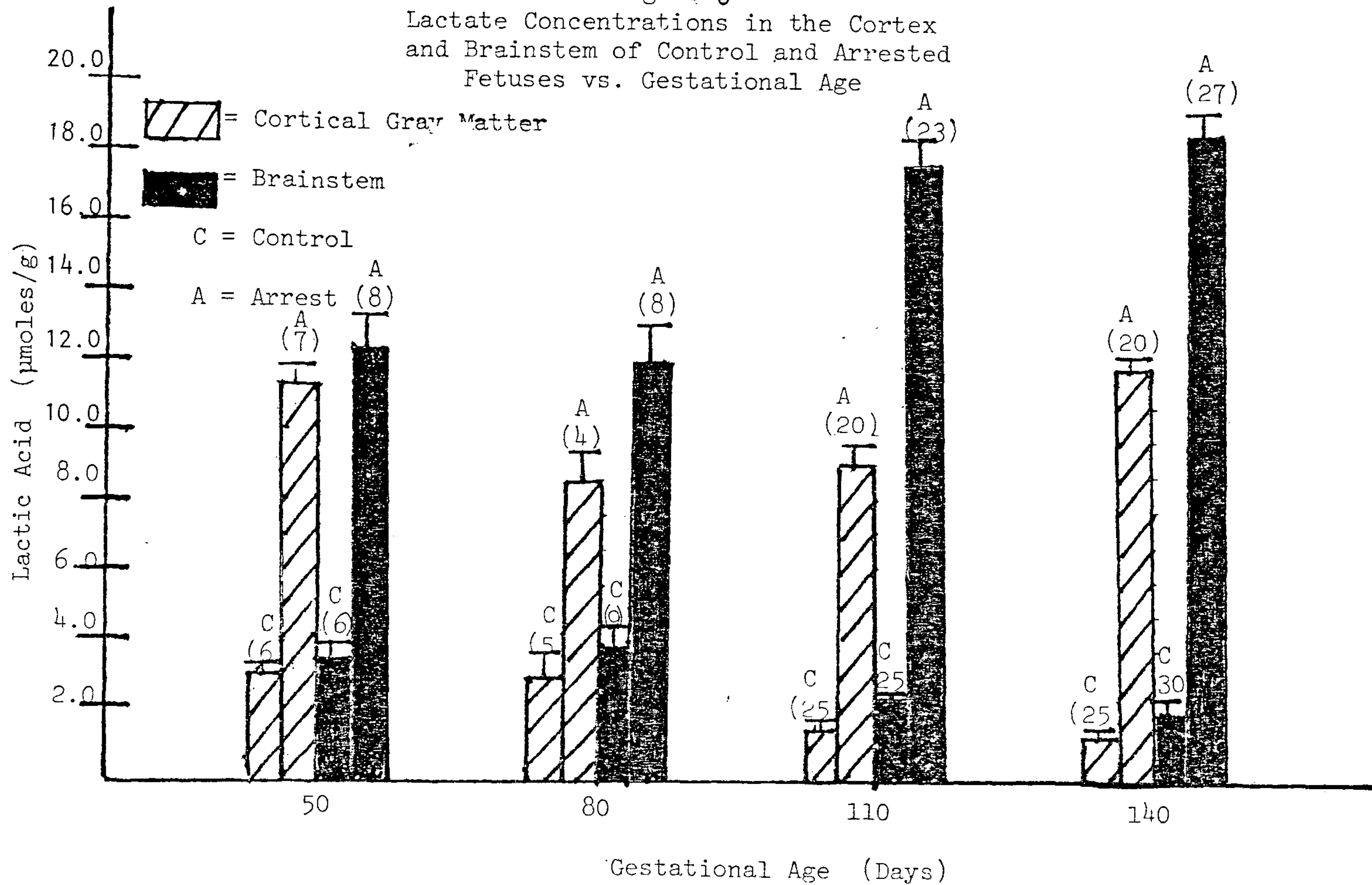
TABLE 6

Lactate Concentrations in Control and Arrested Fetuses at Four Gestational Ages*

Lactate - Controls				
Structure	50 Day	80 Day	110 Day	140 Day
Cortex	3.25+0.14 (6)	2.84+0.72 (5)	1.51+0.10 (25)	1.30+0.14 (25)
White Matter		2.61+0.24 (10)	1.66+0.15 (5)	1.56+0.17 (5)
Brain stem	3.64+0.27 (6)	3.71+0.47 (8)	2.20+0.17 (25)	2.05+0.15 (30)
Lactate - Arrest				
Structure	50 Day	80 Day	110 Day	140 Day
Cortex	11.40+0.49 (7)	8.51+0.72 (4)	8.85+0.62 (20)	11.81+0.29 (20)
White Matter		9.47+0.48 (7)	10.72+0.52 (3)	10.38+0.24 (5)
Brain stem	12.59+0.78 (8)	11.98+0.86 (8)	17.29+0.82 (23)	18.58+0.57 (27)

*Values expressed as mean ± SEM. Units = μmoles lactate/g brain tissue
( ) = Number of samples.

Figure 6
Lactate Concentrations in the Cortex and Brainstem of Control and Arrested Fetuses vs. Gestational Age



lactate concentrations (Table 6). Later, at 140 days gestation, the cortical gray matter lactic acid concentration exceeded the cerebral white matter concentrations ($p<0.05$).

Because lactate accumulation following circulatory arrest depends upon substrate availability (18), glycogen, glucose and fructose were assayed. Glycogen concentrations increased dramatically while glucose and fructose concentrations were variable with gestational age in control fetuses (Table 7, 8, 9). However, all three substrate concentrations decreased significantly in the 110 and 140 day fetuses exposed to anoxia (Figure 7, 8, 9). These changes in substrates with both gestational age in control fetuses, as well as between control and arrested fetuses indicate that all three substrates must contribute to lactate production (Table 10).

The glycogen concentrations in all brain regions increased significantly from 50 days (80 days in white matter) to 140 days of gestation in control fetuses (Table 7). Glycogen levels increased to the greatest extent in the brain stem reaching their maximal concentration of 9.24 ± 0.51 µmoles/g at 140 days (Table 7, Fig. 7). At each gestational age, the glycogen concentrations in the brain stem were significantly higher ($p<0.05$) than the corresponding cortical gray matter levels (Fig. 7). Glycogen levels were not significantly different between white matter and cortex.

A comparison of glycogen concentrations between control and arrested brain structures revealed differences in the amount of glycogen utilized, during anoxia, with gestational age in each brain structure (Fig. 7). Glycogen levels in brain stem structures decreased significantly following

TABLE 7

Glycogen Concentrations in Control and Arrested Fetuses at Four Gestational Ages*

Glycogen - Controls				
Structure	50 Day	80 Day	110 Day	140 Day
Cortex	2.18±0.36 (6)	2.23±0.40 (4)	3.21±0.23 (22)	4.90±0.17 (23)
White Matter		1.33±0.35 (9)	2.45±0.52 (4)	4.41±0.56 (5)
Brain stem	3.81±0.60 (6)	3.75±0.52 (9)	5.11±0.35 (27)	9.24±0.51 (29)
Glycogen - Arrest				
Structure	50 Day	80 Day	110 Day	140 Day
Cortex	1.65±0.54 (7)	1.95±0.17 (4)	1.85±0.23 (11)	2.04±0.36 (20)
White Matter		0.72±0.14 (7)	1.68±0.06 (3)	1.87±0.67 (4)
Brain stem	1.33±0.17 (7)	0.55±0.15 (6)	1.57±0.22 (20)	1.43±0.23 (26)

*Values expressed at mean ± SEM. Units = μmoles of glucosyl units/g brain tissue.
( ) = Number of samples.

TABLE 8

Glucose Concentrations in Control and Arrested Fetuses at Four Gestational Ages*

Glucose - Controls				
Structure	50 Day	80 Day	110 Day	140 Day
Cortex	1.07±0.11 (6)	1.04±0.21 (4)	1.49±0.07 (25)	0.95±0.09 (25)
White Matter		1.27±0.26 (8)	1.27±0.25 (4)	0.68±0.18 (5)
Brain stem	1.41±0.25 (6)	0.96±0.39 (9)	0.77±0.10 (26)	0.90±0.14 (30)

Glucose - Arrest				
Structure	50 Day	80 Day	110 Day	140 Day
Cortex	0.35±0.11 (7)	0.60±0.14 (4)	0.66±0.23 (20)	0.58±0.14 (20)
White Matter		0.51±0.14 (7)	0.43±0.17 (3)	0.19±0.05 (4)
Brain stem	0.25±0.08 (7)	0.31±0.10 (8)	0.39±0.08 (22)	0.31±0.08 (23)

*Values expressed as mean ± SEM. Units = μmoles glucose/g brain tissue.
( ) = Number of samples.

TABLE 9

Fructose Concentrations in Control and Arrested Fetuses at Four Gestational Ages*

Fructose - Controls				
Structure	50 Day	80 Day	110 Day	140 Day
Cortex	0.83±0.08 (6)	0.71±0.27 (3)	1.28±0.15 (25)	0.52±0.11 (25)
White Matter		1.36±0.50 (6)	0.75±0.30 (4)	0.00±0.00 (4)
Brain stem	1.13±0.13 (6)	0.75±.14 (6)	0.79±0.11 (32)	0.33±0.08 (30)
Fructose - Arrest				
Structure	50 Day	80 Day	110 Day	140 Day
Cortex	0.43±0.08 (7)	1.07±0.06 (3)	0.20±0.06 (25)	0.12±0.05 (20)
White Matter		1.34±0.19 (6)	0.28±0.11 (4)	0.14±0.09 (4)
Brain stem	0.58±0.28 (8)	0.32±0.10 (6)	0.15±0.02 (24)	0.08±0.05 (27)

*Values expressed as mean ± SEM. Units = μmoles of fructose/g of brain tissue.
( ) = Number of samples.

TABLE 10

Relationship of Carbohydrate Available in Control Fetuses with Lactate Produced in Arrested Fetuses for Four Gestational Ages*

50 Day			
Structure	Carbohydrate Available	Theoretical Lactate	Actual Lactate
Cortex	4.09±0.49 (6)	5.06-11.98	11.40±0.49 (7)
Brain stem	6.36±0.70 (6)	8.64-17.26	12.59±0.78 (8)
80 Day			
Structure	Carbohydrate Available	Theoretical Lactate	Actual Lactate
Cortex	3.82±0.94 (3)	3.08-10.42	8.51±0.72 (4)
White Matter	4.79±0.55 (4)	6.76-12.64	9.47±0.48 (7)
Brain stem	5.06±0.35 (6)	7.32-12.94	11.98±0.86 (8)

TABLE 10 (con't)

110 Day			
Structure	Carbohydrate Available	Theoretical Lactate	Actual Lactate
Cortex	6.48±0.39 (25)	7.92-22.22	8.85±0.62 (20)
White Matter	4.47±0.67 (4)	5.38-12.76	10.72±0.52 (3)
Brain stem	7.84±0.66 (32)	7.66-35.50	17.29±0.82 (23)
140 Day			
Structure	Carbohydrate Available	Theoretical Lactate	Actual Lactate
Cortex	6.83±0.34 (25)	9.10-23.88	11.81±0.29 (20)
White Matter	5.26±0.37 (5)	8.94-13.48	10.38±0.24 (5)
Brain stem	11.11±0.59 (31)	14.62-44.74	18.58±0.57 (27)

Note: Carbohydrate Available = Glycogen + Glucose + Fructose concentrations in control fetuses
Theoretical Lactate = This is the amount of carbohydrate X 2.
The range indicates the lowest and highest individual carbohydrate levels within each structure. The values indicate the amount of lactate which all the carbohydrates could produce.
Actual Lactate = Lactate values measured in arrested fetuses.

*Values expressed as mean ± SEM. Units = μmoles/g of brain tissue.
( ) = Number of samples.

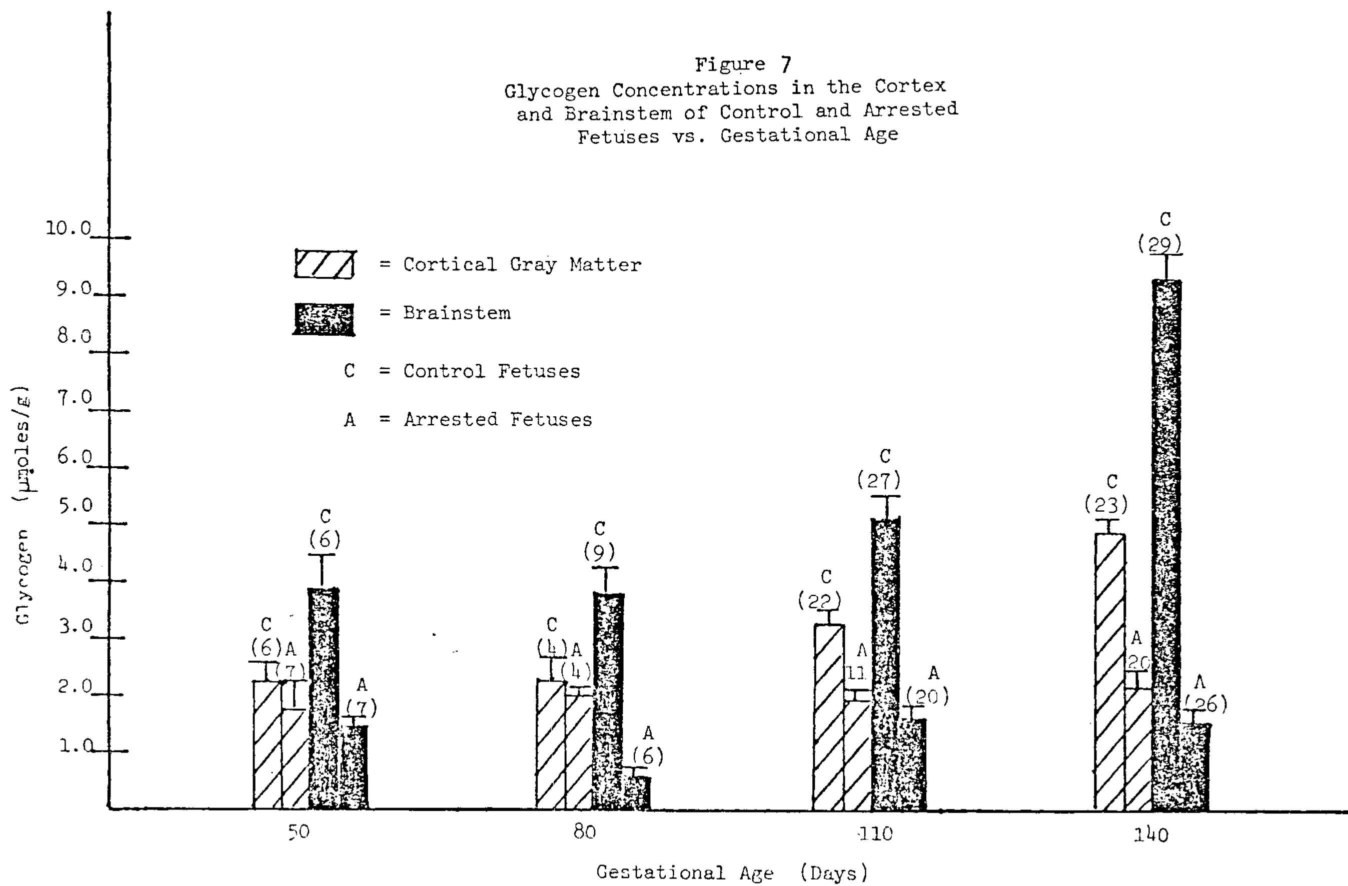


Figure 7
Glycogen Concentrations in the Cortex and Brainstem of Control and Arrested Fetuses vs. Gestational Age

arrest ($p<0.001$) in each age group (Table 7). Cortical glycogen concentrations also decreased following arrest at each gestational age although no significant changes occurred until 110 and 140 days of gestation. White matter glycogen concentrations showed no significant decreases with anoxia until 140 days. The overall decrease in glycogen between control and arrested fetuses was larger in the brain stem than cortex or white matter in each age group (Table 7, Fig. 7). Thus, the change in glycogen contributed to the higher lactic acid levels in the brain stem compared to the cortex and white matter.

Glycogen concentrations in the brain following circulatory arrest did not change significantly with gestational age in the cortex. White matter and brain stem glycogen concentrations following circulatory arrest were significantly lower in 80 day fetuses ($p<0.05$) while the concentrations in 50, 110 and 140 day fetuses were similar (Table 7). The glycogen concentrations following circulatory arrest were not significantly different between cortex and white matter or cortex and brain stem except for the lower concentration in the brain stem of 80 day fetuses (Table 7).

Brain glucose concentrations also changed with gestational age in control fetuses (Table 8, Fig. 8). In cortex, the control glucose levels were significantly higher at 110 days of gestation ($p<0.05$) than 50, 80 or 140 day fetuses which had similar glucose concentrations (Table 8, Fig. 8). Glucose levels tended to decrease with gestational age in the white matter of control fetuses although the changes were not significant (Table 8). The concentration of glucose in the brain stem of control fetuses decreased significantly between 50 and 110 days ($p<0.05$). However, the

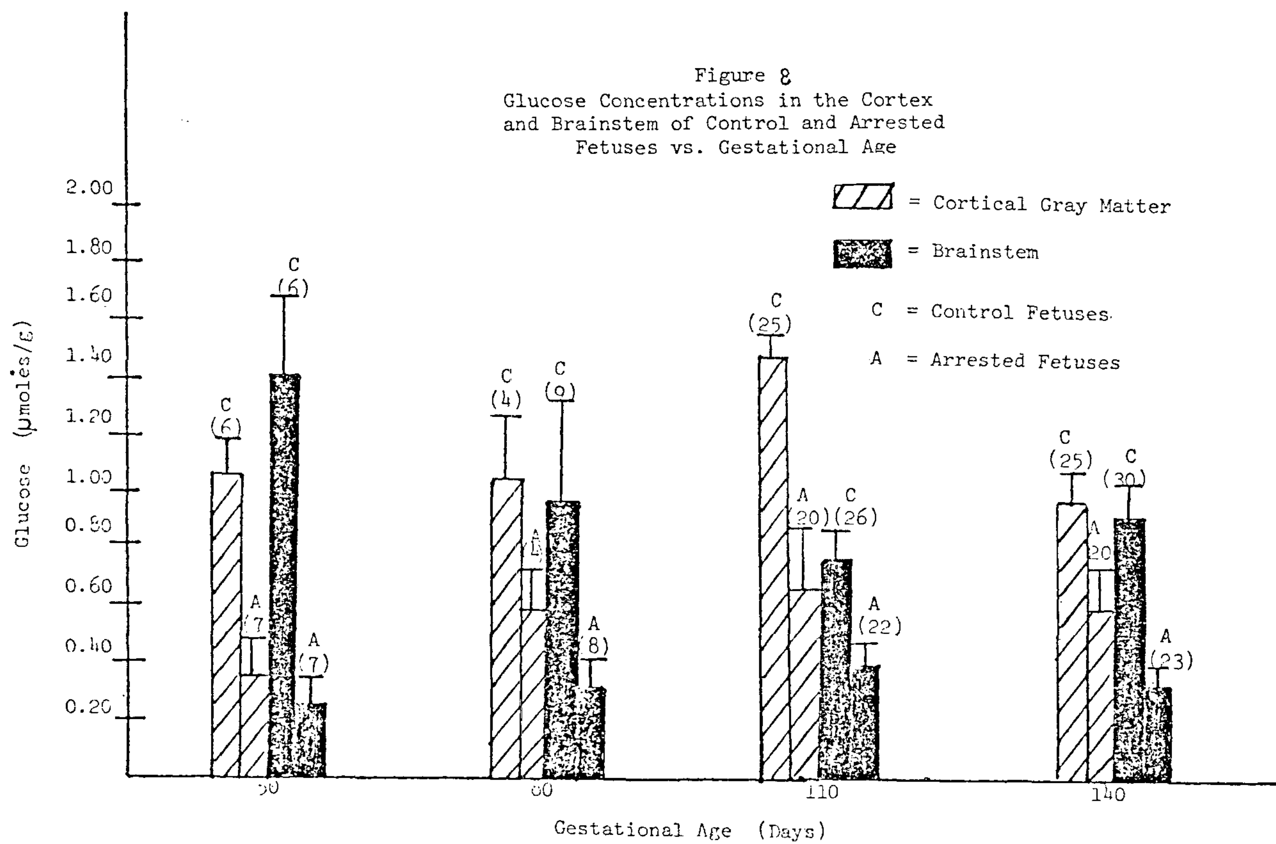


Figure 8
Glucose Concentrations in the Cortex and Brainstem of Control and Arrested Fetuses vs. Gestational Age

glucose concentrations present in the brain stem of 80, 110 and 140 day control fetuses were not significantly different (Table 8, Fig. 8). Cortical gray matter glucose concentrations were not significantly different from white matter levels in control fetuses. A comparison of brain stem and cortex showed that only 110 day fetuses exhibited higher glucose levels in cortex compared to brain stem ($p<0.05$).

Following cardiac arrest, the glucose concentrations in the cortex, white matter and brain stem of 50, 110 and 140 day fetuses decreased significantly from control levels (Table 8, Fig. 8, $p<0.05$). In these fetuses the glucose contributed a smaller proportion of substrate toward lactic acid production than glycogen, but the contribution was still significant. In 80 day fetuses exposed to anoxia, glucose levels were not significantly lower in cortex and brain stem than in control fetuses (Fig. 8). Cortex and white matter glucose concentrations did not vary significantly in arrested fetuses. Cortex and brain stem glucose levels also did not vary significantly in fetuses exposed to anoxia. The final glucose concentrations following circulatory arrest in all three brain regions did not change significantly with gestational age (Table 8, Fig. 8).

In general, the fructose concentrations in the brains of control fetuses tended to follow glucose levels (Table 8, 9). As with glucose, the fructose in the cortex of control fetuses peaked at 110 days (Fig. 9). This peak fructose concentration was significantly higher than 140 day fructose levels ($p<0.05$) but not significantly higher than 50 and 80 day levels (Table 9). The fructose levels in the white matter and brain

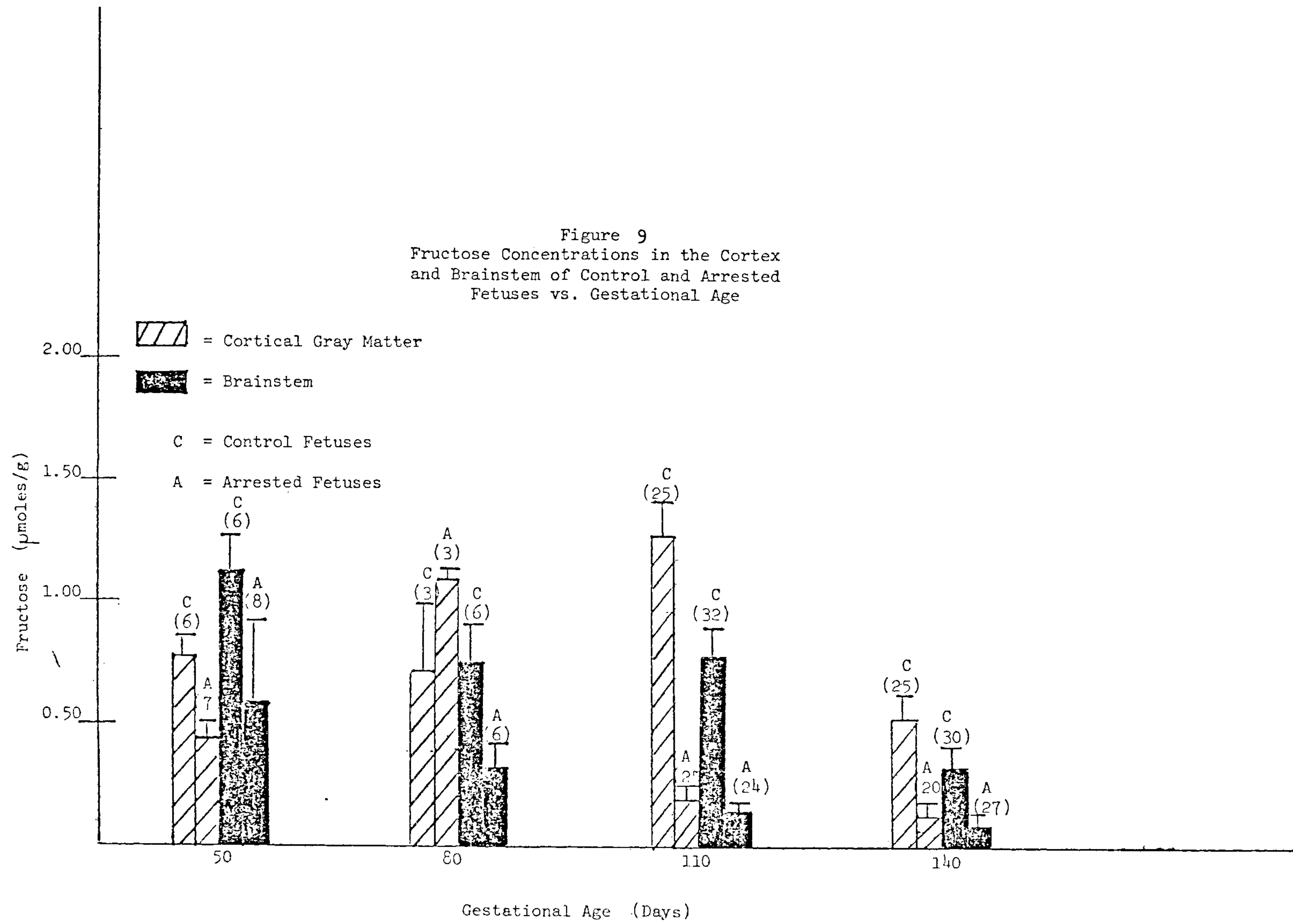


Figure 9
Fructose Concentrations in the Cortex and Brainstem of Control and Arrested Fetuses vs. Gestational Age

stem of control fetuses also followed glucose levels in that both metabolites decreased with age (Table 9, Fig. 9). In both structures the fructose concentrations were significantly lower ($p<0.05$) in 140 day fetuses compared to 50, 80 and 110 day control fetuses (Table 9). As with glucose, only the fructose levels in the cortex of 110 day fetuses were significantly higher than brain stem concentrations ($p<0.05$) (Fig. 8, 9). White matter and cortex fructose levels were not significantly different in control fetuses.

The fructose concentrations following arrest were significantly lower ($p<0.05$) than control levels in 110 and 140 day cortex and brain stem (Fig. 9). The data indicates glucose and fructose contribute similar amounts of substrate toward lactic acid production during anoxia in these fetuses. Cortex and brain stem levels at 50 and 80 days were either similar or slightly lower in fetuses exposed to circulatory arrest compared to the control group (Table 9, Fig. 9). The fructose in cortical white matter did not decrease significantly between control and arrested fetuses at 80, 110 and 140 days of gestation. Fructose levels within the arrested group decreased significantly with gestational age in cortex, brain stem and white matter (50 vs. 140 day; Fig. 9, $p<0.05$). In cortex, however, the fructose levels initially increased from 50 to 80 days followed by a decrease a 110 and 140 days of gestation (Table 9).

Concentrations of ATP and PCr, as well as energy charge values fell significantly ($p<0.05$) during arrest at all gestational ages (Table 11, 12, 13, Fig. 10). However, the changes between control and a rested fetuses were similar in cortex and brain stem at each gestational age for all three metabolites.

TABLE 11

ATP Concentrations in Control and Arrested Fetuses at Four Gestational Ages*

ATP - Controls				
Structure	50 Day	80 Day	110 Day	140 Day
Cortex	2.12±0.06 (5)	1.93±0.12 (5)	1.54±0.07 (23)	1.85±0.02 (25)
White Matter		0.89±0.05 (10)	1.20±0.08 (3)	1.78±0.09 (5)
Brain stem	1.91±0.15 (6)	1.52±0.07 (7)	1.48±0.06 (26)	1.66±0.03 (29)
ATP - Arrest				
Structure	50 Day	80 Day	110 Day	140 Day
Cortex	0.08±0.01 (7)	0.11±0.03 (4)	0.09±0.003 (20)	0.04±0.01 (20)
White Matter		0.07±0.02 (7)	0.08±0.01 (4)	0.04±0.01 (4)
Brain stem	0.10±0.01 (8)	0.03±0.01 (7)	0.08±0.01 (22)	0.05±0.01 (27)

*Values expressed as mean ± SEM. units = μmoles of ATP/g of brain tissue.
( ) = Number of samples.

TABLE 12

PCr Concentrations in Control and Arrested Fetuses at Four Gestational Ages*

PCr - Controls				
Structure	50 Day	80 Day	110 Day	140 Day
Cortex	2.47±0.19 (6)	2.06±0.29 (5)	2.23±0.14 (24)	3.49±0.15 (24)
White Matter		0.79±0.05 (8)	1.04±0.35 (5)	2.22±0.27 (5)
Brain stem	2.00±0.31 (5)	0.95±0.13 (6)	2.45±0.13 (31)	3.72±0.11 (27)

PCr - Arrest				
Structure	50 Day	80 Day	110 Day	140 Day
Cortex	0.62±0.05 (7)	0.41±0.14 (4)	0.22±0.15 (18)	0.39±0.05 (19)
White Matter		0.17±0.06 (7)	0.12±0.06 (40)	0.16±0.05 (4)
Brain stem	0.49±0.08 (8)	0.14±0.05 (8)	0.48±0.07 (23)	0.40±0.05 (27)

*Values expressed as mean ± SEM. Units = μmoles of PCr/g of brain tissue.
( ) = Number of samples.

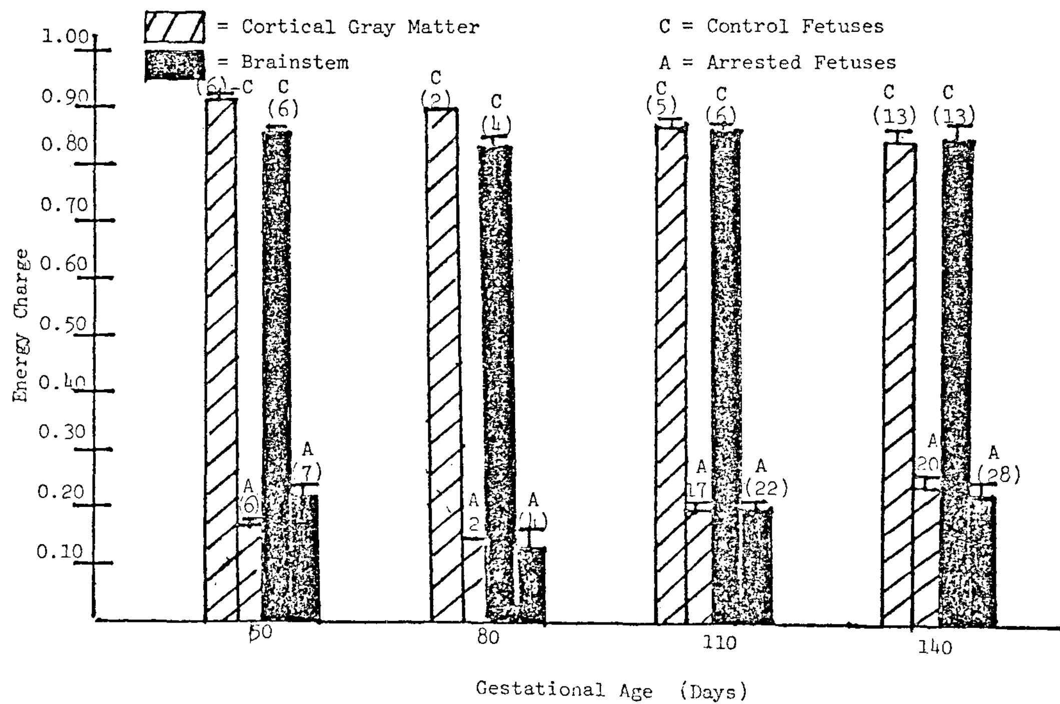
TABLE 13

Energy Charge in Control and Arrested Fetuses at Four Gestational Ages*

Energy Charge - Control				
Structure	50 Day	80 Day	110 Day	140 Day
Cortex	0.92±0.01 (6)	0.91 (2)	0.88±0.01 (5)	0.85±0.02 (13)
White Matter		0.87±0.04 (4)	0.88 (2)	0.89±0.01 (3)
Brain stem	0.86±0.01 (6)	0.84±0.01 (4)	0.87±0.01 (6)	0.86±0.02 (17)
Energy Charge - Arrest				
Structure	50 Day	80 Day	110 Day	140 Day
Cortex	0.17±0.01 (6)	0.15 (2)	0.20±0.01 (17)	0.24±0.02 (20)
White Matter		0.13±0.03 (4)	0.23±0.03 (4)	0.15±0.01 (4)
Brain stem	0.22±0.02 (7)	0.13±0.03 (4)	0.20±0.01 (22)	0.23±0.02 (28)

*Values expressed as mean ± SEM.
( ) = Number of samples.

Figure 10
Energy Charge Concentrations in
the Cortex and Brainstem of Control
and Arrested Fetuses vs.
Gestational Age



The ATP levels in control fetuses changed with gestational age although the changes did not follow the same pattern in cortex, brain stem and white matter. Control ATP levels in cortex decreased significantly between 50 and 110 days ($p<0.05$) and then increased significantly between 110 and 140 days (Table 11, $p<0.05$). A similar pattern occurred in the brain stem of control fetuses. White matter ATP levels increased significantly ($p<0.05$) with age in control fetuses (Table 11). White matter and brain stem had significantly lower ATP levels ($p<0.05$) in 80 day control fetuses than the corresponding cortex but not at other gestational ages.

The levels of ATP in fetuses exposed to circulatory arrest also varied with gestational age. In cortex, the ATP concentrations were similar for 50, 80 and 110 day fetuses but significantly lower ($p<0.05$) for 140 day fetuses (Table 11). Similarly, ATP concentrations in the white matter of arrested fetuses decreased significantly between 110 and 140 days ($p<0.05$). Brainstem ATP concentrations in arrested fetuses decreased significantly between 50 and 80 days as well as between 110 and 140 days of gestation (Table 11, $p<0.05$). Between 80 and 110 days the ATP levels increased. Only the brain stem in 80 day arrested fetuses exhibited a significantly lower ATP concentration ($p<0.05$) than cortex (Table 11).

Phosphocreatine concentrations tended to start at higher levels in control fetuses and decrease to a lesser extent in fetuses exposed to anoxia than ATP concentrations (Table 11, 12). In cortex, PCr tended to decrease from 50 to 110 day control fetuses, although the changes were not significant. Phosphocreatine levels increased significantly at 140 days

of gestation compared to 50 day fetuses in the cortex of control fetuses (Table 12, $p<0.05$). This is the same pattern observed with ATP in the cortex of control fetuses. As with ATP, the PCr levels, in the white matter of control fetuses, increased with gestational age (Table 11, 12). Brainstem PCr concentrations increased significantly ($p<0.05$) from 80 to 140 days in control fetuses (Table 12). Phosphocreatine levels in white matter were significantly lower ($p<0.05$) than cortex PCr levels in 80, 110 and 140 day control fetuses (Table 12). However, only the PCr concentration in the brain stem of 80 day control fetuses was significantly lower than the cortical PCr level.

Changes in PCr concentrations with gestational age also occurred in fetuses exposed to anoxia. As with control cortex PCr levels, the PCr in the cortex of fetuses exposed to circulatory arrest decreased from 50 days to 110 days ($p<0.05$) but increased significantly in 140 day fetuses (Table 12, $p<0.05$). White matter PCr concentrations did not change with gestational age (Table 12). The white matter of arrested fetuses also did not vary significantly from cortex with respect to PCr levels. Only 80 day PCr levels in the brain stem of arrested fetuses were significantly different (i.e. lower) ($p<0.05$) from other age groups. Both 80 and 110 day fetuses exhibited significantly ($p<0.05$) lower PCr values in brain stem than the corresponding cortex in arrested fetuses.

The energy charge values calculated for control sheep fetuses were similar in most age groups with two exceptions (Table 13). In cortex, the energy charge in 50 day fetuses was significantly higher than 110 or 140 day fetuses. Brainstem energy charge in 80 day fetuses was lower than 110

day values ($p<0.05$). The cortex, white matter and brain stem energy charge values in control fetuses were not significantly different when other gestational age fetuses were compared. White matter also was not significantly different from cortex in control fetuses with respect to energy charge. However, the energy charge calculated for the brain stem of 50 day control fetuses was significantly lower than the energy charge for cortical gray matter ($p<0.05$).

Energy charge in fetuses exposed to anoxia did not change much with gestational age (Table 13, Fig. 10). Cortical energy charge did not change with gestational age. In white matter, the only significant difference noted between energy charge values was that 110 day values were significantly higher than 140 day values ($p<0.05$). White matter and cortex energy charge levels were not statistically different at any age. The energy charge in the brain stem of 80 day fetuses was significantly lower than other age groups. Only the energy charge in the cortex of 50 day arrested fetuses was significantly higher than brain stem values (Fig. 10, $p<0.05$).

DISCUSSION

I. The Significance of Metabolite Changes in the Brains of Sheep Fetuses Exposed to Anoxia.

Fetuses and neonates of several animal species are more tolerant than adults to brain injury following oxygen deprivation (5, 13, 15, 24). Many investigators postulate that the tolerance of the fetal brain can be explained on the basis of differences between fetal and adult brain metabolism (7, 13, 15, 43, 44). More specifically, they postulate that the decrease in energy utilization by the fetal brain during glycolysis protects the fetus during oxygen deprivation. However, previous experiments do not prove that the differences between adult and fetal carbohydrate or energy utilization are the basis for the tolerance of the fetal brain to injury (7, 13).

Previous experiments from Dr. Myers' laboratory established that the accumulation of lactic acid above a threshold level of 17-20 µmoles/g in brain tissue results in brain injury in adult animals exposed to oxygen deprivation (24, 26, 29, 30). However, the hypothesis that fetuses are more tolerant to brain injury because lactic acid does not accumulate to threshold levels or accumulates more slowly has not been tested in fetuses. In addition, the relationship between an increased vulnerability of some brain stem nuclei to injury with gestational age and lactic acid accumulation above threshold concentrations has not been examined. Thus,

the purpose of this study is to determine whether the fetal brain accumulates less lactic acid than adults and whether less substrate is available for the production of lactic acid.

Previous pathology studies with sheep fetuses exposed to anoxia have not been done. However, pathology studies of monkey fetuses exposed to anoxia have been performed (24, 25). Monkey fetuses resemble sheep fetuses in that both species have a similar length of gestation (170 days in monkeys and 158 days in sheep) and both species are born as physiologically well developed individuals.

The results of this study established that lactic acid accumulated to threshold levels only in the brain stem of 110 and 140 day sheep fetuses exposed to 1 hour of anoxia (Table 6, Fig. 6). Previous pathology studies with late gestational age monkey fetuses (90-140 days) also found that selective brain stem nuclei were injured following anoxia (24). The brain stem structures of 50 and 80 day sheep fetuses did not acccumulate lactic acid above threshold levels (Table 6, Fig. 6). Monkey fetuses of a similar gestational age (less than 80 days) survived and did not develop brain injury following 30 minutes of total asphyxia (24). Structures, such as cortical gray and white matter of 50, 80, 110 and 140 day sheep fetuses exposed to 1 hour of anoxia accumulated lactic acid although the concentrations did not reach the threshold for injury described by Myers and coworker (25, 26, 30). In monkey fetuses of different gestational ages, the cortical gray and white matter were not damaged following anoxia. Thus, there is an excellent correlation between lactate accumulation in late gestational age sheep fetuses and the development of

a brain stem pattern of injury following anoxia in monkey fetuses. Furthermore, recent studies with sheep fetuses exposed to hypoxia demonstrated a good correlation between lactic acid acccumulation and the pattern of brain pathology (unpublished results - Wagner Ting, Myers).

The level to which lactic acid accumulates in brain depends upon substrate availability during anoxia, if the length of exposure is not a limiting factor. This study examines the concentration of substrates in the brains of control and arrested fetuses, in order to determine the amount and the carbohydrate substrates utilized by the sheep fetuses. Glycogen, glucose and fructose all were used as substrates for lactic acid production in fetal sheep brain tissue during anoxia.

The glycogen concentrations in control fetuses increased significantly with gestational age in all brain structures (Table 7, Fig. 7). Thus, more substrate became available with increasing gestational age for lactic acid production during anoxia. Following arrest, the glycogen levels decreased significantly reaching similarly low levels at all gestational ages (Table 7, Fig. 7). Since glucose and fructose remain at relatively low levels throughout gestation (Table 8, 9), and practically all glycogen is broken down during anoxia, glycogen contributes the greater proportion of substrate for lactic acid production with increasing gestational age (Table 7).

The glycogen concentration in each brain structure of control fetuses correlated closely with the lactic acid concentrations following arrest (Table 7, 10). The brain stem of 110 and 140 day sheep fetuses had significantly higher glycogen levels prior to anoxia compared to 50 and 80

day fetuses which explains the higher level of lactic acid accumulation in these late gestational age fetuses (Table 6, 7, Fig. 6, 7). The glycogen content in the brain stems of the late gestational age fetuses also was significantly higher than the levels in the corresponding cortex and white matter. However, glycogen by itself, does not account for all the lactic acid produced in each structure. Thus, other carbohydrates, such as glucose and fructose, must contribute smaller but significant amounts.

Glucose levels in all brain structures of 50, 110 and 140 day fetuses decreased significantly following circulatory arrest (Table 8, Fig. 8). Fructose concentrations also decreased significantly in the brain structures of 110 and 140 day fetuses following circulatory arrest (Table 9, Fig. 9). However, glucose and fructose contributed less to lactic acid production than did glycogen because of lower initial glucose and fructose concentrations (Tables 7, 8, 9). Thus, although glucose and fructose contribute only a small proportion to total lactic acid accumulation during anoxia, they can provide the substrate to boost lactic acid over the threshold concentration for injury.

In control fetuses, the glucose and fructose concentrations within different brain structures were variable with gestational age. Cortical gray matter glucose levels peaked at 110 days while cortical white matter and brain stem concentrations tended to decrease with gestational age (Table 8, Fig. 8). Similar results were found with fructose in cortex and brain stem, although fructose concentrations were lower than glucose concentrations in each structure (Table 9, Fig. 9). Glucose levels in all brain structures decreased significantly following circulatory arrest and

were not significantly different with gestational age (Table 8, Fig. 8). In fetuses exposed to circulatory arrest, fructose concentrations decreased with gestational age although the decrease was less than 1 μmole/g (Table 9, Fig. 9). The contribution of glucose and fructose to lactic acid production also was not significantly different between cortex and brain stem (Tables 8, 9, Fig. 8, 9) although lactic acid levels in these two structures were significantly different in late gestational age fetuses (Table 6, Fig. 6).

The utilization of fructose by the brain during anoxia is of interest because it does not appear to be used in normally oxygenated ewes and fetuses (14, 50). Although fructose may not be used by normally oxygenated fetuses, serum fructose concentrations are much higher than serum glucose concentrations (Table 4, 5, Fig. 4, 5)(14, 50). In the ewe, the serum glucose concentration is significantly higher than in the fetus (Table 3, 4). Some of the glucose crossing the placenta to the fetus is converted to fructose (6, 14, 40, 41, 50). This conversion results in high serum fructose levels in the fetus. However, fructose is only utilized by the fetus when the ewe is exposed to a stressful situation (e.g. food deprivation) (14, 40, 41, 50). In the present study, the results show that fructose levels decreased significantly in the cortical gray matter and brain stem of 110 and 140 day fetuses following cardiac arrest (Table 9, Fig. 9). Thus, the stressful situation of anoxia stimulates fructose utilization by the brain.

When fructose is metabolized by the brain, it enters the glycolytic pathway at a different point than glucose (16, 53). Initially, fructose is phosphorylated by fructokinase to form fructose-1-phosphate (F1P)

(16). Fructose diphosphate aldolase cleaves the F1P to dihydroxyacetone phosphate (DHAP) and glyceraldehyde. Subsequently, the glyceraldehyde is phosphorylated to glyceraldehyde-3-phosphate (G3P). DHAP and G3P can then be metabolized to lactic acid by the glycolytic pathway during anoxia.

Although sorbitol was not measured, a previous study showed that sorbitol is present in the serum of 80 day sheep fetuses (0.90 ± 0.29 mM) and is used by fetuses (unpublished results - deCourten, Hirsch, Myers). The contribution of sorbitol to lactate production probably was very small because the serum levels are lower than glucose and fructose (glucose = 1.42-1.86 mM, fructose = 6.17-9.70 mM in 80 day fetuses), which only contribute a small amount to lactate production.

Several investigators have proposed that energy availability determines the survival of brain tissue during oxygen deprivation (7, 44, 45). Thus, I performed assays to determine the concentrations of three adenine nucleotides (AMP, ADP, ATP) and phosphocreatine (PCr) and calculated the energy charge. The results indicated that ATP, PCr and the calculated value of energy charge decreased significantly during anoxia in cortex, white matter and brain stem (Tables 11, 12, 13). Brainstem PCr levels were also lower than cortical gray matter concentrations in 110 day arrested sheep fetuses (Table 12). Since brain stem nuclei of late gestational (i.e. 110 and 140 day) age fetuses are injured after 1 hour of anoxia (24), ATP, PCr and energy charge should be lower in the brain stem as compared to cortex of 110 and 140 day fetuses, if these parameters of energy availability are critical determinants of brain injury. However, the ATP levels and energy charge were not significantly different between

cortex and brain stem in 110 and 140 day arrested fetuses (Table 11, 13). Phosphocreatine levels were not different in the brain stem and cortex of 140 day arrested fetuses (Table 12). These results suggest that although the energy state decreased significantly following arrest at all gestational ages, there was no correlation between energy state and the development of brain injury following arrest.

The results from the present study show that:

1. Lactate accumulation above threshold levels in the brain stem of late gestational age sheep fetuses correlates well with the brain stem pattern of injury seen in late gestational age monkey fetuses exposed to anoxia;

2. Increased carbohydrate stores account for increased levels of lactic acid in late gestational age fetuses compared to early or mid-gestational age fetuses;

3. Glycogen contributes a greater proportion of substrate for lactic acid production with increasing gestational age. Glucose and fructose also are utilized by sheep fetus brain tissue during anoxia: however, lower initial concentrations of these two metabolites result in a smaller contribution of substrate to lactic acid production compared to glycogen; and

4. ATP, PCr and energy charge decreased significantly during anoxia, but these three parameters did not correlate with the pattern of brain pathology previously observed in monkey fetuses exposed to anoxia.

II. The Importance of pH, Blood Gas and Serum Metabolite Values in Sheep Fetuses During the Control Period.

The pH, blood gas and serum metabolites were not important during the anoxia portion of this particular study because there was no blood flow to the brain during exposure. However, they were extremely important indicators of the physiological condition of the mother and fetus during the control period. The arterial pH, blood gas and serum metabolites were not significantly different in the ewes of control and ewes of fetuses subsequently exposed to circulatory arrest, during the control period (Table 3). Similar results were observed in a comparison of fetuses subsequently exposed to circulatory arrest and control fetuses (Table 4, 5). The few differences observed between the 2 groups of fetuses did not indicate significant physiologic differences because the other parameters specified earlier (see Materials and Methods) were within the previously defined range of values. Thus, the results from the ewes and fetuses suggested that control and arrested fetuses were not significantly different during the control period. As a result, any differences between control and arrested brain metabolites should indicate changes due to anoxia.

The serum metabolites changed significantly with gestational age (Table 4, 5, Fig. 2-4). These changes were important because they indicate physiological differences between fetuses of different gestational ages.

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