In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Animal Science

Department of _____________________________________________

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date March 1981
Abstract

The turnover, interconversion and oxidation of substrates in the ovine conceptus in utero were studied making use of isotope dilution techniques. In Experiment 1, surgical techniques were standardized for the introduction of vascular catheters into the fetuses at approximately 120-130 days of gestation. Based on maternal and fetal blood acid base parameters and metabolite and hormone levels it was possible to obtain chronic fetal preparations which were physiologically stable.

In Experiment II, radioactive labelled substrates were injected intravenously into the fetus and the disappearance of the label from the fetal circulation was monitored against time. Kinetic parameters of substrate metabolism were calculated by graphic analysis of the specific radioactivity-time curves. The pool size, irreversible rate of disposal and volume of distribution of glucose, lactate, and amino acids were estimated. The single injection technique employed in this study facilitated the calculation of 2 additional kinetic parameters not reported hitherto in the literature. These include the mean total residence time and number of cycles the labelled substrates made before being irreversibly lost from the fetal circulation. The finding that lactate and amino acids make more number of cycles into and out of the fetal circulation than glucose provides support to the concept that the placenta on the fetal side is relatively impermeable to the former two substrates. Though the rapid disappearance of isotopes administered into the fetus was recognized by earlier workers, the results of this study have brought to light the significance of recycling of substrates. It is suggested that this unique dynamic feature serves as a physiological control mechanism to modulate fuel consumption according to nutrient and
oxygen availability. On the other hand, there was very little difference in
the irreversible rate of disposal when [2-3H] or [U-14C] glucose was injected
indicating that there is only approximately 12.5% of recirculation of glucose
within the fetal tissues.

The appearance in maternal circulation of only labelled glucose
injected into the fetus, but not lactate indicates the inability of lactate
to cross the placenta from the fetal side. Though 36% of the administered
glucose label appeared in lactate, the methodology used in this study does
not differentiate whether the conversion of glucose into lactate occurred
in the fetus itself or in the placenta. The recovery of 8.8% of alanine C
into glucose, though suggestive of gluconeogenic potential, may have occurred
by isotopic cross over rather than true metabolic conversion.

In experiment III, the CO2 production rates were estimated from
the plateau specific activity of blood 14CO2 after the continuous infusion
of [14C] NaHCO3. The most important findings in this study pertains to
the contribution of substrates to oxidative metabolism in the fetus.
Contrary to the conclusions based on the Fick principle, the recovery of
administered label into 14CO2 indicates that glucose, lactate, alanine,
acetate and amino acids contribute 15.2, 14.0, 6.8, 1.7 and 8.2% respectively
to fetal oxidative metabolism. Though the metabolism of placental tissues
may have influenced the above values, the results suggest that the metabolic
fuel requirements of the fetus warrant reassessment.

The results of these experiments are discussed with reference to
the metabolism of the fetus during a period of gestation where the greatest
increment in fetal growth occurs.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Title page</td>
<td>i</td>
</tr>
<tr>
<td>Abstract</td>
<td>ii</td>
</tr>
<tr>
<td>Table of Contents</td>
<td>iv</td>
</tr>
<tr>
<td>List of Tables</td>
<td>vii</td>
</tr>
<tr>
<td>List of Figures</td>
<td>ix</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>xi</td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>Review of Literature</td>
<td>2</td>
</tr>
<tr>
<td>1. Maternal nutrition during pregnancy</td>
<td>2</td>
</tr>
<tr>
<td>2. Placental functions</td>
<td>5</td>
</tr>
<tr>
<td>3. Fetal growth and development</td>
<td>7</td>
</tr>
<tr>
<td>4. Fetal growth and endocrinology</td>
<td>8</td>
</tr>
<tr>
<td>A. Growth hormones (OGH and OCS)</td>
<td>8</td>
</tr>
<tr>
<td>B. Thyroid hormones</td>
<td>11</td>
</tr>
<tr>
<td>C. Adrenal corticosteroids</td>
<td>12</td>
</tr>
<tr>
<td>D. Insulin and glucagon</td>
<td>12</td>
</tr>
<tr>
<td>E. Hormonal enzyme induction</td>
<td>16</td>
</tr>
<tr>
<td>5. Fetal metabolism</td>
<td>17</td>
</tr>
<tr>
<td>A. Techniques for metabolic studies in the fetus</td>
<td>17</td>
</tr>
<tr>
<td>B. Fetal caloric requirements</td>
<td>19</td>
</tr>
<tr>
<td>i) Oxygen consumption and carbon dioxide production</td>
<td>20</td>
</tr>
<tr>
<td>ii) Glucose</td>
<td>23</td>
</tr>
<tr>
<td>iii) Fetal gluconeogenesis</td>
<td>26</td>
</tr>
<tr>
<td>iv) Fructose</td>
<td>32</td>
</tr>
<tr>
<td>v) Lactate</td>
<td>34</td>
</tr>
<tr>
<td>vi) Amino Acids</td>
<td>36</td>
</tr>
</tbody>
</table>
vii) Carbon-nitrogen balance

Experiment I: Surgical technique for the cannulation of fetal saphenous vein and post-surgical changes in blood parameters of the ovine fetus in utero.

Introduction 43
Materials and methods 44
Results 49
Discussion 53
Conclusions 57

Experiment II: Substrate turnover and interrelationship in the ovine fetus in utero.

Introduction 59
A. Metabolism of Glucose and Lactate 59
Materials and methods 60
Results 71
Discussion 85
Conclusion 93
B. Metabolism of Amino Acids 94
Materials and methods 94
Results 99
Discussion 107
Conclusion 111

Experiment III: Measurement of carbon dioxide production and substrate oxidation by the ovine conceptus in utero using 14C-labelled compounds.

Materials and methods 113
Results 114
Discussion 118
Conclusions 125
<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Oxygen consumption rates of adults and fetuses of different size.</td>
<td>22</td>
</tr>
<tr>
<td>2. Gestational age and body weights of ewes and fetuses.</td>
<td>50</td>
</tr>
<tr>
<td>3. Maternal and fetal physiological parameters during the experimental period.</td>
<td>72</td>
</tr>
<tr>
<td>4. Kinetic parameters of glucose metabolism in the ovine fetus, estimated using single injection of a mixture of [U-14C] and [2-3H] glucose or [U-14C] glucose alone.</td>
<td>80</td>
</tr>
<tr>
<td>5. Kinetic parameters of glucose and lactate metabolism in the ovine fetus, estimated by using single injection of [U-14C] glucose or [U-14C] lactate.</td>
<td>82</td>
</tr>
<tr>
<td>6. Glucose-lactate conversions in the ovine fetus in utero.</td>
<td>83</td>
</tr>
<tr>
<td>7. Estimation of recycling and recirculation of glucose and lactate in the ovine fetus in utero.</td>
<td>84</td>
</tr>
<tr>
<td>8. Maternal and fetal physiological parameters during the experimental period.</td>
<td>100</td>
</tr>
<tr>
<td>9. Kinetic parameters of substrate metabolism in the ovine fetus.</td>
<td>103</td>
</tr>
<tr>
<td>10. Recycling of amino acids and alanine in the ovine fetus in utero.</td>
<td>105</td>
</tr>
<tr>
<td>11. Conversion of alanine to lactate and glucose in the ovine fetus in utero.</td>
<td>106</td>
</tr>
<tr>
<td>12. Substrate oxidation rates in the ovine fetus in utero.</td>
<td>119</td>
</tr>
</tbody>
</table>
Appendix Table.

1. Per cent recovery of $^{14}$C-labelled compounds after treatment with glucose oxidase and anion exchange chromatography.

2. Per cent recovery of $[1-^{14}$C$]$ lactate following anion exchange chromatography and thin layer chromatography.

3. Per cent recovery of $[U-^{14}$C$]$ amino acid mixture and alanine following cation exchange chromatography and enzymatic conversion of alanine to lactate.

4. Per cent recovery of $^{14}$C$\text{NaHCO}_3$ in saline and whole blood.

5. Mean PO$_2$, PCO$_2$ and pH in maternal and fetal blood of conscious ewes during and following surgery.

6. Mean hematocrit, blood glucose, lactate, $\beta$-hydroxybutyrate and alpha amino nitrogen in fetal blood, following surgery.
List of Figures

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Post surgical changes in blood gas parameters and hematocrit in the ovine fetus in utero.</td>
<td>51</td>
</tr>
<tr>
<td>2.</td>
<td>Post surgical changes in plasma metabolite levels in the ovine fetus in utero.</td>
<td>52</td>
</tr>
<tr>
<td>3.</td>
<td>Post surgical changes in plasma cortisol levels in the ovine fetus in utero.</td>
<td>54</td>
</tr>
<tr>
<td>4.</td>
<td>Model of glucose and lactate metabolism in the ovine fetus.</td>
<td>56</td>
</tr>
<tr>
<td>5.</td>
<td>Semilogarithmic plot of glucose specific activity versus time following injection of [U-14C] glucose.</td>
<td>74</td>
</tr>
<tr>
<td>6.</td>
<td>Semilogarithmic plot of glucose specific activity and lactate formation versus time following the injection of [U-14C] and [2-3H] glucose.</td>
<td>75</td>
</tr>
<tr>
<td>7.</td>
<td>Linear regression of fetal irreversible disposal rate of glucose versus blood glucose concentration.</td>
<td>77</td>
</tr>
<tr>
<td>8.</td>
<td>Linear regression of fetal irreversible disposal rate of glucose versus fetal body weight.</td>
<td>78</td>
</tr>
<tr>
<td>9.</td>
<td>Semilogarithmic plot of lactate specific activity versus time following the injection of [1-14C] lactate.</td>
<td>79</td>
</tr>
<tr>
<td>10.</td>
<td>Model of amino acid metabolism in the ovine fetus.</td>
<td>97</td>
</tr>
<tr>
<td>12.</td>
<td>Specific radioactivity of fetal and maternal blood 14CO2 following primed dose-infusion of NaH14CO3.</td>
<td>120</td>
</tr>
<tr>
<td>14.</td>
<td>Specific radioactivity of blood 14CO2 after injection of 14C-labelled substrates.</td>
<td>123</td>
</tr>
<tr>
<td>15.</td>
<td>Relationship between rates of oxidation and irreversible disposal of glucose and lactate.</td>
<td>124</td>
</tr>
<tr>
<td>16.</td>
<td>Composite picture of fetal substrate metabolism.</td>
<td>133</td>
</tr>
</tbody>
</table>
Appendix Figure.

1. Separation of metabolites by descending paper chromatography (Phenol:water:NH₃; 40:40:1; w/v/v/)  
Page 164

2. Quench curve for ³H and ¹⁴C isotopes.  
Page 165

Page 166
Acknowledgements.

I wish to express my gratitude to the many individuals that assisted me during the course of this study. In particular, I would like to acknowledge the Department of Animal Science for the use of the animal and laboratory facilities. Special thanks is given to Mr. J. Ciok, animal technician, whose expert assistance was called upon many times.

I wish to express my sincere gratitude to Dr. C. R. Krishnamurti, Professor, Animal Science for his dedicated participation with the animal surgery and assistance with the preparation of this thesis. I am also grateful for his encouragement and friendship during my tenure in the department.

I would like to record my gratitude to Mr. G.J. Tompkins for his time spent in assisting me with animal surgery and experiments, and Ms. Madonna Chan for her conscientious help with animal experiments and laboratory work. I would like to acknowledge Mr. G. Galzy, for his technical assistance and Mr. R. Burton (Pharmaceutical Sciences) for help with the computer programs. A sincere thank you is given to Ms. Sannifer Louie for her special attention in typing this manuscript.

I would like to thank my family for their support and understanding during the preparation of this manuscript.

Finally I wish to acknowledge my wife Elizabeth, whose patience and understanding, benefaction and devoted assistance enabled me to pursue my objectives. This thesis is dedicated to Elizabeth and my daughter Katharine Heather.
Introduction

The growth of the ruminant fetus, particularly during the later stages of gestation, has been the subject of extensive investigation and review. Since fetal metabolism has been shown to depend on a continuous supply of nutrients, the pregnant mother must undergo specific metabolic alterations to ensure that the fetal metabolic demands are met. Under normal practical feeding conditions the maternal ruminant is capable of providing a nutritional environment for the fetus which will result in viable offspring. However identification and utilization of nutrients by the fetus in regard to its caloric requirements have not been considered until recently.

The major objectives of this study were to quantitate the metabolism of specific fetal substrates, using a chronic fetal catheterization procedure and isotopic tracer methodologies. In particular, the contribution of substrates towards fetal oxidative metabolism was examined.
Review of the Literature

1. Maternal nutrition during pregnancy

Hammond (1943) originally advanced the theory that available nutrients are divided between maternal and fetal tissues according to their metabolic needs. The apparent partition of nutrients for maternal maintenance and fetal growth requirements was postulated to be especially significant when the nutrient supply was limited. The suggestions brought forth by Hammond were no doubt instrumental in the initiation of numerous studies designed to examine the importance of adequate nutrition during pregnancy on conceptus birth weights and fetal viability (Wallace, 1948; Everitt, 1966; Robinson, 1977; Robinson et al. 1977a).

Nutritional stress imposed on the dam during early pregnancy has been shown to have a more profound direct effect on embryo mortality (Edey, 1976) and placental growth (Alexander, 1964) than on absolute fetal birth weight (Hulet et al., 1969). While a small amount of absolute fetal growth (e.g. total body growth) occurs during early pregnancy, the specific growth rate (e.g. individual organ growth) is very high (16% per day, Robinson and McDonald, 1979). The placenta, on the other hand, is an actively growing tissue in early pregnancy. Robinson et al. (1979) reported that a restriction in placental development beyond a critical threshold (e.g. 160 g.) resulted in significant fetal growth retardation. Alexander (1964;1974) had previously reported high correlations between ovine fetal birth weights and placental size.

During the 2nd and 3rd months of pregnancy, fetal and placental growth characteristics change significantly (Robinson et al., 1977a). Fetuses of ewes fed a diet below maintenance at time of conception were most vulnerable to maternal under-nutrition at this stage of gestation.
The fetus makes its greatest metabolic demands upon the ewe during the last 8 weeks of pregnancy. Robinson et al. (1977a) reported that at four and two weeks prepartum, 50 and 75 percent respectively, of fetal birth weight is obtained. Variability in fetal birth weight observed within common breeds has been attributed to varying levels of maternal nutrition during late pregnancy (Wallace, 1948; Alexander, 1974; Mellor and Matheson, 1980). Reid (1968) and Koong et al. (1975) have also reported that the number of fetuses present in utero affect fetal birth weights more so than maternal under-nutrition at 115-120 days gestation. Mellor and Matheson (1980) reported a 30 to 44 per cent decrease in fetal growth rates three days after the introduction of maternal under-nutrition.

Data obtained from chronic fetal preparations have shown significant changes in fetal metabolite and hormone concentrations during maternal under-nutrition (Tsoulos et al., 1971; Bassett and Madill, 1974a; Mellor et al., 1977). Prior et al. (1979) and Prior and Lister (1979) demonstrated in the bovine, that although maternal metabolism was significantly affected by the restriction of dietary metabolizable energy to maintenance levels there was no significant effect on fetal birthweight, fetal muscle protein or RNA and DNA content. A similar restriction on the level of maternal dietary energy intake has been shown to have little effect on maternal and fetal gluconeogenic enzymatic activity in vitro (Prior and Scott, 1977).

Several investigators have documented the specific changes in maternal intermediary metabolism occurring during pregnancy (Bergman, 1963; Herrera et al., 1969; Prior and Christenson, 1978). Earlier studies have also shown that the ruminant is susceptible to severe hypoglycemia and ketosis during the last several weeks of pregnancy and in fact a reduction in food intake may reproduce most features of clinical ketosis in the bovine and
pregnancy toxemia in the ovine species (Kronfeld, 1958; Reid, 1968; Bergman, 1973).

The net metabolism of various substrates and the subsequent partition of energy utilization by the pregnant ruminant receiving an adequate plane of nutrition do not appear substantially altered during this time. Christenson and Prior (1978) reported no significant changes in arterial plasma glucose concentrations during gestation in sheep. Significant decreases in maternal whole blood concentration of several amino acids have, however, been observed in sheep during this time (Morriss et al., 1979). These results are of particular interest in view of in vivo studies that have disclosed a maximal rate of maternal gluconeogenesis during the 3rd to 4th month of gestation (Prior and Scott, 1977). Curet et al. (1970) have attributed the decline in alpha amino nitrogen levels observed during gestation to the high circulating levels of estrogen, progesterone and cortisol in the pregnant animal.

Numerous studies have demonstrated that the pregnant uterus during the latter stages of gestation consumes large quantities of glucose (Kronfeld, 1958; Bergman, 1963; Reid, 1968; Prior and Christenson, 1978). Morriss et al. (1974) reported logarithmic increases in both uterine oxygen and glucose uptakes as gestation progresses. Significant increases in the uptake of glucose and alpha amino nitrogen by the pregnant uterus have been reported in both ovine (Christensen and Prior, 1978) and bovine (Ferrell and Ford, 1980) species. Bergman (1963) comparing glucose turnover rates in pregnant and nonpregnant sheep, estimated that uterine glucose metabolism accounts for 20 to 40 percent of the total glucose turnover rate in ewes with twin pregnancies. Setchell et al. (1972) reported that 70 percent of maternal glucose turnover was accounted for by the uterus and its contents. Prior and Christensen (1978)
demonstrated further that the number of fetuses in utero significantly increased the proportion of maternal glucose turnover rate to 42 and 62 per cent respectively for twins and triplets. They reported also that insulin markedly reduced uterine glucose uptake primarily by decreasing the plasma glucose concentrations. These results correspond directly with the increased maternal glucose entry rates and turnover time of the glucose pool in pregnant sheep (Bergman, 1964; Steel and Leng, 1968). Steel and Leng (1968) working with pregnant ewes fed ad libitum, attributed the increase in glucose entry rates to voluntary increases in feed intake during pregnancy. Results concerning the utilization of metabolizable energy by the pregnant ewe and conceptus confirmed this conclusion (Rattray et al., 1973). No significant differences in the amount of metabolizable energy utilized for body maintenance and conceptus development were established in singleton and twin pregnancies. However, significant differences were noticed with the total feed requirements at 140 days gestation. Furthermore, the efficiency at which the 140 day conceptus utilized maternal metabolizable energy for development was significantly greater in ewes with a level of nutrition that was 2x maintenance levels.

2. Placental functions

Prior and Lister (1979) reported that neither fetal, placental nor uterine weights correlate with cotyledon number, primarily because of the variable sizes of individual cotyledons. Further it was shown that fetal, placental and uterine weights strongly correlated with cotyledon weights
reflecting the potential for utero-placental compensatory growth. This conclusion is further exemplified with data from single and twin pregnancies and cotyledonary weights (Alexander, 1964). Although the number of cotyledons in placentas of single fetuses was greater than that of twin fetuses there was no significant difference in individual placental weights. Marked histological and morphologic changes occur in the placenta during gestation (Adherne and Dunnill, 1966). Progressive and distinct reductions of the trophoblast and capillary membrane thicknesses as well as a proliferation of the fetal villus capillary system have been attributed to the increased ability of the placenta to transfer nutrients to the fetus (Rosso, 1980). In addition, an increased activity of membrane bound ribosomes (Wunderlick et al., 1974) and the subsequent synthesis of placental peptide hormones important for the regulation of placental substrate metabolism are important functional alterations occurring during the latter stages of gestation.

Maturation of the placenta is characterized by a plateauing and eventual reduction in cellular growth of the placenta. This is reflected by a significant decline in the instantaneous growth rate of placental, uterine and cotyledonary tissues at approximately 90 days gestation in the ewe (Alexander, 1964) and 200 days in the cow (Prior and Lister, 1979). Functional aspects of the placental tissues increase however at this time. Kulhanek et al. (1974) demonstrated a five-fold increase in the permeability to urea when expressed as a fraction of placental DNA content.
3. **Fetal growth and development**

Fetal growth is a complex phenomenon, dependent upon a proper balance of maternal, placental and fetal factors. The factors controlling fetal intrauterine growth have been studied by many workers. Prior to the availability of chronically catheterized fetal sheep preparations, the majority of the information concerning intrauterine development was obtained from growth measurements using comparative slaughter techniques. Mathematical equations obtained from these studies have attempted to describe fetal growth during the gestational period in a number of mammalian species, most notable the ovine species (Huggett and Widdas, 1951; Langlands and Sutherland, 1968; Koong et al., 1975; Robinson and McDonald, 1979). The observation that the weight of the avian and mammalian fetus conforms to a cubic law of growth has been reported since antiquity (Roberts, 1906; Huggett and Widdas, 1951; Langlands and Sutherland, 1968). This relationship of fetal weight and chronological age can be expressed in the form of a general formula:

\[ Wt^{1/3} = \alpha (t-t_0) \]

where \( \alpha = \text{growth rate, derived from slope of the growth curve} \)

\( t = \text{gestation time constant after conception} \)

\( t_0 = \text{specific time constant reduced after conception} \)

The results of this equation when applied to numerous mammalian species illustrate the remarkable similarity in the rate at which intrauterine growth proceeds in domestic animals.

The changes in fetal crown-rump length (CRL) and weight of individual organs have also been studied extensively,
These studies have concluded that the fetal growth gradient occurs antero-posteriorly along the main axis of the fetus and centripetally along the limb axis. Meller and Matheson (1980) reported a linear relationship between fetal CRL and fetal weight after 100 days gestation; however, a curvi-linear relationship was observed when the total gestational period was considered. This is attributed to the markedly different rates at which individual organs grow and the continuous changes in fetal conformation (Wallace, 1948; Rattray et al., 1975). Richardson and Hebert (1978), with a limited number of fetuses, supported this conclusion with observations made on organs of the nervous system. Although the cube root of body and total organ weights gave a linear regression with fetal age throughout gestation, the cube root of the brain, cerebellum and spinal cord weights resulted in a sigmoidal trend. Completion of organogenesis in vital organs such as those of the nervous system has been postulated to be the major factor limiting the gestational length periods (Sacher and Straffeldt, 1974).

Hyperplasia increases as well throughout gestation in mammalian fetuses (Winnick and Noble, 1965). Prior et al. (1979) have reported significant increases in total fetal DNA and DNA/protein and RNA/DNA ratios near the end of gestation in the bovine fetus. Hypertrophy as reflected by DNA/protein and RNA/DNA ratios also increases continuously with fetal age.

4. **Fetal growth and endocrinology**
   a) **Growth hormones (OGH and OCS)**

The source of fetal ovine growth hormone (OGH) is the
fetal pituitary, as evidenced from the fetal hypophysectomized studies and the resulting low circulation of growth hormone in fetal plasma (Wallace et al., 1972). In this study it was shown that isotopic labelled growth hormone when administered into the maternal circulation was not detected in fetal plasma. The pattern of OGH concentration during gestation is triphasic with high concentration at 100-110 days of gestation (Gluckman et al., 1979). Dramatic increases in the fetal circulating hormone concentrations occur in the last month of pregnancy to levels three times the concentrations at 100 days of gestation and ten times the concentration found postnatally (Bassett et al., 1970).

Bassett and Madill (1974b) attempted to determine the regulatory mechanism of fetal OGH secretion by infusing glucose continuously for a prolonged period of time. Although glucose is known to depress OGH in adult sheep, these workers were unable to achieve the same result in the fetus. Liggins and Kennedy (1968) reported that total hypophysectomy was associated with a retardation of somatic development, most notably in bone tissue. Underdevelopment of the thyroid and adrenals was also reported in this study, suggesting that the observed developmental retardation may be a result of hypothyroidism rather than hypophysectomy. No growth retardation has been reported in hypophysectomized porcine fetuses at 40-50 days of gestation (Stryker and Dzuick, 1975).

Wyk et al. (1974) demonstrated that growth hormone does not directly regulate skeletal growth, but rather acts indirectly through a generation of intermediate hormones called somatomedins. Somatomedin
is a peptide hormone reported to influence peripheral action of growth hormone and regulate fetal growth (Falkner et al., 1979). Hintz et al. (1977) working with infants suffering from a protein-calorie deficiency reported low serum levels of somatomedin in spite of elevated levels of growth hormone. A similar observation regarding these two hormones has been reported by Robinson et al. (1977b) in fetal sheep. Plasma somatomedin concentration in fetuses from ewes that had undergone endometrial carunculectomy was lower though normal concentrations of fetal growth hormone was observed. It would appear from results reported by Falkner et al. (1979) in hypophysectomized and nephrectomized fetuses, that somatomedin activity in fetal sheep is regulated by similar mechanisms in the adult. No significant changes in somatomedin activity was observed in control fetuses throughout the gestational period though reduced fetal somatomedin activity was reported in hypophysectomized and nephrectomized fetuses.

In spite of the exhaustive studies designed to monitor fetal OGH throughout gestation there is no direct relationship between fetal OGH and intrauterine weight changes in utero. The isolation and characterization of placental extract, known as ovine chorionic somatomammotrophin, (OCS) stimulated interest in the area of placental regulation and fetal growth (Martal and Djiane, 1975). OCS is a polypeptide hormone, similar to OGH in function and possesses both growth and lactogenic properties. This hormone is secreted primarily in the maternal circulation, and is predominantly active as early as day 16 in the trophoblast (Martal and Djiane, 1977), and reaches a maximum at 120 days of gestation.
Martal (1978) reported that the sum of OGH and OCS is closely related to fetal intrauterine weight changes. This is contrary to the observations of the adult where the sum of growth promoting activities (OGH and OCS) remain constant during the gestational period. Martal concluded from these results that OCS controlled fetal growth during the first half of gestation and that the combined activities of OGH and OCS regulated fetal growth during the second half of gestation.

b) **Thyroid Hormones**

The thyroid axis of the fetal sheep is active and independent of the maternal axis. Experiments in thyroidectomized ovine fetuses have reported undetectable quantities of thyroid hormones, confirming that a maternal source does not contribute to the basal levels of fetal plasma thyroxine concentrations (Hopkins *et al*., 1971; Erenberg *et al*., 1973). Morphological changes in the fetal thyroid gland and detectable levels of thyroxine ($T_4$) have been demonstrated as early as 50 days of gestation (Thornburn and Hopkins, 1973). The significance of fetal thyroid hormones on fetal intrauterine growth is related to the stage of fetal maturity at birth (Hopkins *et al*., 1972; Erenberg *et al*., 1973). Thyroidectomized fetal lambs have shown significant growth retardation in ossification centers of hind limbs, reduced muscle development and thymus weights and reduced differentiation of wool follicles (Hopkins, 1975).
c) **ADRENAL CORTICOSTEROIDS**

Studies with adrenalectomized fetuses have shown increased fetal growth without hepatic glycogen deposition and high mortality after birth (Barnes et al., 1977). In addition to the induction of several enzymes critical for fetal metabolic homeostasis, the glucocorticoids ensure optimal fetal maturation at birth despite gestational age variation (Liggins, 1976).

Jost (1961) first reported the importance of the adrenal cortical hormones in inducing fetal liver glycogen synthesis in the fetal rabbit. Dramatic increases in fetal plasma corticosteroids, most notably cortisol, in the last few days prepartum have been reported in ruminant (Bassett and Thorburn, 1969) and porcine (Dvorak, 1972) fetuses. There is a close temporal relationship between the observed increase in fetal plasma cortisol and hepatic glycogen content (Barnes et al., 1978), which indicates that the adrenal cortex is as essential organ in the control of hepatic glycogen storage in the ovine fetus.

d) **INSULIN AND GLUCAGON**

The fetal pancreas plays a vital role in the metabolism of nutrients delivered to the fetus by the maternal organism. The precise role that insulin and glucagon play in regulating fetal growth has been studied extensively in the rat and sheep (Alexander et al., 1971, 1972, 1973, 1976; Girard et al., 1973; Bassett et al., 1973): Insulin and glucagon influence fetal growth indirectly by influencing the establishment of energy reserves (Milner, 1979) and consequently have been considered to be the most important growth promoting factors of the fetus.
The ovine placenta is impermeable to insulin and glucagon, (Alexander et al., 1972, 1973 and Sperling et al., 1973), indicating that substrate metabolism is regulated by fetal pancreatic activity. Detectable levels of fetal plasma insulin and glucagon during most of the gestational period have been reported (Willes et al., 1969 and Alexander et al., 1971). The secretory response of the pancreatic beta cell matures earlier than that of the alpha secretory cells (Fiser et al., 1974) which in part explains the large circulating insulin to glucagon molar ratio reported by Girard et al. (1974).

A positive relationship exists between insulin secretion and glucose homeostasis (Bassett and Madill 1974a,b; Fiser et al. 1974; Simmons et al., 1978). Willes et al. (1969) concluded from studies on ovine fetuses, with comparatively short postoperative recovery periods, that the fetal pancreas does not respond to a glucose or fructose intravenous challenge by secreting insulin. This result was in direct conflict to earlier observations reported by Alexander et al. (1969), who reported a significant insulin secretion in response to a glucose infusion. Fiser et al. (1974) confirmed Alexander's original observation and added that the fetal insulin response increased as pregnancy proceeded, though the magnitude of this response was significantly lower than in the adult. Bassett et al. (1973) and Bassett and Madill (1974b) demonstrated that insulin release by the fetal pancreas was stimulated by glucose at concentrations present in fetal plasma. Philipps et al. (1978) concluded from slopes of the insulin response curve that the pancreatic β-cell was sensitive to alterations in fetal glucose concentrations. It was also reported that fetal sensitivity to
glucose was equivalent to maternal responses, though a significant lag time existed before a response was noted. Similar insulin induced responses were observed in fetuses from fed and starved ewes (Schreiner et al., 1980). Although maternal starvation was noted to cause a 50% reduction in fetal plasma glucose concentrations, the kinetics of fetal insulin secretion were not affected.

The apparent biphasic secretory pattern of insulin reported by Philipps et al. (1978), warrants the use of a continuous infusion technique rather than an acute injection of glucose for sustaining a physiological response to insulin. Similar inconsistencies in the literature regarding fructose and alanine as stimulators of fetal insulin secretion can be explained on the basis of different experimental protocols. Davis et al. (1971) and Bassett and Madill (1974b) reported positive responses in fetal insulin secretion following fructose infusions; however, this result was not obtainable in a more recent study by Philipps et al. (1978). Similarly, Fiser et al. (1974) reported no change in fetal glucose, insulin or glucagon levels following an acute injection of alanine to the fetus. Philipps et al. (1980) employing a square wave infusion technique, reported an induced elevation of insulin, with maximal response 60 minutes after the start of the amino acid infusion.

The biological activity of fetal insulin has been equally difficult to define. Colwill et al. (1970) infused pharmacological dosages of insulin to the fetus and reported minimal decreases in the concentration of fetal plasma glucose, which led them to conclude that there was a lack of control in the rate of glucose utilization by the
fetus. Simmons et al. (1978) infused insulin over a longer period of time and reported an increase in fetal glucose utilization, independent of any changes in umbilical blood flow, fetal oxygen consumption and placental clearance. This report disproved previous evidence reported by Alexander et al. (1970), describing little or no effect of insulin on fetal glucose utilization. Recently Carson et al. (1980) demonstrated a dose related increase in arterial venous differences of whole blood glucose and oxygen across the umbilical circulation during a sustained infusion of insulin to the ovine fetus. Fetal hypoxia was also noticed to occur in this study and it was speculated that insulin, by increasing the utilization of glucose by fetal tissues compromised fetal oxygenation.

The functional secretory mechanisms of glucagon and its biological activity during fetal life are not well understood. The relative importance of glucagon may be of greatest significance during the immediate neonatal period. The sudden fall in plasma insulin at birth and the rise in glucagon are responsible for the triggering of glycogen mobilization and initiation of gluconeogenesis in the neonate (Snell and Walker, 1978). During this time the development of glycogenolysis and gluconeogenesis is critical in order to maintain blood glucose levels.

No correlation appears to exist between fetal glucose and glucagon concentrations in the ovine fetus (Fiser et al., 1974). This is supported by results of Alexander et al. (1976) who also observed no apparent change in plasma glucagon concentrations following insulin induced fetal hypoglycemia. Failure to stimulate glucagon release by
infusion of alanine in vivo has been reported in both the rhesus monkey fetus (Chez et al., 1974) and the ovine fetus (Fisher et al., 1974). However, independent studies with fetal rats (Girard et al., 1971) and fetal sheep (Alexander et al., 1976) have reported significant pancreatic release of glucagon with arginine infusions.

Girard et al. (1973) demonstrated in the rat fetus that exogenous noradrenalin stimulated glucagon and inhibited insulin release from the pancreas. These workers proposed that fetal stress induced at the time of birth was a potential triggering mechanism for the release of noradrenalin at the pancreatic nerve endings, and would in turn stimulate the release of glucagon and inhibit the release of insulin. It was further concluded that fetal rat pancreas does not respond to acute changes in blood glucose by increasing glucagon release, hence ruling out the possibility that postnatal hypoglycemia was a physiological stimulator of glucagon release.

e) HORMONAL ENZYME INDUCTION

Glucocorticoids, catecholamines and glucagon are potential stimulators of enzyme induction whereas insulin is an active antagonist.

Glucocorticoids have been reported to be potent regulators of urea formation (argininosuccinate synthetase E.C.6.3.4.5., Raiha and Suihkonen, 1968), amino acid metabolism (tyrosine aminotransferase E.C.2.6.1.5., Holt and Oliver, 1969) and carbohydrate
metabolism (P.E.P. carboxykinase E.C.4.1.1.3.2., Kirby and Hahn, 1973) in specific mammalian species. Glucagon and catecholamines are positive stimulators of tyrosine aminotransferase activity and can reverse the effect of adrenalectomy in the rat fetus (Holt and Oliver, 1971). Catecholamines have also been reported to stimulate gluconeogenesis by stimulating phosphoenolpyruvate carboxykinase (Holt and Oliver, 1968). Significant enzyme induction following parturition in the neonate (Warnes et al., 1977b) can be attributed to glucagon release triggered by elevated fetal catecholamines (Girard et al., 1973). It is thus evident that glucocorticoids, glucagon, insulin and catecholamines participate in a complex interplay which determines the process of enzyme induction in the fetal liver and possibly kidney.

5. Fetal metabolism

a) TECHNIQUES FOR METABOLIC STUDIES IN THE FETUS

The pregnant sheep has been a useful animal model for studying fetal physiology. The modern era of fetal physiology research began with the work of Huggett (1927) with exteriorized animal preparations. The delivery of a pregnant goat by caesarean section into a bath tub filled with warm saline enabled Huggett to measure a variety of fetal physiological parameters. Modification of this procedure was performed by Barcroft et al. (1939) and Barcroft and Baron (1946) in attempts to elucidate further the various aspects concerning the metabolism of the fetus in utero.
In view of the obvious shortcomings of these procedures, primarily the relatively short time period during which experiments could be performed, fetal perfusion procedures were developed. Alexander et al. (1955), employing umbilical perfusion of the placenta and Andrews et al. (1961) using perfused livers attempted to maintain a physiological environment for longer durations for experimental purposes. Alexander et al. (1964) further modified these procedures by isolating the sheep fetus and connecting it to the umbilical circulation through an extracorporeal circuit. This development enabled workers to observe the fetus for longer periods of time after separating it from the placenta. Numerous aspects of fetal metabolism were investigated by Alexander and coworkers with this procedure.

The possibility of cannulating ovine fetal blood vessels to facilitate physiological studies in unrestrained animals and without the influence of anesthetics and surgical stress was first demonstrated by Blechner et al. (1960). However, the vascular catheters remained functional only for a short period of time and this necessitated improvements to be made in the surgical procedures. Meschia et al. (1965a) and Kraner (1965) reported procedures for chronic catheterization of fetal blood vessels in unstressed animals for the purpose of sampling the fetus for longer periods of time. The question of how long one must wait following surgery to be assured that the nutrition and metabolic status of operative fetuses represented normal, unoperated
fetuses was examined by Clapp et al., 1977; Slater and Mellor, 1977; Kitts et al., 1979. Recommendations from these studies indicated that it was insufficient to simply rely on blood gas and pH measurements to determine animal normality since metabolic correlates of the fetus are of equal concern. Further it was suggested that a minimum of 5 postoperative days be given for fetal and maternal recovery from surgical trauma before experiments were initiated.

Several sophisticated versions of these techniques are currently in use in studying fetal metabolism in the undisturbed physiological state, and consequently have led to the tremendous wealth of information on fetal physiology that has been generated from the ovine fetus in utero.

b) FETAL CALORIC REQUIREMENTS

The total fetal caloric requirement has been evaluated from calculations based on substrate and oxygen consumption measurements and the determination of caloric requisites for new tissue accretion, by bomb calorimetry (Rattray et al., 1974; Battaglia and Meschia, 1978). A majority of the information available concerning fetal substrate utilization has originated from studies focused on a time period near the end of gestation when the greatest increment of fetal substrate requirement occurs. Battaglia and Meschia (1978) proposed that the fetal lamb oxidizes a mixture of carbohydrates and amino acids. Assuming that the caloric yield of 1 litre of oxygen necessary to combust this mixture in toto is 4.9 kcal, the daily oxidative requirements of the ovine fetus consuming 6 to 9 mls oxygen/min/kg at STP was approximated to be 56 kcal/day/kg. Rattray et al. (1974) disclosed that the 130 day ovine fetus contains a caloric value of 0.9 kcal/g and gains weight at a rate of 36 g/day/kg. The caloric requirement for the
formation of new tissue was computed to approximate 32 kcal/g/day. The total caloric requirements of the ovine fetus has therefore been reported to approximate 88 kcal/day/kg of which 64% is used for oxidative purposes and 36% for tissue growth (Battaglia and Meschia, 1978).

i) **OXYGEN CONSUMPTION AND CARBON DIOXIDE PRODUCTION**

Since the presence of oxygen is essential for the oxidative metabolism of substrates, a tremendous amount of interest has been directed towards accurately defining the mechanisms of oxygen and carbon dioxide exchange between maternal and fetal compartments (Meschia et al., 1965a,b; Motoyama et al., 1967; Matalon et al., 1978). Respiratory gases cross the placenta by simple diffusion (Faber, 1977) and the high affinity for oxygen in fetal blood (Naughton et al., 1963) is common to all mammalian species. This is attributed to the quantity and unique type of fetal hemaglobin (Metcalfe et al., 1972). These characteristics of fetal blood account for the differences in the carrying capacity and dissociation curves of maternal and fetal blood and facilitate the transfer of respiratory gases across the placenta. Notable differences between maternal and fetal $P_{50}$, (oxygen tension at which 50 percent saturation occurs at pH 7.4), have been reported in a number of mammalian species (Silver and Comline, 1975) and are largely responsible for the magnitude of the transplacental gradient for $PO_2$. In ruminant and porcine species, the transplacental gradient of oxygen is 10-20 times greater than in equine species. This is an interesting observation when considering that all three species possess a similar epitheliochorial placenta (Silver and Comline, 1975). Structural differences in the
arrangement of the placental vascular circulation are responsible for the large differences in maternal-fetal substrate gradients in these animals (Silver et al., 1973). The \( \text{PO}_2 \) in fetal arterial blood is lower than in the ewe. This characteristic, together with the high fetal \( \text{PCO}_2 \) and pH, previously led investigators to suggest that the fetus exists in a hypoxic and acidotic environment (Vaughn et al., 1968). More recently, reports have disputed this concept and strongly suggested that anaerobic metabolism does not play an important role in fetal metabolic activities (Battaglia and Meschia, 1978). Battaglia et al. (1968) demonstrated that fetal oxygenation did not alter significantly following increased oxygen availability. Furthermore, it was shown that although maternal \( \text{PO}_2 \) was altered by increasing the amount of oxygen, the increase was not proportionally transmitted to the fetus.

The study of fetal uptake and excretion of substances, such as oxygen, has relied on simultaneous measurements of arterial-venous differences crossing the umbilical and uterine circulations, the blood flow of these circulations, and the application of the Fick principle (Meschia et al., 1965b, 1967b; James et al., 1972). Oxygen consumption in chronically catheterized unanesthetized fetuses varies between 6 and 9 ml/min/kg fetus. This accounts for approximately 60% of the total uterine uptake of oxygen (Meschia et al., 1980). An inverse relationship exists between the arterial-venous difference of oxygen and the blood flow in the uterine and umbilical circulations, thereby facilitating a relatively constant fetal oxygen consumption despite fluctuations
in blood flow (Meschia et al., 1967a, Comline and Silver, 1976).
The rate of fetal oxygen consumption when expressed per unit body
weight is greater than the basal level of the adult (Table 1).
Battaglia and Meschia (1978) have also pointed out that fetal
oxygen consumption values, when expressed on a basis of fetal body
weight, are similar in species that differ in body size. This
is contrary to what is true in adult animals (Kleiber, 1947).

**TABLE 1: OXYGEN CONSUMPTION RATES OF ADULTS AND FETUSES
OF SPECIES OF DIFFERENT SIZE**

<table>
<thead>
<tr>
<th>Animal</th>
<th>Oxygen Consumption (ml/min/kg BWT)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adult</td>
</tr>
<tr>
<td>Horse</td>
<td>2.0</td>
</tr>
<tr>
<td>Cattle</td>
<td>2.2</td>
</tr>
<tr>
<td>Sheep</td>
<td>4.0</td>
</tr>
<tr>
<td>Rhesus Monkey</td>
<td>7.0</td>
</tr>
<tr>
<td>Guinea Pig</td>
<td>9.7</td>
</tr>
</tbody>
</table>

* from Battaglia and Meschia (1978)

Similar approaches have been taken to assess fetal CO₂
production rates across the placenta in chronically catheterized
ovine fetuses (James et al., 1972). The carbon dioxide production
reported by these workers utilizing the Fick principle, was 5.65
ml/min/kg by fetus. Single fetuses yielded a slightly higher CO₂
production rate than twins (5.4 vs 5.1 ml/min/kg fetus) and the
time following surgery before experiments were initiated appeared
significant. No significant changes are observed in the fetal
blood PCO₂, pH or bicarbonate levels at different stages of
gestation in sheep (Comline and Silver, 1972). Fetal PCO₂ levels
in the fetal artery are higher than maternal arterial PCO₂ and can
change in direct proportion to maternal PCO₂. The pH of fetal blood is always slightly lower than that of the mother, although standard bicarbonate concentrations are similar (Meschia et al., 1970).

ii) **GLUCOSE**

Huggett et al. (1951) first reported the transfer of monosaccharides across the ovine placenta and demonstrated the preferential permeability of the placenta to glucose. Calculations made by Widdas (1952) on the data of Huggett et al. (1951) indicated that the transfer of glucose across the placenta of sheep could not be explained by simple diffusion mechanisms alone. The chemical and stereo-specific characteristics regulating placental transfer of mono and disaccharides were examined by Walker (1960). It is now believed that the transfer of glucose from the mother to the fetus is mediated by a process of facilitated diffusion, analagous to the mechanisms in the erythrocyte (Widdas 1961; Boyd et al., 1976). Simmons et al. (1979) recently concluded from determinations on the rate of placental glucose transfer in chronically catheterized fetuses, that the rate of glucose transfer was not only limited by transport characteristics but was also affected by the utilization rate of glucose by placental tissues.

It was not until the advent of chronically catheterized fetal preparations that the relationship between fetal and maternal glucose concentrations was accurately assessed (James et al., 1972, Shelley, 1973; Bassett and Madill, 1974a). The difference between fetal and maternal concentration is common to all animal species
and does not change significantly with gestation (Silver, 1977). This observation has been attributed to a combination of placental tissues (Silver, 1977; Shelley, 1979). Data obtained from the cow, sheep and horse have indicated that the placental morphology influences the fetal to maternal glucose gradients (Silver et al., 1973). Although all three species possess similar placentas, the relative inefficiency of the ruminant placenta, resultant of its vascular morphology, is responsible for the low maternal to fetal substrate gradients reported in the ruminant (Silver et al., 1973). The metabolism of glucose by the placenta is an additional factor controlling fetal glucose metabolism. Alexander et al. (1955) demonstrated in exteriorized, perfused ovine fetuses a significant proportion of fructose was derived from glucose by placental tissues. Battaglia et al. (1961) further demonstrated that ovine placental cotyledons were potential consumers of glucose. Silver (1977) reported that significantly greater uptakes of glucose occur in the ovine uterus than the fetus alone, and Setchell et al. (1972) suggested that a considerable amount of glucose removed by the uterus was not oxidized but rather could be used for synthetic purposes.

The relationship between fetal glucose uptake and maternal concentration of glucose has also been studied in detail. Alexander et al. (1955) first disclosed that an artificial elevation of maternal glucose concentration resulted in an increased transfer of glucose to the fetus. Later studies by Alexander et al. (1969), in exteriorized fetuses, showed that glucose uptake by the fetus was approximately 6 mg/min/kg fetus, similar to reported values in the newborn lamb (5 mg/min/kg lamb, Jarrett et al. 1964).
James et al. (1972) reported that umbilical uptake values of glucose from chronically catheterized ovine fetuses ranged from 1 to 6 mg/min/kg fetus and a linear relationship existed between umbilical glucose uptake and maternal arterial glucose concentrations. Comparative studies in the cow, sheep, and horse have also shown a positive correlation between maternal arterial glucose concentration and transplacental transfer rate of glucose to the fetus (Boyd et al., 1973; Comline and Silver, 1976). There would appear to be an upper limit to the amount transferred to the fetus from the mother, as evidenced from data published on the bovine fetus (Comline and Silver, 1976). It has also been shown in experimentally induced maternal hyperglycemia, that fetal glucose uptake will plateau to 40-60% of the basal levels (Shelley, 1973; Silver et al., 1973). No net change in fetal glucose uptake or maternal-fetal glucose gradients is observed with advancing gestation, although there are notable increases in net fetal oxygen consumption and umbilical blood flow (Boyd et al., 1973). The level of hypoxia, as shown in studies with spontaneous hypoxemic lambs also affects fetal glucose and transplacental glucose gradients (Char and Creasy, 1977).

With the onset of maternal hypoglycemia there is a dramatic fall in both the maternal and fetal glucose concentrations, to 50 and 35% of fed state respectively (Boyd et al., 1973; Schreiner et al., 1978; Anand et al., 1980). The maternal-fetal arterial glucose concentration gradient also falls significantly, and consequently leads to a reduced transfer rate of glucose to the
fetus (Schreiner et al., 1978). Schreiner et al. (1978) proposed that the decline in fetal glucose concentration to a relatively constant level, tends to restore the glucose concentration gradient across the placenta and thus increases the umbilical glucose uptake towards normal levels observed in the nonstarved state. Further, the fetus reduces its consumption of exogenous glucose as evidenced by the reduction in plasma insulin levels 2 days after maternal fasting (Bassett and Madill, 1974b).

iii) **FETAL GLUCONEOGENESIS**

Whereas hepatic gluconeogenesis is the major source of blood glucose in the adult ruminant (Bergman, 1973), the significance of gluconeogenesis in the fetus is not totally resolved.

Incorporation of U-^{14}C pyruvate into ^{14}C glucose was originally reported in liver slices of fetal sheep, embryonic chicks and postnatal rats and sheep but not in fetal rats (Ballard and Oliver, 1965). The activity of gluconeogenic enzymes in the fetal rat is low (Ballard and Hansen, 1967) and although phosphoenopyruvate carboxykinase (PEPCK) a rate limiting enzyme, is inducible prior to parturition with glucagon, the overall pathway is not detectable (Phillippides and Ballard, 1969). The key regulatory enzymes of gluconeogenesis are present in substantial activities in fetal sheep liver (Stephenson et al., 1976; Warnes et al., 1977b) and fetal kidney cortex (Stephenson et al., 1976). Prior to 100 days of gestation there is a limited in vitro production of endogenous glucose due to the low levels of fructose 1, 6 diphosphatase (F.1.6.D.P.) and glucose 6 phosphatase (G.6.P.)
activities (Stephenson et al., 1976). Warnes et al. (1977b) also reported that cytosolic PEPCK activities at mid gestation were only 10 per cent of the activity at birth. Both liver and kidney gluconeogenic enzyme activities are comparable to adult levels just prior to partition (Stephenson et al., 1976; Warnes et al., 1977b; Prior and Scott, 1977). Prior and Scott (1977) further demonstrated the capacity for gluconeogenesis in the bovine fetus as early as 88 days of gestation. These workers concluded that a restriction in maternal dietary energy during late gestation does not significantly alter maternal or fetal gluconeogenic activity. Swiatek (1971) reported that both pyruvate carboxylase (PC) and PEPCK were limiting factors in the absence of gluconeogenesis in the neonatal pig. Jones and Ashton (1976) demonstrated in vitro that the fetal guinea pig has a functional gluconeogenic pathway in both liver and kidney tissues, 10 days prior to birth.

Two aminotransferases, glutamate-oxaloacetate aminotransferase and glutamate-pyruvate aminotransferase are active in bovine fetal liver and kidney tissues as early as 45 days of gestation (Stephenson et al., 1976). Edwards et al. (1975) reported marked increases in glutamate pyruvate aminotransferase activity in fetal heart muscle with advancing gestation.

The incorporation of gluconeogenic precursors into glucose by liver slices and organ culture has given support to the occurrence of gluconeogenesis with the enzyme activities measured in vitro. Spontaneous gluconeogenesis from serine,
glycerol and to a lesser extent alanine and lactate was observed in cultured fetal rat liver (Coufulik and Monder, 1976). Simkins et al. (1978) reported a similar result with galactose added to the medium and further demonstrated that glucocorticoids were potential stimulators of gluconeogenesis from galactose. Results obtained from Prior and Scott (1977) with fetal bovine liver slices, showed a greater amount of gluconeogenesis from lactate and pyruvate than from alanine and aspartate. Furthermore, the incorporation of these substrates into glucose followed a curvilinear pattern with gestational age, with maximal levels occurring after mid gestation.

Although numerous in vitro studies have indicated the presence of a functional gluconeogenic pathway in the ruminant and nonruminant fetal species, the extent to which this capacity is manifested in vivo is uncertain. Boyd et al. (1973) reported that the net fetal uptake of glucose and the maternal-fetal glucose gradient do not change with gestation, although umbilical blood flow and fetal oxygen consumption increase during this time. These workers, therefore, hypothesized the presence of fetal gluconeogenesis. Hay (1979) further speculated that fetal gluconeogenesis could raise fetal arterial blood glucose concentrations to a level that would reduce umbilical glucose uptake. Hodgson and Mellor (1977) estimated from disposal rates of labelled glucose, that 60-82% of glucose requirement was potentially accounted for by gluconeogenesis. Further studies (Hodgson et al. 1980) have reported that 69% of fetal glucose requirements are supplied through fetal gluconeogenesis. Anand et al. (1979; 1980) obtained maternal
fetal specific activity ratios of glucose following the continuous infusion of labelled glucose separately into fetal and maternal circulations. The result was similar to previous work performed with rats (Girard et al., 1977), which demonstrated no significant dilution of maternal glucose specific activities from fetal glucose production.

Prior and Christenson (1977) reported that alanine accounted for 2% of fetal glucose entry rate. This constituted 7% of alanine turnover and 49% of the net uptake of alanine estimated for fetal sheep (Lemons et al., 1976). Warnes et al. (1977a) utilizing single injection of isotopic tracers failed to show any gluconeogenesis from lactate in the fetal lamb. Recently Prior (1980), employing a continuous infusion of radiolabelled lactate, was successful in demonstrating significant gluconeogenesis from lactate. Lactate has been identified as an active gluconeogenic precursor in the newly born lamb (Warnes et al., 1977b).

Although it would appear that there is a potential for fetal gluconeogenesis in most species with the exception of the rat, suggestions have been made that there are additional factors regulating gluconeogenesis in utero. Warnes et al. (1977b) attributed the low fetal arterial P0₂, and resulting redox potential of liver mitochondria (Williamson et al., 1967), to be a potential regulator of gluconeogenesis. Supporting this theory was the appearance of active gluconeogenesis from lactate 2 minutes following natural birth of term lambs. Jones and Ashton (1976) suggested
that high PO₂ of incubation media stimulated gluconeogenesis in vitro in which guinea pig liver slices were used. The apparent stimulator of gluconeogenesis was considered to be a large increase in the phosphoenolpyruvate/pyruvate ratio. The relationship between oxygenation and gluconeogenesis is also indicated by the inhibition of gluconeogenesis by $\beta$-hydroxybutyrate which, by reducing the mitochondrial NAD⁺/NADH ratio, may have diminished mitochondrial PEP production (Jones and Ashton, 1976).

Bohr (1931) originally concluded from an indirect estimation of the fetal respiratory quotient (R.Q.) that glucose was the sole energy substrate of the fetus. Experiments conducted many years later with fetal umbilical perfusion techniques confirmed this conclusion (Alexander et al., 1966). However, because the simultaneous measurement of fetal oxygen consumption was not performed, the fetal respiratory quotient was not made in this study. James et al. (1972) determined the oxygen consumption and CO₂ production in chronically catheterized, unanaesthetized fetuses and reported that the fetal respiratory quotient was significantly less than one.

Meschia and coworkers developed another procedure for identifying substrates utilized by the ovine fetus. Since fetal oxygen consumption is an absolute prerequisite for substrate oxidation, an expression relating the simultaneous uptake of specific substrates and oxygen by the fetal umbilical circulation was formulated. This expression was referred to as the substrate oxygen quotient (Tsuolos et al., 1971; Battaglia and Meschia, 1973).
1) \[ \text{Substrate Quotient} = \frac{n \times V-A \text{ substrate}}{V-A \text{ oxygen}} = \frac{n \times \text{umbilical uptake of substrate}}{\text{umbilical uptake of oxygen}} \]

where \( n \) = number of moles of oxygen required for complete oxidation of substrate to \( \text{CO}_2 \) and water.

This dimensionless quotient represents the fraction of oxygen consumption accounted for by the complete aerobic oxidation of a substrate crossing the umbilical circulation.

Fetal glucose-oxygen quotient values have ranged from 0.41 to 0.64 in the fed ewe (Tsoulos et al., 1971; James et al., 1972; Boyd et al., 1973; Schreiner et al., 1978), 0.57 in the cow (Comline and Silver, 1976) and 0.68 in the horse (Silver and Comline, 1975). This supports the result obtained earlier in sheep that glucose uptake, although an important component of fetal metabolism, does not account for the total oxygen consumption by the fetus.

The wide range observed in the ewe is attributed to the variable levels of dietary energy intake of pregnant ewes in different studies and the apparent dependence of the glucose-oxygen quotient on maternal plasma glucose levels. This is particularly evident with studies performed on starved ewes. Glucose-oxygen quotients obtained during starvation ranged from 0.13 to 0.30 (Tsoulos et al., 1971; Boyd et al., 1973; Schreiner et al., 1978). Schreiner et al. (1978) demonstrated that glucose oxygen quotients decrease rapidly during the first 2 days of maternal starvation and remain constant thereafter. The decrease in placental transfer of glucose during maternal fasting represented a loss of approximately 22% of the normal fetal caloric intake.

The glucose-oxygen quotient measured in individual fetal organs have demonstrated a specificity for substrate oxidation. Tsoulos et al. (1972) estimated a cerebral glucose-oxygen quotient of 1.06, indicating that glucose is the sole source of energy utilized by the fetal brain. Under normal
conditions the cerebral glucose metabolism will account for 15% of the fetal umbilical glucose uptake (Jones et al., 1975). Morriss et al. (1973) reported a similar glucose oxygen quotient for the fetal hind limb and concluded that although the hind limb was less active metabolically than the fetal brain, glucose was the predominant substrate oxidized. It was further concluded from these studies that glucose uptake by individual organs is regulated by fetal arterial glucose concentrations and in the case of the fetal hind limb, glucose uptake exceeded the requirements of oxidation. Reviewing the literature on the subject, Hay (1979) concluded that glucose utilization by individual organs was dependent on an assortment of factors, including glucose availability and rates of glycolysis, oxidation and overall fetal growth.

iv) **FRUCTOSE**

In several mammalian species (sheep, cow and pig) fructose is the principal carbohydrate in fetal blood, and is present in quantities that are 3 to 4 times greater than circulating glucose concentrations (Randall and L'Ecuver, 1976; Comline and Silver, 1976). Bacon and Bell (1948) first identified fructose in blood of fetal sheep and showed that it existed with glucose during the gestation period. Goodwin (1952) demonstrated fructose to be present in the blood of ungulata, but absent in the blood of carnivora or rodents. Hitchcock (1949) observed a gradual disappearance of fructose from the lamb circulation, within 36-72 hours of birth. Huggett et al. (1951) proposed from data obtained from exteriorized fetal sheep preparations
with intact umbilical circulations, that fructose enters the fetal circulation after it is converted from maternal glucose in the placenta and does not return to the maternal circulation. Alexander et al. (1955) further demonstrated that the passage of glucose from mother to fetus resulted in the formation of fructose and occurred at normal blood sugar concentrations. Glucose infused directly into the fetal circulation is actively converted to fructose in fetal sheep (Warnes et al., 1977a) and fetal pig (White et al., 1979). However, it is unclear whether or not there is any interconversion of these two sugars. Setchell et al. (1972) chromatographed blood from fetal lambs that were infused with labelled fructose and reported activity in the glucose molecule in blood obtained from the umbilical vein, and fetal heart, but not from the umbilical artery. Conversely, Warnes et al. (1977a) and White et al. (1979) failed to observe this interconversion. Comline and Silver (1970) have disclosed that fructose is a product of placental metabolism and is dependent on the plasma glucose concentration in both the mother and fetus. This conclusion is particularly evident during cases of maternal starvation, when fetal fructose levels fall to 43% of the pre-starved levels (Tsoulos et al., 1971; Schreiner et al., 1978). Meschia and Battaglia (1978) have speculated from these results that fructose acts as a glucose reserve, especially during periods of maternal starvation and hypoglycemia.

No detectable umbilical uptakes of fructose (Tsoulos et al., 1971), glycerol and fatty acids (James et al., 1971) and ketone bodies (Morriss et al., 1974) have been reported, thus demonstrating that these substrates are not major metabolic fuels of the sheep fetus under normal conditions.
v) LACTATE

Fetal lactate concentrations are 2-3 times higher than maternal concentrations reflecting the high glycolytic capacity of fetal and placental tissues (Char and Creasy, 1976a; Demigne and Ramesy, 1979). Significant umbilical venous-arterial differences of lactate, however, have been reported in ovine (Burd, et al., 1975; Char and Creasy, 1976a) and bovine (Comline and Silver, 1976) fetuses, indicating that lactate is taken up by the fetus across the placental circulation. Higher uterine venous-arterial differences of pyruvate are consistent with higher umbilical arterial-venous differences, indicating that pyruvate is returned to the ewe and is not utilized by the fetus (Char and Creasy, 1976a). These workers also demonstrated significant correlations between fetal umbilical and arterial lactate and pyruvate concentrations with maternal arterial levels.

Initially it was believed that the high fetal lactate levels observed in fetal plasma were the result of anaerobic metabolism. However, the appreciable umbilical uptake of lactate suggests the fetus is a consumer rather than a producer of lactate (Burd et al., 1975; Char and Creasy, 1976a; Comline and Silver, 1976). The large lactate-pyruvate ratios observed in ovine (Burd et al., 1975; Char and Creasy, 1976a), and bovine (Demigre and Ramesy, 1979) fetuses are similar to maternal lactate-pyruvate ratios indicating that lactate is not produced in excess by the fetus.

The source of lactate available for fetal utilization remains questionable. Significant correlations reported between
fetal arterial lactate and maternal arterial lactate concentrations suggest that maternal transfer of lactate occurs in utero. Démigne Ramesy (1979), however, observed that maternal hyperlactemia did not influence fetal lactate levels. This result confirmed a similar observation which showed no transfer of lactate from fetus to ewe under hypoxic conditions (Britton et al., 1967). However, Char and Creasy (1976a) reported positive uterine venous-arterial differences, thereby indicating that the sheep placenta deposits lactate into the maternal circulation. Warnes et al. (1977a) reported rapid labelling of lactate molecules following the injection of U-14C glucose into the fetal circulation. The rate of irreversible disposal of lactate was similar to that of glucose and suggests that placental metabolism as well as fetal metabolism of this substrate occurred. Comline and Silver (1976) previously reported the net lactate production by uteroplacental tissue in bovine fetuses was a function of the rate of glucose utilization by the uterus. It was demonstrated previously by Villee (1954) that the amount of lactate produced by fetal tissue slices in vitro did not change appreciably during the later stages of gestation. Furthermore, it was concluded from these studies that heart tissue produced more lactate than brain and liver, and the amount of lactate produced was not strictly dependent on the type of substrate employed for incubation purposes.

Burd et al. (1975) demonstrated from simultaneous measurements of umbilical uptake of lactate and oxygen that the placenta produced lactate in sufficient quantities to account for approximately 25% (range 0-79%) of fetal oxidative metabolism. The consumption
of lactate by the fetus in utero was confirmed by Char and Creasy (1976a) (32% in ovine fetus) and Comline and Silver (1976), (43% in bovine fetus). Since the production of placental lactate is a function of the high maternal glucose entry rates reaching the uterus (Setchell et al., 1972), discrepancies reported in the stoichiometric measurements of lactate-oxygen quotients can be attributed to dissimilar maternal glucose concentrations. Schreiner et al. (1978) demonstrated this with significant decreases in the fetal lactate-oxygen quotients obtained from fasting ewes. Pyruvate, on the other hand, is not taken up by the fetus and, therefore, makes no contribution as a fetal oxidative substrate (Char and Creasy, 1976a).

Acetate, an important volatile fatty acid in adult ruminant metabolism, is taken up by the umbilical circulation in significant quantities and accounts for 10% of the fetal oxygen consumption (Char and Creasy, 1976b). Comline and Silver (1976) reported a slightly higher respiratory quotient for acetate (16%) in the bovine fetus.

vi) AMINO ACIDS

The ovine fetus is a rapidly growing organism between 100 and 147 days of gestation, increasing in body weight at a rate of 1.2-2 g/hour. This is equivalent to 120-210 mg/g/hour of protein accretion into the fetal body (Alexander et al., 1970b). Southgate (1971) demonstrated from fetal rat carcasses that the amino acid composition of fetal protein was similar in proportions as weaning and adult carcass protein content. It has been well established that the primary source of nitrogen for growing mammalian fetuses is
derived from a circulating maternal free amino acid pool (Young and McFayden, 1973; Smith et al., 1977; Lemons et al., 1976). A majority of the amino acids transferred across the umbilical circulation reaches the fetus in quantities sufficient for adequate growth (Young and McFayden, 1973 and Lemons et al., 1976). Comparison of amino acid content of lamb carcasses together with placental transfer of neutral amino acids led Lemons et al. (1976) to conclude that some amino acids are transferred in quantities in excess of the growth requirements. The requirements of lysine and histidine are, however, similar to respective placental transfer rates. Umbilical venous-arterial concentration differences of 22 amino acids in unstressed ovine fetuses have allowed workers to estimate the fetal uptake of amino acid nitrogen. This uptake (1.5g N/kg/day), is actually somewhat greater than the amount of nitrogen required by the fetus for growth and urea production (1.0 g/kg/day), (Lemons et al., 1976, Holzman et al., 1979).

Numerous studies have attempted to identify and clarify the mechanisms that regulate amino acid transfer from mother and placenta. The placenta has been shown to concentrate a large number of amino acids intracellularly, from maternal blood (Hill and Young, 1973). Fetal plasma levels of individual amino acids exceed those of the mother by as much as 4 to 5 times (Hopkins et al., 1971; Lemons et al., 1976; Smith et al., 1977). A similar observation has been reported with fetal plasma alpha amino nitrogen concentrations (Alexander et al., 1970b). Maternal-fetal plasma concentration gradients are similar for the acidic, branched chain and basic amino acids. However, the
concentration gradient between both the maternal and fetal plasma and the placenta is high for the acidic amino acids. The fetal-maternal concentration gradients are relatively higher for the straight chain amino acids. Young and McFayden (1973) have suggested that the high amino acid levels observed in fetal plasma were a reflection of the high turnover rate of protein in fetal tissues and the relative differences in fetal organ size, as compared to the mother. These workers also concluded that a constant transfer of amino acids occurred independently of maternal plasma concentrations. Holzman et al. (1979) recently demonstrated a strong correlation between amino acid umbilical venous-arterial differences and arterio-venous differences across the uterine circulation. Furthermore, the arterio-venous differences of neutral and basic amino acids were related to the maternal arterial concentration.

Amino acid uptake by the placenta is mediated by diffusion and active (Na pump and oxygen dependent) transport systems. Hill and Young (1973) demonstrated in the guinea pig, that transfer of amino acids from placental parenchyma to fetal plasma was blocked when fetal plasma amino acid concentrations exceeded the free amino acid concentrations of the placental parenchyma. Longo et al. (1973) reported that placental amino acid transport occurs under anaerobic conditions, and is dependent upon glycogenolysis. Christensen and Streicher (1948) first demonstrated that placental transfer of individual amino acids was specific to groups of amino acids. This concept was confirmed in part by Hopkins et al. (1971) who reported that neutral, branched chain amino acids belonging to the "L" preferring transport system were transferred readily across the placental
membrane. Enders et al. (1976) attempted to clarify the mechanisms that regulate amino acid transfer from the placenta, by determining the specificity of the principal placental transport systems. Three transport systems for neutral amino acids were established in human placenta, and were reported to represent protein complexes of the syncytiotrophoblast plasma membrane. With the use of competitive inhibition techniques, these workers were able to identify separately the transport pathways of specific amino acids. Recently Holzman et al. (1979) reported that 65 percent of the total amino acid uptake was represented by eight neutral amino acids (alanine, threonine, serine, valine, leucine, isoleucine, glutamine, and proline). Although glycine is present in very high concentrations and exhibits abnormally large venoarterial differences, it is not taken up by the uterus in significant quantities (Morriss et al., 1979; Holzman et al., 1979). Twenty percent of total amino acid uptake was accounted for by basic amino acids, lysine, histidine, and arginine. Lemons et al. (1976) reported no significant umbilical uptake of acidic amino acids, glutamate and aspartate, but observed a net flux of glutamate out of the fetus into the placenta, indicating de novo synthesis by fetal tissues. Smith et al. (1977) concluded from umbilical venous arterial differences of amino acids that fetal tissues synthesize sufficient glutamate, aspartate, and serine to sustain the synthesis of RNA, DNA and protein but that the remainder of the amino acids needed for protein synthesis was supplied from the maternal circulation.

Due to the relatively small arterial-venous difference of several amino acids and urea, the determination of the umbilical uptake of amino acids by the Fick principle proved difficult. Consequently, amino acid catabolism in the fetus was estimated by a
predetermined value of transplacental clearance of urea and the mean plasma urea differences between fetus and mother (Gresham et al., 1972a; Battaglia and Meschia, 1973).

2) Clearance of urea = \( \frac{\text{Excretion rate of urea}}{a_\text{urea} - A_\text{urea}} \)

where \( a_\text{urea} \) = fetal arterial urea concentration

\( A_\text{urea} \) = maternal arterial urea concentration

The rate of ureogenesis in the ovine and bovine fetus determined by this procedure was 0.54 mg/min/kg and 0.21 mg/min/kg and accounts for 25 and 9% of the fetal oxygen consumption respectively (Gresham et al., 1972a; Comline and Silver, 1976). These comparative differences in the percent of oxygen consumed for amino acid catabolism have been attributed to the lower rate of amino acid breakdown in the bovine fetus (Comline and Silver, 1976). Simmons et al. (1974) reported that amino acid catabolism in the ovine fetus was the initial adjustment of the fetus to maternal starvation and at the peak of urea production could account for 80% of the fetal oxygen consumption.

The relative contribution to total oxidative metabolism by a mixture of fetal fuels has recently been investigated (Shambaugh et al., 1977a,b). Evidence resulting from in vitro studies with the fetal rat indicated a competitive oxidative interaction of specific fetal substrates. An alteration in fetal fuel mixture due to maternal fasting decreased the relative CO₂ production from glucose and lactate to 45 and 75% respectively of the original values. Shambaugh et al. (1977 a,b) have further demonstrated that the addition of betahydroxybutyrate to the incubation medium resulted in a significant reduction
in the CO$_2$ produced from lactate and glucose. It was concluded from these studies that the preferential oxidation of substrates is determined by the ambient concentration of fuels rather than by an intrinsic adaptation of the tissue.

vii) **CARBON-NITROGEN BALANCE**

The measurement of umbilical substrate uptake coupled with fetal carcass analysis enabled investigators to estimate the carbon and nitrogen balance of the near term fetus and the contribution of individual substrates to this balance.

Fetal growth rate in utero is approximately 35 g/kg/day (Gresham et al., 1972b; Rattray et al., 1974). Based on a fetal carcass carbon content of 9 percent of fetal wet weight, the net accumulation of carbon in the fetus was estimated to be 3.15 g C/kg/day (James et al., 1972). Carbon excreted as carbon dioxide and urea amounted to 4.38 gC/kg/day and 0.15 gC/kg/day respectively, representing 60% of the total umbilical carbon flux crossing the placenta to the fetal lamb (James et al., 1972 and Battaglia and Meschia, 1973).

The relative daily amount of substrate carbon contributing to the total carbon flux has been estimated from umbilical substrate uptake measurements. Amino acids, based on a C/N ratio of an average protein supply the majority of the carbon (3.16 gC/kg/day), (Gresham et al., 1972a; Battaglia and Meschia, 1973). The contribution of carbon by glucose (1.76 gC/kg/day; James et al., 1972), lactate (1.22 gC/kg/day; Burd et al., 1975; Char and Creasy 1976a) and acetate (0.56 gC/kg/day; Char and Creasy, 1976b) is relatively small.
The percent contribution of amino acids, glucose, lactate, and acetate to the total carbon balance of the fetus is 41%, 23%, 15%, 9% respectively and totals approximately 88% of the fetal carbon balance. This value therefore indicates that other substrates, possibly under specific environment conditions, cross the sheep placenta in significant quantities.
Experiment 1

Surgical technique for the cannulation of fetal saphenous vein and post-surgical changes in blood parameters of the ovine fetus in utero

Introduction

The possibility of cannulating ovine blood vessels to facilitate physiological studies in unrestrained animals was demonstrated by early workers in this field (Meschia et al., 1965a). The techniques and complexities of intrauterine fetal surgery have been reviewed by Kraner (1965), and Mellor and Slater (1973) in sheep and Randall (1977) in pigs. Successful cannulation of the fetal aorta and vena cava (Comline and Silver, 1972), carotid artery and jugular vein (Bassett and Madill, 1974b) and umbilical vein (Mellor and Matheson, 1975 and Schreiner et al., 1978) have been reported.

For nutritional and physiological studies, it is essential to have chronic fetal preparations which have recovered from the stress of surgery. Mellor and Slater (1973) have stated that the results of experiments done on chronically catheterized fetuses should be interpreted with caution and emphasized that the delivery of full-term lambs following surgery is not absolute proof of intrauterine normality. The minimum time that should elapse before experiments may be undertaken would depend upon the extent of stress imposed during surgery and the maintenance of the patency of vascular catheters.

In this experiment, the technique of ovine fetal surgery was standardized, and the post-surgical status of the fetus was monitored daily to determine the time required for the fetus to return to stable levels. Changes in blood acid-base parameters and metabolite and cortisol concentrations in plasma were measured as criteria of intrauterine fetal normality.
Materials and Methods

Animals

Twenty-five, 1-2 year old pregnant Dorset Horn and Suffolk ewes, weighing 50 to 70 kg were used in this study. The estrous cycles in these ewes were synchronized by the application of progestagen-impregnated intravaginal pessaries (Synchromate; G.D. Searle and Co., Chicago). Intravaginal sponges containing 800 mg progesterone (Sigma, St. Louis) were also used in some ewes. At 90 to 100 days after mating, an ultrasonic detector (Sheepreg, Animark) was used to confirm pregnancy. The ewes were housed in individual pens and fed 650 g of alfalfa cubes (19.6% crude protein; 27.5% crude fiber; 18.58 kJ/g gross energy) twice daily, at 0700 and 1500 hours respectively. Water and salt were made available at all times.

Neonatal lambs were rubbed dry of after birth moisture and weighed as soon as possible after birth. Crown to rump measurements were also taken at this time. The weights of fetuses at the time of experimentation were estimated from fetal birth weights by the regression equation developed by Gresham et al. (1972b).

Animal Preparation

The ewes were starved for 24 hours and were given intramuscularly 5 ml of an antibiotic preparation (Penlong-S Plus, Rogar/5TB) containing 200,000 i.u. Penicillin G and 250 mg dihydrostreptomycin/ml on the day prior to surgery. Atropine sulphate (BDH Pharmaceuticals) was administered subcutaneously (0.06 mg/kg) 15 minutes prior to surgery to reduce excessive salivation.
Surgical Procedure

Anaesthesia was induced by the intravenous administration of thiopental sodium (Abbot) at the rate of 20 mg/kg body weight, and maintained with Halothane (Fluothane, Ayerst) at a concentration of 1.0-1.75% in a closed system. The anesthetized animal was positioned in a supine position with the head turned laterally and tilted slightly downwards to prevent aspiration of saliva or ruminal fluids. The abdominal area was disinfected with surgical soap (Surgidine, Ingram and Bell Ltd.) followed by Tincture of Zephiran, and covered with sterile drapes. An intravenous drip of Ringer's solution was administered to replace loss of electrolytes during surgery. Strict aseptic precautions were maintained throughout.

A midline incision (10-15 cm) starting below the umbilicus and terminating close to the upper margin of the mammary gland was made. After incising the peritoneum the gravid uterus was palpated to determine the position of the fetus. The appropriate segment of the uterine horn containing the fetal hind limbs was brought through the abdominal incision with as little handling as possible. An assistant held the hoof of one of the hind limbs against the uterus and an incision (1-2 cm) was made on the least vascular area of the uterine wall exposing the uterine mucosa and the fetal membranes. The allantoic and amniotic membranes were cautiously cut through and the fetal hind limb pulled through the uterine opening. The uterus was held in such a position which prevented loss of amniotic fluid. The fetal membranes were secured to the wall of the uterus with a chromic 3/0 (Ethicon) purse string suture. An incision (2-3 cm) was made through fetal skin on the hock joint and the external
saphenous vein exposed by blunt dissection and freed of adjacent fascia. Sterile 2% lidocaine (Sterilab) was sprayed on the region to reduce spasms. Fetal catheters were made of polyethylene tubing (0.86 mm i.d. x 1.27 mm o.d.) and enclosed in a polyvinyl collar designed to include silk ligatures used to anchor the catheter to the leg and prevent the catheter tubing from kinking. The catheters were previously sterilized in Cidex (Arbrook Ltd.) and filled with sterile saline (0.15 M) containing heparin (100 U/ml). Silk ligatures were passed beneath the cleared area of the vein at the proximal and distal ends of the incision and made into a loose knot around the vein to facilitate handling during cannulation and prevent the flow of blood whenever necessary. A small opening was made on the wall of the vein and a cannula was introduced 18 to 20 cm proximally from the point of entry into the vein. The silk ligatures (2/0 braided silk, Ethicon) on the collar were anchored to the fascia on either side at the point of insertion of the cannula. Additional collars on the cannula served to anchor it on the fetal skin approximately 5 and 8 cm down the leg.

The fetal incision was closed using 2/0 braided silk and the limb was returned to the uterus taking care to place the fetus in its original position. Ampicillin (Ayert, 500 mg) was instilled into the amniotic cavity and the fetal membranes and the uterus were closed with continuous sutures using chromic catgut 2/0 (Ethicon). The catheter was pushed into the uterus for a distance of approximately 25 cm to allow for fetal movement. The sutures were then buried by a continuous suture to ensure that there was no leak of amniotic fluid. The peritoneum,
adjacent muscle layers and the skin were sutured separately using Echiflex 0, Plain 0, and Proline 0 (Ethicon), respectively.

The catheter was exteriorized by passing the free end through a hollow plastic tube placed subcutaneously on the right flank of the animal. Catheters were coiled in sterile cotton gauze and placed inside a sterile handstitched canvas pouch sutured to the animal.

Immediately following surgery, all animals received 5.0 ml of an antibiotic preparation (Penlong-S Plus) intramuscularly. They were then returned to their metabolism cages where they were allowed to recover quietly. The administration of the antibiotic was continued daily for 3 days following surgery. Catheters were wrapped within sterile gauze daily and free ends were cleansed with an antiseptic solution prior to and following sampling. The patency of catheters was maintained daily by removing heparin from the catheter, flushing with sterile 0.15 M saline and refilling with sterile heparinized saline (100 u/ml).

**Analytical Methods**

Oxygen and carbon dioxide tensions ($P_{O_2}$ and $P_{CO_2}$) and pH of maternal and fetal venous whole blood were determined anaerobically using a Radiometer pH meter (pHM 72) equipped with respective modules and electrodes. The $P_{CO_2}$, $P_{O_2}$ and pH electrodes were thermostatically controlled at 37.5°C. Calibration of the $P_{CO_2}$ and $P_{O_2}$ electrodes was accomplished by bubbling a gas mixture with know partial pressures of CO$_2$ and O$_2$, corrected for daily barometric pressure through the electrodes. Blood gas values were corrected for body temperature using appropriate correction factors (Severinghaus, 1966). Fetal hematocrits were
determined on a microcapillary centrifuge. Oxygen saturation ($S_O^2$) of fetal blood was estimated from $P_O^2$ and pH using the nomogram of Meschia et al. (1961).

**Chemical Analysis**

Fetal blood was collected in ice-chilled test tubes containing EDTA crystals. Samples were centrifuged for 10 minutes at 1000 xg and plasma was removed and stored at $-20^o$ C.

Plasma cortisol was determined by radioimmunoassay (Clinical Assays; Travenol Labs., Inc., Cambridge, Mass.). The antiserum used in the assay kit had 100% crossreactivity for cortisol. For the determination of glucose and betahydroxybutyrate, the plasma was deproteinized by the addition of 0.2 N perchloric acid and neutralized with potassium hydrogen carbonate. After the removal of the potassium perchlorate precipitate, glucose was determined by glucose oxidase (Glucostat, Worthington Biochemical Corp., Freehold), and betahydroxybutyrate by betahydroxybutyrate dehydrogenase (Williamson and Mellanby, 1974). Fetal plasma lactate concentrations were determined on deproteinized filtrates using lactate dehydrogenase (Gutmann and Wahlefeld, 1974). Total alpha amino nitrogen was determined colourimetrically after deproteinizing the plasma with tungstic acid (Mason et al., 1973).

Samples were analyzed in duplicate and presented as means ($\pm$ SEM). Student's t-test was used to test the statistical significance of the changes observed during surgery and day 1 after surgery. To determine the time required for the fetus to return to stable levels, an analysis of variance was done on values between days 1-4 and 5-9 following surgery.
Results

The gestational age when surgery was performed, the body weight of the ewes and the morphometric measurements of fetuses are given in Table 2. During the course of this experiment, 23 fetuses were born alive, 3 were experimentally terminated and 8 were born dead. The average gestational age in the operated ewes was 139.3 ± 0.8 days. At the time of weaning the mean body weight was 22.6 ± 0.7 kg as compared to 18-23 kg of those born naturally and reared under identical conditions.

Fetal and maternal pH, PCO$_2$ and PO$_2$ values are presented in Figure 1 and Appendix Table 5. During the course of surgery maternal and fetal blood gas parameters were altered substantially. It was noticed that maternal and fetal pH values were significantly (P< 0.05) lower during surgery, and were associated with a significant (P< 0.05) increase in fetal PO$_2$. The average increase in fetal PO$_2$ was 2.9 mm Hg/0.1 pH unit change in maternal blood. The fetal venous S0$_2$ also increased from 54 to 68% during surgery. In the post-surgical period a rapid return of the blood gas values to stable levels within 24 hours after surgery was observed (Figure 1 and Appendix Table 5). There were no significant differences in the fetal blood gas parameters between days 1-4 and 5-9 following surgery, indicating that these parameters remained relatively stable for 9 days.

The relative changes in fetal hematocrit, plasma metabolites and cortisol concentrations were used as additional criteria of intrauterine fetal normality (Figures 2, 3 and Appendix Table 6). Fetal hematocrits were elevated during the surgical period and did not return to stable levels until 5 days after surgery. Similarly, fetal plasma glucose, lactate, alpha amino nitrogen and beta-hydroxybutyrate increased by 60.2,
Table 2: Gestational age and body weights of ewes and fetuses (Mean ± S.E.M.)

<table>
<thead>
<tr>
<th>Gestational age at surgery (days)</th>
<th>Gestational age at birth (days)</th>
<th>Ewe body weight (Kg)</th>
<th>Recorded birth weight (Kg)</th>
<th>Curved crown to rump length (cm)</th>
<th>Weaning Weight at 60 days (kg)</th>
<th>Fetal outcome at birth</th>
</tr>
</thead>
<tbody>
<tr>
<td>122.7</td>
<td>139.3</td>
<td>63.73</td>
<td>3.42</td>
<td>40.2</td>
<td>22.6</td>
<td>8 born dead</td>
</tr>
<tr>
<td>+ 1.2</td>
<td>+ 0.8</td>
<td>+ 2.18</td>
<td>+ 0.20</td>
<td>+ 0.9</td>
<td>+ 0.71</td>
<td>23 born alive</td>
</tr>
</tbody>
</table>
Fig. 1 Post-surgical changes (± SEM) in blood gas parameters and hematocrit in the ovine fetus in utero. (●) indicates day of surgery; F.PO₂ = fetal PO₂ (●); M.PO₂ = maternal PO₂ (o); n=10
Fig. 2  Post-surgical changes (± SEM) in plasma metabolite levels in the ovine fetus in utero (↑ indicates day of surgery; n=5)
16.8, 27.7 and 5.8% respectively, over the levels in the post-surgical period (Figure 2). Hematocrit and plasma metabolite concentrations were significantly different (P< 0.05) between post-operative days 1-4 and 5-9, indicating that metabolite concentrations take longer to reach stable levels than blood gas parameters.

Fetal plasma cortisol concentrations were relatively high (4.8 ug/100 ml) during surgery as compared to the post-surgical period (Figure 3). Stable levels (1.8 ug/100 ml) were reached by the third day and significant changes were not observed thereafter until 4-2 days prior to parturition when steep increases were noticed.

Discussion

Experiment 1 was undertaken to determine the standard surgical techniques to cannulate blood vessels in the ovine fetus in utero for the purpose of studying fetal metabolism. Results indicate that fetal development in utero is not significantly altered following intrauterine fetal surgery. The fetal body weights and crown to rump lengths recorded at birth correspond to published values at this time of gestation (Joubert, 1956). Based on physical growth parameters it appears that both intrauterine development and postnatal growth up to weaning are not affected by the stress of surgical procedures used in this study.

Several workers have preferred to employ spinal or epidural anaesthesia to minimize the effects of general anaesthetic agents on the fetus (Meschia et al., 1965a; Towell and Liggins, 1976; and Clapp et al., 1977). Pentobarbital sodium (Nembutol) has been found to be satisfactory by Comline and Silver (1970) and Pearson and Mellor (1975). Halothane was not found suitable by Comline and Silver (1970). However, others (Willes et al., 1969; Barnes et al., 1977) have found it useful. In the
Fig. 3 Post-surgical changes (± SEM) in plasma cortisol levels in the ovine fetus in utero. (n=3)
present study, the use of Halothane at a concentration of 1.0-1.75% with an oxygen flow rate of 1.0 l/minute for maintenance gave adequate depth of anaesthesia and rapid recovery.

The increases in fetal PO$_2$ observed during surgery correlate positively with changes in maternal PCO$_2$ and negatively with changes in maternal and fetal pH. This finding is in agreement with the observations made by Motoyama et al. (1967), who attributed this to combined changes in maternal-fetal oxygen transfer within the placenta and variations in maternal pH. Increased fetal PO$_2$ can be attributed to maternal acidosis (pH 7.258), which decreases the affinity of the maternal blood for oxygen thus increasing the amount available for transplacental exchange. Furthermore, the fetal PO$_2$ will also increase due to the shift in the fetal oxygen disassociation curve because of the existing fetal acidosis (pH 7.277) during surgery. The increases in PO$_2$ values during halothane anaesthesia in ruminants has also been attributed to depressed body metabolism and adequate oxygenation (Gates et al., 1971). Acute changes in maternal blood gas parameters have been reported to result in corresponding variations in fetal circulation, the magnitude of which would depend on the initial maternal levels (Motoyama et al., 1967; Matalon et al., 1978).

In the post surgical period, maternal and fetal blood gas parameters were within the range reported by other investigators (Comline and Silver, 1972; Shelley, 1973). The rapid return of blood gas values to stable levels within 24 hours after surgery indicates that the fetal oxygenation has not been affected by the level of halothane used or surgical trauma.

Fetal metabolite and cortisol in fetal plasma were used as additional criteria of intrauterine normality. The high PO$_2$ levels
observed in this study (Figure 1) precludes hypoxia as the cause of elevated metabolite levels. Increased fetal cortisol output observed in this study (Figure 3) and decreased metabolism due to anaesthetic agents (Gates et al. 1971) may account for the elevated metabolites during surgery.

The metabolite levels in the post-surgical period are similar to those reported by Shelley (1973) and Jones et al. (1977). The rate at which specific metabolites return to stable levels would depend upon homeostatic mechanisms operating in the mature fetus and maternal metabolic changes. The low permeability of the ovine placenta to lactate and the high Km for lactate dehydrogenase isoenzyme I in the ovine fetal heart and liver probably account for the delayed return of lactate to basal levels. The increase in fetal alpha amino nitrogen following surgery may be ascribed to increased protein breakdown due to the surgical trauma (Clapp et al. 1977; Young et al. 1975). The return of the concentrations of alpha amino nitrogen to stable levels 5-6 days following surgery supports similar conclusions from studies on other parameters of nitrogen metabolism such as urea production rates (Gresham et al. 1972a). In the light of these findings the interval of 10-12 days suggested by Slater and Mellor (1977) for the initiation of metabolic studies appears too long. The fluctuations in fetal plasma betahydroxybutyrate in the post surgical period are difficult to explain because the effects of surgical stress may be masked by increasing gestational age occurring simultaneously, particularly in older fetuses (Jones, 1977).

The observed increase in fetal cortisol concentrations during surgery cannot be attributed to hypoxia since there is evidence of adequate oxygenation as indicated by high P0₂ values. The fact that the fetal adrenal gland is relatively unresponsive to ACTH stimulation (Liggins,
1977; Jones et al., 1977) and the placenta is permeable to cortisol suggests that a significant proportion of fetal cortisol may be derived from maternal blood (Jones et al., 1977). Fetal acidosis may also be responsible for the increased cortisol levels as shown by Nathanielsz et al. (1972). The stable levels of cortisol after 3 days following surgery provide additional support that the fetus and the ewe have recovered from surgical stress. The rise in cortisol levels 4 days prior to parturition is in agreement with the observations of Bassett and Thorburn (1969) and may be related to increased adrenal cortex size and maternal hormonal changes which occur shortly prior to parturition (Liggins, 1977).

Conclusions

Experiment 1 was undertaken to standardize a surgical technique that would allow for chronic experimentation of the ovine fetus in utero. Evaluation of the metabolic status of chronic fetal preparations for future physiological studies was also made. The successful cannulation of fetal blood vessels and maintenance of blood catheters for a prolonged period of time, without interfering with the normal growth and development of the fetus, was accomplished.

From the results of maternal and fetal blood gas parameters and fetal plasma metabolite and cortisol concentrations it has been shown that fetal metabolite concentrations take substantially longer to return to stable levels than blood gas partial pressures and pH. Therefore, the normality of chronically catheterized fetuses cannot be assessed only by blood gas and pH measurements.

Using fetal blood metabolites in conjunction with blood gas parameters as criteria, it is concluded that under the conditions of the
surgical technique used in this study, intrauterine fetal normality is reached by the 5th postoperative day.
Experiment II

Substrate turnover and interrelationship in the ovine fetus in utero

Introduction

Utilization of metabolic substrates by the ovine fetus has received considerable attention in an attempt to evaluate the caloric requirements of the ovine fetus in utero. Since the initial conclusions made by Bohr (1931) that glucose was the sole oxidative substrate of the ovine fetus, particular interest has been directed at quantitating the metabolism of glucose in the fetus.

Current knowledge on substrate metabolism in the ovine fetus in utero may be ascribed largely to the pioneering work of Battaglia, Meschia and coworkers (Tsoulos et al., 1971; James et al., 1972; Boyd et al., 1973; Morriss et al., 1973). These workers developed the technique of umbilical catheterization and from venoarterial concentration difference and blood flow, estimated the umbilical uptake of substrates based on the Fick principle. The literature on this subject has been reviewed exhaustively (Battaglia and Meschia, 1978).

Recently, the use of tracer methodology for studying in vivo fetal substrate metabolism has been reported by many workers. Warnes et al. (1977a) injected labelled substrates into the fetal circulation and determined kinetic parameters from the disappearance of the label from the circulation. Others infused labelled substrates continuously into the fetal circulation and estimated substrate utilization and interconversion from
the specific activity after it reached plateau levels (Hodgson et al., 1980; Prior, 1980; Anand et al., 1979, 1980).

The objectives of the present experiments were to examine the utilization of substrates in the ovine fetus by tracer techniques. The irreversible disposal rate provides an estimate of fetal substrate utilization in chronically catheterized ovine fetuses. Because of the short experimental period required and the availability of computer programs for the analysis of data, single injection techniques have particular advantages in fetal metabolic studies. In the present study, this technique was therefore employed to study substrate metabolism and interrelationship with particular emphasis on the re-entry of the tracer into the fetal circulation.

EXPT II A) METABOLISM OF GLUCOSE AND LACTATE

Materials and Methods

Animals

Dorset horn ewes were used in these experiments. The maintenance of pregnant ewes and intrauterine surgical procedures were performed as detailed in Experiment 1. Cannulae were placed in the jugular vein of the ewe the morning prior to the experiment.

Metabolic Studies

All metabolic studies were performed 5-6 hours after the morning feeding to minimize variability associated with differences in the postprandial interval. Adequate recovery from surgery was ensured by monitoring blood gas and pH of both the ewe and fetus. Prior to the experiment, the radioactive substrate was made to volume in 1.0 ml of sterile saline.
(0.15 M). The ewe was kept standing during the course of the 3-hour experiment.

Fetal lambs were given a single intravenous injection of 50 μCi of a mixture of \([U^{-14}C]\) and \([2-^3H]\) glucose and the cannulae were flushed immediately with 1.5 ml of sterile saline. In some fetuses, \([U^{-14}C]\) glucose or \([1-^14C]\) lactate was injected separately. To monitor rapid changes in the early phase of the specific activity-time curve, fetal blood sampling was initiated 2 minutes following the injection of labelled substrates and was continued uninterruptedly for 5 minutes. This was followed by sampling at five minute intervals up to 30 minutes. Thereafter samples were obtained less frequently up to 3 hours. Maternal blood samples were collected from 4 minutes following the injection, on a less frequent basis up to 3 hours. Fetal and maternal blood gas and pH values were monitored, as described in Experiment 1, periodically during the course of the experiment.

Whole blood samples were immediately transferred to ice chilled test tubes containing EDTA crystals. Two hundred microliters of whole blood were deproteinized with 3.0 ml of 0.33 N perchloric acid at 0°C. The protein precipitate was removed by centrifugation at 7,000 x g for 15 minutes in a Sorval refrigerated centrifuge. The supernatant was pipetted into test tubes and stored at -40°C.

**Chemical Methods**

Frozen deproteinized blood filtrates were thawed in cold tap water and recentrifuged prior to analysis. Supernatants were neutralized with KOH and allowed to stand in ice for 45 minutes. The insoluble KC\(_{104}\) precipitate was removed by centrifugation at 0°C and the neutralized supernatant was transferred to freeze drying flasks. The KC\(_{104}\) precipitates were washed...
twice with ice cold water and the combined supernatants and washes were pooled in respective freeze drying flasks and lyophilized (Lab Conco freeze drier). Samples were reconstituted in 1.5 ml of demineralized, distilled water.

Initial fractionation of blood metabolites into basic, neutral and acidic fractions was accomplished by passing the neutralized supernatant successively through cation (AG 50X-8 [H⁺] 200-400 mesh) and anion (AG 1X-8 [formate] 200-400 mesh) (Biorad) exchange resins packed in plastic columns, 0.7 x 5.0 cm (Econocolumns, Biorad). Ion exchange columns were arranged in series with a common deionized water reservoir supplying each column. Cation and anion resins were prepared by the application of 20 volumes of 2N HCl and 1.0 N formic acid respectively, followed by a thorough washing with CO₂ free demineralized water. Resins were stored in sealed containers at 4°C. The bottom of the columns packed with these ion exchange resins was equipped with a small tygon tubing to which a screw clamp was attached to control the flow rate.

1. Assay of Glucose Specific Activity
   a) Radiochemical purity of glucose in fetal whole blood

   The neutral fraction eluted from the anion exchange column was reconstituted in 1.5 ml of demineralized distilled water. For measurement of radioactivity, glucose was quantitatively converted to gluconic acid by incubating the neutral fraction with glucose oxidase (50 units, specific activity 20,000 units/g, Sigma, St. Louis) and catalase (3,000 units, specific activity 65,000 units/mg, Böhringer Mannheim Dorval) at 37°C for 2 hours. The incubation mixture was then passed through a reconditioned anion exchange column and the adsorbed gluconic acid eluted with 8N formic acid. The
recovery of labelled glucose under these conditions was $92.09 \pm 0.71\%$ (Appendix Table 1). To eliminate possible contamination of $[^{14}\text{C}]-\text{glucose}$ with other neutral compounds, particularly fructose which is present in significant amounts in fetal blood, $[^{14}\text{C}]-\text{fructose}$ and $[^{14}\text{C}]-\text{glycerol}$ were subjected to ion exchange chromatography separately and in combination with $[^{14}\text{C}]-\text{glucose}$. Radioactivity in the gluconic acid fraction appeared only when $[^{14}\text{C}]-\text{glucose}$ was added indicating the absence of contamination with fructose or glycerol.

b) Determination of glucose and fructose concentration in the neutral fraction

Glucose in the neutral fraction was determined by a modification of the glucose oxidase procedure. Fructose in the neutral fraction was determined by the anthrone procedure (Nixon, 1969).

2. Assay of Lactate Specific Activity

a) Radiochemical purity of lactate in fetal whole blood

The acidic components adsorbed on the anion exchange resin were eluted with 4 N formic acid, neutralized with NaOH and evaporated. Samples were reconstituted in 1.0 ml of demineralized water and an aliquot was used for radioactive measurements. The validity of this procedure for determining $[^{14}\text{C}]-\text{lactate}$ radioactivity was confirmed by thin layer chromatography. Standard $[^{14}\text{C}]-\text{lactate}$ solutions and samples were applied in 4 cm streaks on Eastman Silica gel Chromatogram Sheets (Eastman, Kodak) and developed twice in acetone:n-propanol:water (6:3:1 v/v) in the same direction. The chromatograms were scanned on an Actigraph III radiochromatograph system (Nuclear Chicago). Radioactive areas corresponding to lactate standards were cut out, scraped into scintillation vials and suspended in 10 ml of scintillation fluid for counting. No contamination of glucose in the lactate fraction was detected. The combined recovery of added $[^{14}\text{C}]-\text{lactate}$ for both procedures was $83.0 \pm 0.63\%$ (Appendix Table 2).
b) Determination of lactate concentration in the acidic fraction

Lactate in the acidic fraction was determined by the enzymatic procedure described by Gutmann and Wahlefeld (1974).

Radioactive Chemicals

[U-14C] glucose, (304 mCi/mmole), [2-3H] glucose, (17.9 Ci/mmole), [1-14C] lactate (51 mCi/mmole), and [U-14C] lactate (60 mCi/mmole) were obtained from Amersham Searle Corp. (Oakville, Ont.). The liquid scintillation solution used was composed of 0.5 ml of aqueous sample and 10 ml of scintillation cocktail (PCS, Amersham Searle Corp.). The scintillation fluid used for counting radioactivity present on silica gel thin layer sheets was prepared by mixing 3.85 litres of 1,4 dioxane, 3.85 litres of xylene, 2.3 litres of absolute ethanol, 800 g of naphthalene, 50 µg of PPO and 0.5 µg dimethyl-POPOP (Amersham Searle Corp.).

Radioactive Counting Procedures

Radioactivity was measured by liquid scintillation spectrometry (LKB, Rack Beta 1215). Quenching was determined by external standard ratios. Automatic quench calibration and calculation of dpm values were performed for both single and doubly labelled samples. Counting efficiencies were 35-40% and 50-60% for [3H] and [14C] respectively, with 30% of the [14C] radioactivity appearing in the [3H] channel.

Analysis of Data

Specific activity values at each sampling time are expressed as a fraction of the initial dose of tracer injected at zero time (Shipley and Clark, 1972). Initial kinetic parameter estimates were obtained using a Fortran computer program, Autoan, (Sedman and Wagner, 1976) to fit the data to the equation
\[ S.A._t = \sum_{i=1}^{n} A_i e^{-a_i t} \]

where

S.A. = specific activity at time t (in fraction dose/mg C);
A = zero time intercept of each exponential component (fraction dose/mgC);
n = number of components; i = component identification;
a = rate constant for each component (min\(^{-1}\)); t = time (min).

The F value and the sum of square deviations were used to evaluate the goodness of fit of the estimates.

\[ F = \sum_{i=1}^{n} (S_{Ai} - \hat{S}_{Ai})^2 \]

where

\( S_{Ai} \) = observed specific activity
\( \hat{S}_{Ai} \) = estimated specific activity

The set of parameters with the minimal F value was chosen as the initial estimate. The number of exponentials increased until the per cent improvement in the goodness of fit was no longer significant. The non-linear program of Metzler (1969), (NONLIN Michigan) was used to give the least squares estimate of the parameters. The squared correlation coefficient \( (R^2) \) indicating the goodness of fit of the data was calculated as follows:

\[ R^2 = \frac{W_{ys} - W_s}{W_{ys}} \]

where

\( W_{ys} \) = weighted sum of squared observations = \( \sum_j W_{ij}(Y_{ij} - \bar{Y}_i)^2 \)
\( W_s \) = sum of weighted squared deviation = \( \sum_j (Y_{ij} - Y_{calcij})^2 \cdot W_{ij} \)
From the result of the final least squares estimates, the observed data was found to be best described by a biexponential equation.

Model Description

In this study the term compartment refers to anatomically identifiable space (e.g., fetal and extrafetal compartments, Fig. 4). The term pool has been used to denote different locations within the fetal compartment (blood; primary pool (a) and tissue, secondary pool (b)) or chemically identifiable spaces (e.g., glucose, lactate, etc.)

Fig. 4 Model of glucose and lactate metabolism in the ovine fetus.
where

\( k_{ba} \) = rate constant (pool a to pool b)

\( k_{ab} \) = rate constant (pool b to pool a)

\( k_{oa} \) = irreversible disposal rate constant

\( \text{---} \) = recirculation (tracer re-entering primary pool after transformation)

\( \text{---} \) = recycling (tracer re-entering primary pool without transformation).

Since the basic objective of this experiment is to study the disappearance of the tracer from the fetal blood pool, the biexponential parameters were formulated into a two pool open exchange system to depict the transfer of the label into and out of the primary fetal blood pool. No attempts were made to identify or sample the large number of pools and/or compartments in which the fetal and extrafetal compartments may be embedded or are in communication with each other.

Calculations

The following equations were used for estimation of kinetic parameters of substrate metabolism:

1. Substrate pool \((Q) = \frac{1^*}{\sum_{i=1}^{n} (A_i)} \) (White et al., 1969)

\((\text{mgC/kg})\)

where

\( A \) = zero time intercept of specific activity (S.A.);

\( i \) = exponential component number; \( n \) = number of exponential components; \( 1^* \) = normalized dose.
2. Irreversible rate of disposal (D.R.) = $\frac{1^*}{\int_0^\infty S.A. \, (dt)}$ (mgC/min/kg)

(Shippley and Clark, 1972)

3. Volume of distribution ($V$) = $\frac{Q \times 100}{\text{blood conc.} \times \text{body weight}}$ (% body weight)

(White et al., 1969)

4. Metabolic clearance rate (M.C.R.) = $\frac{\text{D.R.}}{\text{blood conc.}}$ (ml/min)

(Shippley and Clark, 1972)

5. Half life $T_{1/2} = \frac{0.693}{\alpha}$ (minutes)

$T_{1/2} = \frac{0.693}{\beta}$

where $\alpha$ and $\beta$ are rate constants. $T_1$ and $T_2$ are rapid and slow components respectively.

The transfer of tracer between the fetal and maternal compartments was estimated from the ratio of the area under the specific-activity-time curves integrated to time infinity in these two compartments.

6. Per cent of maternal glucose carbon derived from fetal glucose carbon

$$\int_0^\alpha S.A. \, \text{Maternal glucose C} \, (dt) \times 100$$

$$\int_0^\alpha S.A. \, \text{Fetal glucose C} \, (dt)$$
The re-entry of the label back into the fetal circulation may occur by the tracer leaving the fetal blood pool and, after a sojourn in some other compartment, returning in its original form. This will be referred to as "recycling". If the tracer returns to the primary fetal blood pool after chemical transformation and reincorporation into newly formed molecules, the process will be referred to as "recirculation" (Hetenyi and Norwich, 1974).

Recirculation was estimated according to Dunn et al., 1969 by injecting a mixture of glucose labelled with [2-$^3$H] and [U-$^{14}$C] and measuring the half life with each isotope.

7. Recirculation = 1 - \left( \frac{T_{1/2}^{[3H]}}{T_{1/2}^{[14}C]} \right)
   \hspace{1cm} \text{(Fraction of irreversible disposal rate)}
   \hspace{1cm} \text{where } T_{1/2} = \text{total half life}

Recycling is expressed as the fraction of total turnover which re-enters the circulation after a sojourn in some other part of the system.

8. Fraction lost irreversibly (\phi) = \frac{MCR}{K_T \cdot V} \hspace{1cm} \text{(Gurpide and Mann, 1970)}
   \hspace{1cm} \text{where MCR = metabolic clearance rate (ml/min)}
   \hspace{1cm} K_T = \text{total turnover rate constant}
   \hspace{1cm} V = \text{volume of distribution}
   \hspace{1cm} \phi = \text{Fraction of total turnover irreversibly lost.}
9. The fraction 1-\( \phi \) therefore indicates the fraction of total turnover which returns to the circulation through recycling.

In the dynamic state of transfer of metabolites between the fetus and the mother, molecules differ in their rates of exchange and mechanisms of transport across the placental barrier with the result that different compounds vary in the time spent within and outside the fetal vascular space. Therefore the time of residence during one (mean transit time, \( \overline{t} \)) or all (mean total residence time, \( \overline{T} \)) passages of the labelled substrate through the fetal circulation and the number of times a particle returns to the circulation after its initial passage through it (number of cycles, \( \gamma \)) were calculated according to Rescigno and Gurpide (1973).

10. Mean transit time (\( \overline{t} \)) = \( \frac{1}{K_T} \)

where \( K_T \) = total turnover rate constant.

11. Mean total residence time (\( \overline{T} \)) = \( \frac{Q}{D.R.} \)

where \( Q \) = pool size

\( D.R. \) = irreversible disposal rate.

12. Number of cycles (\( \gamma \)) = \( \left( \frac{T_1}{t_1} \right) - 1 \)

The conversion of glucose to lactate in the fetal compartment was estimated by the ratio of integrals of the specific activity-time curves of these metabolites taken to time infinity (Shipley and Clark, 1972).
13. Fraction of lactate carbon derived from glucose carbon =

\[
\frac{\int_{0}^{\infty} S.A. \text{ lactate C (dt)}}{\int_{0}^{\infty} S.A. \text{ glucose C (dt)}}
\]

14. Rate of lactate C derived from glucose (mgC.min/kg) =

Equation 13 \times \text{lactate irreversible disposal rate}

15. Per cent of glucose irreversible disposal rate going to lactate =

\[
\frac{\text{Equation 14}}{\text{Glucose irreversible disposal rate}} \times 100
\]

All results were expressed as means (+ SEM). Statistical analysis of the data was performed using the paired and independent t-test, where appropriate. The linear regression lines and correlation coefficients were calculated by the method of least squares.

Results

The mean (+ SEM) gestational age, maternal and fetal body weight, blood gas and metabolite measurements recorded at the time of each experiment are presented in Table 3. Very small differences were noted among maternal and fetal body weights and metabolite concentrations showing uniformity of the preparations used. Blood gas parameters and metabolite concentrations were within the range reported previously for well...
Table 3: Maternal and fetal physiological parameters during the experimental period.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>[U-(1^4)C] glucose</th>
<th>[U-(1^4)C][2-(3^)H] glucose</th>
<th>[1-(1^4)C] lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M (n=7)</td>
<td>F (n=8)</td>
<td>F (n=8)</td>
</tr>
<tr>
<td>Body weight (Kg)</td>
<td>60.9 ±1.77</td>
<td>2.36 ±0.10</td>
<td>65.6 ±2.97</td>
</tr>
<tr>
<td>Gestational Age (Days)</td>
<td>- 129.14 ±1.99</td>
<td>- 137.5 ±0.98</td>
<td>- 127.1 ±3.113</td>
</tr>
<tr>
<td>pH</td>
<td>7.502 ±0.02</td>
<td>7.380 ±0.02</td>
<td>7.466 ±10.02</td>
</tr>
<tr>
<td>pCO(_2) (mm Hg)</td>
<td>30.17 ±2.64</td>
<td>38.09 ±1.36</td>
<td>31.27 ±0.89</td>
</tr>
<tr>
<td>pO(_2) (mm Hg)</td>
<td>33.35 ±1.13</td>
<td>18.61 ±0.47</td>
<td>32.8 ±2.04</td>
</tr>
<tr>
<td>TCO(_2) (meg/L.)</td>
<td>- 23.60 ±1.02</td>
<td>- 22.81 ±0.75</td>
<td>- 23.71 ±1.65</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>34.50 ±1.02</td>
<td>34.65 ±1.31</td>
<td>31.27 ±0.89</td>
</tr>
<tr>
<td>Glucose (mM)</td>
<td>3.390 ±0.12</td>
<td>0.842 ±0.03</td>
<td>3.750 ±0.09</td>
</tr>
<tr>
<td>Fructose (mM)</td>
<td>N.D. ±0.31</td>
<td>3.86 ±0.15</td>
<td>N.D. ±0.15</td>
</tr>
<tr>
<td>Lactate (mM)</td>
<td>1.18 ±0.08</td>
<td>1.81 ±0.06</td>
<td>1.55 ±0.14</td>
</tr>
</tbody>
</table>

1 Values are means (+ SEM)
M = Maternal
F = Fetal
oxygenated near-term fetuses (Experiment 1), indicating recovery from surgical procedures by the ewe and fetus.

1. Description of specific activity-time curves for glucose and lactate

Specific activity-time curves, plotted on semilogarithmic coordinates following the single injection of labelled substrates are presented in Figs. 5, 6 and 9 along with whole blood glucose or lactate concentrations during the course of the experiments. The concentrations of glucose and lactate were relatively constant during the experimental period. In all experiments performed with radioactive labelled glucose or lactate, the observed data was found to fit ($R^2 < 0.985$) a biexponential equation. After the injection of labelled substrates there was an initial rapid decline in the specific activity. A slower linear decline in the specific activity followed the initial decay for the duration of the experiment. The specific activity of tritiated glucose was lower than that of $[U^{14}C]$ glucose, which was particularly marked at the latter time periods (Fig. 6).

2. Kinetic parameters of glucose and lactate metabolism

a) Glucose metabolism

The kinetic parameters of glucose metabolism calculated from specific activity-time curves when a mixture of $[U^{14}C]$ and $[2^{-3}H]$ glucose or $[U^{14}C]$ glucose alone was injected are summarized in Table 4. No significant differences in the glucose pool size and volume of distribution were noted with $[U^{14}C]$ and $[2^{-3}H]$ labelled glucose, when tested by a paired t-test ($P < 0.05$). The mean (+ SEM) irreversible disposal rates and metabolic clearance rates calculated from $[U^{14}C]$ glucose were $3.541 \pm 0.25 \text{ mg C/min/kg}$ and $140.80 \pm 4.84 \text{ m1/min}$ respectively. These
Fig. 5 Semilogarithmic plot of glucose specific activity (S.A.) versus time following injection of [U-¹⁴C] glucose and whole blood glucose concentration. Values are means (± SEM; n=7)
Fig. 6  Semilogarithmic plot of glucose specific activity (S.A.) versus time following the injection of a mixture of [U-14C] and [2-3H] glucose and whole blood glucose concentration. Values are means (± SEM; n=8).
values were significantly (P < 0.05) lower than the values obtained with
\[^{3}H\]-glucose, (4.07 ± 0.16 mgC/min/kg and 155.10 ± 5.30 ml/min respectively).
The metabolic half lives of the slower decaying components obtained from
\[^{3}H\]-glucose (44.34 ± 2.21 min) were significantly (P < 0.05) lower than
from \[^{14}C\]-glucose (50.66 ± 2.69 min).

In an attempt to determine if glucose metabolism was correlated
with fetal development, [U-\(^{14}\)C] glucose was injected separately into younger
fetuses of lower body weight. The specific activity-time curves obtained
from [U-\(^{14}\)C] glucose in these fetuses were similar to those obtained in
heavier fetuses (Fig. 5). The mean (± SEM) irreversible disposal rates
and metabolic clearance rates estimated in these fetuses were 2.251 ±
0.15 mgC/min/kg and 99.08 ± 2.83 ml/min respectively (Table 4). These value
were significantly (P < 0.05) lower than those obtained with fetuses of
higher body weight. The mean apparent volume of distribution of glucose
noted in these experiments was 41.38 ± 2.4% and was significantly greater
(P < 0.05) than that observed previously with [U-\(^{14}\)C] glucose in older
fetuses.

There was a positive correlation between fetal plasma glucose
concentration and irreversible disposal rate (r = 0.67, P < 0.05,
Fig. 7). A similar positive correlation was observed between fetal body
weight and glucose irreversible disposal rate (r = 0.61, P < 0.05, Fig. 8).

The maternal-fetal ratio of the area under the specific activity-
time curves was 0.092 ± 0.002 in the case of [U-\(^{14}\)C] glucose whereas it
was 0.141 ± 0.002 in the case of [2-\(^{3}\)H] glucose.
Fig. 7 Linear regression of fetal irreversible disposal rate of glucose (mgC/min/kg) versus blood glucose concentration (mg/100 ml) after single injection of [U-14C] glucose. (Y=0.671x - 3.86; P < 0.05; r=0.66; n=15)
Fig. 8 Linear regression of fetal irreversible disposal rate of glucose (mgC/min/kg) versus body weight (kg) after single injection of [U-14C] glucose. ($Y = 2.635x + 0.094; P < 0.05; r = 0.600; n = 15$)
Fig. 9 Semilogarithmic plot of lactate specific activity (S.A.) versus time following the injection of [1-14C] lactate and whole blood lactate concentrations. Values are means (± SEM; n=8).
Table 4  Kinetic parameters of glucose metabolism in the ovine fetus, estimated using single injection of a mixture of [U-\(^{14}\)C], [2-\(^{3}\)H] glucose or [U-\(^{14}\)C] glucose alone

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Age (days) and Body weight (Kg)</th>
<th>Irreversible Rate of Disposal (mgC/min/Kg)</th>
<th>Metabolic Clearance Rate (MCR) (ml/min)</th>
<th>Volume of distribution v. (% Bwt.)</th>
<th>Pool Size mgC/Kg (Q)</th>
<th>Half lives (T1/2) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[U-(^{14})C]</td>
<td>137.5(^{a}) ±0.98</td>
<td>3.541(^{a}) ±0.251</td>
<td>140.8(^{a}) ±4.8</td>
<td>30.0(^{a}) ±2.65</td>
<td>21.72(^{a}) ±2.16</td>
<td>1.16 ±0.10</td>
</tr>
<tr>
<td>[2-(^{3})H] glucose (n=8) (2.91 Kg)</td>
<td></td>
<td>4.070(^{b}) ±0.162</td>
<td>155.1(^{b}) ±5.3</td>
<td>32.0(^{a}) ±3.80</td>
<td>25.58(^{a}) ±3.12</td>
<td>1.07 ±0.15</td>
</tr>
<tr>
<td>[U-(^{14})C]</td>
<td>129.1(^{b}) ±1.99</td>
<td>2.251(^{c}) ±0.15</td>
<td>99.08 (^{c}) ±2.83</td>
<td>41.38(^{b}) ±2.40</td>
<td>24.66(^{a}) ±1.32</td>
<td>1.47 ±0.13</td>
</tr>
</tbody>
</table>

\(^{1}\) Values = means (± SEM)
\(^{a, b, c}\) Superscripts with different alphabets in the column denote statistical significance (P < 0.05)
T\(_{1}\), T\(_{2}\) - denote half life of fast and slow decaying components respectively.
b) Lactate metabolism

The metabolic parameters of lactate calculated from [1-14C] lactate specific activity-time curves are summarized along with the pooled [U-14C] glucose data in Table 5. The lactate pool size was 29.10 ± 2.69 mgC/kg which was significantly (P < 0.05) larger than the glucose pool size. The irreversible rate of disposal of lactate (2.576 ± 0.182 mgC/min/kg) was less than that observed with [U-14C] glucose, which however, was not significantly different. Lactate metabolic clearance rate (82.03 ± 5.62 ml/min) was significantly (P < 0.05) less than that of glucose. The apparent volume of distribution of lactate (43.93 ± 5.11% of fetal body weight) was significantly larger (P < 0.05) than that of glucose. No radioactivity was observed in fetal blood glucose when [1-14C] lactate was injected into the fetus. Similarly, lactate label was not detected in the maternal circulation following [1-14C] lactate injection into the fetus. The parameters obtained in the single experiment where [U-14C] lactate was injected are very similar to those obtained using [1-14C] lactate (Table 5).

The extent of glucose conversion to lactate is given in Table 6. The per cent of lactate carbon derived from glucose carbon is 44.16 ± 5.40, which results in the formation of 1.223 mgC/min/kg of lactate from glucose. The per cent of glucose irreversible disposal rate which goes to lactate is 35.92 ± 4.30.

c. Re-entry of glucose and lactate carbons

The recycling of glucose and lactate which represents the return of metabolic tracer to the sampled compartment after a sojourn in some other part of the system was 69.6, 66.1 and 80.2 per cent for [U-14C] and [2-3H] glucose and [1-14C] lactate respectively (Table 7). In the
Table 5 Kinetic parameters of glucose and lactate metabolism in the ovine fetus, estimated by using single injections of \([U^{-14}C]\) glucose or \([1^{-14}C]\) lactate.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Pool Size (Q) (mgC/kg)</th>
<th>Irreversible Rate of Disposal (D.R.) (mgC/min/Kg)</th>
<th>Metabolite Clearance Rate (MCR) (ml/min)</th>
<th>Volume of Distribution (v) (% Bwt)</th>
<th>Half life (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>([U^{-14}C]) glucose(^2)</td>
<td>23.10 ±1.32</td>
<td>2.935 ±0.210</td>
<td>121.56 ±9.03</td>
<td>35.27 ±1.33</td>
<td>1.30 ±0.112</td>
</tr>
<tr>
<td>(n=15)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>([1^{-14}C}) lactate</td>
<td>29.10 ±2.69</td>
<td>2.576 ±0.182</td>
<td>82.03 ±5.62</td>
<td>43.93 ±5.11</td>
<td>1.36 ±0.17</td>
</tr>
<tr>
<td>(n=8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>([U^{-14}C}) lactate</td>
<td>27.87 ±1.173</td>
<td>2.171</td>
<td>75.36 ±5.11</td>
<td>45.10 ±4.11</td>
<td>1.62 ±0.17</td>
</tr>
<tr>
<td>(n=1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Values = mean (± SEM)  
2 Includes all \([U^{-14}C]\) glucose experiments from table 4  
\(^a,b\) Superscripts with different alphabets in the column denote statistical significance (P < 0.05)  
\(T_1\) and \(T_2\) = denotes the half life of fast and slow decaying components respectively.
Table 6  Glucose-lactate conversions in the ovine fetus in utero.

<table>
<thead>
<tr>
<th>Fetus</th>
<th>% lactate C derived from glucose C¹</th>
<th>Rate of formation of lactate from glucose (mgC/min/kg)²</th>
<th>% glucose going to lactate³</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>35.5</td>
<td>0.915</td>
<td>26.4</td>
</tr>
<tr>
<td>2</td>
<td>29.7</td>
<td>0.765</td>
<td>21.5</td>
</tr>
<tr>
<td>3</td>
<td>33.2</td>
<td>0.855</td>
<td>42.0</td>
</tr>
<tr>
<td>4</td>
<td>52.2</td>
<td>1.345</td>
<td>32.7</td>
</tr>
<tr>
<td>5</td>
<td>44.8</td>
<td>1.154</td>
<td>30.3</td>
</tr>
<tr>
<td>6</td>
<td>71.6</td>
<td>1.844</td>
<td>44.5</td>
</tr>
<tr>
<td>7</td>
<td>42.11</td>
<td>1.085</td>
<td>54.0</td>
</tr>
<tr>
<td>Mean (± SEM)</td>
<td>44.16 ± 5.40</td>
<td>1.223 ± 0.159</td>
<td>35.92 ± 4.30</td>
</tr>
</tbody>
</table>

¹ Equation 13  
² Equation 14  
³ Equation 15
Table 7 Estimation of recycling and recirculation of glucose and lactate in the ovine fetus.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Isotopes injected</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[U-(^{14})C] glucose (n=8)</td>
</tr>
<tr>
<td>Fraction of total turnover irreversibly lost</td>
<td>0.299(^a) ±0.051</td>
</tr>
<tr>
<td>Fraction of total turnover recycled</td>
<td>0.696(^a) ±0.062</td>
</tr>
<tr>
<td>Mean transit time ((T)) (min)</td>
<td>1.71(^a) ±0.35</td>
</tr>
<tr>
<td>Mean total residence time ((T)) (min)</td>
<td>6.21(^a) ±0.90</td>
</tr>
<tr>
<td>Number of cycles) ((\tau))</td>
<td>3.30(^a) ±0.46</td>
</tr>
<tr>
<td>Recirculation (% of irreversible disposal)</td>
<td>12.6±5.44</td>
</tr>
</tbody>
</table>

\(^1\) Values=means (± SEM)

\(^a, b\) superscripts with different alphabets in rows denote statistical significance (P<0.05)
single experiment where \([U-^{14}C]\) lactate was administered to the fetus, the fraction recycled was 79.9 per cent. No significant difference was noted in the mean transit or total residence times and the number of cycles to the primary pool between \([U-^{14}C]\) and \([2-{^3}H]\) glucose. The mean total residence time observed with \([1-{^4}C]\) lactate was significantly greater (\(P<0.05\)) than with \([U-^{14}C]\) or \([2-{^3}H]\) glucose. The number of cycles made by the lactate tracer to the primary pool was higher than that for glucose; however, this was not significant. The extent of glucose recirculation using \([{^3}H]\) and \([^{14}C]\) glucose was 12.62 ± 5.44% of the irreversible disposal rate (Table 7).

**Discussion**

The mathematical validity of compartmental analysis involving isotopic tracers used in metabolism has been discussed elsewhere (White et al., 1969; Judson and Leng, 1972; Hetenyi and Norwich, 1974; Atkins, 1980). A fundamental assumption inherent in the analysis of tracer kinetic data is that steady state conditions prevail during the experimental period. The constant level of blood glucose and lactate during the course of these experiments (Fig. 5, 6 and 9) indicate that steady state conditions prevailed.

The volume of distribution of glucose noted in this study (35.27%) exceeds the extracellular space in the fetus. The present value is lower than the estimate of 57.4% reported by Warnes et al. (1977a) using similar injection techniques. The lactate pool size and apparent volume of distribution are also lower than those reported by Warnes et al. (1977a). These differences in glucose and lactate space may be due to the lower blood glucose
and higher lactate in the fetuses used by Warnes et al. (1977a) than those used in the present study. Although the fetal blood glucose concentrations are substantially lower than maternal levels (Table 3), the volume of distribution in the fetus exceeds the extracellular fluid space of 18% reported in adult ruminants (Kronfeld and Simenson, 1961; White et al., 1969; 1980). This difference may be explained by the high glucose content and metabolic activity of fetal erythrocytes (Jarrett et al., 1964).

The irreversible disposal rates of glucose and lactate obtained in this experiment (Table 5) are similar to the values reported by Warnes et al. (1977a), using single injection techniques and to recent estimates obtained from the continuous infusion of $^{14}$C labelled glucose (Anand et al., 1979, Hodgson et al., 1980) and lactate (Prior, 1980). However, these values are considerably higher than the estimates obtained by umbilical venous-arterial differences and blood flow measurements (Tsoulos et al., 1971; James et al., 1972; Boyd et al., 1973; Comline and Silver, 1976). The major limitation of the latter procedure is that it does not take into account endogenous substrate production and thus underestimates unidirectional utilization. On the other hand, the contribution of placental metabolism and improper mixing and exchange of $^{14}$C with other substrate pools may have resulted in an overestimation of irreversible loss in the single injection technique employed.

Results from this experiment also indicate that the metabolism of glucose and lactate within the ovine fetus is more rapid than in the post-natal life. For example, White et al. (1980) have reported a glucose pool turnover time in postabsorptive lambs of 40-50 minutes. The mean total residence time of glucose from its entry into the fetal blood pool to its final exit was only $6.21 \pm 0.90$ minutes (Table 7). Similarly,
in the case of lactate the mean total residence time in the fetus was only 12.04 minutes as compared to the value of 20-30 minutes reported in the adult monogastric (Searle and Cavaleri, 1972).

The data also demonstrate that the irreversible disposal rate of glucose is proportional to the fetal blood glucose concentration (Fig. 7). It has been shown by James et al. (1972) that the fetal blood glucose concentration is directly correlated with maternal arterial glucose concentration and umbilical uptake. This relationship may be particularly significant in fetuses of starved ewes, where it has been documented that maternal starvation results in a 35% reduction in fetal plasma glucose levels (Tsoulos et al., 1971, Schreiner et al., 1978). Such an effect would potentially reduce fetal glucose utilization and impair fetal growth under conditions of reduced feed intake.

Comline and Silver (1976) reported that the availability of metabolic substrates to the fetus would depend not only on the rate of supply but also on the tissue mass involved. Fetal body mass as a potential regulator of fetal metabolism was observed in this study. A significant correlation (P < 0.05) was observed between the irreversible disposal rate of glucose and fetal body weight (Fig. 7). James et al. (1972) failed to demonstrate a correlation between umbilical glucose uptake and fetal body weight, which may be attributed to the wide variation in the measured umbilical glucose uptakes in their study. White et al. (1980) reported a significant correlation between the irreversible rate of glucose disposal and preweaning body weight. Similarly, Flecknell et al. (1980) have recently reported that the disposal rate of glucose in the neonatal pig was proportional to both the total body weight and individual organ weights. The positive relationship between fetal oxygen consumption and body weight
reported by James et al. (1972) also supports the increased utilization of glucose observed in this study in fetuses of higher body weight. It is likely that if maternal glucose transfer to the fetus is restricted during undernutrition, the fetus may have to rely on endogenous sources of glucose production to compensate for the increased metabolic demands during advanced stages of gestation.

Glucose recirculation

The reincorporation of isotopic label into endogenously produced compounds has been referred to as recirculation (Zilversmit et al., 1943; Hetenyi and Norwich, 1974). Recirculation of a labelled substrate therefore underestimates its true rates of irreversible disposal.

The simultaneous injection of \([^3\text{H}]\) and \([^{14}\text{C}]\) glucose was employed in this study to quantitate the extent of recirculation of \(^{14}\text{C}\) labelled glucose returning to the blood pool. The metabolic fate of tritium atoms has been discussed by many workers (Katz and Dunn, 1967; Judson and Leng, 1972; Katz and Rognstad, 1976). Briefly, when \([2-^3\text{H}]\) glucose is used as the tracer, tritium from carbon 2 is liberated in the glucose phosphate isomerase reaction between glucose-6-phosphate and fructose 6-phosphate and is rapidly lost through the exchange with body water (Rose and O'Connell, 1961). The use of \([2-^3\text{H}]\) glucose in combination with \([\text{U-}^{14}\text{C}]\) glucose thus yields a turnover rate which gives an estimate of recirculation through both the Cori cycle and glycogenolysis (Katz and Dunn, 1967; Judson and Leng, 1972).

The small proportion of glucose recirculation (12.64 ± 5.1%) is similar to the observations of Anand et al. (1979) who reported very little
difference in turnover rate between $[2^{-3}H]$ and $[U^{14}C]$ glucose in their continuous infusion study. The present values closely resemble those reported in adult ruminants, (10%, Annison et al., 1963; 13%, Judson and Leng, 1972) but are substantially lower than the value of 26% reported in neonatal lambs (Makamatsu et al., 1974). In rats (Hetenyi and Mak, 1970) and dogs (Issekutz et al., 1972) the extent of glucose recirculation has been reported to be 30% and 40% respectively.

**Glucose-lactate conversions**

Results of this experiment show that 36% of the glucose pool is metabolized to lactate, which accounts for 44% of lactate irreversible disposal rate (Table 6). Warnes et al. (1977a) also demonstrated a rapid labelling of lactate following the injection of $[U^{14}C]$ glucose into the fetal circulation. This proportion of lactate produced from glucose closely resembles the value of 40% reported in adult sheep. However, it is possible that part of the glucose conversion to lactate may have occurred in the placenta as shown by Burd et al. (1975) and Char and Creasy (1976a). The present experimental set up is not adequate to differentiate between fetal and placental metabolism and in the absence of umbilical catheterization no definite quantitative conclusions can be drawn on the extent of glucose conversion to lactate by the fetus alone.

When $[1^{14}C]$ lactate was injected no radioactivity could be recovered in fetal blood glucose which agrees with the results of Warnes
et al. (1977a). On the other hand, using continuous infusion of $[\text{U-}^{14}\text{C}]$ lactate, Prior (1980) has recently reported that 22% of the lactate returns to the fetal glucose pool. It is possible that in the single injection technique of $[\text{L-}^{14}\text{C}]$ lactate used in this study, the $^{14}\text{C}$ carboxyl carbon was either lost through decarboxylation to acetyl COA and oxidized or diluted beyond detectable levels by its passage through the oxaloacetate pool. Further the metabolism of lactate by the fetus may be too rapid to detect measurable activity in glucose with single injection techniques.

The ovine fetus, unlike the adult, synthesizes large quantities of glycogen in liver, heart and muscle tissues, during late gestation (Shelley, 1960). However, the apparent low turnover of the fetal glycogen pool (Setchell et al., 1972) suggests that glycogen may not contribute significantly to fetal glucose turnover during the relatively short experimental period employed in this study. Glycogenolysis therefore may not be a major source of fetal glucose recirculation.

**Glucose recycling**

In dynamic studies with tracers, the term recycling refers to the return of the tracer to the sampled pool after a sojourn in other parts of the system. (Gurpide and Mann, 1970; Rescigno and Gurpide, 1973; Hetenyi and Norwich, 1974). In the case of the fetus the blood (pool a) and tissues (pool b) may be considered to be embedded in a multicompartmental system made up of fetal fluids, placenta and maternal tissues. The large fraction of glucose and lactate recycled (70-80%) may be attributed to the possible contribution of placental metabolism of substrates and the inadequate mixing of the label with substrate pools in the fetus in the single injection techniques (Warnes et al., 1977a). The fact that the extent of recycling
was not different when $[^{14}\text{C}]$ or $[^3\text{H}]$ labelled glucose was used suggests that the glucose molecules re-enter the fetal circulation without undergoing transformation. The significantly greater fraction of lactate recycled coupled with the longer mean total residence time (Table 7) than glucose and the observation that no lactate radioactivity was found in the maternal compartment suggest that lactate is recycled in the fetal compartment until it is metabolized. This is in partial agreement with the recent report of Kastendieck et al. (1980) that up to 85% of lactate was eliminated by utilization in fetal sheep. The impermeability of the placenta to lactate (Britton et al., 1967) would also be conducive for recycling. The longer mean total residence time of lactate than glucose may also be ascribed to its significantly larger volume of distribution. The fact that the recycling of $[U-^{14}\text{C}]$ lactate did not differ from that of $[1-^{14}\text{C}]$ lactate is additional evidence that this phenomena is characteristic of the lactate molecule as a whole. On the other hand, the observation that glucose returned to the maternal circulation suggests that fetal glucose recycling is a process active outside and within the fetus.

**Fetal-maternal glucose transfer**

The transfer of glucose from the mother to the fetus has been considered to depend on a stereospecific process of facilitated diffusion which is affected by the glucose consumption rate of placental tissues (Widdas, 1961; Boyd et al., 1976; Simmons et al., 1976, 1979). It is evident, however from the results reported in this experiment that a substantial amount of fetal glucose is also transferred back to the
maternal circulation. From the per cent of maternal glucose carbon derived from fetal glucose carbon (9.2%), the transfer rate of fetal glucose to the mother can be estimated assuming a maternal irreversible disposal rate of glucose of 108 mg glucose/min in late gestation (Steele and Leng, 1973). This would amount to 9.95 mg glucose/minute, representing 52% and 10% of the fetal and maternal glucose irreversible disposal rates respectively. Anand et al. (1979) demonstrated a positive correlation between the fetal-maternal transfer of glucose and fetal glucose concentration. Since the rate of umbilical glucose uptake is dependent upon the maternal-fetal glucose gradient (James et al., 1972; Schreiner et al., 1978), the return of glucose from the fetal circulation to the mother may represent a mechanism that ensures an optimal rate of glucose supply to the fetus.

Although no experiments were done to test the effect of varying levels of fetal oxygen consumption on the reverse transfer of glucose to the maternal circulation, it may be reasonable to suggest that glucose made available to the fetus in excess of the oxygen required for oxidative metabolism would be returned to the mother. This would be a preventative measure against possible hypoxia in the fetus consequent to a sustained increase in umbilical uptake of glucose (Carson et al., 1980).

Charlton et al. (1979) reported large volumes of amniotic fluid swallowed by the ovine fetus add significantly to the overall fetal metabolism. The quantitative contribution of fetal fluids to fetal glucose recycling needs to be studied in order to describe adequately the recycling of glucose within the feto-maternal system.
Conclusions

Experiment II a was conducted to study the metabolism of glucose and lactate in the ovine fetus using radioisotope dilution techniques. It is evident from the results that these techniques complement conclusions based on venous arterial umbilical differences.

It can be concluded that glucose and lactate are utilized at a rapid rate as evidenced by the high rates of irreversible disposal and metabolic clearance of these compounds. There is a correlation observed between the fetal glucose irreversible disposal rate and blood glucose concentration demonstrating that fetal metabolism of glucose is regulated by the fetal blood glucose concentration. Glucose utilization was found to be higher in older fetuses with a higher body weight than younger ones.

The recirculation of glucose has been estimated to be 12.62% of the irreversible disposal rate of glucose. This is less than reported in other nonruminant species. Approximately, 36% of glucose is converted to lactate.

Recycling appears to be an important feature of glucose and lactate metabolism in the fetus occurring to an extent of 70-80% of total turnover of these compounds. The absence of radioactivity in maternal blood following lactate injection indicates that lactate recycling occurs within the fetal compartment, due probably to impermeability of the placenta to lactate.

The fact that approximately 10% of maternal glucose carbon was derived from the fetal glucose carbon indicates that glucose recycling occurs both within and outside the fetal compartment.
EXPT II B) METABOLISM OF AMINO ACIDS

Materials and Methods

Animals

The animals used in this experiment were surgically prepared and maintained as described in Experiment 1.

Metabolic Studies

The return of the ewe’s appetite to presurgical levels and the daily monitoring of fetal and maternal blood gas and pH parameters were used as criteria for determining the physiological condition of the animal on the day of experimentation. The housing and maintenance of the ewes were as described in Experiment 1. All experiments were initiated at approximately 5-6 hours after the morning feeding. The experimental protocol for each study was as follows:

a) [U-\textsuperscript{14}C] amino acid mixture

Approximately 50 μCi of [U-\textsuperscript{14}C] amino acid mixture were introduced into the fetal circulation as a bolus injection in sterile phosphate buffer (pH 6.9), and the cannula dead space was flushed immediately thereafter with 2.0 ml of sterile 0.15 N NaCl. Maternal and fetal whole blood were sampled at frequent intervals up to 3 hours as detailed in Experiment II and transferred to ice chilled test tubes containing EDTA crystals. Blood was centrifuged and the plasma was removed and stored at -40°C. Plasma (500 μl) was deproteinized with double the volume of absolute ethanol and the solution was kept on ice for 30 minutes before centrifugation. The deproteinized supernatant was evaporated to dryness in a warm water bath under a stream of nitrogen.
b) $[\text{U}^{14}\text{C}]$ alanine and $[2-^{3}\text{H}]$ glucose

In experiments where $[\text{U}^{-14}\text{C}]$ alanine and $[2-^{3}\text{H}]$ glucose were administered simultaneously, 50 μCi of each isotope were dissolved in 2.5 ml of sterile phosphate buffer and injected as a bolus. Whole blood (400 μl) were deproteinized with 6.0 ml of 0.33 N perchloric acid. The supernatants were neutralized with KOH and freeze dried as described in Experiment II a.

Analytical Procedures

Chemical

1) Determination of amino acid specific activity

Initial fractionation of blood metabolites into acidic, neutral and basic fractions was performed by ion exchange chromatography as previously described in Experiment II a.

The amino acids adsorbed on the cation exchange resin were eluted with 2N triethanolamine in 20% acetone in water (Harris et al., 1961) and evaporated to dryness under vacuum. After reconstituting in 1.5 ml of 0.1 N HCl, a 500 μl aliquot was taken for radioactive measurements. Recovery of $^{14}\text{C}$-amino acids applied to the columns was 99.1 ± 0.96% (Appendix Table 3).

An additional 500 μl aliquot was diluted with equal volume of CO$_2$-free water and the total organic carbon content was quantitated on an infrared carbon analyzer (Beckman). Potassium biphthalate standards ranging from 0-1000 ppm were prepared in CO$_2$-free water (Appendix Fig. 3).
2) Determination of alanine specific activity

For determining the radioactivity of [U-\(^{14}\)C] alanine, the basic fraction was evaporated to dryness and dissolved in 1.0 ml 0.05 M Tris buffer, pH 7.8. Alanine was quantitatively converted to lactate in a coupled enzymatic reaction involving transamination and dehydrogenation by the addition of alanine amino transferase (EC. 2.6.1.2, 10 U/ml, Sigma), lactate dehydrogenase (EC. 1.1.1. 27, 6 U/ml, Sigma), 2-oxoglutarate (6.7 mM) and NADH (0.2 mM, Sigma). After incubation at 30°C for 2 hours, the mixture was passed through a cation exchange column and the radioactivity in the eluate containing lactate was counted. The recovery of labelled alanine in this procedure was 82.4 ± 0.64% (Appendix Table 3).

The concentration of alanine was determined by monitoring the extinction of NADH at 340 nm between 40 and 60 minutes (Grassl, 1974).

3) Determination of glucose and lactate specific activity

The specific activity of glucose and lactate was determined as described previously (Experiment II a).

4) Plasma protein determinations

The total plasma protein content in maternal and fetal plasma was determined by the procedure of Lowry et al. (1951). Bovine albumin standards ranged from 0-100 mg protein/100 ml. Samples were read against a Folin-phenol reagent blank at 600 nm.

Radiochemicals

[U-\(^{14}\)C] amino acid mixture\(^1\) (50 mCi/mmole) and [U-\(^{14}\)C] alanine (156 mCi/mmole), were supplied by ICN Pharmaceuticals (Montreal). [2,\(^3\)H]

---

\(^1\) Appendix (p. 159)
glucose (17.9 Ci/mmole) was obtained from Amersham Searle Corp., Oakville, Ontario.

**Analysis of Data**

The 3-hour sampling time was not sufficient to describe the terminal slope of the [U-\(^{14}\)C] amino acid decay curve accurately. The observed data were therefore plotted on semilogarithmic coordinates and extrapolated to zero specific activity manually. Initial least squares estimates of the kinetic parameters were calculated using a Fortran computer program (AUTOAN). The final least squares estimates were obtained using NONLIN (Michigan) as described in Experiment II a.

**Model Description**

The data were fitted to a three-pool model (Waterlow et al., 1978) in which amino acids are irreversibly lost only through the primary blood pool (Fig. 10). The other two pools, which were not sampled are represented
by intracellular free amino acid pool and body proteins. The transfer of amino acids from the maternal circulation across the placenta and from degradation of body protein through the intracellular pool was assumed to be the sources of amino acids entering the blood pool.

Calculations

In experiments where [U-\(^{14}\)C] alanine and [2-\(^{3}\)H] glucose were administered simultaneously, the conversion of alanine to lactate and to glucose was estimated by the ratio of integrals of the specific activity-time curves as described in Experiment II a.

1. Fraction of lactate C derived from alanine C

\[
\frac{\int_0^\infty S.A. \text{lactate C} (dt)}{\int_0^\infty S.A. \text{alanine C} (dt)}
\]

2. Rate of lactate C derived from alanine C (mgC/min/kg)

\[= \text{Equation 1} \times \text{lactate irreversible disposal rate}\]

3. Percent of alanine C going to lactate C

\[= \text{Equation 2} \times 100\]

\[\text{alanine irreversible disposal rate}\]

4. Fraction of glucose C derived from alanine C

\[
\frac{\int_0^\infty S.A. \text{glucose C} (dt)}{\int_0^\infty S.A. \text{alanine C} (dt)} \times \frac{6}{5}
\]
where \( \frac{6}{5} \) = the correction factor to account for the mean number of glucose carbons labelled from alanine (Chochinov et al., 1978).

5. Rate of glucose C derived from alanine C (mgC/min/kg)

\[
= \text{Equation 4} \times \text{glucose irreversible disposal rate.}
\]

where glucose irreversible disposal rate was determined simultaneously using [2-\(^3\)H] glucose.

6. Per cent of alanine C going to glucose C

\[
= \frac{\text{Equation 5}}{\text{Alanine irreversible disposal rate}} \times 100
\]

Additional calculations pertaining to the irreversible disposal rates and substrate recycling were performed as described in Experiment II a.

All results are expressed as means (+ SEM). Statistical analysis of the data was performed by students independent t-test.

**Results**

The body weight of ewes and their fetuses as well as blood gas and metabolite parameters recorded at the time of the experiment are presented in Table 8. The physiological parameters of the animals were similar in the series of experiments in which [U-\(^{14}\)C] amino acid mixture or [U-\(^{14}\)C] alanine/[2-\(^3\)H] glucose was injected.
Table 8  Maternal and fetal physiological parameters during the experimental period.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Isotope(s) injected</th>
<th>[U-(^1^4)C] Amino Acid Mixture (n=4)</th>
<th>[U-(^1^4)C] Alanine and [2-(^3^H)] glucose (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maternal</td>
<td>Fetal</td>
<td>Maternal</td>
</tr>
<tr>
<td>Gestational age (days)</td>
<td>135.6±1.86</td>
<td>133.8±6.5</td>
<td>135.6±1.86</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>62.96±3.30</td>
<td>2.99±0.18</td>
<td>61.30±2.40</td>
</tr>
<tr>
<td>(P_0_2) (mm Hg)</td>
<td>33.10±0.90</td>
<td>20.09±1.04</td>
<td>35.07±1.10</td>
</tr>
<tr>
<td>(P_CO_2) (mm Hg)</td>
<td>34.45±2.54</td>
<td>39.10±1.47</td>
<td>28.93±0.90</td>
</tr>
<tr>
<td>pH</td>
<td>7.502±0.01</td>
<td>7.376±0.01</td>
<td>7.479±0.01</td>
</tr>
<tr>
<td>Total (CO_2) (meq/L)</td>
<td>-</td>
<td>23.51±0.52</td>
<td>-</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>33.10±0.87</td>
<td>34.50±1.29</td>
<td>32.89±0.69</td>
</tr>
<tr>
<td>Whole blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>alanine (mM)</td>
<td>-</td>
<td>-</td>
<td>0.133±0.01</td>
</tr>
<tr>
<td>lactate (mM)</td>
<td>-</td>
<td>-</td>
<td>1.14±0.04</td>
</tr>
<tr>
<td>glucose (mM)</td>
<td>3.74±0.03</td>
<td>1.00±0.02</td>
<td>3.37±0.03</td>
</tr>
<tr>
<td>Plasma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma Protein (mg/%)</td>
<td>7.03±0.13</td>
<td>3.34±0.26</td>
<td>6.02±0.11</td>
</tr>
<tr>
<td>Total carbon (2)</td>
<td>0.19±0.02</td>
<td>0.42±0.02</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^1\) Values = mean (± SEM)
\(^2\) moles carbon/liter
Specific activity-time curves for the amino acid mixture and alanine are presented in Fig. 11. The observed data are best described by a three exponential equation with a corresponding mean $R^2$ value of 0.986 and 0.988 for $\text{[U-}^{14}\text{C]}$ amino acid mixture and alanine respectively.

The kinetic parameters obtained from the single injection of a mixture of amino acids and alanine are presented in Table 9. The metabolic half life of the slow component of the amino acid mixture was significantly ($P < 0.05$) higher than those observed with alanine. The half life representing the fast component of the specific activity time curve of the amino acid mixture was significantly less ($P < 0.05$) than that of alanine, indicating a more rapid removal from the primary free amino acid pool.

The alanine pool size was $7.67 \pm 0.39 \text{ mgC/kg}$ and was not significantly different from the plasma pool size of the amino acid mixture. The apparent volume of distribution of alanine was $66.05 \pm 4.09\%$ of fetal body weight and was significantly ($P < 0.05$) greater than that of the amino acid mixture (1%). The irreversible disposal rates of amino acid mixture and alanine were $2.30 \pm 0.277 \text{ mgC/min/kg}$ and $2.021 \pm 0.34 \text{ mgC/min/kg}$ respectively. The total entry rate for the amino acid mixture was significantly greater ($P < 0.05$) than that observed with alanine.

The extent of recycling of the amino acid mixture and alanine was $85.3 \pm 0.03$ and $71.1 \pm 0.05\%$ respectively (Table 10). No significant differences were noted between the mean total residence time for the amino acid mixture and alanine; however the number of cycles made by the amino acid carbons was significantly ($P < 0.05$) greater than by alanine. No $^{14}\text{C}$ activity from alanine or amino acids was detected in the maternal circulation following the administration of these labelled substrates.
Fig. 11. Semilogarithmic plot of $^{14}$C amino acids and $^{14}$C alanine specific activity (S.A.) versus time. Values are means (± SEM); Amino Acid (n=4); Alanine (n=10).
Table 9  Kinetic parameters of amino acid metabolism in the ovine fetus in utero.

<table>
<thead>
<tr>
<th>Labelled substrate injected</th>
<th>Pool size (Q) (mgC/kg)</th>
<th>Irreversible rate of disposal (D.R.) (mgC/min/kg)</th>
<th>Total entry rate (TER) (mgC/min/kg)</th>
<th>Volume of distribution (v) (% Bwt)</th>
<th>Half life (min)</th>
<th>T&lt;sub&gt;1&lt;/sub&gt;</th>
<th>T&lt;sub&gt;2&lt;/sub&gt;</th>
<th>T&lt;sub&gt;3&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>[U-&lt;sup&gt;14&lt;/sup&gt;C] amino acid mixture (n=4)</td>
<td>8.25&lt;sup&gt;a&lt;/sup&gt; ±1.15</td>
<td>2.301&lt;sup&gt;a&lt;/sup&gt; ±0.277</td>
<td>15.82&lt;sup&gt;a&lt;/sup&gt; ±1.30</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.30&lt;sup&gt;a&lt;/sup&gt; ±0.03</td>
<td>1.428&lt;sup&gt;a&lt;/sup&gt; ±0.47</td>
<td>81.8&lt;sup&gt;a&lt;/sup&gt; ±2.77</td>
<td></td>
</tr>
<tr>
<td>[U-&lt;sup&gt;14&lt;/sup&gt;C] alanine (n=10)</td>
<td>7.67&lt;sup&gt;a&lt;/sup&gt; ±0.39</td>
<td>2.021&lt;sup&gt;a&lt;/sup&gt; ±0.34</td>
<td>8.69&lt;sup&gt;b&lt;/sup&gt; ±1.14</td>
<td>66.05&lt;sup&gt;b&lt;/sup&gt; ±4.09</td>
<td>0.88&lt;sup&gt;b&lt;/sup&gt; ±0.14</td>
<td>2.99&lt;sup&gt;b&lt;/sup&gt; ±0.42</td>
<td>52.88&lt;sup&gt;b&lt;/sup&gt; ±1.78</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> Values = mean (+ SEM)

<sup>a,b</sup> Superscripts with different alphabets in the column denote statistical significance (P < 0.05)
Table 10 Recycling of amino acids and alanine in the ovine fetus in utero.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>[U-¹⁴C] Amino Acid Mixture (n=4)</th>
<th>[U-¹⁴C] Alanine (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction of total turnover irreversibly lost</td>
<td>0.146 ± 0.021&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.271 ± 0.041&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fraction of total turnover recycled</td>
<td>0.853 ± 0.030&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.711 ± 0.052&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean total residence time (T) (min)</td>
<td>7.22 ± 0.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.13 ± 1.72&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Number of cycles (v)</td>
<td>6.46 ± 0.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.27 ± 0.74&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Values = means (± SEM)
<sup>a,b</sup> Superscripts with different alphabets in the rows denote statistical significance (P < 0.05)
Table 11  Conversion of alanine to lactate and glucose in the ovine fetus in utero.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Alanine → Lactate (precursor) (product)</th>
<th>Alanine → Glucose (precursor) (product)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% product C derived from alanine C^5</td>
<td>27.07 ±4.85</td>
<td>4.11 ±1.09</td>
</tr>
<tr>
<td>Rate of alanine C going to product C (mgC/min/kg)</td>
<td>0.696 ±0.131</td>
<td>0.161 ±0.040</td>
</tr>
<tr>
<td>% of alanine irreversible disposal going to product C^7</td>
<td>34.95 ±4.40</td>
<td>8.85 ±0.36</td>
</tr>
</tbody>
</table>

1 Values = means (± SEM)
2 Alanine irreversible disposal rate = 2.021 ± 0.34 (mgC/min/kg)
3 Lactate irreversible disposal rate = 2.576 ± 0.18 (mgC/min/kg)
4 Glucose irreversible disposal rate = 4.05 ± 0.16 (mgC/min/kg)
5 \[
\frac{\int_{S.A. \text{ product (dt)}}}{\int_{S.A. \text{ alanine (dt)}}} \times 100
\]
6 5 x Product irreversible disposal rate
7 \[
\frac{6}{\text{alanine irreversible disposal rate}} \times 100
\]
In experiments where \([U-^{14}C]\) alanine and \([2-^{3}H]\) glucose were injected simultaneously into the fetus, attempts were made to quantitate the conversion of alanine carbon into lactate and glucose carbons. The mean (± SEM) irreversible disposal rate of \([2-^{3}H]\) glucose obtained in this experiment was 4.05 ± 0.16 mgC/min/kg and compared favourably with the result obtained in Experiment II a. A rapid transfer of alanine \([^{14}C]\) was observed in lactate and glucose, with peak activity occurring 5-10 minutes after the injection of alanine. The proportion of lactate C derived from alanine C was 27.07 ± 4.85% (Table 11). The rate of conversion of alanine C going to lactate C was 0.696 ± 0.13 mgC/min/kg and was equivalent to 34.95 ± 4.4% of the alanine irreversible disposal rate. The fraction of glucose C derived from alanine C was 4.11 ± 1.09% (Table 11). Based on the irreversible disposal rate of glucose determined simultaneously using \([2-^{3}H]\) glucose, the conversion of alanine C going to glucose C occurred at a rate of 0.161 ± 0.04 mgC/min/kg. This was equivalent to 8.85 ± 0.36% of the alanine irreversible disposal rate.

**Discussion**

In this study an attempt was made to assess the utilization of amino acids by the ovine fetus in utero using isotopic dilution techniques. The single injection of a mixture of \([U-^{14}C]\) amino acids was used to estimate the disposal rates from the plasma free amino acid pool. The simultaneous injection of \([U-^{14}C]\) alanine and \([2-^{3}H]\) glucose was made to quantitate the conversion of alanine carbon to other compounds and its potential contribution to gluconeogenesis.
Amino acid metabolism

A major concern with the single injection or continuous infusion of a mixture of [U-\(^{14}\)C] amino acids is that there are some amino acids that are not included in the mixture and yet account for a significant proportion of ninhydrin positive compounds in plasma (Wolff and Bergman, 1972). Although glutamine, arginine, citrulline, onithine, N- and 3-methyl histidine and carnosine are excluded from the 15 amino acids injected into the fetal circulation, it can be calculated from their umbilical venous-arterial difference and blood flow (Lemons et al., 1976), that these amino acids contribute to only 8% of the umbilical amino acid uptake. Thus, it was assumed that the radioactive mixture used in this experiment closely represents the mixture of amino acids that are transferred to the fetus from the umbilical circulation.

Multicompartmental analysis yielded three exponents which best described the specific activity-time curves of amino acid mixture and alanine. Similar results have been reported with a single [\(^{14}\)C] amino acid injection by Henriques et al. (1955) in the rabbit or a mixture of [\(^{14}\)C] amino acids by Reilly and Green (1975) in the rat.

Though the pool size of alanine and amino acid mixture was similar, the apparent volume of distribution of the amino acid mixture is less than 1% of the fetal body weight, which is less than the plasma space. This is similar to the value of 2% that can be calculated from the data of Reilly and Green (1975). On the other hand, the volume of distribution of alanine was 66.05%, approximately equivalent to the total body water of the fetus. The low volume of distribution observed with the amino acid mixture can be explained on the basis that amino acids are not present in equal concentrations throughout the body water pool but rather are
concentrated in certain organs to a greater extent than in the blood (Munro, 1970). The use of whole blood for alanine specific activity determinations and plasma for amino acid mixture could have also contributed, to some extent to the observed differences in the volume of distribution.

The irreversible disposal rate of the amino acid mixture (2.301 mgC/min/kg) was similar to the umbilical uptake measurement (2.73 mgC/min/kg) reported by Lemons et al. (1976). The irreversible disposal rate of alanine is approximately 40% greater than that reported by Prior and Christenson (1977) using continuous infusion procedures and 30% greater than Lemons et al. (1976) using umbilical venous arterial differences. The exchange of alanine $[^{14}\text{C}]$ atoms with $[^{12}\text{C}]$ atoms of intermediates formed from alanine would result in isotope dilution and thus overestimate the disposal rate of alanine. This may not necessarily lead to a veno-arterial difference.

The extent of recycling of amino acids observed in this experiment (Table 10) is greater than the estimates of 36% reported in adult rats (Reilly and Green, 1975). Wolff and Bergman (1972) have indicated that amino acid recycling involves the intracellular pool and tissue protein pool. It is unlikely that the large amount of recycling observed during the 3-hour experimental period could be attributed to the protein pool, the fastest of which has been reported to have a half life of approximately 24 hours (Young, 1979). It, therefore, appears that recycling involves the intracellular tissue pool, which has been shown to turnover very rapidly in order to sustain the rapid growth rate of the fetus (Young, 1979). This is further supported by the relatively greater number of cycles made by the mixture of amino acids than by lactate or glucose (Experiment II a), though the total residence time of these compounds is similar (Table 10).
It has been shown that the placenta delivers to the mother a significant amount of nitrogen derived from amino acids in the form of urea and ammonia (Gresham et al., 1972a; Holzman et al., 1979). The failure to detect amino acid carbon radioactivity in the maternal circulation following the injection of $^{14}$C amino acids to the fetus raises the question whether amino acids of fetal origin are excreted across the placental barrier into the maternal circulation or are metabolized by the fetus and or placenta. The permeability of the ovine placenta on the maternal side has not been completely defined, however, it would appear from the results of this experiment that the maternal side of the placenta is relatively impermeable to amino acids.

**Alanine conversion to lactate and glucose**

The use of integral equations and ratio of the area under the precursor-product specific activity-time curves were used in this study as a measure of quantitating the conversion of alanine carbon to lactate and glucose carbon in the ovine fetus.

The proportion of alanine C going to lactate C (27%) compares favourably with the result (23%) reported by Prior and Christenson (1977), using a continuous infusion technique. Foster et al. (1980) have recently shown in dogs that carboxyl carbon of alanine exchanges rapidly with lactate. The rapid exchange between alanine and lactate is a reflection of glutamate-pyruvate transaminase activity in fetal tissues (Stevenson et al., 1976) and contribution of alanine to oxidative metabolism in the fetus.

The evidence for the existence of gluconeogenesis in the ovine fetus has been a controversial topic. The presence of gluconeogenic
enzymes in fetal ruminant liver (Ballard et al., 1965) and the sharp increases in phosphoenol pyruvate carboxykinase (EC. 4.1.1.3.2.) activity between 130-140 days of gestation in the ovine fetus (Warnes et al., 1977b) prompted many workers to find the functional significance of these enzymes. Anand et al. (1980) recently reported that no gluconeogenesis was observed in the fetus following induced fetal hypoglycemia with insulin infusions. This may not be surprising, as insulin is an antagonist of gluconeogenesis. On the other hand, Hodgson et al. (1980) have estimated that 69% of fetal glucose requirements are supplied through gluconeogenesis. Prior (1980) recently has reported, using a continuous infusion of $[\text{U}^{-14}\text{C}]$ lactate, that 22% of the glucose turnover was derived from lactate.

In this experiment 4% of the glucose disposal rate was derived from alanine C (Table 10), and is similar to the value of 2.3% obtained by Prior and Christenson (1977) in the fetus and 3.5% by Brockman and Berman (1975) in the adult ewe. Anand and Sperling (1978) have proposed that the apparent gluconeogenic activity originating from $[\text{U}^{-14}\text{C}]$ alanine, in the experiments of Prior and Christenson (1977) was due to the the $[^{14}\text{C}]$ alanine returning to the mother and then recirculating back to the fetus as $[^{14}\text{C}]$ glucose. The fact that no $[^{14}\text{C}]$ activity was detected in the maternal circulation following the injection of $[\text{U}^{-14}\text{C}]$ alanine to the fetus in this experiment, is sufficient evidence to contradict this hypothesis.

The proportion of alanine C (8.8%) going to glucose C in this study when expressed as a % of the alanine irreversible disposal rate is similar to the value (7-9%) obtained in adult monogastrics (Chockinov et al., 1978; Foster et al., 1980), but is almost half as much (15-20%) reported in adult ruminants (Brockman and Bergman, 1975). A similar estimate (7.3%) was reported by Prior and Christenson (1977) in the ovine fetus in their
continuous infusion studies. It should be noted however that the transfer of carbon atoms from alanine to glucose may not be a quantitative measure of the true rate of gluconeogenesis because of the "metabolic exchange" or "cross over" of $^{12}\text{C}$ atoms with $^{14}\text{C}$ atoms in the oxaloacetate pool (Krebs et al., 1966).

The lack of incorporation of lactate label into glucose in the single injection studies, (this study and Warnes et al., 1977a) is contrary to the findings of Prior (1980) in a continuous infusion study in which 22% of glucose turnover was reported to be derived from lactate. The use of $[1^{-14}\text{C}]$ rather than $[U^{-14}\text{C}]$ lactate in this experiment may partly explain the lack of labelling in glucose.

As shown in a subsequent experiment (Expt III, p.128), approximately 63% of $[U^{-14}\text{C}]$ lactate was found to be oxidized. The randomization of lactate carbons during passage through the Krebs cycle would reduce the extent of labelling in glucose even if lactate conversion to glucose did take place. These dilution effects are likely to be more pronounced in single injection than continuous infusion studies. Further work is needed to clarify the role of lactate as a gluconeogenic precursor in the fetus.

Conclusions

Experiment II b was undertaken to study metabolism of amino acids in the ovine fetus in utero.

The kinetic parameters of amino acid metabolism were obtained following the single injection of $[U^{-14}\text{C}]$ amino acid mixture or $[U^{-14}\text{C}]$ alanine $[2^{-3}\text{H}]$ glucose. The specific activity-time data were fitted to a three exponential curve. Based on the rates of total entry and irreversible
disposal, 85% of the amino acid turnover was recycled in this study. The relative impermeability of the placenta to amino acids may account for the amount of recycling observed in this study. The frequency at which amino acids returned to the blood pool was shown to be greater than other metabolic compounds; however the mean total residence time was not longer. This may be due to a rapid turnover of the plasma amino-acid pool.

The extent of conversion of alanine C to lactate and glucose C was determined. Based on the area under the specific activity-time curves of the precursor and the product it was estimated that 8 and 27% of the irreversible disposal rate of alanine C were converted to glucose C and lactate C respectively. Though this conversion indicates that the ovine fetus was capable of gluconeogenesis, the transfer of C atoms from alanine to glucose may not be a quantitative measure of the rate of gluconeogenesis due to the metabolic exchange of $^{12}\text{C}$ atoms with $^{14}\text{C}$ atoms in the oxaloacetate pool.
Experiment III
Measurement of carbon dioxide production and substrate oxidation by the ovine fetus in utero using $^{14}$C-labelled compounds

Introduction

The metabolic fuels for oxidative metabolism of the fetus have been reviewed extensively by Battaglia and Meschia (1978). Using the elegant technique of umbilical catheterization, Tsoulos et al. (1971), James et al. (1972) and Morriss et al. (1973) developed the concept of substrate-oxygen quotient procedure according to which glucose, lactate and amino acids have been reported to contribute approximately 46, 20 and 25% respectively to the total oxygen consumed by the ovine fetus.

To provide a direct measurement of substrate turnover, isotope dilution techniques have recently been employed in fetal metabolic studies (Warnes et al., 1977a; Prior and Christenson, 1977; Anand et al., 1979, 1980; Hodgson et al., 1980; Prior, 1980). However, quantitative measurements of carbon dioxide output from labelled substrates in chronically catheterized conceptus have not been reported. In the post-natal life the excretion of $^{14}$CO$_2$ in breath following the administration of $^{14}$C-labelled substrates has been used to determine substrate oxidation rates in sheep (Lindsay and Ford, 1964; Annison et al., 1967). Carbon dioxide production rates have also been measured in adult sheep by the isotope dilution procedure from specific radioactivity of $^{14}$CO$_2$ in blood, urine or expired air (Whitelaw, 1974). A high correlation between the specific activity of plasma $^{14}$CO$_2$ and respiratory $^{14}$CO$_2$ during the oxidation of labelled substrates in man was reported recently by Clague and Keir (1979). This
coupled with the fact that fetal respiration occurs only through the placental circulation prompted the use of isotope dilution procedures based on $^{14}\text{CO}_2$ activity in fetal blood. The measurement of fetal CO$_2$ production rates and the quantitation of the oxidation of substrates, by the ovine fetoplacental tissues in utero were investigated in this experiment.

Materials and Methods

Animals

The animals, surgery and the maintenance practices have been described previously. In experiments where [U-$^{14}$C] NaHCO$_3$ was infused, two catheters were introduced into the fetal circulation. The catheter used for sampling fetal blood was introduced to a distance of 6-8 cm into the external saphenous vein in one of the hind limbs. Another catheter was passed deeply into the external saphenous vein in the other leg for infusing radioactive compounds. The tip of this catheter was found at autopsy to lie in the inferior vena cava approximately 10 cm from the heart. A minimum of 5 post-operative days elapsed before tracer experiments were undertaken. Cannulae were also placed in the jugular vein of the ewes one day prior to the experiment.

Metabolic Studies

A) Irreversible disposal rate of CO$_2$

The irreversible disposal rate of CO$_2$ was determined using a primed dose-continuous infusion technique. The priming dose consisted
of 30 μCi of NaH$^{14}$CO$_3$ followed by a continuous infusion at the rate of 0.5 μCi/min. Solutions of NaH$^{14}$CO$_3$ were prepared in sterile saline and made slightly alkaline by addition of 0.1 N NaOH. Fetal and maternal blood samples for CO$_2$ and specific activity determination were taken at 30 minute intervals prior to and during the period of isotope equilibrium. The total CO$_2$ content of whole blood was calculated using the Henderson-Hasselbalch equation from PCO$_2$ and pH values which were determined within 5 min after collection (Radiometer). The radioactivity of $^{14}$CO$_2$ in blood was determined by adding 0.5 ml of 6 M perchloric acid from the side arm of Warburg flasks to 0.5 ml of whole blood in the main chamber. The $^{14}$CO$_2$ which was released was trapped in hyamine hydroxide (Don Mills, Ont.) placed in the central well. After standing for 1 hour at 25°C an aliquot of hyamine hydroxide was added to 10 ml of scintillation fluid (PCS, Amersham, Oakville, Ont.) and the radioactivity counted by liquid scintillation spectrometry (LKB, Rack Beta 1215). This procedure gave a recovery of 92.0 ± 2.0% of known amount of NaH$^{14}$CO$_3$ added to blood (Appendix Table 4). To correct for the retention of $^{14}$CO$_2$ in slowly mixing pools such as bone, NaH$^{14}$CO$_3$ was injected into 3 fetuses in separate experiments. Blood $^{14}$CO$_2$ radioactivity was determined at intervals until no further activity was detected. From the $^{14}$CO$_2$ radioactivity per ml of fetal blood at each time of collection the total activity in the entire blood volume of the fetus was obtained. The procedure of Faber et al. (1973) was used to estimate the blood volume at the time of experimentation based on body weight at birth using the regression equation of Gresham et al. (1972b). From the plot of total blood $^{14}$CO$_2$ radioactivity against time the area under the curve at different times was computed and expressed as a per cent of administered NaH$^{14}$CO$_3$ appearing in $^{14}$CO$_2$. 
Oxidation of substrates

The oxidation of substrates was determined in conjunction with experiments II a and b. Approximately 50 μCi of $^{14}$C labelled glucose, lactate, alanine, amino acid mixture and acetate were injected as a bolus into the fetal circulation and the specific radioactivity of blood $^{14}$CO$_2$ was monitored. The specific activity of the labelled substrates in blood was determined by ion-exchange chromatography as described in Experiment II. Normalized specific radioactivity-time curves of labelled precursors and $^{14}$CO$_2$ in blood were described by biexponential equations. Initial estimates of kinetic parameters were calculated using AUTOAN Fortran computer program and the final least squares fitting of the data was done using NONLIN (Michigan), as described in Experiment II a.

Radiochemicals

$[^{14}$C] acetate (51 mCi/mmole) was obtained from ICN Pharmaceuticals, Montreal. NaH$^{14}$CO$_3$ (58 mCi/mmole) was supplied by Amersham Corp., Oakville, Ont. The details of $^{14}$C-glucose, lactate, alanine and amino acid mixture are given in Experiment II a and b.

Calculations

The irreversible disposal rate of carbon dioxide was determined using a continuous infusion of NaH$^{14}$CO$_3$.

1. Irreversible disposal rate of CO$_2$ = \( \frac{\text{Rate of NaH}^{14}\text{CO}_3 \text{ infusion (nCi/min)}}{\text{Blood}^{14}\text{CO}_2 \text{ plateau specific activity (nCi/mgC)}} \)
2. The rate of oxidation of substrates was calculated by 2 procedures following single injections of labelled components:

A) From the isotopic yield in the product (Heath and Barton, 1973).

(i) Isotopic yield in product \( (\text{CO}_2) \) = Irreversible disposal rate of \( \text{CO}_2 \times \frac{A_{\text{ps}}}{\text{Dose}} \)

(ii) Fraction of substrate oxidized = \( \frac{\text{Isotopic yield in } \text{CO}_2 \text{(uCi)}}{-\text{substrate injected} \text{(uCi)}} \)

(iii) Rate of substrate oxidation (mgC/min/kg) = Irreversible disposal rate of substrate \times \text{Fraction of substrate oxidized.}

where,

\( A_{\text{ps}} \) = Area under the specific radioactivity-time curve in the product \( (\text{CO}_2) \) following injection of labelled substrates.

\( \text{Dose} \) = uCi of substrate injected.

B) From carbon transfer quotient ratio (Kleiber et al., 1956).

(i) Rate of substrate oxidation (mgC/min/kg) = Fraction of \( \text{CO}_2 \) from substrate oxidation \( \times \) Irreversible disposal rate of \( \text{CO}_2 \)

(ii) Fraction of \( \text{CO}_2 \) from substrate oxidation = \( \int_{0}^{\infty} \frac{14\text{CO}_2 \text{ specific activity } (dt)}{14\text{C-substrate specific activity } (dt)} \)

(iii) Per cent \( \text{CO}_2 \) derived from substrate = \( \frac{\text{Rate of substrate oxidation}}{\text{Substrate Irreversible disposal rate}} \times 100 \)
3. Time ($T_{max}$) for specific activity of blood $^{14}$CO$_2$ to reach a maximum following injection of labelled substrates was estimated as follows:

$$T_{max}(^{14}$CO$_2) = \frac{2.303 \cdot \log \frac{\alpha}{\beta}}{\alpha - \beta}$$

where

$\alpha$ and $\beta$ are formation and elimination rate constants of $^{14}$CO$_2$

specific activity-time curves (Ritschel, 1976)

Data were subjected to an analysis of variance and where a significant difference was noted means were compared using Newmann Keuls multiple range test. Linear regression lines and correlation coefficients were calculated by the method of least squares.

**Results**

The body weights of ewes and fetuses in the acetate and bicarbonate experiments were 60.0 ± 0.9; 2.67 ± 0.17 and 63.2 ± 1.9; 3.59 ± 0.20 kg respectively. In conjunction with the studies in Experiment II, several measurements of total CO$_2$ concentration in fetal whole blood were made. The total CO$_2$ content of fetal venous whole blood ranged from 22 to 24 meq/liter in all experiments.

Changes in the specific activity of fetal and maternal blood $^{14}$CO$_2$ with time following primed dose-continuous infusion of NaH$^{14}$CO$_3$ are shown in Figure 12. The specific activity of fetal blood $^{14}$CO$_2$ reached a plateau approximately 90 minutes after the infusion commenced and remained constant thereafter. The mean irreversible disposal rate
Table 12  Substrate oxidation rates in the ovine fetus in utero.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>T&lt;sub&gt;max&lt;/sub&gt;(&lt;sup&gt;14&lt;/sup&gt;CO&lt;sub&gt;2&lt;/sub&gt;) (min)</th>
<th>Procedure</th>
<th>% Substrate Oxidized</th>
<th>Rate of Oxidation (mgC/min/kg)</th>
<th>% CO&lt;sub&gt;2&lt;/sub&gt; from Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>19.60 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>30.50 ± 4.20</td>
<td>30.70 ± 4.40</td>
<td>1.06 ± 0.18</td>
<td>1.09 ± 0.20</td>
</tr>
<tr>
<td>(N = 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lactate</td>
<td>2.90 ± 0.19&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.83 ± 3.92</td>
<td>36.13 ± 3.91</td>
<td>0.96 ± 0.14</td>
<td>0.98 ± 0.13</td>
</tr>
<tr>
<td>(N = 8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>19.90 ± 0.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.52 ± 3.99</td>
<td>26.93 ± 3.08</td>
<td>0.549 ± 0.09</td>
<td>0.511 ± 0.09</td>
</tr>
<tr>
<td>(N = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amino acid</td>
<td>9.30 ± 1.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>-</td>
<td>23.55 ± 3.22</td>
<td>-</td>
<td>0.59 ± 0.09</td>
</tr>
<tr>
<td>mixture</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetate&lt;sup&gt;2&lt;/sup&gt;</td>
<td>10.30 ± 1.39&lt;sup&gt;c&lt;/sup&gt;</td>
<td>15.82 ± 2.81</td>
<td>-</td>
<td>0.10 ± 0.20</td>
<td>1.70 ± 0.20</td>
</tr>
<tr>
<td>(N = 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a,b,c</sup> Superscripts with different alphabets in the column denote statistical significance P < 0.05.

1 Values ± standard error of the mean.

2 Calculated values based on umbilical uptake of 1.39 g/kg/day.

I.Y. = Isotopic yield  T.Q. = Transfer quotient.
Fig. 12 Specific radioactivity of fetal and maternal blood $^{14}\text{CO}_2$ following primed dose-infusion of $\text{NaH}^{14}\text{CO}_3$.
of carbon dioxide in 6 fetuses was 13.47 ± 1.07 ml/min/kg. In the 3 single injection experiments designed to estimate the retention of $^{14}$CO$_2$ in slowly mixing pools, it was found that only 82.5 ± 5.4% of the administered radioactivity in NaH$^{14}$CO$_3$ was recovered in fetal blood at the end of 3 h (Fig. 13). Correction factors were therefore applied to the values of specific radioactivity of blood $^{14}$CO$_2$ when $^{14}$C-labelled substrates were injected intravenously. The specific activity of maternal blood $^{14}$CO$_2$ closely followed the increase in the fetus and reached a plateau after 90 minutes (Fig. 12). At steady state conditions, the ratio of the specific activity of maternal and fetal blood $^{14}$CO$_2$ was 0.14 ± .01 indicating the 14% of maternal CO$_2$ production can be accounted for by the placental transfer of CO$_2$.

The changes in the specific activity of blood $^{14}$CO$_2$ following the injection of $^{14}$C-labelled substrates are shown in Fig. 14. The specific activity of blood $^{14}$CO$_2$ reached a maximum within the first 3 minutes (Tmax) of lactate injection which was significantly (P < 0.05) more rapid than in the case of other substrates (Table 12). The peak specific activity of blood $^{14}$CO$_2$ appeared 19.6, 19.9, 10.3 and 9.3 minutes after the injection of labelled glucose, alanine, acetate and amino acid mixture respectively. Secondly, the peak specific activity of blood $^{14}$CO$_2$ was also higher (P < 0.05) in lactate than in all other substrates studied.

The rates of substrate oxidation as well as their contribution to total carbon dioxide production calculated by the two procedures employed were very similar and not statistically different (Table 12). It is noteworthy that, on an average only 30.60 and 36.48% of glucose and lactate carbons respectively appeared in CO$_2$. The extent of oxidation of alanine or amino acid carbons was 27.73 and 23.55% respectively. The
Fig. 13 Recovery of radioactivity of blood $^{14}\text{CO}_2$ after single injection of NaH$^{14}\text{CO}_3$. 
Specific radioactivity (S.A.) of blood 14CO2 after injection of 14C-labeled substrates into fetal circulation: L=1-14C-lactate; A=U-14C-acetate; AL=U-14C-alanine; G=U-14C-glucose; AA=U-14C-amino acid mixture. The curves are reproduced from a Calcomp plotter.
Fig. 15 Relationship between rates of oxidation and irreversible disposal rates of glucose and lactate (●, y = 0.40x - 0.347; r = 0.641; p < 0.10; n = 8; □, y = 0.283x + 0.291; r = 0.604; p < 0.10; n = 8).
fraction of CO₂-carbon derived from the oxidation of glucose, lactate, alanine and amino acids was 15.00, 14.15, 6.66 and 8.19% respectively. Only 15.80% of acetate carbon was oxidized contributing to 1.70% of total CO₂ production. The linear regression of rates of glucose and lactate oxidation on the irreversible rates of disposal is shown in Fig. 15.

**Discussion**

CO₂ Disposal in the Ovine Fetus

The CO₂ disposal rates (13.47 ± 1.07 ml/min/kg) are comparable to the oxygen consumption of 17.5 ml/min/kg by the uterus and its contents reported by Setchell et al. (1972) using isotopic tracers and 14.2 ml/min/kg by Graham (1964) using calorimetric techniques. In this study attempts were not made to cannulate the umbilical blood vessels and the estimated irreversible disposal rates of CO₂ would therefore represent the metabolic activity of the fetus plus contribution from the placental mass. The determination of the fetal CO₂ irreversible disposal rate by single injection techniques was found to be unsatisfactory. It was evident that because the label was leaving the fetal blood pool with such rapidity, the initial slope and intercept describing this pool were subjected to considerable error. The CO₂ production rates obtained from the continuous infusion of NaH¹⁴CO₃ are slightly higher than the value of 11.19 ml/min/kg for the fetus plus the utero-placental tissues estimated from uterine and umbilical arterio-venous differences coupled with blood flow (Meschia et al., 1980). The latter procedure does not take into account the retention and utilization of metabolic CO₂ by the tissues.
and is likely to underestimate true production. In this study, the CO₂ disposal rates have been corrected for these factors which results in slightly higher values. The retention of 17.5% of ¹⁴CO₂ in fetal tissues during the administration of NaH¹⁴CO₃ closely resembles the values of 17-20% in adult sheep (Bergman and Hogue, 1967; Annison et al., 1967).

On the other hand, in adult sheep, it has been reported that CO₂ disposal rates estimated by isotope dilution procedures tend to overestimate actual production determined by calorimetric techniques (Whitelaw, 1974). This has been ascribed to low specific activities resulting from slowly mixing pools and inadequate length of infusion. Though a plateau of ¹⁴CO₂ specific activity was reached within 90 min, the NaH¹⁴CO₃ infusions were continued for periods up to 4 hours with no increase in specific activity indicating that the duration of infusion is not a major factor for the higher values observed. Further support of the validity of the irreversible disposal rates of CO₂ may be obtained from the ratio (0.14) of the specific activity of ¹⁴CO₂ in maternal blood to that in fetal blood after a plateau has been reached (Fig. 12). Assuming a maternal CO₂ production rate of 362 ml/min during late pregnancy (Whitelaw et al., 1972), the rate of placental transfer of CO₂ amounts to 13.06 ml/min/kg which is similar to the value of 13.47 ml/min/kg calculated from the fetal compartment alone.

Substrate Oxidation

The shorter T_max and the higher specific activity of ¹⁴CO₂ from labelled lactate than from labelled glucose are similar to the findings of Shambaugh et al. (1977a) in the tissues of fetal rats under in vitro
conditions. In one experiment where [U-\textsuperscript{14}C]-lactate was injected instead of [\textsuperscript{1-14}C]-lactate, the rate of oxidation was 1.367 mgC/min/kg contributing to 18.94% of CO\textsubscript{2} production. This indicates that the higher rate of CO\textsubscript{2} production from lactate than from glucose is not merely due to preferential oxidation of C-1 of lactate. The absence of hepatic glucokinase in the fetus (Ballard et al., 1969) and the oxidation of glucose through an intermediary pool such as lactate having more carbon than the glucose pool (Table 5; Warnes et al., 1977a) may explain the slower CO\textsubscript{2} release from glucose than from lactate.

Two methods of calculation were used to quantitate the transfer of substrate carbon to CO\textsubscript{2}-carbon. In the isotopic yield procedure (Heath and Barton, 1973) the total recovery of labelled carbon in the product is measured. This would give an estimation of the total fraction of the substrate oxidized by direct and indirect pathways. From the irreversible disposal rates of the substrates and the fraction oxidized, the rate of oxidation and contribution to CO\textsubscript{2} production are calculated. The transfer quotient ratio expresses the transfer of carbon between the precursor and the product but is independent on the number and size of intermediary pools and rates of intermediary pathways (Kleiber et al., 1956; Searle et al., 1975). It is of interest that the rate of substrate oxidation and contribution to CO\textsubscript{2} production calculated by the two procedures (Table 12) were very close to each other.

A particularly significant finding in this study pertains to the extent of substrate oxidation and contribution to CO\textsubscript{2} production. Oxidation accounts only for 15.80 - 36.48% of the irreversible rates of disposal of the carbon from substrates studied. In the single experiment
where \[^{14}\text{C}}\]lactate was injected; 62.3\% was found to be oxidized. Even these values may be considered to be a maximum since oxidation rates based on \[^{14}\text{CO}_{2}\] production are likely to include exchange reactions without net oxidation taking place (Chang and Goldberg, 1978). This would mean that a major proportion of substrate carbon extracted by the fetal tissues is utilized for anabolic purposes than for oxidative metabolism. The positive relationship between oxidation rates and irreversible rates of disposal (Fig. 15) indicates that the magnitude of uptake and utilization of glucose by the fetal tissues depends largely on the materno-fetal transfer confirming previous reports (Boyd et al., 1973). The fact that lactate may be formed from glucose in the placenta (Experiment II a; Warnes et al., 1977a) may explain the similarity in the oxidative metabolism of these compounds in relation to their irreversible disposal rates.

The fraction of total \(\text{CO}_{2}\) derived from the oxidation of glucose (15.0\%) is similar to the value of 22\% obtained by Setchell et al. (1972) in fetal sheep using tracer techniques. Kronfeld and Van Soest (1976) have summarized available data on substrate contribution to \(\text{CO}_{2}\) production and have concluded that approximately 8-11\% of \(\text{CO}_{2}\) was derived from oxidation of glucose in ruminants and 10-50\% in non-ruminants. The mean value of 15\% contribution by glucose to total \(\text{CO}_{2}\) production is within the range reported for adult monogastric animals.

Though the relative importance of various substrates to overall fetal oxidative metabolism would depend on the metabolic fuel mixture available (Shambaugh et al., 1977a,b) contribution of lactate to \(\text{CO}_{2}\) production at a level equal to that of glucose indicates that the fetus relies on other
substrates, in addition to glucose, for oxidation. A major proportion of glucose carbon is therefore used for alternative purposes. A similar explanation would hold good for the small contribution (8.19%) of amino acid carbon to CO₂ production. It is also noteworthy that, based on the direct oxidation of the substrate used in this study, approximately 40% of total CO₂ production by the fetoplacental tissues can be detected. This suggests that, in addition to glucose, lactate, amino acids and acetate, other substrate(s) may be involved in fetal metabolism. Though the present experiment have been designed to study the oxidative metabolism of the fetoplacental unit as a whole it is possible to obtain quantitative information on the unidirectional metabolism of the fetus and placenta separately by the simultaneous use of isotopic tracers and umbilical venoarterial concentration differences coupled with blood flow. This approach has been used in the metabolism of liver (Brockman and Bergman, 1975).

Conclusion

Experiment III was initiated to measure the CO₂ production rate and the relative oxidation rates of specific substrates in the ovine fetus in utero. The rate of CO₂ production noted in this experiment represents the metabolic activity of the fetus and placental tissues.

It was observed that 14% of the labelled bicarbonate infused into the fetal circulation was collected in the mother. It was noted that placental transfer of CO₂ resembled the CO₂ production rate from the fetal placental compartment alone.
From the results of the oxidation of specific substrates, it has been shown that only 15-36% of substrate irreversible disposal rates can be accounted for by oxidation. It can be concluded that a greater proportion of substrate utilization is made available for synthetic purposes than was hitherto reported. Using both the isotopic yield and transfer quotient procedures to estimate the per cent of CO$_2$ produced from these substrates, it was concluded that other substrate(s) contribute to the total fetal oxidative metabolism.

Although the procedure used in this study estimates the oxidative metabolism of the entire fetoplacental unit rather than the fetus itself, the fact that only a small proportion of the substrates utilized by the fetus is oxidized suggests that the quantitative contribution of substrates to total oxidative metabolism should be reassessed.
General Conclusions

Surgical techniques were developed and standardized for introducing vascular catheters into the fetus so that the metabolism of substrates may be studied in vivo without stress.

Using single injections of $[\text{U-}^{14}\text{C}]$ and $[2-^{3}\text{H}]$ glucose the irreversible disposal rates, pool size, volume of distribution and other kinetic parameters were determined. The utilization of glucose was also shown to be related to blood glucose concentration and body mass. This indicated that the rate of utilization of glucose is dependent on the umbilical uptake of this substrate from the maternal circulation and that fetal glucose requirements increase with increases in fetal body mass. Glucose label-recirculated within the fetus to an extent of 12.6 per cent of the irreversible disposal rate.

A substantial amount of fetal glucose was found to be recycled through the maternal circulation. Lactate on the other hand, was not detected in the maternal circulation following injection into the fetus, indicating a low permeability of lactate in the ovine placenta. Recycling of glucose and lactate in the fetus occurred to an extent of 70-80% of the total turnover of these compounds and it was concluded that this was an important feature of fetal glucose and lactate metabolism.

Studies on the metabolism of a mixture of amino acids and alanine were performed to quantitate further the utilization of amino acids by the fetoplacental unit. The rate of irreversible disposal of $[\text{U-}^{14}\text{C}]$ amino acid mixture was similar to that of $[\text{U-}^{14}\text{C}]$ glucose and $[1-^{14}\text{C}]$ lactate; however, the per cent of substrate turnover recycled was greater. This coupled
with the observation that the number of cycles made by amino acids was twice as much as glucose and lactate leads to the conclusion that the fetal plasma amino acid pool is being replaced at a rapid rate.

From the single injection of [U-\textsuperscript{14}C] alanine, it was calculated that 4 per cent of the glucose disposal rate was derived from alanine carbon, indicating the presence of gluconeogenesis. However, further investigation is required before conclusions can be drawn on the physiological circumstances under which gluconeogenesis is manifested.

Carbon dioxide rates were determined by a primed dose, continuous infusion technique. It was concluded from the ratio of fetal to maternal \textsuperscript{14}CO\textsubscript{2} specific activity, measured at plateau specific activity that 14\% of the maternal CO\textsubscript{2} production rate could be accounted for by fetal and placental metabolism. In studies where substrate oxidation was performed in conjunction with the determination of the irreversible disposal rates, it was shown that only 30, 62, 24, 15 and 26\% of [U-\textsuperscript{14}C] glucose, lactate, amino acids, acetate and alanine were oxidized to CO\textsubscript{2}. Substrate oxidation observed in this study accounts for only 40\% of the total CO\textsubscript{2} production of the fetoplacental tissues.

The total inflow and outflow of carbon in the fetoplacental unit were estimated from the rates of irreversible disposal, oxidation and interconversion among metabolites and are presented in Fig. 16. Using the values indicated in Fig. 16 with respect to oxidation and interconversion, the amount of substrate carbon used for anabolic purposes may be estimated. For the purpose of these calculations, the following substrates were assumed to be the major ones involved in fetal metabolism (Battaglia and Meschia, 1978).
Fig. 16 Composite picture of fetal substrate metabolism. Values inside brackets denote ml/min/Kg and those outside mgC/min/Kg.
## Carbon balance in the fetus

<table>
<thead>
<tr>
<th></th>
<th>Glucose</th>
<th>Lactate&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Amino Acids</th>
<th>Total Carbon mgC/min/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irreversible disposal rate (mgC/min/kg) (A)</td>
<td>3.541</td>
<td>2.171</td>
<td>2.301</td>
<td>8.013</td>
</tr>
<tr>
<td>Oxidation rate (mgC/min/kg)</td>
<td>1.087</td>
<td>1.353</td>
<td>0.543</td>
<td>2.983</td>
</tr>
<tr>
<td>Other disposals (mgC/min/kg) (B)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>to fructose&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>to mother</td>
<td>0.576</td>
<td>0.085</td>
<td></td>
<td>2.432</td>
</tr>
<tr>
<td>Used for synthesis (mgC/min/kg) (C = A-B)</td>
<td>0.110</td>
<td>0.821</td>
<td>1.674</td>
<td>2.598 or 3.741 gC/day/kg</td>
</tr>
</tbody>
</table>

<sup>1</sup> [U-<sup>14</sup>C] lactate

<sup>2</sup> 1 mole amino acid yields 0.69 mole urea (Schulz, 1978)

<sup>3</sup> Warnes et al. (1977a)

The net retention of carbon in the fetal tissues (3.74 gC/day/kg) compares favourably with the value of 3.20 gC/day/kg obtained on the basis of carbon content of the ovine fetus (James et al., 1972). It is noteworthy that most of the glucose carbon has been used for oxidation or returned to the mother. Carbon from other substrates, notably amino acids has been largely used for synthesis of fetal tissues.
It is also pertinent to discuss the contribution of substrate oxidation to the overall caloric requirement of the fetus. Depending on the technique employed, heat production in the fetus has been reported to be 42-52 (James et al., 1972; Meschia et al., 1967a, oxygen consumption), 65 (Abrams et al., 1970, differential spirometry) and 90 kcal/day/kg (Graham, 1964, indirect calorimetry). The minimum oxygen consumption needed for the proportions of substrates found to be oxidized in this study is 6.41 ml/min/kg. Assuming that the calorific value of oxygen is 4.9 cal/ml, this would result in the production of 45 kcal/day/kg from oxidative metabolism. In addition energy is also stored in the form of new tissues to the extent of 32 kcal/day/kg (Rattray et al., 1974). Thus, the total caloric requirement of the fetus would amount to 77 kcal/day/kg, a value which falls in the middle of the range reported in the literature. This may suggest that oxidative metabolism alone is not sufficient to account for the total caloric requirement of the fetus. On the other hand, the oxygen uptake of the fetoplacental unit is approximately 11-12 ml/min/kg (Meschia et al., 1980) and would result in the production of 78-85 kcal/day/kg from oxidative metabolism sufficient to meet the total caloric requirement of the fetus. The oxygen consumption of 6.41 ml/min/kg used in the present calculations represents a minimal value to oxidize only those substrates studied in this investigation. Therefore, it is suggestive that other substrate(s) may be involved in fetal oxidative metabolism.

In spite of the failure to identify all the metabolic sources of fetal heat production, it is of interest that, in their review of fetal metabolism, Battaglia and Meschia (1978) have concluded that "only a negligible amount of heat liberated by the fetal carcass in the bomb
calorimeter represents energy formerly derived from oxidative metabolism". It has been suggested that "virtually all the energy used to fuel fetal oxygen consumption is ultimately dissipated as heat". Their conclusions are based on the observations of Abrams (1970) that heat is transferred from the fetus to the mother. Though this may be true to the extent that the energy expended in differentiation and maintenance in the prenatal period may be dissipated as heat (Brody, 1945) it does not imply that virtually all the chemical energy released during oxidation is dissipated as heat and not used for anabolic purposes. The metabolic principles of energy production and utilization discussed thoroughly by Milligan (1971) would argue against such a hypothesis. It may therefore be appropriate to conclude that the maintenance energy cost of the growing fetus is relatively very high and increases with net increase in tissue mass.

Conclusions based on the Fick principle tend to underestimate the true utilization of metabolites by the fetus. On the other hand, tracer techniques, particularly the single injection procedure, may overestimate disposal rates of substrates. Therefore isotopic tracer techniques conducted in conjunction with umbilical veno-arterial differences of metabolites and blood gases would appear to be more appropriate that either technique alone in assessing fetal metabolic requirements.
Bibliography


Alexander, G. Studies on the placenta of the sheep (Ovis ariest); placental size. J. Reprod. Fert. 7:289-305, 1964.


Barnes, R.J., Comline, R.S. and Silver, M. Effect of cortisol on liver glycogen concentrations in hypophysectomized, adrenalectomized and normal foetal lambs during late or prolonged gestation. J. Physiol. 275:567-579, 1978.


Huggett, A.St.G. The foetal blood gas tensions and the gas transfusion through the placenta of the goat. J. Physiol. 62:373-384, 1927.


Mellor, D.J. and Matheson, I.C. Daily changes in the curved crown-rump length of individual sheep fetuses during the last 60 days of pregnancy and effects of different levels of maternal nutrition. Q. J. Exp. Physiol. 64:119-131, 1979.


Appendix
Validation of Methods

The following procedures were used for the separation and quantitation of labelled metabolites:

1. Isolation and recovery of glucose.

Glucose specific activity measurements have routinely been performed by the formation of glucose derivatives. However, due to the nonspecificity of these procedures and the relatively high concentration of fructose in fetal blood, anion exchange chromatographic procedure followed by the conversion of glucose to gluconic acid enzymatically was standardized. Glucose, in the presence of other neutrally charged metabolites was converted to gluconic acid by an excess of glucose oxidase and catalase and the resulting mixture separated by anion exchange chromatography. To test the purity and recovery of glucose in this procedure, aliquots of the neutral and acidic fractions were applied to paper chromatograms (descending) and developed in a solvent consisting of phenol:H₂O::NH₃; 40:40:1; w/v/v for 18 hours. The separation of labelled glucose from fructose is given in Appendix Fig. 1. The recovery of labelled compounds added to the column is given in Appendix Table 1.

The modification of the glucose oxidase procedure was as follows: Glucose oxidase reagents were prepared by mixing 25 mg of glucose oxidase (specific activity, 20,000 units/g, Sigma, St. Louis) and 10 mg of peroxidase (specific activity, 120 units/mg, Sigma, St. Louis) in 10 ml of demineralized water. The colour reagent, o-dianisidine-HCl (Sigma) was prepared by weighing 100 mg
of dry crystals and dissolving them in 10 ml of distilled water. To each reaction flask containing 400 ul of the neutral fraction was added 100 ul of the glucose oxidase-peroxidase and colour reagent. Flasks were mixed and placed in a water bath at 37°C for 40 minutes. The absorbance was then determined at 406 nm. A reagent blank and a set of standards ranging in concentration from 5 to 20 ug D-glucose per flask were run with each series of samples.

2. Isolation and recovery of lactate.

To avoid contamination of lactate with unknown labelled metabolites, ion exchange chromatography was employed in conjunction with thin layer chromatography. Results are given in Appendix Table 2.

3. Isolation and recovery of alanine.

[U-\textsuperscript{14}C] amino acids and alanine in particular were separated by cation exchange chromatography. The separation of alanine from other amino acids was performed by converting alanine to lactate enzymatically and passing the latter through a cation exchange column. Results are given in Appendix Table 3.

4. Recovery of \textsuperscript{14}CO\textsubscript{2}.

The oxidation of labelled substrates was monitored by measuring \textsuperscript{14}CO\textsubscript{2} in whole blood. Whole blood was acidified with perchloric acid and the liberated \textsuperscript{14}CO\textsubscript{2} was collected in Hyamine Hydroxide. Results are given in Appendix Table 4.

5. Amino acid mixture (15 L-amino acids, in same proportions as a typical algal protein hydrolysate) - glycine, alanine, serine, threonine, proline, valine, isoleucine, leucine, phenylalanine, tyrosine, aspartic acid, glutamic acid and lysine.
Table 1: Per cent recovery of $^{14}$C-labelled compounds after treatment with glucose oxidase and anion-exchange chromatography

<table>
<thead>
<tr>
<th>$^{14}$C-metabolite</th>
<th>Initial Activity (Dpm)</th>
<th>Neutral Fraction (Dpm)</th>
<th>Recovery (%)</th>
<th>Acidic Fraction (Dpm)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{14}$Cgluconic acid (N = 6)</td>
<td>19,432 ± 241</td>
<td>-</td>
<td>-</td>
<td>19,093 ± 259</td>
<td>98.11 ± 0.91</td>
</tr>
<tr>
<td>$^{14}$C]glucose$^2$ (N = 10)</td>
<td>161,120 ± 398</td>
<td>2,509 ± 167</td>
<td>1.55 ± 0.34</td>
<td>148,484 ± 1196</td>
<td>92.09 ± 0.71</td>
</tr>
<tr>
<td>$^{3}$Hglucose (N = 6)</td>
<td>42,180 ± 11.82</td>
<td>210 ± 21</td>
<td>0.50 ± 0.02</td>
<td>39,793 ± 665.06</td>
<td>93.72 ± 1.56</td>
</tr>
<tr>
<td>$^{14}$Cfructose$^2$ (N = 6)</td>
<td>78,388 ± 618</td>
<td>77,119 ± 667</td>
<td>98.38 ± 0.46</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$^{14}$Cglycerol (N = 6)</td>
<td>59,702 ± 8.84</td>
<td>56,564 ± 1,431</td>
<td>96.99 ± 2.47</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

1 Values = means (± SEM)
2 Rf values are shown in Appendix Fig 1
Table 2: Per cent recovery of $1^{-14}C$ lactate following anion exchange chromatography and thin layer chromatography.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Initial Activity</th>
<th>Recovery (%)</th>
<th>Contamination from glucose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anion Exchange chromatography</td>
<td>15,669 ±252</td>
<td>93.09 ±0.50</td>
<td>N.D.</td>
</tr>
<tr>
<td>Thin Layer Chromatography</td>
<td>14,489 ±208</td>
<td>89.07 ±0.68</td>
<td>0.8 ±0.01</td>
</tr>
<tr>
<td>(n-propanol:acetone:H$_2$O 6:3:1 v/v)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anion Exchange plus Thin Layer Chromatography</td>
<td>13,857 ±258</td>
<td>83.01 ±0.63</td>
<td>N.D.</td>
</tr>
</tbody>
</table>

¹ Value = means (± SEM)
Table 3: Per cent recovery of [U-$^{14}$C] amino acid mixture and alanine following cation exchange chromatography and enzymatic conversion of alanine to lactate$^1$.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Recovery in Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Neutral</td>
</tr>
<tr>
<td>Cation exchange chromatography</td>
<td></td>
</tr>
<tr>
<td>1) Amino Acid mixture (N = 12)</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>2) Alanine (N = 12)</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Cation exchange and enzymatic conversion of alanine to lactate (N = 12)</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$ Values = means (± SEM)
Table 4: Per cent recovery $[^{14}\text{C}]\text{NaHCO}_3$ in saline and whole blood.\footnote{Values = mean (± SEM)}

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>Initial Activity (Dpm)</th>
<th>Activity recovered in Hyamine Hydroxide (Dpm)</th>
<th>% Recovered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline (n = 8)</td>
<td>168,000 ± 281</td>
<td>154,768 ± 201</td>
<td>91.4 ± 0.8</td>
</tr>
<tr>
<td>Whole Blood (n = 8)</td>
<td>171,080 ± 310</td>
<td>159,446 ± 240</td>
<td>92.3 ± 1.4</td>
</tr>
</tbody>
</table>

\footnote{Values = mean (± SEM)}
Appendix fig. 1  Separation of metabolites by descending paper chromatography 
(phenol:water:NH₃; 40/40/1; w/v/v.)
Appendix Fig. 2  Quench curve for $^3$H and $^{14}$C isotopes.
Appendix fig. 3 Standard curve for total organic carbon determined by infra-red carbon analyzer.
Appendix table 5. Mean PO₂, PCO₂ and pH in maternal and fetal blood of conscious ewes during and following surgery

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Days After Surgery</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>pH</td>
<td>7.258±0.02</td>
<td>7.77±0.02</td>
<td>7.452±0.02</td>
<td>7.348±0.02</td>
<td>7.439±0.02</td>
<td>7.371±0.02</td>
<td>7.438±0.02</td>
<td>7.338±0.02</td>
<td>7.451±0.02</td>
<td>7.358±0.02</td>
</tr>
<tr>
<td></td>
<td>7.450±0.01</td>
<td>7.439±0.02</td>
<td>7.451±0.01</td>
<td>7.439±0.02</td>
<td>7.450±0.01</td>
<td>7.353±0.02</td>
<td>7.450±0.01</td>
<td>7.438±0.02</td>
<td>7.451±0.01</td>
<td>7.353±0.02</td>
</tr>
<tr>
<td></td>
<td>(10)</td>
<td>(11)</td>
<td>(11)</td>
<td>(11)</td>
<td>(10)</td>
<td>(13)</td>
<td>(9)</td>
<td>(10)</td>
<td>(4)</td>
<td>(6)</td>
</tr>
<tr>
<td>PCO₂ (mm Hg)</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>PCO₂ (mm Hg)</td>
<td>39.18±2.20</td>
<td>35.40±0.9</td>
<td>35.85±1.05</td>
<td>7.345±0.02</td>
<td>32.76±1.40</td>
<td>7.399±1.03</td>
<td>32.37±1.00</td>
<td>41.57±1.00</td>
<td>30.10±1.50</td>
<td>40.58±1.00</td>
</tr>
<tr>
<td></td>
<td>42.07±1.85</td>
<td>42.78±0.58</td>
<td>40.21±1.12</td>
<td>42.42±0.77</td>
<td>40.51±1.00</td>
<td>42.76±1.00</td>
<td>42.78±1.00</td>
<td>40.58±1.00</td>
<td>42.17±1.00</td>
<td>41.61±1.00</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(9)</td>
<td>(10)</td>
<td>(11)</td>
<td>(13)</td>
<td>(9)</td>
<td>(10)</td>
<td>(9)</td>
<td>(10)</td>
<td>(6)</td>
</tr>
<tr>
<td>PO₂ (mm Hg)</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>PO₂ (mm Hg)</td>
<td>104.67±8.20</td>
<td>25.80±0.76</td>
<td>42.07±1.85</td>
<td>20.18±0.78</td>
<td>40.33±2.11</td>
<td>22.06±0.62</td>
<td>42.99±1.12</td>
<td>20.01±0.94</td>
<td>43.67±1.12</td>
<td>21.80±1.07</td>
</tr>
<tr>
<td></td>
<td>43.55±1.95</td>
<td>22.03±0.94</td>
<td>43.67±1.33</td>
<td>21.80±1.07</td>
<td>41.55±1.33</td>
<td>21.86±1.07</td>
<td>41.55±1.33</td>
<td>21.86±1.07</td>
<td>41.55±1.33</td>
<td>21.86±1.07</td>
</tr>
<tr>
<td></td>
<td>(6)</td>
<td>(9)</td>
<td>(10)</td>
<td>(11)</td>
<td>(12)</td>
<td>(8)</td>
<td>(6)</td>
<td>(8)</td>
<td>(4)</td>
<td>(6)</td>
</tr>
<tr>
<td></td>
<td>47.75±1.40</td>
<td>22.29±1.07</td>
<td>45.30±1.22</td>
<td>17.48±1.29</td>
<td>47.75±3.64</td>
<td>22.29±1.07</td>
<td>45.30±1.22</td>
<td>17.48±1.29</td>
<td>47.75±3.64</td>
<td>22.29±1.07</td>
</tr>
</tbody>
</table>

1 Data presented as means ± S.E.M.

Values in parenthesis are number of observations.
Table 6: Mean hematocrit, blood glucose, lactate, β-hydroxybutyrate and alpha amino nitrogen in fetal blood of conscious ewes.

<table>
<thead>
<tr>
<th>Days After Surgery</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>38.2 ± 1.02</td>
<td>37.83 ± 1.3</td>
<td>35.10 ± 1.10</td>
<td>33.72 ± 1.00</td>
<td>34.80 ± 1.12</td>
<td>34.05 ± 1.12</td>
<td>34.50 ± 1.25</td>
<td>33.05 ± 1.25</td>
<td>34.64 ± 1.26</td>
<td>34.00 ± 1.50</td>
</tr>
<tr>
<td>Glucose * (mM)</td>
<td>0.602 ± 0.08</td>
<td>0.602 ± 0.02</td>
<td>0.710 ± 0.02</td>
<td>0.731 ± 0.02</td>
<td>0.901 ± 0.02</td>
<td>0.810 ± 0.13</td>
<td>0.880 ± 0.13</td>
<td>0.901 ± 0.02</td>
<td>0.901 ± 0.02</td>
<td>0.870 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>(3)</td>
<td>(5)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>Lactate * (mM)</td>
<td>1.90 ± 0.17</td>
<td>1.80 ± 0.02</td>
<td>1.83 ± 0.04</td>
<td>1.85 ± 0.02</td>
<td>1.78 ± 0.03</td>
<td>1.58 ± 0.02</td>
<td>1.62 ± 0.03</td>
<td>1.66 ± 0.03</td>
<td>1.70 ± 0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(3)</td>
<td>(4)</td>
<td>(4)</td>
<td>(5)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>β-Hydroxybutyrate * (mM)</td>
<td>0.198 ± 0.001</td>
<td>0.187 ± 0.008</td>
<td>0.172 ± 0.001</td>
<td>0.167 ± 0.002</td>
<td>0.160 ± 0.001</td>
<td>0.165 ± 0.003</td>
<td>0.176 ± 0.002</td>
<td>0.173 ± 0.002</td>
<td>0.159 ± 0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(3)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(5)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
</tr>
<tr>
<td>Alpha amino nitrogen * (mg/100 ml)</td>
<td>15.76 ± 3.20</td>
<td>14.40 ± 0.71</td>
<td>12.43 ± 0.27</td>
<td>11.02 ± 0.45</td>
<td>12.11 ± 0.16</td>
<td>11.39 ± 0.94</td>
<td>10.98 ± 0.39</td>
<td>10.48 ± 0.10</td>
<td>10.57 ± 1.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(3)</td>
<td>(4)</td>
<td>(4)</td>
<td>(5)</td>
<td>(4)</td>
<td>(5)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
<td>(4)</td>
</tr>
</tbody>
</table>

1 Data presented as means ± S.E.M. 
Values in parenthesis are number of observations 
* Differences between days 1-4 and 5-9 are statistically significant (P < 0.05).