GLUCONEOGENESIS IN THE DEVELOPING LAMB

by

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Preface

The abbreviations used in this thesis are defined in 'Instructions to authors' in Biochem.J. (1975) 145, 1-20. Non-standard abbreviations are defined in the text.

The following enzymes are quoted in the text:-

aldose reductase	E C 1.1.1.21
aspartate aminotransferase	E C 2.6.1.1
ATP-citrate lyase	E C 4.1.3.8
diaphorase	E C 1.6.4.3
fructokinase	E C 2.7.1.4
glucose 6-phosphatase	E C 3.1.3.9
glucose 6-phosphate dehydrogenase	E C 1.1.1.49
hexokinase	E C 2.7.1.1
hexose diphosphatase	E C 3.1.3.11
lactate dehydrogenase	E C 1.1.1.27
malate dehydrogenase (NAD^+)	E C 1.1.1.37
malate dehydrogenase ($NADP^+$)	E C 1.1.1.82
phosphofructokinase	E C 2.7.1.56
phosphoenolpyruvate carboxykinase (GTP)	E C 4.1.1.32
phosphoglucoseisomerase	E C 5.3.1.9
pyruvate carboxylase	E C 6.4.1.1
pyruvate kinase	E C 5.3.1.9
sorbitol dehydrogenase	E C 1.1.1.14

Declaration

I declare that the experiments reported in this thesis were carried out by myself. Any assistance received from others is specifically acknowledged.

No part of this dissertation has been submitted to any other university for any degree or diploma.

A preliminary report of some of the work has been published:

Warnes, Deirdre, Ballard, F.J. and Seamark, R.F. (1974): "Gluconeogenesis in fetal and neonatal lambs", J.Reprod.Fertil. 36, 471-472.

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Summary

(1) The development of gluconeogenic activity in the fetal and postnatal lamb has been investigated both *in vitro* and *in vivo*.

(2) Measurement of the activities of the key enzymes of gluconeogenesis in liver homogenates from fetal and postnatal lambs revealed that all enzymes were active before birth. The activity of the enzymes glucose 6-phosphatase, hexose diphosphatase, phosphoenolpyruvate carboxykinase (soluble) and pyruvate carboxylase increased with gestational age. The particulate phosphoenolpyruvate carboxykinase did not show this pattern of prenatal development. Activity was high early in gestation and remained constant until birth. After birth the activities of all enzymes increased to values in excess of the activities measured in liver homogenates from adult sheep.

(3) The activity of the gluconeogenic pathway was assessed in the chronically cannulated fetal lamb *in utero*. Cannulae were surgically placed in the umbilical vein and artery or the femoral vein and artery of the fetal lamb. After a period of recovery, normally five days, 50 μCi of [U-^{14}C]lactate, a known gluconeogenic substrate, was administered as a single intravenous injection to the fetal lamb. Conversion to glucose was measured in a series of blood samples taken after injection of isotope.

(4) No glucose formation from lactate was detected in any blood sample indicating that gluconeogenic activity is minimal in the fetal lamb *in utero*. Formation of fructose from lactate did not occur.

(5) Parameters of lactate metabolism by the ovine fetus were calculated from the specific radioactivity disappearance curve of blood lactate by multicompartmental analysis.

The irreversible loss of lactate from the fetus was 0.074 ± 0.005 mmol/kg/min, the lactate pool was 1.681 ± 0.080 mmol/kg and the lactate space was 65% of the body weight.

(6) In contrast, the gluconeogenic pathway was active in the postnatal lamb. Rapid conversion of lactate to glucose followed the intravenous injection of 50 μCi [U-^{14}C]lactate The irreversible loss of lactate from the postnatal lamb was 0.042 ± 0.003 mmol/kg/min, which is significantly lower than the irreversible loss of lactate from the fetus ($P<0.05$). The lactate pool of the postnatal lamb, 0.592 ± 0.059 mmol/kg and the space, 31% of the body weight, were also significantly less than those parameters of the fetus. 35± 8% of the ^{14}C-labelled lactate was incorporated into the glucose pool of the postnatal lamb.

(7) The metabolism of [U-^{14}C]fructose by the chronically cannulated fetal lamb in utero was also studied. [U-^{14}C]fructose (50 μCi) was administered as a single intravenous injection and analysis of the blood samples showed that the isotope disappeared only slowly from the fructose pool of the fetus. Fructose made no significant contribution to the glucose or lactate pools of the fetus.

(8) By multicompartmental analysis of the specific radioactivit disappearance curve an irreversible loss of fructose of 0.008 ± 0.001 mmol/kg/min was calculated. The fructose pool was 1.452 ± 0.049 mmol/kg and the space was 30% of the body weight.

(9) Glucose metabolism in the ovine fetus was measured following the single injection of 50 μCi [U-^{14}C]glucose to the fetal lamb in utero. ^{14}C-glucose was rapidly metabolized by the fetus forming lactate and fructose. 39 ± 6% of the ^{14}C-labelled glucose was incorporated into the lactate pool and 31 ± 3% was incorporated into the fructose pool of the fetus.

The irreversible loss of glucose from the fetus was found to be 0.041 ± 0.002 mmol/kg/min, the pool was 0.398 ± 0.051 mmol/kg and the space was 57% of the body weight.

(10) Similar experiments conducted with postnatal lambs showed that lactate was rapidly formed from the injected glucose. 39 ± 8% of the ^{14}C-labelled glucose was incorporated into the lactate pool of the postnatal lamb. The irreversible loss of glucose from the postnatal lamb was 0.033 ± 0.002 mmol/kg/min, the pool was 0.998 ± 0.033 mmol/kg and the space was 29% of the body weight.

(11) A model is proposed for the metabolism of glucose by the ovine fetus which shows irreversible conversion of glucose to lactate. In contrast , glucose and lactate are readily interconvertible in the postnatal lamb.

(12) The activity of the gluconeogenic pathway in the ovine fetus was assessed after the intravenous administration of adrenaline and glucagon to the chronically cannulated fetal lamb in utero. Elevation of blood glucose and lactate concentrations were observed to follow adrenaline or glucagon infusion. This hyperglycaemia did not result from induction of gluconeogenesis.

(13) The activity of the gluconeogenic pathway was monitored during parturition. Gluconeogenic activity was not detected in any lamb until after birth , either natural or following Caesarian section. The rapid onset of gluconeogenic activity in the lamb at birth is discussed in terms of the biochemical controls which may operate during the transition from fetal to neonatal life.

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CHAPTER I

INTRODUCTION

An important feature of ruminant nutrition is that little carbohydrate is obtained directly from the digestive tract. The bulk of the ingested carbohydrate is rapidly fermented by the microbial population of the rumen to the volatile fatty acids; acetic, propionic and butyric acids and to carbon dioxide and methane (Phillipson and McAnally, 1942; Elsden, 1945; Heald, 1951; Weller and Gray, 1954; Annison, Hill and Lewis, 1957; Bensadoun, Palladines and Reid, 1962; Porter and Singleton, 1965). For its supply of glucose the adult ruminant is completely dependant upon gluconeogenesis, the process by which glucose is synthesized from non-carbohydrate precursors such as propionate, lactate, glycerol and certain amino acids.

Gluconeogenesis is essentially the reverse of glycolysis with many of the enzymes common to both pathways (Fig 1). There are however several reactions in the glycolytic sequence which are essentially irreversible. These are :-

(1) the hexokinase-catalysed phosphorylation of glucose to glucose 6-phosphate,

(2) the phosphofructokinase -catalysed phosphorylation of fructose 6-phosphate to fructose 1,6-diphosphate and,

Figure 1: The Gluconeogenic Pathway

An outline of the gluconeogenic pathway (broad arrows) showing the sites of entry of the glucose precursors ; lactate, propionate, glycerol and the 'glucogenic' amino acids, alanine, glutamate and aspartate.

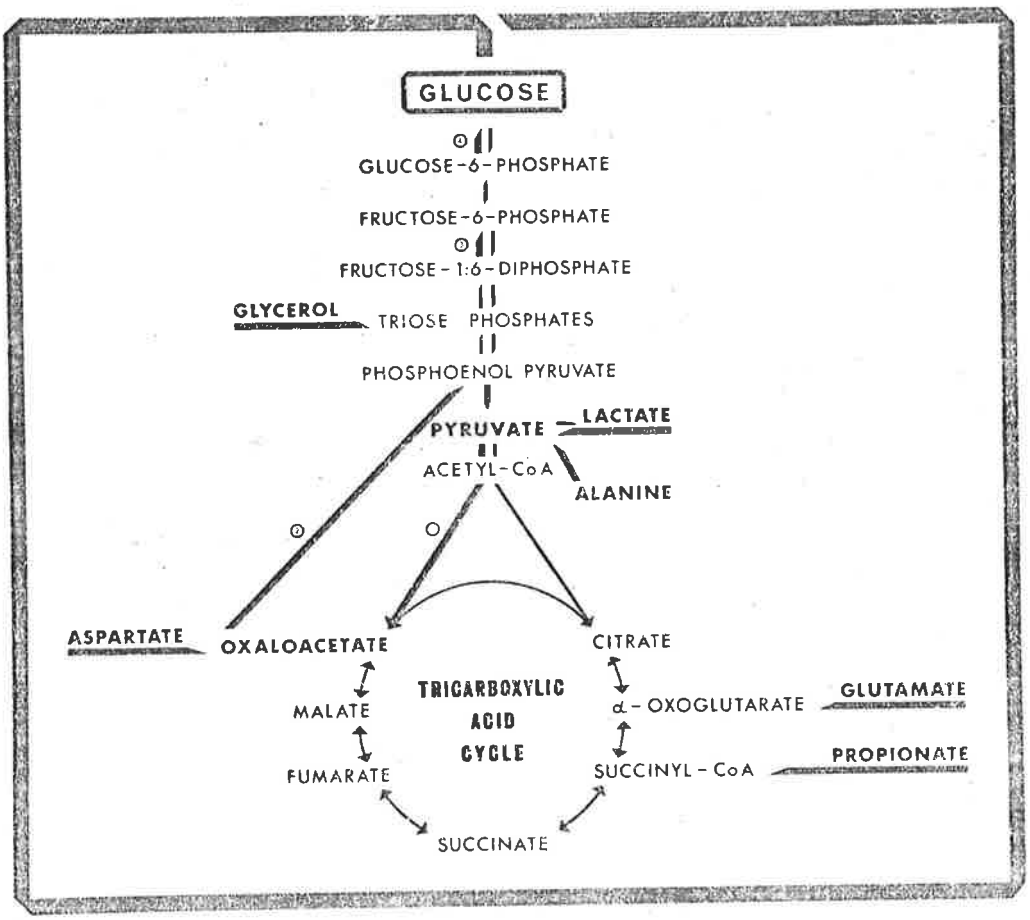
The key regulatory enzymes of gluconeogenesis are:

(1) pyruvate carboxylase

(2) phosphoenolpyruvate carboxykinase

(3) hexose diphosphatase

(4) glucose 6-phosphatase


GLUCOSE
GLUCOSE-6-PHOSPHATE
FRUCTOSE-6-PHOSPHATE
FRUCTOSE-1:6-DIPHOSPHATE
GLYCEROL
TRIOSE PHOSPHATES
PHOSPHOENOL PYRUVATE
PYRUVATE
LACTATE
ACETYL-CoA
ALANINE
ASPARTATE
OXALOACETATE
CITRATE
TRICARBOXYLIC ACID CYCLE
MALATE
α-OXOGLUTARATE
GLUTAMATE
FUMARATE
SUCCINYL-CoA
PROPIONATE
SUCCINATE

(3) the conversion of phosphoenolpyruvate to pyruvate, catalysed by pyruvate kinase.

In gluconeogenesis these irreversible reactions are bypassed by :-

(1') glucose 6-phosphatase which catalyses the conversion of glucose 6-phosphate to glucose,

(2') hexose diphosphatase which catalyses the conversion of fructose 1,6-diphosphate to fructose 6-phosphate and,

(3') the conversion of pyruvate to phosphoenol-pyruvate catalysed by pyruvate carboxylase and phosphoenol-pyruvate carboxykinase.
For further details see Scrutton and Utter (1968).

The liver is the major site of gluconeogenesis in the adult sheep, producing 85% of the glucose synthesized in the entire animal (Bergman, Katz and Kaufman, 1970). Another site of gluconeogenesis is the kidney, a tissue which makes a small, but significant, contribution to the glucose pool of the adult sheep. Kaufman and Bergman (1971) have calculated that net renal glucose production is 5-15% of the total glucose synthesized by the adult sheep. Lactate, from which 40-60% of the renal glucose output is synthesized (Kaufman and Bergman, 1974) and amino acids (Bergman, Kaufman, Wolff and Williams, 1974)

are the major gluconeogenic substrates used by the kidney.

In the fed, resting adult sheep the principal hepatic glucose precursor is propionate from which 20-50% of the glucose is derived (Bergman, Roe and Kon, 1966; Leng, Steel and Luick, 1967; Judson, Anderson, Luick and Leng, 1968). An estimation of the contribution of propionate to the glucose pool was obtained by infusion of ^{14}C-labelled propionate, then the percentage of ^{14}C-incorporated into glucose was measured. The route by which the propionate was administered had a profound effect upon the calculated value for glucose production. Bergman et al (1966) infused ^{14}C-propionate via the ruminal vein, whilst Leng et al (1967) and Judson et al (1968) administered ^{14}C-propionate directly to the rumen. Since propionate is converted to lactate during passage across the rumen epithelium (Pennington and Sutherland, 1956), administration of propionate via the ruminal vein gave a significantly lower estimate of glucose synthesis. Leng et al (1967) have estimated that up to 70% of propionate absorbed from the rumen may be converted to lactate before conversion to glucose.

In the adult sheep direct absorption of lactate from the rumen is negligible since little lactate escapes microbial fermentation (Jayasuriya and Hungate, 1959; Satter and Elsdale, 1968; MacKenzie, 1967). However, lactate for gluconeogenesis is available from anaerobic

glycolysis in peripheral tissues such as working muscles. This makes quantitation of the lactate contribution to gluconeogenesis difficult because the reconversion of lactate to glucose (i.e. the Cori cycle, Cori, 1931) does not represent a net synthesis of glucose. Annison, Lindsay and White (1963) have calculated that a minimum of 15% of the glucose turnover of the fed, resting adult sheep is synthesized from lactate and, whilst 40% of the lactate pool is derived from glucose, only 6% of the glucose pool is recycled via lactate.

After propionate, the glucogenic amino acids are the most quantitatively significant precursors of glucose in the fed adult sheep. They are primarily derived from microbial protein (Kay, 1969) and become available following proteolysis and absorption from the small intestine. Sheep liver removes 2-4 mmol amino acids per hr from portal blood (Wolff and Bergman, 1972a; Wolff, Bergman and Williams, 1972). Glucose synthesis from glucogenic amino acids supplies up to 30% of the daily glucose requirement of the adult sheep (Nolan and Leng, 1968; Ford and Reilly, 1969; Reilly and Ford, 1971a; Wolff and Bergman, 1972b).

Glycerol is another potential precursor of glucose in the ruminant, but in fed sheep only 5% of the glucose synthesized originates from glycerol. During fasting, however, glycerol availability increases as mobilization

of free fatty acids occurs. Under these conditions glycerol can account for up to 40% of the glucose synthesized (Bergman, 1968; Bergman, Starr and Reulein, 1968).

In the adult sheep glucose synthesis (i.e. gluconeogenic flux) increases after feeding. For example Judson and Leng (1968) have observed that glucose synthesis increases with the intake of digestible energy and Katz and Bergman (1969) have reported that a 50% increase in hepatic glucose output accompanies feeding in the adult sheep. This is in contrast to the situation which occurs in the fed adult monogastric (e.g. the rat) in which gluconeogenesis is negligible. In starved sheep net hepatic and net renal glucose output decreases, primarily because of a decrease in the availability of glucose precursors Annison, Brown, Leng, Lindsay and West, 1967; Bergman, 1963, 1964; Kaufman and Bergman, 1971, 1974), while in the adult rat gluconeogenic activity increases during periods of low carbohydrate intake or starvation.

On examination of the activities of the rate-limiting enzymes of gluconeogenesis (see Fig 1), the reasons for the different response of the ruminant and the monogastric to starvation become apparent. In the rat the activities of all four key enzymes of gluconeogenesis increase during starvation (Scrutton and Utter, 1968;

Wimhurst and Manchester, 1970; Filsell, Jarrett, Taylor and Keech, 1969), and there is an overall increase in gluconeogenic capacity. Refeeding is accompanied by a decrease in the activities of these enzymes (Shrago, Lardy, Nordlie and Foster, 1963; Foster, Ray and Lardy, 1966). However, in the adult sheep there is no change in the activity of phosphoenolpyruvate carboxykinase during starvation although the activities of glucose 6-phosphatase, hexose diphosphatase and pyruvate carboxylase increase. Furthermore, there are only small changes in the activity of phosphoenolpyruvate carboxykinase in pregnant, starved pregnant or phlorrhizinized sheep (all conditions in which glucose demand is increased) and in lactating, starved or ketotic cows (Filsell et al, 1969; Baird, Hibbitt, Hunter, Lund, Stubb and Krebs, 1968; Ballard, Hanson, Kronfeld and Raggi, 1968).

Changes in the activities of the key enzymes of gluconeogenesis are also associated with hormonal fluctuations. Gluconeogenesis and phosphoenolpyruvate carboxykinase activity of the rat are increased after the administration of glucagon or dibutyryl cyclic AMP (Shrago et al, 1963; Wicks, Kenney and Lee, 1969; Reshef and Hanson, 1972; Hanson, Fisher, Ballard and Reshef , 1973; Tilghman, Hanson, Reshef, Hopgood and Ballard, 1974). Gluconeogenesis is also increased during diabetes where an increase in phosphoenolpyruvate carboxykinase activity occurs (Shrago et al, 1963; Foster et al, 1966).

Treatment of diabetic animals with insulin results in a decrease in hepatic phosphoenolpyruvate carboxykinase activity (Weber, Singhal and Srivastava, 1965; Freedland, Cunliffe and Zinkl, 1966), and is caused by a decrease in the rate of enzyme synthesis (Wicks et al, 1974; Tilghman et al, 1974). Insulin lowers the hepatic concentration of cyclic AMP (Park and Exton, 1972; Park, Lewis and Exton, 1972) and the decrease in hepatic phosphoenolpyruvate carboxykinase can be blocked by dibutyryl cyclic AMP (Tilghman et al , 1974).

Ovine phosphoenolpyruvate carboxykinase and pyruvate carboxylase activities are similarly increased during diabetes induced by alloxan or by pancreatectomy (Filsell et al, 1969; Taylor, Wallace and Keech, 1971).

Glucocorticoids increase the rate of gluconeogenesis in the perfused liver and have been reported to increase the activities of hepatic phosphoenolpyruvate carboxykinase and pyruvate carboxylase in fed intact or adrenalectomized rats (Lardy, Foster, Shrago and Ray, 1964; Freedman and Kohn, 1964; Henning, Seiffert and Seubert, 1963; Shrago et al, 1963; Foster et al, 1966, Wicks et al, 1969). However, these observations are in conflict with those of Reshef, Ballard and Hanson (1969) who found that triamcinolone, a synthetic glucocorticoid, had no effect on phosphoenolpyruvate carboxykinase activity in fed rats, while it decreased hepatic phosphoenolpyruvate carboxykinase activity in starved rats (Reshef, Hanson and Ballard, 1970).

Gunn, Hanson, Meyuhas, Reshef and Ballard (1975) have clarified the situation by the determination of the rate of phosphoenolpyruvate carboxykinase synthesis and degradation *in vivo* after glucocorticoid administration. In both fed and starved rats the rate of synthesis of phosphoenolpyruvate carboxykinase decreased when triamcinolone or cortisol was injected. Only in diabetic rats did glucocorticoids increase the rate of phosphoenolpyruvate carboxykinase synthesis. Since the increases in blood glucose and insulin concentrations were found to accompany triamcinolone injection in starved rats (Gunn *et al*, 1975) it was proposed that the action of glucocorticoids to decrease phosphoenolpyruvate carboxykinase activity in these animals was mediated by insulin. Since insulin could not be released in diabetic animals phosphoenolpyruvate carboxykinase synthesis was increased by the administration of glucocorticoids.

Administration of glucocorticoids to the adult sheep results in hyperglycaemia (Bassett, 1963; Bassett, Mills and Reid, 1966) but are without effect on the activity of the enzymes of gluconeogenesis (Filsell *et al*, 1969). Therefore, it is not possible for the hyperglycaemia which follows the administration of glucocorticoids to the adult sheep to result from an increase in gluconeogenic capacity. An increase in substrate availability (e.g. amino acids) preceded the increase in glucose concentration (Reilly and Ford, 1971b, 1974) and indicated

an increase in gluconeogenic flux. No increase in propionate conversion to glucose was brought about by glucocorticoid administration (Ford and Winchester, 1974).

From the foregoing statements it is apparent that gluconeogenesis normally functions at close to maximum capacity in the fed adult ruminant and that during conditions of increased glucose demand no marked increase in gluconeogenic capacity occurs. This has important implications during pregnancy when extra glucose is required to meet the demands of the fetus. Under these conditions glucose production increases, presumably as a result of increased gluconeogenic flux due to a greater availability of substrates (Bergman, 1963, 1964; Katz and Bergman, 1969).

Setchell, Bassett, Hinks and Graham (1972) have calculated that the average glucose uptake of the pregnant uterus is equivalent to 70% of the glucose production of the ewe. Of this glucose the fetal lamb has been estimated to require 32 g per day (Kronfeld, 1958) or about 30% of the glucose available to the ewe (Lindsay,1971). Furthermore, Curet, Crenshaw, Mann, Abrams and Barron (1970) have calculated that up to 40 g amino acids per day are removed by the pregnant uterus, thereby depleting the pool of maternal gluconeogenic substrates. It may be concluded that pregnancy presents an extraordinary demand on the gluconeogenic pathway of the ewe . An inability to maintain glucose production would be detrimental to

both ewe and fetus, a situation exemplified in ovine pregnancy toxaemia (i.e. twin lamb disease) (Reid, 1968; Patterson and Cunningham, 1969).

Gluconeogenesis has been shown to play a vital role in the supply of glucose in the adult sheep, but little is known of the gluconeogenic activity of the fetal lamb. In the fetal lamb glucose is not only oxidised (Tsoulos, Colwill, Battaglia, Makowski and Meschia, 1971; James, Raye, Gresham, Makowski, Meschia and Battaglia, 1972) but it is also utilized for the synthesis of glycogen, lipids, fructose etc. As gestation progresses glycogen accumulates in the fetal liver, skeletal muscle and cardiac muscle, until at term the glycogen content of these tissues is higher than found in the adult. At term the liver glycogen concentration is 80 mg per g wet wt which is twice the adult value. In skeletal muscle and cardiac muscle at term glycogen is present at 38 mg per g wet wt and 16 mg per g wet wt respectively, values which are both 5 times the adult values (Shelley, 1961). During normal intra-uterine development glycogen mobilization is low, but utilization of the glycogen reserves has been shown to occur readily during stresses such as induced asphyxia (Dawes, Mott and Shelley, 1959; Shelley, 1961; Britton, Nixon and Wright, 1967). After birth glycogen is a ready source of glucose and concentrations fall to 10% of fetal values within the first day of two (Shelley, 1961; Edwards and Silver, 1969).

Glucose can be converted to lipid in the fetal lamb for, unlike the adult sheep, the enzymes ATP-citrate lyase and NADP-malate dehydrogenase are active in the liver of the fetal ruminant (Hanson and Ballard, 1967, 1968). During fetal development there is a substantial synthesis of lipids, but after birth much of the lipid is mobilized. This leads to a 6 fold increase in plasma fatty acids during the first 2 hr of postnatal life (Van Duyne, Parker, Havel and Holm, 1960; Alexander and Mills, 1968; Comline and Silver, 1972). The delayed utilization of lipid suggests that their potential as an alternative source of energy to glucose is not realised until after birth.

Fructose is another important product of fetal glucose metabolism. It is the predominate carbohydrate found in the blood of the fetal ruminant and represents up to 70% of the total carbohydrate present (Bacon and Bell, 1946, 1948; Cole and Hitchcock, 1946). Fructose is absent from the blood of the adult ruminant. Synthesis of fructose is apparently restricted to the placenta (Alexander, Huggett, Nixon and Widdas, 1955; Andrews, Britton, Huggett and Nixon, 1960) although aldose reductase, which converts glucose to sorbitol is located in the placenta and sorbitol dehydrogenase, which catalyses the conversion of sorbitol to fructose is located in the liver.

Fructose utilization by the fetal lamb is very low (Alexander, Britton and Nixon, 1966, 1970; Setchell *et al*, 1972). The capacity to metabolize substantial amounts of fructose does not appear until 5 days after birth and is dependant on the appearance of hepatic fructokinase (Andrews *et al*, 1960; Andrews, Britton and Nixon, 1961; Ballard and Oliver, 1965).

Since glucose plays a central role in the metabolism of the fetus it would be advantageous if the fetus was able to maintain or supplement its glucose supply through gluconeogenesis. However, in the fetal rat both *in vitro* and *in vivo* experiments have demonstrated that gluconeogenesis is absent (Ballard and Oliver, 1965; Philippidis and Ballard, 1969). Activity develops after birth and parallels the dramatic increase in phosphoenolpyruvate carboxykinase activity (Ballard and Hanson, 1967). The activity of cytosol phosphoenol-pyruvate carboxykinase is very low in fetal rat liver and the absence of gluconeogenic activity in the fetus is largely dependant upon this fact. Administration of glucagon to the fetal rat *in utero* will promote a 10-15 fold increase in phosphoenolpyruvate carboxykinase activity. Even so, *in vivo* gluconeogenesis is not induced (Philippidis and Ballard, 1970), but gluconeogenic activity in liver slices from these fetuses increased to 50% of adult values.

Philippidis and Ballard (1970) postulated that the failure to induce gluconeogenesis in fetal rats, with a full complement of key enzymes, was associated with the hypoxia which invariably accompanies maternal sedation. This hypothesis was supported by Ballard (1971a) who reported inhibition of gluconeogenesis in suckling neonatal rats exposed to a N_2 atmosphere. These experiments illustrate the problems inherent in extrapolation from the *in vitro* to the *in vivo* situation.

It has not been possible to repeat the experiments of Philippidis and Ballard (1970) with non-anaesthetised, chronically cannulated fetal rats because of the technical problems associated with the cannulation of the minute blood vessels. Further studies of the development of gluconeogenesis in the fetal rat are therefore limited.

Due to its size and availability the sheep is particularly suited to the study of fetal physiology and biochemistry during the latter stages of gestation. Surgical techniques are available for the chronic cannulation of the fetal lamb, consequently the development of gluconeogenesis, a pathway so important to the adult sheep, may be studied without the limitations imposed by anaesthesia.

It is the aim of this dissertation to examine

the development of gluconeogenesis in the sheep. Chapter III describes the measurement of the activities of the key enzymes of gluconeogenesis; glucose 6-phosphatase, hexose diphosphatase, phosphoenolpyruvate carboxykinase and pyruvate carboxylase. In Chapter IV *in vivo* experiments are presented which are designed to measure the availability of lactate and fructose for glucose synthesis and to assess the contribution of glucose to these pools. Finally the response of fetal gluconeogenesis to adrenaline and glucagon infusions and the changes at parturition are described in Chapters V and VI.

CHAPTER II

MATERIALS

II.1. Chemicals

All chemicals were of analytical grade and all solutions were prepared in glass distilled water. Before use all solvents, with the exception of toluene used for liquid scintillation counting, were redistilled.

Phosphoenolpyruvate, sodium pyruvate, fructose-1,6-diphosphate, inosine diphosphate, malate dehydrogenase (NAD^+), phosphoglucoseisomerase, hexokinase, glucose 6-phosphate dehydrogenase and lactate dehydrogenase were obtained from Boehringer (Mannheim), Germany. Sigma Chemical Co., St. Louis, Mo., U.S.A. were the suppliers of glucose 6-phosphate, acetyl CoA, dithiothreitol, lithium lactate, 2(-p-iodo-phenyl)-3-p-nitrophenyl-5-phenyl tetrazolium chloride, NAD^+, NADH, $NADP^+$, ATP and diaphorase from Cl. kluyveri, Type II L. Koch-Light Laboratories Ltd., Colnbrook, Bucks., England were the suppliers of 2-mercaptoethanol.

II.2. Surgery

Portex polyvinyl tubing for cannulae was obtained from Boots Co.,(Aust) Pty., Ltd. Steridrapes were obtained from Minnesota Mining and Manufacturing Co., Minnesota, U.S.A. Ethicon suture materials were

purchased from Ethnor Pty., Ltd., Sydney, Australia. Veet hair removing cream was obtained from Dae Health Laboratories Ltd., England. Chlorhexidine solution (chlorhexidine, 0.05%: cetrimide, 0.5% : sodium nitrite, 0.4% in ethanol) and Hibitane cream were purchased from I.C.I., Australia. Ceporan (Cephaloridine (∝-form)) was purchased from Glaxo Lab., Ltd., England and Garamycin (Gentamycin sulphate) was purchased from Schering Corp., New Jersey, U.S.A. Astra Chemical Pty., Ltd., N.S.W., Australia supplied Lignocaine (Xylocaine 2%). Rompun (2% Xylazine) was obtained from Bayer, Australia Ltd. Heparin (mucous) was obtained from Allen and Hanburys, Vic., Australia. Adrenaline tartrate (Hermette) was purchased from David G. Bull, Lab., Pty., Ltd., Canterbury, Australia. Glucagon was purchased from Eli Lilly Laboratories, Indianapolis, Ind., U.S.A.

II.3. Isotopes

D-(U-C^{14})-glucose, specific radioactivity 230 mCi/mmol, D-(U-C^{14})-fructose, specific radioactivity 50-500 mCi/mmol, L-(U-C^{14})-lactic acid, sodium salt, specific radioactivity 20-50 mCi/mmol and sodium bicarbonate ($NaH^{14}CO_3$), specific radioactivity 40 mCi/mmol, were obtained from the Radiochemical Centre, Amersham, England.

Hydrocortisone [1,2,6,7-^3H], specific radioactivity 82.7 Ci/mM was obtained from New England Nuclear, Mass, U.S.A.

The fluors, 2,5-diphenyloxazole (PPO) and 1,4-di-2-(5-phenyloxazolyl)-benzene (POPOP) were obtained from Packard Instrument Co., Inc., U.S.A. The toluene and toluene-triton X-100 (2:1, v/v) scintillation fluids contained 3 g PPO and 0.5 g POPOP per litre toluene.

CHAPTER III

MEASUREMENT OF HEPATIC GLUCONEOGENIC ENZYME ACTIVITIES

III.1. Introduction

The enzymes glucose 6-phosphatase, hexose diphosphatase, phosphoenolpyruvate carboxykinase (cytosolic and mitochondrial) and pyruvate carboxylase are obligatory for gluconeogenesis (Scrutton and Utter, 1968). In the fetal rat cytosol phosphoenolpyruvate carboxykinase activity is very low and gluconeogenesis is inactive. In this species the gluconeogenic capacity increases after birth and parallels the postnatal increase in cytosol phosphoenolpyruvate carboxykinase activity (Ballard and Hanson, 1967; Philippidis and Ballard, 1969). Accordingly it has been argued that phosphoenolpyruvate carboxykinase regulates gluconeogenesis during development. It is my aim in this chapter to report the activities of the four key enzymes of gluconeogenesis in liver extracts from developing lambs.

III.2. Methods

1. Collection of samples

Fetal lamb livers and liver samples from adult sheep were obtained through the cooperation of the local abattoir, SAMCOR. Livers were immediately placed in ice-cold 0.15 M NaCl for transport to the laboratory. The crown-rump length and weight of each fetus were

measured for estimation of fetal age using the nomogram shown in Appendix 1.

Livers from postnatal lambs were collected from freshly slaughtered lambs of known age and immediately placed in ice-cold 0.15 M NaCl. Lambs were kindly donated by the C.S.I.R.O., Division of Nutritional Biochemistry, Glenthorne Experimental Field Station, O'Halloran Hill, South Australia.

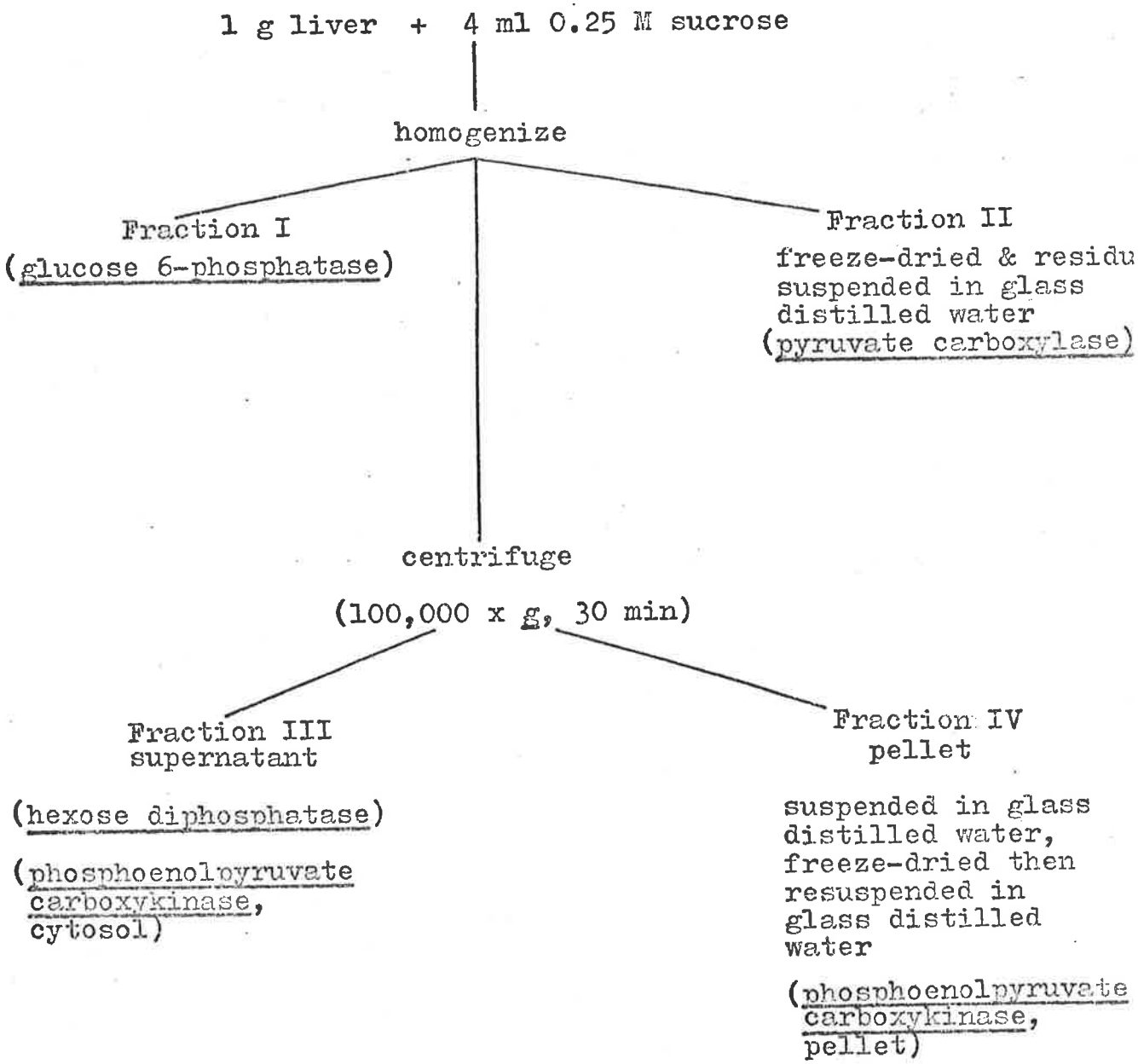
2. Preparation of liver extracts

All steps were carried out at 4°C.

Livers were chopped into small pieces and 1 g portions taken for homogenization in 4 ml of 0.025M sucrose using a teflon and glass coaxial homogenizer. A portion of the crude homogenate was retained for assay of glucose 6-phosphatase activity (Scheme 1, Fraction I). A second portion (0.5 ml) of the crude homogenate was freeze-dried and then suspended in water for immediate assay of pyruvate carboxylase activity (Scheme 1, Fraction II The remaining homogenate was centrifuged at 100,000 x g for 30 min and the supernatant collected (Scheme 1, Fraction III). Hexose diphosphatase and phosphoenol-pyruvate carboxykinase activity were assayed in this fraction. The pellet was suspended in water, freeze-dried and re-suspended in water for immediate analysis of particulate phosphoenolpyruvate carboxykinase activity (Scheme 1, Fraction IV).

Scheme 1

Preparation of Liver Homogenates



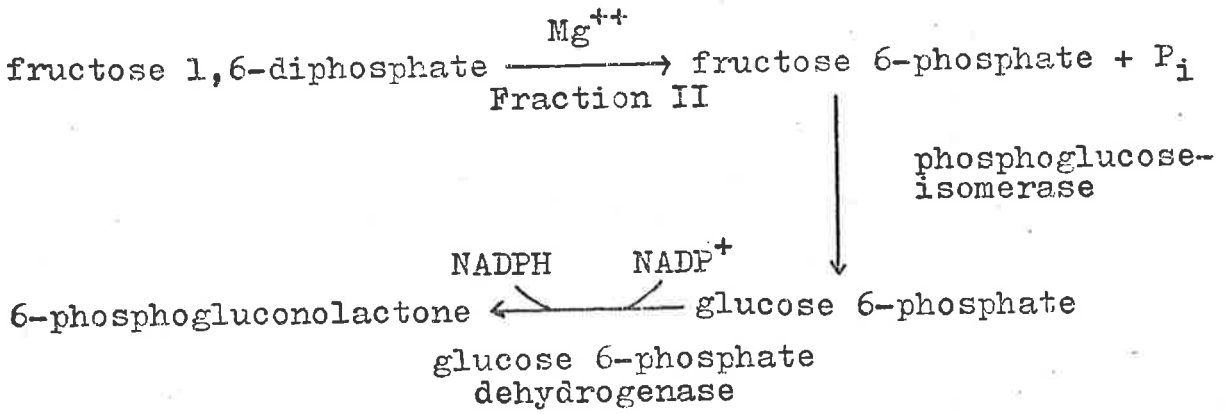
3. Glucose 6-phosphatase assay

Glucose 6-phosphatase activity was measured as the amount of inorganic phosphate released on incubation of Fraction I with glucose 6-phosphate, using the method described by Shull, Ashmore and Mayer (1956). Enzyme activity is expressed as that amount of enzyme which catalysed the release of 1 µmol P_i/ g wet wt/ min at 37°C. The assay mixture contained : 100 µmol glucose 6-phosphate and an aliquot of Fraction I to give a total volume of 150 µl. Enzyme and buffer blanks were included with each group of samples. After incubation for 0, 15 or 30 min the reaction was stopped by the addition of 0.9ml of 10% trichloroacetic acid (w/v) and the protein precipitate removed by centrifugation.

A portion of the supernatant (0.5 ml) was mixed with 1 ml of ferrous-ammonium molybdate solution which contained 5.0 g $FeSO_4.7H_2O$ and 10 ml of a 10 % solution of $(NH_4)_6Mo_7O_{24}.4H_2O$ in 10 N H_2SO_4 per 100 ml H_2O. After standing for 15 min the absorbance at 660 nm was measured and compared to phosphate standards (Taussky and Shorr, 1953).

4. Hexose diphosphatase assay

The assay of hexose diphosphatase activity was based upon the method of Taketa and Pogell (1965). The reaction sequence is as follows:-



The rate of reduction of $NADP^+$ was followed spectrophotometrically at 340 nm in a Zeiss PMQ II spectrophotometer. Enzyme activity is expressed in units of µmol NADPH formed per g wet wt per min at 37°C.

Each cuvette contained 100 µmol tris-HCl buffer, pH 7.5, 0.25 µmol fructose 1,6-diphosphate, 2.5 µmol $NADP^+$, 20 µmol $MgSO_4.7H_2O$, 0.05 µmol 2-mercaptoethanol, 0.03 µmol EDTA, 25 µg glucose 6-phosphate dehydrogenase and 5 µg phosphoglucoseisomerase in a total volume of 0.9 ml. The reaction was started by the addition of 10 µl of Fraction III. Enzyme and buffer blanks were included with each group of samples.

5. Phosphenolpyruvate carboxykinase assay

Phosphoenolpyruvate carboxykinase activity was measured according to the method of Chang and Lane (1966) with modifications as reported by Ballard and Hanson (1967). The assay measured the incorporation of ^{14}C-bicarbonate into oxaloacetate.

The reaction mixture contained : 60 µmol imidazole-HCl buffer, pH 6.6, 0.6 µmol $MnCl_2$, 0.6 µmol dithiothreitol, 1.3 µmol NADH, 1.1 µmol phosphoenolpyruvate, 30 µmol $KHCO_3$ (1.25 µCi $NaH^{14}CO_3$) and 20 µg malate dehydrogenase (NAD^+) in a final volume of 0.5 ml. Each tube contained 25 µl of enzyme , Fraction III or IV and all, except the blanks, contained 0.5 µmol IDP. The reaction was started by the addition of either Fraction III or IV and stopped after 0, 5 or 10 min by the addition of 1 ml of 10% trichloroacetic acid. The blanks were stopped after 7.5 min. Free ^{14}C-bicarbonate was removed by gassing the mixture with CO_2 for 3 min and 0.75 ml of the acid solution was taken for liquid scintillation counting in 10 ml of toluene-Triton X-100 scintillation fluid.

Enzyme activity is expressed as that amount of enzyme which catalysed the fixation of 1 µmol of ^{14}C-bicarbonate into oxaloacetate per g wet wt per min at 37°C after blank values had been subtracted.

6. Pyruvate carboxylase assay

Pyruvate carboxylase activity was assayed according to the method of Utter and Keech (1963) in which the incorporation of ^{14}C-bicarbonate into oxaloacetate was measured.

The reaction mixture contained: 12 μmol tris-HCl buffer, pH 7.4, 0.6 μmol sodium ATP, 2.5 μmol $MgCl_2$, 12 μmol $KHCO_3$ (0.5 μCi $NaH^{14}CO_3$) and 2 μmol sodium pyruvate in a final volume of 0.27 ml. Each tube, except the blanks, contained 0.75 μmol acetyl CoA. The reaction was started by the addition of 20 μl of Fraction II and was stopped by the addition of 1 ml of 10 % trichloroacetic acid at 0, 5, 10 or 15 min. Blanks were stopped at 10 min. Excess ^{14}C-bicarbonate was removed by gassing with CO_2 for 3 min and 0.6 ml of the acid solution was taken for liquid scintillation counting in 10 ml of toluene-Triton X-100 scintillation fluid.

Enzyme activity is expressed as that amount of enzyme which catalysed the fixation of 1 μmol of ^{14}C-bicarbonate into oxaloacetate per g wet wt per min at 37°C after blank values were subtracted.

III.3. Results

The hepatic activities of the four key enzymes of gluconeogenesis of fetal and postnatal lambs are presented in Fig 2, 3, 4, 5 and 6. The activities of the fetal enzymes are presented as both a function of crown-rump length and gestational age.

All enzyme activities quoted in the following section are the mean ± S.E.M., compiled from the data grouped as crown-rump length for fetal lambs, postnatal

age in days for lambs or as adult sheep.

Glucose 6-phosphatase activity increased from 6.83 ± 0.08 μmol/ g wet wt/ min in livers of fetal lambs of 40 days gestation to 9.99 ± 1.05 μmol / g wet wt /min in the livers of term lambs (Fig 2). Activity increased to 15.8 μmol / g wet wt / min 5 days after birth then decreased to a value of 8.73 ± 0.75 μmol / g wet wt / min in the liver of adult sheep.

Hexose diphosphatase activity in the liver of fetal lamb at 40 days gestation increased from 3.10 ± 0.15 μmol / g wet wt / min to a value of 12.80 ± 0.30 μmol / g wet wt /min in the livers of term lambs (Fig 3). The activity of hexose diphosphatase was 10.80 ± 0.070 μmol / g wet wt / min in the adult liver. The activity found in term and suckling lambs was similar to the activity in the livers of adult sheep.

Cytosol phosphoenolpyruvate carboxykinase activity had an activity of 0.19 ± 0.002 μmol / g wet wt/min in fetal lamb liver at 40 days gestation (Fig 4). The activity increased with gestational age to a value of 0.90 ± 0.10 μmol / g wet wt / min in the liver of term lambs and further increased to an activity of 1.60 ± 0.09 μmol / g wet wt / min in adult sheep.

The activity of particulate phosphoenolpyruvate carboxykinase showed a different pattern of development

Figure 2: Hepatic Glucose 6-phosphatase Activity

Glucose 6-phosphatase activity is presented as a function of crown-rump length (cm) and gestational age (days) for fetal lambs and as postnatal age (days) for lambs. 'A' represents the activity found in the adult sheep liver.

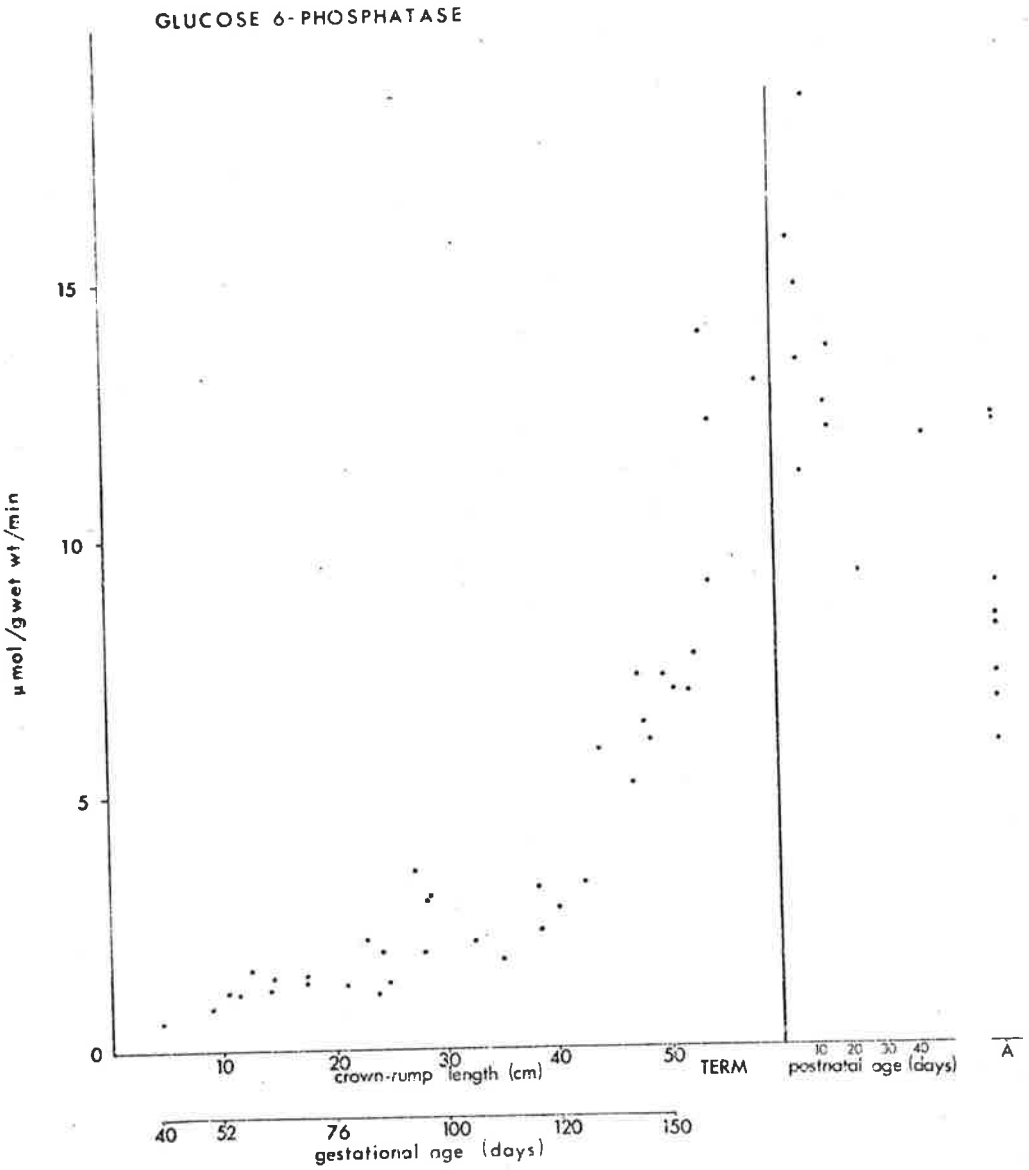

GLUCOSE 6-PHOSPHATASE
μmol/g wet wt/min
15
10
5
0
10
20
30
40
50
crown-rump length (cm)
TERM
10 20 30 40
postnatal age (days)
A
40
52
76
100
120
150
gestational age (days)

Figure 3 : Hepatic Hexose Diphosphatase Activity

Hexose diphosphatase activity is presented as a function of crown-rump length (cm) and gestational age (days) for fetal lambs and as post-natal age (days) for lambs.

'A' represents the activity found in the adult sheep liver.

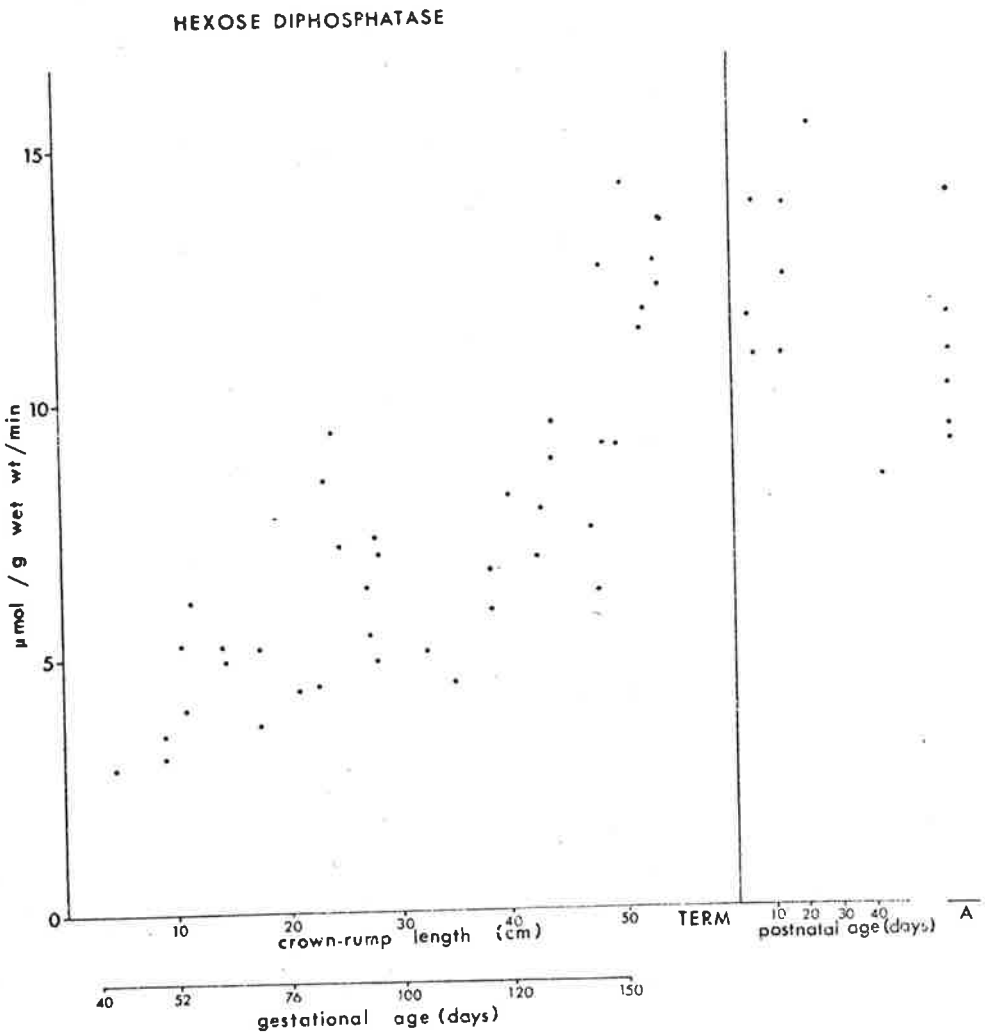
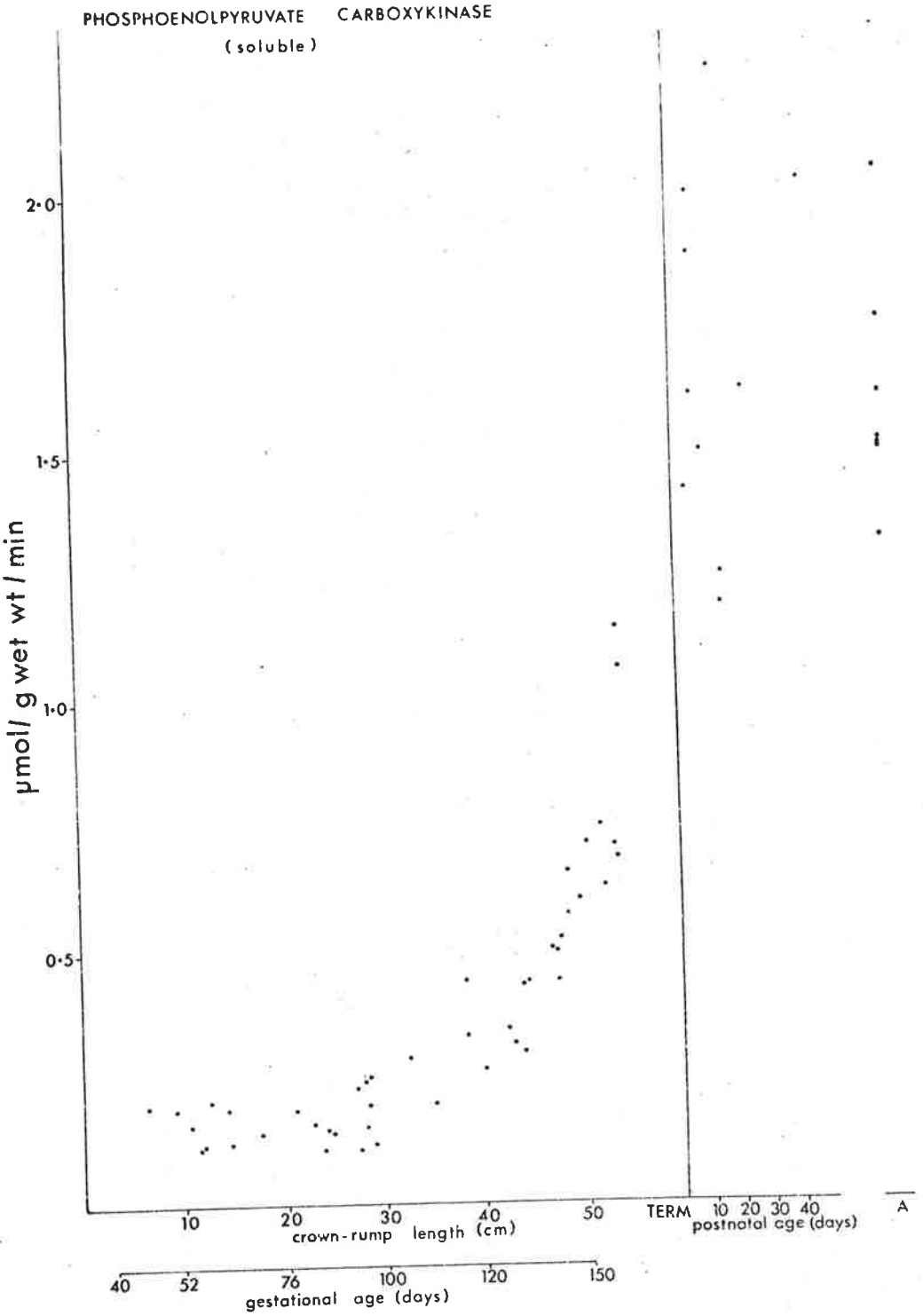

HEXOSE DIPHOSPHATASE
15
10
5
0
µmol / g wet wt / min
10
20
30
40
50
TERM
10
20
30
40
A
crown-rump length (cm)
postnatal age (days)
40
52
76
100
120
150
gestational age (days)

Figure 4 : Hepatic Phosphoenolpyruvate Carboxy-kinase Activity (soluble)

Phosphoenolpyruvate carboxykinase (cytosol) activity is presented as a function of crown-rump length (cm) and gestational age (days) for fetal lambs and as postnatal age (days) for lambs.

'A' represents the activity found in the adult sheep liver.


PHOSPHOENOLPYRUVATE CARBOXYKINASE
(soluble)
μmol/ g wet wt / min
2·0
1·5
1·0
0·5
10
20
30
40
50
TERM
10 20 30 40
crown-rump length (cm)
postnatal age (days)
A
40
52
76
100
120
150
gestational age (days)

to the cytosol enzyme (Fig 5). There was little change in activity from 40 days gestation (1.30 ± 0.28 µmol / g wet wt / min) to term (1.43 ± 0.07 µmol/ g wet wt / min). After birth the activity increased to a value of 2.95 ± 0.05 µmol / g wet wt / min at 7 days. The activity of particulate phosphoenolpyruvate carboxykinase in adult liver was 1.47 ± 0.17 µmol / g wet wt / min.

Pyruvate carboxylase activity increased from 1.30 ± 0.10 µmol / g wet wt /min at 40 days gestation to 2.92 ± 0.30 µmol / g wet wt / min at term (Fig 6). After birth the activity increased to 5.23 ± 0.10 µmol / g wet wt / min at 7 days. The activity of pyruvate carboxylase in the adult sheep liver was 3.72 ± 0.30 µmol / g wet wt / min.

III.4. Discussion

The results show that the key regulatory enzymes of gluconeogenesis : glucose 6-phosphatase, hexose diphosphatase, cytosol and particulate phosphoenol-pyruvate carboxykinase and pyruvate carboxylase were all active in the liver of the fetal lamb. With the exception of particulate phosphoenolpyruvate carboxykinase the activity of these enzymes increased during fetal development, particularly during the last 30 days of gestation when growth accelerates (Cloette, 1939; Wallace, 1948).

Figure 5 : Hepatic Phosphoenolpyruvate Carboxykinase Activity (particulate)

Phosphoenolpyruvate carboxykinase (particulate) activity is presented as a function of crown-rump length (cm) and as gestational age (days) for fetal lambs and as postnatal age (days) for lambs.

'A' represents the activity found in the adult sheep liver.

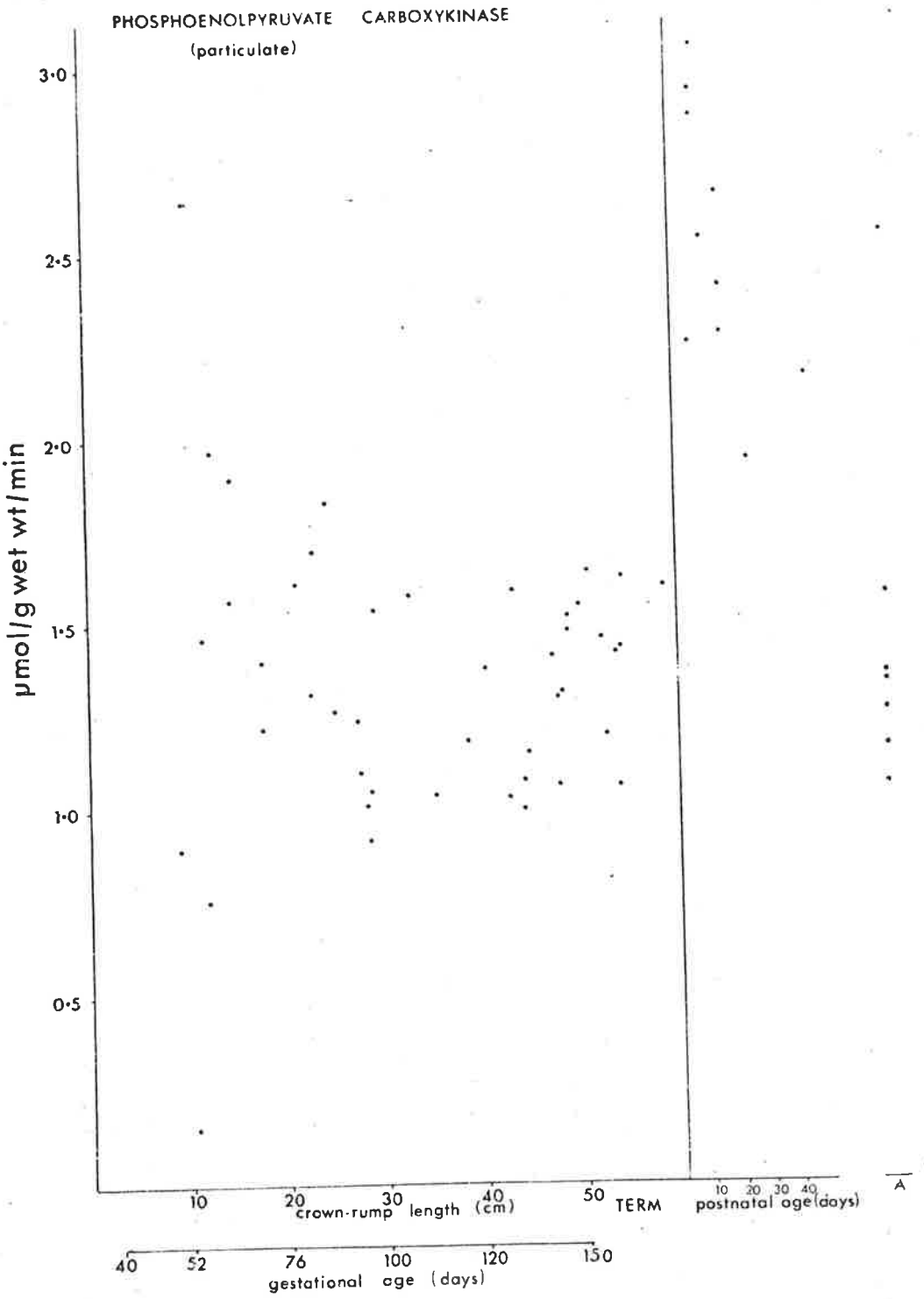
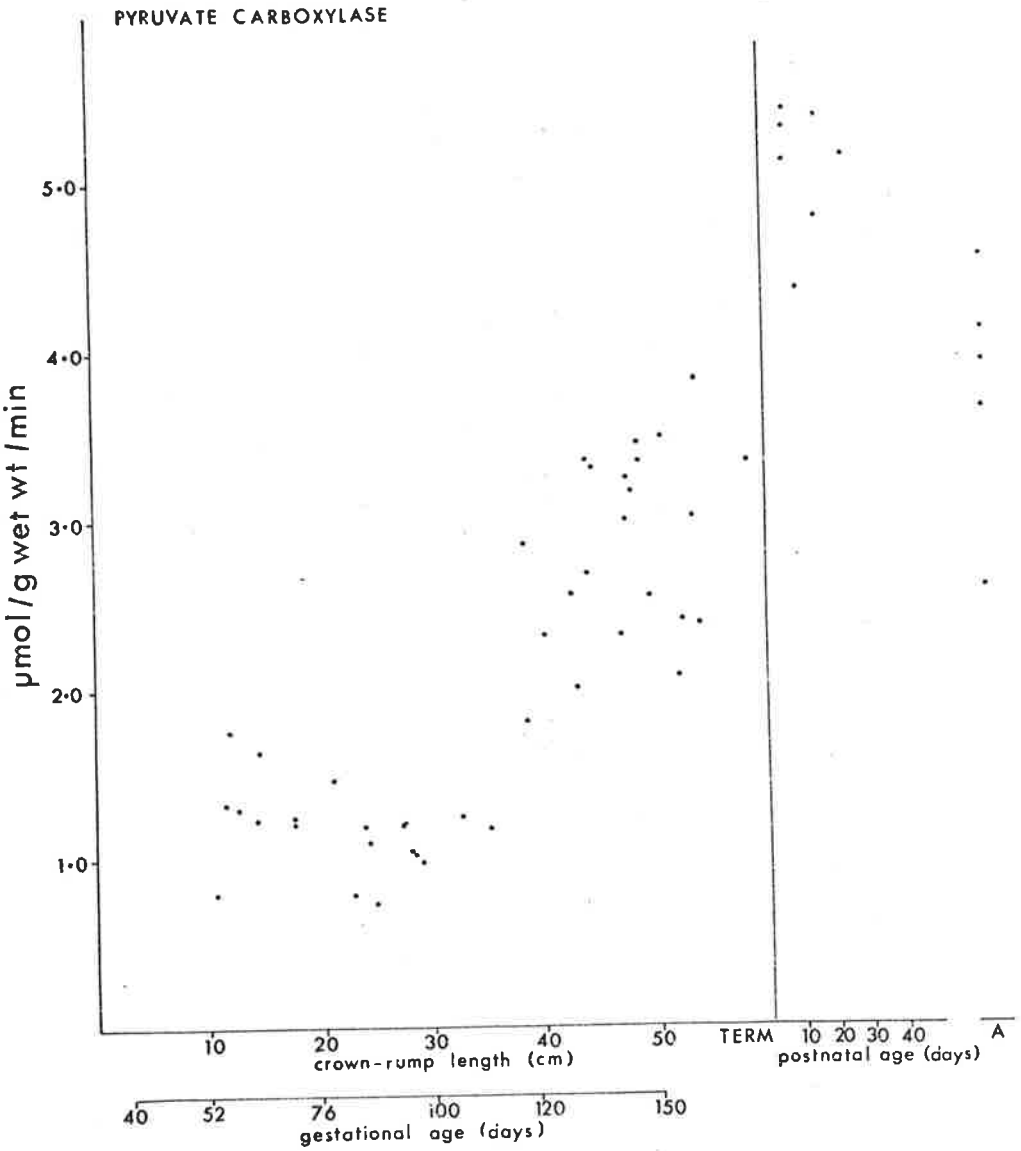

PHOSPHOENOLPYRUVATE CARBOXYKINASE
(particulate)
3·0
2·5
2·0
1·5
1·0
0·5
μmol/g wet wt/min
10
20
30
40
50
crown-rump length (cm)
TERM
10 20 30 40
postnatal age(days)
A
40
52
76
100
120
150
gestational age (days)

Figure 6 : Hepatic Pyruvate Carboxylase Activity

Pyruvate carboxylase activity is presented as a function of crown-rump length (cm) and as gestational age (days) for fetal lambs and as postnatal age (days) for postnatal lambs.

'A' represents the activity found in the adult sheep liver.


PYRUVATE CARBOXYLASE
μmol/g wet wt /min
5·0
4·0
3·0
2·0
1·0
10
20
30
40
50
TERM
10 20 30 40
A
crown-rump length (cm)
postnatal age (days)
40
52
76
100
120
150
gestational age (days)

A further increase in activity occurred after birth when levels of activity were as high or higher than observed in adult liver. In contrast, the activity of particulate phosphoenolpyruvate carboxykinase was high throughout gestation. After birth the activity of this enzyme increased in a similar fashion to the other enzymes.

In the fetal lamb the pattern of development of activity of glucose 6-phosphatase and hexose diphosphatase has been previously described by Dawkins (1961) and Ballard and Oliver (1965). The data presented by these authors showed that both glucose 6-phosphatase and hexose diphosphatase activity was detectable in the liver of the fetal lamb as early as 3 months gestation. Ballard and Oliver (1965) reported the activity of glucose 6-phosphatase to be 1.15 μmol / g / min and the activity of hexose diphosphatase to be 1.11 μmol / g / min in 3 month fetuses. Glucose 6-phosphatase and hexose diphosphatase activity increased to 9.08 μmol / g / min and 6.08 μmol / g / min respectively at term. After birth further increases in enzyme activities occurred reaching maxima at 4-7 weeks when values of 13.33 μmol / g / min for glucose 6-phosphatase and 12.4 μmol / g /min for hexose diphosphatase were attained.

The activity of glucose 6-phosphatase found in this study was of the same order of magnitude as that reported by Ballard and Oliver (1965), while fetal

hexose diphosphatase activity was higher. The discrepancy is probably explained by the more suitable hexose diphosphatase assay employed in this study. Despite the quantitative difference the general pattern of development of both enzymes are similar to that previously reported (Ballard and Oliver,1965) and is similar to the pattern reported for many other species (Table 1).

Cytosol and particulate phosphoenolpyruvate carboxykinase and pyruvate carboxylase activities in the developing sheep liver have not previously been reported. The pattern of development of these enzymes shown by the lamb is generally similar to other species (Table 1), with the exception of the rat,in which hepatic cytosol phosphoenolpyruvate carboxykinase increases dramatically after birth (Ballard and Hanson, 1967). The activities of these enzymes in fetal liver is generally less than in the adult. Maximum activities are usually reached several days after birth, thereafter decreasing to adult values. The activity of particulate phosphoenolpyruvate carboxykinase remained constant throughout gestation in the lamb; similar to the rat and guinea pig (Ballard and Hanson, 1967; Arinze, 1975). This is of interest as the particulate enzyme is not inducible in the adult animal (Lardy et al, 1964 ; Nordlie, Varrichio and Holten, 1965; Taylor et al, 1971). In the rat immunochemical studies have shown that cytosol

and particulate phosphoenolpyruvate carboxykinase are distinct enzymes although they have somewhat similar physical and kinetic properties (Ballard and Hanson, 1967, 1969).

Much research has focussed upon the factors which may initiate the perinatal increase in enzyme activity. Soluble phosphoenolpyruvate carboxykinase activity may be induced in the fetal rat by premature delivery, glucagon, adrenaline, nor-adrenaline or cyclic AMP (Yeung and Oliver, 1967, 1968 a&b; Philippidis and Ballard, 1969; Girard, Caquet, Bal and Guillet, 1973; Hanson, Fisher, Ballard and Reshef, 1973). Triamcinolone, a synthetic glucocorticoid, was without effect and did not induce glucose 6-phosphatase, hexose diphosphatase or pyruvate carboxylase (Yeung, Stanley and Oliver, 1967). Glucose 6-phosphatase activity in the fetal rat also increased in response to glucagon, adrenaline, thyroxine and premature delivery (Dawkins, 1961; Greengard and Dewey, 1967; Greengard, 1969). Insulin will inhibit the postnatal increase in phosphoenolpyruvate carboxykinase and glucose 6-phosphatase activity (Yeung and Oliver, 1968; Girard et al, 1973).

The increase in enzyme activity produced by the administration of hormones is generally associated with the synthesis of new enzyme protein since inhibitors of mRNA or protein synthesis (Actinomycin D, puromycin or

ethionine) prevent increases in enzyme activity (Dawkins, 1963; Yeung and Oliver, 1968b; Hanson et al, 1973). Furthermore, the increase in phosphoenolpyruvate carboxykinase activity is accompanied by a commensurate increase in immunoprecipitable enzyme protein (Philippidis, Hanson, Reshef, Hopgood and Ballard, 1972).

Although the intra-uterine administration of the hormones glucagon, adrenaline, nor-adrenaline or thyroxine will induce phosphoenolpyruvate carboxykinase and glucose 6-phosphatase activity in fetal rat liver it is only possible to speculate as to the extent of involvement of these hormones in the natural increase in the activities of these enzymes.

The adrenal gland of the fetal lamb is mature before birth. As early as 80-90 days gestation the adrenal medulla will secrete catecholamines in response to hypoxia or acetyl choline (Comline, Silver and Silver, 1965). Nor-adrenaline is the predominate catecholamine secreted at this age. As the adrenal gland matures innervation increases and the direct response to hypoxia diminishes . Adrenaline, secreted in response to nervous stimulation, is the major catecholamine secreted during the latter stages of gestation (Comline and Silver, 1966).

The pancreas is functional early in fetal development. Insulin and glucagon have been detected in the

pancreas and plasma of the fetal lamb early in gestation Alexander, Britton, Cohen, Nixon and Parker, 1968; Alexander, Assan, Britton and Nixon, 1971). The fetal pancreas will secrete insulin in response to hyperglycaemia *in vivo* and *in vitro* (Alexander *et al*, 1968 ; Alexander, Britton, Mashiter, Nixon and Smith, 1970; Davis, Beck, Colwill, Makowski, Meschia and Battaglia, 1971; Bassett and Thorburn, 1971; Bassett, Thorburn and Nicol, 1973; Bassett, Madill, Nicol and Thorburn, 1973; Bassett and Madill, 1974 a&b). The pancreatic α-cell of the newborn calf will secrete glucagon in response to sympathetic stimulation (Bloom , Edwards and Vaughan, 1974), a response which can be reproduced in the rat fetus with acetyl choline or nor-adrenaline (Girard *et al*, 1973). In the adult rat, glucagon secretion is evoked by hyperglycaemia (Unger and Lefebvre, 1972; Pagliara, Stillings, Hiver, Martin and Matschinsky, 1974; Gerich, Charles and Grodsky, 1974) and the *in vitro* secretion of glucagon from the adult rat pancreas is enhanced by adrenaline, while insulin release is inhibited (Mallaisse, Mallaisse-Lagae, Wright and Ashmore, 1967; Porte, 1967; LeClerq-Meyer, Brisson and Mallaisse, 1971).

The postnatal increase in enzyme activity may be triggered by hormonal or nutritional factors. Girard and his associates have demonstrated that an increase in plasma glucagon concentrations and a decrease in plasma insulin accompanies birth in the rat (Girard,

Bal and Assan, 1971,1972; Girard et al, 1973; Girard, Kervran, Soufflet and Assan, 1974). Blasquez, Montoya and Quijada (1970) have also reported a decrease in plasma insulin concentration in newborn rats. The changes in plasma content of these hormones may relate to an increase in nervous activity which accompanies birth, and although it has yet to be demonstrated that birth is accompanied by an increase in adrenaline output from the adrenal medulla , the physical and physiological stresses which accompany birth are probably conducive to adrenaline secretion.

Since birth is accompanied by an abrupt cessation of the placental nutrient supply, hypoglycaemia is often reported after the umbilical cord is severed (Snell and Walker, 1973a). Yeung and Oliver (1968a) have found that the administration of hexoses will delay the development of phosphoenolpyruvate carboxykinase activity in the neonatal rat. Glucose 6-phosphatase responds in a similar manner (Dawkins, 1966). Furthermore, Vernon and Walker (1968) have shown that weaning rats on to diets rich in carbohydrate produces a decrease in phosphoenolpyruvate carboxykinase activity. The extent that these factors contribute to the postnatal increases in gluconeogenic enzyme activities in the lamb is unknown . In the adult sheep fluctuations in nutritional and hormonal status, while increasing glucose 6-phosphatase activity, have little

Table 1

Activities of key enzymes in the gluconeogenic pathway: Changes in activity in fetal and suckling liver.

Activities are presented relative to adult values (= 1).

Enzyme	Species	Activity		Reference
		term fetus	suckling	
glucose 6-phosphatase	rat	0.80	4.75	Dawkins (1961) Schaub et al (1972)
	mouse	0.13	0.34	Thorndike (1972)
	guinea pig	0.40	3.50	Nemeth (1954) Dawkins (1961)
	rabbit	0.50	3.00	Dawkins (1961)
	rhesus monkey	0.80	2.75	Dawkins (1961)
	sheep	1.00	1.80	Ballard & Oliver (1965) this thesis
	pig	0.33	2.11	Mersmann (1971)
	human	0.75	-	Aurrichio & Rigillo (1960) Dawkins (1961)
hexose diphosphatase	rat	0.80	3.33	Ballard & Oliver (1962) Schaub et al (1972)
	sheep	0.62	1.26	Ballard & Oliver (1965) this thesis
	pig	0.60	3.20	Mersman (1971)
	human	0.20	-	Raiha & Lindros (1969)
* cytosol phosphoenol-pyruvate carboxykinase	rat	0.02	1.40	Ballard & Hanson (1967)
	guinea pig	0.60	6.00	Arinze (1975)
	sheep	0.56	1.00	this thesis
	human	0.10	-	

Table 1 (cont)

Enzyme	Species	Activity		Reference
		term fetus	suckling	
* particulate phosphoenol-pyruvate carboxykinase	rat	1.00	1.00	Ballard & Hanson (1967)
	guinea pig	1.00	1.40	Arinze (1975)
	sheep	1.00	2.00	this thesis
* pyruvate carboxylase	rat	0.43	1.00	Ballard & Hanson (1967) Thorndike (1972)
	mouse	0.72	1.33	Thorndike (1972)
	sheep	0.78	1.40	this thesis
	pig	0.78	0.86	Mersman (1971)
	human	0.66	-	Raiha & Lindros (1969)

* values are expressed relative to fed adult activity.

effect on phosphoenolpyruvate carboxykinase activity (Filsell *et al*, 1969; Taylor *et al*,1971).

CHAPTER IV

THE KINETICS OF LACTATE, FRUCTOSE AND GLUCOSE METABOLISM IN THE DEVELOPING LAMB *in vivo*.

IV.1. Introduction

The isotope dilution method for studying metabolic rates has been extensively used in ruminants (Annison, Brown, Leng, Lindsay and West, 1967; Leng *et al*, 1967; Bergman *et al*, 1968; White, Steel, Leng and Luick, 1969). Previous studies of glucose and lactate turnover in the adult ruminant have mainly employed either the continuous infusion or primed infusion of isotope (Annison and White, 1961; Annison, Lindsay and White, 1963; Jarrett, Jones and Potter, 1964; Leng *et al*, 1967; White *et al*, 1969). The alternative approach using a single injection of isotope was regarded as unreliable (Searle, Strisower and Chaikoff, 1956, Steele, Wall, de Bodo and Altszuler, 1956; Annison and White, 1961; Bergman , 1963). However, with the development of mathematical methods for multicompartmental analysis of substrate disappearance and product appearance it is now accepted that the single injection procedure yields values comparable to the other techniques (Barker, 1969; White *et al*, 1969; Searle and Cavalieri, 1972; Heath and Barton, 1973). I have used the single injection approach in this study.

It is the aim of this chapter to describe experiments to measure the kinetics of lactate, fructose

and glucose metabolism in the developing lamb *in vivo*, and to derive a model to describe the interrelation of lactate, glucose and fructose metabolism in the fetal lamb and glucose and lactate metabolism in the postnatal lamb.

IV.2. Methods

IV.2.1. Animals

Merino crossbred ewes of known mating date were obtained from the C.S.I.R.O., Division of Nutritional Biochemistry, South Australia or from the University of Adelaide Experimental Farm at Mintaro, South Australia. During the period of experimentation the ewes were housed under conditions of 12 hr light and 12 hr dark, with a constant temperature of 25°C. They were fed a daily ration of 800 g lucerne chaff and were provided with an unlimited supply of water. Before surgery the ewes were starved for 24 hr, but allowed free access to water.

IV.2.2. Pregnancy diagnosis

Pregnancy was confirmed by either abdominal palpation , maternal plasma progestin concentration (Ch. IV.2.5.2.) or by ultrasonic detection of fetal heart beats using a Model 802 Doppler (Parks Electronics Lab., Oregan, U.S.A.). The gestational age of each fetus at the time of experimentation was known from the mating date of the ewe. Fetal weights were estimated from the nomogram, Appendix 1.

IV.2.3. Surgical Procedure

(a) Preparation of animals

Starved ewes, 110-120 days gestation, were sedated with 0.2 ml Rompun (0.08 ml/100lb), injected intramuscularly. Lumbosacral epidural anaesthesia was then induced with 10 ml Lignocaine (2% Xylocaine) following the technique described by Hopcroft (1967). The abdominal wool was removed with coarse and fine clippers, followed by an application of Veet hair removing cream. The abdomen was then scrubbed with soap, rinsed with water and then the whole area was liberally soaked with Chlorhexidine solution. The ewe was taken to the operating theatre in the supine position and placed on the operating table. The abdomen was again liberally soaked with Chlorhexidine solution and the area swabbed with sterile cloths.

(b) Cannulation of fetal vessels

Strict aseptic techniques were maintained during all procedures. All cannulae were sterilized with ethylene oxide and before surgery were filled with sterile 0.15 M NaCl containing 100 I.U. heparin per ml.

The abdomen of the ewe was covered with a plastic Steridrape (size 18" x 36") and sterile cloth drapes. The abdomen was opened by a midline incision and the pregnant tip of the uterine horn exposed. At this point

the instruments used for the skin incision were discarded. The position of the fetus was located by uterine palpation and a 4 cm incision made in the least vascular area of the uterus. Through this incision the fetal hind limb was delivered. The femoral artery was palpated and a 4 cm incision made. By blunt dissection the femoral artery, vein and nerve were located and separated. The femoral artery and vein were then cannulated using Portex polyvinyl tubing No. 2 (o.d. 2.0 mm and i.d. 1.0mm) and No. 1 (o.d. 1.4 mm and i.d. 0.63 mm) respectively. The cannulae were tied in place with 2/0 black braided silk and the incision closed with Chromic 2/0 suture material (Ethicon). In some fetuses the umbilical vein and artery were cannulated. This involved placement of the cannulae in the major vessels via cotyledonary vessels. Portex polyvinyl tubing No.1 was used for both umbilical vein and artery cannulations. These cannulae were held in place by a suture of 2/0 black braided silk. The uterus was then closed, but before return to the abdominal cavity a cannula of dimensions 1 mm i.d. and 2 mm o.d. was placed in the uterine vein. The cannulae were individually exteriorized through the skin incision closure and held in place by suturing to the abdominal skin and to the flank of the ewe. In ewes in which the uterine vein was not cannulated a cannula (o.d. 2 mm and i.d. 1 mm) was inserted in the femoral artery through a subcutaneous vessel. After flushing with heparinized,sterile 0.15 M NaCl, the cannula was sealed with a metal plug.

(c) Post-operative care

Immediately after surgery the ewes were placed in metabolism cages with free access to food and water. The main cause of fetal death was found to be due to infection by Strepococcus faecalis and Pseudomonas aeruginosa. As a precaution, 50 mg Ceporan and 20 mg Garamycin were given to the fetus intramuscularly before closure of the uterus. The ewe received 450 mg Ceporan and 60 mg Garamycin intramuscularly. To minimize infection during cannula maintenance, aseptic techniques were strictly adhered to. Disposable syringes containing 10 ml of sterile 0.15 M NaCl containing 100 I.U. heparin per ml were attached to the cannulae via disposable needles and kept submerged in Chlorhexidine solution. The syringes were renewed daily and the cannulae were flushed with sterile heparinized saline.

(d) Cannulation of postnatal lambs

On the day before an experiment polyvinyl cannulae (o.d. 2 mm and i.d. 1 mm) were placed in the femoral artery and jugular vein of the lamb. The lamb was sedated with Rompun and Lignocaine was injected subcutaneously at the site of the incision. Before the incision was made, the skin was washed as described for the ewes. The cannula was placed in the femoral artery via a subcutaneous vessel . After closure of the

incision the wound was coated with Hibitane cream and the cannula flushed and kept filled with sterile 0.15 M NaCl containing 100 I.U. heparin per ml. It was sealed with a metal plug.

Placement of the cannula in the jugular vein of the lamb was acheived by threading it through a 14 gauge needle inserted in the jugular vein . After placement, the needle was withdrawn and the cannula held in place with a suture. After flushing with the heparinized 0.15 M NaCl the cannula was sealed with a metal plug. Cloth bandages were necessary to protect the cannulae from damage by the ewe.

The lambs were kept with the ewe in a pen until isotope injection at which time they were put in a wire cage (36" x 36" x 36") and removed from the ewe.

IV.2.4. General Experimental Design

Except where otherwise stated all experiments in which fetal lambs were used were conducted at least five days after the implantation of cannulae. This time allowed for recovery of ewe and fetus from surgical stress (Bassett and Thorburn, 1969; Mellor, Slater and Cockburn, 1971; Comline and Silver, 1970; Mellor and Slater, 1971, 1972). Viability of each fetus was assessed from blood glucose, fructose and lactate concentration and blood pO_2 and pCO_2 analyses during the

recovery period .

Fetal lambs of gestational age from 120 days to term were given 50 μCi of either [U-^{14}C] lactate, [U-^{14}C] fructose or [U-^{14}C] glucose as a single injection. The isotope was injected in 2 ml of sterile 0.15 M NaCl and immediately after injection the cannula was flushed with 2 ml sterile 0.15 M NaCl. Sequential blood samples were taken from the fetus and ewe. The blood samples were immediately deproteinized in ethanol and prepared for determination of specific radioactivity. A portion of the injected isotope was retained and the precise quantity of isotope injected was measured. The purity of each injected isotope was assessed from this sample using the electrophoretic technique described in Ch. IV.2.5.5. No impurities were detected.

The postnatal lambs, ranging in age from 5-28 days were given 50 μCi of [U-^{14}C] lactate or [U-^{14}C] glucose as a single intravenous injection. This age group was selected for the following reasons: By 5 days after birth the large fetal store of glycogen has been consumed (Shelley, 1961). Fructose has disappeared from the blood of the lamb, either as a result of renal excretion (Dawes and Shelley, 1962) or synthesis of fructokinase, the rate limiting enzyme of fructose metabolism (Andrews et al, 1960 ; Ballard and Oliver, 1965). The rapid changes in the activity of gluconeogenic enzymes observed after birth are also complete by 5 days after birth.

During all experiments disturbance of the ewe or the lamb was minimized since it is well recognised that stress can cause changes in carbohydrate metabolism. Lambs were gently restrained, but even so they fretted after removal from their mothers. It is possible that this may have biased the estimates of glucose and lactate metabolism .

IV.2.5. Analytical Techniques

1. Collection and preparation of blood samples

Two ml samples of fetal arterial blood were collected in sterile disposable syringes. The blood was immediately haemolysed and deproteinized by addition to 8 ml of ethanol in a tared plastic centrifuge tube. The supernatant, collected after centrifugation, was shaken with 25 ml of chloroform, the upper phase (aqueous) recovered and used for specific radioactivity determination and for measurement of glucose, lactate and fructose. It was necessary to deproteinize blood samples using this procedure for the electrophoretic separation of glucose, lactate and fructose. Deproteinization with perchloric acid, followed by neutralization, proved unsuitable since considerable streaking occurred during electrophoresis, suggesting that salts or lipids present in perchlorate extracts interferred with the separation process.

2. Plasma progestin determination

Plasma progestin concentrations (ng progesterone equivalents per ml) were determined in samples taken from the jugular vein of pregnant ewes, using the competitive protein binding assay of Thorburn, Bassett and Smith (1969). Micro-sephadex columns containing Sephadex G-25 (fine) were used to separate the bound from free steroid (Bassett and Hinks, 1969).

3. Analysis of blood pO_2, pCO_2 and pH

These assays were carried out with the co-operation of the Biochemistry Department of the Queen Elizabeth Hospital, Woodville, South Australia . Two ml blood samples, collected in heparinized disposable plastic syringes, were assayed immediately for oxygen, carbon dioxide and pH using the Radiometer (Copenhagen) Blood Micro System MK I. Care was taken to minimize exposure of the collected blood samples to air.

4. Haematocrit determinations

The haematocrit determinations were carried out using a micro-haematocrit centrifuge, Hawksby, England.

5. Determination of specific radioactivity

(a) Quantitation of radioactivity

Radioactivity was quantitated using an Isocap 300

liquid scintillation counter (Nuclear Chicago). Aqueous samples were counted in 10 ml toluene-triton X-100 scintillation fluid (Ch. II.3.). Paper strips were counted in 10 ml of toluene scintillation fluid. Efficiency of counting was measured by channels ratio technique using serially quenched samples of isotopes (Davidson ,1970). From this data the absolute radioactivity was calculated.

(b) Separation of glucose, fructose and lactate

Glucose, fructose and lactate were separated by high voltage electrophoresis in 0.1 M sodium arsenite buffer, pH 9.6 (Frahn and Mills, 1959), using an apparatus with a solid heat exchanger. Radioactive areas were located using a Packard Radiochromatogram Scanner Model 7200. Fig 7 shows the separation of these compounds using this electrophoretic system.

Standard glucose and fructose were detected as brown spots on a white background after gentle heating . Lithium lactate ($\geqslant$ 15 µmol) was detected by spraying the paper with 0.5 M aqueous copper sulphate. The lactate area was pale blue on a green background. These standards were run on separate lanes to the radioactive samples.

For electrophoresis, 0.5 ml of the aqueous sample was placed on Whatman 3 MM paper in a 2 cm streak and dried under a stream of warm air. The paper was

Figure 7 : Electrophoretic Separation of ^{14}C-labelled glucose, fructose and lactate

For electrophoresis a mixture of ^{14}C-labelled glucose, fructose and lactate was placed on Whatman 3MM paper and electrophoresed in 0.1 M sodium arsenite buffer, pH 9.6, for 2hr at 1.5kV (17V/cm). Radioactive peaks were detected with a Packard Radiochromatogram Scanner, Model 7200.
The hatched areas show the migration rates of unlabelled compounds .
Glucose and fructose were detected as brown spots on a white background after gentle heating and lactate as pale blue spot on a green background, after spraying with 0.5 M aqueous $CuSO_4$.

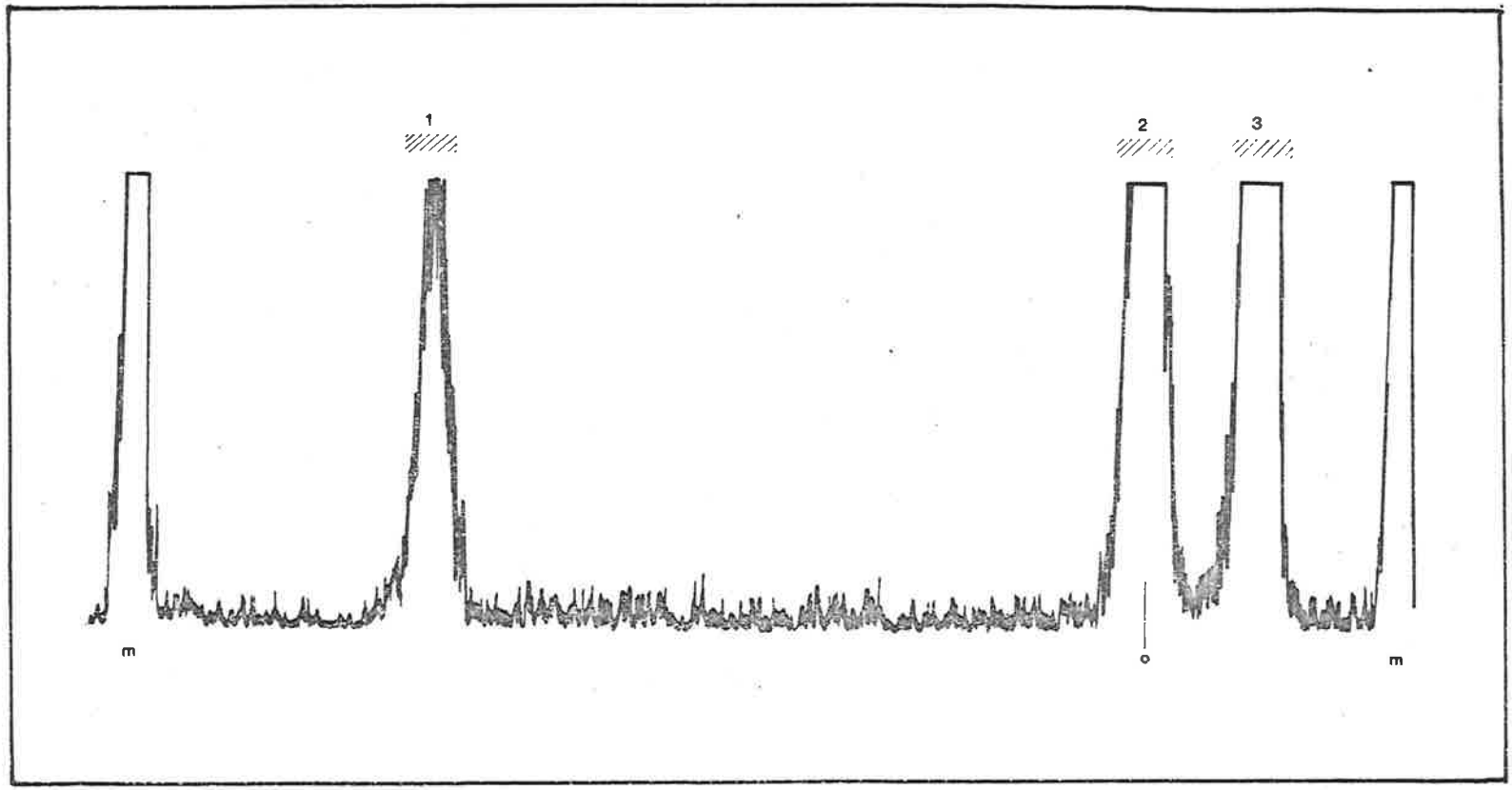
M = radioactive marker

o = origin

1 = lactate

2 = fructose

3 = glucose


1
2
3
m
o
m

carefully soaked with 0.1 M sodium arsenite buffer, pH 9.6 and excess buffer was removed by gentle blotting using a roller with even pressure. Papers were electrophoresed at 1.5 kV (17 V/cm) for 2 hr to achieve maximum separation of glucose and fructose. The areas corresponding to the standards were cut into strips (1 cm x 4 cm) and the radioactivity was measured.

6. Glucose determination

Glucose was measured spectrophotometrically with $NADP^+$, hexokinase and glucose 6-phosphate dehydrogenase by following the increase in absorbance at 340 nm in a Zeiss PMQ II recording spectrophotometer. Each cuvette contained 76 µmol tris-HCl buffer, pH 7.6, 3.8 µmol $MgCl_2$, 0.5 µmol ATP, 0.5 µmol $NADP^+$, 2 µmol dithiothreitol, 12 µg glucose 6-phosphate dehydrogenase, 5 µg hexokinase and 0.5 ml of a suitably diluted sample, in a total volume of 0.77 ml. The reaction temperature was 37°C. The reaction was started by the addition of hexokinase. The aqueous blood extracts were compared to glucose standards. The standards were prepared in a manner similar to the blood samples to correct for any possible effects or losses due to contamination with either residual ethanol or chloroform. Contamination was not found to be a problem and glucose concentrations measured using standards correlated well with the calculated values based on the extinction coefficient of NADPH.

7. Lactate determination

Lactate concentrations were determined in blood extracts using the automated method of Asrow (1969). Fig 8 shows the autoanalyser flow diagram and the associated flow rates. One millilitre of enzyme-cofactor reagent contained 0.45 mmol tris-HCl buffer, pH 7.4, 300 mg lactate dehydrogenase, 5 mg diaphorase and 25 μmol NAD^+. The dye, 2(-p-iodo-phenyl)-3-p-nitrophenyl-5-phenyl tetrazolium chloride, was present at a concentration of 2 mM. The 0.1 M glycine-NaOH buffer, pH 9.6 used, contained 2% triton X-100. The reaction temperature was 37°C and a 15 mm colorimeter flow cell was used with a 505 nm filter. Absorbance was compared to L-lactate standards. The standards were prepared in a manner similar to the blood samples.

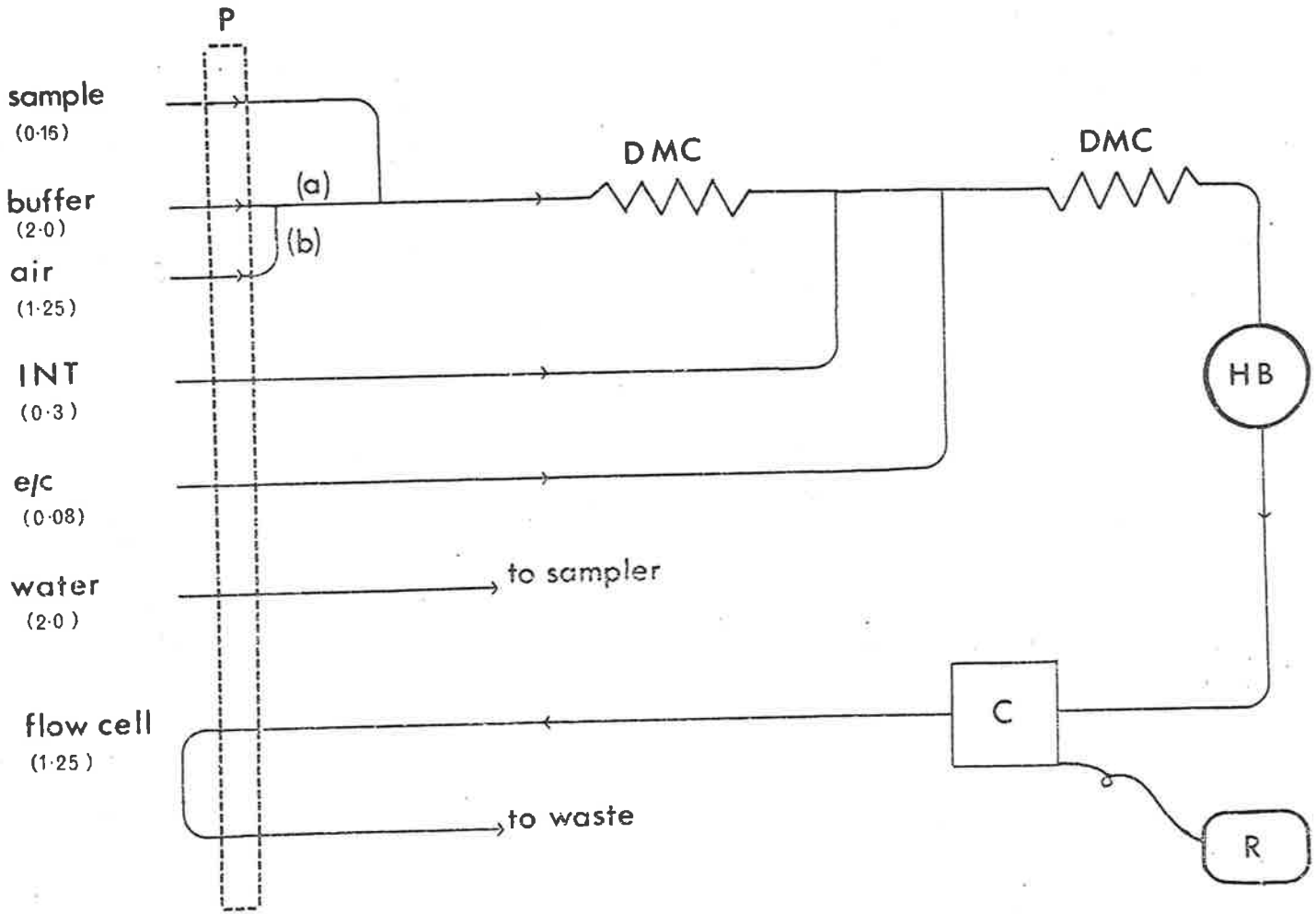
8. Fructose determination

Fructose was measured colorimetrically in blood extracts by the method of Bacon and Bell (1948). Each cuvette contained 0.5 ml of standard fructose or blood extract, 0.8 ml of 0.15% resorcinol in ethanol and 0.8 ml of concentrated HCl (specific gravity 1.16) containing 7.5 mg $FeCl_3$ per litre. Colour formation was complete after heating the mixture at 80°C for 30 min in a water bath. The standards and samples were read at 514 nm. Standards were prepared in a similar manner to the blood samples.

Figure 8 : Autoanalyser Flow Diagram for Lactate Analysis

Lactate concentration in blood extracts was measured by the procedure of Asrow (1969).

The autoanalyser flow rates (ml/min) are presented in parentheses. The buffer was 0.1 M glycine-NaOH, pH 9.6, containing 2% Triton-X100; the dye (INT), 2(-p-iodo-phenyl)-3-p-nitrophenyl-5-phenyl tetrazolium chloride; e/c is the enzyme-cofactor reagent containing 0.45 mmol tris-HCl buffer, pH 7.4, 300 mg lactate dehydrogenase, 5 mg diaphorase and 25 μmol NAD^+. The reaction temperature of the heating bath (HB) was 37°C. The internal diameters of the pulse suppressors were (a) 0.005 mm and (b) 0.015 mm. There were two double mixing coils (DMC). The colorimeter flow cell (C) was 15 mm and was used with a 505 nm filter. A recorder (R) was attached to the colorimeter.


P
sample
(0·16)
buffer
(2·0)
air
(1·25)
INT
(0·3)
e/c
(0·08)
water
(2·0)
flow cell
(1·25)
(a)
(b)
DMC
DMC
HB
C
R
to sampler
to waste

IV.2.6. Mathematical Analysis of Data

The direct intravenous injection of radioactive tracer results in a rapid distribution of radioactivity, essential for subsequent compartmental analysis. Because of the rapid mixing of isotope, the position of the cannula through which the isotope was administered should be irrevelant.

Before the parameters of glucose, fructose and lactate turnover in the lamb are obtained by compartmental analysis (Shipley and Clark, 1972) it is first necessary to describe mathematically the changes in the specific radioactivity of the substrate in the blood. The specific radioactivities (dpm / μmol) were calculated by dividing the dpm / g blood by μmol substrate/ g blood. In all experiments the specific radioactivity was corrected to 50 μCi isotope injected. Since the single injection technique was employed in all experiments the isotope disappearance curve is described by the equation:-

$$SR_t = \sum_{i=1}^{n} A_i e^{-m_i t} \qquad \text{(equation 1)}$$

which represents the sum of all the exponential terms (White et al, 1969). In this equation :-

SR_t = the specific radioactivity of the substrate assayed in whole blood of the fetus or lamb at time t (dpm / μmol substrate).

A_i = the zero time intercept of each component (dpm / μmol substrate).

m_i = the rate constant of each component (min^{-1}).

n = the number of exponential components.

i = the exponential component number.

t = time (min).

A computer program was used to calculate the line of best fit to the equation. The program was designed to fit non-linear functions by the method of least squares (Bevington, 1969; McIntosh and Lutwak-Mann, 1972) and incorporated an itterative procedure to produce values of the coefficients which minimized the sums of squares of the deviation about the fit (Marquant, 1963). The program gave optimized values for each coefficient in the fitting of the function, together with an estimation of standard deviation.

Before fitting the curve by computer, initial estimates of the slope and intercept for each component were calculated by graphical analysis. The number of components of the curve was resolved when the experimental data were plotted on semilogarithmic paper. After the conversion of the data to natural logarithms the regression line for each component was obtained by least squares analysis, in conjunction with the usual 'curve peeling' techniques.

The slope and intercept were thus obtained for each component of the curve. Exponential functions are very susceptible to parameter interactions. However, when initial estimates of the coefficients were fitted the values did not change by more than 0.04%. Data points and fitted functions were drawn by a plotter controlled by the computer.

The following parameters, defined below, were calculated (see Leng, 1970).

The pool, Q, is the quantity of substrate, measured in mmol, with which the injected isotopically labelled substrate mixes in the body. It is calculated from the dilution of the injected dose of radioactivity (P) by the following equation:

$$Q = \frac{P}{\sum_{i=1}^{n} A_i} \qquad \text{(equation 2)}$$

The sum of the zero time intercepts (A_i) of each component of the curve gives the specific radioactivity at the time of injection.

The space is the volume of fluid, measured in ml, through which the substrate pool is distributed. It represents the pool (Q) divided by the mean concentration of substrate (mmol / ml).

The irreversible loss is the rate of removal of substrate not returning to the substrate pool during the

course of the experiment. The net irreversible loss obtained is contributed to by all routes of disposal and is independant of any assumption about the distribution of substrate between body pools. It is represented by the pool (Q) divided by the area under the decay curve (Shipley and Clark, 1972). The area under the curve (AUC) was obtained from the measured exponential coefficients of the curve. An estimate was made of the area under the curve from the last collection to infinity by fitting a single exponential decay to the final part of the curve:

$$\text{irreversible loss} = \frac{Q}{\sum_{i=1}^{n} \frac{A_i'}{m_i}} \qquad \text{(equation 3)}$$

$$\text{where} \quad \sum_{i=1}^{n} A_i' = 1$$

The use of equation 3, known as the Stewart-Hamilton equation, in the calculation of the kinetics of the specific radioactivity disappearance curve has the following advantages: (i) it is independant of assumptions about the distribution of substances between body pools; (ii) it includes all routes of disposal; (iii) an accurate estimate of the zero-time intercepts is not critical and any error introduced by truncating the curve when it has fallen to a few percent of the zero-time value is not critical. In this study the errors of some

of the fitted curves were higher than desirable due to the small number of samples which could safely be taken from the fetus.

Pool size and the irreversible loss have been calculated in terms of conceptus weight in the experiments with fetal lambs. The weight of tissues other than fetus was estimated from data of Cloette (1939) and Wallace (1948). Their studies showed that the sheep placenta reaches its maximum weight about mid-gestation. The mean weight of placenta at this time, 390 g, was taken as an estimate of the metabolic tissue of the conceptus other than fetus. Fetal weight was estimated from the nomogram reproduced in Appendix 1.

IV.3 Results and Discussions

IV.3.1. Lactate Metabolism by the Ovine Fetus.

The utilization of lactate by five fetal lambs, aged between 125 and 135 days gestation, was measured by the experimental and mathematical approach described above . The results of a typical experiment, conducted on fetus 114, will be described in detail.

Fetus 114, gestational age 125 days, with cannulae placed in the femoral artery and vein was given 50 μCi [U-^{14}C]lactate as a single injection via the femoral vein. Blood samples were collected from the femoral artery of the fetus and the femoral artery of the ewe at various time intervals up to 150min from injection of the isotope. The specific radioactivity was determied at each sampling time (Table 2). During the experimental period, blood pO_2, pCO_2 and haematocrit were measured in fetal arterial blood to assess the well-being of the fetus (Table 3; fetus 114). At the commencement of the experiment blood pO_2 was 21.9 mm Hg and blood pCO_2 was 46.0 mm Hg. At the end of the experiment blood pO_2 was 20.0 mm Hg whilst blood pCO_2 was 46.5 mm Hg. The haematocrit fell slightly from 33 .0% at the start of the experiment to 31.5 % at the conclusion.

Blood concentration of glucose (mean 0.56 ± 0.03 μmol/g blood), lactate (mean 3.2 ± 0.11 μmol/ g blood) and fructose (mean 6.02 ± 0.26 μmol / g blood) were steady throughout the experiment indicating that steady state

Table 2

Lactate metabolism by the ovine fetus : Data for fetus 114.

[U-^{14}C] lactate was rapidly injected via the cannula placed in the femoral vein. Blood samples were collected from the femoral artery over a period of 150 min. Blood lactate, glucose and fructose concentrations were determined as described in Ch. IV.2.5. Specific radioactivity data are normalized to an injection of 50 μCi.

time (min)	lactate (mM)	glucose (mM)	fructose (mM)	specific radioactivity (dpm x 10^{-3}/μmol)
2	2.75	0.45	4.64	26.18
5	2.64	0.47	4.31	20.47
15	2.98	0.56	5.66	10.85
30	3.17	0.72	5.80	4.63
45	3.00	0.57	6.90	3.85
60	3.04	0.59	6.04	2.55
75	3.17	0.48	6.04	2.06
90	3.76	0.75	7.06	1.36
105	3.60	0.56	5.74	0.88
120	3.52	0.52	6.74	0.54
150	3.76	0.57	6.98	0.30
mean	3.21	0.56	6.02	
S.E.M.	0.11	0.03	0.26	

Table 3

Lactate Metabolism by the Ovine Fetus: Experimental Details.

Each fetus was rapidly injected with 50 μCi [U-^{14}C lactate] via the cannula placed in either the umbilical vein (UV) or the femoral vein (FV). Blood samples were taken from the umbilical artery (UA) or the femoral artery (FA) of the fetus or the uterine vein (UtV) or femoral artery of the ewe. Blood glucose, fructose and lactate determinations were carried out as described in Ch.IV.2.5. and are the mean ± S.E.M. of the number of determinations expressed in parentheses. Fetal blood pO_2, pCO_2 and haematocrit (%PCV) were measured at the beginning and end of each experiment.

Fetus	1541	LT 48	164	44	114
vessel cannulated fetal					
injected	UV	UV	FV	FV	FV
sampled	UA	UA	FA	FA	FA
maternal sampled	UtV	FA	FA	FA	FA
sampling period (min)	0-120	0-120	0-150	0-120	0-150
fetal					
lactate (mM)	5.6±0.19 (15)	2.7±0.14 (7)	2.3±0.07 (7)	2.2±0.07 (7)	3.2±0.11 (11)
glucose (mM)	0.5±0.03 (16)	0.4±0.02 (8)	0.4±0.01 (7)	0.4±0.02 (7)	0.5±0.02 (11)
fructose (mM)	6.8±0.50 (16)	4.2±0.10 (8)	5.0±0.06 (7)	6.6±0.23 (7)	6.0±0.26 (11)
pO_2 start	16.4	19.0	20.0	20.4	21.9
pO_2 end	10.0	21.2	19.2	22.1	20.0
pCO_2 start	44.4	49.8	43.0	45.6	46.0
pCO_2 end	37.9	40.0	41.5	41.6	46.5
%PCV start	38.0	35.6	37.0	37.0	33.0
%PCV end	28.5	35.0	36.5	35.0	31.5
maternal					
glucose (mM)	2.03±0.14 (3)	2.3±0.23 (4)	2.83±0.21 (3)	2.23±0.16 (3)	2.69±0.18 (3)

conditions prevailed. The mean maternal arterial glucose concentration was 2.69 $\pm$ 0.18 µmol / g blood.

The determination of the specific radioactivity of blood lactate over the experimental period of 150 min showed that ^{14}C-lactate disappeared rapidly from the blood of the fetal lamb. When specific radioactivity of blood lactate was plotted semilogarithmically it was evident that the disappearance curve was composed of two exponents (Fig9). Computer analysis of the curve confirmed that the simplest description was given by a function composed of the sum of two exponential terms. The value of the derived coefficients for this fetus are presented in Table 4. The zero time intercept and the slope of the initial fast component of this curve were 23.16 x 10^3 dpm / µmol and 0.106 min^{-1} respectively, and of the second slower component they were 7.66 x 10^3 dpm / µmol and 0.019 min^{-1} respectively.

The sum of the zero time intercepts, 30.8 x 10^3 dpm / µmol , gave the specific radioactivity at the time of isotope injection. The injected dose of ^{14}C-lactate was 50 µCi (i.e. 110 x 10^6 dpm), so from equation 2 the lactate pool is:

$$\frac{110 \times 10^6}{30.8 \times 10^3} = 3.57 \text{ mmol lactate}$$

or 1.630 mmol/ kg conceptus weight (Table 5).

The lactate space, 1115 ml, was obtained by dividing

Figure 9 : Semilogarithmic Plot of Blood Lactate Specific Radioactivity in Fetus 114.

Specific radioactivities are normalized to an injection of 50 μCi [U-^{14}C]lactate, and are expressed as dpm x 10^3/μmol.

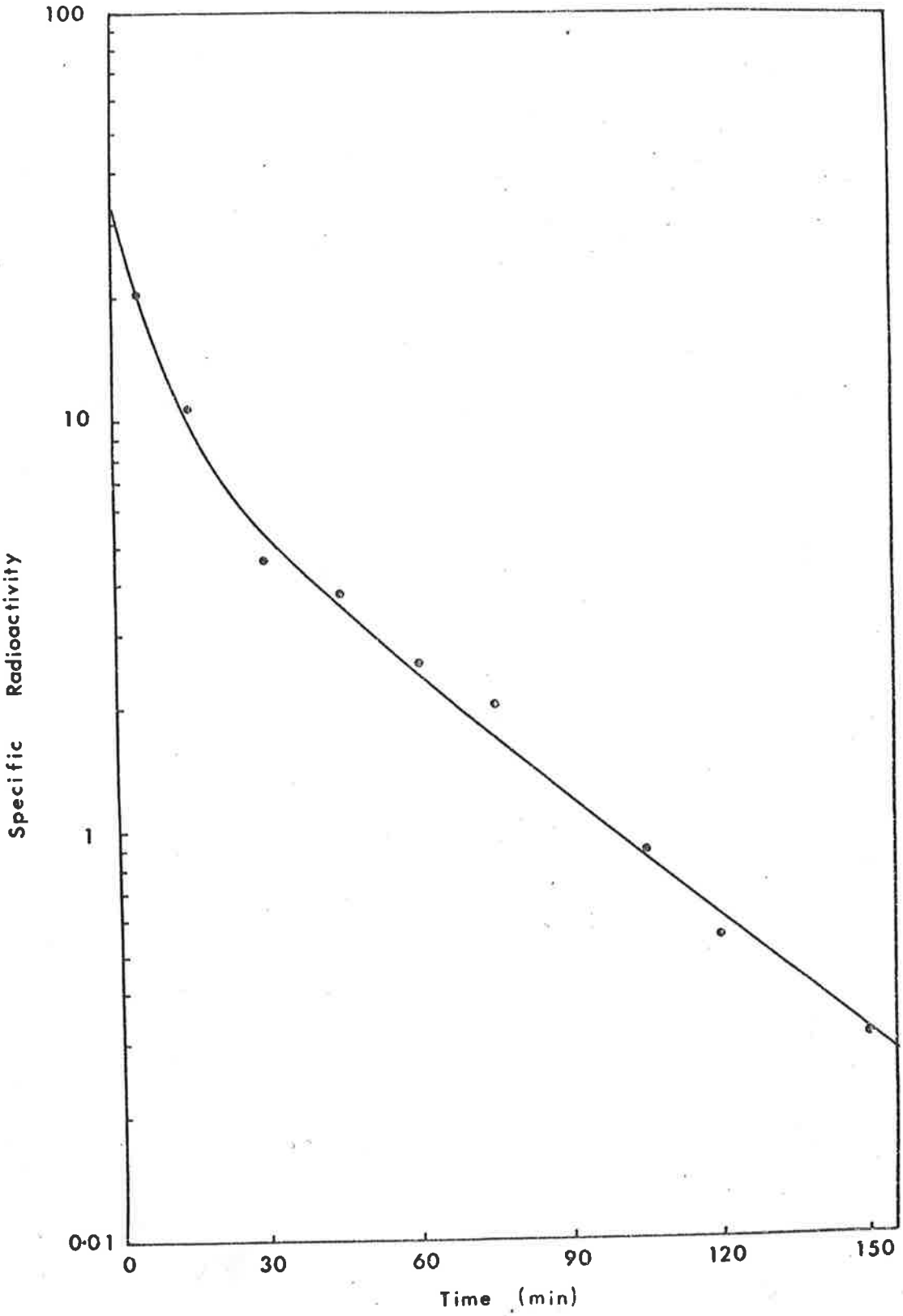

100
10
1
0·01
Specific Radioactivity
0
30
60
90
120
150
Time (min)

Table 4

Lactate metabolism by the ovine fetus : Coefficients of the lactate specific radioactivity curve.

Each fetus was injected intravenously with 50 μCi [U-^{14}C] lactate at zero time (refer Table 3). Specific radioactivity (SR_t) was determined as described in Ch.IV.2.5. and mathematical analysis carried out as detailed in Ch.IV.2.6. Specific radioactivities are normalized to an injection of 50 μCi. A_i represents the zero time intercept (x 10^3 dpm/μmol) and m_i the slope (/min) of each component of the curve. The standard deviation for each coefficient is shown in parenthesis.

Fetus	1541	LT 48	164	44	114
initial SR_t	63.50	27.02	21.72	26.50	30.80
first decay component					
A_1	53.40 (0.35)	20.95 (2.64)	18.77 (0.78)	18.22 (0.14)	23.16 (1.60)
m_1	-0.181 (0.003)	-0.148 (0.047)	-0.160 (0.013)	-0.148 (0.002)	-0.106 (0.013)
second decay component					
A_2	10.08 (0.490)	6.07 (2.880)	2.95 (0.530)	8.30 (0.170)	7.66 (1.830)
m_2	-0.034 (0.0007)	-0.013 (0.008)	-0.005 (0.002)	-0.026 (0.0004)	-0.019 (0.004)
variance	0.0014	1.070	0.174	0.0006	0.147

the pool (mmol) by the mean lactate concentration (0.0032 mmol/ g blood). The lactate space represented approximately 50 % of the conceptus weight. Irreversible loss of lactate was calculated from equation 3 and was 0.079 mmol/ kg conceptus/min.

Separation of blood metabolites by high voltage electrophoresis allowed simultaneous measurement of the incorporation of ^{14}C-label into glucose and fructose. However, no formation of either glucose or fructose from lactate was detected in any blood sample taken from fetus 114. Radioactivity was not detected in maternal blood.

Similar experiments were conducted on four other fetal lambs. The general experimental design is summarized in Table 3 which also shows mean glucose, fructose and lactate concentration, blood pO_2, pCO_2 and haematocrit for each fetus. Blood gases did not show any marked fluctuations during the experimental period , with the exception of fetus 1541.

The experiment conducted on fetus 1541 was performed on the day of fetal cannulation. This fetus illustrates the problems associated with experimentation without allowing the ewe and fetus adequate time for recovery from surgical stress. Although blood glucose and fructose concentrations remained relatively constant throughout the experiment, blood lactate concentrations increased

from 2.30 µmol/ g blood to 13.0 µmol/g blood, with a mean of 5.60± 0.19 µmol/ g blood. Towards the end of the experiment blood pO_2 decreased to 10 mm Hg. The high blood lactate concentration and the low pO_2 value indicated that the fetus was severely stressed. The following day the fetus was aborted. Data from this animal were not included in the calculations of the means listed in Table 5.

Three of the ewes (numbers 44, 164 and 114) delivered live lambs at term, which was approximately 148 days gestation for this breeding flock. The fetus of LT 48, in which the umbilical vein and artery were cannulated was dead at term (i.e 23 days after experimentation). It was difficult to maintain patency of the cannulae placed in the blood vessels of this fetus and during daily cannula maintenance an infection was probably introduced to the fetus.

The shape of the specific radioactivity disappearance curve of blood lactate is shown in Fig 10. The curve, fitted by computer from the data of fetuses LT 48, 164, 44 and 114, shows the normalized data points ± S.E.M. Normalization has been carried out by making the specific radioactivity at zero time equal to unity. From the coefficients of the disappearance curves (Table 4), the parameters of lactate metabolism in the four fetuses were calculated (Table 5).

The mean lactate <u>pool</u> of the fetal lamb was 1.681 ± 0.08 mmol/ kg conceptus wt (n=4)

Figure 10 : Mean Specific Radioactivity of Lactate in Fetal Lambs

Specific radioactivities are plotted semilogarithmically against time, and are the mean ± S.E.M. of 4 fetal lambs given 50 μCi [U-^{14}C]lactate. Fetus 1541 is omitted from the mean determinations.

Specific radioactivities are normalized to a base of one where the sum of the zero time intercepts of the components of the lactate disappearance curve equals unity.

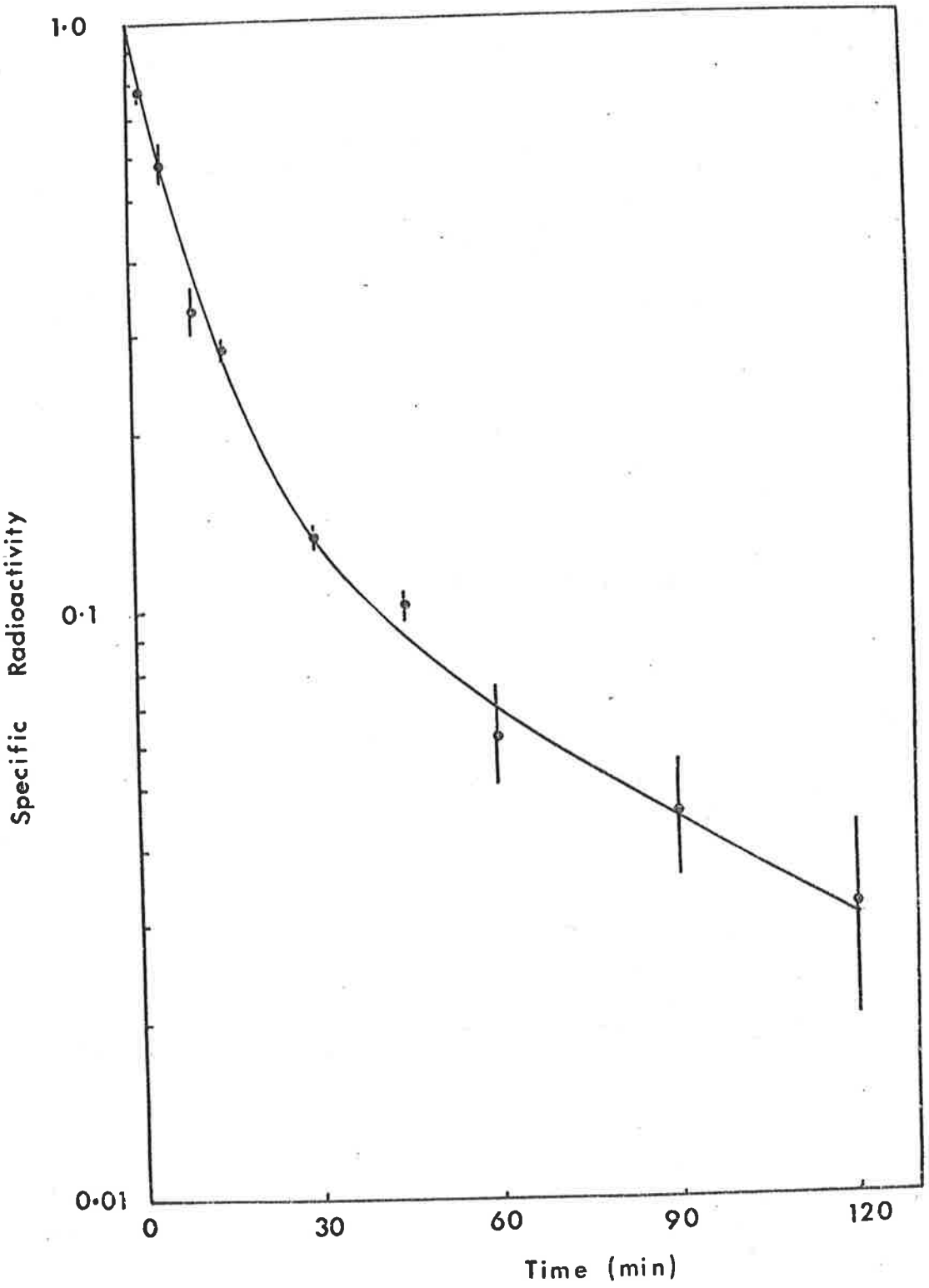

1·0
0·1
0·01
Specific Radioactivity
0
30
60
90
120
Time (min)

Table 5

Parameters of Lactate Metabolism by the Ovine Fetus.

The parameters, pool, space and irreversible loss are defined in Ch. IV.2.6. The pool size and irreversible loss are calculated in terms of conceptus weight (i.e. fetus + placenta) Fetus 1541 is omitted from mean determinations.

Fetus	1541	LT 48	164	44	114	mean	S.E.M.
age (days)	123	125	135	133	125		
weight (kg)	2.14	2.19	2.89	2.79	2.19		
pool							
(mmol)	1.732	4.070	5.064	4.147	3.570		
(mmol/kg)	0.809	1.858	1.752	1.486	1.630	1.681	0.080
lactate (mM)	5.6	2.7	2.3	2.2	3.2		
space (ml)	692	1507	2201	1885	1115	1677	234
% body wt	52.3	68.8	76.0	67.5	50.9	65.8	5.3
irreversible loss (mmol/kg/min)	0.087	0.067	0.062	0.089	0.079	0.074	0.005

The mean lactate space was 1677 ± 234 ml (n=4), or 65% of the mean conceptus weight.

The irreversible loss was 0.074 ± 0.005 mmol/kg conceptus weight/min (n=4).

No glucose or fructose formation was detected in any blood sample taken from the fetus and it is concluded that gluconeogenesis from lactate is negligible in the fetal lamb in utero .

No radioactivity was detected in any blood sample taken from the femoral artery of ewe 44, 164 or 1541, nor in any blood sample taken from the uterine vein of ewe LT 48.

IV.3.2. Lactate metabolism by the postnatal lamb.

The metabolism of [U-^{14}C]lactate by five postnatal lambs aged from 5-10 days was studied using the experimental procedure described in the previous section (Table 7). The detailed results of the experiment with lamb 4 are shown in Table 6. This animal was injected with 50 μCi [U-^{14}C]lactate via the cannula placed in the femoral artery . Blood samples were taken from the femoral artery for a period of 180 min after injection of the isotope. Blood lactate concentration (mean 1.62 ± 0.04 μmol/ g blood) and glucose concentration (mean 4.31 ± 0.09 μmol/ g blood) remained steady during the experimental period (Table 6).

Table 6

Lactate metabolism by the postnatal lamb : Data for lamb 4.

[U-^{14}C]lactate was injected via the cannula placed in the femoral artery. Blood samples were collected from the femoral artery over a period of 180 min. Blood lactate and glucose concentrations were measured as described in Ch. IV.2.5. Specific radioactivity data are normalized to an injection of 50 µCi.

time (min)	lactate (mM)	glucose (mM)	specific radioactivity lactate (dpm x 10^3/ µmol)	specific radioactivity glucose (dpm x 10^3/ µmol)
2	1.66	4.68	56.28	1.61
4	1.76	3.83	39.39	2.38
6	1.50	4.06	32.93	2.67
8	1.32	3.88	26.63	-
10	1.64	4.03	20.37	5.21
15	1.52	4.21	11.29	6.40
30	1.41	4.91	5.46	4.39
45	1.50	4.67	4.73	3.15
60	1.84	4.41	2.69	2.97
90	1.72	4.50	2.15	2.06
120	1.74	4.20	1.68	1.39
150	1.77	4.59	1.09	1.17
180	1.76	4.54	1.07	1.10
mean	1.62	4.31		
S.E.M.	0.04	0.09		

Table 7

Lactate Metabolism by the Postnatal Lamb: Experimental Details.

Each lamb was rapidly injected with 50 μCi [U-^{14}C] lactate via the cannula placed in the femoral vein (FV), jugular vein (JV) or the femoral artery (FA). Blood samples were taken from the femoral artery or the jugular vein. Blood glucose and lactate were measured as described in Ch.IV.2.5. and are means ± S.E.M. of the number of samples expressed in parentheses.

Lamb	155	96	77	4	5
vessel cannulated					
injected	FV	JV	JV	FA	JV
sampled	FA	FA	FA	FA	JV
sampling period (min)	0-90	0-120	0-90	0-180	0-120
lactate (mM)	1.70±0.10 (7)	2.0±0.03 (8)	2.6±0.11 (8)	1.62±0.04 (13)	1.60±0.60 (13)
glucose (mM)	3.4±0.21 (8)	4.2±0.19 (8)	2.8±0.08 (8)	4.3±0.09 (13)	3.5±0.10 (13)

The variation in specific radioactivity of blood lactate with time for lamb 4 is shown in Fig 11 as a semilogarothmic plot over the sampling period of 180 min. The specific radioactivity of blood lactate decreased rapidly during the first 15 min after the injection of the isotope; thereafter it decreased at a slower rate. Computer analysis showed that the curve was best represented by the sum of two exponential functions. The zero time intercept of the initial fast component was 66.09×10^3 dpm / µmol and the slope was 0.157 min^{-1}. For the second slower component the zero time intercept was 7.09×10^3 dpm / µmol and the slope was 0.012 min^{-1} (Table 8).

The parameters of lactate metabolism were calculated using equations 2 and 3. The lactate pool, calculated from the extent of dilution of the isotope at the time of injection was 0.536 mmol/kg. The space was 837 ml or 29.8 % of the body weight. The irreversible loss of lactate was 0.039 mmol/kg/min (Table 9).

In contrast to the fetus, the postnatal lamb rapidly synthesized glucose from lactate (Table6; Fig 11). The blood glucose specific radioactivity curve , fitted by computer program, is composed of an initial rising function with a slope of 0.119 min, followed by a decreasing function with a zero time intercept of 6.93×10^3 dpm/ µmol and a slope of 0.013 min^{-1} (Table 8; lamb 4). Comparison of the area under the glucose incorporation curve with the area under the lactate disappearance curve

Figure 11 : Semilogarithmic Plot of Blood Lactate and Glucose Specific Radioactivity in Lamb 4

Specific radioactivities are normalized to an injection of 50 μCi [U-^{14}C]lactate, and are expressed as dpm x 10^3/ μmol.

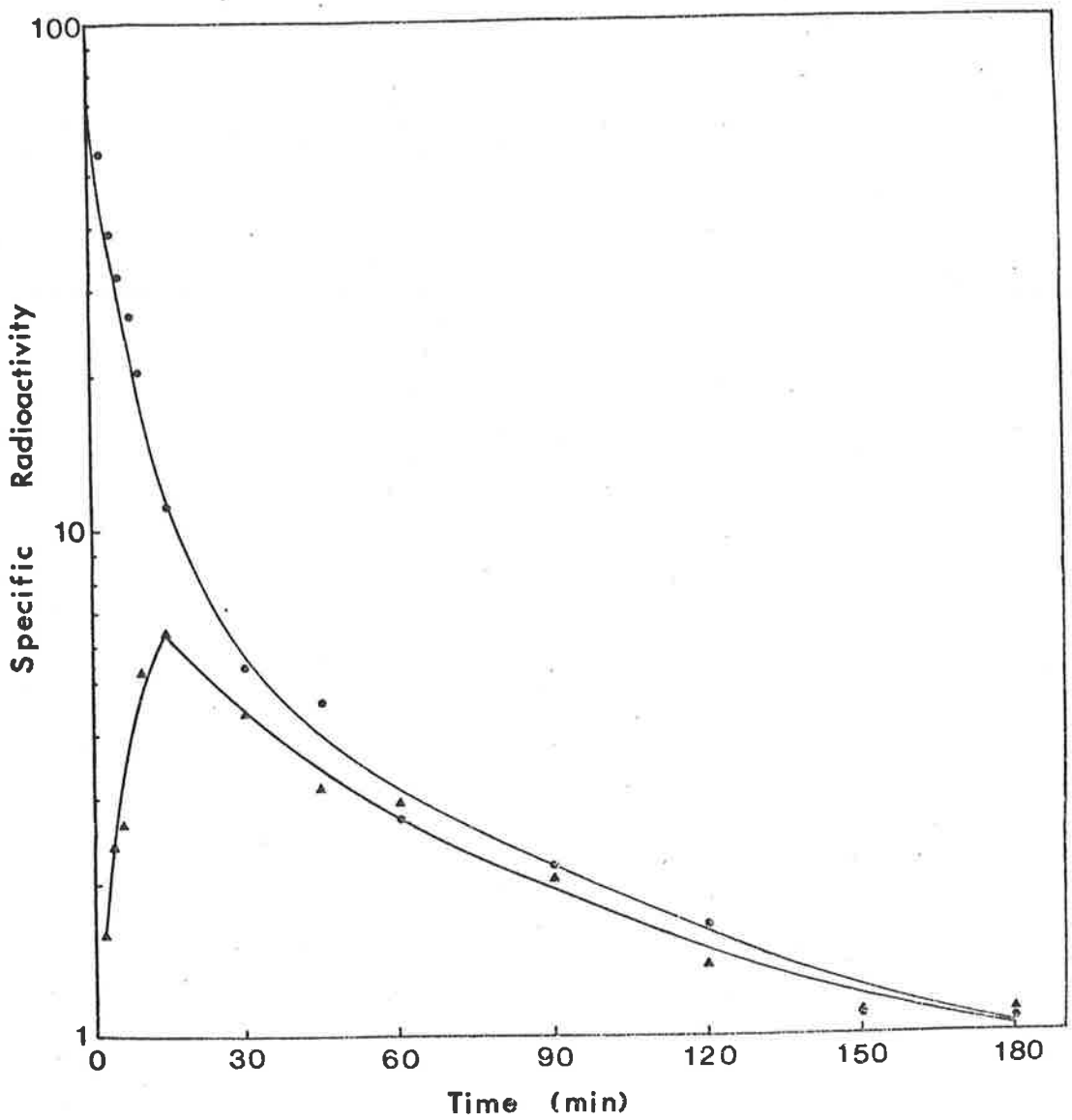

100
10
1
Specific Radioactivity
0
30
60
90
120
150
180
Time (min)

Table 8

Lactate metabolism by the postnatal lamb : Coefficients of lactate and glucose specific radioactivity curves.

Each lamb was injected intravenously with 50 μCi [U-^{14}C]lactate at zero time (refer Table 7). Specific radioactivity (SR_t), expressed as x 10^3 dpm / μmol, was determined as described in Ch.IV.2.5. and mathematical analysis carried out as detailed in Ch.IV.2.6. Specific radioactivities are normalized to an injection of 50 μCi. A_i represents the zero time intercept (x 10^3 dpm/μmol) and m_i the slope (min) of each component of the curves. The standard deviation for each coefficient is shown in parenthesis.

Lamb	155	96	77	4	5
initial SR_t	27.59	19.43	18.59	73.18	52.80
	^{14}C-lactate disappearance				
first decay					
A_1	20.02 (1.772)	17.25 (0.511)	17.89 (1.900)	66.09 (2.450)	46.19 (11.040)
m_1	-0.262 (0.057)	-0.336 (0.016)	-0.104 (0.026)	-0.157 (0.012)	-0.207 (0.106)
second decay					
A_2	7.499 (1.690)	2.185 (0.167)	0.613 (1.880)	7.091 (2.220)	6.611 (14.100)
m_2	-0.029 (0.007)	-0.010 (0.002)	-0.003 (0.053)	-0.012 (0.005)	-0.037 (0.050)
variance	0.264	0.012	0.789	1.350	6.280
	^{14}C-glucose appearance				
rising					
A'_1	3.520 (3.390)	1.920 (0.272)	4.000 (0.214)	6.950 (1.830)	1.360 (0.187)
m'_1	0.717 (0.506)	0.294 (0.076)	0.173 (0.029)	0.119 (0.062)	0.192 (0.057)
decay					
A'_1	2.520 (1.014)	1.810 (0.107)	4.550 (0.220)	6.930 (1.89)	1.440 (0.192)
m'_2	-0.006 (0.00008)	-0.012 (0.001)	-0.012 (0.0008)	-0.013 (0.004)	-0.016 (0.003)
variance	0.015	0.005	0.008	0.606	0.006

Table 9

Parameters of Lactate Metabolism by the Postnatal Lamb

The parameters, pool, space and irreversible loss are defined in Ch.IV.2.6.

Lamb	155	96	77	4	5	mean	S.E.M.
age (days)	5	10	20	5	5		
weight (kg)	7.0	7.9	8.1	2.8	5.1		
pool							
(mmol)	3.997	5.656	5.913	1.508	2.080		
(mmol/kg)	0.571	0.716	0.730	0.536	0.408	0.592	0.059
lactate (mM)	1.7	2.0	2.6	1.6	1.6		
space (ml)	2351	2829	2274	837	1300	1918	367
% body wt	33.6	35.8	28.0	29.8	25.5	30.5	1.85
irreversible loss (mmol/kg/min)	0.046	0.074*	0.036	0.039	0.048	0.042	0.003

* omitted from mean determination

showed that 23% of the lactate carbon was incorporated into glucose.

Similar experiments were carried out on four other lambs and the results are summarized in Tables 7 and 8. In all experiments the lactate disappearance curve was best represented as the sum of two exponential terms. The glucose incorporation curve was also represented as the sum of two exponential terms, one rising and the other decreasing. Maximum incorporation of ^{14}C-label into glucose occurred 10-15 min after the injection of [U-^{14}C]lactate. Normalized mean values for lactate disappearance and glucose formation are shown in Fig 12.

By comparison of the areas under the glucose formation and lactate disappearance curves it was calculated that between 23 and 44% of the lactate carbon was incorporated into the glucose pool of the lamb (mean 35$\pm$8%).

The parameters of lactate metabolism for the five lambs are presented in Table 9. The average lactate pool was 0.592 $\pm$ 0.059 mmol/kg, the space was 1918 $\pm$ 367 ml., 31% of the body weight. The irreversible loss was 0.042 $\pm$ 0.003 mmol/kg/min.

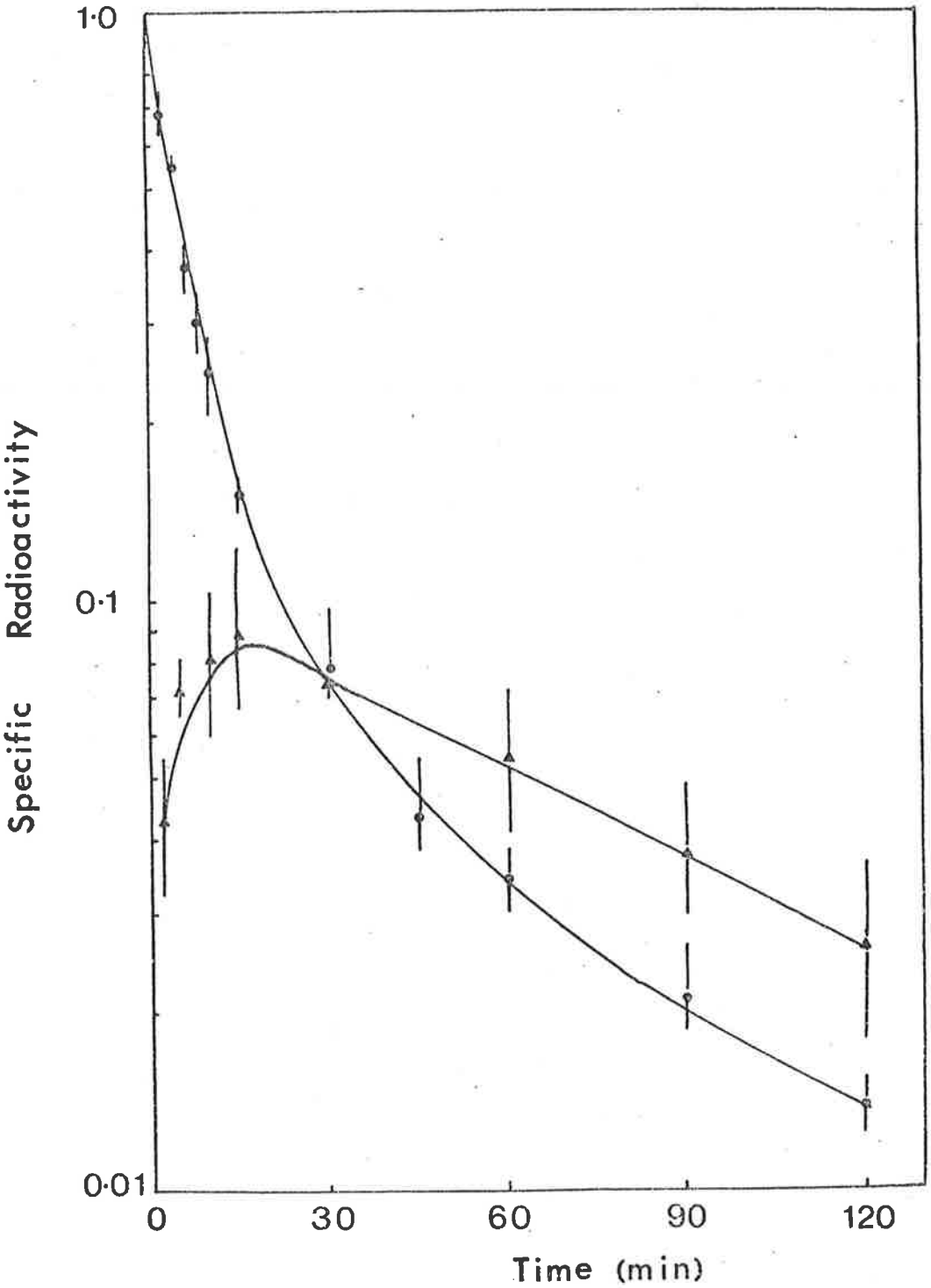
Statisitical comparison of the parameters of lactate metabolism of the ovine fetus with the postnatal lamb (using Student's t-test showed that:-

(1) the lactate pool of the fetus was significantly greater than that of the postnatal lamb ($P \leqslant 0.05$);

Figure 12 : Mean Specific Radioactivity of Lactate and Glucose in Postnatal Lambs

Specific radioactivities are plotted semilogarithmically against time, and are the means ± S.E.M. of 5 postnatal lambs given 50 μCi [U-^{14}C]lactate.

Specific radioactivities are normalized to a base of one where the sum of the zero time intercepts of the components of the lactate disappearance curve equals unity.


1·0
0·1
0·01
Specific Radioactivity
0
30
60
90
120
Time (min)

(2) the blood lactate concentration was significantly greater in the fetus than in the postnatal lamb ($P \leqslant 0.05$);

(3) there was no significant difference in the volume of fluid in which the lactate pool was distributed in the fetus and the postnatal lamb, however, when compared as the % body weight the fetal space was significantly greater than the postnatal lamb ($P \leqslant 0.05$);

(4) the irreversible loss of lactate was significantly greater in the fetus than in the postnatal lamb ($P \leqslant 0.05$);

(5) lactate was converted to glucose in the lamb but <u>not</u> the fetus.

IV.3.3. Discussion

The failure of the ovine fetus to synthesize glucose from lactate clearly demonstrated the absence of gluconeogenesis. In this respect the sheep fetus ressembles the fetal rat; but whilst the absence of gluconeogenesis in the rat fetus may be partly attributed to the low cytosol phosphoenolpyruvate carboxykinase activity (Ballard and Hanson, 1967), in the fetal lamb all gluconeogenic enzymes are active (Ch. III). The lack of fructose synthesis from lactate in the ovine fetus showed that the gluconeogenic pathway was blocked before glucose 6-phosphatase.

In the fetus, lactate is present in higher concentrations than in the neonate or the adult

(see also Annison et al, 1963) . Further, the high irreversible loss of lactate in the fetal lamb suggests that lactate is an important intermediary metabolite at this age. Assuming steady state conditions exist and recycling is negligible, the large irreversible loss is indicative of a high lactate production rate. The availability of lactate as an oxidative substrate in the fetal lamb has been recently discussed by Burd, Jones, Simmons, Makowski, Meschia and Battaglia (1975) who, from calculation of the lactate: oxygen quotient of the fetus, argued that lactate oxidation could account for up to 25% of the oxygen consumption of the fetal lamb.

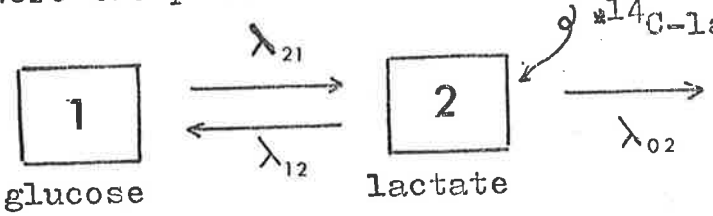
Transfer of lactate across the placenta to the maternal circulation was once widely accepted as the main route of lactate removal from the fetus. This hypothesis was based upon the assumption that the lower concentration of maternal lactate would favour diffusion of fetal lactate across the placenta. However, in one experiment (ewe LT 48) radioactivity was measured in blood samples taken from the uterine vein. No radioactivity was detected in any maternal blood sample, indicating that the transfer of lactate from fetus to mother was negligible. Further support for this observation is presented by Barker and Britton (1958), Britton, Nixon and Wright (1967), Mann (1970) and Burd et al (1975).

In contrast to the fetal lamb, the postnatal lamb possesses an active gluconeogenic pathway. Since recycling between lactate and glucose occurs, irreversible

loss cannot be assumed to be equivalent to production rate. Irreversible loss would be an underestimate of lactate production rate as an alternative route for lactate disposal is available.

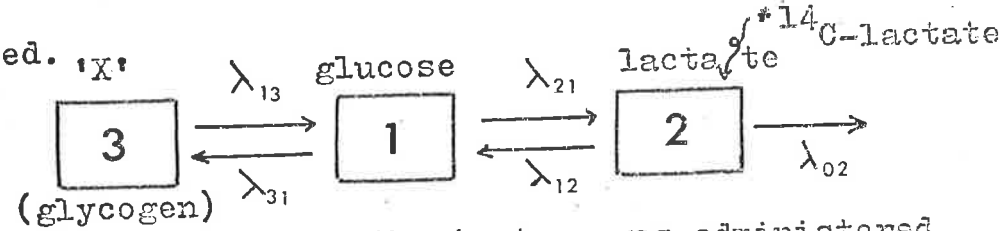
It was the aim of this chapter to derive a model to describe the interrelation of lactate and glucose metabolism in the developing lamb. Several computer programs, incorporating the fitting of non-linear functions, are available for the testing of models and the calculation of rate constants. I have used 'Non-Lin' (Metzler, 1969). The shape of the lactate disappearance curve indicated that there were two pools reversibly interconnected.

i.e.



Lactate carbon was either recycled via glucose or irreversibly lost from the system through oxidation (λ_{02}). However, when tested this model did not satisfactorily fit the data and a second model consisting of three pools was proposed.

i.e.



The lactate pool into which the isotope was administered was reversibly interconnected with the product pool, glucose (sampled). The glucose pool was also reversibly interconnected with an unknown pool (not sampled), but

possibly glycogen.

Neither model provided a satisfactory solution to the kinetics of lactate metabolism in the lamb. Although the procedure aimed at finding the simplest mathematical model to describe the behaviour of the tracer it appears that the models were too simple. Without additional data, provided by a longer sampling time and sampling from more pools, it is not possible to derive a more suitable model and hence rate constants for lactate metabolism in the lamb.

In some cases calculation of the irreversible loss of the product, using the Stewart-Hamilton equation (equation 3) and the coefficients derived for the product specific radioactivity curve, will give an estimate of the irreversible loss of that compound. This procedure is not valid for calculation of input-ouput for all pools to which the tracer enters and leaves (Shipley and Clark, 1972). It is true only if there is a single point of output. Since we are dealing with a metabolic pathway composed of a series of substrate-product reactions there are numerous sites for loss of lactate-derived isotope before the major product, glucose, is reached. Thus calculation of glucose irreversible loss cannot be made from the experiments where ^{14}C-lactate was injected. An independant estimate of glucose irreversible loss in the postnatal lamb was obtained in Chapter IV.3.7.

Annison et al (1963) found that rapid recycling between glucose and lactate occurred in fed, resting adult sheep. They estimated that 15% of the glucose pool was derived from lactate, a value that should be compared to the 30% calculated in my experiments. The sheep used by Annison et al (1963) were anaesthetised, so that muscle activity was low. The lambs used in this study were not anaesthetised, although they were restrained in a wire cage. Muscle activity and stress were apparent.

Irreversible loss, blood lactate concentration and lactate pool were significantly less in the newborn than in the fetal lamb. Comparison of the results of Annison et al (1963) with those presented here show that a further decrease in lactate utilization accompanies maturity. In the fed, resting adult sheep, lactate utlization rates (i.e. irreversible loss) were 0.012-0.019 mmol/kg/min, values which should be compared to 0.042 mmol/kg/min in lambs (Table 9) and 0.075 mmol/kg/min in fetuses (Table 5).

No comparable studies of lactate metabolism are available between pre- and post-natal animals of other species, so an inter-species comparison cannot be made. However, Vernon and Walker (1972b) and Snell and Walker (1973a) have shown that suckling rats have a higher rate of lactate utilization than 30 day old weaned rats. Vernon and Walker (1972b) therefore concluded that the rate of gluconeogenesis in 2-10 day old rats was twice

that of 30 day olds.

The lower irreversible loss of lactate in the post-natal lamb than in the fetal lamb is probably associated with a decrease in production brought about by the removal of the placenta, a major site of lactate synthesis in the conceptus (Burd et al, 1975). The increase in oxygenation which occurs at birth would also contribute to the decrease in lactate production, since the equilibrium of the lactate-pyruvate reaction would no longer favour extensive lactate production (discussed in Chapter VI). The decrease in lactate utilization and production may also be associated with the change in the composition of available nutrients after birth. The placental circulation is a rich source of carbohydrate to the fetus but milk is low in carbohydrate and rich in fat and protein, which are additional and alternative sources of energy. Furthermore, weaning in the ruminant is accompanied by a further decrease in the dietary availability of carbohydrate since these are converted to volatile fatty acids in the rumen.

IV.3.4. Fructose metabolism by the ovine fetus

The metabolism of fructose was studied in five fetal lambs *in utero* ranging in age from 125 days to term.

The results of a typical experiment are presented in Table 10. Fetus 1465 was rapidly injected with 50 μCi [U-^{14}C]fructose via the cannula placed in the umbilical vein. Blood samples were taken from the umbilical artery for a period of 135 min after injection of isotope and specific radioactivities determined. During the experiment the blood concentration of fructose (mean 4.66 ± 0.14 μmol/g blood), glucose (mean 0.73 ± 0.014 μmol/g blood) and lactate (mean 2.50 ± 0.04 μmol/ g blood) did not alter appreciably. Mean maternal glucose concentration was 2.31 ± 0.17 μmol/ g blood. Haematocrit, pO_2 and pCO_2 were constant throughout the experiment (Table 11). The lamb was delivered live at term.

Blood fructose specific radioactivity decreased slowly during the experimental period (Fig 13; Table 10). The specific radioactivity disappearance curve was best described as the sum of two exponential functions. The zero time intercept of the initial component was 13.1 x 10^3 dpm/ μmol and the slope was 0.273 min^{-1}. For the second slower component the zero time intercept was 23.4 x 10^3 dpm/ μmol and the slope was 0.006 min^{-1}. (Table 12; fetus 1465). The fructose pool, derived from equation 2, for fetus 1465 was 1.407 mmol/ kg conceptus weight and the fructose space was 669 ml, 31% of the

Figure 13 :: Semilogarithmic Plot of Blood Fructose Specific Radioactivity in Fetus 1465. Specific radioactivities are normalized to an injection of 50 μCi [U-^{14}C]fructose, and are expressed as dpm x 10^3/ μmol.

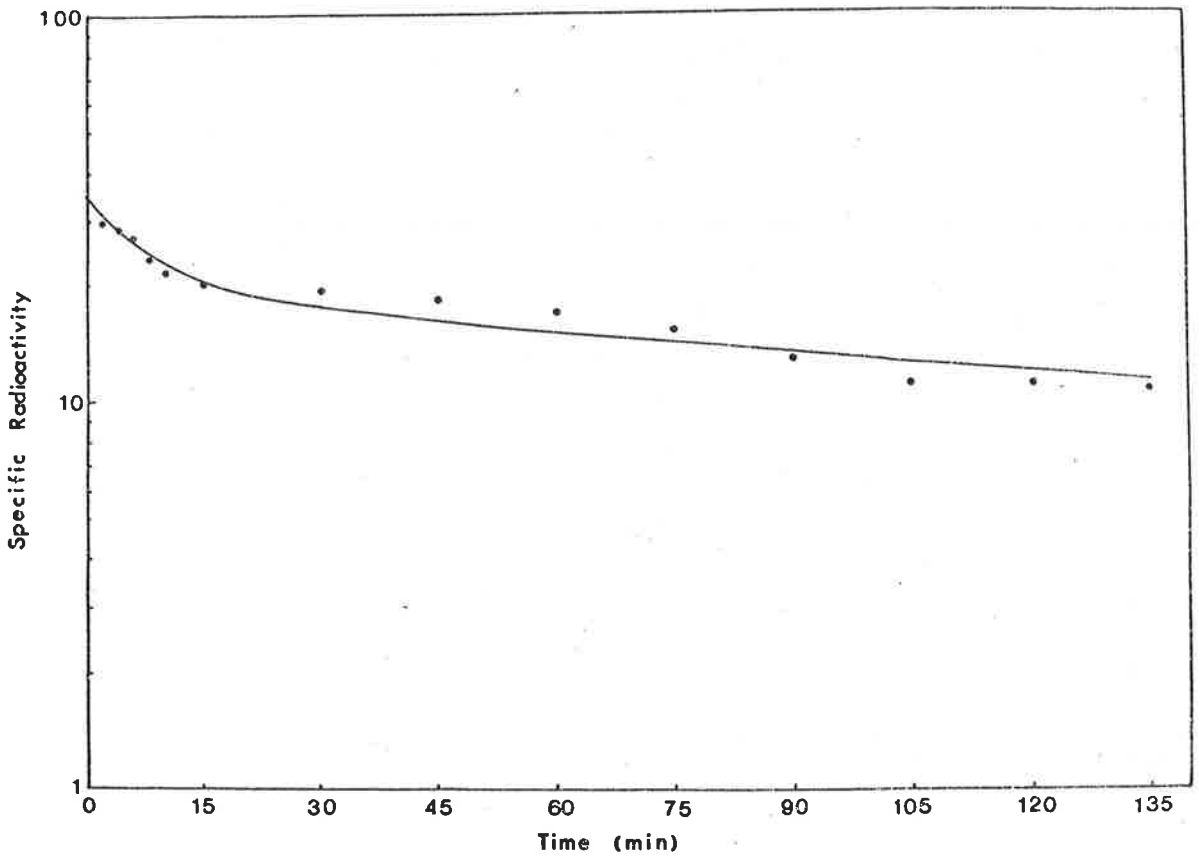

100
10
1
Specific Radioactivity
0
15
30
45
60
75
90
105
120
135
Time (min)

Table 10

Fructose metabolism by the ovine fetus : Data for fetus 1465.

[U-^{14}C] fructose was rapidly injected via the cannula placed in the umbilical vein. Blood samples were taken from the umbilical artery over a period of 135 min. Blood fructose, glucose and lactate concentrations were determined as described in Ch.IV.2.5. Specific radioactivity data are normalized to an injection of 50 µCi.

time (min)	fructose (mM)	glucose (mM)	lactate (mM)	specific radioactivity (dpm x 10^3/ µmol)
2	4.80	0.627	2.57	29.83
4	4.35	0.694	2.33	28.08
6	4.98	0.766	2.21	27.68
8	4.28	0.738	2.42	23.20
10	4.15	0.672	2.33	21.52
15	4.25	0.666	2.41	20.34
30	4.42	0.733	2.41	19.92
45	4.67	0.700	2.56	18.60
60	4.89	0.750	2.52	17.83
75	6.00	0.705	2.65	15.91
90	4.68	0.788	2.67	13.23
105	3.92	0.755	2.63	11.23
120	5.32	0.780	2.65	10.82
135	4.60	0.855	2.61	10.32
mean	4.66	0.730	2.50	
S.E.M.	0.14	0.014	0.04	

Table 11

Fructose Metabolism by the Ovine Fetus: Experimental Details.

Each fetus was injected with 50 μCi [U-^{14}C] fructose via the cannula placed in the umbilical vein (UV) or femoral vein (FV). Blood samples were taken from the umbilical artery (UA) or femoral artery (FA) of the fetus or the uterine vein (UtV) or femoral artery of the ewe. Blood glucose, fructose and lactate determinations are described in Ch. IV.2.5. and are means ± S.E.M of the number of samples expressed in parentheses. Fetal pO_2, pCO_2 and haematocrit were determined at the start and finish of each experiment.

Fetus	1465	A4-251	14	154	248
vessel cannulated fetal: injected	UV	UV	FV	FV	FV
sampled	UA	UA	FA	FA	FA
maternal: sampled	UtV	FA	FA	FA	FA
sampling period (min)	0-135	0-120	0-120	0-180	0-150
fructose (mM)	4.66±0.14 (14)	6.90±0.50 (6)	4.00±0.08 (8)	5.00±0.02 (12)	4.30±0.11 (12)
glucose (mM)	0.73±0.01 (14)	0.41±0.01 (7)	0.52±0.005 (7)	0.60±0.005 (12)	0.87±0.03 (12)
lactate (mM)	2.50±0.04 (14)	2.02±0.10 (7)	2.00±0.07 (7)	1.73±0.03 (12)	1.65±0.06 (12)
pO_2 start	20.1	-	18.3	20.0	22.6
pO_2 end	19.0	-	19.4	22.0	22.6
pCO_2 start	43.1	-	40.0	39.0	30.3
pCO_2 end	43.2	-	41.1	46.5	25.0
%PCV start	35.5	-	33.1	30.0	27.8
%PCV end	36.7	-	32.9	28.5	26.5
maternal glucose (mM)	2.31±0.17 (3)	2.15±0.21 (3)	3.03±0.25 (3)	3.13±0.25 (3)	2.76±0.19 (4)

conceptus weight. The irreversible loss of fructose was 0.012 mmol/kg conceptus wt/min and represents very slow turnover of the fructose pool.

No incorporation of ^{14}C-labelled fructose into either glucose or lactate was detected.

No radioactivity was detected in blood from the uterine vein of the ewe.

Similar experiments were conducted on four other fetuses. The experimental design is summarized in Table 11. Blood fructose concentration varied from 4.00 μmol/ g blood to 6.90 μmol/ g blood and blood glucose concentrations ranged from 0.41-0.87 μmol/ g blood. Blood lactate concentrations were 1.65-2.50 μmol/ g blood. The specific radioactivity of blood fructose decreased slowly in all fetuses and is described by the sum of two exponential functions, except for fetus 64 in which the disappearance of fructose was best fitted by one term. The coefficients for each curve are presented in Table 12, while the normalized curve is shown in Fig 14.

The parameters of fructose metabolism were calculated from the data in Table 12 and mean values are reported in Table 13

The fructose pool was 1.452 ± 0.049 mmol/kg conceptus

Figure 14 : Mean Specific Radioactivity of Fructose in Fetal Lambs.

Specific radioactivities are plotted semilogarithmically against time, and are the mean ± S.E.M. of 5 fetal lambs given 50 μCi [U-^{14}C]fructose. Specific radioactivities are normalized to a base of one where the sum of the zero time intercepts of the components of the fructose disappearance curve equals unity.

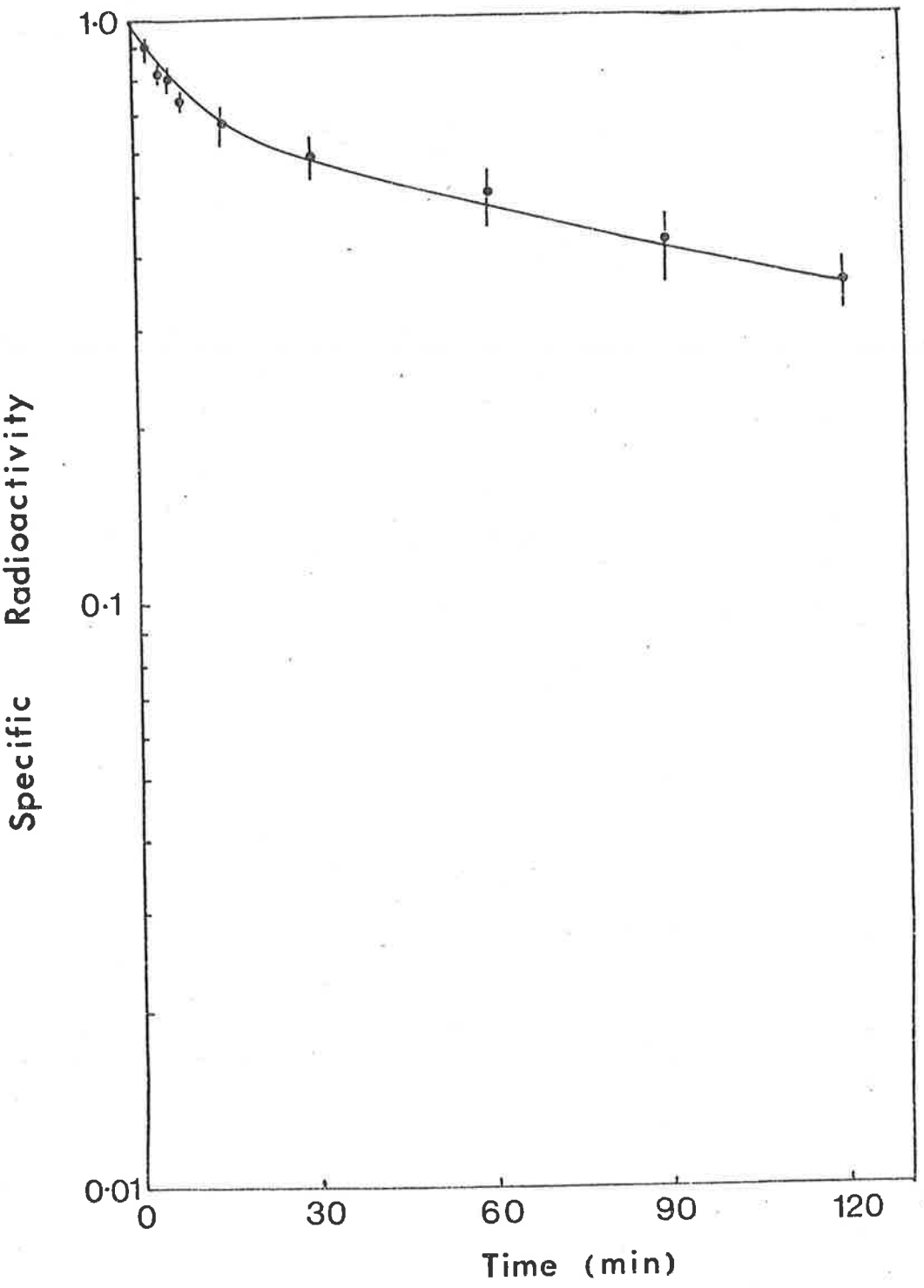

1·0
0·1
0·01
Specific Radioactivity
0
30
60
90
120
Time (min)

Table 12

Fructose metabolism by the ovine fetus : Coefficients of the fructose specific radioactivity curve.

Each fetus was injected intravenously with 50 μCi [U-^{14}C] fructose at zero time (refer Table 10). Specific radioactivity (SR_t) was determined as described in Ch.IV.2.5. and is expressed in units $x10^3$ dpm/μmol. Mathematical analysis was carried out as detailed in Ch.IV.2.6. Specific radioactivities are normalized to an injection of 50 μCi. A_i represents the zero time intercept ($x10^3$ dpm/μmol) and m_i the slope (min) of each component of the curve. The standard deviation for each coefficient is shown in parenthesis.

Fetus	1465	A4-251	14	154	248
initial SR_t	36.50	15.54	28.83	23.04	30.99
first decay					
A_1	13.100 (3.430)	9.147 (1.289)	16.330 (0.902)	23.040 (0.539)	10.180 (3.010)
m_1	-0.273 (0.113)	-0.067 (0.019)	-0.174 (0.030)	-0.005 (0.0004)	-0.110 (0.085)
second decay					
A_2	23.400 (1.620)	6.398 (1.405)	12.500 (0.768)		20.810 (2.280)
m_2	-0.006 (0.0009)	-0.0009 (0.002)	-0.004 (0.0007)		-0.005 (0.0013)
variance	2.137	0.266	0.162	0.943	2.760

Table 13

Parameters of Fructose Metabolism by the Ovine Fetus

The parameters, pool, space and irreversible loss are defined in Ch. IV.2.6. The pool size and irreversible loss are calculated in terms of conceptus weight (i.e. fetus + placenta).

Fetus	1465	A4-251	14	154	248	mean	S.E.M.
age (days)	125	term	135	130	130		
weight (kg)	2.14	5.05	2.89	2.39	2.39		
<u>pool</u>							
(mmol)	3.013	7.070	3.815	4.770	3.549		
(mmol/kg)	1.407	1.400	1.320	1.650	1.484	1.452	0.049
fructose (mM)	4.66	6.90	4.00	5.00	4.30		
space (ml)	669	1024	953	954	825	885	56
% body wt	31.2	20.2	32.9	33.0	34.5	30.3	2.3
irreversible loss (mmol/kg/min)	0.012	0.002	0.011	0.008	0.010	0.008	0.001

Fructose space was 885 ± 56 ml or 30% of the conceptus weight.

Irreversible loss was 0.008 ± 0.001 mmol / kg conceptus wt/min.

The femoral arteries of ewes A4-251, 14,154 and 248 were cannulated. No radioactivity was detected in any blood sample taken from the maternal artery after the injection of isotope. It is concluded that no significant amount of fructose crosses the placenta in the direction of fetus to mother.

Ewe 14 delivered a live lamb at term and a live lamb was delivered after Caesarian section was performed on ewe 248. Although the fetus of ewe 154 was dead at term, all cannulae were functional for 17 days after the experiment and the sudden death suggests that an infection was introduced during routine daily cannula maintenance.

IV.3.5. Discussion

Research during the last few years has failed to show that there is any significant fructose utilization by the fetal lamb (Alexander etal, 1970; Setchell et al, 1972; Tsoulos et al, 1971). In perfused fetal lambs little ^{14}C-fructose is metabolized although a little $^{14}CO_2$ was detected (Alexander et al, 1970). Some ^{14}C-label has also been found in lipids after the in vivo administration of ^{14}C-fructose to fetal lambs (Scott et al, 1967) while Ballard and Oliver (1965) have found

incorporation of ^{14}C-label into glycogen on incubation of fetal lamb liver slices with ^{14}C-fructose. The experiments reported in this section have also failed to show substantial utilization of fructose by the fetal lamb *in utero*.

Alexander *et al* (1955) found that the production rate of fructose by the perfused placenta was 0.027-0.050 mmol/min which, for a 4 kg fetus, would be equivalent to 0.007-0.014 mmol/kg/min. Assuming steady state conditions prevail and metabolic recycling is minimal, then irreversible loss of fructose appears to be a reliable estimate of production rate. However, production rate, calculated from the perfused placenta, must be cautiously interpretted because fructose production is self-limiting. High plasma fructose concentration would cause a decrease in production rate (Nixon, 1963; Nixon *et al*, 1966).

Loss of fructose to the mother does not occur because the placenta is impermeable to fructose (Alexander *et al*, 1955; Setchell *et al*, 1972). In the fetus the major proportion of fructose would be lost by renal excretion to the amniotic fluid (Alexander and Nixon, 1963; Alexander, Britton and Nixon, 1966). Some of the fructose found in the amniotic fluid may be absorbed from the gastrointestinal tract as a result of the swallowing action of the lamb *in utero* (Nixon and Wright, 1964). This recycling would not be of significance

during the short period in which these experiments were performed.

It has been suggested that fructose may act as an alternative source of fetal energy under conditions in which availability of glucose is restricted (Huggett, Warren and Warren, 1951; Alexander et al, 1955). If this were the case then it would be necessary for fructose to enter the glycolytic sequence through glucose or fructose 6-phosphate. No incorporation of radioactivity into either glucose or lactate was detected in any blood samples taken after ^{14}C- fructose administration. Britton et al,(1967) were also unable to find evidence for fructose conversion to lactate. It therefore seems unlikely that fructose is a readily available source of energy to the fetus.

Although there is no significant fructose metabolism until after birth, fructose rapidly disappeared from the blood of the neonatal lamb (Huggett et al, 1951). Dawes and Shelley (1962) have shown that this rapid loss of blood fructose must be accounted for by renal excretion since fructose metabolism is not detected until 5 days after birth (Andrews et al, 1960) and coincides with the increase in fructokinase activity (Ballard and Oliver, 1965). Premature delivery of lambs will accelerate the development of the fructose metabolizing system (Andrews et al, 1961).

IV.3.6. Glucose metabolism by the ovine fetus

Glucose metabolism and its contribution to the lactate and fructose pools was studied in four fetal lambs with cannulae chronically implanted in either the umbilical vein and artery or the femoral vein and artery (see Table 15 for experimental design).

The results of a typical experiment conducted on fetus 166 are presented in Table 14. [U-^{14}C] glucose (50 μCi) was injected into the fetus via the cannula implanted in the femoral vein. Blood samples were taken from the femoral artery of the fetus and the ewe over a period of 120 min. Fetal blood glucose, fructose and lactate concentrations and maternal blood glucose concentrations were measured in these samples and the specific radioactivity was calculated. During the experimental period the blood concentration of glucose (mean 0.75 ± 0.008 μmol/ g blood) , fructose (mean 5.3 ± 0.14 μmol/ g blood) and lactate (3.2 ± 0.06 μmol/ g blood) remained constant. Blood pO_2 and pCO_2 values, measured at the beginning and end of the experiment, showed no large variations and were similar to the fetuses of previous experiments. Haematocrit readings taken at these times were also constant.

The fall in the specific radioactivity of blood glucose with time is plotted semilogarithmically in Fig 15. There was a large and rapid fall in specific radioactivity

Figure 15 : Semilogarithmic Plot of Blood Glucose Lactate and Fructose Specific Radioactivity in Fetus 166.

Specific radioactivities are normalized to an injection of 50 μCi [U-^{14}C] glucose and are expressed as dpm x 10^3/μmol.

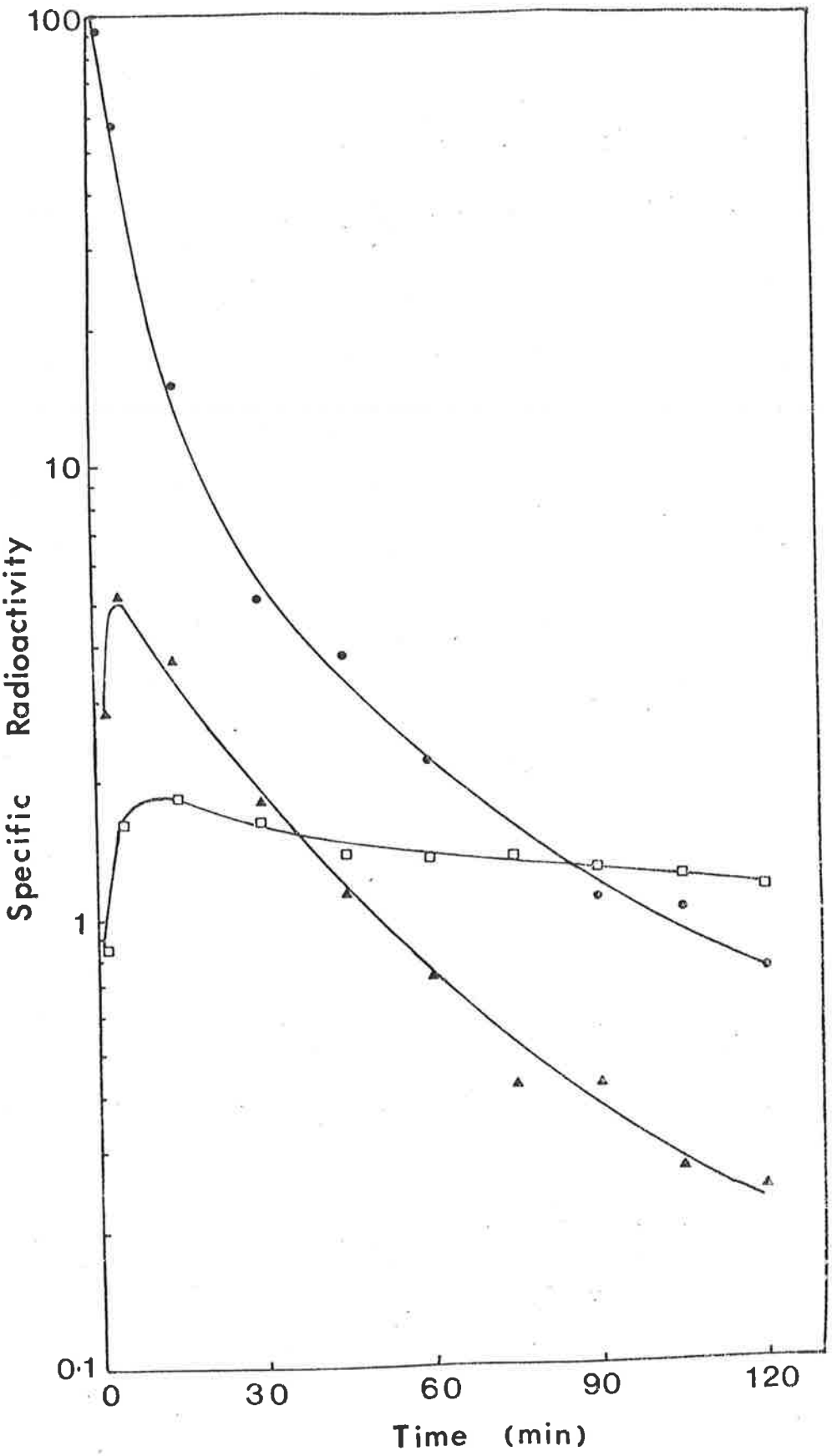

100
10
1
0·1
Specific Radioactivity
0
30
60
90
120
Time (min)

Table 14

Glucose metabolism by the ovine fetus : Data for fetus 166.

U-^{14}C glucose was rapidly injected via the cannula placed in the femoral artery. Fetal blood samples were collected from the femoral artery over a period of 120 min. Blood glucose, lactate and fructose concentrations were determined as described in Ch. IV.2.5. Specific radioactivity data are normalized to an injection of 50 uCi.

time (min)	glucose (mM)	lactate (mM)	fructose (mM)	specific radioactivity glucose (dpm x 10^3 / µmol)	lactate	fructose
2	0.71	3.14	5.45	92.81	2.85	0.85
5	0.71	3.14	5.31	57.38	5.21	1.60
15	0.76	3.16	6.06	15.13	3.71	1.86
30	0.78	3.22	4.90	5.07	1.78	1.62
45	0.78	3.23	5.21	3.80	1.13	1.49
60	0.75	3.14	5.66	2.24	0.72	1.47
75	0.73	3.15	4.94	1.39	0.41	1.47
90	0.76	3.31	5.77	1.07	0.42	1.40
105	0.76	3.71	5.66	1.05	0.27	1.27
120	0.78	3.60	4.44	0.75	0.25	1.17
mean	0.75	3.28	5.34			
S.E.M.	0.008	0.06	0.14			

Table 15

Glucose Metabolism by the Ovine Fetus: Experimental Details.

Each fetus was injected with 50 μCi [U-^{14}C] glucose via the cannula placed in the umbilical vein (UV) or the femoral vein (FV). Blood samples were taken from either the umbilical artery (UA) or femoral artery (FA) of the fetus and the femoral artery of the ewe. Blood glucose, fructose and lactate determinations are described in Ch. IV.2.5. and are means ± S.E.M of the number of samples expressed in parentheses.

Fetus	146	145	64	166
vessel cannulated				
fetal: injected	UV	UV	FV	FV
sampled	UA	UA	FA	FA
maternal: sampled	FA	FA	FA	FA
sampling period (min)	0-120	0-150	0-120	0-120
fetal glucose (mM)	0.69±0.01 (17)	0.63±0.05 (7)	0.69±0.02 (7)	0.75±0.008 (10)
lactate (mM)	2.1±0.09 (17)	1.5±0.10 (7)	1.1±0.005 (7)	3.2±0.06 (10)
fructose (mM)	4.0±0.10 (13)	4.8±0.08 (7)	5.1±0.11 (7)	5.3±0.14 (10)
pO_2 start	21.3	19.8	23.1	20.0
pO_2 end	20.7	21.3	22.9	19.0
pCO_2 start	44.8	40.0	42.0	48.0
pCO_2 end	46.7	39.0	40.0	49.0
%PCV start	35.6	25.0	39.0	29.5
%PCV end	34.3	25.3	39.0	27.0
maternal glucose (mM)	2.73±0.30 (3)	2.67±0.28 (3)	2.94±0.21 (3)	2.90±0.17 (3)

during the first 15 min after the injection of [U-^{14}C] glucose; thereafter specific radioactivity decreased slowly. Compartmental analysis of the specific radioactivity curve over this time period showed that the curve was best represented as the sum of two exponential terms with zero time intercepts of 119.4 x 10^3 dpm/ µmol and 9.72 x 10^3 dpm/µmol and slopes of 0.179 min^{-1} and 0.023 min^{-1} respectively (Table 16). The sum of the zero time intercepts gave the specific radioactivity at the time of injection, and from the extent of dilution of the dose of isotope administered the glucose pool of the fetus was calculated to be 0.427 µmol / kg conceptus wt and the space was 1105 ml which represented 55% of the conceptus weight. The irreversible loss , calculated using equation 3, was 0.046 mmol/ kg conceptus wt/min (Table 17).

Within 2 min of administration of [U-^{14}C] glucose, radioactivity was detected in both lactate and fructose (Table 14, Fig 15). Maximum labelling in blood lactate was detected 5 min, and in fructose 15 min, after injection of isotope. The specific radioactivity of blood lactate declined rapidly after maximum incorporation was reached,. However, the specific radioactivity of blood fructose declined very slowly reflecting the slow metabolism (Ch. IV.3.4.). Compartmental analysis revealed that the incorporation of ^{14}C-label into blood lactate was best described by the sum of three exponential terms,

Table 16

Glucose metabolism by the ovine fetus: Coefficients of glucose , lactate and fructose specific radioactivity curves.

Each fetus was injected intravenously with 50 μCi [U-^{14}C] glucose at zero time (refer Table 15). Specific radioactivity (SR_t), expressed in units x10^3 dpm/μmol, was determined as described in Ch.IV.2.5. and mathematical analysis was carried out as detailed in Ch.IV.2.6. Specific radioactivities are normalized to an injection of 50 μCi. A_i represents the zero time intercept (x10^3 dpm/μmol) and m_i the slope (min) of each component of the curve . The standard deviation for each component is shown in parenthesis.

Fetus	146	145	64	166
initial SR_t	135.73	77.16	83.91	129.16
		^{14}C-glucose disappearance		
first decay				
A_1	135.5 (7.400)	72.89 (1.470)	82.38 (4.310)	119.4 (0.861)
m_1	-0.186 (0.015)	-0.251 (0.011)	-0.167 (0.012)	-0.179 (0.003)
second decay				
A_2	0.234 (1.290)	4.270 (1.160)	1.530 (1.230)	9.725 (0.930)
m_2	-0.010 (0.062)	-0.020 (0.006)	-0.0018 (0.009)	-0.023 (0.002)
variance	17.280	0.291	0.605	0.075
		^{14}C-lactate appearance		
rising				
A'_1	68.450 (60.110)	4.590 (1.540)	114.712 (48.011)	12.023 (0.484)
m'_1	1.128 (0.528)	0.038 (0.0063)	0.597 (0.122)	0.442 (0.070)
first decay				
A'_2	13.560 (1.830)	4.593 (1.551)	23.824 (10.785)	7.260 (0.622)
m'_2	-0.116 (0.027)	-0.021 (0.002)	-0.260 (0.044)	-0.071 (0.012)
second decay				
A'_3	0.922 (1.063)		1.850 (0.218)	1.551 (0.579)
m'_3	-0.006 (0.017)		-0.023 (0.003)	-0.015 (0.004)
variance	0.112	0.021	0.002	0.003

Table 16 (continued)

Fetus	146	145	64	166
		^{14}C-fructose appearance		
rising				
A''_1	8.810 (4.100)	0.919 (0.109)	2.771 (0.465)	1.546 (0.295)
m''_1	0.763 (0.245)	0.177 (0.043)	0.215 (0.039)	0.426 (0.025)
decay				
A''_2	3.093 (0.095)	0.889 (0.087)	1.301 (0.068)	2.320 (0.309)
m''_2	-0.0019 (0.00064)	-0.0054 (0.0016)	-0.0028 (0.0006)	-0.0053 (0.0015)
variance	0.013	0.002	0.001	0.017

Table 17

Parameters of Glucose Metabolism by the Ovine Fetus

The parameters, pool, space and irreversible loss are defined in Ch. IV.2.6. The pool size and irreversible loss are calculated in terms of conceptus weight (i.e. fetus + placenta).

Fetus	146	145	64	166	mean	S.E.M.
age (days)	127	term	130	124		
weight (kg)	2.29	5.39	2.39	1.99		
pool						
(mmol)	0.814	1.425	1.301	0.851		
(mmol/kg)	0.355	0.264	0.548	0.427	0.398	0.051
glucose (mM)	0.69	0.63	0.69	0.75		
space (ml)	1292	2065	1898	1105	1590	200
% body wt	56.4	28.3	79.4	55.5	57.4	7.3
irreversible loss (mmol/kg/min)	0.045	0.041	0.034	0.046	0.041	0.002

whilst fructose formation was described by the sum of two exponential terms, (Table 16; fetus 166). Comparison of the area under the lactate or fructose incorporation curves with the area under the glucose disappearance curve showed that 39% of the ^{14}C-glucose was incorporated into the lactate pool and 31% was incorporated into the fructose pool.

Similar experiments were performed on three other fetal lambs and the results are summarized in Tables 15, 16 and 17). Since difficulty was experienced in maintaining patency of the cannulae in the umbilical vein and artery of fetus 145 the experiment was conducted 2 days after surgery. Two ewes, 64 and 166, delivered live lambs at term. Fetus 145 was aborted the day following the experiment and fetus 146 was dead at term.

In the four ewes where the femoral artery was cannulated no radioactivity was detected in any of the blood samples.

The specific radioactivity curve of blood glucose was, in all cases, best described by the sum of two exponential functions. In ewe 146 glucose specific radioactivity declined so rapidly that it was difficult to accurately calculate the coefficients of the slow component of the curve. This is evident both by the large standard deviation for the intercept and the large variance of the curve. However, as mentioned before, calculation of utilization rates using the Stewart-Hamilton equation

is independant of the errors associated with such coefficients.

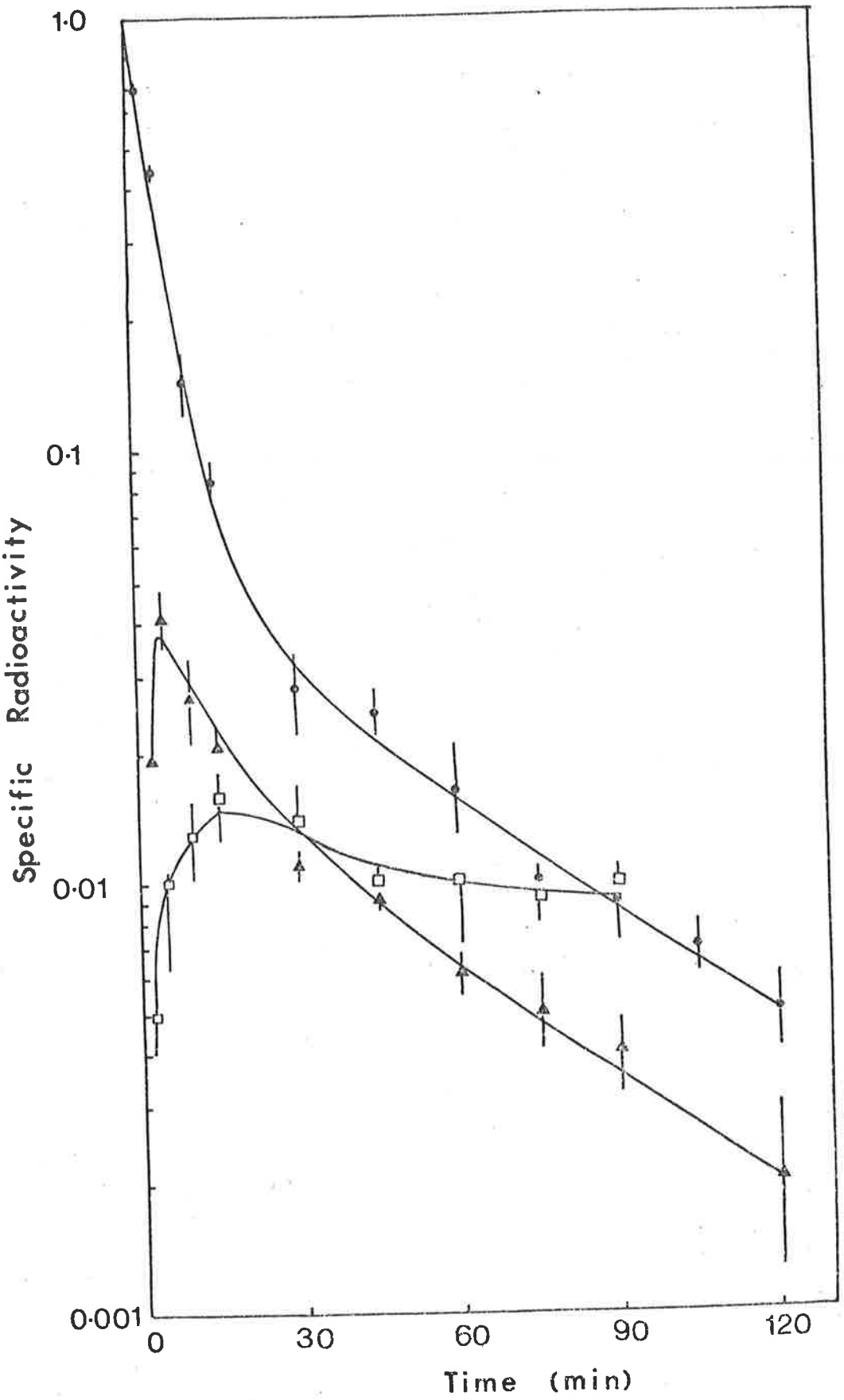
The lactate specific radioactivity incorporation curve was composed of 3 exponential functions in fetus 146, 64 and 166; but the sum of two exponentials best described the curve of fetus 145. The fructose specific radioactivity incorporation curve for all fetuses was best described by the sum of two exponential functions, one rising and one falling. The values of the derived coefficients for all specific radioactivity curves are given in Table 16. The changes in specific radioactivity of glucose, fructose and lactate are shown in Fig 16 where the data points are means ± S.E.M. for the normalized values.

It was calculated by comparison of the areas under the lactate or fructose production curves with the area under the glucose disappearance curve that from 25% to 59% of the ^{14}C-glucose was incorporated into the lactate pool and from 22% to 37% was incoporated into the fructose pools, with means of 39 ± 6% and 31 ± 3 % respectively.

The glucose pool was 0.398 ± 0.05 mmol/ kg conceptus weight, the space was 1590 ± 200ml and represented 57% of the conceptus weight. The irreversible loss was 0.041 ± 0.002 mmol/kg conceptus wt/min, (Table 17) .

Figure 16 : Mean Specific Radioactivity of Glucose Lactate and Fructose in Fetal Lambs

Specific radioactivities are plotted semilogarithmically against time, and are the mean ± S.E.M. of 4 fetal lambs given 50 μCi [U-^{14}C]glucose. Specific radioactivities are normalized to a base of one where the sum of the zero time intercepts of the components of the glucose disappearance curve equals unity.


1·0
0·1
0·01
0·001
Specific Radioactivity
0
30
60
90
120
Time (min)

IV.3.7. Glucose metabolism by the postnatal lamb

Glucose metabolism was studied in five postnatal lambs aged from 5 to 28 days. The experimental procedure is summarized in Table 19. The results of a typical experiment are presented in Table 18.

Lamb I was injected with 50 μCi of [U-^{14}C] glucose via the jugular vein and blood samples were collected from the femoral artery over a period of 0-150 min. Blood glucose concentration (mean 3.98 ± 0.11 μmol/ g blood) and blood lactate (mean 1.73 ± 0.037 μmol/ g blood) remained constant throughout the experimental period (Table 18 and 19). Compartmental analysis of the specific radioactivity disappearance curve showed that the simplest description of the changes in specific radioactivity in blood was given by a function consisting of the sum of two exponential terms (Fig 17). The derived coefficients of the glucose specific radioactivity curve are presented in Table 20. The zero time intercept and slope of the initial fast component of the curve for lamb I were 8.86×10^3 dpm/μmol and 0.080 min^{-1} respectively and the second slower component were 3.55×10^3 dpm /μmol and 0.015 min^{-1}. From these coefficients the glucose pool of the lamb was calculated as 1.107 mmol/kg, the space in which this pool is distributed was 1029ml which was 25.6% of the body wt. The irreversible loss was 0.034 mmol/kg/min (Table 21).

Figure 17 : Semilogarithmic Plot of Blood Glucose and Lactate Specific Radioactivity in lamb I

Specific radioactivities are normalized to an injection of 50 μCi [U-^{14}C]glucose and are expressed as dpm x 10^3/μmol.

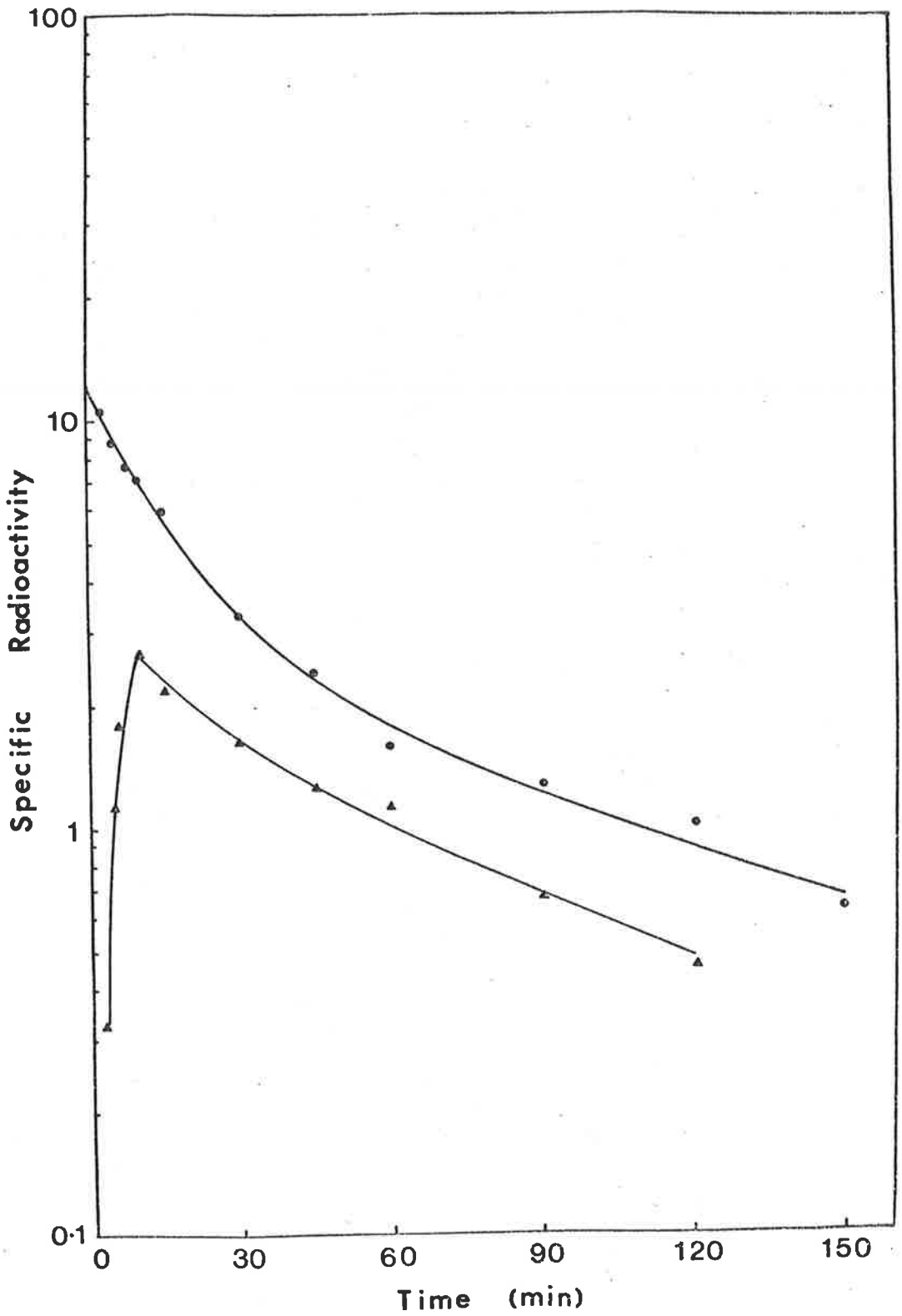

100
10
1
0·1
Specific Radioactivity
0
30
60
90
120
150
Time (min)

Table 18

Glucose metabolism by the postnatal lamb : Data for lamb I.

[U-^{14}C] glucose was injected via the cannula placed in the jugular vein. Blood samples were collected from the femoral artery over a period of 150 min. Blood glucose and lactate concentrations were determined as described in Ch.IV.2.5. Specific radioactivities are normalized to an injection of 50 µCi.

time (min)	lactate (mM)	glucose (mM)	specific radioactivity glucose (dpm x 10^3 / µmol)	specific radioactivity lactate (dpm x 10^3 / µmol)
3	4.48	1.56	10.77	0.32
5	4.61	1.97	8.92	1.16
8	4.42	1.63	7.57	-
10	4.06	1.78	7.05	2.74
15	3.86	1.74	6.08	2.15
30	3.62	1.68	3.26	1.61
45	4.16	1.81	2.42	1.28
60	3.60	1.84	1.58	1.18
90	3.51	1.72	1.31	0.67
120	3.74	1.65	1.06	0.45
150	3.76	1.71	0.61	-
mean	3.98	1.73		
S.E.M.	0.11	0.03		

Table 19

Glucose Metabolism by the Postnatal Lamb : Experimental Details.

Each lamb was injected with 50 μCi [U-^{14}C] glucose via the cannula placed in either the jugular vein (JV) or the femoral artery (FA). Blood samples were taken from either the femoral artery or the jugular vein.
Blood glucose and lactate were measured as described in Ch. IV.2.5. and are the mean ± S.E.M. of the number of determinations expressed in parentheses.

Lamb	274	266	147	I	II
vessel cannulated					
injected	JV	JV	JV	JV	FA
sampled	FA	FA	FA	FA	JV
sampling period (min)	0–150	0–120	0–120	0–150	0–120
glucose (mM)	2.8±0.09 (9)	3.6±0.20 (8)	2.8±0.21 (8)	3.9±0.11 (11)	4.3±0.10 (12)
lactate (mM)	0.9±0.05 (9)	1.6±0.06 (7)	2.4±0.15 (8)	1.7±0.03 (11)	1.5±0.11 (12)

Table 20

Glucose metabolism by the postnatal lamb : Coefficients of glucose and lactate specific radioactivity curves.

Each lamb was injected intravenously with 50 μCi [U-^{14}C]glucose at zero time (refer Table 19). Specific radioactivity (SR_t), expressed as $x10^3$ dpm/μmol, was determined as described in Ch.IV.2.5. and mathematical analysis carried out as detailed in Ch. IV.2.6. Specific radioactivities are normalized to an injection of 50 μCi. A_i represents the zero time intercept ($x10^3$ dpm/μmol) and m_i the slope (min) of each component of the curve . The standard deviation for each coefficient is shown in parenthesis.

Lamb	274	266	147	I	II
initial SR_t	11.3	20.9	12.7	12.4	24.8
	^{14}C-glucose disappearance				
first decay					
A_1	7.013 (6.098)	13.660 (1.410)	6.190 (0.782)	8.862 (0.870)	24.840 (0.860)
m_1	-0.058 (0.033)	-0.177 (0.042)	-0.218 (0.068)	-0.080 (0.146)	-0.031 (0.002)
second decay					
A_2	4.353 (6.296)	7.297 (1.610)	6.560 (0.731)	3.550 (0.981)	
m_2	-0.019 (0.015)	-0.019 (0.005)	-0.018 (0.002)	-0.015 (0.003)	
variance	0.114	0.245	0.086	0.086	0.169
	^{14}C-lactate appearance				
rising					
A'_1	1.060 (0.322)	1.669 (0.301)	1.510 (0.352)	5.290 (1.880)	2.870 (1.156)
m'_1	0.314 (0.181)	0.176 (0.086)	0.628 (0.125)	0.240 (0.145)	0.244 (0.092)
decay					
A'_2	0.801 (0.192)	1.754 (0.299)	0.588 (0.019)	3.000 (0.750)	6.245 (1.353)
m'_2	-0.015 (0.0054)	-0.016 (0.003)	-0.012 (0.0009)	-0.017 (0.006)	-0.035 (0.007)
variance	0.010	0.014	0.00036	0.139	0.133

Incorporation of ^{14}C-label into lactate occurred rapidly after injection of ^{14}C-glucose into lamb I, with maximum incorporation reached after 10 min (Fig 17). The lactate specific radioactivity curve was best described as the sum of two exponential terms. The slope of the rising function was 0.240 min and of the decreasing function 0.017 min^{-1}. The zero time intercept of the decreasing function was 3.00×10^3 dpm / μmol. From comparison of the area under the lactate specific radioactivity curve with the area under the glucose specific radioactivity curve it was calculated that 28% of the ^{14}C-glucose was incorporated into the lactate pool of the lamb.

Similar experiments were conducted on four other lambs and the results are summarized in Tables 19, 20 and 21. With the exception of lamb II the specific radioactivity disappearance curve of glucose from the blood of the lamb was described as the sum of two exponentials (Table 20). For lamb II only one term was derived after computer analysis. In all cases the specific radioactivity incorporation curve was described as the sum of two exponential terms, one rising and one falling. The changes in specific radioactivity for all lambs is shown in Fig 18. The glucose and lactate specific radioactivities were normalized to one and the data points are presented as means ± S.E.M.

Figure 18 : Mean Specific Radioactivity of Glucose and Lactate in Postnatal Lambs

Specific radioactivities are plotted semilogarithmically against time, and are the mean ± S.E.M. of 5 postnatal lambs given 50 μCi [U-^{14}C]glucose.

Specific radioactivities are normalized to a base of one where the sum of the zero time intercepts of the components of the glucose disappearance curve equals unity.

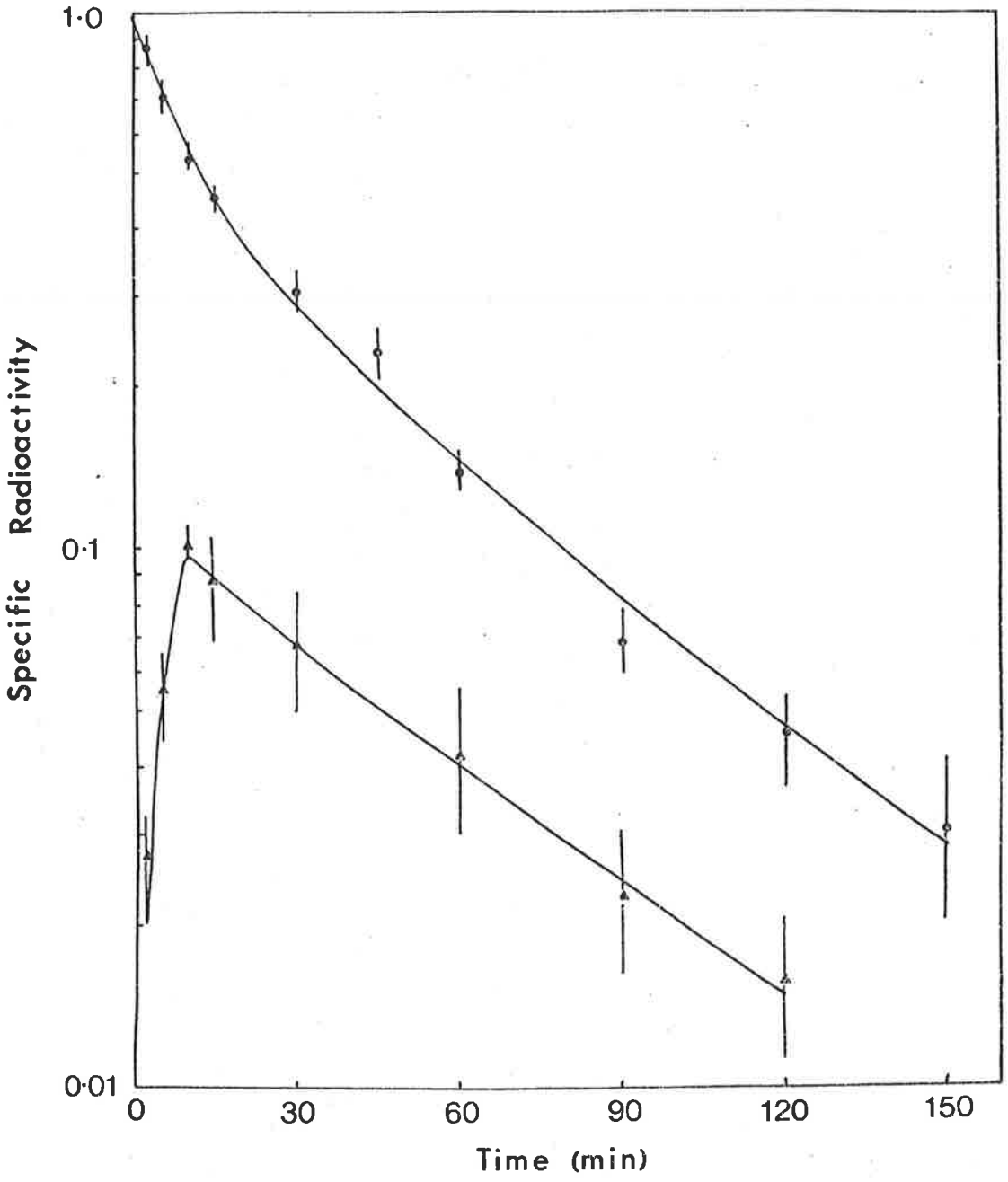

1·0
0·1
0·01
Specific Radioactivity
0
30
60
90
120
150
Time (min)

Table 21

Glucose Metabolism by the Postnatal Lamb

The parameters, pool, space and irreversible loss are defined in Ch. IV.2.6.

Lamb	274	226	147	I	II	mean	S.E.M.
age (days)	12	6	12	5	28		
weight (kg)	10	5.6	9.4	4.0	8.1		
pool							
(mmol)	9.677	5.248	8.627	4.428	8.863		
(mmol/kg)	0.967	0.937	0.917	1.107	1.065	0.998	0.033
glucose (mM)	2.8	3.6	2.8	3.9	4.3		
space	3456	1457	3081	1029	2061	2294	371
% body wt	34.5	26.0	32.7	25.6	25.4	29.3	1.81
irreversible loss (mmol/kg/min)	0.031	0.043	0.029	0.034	0.030	0.033	0.002

Comparison of the area under the lactate specific radioactivity curve with the area under the glucose specific radioactivity curved showed that between 23 % and 44 % of the lactate pool was derived from ^{14}C-glucose (mean 39 ± 8%).

The parameters of glucose metabolism, calculated from the derived coefficients of Table 20, are presented in Table 21. The mean glucose pool was 0.998 ± 0.033 mmol/kg, the space was 2294 ± 371 ml representing 29.3% of the body weight. The mean irreversible loss was 0.033 ± 0.002 mmol/kg/min.

Statistical comparison (Student's t-test) of the glucose metabolism in the fetus with the postnatal lamb showed:-

(1) the glucose pool of the fetus was significantly less than in the postnatal lamb ($P \leqslant 0.05$);

(2) the blood glucose concentration of the fetus was significantly less than in the postnatal lamb ($P \leqslant 0.05$);

(3) the space in which the glucose pool was distributed was not significantly different ($P \leqslant 0.05$). On the basis of body weight, the glucose space was significantly greater in the conceptus than in the postnatal lamb ($P \leqslant 0.05$);

(4) the irreversible loss of glucose did not differ significantly ($P \geqslant 0.05$).

IV.3.8. Discussion

Glucose metabolism by the fetal lamb has been studied by the techniques listed in Table 22. Early estimations of glucose metabolism employed isolated perfused fetuses (Alexander *et al* , 1955; Alexander, Britton and Nixon, 1964). The glucose difference across the perfusion circuit was measured. Glucose utilization by the perfused lamb after administration of ^{14}C-glucose has also been measured (Alexander *et al*, 1970) , but detailed mathematical analysis as carried out in this dissertation was not possible. Tsoulos *et al*, (1971) and James *et al* (1972) measured glucose turnover in the chronically cannulated fetal lamb *in utero*. However, these experiments have been confined to measurement of the umbilical venous-arterial difference for blood glucose. A further requirement for accurate measurements of glucose uptake is the accurate measurement of umbilical blood flow. Crenshaw *et al* (1973) found considerable variability in blood flow between fetuses and reported values ranging from 111 ml/kg/min to 624 ml/kg/min. On the other hand, James *et al* (1972) reported less variability.

With the different techniques used and the different amounts of stress that accompany each procedure a comparison of the estimates of glucose metabolism by the fetal lamb is difficult. For example, glucose turnover

Table 22

Comparison of results of glucose metabolism by the fetal lamb.

glucose uptake (mmol/kg/min)	technique	reference
0.038	isolated, perfused fetus	Alexander et al (1955;1964;1970)
0.060	chronically cannulated (hypoxic)	Mann et al (1970)
0.016	chronically cannulated (unstressed)	Tsoulos et al (1971); James et al (1972)
0.031	chronically cannulated (unstressed)	Crenshaw et al (1973)
0.041	chronically cannulated (unstressed)	this thesis

generally increases with increased stress. This is illustrated by the data of Mann et al (1970). Difficulties also arise when comparing glucose utilization in the adult sheep, for different feeding regimes and physiological states produce variable values for glucose utilization. Therefore, I have not attempted a detailed comparison.

For the sake of discussion I shall take as an example the experiments of Jarrett et al (1964) in which glucose turnover in the postnatal lamb and adult sheep was measured by both the single injection and continuous infusion techniques. They found a glucose utilization rate of 0.04mmol/kg/min in lambs two weeks old, a value similar to that calculated for the fetus and postnatal lamb in this study. By eight weeks of age glucose utilization rate had decreased to 0.02 mmol/kg/min. A further decrease in glucose utilization rate accompanied maturity since sheep 1-3 yr old had a glucose utilization rate of 0.01 mmol/kg/min.

For an accurate estimation of glucose turnover in the postnatal lamb and adult sheep it is necessary to measure the extent of recycling between glucose and lactate. It is usual to use a double labelled tracer, i.e. glucose with ^{3}H at the 2 or 6 position and ^{14}C at the 6 position on the molecule. The tritium label is lost during metabolism whilst the ^{14}C may be recycled. A comparison of the area under

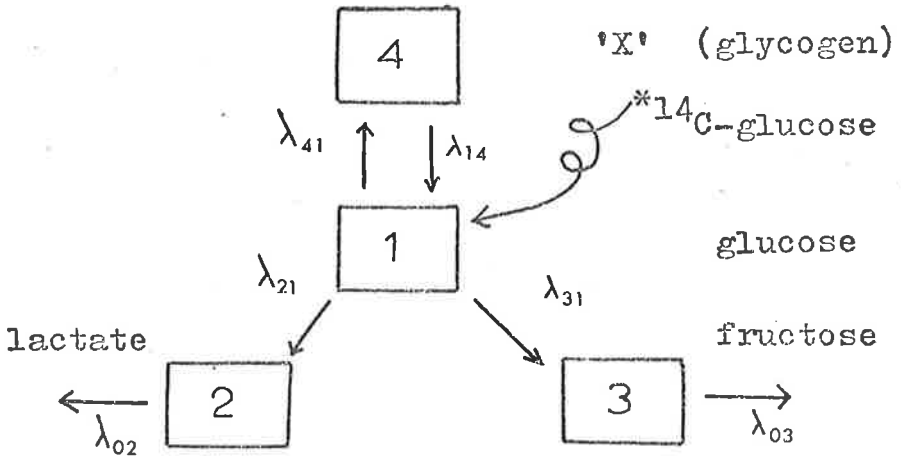
the two specific radioactivity curves gives the amount of recycling. In the present study I used the more direct approach of following ^{14}C-lactate and ^{14}C-glucose metabolism. The electrophoretic separation technique chosen allowed the simultaneous estimation of the specific radioactivity of blood glucose , fructose and lactate in a small portion of the blood extract. Other techniques for the estimation of specific radioactivity of glucose and lactate involve considerable dilution of the sample such as resolution of glucose and lactate by ion exchange chromatography. Separation of glucose and fructose is not readily acheived by such a process. A further reason against using double labelled glucose is the inability to accurately measure specific radioactivity caused by the low counting efficiency for this isotope (i.e ^{3}H). Very large amounts of ^{3}H-glucose would have to be injected.

The decrease in glucose utilization in the sheep with maturity is probably associated with the dietary changes which accompany the development of rumination and the increase in blood volatile fatty acids. This is exemplified by the study of Jarrett et al (1964) in which it was shown that glucose utilization rate decreased with age. In eight week old lambs Wardrop and Coomb (1961) and Boda, Riley and Wegner (1962) found that blood volatile fatty acid concentrations had reached adult concentrations. At this age, therefore, substrates other than glucose are available for energy production.

A contrasting situation occurs in the rat in which the glucose utilization rate decreases from 15.2 μmol/min/100 g in the immediate newborn to 5.2 μmol/min/100 g at 2 hr after birth. There is a slight increase to 6.37 μmol/min/100 g in animals 2-10 days old and a further large increase to 11.6 μmol/min/100g g after weaning. This illustrates the difference between ruminants and monogastrics, for the latter group of animals have large amounts of glucose and other carbohydrates available from the digestive tract after weaning. Indeed, Reid (1952) noted that the ability of extrahepatic tissues to utilize glucose is lower in ruminants than in non-ruminants. The suckling rat ressembles the ruminant in that its diet contains a high concentration of fat and protein and is low in carbohydrate. The lower rate of glucose utilization and higher rate of gluconeogenesis in the milk-fed rat suggests conservation of glucose (Vernon and Walker, 1972 a and b).

I was unsuccessful in my attempts to derive a model describing the interrelationship of glucose, lactate and fructose metabolism in the fetal lamb using the computer program 'Non-lin' (Metzler, 1969). The model proposed was based on the data obtained from the experiments in which ^{14}C-lactate, ^{14}C-fructose and ^{14}C-glucose were administered to the fetus <u>in utero</u>.

It consisted of four pools :-



The glucose pool, into which the isotope was administered, was reversibly interconnected to an unknown pool (λ_{14} , λ_{41}), and irreversibly interconnected with the lactate (λ_{21}) pool and fructose (λ_{31}). Irreversible loss from the system occurred via lactate (λ_{02}) and fructose (λ_{03}).

The fetal model is complex and the amount of data which can be obtained without killing the fetus is limited, so my attempts to confirm or modify this model were unsuccessful. Consequently no values for the rate constants are presented.

As discussed in Chapter IV.3.3. it is not possible to use the Stewart-Hamilton equation (equation 3) to to calculate the irreversible loss of lactate and fructose from the specific radioactivity product curves obtained after injection of ^{14}C-glucose. On the basis my independant estimates of the irreversible loss of glucose, lactate and fructose in the fetus, a simple model describing the

the metabolism of glucose, lactate and fructose is presented in Fig 19). Some values from other studies have been included. The model is based upon the following assumptions:-

(1) An approximate steady state exists ;

(2) Gluconeogenesis is zero and so recycling of isotope from lactate to glucose to lactate cannot occur.

The following reactions predominate:

glucose ⟶ lactate

glucose ⟶ fructose

glucose ⟶ glycogen

For this model the irreversible loss of substrate gives a good estimate of production rate.

The mean irreversible loss of glucose from the ovine fetus was 0.041 mmol/kg/min (Table 17), which should be equivalent to the sum of the irreversible losses of lactate and fructose, the two major output fluxes. The irreversible loss of lactate was 0.075 mmol/kg/min (Table 5), i.e. 0.037 mmol glucose equivalents/kg/min and for fructose it was 0.008 mmol/kg/min. The irreversible loss of glucose includes oxidation , the production of lactate, fructose and lipids (Table 23 a). Glycogen could be a reversible or irreversible loss of glucose radioactivity depending on the physiological state of the fetus.

Figure 19

Interrelationship of glucose, lactate and fructose in the ovine conceptus.

All rates are expressed as mmol glucose equivalents/kg/min, refer Table 23 (a,b,c)

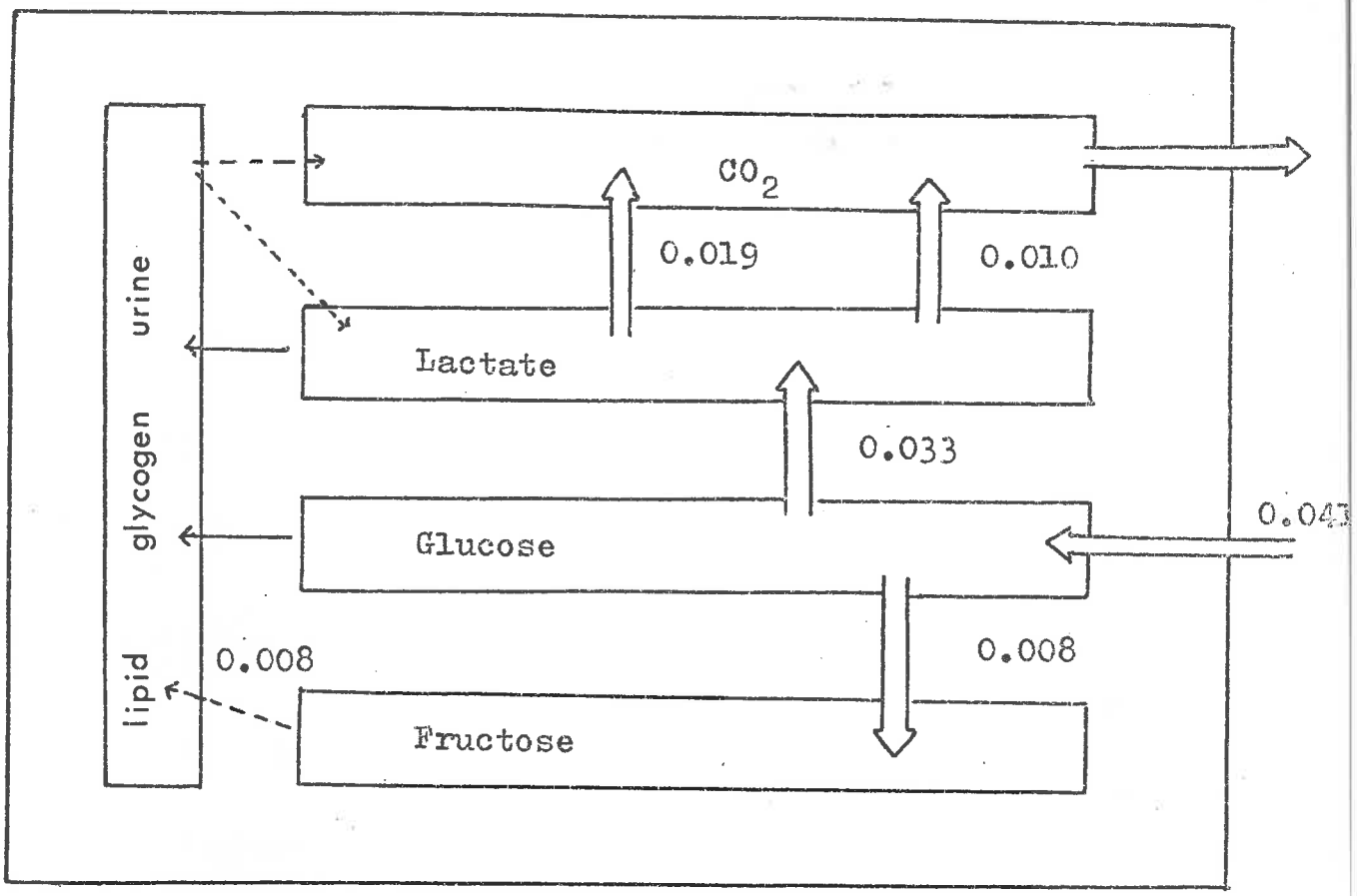


Table 23 (a)

Glucose, lactate and fructose metabolism in the ovine fetus: Glucose metabolism.

Utilization	Rate*	Reference
total irreversible loss.	0.041	this thesis
made up of:		
(1) oxidation (via lactate = 50%)	0.020	Tsoulos et al, 1971 James et al, 1972 Setchell et al, 1972
(2) placental lactate production (i.e 25% oxygen consumption)	0.010	Burd et al, 1975
(3) fructose production	0.008	this thesis Alexander et al, 1955
sub-total	0.038	
(4) glycogen } lipids }	balance	Alexander et al, 1970 Setchell et al, 1972 Hanson and Ballard, (1968)

* Rates are expressed as mmol glucose equivalents/kg / min.

Tsoulos et al (1971) and James et al (1972) have calculated that fetal glucose uptake accounts for about 50% of the fetal oxygen consumption . This represents an oxidation of 0.02 mmol glucose/kg/min (half of 0.041 mmol/kg/min). Lactate is an intermediate in this pathway.

The irreversible loss of lactate was 0.075 mmol/kg/min (0.037 mmol glucose equivalents/kg/min) (Table 5). Now if 50% of this represents the production of lactate as an intermediate of fetal glucose oxidation then 0.019 mmol/ glucose equivalents/kg/min has been derived from glucose. Burd et al (1975) argue that a further 25% of the irreversible loss of lactate results from oxidation by the placenta. This would account for a further 0.01 mmol glucose equivalents/ kg/min. The remaining lactate (0.008 mmol glucose equivalents/kg/min if calculated from the lactate data or, 0.004 mmol glucose equivalents/kg/min if calculated from the glucose irreversible loss, after allowing for fructose production) represents renal loss and lipid synthesis,.(Table23b). Apparently lactate cannot cross the placenta and be utilized by the mother.

In addition to its conversion to lactate, glucose is also the precursor of fructose in the ovine fetus. Under the steady state conditions, the irreversible loss of fructose (0.008 mmol/kg/min) will be equivalent to the fructose production rate. This means that 0.008 mmol glucose/kg/min will be consumed by the placenta for fructose synthesis.

Table 23 (b)

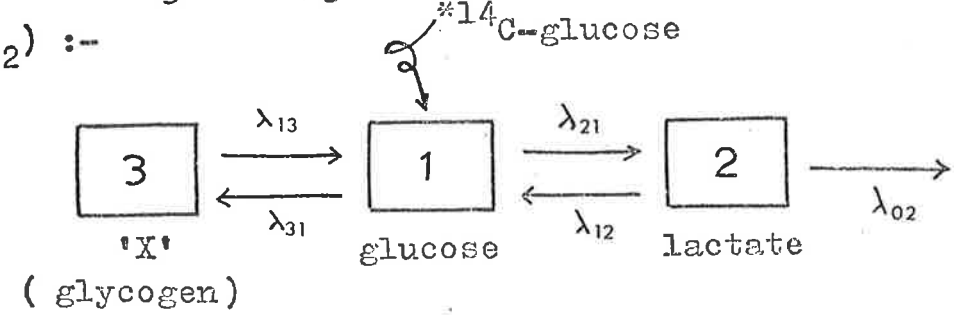
Glucose, lactate and fructose metabolism in the ovine fetus: Lactate metabolism.

Utilization	Rate*	Reference
total irreversible loss	0.037	this thesis
made up of:		
(1) oxidation of fetal lactate i.e 50% O_2 consumption	0.019	Tsoulos et al, 1971 James et al, 1972 Setchell et al, 1972
(2) oxidation of placental lactate (i.e. 25% O_2 consumption)	0.010	Burd et al, 1975
sub-total	0.029	
(3) lipids	balance	

* Rates are expressed as mmol glucose equivalents/kg /min.

This value agrees well with the range of 0.007-0.014 mmol/kg/min estimated as placental fructose production for a 4 kg fetus (Alexander et al, 1955). Although fructose is present in fetal blood at several times the glucose concentration, the rate of fructose metabolism is only one fifth of the glucose utilization rate. Fructose utilization includes oxidation, incorporation into lipids and glycogen and loss to the amniotic fluid (Table 23 c). My inability to detect lactate formation from fructose suggests that oxidation is minimal.

No model has been presented for the metabolism of glucose and lactate in the postnatal lamb . The most acceptable model would be based upon the Cori cycle (Cori, 1931) and involves the reversible interconversion of glucose and glycogen (λ_{31} , λ_{13}) and glucose and lactate (λ_{21} ,λ_{12}) :-



Verification of this model by computer program and the calculation of the rate constants was unsuccessful. Without slaughtering the lamb, quantitation of the incorporation of glucose into hepatic glycogen was not possible, and as discussed above it is necessary to use double labelled tracer to calculate the amount of recycling between lactate and glucose.

Table 23 (c)

Glucose, lactate and fructose metabolism in the ovine fetus: Fructose metabolism .

Utilization	Rate*	Reference
total irreversible loss	0.008	this thesis
made up of:		
oxidation lipid glycogen renal excretion	?	Alexander et al, 1970 Setchell et al, 1972 Scott et al, 1967 Alexander and Britton, (1963.
placental production	0.007-0.014	Alexander et al, 1955

* Rates are expressed as mmol glucose equivalents/kg/min

CHAPTER V

THE EFFECT OF ADRENALINE OR GLUCAGON INFUSIONS ON GLUCONEOGENIC ACTIVITY IN FETAL LAMBS *in utero*

V.1. Introduction

Gluconeogenesis may be stimulated by the administration of adrenaline or glucagon *in vivo* or *in vitro* (Exton and Park, 1966, 1969; Exton, Mallette, Wong, Friedmann, Miller and Park, 1970; Exton, 1972; Tews, Woodcock and Harper, 1970; Eisenstein, Strack and Steiner, 1974). The proposed modes of action of these hormones on gluconeogenesis are by (i) increasing the hepatic uptake of gluconeogenic substrates (e.g. amino acids); (ii) decreasing the peripheral uptake of gluconeogenic substrates; (iii) increasing tissue mobilization of gluconeogenic substrates or; (iv) by increasing the activity of the key enzymes of gluconeogenesis (Mallette, Exton and Park, 1969; Tews *et al*, 1970; Shrago *et al*, 1963; Weber, Lea, Hird Convery and Stamm, 1967; Taunton, Stifel, Greene and Herman, 1972; Greene, Taunton, Stifel and Herman, 1974; Wicks, 1969, 1971).

Bassett (1971) has demonstrated that an increase in blood glucose concentration follows glucagon or adrenaline administration to the adult sheep. It is unlikel that such hyperglycaemia resulted from an increase in gluconeogenic capacity for Filsell *et al* (1969)

failed to show increases in the activities of the obligatory enzymes of gluconeogenesis with various hormonal stimuli (see Ch I). It is more likely that these hormones act by either increasing the availability of gluconeogenic substrates (Reilly and Ford, 1974) or they stimulate glycogenolysis.

Comline and Silver (1972), Dawkins (1964) and Alexander, Mills and Scott (1968) have reported that fetal and newly-born lambs become hyperglycaemic after administration of adrenaline. In view of this response and to distinguish between effects on gluconeogenesis and glycogenolysis I have measured the rate of the fetal gluconeogenic pathway after infusions of glucagon or adrenaline.

V.2. Methods

Polyvinyl cannulae were surgically implanted in the femoral artery and vein of three fetal lambs (Ch IV.2.3). A minimum of five days elapsed between surgery and experimentation. During this period the viability of the fetus was assessed from blood glucose, fructose and lactate concentrations and blood pO_2 and pCO_2 (Ch IV.2.5.). All three fetuses used in these experiments were delivered live at term.

A constant infusion, via the femoral vein, of adrenaline, glucagon or 0.15 M NaCl (sterile) was

maintained with a constant injection apparatus (Handley intravenous injections, Southdown Medical Developments, Harpenden, Herts., England). The following procedure was adopted:-

(a) Sterile 0.15 M NaCl

Sterile, 0.15 M NaCl was infused into the femoral vein of fetus 192 (134 days gestation at a rate of 0.1 ml / min.

(b) Adrenaline

Adrenaline, prepared in sterile, 0.15 M NaCl, was infused into the femoral vein of fetuses 180 and 91 (aged 121 days and 119 days gestation respectively), at a rate of 1.75 µg / min, after they were each injected with a priming dose of 10 µg adrenaline. The infusion was continued for 35 min in fetus 180 and 22 min in fetus 91.

(c) Glucagon

After an initial priming dose of 50 µg glucagon, prepared in 0.15 M sterile NaCl, infusion was continued at a rate of 5 µg / min in fetus 192 (144 days gestation) and fetus 91 (131 days gestation). The infusion was stopped after 75 min in fetus 192 and 50 min in fetus 91.

In each experiment three blood samples were taken at intervals of 5 min before the commencement of the

infusions. The average concentration of glucose, lactate and fructose in these samples was taken as representative of the pre-infusion concentration. During the hormone infusions, 10 µCi pulses of [U-^{14}C] lactate (prepared in 0.15 M sterile NaCl) were administered to the fetuses via the femoral artery. The cannula was immediately flushed and kept filled with 0.15 M sterile NaCl. Blood samples were taken from the femoral artery 2 min after the injection of [U-^{14}C] lactate and immediately deproteinized in ethanol (Ch IV.2.5.). Between samples the cannula was kept filled with 0.15 M NaCl (sterile). Preliminary experiments with newborn lambs of ewes 44 and 64 established the feasibility of measuring ^{14}C-incorporation into glucose within 2 min of ^{14}C-lactate injection. Further, this short interval represents an early part of the glucose labelling as shown with postnatal lambs (Table 6; Fig 11 and 12).

The percentage conversion of ^{14}C-lactate into glucose was measured after separation of these compounds by high voltage electrophoresis (Ch IV.2.5.5.). The value obtained, after specific radioactivity determination was compared to the conversion of ^{14}C-lactate to ^{14}C-glucose in postnatal lambs (Ch. IV.3.2.). For quantitation, the incorporation of ^{14}C-lactate into glucose, 2min after administration of isotope in postnatal lambs was taken as 100 and all values are

expressed relative to this base. Blood glucose, lactate and fructose were measured as described in Ch.IV.2.5.

V.3. Results

A sterile, 0.15 M NaCl infusion was maintained for 20 min in fetus 192. During this period a total of 5 blood samples were taken and the glucose, fructose and lactate concentrations determined. Blood glucose, fructose and lactate concentrations did not show any significant changes during the infusion of 0.15 M NaCl, (Table 24).

The results of adrenaline infusion to fetus 180 are shown in Fig 20 a. The infusion was continued for 35 min and 6 blood samples were taken during this period. Each blood sample was taken 2 min after the injection of 10 μCi of [U-^{14}C] lactate. During the infusion the blood glucose concentration increased slowly from 0.714 mM at zero time to 1.372 mM after 44 min of infusion. A large increase in blood lactate concentration also occurred. At zero time blood lactate concentration was 1.603 mM and at the termination of the infusion it had reached 4.620 mM. Blood fructose concentrations, 3.560 mM at the start of the infusion and 3.733 mM at the end , did not show any significant change.

No incorporation of ^{14}C-lactate into glucose or fructose was detected in any blood sample taken.

Figure 20 : Adrenaline Infusion in the Fetal Lamb

(a) Adrenaline infusion in fetus 180. Infusion was continued at a rate of 1.75 µg/min after a priming dose of 10 µg adrenaline. Each data point represents a blood sample taken 2 min after the injection of 10 µCi [U-^{14}C] lactate. The change in glucose concentration is represented by: —△— lactate concentration : —○— fructose concentration: —□—

(b) Adrenaline infusion in fetus 91. Experimental detail is the same as for fetus 180.

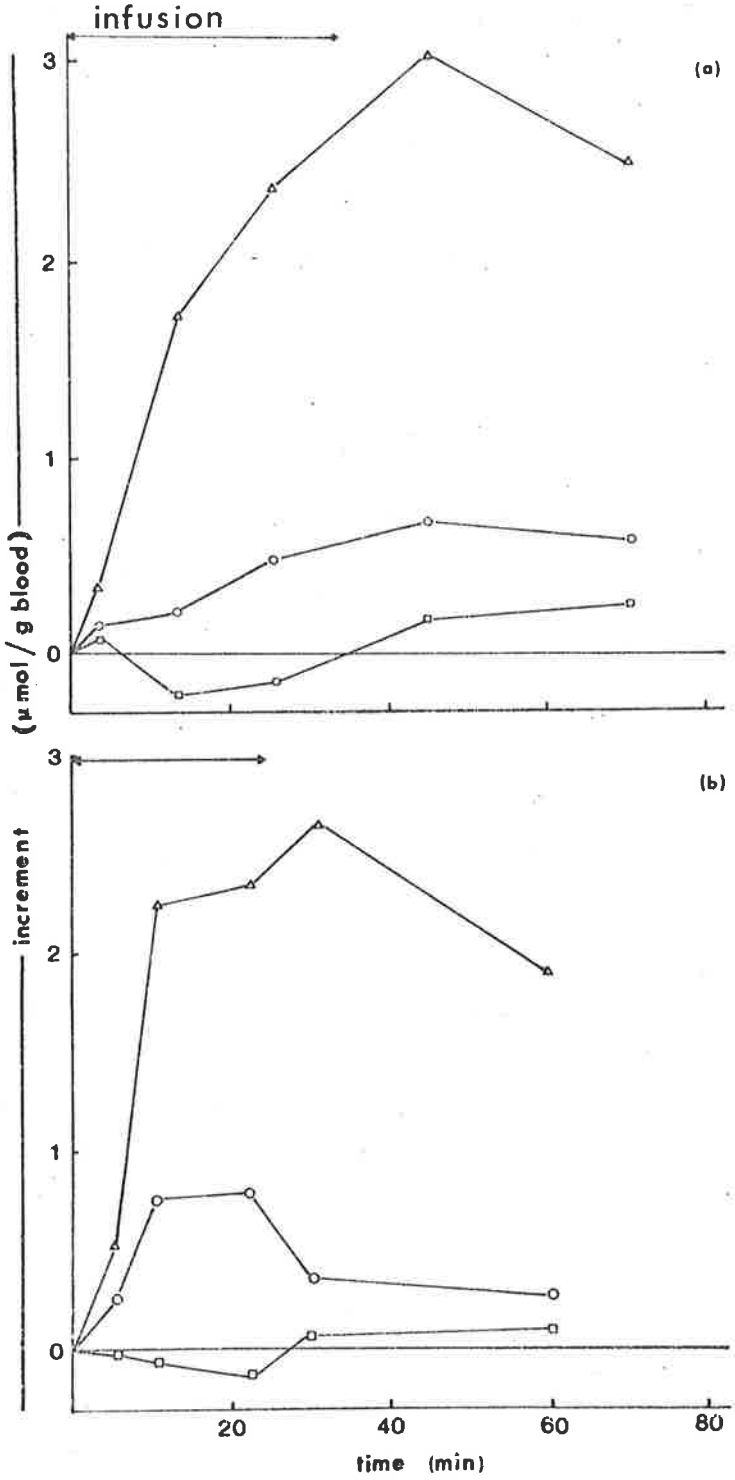

infusion
(a)
3
2
1
0
(µ mol / g blood)
(b)
increment
20
40
60
80
time (min)

Table 24

Effect of 0.15 M NaCl infusion in the Ovine Fetus

Sterile 0.15 M NaCl was infused at a rate Of 0.1 ml/min in fetus 192. Blood glucose, fructose and lactate determinations are described in Ch.IV.2.5. Zero time values are means of 3 blood samples.

Time of infusion (min)	glucose (mM)	fructose (mM)	lactate (mM)
0	0.477	3.531	2.077
5	0.527	3.862	2.233
10	0.500	3.288	2.155
15	0.498	3.877	2.188
20	0.499	3.591	2.222

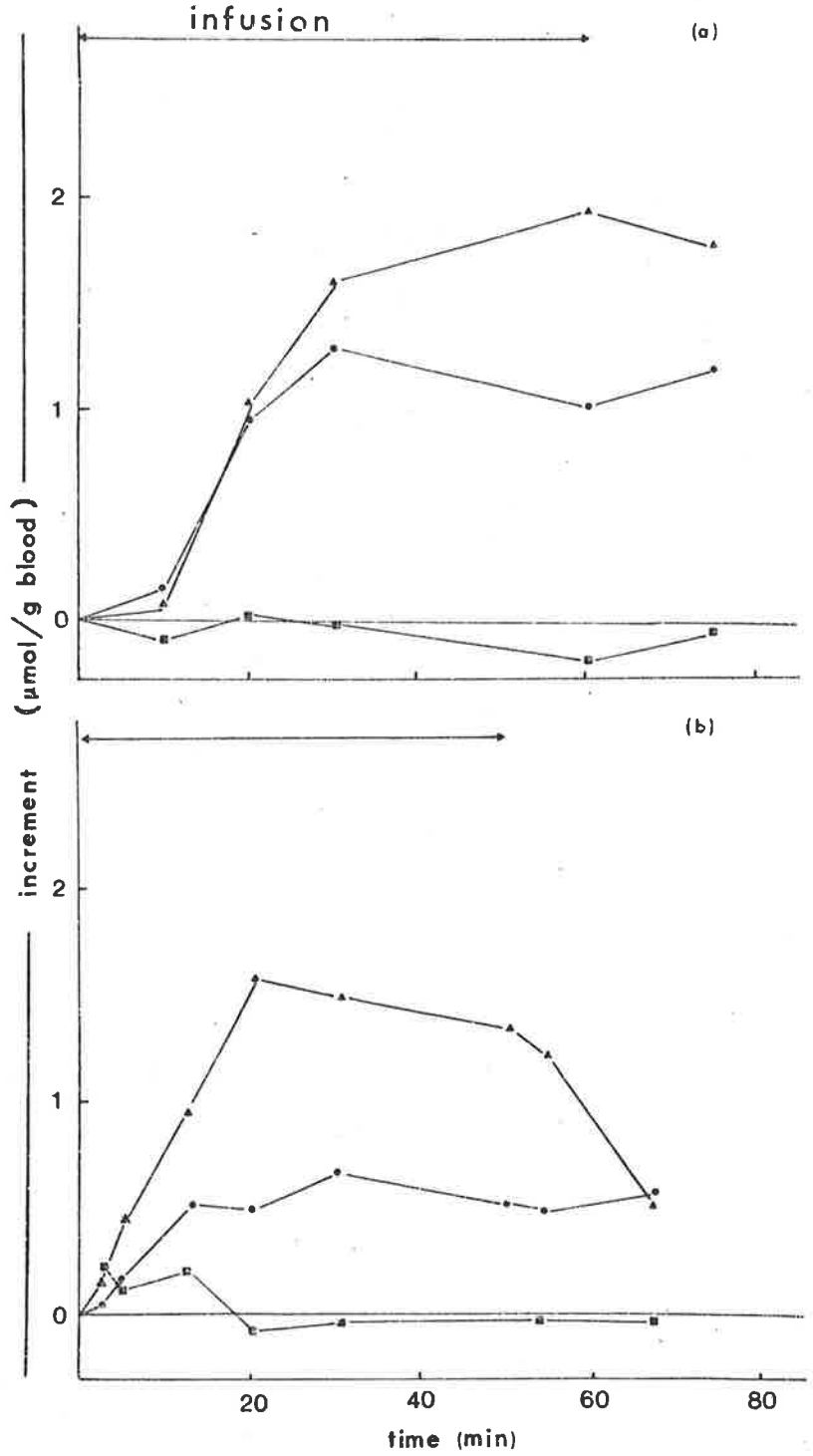
Blood glucose and lactate concentrations also increased in fetus 91 during the infusion of adrenaline (Fig 20 b). Six 10 μCi pulses of [U-^{14}C] lactate were given to fetus 91 during the 20 min infusion and blood samples were taken 2 min after the injection of isotope. Glucose concentration at zero time was 0.430 mM and increased to a maximum of 1.220 mM, 22 min after the commencement of adrenaline infusion. Blood lactate concentration increased from 1.612 mM to 2.610 mM at the end of the infusion. Blood fructose concentration did not change significantly during the infusion. As with fetus 180, ^{14}C-glucose or ^{14}C-fructose synthesis were not detected in any blood sample taken after the administration of ^{14}C-lactate.

During the 75 min glucagon infusion in fetus 192, six blood samples were taken. Each blood sample was taken 2 min after the injection of 10 μCi [U-^{14}C] lactate. The glucagon infusion was accompanied by an increase in blood glucose and lactate concentration (Fig 21a). Glucose concentration increased steadily during the infusion period from 0.479 mM to 1.730 mM, 30 min after the commencement of the glucagon infusion. Blood lactate concentration increased from 1.58 mM to 3.530 mM, 60 min after the start of the infusion. Blood fructose concentration did not show any significant change during the glucagon infusion. No incorporation of ^{14}C-label into either glucose or fructose was detected in any blood sample taken after the administration of

Figure 21 : Glucagon Infusion in the Fetal Lamb.

(a) Glucagon infusion in fetus 192. Infusion was continued at a rate of 5 μg/min after a priming dose of 50 μg glucagon. Each data point represents a blood sample taken 2 min after the injection of 10 μCi [U-^{14}C]lactate. The change in blood glucose concentration is represented by: —●— ——▲—— for lactate concentration; ——■—— for fructose concentration.

(b) Glucagon infusion in fetus 91. Experimental detail is the same as for fetus 192.


infusion
(a)
2
1
0
(µmol/g blood)
(b)
increment
2
1
0
20
40
60
80
time (min)

[U-^{14}C] lactate.

The glucagon infusion in fetus 91 was continued for 50 min (Fig 21 b) and 9 blood samples were taken during this period. Each blood sample was taken 2 min after the injection of 10 μCi [U-^{14}C] lactate. Blood glucose concentration increased during the infusion period from 0.520 mM to 1.150 mM and blood lactate concentration increased from 1.714 mM to 3.260 mM, 30 min after the start of the infusion. There was no significant change in blood fructose concentration during the infusion of glucagon. ^{14}C-glucose or ^{14}C-fructose were not detected in any blood sample taken after the administration of [U-^{14}C] lactate.

V.4. Discussion

The increase in blood glucose and lactate concentratic in the fetal lamb in response to adrenaline has been described by several research groups. Thus, Comline and Silver (1972)noted an increase in blood glucose of up to 0.66 mM when adrenaline was infused at 2 μg per kg per min. Blood lactate concentration also increased. Similarly, Dawkins (1964) reported a hyperglycaemic effect with adrenaline (dose 25 μg per kg) administered to neonatal lambs delivered at term by Caesarian section. In Dawkins' experiment the hyperglycaemic response was age dependant. Fetal lambs were less responsive to adrenaline than neonatal lambs. By 24 hr after birth the response to adrenaline was of the same order of magnitude as found in adult sheep; an increase of up to

3 mM. Alexander, Mills and Scott (1968) also observed a 2 mM increase in blood glucose concentration in young lambs given 25 µg adrenaline per kg. A similar response occurred when nor-adrenaline was injected.

I have not found any other reports of hyperglycaemia following glucagon infusion into fetal lambs. However, Bassett (1971) reported an increase in glucose concentration of 8 mM in adult sheep given 5 µg glucagon per min.

Since there was no induction of gluconeogenesis in the fetal lamb during the period of glucagon or adrenaline infusion, the accompanying hyperglycaemia can only result from increased glycogen mobilization or decreased peripheral utilization of glucose. A distinction between these alternatives cannot be made on the basis of the present experiments, although it is known that the fetal lamb has the capacity to mobilize glycogen reserves during stress (Dawes et al, 1959; Shelley, 1961; Britton et al, 1967).

Hyperglycaemia has been shown to accompany the administration of glucagon to the fetal rat (Hunter, 1969; Girard and Bal , 1970; Girard et al, 1973), but unlike the fetal lamb the hyperglycaemic response was restricted to term fetuses (i.e. 21.5 days gestation). In the fetal rat (20.5 days gestation) a decrease in hepatic glycogen concentration occurred after the administration of glucagon

or cyclic AMP (Hunter, 1969; Girard et al, 1973) . However, newly-born rats do not appear to respond to glucagon or dibutyryl cyclic AMP as rapidly as fetal rats (Snell and Walker, 1973a). Snell and Walker (1973b) have reported a delay of up to 3 hr before the onset of glycogenolysis in Caesarian born rats treated with glucagon or dibutyryl cyclic AMP. They proposed that the increase in blood glucose, which accompanies the administration of glucagon, resulted from either an increase in glucose formation or a decrease in glucose utilization, which may have resulted from the antagonistic effect of glucagon on insulin secretion. A similar process may occur in sheep fetuses.

CHAPTER VI

THE EFFECT OF PARTURITION ON GLUCONEOGENIC ACTIVITY IN THE FETAL LAMB

VI.1. Introduction

Gluconeogenesis is active in the postnatal lamb but the pathway is not functional in the fetus. In Chapter III it was shown that the four enzymes obligatory for gluconeogenesis were all active in the fetal lamb. Furthermore, neither adrenaline nor glucagon infusions were able to initiate gluconeogenesis in fetal sheep (Chapter V), although blood glucose concentrations were increased. It is the aim of this chapter to describe the changes in gluconeogenic activity in the developing lamb which accompany parturition.

VI.2. Methods

(a) Natural birth

The approach of parturition was shown by the increase in fetal plasma cortisol which occurred during the last days of gestation (refer Bassett and Hinks , 1969). Behavioural changes in the ewe were also noted as parturition became imminent and the ewe was closely observed during this period.

Two lambs, 114 and 91, with cannulae placed in the femoral artery and vein were monitored for the conversion of ^{14}C-lactate to glucose during parturition.

Blood samples were taken from the femoral artery 2 min after the injection of 10 μCi pulses of [U-^{14}C] lactate into the femoral vein. The [U-^{14}C] lactate was made up in 0.15 M sterile NaCl and administered in a volume of 0.4 ml. After injection of the isotope the cannula was flushed with 0.15 M sterile NaCl. Between samples the cannula was filled with 0.15 M NaCl containing 100 I.U. heparin per ml. All blood samples used for glucose, lactate and fructose determinations and for electrophoresis were immediately deproteinized in ethanol and prepared as described in Ch IV.2.5.

After delivery of the lamb a three-way stopcock was attached to the cannula, the umbilical cord ligated and the experiment continued . The mother was allowed free access to the newborn lamb, but the lambs made no attempt to suckle during the experimental period.

(b) Caesarian delivery

Gluconeogenic activity was studied in four lambs delivered by mid-line laparotomy after implantation of cannulae in the femoral artery and vein (Ch. IV.2.3.). [U-^{14}C] lactate (10 μCi) was injected via the femoral vein at various time intervals before and after delivery and blood samples were taken 2 min after the administration of isotope (see above). The umbilical cord was ligated after the head was delivered and breathing established. The lambs were vigorously dried and kept in a draught-free

basket in a warm place.

(c) Analytical techniques

Blood pO_2, pCO_2 and pH were measured in blood samples taken from lamb 91 and from the four Caesarian delivered lambs (Ch.IV.2.5.3.). Blood glucose, lactate and fructose concentrations were measured using the procedures described in Ch IV.2.5.6,7,8 . The percentage incorporation of ^{14}C-label into glucose from lactate was estimated after separation of glucose and lactate by high voltage electrophoresis (Ch IV.2.5.5.). The incorporation of ^{14}C-lactate into glucose in postnatal lambs, 2 min after the injection of isotope, was set at 100% and all incorporation rates have been related to this.

VI.3. Results

The results showing the effects of natural birth on gluconeogenic activity are presented in Table 25 . No incorporation of ^{14}C-label from lactate into glucose was detected in blood samples taken prior to birth. Within 2 min of the birth of lamb 91 glucose synthesis was detected, whereas glucose synthesis was not detected until 8 min after the birth of lamb 114. The percentage of ^{14}C-incorporated into glucose increased steadily after birth. In both fetuses blood glucose and lactate concentrations increased during the sampling period before and after birth. Blood fructose concentration did not alter appreciably during the experimental period. The blood parameter which showed

Table 25

Gluconeogenic Activity in the Ovine Fetus: Effect of Natural Birth

Gluconeogenic activity was determined during parturition by measuring the percentage conversion of ^{14}C-lactate to glucose. The percentage conversion of lactate to glucose is expressed relative to the postnatal lamb = 100% (Ch. IV.3.2.). Blood samples were taken 2 min after the injection of ^{14}C-lactate (10 µCi pulses). Glucose, fructose, lactate , pO_2, pCO_2 and pH were determined as described in Ch.IV.2.5.

Fetus	time(min) from delivery injected	sampled	% conversion	glucose (mM)	fructose (mM)	lactate (mM)	pO_2	pCO_2	pH	comments
91	-208	-206	0	0.383	2.600	3.110	13	49	7.42	wt = 3.75 kg
	- 72	- 70	0	0.988	2.380	9.800	20	48	7.34	
	0	2	6.5	1.533	2.340	11.830	44	51	7.25	
	12	14	20.2	1.800	2.320	13.230	44	35	7.28	
	46	48	46.6	1.338	2.250	12.150	44	35	7.36	
114	- 30	- 28	0	0.638	2.180	5.333				
	- 22	- 20	0	0.955	2.050	5.133				
	- 19	- 17	0	1.155	2.150	6.044				obvious colour
	- 10	- 8	0	1.183	1.927	5.944				change in blood,
	6	8	10.9	1.066	1.755	8.588				indicating improved
	11	13	11.6	1.277	1.683	7.933				oxygenation
	19	21	14.9	2.622	2.161	7.800				
	76	78	35.8	2.855	1.700	8.711				

the most dramatic change with birth was blood oxygen. Within 2 min of birth blood oxygen had increased to 44 mm Hg from 13 mm Hg 3 hr before birth of fetus 91. Blood gas data was not available for lamb 114, but an increase in oxygenation was evident from the change in blood colour from this neonate. Blood carbon dioxide in lamb 91 did not alter appreciably and blood pH did not change significantly during the experimental period.

The effects of Caesarian delivery on gluconeogenic activity are presented in Table 26. Glucose synthesis was detected between 4 and 11 min after the umbilical cord was tied, and effective breathing started in lambs 109 and 248. Rapid oxygenation of blood occurred in both these neonates, which were estimated to be close to term.

The onset of gluconeogenesis was delayed by about 30 min in lambs 20 and 151. These lambs were 144 days and 139 days gestation respectively and experienced considerable difficulty breathing . This was reflected in the lower blood pO_2 levels during the first hour after delivery. In all four Caesarian delivered lambs the blood glucose and lactate concentrations were greater than in the natural born lambs. This difference was probably the result of experimental and surgical stress .

Table 26

Gluconeogenic Activity in the Ovine Fetus: Effect of Premature Delivery.

Lambs were delivered by Caesarian section following cannulation of the femoral artery and vein. The umbilical vessels were ligated after effective breathing had commenced. Gluconeogenic activity was measured as the percentage conversion of ^{14}C-lactate to glucose relative to the postnatal lamb (100%), refer Ch. IV.3.2. Blood samples were taken 2 min after the injection of 10 µCi pulses of ^{14}C-lactate. Glucose, fructose, lactate pO_2, pCO_2 and pH measurement is described in Ch.IV.2.5.

Fetus	time (min) from delivery injected	sampled	% conversion	glucose (mM)	fructose (mM)	lactate (mM)	pO_2	pCO_2	pH	comments
109	- 2	0	0	1.338	3.555	5.366	16	40	7.39	wt = 4.23 kg
	2	4	15.7	1.361	2.778	5.166	54	41	7.36	
	17	19	28.1	1.277	2.961	3.944	44	40	7.39	age = 148 days
	38	40	73.4	1.188	2.883	3.922	51	41	7.39	
	65	67	71.2	1.150	2.688	4.455	54	35	7.44	
151	- 13	- 11	0	2.855	3.550	4.600	19	37	7.38	wt = 3.34 kg
	- 2	0	0	3.216	3.956	5.733	12	49	7.34	
	9	11	0	3.938	4.733	6.955	24	57	7.19	age = 139 days
	31	33	12.5	4.500	3.850	5.444	29	53	7.17	
	54	56	20.5	4.944	4.122	5.222	37	55	7.15	
	123	125	28.2	5.833	2.788	6.911	49	50	7.25	
	207	209	29.0	5.111	2.516	3.833	45	49	7.25	
20	- 10	- 8	0	0.866	3.955	2.888	19	40	7.43	wt = 3.51 kg
	- 8	- 6	0	0.700	2.894	3.455	14	54	7.24	
	13	15	0	1.300	3.355	3.622	24	50	7.23	age = 144 days
	32	34	20.8	1.515	3.122	2.544	39	54	7.25	
	64	66	20.9	1.755	3.062	2.022	31	45	7.31	
	93	95	24.3	1.166	2.688	2.110	50	42	7.35	
	118	120	22.1	0.766	2.477	2.377	54	42	7.38	
	133	135	16.8	1.066	3.072	3.700				
	143	145	12.9	1.027	3.055	3.177				

Table 26 (continued)

Fetus	time (min) from delivery injected	sampled	% conversion	glucose (mM)	fructose (mM)	lactate (mM)	pO_2	pCO_2	pH	comments
248	- 8	- 6	0	2.083	2.611	7.322	21	47	7.41	wt = 4.35 kg
	- 2	0	0	1.905	2.822	9.277	19	54	7.31	
	4	6	2.37	2.083	2.722	9.377	51	45	7.34	age = term
	68	70	15.09	3.955	2.250	7.622				
	140	142	18.91	4.683	2.927	6.955				

VI.4. Discussion

The transition from fetal to neonatal life involves many physiological and biochemical changes. In the lamb this developmental phase is accompanied by the rapid onset of gluconeogenesis. The appearance of gluconeogenesis after birth also occurs in the rat (Ballard, 1971b). The rapid onset of gluconeogenesis after birth in this species is also illustrated by the experiments of Snell and Walker (1973a) in which recycling of ^{14}C-glucose was detected in Caesarian-delivered animals within the first hour of birth.

As mentioned in Chapter III, the low cytosol phosphoenolpyruvate carboxykinase activity in the fetal rat limits gluconeogenesis. After birth the increase in gluconeogenic activity parallels the increase in activity of this enzyme (Ballard and Hanson, 1967). However, Philippidis and Ballard (1970) failed to show any prenatal initiation of gluconeogenesis *in vivo* when fetal phosphoenolpyruvate carboxykinase activity was induced by glucagon . In contrast, liver slices from these glucagon-treated fetuses synthesized glycogen from pyruvate or lactate.

The fetal lamb *in utero* ressembles the glucagon-treated rat in that all four enzymes of gluconeogenesis are active (Ch. III), but there is no active gluconeogenesis. Incubations of fetal lamb liver slices

with ^{14}C-pyruvate or ^{14}C-propionate have shown that active gluconeogenesis occurs (Ballard and Oliver, 1965; Garber and Ballard, as cited by Ballard et al, 1969). It seems likely that in the fetal lamb, as in the fetal rat, the intra-uterine conditions are inhibitory to gluconeogenesis.

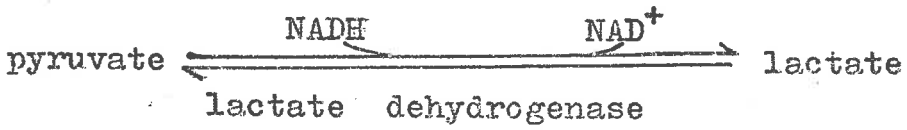
Philippidis and Ballard (1970) postulated that the hypoxia associated with maternal sedation (i.e. ether) inhibited gluconeogenesis in the fetal rat in utero. Subsequent measurement of the redox state of the fetal liver showed that the ether anaesthesia was accompanied by a three fold decrease in the [free NAD^+]:[free NADH] ratio . The liver of the immediate newborn was also found to have a highly reduced redox state relative to the suckling and adult rat. The ratio of [free NAD^+]: [free NADH] in the fetal liver was 40, compared to 700 in the liver of the one-hour newborn rat (Philippidis and Ballard, 1970). Williamson, Lund and Krebs (1967) have reported a value of 725 for the [free NAD^+]:[free NADH] ratio of the adult rat liver.

That the redox state of the liver played an important role in the regulation of gluconeogenesis is further indicated from the study of Ballard (1971a) in which newborn suckling rats were exposed to an atmosphere of nitrogen. The hepatic lactate: pyruvate ratio increased in these animals (i.e. the redox state was

reduced), and gluconeogenesis was suppressed. Furthermore, the degree of phosphorylation of adenine nucleotides was reduced in the liver of newborn rats exposed to nitrogen (Ballard, 1970). A shift towards a higher concentration of ATP occurred at birth; the ATP : AMP ratio increased from 4.48 in fetuses to 29.6 six hours after birth (Philippidis and Ballard, 1970; Ballard, 1970).

Neblett, Green, Exton and Park (as cited by Exton, 1972) have also reported a decrease in the [free NAD^+] : [free NADH] ratio in the anoxic rat liver, with subsequent inhibition of gluconeogenesis. A decrease in ATP concentration and an increase in AMP + P_i was also found.

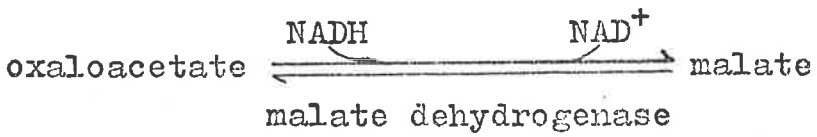
There are three reactions in the gluconeogenic sequence in which a low redox state (i.e. a low[free NAD^+]: [free NADH]) would be unfavourable for glucose synthesis. Firstly there is the lactate dehydrogenase reaction in the cytosol :



in which a low [free NAD^+] : [free NADH] ratio would favour lactate production rather than utilization.

A second reaction where the ratio of [free NAD^+]: [free NADH] influences the equilibrium is the interconversion

of oxaloacetate and malate by malate dehydrogenase:



Oxaloacetate may also be formed from the transamination of aspartate to glutamate, catalysed by aspartate aminotransferase. Rognstad and Katz (1970) have shown that as the ratio of [free NAD^+] : [free NADH] increases, the synthesis of oxaloacetate decreases. Since oxaloacetate is an obligatory intermediate in glucose formation from most precursors, a reduction in oxaloacetate production would probably depress gluconeogenesis.

Reactions which remove oxaloacetate for non-gluconeogenic pathways (such as lipogenesis) will also tend to reduce the gluconeogenic flux. This is particularly relevant in the fetal ruminant in which the hepatic enzymes, ATP-citrate lyase and NADP malate dehydrogenase, necessary for the synthesis of lipid from glucose, are active (Hanson and Ballard, 1967, 1968).

The availability of oxaloacetate for gluconeogenesis is also governed by its intracellular location. The production of oxaloacetate from pyruvate, catalysed by pyruvate carboxylase, occurs within the mitochondria, whilst the subsequent conversion of oxaloacetate to phosphoenolpyruvate takes place in both the cytosol and the mitochondria. The mitochondrial membrane

has restricted permeability for oxaloacetate (Lardy, Paetkau and Walter, 1965) and transport of the oxaloacetate carbon probably occurs via malate or aspartate (Haynes, 1965; Shrago and Lardy, 1966). This becomes especially important in species such as the rat where phosphoenolpyruvate carboxykinase activity is located exclusively in the cytosol (Nordlie and Lardy, 1963).

In the liver of the adult sheep regulation of the rate of oxaloacetate transfer across the mitochondrial membrane is probably less important, since phosphoenolpyruvate carboxykinase activity is found in both the cytosol and mitochondria (Taylor et al, 1971). I have shown that both phosphoenolpyruvate carboxykinase enzymes are active in the liver of the fetal sheep.

In contrast to the reactions in which a low ratio of [free NAD^+] : [free NADH] restricts the flow of carbon to glucose, the glyceraldehyde 3-phosphate dehydrogenase reaction in the liver cytosol:

$$\text{1,3-phosphoglycerate} \xrightarrow{NADH \quad NAD^+} \text{3-phosphoglyceraldehyde} + P_i$$

is favoured by a reduced redox state. However, if oxaloacetate generation is rate-limiting for gluconeogenesis and the redox state of the fetal liver is low, then the hepatic environment would be less favourable for the overall conversion of lactate, pyruvate or amino acids to glucose than the more oxidised environment of the newborn rat.

Rapid changes in the oxygenation state of the fetal lamb at birth are implied from my experiments and from other studies. In the fetal lamb, blood lactate concentrations increased before birth, while oxygen and carbon dioxide levels remained relatively constant. A decrease in pH followed the increase in blood lactate. During the period before breathing was effective blood oxygen levels declined and this was accompanied by a small decrease in blood carbon dioxide and pH (see also Comline and Silver, 1972). I found a rapid rise in blood oxygen following the initiation of effective breathing. This increased oxygenation may be responsible for the rapid onset of gluconeogenic activity observed in the neonatal lamb, through a change in the redox state of the hepatocyte.

It remains for further investigations to define the redox state of the fetal lamb. The high blood lactate concentration of the fetal lamb is suggestive of a highly reduced environment in which pyruvate conversion to lactate is favoured. Measurement of lactate : pyruvate ratios of fetal and newborn lamb blood were inconclusive (see also Mann, 1970). Generally the ratio was lower in the adult and newborn, but the difference was not as great as that noted between fetal and neonatal rat liver.

To describe precisely the redox state of the fetal lamb a technique for the rapid withdrawal of fetal liver

samples with minimal disturbance to the mother and fetus is necessary. With sheep, the technical problems with this approach are considerable. The usual freeze clamping technique, successfully used for small laboratory animals, would not be reliable. Further studies on the regulation of gluconeogenesis in the fetal lamb could be initiated with isolated fetal hepatocytes (Seglen, 1972). Seglen (1974) has shown that gluconeogenesis is inhibited in isolated hepatocytes incubated under anaerobic conditions, but these experiments would suffer the same limitations as those of Ballard and Oliver (1965) and Garber and Ballard (as cited by Ballard *et al*, 1969) in which fetal lamb liver slices were incubated under an atmosphere of 95% O_2 and 5% CO_2. Under this gas phase gluconeogenesis was rapid, probably because of the unphysiological and rapid penetration of O_2 to the inner cells. My experiments indicate that the gluconeogenesis demonstrated by these authors was an artifact of experimentation *in vitro*. Although it might be possible to super-oxygenate the fetus *in utero* (Kolobow *et al*, 1968), such preparations would be highly stressed and any results questionable.

CHAPTER VII

GENERAL DISCUSSION

During recent years there has been much research devoted to the study of the development of the mammalian fetus *in utero* and the adaptation of the newborn to extra-uterine life. One of the major and most significant adaptations in the neonatal ruminant is the onset of gluconeogenesis. The fetal lamb *in utero* has the mechanisms for glucose synthesis, but as this study has shown, it is not until after birth that glucose is actually synthesized.

The virtual absence of gluconeogenic activity *in utero* makes the fetus dependant upon its mother for its supply of glucose. To the ruminant fetus this means a total reliance upon maternal gluconeogenic activity, as well as the efficient transplacental passage of glucose. Although glycogen is synthesized during fetal life it appears that the fetal lamb can only utilize these glycogen reserves during stress. Normally the synthetic processes are directed towards glycogen production, so this alternative source of glucose can make only a negligible contribution to the fetal glucose pool. The glycogen is required to meet the glucose and energy demand after birth.

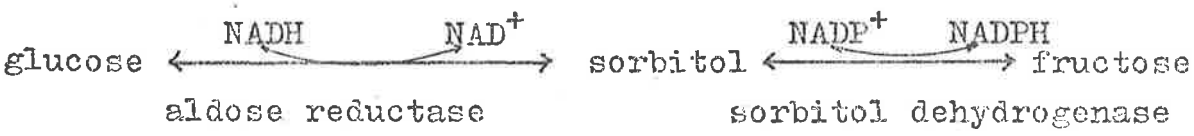
Whilst gluconeogenesis is essential to the suckling lamb with its intermittant food supply and to the

adult ruminant with virtually no dietary carbohydrate available from the digestive tract, the absence of gluconeogenic activity in the fetus is not altogether surprising. Gluconeogenesis is only required if other sources of glucose are not available and the fetus has a constant supply of glucose from the mother. Furthermore, gluconeogenesis is an energy consuming pathway that would represent an unnecessary diversion of energy from metabolic processes directed to the growth and development of the fetus. In the absence of any gluconeogenic activity energy substrates may be conserved. For example , lactate, produced as an end product of glycolysis, can be further oxidized and so contribute to the fetal energy supply (Burd et al, 1975). Similarly the large quantity of amino acids available to the fetus, estimated to be 40 g per day (Curet et al, 1970), can be directed largely towards protein synthesis, without competition from an active gluconeogenic pathway. This is of particular importance in the latter stages of gestation when growth accelerates (Cloette, 1939; Wallace, 1948). Finally, lipogenesis and other reactions compete with gluconeogenesis for a common intermediate, oxaloacetate. The absence of gluconeogenesis therefore favours lipogenesis and pyruvate oxidation.

Fructose is still a mystery product of glucose metabolism. It has been suggested that fructose may

act as an alternative source of glucose and energy during fetal stress (Huggett et al, 1951; Alexander et al, 1955), but such extreme conditions would not be encountered in the normal intra-uterine environment, for even the fetus of the starved ewe (a not uncommon event in the average flock) failed to show significant fructose uptake (Tsoulos et al, 1971

The proposed pathway of fructose synthesis in the fetal lamb is similar to the mechanism proposed by Hers (1957) for the seminal vesicle:-



The highly reduced environment of the fetal lamb would favour sorbitol synthesis and the NADPH produced on dehydrogenation of sorbitol could supply the necessary reducing equivalents for lipogenesis and other synthetic reactions. Although the fructose synthesizing enzymes are located in the placenta (aldose reductase) and the fetal liver (sorbitol dehydrogenase), there is strong evidence to suggest that the placenta is the sole site of fructose synthesis (Huggett et al, 1951; Alexander et al, 1955). There also appears to be very little fetal uptake of sorbitol and so hepatic production of NADPH from this source would be restricted.

In conclusion, it appears that the lack of gluconeogenesis in the fetal lamb is consistent with the general impression of fetal metabolism; one that is well adapted for the maximum and efficient use of the available energy. Energetically extravagant reactions are not favoured.

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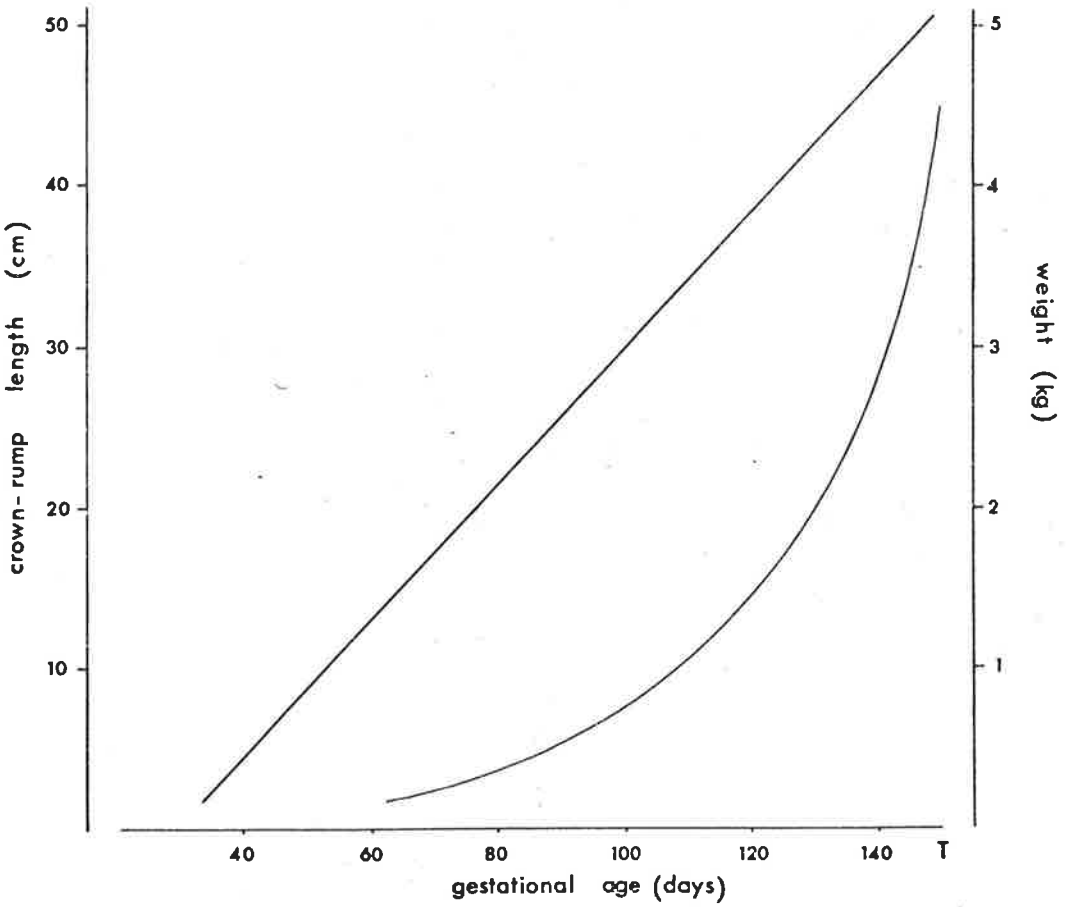
APPENDIX 1

Appendix 1 : The Relationship of Fetal Crown-rump Length and Weight to Gestational Age in the Ovine Fetus

Fetal crown-rump length (cm) and gestational age (days) show a linear relationship. Fetal weight (kg) and gestational age (days) show a curvi-linear relationship.


50
40
30
20
10
crown-rump length (cm)
5
4
3
2
1
weight (kg)
40
60
80
100
120
140
T
gestational age (days)