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Metabolism of Glucose, Fructose and Lactate *in vivo* in Chronically Cannulated Foetuses and in Suckling Lambs

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1. Chronically cannulated sheep foetuses and suckling lambs were injected with ^{14}C-labelled glucose, fructose or lactate, and sequential blood samples taken under conditions of minimal stress and without anaesthesia. 2. Gluconeogenesis from lactate was not detectable in foetal sheep, but the pathway was active in suckling lambs. 3. Fructose utilization rates were low in foetal sheep, with no measurable conversion into glucose or lactate. 4. The high rates of irreversible loss of both glucose and lactate in the foetus were decreased in suckling lambs. Radioactivity from labelled glucose entered both the lactate and fructose pools in foetal sheep, and entered the lactate pool in suckling lambs. 5. A model is proposed in which carbon flow between glucose, fructose and lactate has been quantified in foetal sheep.

Glucose is oxidized in the foetal lamb, is utilized for the synthesis of glycogen and fructose, and is probably the source of the large amount of lactate present in foetal blood (Tsoulos *et al.*, 1971; James *et al.*, 1972; Burd *et al.*, 1975). However, the interrelationships between glucose, fructose and lactate are poorly quantified, even though the nutritional importance of these compounds changes markedly during development. Of particular interest is the activity of the gluconeogenic pathway in foetal sheep. Measurements with sheep liver slices show a capacity to convert pyruvate or propionate into glucose and glycogen (Ballard & Oliver, 1965), but evidence from other species suggests that other factors may restrict flux *in vivo*. Thus gluconeogenesis in foetal rat liver is limited by the near-absence of phosphoenolpyruvate carboxykinase (GTP) (EC 4.1.1.32) in that tissue (Ballard & Hanson, 1967), and even when the enzyme is induced with glucagon, no gluconeogenesis can be measured *in vivo* (Philippidis & Ballard, 1969, 1970).

Chronic cannulation of blood vessels in foetal sheep permits the injection of radioactive compounds to unstressed unanaesthetized animals for the assessment of gluconeogenesis and other pathways. By using this technique we have shown that lactate is not converted into glucose and that fructose is not metabolized to either lactate or glucose in foetal sheep. However, both lactate and fructose are labelled after injection of radioactive glucose into foetuses. Calculations of carbon flow rates have been used to describe a pattern of carbohydrate metabolism in foetal sheep, although rates are not readily obtained in suckling lambs.

Materials and Methods

Chemicals

Hexokinase, glucose 6-phosphate dehydrogenase and lactate dehydrogenase were obtained from Boehringer, Mannheim, Germany; NAD^+, 2-(*p*-iodophenyl) - 3 - *p* - nitrophenyl - 5 - phenyltetrazolium chloride, ATP, $NADP^+$ and diaphorase (type 2L) were from Sigma Chemical Co., St. Louis, MO, U.S.A.; D-[U-^{14}C]glucose, D-[U-^{14}C]fructose and L-[U-^{14}C]lactate were from The Radiochemical Centre, Amersham, Bucks., U.K.

Polyvinyl tubing for cannulae was obtained from Boots Aust., Clovelly Park, South Australia, Australia; steridrapes were from Minnesota Mining and Manufacturing, St. Paul, MN, U.S.A.; suture thread was from Ethnor, Sydney, N.S.W., Australia; chlorhexidine solution (0.05% chlorhexidine, 0.5% cetrimide, 0.4% $NaNO_2$ in ethanol) and Hibitane cream were purchased from I.C.I., Sydney, Australia; cephaloridine (α-form) was from Glaxo Laboratories, Norwood, South Australia, Australia and gentamycin was from Schering, Kenilworth, NJ, U.S.A. Heparin was obtained from Allen and Hanburys, Welland, South Australia, Australia; 2% xylocaine solution was from Astra Chemicals, Sydney, N. S. W., Australia and 2% xylazine solution was from Bayer, Hindmarsh, South Australia, Australia.

Animals

Merino crossbred ewes of known mating dates were obtained from either the C.S.I.R.O. Experimental Station, Glenthorne, South Australia, or the University of Adelaide Experimental Farm at Mintaro, South Australia, Australia. During the experimental period, sheep were housed under conditions of 14h light and 10h dark, with a constant room temperature of 25°C. Animals were fed on 800g of lucerne daily but were starved for 24h before surgery.

Lambs were kept with their mothers except during the period of radioisotope injection and sampling. They had free access to food.

Surgery

Ewes were sedated with an intramuscular injection of 2% xylazine, and epidural anaesthesia was induced with xylocaine (Hopcroft, 1967). Abdominal wool was removed and the incision area scrubbed, rinsed and soaked with chlorhexidine solution. Strict aseptic techniques were maintained during all subsequent steps. Cannulae were sterilized with ethylene oxide and were filled with sterile 0.15M-NaCl containing 100 units of heparin/ml.

The abdomen was opened by a midline incision and the tip of the pregnant uterine horn exposed. A 4cm incision was made in the least vascular area of the uterus and a foetal hind limb exposed. The femoral artery and vein were separated by blunt dissection and cannulated by using polyvinyl tubing size 2 (outside diam. 2mm, inside diam. 1mm) and size 1 (outside diam. 1.4mm, inside diam. 0.63mm) respectively. The cannulae were tied in place with 2/0 braided silk and the incision closed with 2/0 suture material. In some foetuses the umbilical vessels instead of femoral blood vessels were cannulated. Size-1 cannulae were placed in a major vein and artery via cotyledonary vessels and held in place with 2/0 braided silk. Each foetus was then injected intramuscularly with 20mg of gentamycin and 50mg of cephaloridine. The uterus was closed and a size-2 cannula placed in the uterine vein or in a maternal femoral artery. Foetal and uterine cannulae were individually exteriorized through the skin-incision closure and held in place by suturing to the abdominal skin of the ewe. Immediately after surgery the ewe was placed in a metabolism cage with access to food and water.

Postnatal lambs were sedated with 2% xylazine, after which size-2 polyvinyl cannulae were inserted in the femoral artery and jugular vein under local xylocaine anaesthesia. Each cannula was sealed with a metal plug. After closure of the limb incision, the wound was coated with Hibitane cream.

The main cause of foetal death was due to infection by *Streptococcus faecalis* and *Pseudomonas aeruginosa*. Accordingly, each foetus was injected with gentamycin and cephaloridine as indicated above, and the ewe received 60 and 450mg respectively of these antibiotics. Disposable syringes containing 0.15M-NaCl and 100 units of heparin/ml were attached to foetal and uterine cannulae and kept submerged in chlorhexidine solution. The cannulae were flushed daily with the NaCl/heparin solution and the syringes replaced.

Radioisotope injection and sampling

Except where otherwise stated, all experiments in which foetal lambs were used were conducted at least 5 days after the implantation of cannulae. This time allowed for recovery of ewe and foetus from surgical stress, as assessed from measurements of blood glucose, fructose, lactose, pO_2 and pCO_2 during the recovery period.

Foetal or suckling lambs were given a single intravenous injection of 2ml of 0.15M-NaCl containing 50μCi of [^{14}C]lactate, [^{14}C]glucose or [^{14}C]fructose, and the cannula was immediately flushed with 2ml of 0.15M-NaCl. Sequential blood samples (2ml) were taken via the arterial cannula and also from the ewe. The blood was immediately haemolysed and deproteinized by addition to 8ml of ethanol, mixed and centrifuged. The supernatant was extracted with 25ml of chloroform and the aqueous phase from this extraction used for specific-radioactivity determinations.

O_2, CO_2 and pH

Blood was collected in plastic syringes rinsed with 0.15M-NaCl containing 100 units of heparin/ml and assayed immediately for pO_2, pCO_2 and pH by using the Radiometer (Copenhagen) Blood micro system MKI. Care was taken to avoid exposure of the samples to air.

Measurements of lactate, glucose and fructose specific radioactivities

For electrophoresis, 0.5ml of the aqueous blood extract was placed on Whatman 3MM paper in a 2cm streak and dried under a stream of warm air. The paper was soaked with 0.1M-sodium arsenite buffer, pH9.6, blotted, and placed on a high-voltage electrophoresis apparatus with a solid heat-exchanger (Frahn & Mills, 1959). Separation of glucose, fructose and lactate occurred after 2h electrophoresis at 1.5kV (17V/cm). Standards were run on separate lanes in which glucose and fructose were detected after heating, and lactate was located by spraying the paper with 0.5M-$CuSO_4$. Areas on the test lanes corresponding in mobility to the standards were cut into strips (1cm×4cm), transferred to

vials containing 20ml of a scintillation fluid comprising 4g of 2,5-diphenyloxazole and 100mg of 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene per litre of toluene and the radioactivity was measured by liquid-scintillation spectrometry. The counting efficiency was approx. 65%.

Glucose was measured spectrophotometrically with hexokinase and glucose 6-phosphate dehydrogenase (Lamprecht & Trautschold, 1965), and lactate by the automated method of Asrow (1969) with lactate dehydrogenase and diaphorase. The colorimetric analysis of fructose was performed as described by Bacon & Bell (1948).

Calculations

In all experiments the specific radioactivities of glucose, fructose and lactate were calculated on the basis of an injection of 50μCi of label and expressed as d.p.m./mg-atom of carbon. Radioisotope disappearance from the injected pool is described by the sum of all exponential terms (White *et al.*, 1969):

$$SR_t = \sum A_i e^{-m_i t}$$

where SR_t is the specific radioactivity of the injected pool at time t, A_i is the zero-time intercept of each component, m_i is the rate constant of each component (min^{-1}), t is the time (min) and i is the exponential component number.

For each specific-radioactivity curve the number of exponential components was resolved graphically by using 'curve-peeling' techniques (Shipley & Clark, 1972). The precise regression line and intercept for each exponential were computed by a least-squares method by using an iterative procedure (Bevington, 1969; Marquardt, 1963). The following parameters were also calculated (White *et al.*, 1969).

The pool, Q, is the quantity of substrate, measured in mg-atoms of carbon, with which the tracer mixes in the animal. It is calculated as the ratio between the injected dose of radioactivity (P) and the calculated specific radioactivity at zero time:

$$Q = \frac{P}{\sum A_i}$$

The space is the volume of fluid (ml) through which the substrate pool is distributed. It represents the pool (Q) divided by the mean concentration of substrate (mg-atoms of carbon/ml) and has been expressed as a percentage of body weight.

The irreversible loss is the rate of removal of substrate not returning to the substrate pool during the course of an experiment. It is represented by the pool (Q) divided by the area under the specific-radioactivity curve from zero time to infinity:

$$\text{Irreversible loss} = \frac{Q}{\sum \frac{A'_i}{m_i}}$$

where A'_i are fractional zero-time intercepts so that

$$\sum A'_i = A'_1 + A'_2 + \cdots A'_n = 1$$

The equation for irreversible loss is a modification of the Stewart–Hamilton equation (Shipley & Clark, 1972) and is particularly useful in tracer kinetic studies, since all routes of radioisotope disposal are included without making assumptions about the number of metabolite pools.

To permit comparisons between foetuses and lambs of different weights, measurements of pool size and irreversible loss are expressed on a per-kilogram-bodyweight basis. To accomplish this, placenta plus foetal weight has been determined from known foetal age by reference to a nomogram compiled from data on sheep of a similar breed.

Results

Physiological parameters

Measurements of foetal blood lactate, glucose, fructose, pO_2 and pCO_2 and the maternal blood glucose are listed in Table 1. Although small differences occurred between individual foetuses, none of the measurements correlated with foetal age. In all experiments reported, the foetal arterial pO_2 was normal and did not show any change between the sampling periods. Similarly, blood glucose, lactate and fructose were relatively constant during the sampling period for each foetus, an important situation if steady-state conditions are being inferred.

Most foetal lambs showed normal physiological parameters not only during the sampling period but for many days subsequently. Thus foetuses numbered 2, 3, 4, 10, 12, 14, 17 and 18 were delivered live at term and all others except no. 16 appeared normal for several days after the injection experiment. Indeed all foetuses in which the femoral vessels were cannulated were born live except for number 13, and both cannulae in this foetus were functional for 17 days after the experiment.

Lactate concentrations were lower in suckling (Table 2) as compared with foetal lambs (Table 1), possibly because of a higher rate of oxidative metabolism by postnatal animals. No fructose could be detected in the blood of the suckling animals, whereas the glucose concentration was five- to ten-fold greater than in foetal sheep. Radioisotope was injected into suckling rather than immediately postnatal animals to avoid problems of a non-steady-state condition caused by the rapid decrease in fructose and the rapid increase in glucose which occurs during the first 2 days after birth (Shelley, 1960).

Injection of [^{14}C]lactate

Labelled lactate was injected into four foetal lambs (nos. 1–4, Table 1) and five suckling animals (nos. 5–9,

Table 1. *Physiological parameters for foetal lambs*

Values are the means ± S.E.M. for between 7 and 16 determinations made in arterial blood at different times after radioisotope injection. The two sets of data for pO_2 and pCO_2 represent measurements (expressed in mmHg) at the beginning (1) and at the end (2) of the sampling period respectively. Foetuses nos. 1–4 were injected with [^{14}C]lactate, nos. 10–14 with [^{14}C]fructose and 15–18 with [^{14}C]glucose.

			Foetal blood							Maternal blood
						pO_2		pCO_2		
Foetus no.	Estimated weight (kg)	Age (days)	[Lactate] (mM)	[Glucose] (mM)	[Fructose] (mM)	(1)	(2)	(1)	(2)	[Glucose] (mM)
1	2.19	125	2.74±0.14	0.43±0.02	4.21±0.18	19.0	21.2	49.8	40.0	2.30±0.23
2	2.89	135	2.32±0.07	0.40±0.01	5.08±0.06	20.0	19.2	48.0	41.5	2.83±0.21
3	2.79	133	2.23±0.07	0.41±0.02	6.63±0.20	20.4	22.1	45.6	41.6	2.23±0.16
4	2.19	125	3.21±0.11	0.56±0.02	6.02±0.26	21.9	20.0	46.0	46.5	2.69±0.18
10	2.14	125	2.50±0.04	0.73±0.01	4.66±0.14	20.1	19.0	43.1	43.2	2.31±0.17
11	5.05	Term	2.02±0.10	0.41±0.01	6.90±0.50	—	—	—	—	2.15±0.21
12	2.89	135	2.00±0.07	0.52±0.01	4.00±0.08	18.3	19.4	40.0	41.1	3.03±0.25
13	2.39	130	1.73±0.03	0.60±0.01	5.00±0.02	20.0	22.0	39.0	46.5	3.13±0.25
14	2.39	130	1.65±0.06	0.87±0.03	4.30±0.11	22.6	22.6	30.3	25.0	2.76±0.19
15	2.29	127	2.15±0.09	0.69±0.01	4.10±0.10	21.3	20.7	44.8	46.7	2.73±0.30
16	5.39	Term	1.56±0.10	0.63±0.05	4.84±0.08	19.8	21.3	40.0	39.0	2.67±0.28
17	2.39	130	1.13±0.01	0.69±0.02	5.11±0.11	23.1	22.9	42.0	40.0	2.94±0.21
18	1.99	124	3.28±0.06	0.75±0.01	5.34±0.14	20.0	19.0	48.0	49.0	2.90±0.17

Table 2. *Physiological parameters for suckling lambs*

Lactate and glucose concentrations in blood are expressed as means ± S.E.M. for between 8 and 13 determinations at different times after radioisotope injection. Lambs nos. 5–9 were injected with [^{14}C]lactate, and 19–23 with [^{14}C]glucose.

Lamb no.	Weight (kg)	Age (days)	[Lactate] (mM)	[Glucose] (mM)
5	7.0	5	1.70±0.10	3.47±0.23
6	7.9	10	2.06±0.03	4.27±0.19
7	8.1	20	2.64±0.11	2.86±0.08
8	2.8	5	1.62±0.04	4.34±0.09
9	5.1	5	1.60±0.60	3.52±0.10
19	10.0	12	0.90±0.05	2.87±0.09
20	5.6	6	1.60±0.06	3.67±0.21
21	9.4	12	2.40±0.15	2.81±0.12
22	4.0	5	1.73±0.03	3.98±0.11
23	8.1	28	1.55±0.11	4.28±0.17

Table 2). Label was injected into the umbilical vein of foetus 1 and into the femoral vein of the other animals. Sampling was from the umbilical artery in foetus 1 and from the femoral artery of the other animals.

Analysis of glucose, fructose and lactate in blood after the [^{14}C]lactate injection into foetuses showed that label occurred only in lactate (Fig. 1*a*). No radioisotope was detected in maternal blood. The shape of the specific-radioactivity disappearance curve of blood lactate is shown in Fig. 1(*a*). This curve was fitted by the least-squares program (Marquardt, 1963; Bevington, 1969), and shows the normalized data ± S.E.M. at each time-period. Normalization of this and the other curves for changes in specific radioactivities of the injected radioisotope (Figs. 1–3) was done by making the specific radioactivity at zero time equal to unity. In each case the zero-time value has been obtained by resolution of the specific-radioactivity curve into two decay components, followed by summation of the extrapolated values of specific radioactivity at zero time in conjunction with 'curve-peeling' techniques. Normalization of product radioactivity was obtained by dividing the measured specific radioactivity by the zero-time specific radioactivity of the injected radioisotopes. By using these methods we found that the lactate pool in foetal sheep was 5.04 mg-atoms of carbon/kg conceptus weight and the lactate space was 65.8% of body weight (Table 3). Irreversible loss of lactate was 222 μg atoms of carbon/min per kg. Since label did not occur in glucose, it is evident that gluconeogenesis was not active.

The decrease in lactate specific radioactivity in suckling lambs was more rapid than in foetal animals, but since the lactate concentration and pool size were much lower in suckling animals, the rate of irreversible loss of lactate, 147 μg-atoms of carbon/min per kg, was lower than in the foetus (Table 3). Substantial conversion of lactate into glucose was evident (Fig. 1*b*) with a maximum glucose radioactivity at 15 min.

Injection of [^{14}C]*fructose*

Foetuses nos. 10 and 11 had radioactive fructose injected into the umbilical vein with sampling from the umbilical artery, whereas radioisotope was injected

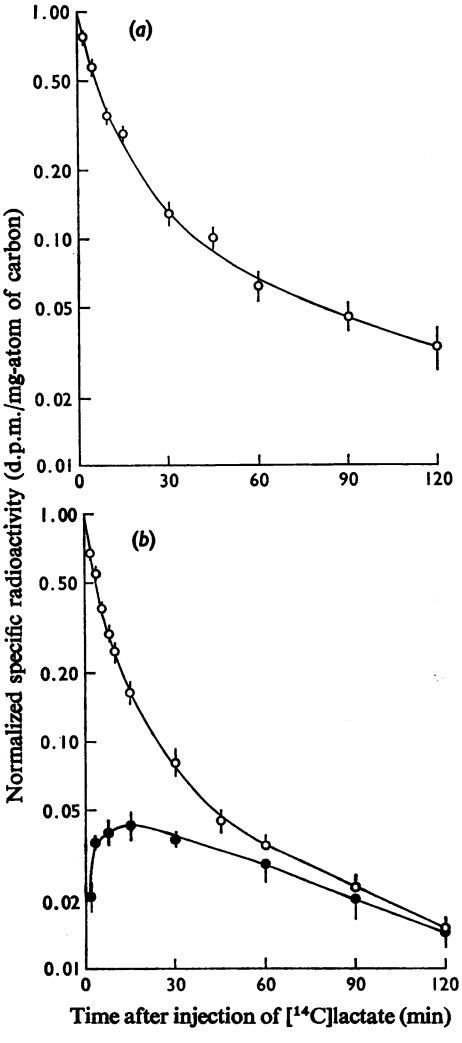

Fig. 1. *Normalized specific radioactivities of lactate* (○) *and glucose* (●) *in blood after injection of* 50 μ*Ci of* [^{14}*C*]*-lactate into* (*a*) *foetuses or* (*b*) *lambs*

Specific radioactivities have been normalized to a calculated lactate specific radioactivity of 1 at the time of injection. Means ± S.E.M. have been calculated at each time-point from data on four foetuses and five suckling lambs.

into the femoral vein and blood taken from the femoral artery of foetuses nos. 12, 13 and 14. The specific-radioactivity-disappearance curves were similar for all five foetuses (see Fig. 2), and could be resolved into two decay components by 'curve-peeling'. Parameters of fructose metabolism calculated from these experiments indicated a pool of 8.70 mg-atoms of carbon/kg distributed over 30.3% of the conceptus weight. The rate of irreversible loss of fructose was 48 μg-atoms of carbon/min per kg, but the fate of this radioactivity was not apparent, since radioisotope was not detected in foetal glucose, foetal lactate or in the maternal blood. Accordingly, glucogenesis from fructose did not occur, and if fructose carbon was oxidized, it did not appear to pass through an intermediate equilibrating with the lactate pool.

Injection of [^{14}*C*]*glucose*

Glucose metabolism and its contribution to the lactate and fructose pools was followed in four foetal lambs with cannulae implanted in either the umbilical vein and artery (nos. 15, 16) or the femoral vein and artery (nos. 17, 18). The change in the specific radioactivity of blood glucose with time in these experiments shows a large and rapid fall during the first 15 min, followed by a slower decrease in specific radioactivity (Fig. 3*a*). The radioisotope-disappearance curve for each foetus was best represented as the sum of two exponential components that were readily resolved by 'curve-peeling' techniques. From the glucose specific-radioactivity curves we obtained values of glucose pool, space and irreversible loss of 2.40 mg-atoms of carbon/kg, 57.4% of conceptus weight and 249 μg-atoms of carbon/min per kg respectively.

Within 2 min after injection of [^{14}C]glucose, radioactivity was detected in both lactate and fructose (Fig. 3*a*). Maximum labelling in lactate occurred at 5 min, and in fructose 15 min after radioisotope administration. The specific radioactivity of lactate declined rapidly after the maximum incorporation was reached, with a terminal slope similar to that of the glucose-radioactivity curve. On the other hand, the specific radioactivity of fructose declined very slowly, reflecting the slow metabolism of fructose noted in the previous section. No radioactivity was detected in maternal blood.

Glucose metabolism was studied in five suckling lambs aged from 5 to 28 days (animals nos. 19–23, Table 2). Compartmental analysis of the specific-radioactivity curves after injection of radioactive glucose showed two exponential disappearance terms in each case. Although the rate of radioisotope loss from the glucose pool was much slower than in foetal sheep (cf. Figs. 3*b* and 3*a*), this was caused by the large increase in glucose pool in the suckling animal rather than by a substantial fall in the rate of irreversible loss of glucose carbon (Table 3). The glucose space, 29.3% of body weight, was substantially lower than in foetal lambs (Table 3). Incorporation of label into lactate occurred rapidly after injection of [^{14}C]glucose, with a maximum incorporation after 10 min (Fig. 3*b*).

Table 3. *Calculated metabolic parameters in foetal and suckling sheep*
Values are means ± S.E.M. for determinations on the numbers of animals indicated in parentheses. The calculations are described in the text.

	Foetus	Suckling
Lactate		
Pool (mg-atoms of carbon/kg)	5.04 ± 0.24 (4)	1.77 ± 0.18 (5)
Space (% body wt.)	65.8 ± 5.3 (4)	30.5 ± 1.9 (5)
Irreversible loss (μg-atoms of carbon/min per kg)	222 ± 15 (4)	147 ± 21 (5)
Glucose		
Pool (mg-atoms of carbon/kg)	2.40 ± 0.30 (4)	5.99 ± 0.18 (5)
Space (% body wt.)	57.4 ± 7.3 (4)	29.3 ± 1.8 (5)
Irreversible loss (μg-atoms of carbon/min per kg)	249 ± 12 (4)	198 ± 11 (5)
Fructose		
Pool (mg-atoms of carbon/kg)	8.70 ± 0.25 (5)	—
Space (% body wt.)	30.3 ± 2.3 (5)	—
Irreversible loss (μg-atoms of carbon/min per kg)	48 ± 10 (5)	—

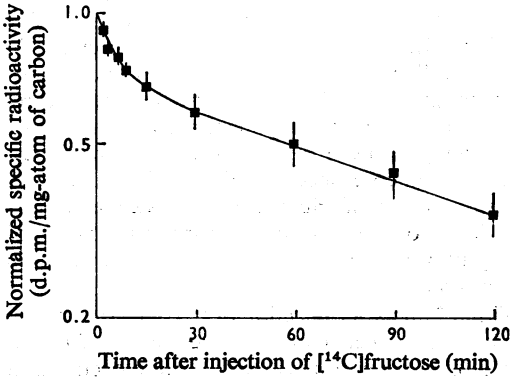

Fig. 2. *Normalized specific radioactivities of fructose after injection of 50 μCi of [14C]fructose into foetuses*
Specific radioactivities have been normalized to a calculated fructose specific radioactivity of 1 at the time of injection. Means ± S.E.M. have been calculated at each time-point from data on five foetuses.

Discussion

Lactate metabolism

The failure of the foetal lamb to synthesize glucose from lactate clearly demonstrates the absence of gluconeogenesis at this developmental stage. In this respect the foetal lamb resembles the foetal rat, but whereas the absence of gluconeogenesis in the rat foetus can be attributed to the extremely low activity of hepatic phosphoenolpyruvate carboxykinase (GTP) (Ballard & Hanson, 1967), all key gluconeogenic enzymes are present at substantial activities in foetal sheep liver [see the following paper, Warnes *et al.* (1977)].

Lactate is present in the foetus at higher concentrations than in suckling lambs or adult sheep (Tables 1 and 2; see also Annison *et al.*, 1963). Further, the large irreversible loss of lactate in the foetal lamb implies an important metabolic flux via this intermediate. Transfer across the placenta has been inferred as a major fate of lactate produced by glycolysis in foetal tissues. However, lactate transport from foetus to mother has not been observed even under conditions of foetal hypoxia (Britton *et al.*, 1967; Mann, 1970), and we were unable to detect radioactive lactate in uterine (ewe no. 1) or femoral blood of the mother. A more likely route for lactate removal is oxidation by foetal tissues. Indeed, Burd *et al.* (1975) have calculated from oxygen- and lactate-utilization rates that lactate can account for approx. 25% of the oxygen consumption of the sheep foetus.

In contrast with the foetus, the suckling lamb possesses an active gluconeogenesis pathway, as indicated by the rapid and substantial labelling of the glucose pool after injection of [14C]lactate. The irreversible loss of lactate is lower in suckling lambs than in the foetus, but, unlike the foetus, the suckling animal has a total lactate-production rate higher than the rate of irreversible loss. The difference occurs because the existence of both gluconeogenesis and glycolysis permits radioactivity to return to the lactate pool after glucose is labelled.

In addition to a fall in the irreversible loss of lactate after birth, the blood-lactate concentration, the lactate pool and the lactate space are also less than in the foetal lamb. Comparison of the results of Annison *et al.* (1963) with those presented here show a further decrease in lactate utilization on maturity. In fed resting adult sheep the irreversible loss of lactate was approx. 50 μg-atoms of carbon/min per kg. The decrease in lactate utilization with age may be associated with changes in the nutrients available to the developing sheep. Thus the placental circulation is a rich source of carbohydrate for the

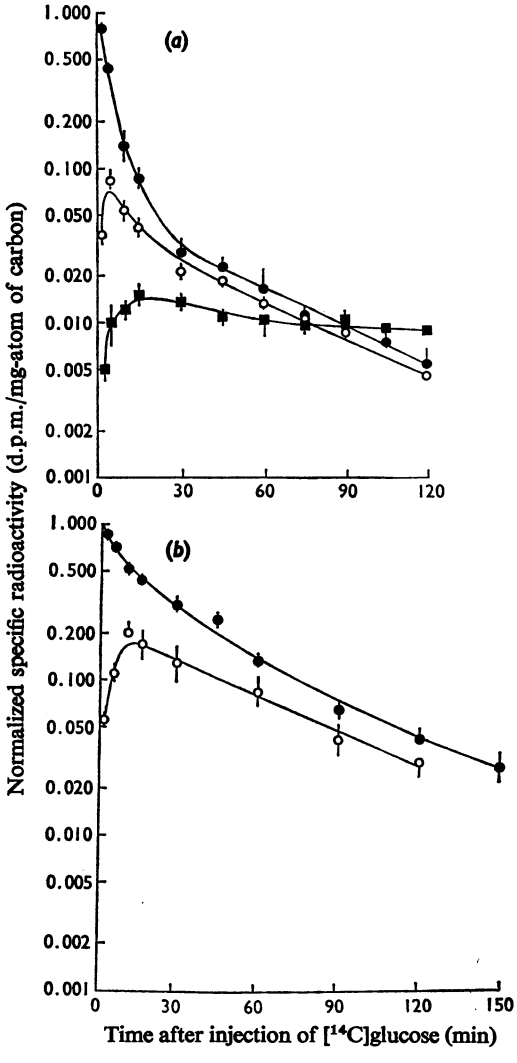

Fig. 3. *Normalized specific radioactivities of glucose* (●), *lactate* (○) *and fructose* (■) *after injection of* 50 μ*Ci of* [^{14}*C*]*glucose into* (*a*) *foetuses or* (*b*) *lambs*

Specific radioactivities for each blood metabolite have been normalized to a calculated glucose specific radioactivity of 1 at the time of injection. Means ± S.E.M. at each time-point have been calculated from data on four foetuses and five lambs.

foetus, whereas the milk available to the newborn is essentially a high-fat high-protein diet. Subsequently the development of rumen function is accompanied by fermentation and removal of dietary carbohydrate, so that the diet of the mature ruminant is also low in carbohydrate.

Fructose metabolism

The low rate of irreversible fructose loss in the sheep foetus is indicative of a relatively inert metabolite. This interpretation agrees with other studies where only small amounts of [^{14}C]fructose were converted into $^{14}CO_2$ (Alexander *et al.*, 1970), lipids (Scott *et al.*, 1967) or glycogen (Ballard & Oliver, 1965) by the sheep foetus. Loss of fructose to the mother does not occur, because the placenta is impermeable to fructose (Alexander *et al.*, 1955). The small irreversible loss of fructose probably represents renal excretion to amniotic fluid (Alexander & Nixon, 1963), since no radioactivity was found in glucose, lactate or other blood metabolites after injection of [^{14}C]fructose. These negative findings also argue against the hypothesis that fructose can act as a reserve of foetal energy in an analogous way to glycogen (Huggett *et al.*, 1951).

Fructose is synthesized in the placenta by reduction of glucose to sorbitol, followed by oxidation of sorbitol (Hers, 1960). Calculations of the placental fructose-production rate give a value of approx. 50 μg-atoms of carbon/min per kg of conceptus (Alexander *et al.*, 1955), close to the irreversible loss reported in Table 3. These low and closely similar rates of production and irreversible loss are to be expected for a compound that is poorly metabolized and slowly excreted.

Glucose metabolism

Although the glucose concentration in the blood of foetal sheep is low compared with the postnatal lamb or with foetuses of mammals other than ungulates, glucose has a substantial irreversible utilization rate. The rate found in the present study is somewhat higher than that calculated from umbilical arteriovenous differences and blood-flow measurements (Tsoulos *et al.*, 1971; James *et al.*, 1972; Crenshaw *et al.*, 1973), but such studies do not take into account placental oxidation or conversion of glucose into fructose. These placental reactions could account for the difference between the present results and those of Crenshaw *et al.* (1973).

The decrease in glucose utilization in suckling lambs and the further decrease in the adult (Jarrett *et al.*, 1964; Muramatsu *et al.*, 1974) are probably related to a decreased availability of glucose from the diet. Our values of irreversible loss in suckling lambs are slightly lower than those reported by Jarrett *et al.* (1964) and substantially lower than found by Muramatsu *et al.* (1974). However, in both these studies the earliest measurement of glucose specific radioactivity was 15–20 min after injection of radioisotope, but the subsequent decay of radioactivity showed a single first-order exponential component. Such procedures amount to an omission of the

initial fast-decay component and will give a larger apparent pool size and a lower calculated value of irreversible loss of glucose carbon than found in the present studies (White *et al.*, 1969).

Conservation of glucose in the adult ruminant contrasts with the situation in the rat. Vernon & Walker (1972*a*,*b*) report a decrease in glucose utilization rate from 900 μg-atoms of carbon/min per kg in rats immediately after birth to 300 μg-atoms of carbon/min per kg in rats 2h after birth. Suckling animals have a similarly low rate, but after weaning the rate of glucose utilization increases approximately twofold. These changes in the rat reflect a declining availability of glucose during suckling, followed by a high availability in rats weaned on to a high-carbohydrate diet. The suckling rat resembles the postnatal ruminant in both diet and low rate of glucose utilization.

Interrelationships between glucose, lactate and fructose

The aims of the present experiments were to measure the overall flux rates between glucose, fructose and lactate and the rates of production and irreversible loss of each compound. Such rates should be attainable by sampling the specific radioactivities of each metabolite after radioisotope is introduced to one of the pools. To accomplish these calculations it is necessary either to have independent information on the number and nature of radioactive pools or to be able to obtain such information from the radioactivity-decay curves. Unfortunately, numerous samples are required to define accurately the shape of each specific-radioactivity curve and to distinguish and quantify the various exponential components. Taking large numbers of blood samples is physiologically undesirable, since not only is the animal exposed to stress, but significant amounts of blood must be withdrawn. With the available data, we have not been successful in attempts to derive a model describing the interrelationships between glucose, lactate and fructose that is based on specific-radioactivity curves from a single experiment. In these attempts we tested various two-, three- or four-pool models for foetal and suckling lambs by using the computer program 'Non-Lin' (Metzler, 1969). Part of the difficulty is caused by lack of precision for the exponential terms and part by the uncertainty in assigning physiological sense to each pool.

Parameters such as the total entry rate of the compound in the initially labelled pool are very sensitive to the decay of specific radioactivity during the first few minutes after injection of radioisotope, as indicated by the relationship (White *et al.*, 1969):

$$\text{Total entry rate} = Q(\sum A_i' m_i)$$

The problem is readily seen from the fructose-labelling experiments. Since fructose label is not transferred to glucose, lactate or other blood metabolites, and since arteriovenous-difference studies have independently shown that fructose metabolism in foetal sheep is extremely low (Tsoulos *et al.*, 1971), it is reasonable to assume that the total entry rate of fructose should be equal to, or only slightly greater than, the rate of irreversible loss of fructose. However, application of the above equation to the data on fructose utilization gives a total entry rate of 0.63 mg-atom of carbon/min per kg, some 13-fold higher than the rate of irreversible loss. The difference is probably a result of the initial rapid fall in fructose specific radioactivity (Fig. 2) being caused by slow distribution of fructose among extracellular fluid rather than by metabolism.

Accordingly we report a model of carbohydrate metabolism in foetal sheep (Fig. 4) that is based completely on pool sizes, rates of irreversible loss of carbon and on the observed metabolic restrictions that: (1) lactate cannot be converted into glucose, and (2) fructose cannot be converted into either glucose or lactate. Entry into the glucose pool is taken as equal to the irreversible loss of glucose, 249 μg-atoms of carbon/min per kg, although it is recognized that the true value may be a little higher if reversible labelling of a glycogen pool occurs. Fructose is considered to be in a steady-state, with the rate of synthesis equal to the measured rate of irreversible loss, 48 μg-atoms of carbon/min per kg. The measured irreversible loss of lactate carbon, 222 μg-atoms of carbon/min per kg, is higher than the calculated glycolytic rate, and it must therefore be assumed that there is a second entry into the lactate pool accounting for the difference of 21 μg-atoms of carbon/min per kg. This value would be larger if any of the irreversible loss of glucose represented irreversible labelling of the glycogen pool.

The model for carbon flux in the sheep foetus is simplified by the restrictions that neither lactate nor fructose is metabolized to glucose. Comparable calculations cannot be made from the data on suckling sheep, because lactate is converted into glucose. A simple two-pool model is not appropriate, because the finding that the irreversible loss of glucose is greater than the irreversible loss of lactate implies a substantial non-glycolytic irreversible metabolism of glucose, a restriction that is probably untenable.

The absence of gluconeogenesis makes the foetus totally dependent on its mother for glucose. To the ruminant foetus this means a complete reliance on maternal gluconeogenesis, since glucose fed to the mother is destroyed by rumen fermentation. Although all the enzymes needed for gluconeogenesis are present in the foetal sheep [see the following paper, Warnes *et al.* (1977)], the absence of gluconeogenic

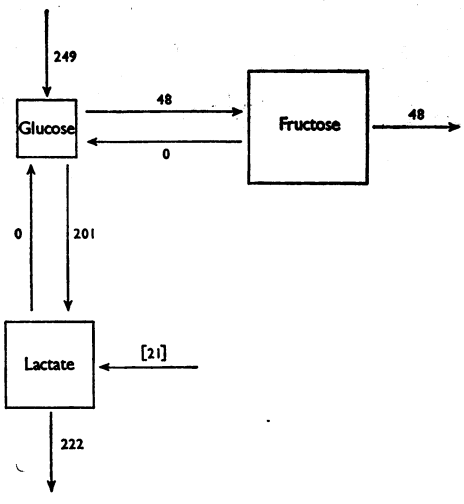

Fig. 4. *Model describing the interrelationships between glucose, fructose and lactate in foetal sheep*
Assumptions and limitations of the model are described in the text. Areas are proportional to the pool sizes of lactate, fructose and glucose, and flow rates are expressed as μg-atoms of carbon/min per kg. The value in brackets showing entry into the lactate pool is an assumed rate reflecting the difference between lactate formation from glucose and lactate irreversible loss.

flux is not surprising. The pathway is only required if adequate glucose is not available, and it would represent an unnecessary diversion of energy from metabolic processes directed to the growth and development of the foetus. It appears, therefore, that the lack of gluconeogenesis in foetal sheep as well as rats (Philippidis & Ballard, 1969, 1970) is consistent with a foetal metabolism that is well adapted for the maximum and efficient use of available energy. Energetically extravagant reactions and redundant homoeostatic processes are not active.

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