Whole body and tissue fractional protein synthesis in the ovine fetus *in utero*

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(*Received 9 August 1983 – Accepted 12 April 1984*)

1. Whole-body and tissue fractional protein synthesis rates were determined in chronically-catheterized ovine fetuses at 120–130 d of gestation following an 8 h continuous infusion of L-[U-^{14}C]- or L-[2,3,5,6-^{3}H]tyrosine.

2. From the net utilization of tyrosine by the fetus, corrected for apparent oxidation, and tyrosine concentration in the fetal carcass protein, whole-body protein synthesis was estimated to be 63 g/d per kg.

3. Following 8 h of infusion of labelled tyrosine the ewes were killed and fetal tissues were removed for the determination of tyrosine specific activity. The fractional rate of protein synthesis (k_s) was calculated from the specific activity ratio, protein bound:intracellular free tyrosine. Tissue k_s values for the liver, kidney, lungs, brain, skeletal muscle and small intestine were 78, 45, 65, 37, 26 and 93% /d respectively.

4. The absolute rate of synthesis was calculated by multiplying the tissue protein content by k_s. Muscles, gastrointestinal tract, liver and lungs contributed approximately 20·5, 20·5, 14·4 and 9·4% respectively to whole-body protein synthesis.

5. The efficiency of protein synthesis as expressed by the RNA activity was higher in liver, lung and brain followed by kidney, skeletal and cardiac muscle.

The importance of maternal nutrition and environmental conditions on the growth of the fetus and postnatal development (Robinson, 1977, 1981; Richardson, 1978; Arthur, 1981; McDonald *et al.* 1981; Mellor & Murray, 1981, 1982) has led to the development of techniques for the measurement of intra-uterine growth. Morphometric changes in the sheep fetus have been measured at different stages of gestation under chronic (Mellor & Matheson, 1979) or acute (Koong *et al.* 1975; Robinson & McDonald, 1979) conditions. The rate of protein deposition in the sheep fetus has been determined by the comparative slaughter technique (Lodge & Heaney, 1973; Rattray *et al.* 1974, 1975; Broad & Davies, 1981). In addition, the retention of α-amino-nitrogen was estimated in chronically-catheterized fetuses from umbilical veno-arterial concentration differences (Lemons *et al.* 1976; Faber & Woods, 1981).

The rate of protein synthesis has also been estimated in the fetus *in utero* by the use of isotopic-dilution techniques (Meier *et al.* 1981; Noakes & Young, 1981; Schaefer & Krishnamurti, 1982*a*). This technique has been extensively used for studying the rate of whole-body protein synthesis under in vivo conditions in postnatal life in man (James *et al.* 1976), pig (Garlick *et al.* 1976; Simon *et al.* 1978; Reeds *et al.* 1980), sheep (Buttery *et al.* 1977; Davis *et al.* 1981; Bryant & Smith, 1982) and cattle (Lobley *et al.* 1980).

Basically, a labelled amino acid is infused continuously into the venous system until the specific activity of the amino acid in the plasma reaches a plateau. The rate of protein synthesis is estimated from the amino acid flux (irreversible loss) after adjusting for oxidative loss. The validity of the assumptions made in the calculations and the application of the technique under a variety of nutritional states have been critically reviewed (Waterlow *et al.* 1978; Reeds & Lobley, 1980).

Apart from the inherent limitation of using the plateau specific radioactivity for calculating protein synthesis, the application of this technique to the fetal system is further

* For reprints.

complicated by the bidirectional transfer of nutrients between the mother and the fetus (Anand *et al.* 1979; Kitts & Krishnamurti, 1982*a*). Under these conditions the flux, calculated from the plasma specific activity of compounds in the fetal compartment alone, would not truly reflect the unidirectional utilization by the fetus. To overcome this problem, a technique based on a two-pool kinetic model was proposed by Hodgson *et al.* (1980) in which differently labelled isotopes are introduced simultaneously into the maternal and fetal systems. The net utilization of substrate by the fetus is obtained from the sum of the net placental exchange and endogenous production.

Recently, we have published information (Schaefer & Krishnamurti, 1982*b*) on the kinetic indices of tyrosine utilization by the sheep fetus *in utero* using the procedure of Hodgson *et al.* (1980). The objective of the present study was to use these indices in conjunction with the extent of tyrosine oxidation to estimate the whole-body and fractional (k_s) rates of protein synthesis in the ovine fetus under *in utero* conditions.

MATERIALS AND METHODS

Animals

Fifteen Dorset × Suffolk ewes of known gestational age were used in the present study. They weighed 55–80 kg and were given lucerne (*Medicago sativa*) cubes (g/kg: 196 crude protein (nitrogen × 6·25), 275 crude fibre) twice daily. On average the ewes consumed 1·94 kg cubes/d which provided 18·58 kJ gross energy/g. Iodized block salt and water were provided *ad lib.* Constant fluorescent lighting was maintained and the room temperature was kept at 16° (dry bulb).

Surgical procedure

To facilitate the infusion of radioactive tracers into the fetus and the ewe and withdrawal of blood, polyethylene catheters with silastic tips were inserted into the fetal inferior vena cava, pedal artery and umbilical vein as well as into the uterine vein, femoral artery and jugular vein of the ewe using the surgical procedures described previously (Kitts *et al.* 1979; Schaefer & Krishnamurti, 1982*a*).

Protein-turnover studies

To avoid the influence of postsurgical trauma on protein turnover, infusions were carried out after a minimum of 5 d following surgery. The physiological stability of the preparations was verified before and during the experiment by monitoring blood gas, pH, packed cell volume (PCV), haemoglobin and plasma metabolites (Table 1, see p. 363). The mean values reported in Table 1 are from eleven animals with triplicate observations taken for blood gas and pH, duplicate observations for PCV and haemoglobin and single observations for body-weight and temperature. For blood glucose, α-NH_2-N and plasma lactate, values are from eight animals with duplicate observations per index. These observations were taken over an 8 h period during the experiment. The protocol of the isotopic infusions has been described in detail previously (Schaefer & Krishnamurti, 1982*b*). Briefly, L-[U-^{14}C]tyrosine (50 μCi) or L-[2,3,5,6^{3}H]tyrosine (300 μCi) (New England Nuclear, Quebec) in 20 ml sterile physiological saline (9 g sodium chloride/l) was infused into the central venous system of the fetus for 8 h without a priming dose at the rate of 2·2 ml/h. In five preparations, when the fetus was infused with [^{14}C]tyrosine, a simultaneous infusion of [^{3}H]tyrosine was made into the ewe. Blood samples were withdrawn at 1·5, 3, 4·5, 6, 7, 7·5 and 8 h from the start of infusion for the determination of plasma tyrosine specific activity.

Analysis of tissues

For estimating the tissue k_s, four of the fetuses were killed at the end of the infusion of tyrosine into the inferior vena cava of the fetus. The ewe was anaesthetized with sodium pentothal and the fetuses were delivered by laparotomy. The infusion was continued until the umbilical cord was ligated. The fetus was towel-dried, weighed and the whole carcass transferred to a liquid N_2 container for quick freezing. The laparotomy and transfer of the fetal carcass to liquid N_2 was performed as quickly as possible and the interval between the end of infusion and freezing of the fetal carcass was approximately 5 min in two of the preparations and less than 10 min in the other two. The frozen fetal carcass was placed in a container of crushed ice and tissue samples of between 3–5 g were dissected with bone scissors from the still frozen carcass and rinsed briefly in saline to remove any possible contamination with blood. For the gastrointestinal samples, care was taken to rinse the lumen side also of the tissue to remove any contamination with the lumen contents. The samples were then sectioned with dissecting scissors and scalpel to obtain subsamples which were weighed accurately and immediately placed in chilled glass homogenizing tubes to which 2 ml refrigerated trichloroacetic acid (100 g/l; TCA) were added. The glass homogenizing tubes containing the tissue subsamples were kept in 500-ml glass beakers of crushed ice during tissue homogenization to minimize proteolysis. The homogenates were centrifuged at 3000 *g* for 5 min after which the supernatant fraction, containing the intracellular free amino acids, was transferred to chilled 15-ml Pyrex test tubes. The pellet was washed twice with 1 ml portions of TCA, rehomogenized and centrifuged between each washing. The combined supernatant fractions were then analysed for tyrosine concentration and radioactivity. For the determination of specific activity of tyrosine in the protein-bound amino acids, the tissue pellet was acid-hydrolysed in screw-capped test-tubes by the addition of 2 ml 6 M-hydrochloric acid and placed in a 110° oven for 20 h before tyrosine analysis. For determining the fetal whole-carcass tyrosine content, four fetuses obtained by laparotomy, were separately homogenized in an industrial blender and weighed portions of carcass homogenates were analysed for tyrosine.

Analytical methods

Haemoglobin was determined by the cyanomethaemoglobin method (Hycell Inc. Houston) and PCV by the microhaematocrit procedure. Blood glucose was analysed by the glucose oxidase (*EC* 1.1.3.4) procedure (Sigma Chemical Co., St Louis, Missouri). Plasma lactate was determined by the lactate dehydrogenase (*EC* 1.1.1.27) mehtod (Kit no. 510 and 826 UV; Sigma Chemical Co.), and α-NH_2-N according to the dinitrofluorobenzene-derivative method of Goodwin (1968). The total N in the tissue samples was determined in a Technicon AutoAnalyzer (Model II; Technicon Instrument Corp., Terrytown, N.Y.) following wet acid-digestion. DNA concentration in tissue samples was determined according to the method of Gold & Shochat (1980) and RNA by the modified Schmidt-Thannhauser technique (Cheek, 1975). DNA standards were prepared from calf-thymus DNA (Mann Research Laboratories, N.Y.) and RNA standards were obtained from the Sigma Chemical Co.

L-Tyrosine was quantitatively converted to tyramine by the addition of tyrosine decarboxylase (*EC* 4.1.1.25; Sigma Chemical Co.). The tyramine was extracted by the procedure of Garlick & Marshall (1972) with modifications as described by Schaefer & Krishnamurti (1982*c*) which improved the precision, accuracy and sensitivity. Fluorometric determination was carried out according to the procedure of Ambrose (1974). Radioactivity of tyrosine was measured by placing 1 ml of the extracted tyramine solution into 10 ml scintillation fluor (PCS; Amersham, Arlington Heights, Ill.) and counted in a Packard

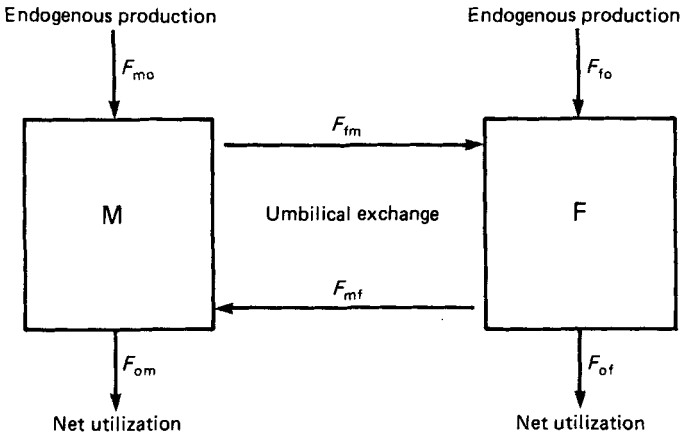

Fig. 1. Two pool model describing the kinetics of maternal – fetal tyrosine transfer. M, ewe (maternal tissues plus placenta); F, fetus; F_{fo} (fetus) and F_{mo} (ewe), endogenous production rates; F_{of} (fetus) and F_{om} (ewe), net utilization rates; F_{fm} and F_{mf}, placental exchange from ewe to fetus and from fetus to ewe respectively (after Schaefer & Krishnamurti, 1982*b*). Net placental exchange $= F_{fm} - F_{mf}$. Fetal net utilization rate $= (F_{fm} - F_{mf}) + F_{fo}$.

liquid-scintillation counter (model A 300 C). The determination of specific activity of blood $^{14}CO_2$ was estimated according to procedures previously described (Kitts & Krishnamurti, 1982*b*; Schaefer & Krishnamurti, 1982*b*). The retention of labelled bicarbonate and other intermediates from tyrosine oxidation in the fetal tissues was not considered and the values of tyrosine catabolism calculated from blood $^{14}CO_2$ refer to apparent tyrosine oxidation.

Calculations

The rate of whole-body protein synthesis was calculated by dividing the net rate of utilization of tyrosine, corrected for apparent oxidation, by the tyrosine content of the fetal carcass protein.

$$\text{Rate of protein synthesis (g/d per kg)} = \frac{\text{Net utilization of tyrosine} - \text{tyrosine oxidized}}{\text{Tyrosine content of fetal carcass protein}}.$$

The net utilization rate is the sum of net placental exchange and endogenous production (Fig. 1) and was calculated from the plateau specific activity of tyrosine in the maternal and fetal compartments following the continuous infusion of the [^{3}H]tyrosine in the ewe and [^{14}C]tyrosine in the fetus. The details of the kinetic procedure and determination of extent of tyrosine oxidation have been described (Schaefer & Krishnamurti, 1982*b*).

The k_s in the tissues was calculated according to two procedures. The first procedure was according to the method of Waterlow *et al.* (1978) using the following equations:

for muscle

$$S_B/S_i = \frac{R}{R-1} \cdot \frac{1-e^{-k_s t}}{1-e^{-Rk_s t}} - \frac{1}{R-1}, \quad (1)$$

for liver, brain, kidney, gastrointestinal and lung tissues

$$S_B/S_i = \frac{\lambda i}{\lambda i - k_s} \cdot \frac{1-e^{-k_s t}}{1-e^{-\lambda i t}} - \frac{k_s}{\lambda i - k_s}, \quad (2)$$

where S_B is the specific activity of protein-bound tyrosine, S_i is the specific activity of intracellular free tyrosine, λi is the rate constant of turnover of tyrosine in the intracellular

Table 1. *Mean physiological indices for the fetus and ewe*

Index	Fetus*		Ewe†	
	Mean	SE	Mean	SE
Blood pH	7·39	0·03	7·49	0·04
Blood P_{CO_2} (mmHg)	33·72	1·80	28·31	1·25
Blood P_{O_2} (mmHg)	16·46	1·75	24·98	0·60
PCV	0·3440	0·0143	0·3207	0·0106
Hb (g/l)	101·2	4·8	113·2	4·1
Body temperature(°)	—	—	38·84	0·12
Body-wt (kg)‡	1·78	0·18	65·72	2·71
Plasma glucose (mg/l)	125·5	15·6	601·8	52·1
Plasma α-amino-nitrogen (mg/l)	133·8	7·0	106·8	4·2
Plasma lactate (mg/l)	129·8	12·6	55·8	7·3

PCV, packed cell volume; Hb, haemoglobin.
* Fetal blood samples were obtained from the inferior vena cava.
† Ewe blood samples were obtained from the jugular vein.
‡ Predicted fetal body-weight at date of experiment based on the regression formula of Gresham *et al.* (1972).

free pool (in liver $\lambda i = \lambda p$ or the rate constant of turnover of the infused tyrosine in the plasma; in muscle $\lambda i = Rk_s$), k_s is the fractional synthetic rate, t is the time of infusion (d), $e^s = 2{\cdot}7182$, R is the ratio protein-bound:intracellular free tyrosine, S_p is the specific activity in plasma.

In the second procedure the values for tissue k_s were calculated by substituting S_B/S_p for S_B/S_i in eqns (1) and (2). These methods of calculating tissue k_s provide estimates of maximum and minimum rates respectively as shown in Table 3 (see p. 364). The advantages and disadvantages of both methods are discussed later.

Statistical analysis

The differences among tissue indices were determined using the analysis of variance and the differences tested for significance by the Newman-Keuls multiple range test (Steel & Torrie, 1960). Bartlett's test of homogeneity of variances (Sokal & Rohlf, 1969) revealed that heterogeneity was evident in the data for tissue protein-bound tyrosine concentration (mg/g) and RNA concentration (mg/g). Therefore, these particular data were statistically analysed using appropriate ANOVA tests for data with heterogeneous variances as per the procedures of Sokal & Rohlf (1969).

RESULTS

The blood indices of the fetus and ewe at the time of experimentation (Table 1) are within the physiological range reported for fetuses which have been catheterized (Comline & Silver, 1970; Battaglia & Meschia, 1978; Kitts *et al.* 1979).

The plateau specific activity ratio, intracellular free tyrosine (S_i):plasma free tyrosine (S_p) was low (0·14–0·19) in liver, small intestine, lung, brain and kidney and high (0·45–0·62) in skeletal and cardiac muscles (Table 2). S_B:S_p in all tissues was lower than the corresponding S_B:S_i values. The protein turnover in the liver and small intestine was more rapid than in the other tissues as indicated by the shorter half-life of mixed proteins (Table 3).

The net utilization of tyrosine by the fetal tissues as reported by Schaefer & Krishnamurti

Table 2. *Relative plateau specific activity of [^3H]tyrosine in different tyrosine pools*
(Mean values with their standard errors)

Tissue	$S_i:S_p$ Mean	SE	$S_B:S_p$ Mean	SE	$S_B:S_i$ Mean	SE
Liver	0·139	0·056	0·021	0·009	0·201	0·027
Small intestine	0·152	0·055	0·031	0·009	0·238	0·062
Lung	0·139	0·059	0·022	0·011	0·174	0·047
Brain	0·189	0·042	0·018	0·007	0·103	0·036
Kidney	0·152	0·048	0·021	0·012	0·126	0·032
Skeletal muscle	0·449	0·209	0·013	0·006	0·029	0·008
Cardiac muscle	0·619	0·181	0·019	0·005	0·032	0·006

S_i, specific activity of intracellular free tyrosine; S_B, specific activity of protein-bound tyrosine; S_p, specific activity of plasma tyrosine.

Table 3. *Fractional (% /d) and absolute rate of protein synthesis in fetal tissues*

Tissue	Mean wt (g) Mean	SE	Protein (mg/g) Mean	SE	Total protein (g)	Fractional (k_s) synthetic rate (%/d) Min	SE	Max	SE	Half-life (d)	Absolute rate‡ of synthesis (g/d)	Percentage of whole-body synthesis
Heart	20·1	2·6	141	4	2·8	11·2	3·0	14·0	3·0	4·950	0·40	0·6
Muscle*	374	—	133	3	49·7	10·3	3·4	26·0	7·0	2·665	12·93	20·5
Liver	72·1	7·0	160	4	11·6	9·5	5·2	78·0	15·0	0·888	9·06	14·4
Brain	38·2	2·8	71	4	2·7	14·2	3·1	37·3	18·7	1·690	1·01	1·6
Lung	67·5	7·3	135	8	9·1	12·8	6·9	65·0	16·0	1·066	5·92	9·4
Kidney	20·0	3·5	114	6	2·3	10·5	4·9	44·6	11·8	1·540	1·02	1·6
GIT†	122	—	148	4	18·1	17·6	4·1	71·2	25·7	0·707	12·90	20·5

GIT, gastrointestinal tract; Min, Max, minimum and maximum rates of tissue fractional protein synthesis using specific activity ratios, protein-bound tyrosine:plasma tyrosine or protein-bound tyrosine:intracellular free tyrosine.

* Based on fetal carcass dissection, muscles were found to contribute 21% of body-weight.

† GIT weight includes reticulum, rumen, omasum, abomasum, small intestine and large intestine. k_s For GIT refers to mean values of small intestine and reticulum-rumen only.

‡ Total protein × k_s Max.

Table 4. *The concentration of tyrosine and nucleic acids in fetal tissues*

Tissue	Protein-bound tyrosine (mg/g) Mean	SE	RNA (mg/g) Mean	SE	DNA (mg/g) Mean	SE
Liver	5·49^{a}	0·474	8·80^{a}	0·95	2·62^{c}	0·40
Lung	2·52^{cd}	0·277	5·94^{b}	0·38	4·45^{ab}	0·69
Kidney	2·19^{d}	0·258	4·69^{bc}	0·55	5·15^{a}	0·63
Brain	2·24^{d}	0·072	1·90^{d}	0·08	3·11^{bc}	0·43
Skeletal muscle	3·66^{bc}	0·078	3·36^{cd}	0·15	2·43^{c}	0·37
Cardiac muscle	3·33^{bcd}	0·271	3·60^{c}	0·28	3·61^{abc}	0·29

$^{a, b, c, d}$ Means with different superscript letters within vertical columns were significantly different ($P < 0·01$).

Table 5. *Protein synthetic capacity and activity in fetal tissues*

Tissue	RNA:DNA	Protein:DNA	Protein:RNA	RNA activity (g protein/g RNA per d)
Liver	3·36[a]	61·11[a]	18·19	14·3
Lung	1·34[bc]	30·40[b]	22·76	14·8
Kidney	0·91[bcd]	22·17[b]	24·33	10·9
Brain	0·61[cd]	22·88[b]	37·47	15·3
Skeletal muscle	1·38[b]	54·41[a]	39·43	10·3
Cardiac muscle	0·99[bcd]	39·81[b]	39·89	5·6

[a, b, c, d] Means with different superscript letters within vertical columns were significantly different ($P < 0·05$).

(1982*b*) was 6·536 mmol/d per kg of which 5·2 (SE 1·65)% was used for apparent oxidation leaving 6·196 mmol/d per kg for synthesis of proteins. From the tyrosine content of the entire fetal carcass protein (15·71 (SE 1·30) mmol/kg) the whole-body rate of protein synthesis can be calculated to be 63 g/d per kg.

The fractional rate of protein synthesis (Table 3) was relatively low in cardiac and skeletal muscles and high in small intestine, liver and lung with the kidney and brain occupying an intermediate position. The absolute rate of protein synthesis in individual tissues (g/d) was calculated by multiplying the total protein content of the tissue by k_s (Table 3). Though k_s of skeletal muscles was only 26%/d, the total amount of protein synthesized in the muscles (12·93 g/d) was higher than in any other single tissue. The absolute rates were also high in liver and gastrointestinal tract which synthesized 9·06 and 12·90 g/d respectively.

The tyrosine and nucleic acid concentrations in fetal tissues are presented in Table 4. The concentrations of tyrosine and RNA were higher in liver than in any other tissue, while the DNA concentration was relatively low. Skeletal and cardiac muscles also had a high concentration of tyrosine. The RNA concentrations in the lung and kidney were higher than in the muscles and brain. Kidney and lung had the highest concentration of DNA among the tissues analysed. The efficiency of protein synthesis was expressed as the amount of protein synthesized per unit RNA per d (Table 5). The efficiency was high in liver, lung and brain followed by kidney, skeletal and cardiac muscle.

DISCUSSION

Protein synthesis in the fetus has recently been studied by the continuous infusion of labelled lysine (Noakes & Young, 1981; Meier *et al.* 1981). However, the use of labelled lysine necessitates a rather lengthy infusion period of between 9 and 13 h as reported by Meier *et al.* (1981). The choice of labelled tyrosine in the current study was based on its smaller pool size than lysine in the fetal plasma (Lemons *et al.* 1976) so that the plateau specific activity is reached more rapidly. As reported earlier (Schaefer & Krishnamurti, 1982*b*) the slope of the specific activity *v.* time curve was approximately zero after 6 h of infusion. It was considered important to minimize the stress on the chronic fetal preparation by keeping the duration of infusion as short as possible. In addition, under our conditions, the modified fluorometric analysis procedure for tyrosine (Schaefer & Krishnamurti, 1982*c*) was found to be more accurate and precise than the original method of Garlick & Marshall (1972).

The limitations of using the tyrosine flux based on plasma specific activity for the estimation of whole-body protein synthesis have been reviewed (Waterlow *et al.* 1978). The fetal system is further confounded by the high turnover as well as the possibility of bidirectional transfer of the label across the placenta. In order to overcome this problem,

the net utilization of tyrosine by the fetus was estimated by adding the net placental transfer and endogenous production using the two-pool kinetic model as described by Hodgson *et al.* (1980). From the net utilization rate, the amount of tyrosine used for apparent oxidative purposes is deducted and the remainder is assumed to be used for protein synthesis. While some of the tyrosine-C will undoubtedly be used for non-oxidative, non-protein purposes, this quantity has been suggested to be quite small (Faber & Woods, 1981). Furthermore, the use of tyrosine for catecholamines, thyroid hormones and melanin production, which was not measured, is assumed to be quantitatively negligible (Garlick *et al.* 1976).

In order to calculate tissue k_s values accurately the use of the immediate precursor amino acyl tRNA specific activity is desired. However, technical limitations to this approach have encouraged workers to make use of alternative procedures (Garlick & Marshal, 1972; Waterlow *et al.* 1978) whereby the specific activity of the labelled amino acid in the plasma or the intracellular free compartments is used. As discussed by Waterlow *et al.* (1978) and Zak *et al.* (1979) the most conventional approach in this regard is to use the intracellular free specific activity as representing the precursor specific activity. However, depending on the tissue concerned, the precursor pool may in fact be more accurately represented by the extracellular specific activity in the plasma as certain tRNA species appear to be charged preferentially from the plasma extracellular pool rather than the intracellular free pool. Furthermore, because of the unavoidable time-lag between the cessation of the isotope infusion and the freezing of tissues, there could possibly be a loss of radioactivity from the intracellular free amino acid pool which may bias the subsequent protein synthesis calculations. In view of the uncertainty in general of the precise precursor specific activity pool that is considered most representative and also the possible loss of intracellular radioactivity following the cessation of the infusion, estimates were made of minimum and maximum tissue k_s using $S_B:S_p$ and $S_B:S_i$ values respectively at steady-state (Table 3). The true value is likely to lie between these two extremes as has been reported by Davis *et al.* (1981).

The ratio, $S_i:S_p$ indicates the extent of dilution of the infused label by unlabelled tyrosine arising from protein degradation. It is noteworthy that the extent of dilution varies in different tissues. In muscles, for example, the specific activity ratio, intracellular free tyrosine: plasma free tyrosine was 0·45–0·62 which is similar to the value of 0·46 in the fetus (Noakes & Young, 1981) and 0·49 in the mature sheep (Bryant & Smith, 1982). On the other hand, the ratio, $S_i:S_p$ in the liver (0·14) is much lower than the values reported in the fetus (0·42; Noakes & Young, 1981) or in growing lambs (0·30; Davis *et al.* 1981). The short half-life of the mixed proteins in the liver and small intestine (Table 3) indicates a rapid turnover in these tissues resulting in greater dilution of the label in the intracellular pool. The intracellular pool in the skeletal and cardiac muscles undergoes less dilution because of the slower turnover of proteins.

There was a wide variation in k_s (Table 3) among the tissues, an observation similar to that reported by Meier *et al.* (1981) and Noakes & Young (1981). Among the tissues, the small intestine displayed the highest k_s. A high degree of metabolic activity and protein turnover in gastrointestinal tissue might be expected considering the swallowing of amniotic fluid by the fetus as well as the absorption of nutrients from the gastrointestinal tract as shown by Pitkin & Reynolds (1975) and Char & Rudolph (1979). This would suggest that in late gestation, even under *in utero* conditions, the digestive organs of the fetus are already functioning to a certain extent.

The high k_s values and total RNA concentration in the liver indicate the high protein synthetic capacity and turnover of this tissue. Although the protein: RNA value in this tissue was comparatively lower than in the other tissues (Table 5) the RNA activity, which represents

the extent to which the protein synthetic capacity is expressed (g protein/g RNA per d; Table 5) was high. This possibility indicates that much of the synthesized protein is exported. The lungs also resemble the liver in this regard, suggesting the synthesis of extracellular surfactant proteins. Brain and kidney occupy an intermediate position (Table 3) with respect to k_s. Brain tissue showed the lowest RNA:DNA value (Table 5) but a higher protein:RNA value and RNA activity than liver and lung, indicating that the protein synthetic capacity is utilized to the maximum in this tissue. Compared with other tissues, both skeletal and cardiac muscles showed relatively low k_s values (14–26%). These values are comparable to the k_s of 23–35%/d reported in newborn or young lambs (Soltesz *et al.* 1973; Arnal, 1977). As the lambs grow older the muscle k_s drops to 2–7%/d (Buttery *et al.* 1975; Arnal, 1977; Davis *et al.* 1981). The DNA unit size (protein:DNA) is, however, highest in the muscles and liver, indicative of hypertrophy of the cells. In spite of the high protein:RNA value in the muscles which represents a high protein synthetic capacity, the actual expression of this ability appears to be low as indicated by the relatively low RNA activity (Table 5) and k_s (Table 3).

The whole-body rate of protein synthesis of 63 g/d per kg observed in the present study is higher than the values of 38·6 g/d per kg reported by Noakes & Young (1981) or 15 g/d per kg reported by Meier *et al.* (1981) for fetuses in late gestation. Nevertheless, these values from all three studies on fetal sheep contrast sharply with the low rate of whole-body protein synthesis of 6·9 g/d per kg reported in adult sheep (Reeds & Lobley, 1980). Values ranging from 45 to 78·9 g/d per kg have been reported for immature rats (Garlick, 1980) and 33 g/d per kg for newborn lambs (Soltesz *et al.* 1973). When the present values are expressed on a metabolic body size basis, the whole-body protein synthetic rate amounts to 52 g/kg body-weight$^{0.75}$ per d which is close to the value of 49 g/kg body-weight$^{0.75}$ per d reported by Soltesz *et al.* (1973) for newborn lambs.

Apart from the inherent variables associated with the physiological condition of the preparations, the major difference appears to lie in the isotopes employed and the methodology used in estimating the kinetic indices. The fetuses used by Noakes & Young (1981) were infused 24–48 h after surgery which may not provide adequate time for recovery from the surgical trauma (Kitts *et al.* 1979). This might influence the dynamics of protein turnover.

It is also noteworthy that although [^{14}C]lysine was used for infusion in both cases, the lysine flux values and whole-body rates of protein synthesis reported by Meier *et al.* (1981) are approximately three times lower than those obtained by Noakes & Young (1981) for fetuses of similar age. Methodological and analytical differences and variability in the physiological status of the preparations may account for the differences observed.

Energy cost of protein synthesis in the fetus

The efficiency of whole-body protein synthesis is the ratio, rate of protein deposition: total rate of protein synthesis. Based on the comparative slaughter results of Rattray *et al.* (1974) it can be determined that the sheep fetus at 120 d of gestation gains approximately 6–9 g/d per kg. This is similar to the rate of 9·5 g/d per kg reported by Lemons *et al.* (1976) based on the net α-NH_2-N uptake by the fetus. In the present study the net α-NH_2-N uptake by the fetus was determined in one preparation by multiplying the arterio-venous concentration difference by the umbilical blood flow. It was found that 1·59 g α-NH_2-N/d per kg was retained by the fetus which is equivalent to a protein-N retention of 9·9 g/d per kg.

Thus the rate of whole-body protein synthesis in the fetus (63 g/d per kg) is at least six to eight times more than the rate of protein deposition (6–10 g/d per kg). However, the significance of protein turnover in the fetus should be considered in relation to its contribution to the energy economy of the fetus. Using the value of 4·5 kJ/g as the cost

of protein synthesis (Webster, 1977), and a heat production of 368 kJ/d per kg (Battaglia & Meschia, 1978), it may be calculated that whole-body protein turnover contributes at least 55% to total heat production. This is similar to the values reported for lambs (25–42%; Soltesz *et al.* 1973; Davis *et al.* 1981) and much higher than that in older sheep (4·5–14%; Buttery *et al.* 1975; MacRae & Reeds, 1977). Further, as suggested by Davis *et al.* (1981), because of the high rate of protein turnover as compared to the rate of deposition, even small changes in the former are likely to result in larger changes in protein deposition. The methodology used in this investigation can be used to investigate intrauterine growth retardation by monitoring changes in fetal rates of protein synthesis as influenced by nutritional and endocrine status.

The authors wish to thank the Natural Sciences and Engineering Research Council of Canada for financial support of this project and Miss Sandy Heindze for technical assistance.

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