J. theor. Biol. (1999) **198,** 121–133
Article No. jtbi.1999.0897, available online at http://www.idealibrary.com on IDEAL

An Isotope Dilution Model for Partitioning Leucine Uptake by the Liver of the Lactating Dairy Cow

J. France*†, M. D. Hanigan‡, C. K. Reynolds*, J. Dijkstra§, L. A. Crompton*, J. A. Maas*, B. J. Bequette¶, J. A. Metcalf‖, G. E. Lobley¶, J. C. MacRae¶ and D. E. Beever*

**Department of Agriculture, The University of Reading, Earley Gate, P.O. Box 236, Reading RG6 6AT, U.K., ‡Purina Mills Inc., P.O. Box 66812, St. Louis, MO 63166-6812, U.S.A., §WIAS Animal Nutrition, Wageningen Agricultural University, Marijkeweg 40, P.O. Box 338, 6700 AH Wageningen, The Netherlands, ¶Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB, U.K. and ‖Borregaard UK Ltd, Clayton Road, Risley, Warrington, Cheshire WA3 6QQ, U.K.*

(*Received on* 21 *August* 1998, *Accepted in revised form on* 7 *January* 1999)

An isotope dilution model for partitioning leucine uptake by the liver of the lactating dairy cow is constructed and solved in the steady state. If assumptions are made, model solution permits calculation of the rate of leucine uptake from portal and hepatic arterial blood supply, leucine export into the hepatic vein, leucine oxidation and transamination, and synthesis and degradation of hepatic constitutive and export proteins. The model requires the measurement of plasma flow rate through the liver in combination with leucine concentrations and plateau isotopic enrichments in arterial, portal and hepatic plasma during a constant infusion of [1-^{13}C]leucine tracer. The model can be applied to other amino acids with similar metabolic fates and will provide a means for assessing the impact of hepatic metabolism on amino acid availability to peripheral tissues. This is of particular importance when considering the dairy cow and the requirements of the mammary gland for milk protein synthesis.

1. Introduction

The ability to predict accurately dietary nitrogen utilization for production in the ruminant depends on a clear understanding of amino acid flows from the gut to productive tissues. In other words, it depends on an accurate description of the regulation of intestinal amino acid supply, absorption of these products from the gut, metabolism by gut and liver tissues and subsequent use by peripheral tissues. Whilst each of these processes is important, a major site of net loss of digested and absorbed amino acids (and peptides) in a lactating dairy cow is the liver (Reynolds *et al.*, 1988; Danfaer, 1994). In many cases, up to 80% of the free α-amino nitrogen appearing in the portal vein is removed by ruminant liver metabolism (Reynolds *et al.*, 1991, 1992a; Huntington, 1989; Seal & Reynolds, 1993). Whilst the liver is a major site of oxidation, some of the extracted amino acids can be used for protein synthesis and other

†Author to whom correspondence should be addressed. E-mail: j.france@reading.ac.uk

0022–5193/99/009121 + 13 $30.00/0

synthetic processes. The first step in amino acid oxidation is generally deamination, after which some of the carbon skeletons can be used subsequently for glucose synthesis. The nitrogen arising from deamination can be used for *de novo* synthesis of non-essential amino acids or in urea synthesis (Seal & Reynolds, 1993; van der Walt, 1993).

There is a variety of mechanisms that exert a coordinated effort to control the rate of amino acid extraction and deamination in the liver. Energy and amino acid supply relative to body requirements represents one major influence on the rate of amino acid deamination (Elwyn, 1970; Munro, 1982; Meijer *et al*., 1990; Reynolds *et al*., 1992b; Benevenga *et al*., 1993; Reynolds, 1995) and numerous experiments have been conducted using radio-isotopes to determine the partitioning of particular amino acids taken up by the liver into oxidation, glucose synthesis, protein synthesis, etc. (Wolff & Bergman, 1972; Heitmann & Bergman, 1978, 1981; Pell *et al*., 1986). More recently, stable isotopes have been used to describe unidirectional amino acid uptake and use for protein synthesis in the liver (Lobley *et al*., 1995, 1996; Reeds, 1992).

Models of ruminant liver metabolism have been developed that are useful in analysing and interpreting data for non-nitrogenous nutrients and non-protein N (Waghorn, 1982; Freetly *et al*., 1993; Danfaer, 1994); however, to our knowledge, a similar model has not been developed for the analysis of isotopic data for individual amino acids. The primary objective of this work was to develop a steady-state model of liver leucine metabolism adequate to analyse *in vivo* isotopic data in the lactating dairy cow.

2. The Model

2.1. BIOCHEMICAL BACKGROUND

Leucine (LEU) taken up by the liver is available for protein synthesis and for catabolism to provide N for non-essential amino acid synthesis and carbon skeletons in support of energy requirements. LEU used for protein synthesis can be incorporated into albumin, lipoproteins, fibrins and other proteins which are subsequently exported from the liver, or incorporated into constitutive liver proteins such as structural proteins and enzymes.

LEU catabolism involves a series of steps (Fig. 1), the first of which is reversible and is catalysed by LEU transaminase which transfers the NH_2 group of LEU to α-ketoglutamate yielding one mole each of glutamate and α-ketoisocaproate (KIC) from a mole each of α-ketoglutarate and LEU, respectively. As the carbon skeleton for the synthesis of glutamate is derived entirely from α-ketoglutarate, labelled carbon is not transferred from [1-^{13}C]LEU into the glutarate pool at this step. A subsequent series of reactions results in the generation of one mole each of acetoacetate, acetyl-CoA, ADP, NADH and $FADH_2$. The labelled carbon in the number one position is lost as CO_2, but a mole of CO_2 is also utilized in the catabolic process resulting in no net change in CO_2 production. However, labelled carbon in the number one position would exchange with the whole-body CO_2 pool (Pell *et al*., 1986).

Acetyl-CoA can enter the tricarboxylic acid (TCA) cycle or be used for *de novo* fatty acid synthesis. The majority of the labelled carbon in the acetyl-CoA that enters the TCA cycle would be recovered in CO_2, although some label would also appear in α-ketoglutarate and oxaloacetate and ultimately in other metabolites such as glucose, glutamate, glutamine, aspartate and asparagine. As these amino acids are also used for protein synthesis, additional label could be incorporated into protein thereby resulting in a slight overestimate of protein synthesis when only considering LEU as a source of label for protein synthesis (Reeds, 1992).

Acetoacetate can be activated to acetoacetyl-CoA and subsequently enter the TCA cycle as acetyl-CoA or can escape the cell as acetoacetate or β-hydroxybutyrate (Lehninger, 1975). The ratio of acetoacetate:β-hydroxybutyrate leaving the liver is dependant upon the NAD:NADH ratio in the liver, i.e. greater NADH concentrations result in greater β-hydroxybutyrate concentrations. The rate of entry of acetoacetate into the TCA cycle is thought to be dependant upon the availability of CoA and the concentration of ATP (substrates for the reaction).

Catabolism of uniformly labelled [^{13}C]LEU would therefore result in the transfer of label into protein, KIC, CO_2, acetoacetate, β-hydroxybutyrate, long chain fatty acids and other metabolites such as glutamate, aspartate and glucose. However, the label would be captured predominantly in the protein, KIC and CO_2. The model is therefore constructed in terms of LEU, protein, KIC and CO_2.

2.2. MODEL CONSTRUCTION

The simplified scheme based on carbon flows adopted for the proposed model of LEU metabolism by the liver is shown in Fig. 2(a). It

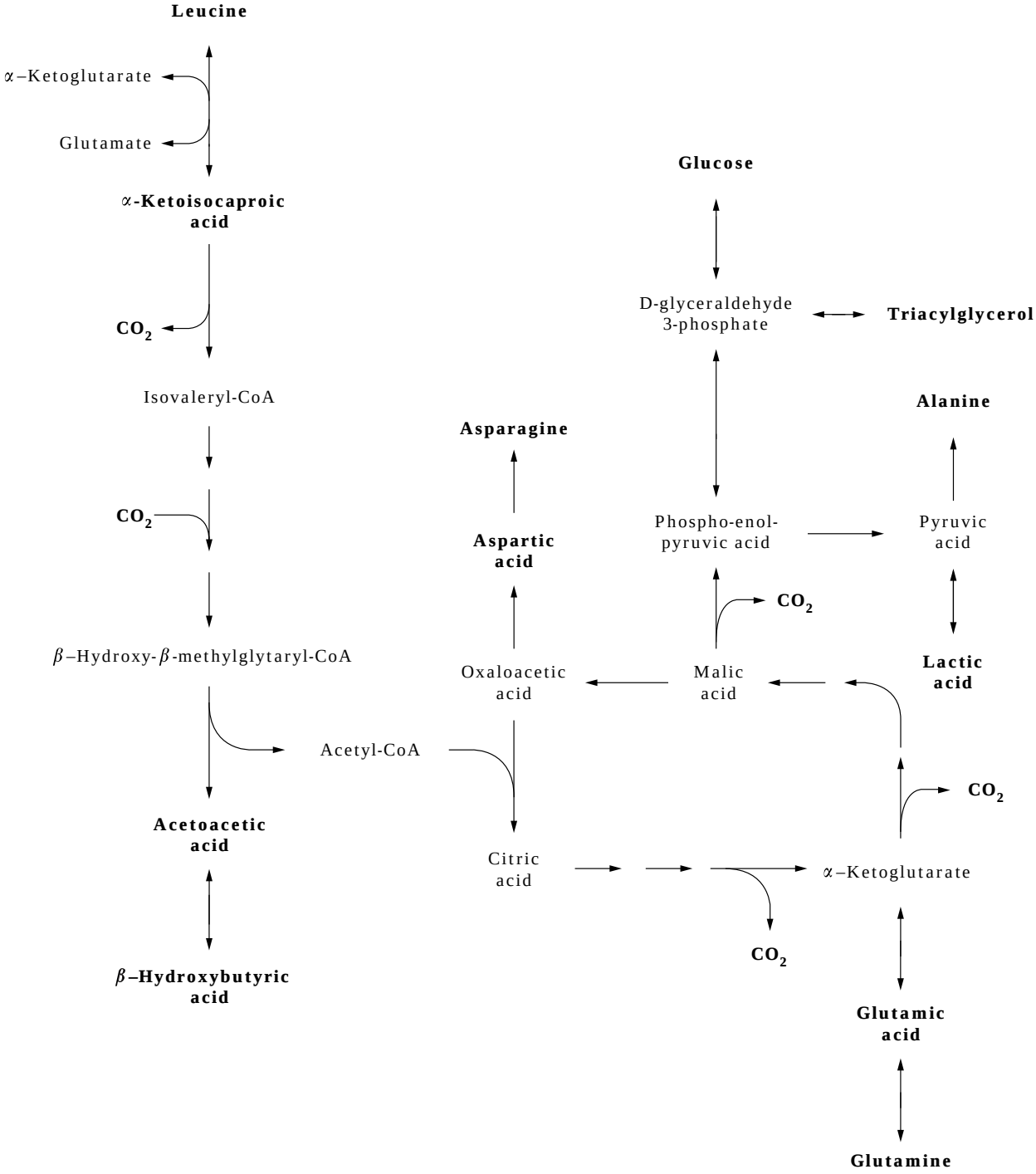

FIG. 1. Pathway for LEU degradation. Bold items are the significant points of carbon loss from the pathway.

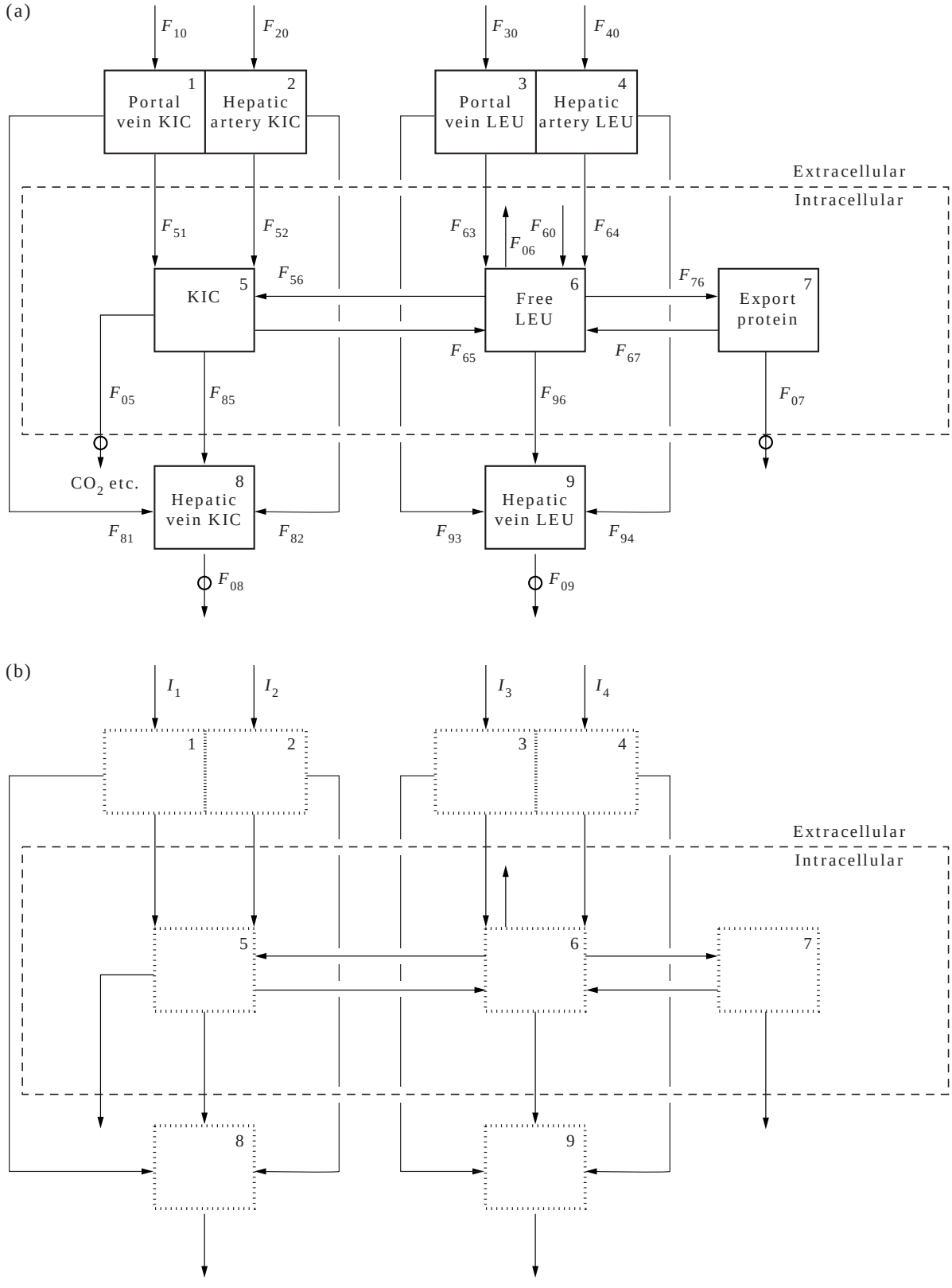

FIG. 2. Scheme for the uptake and partition of LEU by the liver (a) total LEU, (b) labelled. The small circles in Fig. 2(a) indicate fluxes which need to be measured experimentally.

contains three intracellular and six extracellular pools. The intracellular pools are KIC, free LEU and LEU in export protein (pools 5, 6 and 7, respectively) while the extracellular ones represent portal vein, hepatic artery and hepatic vein KIC and LEU (pools 1–4, 8 and 9). The fluxes of KIC and LEU between pools and into and out of the system are shown as arrowed lines. The intracellular KIC pool has three influxes, from the portal pool, F_{51}, from the arterial pool, F_{52}, and from free LEU, F_{56}, and three effluxes, to oxidation and KIC degradation products, F_{05}, to free LEU, F_{65}, and to the hepatic vein pool, F_{85}. The free LEU pool has five influxes, from degradation of constitutive liver protein and undefined sources such as import proteins and peptides, F_{60}, from the portal pool, F_{63}, from the arterial pool, F_{64}, from KIC, F_{65}, and from degradation of export protein, F_{67}, and four effluxes, synthesis of constitutive liver protein, F_{06}, to KIC, F_{56}, incorporation into export protein, F_{76}, and to the hepatic vein pool, F_{96}. The export protein-bound LEU pool has one influx, from free LEU, F_{76}, and two effluxes, secretion of export protein, F_{07}, and degradation, F_{67}. The extracellular portal vein KIC pool also has a single influx, entry from the portal blood supply, F_{10}, and two effluxes, uptake by the liver, F_{51}, and passage to the hepatic vein pool, F_{81}. Similarly for the extracellular hepatic artery KIC pool with entry F_{20}, uptake F_{52} and passage F_{82}. The same description applies to the corresponding LEU pools, i.e. pools 3 and 4 with fluxes F_{30}, F_{63}, F_{93} and F_{40}, F_{64}, F_{94}, respectively. The hepatic vein KIC pool has three influxes, from the portal pool, F_{81}, from the arterial pool, F_{82}, and from the liver, F_{85}, and one efflux, outflow in hepatic vein blood, F_{08}. The same description applies to the corresponding LEU pool with fluxes F_{93}, F_{94}, F_{96}, and F_{09}, respectively.

The scheme adopted for movement of label is shown in Fig. 2(b). Labelled [1-^{13}C]LEU is infused systemically at a constant rate and the enrichment of the pools monitored. The scheme assumes that the only entries of label into the system are into the KIC and LEU portal vein pools via fluxes I_1 and I_3, respectively, and into the hepatic artery pools via fluxes I_2 and I_4, and that the duration of the infusion is such that the enrichment of constitutive protein can be regarded as negligible.

Conservation of mass principles can be applied to each pool in Fig. 2 to generate differential equations which describe the dynamic behaviour of the system. For total (isotopic plus non-isotopic) LEU, these differential equations are (mathematical notation is defined in Table 1):

$$dQ_1/dt = F_{10} - F_{51} - F_{81} \quad (1)$$

$$dQ_2/dt = F_{20} - F_{52} - F_{82} \quad (2)$$

$$dQ_3/dt = F_{30} - F_{63} - F_{93} \quad (3)$$

$$dQ_4/dt = F_{40} - F_{64} - F_{94} \quad (4)$$

$$dQ_5/dt = F_{51} + F_{52} + F_{56} - F_{05} - F_{65} - F_{85} \quad (5)$$

$$dQ_6/dt = F_{60} + F_{63} + F_{64} + F_{65} + F_{67} - F_{06} - F_{56} - F_{76} - F_{96} \quad (6)$$

TABLE 1
Principal symbols

F_{ij}	Total flux of LEU (or KIC) to pool i from j; F_{i0} denotes an external flux into pool i and F_{0j} a flux from pool j out of the system; $\mathscr{F}_{ij}$ indicates a flux which can be measured experimentally; μmol min^{-1}
I_i	Constant rate of infusion of labelled LEU (or KIC) into pool i: μmol min^{-1}
Q_i	Total quantity of LEU (or KIC) in pool i: μmol
q_i	Quantity of labelled LEU (or KIC) in pool i: μmol
e_i	Enrichment of pool i ($= q_i/Q_i$): μmol label (μmol total LEU or KIC)$^{-1}$
t	Time: min

$$dQ_7/dt = F_{76} - F_{07} - F_{67} \quad (7)$$

$$dQ_8/dt = F_{81} + F_{82} + F_{85} - F_{08} \quad (8)$$

$$dQ_9/dt = F_{93} + F_{94} + F_{96} - F_{09} \quad (9)$$

and for label:

$$dq_1/dt = I_1 - e_1(F_{51} + F_{81}) \quad (10)$$

$$dq_2/dt = I_2 - e_2(F_{52} + F_{82}) \quad (11)$$

$$dq_3/dt = I_3 - e_3(F_{63} + F_{93}) \quad (12)$$

$$dq_4/dt = I_4 - e_4(F_{64} + F_{94}) \quad (13)$$

$$dq_5/dt = e_1F_{51} + e_2F_{52} + e_6F_{56} - e_5(F_{05} + F_{65} + F_{85}) \quad (14)$$

$$dq_6/dt = e_3F_{63} + e_4F_{64} + e_5F_{65} + e_7F_{67} - e_6(F_{06} + F_{56} + F_{76} + F_{96}) \quad (15)$$

$$dq_7/dt = e_6F_{76} - e_7(F_{07} + F_{67}) \quad (16)$$

$$dq_8/dt = e_1F_{81} + e_2F_{82} + e_5F_{85} - e_8F_{08} \quad (17)$$

$$dq_9/dt = e_3F_{93} + e_4F_{94} + e_6F_{96} - e_9F_{09} \quad (18)$$

When the system is in steady state with respect to both total and labelled matter, the derivative terms in eqns (1)–(18) are zero and the equations become:

$$F_{10} - F_{51} - F_{81} = 0 \quad (19)$$

$$F_{20} - F_{52} - F_{82} = 0 \quad (20)$$

$$F_{30} - F_{63} - F_{93} = 0 \quad (21)$$

$$F_{40} - F_{64} - F_{94} = 0 \quad (22)$$

$$F_{51} + F_{52} + F_{56} - F_{05} - F_{65} - F_{85} = 0 \quad (23)$$

$$F_{60} + F_{63} + F_{64} + F_{65} + F_{67} - F_{06} - F_{56} - F_{76} - F_{96} = 0 \quad (24)$$

$$F_{76} - F_{07} - F_{67} = 0 \quad (25)$$

$$F_{81} + F_{82} + F_{85} - F_{08} = 0 \quad (26)$$

$$F_{93} + F_{94} + F_{96} - F_{09} = 0 \quad (27)$$

$$I_1 - e_1(F_{51} + F_{81}) = 0 \quad (28)$$

$$I_2 - e_2(F_{52} + F_{82}) = 0 \quad (29)$$

$$I_3 - e_3(F_{63} + F_{93}) = 0 \quad (30)$$

$$I_4 - e_4(F_{64} + F_{94}) = 0 \quad (31)$$

$$e_1F_{51} + e_2F_{52} + e_6F_{56} - e_5(F_{05} + F_{65} + F_{85}) = 0 \quad (32)$$

$$e_3F_{63} + e_4F_{64} + e_5F_{65} - e_6(F_{06} + F_{56} + F_{76} - F_{67} + F_{96}) = 0 \quad (33)$$

$$e_1F_{81} + e_2F_{82} + e_5F_{85} - e_8F_{08} = 0 \quad (34)$$

$$e_3F_{93} + e_4F_{94} + e_6F_{96} - e_9F_{09} = 0 \quad (35)$$

Note that, for the scheme assumed, the enrichments of the intracellular LEU and export protein pools equalise in steady state (i.e. $e_6 = e_7$). Therefore, eqns (7) and (16) both yield eqn (25), and e_7 can be written as e_6 in eqn (15) yielding eqn (33). To obtain steady-state solutions to the model, it is assumed that (i) CO_2 production, export protein secretion and the hepatic vein outflows [i.e. the fluxes F_{05}, F_{07}, F_{08} and F_{09}, respectively in Fig. 2(a)] are measured experimentally, and (ii) percentage KIC extraction by the liver is the same from the portal vein and hepatic artery supplies and similarly for LEU extraction, i.e.

$$F_{51}/F_{10} = F_{52}/F_{20} \quad (36)$$

and

$$F_{63}/F_{30} = F_{64}/F_{40} \quad (37)$$

Algebraic manipulation of eqns (19)–(37), which enables the model to be solved for the unknown fluxes steady-state values, gives:

$$F_{10} = I_1/e_1 \quad (38)$$

$$F_{20} = I_2/e_2 \quad (39)$$

$$F_{30} = I_3/e_3 \quad (40)$$

$$F_{40} = I_4/e_4 \quad (41)$$

$$F_{76} - F_{67} = \mathscr{F}_{07} \quad (42)$$

$$F_{51} = F_{10}[(e_1 - e_5)F_{10} + (e_2 - e_5)F_{20} - (e_8 - e_5)\mathscr{F}_{08}]/[(e_1 - e_5)F_{10} + (e_2 - e_5)F_{20}] \quad (43)$$

$$F_{52} = F_{20}F_{51}/F_{10} \quad (44)$$

$$F_{81} = F_{10} - F_{51} \quad (45)$$

$$F_{82} = F_{20} - F_{52} \quad (46)$$

$$F_{85} = \mathscr{F}_{08} - F_{81} - F_{82} \quad (47)$$

$$F_{56} = [(e_1 - e_5)F_{51} + (e_2 - e_5)F_{52}]/(e_5 - e_6) \quad (48)$$

$$F_{65} = F_{51} + F_{52} + F_{56} - \mathscr{F}_{05} - F_{85} \quad (49)$$

$$F_{63} = F_{30}[(e_3 - e_6)F_{30} + (e_4 - e_6)F_{40} - (e_9 - e_6)\mathscr{F}_{09}]/ [(e_3 - e_6)F_{30} + (e_4 - e_6)F_{40}] \quad (50)$$

$$F_{64} = F_{40}F_{63}/F_{30} \quad (51)$$

$$F_{93} = F_{30} - F_{63} \quad (52)$$

$$F_{94} = F_{40} - F_{64} \quad (53)$$

$$F_{96} = \mathscr{F}_{09} - F_{93} - F_{94} \quad (54)$$

$$F_{06} = (e_3F_{63} + e_4F_{64} + e_5F_{65})/e_6 - F_{56} - (F_{76} - F_{67}) - F_{96} \quad (55)$$

$$F_{60} = F_{06} + F_{56} + (F_{76} - F_{67}) + F_{96} - F_{63} - F_{64} - F_{65} \quad (56)$$

where the symbol $\mathscr{F}$ denotes an experimentally-determined flux.

The constitutive protein synthesis and export protein secretion fluxes, F_{06} and F_{07}, respectively (both μmol min^{-1}), can be used to calculate a fractional synthesis rate for total liver protein (based on albumin) by assuming a molecular weight for LEU of 131 Da, a LEU concentration in albumin of 10.48% by weight (Peters, 1985), and a total hepatic protein content for a Holstein-Friesian in mid-lactation of 2.13 kg (Gibb *et al*., 1992).

Model solution does not allow separation of the export protein synthesis and degradation fluxes F_{76} and F_{67}, but merely permits calculation of their difference, i.e. net synthesis [eqn (42)]. However, separation could be achieved by assuming that a fixed proportion (~ 0.1) of the nascent protein is cleaved and degraded during the docking and secretory processes (Razooki Hasan *et al*., 1982).

The model is applied by using eqn (38) *et seq*. to compute the steady-state flux values.

3. Application

Application of the model is illustrated using data from two experiments conducted at our laboratories with multicatheterised, mid-lactation cows.

The data from the first (a preliminary) experiment (C11) relates to a pluriparous Holstein-Friesian cow (liveweight c. 670 kg). The cow was adapted to a diet consisting of 40% grass silage and 60% concentrate, on a dry matter (DM) basis, for a period of 3 weeks and fed hourly by autofeeder. Daily intake was 17.5 kg DM and dietary crude protein (CP) level was 155 g kg^{-1} DM. Blood samples were taken on one day in the week following dietary adaptation. During the sampling period, [1-^{13}C]LEU was infused for 8 hr into the jugular vein and blood samples were taken from the carotid artery, and the portal and hepatic veins (Reynolds *et al*., 1988).

The data from the second experiment (C16) relates to multiparous Friesan cows (average weight c. 550 kg) fed grass silage (16% CP) and concentrate (12% CP) hourly in a 40:60 DM ratio. Average daily intakes were 16.1 kg DM. The cows were given constant mesenteric vein infusions (2.2 ml min^{-1}) of saline for 3 d, followed by a sterile, buffered mixture of total amino acids for a further 3 d. The total amino acids were administered at a daily rate equivalent to the amino acids in 600 g milk protein. On day 3 of both saline and amino acid infusions, the animals received a constant jugular vein infusion of [1-^{13}C]LEU (700 mg hr^{-1}) for 8 hr and blood samples were taken simultaneously from catheters in the dorsal aorta and the portal and hepatic veins for the measurement of blood flow rate (by PAH infusion) and nutrient metabolism by the portal drained viscera and liver.

The relevant experimental measurements are given in Table 2. They are reported for the one animal in the preliminary experiment, and for four animals during the saline infusion and two animals during the amino acid infusion in the second experiment. They are based on plasma rather than whole blood values. LEU measurements are based on free rather than total (i.e. free plus bound) plasma LEU. The effective isotope flows to the liver I_1 to I_4 were obtained from portal/arterial concentration and enrichment of KIC and LEU and portal/arterial plasma flow. The fluxes $\mathscr{F}_{08}$ and $\mathscr{F}_{09}$ were determined from hepatic measurements of plasma flow and KIC and LEU concentration.

Four of the measurements shown in Table 2, namely the intracellular enrichments e_5 and e_6 and the fluxes $\mathscr{F}_{05}$ and $\mathscr{F}_{07}$, were not made in the experiment and therefore had to be estimated. The intracellular enrichment of KIC, e_5, was set equal to the corresponding hepatic

TABLE 2
Experimental measurements

Cow		VM	6044/3	6188/4	6188/5‡	6093/6	6093/7‡	6062/8
experiment		C11	C16	C16	C16	C16	C16	C16
mild yield (kg/d)†		26.8	24.6	24.6	24.6	24.6	24.6	24.6
Plateau enrichment (%):	e_1	3.92	5.63	5.37	4.22	6.60	5.29	5.92
	e_2	3.79	5.57	5.41	4.28	6.61	5.30	5.86
	e_3	3.10	4.47	4.84	3.63	5.70	3.96	4.81
	e_4	4.06	7.05	6.92	4.99	7.87	5.41	6.93
	e_5*	3.88	5.54	5.33	4.22	6.53	5.26	5.79
	e_6*	1.46	2.54	2.49	1.80	2.83	1.95	2.49
	e_8	3.88	5.54	5.33	4.22	6.53	5.26	5.79
	e_9	2.47	4.40	4.53	3.57	5.30	3.81	4.65
Flux (μmol min^{-1}):	I_1	10.1	7.9	7.2	6.9	8.9	6.1	5.1
	I_2	0.9	1.4	1.7	1.4	0.9	0.8	1.5
	I_3	156.8	146.9	122.0	185.6	140.9	141.4	113.9
	I_4	19.0	39.4	37.9	45.3	18.0	23.2	47.6
	F_{05}*	15.7	0.0	0.0	8.0	12.9	6.2	15.7
	F_{07}*	58.2	58.2	58.2	58.2	58.2	58.2	58.2
	F_{08}	246.2	137.1	154.9	187.0	125.7	112.8	99.2
	F_{09}	7022.0	3895.3	3215.1	6154.3	2753.3	4003.1	3327.0

*Estimated value; †average value value (for C16 cows); ‡total amino acid mixture infused.

measurement, e_8, following Harris *et al*. (1992) for the tissues of the hindlimb. Eisemann *et al*. (1989) measured liver intracellular free LEU specific radioactivies (SRA) in beef steers after 6 hr of infusion of [1-^{14}C]LEU into the jugular vein. The samples were obtained while the animals were anaesthetized and whilst maintaining the isotope infusion. The ratio of hepatic intracellular LEU SRA to plasma free LEU SRA at the jugular vein was 0.36 with a standard error of 0.04. Therefore the missing intracellular free LEU enrichment, e_6, was estimated as 0.36 times the corresponding arterial enrichment, e_4. The flux $\mathscr{F}_{05}$ was obtained from labelled CO_2 elevation in plasma flow across the liver and intracellular KIC enrichment, by assuming 0.45 of the increase in labelled CO_2 is derived from intracellular oxidation and degradation of KIC (Pell *et al*., 1986; for maintenance-fed sheep). The export protein flux, $\mathscr{F}_{07}$, was asigned the value 58.2 μmol min^{-1}, estimated for a lactating Holstein cow by Freetly *et al*. (1993) using data from rats.

The model calculations obtained from these experiments are presented in Table 3, and the sensitivity to perturbations ($\pm 50\%$) in the measurements which had to be estimated (i.e. e_5, e_6, $\mathscr{F}_{05}$ and $\mathscr{F}_{07}$) are shown in Table 4 for one cow.

LEU extraction by the liver from the portal vein and hepatic artery supplies is 17.7% on average (Table 3). However, the influx into the intracellular free LEU pool from the portal and arterial pools is, however, markedly sensitive to e_6, the intracellular free LEU enrichment (Table 4). Doubling the estimate of e_6 requires, in particular, an increase in the amount of LEU extracted and a decrease in the turnover of constitutive liver protein. The degradation of constitutive protein plus undefined sources (F_{60}), and the portal and arterial supply (F_{63} and F_{64}), contribute almost equal amounts of LEU to the intracellular free LEU pool (between 49.1 and 54.6% from F_{60}). The hepatic vein outflow of LEU is slightly lower than LEU inflow for the cows in the second experiment, but in the first experiment, LEU hepatic outflow is much larger than LEU inflow (7022 vs. 5526 μmol min^{-1}). This net release of LEU is contrary to the general observation that the liver is a net utilizer of amino acids, and is probably due to feeding a low protein diet.

Only a small part of the LEU uptake is catabolized in the liver (F_{56}; between 0.8 and 4.5 μmol min^{-1} in Table 3), in line with the assertion that branched chained amino acids are catabolized in the muscle tissues (Pell *et al*., 1986). The calculated portion of LEU

catabolized is varied by changing the liver intracellular KIC enrichment, e_5 (which is set to the corresponding hepatic measurement, e_8, herein), and a reduction of this value increased the net oxidation of intracellular LEU in the liver by an order of magnitude (Table 4). Both positive and negative perturbation of e_5 produces significant divergence between venous and intracellular KIC enrichment, causing a decrease in the KIC uptake flux F_{51} and consequently an increase in the bypass flux F_{81}.

The calculated fluxes are not sensitive to the value of the export protein flux, $\mathscr{F}_{07}$, apart from the net incorporation of LEU into export protein, $F_{76} - F_{67}$, and the incorporation into constitutive protein, F_{06}. Equally, sensitivity to the KIC oxidation flux, $\mathscr{F}_{05}$, is small, and only LEU incorporation into constitutive protein (F_{06}), degradation of constitutive protein (F_{60}), and LEU influx from KIC (F_{65}) changed. Thus, the sensitivity analysis demonstrates that in contrast to e_5 and e_6, it is not paramount to determine experimentally the export protein flux or the KIC oxidation and degradation flux.

The constitutive protein synthesis rate is always much larger than the export protein synthesis rate, the contribution of the former ranging between 72.5 and 91.1% of the total rate of protein synthesis rate (Table 3). FSR for liver protein is on average 42.2%/d, but varies widely (17.9 to 55.5%/d). This is consistent with liver protein FSRs reported for other ruminants, for example, 9 to 31%/d for lactating goats (Baracos *et al*., 1991; Champredon *et al*., 1990), 7 to 39%/d for (non-lactating) cattle (Lobley *et al*., 1980), and 15 to 57%/d for sheep (Lobley *et al*., 1992). These values, however, are generally exclusive of export protein. Constitutive protein synthesis rate, and consequently FSR of liver protein, is highly sensitive to LEU intracellular enrichment (Table 4), and doubling this value significantly reduces the constitutive protein synthesis rate and the liver protein FSR.

4. Discussion

4.1. OPENING REMARKS

While measurements of unidirectional LEU metabolism are of interest, labelled LEU is, in most cases, used as an indicator of tissue or whole body protein metabolism. The present model refines the calculation of liver protein

TABLE 3
Model calculations

Cow		VM	6044/3	6188/4	6188/5	6093/6	6093/7	6062/8
Flux (μmol min^{-1})	F_{10}	256.8	140.9	135.0	162.7	134.1	116.1	85.9
	F_{20}	22.6	25.1	31.2	32.0	13.1	15.7	25.6
	F_{30}	5057.8	3284.7	2519.7	5113.4	2474.2	3571.6	2368.1
	F_{40}	467.7	558.6	547.2	909.3	229.1	428.3	686.3
	$F_{75} - F_{67}$	58.2	58.2	58.2	58.2	58.2	58.2	58.2
	F_{51}	256.8	140.9	135.0	162.7	134.1	116.1	85.9
	F_{52}	22.6	25.1	31.2	32.0	13.1	15.7	25.6
	F_{81}	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	F_{82}	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	F_{85}	246.2	137.1	154.9	187.0	125.7	112.8	99.2
	F_{56}	3.4	4.5	2.8	0.8	2.8	1.2	3.9
	F_{65}	20.9	33.4	14.1	0.5	11.5	14.1	0.5
	F_{63}	1288.6	596.6	540.7	560.7	437.6	499.4	381.3
	F_{64}	119.1	101.5	117.4	99.7	40.5	59.9	110.5
	F_{93}	3769.2	2688.1	1979.0	4552.8	2036.6	3072.2	1986.8
	F_{94}	348.5	457.1	429.8	809.6	188.6	368.4	575.8
	F_{96}	2904.3	750.0	806.3	792.0	528.1	562.4	764.4
	F_{06}	153.3	593.4	540.5	560.7	429.6	599.0	216.9
	F_{60}	1690.8	674.6	735.6	750.8	529.1	647.5	551.0
Protein synthesis (g d^{-1})								
Constitutive		276.3	1068.1	973.0	1009.2	773.3	1078.1	390.4
Export		104.8	104.8	104.8	104.8	104.8	104.8	104.8
FSR (%/d)		17.9	55.1	50.6	52.3	41.2	55.5	23.2

TABLE 4
Sensitivity of estimated measurements for cow 6093/6

			Perturbed*							
			e_5		e_6		F_{05}		F_{07}	
		Unperturbed	−50%	+50%†	−50%	+50%	−50%	+50%	−50%	+50%
Flux (μmol min^{-1})	F_{10}	134.1								
	F_{20}	13.1								
	F_{30}	2474.2								
	F_{40}	229.1								
	$F_{76} - F_{67}$	58.2								
	F_{51}	134.1	22.1	17.2						
	F_{52}	13.1	2.2	1.7						
	F_{81}	0.0	112.0	117.0						
	F_{82}	0.0	11.0	11.5						
	F_{85}	125.7	2.7	−2.8						
	F_{56}	2.8	187.7	−8.6	2.0	4.6				
	F_{65}	11.5	183.4	4.4	10.7	13.3	18.0	5.1		
	F_{63}	437.6			284.2	857.9				
	F_{64}	40.5			26.3	79.4				
	F_{93}	2036.6			2190.0	1616.3				
	F_{94}	188.6			202.8	149.7				
	F_{96}	528.1			360.5	987.3				
	F_{06}	429.6			917.5	267.0	444.5	414.7	458.7	400.5
	F_{60}	529.1			1017.0	366.5	537.6	520.7		
Protein sythesis (g d^{-1})										
Constitutive		773.3			1651.5	480.6	800.2	746.5	825.7	721.0
Export		104.8							52.4	157.1
FSR (%/d)		41.2			82.5	27.5	42.3	40.0		

*Only flux values which differ from the unperturbed values are shown; †this perturbation results in some flux values becoming negative (i.e. non-physiological).

synthesis into constitutive tissue and export but as with any continuous infusion approach, requires an accurate estimate of the enrichment in the precursor pool. The model assumes a single homogenous pool from which both constitutive and export proteins are synthesised. This is possibly an over-simplification that has consequences for the estimation of protein synthesis. There is limited evidence which suggests that proteins destined for export are probably synthesised on the rough endoplasmic reticulum (ER) and preferentially utilise amino acids as they enter the cell (Fern & Garlick, 1976). Such cellular compartmentalisation is achieved, at least in part by the selectivity conferred by the 3′ untranslated region of mRNAs (Hesketh, 1994). As the ER is associated with extracellular components of the cell, the isotopic activity of free amino acids in this part of the cell will be closer to that of blood. Recent data in ruminants has supported this theory, demonstrating that the enrichment of hepatic apolipoprotein B100 is most closely represented by the enrichment in the hepatic vein plasma pool (Connell *et al*., 1997). In contrast, constitutive proteins are synthesised on polysomes within the cell cytosol and the precursor pool would include intracellular free amino acids or perhaps preferential use of amino acids released from protein degradation (e.g. Smith & Sun, 1995). The concept of cellular compartmentalisation may need to be incorporated into compartmental models of protein turnover in the future.

4.2. DIRECT MEASUREMENT OF PRECURSOR POOL ENRICHMENT

The choice of which precursor pool best represents the enrichment of the true precursor has to be made from the accessible free amino acid pools, e.g. vascular inflow or outflow, tissue homogenate, etc. For the present model, intracellular free LEU enrichment was assumed to be the best indicator of precursor pool enrichment, though it had to be estimated. As discussed previously (e.g. France *et al*., 1988; Crompton & Lomax, 1993), direct measurement of the aminoacyl-tRNA would provide the true precursor enrichment, but in practice, requires a sample of tissue and has the accompanying concern that the turnover of the aminoacyl-tRNA species is more rapid than extraction techniques (0.3–3 s for rodent valyl-tRNA; Smith & Sun, 1995). Furthermore, the values obtained represent only a mean value for the tissue and do not distinguish between different aminoacyl-tRNA pools which may support constitutive and export protein synthesis.

In the present study, intracellular free LEU enrichment was not measured directly and estimated as 0.36 times the corresponding free LEU enrichment in the arterial pool (e_4), following Eisemann *et al*. (1989) for cattle. Recent studies across the splanchnic tissues of sheep using the continuous infusion approach have reported that the free [1-^{13}C]LEU enrichments in the liver homogenate pool were 0.69 that of the arterial pool and 0.85 that of the hepatic vein pool in fed sheep (Connell *et al*., 1997). Sensitivity analysis has indicated that intracellular free LEU enrichment has the greatest influence on LEU flow in the model and consequently on the estimates of constitutive protein synthesis rate (Table 4). A concern with our estimate of intracellular LEU enrichment is that the ratio of free LEU enrichment in vascular to intracellular pools appears to differ between animal species and their physiological state. Other workers have used LEU enrichment in the arterial supply and venous drainage to provide a range of estimates (e.g. Lobley *et al*., 1995), as indicated above.

4.3. INDIRECT MEASUREMENT OF PRECURSOR POOL ENRICHMENT

Another approach is to use the venous enrichment of KIC as an indicator of intracellular LEU enrichment, as the enrichment of KIC produced by the tissues studied is believed to reflect the intracellular LEU enrichment of those tissues (Harris *et al*., 1992). A concern with this approach is the influence of arterial KIC on venous enrichment, i.e. pool 8 is dominated by F_{81} and F_{82} rather than F_{85}. There is only a small change in enrichment of KIC between the portal vein pool (e_1) and the hepatic vein pool (e_8) which may be the result of little exchange (both uptake and release) across the liver. Indeed our estimate of intracellular KIC enrichment (e_5) (set equal to

hepatic vein KIC enrichment e_8) differs appreciably from our estimate of intracellular LEU enrichment (set at 0.36 times arterial enrichment). An alternative approach, which has been applied for muscle studies, is to set intracellular KIC enrichment equal to intracellular free LEU enrichment. The relative inflows of bypass vs. intracellular outflow can then be calculated from the enrichment of KIC in the hepatic vein pool (e_8). These data emphasise the importance of obtaining a direct measurement of liver intracellular free LEU and KIC enrichment at the same time as the vascular samples are being taken.

An alternative approach to measuring the intracellular enrichment directly, would be to isolate specific plasma proteins synthesised in the liver and measure their enrichment. The protein would ideally have a fairly rapid turnover in order to achieve plateau enrichment during a relatively short-term infusion. Recent studies in ruminants have used apolipoprotein B-100 as an indicator of hepatic protein synthesis during a 12 hr infusion of isotopically labelled amino acids (Connell *et al*., 1997). Another option would be to isolate a plasma protein with a much longer half-life from the samples taken during the course of the isotope infusion and predict plateau enrichment from the rate of increase during the infusion.

4.4. CONCLUDING REMARKS

A notable observation from the present modelling exercise is the magnitude of the contribution of degradation from constitutive protein and undefined sources, F_{60}, to the intracellular free LEU pool required to resolve the model. The fact that the FSRs for liver protein for lactating dairy cows obtained using the model are consistent with estimates reported in the literature for other ruminants lends more confidence to the calculated flux values.

Just as accurate measurements of precursor pool enrichment are critical for the use of labelled amino acids to determine tissue protein synthesis generally, the measurement of intracellular free LEU and KIC enrichment are crucial components of the present model, yet had to be estimated along with liver export protein in order to apply the model using the data available from the two experiments. If the model is to be applied more rigorously, future *in vivo* studies should measure simultaneously the intracellular free LEU and KIC pools and liver export protein, along with the plasma enrichments and flow rates measured in the present work.

The work reported here forms part of a collaborative project funded by a consortium comprising government (MAFF, BBSRC, SOAFD) and agribusiness (MDC, Purina Mills, Hendrix).

REFERENCES

Baracos, V. E., Brun-Bellut, J. & Marie, M. (1991). Tissue protein synthesis in lactating and dry goats. *Brit. J. Nutr*. **66,** 451–465.

Benevenga, N. J., Gahl, M. J. & Blemings, K. P. (1993). Role of protein synthesis in amino acid catabolism. *J. Nutr*. **123,** 332–336.

Champredon, C., Debras, E., Mirand, P. P. & Arnal, M. (1990). Methionine flux and tissue protein synthesis in lactating and dry goats. *J. Nutr*. **120,** 1006–1015.

Connell, A., Calder, A. G., Anderson, S. E. & Lobley, G. E. (1997). Hepatic protein synthesis in the sheep: effect of intake as monitored by use of stable-isotope-labelled glycine, leucine and phenylalanine. *Brit. J. Nutr*. **77,** 255–271.

Crompton, L. A. & Lomax, M. A. (1993). Hindlimb protein turnover and muscle protein synthesis in lambs: a comparison of techniques. *Brit. J. Nutr*. **69,** 345–358.

Danfaer, A. (1994). Nutrient metabolism and utilization in the liver. *Livestock Prod. Sci*. **39,** 115–117.

Eisemann, J. H., Hammond, A. C. & Rumsey, T. S. (1989). Tissue protein synthesis and nucleic acid concentration in steers treated with somatotropin. *Brit. J. Nutr*. **62,** 657–671.

Elwyn, D. H. (1970). The role of the liver in regulation of amino acid and protein metabolism. In: *Mammalian Protein Metabolism*, Vol. 4 (Munro, H. N., ed.) pp. 523–557. New York: Academic Press.

Fern, G. D. & Garlick, P. J. (1976). Compartmentation of albumin and ferritin synthesis in rat liver *in vivo*. *Biochem. J*. **156,** 189–192.

France, J., Calvert, C. C., Baldwin, R. L. & Klasing, K. C. (1988). On the application of compartmental models to radioactive tracer kinetic studies of *in vivo* protein turnover in animals. *J. theor. Biol*. **133,** 447–471.

Freetly, H. C., Knapp, J. R., Calvert, C. C. & Baldwin, R. L. (1993). Development of a mechanistic model of liver metabolism in the lactating cow. *Agric. Systems* **41,** 157–194.

Gibb, M. J., Ivings, W. E., Dhanoa, M. S. & Sutton, J. D. (1992). Changes in body components of autumn-calving Holstein-Friesian cows over the first 29 weeks of lactation. *Anim. Prod*. **55,** 339–360.

Harris, P. M., Skene, P. A., Buchan, V., Milne, E., Calder, A. G., Anderson, S. E., Connell, A. & Lobley, G. E. (1992). Effect of food intake on hind-limb and wholebody protein metabolism in young growing sheep:

chronic studies based on arterio-venous techniques. *Brit. J. Nutr.* **68,** 389–407.

Heitmann, R. N. & Bergman, E. N. (1978). Glutamine metabolism, interorgan transport & glucogeneity in the sheep. *Amer. J. Physiol.* **234,** E197–E203.

Heitmann, R. N. & Bergman, E. N. (1981). Glutamate interconversions and glucogeneity in the sheep. *Amer. J. Physiol.* **241,** E242–E247.

Hesketh, J. (1994). Translation and the cytoskeleton: a mechanism for targeted protein synthesis. *Mol. Biol. Rep.* **19,** 233–243.

Huntington, G. B. (1989). Hepatic urea synthesis and site and rate of urea removal from blood of beef steers fed alfalfa hay or a high concentrate diet. *Can. J. Anim. Sci.* **69,** 215–223.

Lehninger, A. L. (1975). *Biochemistry*, 2nd Edn, pp. 443–476. New York: Worth Publishers.

Lobley, G. E., Milne, V., Lovie, J. M., Reeds, P. J. & Pennie, K. (1980). Whole body and tissue protein synthesis in cattle. *Brit. J. Nutr.* **43,** 491–502.

Lobley, G. E., Harris, P. M., Skene, P. A., Brown, D., Milne, E., Calder, A. G., Anderson, S. E., Garlick, P. J., Nevison, I. & Connell, A. (1992). Responses in tissue protein synthesis to sub- and supra-maintenance intake in young sheep: comparison of large dose and continuous infusion techniques. *Brit. J. Nutr.* **68,** 373–388.

Lobley, G. E., Connell, A., Lomax, M. A., Brown, D. S., Milne, E., Calder, A. G. & Farningham, D. A. H. (1995). Hepatic detoxification in the ovine liver; possible consequences for amino acid catabolism. *Brit. J. Nutr.* **73,** 667–685.

Lobley, G. E., Connell, A., Revell, D. K., Bequette, B. J., Brown, D. S. & Calder, A. G. (1996). Splanchnic-bed transfers of amino acids in sheep blood and plasma, as monitored through the use of a multiple U-^{13}C-labelled amino acid mixture. *Brit. J. Nutr.* **75,** 217–235.

Meijer, A. J., Wouter, H. L. & Chamuleau, R. A. F. M. (1990). Nitrogen metabolism and ornithine cycle function. *Physiol. Rev.* **70,** 701–748.

Munro, H. M. (1982). Interaction of liver and muscle in the regulation of metabolism in response to nutritional and other factors. In: *The Liver*: *Biology and Pathobiology* (Arias, I., Popper, H., Schachter, D. & Shafritz, D. A. eds) pp. 677–691. New York: Raven Press.

Pell, J. M., Caldarone, E. M. & Bergman, E. N. (1986). Leucine and α-ketoisocaproate metabolism and interconversions in fed and fasted sheep. *Metab.* **35,** 1005–1016.

Peters, T. (1985). Serum albumin. *Adv. Prot. Chem.* **37,** 191–245.

Razooki Hasan, H., White, D. A. & Mayer, R. J. (1982). Extensive destruction of newly synthesized casein in mammary explants in organ culture. *Biochem. J.* **202,** 133–138.

Reeds, P. J. (1992). Isotopic estimation of protein synthesis and proteolysis *in vivo*. In: *Modern Methods in Protein Nutrition and Metabolism* (Nissen, S., ed.) pp. 249–273. New York: Academic Press.

Reynolds, C. K. (1995). Quantitative aspects of liver metabolism in ruminants. In: *Ruminant Physiology*: *Digestion*, *Metabolism*, *Growth and Reproduction*. *Proc. of the* 8*th Int. Symp. on Ruminant Physiology* (Engelhardt, W. v., Leonard-Marek, S., Breves, G. & Giesecke, D., eds) pp. 351–371. Stuttgart: Ferdinand Enke Verlag.

Reynolds, C. K., Huntington, G. B., Tyrrell, H. F. & Reynolds, P. J. (1988). Net portal-drained visceral and hepatic metabolism of glucose, L-lactate & nitrogenous compounds in lactating Holstein cows. *J. Dairy Sci.* **71,** 1803–1812.

Reynolds, C. K., Tyrrell, H. F. & Reynolds, P. J. (1991). Effects of diet forage-to-concentrate ratio and intake on energy metabolism in growing beef heifers: net nutrient metabolism by visceral tissues. *J. Nutr.* **121,** 1004–1015.

Reynolds, C. K., Casper, D. P., Harmon, D. L. & Milton, C. T. (1992a). Effect of CP and ME intake on visceral nutrient metabolism in beef steers. *J. Anim. Sci.* **70 (Suppl. 1),** 315.

Reynolds, C. K., Lapierre, H., Tyrrell, H. F., Elsasser, T. H., Staples, R. C., Gaudreau, P. & Brazeau, P. (1992b). Effects of growth hormone-releasing factor and feed intake on energy metabolism in growing beef steers: net nutrient metabolism by portal-drained viscera and liver. *J. Anim. Sci.* **70,** 752–763.

Seal, C. J. & Reynolds, C. K. (1993). Nutritional implications of gastrointestinal and liver metabolism in ruminants. *Nutr. Res. Rev.* **6,** 185–208.

Smith, C. B. & Sun, Y. (1995). Influence of valine flooding on channelling of valine into tissue pools and on protein synthesis. *Amer. J. Physiol.* **268,** E735–E744.

van der Walt, J. G. (1993). Nitrogen metabolism by the ruminant liver. *Aust. J. Agric. Res.* **44,** 381–403.

Waghorn, G. (1992). Modelling analysis of bovine mammary and liver metabolism. Ph.D. Thesis, University of California, Davis.

Wolff, J. E. & Bergman, E. N. (1972). Gluconeogenesis from plasma amino acids in fed sheep. *Amer. J. Physiol.* **223,** 455–460.