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Glucose and [^{13}C]leucine metabolism by the portal-drained viscera of sheep fed on dried grass with acute intravenous and intraduodenal infusions of glucose

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The effect of exogenous glucose supply by either intrajugular (IJG) or intraduodenal (IDG) infusion at 2·0 mg glucose/kg body weight per min was investigated in four wether sheep (average weight 44 (SD 4) kg) chronically catheterized in the carotid artery and portal veins. Sheep were fed on a dried grass pellet diet hourly using continuous belt feeders. Whole-body glucose irreversible loss (IL) rate, measured with [6-^{3}H]glucose, was increased by 0·5 and 0·8 of exogenous supply for IJG and IDG infusions respectively. Portal glucose utilization, measured by isotope dilution across the portal-drained viscera, was unaffected by additional glucose regardless of the route of glucose supply ($P = 0·76$ for control *v.* glucose infusions) and was a constant proportion of glucose IL (0·28) for all treatments. Portal plasma flow was higher during IDG infusions compared with IJG infusions (1·65 *v.* 1·44 litres/min, $P = 0·055$). Circulating total free amino acid concentrations fell during glucose infusions (2146, 1808 and 1683 μmol/l for control, IJG and IDG treatments respectively, $P = 0·067$ for treatment effect) but net portal absorption was not affected by increased glucose supply. Recovery in the portal vein of [1-^{13}C]leucine infused into the duodenum averaged 0·65 and was not affected by increasing glucose supply to the gut tissues. The results show that glucose utilization by gut tissues is responsive to changes in both vascular and luminal glucose supply. The effects of changing gut tissue use of glucose and increased whole body glucose IL on metabolism of nutrients is discussed.

Glucose metabolism: Leucine absorption: Gut tissue metabolism: Sheep

The central role of the portal-drained viscera (PDV) in influencing the supply of nutrients to the liver is well established. In addition to the digestion, absorption and metabolism of ingested nutrients, the gut maintains a high rate of tissue turnover and synthesis of secretory products. These activities are associated with high rates of energy expenditure accounting for a disproportionate amount of whole-body O_2 consumption (for review, see Seal & Reynolds, 1993) and a significant requirement for amino acids in order to maintain the high rates of protein synthesis seen in these tissues (Lobley *et al.* 1980). Gut tissue requirements may be met from both absorbed nutrients and those of arterial origin. The relative contributions from these sources are dependent on the nutrients available in different regions of the intestine. Acetate utilization by ruminal tissues, for example, will primarily be of luminal origin whereas for small-intestinal tissues acetate is removed from the arterial supply (Bergman & Wolff, 1971; Reynolds & Huntington, 1988; Seal *et al.* 1992; Seal & Parker, 1994). In ruminants, under most dietary situations the amount of

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starch or glucose available in the lumen is very low (Armstrong & Smithard, 1979). Glucose requirements for gut tissue metabolism must be met from arterial sources and often result in net extraction of this nutrient across both the mesenteric-drained viscera and PDV (Parker, 1990). The present experiment was designed to investigate the effects of changing glucose supply to intestinal tissues either at the apical membrane by infusion directly into the duodenum or at the basolateral membrane by infusion of glucose into the peripheral circulation. Changes in glucose and amino acid uptake across the vascular bed were assessed by arterio–venous difference techniques and involved the use of [6-^{3}H]glucose and [1-^{13}C]leucine as tracers to monitor nutrient metabolism. Preliminary reports from the experiment have been published in abstract form (Piccioli Cappelli *et al.* 1993*a,b*).

MATERIALS AND METHODS

Animals and diets

Four Suffolk-cross wether sheep, average weight 43·6 (SD 3·85) kg, were used in the experiment. Chronic indwelling Silastic catheters (Dow Corning Corporation, Midland, MI, USA) were inserted in the portal vein, distal mesenteric vein and a carotid artery as previously described (Balcells *et al.* 1995). An additional catheter (1·016 mm i.d., 2·159 mm o.d.) was inserted directly into the duodenum via a purse-string suture 100 mm from the pylorus with a 50 mm tip inside the intestine. All surgery was completed at least 14 d before the start of the experiment and all sheep were back on full feed with *ad libitum* access to drinking water before the experiment began. The sheep were housed in individual metabolism cages and were fed at a metabolizable energy (ME) intake calculated at 1·3 × requirement for maintenance plus 50 g/d growth (Agricultural and Food Research Council, 1993), equivalent to 924 g DM/d of a dried grass pellet diet (g/kg DM: total N, 31·0; crude fibre, 234; gross energy 19·8 MJ/kg DM) in twenty-four equal portions fed hourly throughout the day using continuous belt feeders. There were no feed refusals throughout the whole experimental period. Temporary jugular catheters were inserted 24 h before sampling days for isotope and intravascular glucose infusions.

Infusions and sampling procedure

At 09.00 hours on sampling days each sheep received the following treatments for 6·5 h in random order, allowing 7 d between each treatment to ensure no carry-over between treatments; (a) control; intrajugular infusion of physiological saline (9 g NaCl/l, 1 ml/min) and intraduodenal infusion of distilled water (1 ml/min); (b) intrajugular glucose (IJG); intrajugular glucose infusion (0·5 M-glucose in physiological saline, 1 ml/min) and intraduodenal infusion of distilled water; (c) intraduodenal glucose (IDG); intrajugular infusion of physiological saline and intraduodenal glucose infusion (0·5 M-glucose in distilled water, 1 ml/min). For each treatment whole-body glucose irreversible loss (IL) rate was measured by intravenous infusion of [6-^{3}H]glucose. Simultaneous measurements of recovery of [1-^{13}C]leucine infused into the duodenum, net nutrient absorption rates and blood flow in the portal vein were determined for each treatment. A time-line for the experiment is shown in Fig. 1. All infusions of labelled and unlabelled substrates were non-primed and continued at a constant rate throughout the infusion period.

Measurement of whole-body glucose irreversible loss rate. A solution of [6-^{3}H]glucose containing 29·6 MBq [6-^{3}H]glucose/ml (Amersham International plc, Aylesbury, Bucks.) was prepared in autoclaved physiological saline. In the control and IDG treatments 1 mg

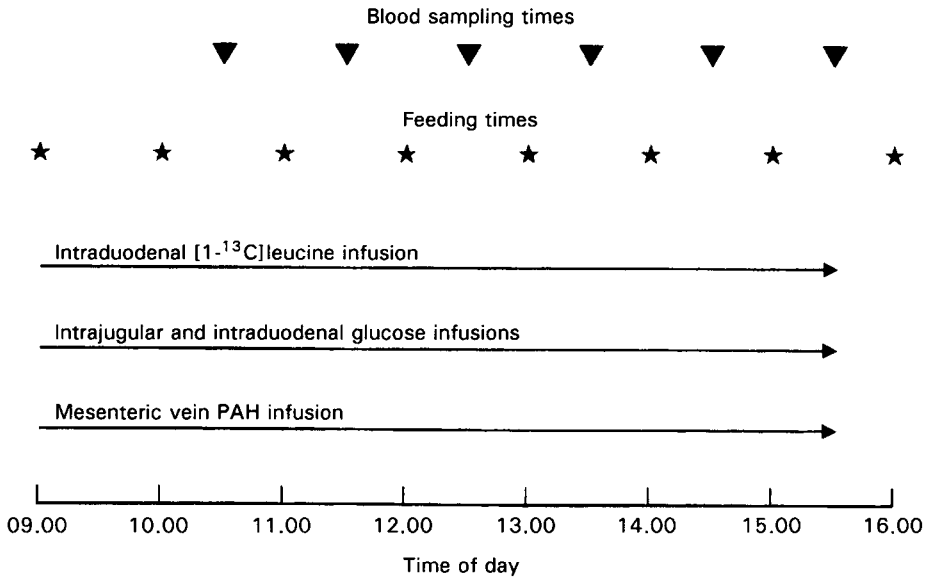

Fig. 1. Time line for infusion and sampling period. PAH, *p*-amino hippuric acid. For details of infusion procedure and composition of infusates, see pp. 932–933.

carrier glucose/ml was included in the solution which was infused for 6·5 h at approximately 1·0 ml/min using a Watson Marlow Model 501 peristaltic pump (Watson Marlow Ltd, Falmouth, Cornwall) via the temporary jugular catheter. In the IJG treatment the labelled glucose was added to the glucose infusion solution. At each sampling time 5 ml portions of whole blood from each catheter were frozen immediately for whole blood glucose specific radioactivity determinations.

[1-^{13}C]leucine uptake into the portal vein. [1-^{13}C]leucine (Tracer Technologies, Somerville, MA, USA) was added to duodenal infusates (0·833 mg [1-^{13}C]leucine 99 atoms percent /ml) immediately before starting infusions and was infused at approximately 1 ml/min using the duodenal infusion catheter. At each sampling time plasma from blood samples taken from each catheter was stored frozen at $-70°$ before analysis.

Blood flow. Blood flow in the portal vein was determined by downstream dye dilution using *p*-amino hippuric acid (PAH; Katz & Bergman, 1969). Blood samples (10 ml) were taken at the midpoint between feeds from the portal vein and carotid artery during the last 6 h of a 6·5 h continuous infusion of PAH into the distal mesenteric vein (15 mg PAH/ml per min infusion rate). Samples were kept on ice and processed immediately for subsequent metabolite analyses.

Analytical Methods

Blood metabolites and p-amino hippuric acid. Plasma glucose, lactate, NH_3, urea and 3-hydroxybutyrate concentrations were determined by standard enzymic procedures using commercial kits (Roche Diagnostics, Welwyn Garden City, Herts and Sigma Chemicals, Poole, Dorset). Volatile fatty acid concentrations in plasma were determined in the supernatant fraction from plasma deproteinized with perchloric acid as described previously (Seal & Parker, 1994). Plasma insulin concentrations were determined by specific radioimmunoassay by the method of Basset & Thornburn (1971) as modified by

Fuller *et al.* (1977). Plasma gastrin concentrations were determined by specific radioimmunoassay by the method of Perry *et al.* (1988). Plasma free amino acid concentrations were determined by reversed-phase HPLC after filtration through 10 K molecular mass filters (Millipore UK Ltd., Watford, Herts.) and pre-column derivatization with phenylisothiocyanate using a Waters Pico-Tag system (Waters Chromatography Division, Millipore Corporation, Milford, MA, USA). PAH in the supernatant fraction from whole blood samples deproteinized immediately after samples were obtained from each catheter (whole blood–TCA (100 g/l), 1:10, v/v) was determined colorimetrically (Katz & Bergman, 1969) after deacetylation by boiling in stoppered tubes for 30 min.

Analysis of glucose specific radioactivity. The level of radioactivity of blood glucose was determined after separation of metabolites by tandem ion-exchange resin columns (Janes *et al.* 1985). Lactate fractions isolated by this procedure were found to be contaminated with a ^{3}H-labelled compound which co-eluted with the lactate fraction (Neely *et al.* 1990; Virkamaki *et al.* 1990) and were not used for subsequent analyses or calculations. Glucose in isolated fractions was determined enzymically as described earlier before liquid scintillation counting on a Beckman LS 8100 Liquid Scintillation Counter (Beckman Instruments, Irvine, CA, USA) to determine the glucose specific radioactivity (disintegrations/min per mmol glucose). Counting efficiency was determined by the H-number method of quench monitoring, using the inflection point of the Compton edge with an external ^{135}Cs source.

Analysis of plasma [^{13}C]leucine, [^{13}C]ketoisocaproic acid and blood $^{13}CO_2$. Preparation of plasma for measurement of isotopic enrichment of free leucine and ketoisocaproic acid (KIC) was as described by Calder & Smith (1988). Leucine and KIC enrichments were determined after separation of the tertiary butyl-dimethylsilyl derivatives with a 30 m DB5 capillary column (0·25 mm i.d., 0·25 μm coating, J & W Scientific, Folsom, CA, USA) and electron-impact selective ion monitoring of mass fragments using a Finnigan MAT 1050 GC mass spectrometer (Finnigan MAT Ltd, Paradise, Hemel Hempstead, Herts.). Plasma KIC concentrations were determined under the same analytical conditions using *n*-hexanoic acid as internal standard. The isotopic enrichment of CO_2 in whole blood was determined by isotope ratio mass spectroscopy with a Europa ANCA 20:20 mass spectrometer (Europa Scientific, Crewe, Ches.) after release of dissolved CO_2 with H_3PO_4 using the method described by Scrimgeour & Rennie (1988). Whole-blood CO_2 concentrations were measured using a Nova Stat Profile 5 Analyser (Nova Biomedical, Waltham, MA, USA).

Calculations and statistical analysis

Values for metabolite concentrations, blood flow and net portal flux rates were averaged for the last four samples taken during the infusion period. Blood flow through the PDV was calculated using the equations of Katz & Bergman (1969) as described previously (Seal & Parker, 1994). Plasma flow was calculated as the measured value for whole blood flow corrected for packed cell volume. Net nutrient flux rates into plasma were calculated as the product of plasma flow rate and plasma venous–arterio (V–A) concentration differences for the PDV. Positive values indicate net appearance or absorption into the portal circulation and negative values indicate net extraction by the PDV tissues.

Net movement of isotopically labelled leucine metabolites was determined across the PDV using the following equation:

$$\text{net flux (mmol/min)} = (M_P \times E_P - M_A \times E_A) \times \text{portal plasma flow}, \quad (1)$$

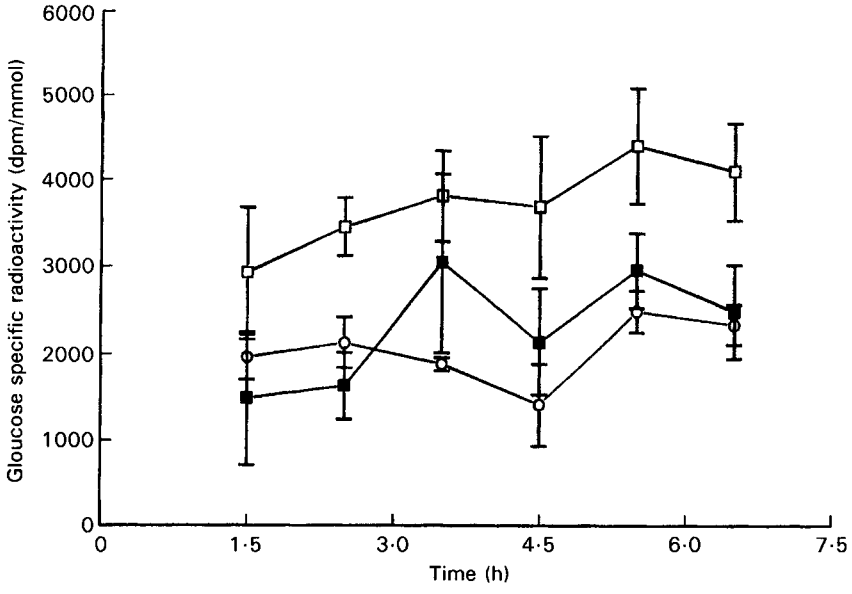

Fig. 2. Change in plasma glucose specific radioactivity (dpm/mmol) in sheep fed on a dried grass pellet diet during control (□), intrajugular glucose (■) and intraduodenal glucose (○) infusion periods. Values are means for four sheep, with their standard errors represented by vertical bars.

where M_P and M_A are the concentrations of each metabolite in portal and arterial plasma respectively and E_P and E_A are the enrichments of the metabolite (in atoms percent excess above background abundance) in the corresponding portal and arterial plasma. These calculations represent minimal values for net flux as they do not account for sequestration of label within gut tissues.

Whole-body glucose IL rate was determined by standard isotope dilution procedures (White *et al.* 1969) using the appropriate ratio of isotope infusion rate and pseudo-plateau glucose specific radioactivities which were achieved for all treatments during the last 3 h of [^{3}H]glucose infusions (Fig. 2). Portal glucose utilization rates were calculated using the isotope extraction ratio method (equation 2, Bergman *et al.* 1970) as described previously (Seal & Parker, 1994; Balcells *et al.* 1995):

$$\text{portal glucose utilization (mmol/min)} = ((S_A - S_P)/S_A) \times C_A \times \text{portal plasma flow.} \quad (2)$$

Where S_A and S_P are radioactivity concentrations (MBq/l) in arterial and portal blood respectively and C_A is the arterial glucose concentration. A simplified model based on that described by Seal & Parker (1994) and used by Balcells *et al.* (1995) was used to quantify glucose metabolism within the PDV. The model predicts values for the rates of arterial supply of glucose to the PDV (R1, equation 3), glucose leaving the PDV (R2, equation 4) and net glucose supply from the intestine (representing the balance of glucose absorbed from the lumen and extracted from arterial supply (R3, equation 5)):

$$\text{R1(mmol/min)} = \text{arterial glucose concentration} \times \text{portal plasma flow,} \quad (3)$$

$$\text{R2(mmol/min)} = \text{portal venous glucose concentration} \times \text{portal plasma flow,} \quad (4)$$

$$\text{R3(mmol/min)} = (\text{R2} - \text{R1} + \text{portal glucose utilization}). \quad (5)$$

The experiment was a four animal × three treatment completely randomized block design. Statistical analysis of the difference between means for each treatment was by ANOVA fitting animal and treatment effects into a linear model using the general linear models procedure (Minitab, State College, PA, USA). Treatment sums of squares were subdivided into two orthogonal contrasts *a priori*; contrast 1, control *v.* (IJG + IDG) and contrast 2, IJG *v.* IDG. Tables of results show mean values with probability values for each contrast; variables were considered unaffected by glucose infusion if $P > 0{\cdot}10$.

RESULTS

Glucose metabolism

Carotid arterial and portal venous plasma glucose concentrations were increased significantly during IJG and IDG infusions (Table 1, $P = 0{\cdot}024$ and $P = 0{\cdot}039$ respectively). On average there was no significant effect of glucose infusion on net portal flux compared with the control treatment. However, net portal absorption of glucose was lower during IJG infusions indicating increased tissue extraction which was higher than during IDG infusions (Table 1, $P = 0{\cdot}04$ for contrast 2). Glucose IL rate, as measured by dilution of infused [6-^{3}H]glucose, was increased by 0·5 and 0·8 of exogenous supply for IJG and IDG respectively (Table 1, $P = 0{\cdot}001$ for overall treatment effect). Portal glucose utilization (PGU) was numerically higher during IDG infusions but was not significantly affected by treatment and remained a constant proportion of glucose IL (average 0·28, Table 1) for all treatments. As a result of increased arterial and venous plasma glucose concentrations, the rates of glucose supply to, and leaving from, the PDV were significantly higher during glucose infusions (Table 1, contrast 1; $P = 0{\cdot}01$ and $P = 0{\cdot}01$ for R1 and R2 respectively) and were higher during IDG than during IJG infusions (Table 1, contrast 2; $P = 0{\cdot}01$ and $P = 0{\cdot}004$ for R1 and R2 respectively). Net glucose supply from the intestine

Table 1. *Carotid arterial and portal venous plasma glucose concentrations, net portal absorption rate and glucose kinetic data derived from isotope infusions in sheep (*n *4) fed on a dried grass pellet diet with intrajugular (IJG) and intraduodenal (IDG) glucose infusions**

	Treatment				*P*†	
	Control	IJG	IDG	ems‡	1	2
Plasma glucose (mmol/l)						
Carotid	4·18	4·86	4·68	0·067	0·01	0·37
Portal	4·12	4·68	4·68	0·070	0·01	0·99
Net portal absorption§ (mmol/min)	−0·10	−0·27	−0·01	0·021	0·70	0·04
Glucose kinetics (mmol/min)						
Irreversible loss (IL) rate	0·57	0·80	0·95	0·006	0·001	0·04
Portal glucose utilization (PGU)	0·25	0·21	0·37	0·039	0·76	0·28
PGU/glucose IL	0·43	0·25	0·39	0·074	0·52	0·49
R1‖	6·60	6·94	7·73	0·098	0·01	0·01
R2	6·50	6·70	7·73	0·105	0·01	0·004
R3§	0·16	−0·05	0·37	0·099	0·98	0·11

* For details of infusion procedure, see pp. 932–933.

† Significance probability values for contrast 1; control *v.* (IJG + IDG); contrast 2, IJG *v.* IDG.

‡ Error mean square, 6 df.

§ Negative values indicate net utilization of metabolite by the gut tissues, positive values indicate net uptake into portal blood.

‖ R1, arterial glucose supply to the portal-drained viscera; R2, glucose leaving the portal-drained viscera; R3, net glucose supply from intestine (R2 − R1 + PGU).

Table 2. *Total and ^{13}C-labelled carotid arterial leucine and ketoisocaproate concentrations, portal–arterial concentration difference and net portal absorption rate into plasma of sheep (n 4) fed on a dried grass pellet diet with intrajugular (IJG) and intraduodenal (IDG) glucose infusions**

	Treatment				*P*†	
	Control	IJG	IDG	ems‡	1	2
Arterial concentration (μmol/l)						
Leucine	227	184	143	1650·0	0·04	0·20
Ketoisocaproic acid	31·1	19·7	20·7	41·42	0·02	0·83
[^{13}C]leucine	9·41	8·08	4·68	6·25	0·10	0·10
[^{13}C]ketoisocaproic acid	0·83	0·60	0·52	0·03	0·84	0·30
Portal–arterial difference (μmol/l)						
Leucine	26·5	25·5	16·0	275·9	0·59	0·45
Ketoisocaproic acid	1·3	2·8	1·8	12·03	0·67	0·72
[^{13}C]leucine	2·16	3·17	1·88	1·120	0·59	0·14
[^{13}C]ketoisocaproic acid	0·13	0·13	0·19	0·065	0·85	0·72
Net portal absorption (μmol/min)§						
Leucine	41·5	39·0	27·0	615·5	0·60	0·52
Ketoisocaproic acid	1·9	4·1	3·1	27·68	0·64	0·80
[^{13}C]leucine	3·42	4·73	3·13	3·090	0·65	0·25
[^{13}C]ketoisocaproic acid	0·21	0·18	0·33	0·158	0·86	0·61
$^{13}CO_2$	0·04	0·06	0·04	0·0006	0·69	0·20
Recovery of [^{13}C]leucine infused as						
[^{13}C]leucine	0·60	0·82	0·52	0·099	0·73	0·23
[^{13}C]ketoisocaproic acid	0·04	0·03	0·06	0·005	0·91	0·64
$^{13}CO_2$	0·008	0·011	0·007	0·00002	0·69	0·18

* For details of infusion procedure, see pp. 932–933.
† Probability values for contrast 1; control *v.* (IJG + IDG); contrast 2, IJG *v.* IDG.
‡ Error mean square, 6 df.
§ Positive values indicate net uptake of metabolite into portal blood.

(R3) became negative during IJG infusions but rose when glucose was infused into the duodenum (IDG) (Table 1, contrast 2; $P = 0{\cdot}11$).

Leucine metabolism across the portal-drained viscera

Arterial leucine, KIC and [^{13}C]leucine concentrations were all significantly reduced during glucose infusions compared with the control sheep (Table 2). There was a similar fall in [^{13}C]KIC concentration but this was not statistically significant. There was a corresponding fall in portal venous concentration for all these metabolites resulting in no change in either portal–arterial concentration difference or net flux of the metabolites across the PDV. Averaged across all treatments 0·65 of [^{13}C]leucine infused into the duodenum was recovered as [^{13}C]leucine in the portal vein, 0·04 as [^{13}C]KIC and 0·006 as $^{13}CO_2$. There was no change in metabolism of leucine across the PDV as a result of glucose infusion.

Plasma metabolite concentrations and net portal absorption rates

Arterial packed cell volume values and portal plasma flow rates are shown in Table 3. Packed cell volume was not affected by treatment and did not change during sampling days or during the course of the experiment. Portal plasma flow was significantly higher during IDG infusions than during IJG infusions ($P = 0{\cdot}001$ for contrast 2). Plasma urea

Table 3. *Arterial packed cell volume and portal plasma flow, carotid arterial metabolite concentrations, portal–arterial concentration difference and net portal absorption rate into plasma for sheep* (n 4) *fed on a dried grass pellet diet with intrajugular (IJG) and intraduodenal (IDG) glucose infusions**

	Treatment				*P*†	
	Control	IJG	IDG	ems‡	1	2
Packed cell volume	0·27	0·28	0·27	0·0003	0·80	0·43
Portal plasma flow (litres/min)	1·58	1·44	1·65	0·003	0·36	0·001
Arterial concentration (mmol/l)						
Lactate	0·75	0·75	0·82	0·015	0·39	0·18
Ammonia	0·06	0·04	0·03	0·0003	0·12	0·48
Urea	4·43	4·34	3·95	0·076	0·14	0·09
Acetate	2·32	2·10	2·26	0·118	0·55	0·55
Propionate	0·14	0·14	0·13	0·0003	0·55	0·53
β-Hydroxybutyrate	0·40	0·36	0·36	0·008	0·51	0·91
Gastrin (pg/ml)	69	66	80	37·8	0·34	0·02
Insulin (μU/ml)	35	75	62	600·3	0·07	0·48
Portal–arterial difference (mmol/l)‖						
Lactate	0·11	−0·04	0·16	0·025	0·64	0·13
Ammonia	0·13	0·15	0·15	0·0002	0·11	0·80
Urea	0·08	0·05	0·01	0·005	0·28	0·49
Acetate	0·98	0·99	1·12	0·019	0·38	0·24
Propionate	0·33	0·35	0·37	0·004	0·41	0·65
Butyrate§	0·07	0·08	0·07	0·0004	0·71	0·75
β-Hydroxybutyrate	0·07	0·06	0·08	0·0004	0·97	0·25
Gastrin (pg/ml)	5	11	−4	41·3	0·78	0·02
Insulin (μU/ml)	12	30	41	109·6	0·01	0·20
Net portal absorption (mmol/min)‖						
Lactate	0·19	−0·04	0·27	0·057	0·63	0·11
Ammonia	0·22	0·22	0·26	0·0001	0·10	0·003
Urea	0·13	0·07	0·02	0·016	0·30	0·55
Acetate	1·55	1·43	1·84	0·054	0·59	0·04
Propionate	0·52	0·51	0·62	0·008	0·50	0·15
Butyrate	0·11	0·11	0·12	0·001	0·83	0·73
β-Hydroxybutyrate	0·12	0·09	0·13	0·001	0·78	0·11
Gastrin (pg/min)	8	16	−6	113·3	0·69	0·03
Insulin (μU/min)	19	43	68	288·8	0·01	0·09

* For details of infusion procedure see pp. 932–933.
† Probability values for contrast 1; control *v.* (IJG + IDG); contrast 2, IJG *v.* IDG.
‡ Error mean square, 6 df.
§ Not detected in carotid arterial plasma.
‖ Negative values indicate net utilization of metabolite by the gut tissues, positive values indicate net uptake into portal blood.

concentrations fell as a result of glucose infusions and were significantly lower during IDG compared with IJG ($P = 0{\cdot}09$ for contrast 2); however, there was no effect on portal–arterial difference or net flux of urea across the PDV as a result of increased glucose supply. Net acetate appearance into portal blood was increased during IDG infusions ($P = 0{\cdot}04$ for contrast 2) as a consequence of a small increase in portal–arterial concentration difference and the increased blood flow observed for this group. There were similar numerical increases observed for propionate and butyrate, but these did not attain statistical significance.

Arterial plasma gastrin concentrations were significantly higher during IDG compared with either control or IJG treatments and there was net removal of the hormone across the PDV during this treatment (P= 0·03 for contrast 2, Table 3). Circulating insulin

Table 4. *Carotid arterial plasma free amino acid (AA) concentrations, portal–arterial concentration difference and net portal absorption rate into plasma of sheep (n 4) fed on a dried grass pellet diet with intrajugular (IJG) and intraduodenal (IDG) glucose infusions**

	Treatment				*P*†	
	Control	IJG	IDG	ems‡	1	2
Arterial concentration (μmol/l)						
Total AA	2146	1808	1683	52273·0	0·029	0·468
Essential AA	1027	797	785	16523·0	0·024	0·905
Non-essential AA	1120	1012	898	13829·0	0·062	0·220
Branched-chain AA	651	478	451	7062·0	0·011	0·668
Portal–arterial difference (μmol/l)						
Total AA	221	277	278	4837·0	0·234	0·984
Essential AA	121	111	124	2474·0	0·913	0·738
Non-essential AA	100	166	154	2143·0	0·080	0·740
Branched-chain AA	50	63	65	692·5	0·430	0·901
Net portal absorption (μmol/min)§						
Total AA	338	449	480	16538·0	0·160	0·737
Essential AA	187	189	215	9831·0	0·819	0·721
Non-essential AA	151	260	265	3902·0	0·027	0·902
Branched-chain AA	79	107	112	2436·0	0·345	0·901

* For details of infusion procedure, see pp. 932–933.
† Probability values for contrast 1; control *v.* (IJG + IDG); contrast 2, IJG *v.* IDG.
‡ Error mean square, 6 df.
§ Positive values indicate net uptake of metabolite into portal blood.

concentrations were doubled during glucose infusions (Table 3, $P = 0{\cdot}07$ for contrast 1). Net secretion of insulin into portal blood was increased during glucose infusions and was higher during IDG compared with IJG ($P = 0{\cdot}01$ and $P = 0{\cdot}09$ for contrasts 1 and 2 respectively).

Arterial total plasma free amino acid concentrations fell significantly during glucose infusions (Table 4, $P = 0{\cdot}03$) and the effects were similar for essential (EAA, $P = 0{\cdot}02$), non-essential (NEAA, $P = 0{\cdot}06$) and branched-chain (BCAA, $P = 0{\cdot}01$) groupings of amino acids. There was no effect of site of glucose infusion ($P > 0{\cdot}10$ for contrast 2 for all groups) although arterial concentrations were numerically lower during IDG compared with IJG. The fall in arterial total free amino acid concentrations was greater than that in portal venous plasma, resulting in an apparent increase in portal–arterial difference and net portal amino acid absorption during glucose infusions (Table 4). Unlike the changes in arterial amino acid concentrations, which were consistent across all amino acid groups, the increased portal–arterial difference and net portal absorption was only found in the NEAA group ($P = 0{\cdot}08$ and $P = 0{\cdot}03$ for contrast 1 for portal–arterial difference and net portal absorption respectively). Data for individual NEAA are shown in Table 5 and these show that within this group of amino acids net portal absorption of citrulline ($P = 0{\cdot}02$ for contrast 1), glycine ($P = 0{\cdot}01$ for contrast 1) and ornithine ($P = 0{\cdot}03$ for contrast 2) was significantly affected by increased glucose supply.

DISCUSSION

For ruminants fed on a mixed diet high in forages, the amount of α-linked glucose polymer flowing into the small intestine is low and the contribution of absorbed glucose to whole-body glucose supply is thought to be minimal. As the content of cereal or protected starch is increased in the diet, however, significant quantities of starch may escape fermentation

Table 5. *Carotid arterial non-essential free amino acid concentrations, portal–arterial concentration difference and net portal absorption rate into plasma for sheep* (n 4) *fed on a dried grass pellet diet with intrajugular (IJG) and intraduodenal (IDG) glucose infusions**

	Treatment				*P*†	
	Control	IJG	IDG	ems‡	1	2
Arterial concentration (μmol/l)						
Alanine	102·5	93·4	103·9	165·57	0·64	0·29
Asparagine	27·1	20·1	26·7	32·97	0·33	0·16
Aspartic acid	12·1	12·3	7·3	8·39	0·24	0·05
Citrulline	128·2	105·2	88·6	314·93	0·03	0·23
Glutamic acid	85·1	82·0	60·9	115·70	0·08	0·03
Glutamine	189·1	175·7	153·2	725·92	0·19	0·28
Glycine	268·2	254·8	214·2	1353·67	0·19	0·17
Ornithine	54·9	44·2	44·1	172·70	0·23	0·99
Proline	89·7	82·9	68·7	189·47	0·15	0·20
Serine	34·0	34·7	36·1	38·18	0·73	0·76
Portal–arterial difference (μmol/l)						
Alanine	28·8	39·5	24·5	267·82	0·76	0·24
Asparagine	9·6	16·2	5·4	59·37	0·81	0·09
Aspartic acid	0·5	1·8	2·8	5·10	0·23	0·56
Citrulline	1·4	14·8	15·8	57·81	0·03	0·85
Glutamic acid	2·8	2·1	14·5	193·55	0·55	0·26
Glutamine	−3·5	7·1	14·6	230·55	0·18	0·51
Glycine	11·9	34·7	34·3	159·25	0·03	0·96
Ornithine	−2·1	−1·7	6·1	19·01	0·16	0·04
Proline	23·9	13·4	14·8	325·10	0·41	0·92
Serine	16·3	13·7	10·5	51·56	0·38	0·56
Net portal absorption (μmol/min)§						
Alanine	44·5	59·9	42·3	366·70	0·73	0·27
Asparagine	14·5	25·4	9·7	181·48	0·73	0·15
Aspartic acid	0·7	2·8	4·9	11·89	0·12	0·40
Citrulline	1·8	23·7	27·3	138·25	0·02	0·67
Glutamic acid	3·3	2·1	24·5	552·15	0·51	0·23
Glutamine	−5·6	9·1	23·8	683·87	0·22	0·46
Glycine	17·3	56·1	58·4	307·02	0·01	0·86
Ornithine	−2·9	−3·6	10·5	48·86	0·53	0·03
Proline	38·9	21·6	25·8	1103·00	0·48	0·86
Serine	25·1	21·2	18·6	177·68	0·55	0·79

* For details of infusion procedure, see pp. 932–933.
† Probability values for contrast 1; control *v.* (IJG + IDG); contrast 2, IJG *v.* IDG.
‡ Error mean square, 6 df.
§ Negative values indicate net utilization of metabolite by the gut tissues, positive values indicate net uptake into portal blood.

and pass into the duodenum depending on the type of cereal fed (Nocek & Taminga, 1991; Kreikemeier & Harmon, 1995). In experiments in which intestinal infusion rates have been compared with portal recovery of glucose in the portal vein, between 29 and 84 % of the infused glucose has been detected (Kreikemeier *et al.* 1991; Seal *et al.* 1993; Kreikemeier & Harmon, 1995). These results demonstrate that ruminant intestinal tissues have the ability to absorb luminal glucose and separate studies involving the use of phlorizin (Bauer *et al.* 1995) and 2-deoxyglucose (Krehbiel *et al.* 1996) confirm that this process is mediated by the Na^+-dependent glucose co-transporter. None of these studies, however, has measured changes in glucose metabolism of the gut tissues per se in response to increased supply of the metabolite. The aim of the present experiment was to compare the effects of

changing glucose supply at the luminal or serosal surface on metabolism of glucose and leucine across gut tissues using a combination of arterio–venous and tracer techniques.

Glucose metabolism

Although on average for the last 4 h of IDG infusions there was no apparent net appearance of glucose across the PDV compared with control and IJG infusions (Table 1), Fig. 3 shows that there were differences between the treatments in the relationship between portal venous and arterial blood glucose concentrations during the infusions. Between 2·5 and 5·5 h from the start of the IDG infusion portal glucose concentration was higher than that in the arterial blood indicating net absorption of glucose whereas the net uptake of glucose by gut tissues observed during control periods (shown by a negative V–A difference) increased during the IJG infusions. These observations support previous studies which have shown that glucose infused in the small intestine of ruminants is readily absorbed into the portal vein even in animals which have not previously consumed diets in which glucose or

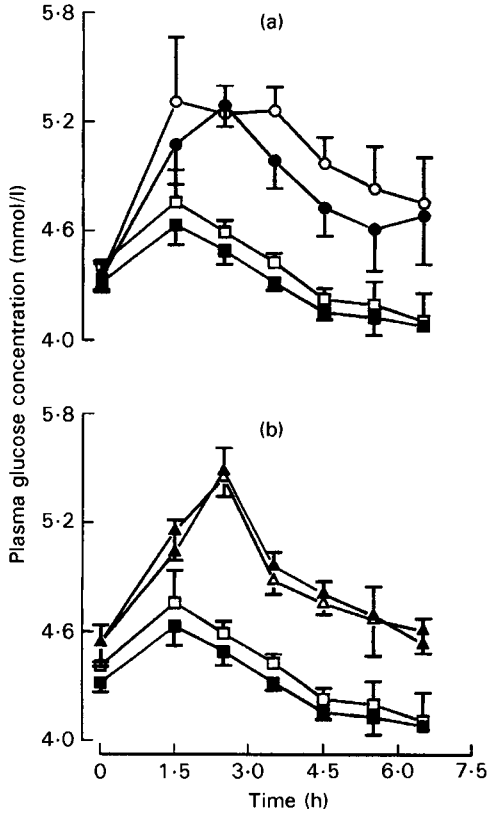

Fig. 3. Plasma glucose concentration (mmol/l) in sheep (*n* 4) fed on a dried grass pellet diet during intrajugular glucose (IJG) and intraduodenal glucose (IDG) infusion periods. (a) Control carotid arterial (□—□), control portal venous (■—■) and IJG carotid arterial (○—○), IJG portal venous (●—●) plasma samples. (b) Control carotid arterial (□—□), control portal venous (■—■) and intraduodenal (IDG) carotid arterial (△—△), IDG portal venous (▲—▲) plasma samples. Values are means with their standard errors represented by vertical bars. For details of infusion procedure, see pp. 932–933.

glucose polymers would be expected to reach this area of the gut (Seal *et al.* 1993; Bauer *et al.* 1995; Kriekemeier & Harmon, 1995).

The measured rate of exogenous glucose infused in the present experiment was similar to basal IL rate based on another experiment from this laboratory (Balcells *et al.* 1995) as intended. The data in Table 1 show that IL rate increased by 0·5 of the exogenous supply during IJG infusions, suggesting that endogenous glucose synthesis (total glucose IL rate minus exogenous supply) was decreased during glucose infusion. This is in contrast to our previous observations (Balcells *et al.* 1995) but is consistent with other studies in cattle and goats (Lomax *et al.* 1979; Baird *et al.* 1980; Chaiyabutr *et al.* 1983) and may reflect insulin-induced increased liver glycogen synthesis either from gluconeogenic precursors at the expense of glucose formation or by direct inhibition of gluconeogenesis via glucose-6-phosphate formation (Stangassinger & Gieseke, 1986). Although PGU increased during IDG infusions (Table 1) it remained at 0·28 of glucose IL rate in agreement with our previous observations (Balcells *et al.* 1995). As a consequence of increased glucose turnover and the elevated circulating concentrations of glucose, the arterial supply of glucose to the PDV (R1) and glucose leaving the PDV in the venous drainage (R2) were both significantly increased during the infusions and were higher during IDG when compared with IJG (Table 1). Net glucose supply from the intestine (R3) was positive during the IDG infusion in contrast to the negative value with IJG administration.

The design of the current experiment in which V–A difference studies were coupled with isotope dilution methods provided data whereby glucose uptake and utilization across the gut could be evaluated using different variables. It is apparent that net absorption during IDG infusions calculated from plasma concentration and/or plasma flow measurements indicate low overall recovery of infused glucose (0·4 if account is taken of arterial glucose utilization). This value can be compared with absorption of 0·7 of the infused glucose based on the isotope exchange data (R3) although this value would also be 0·4 if account were taken of the glucose apparently absorbed during the control glucose infusion period. Conversely, changes in glucose IL rate during IDG infusion suggest a greater contribution of the substrate to whole-body glucose turnover (0·8 if one assumes no change in endogenous supply). Resolution of these discrepancies requires the simultaneous infusion of different isotopically-labelled substances into the gut and peripheral circulation in order to quantify the pathways of uptake and metabolism across the intestine. This approach would, for example, provide evidence for the extent of glucose oxidation during absorption and the relationship between arterial and luminal supply to the tissue.

Leucine metabolism across the portal drained viscera

We have previously reported (Seal *et al.* 1992; Balcells *et al.* 1995; Seal & Parker, 1996) that amino acid flux across the PDV is responsive to changes in diet and the supply of energy-yielding substrates within the gastrointestinal tissues. It is unclear from these studies whether flux rates were affected by modification of transport rates through intestinal tissues into portal blood, sequestration by intestinal tissues or metabolism within the tissues per se. We chose to use [1-^{13}C]leucine in this experiment to quantify amino acid metabolism during absorption across the gastrointestinal tract. This does not take into account possible sequestration of arterial amino acid, which may be quantified by intravenous isotope infusion (MacRae *et al.* 1993). Glucose infusion caused a significant fall in circulating leucine and total free amino acid concentrations compared with control animals but had no effect on net portal flux (Tables 2 and 4). While the concentrations of leucine and [^{13}C]leucine in arterial plasma declined during the IDG infusion, the levels of

enrichment were very similar across treatments. These changes are compatible with an increase in overall leucine uptake by tissues resulting from increased insulin secretion. This is in contrast to our previous observations with sheep receiving comparable amounts of glucose intravenously (Balcells *et al.* 1995) in which circulating free amino acid concentrations were unaffected by glucose infusion and net portal flux fell with increasing glucose supply. These differences may relate to the higher N intake of the forage-fed sheep in this experiment coupled with the lower response to glucose infusion in terms of plasma glucose and insulin concentration in these animals compared with the straw–cereal-fed sheep described previously (Balcells *et al.* 1995). There was no change in net portal flux of [1-^{13}C]leucine and recovery of intraduodenally infused isotope averaged 0·65. Approximately 0·05 of infused [1-^{13}C]leucine was recovered as [^{13}C]KIC and less than 0·01 as $^{13}CO_2$. Label not recovered in the portal vein (0·35 of total infused) may represent unabsorbed amino acid or sequestration of label from the lumen and from arterial blood within the PDV. Comparison of our data with those of Pell *et al.* (1986) provides further support for the hypothesis that in sheep utilization of leucine by the gut is directed primarily at tissue protein synthesis, with only limited transamination of leucine and oxidation to KIC. In the present study, net output of leucine across the PDV averaged 36 μmol/min across the treatment groups compared with 15 μmol/min reported by Pell *et al.* (1986), reflecting the difference in N intake between the two studies (28·6 g N/d and 14·1 g N/d respectively). Despite these differences in leucine flux the net output of KIC across the PDV was the same at 2–3 μmol/min in both studies. The source of this metabolite was previously identified as direct transamination of free leucine and not absorption from the gut (Pell *et al.* 1986). In the present study less than 0·01 of duodenally infused [^{13}C]leucine was recovered as CO_2 in portal blood, in agreement with previous stable isotope infusions (Pell *et al.* 1986) in which there was no significant oxidation of leucine by gut tissues in either fed or fasted sheep. In our experiments the provision of additional glucose either intravenously or intraduodenally had no effect on these variables.

Plasma hormone and metabolite concentrations and net portal absorption rates

The increased plasma gastrin concentration during the IDG infusion is interesting since this represents a hormonal response to luminal glucose in the proximal small intestine. This may be due to stimulation of secretion of duodenal gastrin rather than that of antral origin and changes in extraction of the hormone by the liver. Actions of gastrin include stimulation of gastric blood flow and insulin secretion (Reynolds *et al.* 1991) and both portal plasma flow and insulin concentrations were increased during IDG infusion compared with IJG infusion (Table 3). There were no changes in arterial concentrations for metabolites determined during the experiment, and little change in portal–arterial concentration differences. There were, however, significant increases in the net portal fluxes of NH_3 and acetate during IDG compared with IJG infusions (Table 3). Increased portal acetate appearance may be due to reduced metabolism of this substrate by gastrointestinal tissues in response to increased utilization of glucose, as has been demonstrated previously in steers receiving intraruminal propionate infusion (Seal & Parker, 1994). In contrast to these previous studies with propionate there was no significant increase in net uptake of EAA across the PDV as a consequence of glucose infusion, although there was a significant increase in net uptake of NEAA associated with increased glucose supply (Table 4). Within this group of amino acids (Table 5) there were significant increases in net output of glycine and citrulline across the PDV in addition to changes in the net flux of glutamine indicating a shift from net uptake in the control animals to net

output during glucose infusions. In non-ruminants, glutamine is established as a major energy source for gut tissue metabolism (Windmeuller & Spaeth, 1978) and studies with intact pigs and enterocytes indicate that glutamine utilization was linked to release of citrulline (Wu *et al.* 1994). These observations would appear to be in contrast to results from the present experiment in sheep in which during glucose infusion there was release of both citrulline and glutamine from the PDV. The role of glutamine in the overall metabolism of gut tissues in the ruminant has yet to be established but these observations suggest that it may not have the same quantitative importance in energy metabolism as suggested in non-ruminant species.

The results from this experiment underline the importance of glucose as an energy substrate for the ruminant gastrointestinal tract, and confirm the sensitivity of this metabolism to the availability of the substrate from both luminal and vascular sources. The complex interaction between carbohydrate and N metabolism in the gut further emphasizes the importance of these tissues in controlling the release of nutrients to the liver and peripheral tissues for productive use.

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REFERENCES

Agricultural and Food Research Council (1993). *Energy and Protein Requirements of Ruminants. An Advisory Manual Prepared by the AFRC Technical Committee on Responses to Nutrients.* Wallingford: CAB INTERNATIONAL.

Armstrong, D. G. & Smithard, R. R. (1979). The fate of carbohydrate in the small intestine of the ruminant. *Proceedings of the Nutrition Society* **38**, 283–294.

Baird, G. D., Lomax, M. A., Symonds, H. W. & Shaw, S. R. (1980). Net hepatic and splanchnic metabolism of lactate, pyruvate and propionate in dairy cows *in vivo* in relation to lactation and nutrient supply. *Biochemical Journal* **186**, 47–57.

Balcells, J., Seal, C. J. & Parker, D. S. (1995). Effect of intravenous glucose infusion on metabolism of portal-drained viscera in sheep fed a cereal/straw-based diet. *Journal of Animal Science* **73**, 2146–2155.

Bassett, J. M. & Thornburn, G. D. (1971). The regulation of insulin secretion by the ovine foetus in utero. *Journal of Endocrinology* **50**, 59–74.

Bauer, M. L., Harmon, D. L., McLeod, K. R. & Huntington, G. B. (1995). Adaptation to small intestinal starch assimilation and glucose transport in ruminants. *Journal of Animal Science* **73**, 1828–1838.

Bergman, E. N., Katz, M. L. & Kaufman, C. F. (1970). Quantitative aspects of hepatic and portal glucose metabolism and turnover in sheep. *American Journal of Physiology* **219**, 785–793.

Bergman, E. N. & Wolff, J. E. (1971). Metabolism of volatile fatty acids by liver and portal-drained viscera in sheep. *American Journal of Physiology* **221**, 586–592.

Calder, A. G. & Smith, A. (1988). Stable isotope ratio analyses of leucine and ketoisocaproic acid in blood plasma by gas chromatography/mass spectrometry. *Rapid Communications in Mass Spectrometry* **2**, 14–16.

Chaiyabutr, N., Faulkner, A. & Peaker, M. (1983). Effects of exogenous glucose on glucose metabolism in the lactating goat. *British Journal of Nutrition* **49**, 159–165.

Fuller, M. F., Weekes, T. E. C., Cadenhead, A. & Bruce, J. B. (1977). The protein-sparing effect of carbohydrate 2. The role of insulin. *British Journal of Nutrition* **38**, 489–496.

Janes, A. N., Weekes, T. E. C. & Armstrong, D. G. (1985). Absorption and metabolism of glucose by the mesenteric-drained viscera of sheep fed on dried-grass or ground, maize-based diets. *British Journal of Nutrition* **54**, 449–458.

Katz, M. L. & Bergman, E. N. (1969). Simultaneous measurements of hepatic and portal venous blood flow in the sheep and dog. *American Journal of Physiology* **216**, 946–952.

Krehbiel, C. R., Britton, R. A., Harmon, D. L., Peters, J. P., Stock, R. A. & Grotjan, H. E. (1996). Effects of varying levels of duodenal or midjejunal glucose and 2-deoxyglucose infusion on small intestinal disappearance and net portal glucose flux in steers. *Journal of Animal Science* **74**, 693–700.

Kreikemeier, K. K. & Harmon, D. L. (1995). Abomasal glucose, maize starch and maize dextrin infusions in cattle: small-intestinal disappearance, net portal glucose flux and ileal oligosaccharide flow. *British Journal of Nutrition* **73**, 763–772.

Kreikemeier, K. K., Harmon, D. L., Brandt, R. T. Jr, Avery, T. B. & Johnson, D. E. (1991). Small intestinal starch digestion in steers: effect of various levels of abomasal glucose, corn starch and corn dextrin infusion on small intestinal disappearance and net glucose absorption. *Journal of Animal Science* **69**, 328–338.

Lobley, G. E., Milne, V., Lovie, J. M., Reeds, P. J. & Pennie, K. (1980). Whole-body and tissue protein synthesis in cattle. *British Journal of Nutrition* **43**, 491–496.

Lomax, M. A., Baird, G. B., Mallison, C. B. & Symonds, H. W. (1979). Differences between lactating and non-lactating dairy cows in concentration and secretion rate of insulin. *Biochemical Journal* **180**, 281–289.

MacRae, J. C., Lobley, G. E., Bruce, L. A., Luo, Q., Calder, A. G. & Farningham, D. A. H. (1993). Leucine kinetics across the portal drained viscera of sheep. *Journal of Animal Science* **71**, Suppl. 1, 279 Abstr.

Neely, R. D. G., Rooney, D. P., Atkinson, A. B., Sheridan, S., Ennis, C. N., Trimble, E. R. & Bell, P. M. (1990). Underestimation of glucose turnover determined using [6-^{3}H]glucose tracer in non-steady state. The role of a tritiated tracer impurity. *Diabetologia* **33**, 681–687.

Nocek, J. E. & Taminga, S. (1991). Site of digestion of starch in the gastrointestinal tract of dairy cows and its effect on milk yield and composition. *Journal of Dairy Science* **74**, 3598–3629.

Parker, D. S. (1990). Manipulation of the functional activity of the gut by dietary and other means (antibiotics/probiotics) in ruminants. *Journal of Nutrition* **120**, 639–648.

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

Perry, K. W., Weekes, T. E. C., Rooke, J. A., Parker, D. S. & Armstrong, D. A. (1988). Effect on protein intake of gastrin secretion in the ruminant. *Quarterly Journal of Experimental Physiology* **73**, 985–993.

Piccioli Cappelli, F., Seal, C. J. & Parker, D. S. (1993*a*). Portal glucose absorption and utilisation in sheep receiving exogenous glucose intravascularly or intraduodenally. *Journal of Animal Science* **71**, Suppl. 1, 279 Abstr.

Piccioli Cappelli, F., Seal, C. J. & Parker, D. S. (1993*b*). [1-^{13}C]Leucine uptake into the portal vein of sheep receiving exogenous glucose intravascularly or intraduodenally. *Proceedings of the Nutrition Society* **52**, 192A.

Reynolds, C. K. & Huntington, G. B. (1988). Partition of portal-drained visceral net flux in beef steers. 1. Blood flow and net flux of oxygen, glucose and nitrogenous compounds across stomach and post-stomach tissues. *British Journal of Nutrition* **60**, 539–551.

Reynolds, G. W., Simpson, H. V., Carr, D. H. & McLeay, L. M. (1991). Gastrin: its molecular forms and secretion in sheep. In *Proceedings of the 7th International Symposium on Ruminant Physiology, Sendai, Japan*, pp. 63–87 [T. Tsuda, Y. Sasaki and R. Kawashima, editors]. London: Academic Press.

Scrimgeour, C. M. & Rennie, M. J. (1988) Automated measurement of the concentration and ^{13}C enrichment of carbon dioxide in breath and blood samples. *Biomedical and Environmental Mass Spectrometry* **15**, 365–367.

Seal, C. J. & Parker, D. S. (1994). Effect of intraruminal propionic acid infusion on metabolism of mesenteric- and portal-drained viscera in growing steers fed a forage diet: I. Volatile fatty acids, glucose and lactate. *Journal of Animal Science* **72**, 1325–1334.

Seal, C. J. & Parker, D. S. (1996). Effect of intraruminal propionic acid infusion on metabolism of mesenteric- and portal-drained viscera in growing steers fed a forage diet: II. Ammonia, urea, amino acids and peptides. *Journal of Animal Science* **74**, 245–256.

Seal, C. J., Parker, D. S. & Avery, P. J. (1992). The effect of forage and forage-concentrate diets on rumen fermentation and metabolism of nutrients by mesenteric- and portal-drained viscera in growing steers. *British Journal of Nutrition* **67**, 355–370.

Seal, C. J., Parker, D. S. & Lobley, G. E. (1993). Effect of intraduodenal starch infusion on glucose metabolism of growing steers fed alfalfa. *Journal of Animal Science* **70**, Suppl. 1, 279 Abstr.

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

Stangassinger, M. & Gieseke, D. (1986). Splanchnic metabolism of glucose and related energy substrates. In *Control of Digestion and Metabolism in Ruminants* [L. P. Milligan, W. L. Grovum and A. Dobson, editors]. Englewood Cliffs, NJ: Prentice Hall.

Virkamaki, A., Puhakainen, N., Nurjhan, N., Gerich, J. E. & Yki-Järvinen, H. (1990). Measurement of lactate formation from glucose using [6-^{3}H] and [6-^{14}C]glucose in humans. *American Journal of Physiology* **259**, E397–E404.

White, R. G., Steel, J. E., Leng, R. A. & Luik, J. R. (1969). Evaluation of three isotope-dilution techniques for studying the kinetics of glucose metabolism in sheep. *Biochemical Journal* **114**, 203–214.

Windmeuller, H. G. & Spaeth, A. E. (1978). Identification of ketone bodies and glutamine as the major respiratory fuels *in vivo* for postabsorptive rat small intestine. *Journal of Biological Chemistry* **253**, 69–76.

Wu, G., Borbolla, A. G. & Knabe, D. A. (1994). The uptake of glutamine and release of arginine, citrulline and proline by the small intestine of developing pigs. *Journal of Nutrition* **124**, 2437–2444.