Influence of dietary leucine content on the activities of branched-chain amino acid aminotransferase (*EC* 2.6.1.42) and branched-chain α-keto acid dehydrogenase (*EC* 1.2.4.4) complex in tissues of preruminant lambs

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1. Branched-chain amino acid aminotransferase (*EC* 2.6.1.42; BCAAT) and branched-chain α-keto acid dehydrogenase (*EC* 1.2.4.4; BCKDH) activities were measured in preruminant lamb liver, longissimus dorsi muscle, kidney, jejunum and adipose tissue, 2 h after a meal with or without an excess of leucine.

2. Skeletal muscle contained about 70% of the total basal BCAAT activities of the tissues studied whereas liver contained about 60% of the total BCKDH activities of these tissues.

3. BCAAT activities were very low in preruminant lamb tissues. BCKDH was more phosphorylated in tissues of preruminant lambs than in rats, especially in liver. These low catalytic potentialities might contribute to a low rate of branched-chain amino acid catabolism in sheep.

4. Ingestion of an excess of leucine led to an increase in liver and jejunum BCAAT activities and activation of BCKDH in jejunum.

The first reaction in the catabolism of branched-chain amino acids (BCAA) is a transamination catabolized by BCAA aminotransferase (BCAAT; *EC* 2.6.1.42). This reaction is readily reversible. The branched-chain α-keto acids (BCKA) formed are then irreversibly decarboxylated by the BCKA dehydrogenase (BCKDH; *EC* 1.2.4.4) to form acyl-CoA compounds which are further metabolized by a series of reactions similar to those by which fatty acyl-CoA is degraded. Both enzymes are widespread in the body but muscle is the tissue containing most of the BCAAT, and liver is the organ with the highest content of BCKDH in the rodent (for review, see Harper *et al.* 1984). BCKDH is a multi-enzyme complex composed of a central core of a transacylase component (E2) around which the α-keto acid dehydrogenase (E1) and dihydrolipoyl dehydrogenase (E3) components are arranged. The complex is inactivated by phosphorylation and activated by dephosphorylation (Harris *et al.* 1985). It has been proposed that portal blood BCKA concentrations have an important part to play in the regulation of the activity state of hepatic BCKDH (Harris *et al.* 1986). This is based on observations of inhibition of BCKDH kinase by BCKA, especially α-ketoisocaproate (α-keto acid corresponding to leucine).

Ballard *et al.* (1976) were first to suggest that the degradation of BCAA is impaired in sheep muscle. Recent papers confirm this hypothesis. Wijayasinghe *et al.* (1983) and Busboom *et al.* (1983) demonstrated that BCAAT and BCKDH activities are lower in sheep than in rat tissues. However, in these experiments, L-[1-^{14}C]leucine was used as a substrate not only for BCAAT but also BCKDH assays. Using leucine as substrate for BCKDH assays represents a serious shortcoming in these experiments, i.e. 'lack of control of substrate concentration owing to dependence upon transamination to supply the α-keto acids' (Shinnick & Harper, 1976).

Recently BCAAT and BCKDH activities and the activation state of the latter have been compared in maternal and fetal sheep by Goodwin *et al.* (1987). The most important difference they found between maternal and fetal tissues concerned muscle BCAAT which was low in the adult and high in the fetus. The inter-organ metabolism of BCAA would, therefore, appear to be different in adult pregnant and fetal sheep.

We measured BCAAT and BCKDH activities and activation state in several tissues of the preruminant lamb in order to learn how BCAA metabolism is influenced by metabolic modifications linked to birth and to weaning. Furthermore we studied the effect of an excessive leucine intake on these activities. This should be helpful in understanding the differences in plasma BCAA and BCKA concentrations previously observed in preruminant lambs fed on excess-leucine diets (Papet *et al.* 1988).

MATERIALS AND METHODS

Animals

Eleven male Limousin lambs were removed from their dams at 12–24 h of age. They were fed twice daily (at 09.00 and 16.00 hours) on a commercial milk diet *ad lib.* Between the ages of 2 and 4 weeks their growth rate was about 270 g/d. Animals were separated into two groups when they were 6–7 weeks old and weighed about 13 kg. The control group (n 6) was fed only on the control diet whereas the experimental group (leucine; n 5) was fed on the control diet supplemented with an excess of leucine for three meals (1·5 d).

Diets

The control diet was a commercial milk (Agnodor, Union Univor, Paris, France) whose leucine content was 23 g/kg dry matter (DM) (Table 1). The excess-leucine diet given to the experimental group was made by adding L-leucine (Ajinomoto Co Inc., Tokyo, Japan) to the fresh milk (200 g power/kg water) to give a diet containing 106 g leucine/kg DM. The food intake of lambs was about 340 g DM/d for both groups a few days before the experiment. Increasing dietary leucine content from 23 to 106 g/kg DM led to a significant 30% decrease in the quantity of food consumed by animals from the experimental group on the day preceding the measurements of the enzyme activities. This decrease was a classical manifestation of BCAA antagonism also observed in lambs (Papet *et al.* 1987).

Experiments

The morning meal given to control lambs before slaughter was limited to 400 g fresh milk. The leucine group was fed on the excess-leucine diet (106 g/kg DM) on the day before slaughter and also for the morning meal on the slaughter day. Intake was also limited to 400 g. Lambs consumed their meal in about 15 min and were killed 2 h after the beginning of the meal. Liver, longissimus dorsi muscle, kidneys with perirenal adipose tissue, and jejunum were removed. Liver, kidney and jejunum were rinsed in cold saline (9 g sodium chloride/l). Samples of each tissue were immediately used for BCKDH assays or frozen (−80°) until BCAAT assays were run.

Subcellular tissue fractionation

Homogenization and subcellular fractionation were carried out in the same way for both enzyme assays. However, for BCKDH assays subcellular fractions were prepared without or with 100 mM-sodium fluoride (NaF), which inhibits phosphatase activation of BCKDH, in order to measure total and basal activities respectively. Homogenates containing 200 g liver, kidney, jejunum or adipose tissue/l were prepared in an ice-cold medium (210 mM-mannitol, 60 mM-sucrose, 10 mM-HEPES buffer (pH 7·4 at 4°), 10 mM-potassium chloride

Table 1. *Composition of the commercial diet (g/kg)*

Ingredients	
Spray dried skim milk	690
Animal fat (tallow)	180
Vegetable fat (coconut)	90
Starch	Trace
Mineral and vitamin mixture UCAAB*	30
Analysis	
Gross energy (MJ/kg DM)	22·2
Crude protein (nitrogen × 6·25) (g/kg DM)	240
Leucine (g/kg DM)	23

DM, dry matter.

* Composition (mg/kg mixture): 2520 Mn, 2520 Zn, 5 Se, 9 Co, 2561 Mg, 38 Fe, 750 retinol, 5 cholecalciferol, 740 α-tocopherol, 150 menadione, 3170 ascorbic acid, 240 thiamin, 710 riboflavin, 1520 pantothenic acid, 65 pyridoxine, 216 nicotinic acid, 67500 choline, 30 bacitracin, 10 spiramicyne, 113·7 methionine.

(KCl), 1 mM-ethyleneglycolbis(aminoethylether)tetra-acetate (EGTA)). Liver, kidney and jejunum were homogenized using a Potter-Elvehjem tube with a motor-driven Teflon pestle, whereas for adipose tissue a Sorvall Omni-Mixer 17106 (Du Pont Instruments, Newtown, Ct, USA) was used. Longissiumus dorsi muscle was gently homogenized in a medium containing 100 mM-KCl, 50 mM-Tris-buffer (pH 7·4 at 4°), 5 mM-magnesium chloride and 1 mM-EGTA. A pretreatment with 0·1 g Nagarse (Sigma P 5255)/l at 37° for 6 min was made before homogenization with the device used for liver but at a lower speed. The homogenates were clarified by centrifugation (except for adipose tissue) for 10 min at 4° and 500 ***g*** (Sorvall RC 2-B). Adipose tissue homogenate was filtered through a nylon Bluter cloth. A sample of each clarified homogenate was kept for enzyme assays and the remainder was centrifuged for 10 min at 4° and 10000 ***g*** to separate the cytosolic (supernatant fraction) and mitochondrial fractions.

Enzyme assays

BCAAT and BCKDH were isotopically assayed according to the procedure of Shinnick & Harper (1976) with modifications. Both enzymes were assayed at 37° in a final volume of 400 μl with the assay medium containing 154 mM-mannitol, 49 mM-sucrose, 78 mM-HEPES buffer (pH 7·4 at 37°), 25 mM-potassium dihydrogen phosphate, 8 mM-sodium carbonate, 2 mM-$MgCl_2$, 1·1 mM-EGTA.

For BCAAT, nonidet P-40, pyridoxal 5-phosphate and α-ketoglutarate were present in the medium so that their final concentrations were 5 g/l, 0·1 mM and 5 mM respectively. In the case of liver, 40 g bovine serum albumin (BSA)/l were included in the medium. Activity was linear with respect to the amount of enzyme. After a 5 min preincubation of 100 or 150 μl of each fraction, L-[1-^{14}C]leucine (30 disintegrations/min (dpm) per μmol) was added to the assay medium. Its final concentration was 8 mM. The reaction was stopped with 0·1 ml 4 M-sulphuric acid. Then 0·5 ml of 0·2 M-ceric sulphate in 2 M-H_2SO_4 was used to decarboxylate α-keto[1-^{14}C]isocaproate produced during the incubation.

BCKDH assays were run without and with 100 mM-NaF for the fractions prepared without and with NaF respectively. Cofactors of BCKDH were present in the assay medium as follows: 0·2 mM-thiamin pyrophosphate, 1·0 mM-coenzyme A, 1·9 mM-NAD. For liver, kidney and adipose tissue the medium contained 5 g nonidet P-40/l whereas for muscle and jejunum 1 mM-carnitine was included. After a 5 min preincubation of 100 μl of each fraction, the reaction was started with α-keto[1-^{14}C]isocaproate (90 dpm/nmol) so that its final concentration was 0·5 mM. The reaction was stopped with 0·1 ml 4 M-H_2SO_4.

For both enzymes, all assays were run in quadruplicate and they were linear with respect to time and amount of enzyme.

Radioactive carbon dioxide was collected in 1 ml methylcellosolve–ethanolamine mixture (2:1, v/v) and assayed by liquid scintillation (Packard 460 CD).

Protein content of each fraction was measured using the Biorad Protein Assay with BSA (Sigma) as standard.

Statistical analysis

The statistical significance of difference between the means was assessed by Student's *t* test.

RESULTS

The BCAAT specific activities of control preruminant lamb tissue homogenates ranged from 0·28 to 5·1 nmol/min per mg protein; values were lowest in liver and highest in adipose tissue (Table 2). Activities per g tissue (Table 3) were estimated from the total homogenate activities. Based on these estimations, lamb tissues were ranked for BCAAT activity in the order kidney > jejunum and adipose tissue > muscle and liver.

The subcellular distribution of BCAAT activity in preruminant lamb tissues is shown in Table 3. This enzyme appeared to be more cytosolic (60–80%) than mitochondrial, particularly in adipose tissue.

BCAAT specific activities of jejunum homogenate, and jejunum and liver mitochondrial fractions from the leucine group (160 g leucine/kg diet) were significantly higher (13, 53 and 45% respectively) than in fractions from the control group (23 g leucine/kg diet). No significant change occurred in adipose tissue, kidney or muscle when dietary leucine content was increased.

The total specific activities of BCKDH (completely dephosphorylated) in homogenates of control lamb tissue ranged from 0·07 to 3·2 nmol/min per mg protein and from 0·4 to 18·7 nmol/min per mg protein in the corresponding mitochondrial fractions (Table 4) with jejunum being lowest and kidney highest. Total and basal BCKDH activities estimated per g tissue were respectively about 130, 38 in liver; 11, not detectable (ND) in muscle; 160, 56 in kidney; 3, ND in jejunum; 3, 1 in adipose tissue. This indicated that the most active tissues were kidney and liver.

The percentage of BCKDH in the active form is presented as the ratio, basal activity measured in the presence of NaF: total activity (Table 4). The degree of activation of BCKDH was least in muscle (7%), followed by jejunum (14%) then adipose tissue (25–50%) and liver and kidney (40%). In adipose tissue, individual variation was very high. In preruminant lamb tissue BCKDH activity was mostly in the mitochondrial fraction (80–90%).

Total specific BCKDH activity was not affected by increasing dietary leucine level. This means that, under our experimental conditions, no induction of this enzyme occurred. In contrast, basal specific activity decreased (34%) in kidney homogenate and increased (180%) in jejunum mitochondrial fraction (Table 4). The activation state of kidney BCKDH was significantly lower when calculated from mitochondrial values but was not different when calculated from homogenate values. Concerning jejunum, the increase in basal activity was associated with a significant increase in the activation state of BCKDH (110%).

Table 2. *Specific activity of branched-chain amino acid aminotransferase* (EC *2.6.1.42; BCAAT*) *in subcellular fractions of liver, muscle, kidney, jejunum and adipose tissue of preruminant lambs*

(Mean values with their standard errors; no. of animals in parentheses)

		BCAAT specific activity (nmol/min per mg protein)			
		Control (*n* 6)		Leucine (*n* 5)	
Tissue	Fraction	Mean	SE	Mean	SE
Liver	H	0·28	0·02	0·31	0·02
	S	0·31	0·01	0·33	0·03
	M	0·45	0·04	0·66**	0·03
Muscle	H	0·45	0·07	0·34	0·02
	S	0·43	0·07	0·27	0·01
	M	0·58	0·09	0·38	0·04
Kidney	H	1·00	0·05	1·03	0·05
	S	0·89	0·05	0·97	0·06
	M	1·52	0·07	1·59	0·07
Jejunum	H	0·84	0·06	1·11*	0·05
	S	0·72	0·04	0·79	0·06
	M	1·27	0·12	1·94***	0·11
Adipose tissue	H	5·12	0·54	4·17	0·42
	S	5·27	0·70	4·69	0·73
	M	5·15	0·61	5·50	0·80

H, homogenate; S, supernatant fraction; M, mitochondrial fraction.
Mean values were significantly different from the control values: * $P < 0·01$, ** $P < 0·005$, *** $P < 0·0025$.

Table 3. *Subcellular distribution of branched-chain amino acid aminotransferase* (EC *2.6.1.42; BCAAT*) *activity in preruminant lamb tissues*

(Mean values with their standard errors for six (control group) or five (leucine group) lambs)

		BCAAT activity (nmol/min per g wet tissue)		S/H (%)†		M/H (%)†		(S+M)/H (%)†	
Tissue	Group	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Liver	C	21	3	82	4	39	4	121	7
	L	28	2	70	4	26	3	96	4
Muscle	C	20	2	77	5	31	5	108	4
	L	20	2	62	2	35	2	95	3
Kidney	C	49	2	62	3	50	2	112	4
	L	54	2	68	6	51	5	119	8
Jejunum	C	36	4	77	6	26	2	103	7
	L	47*	2	63	4	31	3	94	4
Adipose tissue	C	36	4	83	2	14	2	97	3
	L	26	3	88	5	11	1	99	5

C, control; L, leucine; S, supernatant fraction; M, mitochondrial fraction; H, homogenate.
* Mean value was significantly different from control value ($P < 0·05$).
† Values are the percentage of the activity found in S or M fractions and the sum of S+M fractions in relation to the activity of H.

Table 4. *Total (without sodium fluoride (NaF)) and basal (with NaF) specific activities of branched-chain α-keto acid dehydrogenase* (EC *1.2.4.4; BCKDH*) *in liver, muscle, kidney, jejunum and adipose tissue in preruminant lambs*

(Mean values with their standard errors for six (control group) or five (leucine group) lambs)

			BCKDH specific activity (nmol/min per mg protein)				Activation state† (%)	
			Without NaF		With NaF			
Tissue	Fraction	Group	Mean	SE	Mean	SE	Mean	SE
Liver	H	C	1·75	0·30	0·50	0·05	32	4
		L	1·76	0·36	0·65	0·13	38	2
	S	C	0·35	0·03	0·16	0·01	49	5
		L	0·38	0·08	0·17	0·02	48	6
	M	C	8·63	0·42	3·86	0·29	46	6
		L	9·56	1·28	3·61	0·67	37	2
Muscle	H	C	0·23	0·02	nd	—	—	—
		L	0·28	0·05	nd	—	—	—
	S	C	nd	—	nd	—	—	—
		L	nd	—	nd	—	—	—
	M	C	0·94	0·15	0·07	0·02	7	1
		L	1·34	0·22	0·12	0·02	8	2
Kidney	H	C	3·23	0·39	1·15	0·11	36	2
		L	2·57	0·58	0·76*	0·09	37	7
	S	C	0·29	0·03	0·12	0·01	43	9
		L	0·29	0·02	0·11	0·01	37	5
	M	C	18·7	1·3	7·38	0·48	40	3
		L	20·4	1·5	6·16	0·66	30*	2
Jejunum	H	C	0·067	0·012	nd	—	—	—
		L	0·072	0·009	0·017	0·001	27	6
	S	C	nd	—	nd	—	—	—
		L	nd	—	nd	—	—	—
	M	C	0·40	0·06	0·05	0·01	14	3
		L	0·47	0·04	0·14*	0·02	29*	3
Adipose tissue	H	C	0·79	0·13	0·35	0·09	50	11
		L	1·13	0·54	0·30	0·14	36	16
	S	C	0·32	0·07	0·16	0·06	58	13
		L	0·53	0·23	0·30	0·11	65	7
	M	C	5·11	1·04	1·16	0·51	25	14
		L	5·62	1·86	2·39	1·28	34	12

H, homogenate; S, supernatant fraction; M, mitochondrial fraction; C, control; L, leucine; nd, not detectable.

* Mean values were significantly different from control values ($P < 0·05$).

† Activation state is the ratio, basal: total specific activities.

DISCUSSION

Measurements on subcellular fractions of lamb tissues indicated that transamination of BCAA occurred in both cytosol and mitochondria with slightly higher activity in the cytosolic fraction. In contrast, BCKDH activity was found to be essentially mitochondrial in lamb tissues. These subcellular distributions are very similar to those reported for rat tissues (Cappucino *et al.* 1978; Wohlhueter & Harper, 1970). However Snell & Duff (1985) showed that BCAAT activity in rat muscle is located in the mitochondrial compartment and that the BCAAT activity found in the cytosol fraction after differential centrifugation

was accompanied by leakage of mitochondrial matrix marker enzymes. BCAAT subcellular distribution should be reinvestigated using marker enzymes of both the mitochondrial-matrix and the mitochondrial membrane. Nevertheless the intracellular localization of these enzymes in lambs cannot contribute to any difference between lamb and rat with respect to the extent of BCAA catabolism.

The rates of BCAA transamination and BCKA oxidative decarboxylation in vivo cannot be deduced from the corresponding enzyme activities because they are measured with saturating concentrations of substrates, and in vivo substrate concentrations at the catalytic sites are unavailable. However, potential contribution of each tissue has been calculated by enzyme activity × mass. Considering that skeletal muscle, adipose tissue, liver, jejunum and kidneys make up 37, 6·5, 2·7, 1·2 and 0·5% total body-weight of preruminant lambs respectively (D. Attaix, personal communication), total muscle and adipose tissue BCAAT activities represented about 70 and 20% respectively of total activity of these tissues. Based on these assessments muscle appeared to be the principal site of BCAA transamination in preruminant lambs as in rats (Shinnick & Harper, 1976). However adipose tissue, in which BCAAT specific activity was the highest in lambs as well as in wethers (Busboom *et al.* 1983), may contribute significantly to whole body BCAA transamination in preruminant and ruminant sheep.

Due to the respective contributions of different tissues to total body-weight of lambs, total BCKDH (completely dephosphorylated) activities of liver and muscle were about the same. Total activity of both tissues was about 85% of the total BCKDH activity of the tissues studied. In contrast, when basal BCKDH activities were estimated for all tissues, liver contained about 60% of the basal activity of all of them whereas muscle and kidneys each contained 15% of the sum of the basal activities. In the rat, 70–90% of total BCKDH activity is located in liver (Shinnick & Harper, 1976; Khatra *et al.* 1977) but in the monkey and human only 50 and 30% respectively of this activity is located in liver (Khatra *et al.* 1977). These studies demonstrated the occurrence of species differences with respect to the organ distribution of BCKDH activity but did not take into account the activation state of BCKDH. Potential contributions of the studied tissues in the two first steps of BCAA catabolism would not seem to be very different in preruminant lambs and simple-stomached animals.

The main difference between rats and preruminant lambs with respect to BCAA catabolism was in total BCAAT activity. This enzyme activity was much lower in preruminant lambs than in rats (Shinnick & Harper, 1976) or chicks (Featherston & Horn, 1973) in all tissues studied. This observation is in accord with the lower rate of leucine and its BCKA interconversion observed in sheep than in pig, human and dog (Nissen & Ostaszewski, 1985). The low BCAAT activity in preruminant lamb tissues also agreed with results reported for tissue homogenates (Busboom *et al.* 1983) or incubated tissues (Wijayasinghe *et al.* 1983) from ruminant sheep. In the latter study the reported values were very low, probably due to limited transport of substrate into the tissue and the low leucine concentration (0·5 mM). In contrast, especially in muscle, BCAAT activity in the preruminant lamb was only about 10% of that in the ovine fetus (Goodwin *et al.* 1987). BCAAT was likely to be more sensitive to metabolic modifications linked to birth than to weaning. High fetal muscle BCAAT activity may indicate that utilization of leucine as an energy source is important during fetal life (Goodwin *et al.* 1987; Van Veen *et al.* 1987).

Total activities of BCKDH in preruminant lamb tissues (expressed per g tissue) were about half those in pregnant sheep (Goodwin *et al.* 1987). This activity was also lower in the lamb than in the ovine fetal tissues with the exception of the kidney (Goodwin *et al.* 1987). Comparison of our measurements of the BCKDH activities in preruminant lambs

with those reported in ruminant sheep (Busboom *et al.* 1983) was made difficult because in the latter study leucine was used as the substrate in the BCKDH assay. Due to the low BCAAT activity in sheep tissues this would result in low BCKA concentrations and underestimation of BCKDH activities. Based on the values reported by Shinnick & Harper (1976), total specific activity of kidney BCKDH was about the same in rats and preruminant lambs. Total specific activity of liver BCKDH in lambs was about 25% of that in rats whereas muscle activity was ten times higher in lambs than in rats.

BCKDH appeared to be more highly phosphorylated in the preruminant lamb than in the ovine fetus, pregnant sheep (Goodwin *et al.* 1987) or rat (especially in liver) (Gillim *et al.* 1983; Wagenmakers *et al.* 1984). This may reflect either a more active kinase or a less active phosphatase or both. This high level of phosphorylation of BCKDH in preruminant lambs probably contributes to the low BCAA catabolism observed in lambs compared with rats.

Although BCAAT activities were very low in preruminant lamb tissues, BCAAT specific activity was equal to (kidney) and higher than (adipose tissue) basal BCKDH specific activity and higher than total BCKDH specific activity (muscle and jejunum). BCAAT specific activity was lower than basal BCKDH specific activity only in liver. Based on these comparisons BCAAT seemed to be more rate-limiting in liver whereas BCKDH is more rate-limiting in muscle, jejunum and adipose tissue in preruminant lambs. So it is impossible to conclude definitively that the low BCAAT activity in sheep in postnatal life directly induces a low rate of BCAA catabolism in the species but it probably contributes towards impairing it. The low rate of BCAA catabolism in the adult sheep is not linked to the ruminant state as suggested by Lindsay & Buttery (1980) but occurs generally in this species. These metabolic conditions favour the utilization of leucine for protein synthesis in lambs and sheep.

Increasing dietary leucine content induced few modifications of either BCAAT or BCKDH activities in preruminant lamb tissues. However BCAAT was increased in jejunum and liver which are the first tissues flooded by leucine excess. Leucine which was added to the milk was probably absorbed before the other amino acids due to milk-protein coagulation in the abomasum (Patureau-Mirand *et al.* 1971). BCAAT activity in rat liver has been shown to be modulated by dietary manipulations such as starvation or protein deprivation (Adibi *et al.* 1975). However addition of 50 g leucine/kg to a diet containing 200 g casein/kg increased neither cytosolic nor mitochondrial hepatic BCAAT in rats (Block *et al.* 1985). To our knowledge no study concerning the dietary manipulation of BCAAT activity has been carried out in the intestine. Total BCKDH specific activity was not affected by dietary leucine content. This means that no induction of BCKDH occurred under our experimental conditions. In contrast, an activation (by dephosphorylation) of BCKDH was observed only in jejunum of preruminant lambs. In rats, addition of 50 g leucine/kg diet led to activation of liver BCKDH only when the dietary protein level was low (Block *et al.* 1985). Actually the activation state of liver BCKDH was already high (74%) in rats fed on the 200 g casein/kg diet. The activation state of BCKDH in rat muscle increases after intraperitoneal or intravenous injection of leucine (Aftring *et al.* 1986) and this increase parallels plasma leucine concentration. Although the activation states of hepatic and muscle BCKDH were low in preruminant lambs, leucine excess failed to activate this enzyme in liver and muscle. Nevertheless, the activation of both enzymes in preruminant lamb jejunum provides further evidence for the involvement of non-hepatic tissues in the response to an excessive leucine intake as has been proposed for rats (Block *et al.* 1985). These observations are also in accord with the hypothesis that portal-drained viscera may be an important site of BCAA transamination in steers (Early *et al.* 1984). The low enzyme adaptation to leucine excess in preruminant tissues may contribute to the

limited decrease in plasma valine and isoleucine concentrations previously observed (Papet *et al.* 1988). Also the 50% decrease in the plasma concentrations of their corresponding α-keto acids may result from competition between these amino acids and leucine for transamination.

CONCLUSIONS

The results of the survey of BCAAT and BCKDH activities reported in the present paper are in accord with reports that catabolism of BCAA is low in sheep and extend the observations to the preruminant state. The results are not in accord with the hypothetical role of nutrients that are energy sources in decreasing BCAA catabolism in ruminants. The low amount of BCAAT and to a lesser extent the high phosphorylation state of BCKDH, especially in liver, may contribute to the low rate of BCAA catabolism in sheep after birth.

The leucine stimulation of both enzymes in jejunum supports the proposal of a role for portal vein-drained viscera in the regulation of BCAA catabolism even when the dietary protein level is adequate.

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