Protein synthesis and growth in the gastrointestinal tract of the young preruminant lamb

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1. In Expt 1, fractional synthesis rates (FSR) of tissue protein were measured along the gastrointestinal tract (GIT) of six 1-week-old, milk-fed lambs by using a large amount of L-[3,4(n)-^{3}H]valine.

2. In Expt 2, eighteen lambs were used to determine the fractional growth rate (FGR) of gastrointestinal tissue protein.

3. $FSR_{Minimum (Min)}$ and $FSR_{Maximum (Max)}$ were calculated assuming plasma or tissue homogenate free valine specific radioactivity was representative of the valine precursor pool for protein synthesis. There were no significant differences between FSR_{Min} and FSR_{Max} in any gastrointestinal tissue of lambs used in Expt 1 ($P > 0.05$). FSR gradually and significantly ($P < 0.05$) increased from the oesophagus (FSR_{Max} 26·5%/d), reticulo-rumen (30·1%/d), omasum (41·0%/d) and abomasum (56·1%/d) to small intestine (87·5%/d), and then declined significantly ($P < 0.05$) towards the caecum (45·2%/d) and the colon (38·4%/d). No significant differences were observed between FSR in the duodenum, jejunum or ileum ($P > 0.05$).

4. FGR ranged from 2·6%/d in the oesophagus to 8·7%/d in the omasum. The ratio, FGR:FSR, which reflected the efficiency of protein deposition, was at a maximum in the stomachs and caecum and at a minimum in the small intestine.

5. The relative contribution of the oesophagus, stomachs, small intestine and large intestine to GIT protein synthesis was 1, 13, 76 and 10% respectively. The GIT accounted for approximately 11·5% of whole-body protein synthesis.

The importance of the contribution of gastrointestinal tissues to whole-body protein synthesis is now well established in rats (McNurlan & Garlick, 1980; Goldspink *et al.* 1984). Fractional synthesis rates (FSR) of tissue protein have been measured in the gastrointestinal tract (GIT) of the ovine fetus (Schaefer & Krishnamurti, 1984) and newborn (Patureau-Mirand *et al.* 1986) and growing lambs (Buttery *et al.* 1977; Arnal *et al.* 1978; Combe *et al.* 1979; Davis *et al.* 1981). Unfortunately all these estimates were obtained after infusion, or injection (Arnal *et al.* 1978; Combe *et al.* 1979), of tracer doses of labelled amino acids. This resulted in great differences between the specific radioactivity of the label in the plasma and in the gastrointestinal tissue homogenates (Garlick, 1980). Consequently, in these experiments, the FSR are very different when calculated using the specific radioactivity of the label in both potential precursor pools of protein synthesis. In addition, labelled proteins are released in infused lambs during the experiments, so that proteins exported by cell exfoliation and secretion are not included in the measurements of FSR (James *et al.* 1971).

Recently, Schaefer *et al.* (1986) determined tissue protein FSR in sheep during sustained elevation of plasma leucine concentration by intravenous infusion. This procedure prevented significant differences between the specific radioactivity of the label in the plasma and the tissue homogenates, but does not solve the problems that result from labelled protein export and secretion during infusion.

The large-dose technique, i.e. the injection of a large amount of amino acid combined with a tracer dose of the same radiolabelled amino acid, overcomes problems arising both from the determination of the specific activity of the precursor of protein synthesis and from protein export or secretion during the experiments (McNurlan *et al.* 1979; Garlick *et al.*

1980). This technique has been used for measuring protein FSR in the intestine of rats, in a wide variety of conditions, and of chickens (Bryan *et al.* 1983; Muramatsu *et al.* 1983). To our knowledge, no attempt has been made in large domestic animals such as lambs.

The aim of the present study was to obtain reliable FSR estimates in the GIT of young preruminant lambs by using the large-dose technique. This was also of interest since there is little information about rates of protein synthesis along the GIT, even in laboratory animals. In addition, we assessed fractional growth rates (FGR) in gastrointestinal tissue proteins for which no information is available.

MATERIALS AND METHODS

Two experiments were conducted with eighteen male crossbred lambs (Ile de France × Romanov–Limousin). The animals, born in the Sheep Production Laboratory flock (INRA, Theix, France), and weighing 2·5–4·0 kg at birth, were separated from their dams 12–24 h post partum. They were then reared in individual metallic cages with mesh floors in a room at 21 °, and given only a commercial milk replacer ('Agnodor'; Univor, Paris, France) *ad lib*. Milk was given by nipple feeders and renewed twice daily. The dry matter intake and the live weight were recorded daily, from the 2nd day of life to slaughter at 6–9 d of age.

In the first experiment, six lambs received a massive dose of L-[3,4(*n*)-³H]valine to estimate the FSR of tissue protein in the GIT. Valine was essentially chosen as a label for its high solubility. Moreover, we have previously shown that valine had no effect on plasma insulin or glucagon release in milk-fed lambs (Attaix *et al.* 1986*a*).

In the second experiment, all lambs were used to measure the FGR of gastrointestinal tissue proteins.

Experimental procedure

Expt 1. Protein synthesis experiment. Protein synthesis was measured in vivo after an intravenous injection of a large amount of L-valine, according to the procedure described elsewhere (Attaix *et al.* 1986*a*). Jugular catheters were inserted bilaterally into each 5 to 6-d-old lamb. At 2 d later, the animals received between 10.00 and 11.00 hours, a single intravenous injection of unlabelled L-valine (17·1 mmol/5 kg body-weight) combined with L-[3,4(*n*)-³H]valine (25–50 Ci/mmol; Amersham International plc, Amersham, Bucks) to give 95 µCi and 0·45 mmol/ml saline (9 g sodium chloride/l). Blood samples were regularly collected until slaughter, centrifuged and plasma isolated. Pairs of lambs were anaesthetized with sodium pentobarbitone to prevent cell sloughing in the GIT (Attaix *et al.* 1984), and bled by severing the vessels of the neck 5, 13 and 30 min after the injections. The GIT was quickly removed and whole or representative parts of the oesophagus, reticulo-rumen, omasum, abomasum, abomasal mucosa and musculosa, small intestine (duodenum: 150 mm long from the pylorus; jejunum: 500 mm long, 1·35 m beyond the pylorus; ileum: 500 mm long, 1 m above the ileo-caecal valve), caecum and colon (sigmoid colon) were rinsed with ice-cold saline. Abomasal mucosa and musculosa were easily separated by means of a surgical blade. Tissue samples were frozen in liquid nitrogen within an average 7 min period following slaughter, and were stored at −15° until analysis.

Plasma (0·5 ml) and tissue samples (2–16 g) were homogenized with 8 vol. ice-cold trichloroacetic acid (100 g/l; TCA). The protein precipitates were collected by centrifugation (12 100 and 5600 *g* for the plasma and tissues respectively) for 10 min, then resuspended in TCA. The homogenization was repeated four times and the four supernatant fractions were mixed. The TCA was removed from supernatant fractions by anion-exchange chromatography (Dowex 2-X8; 20–50 mesh). The hydrochloric acid in soluble fractions was then evaporated and the free amino acids resuspended in 0·2 M-lithium citrate buffer,

pH 2·2. Portions (2·5 ml) were chromatographed on an amino acid analyser. Amino acids were separated on Aminex A-9 resin (Bio-Rad). To determine the specific radioactivity of free L-[3,4(*n*)-³H]valine, the column eluate was diverted to liquid scintillation counting (60%) and to ninhydrin reaction (40%) by a stream splitter (Model ES; Radiomatic Instruments and Chemical Co., Florida, USA) controlling a three-way solenoide valve (Model 1.85.900; General Valve Corporation, New Jersey, USA) within a 5 s time cycle. Splitting reproducibility was ±0·5%. The radioactivity was measured in a liquid-scintillation spectrometer (Flo-One D/R; Radiomatic Instruments and Chemical Co.), using Pico-Fluor 30 scintillation cocktail (Packard Instrument Co., Illinois, USA). Efficiency counting (18%) was calibrated with chromatographed L-[3,4(*n*)-³H]valine standards in 0·2 M-lithium citrate buffer, pH 2·2.

The protein precipitates were delipidated, then dried and pulverized to a powder as previously described (Attaix *et al.* 1986*a*). Duplicate portions of protein precipitates (40–50 mg) were hydrolysed at 60°, in the presence of 0·1 ml water, with 1 ml Soluene-350 (Packard Instruments Co.) in liquid-scintillation vials. The radioactivity was measured, using 10 ml of a toluene-based scintillation cocktail (Glass & Woods, 1971) added to the hydrolysates, with a Packard 460 CD spectrometer. Counting efficiency (15–25%) was determined by external-standard channel ratio. No sample had less than 800 counts/min, 5 min after [³H]valine injection. Protein pellets were analysed for N in duplicate, by a Kjeldahl procedure. Tissue crude protein content was calculated as $N \times 6·25$. The valine content of mixed tissue protein was obtained in duplicate portions (200 mg) of pooled protein precipitates. These pellets were hydrolysed for 48 h at 110° in 5·5 M-HCl, with norleucine added as an internal standard. The HCl was eliminated by evaporation and the free amino acids resuspended in 0·2 M-sodium citrate buffer, pH 2·2, for analysis on an automatic amino acid analyser. The specific radioactivity of protein-bound valine (disintegrations/min (dpm) per μmol; SB) was determined from the specific activity of protein (dpm/mg protein) and the valine content of mixed tissue protein. This approximation was thought to be valid, since we verified that 98% of the radioactivity was valine-bound in jejunum protein hydrolysates of lambs killed 30 min post injection.

An estimate of FSR (%/d) of tissue protein was calculated as the slope of the linear regression of SB (*t*) *v*. (SA × *t*) by using the following equation (Attaix *et al.* 1986*a*):

$$100 \times SB(t) = FSR\,(SA \times t) + 100 \times SB(t_0),$$

where *t* is the incorporation time (d), and SA is the mean specific radioactivity of free valine either in plasma or in tissue homogenates between t_0 and *t*. Individual values of SA were calculated according to Garlick *et al.* (1980). No allowance was made for the time elapsed between killing the lambs and freezing the tissue samples in liquid N_2, as previously discussed (Attaix *et al.* 1986*a*).

Expt 2. FGR estimations. The remaining twelve 6- to 9-d-old lambs were killed. The digestive tract was rapidly excised and the different gastrointestinal tissues were separated. All samples were rinsed with ice-cold saline, blotted and weighed. The same procedures were conducted as those used for the lambs in Expt 1, allowance being made for the tissue samples that had been removed for measuring protein synthesis. As for lambs used in Expt 1, tissue protein content was obtained after homogenization of tissue samples in TCA and analysis for N content of lipid-free protein precipitates. The amount of tissue protein gained per kg body-weight gained was calculated for each lamb used in Expt 1 by linear regression of total tissue protein mass *v*. the empty body-weight of the other seventeen lambs, according to a procedure described by MacDonald & Swick (1981). Empty body-weight was calculated as 96% live weight in the 1-week-old lamb (Bénévent, 1971). Using the empty body-weight growth rate of each lamb, the tissue protein gained per d was

Table 1. *Expts 1 and 2. Live weight (kg), food intake (g dry matter/d), growth rate (g/d) and weight (g) of gastrointestinal tissues in preruminant lambs*

(Mean values with their standard errors)

Expt no.	n	Live wt (kg) Mean	SE	Growth rate* (g/d) Mean	SE	Dry-matter intake* (g/d) Mean	SE	Wt (g) Oesophagus	Stomachs	Small intestine	Large intestine
1	6	4·5	0·2	273	14	189	10	5·3	55·2	127·1	40·6
2	12†	4·6	0·2	253	16	203	12	4·6	54·1	135·8	42·8

* From 2 d of age to slaughter (7–8 d in Expt 1 and 6–9 d in Expt 2).

† Excluding animals used in Expt 1, since all lambs were used for fractional growth rate determinations in Expt 2. For details, see p. 161.

calculated. FGR was then derived from the daily change in tissue protein amount divided by the tissue protein mass present at that time.

Statistical analysis

The standard errors for slopes and intercepts of regression lines were computed according to Sokal & Rohlf (1969). For the comparison of FSR in Expt 1, the significance of differences between the slopes of best-fit were assessed according to Snedecor & Cochran (1971). Bartlett's test of homogeneity of variances (Sokal & Rohlf, 1969) revealed that heterogeneity was evident for FGR. FGR were therefore subjected to the Kruskal–Wallis test (Sokal & Rohlf, 1969) and mean values were compared using the Mann–Whitney U test (Mann & Whitney, 1947).

RESULTS

All animals were healthy and presented no signs of digestive disorders. The mean growth rate and dry matter intake of lambs used in the two experiments (Table 1) were in the range of values obtained in lambs of the same genotype and bred in similar conditions (Villette & Theriez, 1981).

SA in the plasma and in tissue homogenates declined very slowly and not significantly ($P > 0.05$) between 5 and 30 min following the injection of the large amount of L-[3,4(*n*)-^{3}H]valine (Table 2). Only slight differences were obtained between lambs. In the plasma, SA for the six lambs was 403 140 (SE 7467) dpm/μmol, 5 min post-injection. The SA ratios, tissue homogenate:plasma, were always very high and exceeded 0·78 in any gastrointestinal tissue.

The evolution of SB was linear with respect to time (Table 2). Extrapolation of the values to zero time in calculated regression lines of SB *v.* time generally showed positive intercepts, although they were never significantly different from the origin ($P > 0.05$), except in the oesophagus and the reticulo-rumen.

No significant differences were observed between FSR calculated by using the specific activity of free valine either in the plasma ($FSR_{Minimum}$ (FSR_{Min})) or in tissue homogenates ($FSR_{Maximum}$ (FSR_{Max})) (Table 3). The highest values for FSR were observed in the small intestine and the abomasal mucosa, and the lowest in the oesophagus. The FSR increased gradually and significantly ($P < 0.05$) from the oesophagus towards the small intestine and then decreased significantly ($P < 0.05$) from the small intestine towards the rectum.

Table 2. *Regression equations relating free (SA) or protein-bound (SB) [³H]valine specific radioactivity (disintegrations/min per µmol; y) in the plasma and gastrointestinal tissues of lambs to time after valine injection (min; x): $y = a + bx$*

(Mean values with their standard errors for six lambs)

Tissue	Linear coefficient							
	SA				SB			
	a		b		a		b	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Plasma	405044	6062	−517ⁿˢ	392	—	—	—	—
Oesophagus	335200	27124	348**	1420	295*	22	65	1
Reticulo-rumen	346874	8115	−426ⁿˢ	425	367*	101	72	5
Omasum	352445	14543	−701**	762	200ˣˢ	119	100	6
Abomasum	353526	11493	−573ⁿˢ	602	120**	189	136	10
Abomasal mucosa	350783	14394	−1314ⁿˢ	753	−16**	210	169	11
Abomasal musculosa	353098	14457	−798ⁿˢ	757	64**	114	94	6
Duodenum	370286	12639	−1149ⁿˢ	662	179ˣˢ	249	214	13
Jejunum	358098	21972	−1675ⁿˢ	1410	235ˣˢ	415	208	22
Ileum	365983	14859	−1234ⁿˢ	778	77ˣˢ	249	206	13
Caecum	359605	12989	−804ˣˢ	680	159ˣˢ	274	110	14
Colon	362370	14255	−848ˣˢ	746	228ˣˢ	177	94	9

NS, not significantly different from zero.
* Significantly different from zero ($P < 0.05$).

Tissue protein FGR was low in the oesophagus and the colon and high in the omasum and caecum. The reticulo-rumen, abomasum and small intestine occupied an intermediate position (Table 3). The ratio, FGR:FSR, which reflected the efficiency of protein deposition (Table 4), was higher in the stomachs and caecum than in the oesophagus, the colon and the small intestine.

The absolute rates of protein synthesis and deposition (g/d) were calculated by multiplying tissue protein content by FSR_{Max} and FGR respectively (Table 4). The stomachs and the large intestine, which accounted for 20·3 and 16·6% of the GIT protein mass respectively, synthesized only 13·2 and 9·6% of the protein synthesized in the GIT (Table 5). On the other hand, the contribution of the small intestine (60·5% of the GIT protein mass) to GIT protein synthesis amounted to 76·2%.

An estimate of whole-body protein synthesis was calculated as the sum of the absolute rates of protein synthesis in the GIT, the liver and the exsanguinated and eviscerated whole-body, and was found to be 146·4 (SE 5·7) g/d in the lambs used in Expt 1. The absolute rates of protein synthesis in liver and in the eviscerated whole-body were obtained by multiplying FSR_{Max} in these tissues (115·0 and 19·6%/d, respectively) (Attaix *et al.* 1986*b*) by their corresponding protein mass (14·9 (SE 5·7) and 572·7 (SE 19·8) g respectively) (D. Attaix and A. Manghebati, unpublished results). The contribution of the GIT to whole-body protein synthesis was estimated to be 11·5% (Table 5). A similar estimation (10·7%) was obtained when FSR_{Min} were used for this calculation. The small intestine exerted a significant contribution to whole-body protein synthesis (8·8%). By contrast, the relative contributions of the oesophagus (0·1%), the stomachs (1·5%) and the large intestine (1·1%) to whole-body protein synthesis were very low.

Table 3. *Tissue protein fractional synthesis rates (FSR) and fractional growth rates (FGR) in the gastrointestinal tract of the preruminant lamb*

(Mean values with their standard errors for six lambs)

| | FSR (%/d) | | | | FGR (%/d) | |
| | Min* | | Max* | | | |
Tissue	Mean	SE	Mean	SE	Mean	SE
Oesophagus	$23\cdot5^a$	$0\cdot1$	$26\cdot5^a$	$1\cdot8$	$2\cdot6^a$	$0\cdot1$
Reticulo–rumen	$26\cdot2^{ab}$	$1\cdot8$	$30\cdot1^{ab}$	$2\cdot2$	$4\cdot0^b$	$0\cdot1$
Omasum	$36\cdot3^c$	$2\cdot2$	$41\cdot0^c$	$3\cdot0$	$8\cdot7^c$	$0\cdot4$
Abomasum	$49\cdot5^{de}$	$3\cdot7$	$56\cdot1^d$	$3\cdot8$	$6\cdot1^{de}$	$0\cdot4$
Abomasal mucosa	$61\cdot4^{ef}$	$4\cdot1$	$74\cdot1^e$	$4\cdot3$	—	—
Abomasal musculosa	$34\cdot2^c$	$2\cdot4$	$39\cdot1^c$	$1\cdot7$	—	—
Duodenum	$77\cdot9^g$	$4\cdot6$	$86\cdot0^e$	$6\cdot0$	—	—
Jejunum	$75\cdot8^{fg}$	$7\cdot9$	$92\cdot5^e$	$13\cdot3$	—	—
Ileum	$74\cdot6^{fg}$	$5\cdot0$	$84\cdot1^e$	$6\cdot6$	—	—
Small intestine†	$76\cdot1$	—	$87\cdot5$	—	$6\cdot1^e$	$0\cdot3$
Caecum	$40\cdot0^{cd}$	$5\cdot3$	$45\cdot2^{cd}$	$5\cdot4$	$7\cdot6^{cd}$	$0\cdot6$
Colon	$34\cdot1^{bc}$	$3\cdot8$	$38\cdot4^{bc}$	$3\cdot5$	$3\cdot0^{ab}$	$0\cdot4$

a–g, Values within the same column not sharing a common superscript letter were significantly different ($P < 0\cdot05$).

* FSR_{Min} and FSR_{Max} were not significantly different in any tissue ($P > 0\cdot05$).

† Mean of duodenum, jejunum and ileum FSR.

Min, Max, minimum and maximum estimates of FSR obtained using the specific radioactivity of free valine in the plasma and the tissues homogenates respectively.

DISCUSSION

Measurements of protein synthesis

In the present study, FSR were obtained by using the large-dose technique (McNurlan *et al.* 1979; Garlick *et al.* 1980). It is, however, very important to prove that the large amount of amino acid does not affect FSR measurements. We have recently reported that FSR in the jejunum, liver and skeletal muscle were not significantly altered when 1-week-old lambs received a massive amount of $17\cdot1$ mmol L-[3,4(n)-^{3}H]valine/5 kg body-weight compared with a lower massive amount of $12\cdot8$ mmol L-[3,4(n)-^{3}H]valine/5 kg body-weight (Attaix *et al.* 1986*a*). Therefore, it seems very unlikely that the large amount of valine that we used had any effect on FSR determinations.

Since the SA ratios, tissue homogenate:plasma, were very high, FSR calculated by using the SA in both potential precursor pools were not significantly different (Table 3). This contrasted with findings obtained in gastrointestinal tissues of lambs, either by using the constant-infusion technique (Davis *et al.* 1981; Schaefer & Krishnamurti, 1984) or by a pulse injection of L-[U-^{14}C]lysine (Arnal *et al.* 1983), and demonstrated the advantages of a massive injection of amino acid. However, there is also evidence that lumen amino acids are preferentially utilized for protein synthesis in the small intestinal mucosa (Hirschfield & Kern, 1969; Alpers, 1972). To our knowledge, it has not been verified in large-dose experiments that the specific radioactivity of the label in the lumen amino acid pool approached the specific radioactivity of the precursor in the mucosal tissue homogenate.

Table 4. *Protein-bound valine (mg/g), protein content (g), absolute rates of protein synthesis and of protein deposition (g/d) and efficiency of protein deposition (%) in the gastrointestinal tract of preruminant lambs*

(Mean values with their standard errors for six lambs)

Tissue	Protein-bound valine (mg/g)	Protein content (g)		Protein synthesis* (g/d)		Protein deposition* (g/d)		Efficiency of protein deposition† (%)
		Mean	SE	Mean	SE	Mean	SE	
Oesophagus	51	0·62	0·04	0·16	0·01	0·016	0·001	9·8
Reticulo-rumen	57	1·88	0·11	0·57	0·03	0·076	0·004	13·3
Omasum	61	0·37	0·02	0·15	0·01	0·032	0·002	21·2
Abomasum	64	2·66	0·25	1·49	0·14	0·158	0·009	10·9
Small intestine	44	14·66	0·65	12·83	0·57	0·887	0·047	7·0
Caecum	59	1·09	0·09	0·49	0·04	0·081	0·006	16·8
Colon	58	2·94	0·36	1·13	0·14	0·085	0·010	7·8
Gastrointestinal tract	—	24·22	1·04	16·82	0·71	1·335	0·077	—

* Absolute rates of protein synthesis or deposition were calculated from tissue protein content multiplied by maximum fractional synthesis rate (FSR_{Max}) or by fractional growth rate (FGR) respectively (see Table 3).

† (FGR × 100)/FSR_{Max}.

Table 5. *Percentage contributions of gastrointestinal tissues to gastrointestinal tract (GIT) protein mass (GITPM) or synthesis (GITPS), and to whole-body protein mass (WBPM) or synthesis (WBPS)*

Tissue	GITPM	GITPS*	WBPM†	WBPS‡
Oesophagus	2·6	1·0	0·1	0·1
Reticulo-rumen	7·8	3·4	0·3	0·4
Omasum	1·5	0·9	0·1	0·1
Abomasum	11·0	8·9	0·4	1·0
Small intestine	60·5	76·2	2·4	8·8
Caecum	4·5	2·9	0·2	0·3
Colon	12·1	6·7	0·5	0·8
Gastrointestinal tract	100	100	4·0	11·5

* Contributions of gastrointestinal tissues to GITPS or WBPS were calculated from absolute synthesis rates (see Table 4).

† 611·8 (SE 21·6) g, includes exsanguinated and eviscerated whole-body, liver and GIT protein content. For details, see p. 163.

‡ 146·4 (SE 5·7) g/d, sum of absolute protein synthesis rates (maximum fractional synthesis rate × protein content) in the exsanguinated and eviscerated whole-body, liver and GIT. For details, see p. 163.

Tissue protein FSR

Comparison of our findings and of gastrointestinal tissue protein FSR reported by others in lambs or sheep is particularly difficult and could be misleading for several reasons. First, these FSR crucially depend on the choice of the precursor pool of protein synthesis. Second, in infused animals, the proteins exported by secretion or cell exfoliation are not taken into account in FSR measurements. The same problems are encountered with the recent procedure proposed by Schaefer *et al.* (1986), when the concentration of the radiolabelled amino acid is sustained by simultaneous infusion of the same unlabelled amino acid. Therefore, the large-dose technique gives higher estimates of FSR in the GIT than the continuous-infusion technique (McNurlan *et al.* 1979; Garlick, 1980). Finally, all the published studies differ in the live weight of lambs and methodology used, or both. Consequently, tissue protein FSR varies greatly between experiments (Davis *et al.* 1981). However, we observed among the gastrointestinal tissues the highest values for FSR in the small intestine (Table 3), as previously reported by Buttery *et al.* (1977) in ruminant lambs and by Schaefer & Krishnamurti (1984) in the ovine fetus.

Comparison of our findings with results in other species is also difficult, since information for sucking animals is poorly documented. Our estimates for FSR in the duodenum, jejunum or ileum (84·1–92·5 %/d) compared favourably with the value of 100 %/d reported for the intestine of immature rats by using a large amount of [³H]phenylalanine (Reeds *et al.* 1982). Compared with results obtained in large-dose experiments in weaned rats, our estimates in the small and large intestines (87·5–38·4 %/d) are slightly lower than the values reported in 21-d-old animals (97·5–57·8 %/d) by Goldspink *et al.* (1984) and in 100 g rats (103·4–62·1 %/d) by McNurlan & Garlick (1980). In the stomach of the 100 g rat, McNurlan & Garlick (1980) reported a FSR of 74 %/d which is also higher than our estimate in the abomasum (56·1 %/d).

The positive gradient of FSR from oesophagus to small intestine may correlate with the gradient of metabolic activity in the GIT of preruminant animals. The young preruminant is physiologically a simple-stomached animal. The majority of liquid feed bypasses the reticulo-rumen by means of the oesophageal groove, and goes directly through the omasum to the abomasum (Church, 1969). The rumen mucosa exhibits only small and undeveloped

papillae in milk-fed young ruminants (Warner & Flatt, 1965; Stobo *et al.* 1966; Church, 1969). Therefore, a rather low estimate of FSR could be expected in the reticulo-rumen (Table 3). The significantly higher FSR of the abomasum (56·1 %/d) compared with the omasum (41·0 %/d) probably reflected the synthesis and secretion of digestive enzymes which do not occur in the forestomach. This was supported by the estimate of FSR that we measured in the abomasal mucosa (74·1 %/d) compared with the abomasal musculosa (39·1 %/d). The latter value was in agreement with the oesophageal smooth muscle FSR (41·6 %/d) reported by Lewis *et al.* (1984) in the 21-d-old rat. However, our estimate in the whole oesophagus was very different (26·5 %/d) and in the same range as the 22·9 %/d value determined in the skeletal M. tensor fasciae latae of 1-week-old lambs (Attaix *et al.* 1986*a*). In the rat, the lower two-thirds of the oesophagus contains only smooth muscle, and the 41·6 %/d value reported by Lewis *et al.* (1984) referred only to this portion. By contrast, the oesophagus of the sheep contains essentially striated muscle (Comline *et al.* 1968). Higher FSR in the smooth muscle than in striated muscles have been reported throughout the development of the rat (Lewis *et al.* 1984).

Cell turnover accounts for a significant loss of protein in the gut and therefore could explain the high rates of protein synthesis in the GIT (Alpers & Kinzie, 1973; McNurlan *et al.* 1979). It is noteworthy that the pattern for FSR that we observed, increasing from oesophagus to small intestine and then decreasing towards the rectum, corresponded to a similar pattern for both labelling and mitotic indexes in the GIT of the rat (Sakata & Yajima, 1984). However, these indexes represent a state and do not measure the rate of cell production.

Measurements of cell production rate have not been performed along the GIT of preruminant animals. We have measured an epithelial cell transit time of approximately 50, 91 and 94 h in the duodenum, jejunum and ileum respectively, of lambs of identical genotype and reared in similar conditions as those of the present experiment (Attaix *et al.* 1984). By contrast, our estimates of FSR were not significantly different from duodenum to ileum (Table 3). However, it may be argued that differences in cell transit times did not necessarily imply differences in cell production per unit of small intestine surface. Southon *et al.* (1985*b*) found similarly no relation between the rates of cell production and protein synthesis in mucosal scrapes in zinc-deficient rats. This contrasted with a previous report of the same group that a reduction in the FSR was associated with a decreased mucosal cell proliferation in rats given a semi-synthetic diet when compared with pellet-fed animals (Southon *et al.* 1985*a*). These discrepancies probably resulted from the large-dose technique used for measuring protein synthesis which offers no means of studying the partition of protein synthesis between intracellular turnover, cell secretion and cell renewal. Therefore, the decrease in FSR from the small intestine to the caecum and colon cannot be interpreted only in terms of a reduced cell turnover, as previously suggested in the rat (McNurlan & Garlick, 1980). Although the hypothesis of reduced cell-production rate along the intestines cannot be excluded, it is noteworthy that the rat large intestine exhibited a lower ribosomal capacity than the small intestine 3–105 weeks after birth (Goldspink *et al.* 1984).

Contribution of the GIT to whole-body protein synthesis

As for FSR, comparisons of our estimates with other published values for lambs are difficult to make due to methodological considerations and stage of development of the animals used. However, our estimate of 11·5 % for the contribution of the GIT to whole-body protein synthesis was close to the 13·9 % value obtained by using the constant-infusion technique in the ovine fetus (Schaefer & Krishnamurti, 1984). By contrast, our estimate was lower than the estimates made in growing, ruminant lambs (18–34 %) by Davis *et al.* (1981), and in cattle (32·1–45·9 %) by Lobley *et al.* (1980). However, the contribution of the GIT

to protein synthesis, calculated as the sum of muscles, skin, liver and GIT protein synthesis, has been reported to increase significantly in lambs, between 1 and 16 weeks of age, especially after weaning (Combe *et al.* 1979). The development of the ruminant stomach and the importance of dietary fibre in the diet of ruminants could explain this increase. In a recent report Southon *et al.* (1985a) have shown that giving rats a commercial pelleted diet led to a significant increase in small intestinal FSR compared with that of rats fed on a fibre-free, semi-synthetic diet. On the other hand, compared with the estimates obtained with large-dose experiments in rodents, the contribution of the small and large intestine to whole-body protein synthesis (8·8 and 1·1 % respectively) appeared, to a lesser extent, to be lower than the values reported for the intestines of immature lean Zucker rats (16·2 %) by Reeds *et al.* (1982), and by McNurlan & Garlick (1980) in the 100 g rat (15·2 and 2·5 % for the small and large intestine respectively). The contribution of the stomachs to whole-body protein synthesis (1·5 %) was also in close agreement with the 1·1 % value calculated for the 100 g rat stomach (McNurlan & Garlick, 1980).

CONCLUSIONS

The large-dose technique appeared suitable to obtain reliable estimates for FSR in the GIT of large domestic animals. The present findings demonstrated that a positive gradient for FSR was observed from the oesophagus to the small intestine; thereafter FSR declined from the small intestine to the rectum. Our results also suggested that both in terms of FSR and contribution of GIT to whole-body protein synthesis, there were only slight differences between the preruminant lamb and the immature rat.

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