Protein digestion in the intestine of sheep

By D. BEN-GHEDALIA, H. TAGARI AND A. BONDI

Department of Animal Nutrition and Agricultural Biochemistry, The Hebrew University, Faculty of Agriculture, Rehovot, Israel

AND A. TADMOR

Kimron Veterinary Research Institute, Beth Dagan, Israel

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1. The rate of flow of digesta along the intestinal tract, and particularly the changes occurring in proteins during their passage through the intestine were determined in six rams; each animal was fistulated with three cannulas which involved six different sites of the intestine. Cr_2O_3 was used as a marker substance to measure the rate of flow of the digesta.

2. In the sections of the intestine from 1 to 15 m posterior to the pylorus the amounts of water, dry matter and total nitrogen decreased gradually as a result of their absorption through the intestinal wall. The region of the intestine situated at a distance of 7–15 m from the pylorus was more active with respect to the absorption of N, whereas water and dry matter were adsorbed to a greater extent in the region from 1 to 7 m from the pylorus.

3. The only part of the intestine in which substantial increases of water, dry matter and total N were found was the section immediately distal to the pylorus, and these increases were caused by the inflow of bile, and pancreatic and duodenal juices. The net increase found beyond the entry of the common bile duct was 2·7 g protein N and 2·0 g non-protein N (NPN)/24 h.

4. The activities of trypsin, chymotrypsin and carboxypeptidase A and the ratio α-NH_2–NPN:protein N increased from the pylorus up to a distance of 7 m and decreased again from this point to a distance of 15 m from the pylorus.

5. In the sections of the intestine between 1 and 3 and between 3 and 7 m distant from the pylorus the extent of proteolysis exceeded considerably that of absorption of amino acids through the intestinal wall. This was concluded from the decrease in the rate of flow of protein amino acids (by 31 % between 1 and 3 m distant from the pylorus and by 34 % between 3 and 7 m) and the simultaneous increase in non-protein amino acids (by 20 % in the region between 1 and 3 m) or no change in non-protein amino acids (between 3 and 7 m).

6. The relatively greater decrease in non-protein amino acids (by 57 %) compared with that of protein amino acids (by 41 %) occurring in the section 7 to 15 m distant from the pylorus showed that this is an area of most intensive absorption of amino acids.

7. In the lower section of the intestine, from 15 to 25 m distant from the pylorus, the total amount of amino acids showed almost no change; probably a net effect of loss and gain of amino acids mainly due to microbial activities. Increases in the dehydrogenase activity suggested enhancement of bacterial activity in this lower region of the intestine.

8. The supply of essential amino acids to the tissues of sheep is improved, compared with the amino acid composition of the diet, as the result of ruminal biosynthesis of essential amino acids and ruminal degradation of non-essential amino acids and preferential absorption of essential amino acids through the intestinal wall, particularly in the section of most intensive absorption, 7–15 m distant from the pylorus.

Whereas the transformations of the nitrogenous food components occurring in the reticulo-rumen of ruminant animals have been thoroughly studied, very few quantitative results are available on the course of digestion of proteins in the intestine (Kay, 1969). Since extensive digestion of nitrogen compounds takes place in the intestine and the greater part of the N-containing metabolites is absorbed through the intestinal wall, a study of intestinal digestion in ruminants is of interest.

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Table 1. *Position of intestinal cannulas in the rams nos.* 1–6

Distance from pylorus (m)	No. 1	No. 2	No. 3	No. 4	No. 5	No. 6
0·05	.	+	+	+	.	.
1	.	+	+	.	+	.
3	.	.	+	.	+	+
7	+*	.	.	.	+	+*
15	+	.	.	+	.	+
25 (terminal ileum)	+	+	.	+	.	.

* Rams nos. 1 and 6 rejected these cannulas at the beginning of the experiment.

Whereas a periodical influx of digesta into the intestine is typical for the simple-stomached animal, the passage of the digesta from the reticulo-rumen and omasum is a continuous process. Hence the digesta that reach the intestine in ruminants are of more uniform composition and less dependent on the kind of food ingested than in simple-stomached animals. These digesta consist to a great extent of microbial protein and contain only very small amounts of carbohydrates, since most of the latter have been digested and their metabolites have been absorbed in the rumen. Therefore the digestive processes in the intestinal tract of simple-stomached and ruminant animals may differ. In this work the rate of passage of digesta, and particularly of their nitrogenous constituents, such as non-protein N(NPN), α-NH_2–N, NH_3 and individual amino acids through the different regions of the intestine has been studied. The changes occurring in nitrogenous constituents during passage through the intestine have been examined, the activity of proteolytic enzymes in the different parts of the intestine has been assessed, and the extent of the absorption of the nitrogenous metabolites from the different sections of the intestinal wall has been determined.

Furthermore, in order to examine the possible involvement of micro-organisms in N interactions occurring in the intestine, dehydrogenase activity reflecting microbial biochemical capacity was assayed in the samples of digesta.

EXPERIMENTAL

Animals

Six Awassi rams, 1–1·5 years old and weighing 50–60 kg, were used. Each animal was equipped with 3 T-type Perspex cannulas inserted at different sites of the small intestine; the positions of the cannulas are given in Table 1.

The cannulas consisted of two parts: a straight barrel, 45 mm long, was joined with a screw to a gutter-shaped internal flange, 42 mm long; the flange was inserted into the intestine and the barrels exteriorized through the abdominal wall. The barrels were closed by a combined cap and core which prevented accumulation of digesta in the barrel between sampling times. Two kinds of cannula were used. The first type with a barrel of 11·5 mm internal diameter and with an internal flange of 13 mm internal diameter was inserted into the intestine at 0·05 m distance from the pylorus and into the terminal ileum. The second type with a barrel of 9·5 mm internal diameter and a flange of 10·5 mm internal diameter was inserted at the middle sites of the intestine.

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Table 2. *Composition (g/kg) of the feedstuffs*

Feedstuff	Dry matter	Crude protein	Diethyl ether extract	Crude fibre	Ash	Nitrogen-free extract
Concentrate mixture*	890	100	24·8	124	72·5	568
Vetch hay (*Vicia sativa* L.)	885	144	18·0	273	112·0	338

* Contained (per kg) 250 g barley, 250 g maize, 250 g wheat, 150 g cottonseed hulls, 50 g cottonseed meal, 50 g mineral mixture, 1200 μg retinol and 10 μg ergocalciferol.

Table 3. *Amino acid content (mmol/kg) of the feedstuffs*

Amino acid	Vetch hay (*Vicia sativa* L.)	Concentrate
Essential:		
Threonine	42·9	26·5
Valine	76·5	51·0
Methionine	9·9	5·8
Isoleucine	39·8	29·2
Leucine	62·7	64·5
Phenylalanine	43·2	31·8
Lysine	40·2	22·4
Histidine	15·7	11·4
Arginine	30·8	27·3
Non-essential:		
Aspartic acid	109·0	54·3
Serine	49·4	35·9
Glutamic acid	82·4	123·0
Proline	85·8	65·5
Glycine	92·5	59·2
Alanine	70·3	66·3
Cystine + cysteine	4·6	9·4
Tyrosine	42·4	22·6

The animals recovered quickly and returned to normal food consumption within a month after the operation; the experiment began 3 months later.

Rams nos. 1 and 6 rejected the cannulas inserted at 7 m distance from the pylorus at the beginning of the experiment. The rejection was accompanied by a slight inflammation, after which the site of rejection healed up within 2 weeks, and the experiment, which had been interrupted, began again.

The total length of the small intestine was measured in rams nos. 1, 2, 4 and 6; it was $25 \pm 1{\cdot}2$ m. Very similar values for the length of the intestine have been found for Suffolk Cross and Scottish Blackface sheep by T. E. C. Weekes and R. M. Campbell at the Rowett Research Institute (private communication from Dr Margaret I. Chalmers).

Feeding

Each animal received a daily ration consisting of 400 g vetch hay (*Vicia sativa* L.) and 600 g concentrate mixture. Feeds were given in equal amounts twice daily at 07.30 and 19.30 hours and were completely consumed. The composition of these feeds is given in Table 2. Water was freely available. During the experiment the rams were

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Table 4. *Daily faecal recoveries of Cr_2O_3 given orally as a component of paper to sheep given three different rations*

Ration	Mean daily excretion (g dry faeces/sheep)	Cr_2O_3 recovery g/d	Cr_2O_3 recovery As ratio of intake mean
A	301	2·068	1·02
B	335	2·008	0·99
C	338	1·988	0·98
SEM			0·04

housed in individual pens. Chromic oxide (1 g) impregnated into paper was given orally twice daily before feeding. The amino acid content of the feedstuffs ingested is given in Table 3.

Recovery of Cr_2O_3 and its use for calculating the flow rate of digesta

A separate experiment was done in order to ensure quantitative recovery of Cr_2O_3 from the faeces. Nine 1-year-old rams (average weight 55 kg) were used. Three rations were used, each being given to three animals. The rations and the Cr_2O_3 were given for a preliminary period of 5 d before the start of the collection period, which lasted for 12 d. The virtually complete recovery of Cr_2O_3 in the faeces (Table 4) is in agreement with the findings of Corbett, Greenhalgh, McDonald & Florence (1960) and of MacRae & Armstrong (1969).

Klooster, Rogers & Sharma (1969) and Corse & Sutton (1971) compared the flow of digesta measured by direct continuous collections through re-entrant cannulas with indirect measurements based on the concentration of Cr_2O_3 in spot samples of the digesta. According to these authors there was very little difference between the flow rate estimated by either method. In this work, calculation of the flow rate was based on spot sampling with Cr_2O_3 as indicator.

Sampling technique

The frequency of sampling and size of samples withdrawn had to be limited in order to avoid influencing the flow rate. Sampling of faeces and digesta began at the rectum and continued upwards through the terminal ileum to the proximal duodenum. Before the samples were collected through the cannulas the animals were accustomed for 1 d to the sampling procedure. Samples, each of 25 ml digesta, were withdrawn through each cannula six times daily for 4 successive d, 1, 3, 5, 7, 9 and 11 h after the morning feed. The samples obtained on the 4 successive d from each cannula were pooled for each sheep separately and kept frozen at $-20°$ until analysis. Four or five samples were obtained daily from the terminal ileum cannulas. The flow of digesta through the ileum was characterized by its irregularity, in contrast with the virtually continuous flow through the duodenum. Sometimes there were no digesta present at the site of the ileal cannulas and it was impossible to take samples from this site.

Total N, N-containing fractions, and Cr_2O_3 were determined in the pooled samples.

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Portions of these samples were freeze-dried for determination of the dry-matter content.

At the end of the sampling period, samples were taken simultaneously from all cannulas during 1 d, 1, 3, 5, 7 and 9 h after the morning feed. The samples obtained after each time-interval from all sheep were pooled and used for determination of pH. Activities of trypsin (*EC* 3.4.4.4), chymotrypsin (*EC* 3.4.4.5) and carboxypeptidase A (*EC* 3.4.2.1) were measured in the pooled digesta samples taken 1, 5 and 9 h after the morning feed.

For determination of amino acids, digesta samples of 25 ml each were withdrawn from three rams through each cannula six times daily on 4 successive d. The samples obtained on the 4 successive d from the three sheep were pooled separately for each site of cannulation.

Samples of digesta for the determination of dehydrogenase activity were removed from three rams through each cannula, three times on 1 d, 1, 5 and 9 h after the morning feed. The samples taken from all cannulas were pooled separately for each sampling time.

Analytical procedures

Cr_2O_3 was determined by the method of Stevenson & de Langen (1960). Total N in digesta and faeces was measured by the Kjeldahl method. NPN was determined by the micro-Kjeldahl method in the supernatant fraction of digesta separated by precipitation with trichloroacetic acid (TCA) solution (50 g/l).

Ammonia was determined with a Technicon AutoAnalyzer (Technicon Instrument Co. Ltd) by the method of Clare & Stevenson (1964).

Free α-NH_2–N was measured in the TCA supernatant fraction of the digesta and faeces by the colorimetric ninhydrin procedure (Rosen, 1957). For determination of total α-NH_2–N in NPN, a pooled sample from each site of cannulation was taken from three sheep and protein was precipitated with TCA solution (50 g/l). After removal of TCA by extraction with diethyl ether a portion (5 ml) of the supernatant fraction was freeze-dried and the dry residue hydrolysed by boiling in 6 M-HCl (constant boiling point) for 22 h at 110° under reduced pressure. The hydrolysate was dried and the α-NH_2–N determined by the ninhydrin method.

For the determination of individual free amino acids (non-protein amino acids) samples were prepared similarly. Before hydrolysis, norleucine was added as internal standard. After hydrolysis, HCl was removed on a rotary evaporator under reduced pressure at 60° and the residue dissolved in 0·2 M-citrate buffer, pH 2·2. Individual amino acids were determined by column chromatography using an amino acid analyzer, Bio-Cal, BC-200.

For the determination of individual amino acids (total amounts) in feeds, digesta and faeces, the samples were freeze-dried, ground in a hammermill and passed through a 1 mm sieve; hydrolysis and determination of amino acids were as described above. pH was measured with a Beckman portable pH meter immediately after sampling.

The pooled samples used for determination of trypsin and chymotrypsin activities were kept in crushed ice and centrifuged at 70000 *g* for 15 min at 2°. Trypsin,

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Table 5. *Mean flow rates of total digesta, water and dry matter and pH range of intestinal contents of three sheep sampled at different sites (dry matter ingested: 37-82 g/h)*

Distance of site from pylorus (m)	Adjusted flow-rate mean (g/h)			pH (range)
	Digesta	Water	Dry matter	
0·05	511	487	23·8	2·60–3·00
1	555	526	29·1	3·54–4·65
3	433	408	24·4	4·11–5·15
7†	291	270	20·1	5·95–7·02
15	205	187	17·8	7·80–8·15
25 (terminal ileum)	181	162	18·5	7·70–8·22
Residual SD	37·9	37·3	1·4	
SE of difference between adjusted means:				
Least value	34·0	33·5	1·3	
Greatest value	59·3	58·4	2·2	
	Significance of the factors			
Effects of rams	NS	NS	NS	
Effects of cannulas	***	***	**	

NS, not significant. ** $P < 0.01$. *** $P < 0.001$. † One sheep only.

chymotrypsin and carboxypeptidase A activities in the supernatant fraction were measured titrimetrically with benzoyl-L-arginine ethylester hydrochloride, *N*-acetyl-L-tyrosine ethylester (Neurath & Schwert, 1950) and hippuryl-DL-phenyl-lactic acid (Marchaim & Kulka, 1967) respectively, as substrates, using a Radiometer-Copenhagen pH-stat with automatic titrator 11. The reaction was carried out at 38°; 1 μmol substrate hydrolysed/min was defined as one unit of activity.

Dehydrogenase activity of the digesta was determined with triphenyltetrazolium chloride by the method of Tagari, Dror, Ascarelli & Bondi (1964) as modified by Dror, Tagari & Bondi (1970).

Statistical analysis

An 'incomplete block' analysis was used in order to compare the results obtained at six sites on the basis of samples taken from two or three cannulas in each of six rams. Because the design was not balanced, the standard error of the difference between the adjusted means is not the same for each pair of sites. The least and greatest values are given in Tables 5 and 6. The residual standard deviation is also given as a measure of the variability of the measured values.

RESULTS

Changes occurring in the digesta along the intestinal tract

It is unfortunate that samples taken 7 m distant from the pylorus were available from only one animal, and this should be borne in mind when changes spanning this region are considered.

Water, dry matter and total N. The rates of flow per h of digesta dry matter (g/h), water, total N and N-containing fractions through the different sections of the intestine

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Table 6. *Amounts (adjusted means) (mg/h) of nitrogen and of nitrogenous components in digesta of three sheep, sampled at different sites of the intestinal tract and excreted in faeces (N ingested in food: 785 mg/h)*

Distance of site from pylorus (m)	Total N	Non-protein N	α-NH_2-N			Ammonia N
			Total	Bound	Free	
0·05	743	254	181	123	62	53
1	951	320	199	138	68	64
3	828	277	248	175	75	55
7†	810	281	237	127	120	52
15	449	150	105	22	75	29
25 (terminal ileum)	383	102	57	20	31	23
Faeces	296				13·5	
Residual SD	76·2	11·7			16·0	5·1
SE of difference between adjusted means:						
Least value	68·5	10·5			14·4	4·6
Greatest value	119·3	18·3			25·1	8·0
	Significance of the factors					
Effects of rams	NS	NS			NS	NS
Effects of cannulas	**	***			NS	**

NS, not significant. ** $P < 0.01$. *** $P < 0.001$. † One sheep only.

are presented in Tables 5 and 6. Some of the results were calculated on a 24 h basis to facilitate comparison with results of earlier publications, although it was recognized that this may have introduced a substantial error. The changes in the amounts of constituents of digesta during passage along the intestine represent the balances between the inflows of digesta from the abomasum and the secretion of water and endogenous materials into the gut, on the one hand, and their absorption through the intestinal mucosa, on the other hand. The means, calculated by an 'incomplete block' analysis, showed that the cannulation site had a highly significant effect on the flow of digesta, dry matter and NPN, and a significant effect on that of total N and ammonia N. The effect on α-NH_2–N came close to being significant. There were no significant differences between rams.

The amount of digesta passing the pylorus, 511 g/h (12·3 kg/24 h) (see Table 5) was similar to the mean amount reported by other authors (e.g. Nicholson & Sutton, 1969), who kept sheep on a similar dietary regimen.

In agreement with the general experience of others (Hogan & Phillipson, 1960), it was observed that 40% of the digestible dry matter ingested reached the pylorus; the net loss of 60% was mainly digestible carbohydrate. Only 5% less N than the amount ingested reached the pylorus (Table 5).

The only part of the intestine in which substantial increases of water, dry matter and total N were found was the 1 m section posterior to the pylorus, but the increase was statistically significant only for total N. These increases were caused by the inflow of bile, and pancreatic and duodenal juices. The observed increase of about 44 g/h in the flow is consistent with the reported daily rates of secretion of digestive juices by sheep,

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Table 7. *Nitrogenous fractions expressed as a ratio of total nitrogen in the digesta of sheep, sampled at different sites of the intestinal tract*

Distance of site from pylorus (m)	Protein	Non-protein N	α-NH_2–N		Ammonia N	Unaccounted N
			Bound	Free		
0·5	0·667	0·333	0·164	0·078	0·070	0·021
1	0·649	0·351	0·146	0·064	0·066	0·075
3	0·622	0·378	0·216	0·090	0·064	0·008
7	0·606	0·394	0·171	0·148	0·066	0·009
15	0·681	0·319	0·047	0·183	0·067	0·022
25	0·772	0·228	0·094	0·092	0·065	0·022

namely bile 700 ml (Harrison, 1962), pancreatic juice 300–400 ml (Magee, 1961; Taylor, 1962), duodenal juice 500 ml (Harrison & Hill, 1962).

In the sections of the intestine from 1 to 15 m posterior to the pylorus, however, the amounts of water, dry matter and total N decreased gradually as a result of their absorption through the intestinal wall. The region of the intestine situated at a distance of 7–15 m from the pylorus was more active with respect to the absorption of N compounds, whereas water and dry matter were absorbed to a greater extent in the section 1–7 m from the pylorus (Tables 5 and 6). Only very small changes in the amounts of water, dry matter and total N occurred in the lower part of the intestine (between 15 and 25 m distant from the pylorus).

pH. The pH of the intestinal contents sampled at the pylorus fluctuated between 2·6 and 3·0, increased gradually along the intestine, but remained acid even at a distance of more than 7 m from the pylorus. This agrees with pH measurements made by Lennox & Garton (1968). The fluctuations of the pH values measured in different samples from the same sites of the intestine resulted from the changes in the pH during the day and did not show any regular trend.

N-containing fractions. The amounts of N and N-containing constituents of the digesta passing the different sites of the intestine are presented in Table 6, and the composition of these N-containing fractions is given in Table 7.

The net daily increases found beyond the entry of the common bile duct were 2·7 g protein N and 2·0 g NPN. These were the amounts secreted by one sheep (Table 6). The increase in the NPN content of the digesta in the uppermost region of the intestine, that is the section immediately distal to the pylorus, indicates the presence of non-protein amino acids in the endogenous secretions.

Whereas the amounts of total N decreased considerably at a distance of 1–7 m from the pylorus, the amounts of NPN remained fairly constant in this region, unlike those of free α-NH_2–N, which increased slightly. This tendency indicates that the rate of proteolysis exceeds the rate of absorption of its products, which obviously occurs as free amino acids. The amounts of total N and of all N-containing fractions decreased considerably in the section 7–15 m distant from the pylorus, the area of most intensive N absorption. The decrease of bound α-NH_2–N in the area 7–15 m distant from the pylorus may have resulted from the action of exopeptidases. The highest percentage of free α-NH_2–N was found at a distance of 15 m from the pylorus (Table 7).

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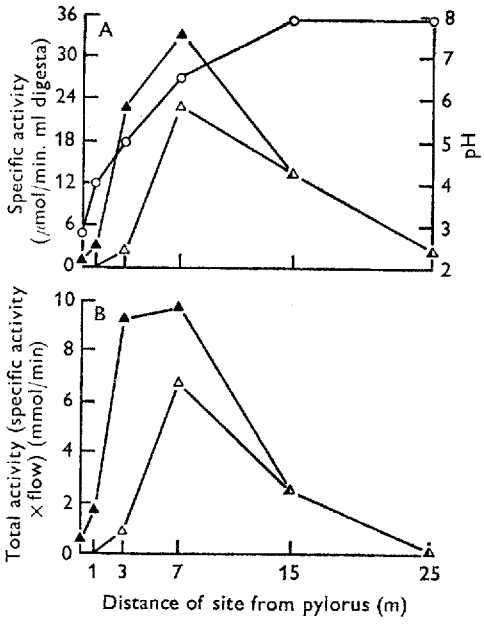

Fig. 1

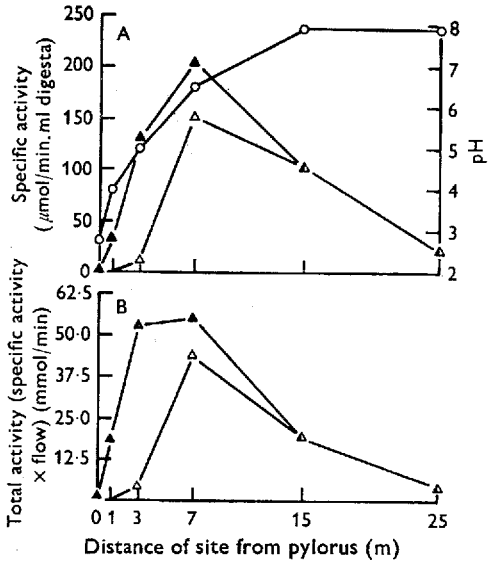

Fig. 2

Fig. 1. Trypsin activity in the digesta of sheep, sampled at different sites of the intestine. △, trypsin activity at pH *in situ*; ▲, trypsin activity at optimal pH (pH 8·0); ○, pH *in situ*.

Fig. 2. Chymotrypsin activity in the digesta of sheep, sampled at different sites of the intestine. △, chymotrypsin activity at pH *in situ*; ▲, chymotrypsin activity at optimum pH (pH 8·0); ○, pH *in situ*.

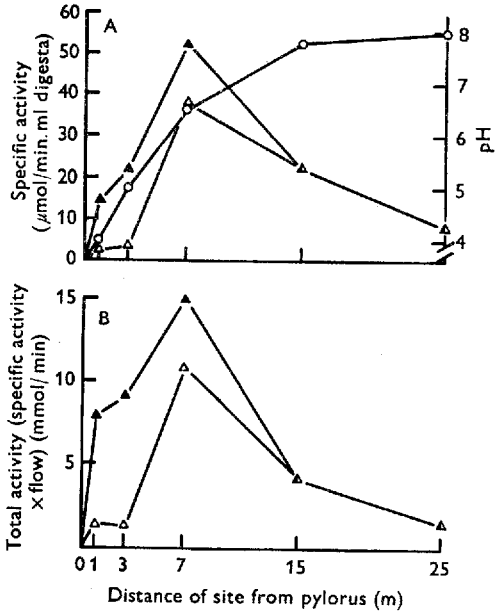

Fig. 3. Carboxypeptidase A activity in the digesta of sheep, sampled at different sites of the intestine. △, carboxypeptidase A activity at pH *in situ*; ▲, carboxypeptidase A activity at optimum pH (pH 7·6); ○, pH *in situ*.

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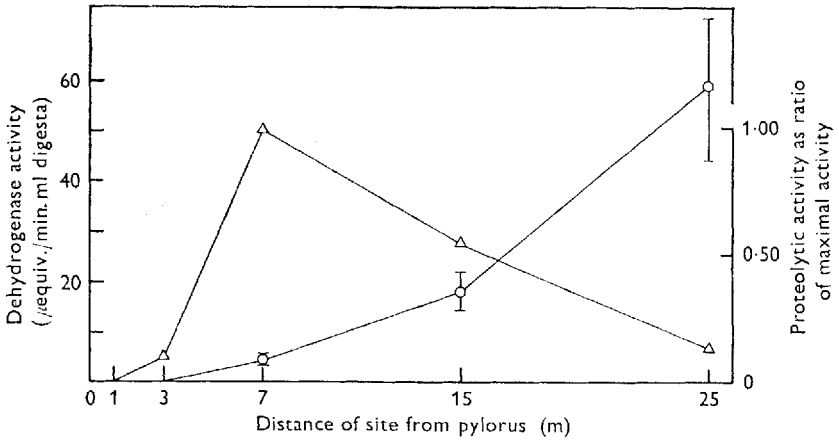

Fig. 4. Dehydrogenase and proteolytic activities in digesta of sheep sampled at different sites of the intestine. ○, dehydrogenase activity; △, proteolytic activities are mean values of the sum of trypsin, chymotrypsin and carboxypeptidase A activities (expressed as ratio of maximal activity found at 7 m distance from the pylorus).

The nitrogenous components of the digesta contain also an unidentified fraction, which includes hexosamines (Badawy & Mackie, 1964) and nucleic acids (Smith & McAllan, 1971). The highest percentage of this unidentified fraction was found in the upper section of the intestine proximate to the entry of the common bile duct.

The small losses of N found in the large intestine were due to the absorption of ammonia from the caecum, as shown by McDonald (1948), whereas the absorption of amino acids from the caecum is unlikely, since they would be destroyed there by bacterial enzymes (Clarke, Ellinger & Phillipson, 1966).

Proteolytic activities. The activities of the proteolytic enzymes trypsin, chymotrypsin and carboxypeptidase A were examined in samples taken from different sites of the intestine, and the measurements were made at the actual pH (*in situ*) and at the pH of optimal activity of the three enzymes. The activities of samples withdrawn at three different time-intervals after the morning feed differed only slightly and did not show any regular trend. The results presented in Figs. 1, 2 and 3 were based on average values of activities determined at three different times of the day. Samples taken 1 m distant from the pylorus showed very low trypsin or chymotrypsin activities, at the pH *in situ* and also at the optimum pH.

Since the conversion of the zymogens into active enzymes takes place above pH 5, the proteolytic activities increase only slowly along the intestine (even when measured at the optimum pH) and maximum activity of these enzymes is reached in the samples withdrawn at a distance of 7 m from the pylorus; since the pH of the digesta increases along the intestine, the difference between the activities measured at the actual and optimum pH became smaller in the sample taken at the site 7 m distant from the pylorus and disappeared in those taken in the lower regions of the intestine. The decrease in activities of all three enzymes in the lower regions of the intestine may be attributed to inactivation of these enzymes in the more alkaline medium.

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Table 8. *Amounts (mmol/h) of total amino acids present in the feed, passing through different sites of the intestine and excreted in the faeces of sheep*

Amino acid	In feed	In digesta at distance from pylorus of (m) 0·05	1	3	7	15	25	In faeces
Essential:								
Threonine	1·39	1·86	2·11	1·76	1·58	0·75	0·77	0·62
Valine	2·55	3·08	3·51	3·06	2·54	1·22	1·19	1·06
Methionine	0·31	0·61	0·76	0·68	0·60	0·27	0·28	0·23
Isoleucine	1·39	1·44	2·03	1·77	1·39	0·63	0·61	0·59
Leucine	2·66	2·11	3·05	2·50	2·00	0·86	0·88	0·86
Phenylalanine	1·53	1·47	1·69	1·54	1·30	0·67	0·53	0·66
Lysine	1·23	1·65	2·33	2·06	1·58	0·47	0·58	0·60
Histidine	0·55	0·50	0·48	0·50	0·45	0·21	0·18	0·20
Arginine	1·20	0·87	1·29	1·11	0·81	0·30	0·36	0·32
Total	12·8	13·6	17·3	15·0	12·3	5·38	5·38	5·14
Non-essential:								
Aspartic acid	3·17	3·40	3·95	3·49	2·96	1·26	1·30	1·11
Serine	1·72	1·31	2·30	1·91	1·80	0·77	0·81	0·64
Glutamic acid	4·45	2·98	4·37	3·94	2·65	1·36	1·42	1·22
Proline	3·07	1·46	2·30	1·72	1·55	0·77	0·66	0·90
Glycine	3·02	2·80	6·25	5·06	3·65	3·07	1·54	1·36
Alanine	2·83	2·59	3·66	3·17	2·51	1·15	1·21	1·10
Cystine + cysteine	0·31	0·30	0·62	0·34	0·41	0·24	0·16	0·17
Tyrosine	1·27	1·12	1·72	1·46	1·07	0·58	0·32	0·23
Total	19·9	16·0	25·2	21·1	16·6	9·20	7·42	6·73

Dehydrogenase activity. An increase of the dehydrogenase activity was observed in the lower part of the intestine (Fig. 4).

Amino acids. The rate of flow of total amino acids through the different sections of the intestines of sheep is given in Table 8, that of non-protein amino acids in Table 9 and that of amino acids bound as protein in Table 10. The values in Table 9 were obtained by hydrolysis of TCA filtrates of digesta and represent the sum of amino acids present in free form and as peptides. The results concerning the flow of amino acids present as proteins given in Table 10 were obtained by subtraction of the values for non-protein amino acids (Table 9) from those for total amino acids (Table 8). Individual amino acids were determined in pooled samples. The trend in their changes along the intestine agreed with those of total N and NPN, which were found to be highly significant (see Table 6). The amino acids designated as 'essential' in this work are those required by the growing rat, since, according to other authors, the same amino acids are also essential for the tissue metabolism of ruminant animals (see Coelho da Silva, Seeley, Thomson, Beever & Armstrong, 1972).

There was a net loss of non-essential amino acids between the mouth and the pylorus; it was considerable for glutamic acid, proline and serine. The total amount of amino acids reaching the small intestine was smaller than that of the ingested amino acids, and the quantity of amino acids lost in the rumen exceeded the amount synthesized there. The 100% increase in methionine during the passage of the food through the reticulo-rumen is comparatively high, but agrees with the results of other authors (Conrad, Miles & Butdorf, 1967), who reported the biosynthesis of considerable

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Table 9. *Amounts (mmol/h) of non-protein amino acids in digesta of sheep passing through different sites of the intestine*

	At distance from pylorus of (m)					
Amino acid	0·05	1	3	7	15	25
Essential:						
Threonine	0·75	0·76	0·92	0·91	0·38	0·26
Valine	1·00	1·06	1·24	1·11	0·50	0·28
Methionine	0·23	0·27	0·31	0·21	0·11	0·08
Isoleucine	0·54	0·55	0·69	0·75	0·19	0·12
Leucine	0·90	0·86	1·04	0·91	0·37	0·19
Phenylalanine	0·59	0·57	0·70	0·68	0·29	0·21
Lysine	0·80	0·88	1·10	1·02	0·32	0·15
Histidine	0·13	0·16	0·19	0·11	0·10	0·05
Arginine	0·34	0·35	0·46	0·42	0·11	0·05
Total	5·28	5·46	6·65	6·12	2·37	1·39
Non-essential:						
Aspartic acid	1·36	1·41	1·74	1·78	0·60	0·40
Serine	0·71	0·76	0·90	0·86	0·40	0·24
Glutamic acid	1·37	1·53	2·01	1·96	0·73	0·40
Proline	0·60	0·66	0·65	0·69	0·28	0·23
Glycine	1·02	1·34	1·59	2·15	1·40	0·36
Alanine	1·28	1·29	1·59	1·30	0·54	0·34
Cystine + cysteine	0·07	0·13	0·08	0·10	0·07	0·04
Tyrosine	0·63	0·81	0·89	0·80	0·42	0·29
Total	7·04	7·93	9·45	9·64	4·44	2·30

Table 10. *Amounts (mmol/h) of protein amino acids in digesta of sheep passing through different sites of the intestine*

	At distance from pylorus of (m)					
Amino acid	0·05	1	3	7	15	25
Essential:						
Threonine	1·11	1·34	0·84	0·67	0·37	0·51
Valine	2·08	2·45	1·82	1·43	0·72	0·91
Methionine	0·38	0·48	0·37	0·39	0·15	0·20
Isoleucine	0·90	1·47	1·08	0·65	0·44	0·49
Leucine	1·21	2·18	1·52	1·09	0·49	0·69
Phenylalanine	0·88	1·11	0·84	0·62	0·39	0·32
Lysine	0·85	1·46	0·95	0·56	0·15	0·43
Histidine	0·36	0·33	0·31	0·33	0·11	0·13
Arginine	0·52	0·95	0·65	0·40	0·18	0·30
Total	8·29	11·77	8·38	6·14	3·00	3·98
Non-essential:						
Aspartic acid	2·03	2·55	1·75	1·18	0·67	0·90
Serine	0·61	1·56	1·01	0·94	0·38	0·58
Glutamic acid	1·61	2·83	1·93	0·69	0·63	1·01
Proline	0·86	1·64	1·07	0·86	0·49	0·43
Glycine	1·78	4·92	3·48	1·50	1·67	1·18
Alanine	1·32	2·34	1·59	1·22	0·61	0·88
Cystine + cysteine	0·22	0·49	0·26	0·31	0·17	0·13
Tyrosine	0·48	0·91	0·57	0·27	0·17	0·03
Total	8·91	17·24	11·66	6·97	4·72	5·14

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Table 11. *Ratio of free to bound α-NH_2–nitrogen in digesta of sheep sampled at different sites of the intestinal tract*

Distance of site from pylorus (m)	Ratio
0·50	0·48
1	0·44
3	0·43
7	0·87
15	3·84
25	1·89

amounts of methionine in the rumen. The proportions of essential amino acids were slightly higher in the digesta reaching the intestines than in the food ingested. This improvement in the amino acid pattern of food proteins brought about by ruminal action agrees with observations of other authors (Neudoerffer, Leadbeater, Horney & Bayley, 1971; Potter, McNeill & Riggs, 1971).

Between one-third and one-half of most amino acids entered the intestine as non-protein amino acid (free- and peptide-bound), but the percentage was much lower for histidine, arginine, proline and cystine (Tables 8 and 9).

The rate of flow of almost all individual amino acids was increased in the section 1 m posterior to the pylorus owing to pancreatic, duodenal and biliary secretions. Their endogenous influx into the uppermost region of the intestine contained mainly proteins and only small amounts of non-protein amino acids. The influx of endogenous juices contributes more non-essential amino acids than essential ones. The very high increase in the flow of glycine (250%) in the region between 0·05 and 1 m distant from the pylorus has to be attributed mainly to the secretion of glycocholic acid, an important constituent of bile acids, and only to a comparatively small extent to endogenous protein (see Nixon & Mawer, 1970).

The changes in rate of flow in the sections between 1 and 15 m distance from the pylorus, measured by analysis of individual amino acids (see Table 8), showed the same trend as that of N and NH_2 groups. In the sections between 1 and 3 m and between 3 and 7 m distant from the pylorus there were considerable decreases (31% and 34% respectively) in the rate of flow of protein-bound amino acids (Table 10), whereas the rate of flow of non-protein amino acids was increased by 20% in the region between 1 and 3 m and did not change in that between 3 and 7 m (Table 9). It might be concluded from these findings that in both these regions the extent of proteolysis exceeded that of absorption. The fate of N compounds in the section 7–15 m distant from the pylorus differed substantially from that in both preceding sections. The relatively greater decrease in non-protein amino acids (57%) compared with that of protein amino acids (41%) that occurred in this region showed that this is an area of intensive absorption of amino acids. The rate of flow of some individual amino acids, such as lysine, decreased here by as much as 70%, while a preferential absorption of essential amino acids was apparent.

The total amount of amino acids (protein and non-protein, Table 8) showed almost no change during passage of the digesta through the lower section of the intestine,

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15–25 m distant from the pylorus. In the lower part of the intestine the dehydrogenase activity was considerably enhanced (Fig. 4).

A few individual amino acids, among them glycine, tyrosine and cysteine, showed changes in the rate of flow in the section of the intestine 15–25 m posterior to the pylorus. Glycine decreased by as much as 50 % during passage through the lower part of the intestine. This decrease in glycine might be attributed in great part to re-absorption of bile acids from the lower small intestine (Garton, 1969). The amounts of most individual amino acids excreted daily in the faeces were somewhat lower than the amounts leaving the terminal ileum (at 25 m distant from the pylorus). This decrease in the amino acid content of the digesta during their passage through the large intestine was apparently caused by the deaminase activity of caecal bacteria (Hecker, 1971). For some amino acids, such as isoleucine, histidine and cystine, the daily amounts passing through the terminal ileum were the same as those excreted in the faeces. The amounts of phenylalanine and proline excreted daily in the faeces exceeded the amounts leaving the terminal ileum.

DISCUSSION

Whereas digestible carbohydrates are degraded to a great extent in the rumen and their metabolites are absorbed through the rumen wall, the net loss of N may be very small in the reticulo-rumen; in this study the equivalent of 95 % of the food N reached the pylorus. Microbial proteins formed by transformation of dietary proteins and undecomposed dietary proteins enter the intestinal tract, where the decisive steps in their digestion and absorption take place. It is remarkable that the amount of N present in the digesta increased considerably (4·7 g/d) after the inflow of pancreatic juice and bile. Apart from their specific functions, these endogenous proteins may be a useful supplement to the dietary and microbial proteins reaching the intestinal tract, particularly when the diet is deficient in protein.

Some of our findings in this work concerning the extent and sites of proteolytic action occurring in the intestine, point to differences between ruminants and simple-stomached animals. One of the reasons for these differences is that neutralization of the digesta entering the intestine is much slower in ruminants than in simple-stomached animals (Kay, 1969) and different pH values prevailing in corresponding sections of the intestine have a great influence on proteolytic activities. The increase in the ratio of α-NH_2–NPN to protein N (see Fig. 5) in samples taken 3 m distant from the pylorus compared with that in samples taken 1 m distant must be attributed partly to the action of pepsin (unpublished results) which continues to act in the weakly acid medium of the upper intestine.

Because of the acidity persisting in the upper sections of the intestinal tract of the sheep, the transformation site of pancreatic zymogens into active enzymes is shifted slightly along the intestinal tract. The highest ratio of α-NH_2–NPN to protein N found at a distance of 7 m from the pylorus (Fig. 5) is consistent with our results from enzymic assays, which showed highest activities of trypsin, chymotrypsin and carboxypeptidase A in samples taken at this site (Figs. 1–3). As seen from Fig. 5, the ratio

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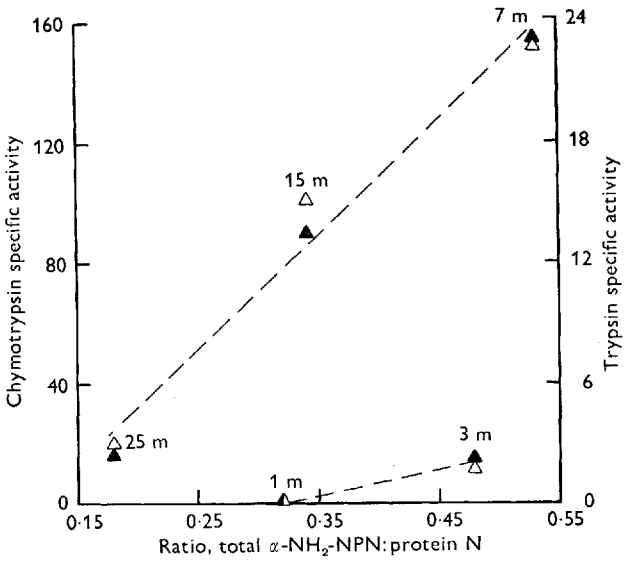

Fig. 5. Relationship between trypsin and chymotrypsin activities and the ratio of α-NH_2-non-protein nitrogen to protein N in digesta of sheep. ▲, trypsin specific activity; △, chymotrypsin specific activity.

between trypsin and chymotrypsin activities is always constant, and the parallel changes in these two enzymic activities are accompanied by corresponding changes in the ratio of α-NH_2–NPN to protein N. In rats, unlike sheep, the amount of chymotrypsin relative to trypsin undergoes a progressive decrease along the small intestine (Pelot & Grossman, 1962).

Unlike trypsin and chymotrypsin activities and the ratio of α-NH_2–NPN to protein N, which are at a maximum at a distance of 7 m from the pylorus (Fig. 5), the ratio of free to bound α-NH_2–N increases gradually along the intestine over a distance of 15 m from the pylorus (Table 11). The increase in the latter ratio along the intestine originates apparently from the action of exopeptidases. In agreement with this conclusion, Symons & Jones (1966) found a peak of activity of dipeptidases in the mid-ileum of the sheep.

According to our results (Tables 6 and 7), intensive absorption of α-NH_2–N takes place in the section between 7 and 15 m distant from the pylorus, but the exopeptidase activity seems to be even greater than that of the absorption, as can be seen from the high concentration of α-NH_2–N, and the high ratio of free α-NH_2–N to bound NH_2–NPN of the digesta sampled in the section between 7 and 15 m distant from the pylorus (Table 11).

The results of this work indicate that the absorption of proteins (as amino acids) and of other nutrients occurs in the area of the intestine 1–15 m posterior to the pylorus. Whereas absorption of amino acids takes place to a greater extent in the section 7–15 m distant from the pylorus, the absorption of other nutrients from the area between 1 and 7 m distant exceeds the extent of their absorption from the 7–15 m section. Results agreeing with our work regarding the sites of absorption of amino acids were obtained

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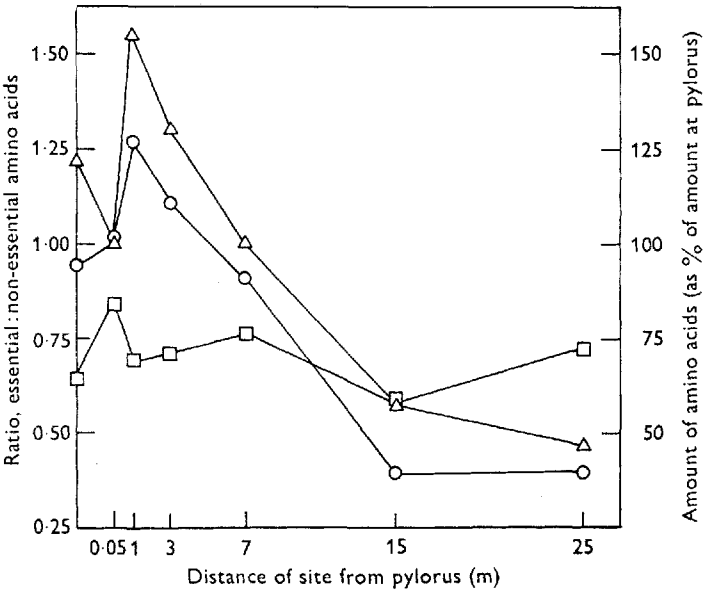

Fig. 6. Changes in the amounts of essential and non-essential amino acids and in the ratio of essential to non-essential amino acids in the digesta of sheep, sampled at different sites along the intestine. ○, total essential amino acids; △, total non-essential amino acids; □, ratio of essential to non-essential amino acids.

by M. I. Chalmers & F. White (private communication). These workers found that absorption of single amino acids, administered either intraruminally or into the abomasum, was maximum over the area drained by the mesenteric vein. The position of the mesenteric vein cannula used for sampling coincides with the area of absorption from the intestinal tract found in this work. Further, Neudoerffer, Duncan & Horney (1971) examined the sites of intestinal uptake of [^{14}C]methionine by incubation in vitro with gut segments of cattle and, in agreement with our results, found that the duodenum and jejunum are the sites of absorption of [^{14}C]methionine.

In agreement with the concepts of other authors (Clarke *et al.* 1966; Coelho da Silva *et al.* 1972), the supply of essential amino acids to the tissues of ruminant animals is somewhat improved compared with the amino acid composition of the diet. This trend is shown by the changes in the ratio of essential to non-essential amino acids along the intestinal tract, as seen in Fig. 6. This ratio was higher in the digesta which passed the rumen and reached the intestine than in the feed ingested, which indicates an improvement in protein quality, caused by ruminal biosynthesis of essential amino acids and microbial degradation of non-essential amino acids.

The supply of essential amino acids required for the tissue metabolism of the host animal is augmented also by preferential absorption through the intestinal wall. In the section of most intensive absorption, 7–15 m distant from the pylorus, the absorption of essential amino acids markedly exceeded that of non-essential amino acids; this is shown by the decrease in the ratio of essential to non-essential amino acids from 0·76 at 7 m distant to 0·58 at 15 m distant from the pylorus. As a net result, 60·5 % of the

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essential amino acids passing through the pylorus and 43 % of the non-essential amino acids were absorbed by the time the digesta had reached the site in the intestine 15 m distant from the pylorus. The very efficient absorption of the essential amino acids, such as methionine, lysine and threonine, in this region (Tables 8 and 10) is of great benefit to ruminants, since under different feeding conditions limiting amounts of these amino acids reach the tissues of the host animal.

The fate of nitrogenous compounds passing the lower intestine (15–25 m distant from the pylorus) was different from that in the higher sections of the intestine, because of inactivation of proteolytic enzymes and enhanced bacterial activity in the lower sections. Since the amounts of essential amino acids did not change in the region between 15 and 22 m distant from the pylorus and those of non-essential amino acids decreased, the ratio of essential to non-essential amino acids increased.

The increase in the amounts of characteristic bacterial metabolites such as α-amino-isobutyrate and of α,ε-diaminopimelic acid in the lower parts of the ileum, as reported by Harrison, Beever & Thomson (1971) and by Mason & White (1971), provide further evidence for bacterial activity in the lower intestine. It appears from Fig. 4, that the microbial activity increases considerably in those lower parts of the intestine where the activity of proteolytic enzymes is limited because of their inactivation (Phillipson, 1971).

Biosynthetic activities of the caecal bacteria apparently balanced the loss of some amino acids or even caused an increase in others. The biosynthesis of amino acids and proteins by the caecal microflora lacks nutritional significance and no differentiation was made therefore between free and bound faecal amino acids.

The net changes in the composition of amino acids in the digesta between entrance and exit from the intestine observed in the present work are in general agreement with the results reported by Clarke *et al.* (1966) and Coelho da Silva *et al.* (1972). These earlier authors used sheep equipped with two fistulas in the proximal duodenum and the terminal ileum. In the present work a clearer picture has been obtained of the sites at which proteolysis and absorption of amino acids occur, and of the relative extents of absorption of essential and non-essential amino acids. This was made possible by the use of sheep equipped with two or three fistulas which involved six different sites in the intestine and separation of protein and NPN amino acids in the digesta passing through the intestine.

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