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Nitrogen and carbon flows between the caecum, blood and rumen in sheep given chopped lucerne (*Medicago sativa*) hay

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1. Experiments involving ^{15}N and ^{14}C tracers were made in sheep consuming 800 g air-dry chopped lucerne (*Medicago sativa*) hay/d and providing 20·4 g N/d to study N and C flows within the caecal digesta and between the caecum, blood and rumen.

2. Continuous infusions of ^{15}N tracers were made into the caecal ammonia, blood urea and rumen NH_3 pools. The concentration and enrichment of caecal digesta NH_3-N, caecal microbial N, caecal digesta non-urea, non-ammonia-N (NU-NAN), faecal NU-NAN, blood urea-N, rumen digesta NH_3-N and rumen bacterial N were estimated at intervals during the infusions. A three-pool open-compartment model was solved to estimate N flows between the caecal digesta NH_3-N, blood urea-N and rumen digesta NH_3-N pools.

3. The rate of irreversible loss from the caecal digesta NH_3-N pool was 2·17 (SE 0·623) g N/d. On average 0·9 (SE 0·56) g N/d of caecal digesta NH_3-N was derived from blood urea and 0·1 (SE 0·08) g caecal digesta NH_3-N/d was apparently derived from the fermentation of undigested rumen microbes in the caecum. The amount of NH_3-N produced by proteolysis and deamination of dietary and endogenous N was 1·1 (SE 0·13) g/d.

4. There was net incorporation of 0·56 (SE 0·306) g caecal digesta NH_3-N/d into caecal microbes. The microbial N synthesized *de novo* in the caecum was not determined, but 2·9 (SE 0·52) g microbial N/d of both rumen and caecal origin flowed out of the caecum and constituted 0·48 of the NU-NAN flow. The majority (mean 0·83 (SE 0·044)) of this microbial N was excreted in faeces.

5. On average 1·8 (SE 0·80) g caecal digesta NH_3-N/d were absorbed. Of this NH_3-N, 0·92 (SE 0·054) was converted to blood urea, contributing 0·10 (SE 0·031) of blood urea-N. Only 0·012 (SE 0·0041) of rumen digesta NH_3-N and 0·005 (SE 0·0009) of rumen bacterial N were derived from caecal digesta NH_3-N.

6. Infusions of ^{14}C tracers were made into the caecal digesta bicarbonate, blood bicarbonate, rumen digesta bicarbonate and blood urea pools, and samples were obtained at intervals to determine the specific radioactivity of each pool. A four-pool open-compartment model was solved to estimate C flows between these pools.

7. The rate of irreversible loss of blood urea estimated with [^{14}C]urea (17·1 (SE 1·18) g N/d) was greater ($P < 0·01$) than that estimated with [^{15}N]urea (14·0 (SE 0·87) g N/d).

8. Transfer of blood urea to the caecal digesta estimated with ^{14}C tracers (1·4 (SE 0·61) g N/d) was greater ($P < 0·01$) than that estimated with ^{15}N tracers (0·9 (SE 0·56) g N/d). The estimate of transfer of blood urea to the rumen digesta was also greater with ^{14}C tracers ($P < 0·05$; 1·7 (SE 0·15) and 1·2 (SE 0·19) g N/d respectively). The urea hydrolysed in the gastrointestinal tract other than in the rumen digesta pool and the caecal digesta pool was 0·56 of total urea hydrolysis when estimated with ^{14}C tracers, or 0·69 when estimated with ^{15}N tracers. Results from previous acute experiments suggested that with three of the four observations made in three sheep in the present experiment the transfer of blood urea to the caecal digesta could have occurred entirely via ileal digesta. Similarly, urea transfer to the rumen digesta could have occurred entirely via saliva.

A considerable amount of ammonia is produced in the large intestine of sheep by hydrolysis of endogenous urea and by proteolysis and deamination of dietary and endogenous materials entering from the small intestine. The apparent absorption of NH_3 and other nitrogeneous materials between the ileum and the rectum has been measured in numerous studies with sheep prepared with cannulas in the ileum (Coelho da Silva *et al.* 1972*a*,*b*; MacRae & Ulyatt, 1974; Beever *et al.* 1976) and also by sampling various parts of the large intestine during acute experiments (Dixon & Nolan, 1982). It is generally thought that there is negligible absorption of amino acids, and that apparent digestion of nitrogen is due to absorption of NH_3 (Ulyatt *et al.* 1975; Hoover, 1978). Since hydrolysis of urea occurs only in the gastrointestinal tract by the action of urease (*EC* 3.5.1.5) of bacterial origin

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(Kornberg & Davies, 1955), the quantity of endogenous urea hydrolysed in the post-ruminal tract has been estimated as the differences between the urea hydrolysed in the rumen digesta pool and that hydrolysed in the entire gastrointestinal tract (Nolan & Leng, 1972; Norton *et al.* 1978; Kennedy, 1980). ^{15}N tracers have been used to estimate the flows of urea, NH_3-N and microbial N in the large intestine in in vivo (Nolan *et al.* 1976) and in acute experiments (Dixon & Nolan, 1983; Dixon & Milligan, 1984), but the former study was based on injection of ^{15}N tracers into the caecum of only one sheep while the latter studies depended on a single sample of caecal digesta obtained at slaughter.

The experiments now reported were undertaken in order to gain a better understanding of the kinetics of N utilization and metabolism in the large intestine of sheep and of the importance of these N flows in relation to the rest of the body. Flows of N in intact sheep consuming lucerne (*Medicago sativa*) hay were measured using in vivo infusions of ^{15}N and ^{14}C tracers into and sampling from the caecum, blood, rumen, urine and faeces. These results were used to solve multi-pool quantitative models describing N and C kinetics in the body. The simultaneous infusion and sampling of ^{15}N and ^{14}C tracers enabled separate estimates of urea hydrolysis in various parts of the gut. ^{51}Cr-EDTA was also administered to estimate the flow of digesta through the caecum.

MATERIALS AND METHODS

Sheep and preparation

Mature Merino wethers (29–35 kg live weight) of similar genetic origin were prepared with a rumen cannula (Hecker, 1969) and with a cannula and an infusion line into the caecum. Polypropylene cannulas (60 mm × 17 mm) were implanted one-third of the distance from the ileo-caecal junction to the caecal pole, and were exteriorized through the lower right flank (MacRae *et al.* 1973). An infusion line (Silastic; Dow Corning, Michigan, USA; internal diameter 1·02 mm) into the caecum was securely attached to the caecal wall midway between the cannula and the pole of the caecum. A post-operative recovery period of at least 8 weeks was allowed before experiments were undertaken. Jugular catheters were inserted into one or both jugular veins 8–12 h before tracer infusions were commenced.

The sheep were held indoors in metabolism crates under continuous lighting and were accustomed to the sampling procedures before experiments commenced. Anthelmintics were administered regularly to control intestinal parasites. The chopped-lucerne-hay diet (800 g/d) was given for at least 60 d before an experiment and given in equal hourly portions for at least 7 d before and also during each experiment. The lucerne hay contained 903 g dry matter (DM)/kg air-dry material, and 920 g organic matter and 28·3 g N/kg DM. Water was available at all times.

Experiments

A series of experiments (Table 1) involving continuous infusions of tracers was carried out with three mature sheep over an interval of 12 months, and some measurements were made twice in two sheep. Infusions of ^{15}N tracers were made into the caecal digesta NH_3-N, blood urea and rumen digesta NH_3-N pools, with sampling during each infusion to determine the concentration and enrichment of caecal digesta NH_3, caecal non-urea, non-ammonia-N (NU-NAN), caecal microbial N, blood urea-N, rumen digesta NH_3-N, rumen bacterial N, urinary urea-N and faecal NU-NAN. Infusions of ^{14}C tracers were also made into the caecal digesta bicarbonate, blood bicarbonate, rumen digesta bicarbonate and blood urea pools, and samples were obtained during each infusion to determine the specific radioactivity (SR) of each of these pools. Also, during each infusion of ^{15}N and ^{14}C tracers, the ^{51}Cr-labelled complex of EDTA (^{51}Cr-EDTA) was infused into the caecum and the ^{51}Cr content in caecal digesta was determined to measure the flow rate of caecal digesta.

Table 1. *Details of tracer infusion experiments giving the sheep used for each experiment, the quantity and infusion site for each tracer, the time-period for which each infusion was made and the number of samples obtained to measure tracer plateau*

Expt no.	Sheep	Tracer	Infusion site	Infusion rate	Infusion period (min)	No. of samples
1	A	[^{15}N]urea	Blood	2·7 mg atoms ^{15}N/d	1460	6
		[^{14}C]urea	Blood	55 μCi/d	1460	7
		^{51}Cr-EDTA	Caecum	30 μCi/d	1390	6
2	A, B, C	[^{15}N]urea	Blood	0·9 mg atoms ^{15}N/d	2030	6
		[^{14}C]urea	Blood	80 μCi/d	2030	6
		^{51}Cr-EDTA	Caecum	100 μCi/d	2500	6
3	A, B	$(^{15}NH_4)_2SO_4$	Caecum	1·1 mg atoms ^{15}N/d	1500	8
		^{51}Cr-EDTA	Caecum	30 μCi/d	1450	8
4	A, B, C	$(^{15}NH_4)_2SO_4$	Caecum	3·3 mg atoms ^{15}N/d	2100	6
		$NaH^{14}CO_3$	Caecum	510 μCi/d	2100	6
		^{51}Cr-EDTA	Caecum	100 μCi/d	2050	6
5	A, B, C	$NaH^{14}CO_3$	Caecum	50 μCi/d	1270	8
		^{51}Cr-EDTA	Caecum	20 μCi/d	1250	8
6	A, B, C	$NaH^{14}CO_3$	Blood	42 μCi/d	1170	8
		^{51}Cr-EDTA	Caecum	15 μCi/d	1100	7
7	A, B, C	$(^{15}NH_4)_2SO_4$	Rumen	3·8 mg atoms ^{15}N/d	2100	5
		$NaH^{14}CO_3$	Rumen	580 μCi/d	2100	5

A total collection of urine and faeces for 8 d was also made from the three sheep plus one additional sheep.

Preparation and administration of tracers

The ^{51}Cr-EDTA was obtained from Lucas Heights, Sydney, Australia; ^{14}C tracers from Amersham International, Amersham, Bucks UK; $(^{15}NH_4)_2SO_4$ from the British Oxygen Co., London (97 atoms %) or from Adlershof, Berlin (96·2 atoms %); and [^{15}N]urea (96 atoms %) from ONIA, Paris. The ^{51}Cr-EDTA was mixed with carrier Cr-EDTA (Binnerts *et al.* 1968; 1–7 mg Cr/μCi). The NaH $^{14}CO_3$ was washed from the ampoule in which it was supplied using a solution containing $NaHCO_3$ as carrier. [^{14}C]urea was mixed with [^{15}N]urea and therefore no additional carrier urea was used.

The intravenous infusions were administered at 0·08–0·12 litres/d in sterile saline (0·15 M-sodium chloride) via a jugular catheter. Infusions into the caecum and into the rumen were in aqueous solution at 0·72–0·78 litres/d. Water or saline solution was infused for 200–700 min before tracer infusion was begun.

Sampling

Experiments were at least 10 d apart, and samples were taken from all sampling sites for analysis for background radioactivity before tracers were administered.

Caecal digesta were sampled after scraping out and discarding digesta in the barrel of the cannula, and then by collecting 30–50 g digesta into a container attached to the cannula. Sometimes digesta were obtained immediately, but often it was necessary to leave the container in position for 15–45 min. The digesta were subsampled to enable analysis for ^{51}Cr-EDTA (1–4 g into a tared γ-counting vial using a glass tube, 7 mm internal diameter), DM (10 g), total N (5 g into 5 ml 0·5 M-sulphuric acid), and the SR of $H^{14}CO_3^-$ (3 g into

a McCartney bottle). Another subsample (10 g) was mixed with 0·5 M-H_2SO_4 (10 ml) and centrifuged (1000 ***g***, 1 min) to remove large particulate matter which was discarded; the resulting supernatant fraction was recentrifuged (16000 ***g***, 20 min) to isolate a microbe-rich fraction and the supernatant fraction was also retained for later analysis. The microbe-rich fraction was also washed (Nolan & Leng, 1972).

Blood (5–30 ml) from the jugular vein was placed into heparinized tubes which were immediately centrifuged (3000 ***g***, 15 min) to separate plasma. Also, during ^{14}C infusions, 5 ml blood were immediately transferred to a McCartney bottle for $H^{14}CO_3^-$ analysis.

Rumen fluid samples (10–20 ml) were withdrawn through a nylon-gauze-covered cage in the ventral sac of the rumen. Subsamples (5 ml) were placed into a McCartney bottle for $H^{14}CO_3^-$ analysis and, where appropriate, a subsample (10 ml) was acidified (0·2 ml 5 M-H_2SO_4), centrifuged (16000 ***g***, 20 min) and the supernatant fraction retained. Bacteria-rich samples were also isolated from the pellet obtained by this centrifugation and washed (Nolan & Leng, 1972).

Urine was collected into glacial acetic acid (20 ml/d) containing 1 g mercuric chloride/l. During ^{15}N infusions, faeces and urine were obtained during the last 240 min of the infusion and 10-g samples of faeces were acidified with 5 ml 0·5 M-H_2SO_4.

All samples awaiting analysis were stored at −20°.

Laboratory procedures

DM, organic matter and total N content of food and digesta were determined by standard procedures (Association of Official Analytical Chemists, 1975). The concentration and enrichment of NH_3-N, total N, NU-NAN and microbial N in digesta, and urea-N in plasma and urine were determined by the procedures described by Dixon & Nolan (1983). The radioactivity of ^{51}Cr-EDTA in digesta and faeces was determined as described by Dixon *et al.* (1982).

The SR of urea in plasma and urine were determined as described by Nolan & Leng (1970) and the SR of $H^{14}CO_3^-$ by the procedures of Leng & Leonard (1965) following isolation of the acid-labile HCO_3^- as $Ba^{14}CO_3$. $NaH^{14}CO_3$ infusate was sampled several times during each infusion, an appropriate volume added to a known weight of sodium carbonate and diluted with bicarbonate-free water, and $Ba^{14}CO_3$ analysed as for the samples. [^{14}C]toluene of known SR (Lucas Heights, Australia) was used to construct efficiency-correction curves for the toluene–triton X scintillation cocktail. The radioactivity of $NaH^{14}CO_3$ infusate was determined by direct counting, and $Ba^{14}CO_3$ standards prepared from this infusate were used to construct an efficiency-correction curve for the scintillation mixture containing thixotropic gel powder (Cab-o-sil, Packard Instrument Co., USA) used to count the $Ba^{14}CO_3$ samples. The differences in SR between groups of $Ba^{14}CO_3$ standards prepared independently were less than 3%. The coefficient of variation of the SR of [^{14}C]urea standards was less than 1%, and of $Ba^{14}CO_3$ standards 1–4%. The SR of blood urea-C determined on protein-precipitated supernatant solutions was found to be 0·30–0·60 greater than the concurrent SR of urinary urea-C during the plateau of $H^{14}CO_3^-$ infusions, presumably because there were ^{14}C-labelled compounds other than urea in the deproteinized plasma. There were no detectable differences between the SR of bood urea-C and urinary urea-C during infusion of [^{14}C]urea into the blood, thereby confirming the validity of the deproteinization procedures of Cocimano & Leng (1967) for determination of the SR of blood urea in [^{14}C]urea infusion experiments. Consequently the SR of blood urea-C during infusions of $H^{14}CO_3$ was estimated from the SR of urinary urea-C collected during the last 240 min of $H^{14}CO_3$ infusion.

Calculations

Results were normalized to infusion rates (/d) of $1{\cdot}44 \times 10^6$ counts/min for ^{51}Cr-EDTA, 100 μCi for [^{14}C]urea and $H^{14}CO_3^-$, and 5 mg atoms for $(^{15}NH_4)_2SO_4$ and [^{15}N]urea.

The time-point at which plateau ^{15}N and ^{14}C tracer concentration was considered to have been reached was determined visually by examining the tracer concentration with time-curves for all sheep in similar experiments. The flow rate of digesta components through the caecum was calculated from the six to ten observations of ^{51}Cr-EDTA concentration in caecal digesta sampled more than 250 min after the commencement of the infusion (Dixon *et al.* 1982). The rate of irreversible loss of traced substance from a primary pool, the proportion of traced substance in a secondary pool derived from a primary pool and the proportion of $(^{15}NH_4)_2SO_4$ infused into the caecum incorporated into blood urea were calculated by the procedures described by Shipley & Clark (1972), Nolan & Leng (1974) and Dixon & Nolan (1983). The blood urea transfer to the gastrointestinal tract other than the rumen and the caecum was calculated as the rate of irreversible loss estimated with [^{14}C]urea minus urea transfer to the rumen digesta and the caecal digesta minus urinary urea excretion.

Model construction

Three-pool and four-pool open-compartment models were used to describe the flows of N and C respectively between the caecum, rumen and blood.

The methods of Mann & Gurpide (1966) and Nolan *et al.* (1976) were used to solve equations representing the rates of flow of N or C in these models. Tracers were infused into each pool in each sheep in independent experiments so that separate models could be constructed from sets of data from each sheep. Data set 1 was developed for sheep A from Expts 1, 3, 5, 6 and 7. Data sets 2, 3 and 4 were developed for sheep A, B and C respectively from Expts 2, 4, 6 and 7. Values derived for data sets 1 and 2 were used to calculate the mean values for sheep A. The standard errors of the individual flows were calculated from the model solutions for the three sheep.

Statistical analysis

Differences between the mean values for flows of digesta or the concentrations of NH_3-N or urea-N for the various sheep on different experimental days were examined by one-way analysis of variance. Differences between means were compared using the 5% Student–Newman–Keuls test. A paired t test was used to compare rates of irreversible loss and transfers of urea measured using ^{15}N and ^{14}C tracers for the four data sets. The slopes and elevations of regression lines were compared using the methods of Snedecor & Cochran (1967).

RESULTS

Variation in concentration and flow of N in the sampled pools

There were significant differences ($P < 0{\cdot}05$, $P < 0{\cdot}01$) in the caecal water flow and in the concentrations of caecal digesta NH_3-N, rumen digesta NH_3-N and blood urea-N among sheep and among tracer infusions within any one sheep (Table 2). However the differences between Expts 3 and 4 for sheep A and B, or between Expts 1 and 2 for sheep A, were associated with measurements 12 months apart, and differences were in almost all cases non-significant within any one sheep measured at intervals over several weeks (e.g. sheep A, B or C in Expts 2, 4 and 7). Within periods of infusion of ^{15}N tracers there were only small changes in concentrations of caecal digesta NH_3-N, rumen digesta NH_3-N and blood urea-N, the variations shown in Fig. 1(*a*) being typical. The coefficient of variation in concentration of caecal digesta NH_3-N was somewhat greater than that of rumen digesta NH_3-N or blood urea.

Table 2. *Flow of water through the caecal digesta pool measured by continuous infusion of ^{51}Cr-EDTA, concentrations of ammonia-nitrogen in caecal and rumen digesta and concentration of blood urea-N measured during continuous infusions of $(^{15}NH_4)_2SO_4$ into the caecum or rumen, or [^{14}C]- and [^{15}N]urea into the blood*

(Mean values for each infusion and the number of observations are given)

Infusion site and Expt no.	Sheep	Caecal water flow (l/d)		Caecal NH_3 concentration (mg N/l)		Blood urea concentration (mg N/l)		Rumen NH_3 concentration (mg N/l)	
		Mean	*n*	Mean	*n*	Mean	*n*	Mean	*n*
Caecum									
3	A	3·15	9	313	11	—	—	108	10
4	A	3·94	6	233	8	237	7	190	8
3	B	2·64	7	295	8	—	—	—	—
4	B	1·82	5	219	8	272	8	198	8
4	C	2·88	5	131	8	244	8	185	8
Blood									
1	A	4·17	6	293	15	257	15	189	16
2	A	3·74	6	226	8	230	7	201	7
2	B	2·39	10	212	12	276	10	193	11
2	C	3·13	6	163	8	242	8	203	8
Rumen									
7	A	—	—	272	8	206	7	171	7
7	B	—	—	248	7	233	7	208	7
7	C	—	—	192	8	211	7	161	6
SEM		0·496		16		6		7	
Statistical signi-ficance: $P <$		0·05		0·01		0·01		0·01	

SEM, standard error of the difference between two means.

Infusions of $(^{15}NH_4)_2SO_4$ into the caecum

The build-up of ^{15}N tracer concentrations with time in primary and secondary pools in one sheep are shown in Fig. 1(*c*); the results for other sheep were similar.

The rate of irreversible loss of NH_3-N from the caecal digesta pool was 2·17 (SE 0·623) g N/d (Table 3). There were differences among sheep from 1·5 to 3·4 g N/d; estimates made 12 months apart were similar in sheep B but differed by 1·3 g N/d in sheep A. On average 0·92 (SE 0·054) of the caecal digesta NH_3-N that was absorbed from the large intestine apparently entered the blood urea pool and contributed 0·10 (SE 0·031) of this pool. The proportion of the rumen digesta NH_3-N pool derived from the caecal digesta NH_3-N was 0·012 (SE 0·0041) and the proportion of rumen bacterial N 0·005 (SE 0·0009).

The loss of NH_3-N from the caecal digesta pool in water flowing to the colon was 0·70 (SE 0·150) g N/d, while 0·56 (SE 0·306) g N/d was lost by net synthesis into microbial N. Microbial N constituted 0·48 of the NU-NAN flowing from the caecum, and 0·83 (SE 0·044) of this microbial N flowing from the caecum appeared in faecal NU-NAN (Table 3).

Infusion of [^{15}N]urea into the blood

The build-up of ^{15}N tracer concentration with time is shown for one sheep in Fig. 1(*b*). The rate of irreversible loss of N from the blood urea pool was 14·0 (SE 0·87) g N/d, and 0·12 (SE 0·013) of rumen digesta NH_3-N was derived from blood urea N (Table 4). The

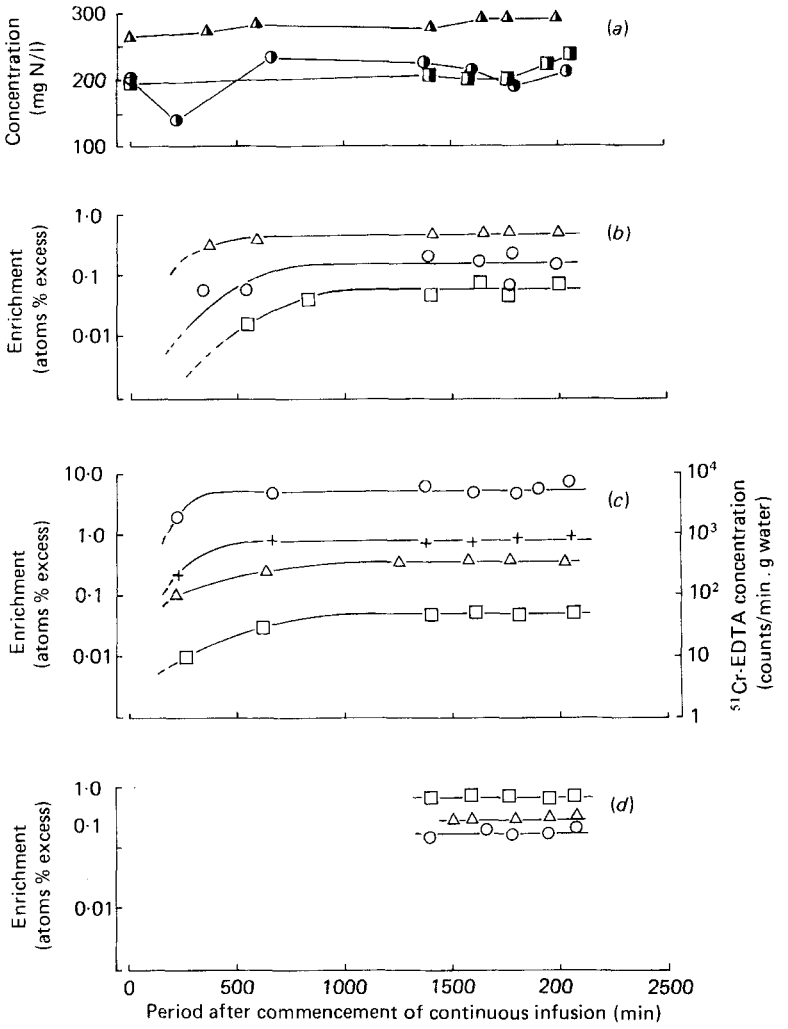

Fig. 1. (*a*) The concentrations (mg nitrogen/l) of blood urea-N (▲), caecal digesta ammonia (◑) and rumen digesta NH_3 (◨) in one sheep when each pool was the primary pool for ^{15}N tracer infusion. The enrichments (atom % excess) of blood urea-N (△), caecal digesta NH_3-N (○) and rumen digesta NH_3-N (□) for one sheep (*b*) during continuous infusion (5 mg atoms ^{15}N/d) of [^{15}N]urea into the blood and during continuous infusions (5 mg atoms ^{15}N/d) of $(^{15}NH_4)_2SO_4$ (*c*) into the caecum and (*d*) into the rumen. The concentration (counts/min per g water) of ^{51}Cr-EDTA in caecal digesta (+) during infusion (/d) of $1{\cdot}44 \times 10^6$ counts/min of ^{51}Cr-EDTA into the caecum.

proportion of the caecal digesta NH_3-N pool derived from blood urea ranged widely among sheep from 0·19 to 0·60 with a mean of 0·38 (Table 4).

Infusion of $(^{15}NH_4)_2SO_4$ into the rumen

The ^{15}N tracer concentration with time for one sheep is shown in Fig. 1(*d*). The rate of irreversible loss from the rumen digesta NH_3-N pool was 9·29 (SE 0·645) g N/d, and the proportions of blood urea and caecal digesta NH_3-N derived from rumen digesta NH_3-N were 0·41 (SE 0·021) and 0·25 (SE 0·012) respectively (Table 5).

Table 3. *Expts 3 and 4. Measurements of caecal ammonia-nitrogen, rumen, blood urea-N and caecal microbial N kinetics measured during continuous infusions of* $(^{15}NH_4)_2SO_4$ *into the caecum in three sheep, two of which were measured twice*

(Mean values with their standard errors)

Measurement	Mean	SE
Rate of irreversible loss from the caecal digesta NH_3-N pool (g N/d)	2·17	0·623
Proportion of blood urea-N derived from caecal digesta NH_3-N	0·10	0·031
Proportion of rumen digesta NH_3-N derived from caecal digesta NH_3-N	0·012	0·0041
Proportion of rumen bacterial N derived from caecal digesta NH_3-N	0·005	0·0009
Proportion of caecal digesta NH_3-N absorbed from the large intestine transferred to blood urea	0·92	0·054
Caecal digesta NH_3-N flowing to the colon in water (g N/d)	0·70	0·150
Caecal digesta NH_3-N flowing to the colon as microbial N (g N/d)	0·56	0·306
Caecal NU-NAN flowing to the colon (g N/d)	6·0	1·06
Caecal microbial N flowing to the colon (g N/d)	2·9	0·52
Proportion of caecal microbial N apparently derived from caecal digesta NH_3-N*	0·17	0·064
Faecal NU-NAN enrichment: caecal NU-NAN enrichment	0·83	0·044

NU-NAN: non-urea, non-ammonia-nitrogen.

* This value underestimates the proportion of caecal microbial N actually derived from caecal digesta NH_3-N by the proportion of caecal microbial N consisting of undigested rumen microbial debris.

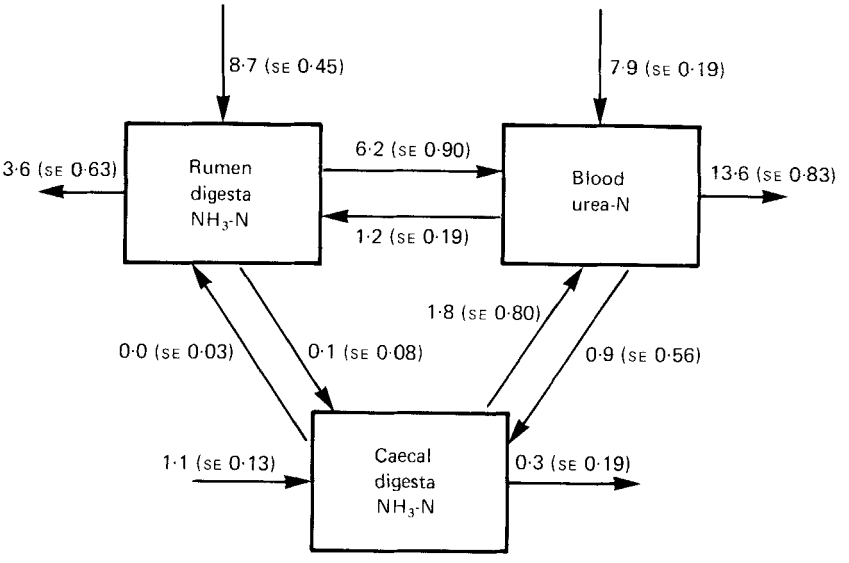

Fig. 2. An open-compartment model of flows of nitrogen (g N/d) between the pools of rumen digesta ammonia-N, blood urea-N and caecal digesta NH_3-N. Values are means with their standard errors for individual solutions of the model for each of the three sheep.

Table 4. *Expts 1 and 2. Measurements of blood urea-nitrogen kinetics measured during four continuous infusions of [^{14}C]urea and [^{15}N]urea into the blood and by total collection of urine from three sheep*

(Mean values with their standard errors)

Measurement	Mean	SE	Statistical significance of difference†; $P <$
Rate of irreversible loss from the blood urea pool (g N/d) determined with:			
[^{14}C]urea	17·1	1·18	0·01
[^{15}N]urea	14·0	0·87	
Proportion of caecal digesta ammonia-N derived from blood urea	0·38	0·120	
Proportion of rumen digesta ammonia-N derived from blood urea	0·12	0·013	
Blood urea transfer to the rumen digesta (g N/d):			
^{14}C tracers	1·7	0·15	0·05
^{15}N tracers	1·2	0·19	
Blood urea transfer to the caecal digesta (g N/d):			
^{14}C tracers	1·4	0·61	0·01
^{15}N tracers	0·9	0·56	
Urinary urea excretion (g N/d)	10·1	0·34	
Blood urea transfer to the gastrointestinal tract other than the rumen digesta and the caecal digesta (g N/d)*:			
^{14}C tracers	3·9	1·37	0·05
^{15}N tracers	4·8	1·32	

* Calculated as the rate of irreversible loss estimated with [^{14}C]urea minus urea transfer to the rumen and the caecum minus urinary urea excretion.

† Compared using a *t* test paired for measurements made within each sheep and twice for one sheep.

Table 5. *Expt 7. The rate of irreversible loss of rumen digesta ammonia-nitrogen and the proportions of the N in various secondary pools derived from rumen digesta ammonia-N estimated by continuous infusion of $(^{15}NH_4)_2SO_4$ into the rumen of each of three sheep*

(Mean values with their standard errors)

Measurement	Mean	SE
Rate of irreversible loss from the rumen digesta NH_3-N pool (g N/d)	9·29	0·645
Proportion of rumen bacterial N derived from rumen digesta NH_3-N	0·41	0·021
Proportion of blood urea-N derived from rumen digesta NH_3-N	0·41	0·027
Proportion of caecal NH_3-N derived from rumen digesta NH_3-N	0·25	0·012
Proportion of caecal microbial N apparently derived from rumen digesta NH_3-N	0·18	0·009
Proportion of caecal NU-NAN apparently derived from rumen digesta NH_3-N	0·13	0·015

NU-NAN: non-urea, non-ammonia nitrogen.

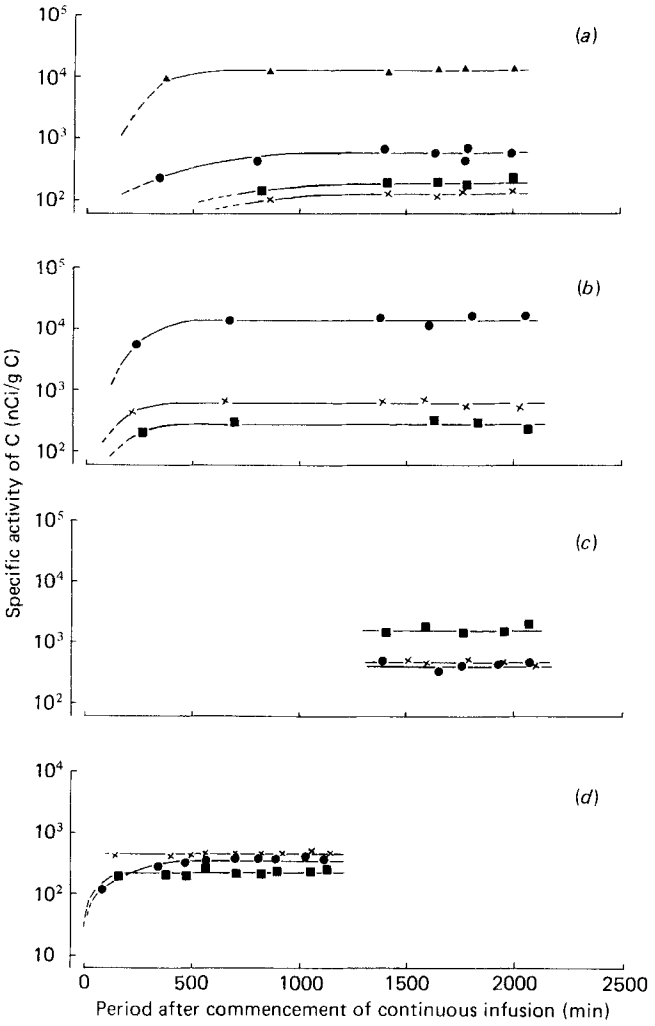

Fig. 3. The specific radioactivity of blood urea-carbon (▲), caecal digesta bicarbonate-C (●), rumen digesta bicarbonate-C (■) and jugular vein blood bicarbonate-C (X) in one sheep (*a*) during infusion (100 μCi/d) of [^{14}C]urea into the blood, and during infusion (100 μCi/d) of $H^{14}CO_3$ into (*b*) the caecum, (*c*) rumen or (*d*) jugular vein.

Three-pool model of N flows derived from ^{15}N tracer results

The three-pool model of mean N flows is shown in Fig. 2.

The production of NH_3-N by proteolysis and deamination of endogenous N and undigested dietary N was 1·1 (SE 0·13) g N/d. Transfer of NH_3-N to the caecal digesta pool from the rumen digesta NH_3 pool was 0·1 (SE 0·08) g N/d. Since the enrichment of rumen bacterial N was only 0·41 of that of rumen digesta NH_3-N (Table 5), while the model estimated the N flow from the rumen digesta NH_3-N to the caecal digesta NH_3-N pools, the actual degradation of rumen microbial N was 2·4 times this flow, i.e. 0·3 g N/d. Transfer of blood urea-N to the caecal digesta NH_3 pool was 0·9 (SE 0·56) g N/d and hence tended to be less than the transfer to the rumen (1·2 (SE 0·19) g N/d; Fig. 2).

Caecal digesta NH_3 entering the blood urea pool by absorption from the large intestine

Table 6. *The rates of irreversible loss of urea-carbon or bicarbonate-C from primary pools and the proportions of C in each secondary pool derived from the primary pool during four continuous infusions of [^{14}C]urea into the blood or of $H^{14}CO_3^-$ into the blood, rumen or caecum of three sheep, one of which was measured twice*

Primary pool (site of ^{14}C tracer infusion)	Rate of irreversible loss (g C/d)		Proportion of secondary pool derived from each primary pool							
			Rumen digesta HCO_3^-		Blood HCO_3^-		Caecal digesta HCO_3^-		Blood urea	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Rumen digesta HCO_3^-	74·8	6·78	1·0	—	0·342*	0·0223	0·312*	0·0246	0·401*	0·0270
Blood HCO_3^-	215	4·8	0·537*	0·0233	1·0	—	0·798	0·0121	0·95†	—
Caecal digesta HCO_3^-	13·6	3·70	0·038	0·0101	0·072	0·0172	1·0	—	0·085	0·0170
Blood urea	7·34	0·506	0·012	0·0019	0·009	0·0011	0·047	0·0058	1·0	—

* Mean of three infusions only.

† Not measured, this value obtained from other $H^{14}CO_3^-$ infusions (R. M. Dixon and J. V. Nolan, unpublished results).

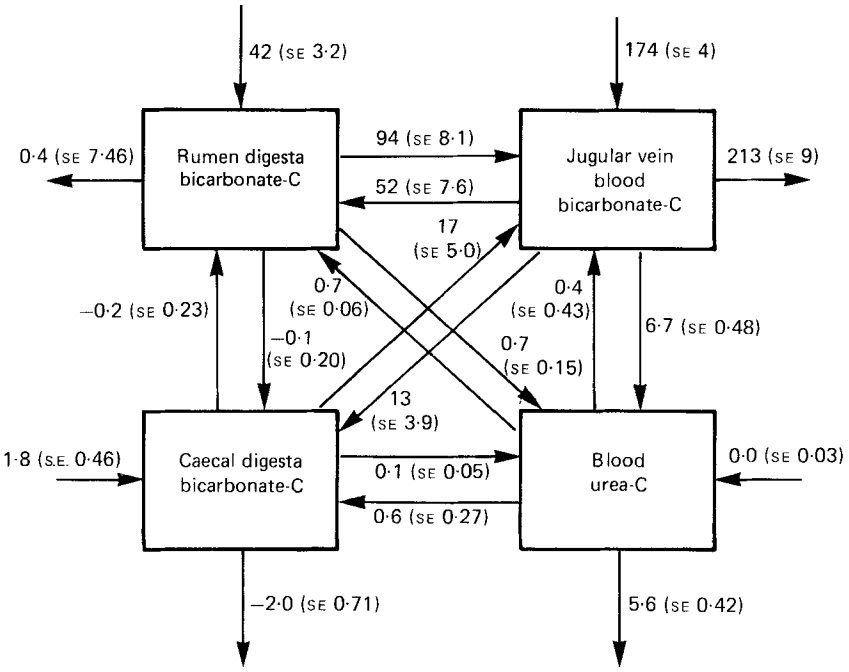

Fig. 4. An open-compartment model of flows of carbon (g C/d) between the pools of rumen digesta bicarbonate-C, jugular vein blood bicarbonate-C, caecal digesta bicarbonate-C and blood urea-C. Values are means with their standard errors for individual solutions of the model for each of the three sheep.

was 2·2 (SE 0·7) g N/d. Direct transfer of caecal digesta NH_3-N to rumen digesta NH_3-N was negligible, indicating that caecal digesta NH_3 was transferred to the rumen only via the blood urea pool.

Continuous infusion of ^{14}C tracers into the caecal, blood and rumen bicarbonate pools and into the blood urea pool

The build-up of ^{14}C tracer concentrations with time in primary and secondary pools during infusions of tracer into the various sites in one sheep are shown in Fig. 3, and the rates of irreversible loss from each primary pool and the proportion of each secondary pool derived from each primary pool are given in Table 6. The four-pool model of C flows representing all sheep is given in Fig. 4.

Bicarbonate-C derived from fermentation of substrate in the caecal digesta was 1·8 (SE 0·46) g C/d, or 4% of the production in the rumen digesta. There were large flows of bicarbonate-C in both directions between the blood bicarbonate and the caecal digesta bicarbonate pools, as well as between the blood bicarbonate and the rumen digesta bicarbonate pools.

The transfer of blood urea-C to the caecal digesta bicarbonate pool was 0·6 (SE 0·27) g C/d, and transfer of blood urea-C to the rumen digesta bicarbonate pool was 0·7 (SE 0·15) g C/d (Fig. 4).

Comparison of the results obtained with ^{15}N and ^{14}C tracers

The estimate of the rate of irreversible loss from the blood urea pool was greater ($P < 0·01$) when measured with [^{14}C]urea (17·1 g N/d) than with [^{15}N]urea (14.0 g N/d) (Table 4).

The estimates of transfer of blood urea measured with ^{14}C tracers to the caecal digesta

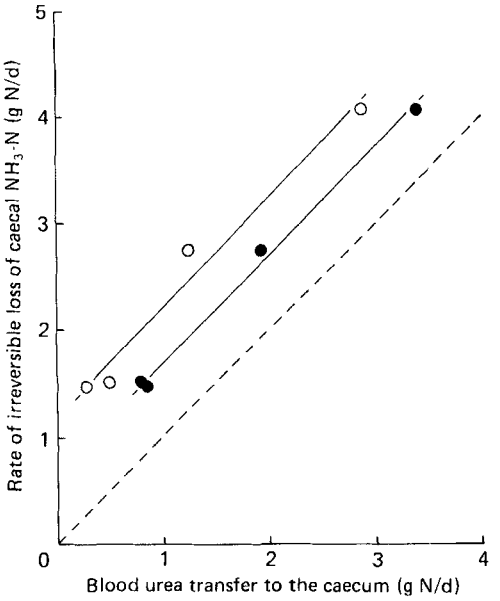

Fig. 5. The relations between the rate of irreversible loss of ammonia-nitrogen from the caecal digesta pool (Y) and the apparent transfer of blood urea into the caecum measured in the three-pool model of N flows in Fig. 2. (X_N, ○), or the four-pool model of C flows in Fig. 4 (X_C, ●). The regression equations are:

$$Y = 1{\cdot}18 + 1{\cdot}04\ (\text{SE}\ 0{\cdot}124)X_N \quad (r\ 0{\cdot}99,\ \text{residual SD (RSD)}\ 0{\cdot}258,\ P < 0{\cdot}05,\ n\ 4)$$
$$Y = 0{\cdot}67 + 1{\cdot}02\ (\text{SE}\ 0{\cdot}056)\ X_C \quad (r\ 0{\cdot}99,\ \text{RSD}\ 0{\cdot}112,\ P < 0{\cdot}01,\ n\ 4)$$

The relation (---) was that expected if all urea entering the caecal digesta were hydrolysed to ammonia-N. Measurements were made once in sheep B and C and twice in sheep A (see Table 2); the two highest values are for sheep A.

and the rumen digesta were greater than these estimates of transfer of blood urea measured with ^{15}N tracers ($P < 0{\cdot}01$, $P < 0{\cdot}05$ respectively; Table 4). Urea hydrolysis in the gastrointestinal tract that did not enter the rumen digesta and the caecal digesta pools accounted for 4·8 g N/d or 0·69 of the total urea hydrolysis when measured with ^{15}N tracers, or 3·9 g N/d or 0·56 of total urea hydrolysis when measured with ^{14}C tracers (Table 4).

The relations between the rate of irreversible loss of NH_3-N from the caecal digesta pool and the rate of transfer of blood urea into the caecal digesta measured with either ^{14}C or ^{15}N tracers were different in elevation ($P < 0{\cdot}05$), while the slopes were identical to that expected on the basis that 1 mol urea is hydrolysed to 2 mol NH_3-N (Fig. 5). The concentration of caecal digesta NH_3-N and the apparent transfer of blood urea to the caecal digesta measured with either ^{15}N or ^{14}C tracers also tended ($P > 0{\cdot}05$) to be related (r 0·84, r 0·79 respectively; n4).

Total collection of faeces and urine

Apparent digestibility of organic matter was 0·58 (SE 0·006). Urinary total N excretion was 12·1 (SE 0·44) g N/d, of which 10·1 (SE 0·34) g N/d consisted of urea-N. Of the intake of 20·4 g N/d, 1·7 (SE 0·17) g N/d was retained.

DISCUSSION

The reliability of the quantitative models

Consideration of concentration in the caecal digesta NH_3-N, rumen digesta NH_3-N and blood urea N pools (Table 2) suggested that metabolic steady-state conditions were satisfactorily achieved within the individual sheep used to provide the data for the three-pool and four-pool models. Differences between successive infusions in individual sheep measured over several weeks were small, and during most infusions there was no change ($P > 0{\cdot}05$) in concentration of NH_3-N or urea with time. This suggested that error resulting from metabolic differences between experiments was likely to be minor. The variability associated with measurements of caecal pools was, as expected from previous marker experiments, greater than in the rumen but was unlikely to have introduced bias (Dixon *et al.* 1982).

The open-compartment model used in the present study provided a complete quantitative description of the flows between the sampled pools during an experiment, but it must be recognized that the estimated flow includes transfer of the traced substance by all possible pathways (Nolan *et al.* 1976; Nolan & Rowe, 1976). For example, the pathways for flow of N from the caecal digesta NH_3 to blood pools in Fig. 2 may have included (1) NH_3-N which was absorbed directly through the caecal wall and converted immediately to urea, (2) NH_3-N which flowed out of the caecal digesta pool to the colon as NH_3-N or microbial N, was absorbed as NH_3-N or amino acids and subsequently converted to urea and (3) caecal digesta NH_3-N incorporated into non-essential amino acids in the liver and which were subsequently deaminated and the amino-N converted to urea. Consequently, care must be taken with the interpretation of the measured flows and additional information is needed to indicate major pathways.

The extent to which flows through the sampled caecal digesta pool indicated the total flows of NH_3-N through the caecum and proximal colon has been discussed previously (Dixon & Nolan, 1983; Dixon & Milligan, 1984); in these experiments the production of NH_3-N by proteolysis and deamination in the caecal digesta pool accounted for 0·76 and 0·60 of the NH_3-N production in the digesta of the entire caecum and proximal colon in sheep given lucerne and bromegrass (*Bromus inermus*) respectively. Hence the measurements of flow through the sampled digesta NH_3-N and digesta bicarbonate-C pools in the present experiments would have underestimated the production in the digesta of the entire caecum and proximal colon.

As expected the estimate of NH_3-N production from sources of N other than urea in the caecal digesta pool in the three-pool model ($1{\cdot}1 + 0{\cdot}1 = 1{\cdot}12$ g N/d; Fig. 2) was similar to that obtained from the *Y*-intercept of the regression line when no [^{15}N]urea-N apparently entered the caecal NH_3-N pool (1·17 g N/d; Fig. 5). This was to be expected since the latter value is that part of the rate of irreversible loss of the caecal digesta NH_3-N pool which could not be attributed to the hydrolysis of blood urea. However, the estimated transfer of blood urea to the caecal digesta pool depended on both the estimates of the proportion of the caecal digesta NH_3-N or caecal digesta bicarbonate-C pools derived from the blood urea made during the tracer infusion into the blood urea pool, and the rate of irreversible loss when caecal digesta was the primary pool, whereas the estimate of the rate of irreversible loss of caecal digesta NH_3-N depended on the infusion into the caecal pool. Hence the linearity of the relation between the rate of irreversible loss from the caecal digesta NH_3-N pool and urea transfer to the caecal pool when measured by either tracer suggested that differences in the urea transfer to the caecal pool were actual differences among sheep and that constant conditions prevailed when the same sheep was studied on different experimental

days. Furthermore, the slope of the regression lines was that expected on the basis that each mol blood urea is hydrolysed to 2 mol NH_3.

Comparison of ^{15}N and ^{14}C tracer techniques for measurement of urea kinetics

The lower rate of irreversible loss of [^{15}N]urea than of [^{14}C]urea (Table 4) was expected, and the latter measurement should be the better estimate of total flux of urea through the blood pool (Nolan & Stachiw, 1979; Dixon & Milligan, 1984). The greater estimate of blood urea transfer to the caecal digesta pool with ^{14}C than with ^{15}N (Table 4) provides an explanation for the differences in elevation of the two regression lines shown in Fig. 5.

The estimated transfer of blood urea to the caecal digesta or rumen digesta measured in the open-compartment models with either ^{14}C or ^{15}N tracers would have been increased if $H^{14}CO_3^-$ or $^{15}NH_4^+$ produced outside the relevant pool entered it without passing through the blood bicarbonate-C pool or the urea pool. Furthermore, the rate of reabsorption of each would be likely to differ depending on the pH and the respective dissociation constants. In the ileum, with a pH 7–8 (Dixon & Nolan, 1982), $H^{14}CO_3^-$ was likely to remain in the digesta and hence flow into the caecum, whereas $^{15}NH_4^+$ was likely to be absorbed rapidly. Rapid absorption of NH_3-N from the ileum has been observed in other species (Mossberg & Ross, 1967). Consequently the finding that transfer to the caecal digesta of blood urea when measured with ^{14}C tracers was 0·52 greater than when measured with ^{15}N tracers may have been the result of differential rates of absorption of the products of urea hydrolysis in the small intestine and, therefore, different flows of these products to the caecal digesta pool via the ileum. Furthermore, observations in the present experiments that caecal digesta bicarbonate SR tended to be maintained for some time after the termination of the infusion of $H^{14}CO_3^-$ into the blood, as it would if a considerable proportion of blood bicarbonate entered the caecum via ileal digesta, supported this hypothesis. Similarly $H^{14}CO_3^-$ from hydrolysis of salivary urea in the buccal regions and oesophagus (Kornberg *et al.* 1954) may have passed to the rumen in alkaline saliva while $^{15}NH_4^+$ was absorbed, and thus caused the apparent transfer of urea to the rumen to be 0·38 greater when measured with the ^{14}C than with the ^{15}N tracers. In addition, blood urea hydrolysis may have occurred in or near the rumen wall and caecal wall with a greater proportion of the $H^{14}CO_3^-$ than of the $^{15}NH_4^+$ thus produced entering the respective rumen or caecal digesta pool (Houpt, 1970; Allen & Miller, 1976; Cheng & Wallace, 1979). The proportions of the $H^{14}CO_3^-$ and NH_4^+ hydrolysis products which mixed with the rumen or caecal digesta pools or were absorbed into the blood may have differed depending on the respective dissociation constants and concentration gradients.

The different estimates obtained with ^{14}C and ^{15}N tracers in the present experiment are in contrast to the results obtained by Nolan & Stachiw (1979) with sheep given low-N diets where no significant difference was observed, and where transfer estimated with ^{14}C tracers even tended to be less than that estimated with ^{15}N tracers. In choosing which tracer to use for similar studies, because NH_3-N is the hydrolysis product of urea that is potentially useful for microbial N synthesis, ^{15}N tracer results are likely to give a better indication of the availability of endogenous urea-N for microbial growth in the rumen or the caecum.

Transfer of blood urea-N to various sections of the gastrointestinal tract

The transfer of blood urea into the caecal digesta (Table 4) could have occurred either via ileal digesta or by direct transfer across the caecal wall. In order to estimate the likely importance of endogenous urea entry to the caecum in ileal digesta, results obtained in previous acute experiments (Dixon & Nolan, 1982; Dixon & Milligan, 1984) were collated. Significant relations were observed between the concentration of urea-N + NH_3-N in ileal

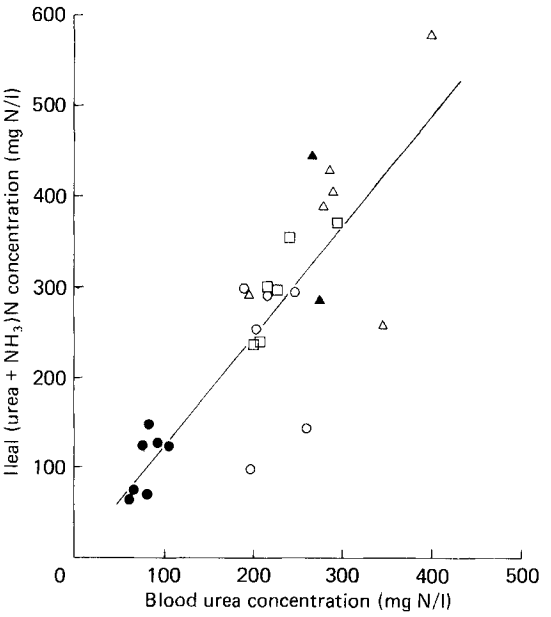

Fig. 6. The relation between ileal (urea + ammonia)-nitrogen concentration (mg N/l) and the blood urea concentration (mg N/l) measured in each of twenty-seven sheep given lucerne hay (*Medicago sativa*) (○), bromegrass (*Bromus inermus*) hay (●), sugar-cane bagasse–sugar-based diets (△), pelleted barley grain and urea (▲) or grazing *Phalaris tuberosa* – white clover (*Trifolium repens*) pasture (□). The equation of the regression is given by

$$Y = 1{\cdot}17 + 1{\cdot}23 \text{ (SE } 0{\cdot}159)X \text{ (}r\ 0{\cdot}84\text{, residual SD } 8{\cdot}50\text{, } P < 0{\cdot}01\text{, } n\ 27\text{)}.$$

digesta and the blood urea concentration (Fig. 6), and also the concentration of NH_3-N in the caecum and proximal colon and the blood urea concentration; the latter relation is described by:

$$Y = 100 + 0{\cdot}69 \text{ (SE } 0{\cdot}148)X \quad (r\ 0{\cdot}68\text{, residual SD } 8{\cdot}21\text{, } P < 0{\cdot}01\text{, } n\ 27\text{)},$$

where Y is the mean NH_3-N concentration in the digesta of the caecum and proximal colon (mg NH_3-N/l), and X is the blood urea concentration (mg N/l). These results for sheep are in agreement with observations of transfer of blood urea into the small intestine in dogs (Hakim & Lipson, 1964) and in sheep (Hecker, 1971*b*; Boda *et al.* 1976). If the concentration of urea in ileal water and blood were equal, on the basis of estimates of ileal digesta flow in similar sheep (Dixon & Nolan, 1982), up to 1·5 g urea-N/d could have entered the caecum in ileal digesta. In sheep B and C 0·27–0·86 g blood urea-N entered the caecal digesta NH_3-N pool, and this transfer could have occurred entirely via ileal digesta. Estimates of transfer of blood urea into the caecal digesta pool of sheep A were 2·1 and 2·7 g N/d for ^{15}N and ^{14}C tracers respectively and, hence, there was apparently some transfer of blood urea across the caecal wall to the caecal digesta in this sheep. The likely absence of transfer of blood urea across the caecal wall into the caecal digesta pool in most instances agrees with measurements using ^{15}N tracers in acute experiments with sheep given either lucerne or bromegrass hay (Dixon & Nolan, 1983; Dixon & Milligan, 1984) and with evidence of low transfer in perfusion experiments in humans (Billich & Levitan, 1969; Wolpert *et al.* 1971). Nevertheless there is evidence that blood urea can pass across the caecal wall under some circumstances (Chalmers *et al.* 1976) and that the rate varies with diet

(Engelhardt & Hinderer, 1976). The mean transfer of blood urea into the caecal digesta NH_3 pool by all routes measured with ^{15}N tracers (0·9 g N/d) was only slightly greater than the estimate of 0·67 g N/d obtained in acute experiments (Dixon & Nolan, 1983) and less than the estimate of 1·3 g N/d obtained by Nolan *et al.* (1976) in sheep given lucerne hay. The wide range (0·19–0·60) in the proportion of caecal digesta NH_3-N derived from blood urea-N is in accord with a wide range (0·14–0·34) observed in the acute experiments of Dixon & Nolan (1983). Also there were differences among sheep, with the two estimates in sheep A (0·56 and 0·64) being greater ($P < 0·05$) than these estimates for sheep B and C (0·34 and 0·19 respectively).

In agreement with previous experiments with sheep given lucerne (Nolan & Leng, 1972; Nolan *et al.* 1976; Kennedy & Milligan, 1977), the hydrolysis of urea in the rumen digesta pool comprised only 0·17–0·24 of total urea hydrolysis. Urea hydrolysis in the gastrointestinal tract other than in the rumen and caecal digesta pools was 0·56 of total urea hydrolysis when determined with ^{14}C tracers, or 0·69 when determined with ^{15}N tracers. This compares with the estimate of Nolan *et al* (1976) of 0·54. Appreciable hydrolysis of blood urea did not occur in the digesta of the distal colon of sheep in the experiments of Dixon & Nolan (1983) and Dixon & Milligan (1984), but did occur in the experiment of Nolan *et al.* (1976).

As discussed (p. 327), it is quite possible that blood urea did pass across the caecal wall or the rumen wall, or both, and that hydrolysis of this urea occurred in or near the wall without the resultant $H^{14}CO_3^-$ or NH_4^+ mixing with the respective sampled digesta pool. Much of the hydrolysis of urea other than in the caecal and rumen digesta pools may have occurred in such a manner. Alternatively the buccal regions, oesophagus, omasum, abomasum and small intestine may also be quantitatively important sites of urea hydrolysis.

Production of NH_3-N in the caecum

The rate of irreversible loss of NH_3-N from the caecal digesta pool for the three sheep in the present study (2·17 (SE 0·623) g N/d) was somewhat less than the estimates of Dixon & Nolan (1983) (3·2 (SE 0·49) g N/d) and Nolan *et al.* (1976) (4·2 g N/d), although the latter measurement was associated with a relatively large transfer of endogenous urea into the caecum. On the basis of recycling of 0·6 g N/d measured by Nolan *et al.* (1976), the total flux rate of NH_3-N through the digesta pool would have been slightly greater than this rate of irreversible loss. Since the caecal digesta NH_3-N pool size was only approximately 88 mg N (Dixon & Nolan, 1983), the turnover of the pool was rapid.

The production of NH_3-N in the caecum by proteolysis and deamination of non-^{15}N-labelled endogenous and dietary N (1·1 (SE 0·13) g N/d; Fig. 2) was less than the estimate from acute experiments (Dixon & Nolan, 1983; 2·3 g N/d) or that by Nolan *et al.* (1976) (2·1 g N/d) in sheep given lucerne hay, but was greater than that in sheep given bromegrass hay (0·7 g N/d; Dixon & Milligan, 1984). Whether or not there were real differences between the groups of sheep given lucerne could not be determined. Hecker (1971*a*) demonstrated that there was extensive degradation of proteins in caecal digesta in vitro, indicating that the necessary levels of enzyme activity occur to account for extensive proteolysis and deamination. Furthermore, degradation in the caecum of endogenous soluble mucins has been demonstrated (Hecker, 1973). The negligible flow from the rumen digesta NH_3-N pool direct to the caecal digesta NH_3-N pool in the three-pool N model (Fig. 2) suggested that little undigested rumen microbial N was degraded to NH_3-N in the caecum.

Utilization of caecal digesta NH_3-N

If recycling of N to the caecal digesta NH_3-N pool were 0·6 g N/d, as estimated by Nolan *et al.* (1976), the total flux through the caecal digesta NH_3-N pool would have been

2·17 + 0·6, i.e. 2·8 g N/d. Hence, since 0·70 g NH_3-N/d flowed to the proximal colon in digesta and 0·56 g NH_3-N/d was incorporated into caecal microbial N (Table 3), by difference 1·5 g NH_3-N/d was absorbed directly through the caecal wall. This estimate of absorption was similar to that made in sheep given lucerne in acute experiments (1·9 g N/d; Dixon & Nolan, 1983). Rapid absorption of NH_3 from the caecum has been observed (Hecker, 1971*b*; Chalmers *et al.* 1976), probably due to the high pH in the caecum of sheep at least when given forage diets. Since virtually all the 0·70 g NH_3-N/d flowing to the proximal colon in digesta would have been absorbed before reaching the rectum (Dixon & Nolan, 1983), total NH_3-N absorption from the large intestine of NH_3-N of caecal origin was at least 2·2 g NH_3-N/d.

In the present study, caecal digesta NH_3-N contributed on average 0·10 of the blood urea pool (Table 3), a value similar to that (0·13) obtained in sheep given lucerne by Nolan *et al.* (1976) and that (0·14) by Dixon & Nolan (1983). The proportion of absorbed caecal digesta NH_3-N which entered the blood urea-N in the present study (0·92, Table 3) and in the experiment of Dixon & Nolan (1983) (0·83) was, however, much greater than that observed by Nolan *et al.* (1976) (0·30–0·40). We can provide no definite explanation for this difference between experiments. However, since the NH_3-N absorbed from the gut that was not synthesized into blood urea was presumably synthesized into non-essential amino acids, the more recent studies do suggest that, in sheep given a high-N forage diet, the contribution of caecal digesta NH_3-N to synthesis of non-essential amino acids is not quantitatively important. The incorporation of only 0·64 of absorbed caecal NH_3-N into blood urea in sheep given a forage diet of moderate N content (Dixon & Milligan, 1984) does, however, suggest that at lower N intake the contribution of caecal NH_3-N is of greater importance. In the present study, 0·41 of blood urea was derived from rumen digesta NH_3-N (Table 5); consequently the caecum was of much lesser importance than the rumen in providing NH_3-N for the synthesis of blood urea.

The principal contribution of the caecum to N retention by the ruminant is likely to be by provision of NH_3-N for urea-N synthesis with recycling of this urea to the rumen and utilization of the N for microbial protein synthesis. In agreement with previous studies with sheep given high-N forage diets (Nolan & Leng, 1972; Nolan *et al.* 1976; Kennedy & Milligan, 1977), transfer of blood urea to the rumen digesta pool in the present study was equivalent to only 0·06–0·08 of N intake. However, if more extensive blood urea transfer to the rumen occurs under some circumstances (Kennedy & Milligan,1980; Norton *et al.* 1982) this pathway may be of importance to the ruminant.

Turnover and synthesis of microbial N in the caecum

The observations that 0·48 of the NU-NAN flowing from the caecum consisted of microbial N, that 0·56 g caecal digesta NH_3-N/d was incorporated into caecal microbial N and that 0·83 of caecal microbial N was excreted in faeces (Table 3) were in agreement with previous experiments in sheep given lucerne (0·57, 0·6 g N/d and 0·89 respectively; Dixon & Nolan, 1983) or bromegrass hay (0·49, 0·4 g N/d and 0·73 respectively; Dixon & Milligan, 1984). The present study supports the concept that there is little net synthesis of microbial N in the large intestine, that approximately half the caecal digesta N is microbial N and that the majority of the microbial N passing from the caecum is excreted as such.

As discussed by Dixon & Nolan (1983), the microbial N in caecal digesta determined by the ^{15}N tracer method would have included microbial N of both rumen and caecal origin. During the $(^{15}NH_4)_2SO_4$ infusion into the rumen the enrichment of caecal microbial N was 0·44 of rumen bacterial N (Table 5). Furthermore, since some microbes would have been synthesized in the caecum from $^{15}NH_3$-N present in the caecal pool during infusion into the rumen NH_3-N pool, the proportion of caecal microbial N consisting of rumen microbial

N would actually have been less than 0·44 i.e. at least 0·56 of caecal microbial N was synthesized in the caecum. This indication of extensive caecal degradation of rumen microbial debris is in agreement with other estimates of digestion of other microbial constituents in the large intestine. In sheep given *Trifolium subterraneum*, Hogan (1973) reported 0·57 apparent digestion of the bacterial marker diaminopimelic acid between the ileum and the rectum and suggested a true digestibility of 0·65. Apparent digestion of bacterial cell-wall constituents of 0·20–0·40 for diaminopimelic acid (Mason & White, 1971), 0·68 for muramic acid (Mason & Milne, 1971) and 0·75–1·00 for α-aminoisobutyrate (Harrison *et al.* 1971) has also been reported for sheep given forage diets. Judson *et al.* (1975) reported an apparent digestibility of 0·31 and a degradation of 0·42 of ^{35}S-labelled rumen bacteria in the large intestine.

Only 0·17 of caecal microbial N was apparently derived from the caecal digesta NH_3-N pool during $(^{15}NH_4)_2SO_4$ infusion into the caecum (Table 3). This value will underestimate the importance of caecal NH_3 as a microbial substrate by the proportion of sampled caecal microbial N consisting of rumen microbial debris, but does suggest that N sources other than caecal NH_3 were used extensively by caecal microbes. The small contribution of rumen microbial N to the caecal digesta NH_3 pool, even though there was probably extensive degradation of rumen microbial debris, suggests that there may have been extensive re-incorporation into caecal microbes of the ^{15}N-labelled degradation products of rumen microbes. The low apparent digestibility of [^{15}N]NU-NAN of 0·25–0·27 between the ileum and the rectum previously reported (Dixon & Nolan, 1983; Dixon & Milligan, 1984) can only be reconciled with extensive degradation of rumen microbial debris if there were extensive re-incorporation into caecal microbes of the ^{15}N-labelled degradation products. This hypothesis is consistent with the finding that in the rumen a considerable fraction of the bacterial N is apparently derived from peptides and amino acids, and this fraction increases as the concentration of peptides and amino acids increases (Nolan & Leng, 1972; Maeng *et al.* 1976; Salter *et al.* 1979). Much higher concentrations of α-amino-N in caecal liquid (20 mg N/l) than in rumen fluid (7 mg N/l) have been observed (Hecker, 1971*b*). Considering the extensive proteolysis and deamination in the caecum, amino acids and peptides may well be available as microbial substrates.

CONCLUSIONS

The present study was largely in agreement with previous experiments in this laboratory with sheep given lucerne hay. Estimates of blood urea transfer to the digesta in the caecum and the rumen were greater when determined with ^{14}C tracers than with ^{15}N tracers. Appreciable hydrolysis of blood urea occurred in the caecal digesta pool, and much of this blood urea probably entered the caecum with ileal digesta. Considerable amounts of NH_3 were also produced by proteolysis and deamination in the caecum. Although most of the NH_3-N produced was absorbed from the large intestine it did not contribute substantially to microbial synthesis in the rumen, and the observation that most of the NH_3-N was transferred to blood urea suggested that it was also not used for the synthesis of non-essential amino acids.

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