Digestibilities of nitrogen compounds in rumen bacteria and in other components of digesta in the small intestine of the young steer

BY D. N. SALTER AND R. H. SMITH

National Institute for Research in Dairying, Shinfield, Reading RG2 9AT

(*Received* 23 *July* 1976 – *Accepted* 2 *November* 1976)

1. Mixed bacteria were obtained from the rumen contents of steers and were taken at different times after the animals had been given different feeds. The feeds contained [^{15}N]urea and in some experiments $Na_2{}^{35}SO_4$ so that the bacteria were labelled with these isotopes.

2. Samples of labelled bacteria were subjected to a simulated abomasal digestion with pepsin and the digests were infused with a non-absorbed marker, polyethylene glycol, into the duodenums of test steers equipped with re-entrant ileal cannulas. Except for samples taken after a 24 h fast, which sometimes gave somewhat lower values, mean values for digestibilities of ^{15}N and ^{35}S in the small intestine were 0·79 and 0·85 respectively.

3. The corresponding value for the digestibility of ^{15}N in similarly-treated wheat-leaf protein concentrate was 0·86.

4. Net digestibilities of total N in the small intestine of the test steers when they were given either a mainly protein-free diet of straw, tapioca and urea or a diet of flaked maize and hay were approximately 0·62.

5. From these and other values it was calculated that approximately 5·2 g intestinally-secreted endogenous N/d flowed through the ileum of a 100 kg steer.

Rumen micro-organisms generally provide the ruminant with more than half the amino acids (free and combined) that enter the duodenum (Smith, 1976). The digestibilities in the small intestine of these, as well as of amino acids from dietary sources, help to determine the nutritional value of a diet to the host animal but have rarely been studied separately. A series of determinations of digestibilities of nitrogen compounds in the intestines of ruminants have been made for the mixture of microbial, dietary and endogenous components entering the duodenum (Clarke, Ellinger & Phillipson, 1966; Smith & McAllan, 1971; Coelho da Silva, Seeley, Beever, Prescott & Armstrong, 1972; Van't Klooster & Boekholt, 1972; Hogan, 1973; Ben-Ghedalia, Tagari & Bondi, 1974) but there is little published information on the extents to which such values are affected by differences in properties of the individual N sources or by the different proportions present.

Although experiments have been done to study the digestibilities of rumen microbial N compounds in the rat (McNaught, Owen, Henry & Kon, 1954; Bergen, Purser & Cline, 1968) and in vitro (Bergen, Purser & Cline, 1967), the only published work in which such a determination in the ruminant intestine was attempted is that of Bird (1972) who infused a sample of ^{35}S-labelled rumen bacteria into the sheep abomasum and estimated the recovery of ^{35}S in the faeces.

In the present studies isotopically-labelled materials were infused into the duodenum of the young steer in an attempt to assess the true digestibilities of rumen bacterial N compounds and a typical food protein in the small intestine. A comparison of these values with estimates of net digestibility of total digesta N compounds was also made. Part of this work has been reported in a preliminary communication (Salter & Smith, 1974).

Table 1. *Daily intakes (kg) of the major components of different diets given to steers weighing* 90–110 *kg**

Ingredients	Diets						
	A§	B§	C	D	E§	F	G
Straw†	1·00	1·00	1·00	1·00	1·00	—	0·30
Hay†	—	—	—	—	—	1·15	—
Flaked maize‡	—	—	—	—	—	1·26	—
Tapioca‡	1·40	1·40	1·20	1·30	1·30	—	1·56
Decorticated, extracted groundnut meal‡	—	—	0·28	0·14	—	—	—
Peptide mixture‖	—	—	—	—	0·10	—	—
Urea‡	0·038	0·066	0·010	0·038	0·038	—	0·066

* These amounts were increased or decreased by about 120 g/kg for each 20 kg change in live weights.
† Straw or hay were given at 17.00 hours only.
‡ Concentrates were given in two equal amounts at 09.00 and 17.00 hours and were supplemented with vitamins and minerals.
§ A small amount of molasses (0·25 kg/d) was sometimes added to diets A, B and E to increase palatability but was omitted from an 09.00 hour feed containing [^{15}N]urea (for details see p. 209) given through the rumen cannula before a bacterial collection was made.
‖ Bacto Peptone; Difco Laboratories Inc., Michigan, U.S.A.

EXPERIMENTAL

Animals and feeding

Seven Friesian bull calves were weaned at 7–11 weeks of age onto a diet consisting of about equal amounts of hay and a concentrate mixture, given twice/d. At about 12–18 weeks they were castrated and equipped either with rumen and simple duodenal cannulas (Smith & McAllan, 1970) (steers nos. 1–4) or with simple duodenal and re-entrant ileal cannulas (Smith & McAllan, 1971) (steers nos. 15–17). Steers nos. 1–4 (donor steers) were used as sources of samples of rumen bacteria which had been labelled in vivo with ^{15}N and, sometimes, ^{35}S. Steers nos. 5–7 (test steers) were used to study the digestibilities of these labelled bacteria and ^{15}N-labelled leaf protein in the small intestine, and were used also to study net N digestibilities in the small intestine. An appropriate experimental diet (Table 1) was given for at least 20 d before an experiment was carried out, and this was not done earlier than 6 weeks after surgery or 10 weeks after weaning.

Preparation of labelled sources for digestibility studies

^{15}N-labelled leaf protein. Wheat-leaf protein was prepared from the leaves of wheat grown for 6–7 weeks on a medium in which the only exogenous source of N was given as ^{15}N-labelled potassium and calcium nitrates (30 atoms % ^{15}N) in equal parts by weight. The fresh, whole-leaf juice, prepared as described by Morrison & Pirie (1961) was passed through a brass tube heated at 60° to form a coagulum which was continually scraped from the surface of the tube. The coagulum was separated by centrifugation, heated to 100° to make it more compact, filtered off and pressed to form a moist cake which was subsequently freeze-dried. The two batches (nos. 1 and 2) contained 112 mg N/g, with 9·2 and 12·5 atoms % excess ^{15}N respectively in their N. They were found to consist mainly of chloroplast protein (N. W. Pirie, private communication).

^{15}N-labelled rumen bacteria (group 1). On the day of the experiment, the morning feed was not given by mouth to a donor steer. Instead, the tapioca and, where appropriate, the decorticated groundnut meal were coarsely ground to a particle size of approximately 1–2 mm and these ingredients together with all but 5 g urea in the diet were added directly

to the rumen through the cannula. At the same time (approximately 09.00 hours) [^{15}N]urea (containing about 30 atoms % excess ^{15}N) dissolved in 500 ml 150 mM-sodium chloride was added to the rumen. After this, food was not given before the samples of rumen contents, from which rumen bacteria were separated, were taken at 7 or 24 h after addition of [^{15}]N-urea. The samples were taken at the end of experiments to study other aspects of N metabolism (in which small samples of rumen contents totalling approximately 2 and 2·5 kg respectively were taken at intervals) by pumping out as much of the rumen contents (6·5–10 *l*) as could readily be obtained. Each sample was strained through layers of cheesecloth and, after adding 0·6 vol. 150 mM-NaCl, was centrifuged at 200 *g* at 1° for 10 min. This centrifugation removed protozoa and food particles, and the supernatant fraction was centrifuged at 30000 *g* at 1° for 10 min. Microscopic examination of Gram-stained peparations showed that the sediment consisted of bacteria essentially free from other particles. Sediments were stored frozen at −20° for not more than 2 d before being freeze-dried.

^{15}N- and ^{35}S-labelled rumen bacteria (group 2). The rumen bacteria were labelled in a similar way to those in 'group 1' experiments except that for 4·5 d before a collection was made approximately 0·25 mCi ^{35}S was included with each feed, and on the morning of an experiment a solution containing both 5 g [^{15}N]urea (containing 30 atoms % ^{15}N) and 0·25 mCi $^{35}SO_4^{2-}$ dissolved in 500 ml 150 mM-NaCl was added to the rumen of a donor steer with the ground ingredients of the feed. As much of the rumen contents as could readily be collected (approximately 6·5 *l*) was removed after 2·5 h (first harvest) and a bacterial sample prepared from it as described previously. The rumen contents removed were replaced by an equal volume of a solution containing sodium acetate (50 mM), sodium bicarbonate (30 mM), potassium acetate (45 mM) and potassium chloride (150 mM) at 39°. After an additional 2 h a similar volume of rumen contents was again removed (second harvest). Replacement solution was once more added and a final collection was made at 24 h without additional food being given (third harvest). Bacteria were prepared from each harvest as described previously.

Determination of digestibilities of N components entering the duodenum

Net digestibilities. Determinations were made essentially by the method described by Smith & McAllan (1971) while the test steers were receiving diets B or F (Table 1). Polyethylene glycol (molecular weight 4000; PEG) (200 g) was given with an evening feed, and then 3·5 h after the feed on the next morning a sample (approximately 50 g) of duodenal contents was taken. Immediately afterwards, 5 ml phenol red solution (20 g/l) was injected into the duodenum via the cannula and after 5–10 min another 50 g sample of duodenal contents was taken. The two duodenal samples were pooled. As soon as the phenol-red marker appeared at the ileum the digesta flowing at this site was collected for 10–30 min (until approximately 100 g was obtained). The values for the ratio, N:PEG in the duodenal and ileal digesta were determined and the net digestibility was calculated as the proportion of N which had disappeared from the small intestine relative to the non-absorbable marker.

Digestibilities of labelled compounds. A sample of leaf protein (containing approximately 0·2 g N with an enrichment of 9·2 (batch 1) or 12·5 (batch 2) atoms % excess ^{15}N, or of freeze-dried rumen bacteria (containing 0·3–1·2 g N with an enrichment of 1·0–6·0 atoms % excess ^{15}N and in some experiments with ^{35}S giving an activity of about 5 μCi/g N) was subjected to a simulated abomasal digestion by suspending it in 50 ml 150 mM-NaCl, adjusting the pH to 2·0 with hydrochloric acid, adding pepsin (from pig stomach mucosa, twice crystallized, freeze-dried powder, 2700 units enzyme activity/mg protein; Sigma (London) Chemical Co. Ltd, Kingston upon Thames, Surrey) at a rate of 30 mg pepsin-protein-N/g substrate N, adjusting the volume to 100 ml with 150 mM-NaCl and shaking the mixture at 37° for 3 h. It is clearly not possible for in vitro and in vivo digestion conditions to be

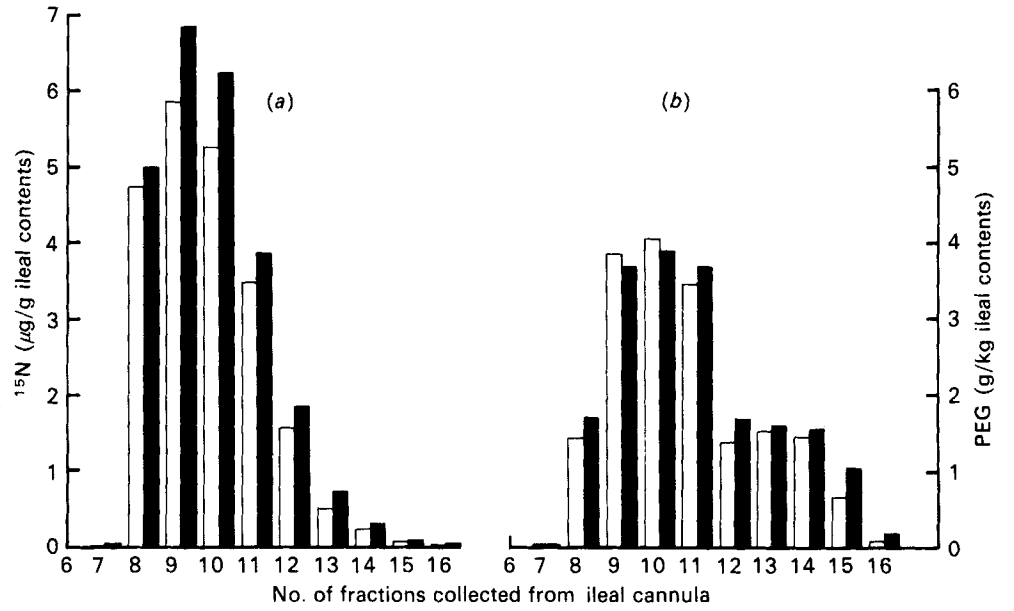

Fig. 1. Pattern of emergence of ^{15}N (■) and polyethylene glycol (PEG) (□) from the terminal ileum of steers nos. 6 (a) and 7 (b). ^{14}N-labelled rumen bacteria that had been subjected to a pepsin treatment (see p. 209) and PEG were infused into the duodenum and the outflow from the terminal ileum was collected in successive 50 g fractions between 1 and 6 h after completion of the infusion.

identical but the concentration of pepsin chosen in our in vitro simulation was within the range that can be calculated from the results of Harrop (1974) to be present in the abomasal contents of sheep. It is known that a marker added to the rumen can be detected at the proximal duodenum in about 1 h and reaches a peak concentration in 3–6 h (Horn, 1975). It seemed reasonable therefore to use an incubation time of 3 h in vitro. After incubation, 13 ml PEG in 150 mM-NaCl (200 g/l) usually containing 20 μCi [U-^{14}C]PEG, were added, the pH of the mixture adjusted to 3·5 and the volume made up to 130 ml with 150 mM-NaCl. [U-^{14}C]PEG was omitted when preparations contained ^{35}S, in order to facilitate determination of the latter isotope, and in these instances it was necessary to determine PEG by the more tedious chemical method.

A flexible plastic tube (Tygon; Norton Plastics and Synthetics Division, Ohio, USA), internal diameter approximately 3 mm, was introduced into the duodenal cannula of a test calf so that the outlet end of the tube was approximately 0·3 m distal to the pylorus. The simulated abomasal digest was pumped into the duodenum through the tube at a rate of 8–10 ml/min. Within 60 min of completing this addition the re-entrant ileal cannula was disconnected and the outflow collected in successive 50 g fractions for the next 6 h. Most of the PEG and tracer (80–98%) appeared in approximately six fractions (see Fig. 1) starting at approximately fraction no. 8 which generally first reached the ileum between 3 and 5 h after starting the infusion.

For each of these fractions the digestibility of ^{15}N between duodenum and ileum was estimated from the ratio, ^{15}N (mg excess):PEG (mg, or disintegrations/min when ^{14}C-labelled PEG was included) in the fraction (R_{ileum}) and the corresponding ratio ($R_{infused}$) in the infusion mixture:

$$^{15}\text{N digestibility} = \frac{R_{infused} - R_{ileum}}{R_{infused}}.$$

Successive fractions gave closely similar values for estimates of ^{15}N digestibility (coefficients of variation for twenty-four experiments with three to six samples for each experiment ranged from 1·9 to 11·6 with an average of 7·6) and statistical analysis showed no consistent change in an estimate with increasing fraction number. Estimates of digestibility for an experiment consisted therefore of mean values derived from the three to six samples analysed. Digestibilities of ^{35}S were estimated by a method analogous to that used for ^{15}N, but in experiments in which ^{35}S was present in the digesta ^{14}C-labelled PEG was not added to the simulated abomasal digesta and estimates were therefore based upon a chemical determination of PEG. Preliminary experiments showed that proportions of inactive and [U-^{14}C]PEG recovered in successive samples at the ileum were virtually identical. As for ^{15}N, values for ^{35}S:PEG in successive fractions showed no consistent change and results were therefore based on mean values of three to six samples for each experiment.

Isotopically-labelled compounds

[^{15}N]urea (30 atoms % ^{15}N (double N label)) was obtained from Prochem, British Oxygen Co., Deer Park Road, London SW19 3UF.

$Na_2{}^{35}SO_4$ (specific activity 90 mCi/mmol) was obtained from the Radiochemical Centre, White Lion Road, Amersham, Bucks. [^{14}C]PEG (specific activity 0·46 μCi/mg) was obtained from NEN Chemicals GmbH, D 6072 Dreieichenhain, Siemenstrasse 1, West Germany.

Analytical methods

N. Samples (1 g) of digesta and infusion mixtures were digested, using a semi-micro-Kjeldahl technique, with 3 ml concentrated sulphuric acid, 100 mg mercuric oxide and 2·4 g potassium sulphate. The ammonium sulphate formed was estimated using an Auto-Analyser (Technicon Instruments Co. Ltd, Basingstoke, Hants) using the procedure described by Ferrari (1960).

PEG. A sample of digesta was centrifuged at 30000 ***g*** for 10 min at 1° and PEG determined as described by Smith (1962) except that 20 min was allowed for development of turbidity. When phenol red was present in the samples it was removed before determination of PEG (Smith, 1964). From estimates of dry-matter content, values for the amount of PEG in total digesta were calculated.

Dry matter. Samples (5 g) of whole ileal fractions or supernatant fractions obtained from them by centrifugation were dried at 105° for 6 h.

Measurement of ^{15}N. Samples containing 0·5–2 mg N were digested by the Kjeldahl technique as described for total N determination. Ammonia was distilled from portions of the diluted digesta (containing about 500 μg N) into 10 ml 0·025 M-HCl to form ammonium chloride. The solutions of NH_4Cl were evaporated to dryness at 70° and the residues redissolved in distilled water to give a concentration of 1 μg N/μl. Samples of these solutions containing 10 μg N were used to prepare pure N_2 gas for determination of ^{15}N enrichment using an optical emission spectrometer (Statron Model NOI-4; C.Z. Scientific Instruments Ltd, Zeiss England House, 93–97 New Cavendish Street, London W1 AZAR) as described previously (Lloyd-Jones, Salter & Adam, 1975).

Measurement of ^{35}S and ^{14}C. [^{14}C]PEG was omitted from infusions that contained ^{35}S-labelled bacteria and in these samples PEG was measured chemically. For the measurement of ^{35}S or ^{14}C, 0·1 g samples of gut contents were digested with 0·7 ml tissue solubilizer (NCS; Amersham-Searle, Illinois 60005, USA) for 1 h at 50°, neutralized with 0·02 ml concentrated acetic acid, and radioactivity measured using a liquid-scintillation spectrometer (for ^{35}S (using an internal standard): Packard Tricarb Model 3003; Packard Instrument Co. Inc., Illinois 60515, USA; for ^{14}C (using an external standard): liquid-scintillation analyser Model PW4510; Phillips, Eindhoven, The Netherlands).

Table 2. *'Group 1' Expt. Digestibilities of the ^{15}N in different samples of labelled rumen bacteria between proximal duodenum and distal ileum of different test steers*

Bacterial sample†						
	Origin					
No.	Donor steer	Diet*	Interval after ^{15}N dose (h)	Test steer no: 5	6	7
1	1	C	24	0·71‡	—	—
2	2	C	24	0·79‡	—	—
3	2	D	24	0·53‡ ± 0·04§	—	—
4	2	B	24	0.62	—	—
5	2	C	24	—	—	0·74
6	2	D	24	—	0·62	0·62
7	3	B	7	—	0·81	0·81
8	3	A	7	—	0·74 ± 0·02§	0·76
9	3	E	7	—	0·80 ± 0·01§	0·82 ± 0·02§
10	4	B	2·5	—	0·74	0·74
11	4	D	2·5	—	0·85	0·85

* For details, see Table 1.
† For details of sample preparation, see p. 208.
‡ A diet of urea and tapioca (diet G) was given, otherwise test steers received a diet of flaked maize and hay (diet F).
§ Mean results from duplicate experiments ± variation between individual values.

RESULTS

Digestibilities of ^{15}N or ^{35}S in rumen bacteria in the small intestine of the steer

Samples from 'group 1' Expt and from the first harvest of 'group 2' Expt. Digestibilities of ^{15}N in bacterial samples of different origin and tested in different steers are shown in Table 2. As it had been shown (see p. 211) that there was no consistent increase in R_{ileum} in consecutive digesta fractions, it could be assumed that there was negligible recycling of ^{15}N into the gut in the course of the experiments. Thus each result indicates an average true digestibility for the N compounds in a bacterial sample that were labelled with ^{15}N. A range of digestibilities was shown for the different bacterial samples and it appeared that this range was similar whichever test steer was used. For example, sample nos. 6 and 11 showed the lowest and highest digestibilities respectively in both test steers, nos. 6 and 7. No clear association was apparent between the diet received by a donor calf and the ^{15}N digestibility of its rumen bacteria. There was some indication that samples taken after a fast of 24 h (sample nos. 1–6) had, on average, lower ^{15}N digestibilities than samples taken 2·5–7 h after a feed (sample nos. 7–11). Although for sample nos. 1–4 test conditions were not the same as for the other samples, low digestibilities were also found for sample nos. 5 and 6 which were tested in calves nos. 6 and 7. If samples taken after a 24 h fast are omitted, mean (± SE) ^{15}N digestibilities for all samples irrespective of their origin were 0·78 ± 0·02 and 0·80 ± 0·02 for test steers nos. 6 and 7 respectively. In order to obtain further information on possible variations in ^{15}N digestibility between different bacterial samples the 'group 2' experiments were carried out.

Samples from 'group 2' Expt. Rumen bacterial samples were labelled with both ^{15}N and ^{35}S. The harvesting procedure described on p. 209 was adopted partly to obtain the maximum yield of labelled material and partly to obtain bacterial samples differing in nutritional status. Results for the different samples obtained are shown in Table 3.

Although once again there appeared to be differences in ^{15}N digestibility between samples,

Table 3. *'Group 2' Expt Digestibilities of the ^{15}N and ^{35}S in different samples of double-labelled rumen bacteria between proximal duodenum and distal ileum of different test steers*

(A single donor steer (steer no. 14) was used. For each diet the different bacterial samples were obtained in a single 24 h period and rumen contents taken at 2·5 and 4·5 h were immediately replaced by an equal volume of a solution containing sodium acetate (50 mM), sodium bicarbonate (30 mM), potassium acetate (45 mM) and potassium chloride (15 mM))

Bacterial sample†			Test steer no.:			
			6		7	
No.	Diet*	Interval after '09.00-hours' feed containing [^{15}N]urea dose (h)	^{15}N	^{35}S	^{15}N	^{35}S
10	B	2·5‡	0·74	0·82	0·74	0·82
12		4·5	0·83	0·75	0·84	0·87
14		24	0·77	0·94	0·80	0·83
11	D	2·5‡	0·85	0·82	0·85	0·85
13		4·5	0·78	0·84	0·77	0·78
15		24	0·79	0·84	0·78	0·84

* The diet for the test steers was diet F; for details of diets, see Table 1.
† For details of experimental procedures, see p. 209.
‡ ^{15}N results shown also in Table 2.

Table 4. *Digestibilities of the ^{15}N in samples of wheat-leaf protein between proximal duodenum and distal ileum of different test steers*

Leaf-protein sample batch no.	Test steer no.: 5	6	7
1	0·85*	—	—
	0·88*	—	—
	0·81*	—	—
	0·84	—	—
	0·82	—	—
2	—	0·86	0·87

* A diet of urea and tapioca (diet G) was given, otherwise steers were given a diet of flaked maize and hay (diet F); for details of diets, see Table 1.

which were shown in each of the test calves, no consistent relationship was apparent between these differences and differences either in diet or time of harvesting. Mean (± SE) values for ^{15}N digestibilities irrespective of sample origin were 0·79 ± 0·02 and 0·80 ± 0·04 for test steers nos. 6 and 7 respectively. Similarly there appeared to be no consistent relationship between ^{35}S digestibilities and differences either in diet or time of harvesting but, on average, digestibilities measured with ^{35}S were higher than those measured with ^{15}N. If results are considered for all samples irrespective of origin then mean (± SE) values for ^{35}S digestibilities were 0·84 ± 0·03 and 0·83 ± 0·01 for test calves nos. 6 and 7 respectively. The difference between the methods, 0·04 ± 0·018 (mean difference ± SE; $P < 0·1$; paired-t test) just failed to reach significance ($P > 0·05$).

Table 5. *Net digestibilities of total digesta N compounds between proximal duodenum and distal ileum of steers given different diets*

(Replicate experiments were made at intervals of about 1–2 weeks over periods of 20–24 weeks)

Diet*	Steer no.	No. of determinations	Digestibility†	
			Mean	SE
G	5	4	0·62	0·03
F	5	3	0·63	0·02
F	6	9	0·61	0·02
F	7	10	0·60	0·03

* For details of diets, see Table 1.
† For details of experimental procedure, see p. 209.

Digestibility of ^{15}N-labelled wheat-leaf protein in the small intestine of the steer

During the series of experiments to determine digestibilities of ^{15}N-labelled bacteria with test steer no. 5 alternate experiments were made to determine in a similar way the digestibility of samples of ^{15}N-labelled wheat-leaf protein (batch 1). The values (Table 4) were considerably higher and less variable than the corresponding values for bacterial ^{15}N (Table 2). Determinations for samples of a different batch of ^{15}N-labelled wheat-leaf protein (batch 2) in test calves nos. 6 and 7 gave closely similar values (Table 4).

The over-all mean value of the ^{15}N digestibility for three steers was 0·85.

Net digestibilities of N compounds entering the duodenums of the test calves

During each series of experiments to study the digestibilities of ^{15}N-labelled bacteria with test steers nos. 5, 6 and 7, periodic determinations were made of the net digestibilities in the small intestine of N compounds entering the duodenum with the digesta flowing from the abomasum. The results given in Table 5 show that for a diet consisting of approximately equal weights of flaked maize and hay (diet F) the mean net digestibility of N between duodenum and ileum was 0·60–0·63 for the three test animals. Similar values were obtained when a diet largely free of protein (diet G) was given to one of the steers.

DISCUSSION

It appeared unlikely that recycling of ^{15}N or ^{35}S into the small intestine would have occurred to an appreciable extent in the short-term collections made in the present experiments, as the single dose of labelled components absorbed would probably have been greatly diluted in a body pool. This view was supported by the absence of a significant trend in values for ^{15}N:PEG or ^{35}S:PEG observed between successive samples obtained during collections of ileal contents (Fig. 1 and p. 211). Thus disappearance of a tracer in the small intestine presumably indicated the average true absorption of components containing this tracer. For ^{15}N-labelled bacteria these components include newly-synthesized amino acids present mainly in proteins, and purines and pyrimidines present mainly in nucleic acids. The extent of labelling is likely to vary for the different components (Daneshvar, Salter & Smith, 1976) while their digestibilities may also differ. There is, for example, evidence that bacterial nucleic acids, particularly RNA, are more digestible than other N compounds in the small intestine of the steer (Smith & McAllan, 1971) and in sheep and cows net digestibilities of different amino acids have been reported to differ (Ben Ghedalia *et al.* 1974; Van't Klooster & Boekholt, 1972). It also appears that certain cell-wall com-

ponents such as α, ϵ-diaminopimelic acid, which is known to be labelled with ^{15}N in mixed rumen bacteria prepared as described in the present studies, (Daneshvar, 1976), are less digestible than other bacterial N components in the small intestine (Mason & Milne, 1971; Hogan, 1973).

Differences observed in ^{15}N digestibility between samples (Table 2) were probably due therefore to a combination of factors difficult to define and, not surprisingly, difficult to relate to different specific conditions obtaining during the development of the rumen bacterial population. It is possible, for example, that the low digestibilities observed for sample nos. 3, 4 and 6 may have been due to relatively greater amounts of cell-wall N in these samples but there is no direct evidence that this was so. It was clear that even without these very low values for sample nos. 3, 4 and 6, digestibility of N was appreciably less in labelled bacteria than in labelled leaf protein. It is possible that this was due mainly to the presence of poorly digested cell-wall N in the former but, once again, direct evidence of this is lacking. The fact that ^{35}S digestibilities tended to be greater than the ^{15}N values (Table 3) offers some support for this belief however as the cell walls of many bacteria contain relatively little S (Salton, 1960).

The present rumen bacterial ^{35}S digestibilities were mostly higher than the value (0·74) reported by Bird (1972) for the sheep. This could have been for many reasons. For example, the value of Bird (1972) was for a single sample of mixed rumen bacteria which was labelled by culturing in vitro and digestibility was measured between abomasum and faeces.

Despite their variability true digestibilities of total non-NH_3-N components in the small intestine can always be expected to be higher than corresponding values for net digestibilities. The latter have been reported to be within the range 0·45–0·71 (usually between 0·60 and 0·70) for sheep (Clarke *et al.* 1966; Coelho da Silva *et al.* 1972; Hogan, 1973; Ben-Ghedalia *et al.* 1974) young steers (Smith & McAllan, 1971) and cows (Van't Klooster & Boekholt, 1972). These results, which are in general agreement with those given in Table 5, undoubtedly varied in part because of differences in dietary components but it can be expected that most would be determined mainly by the digestibility of microbial N compounds (usually the major component at the duodenum) and endogenous secretion of N in the small intestine.

By making certain assumptions it is possible to estimate the amount of N secreted into the small intestine which was not re-absorbed up to the ileum for some of the present experiments. For steers given a largely non-protein diet of similar N content to diet G the mean amount of non-NH_3-N entering the duodenum was about 90% of the total N consumed (unpublished results, A. B. McAllan & R. H. Smith). If it is assumed that the N compounds entering the duodenum have a true digestibility similar to that found for the N compounds in most samples of bacteria (0·79) but that net digestibility of N in the small intestine is 0·62 (Table 4) then for a steer of 100 kg live weight receiving diet G (providing daily approximately 1·8 kg dry matter and 34 g N) it can be calculated that approximately 5·2 g intestinally-derived endogenous N/d survive digestion in the small intestine.

From studies with sheep in which the compositions of abomasal contents and faeces were analysed for different N intakes, Hogan & Weston (1968) and Egan (1974) calculated by regression the contributions of gut secretions to faecal N. Their results indicated true digestibilities of N in the intestines ranging from approximately 0·74 to 0·84 for various forage diets. Although these results were for the whole intestine and for N compounds which included some dietary components, they are compatible with the present estimates for digestibilities of microbial N compounds in the small intestine. It may also be relevant that the present estimates do not differ greatly from values for true digestibilities of rumen bacteria N in the rat (0·73–0·75) reported by McNaught *et al.* (1954) and Bergen *et al.* (1968).

It is difficult to relate in absolute terms the extent of digestion of pure cultures of rumen bacteria in vitro (Bergen *et al.* 1967) with values for digestibility in the alimentary tract of an animal, but the variation (ranging from 0·44 to 0·93) observed by these authors may have reflected differences similar in nature to those found in the present studies.

The authors are indebted to Mr N. W. Pirie, FRS, and his colleagues of Rothamsted Experimental Station, Harpenden, Herts. for the preparation of the ^{15}N-labelled wheat-leaf protein. We are also grateful to Dr H. L. Buttle and Mr S. C. Watson for carrying out all surgical operations, to Miss S. Askew for the care of the animals, to Miss P. E. Lewis and Mrs K. E. A. Smith for skilled technical assistance, and to Dr D. Hewitt for statistical help.

REFERENCES

Ben-Ghedalia, D., Tagari, H. & Bondi, A. (1974). *Br. J. Nutr.* **31**, 125.
Bergen, W. G., Purser, D. B. & Cline, J. H. (1967). *J. Nutr.* **92**, 357.
Bergen, W. G., Purser, D. B. & Cline, J. H. (1968). *J. Dairy Sci.* **51**, 1698.
Bird, P. R. (1972). *Aust J. biol. Sci.* **25**, 195.
Clarke, E. M. W., Ellinger, G. M. & Phillipson, A. T. (1966). *Proc. R. Soc.* B **166**, 63.
Coelho da Silva, J. F., Seeley, R. C., Beever, D. E., Prescott, J. D. H. & Armstrong, D. G. (1972). *Br. J. Nutr.* **28**, 357.
Daneshvar, K. (1976). Studies with ^{15}N of the incorporation in the rumen of non-protein nitrogen into microbial protein and nucleic acids. PhD thesis, University of Reading.
Daneshvar, K., Salter, D. N. & Smith, R. H. (1976). *Proc. Nutr. Soc.* **35**, 54A.
Egan, A. R. (1974). *Aust. J. agric. Res.* **25**, 613.
Ferrari, A. (1960). *Ann N.Y. Acad. Sci.* **87**, 792.
Harrop, C. J. F. (1974). *J. agric. Sci., Camb.* **83**, 249.
Hogan, J. P. (1973). *Aust. J. agric. Res.* **24**, 587.
Hogan, J. O. & Weston, R. H. (1968). *Proc. Aust. Soc. Anim. Prod.* **7**, 364.
Horn, J. (1975). Factors affecting the absorption of magnesium in the stomachs and small intestine of the calf. PhD thesis, University of Reading.
Lloyd-Jones, C. P., Salter, D. N., & Adam, J. (1975). *Analyst, Lond.* **100**, 891.
McNaught, M. L., Owen, E. C., Henry, K. M. & Kon, S. K. (1954). *Biochem. J.* **56**, 151.
Mason, V. C. & Milne, G. (1971). *J. agric. Sci., Camb.* **77**, 99.
Morrison, J. E. & Pirie, N. W. (1961). *J. Sci. Fd Agric.* **12**, 1.
Salter, D. N. & Smith, R. H. (1974). *Proc. Nutr. Soc.* **33**, 42A
Salton, M. R. J. (1960). In *The Bacteria*, vol. 1, p. 120 [I. C. Gunsalus and R. Y. Stanier, editors]. New York and London: Academic Press.
Smith, R. H. (1962). *Biochem J.* **83**, 151.
Smith, R. H. (1964). *J. Physiol., Lond.* **172**, 305.
Smith, R. H. (1976). In *Digestion and Metabolism in the Ruminant*, p. 399 [I. W. McDonald & A. C. I. Warner, editors]. Armidale: University of New England.
Smith, R. H. & McAllan, A. B. (1970). *Br. J. Nutr.* **24**, 545.
Smith, R. H. & McAllan, A. B. (1971). *Br. J. Nutr.* **25**, 181.
Van't Klooster, A. Th. & Boekholt, H. A. (1972). *Neth. J. agric. Sci.* **20**, 272.

Printed in Great Britain