Nucleic acid metabolism in the ruminant

3*. Amounts of nucleic acids and total and ammonia nitrogen in digesta from the rumen, duodenum and ileum of calves

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1. Concentrations of nucleic acid nitrogen and other nitrogenous constituents were estimated in digesta taken from the proximal duodenum of calves which were given, either, one of a number of stall diets or pasture. These concentrations were compared, using polyethylene glycol (PEG) as a non-absorbed marker, with corresponding concentrations in rumen fluid and ileal contents.

2. There was little net change in amounts of RNA or DNA between rumen and duodenum relative to PEG, but there was a marked increase in amounts of total-N. In duodenal digesta, for any one animal given most diets, nucleic acid-N formed a fairly constant percentage (8–11 for different animals) of the total non-ammonia-N. This value was lower (by about 3) than the corresponding percentage in rumen fluid. Comparison of nucleic acid-N: total-N ratios in duodenal contents and bacteria suggested that, for these diets, about 40–55% of the non-ammonia-N in duodenal contents was of microbial origin.

3. During passage of digesta between the duodenum and ileum the mean percentage disappearances of total-N, RNA and DNA were estimated to be about 67, 85 and 75 respectively. There was evidence that these values varied with the amounts of the constituents entering the duodenum.

4. Ammonia was absorbed in the omasum–abomasum only when concentrations in rumen fluid were high (40 mM), but even moderate concentrations of ammonia entering the duodenum (3 mM) were efficiently absorbed (about 90%) in the small intestine.

The quantitative importance of nucleic acids to the nutrient intake of the bovine rests in the amounts of these materials, relative to other nitrogenous substances, which are presented to the small intestine. These amounts bear little or no relationship to the dietary intake of nucleic acids but appear to derive almost entirely from microbial synthesis in the rumen (Smith & McAllan, 1970). Provided that the nucleic acids survive passage through the omasum and abomasum and bearing in mind that the ratio of nucleic acid nitrogen to total nitrogen in rumen microbes is reasonably constant (Smith, 1969), it should be possible to use nucleic acid concentrations in abomasal effluent to estimate the contribution of microbial-N to the digesta entering the duodenum. Temler-Kucharski & Gaussères (1965) have, in fact, reported experiments in which certain nitrogenous constituents (including the adenine and guanine from DNA) were estimated in the duodenal contents of a calf. On the basis of these results, they estimated that 50–70% of the N at this site was microbial.

In the work described here we have investigated the quantitative importance, origin and fate of the nucleic acids entering the small intestine by studying, in a number of calves, interrelationships between RNA nitrogen (RNA-N), DNA nitrogen (DNA-N) and total-N concentrations in digesta taken from the proximal duodenum and the changes in the amounts of these constituents with passage of digesta between the rumen and duodenum and between the duodenum and ileum.

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At the same time observations have been made on the fate of ammonia-N in rumen contents passing into the omasum and abomasum and thence to the duodenum and ileum.

Brief reports on parts of this work have already been published (Smith, McAllan & Hill, 1968, 1969).

EXPERIMENTAL

Animals, feeding and sampling of digesta

Castrated male Friesian calves, aged 13–39 weeks, were used. Some were provided with rumen and simple duodenal cannulas; these animals and their treatment have been described previously (Smith & McAllan, 1970). Other calves (1K, 5K, N123, N133) were weaned at 4–6 weeks of age and at 5–14 weeks of age were fitted with a simple Perspex cannula in the proximal duodenum and a re-entrant cannula in the most distal ileum (within about 15 cm of the ileo-caecal junction). Duodenal cannulas were all inserted within 10 cm of the pyloric sphincter. The ileal cannulas were similar to those described by Ash (1962) but with a slightly greater internal diameter (12·5 mm). Periods of at least 3 weeks after the operation and at least 9 weeks after weaning were allowed before experiments involving the sampling of digesta were begun. During the experimental periods the calves were given one or other of the diets: A (flaked maize + hay), B (flaked maize, decorticated extracted groundnut meal + hay), D (barley, fish meal, molassine meal, starch + straw) or E (flaked maize alone) described in detail previously (Smith & McAllan, 1970). Alternatively they were allowed to graze pasture. At least 8 d were allowed between changing a diet and taking samples.

Samples of strained rumen fluid were obtained as described by Smith & McAllan (1970). Samples from the proximal duodenum were obtained by unplugging the cannula and waiting for gushes of digesta; about 60–100 ml were normally obtained within 5 min. Although back flow of digesta can occur at this site (Singleton, 1961), previous experience has shown that such gushes derived from the abomasum because they were free of bile and had pH values less than 2·5. Ileal effluent was collected by disconnecting the re-entrant cannula and passing the whole flow into a bottle. Flow was irregular but averaged about 200 ml/h.

Changes in digesta constituents along the alimentary tract

Polyethylene glycol (PEG), molecular weight 4000, was used as a reference material to assess net changes in the amounts of constituents between one part of the alimentary tract and another.

When comparison was made between amounts in the rumen and duodenum, 50–100 g PEG in 200–400 ml water were rapidly infused into about four different randomly selected sites within the rumen. Digesta collections were made up to 7 h later (e.g. see Fig. 1). When comparison was made between the amounts in the duodenum and ileum, 100–200 g PEG were added to a concentrate diet on the day preceding that on which digesta collections were to be made. On the day of collection, a sample (about 50 g) was collected from the duodenum about 3 h after the morning feed. Immediately afterwards, 5 ml of a 2% (w/v) phenol red solution (solid phenol red dissolved in the minimum quantity of NaOH and made up to volume in 0·9%

NaCl) were injected into the duodenum and about 10 min later a further 50 g duodenal sample was taken. Ileal effluent was observed until the phenol red appeared and was then collected for about 30 min. The pooled duodenal samples and the ileal sample were analysed and net changes in composition between the two sites were estimated by reference to the amounts of PEG present.

Analytical methods

Methods of determining total-N, ammonia (this term includes both un-ionized ammonia and ammonium ions) and PEG in rumen fluid have been described (Smith & McAllan, 1970). The same methods were used for analysing samples of duodenal and ileal digesta, except that PEG was determined by the method of Smith (1958, 1962) but with a 20 min time for development of turbidity. When phenol red was present in ileal samples it was removed (Smith, 1964) before determination of PEG.

Nucleic acids (RNA and DNA) were determined according to the method of McAllan & Smith (1969).

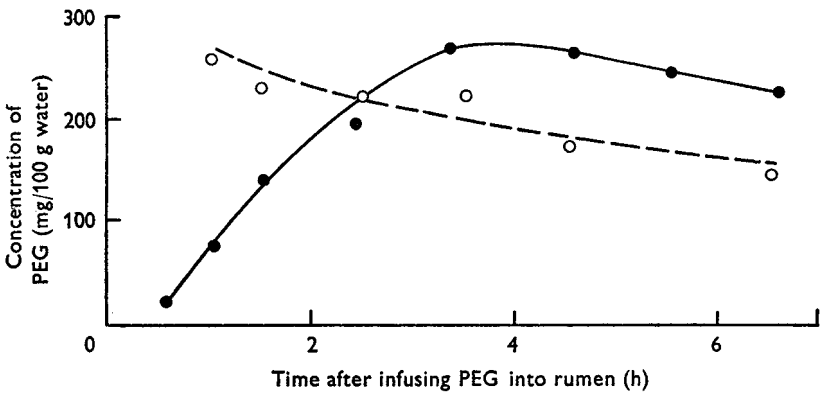

Fig. 1. Changes with time in polyethylene glycol (PEG) concentrations in rumen fluid (-- ○ --) and duodenal contents (—●—) after 50 g in 200 ml water were infused into the rumen of a calf (3K) weighing 125 kg and receiving diet A (see Table 1 of Smith & McAllan, 1970).

RESULTS

Composition of digesta entering the proximal duodenum

Concentrations of PEG in rumen fluid and in duodenal digesta following infusion of a single dose of the non-absorbable marker PEG into the rumen of a calf are shown in Fig. 1. In experiments with two calves given diet A (three experiments), diet B (five experiments) or pasture (three experiments), PEG first arrived at the proximal duodenum some 20–30 min after the infusion and reached a peak concentration at this site $3{\cdot}3 \pm 0{\cdot}2$ h after the infusion. Subsequently the slow fall in PEG concentration in the rumen was accompanied by a parallel fall in the duodenum. During this time, samples were taken and concentrations of various components were determined (Table 1).

Assuming strained rumen fluid to be approximately representative of material passing into the omasum and 1·5 h to represent a reasonable mean time interval for

Table 1. *Composition of duodenal contents taken 4–7 h after giving calves different stall diets or between 14.00 and 17.00 hours for calves at pasture*

(The results for different nitrogenous constituents (given as mean values with their standard errors) are shown both as absolute concentrations and in relation to concentrations in samples of rumen fluid taken 1–2 h earlier. Values for polyethylene glycol (PEG) (previously infused into the rumen as a marker) relate concentrations in duodenal contents with concentrations in rumen fluid taken 1·5 h earlier. The diets are described in Table 1 of Smith & McAllan (1970))

Diet	No. of calves	No. of expts	Concentration in duodenal contents (mg/100 g water)				Ratio, duodenal concentration:rumen fluid concentration				
			Total-N	RNA-N	DNA-N	Ammonia-N	Total-N	RNA-N	DNA-N	Ammonia-N	PEG
A	3	8	125±12	6·5±0·8	4·2±0·6	1·0±0·4	2·18±0·33	1·44±0·17	1·59±0·18	2·41±0·49*	1·24±0·10†
B	2	8	340±20	16·7±2·4	7·8±1·5	11·5±1·4	1·66±0·16	1·36±0·18	1·40±0·25	1·14±0·08	1·08±0·04
A+casein‡	2	4	NM	10·5±3·1	5·0±0·8	22·5±1·6	NM	1·07±0·17	0·98±0·13	0·31±0·07	NM
Pasture	1	3	247±15	17·9±2·3	9·4±1·8	3·8±0·5	1·11±0·15	0·91±0·21	0·99±0·23	0·98±0·47	0·93±0·10

NM, not measured.
* Three values in rumen fluid were too low to measure; these experiments were omitted in calculating the mean.
† PEG added in three experiments only.
‡ Casein (350 g) added to the rumen with each of the three concentrate feeds immediately preceding collection of sample.

Table 2. *Composition of ileal contents taken 5–7 h after giving calves different stall diets*

(Concentrations (mg/100 g water) are given as mean values with their standard errors. The diets are described in Table 1 of Smith & McAllan (1970))

Diet	No. of calves	No. of expts	Total-N	RNA-N	DNA-N	Ammonia-N
A	4	8	163±17	3·7±0·7	3·8±0·8	1·4±0·2
A+casein*	4	10	223±20	2·9±0·4	3·0±0·4	4·7±0·8
B	2	6	309±41	5·9±0·9	4·7±1·1	5·7±1·9

* Casein (350 g) added to the rumen with each of the three concentrate feeds immediately preceding collection of samples.

passage of material through the omasum and abomasum (the validity of these assumptions will be considered in detail on p. 188), the net changes of the constituents between rumen and duodenum could be assessed by reference to PEG. There were only small net changes in amounts of RNA or DNA relative to PEG between the two sites (the significance of the apparent small increase sometimes shown will be discussed later), but total-N showed an appreciable net increase relative to PEG. In almost all individual experiments (including those, also shown in Table 1, in which no PEG was infused) no change in the RNA:DNA ratio but a marked increase in the total-N:nucleic acid-N ratio between rumen and duodenum occurred.

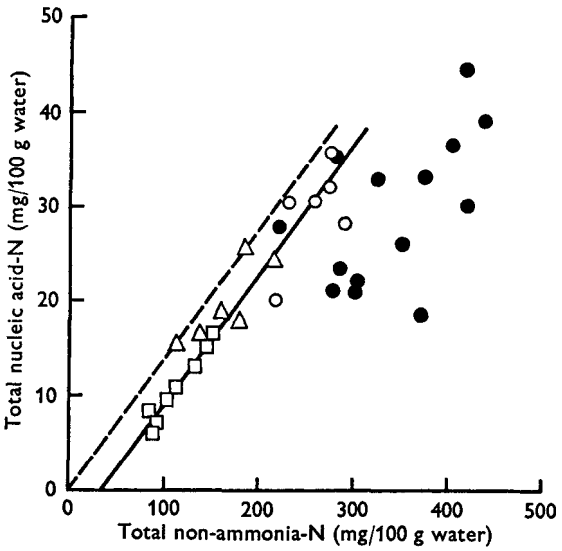

Fig. 2. Relationship between concentrations of nucleic acid nitrogen (RNA+DNA) and total non-ammonia-N in individual samples of duodenal contents from calf N 136 receiving diet A (□), diet B (●) or diet D (△) (Table 1 of Smith & McAllan, 1970) or pasture (○). The linear curve which relates to the experimental points (other than those for diet B) is shown as a continuous line. The broken line represents the corresponding relationship found in rumen fluid (Fig. 1 of Smith & McAllan, 1970).

Consistent with these findings was the fact that the linear relationship between nucleic acid-N and total non-ammonia-N concentrations shown in the rumen fluid of individual calves given several different diets (Smith & McAllan, 1970) was shown also in duodenal contents, except that there was a displacement of the curve corresponding to increased relative amounts of total-N. The relationship, exemplified in Fig. 2, was shown by calves 3K, N122 and N136 when they were given diets A, D or E (Table 1 of Smith & McAllan, 1970) or pasture. Mean values with their standard errors (numbers of samples in parentheses) for the percentage of total non-ammonia-N represented by nucleic acid-N in duodenal contents were 8·0 ± 0·4 (16), 8·6 ± 0·3 (8) and 10·7 ± 0·4 (20) for the three calves respectively. These percentages, like those in rumen fluid (Smith & McAllan, 1970) were, for any one calf, generally lower for diet B (containing extracted decorticated groundnut meal) than for the other diets (cf. Fig. 2).

Table 1 also gives results for ammonia exchanges. With moderate concentrations of ammonia-N (about 5–15 mg/100 g water) in the rumen (diet B and pasture), there appeared to be little net exchange of ammonia between the rumen and duodenum. When diet A was given, concentrations of ammonia-N in the rumen were very low (generally < 0·5 mg/100 g water) and there appeared then to be a small net increase in ammonia between rumen and duodenum. When, however, concentrations of ammonia in the rumen were made very high (60–100 mg/100 g water) by the addition

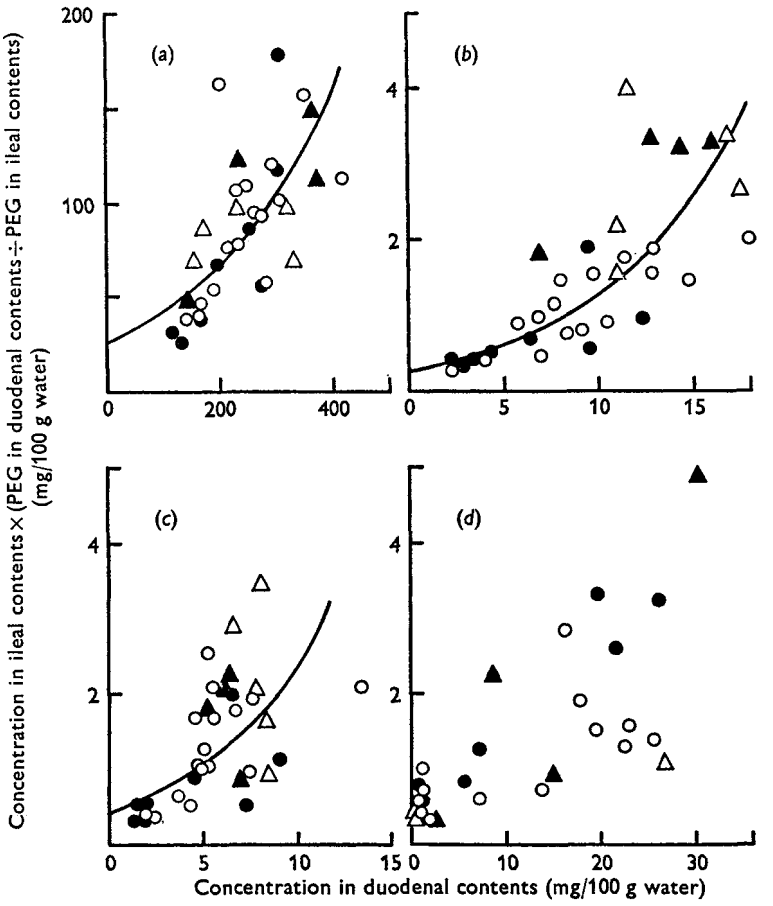

Fig. 3. Relationship for (*a*) total-nitrogen, (*b*) RNA-N, (*c*) DNA-N and (*d*) ammonia-N between concentrations in duodenal contents and concentrations in ileal contents × (PEG in duodenal contents ÷ PEG in ileal contents). Results are for individual experiments with calves 1K (○), 5K (●), N123 (△) and N133 (▲) aged 13–28 weeks and receiving diet A or B (Table 1 of Smith & McAllan, 1970) with, occasionally, the addition of casein (about 350 g) to the rumen during one or two feeds immediately preceding the collection of digesta. The duodenal samples were collected about 3 h after feeding; the ileal samples were collected after a suitable time to allow passage of digesta through the small intestine (see p. 182).

of casein to the rumen, then the concentrations of ammonia decreased very greatly between the rumen and duodenum. Although PEG was not present as a marker in the experiments demonstrating this, the fact that nucleic acid concentrations did not

change greatly suggests that the decrease in ammonia concentration was not due primarily to water exchange but that ammonia was strongly absorbed in the omasum or abomasum or in both.

Change in composition of digesta between proximal duodenum and distal ileum

Samples of ileal contents were collected about 5–7 h after calves (1K, 5K, N123 and N133) received one or other of the stall diets shown in Table 1, or between about 15.00 and 17.00 hours when they were at pasture. In thirty-four samples, concentrations of RNA-N, DNA-N, total-N and ammonia-N, expressed as mg/100 g water, ranged between about 1·5 and 10·0, 1·3 and 9·0, 100 and 500, and 0·5 and 13·0 respectively. These concentrations apparently differed with different diets (Table 2) but there were wide variations between individual samples, even for any one diet. The approximate mean rate of flow of digesta at the ileum (measured in fifteen collections over periods of 2·5–4·5 h for two calves receiving one or other of the stall diets shown in Table 1) was 190 ± 25 g/h. Thus, approximately 3–20 mg of both RNA-N and DNA-N, 0·2–1·0 g of total-N and 1–25 mg of ammonia-N passed the ileum per h. The extent to which these amounts represented endogenous secretion and to what extent they were influenced by the composition of material entering the duodenum was not clear, and experiments were carried out to clarify the situation.

Samples of duodenal and ileal contents, related one to the other by using phenol red to indicate time of passage through the small intestine (see p. 182) and containing PEG as a quantitative marker, were collected and analysed. Results are summarized in Fig. 3. The different diets used gave rise to different duodenal concentrations similar to those shown in Table 1. Apart from this, differences in diet had no apparent effect on relationships between duodenal and ileal concentrations and they are, therefore, not distinguished in Fig. 3.

The amounts of the different constituents which arrived at the ileum are expressed as (ileal concentrations) × (PEG in duodenal contents ÷ PEG in ileal contents). These values (I) for total-N, RNA-N and DNA-N (representing hypothetical concentrations that would have been found at the ileum if no water had been absorbed between duodenum and ileum) were highly significantly ($P < 0.001$) related to duodenal concentrations (D) according to the regression equation $\log I = A + B \times D$ (where A and B are constants for any one constituent. The mean curves shown in Fig. 3*a*, *b* and *c* represented the best fits for such relationships. Thus the net percentage removals $[\{(D - I) \div D\} \times 100]$ of total-N, RNA-N and DNA-N between duodenum and ileum (for which mean values with their standard errors were 67 ± 2, 85 ± 1 and 75 ± 2 respectively) varied with different duodenal concentrations. It appeared also that endogenous contributions were appreciable at the lower concentrations.

Ammonia was efficiently absorbed in the small intestine (Fig. 3*d*) with the amounts surviving to the ileum significantly ($P < 0.001$) related to the concentrations presented to the duodenum. The mean net percentage removal (± standard error) for duodenal concentrations of ammonia-N greater than 4 mg/100 g water was 89 ± 2. When duodenal concentrations of ammonia-N were much smaller than this the net percentage removal of ammonia in the small intestine was appreciably reduced, presumably because endogenous secretion then provided an appreciable proportion of the ammonia found at the ileum.

DISCUSSION

In attempting to estimate net changes in nitrogenous constituents between rumen and duodenum by reference to PEG as a non-absorbed marker, we have made two main assumptions. (1) That rumen fluid, strained through surgical gauze was representative of material passing from the rumen to the omasum with respect to its nucleic acid-N and total-N contents; we have previously presented evidence (Smith & McAllan, 1970) which suggests that this is approximately true. (2) That there was an average interval of 1·5 h between the time at which material left the rumen and that at which it arrived at the duodenum. The latter assumption was necessary as the PEG concentration in the rumen was not kept constant during the experiments. The period chosen seems reasonable in view of the pattern of change in PEG concentration in the duodenum following the introduction of PEG into the rumen (Fig. 1) and, in any event, calculations were not very critically dependent upon the precision of the choice; altering the period by ± 1 h led to mean changes in the PEG ratios given in Table 1 of only ± 11%. Bearing these facts in mind, we consider that, although the relationships given in Table 1 do not allow precise estimations of net changes between rumen and duodenum, they do allow reasonable approximations to be made. They suggest that net water exchange during passage of digesta between these two sites was sometimes slightly positive and sometimes slightly negative. With restricted diets for sheep, Hogan (1964) also found little difference between the volume of digesta entering the omasum and that leaving the abomasum.

The increases in total-N relative to PEG between the rumen and duodenum were apparently due to secretion of nitrogenous materials between the two sites. Corresponding increases for RNA and DNA, which were sometimes observed, were very much smaller and could have been due to sampling errors as described above. The finding that neither RNA nor DNA changed markedly between rumen and duodenum is in agreement with the absence of nucleic acid digestion in the stomach of the simple-stomached animal (Kaldor, 1969). The results for the composition of duodenal contents confirm what we have suggested from results on rumen fluid (Smith & McAllan, 1970), that, for calves given most of the diets that we have studied, nucleic acids form a substantial and, for any one calf, fairly constant proportion (8–11% in three calves) of the total-N entering the duodenum. If it is assumed that these nucleic acids derive almost entirely from the rumen microbes and that the nucleic acid-N : total-N ratio in the microbes is 0·19 (for a discussion of the validity of these assumptions see Smith & McAllan, 1970), it can be calculated that, as mean results, microbial-N contributed 42–56% of the total-N in the duodenal contents of these calves. In the rumen fluid, corresponding values were higher (54–76%) (Smith & McAllan, 1970); presumably, the difference was due mainly to the addition of endogenous N in the omasum–abomasum.

It would be expected that nucleic acids entering the bovine small intestine would be efficiently digested and absorbed. It is well known that this animal contains very high concentrations of ribonuclease in the pancreas (Barnard, 1969) and we have found (unpublished observations) that at least 97% of large doses of pure RNA given to a milk-fed calf were digested and absorbed before the ileum. Thus, although nucleic

acids entering the duodenum of ruminating calves were fairly efficiently removed up to the ileum (85 and 75% for RNA and DNA respectively), the values were sufficiently low to suggest that microbial nucleic acids, as well as microbial protein (discussed by Smith, 1969), may be relatively resistant to digestion in the small intestine.

By analogy with experiments in which RNA and DNA were given to rats and RNA to a milk-fed calf (Smith *et al.* 1969), it seems probable that about 20–30% of the digested nucleic acid-N in the ruminating calf is excreted in the urine as allantoin. Our results are therefore consistent with the observation of Topps & Elliott (1965) that in sheep there was a positive significant correlation between allantoin excretion and nucleic acid concentrations in the rumen. In the rat (Smith *et al.* 1969) most of the remaining digested nucleic acid-N was excreted in the urine as urea, but it is not known whether this is true also for the calf.

In general, the results reported in this paper and in that by Smith & McAllan (1970) show net changes in amounts of nucleic acids along the alimentary tract (increase between normal diet and rumen, little change between rumen and duodenum, decrease of about 80% between duodenum and ileum) similar to those suggested by the results of Ellis & Bleichner (1969), who compared the ratio of adenine to chromic oxide in the diet with ratios in digesta samples from slaughtered sheep.

Our results gave no information on the amounts of ammonia absorbed direct from the rumen, but they showed that moderate or high concentrations of ammonia in digesta passing out of the reticulo-rumen were efficiently absorbed before the ileum. Much of this absorption occurred in the small intestine; efficient ammonia absorption, with some evidence for active transport, has also been reported in the small intestine of other animals (Ewe & Summerskill, 1965; Mossberg & Ross, 1967). However, Hogan & Weston (1967) found with sheep that considerable amounts of ammonia were sometimes absorbed in the omasum–abomasum. Our results (Table 1) showed a similar absorption with calves but only when rumen concentrations of ammonia were high (over about 40 mM). Net absorption between the rumen and duodenum was negligible for ammonia concentrations much lower than this and there was a slight net secretion of ammonia in this region when rumen concentrations were below about 0·4 mM. This was probably due to the presence of ammonia in gastric juice (1·3–5·5 mM in human gastric juice (Vagne, Cille, Martin & Lambert, 1964)).

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