The appearance of re-cycled urea in the digestive tract of goats during the final third of a once daily feeding of a low-protein ration

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(*Received* 23 *August* 1979 – *Accepted* 25 *April* 1980)

1. An experiment was carried out with goats fed on a low-protein ration to clarify the importance of the rumen and significance of saliva in the appearance of re-cycled urea in the digestive tract during the final third of a once daily feeding regimen. The isotope-dilution method with [^{15}N]urea and $^{15}NH_4Cl$ was used.

2. When the serum urea level was 58 mg N/l, the amount of urea transferred from the blood urea pool to the rumen ammonia pool was 48·6 mg N/h, which was estimated to be approximately 43 % of the total amount of urea having appeared in the NH_3 pool of the digestive tract. When the serum urea level was 106 mg N/l, the corresponding amount of NH_3 was 77·7 mg N/h, which was estimated to be approximately 46 % of this total amount.

3. The amount of saliva secreted was measured directly by the oesophageal fistula method. Salivary secretion serves as a mode of transfer of blood urea to the rumen NH_3 pool. Then the ratio, salivary secretion: diffusion through the rumen wall during the final third of the cycle was calculated to be 1: 4–1: 6.

4. In goats fed on a low-protein diet, the rumen is an important site of appearance of blood urea in the digestive tract. It was verified that the principal mode of transfer of blood urea to the rumen was the direct diffusion through the wall of the rumen.

It is known in domestic ruminants that when a nitrogen-deficient ration is ingested, urea does not pass into the urine but is transferred to the digestive tract and converted into microbial protein to be re-utilized (Schmidt-Nielsen *et al.* 1957; Cocimano & Leng, 1967). The rumen has been assumed to be the principal site of appearance of re-cycled urea in the digestive tract. This assumption has been supported by the results of experiments which demonstrated that a large quantity of urea is transferred to the rumen (Juhasz, 1965; Houpt & Houpt, 1968), and by the hypothesis that rumen microbes are responsible for most of the decomposition of urea in the body (Waldo, 1968). Moreover two pathways, salivary secretion and direct diffusion through the rumen wall, have been considered to be involved in the appearance of blood urea in the rumen. Juhasz (1965) and Houpt & Houpt (1968) suggested that direct diffusion through the rumen wall was the principal pathway. Recently, Nolan & Leng (1972) found that the amount of re-cycled urea that appeared in the rumen was approximately 20 % of the total amount of re-cycled urea that appeared in the digestive tract. They suggested that the lower part of the digestive tract was an important site of decomposition of blood urea. Furthermore, they suggested that the main pathway for the appearance of urea in the rumen was not direct diffusion through the rumen wall, but salivary secretion. The latter finding was not in agreement with those of other workers.

The present investigation, using an isotope-dilution method with [^{15}N]urea and $^{15}NH_4Cl$ and an oesophageal fistula during the final third of a once daily feeding regimen, was designed to elucidate first the significance of the rumen in the utilization of re-cycled urea in animals fed on a low-protein diet and secondly the quantitative relationship between

0007/1145/80/3190-2504 $01.00

salivary secretion and diffusion through the rumen wall at the time of appearance of urea in the rumen. Finally, consideration was given on urea metabolism in domestic ruminant animals.

EXPERIMENTAL

Experimental animals

Six female goats were used, of which four had oesophageal fistulas and two had rumen fistulas. They were fed on two rations (rations nos. 1 and 2) once daily at 16.00 hours and given water *ad lib.* Ration no. 1 contained (g/kg body-weight per d) 0·6 digestible crude protein (DCP), 11·1 total digestible nutrients (TDN), so that the serum urea level was approximately 50 mg N/l in animals fed on this ration at the time of experiment. Ration no. 2 contained (g/kg body-weight per d) 1·2 DCP, 11·3 TDN, so that the serum urea level was approximately 100 mg N/l in animals fed on this ration at the time of experiment. The chemical composition of rations nos. 1 and 2 are shown in Table 1. The two rations contained hay and different proportions of two low-protein feeds (A and B) details of which are shown in Table 2. Each ration was fed for 4 weeks.

Table 1. *Composition of rations (g/kg body-weight per d) and chemical composition of the constituents of rations (g/kg) fed to goats*

	Ration no. 1	Ration no. 2
Hay	7·5	7·5
Low-protein feed: A	12·0	6·0
B	—	6·0
Total digestible nutrient	11·1	11·3
Digestible crude protein	0·6	1·2

		Low-protein feed	
	Hay	A	B
Moisture	151	151	110
Crude protein	84	74	186
Crude fat	23	29	40
Crude fibre	267	63	45
N-free extract	402	597	568
Crude ash	64	86	51
Total digestible nutrient	380	690	713
Digestible crude protein	28	32	137

Table 2. *Composition (g/kg) of low-protein feeds fed to goats*

A		B	
Ingredient		Ingredient	
Maize	189	Soyabean meal	100
Molasses	76	Linseed meal	100
Sugar beet pulp	474	Barley	250
Maize starch	189	Maize	160
Soyabean oil	19	Wheat bran	250
Mineral mixture*	38	Rice bran	100
Sodium bicarbonate	10	Calcium carbonate	25
Potassium bicarbonate	5	Sodium chloride	10
Vitamin E supplement†	0·6	Mineral mixture*	4
Vitamin A and D mixture‡	0·2	Vitamin A and D mixture‡	1

* Consisting (mg/kg mixture): 660 iron, 132 copper, 132 cobalt, 132 magnesium, 330 manganese, 496 sulphur, 761 calcium, 225 phosphorus, 965 sodium chloride.

† Containing 100 mg of DL-α-tocopheryl acetate/g.

‡ Containing 3 mg of retinol and 50 μg of cholecalciferol/g.

Design of experiment

Each experiment was carried out in the period 17–24 h after feeding. In Expt 1, two goats with rumen fistulas were used. The animals were placed in a metabolism cage and injected with a single dose of 300 mg [^{15}N]urea (90 atom %) into the jugular vein. Changes in the concentration of [^{15}N]urea in jugular blood and $^{15}NH_3$ in the rumen were examined at 10 min, 0·5, 1, 2, 3, 5 and 7 h after injection. After 1 week both goats were injected simultaneously with a single dose of 250 mg $^{15}NH_4Cl$ (95 atom %) and polyethylene glycol (PEG) into the rumen. Changes in the concentrations of [^{15}N]urea in the jugular blood and $^{15}NH_3$ in the rumen were examined at the previously-mentioned intervals after injection. Changes in the concentrations of PEG in the rumen were examined at regular intervals. For urine analysis a 7 h total urine sample was collected into toluene. In Expt 2 goats with oesophageal fistulas were used. Each goat was placed in a metabolism cage and the oesophageal fistula was removed for saliva collection. At the same time a single dose of [^{15}N]urea was injected into the jugular vein and changes in [^{15}N]urea in venous blood and $^{15}NH_3$ in rumen fluid were monitored. The amount of saliva secreted was measured at hourly intervals using a graduated cylinder. A 5 ml portion of each hourly saliva sample was used for analysis and the remainder was returned to the rumen at given intervals.

Chemical analysis

Urea-N in serum, urine and saliva was estimated by a modified diacetyl-monoxime method (Coulombe & Favreau, 1963). Ammonia in rumen fluid was measured by a colorimetric method (Weatherburn, 1967). To determine [^{15}N]urea in the serum, 0·5 ml of an enzyme solution (6 mg urease (*EC* 3.5.1.5) dissolved in 10 ml 0·04 M-phosphate buffer, pH 7·0) and 0·5 ml 0·2 M-Tris buffer, pH 9·0, were added to 2·0 ml serum and incubated at 37° for 30 min. The enzymic product was then converted to ammonium sulphate by Seligson's method (Seligson & Seligson, 1951) for [^{15}N]urea determination. To determine urea in the urine, NH_3-N was removed from the urine sample using an Amberlite CG120 resin column and subjected to the same procedures as the serum sample. $^{15}NH_3$ in rumen fluid was estimated after conversion to $(NH_4)_2SO_4$ by Seligson's method (Seligson & Seligson, 1951). The sample was then placed in a Rittenberg's tube and treated with potassium hypobromite to liberate N_2 which was introduced into an electric discharge tube under high vacuum. A ^{15}N analyser (model NIA-1; Nippon Bunko Co., Ltd Tokyo) was used for the estimation of ^{15}N. The concentration of PEG in the rumen fluid was determined by Hyden's method (Hyden, 1955).

Mathematical procedures

The results of the ^{15}N tracer experiment were obtained by mathematical treatment of experimental values using Nolan's (1974) method. For goats injected with single doses of [^{15}N]urea and $^{15}NH_4Cl$ into the jugular vein and rumen, respectively, the time-course of changes in ^{15}N (atom % excess) was plotted and the straight line obtained was expressed by the following formula:

$$E_t = \sum_{i=1}^{n} A_i e^{-mit},$$

where t is time, Et is serum [^{15}N]urea and $^{15}NH_3$ (atom % excess) present in the contents of the rumen, A is the section of the Et axis when $t = 0$, m is the velocity constant (/t) of each component, n is the number of exponential components, and i is the exponential component.

The area under the isotope dilution curve up to time t is expressed by the following formula:

$$Xt = \sum_{i=1}^{n} \frac{Ai}{mi} (1 - e^{-mit}).$$

In the present 7 h experiment, computer analysis revealed that each curve was represented by two exponential functions. The border between the two functions was at 3 h after injection. When [^{15}N]urea was injected into the jugular vein, therefore, [^{15}N]urea in the serum (primary pool) was expressed as the sum of the two exponential functions, and $^{15}NH_3$ in the rumen (secondary pool) as the difference between these functions. On the other hand, when $^{15}NH_4Cl$ was injected into the rumen, rumen NH_3 was expressed as the sum of two exponential functions, and the serum urea as the difference between these functions.

Calculations of pool size and irreversible loss rate with respect to the pool into which an isotope was injected were calculated from the equation for the curves by standard procedure (see Nolan, 1974). The proportion (Q) of the N in secondary pool (S) derived from a primary pool (P) was given by:

$$Q = \frac{\text{area under the enrichment curve for pool S}}{\text{area under the enrichment curve for pool P}}.$$

The rates of flow of N (c and d mg N/h) associated with rumen fluid NH_3 and serum urea pool were obtained by a general two-pool open-compartment model as shown in Fig. 1 using the results of the two experiments involving injection of $^{15}NH_4Cl$ into the rumen and [^{15}N]urea into the jugular vein (see Nolan *et al.* 1976).

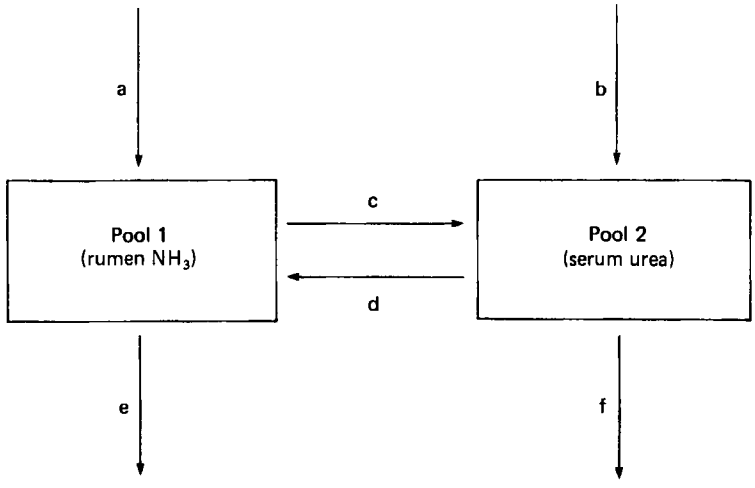

Fig. 1. A general two-pool open-compartment model for nitrogen transactions associated with rumen ammonia (pool 1) and serum urea (pool 2): a, b, c, d, e, f, the rates of flow of material (mg N/h).

RESULTS

Measurements of urea and ammonia metabolism during the final third of a once daily feeding regimen were determined in goats. In Expt 1 urea metabolism in goats injected with a single dose of [^{15}N]urea into the jugular vein was studied (Table 3). The serum urea level was in proportion to the amount of N ingested. The urea pool size in the body was in proportion to the serum urea level. There was no difference in the urea space between the two experimental groups, in which the urea space was approximately 50% of the body-weight. The irreversible loss of urea increased in proportion to the serum urea level. The amount of urea excreted into the urine was 194·0 mg N/h when the serum urea level was 108 mg N/l. The amount of urea decreased markedly to 5·2 and 56·8 mg N/h for the two goats when the serum urea level was reduced to 63 mg N/l.

Table 3. *Expt* 1. *Measures of urea metabolism in goats with rumen fistulas using a single injection of* [^{15}N]*urea into the jugular vein*

Ration no.	Goat no.	Body-wt (kg)	Serum urea level (mg N/l)	Body urea pool size (mg N)	Body urea space (l)	Urea irreversibly lost (mg N/h)	Rumen ammonia from serum urea (%)	Urea excretion (mg N/h)
1	51A	31·0	57	872·0	15·3	134·1	24·3	5·2
	51B	30·0	68	1002·9	14·8	163·8	18·0	56·8
	Mean	30·5	63	937·5	15·1	149·0	21·2	31·0
	SE	0·5	6	65·5	0·3	14·9	3·2	25·8
2	51A	30·0	98	1387·0	14·2	338·0	16·8	168·6
	51B	30·0	117	1861·9	15·9	369·0	16·6	219·3
	Mean	30·0	108	1624·5	15·1	353·5	16·7	194·0
	SE	—	10	237·5	0·9	15·5	0·1	25·4

Table 4. *Expt* 1. *Measures of ammonia metabolism in goats with rumen fistulas using a single injection of* $^{15}NH_4Cl$ *into the rumen*

Ration no.	Goat no.	Serum urea level (mg N/l)	Rumen volume (l)	Rumen ammonia level (mg N/l)	Rumen ammonia pool size		Rumen ammonia irreversibly lost (mg N/h)	Serum urea contributed by rumen ammonia (%)
					PEG* (mg N)	^{15}N†		
1	51A	56	5·8	37	215	272	399·7	7·3
	51B	44	4·3	62	266	318	244·6	24·6
	Mean	50	5·1	50	241	295	322·2	16·0
	SE	6	0·8	10	26	23	77·6	8·6
2	51A	92	5·5	114	627	794	529·5	41·2
	51B	107	5·5	104	572	632	383·0	27·9
	Mean	100	5·5	109	600	713	456·3	34·6
	SE	8	—	5	28	81	73·3	6·7

* Estimated from rumen volume and rumen ammonia level.
† Estimated from ^{15}N isotope-dilution values.

Table 5. *Expt 2. Measures of urea metabolism in goats with oesophageal fistulas using a single injection of* [^{15}N]*urea into the blood*

Ration no.	Goat no.	Body-wt (kg)	Serum urea level (mg N/l)	Urea pool size (mg N)	Urea space (l)	Urea irreversibly lost (mg N/h)	Rumen ammonia from serum urea (%)	Urea excretion (mg N/h)	Saliva secretion rate (ml/h)	Saliva urea level (mg N/l)	Saliva urea secretion (mg N/h)
1	50B	50	69	1229·7	17·8	222·4	14·6	61·8	118	49	5·8
	50C	42	54	959·2	17·8	108·1	9·8	11·9	294	39	11·3
	50D	38	59	1023·0	17·4	190·9	7·5	130·6	143	30	4·3
	Mean	43	61	1070·6	17·6	173·8	10·6	68·1	185	39	7·1
	SE	4	4	81·6	0·2	34·1	2·1	34·4	55	5	2·1
2	50A	47	98	2660·0	27·1	422·0	15·0	324·8	207	70	14·5
	50B	54	125	3054·6	24·4	494·3	25·0	234·5	215	70	15·1
	50C	45	106	2668·5	25·2	400·3	7·2	233·6	302	58	17·5
	Mean	49	110	2794·4	25·6	438·9	15·7	261·0	241	66	15·7
	SE	3	8	130·1	0·8	28·4	5·2	32·1	30	4	0·9

Goats were injected with a single dose of $^{15}NH_4Cl$ into the rumen. Table 4 presents measurements of NH_3 metabolism. The rumen NH_3 levels for the two goats were 50 and 109 mg N/l and almost equal to the serum urea levels. The volume of the rumen was approximately 5 l when estimated by the PEG-dilution method. The rumen NH_3 pool size calculated from the rumen NH_3 level and the volume of the rumen was compared with that estimated by the $^{15}NH_3$-dilution method. The rumen NH_3 pool size seemed to reflect the serum urea level. There was a considerable individual difference in the irreversible NH_3 loss in the rumen. However, in general, there was a relationship between the rumen NH_3 loss and the serum urea level.

In Expt 2 measurements of urea metabolism were obtained for goats with oesophageal fistulas while the extent of salivary excretion was monitored (Table 5). The results showed essentially the same trend as those obtained in Expt 1. The salivary urea level was 60 % of the serum urea level in both experimental groups. The amount of salivary secretion was 185 and 241 ml/h in the groups given rations nos. 1 and 2 respectively. There was a fairly large individual difference in the values obtained for each group. The amounts of urea discharged into the rumen via the saliva were 7·1 and 15·7 mg N/h in the groups given rations nos. 1 and 2 respectively, and this was related to the serum level.

The amounts of N moving within the rumen, blood and lower part of the digestive tract during the final third of a once daily feeding regimen were estimated and are presented in Table 6. The values were derived from the means of the values shown in Tables 3–5. The amount of N transferred from the serum urea pool to the rumen NH_3 pool was 48·6 and 77·7 mg N/h in the groups given rations nos. 1 and 2 respectively. The amount of N transferred to the NH_3 pool of the whole digestive tract was 114·2 and 168·7 mg N/h in the groups given rations nos. 1 and 2 respectively. The amount of blood urea transferred to the rumen NH_3 pool relative to that transferred to the ammonia pool of the whole digestive tract was 42·6 and 46·1 % when the serum urea levels were 58 and 106 mg N/l respectively. On the other hand, the quantitative ratio, salivary secretion:rumen diffusion, which serves as a mode of transfer of blood urea to the rumen NH_3 pool was 1:6 and 1:4, when the serum urea levels were 58 and 106 mg N/l respectively. Therefore, it was demonstrated that the rate of diffusion in the rumen wall was much higher in goats given rations nos. 1 and 2 when the blood urea level was low.

Table 6. *Estimates of the nitrogen flow*

	Ration no. 1	Ration no. 2
Serum urea level (mg N/l)	58	106
Rumen ammonia level (mg N/l)	67	111
Irreversible loss of urea from serum (mg N/h) (A)	163·8	404·7
Irreversible loss of rumen NH_3 (mg N/h)	322·2	456·3
Rumen NH_3 derived from serum urea (%)	14·8	16·1
Serum urea derived from rumen NH_3 (%)	16·0	34·6
Urea excretion rate (mg N/h) (B)	49·6	236·1
Saliva urea secretion (mg N/h) (C)	7·1	15·7
Rate of transfer of serum urea to rumen NH_3 (mg N/h) (D)	48·6	77·7
Rate of transfer of rumen NH_3 to serum urea (mg N/h)	26·8	148·3
Rate of transfer of serum urea to whole digestive tract (mg N/h) (A−B)	114·2	168·7
Rate of transfer of serum urea to lower tract (mg N/h) ((A÷B)−D)	71·3	91·0
Rate of diffusion urea through rumen wall (mg N/h) (D−C)	41·5	62·0
Amount of serum urea transferred to rumen relative to that of whole digestive tract (%) (D÷(A−B)×100)	42·6	46·1
Saiivary secretion:rumen (C:(D−C))	1:6	1:4

DISCUSSION

First, the authors must emphasize that the results of the present experiment were obtained during the final third of a once daily feeding regimen, and therefore cannot be considered on a 24 h basis. When the animals were fed once daily, the serum urea level and the rumen NH_3 concentration were hardly changed during the final third of a 24 h cycle. Assuming that the final third of a 24 h cycle is in a steady-state condition, the isotope-dilution experiment was carried out for 7 h starting 17 h after feeding. However, the present experimental situation might not be considered as steady-state. If so, it is assumed that the experimental results were derived using steady-state kinetics in a non-steady-state situation. Nolan & Leng (1972) divided the daily ration into twenty-four equal portions, which were given to sheep at hourly intervals, so that the blood urea and rumen NH_3 levels might be constant throughout the experimental period. In sheep held in a steady-state, a quantitative study was made of urea metabolism.

Seven samples taken over a 7 h period in the present experiment might not give sufficient values to fit the exponential terms which are necessary to determine the area under the curves in the mathematical treatment. Compared with the experiments of Nolan and his co-workers (Nolan & Leng, 1972; Nolan *et al.* 1976), the rate of transfer between rumen NH_3 and the blood urea pool in the present experiment appeared to be estimated lower. Similarly, possible errors in the calculation of pool size and irreversible loss might be induced in the present experiment. The amount of N transferred could have been estimated more exactly in the experiment of Nolan & Leng (1972), since their work involved sampling for 2–3000 min by which time the last exponent was very well defined.

As indicated in Table 5, the amounts of urea appearing in the whole digestive tract were 114·2 and 168·7 mg N/h, when the serum urea levels were 58 and 106 mg N/l respectively. It was estimated that the relative transfer of urea to the rumen were 43 and 46% of the amounts of urea appearing in the whole digestive tract when the serum urea levels were 58 and 106 mg N/l respectively. Houpt (1959) estimated that the amount of urea-N transferred from the blood to the digestive tract was in the range 7·8–13·0 mmol/h (106–182 mg N/h). In addition, Houpt (1959) reported that when the rumen contents were removed and the rumen filled with artificial saliva, 5·2 mmol urea-N/h (73 mg N/h) was transferred to the rumen, and suggested the importance of this organ in urea metabolism. The latter results were obtained from an experiment conducted with animals under anaesthesia and the same results may not have been obtained with intact animals. Nolan & Leng (1972) pointed out from the results of the isotope-dilution method during a 24 h cycle that the lower part of the digestive tract played a more important role than the rumen in the utilization of re-cycled urea. According to Nolan & Leng (1972), the amount of blood urea-N transferred to the whole digestive tract was 6·3 g/d (262 mg N/h). Of this amount, only 1·2 g/d (50 mg N/h) was introduced into the rumen NH_3 pool. Accordingly, the rate of urea transferred into the rumen was less than 20% of that of the whole digestive tract. These results reported by Nolan & Leng (1972) have raised controversy, since they are not in agreement with the hypothesis of Waldo (1968) that the decomposition of urea may take place in the rumen in the living body, and the result previously reported that a large amount of urea was transferred to the rumen (Houpt, 1959; Juhasz, 1965). In the present experiment with goats given low-protein rations, the rumen was confirmed to be a principal organ of the digestive tract to which re-cycled urea was transferred. It should be noted that Nolan & Leng's (1972) result was obtained from animals in which the plasma urea level was as high as 225 mg N/l. On the other hand, the present authors' results were obtained from animals in which the serum urea level was 58 or 106 mg N/l. Furthermore, the authors estimated that the amounts of urea

appearing in the rumen relative to those appearing in the whole digestive tract in animals given a normal- or high-protein ration were 22 and 14% when the serum urea levels were 175 and 350 mg N/l respectively. In short, the larger the amount of N ingested, the lower was this relative amount (Y. Obara and K. Shimbayashi, unpublished results). In this manner the serum urea level varied with the amount of N ingested. In a ruminant given a low-protein ration in which the amount of N ingested was so small that the serum urea level was low, re-cycled urea was presumed to be utilized in the rumen. In ruminants which had ingested a sufficient or an excessive amount of N, the rumen was assumed to be replaced as the site of appearance of re-cycled urea by the lower part of digestive tract. However, the nutritional physiological significance of the appearance of re-cycled urea in the lower part of the digestive tract is not known.

In the present experiment, the amount of urea discharged into the rumen through saliva was actually measured in goats with an oesophageal fistula. Various methods have been reported for the collection of saliva (Hyden, 1958; Sasaki & Umezu, 1962; Tribe & Peel, 1963). Of these methods, the oesophageal fistula method has been regarded as the best, since it gives less stimulation to the animal at the time of collection than any other method. The salivary urea level obtained from the present experiment was almost 60% of the serum urea level and agreed with the experimental values reported by Somers (1961). The amount of saliva discharged into the rumen ranged from 118 to 302 ml/h, showing the remarkable individual difference. Few experiments have been performed to measure the saliva directly in order to obtain quantitative estimates of urea metabolism. Only Houpt (1959) carried out an experiment with anaesthetized animals in which the oesophageal region had been ligatured to collect saliva. According to Houpt (1959), the amount of urea transferred to the rumen through saliva was only 6% of that of re-cycled urea appearing in this organ. In Houpt's (1959) experiment the rumen contents were removed, and it seems possible that the amount of urea diffused in the rumen may have been over-estimated. Since the present authors could actually measure the amount of urea discharged into the saliva, the quantitative relationship between the amount of the two urea fractions appearing in the rumen could be elucidated. The two fractions were the urea discharged in and transferred by the saliva to this organ, and the urea diffused in the rumen wall. In the present experiment the amount of blood urea appearing in the rumen was determined by the ^{15}N isotope-dilution method and from this value was subtracted the amount of urea discharged into the saliva to obtain the amount of urea diffused in the rumen wall. As a result, the quantitative ratio, salivary urea secretion: urea diffusion in the rumen was approximately 1:4–1:6. It was assumed that the diffusion in the rumen wall might be a principal pathway involved in the appearance of urea in the rumen. In an experiment with animals in which the serum level was 175 or 350 mg N/l, it was estimated that more than 80% of the amount of urea appearing in the rumen might have been derived from urea discharged in saliva (Y. Obara and K. Shimbayashi, unpublished results). The results mentioned previously revealed an outstanding fact; that is, the serum urea level varies with the amount of N ingested, and a reverse occurs in the ratio, salivary secretion:urea diffused in the rumen. Further experiments may be necessary before the mechanism of reversion of this ratio is clarified.

As discussed previously, urea metabolism in domestic ruminants seems to vary greatly with the amount of N ingested and the serum urea level. Such great variation may be useful for the interpretation of the discrepancy in previously-reported experimental results. In animals given a low-protein ration, the rumen plays an important role in the utilization of re-cycled urea in the whole digestive tract. Urea may appear in the rumen chiefly by direct diffusion across the rumen wall. The amount of urea discharged in and transferred by saliva seems to contribute little to that appearing in the rumen. The transfer of intrinsic urea from the blood to the rumen may be influenced not only by the content of N but also by the quality

of carbohydrate and other nutrients of food contained in the rumen at the time. An improvement in techniques seems to be required to make the rumen display its function sufficiently and to economize in the N content of a ration.

The authors wish to express their grateful thanks to Mr Kunio Nagatomo, of the Chuo Animal Hygiene Service Centre, Prefecture of Saga, for technical assistance.

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Printed in Great Britain