The effect of cold exposure of sheep on digestion, rumen turnover time and efficiency of microbial synthesis

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1. Six closely shorn sheep were given brome grass (*Bromus inermis*) pellets at 1 h intervals and maintained at ambient temperatures of −1 to 1° and 18–21° for 28 d. Measurements of digestion were made during the last 10 d of temperature exposure.

2. Cold exposure resulted in a reduction in apparent dry matter (DM) digestibility from 0·482 to 0·450, and of apparent digestibility of organic matter (OM) from 0·511 to 0·477. Neither apparent digestibility nor retention of nitrogen was affected.

3. Apparent digestibility of OM in the rumen decreased from 0·300 to 0·242 with cold exposure, and was highly correlated with turnover time in the rumen of ^{103}Ru, which was used as a particulate marker.

4. The efficiency of microbial synthesis (g N incorporated into microbial cells/kg OM apparently digested) was correlated with the dilution rate of the solute marker (^{51}Cr) and with the turnover time of the particulate marker (^{103}Ru) in the rumen.

5. Digestion in the intestine of DM and OM accounted for significantly more of apparent digestion in the whole gastrointestinal tract for sheep kept in the cold than for sheep kept in the warm. The apparent digestibilities of DM and OM entering the intestines were similar in sheep on both treatments, but significantly more non-ammonia-N was digested in the intestines of cold-exposed sheep.

6. The influence of dilution rate of rumen fluid on the efficiency of synthesis of microbial cells in the rumen is discussed.

Recent results indicate that digestibility of food is reduced in calves and steers exposed to the cold winter temperatures experienced in Western Canada (Christopherson, 1976). Similar results have been obtained for closely-shorn sheep exposed to temperatures of about 10° in the laboratory (Graham, Wainman, Blaxter & Armstrong, 1959; Graham, 1964). The results of experiments with shorn sheep given brome grass (*Bromus inermis*), either as hay or pellets, have indicated that acclimation of sheep to 0·8° for 4 weeks results in significant reductions of the apparent digestibilities of dry matter (DM) and fibre when compared to sheep maintained at 17·7° on the same diet at equivalent intakes (Westra, 1975). Westra (1975) found that these reductions were associated with a significant reduction of the mean retention time of the particulate marker ^{144}Ce in the gastrointestinal tract from 38·5 to 32·5 h, and an increase in the frequency of reticular contractions from 60·1 to 72·5/h.

The present experiment was designed to provide quantitative estimates of digestion in the stomach (reticulo-rumen, omasum and abomasum) and intestines of sheep exposed to cold, and to study the influence of increases in gut motility on the turnover time of digesta in the rumen in relation to the efficiency of microbial cell production and to the breakdown of dietary nitrogen.

EXPERIMENTAL

Sheep and their management

Six Suffolk wethers, 1·5 years old, and weighing 44–58 kg, were fitted with permanent cannulas in the rumen and abomasum several months before the experiment. The sheep were accustomed to sampling procedures, and then were housed in individual metabolism cages with three sheep in each of two climatic chambers, which were continuously illuminated. One chamber was maintained at −1 to 1° (cold), and the other at 18–21° (warm). At intervals of 2 weeks, all sheep were closely shorn, weighed, and injected with retinol, cholecalciferol and α-tocopherol. After 28 d, the three sheep in each chamber were transferred to the other chamber, in a single cross-over experimental design.

Diet

The ration, brome grass pellets, containing approximately 120 g crude protein (N × 6·25)/kg was offered in equal portions (77 g DM) at 1 h intervals, using an automatic feeding apparatus. The hay was ground through a 5 mm screen before pelleting. Water was available *ad lib.*

Procedures

After a temperature adaptation period (days 1–18), faeces were collected twice daily and urine daily for 6 d (days 19–24). Collected urine was stored at pH < 2 using sulphuric acid (to inhibit bacterial action and to fix free ammonia).

The movement of digesta through the abomasum was estimated by reference to ^{51}Cr complexed with ethylenediaminetetraacetic acid (^{51}Cr-EDTA) (Downes & McDonald, 1964) and ^{103}Ru-labelled Tris–1,10-phenanthroline–ruthenium(II) chloride (Tan, Weston & Hogan, 1971) as described by Faichney (1975*b*). These markers were infused into the rumen at a steady rate (55 ml/d; 0·28 μCi ^{103}Ru and 2·2 μCi ^{51}Cr/ml) for 7 d, following a priming dose (55 ml) at 08.00 hours on day 21. Four samples (150 ml) of abomasal digesta were taken daily at intervals of 4 h (08.00–20.00 hours) on days 25–27. The twelve samples of digesta were pooled for each sheep, the particulate matter allowed to settle, and approximately half the supernatant fraction was decanted. The two fractions were assayed for ^{51}Cr and ^{103}Ru, and recombined in proportions to yield a digesta sample representative of abomasal contents (Faichney, 1975*b*), as determined by the infusion rates of the markers. Corrections were made for absorption of ^{51}Cr-EDTA from the stomach, assuming that 37 % of ^{51}Cr excreted in the urine on days 25–27 was absorbed from the stomach (Faichney, 1975*a*).

On day 28, infusion of markers was stopped at 08.00 hours and the rate of disappearance of ^{51}Cr from rumen fluid, and the rate of disappearance of ^{103}Ru in abomasal digesta were determined. Rumen fluid volume, dilution rate of ^{51}Cr (solute marker) in the rumen and the turnover time (the reciprocal of dilution rate) of ^{103}Ru (particulate marker) in the rumen were calculated using the equations given by Shipley & Clark (1972).

Microbial flow was calculated using organic ^{35}S as a marker (Beever, Harrison,

Thomson, Cammell & Osbourn, 1974; Hume, 1974). $Na_2{}^{35}SO_4$ (80 μCi/d) was infused into the rumen with the other radionuclides and the specific radioactivity of organic ^{35}S was measured in abomasal digesta and in microbial samples isolated from abomasal digesta (100 ml) taken from each sheep at 20.00 hours on days 25, 26 and 27.

Samples of rumen fluid (20 ml) were also taken twice daily (10.00 and 14.00 hours) from each sheep on days 25, 26 and 27 to yield composite samples for ammonia and volatile fatty acid (VFA) analyses.

Analytical methods

DM content was determined by heating samples at 95° to constant weight. The samples were subsequently ignited at 550° for 6 h to determine organic matter (OM) content. Sulphate, total S and ^{35}S in abomasal digesta and in microbial samples were determined in triplicate by the methods of Bird & Fountain (1970). N was determined by the Kjeldahl method (Association of Official Analytical Chemists, 1975). Samples of rumen fluid (15 ml plus three drops conc. H_2SO_4 (added to inhibit bacterial action and to fix free ammonia) stored at $-5°$ until analysed) and abomasal filtrate were diluted (5 ml diluted to 50 ml with 0·2 M-sodium hydroxide to increase the pH to $\geqslant$ 11) before ammonia analyses. The concentrations of ammonia were estimated by comparison with aqueous ammonium chloride reference solutions, using a specific ion electrode (model 95–10; Orion Research Corp., Cambridge, Massachusetts, USA) and a digital meter (model 520; Fisher Scientific Co. Ltd, Pittsburgh, Pennsylvania, USA).

Total VFA were determined by titration with 0·1 M-NaOH after steam distillation from acidified magnesium sulphate (Briggs, Hogan & Reid, 1957) and the proportions of individual VFA measured using a gas–liquid chromatograph (model 2500; The Bendix Corp., Ronceverte, West Virginia, USA) with a column packed with 100 g SP-1200 (Supelco Inc., Bellefonte, Pennsylvania, USA) coated with 10 g phosphoric acid/kg Chromasorb W (80/100 mesh; Supelco Inc.). A hydrogen flame-ionization detector was used.

Microbial samples were separated from plant material by filtration through cheese-cloth and differential centrifugation (Beever *et al.* 1974), and were freeze-dried before analysis.

^{51}Cr and ^{103}Ru were estimated using a gamma counter (Biogamma; Beckman Instruments Inc., Fullerton, California, USA). ^{35}S was estimated using a liquid-scintillation counter (Nuclear Chicago, Mark I; Searle Analytic Inc., Des Plaines Illinois, USA), using the channel-ratios method (Bruno & Christian, 1961) to correct for quenching.

The contribution of microbial N to the total N flow from the stomach was calculated from the ratio, N:organic ^{35}S in microbes, and the flow of organic ^{35}S from the stomach, assuming all organic ^{35}S in abomasal digesta was derived from microbes (Hume, 1974). Allowance was made for N in abomasal secretions (1 g non-ammonia-N/d; Weston & Hogan, 1967).

Table 1. *Intake, digestibility, excretion and retention in the whole gastrointestinal tract of dry matter* (DM), *organic matter* (*OM*) *and nitrogen by sheep maintained at different temperatures*

(Mean values with their standard errors for six sheep/treatment)

	Temperature		
	Warm (18–21°)	Cold (−1 to 1°)	SE of mean
Intake (g/d)			
DM	1841	1841	—
OM	1643	1643	—
N	34·9	34·9	—
Digestibility:			
DM	0·482	0·450	0·0036
OM	0·511	0·477	0·0040
N	0·566	0·573	0·0080
Urinary N (g/d)	15·0	16·3	0·60
N retention (g/d)	4·8	3·7	0·58
Mean body-wt (kg)	54·5	52·3	0·24

Table 2. *Digestion in, and flow from stomach of dry matter* (DM), *organic matter* (*OM*) *and nitrogen, and efficiency of microbial synthesis in the rumen of sheep maintained at different temperatures*

(Mean values with their standard errors for six sheep/treatment)

	Temperature		
	Warm (18–21°)	Cold (−1 to 1°)	SE of mean
Flow through abomasum (g/d) of			
Digesta	20 040	20 310	58·5
DM	1 428	1 539	11·1
OM	1 152	1 246	11·5
N	40·2	42·7	0·70
Non-ammonia-N	38·7	41·4	0·69
Apparent loss in stomach (g/d) of			
DM	413	302	11·1
OM	492	397	11·5
N	−5·3	−7·8	0·70
Non-ammonia-N	−3·8	−6·5	0·69
Microbial synthesis			
g DM/d	248	225	7·4
g N/d	23·6	21·4	0·66
g N/kg OM apparent loss in stomach	47·9	53·9	1·4
Food N escaping rumen digestion (g/d)	14·1	19·0	1·25
Turnover time of ^{103}Ru (h)	17·6	10·9	0·17
Dilution rate of ^{51}Cr (/h)	0·0971	0·141	0·0062

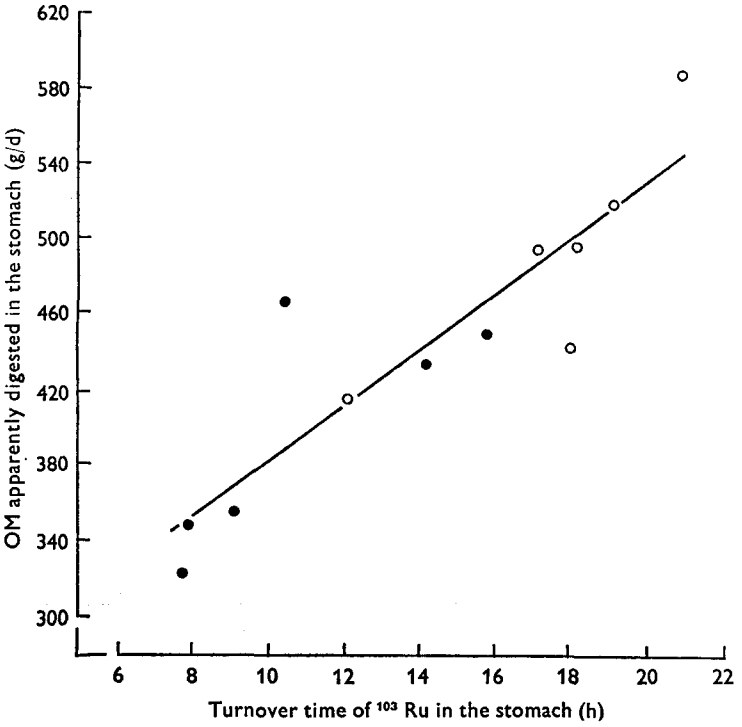

Fig. 1. The relationship between organic matter (OM) apparently digested in the stomach (D, g/d) and turnover time of ^{103}Ru in the stomach (T, h) in sheep exposed to temperatures of -1 to 1° (●) and 18–21° (○); $D = 14{\cdot}5T + 239$.

Statistical analysis

Differences between mean values were analysed statistically by analysis of variance (Steel & Torrie, 1960).

RESULTS

Exposure of shorn wethers to -1 to 1° resulted in a significant ($P < 0{\cdot}01$) reduction in apparent digestibility of DM and OM in the gastrointestinal tract of 0·030–0·035, but the apparent digestibility and retention of N were not affected (Table 1). Sheep maintained their weight when exposed to -1 to 1°, but gained several kg over 28 d when exposed to 18–21°.

Digestion in the stomach

The flow of DM and OM through the abomasum was greater ($P < 0{\cdot}01$) during cold exposure than during warm exposure (Table 2). Furthermore, the apparent digestibilities of DM and OM in the stomach were 0·224 and 0·300 for the sheep kept warm, and 0·163 and 0·242 for the sheep kept cold respectively. The amount of OM apparently digested in the stomach (D, g/d) was related to the turnover time in the rumen of ^{103}Ru (T, h) (Fig. 1) according to the equation:

$$D = 14{\cdot}5T + 239 \quad (r\ 0{\cdot}90,\ \text{SE}\ 32{\cdot}6).$$

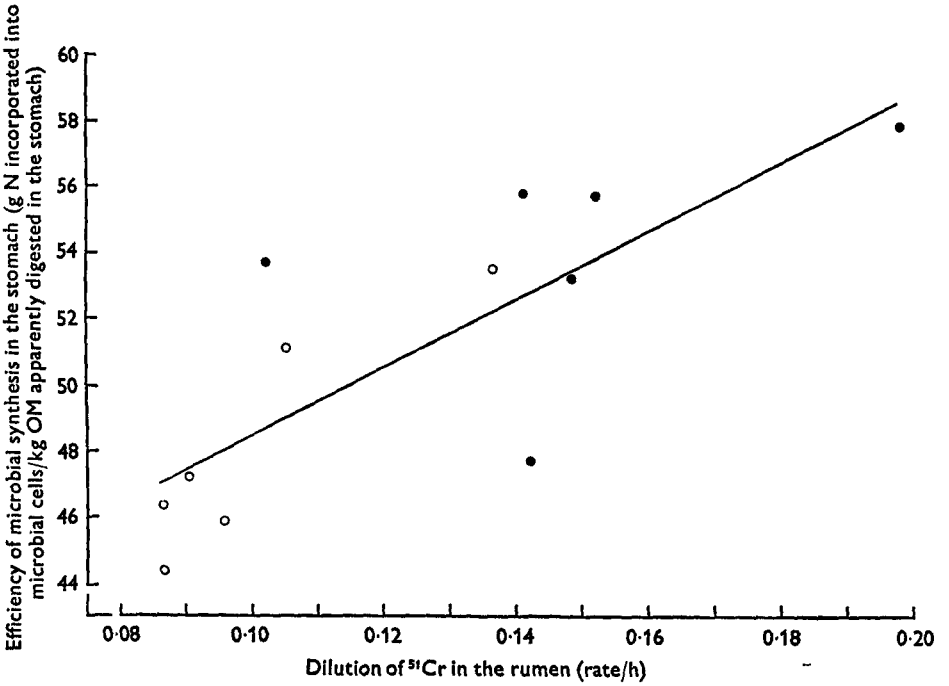

Fig. 2. The relationship between efficiency of microbial synthesis in the stomach (E, g N incorporated into microbial cells/kg organic matter (OM) apparently digested in the stomach) and dilution rate of ^{51}Cr in the rumen (K, /h) in sheep exposed to temperatures of -1 to 1° (●) and 18–21° (○); $E = 102K + 38{\cdot}3$.

The flow of total N and non-ammonia-N from the stomach was similar at both temperatures, and exceeded total N intake by approximately 6 g/d. The quantity of food N escaping digestion in the stomach was calculated to be 19·0 g N/d (54% of intake) in sheep exposed to -1 to 1°, compared with 14·1 g N/d (40% of intake) for sheep exposed to 18–21°. This difference was significant ($P < 0{\cdot}01$) (Table 2).

Synthesis of microbial cells

The quantity of microbial DM or N synthesized in the stomach did not differ significantly between treatments, but the efficiency of microbial synthesis (E, g microbial N/kg OM apparently digested in the stomach) was increased significantly ($P < 0{\cdot}05$) by cold exposure from 47·9 to 53·9 (Table 2), and was related to the dilution rate of ^{51}Cr (K, /h) in the rumen (Fig. 2) according to the equation:

$$E = 102K + 38{\cdot}3 \quad (r\ 0{\cdot}79,\ \text{SE}\ 2{\cdot}48).$$

The efficiency of microbial synthesis was also related to the turnover time of ^{103}Ru in the rumen (T, h) according to the equation:

$$E = -6{\cdot}38T + 60{\cdot}0 \quad (r\ 0{\cdot}67,\ \text{SE}\ 3{\cdot}18).$$

The concentrations of ammonia and VFA, and the molar proportions of individual VFA in rumen fluid did not differ significantly between treatments, but the volume

Table 3. *Rumen fluid volume, and concentrations of ammonia, volatile fatty acids (VFA) and proportions of individual VFA in rumen fluid of sheep maintained at different temperatures*

(Mean values with their standard errors for six sheep/treatment)

	Temperature		
	Warm (18–21°)	Cold (−1 to 1°)	SE of mean
Ammonia (mg N/l)	99·5	87·8	5·27
VFA (mmol/l)	113·0	96·1	5·15
Rumen fluid volume (l)	6·88	5·28	0·25
Proportions of individual VFA (mmol/mol)			
Acetic	615	590	6·8
Propionic	209	241	9·9
Isobutyric	4·9	4·7	0·36
Butyric	151	137	6·4
Isovaleric	4·5	4·2	0·55
Valeric	24	24	1·1

Table 4. *Digestibility of dry matter* (DM), *organic matter* (*OM*) *and nitrogen in the intestines of sheep maintained at different temperatures*

(Mean values with their standard errors for six sheep/treatment)

	Temperature		
	Warm (18–21°)	Cold (−1 to 1°)	SE of mean
DM digestibility	0·258	0·286	0·0039
OM digestibility	0·203	0·235	0·0065
Non-ammonia-N (NAN) apparently digested (g/d)	23·6	26·5	0·65
True digestibility of NAN in intestines (g/g NAN leaving stomach)*	0·788	0·821	0·0064
Apparent digestibility of DM in intestines			
g/g DM leaving stomach	0·332	0·343	0·0064
g/g DM digested in whole gastrointestinal tract	0·536	0·637	0·0294
Apparent digestibility of OM in intestines:			
g/g OM leaving stomach	0·302	0·310	0·0038
g/g OM digested in whole gastrointestinal tract	0·415	0·495	0·0395

* Assuming endogenous faecal N was 6 g N/kg OM reaching duodenum (Egan, 1974).

of rumen fluid, estimated by reference to ^{51}Cr-EDTA, was lower ($P < 0·05$) in the sheep exposed to cold (Table 3).

Digestion in the intestines

The apparent digestibilities of DM and OM in the intestines, when expressed relative to the amounts digested in the whole gastrointestinal tract, were significantly ($P < 0·05$) greater in sheep exposed to cold, but values were similar ($P > 0·05$) for sheep on both treatments when expressed relative to the amount of DM or OM entering

the intestines (Table 4). Significantly more ($P < 0.05$) non-ammonia-N (g/d) was apparently absorbed in the intestines of sheep exposed to cold. Assuming that endogenous faecal N was 6 g N/kg OM reaching the duodenum (Egan, 1974), the true digestibility of non-ammonia-N in the intestine was calculated as about 0·80 for sheep on both treatments.

Variation between sheep

Significant differences between sheep were obtained for the following indices: turnover time in the rumen of ^{51}Cr and ^{103}Ru ($P < 0.01$), flow of DM and OM through the abomasum ($P < 0.05$), apparent digestibility of DM and OM in the stomach and intestines ($P < 0.05$), and flow of ammonia through the abomasum ($P < 0.05$). Most of this variance could be attributed to one sheep, whose over-all value for turnover time of ^{103}Ru was significantly lower (10·6 h) than the mean value (± SEM) for the remaining five sheep (14·9 ± 1·7 h).

DISCUSSION

The effects of exposure of sheep to cold in this experiment were similar to those found when the particle size of forages was reduced by grinding and pelleting (Thomson, 1972). In both situations there was a decrease in the apparent digestibility of DM and OM in the gastrointestinal tract, due principally to an increase in the rate of passage of digesta from the rumen, with a consequent reduction in fermentation rate in the rumen. The increase in apparent digestion in the intestines compensated for only a portion of the reduction in digestion in the rumen. Similar results were obtained by Hemsley, Hogan & Weston (1975) when the rate of passage of digesta from the rumen of sheep was increased by ingestion of sodium chloride. The decrease in apparent DM digestibility (from 0·482 to 0·450). in this experiment induced by exposure of shorn sheep to cold was similar to that (from 0·599 to 0·566) previously reported by Westra (1975), who used a similar diet and similar sheep, but fed them twice daily at intakes approximately half that offered in the present experiment. This decrease was apparently due to a true temperature effect rather than to an artifact in technique, since Fuller & Cadenhead (1969) reported that the results of proximate analysis were not affected by the ambient temperature at which faeces were held between voiding and collection.

Relationship between turnover time and OM digested in rumen and microbial synthesis

For pelleted diets, the digestion of readily fermentable dietary material may be essentially complete in the rumen (Topps, Kay & Goodall, 1968). However, the extent of digestion of structural material may be dependent on the rate of passage of digesta through the rumen (Thomson, 1972). In the present experiment, the turnover time of the particulate marker ^{103}Ru was highly correlated with the amount of OM apparently digested in the stomach. The relationship was linear for the range of turnover times of ^{103}Ru from 8 to 21 h. Clearly, the rate of disappearance of OM during the initial 8 h was greater than that after 8 h. In all likelihood the early, rapid disappearance

of OM was due to fermentation of readily solubilized components of the diet (Hungate, 1968). Consequently, the OM digested after 8 h probably consisted largely of plant structural material, including cellulose. The reduction in the quantity of OM digested in the rumen when sheep were exposed to cold may thus be attributed to a reduction in the quantity of structural material degraded by the microbial population.

The significant increase in the efficiency of microbial DM production per unit OM apparently digested in the stomach of cold-exposed sheep is in accord with the suggestions of Hogan & Weston (1970), Thomson (1972), and Walker, Egan, Nader Ulyatt & Storer (1975) that such efficiency may be positively correlated with dilution rate in the rumen. Hogan & Weston (1970) found, for sheep given two groups of forage diets, that the measured dilution rates of rumen fluid of about 0·1/h and 0·05–0·07/h were associated with estimated production rates of bacterial protein of 37 and 31 g bacterial N/kg OM digested in the stomach respectively. These results are consistent with the concepts presented by Stouthamer & Bettenhaussen (1973) that at increased dilution rates, the maintenance costs of microbes are reduced, and efficiency of cell yield is increased. Other authors have commented on a negative correlation between dilution rate (the reciprocal of turnover time) and numbers of rumen microbes (Potter, Walker & Forrest, 1972) and protozoa (Christiansen, Woods & Burroughs, 1964). Recent publications have associated efficiency of microbial synthesis in the rumen with fermentation patterns of VFA and dilution rate of rumen fluid (Ishaque, Thomas & Rook, 1971; Harrison, Beever, Thomson & Osbourn, 1975; Hodgson & Thomas, 1975). However, the significance of fermentation pattern in these relationships is doubtful, since highly efficient synthesis of microbial cells has been related to both high and low concentrations of propionic acid in rumen fluid (Hume, 1970; Ishaque *et al.* 1971; Harrison *et al.* 1975; Hodgson & Thomas, 1975). In the present experiment, the proportions of individual VFA in rumen fluid from cold-exposed sheep were not significantly different from those from sheep maintained at 18–21°. Although the effect on microbial synthesis of the ingestion of cold water has not been determined, Bailey, Hironaka & Slen (1962) found a transient decrease in intraruminal temperature after sheep drank water at 0°, but the mean intraruminal temperature did not decrease significantly. Cunningham, Martz & Merilan (1964) found that variation of the temperature of the drinking-water did not influence digestibilities of DM, energy or crude protein in cows.

Increasing evidence supports the contention that the relationship between efficiency of microbial growth and dilution rate, found in vitro (e.g. Hobson, 1965; Meers, 1971) is also of significance in the rumen. Any one, or more likely a combination, of the following factors may be involved in causing efficiency of microbial synthesis to be positively related to dilution rate. With increasing dilution rate there may be reduced autolysis of bacteria, reduced engulfment of bacteria by protozoa, or changes in microbial population structure induced by a change in substrate or caused by an increased flow of liquid through the rumen *per se*. The relative importance of these factors has not been determined. However, published estimates of inefficiencies in microbial growth include that of Nolan & Leng (1972) who calculated that 30% of ammonia-N incorporated into rumen bacteria had been derived from breakdown of

microbial protein, and of Abe & Kandatsu (1969) who estimated that up to 40% of rumen bacteria may be engulfed by protozoa.

Although published values for efficiencies of microbial synthesis for sheep given pelleted diets are limited, the values obtained in this study (53·9 and 47·9 g N/kg OM apparently digested in the stomach for cold- and warm-exposed sheep respectively) are similar to values of 47·6 and 63·2 for sheep given pelleted lucerne (*Medicago sativa* L.) and ryegrass, calculated using nucleic acid as a microbial marker from the results of Coelho da Silva, Seeley, Beever, Prescott & Armstrong (1972*a*), and Coelho da Silva, Seeley, Thomson, Beever & Armstrong (1972*b*). These estimates are high when compared with the published range of 15–53 g bacterial N/kg OM apparently digested for sheep given non-pelleted diets (Thomas, 1973).

Endogenous N

The substantial gain of non-ammonia-N in the stomach of sheep given pelleted rations (present results, and Coelho da Silva *et al.* 1972*a*, *b*) presumably reflected incorporation of recycled endogenous N into microbial protein. The relatively low concentrations of rumen ammonia (88–100 mg N/l) in the present experiment may have facilitated entry of endogenous urea into the rumen (Nolan, 1975) where the presence of a rapidly fermented energy source would have lead to a relatively efficient fixation of the resultant ammonia-N.

Intestinal digestion

Of the OM entering the intestines and apparently digested therein, approximately half could be attributed to digestion of crude protein. At least some of the rest would be due to digestion of cellulose since Beever, Coelho da Silva, Prescott & Armstrong (1972) found that up to 60 g cellulose/d could be fermented in the intestines of sheep given pelleted ryegrass. Using published values for the contribution of metabolic N to faecal N, it was calculated that the true digestibility of non-ammonia-N entering the intestines was approximately 0·80 (Table 4). Since the postruminal digestibility of microbial N is also about 0·80 (McNaught, Owen, Henry & Kon, 1954), it appears that the N-containing dietary components that escaped complete degradation in the rumen had a similar postruminal digestibility. However, such calculations may be unreliable since published estimates for the prediction of metabolic N produced in the intestines vary widely (Hogan & Weston, 1970; Egan, 1974), and may not reliably apply to sheep given pelleted diets.

The effect of cold-exposure on digestion in sheep in this experiment could be attributed largely to the increase in the rate of passage of digesta through the rumen. The adaptive significance of this mechanism in cold-exposed ruminants is not clear, but may be to allow increased availability of nutrients to tissues of animals allowed to feed *ad lib.*

Sleeth & van Liere (1937) found that low environmental temperatures decreased the emptying time of the stomach in dogs. In simple-stomached animals thyroid hormones have been implicated in increased gut motility and enhanced gastric emptying (Levin, 1969). Westra (1975) found that the concentration of serum triiodothyronine

was increased from 0·95 μg/l for sheep kept at 21° to 1·56 μg/l for sheep kept at 1°. Serum triiodothyronine concentrations were negatively related to the mean retention time of ^{144}Ce in both the whole gastrointestinal tract and in the reticulo-rumen, and positively related to the frequency of reticular contractions. Increased parasympathetic (vagal) activity is also known to increase motility of the reticulo-rumen (Titchen, 1968), but there is little experimental evidence that this mechanism mediates the stimulatory effect of cold on reticular motility. Further studies of digestion in cold-stressed ruminants are required to clarify the relationships between rate of passage of digesta in the gut, site of digestion, and efficiency of microbial synthesis.

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