

Seasonal Changes in the Ruminal Microflora of the High-Arctic Svalbard Reindeer (*Rangifer tarandus platyrhynchus*)

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The dominant rumen bacteria in high-arctic Svalbard reindeer were characterized, their population densities were estimated, and ruminal pH was determined in summer, when food quality and availability are good, and in winter, when they are poor. In summer the total cultured viable population density was $(2.09 \pm 1.26) \times 10^{10}$ cells ml⁻¹, whereas in winter it was $(0.36 \pm 0.29) \times 10^{10}$ cells ml⁻¹, representing a decrease to 17% of the summer population density. On culture, *Butyrivibrio fibrisolvens* represented 22% of the bacterial population in summer and 30% in winter. *Streptococcus bovis* represented 17% of the bacterial population in summer but only 4% in winter. Methanogenic bacteria were present at 10⁴ cells ml⁻¹ in summer and 10⁷ cells ml⁻¹ in winter. In summer and winter, respectively, the proportions of the viable population showing the following activities were as follows: starch utilization, 68 and 63%; fiber digestion, 31 and 74%; cellulolysis, 15 and 35%; xylanolysis, 30 and 58%; proteolysis, 51 and 28%; ureolysis, 40 and 54%; and lactate utilization, 13 and 4%. The principal cellulolytic bacterium was *B. fibrisolvens*, which represented 66 and 52% of the cellulolytic population in summer and winter, respectively. The results indicate that the microflora of the rumen of Svalbard reindeer is highly effective in fiber digestion and nitrogen metabolism, allowing the animals to survive under the austere nutritional conditions typical of their high-arctic habitat.

Svalbard reindeer (*Rangifer tarandus platyrhynchus*) (Fig. 1A) survive under the most austere nutritional conditions on the high-arctic archipelago of Svalbard (77 to 81° N) (Fig. 1B). At Svalbard there is no daylight for almost 3 months in winter and there is an equally long period of continuous daylight in summer. Plant growth is restricted to about 2 months in summer. In winter most of the range is covered by snow, and unlike other high-arctic regions, at Svalbard the ambient temperature often rises above freezing, even in midwinter. Such episodes of warm weather followed by subzero temperatures produce a crust of solid ice which severely limits the access of the animals to the already poor winter range.

In the summer the animals have access to a number of different plant species. By September many plants, particularly grasses and sedges, have formed seed heads, which make up a major part of the summer diet of the reindeer. In winter, mostly mosses and other fibrous plants such as *Salix polaris* and herbs such as *Dryas octapetala* and *Luzula* sp. are available, whereas lichen is scarce and nutritionally unimportant at Svalbard. The diet of the animals therefore ranges from fresh, young forage in the spring through a highly concentrated diet, including grass seed heads, in September to fibrous plants, including mosses, in winter (38, 41).

Although Svalbard reindeer may deposit up to 30% of their body weight as fat in the autumn (42) (Fig. 1C), this is insufficient to guarantee their survival over the winter. In fact, we have calculated from the data of Nilssen et al. (33, 34) that body fat can only supply 10 to 30% of the daily energy expenditure during 4 months in winter, depending on activity. Therefore, in spring most animals are in poor condition and a significant proportion of the population often

dies from starvation before the onset of plant growth in summer.

Since the ability of the rumen microbial population to digest the poor-quality plants which are available in winter as well as to make maximum use of the summer forage could be crucial for the survival of these animals, we have characterized their dominant culturable rumen bacterial species and investigated their role in fiber digestion both in summer and winter.

MATERIALS AND METHODS

Animals. A total of 12 adult Svalbard reindeer of both sexes were shot, 6 in September (high-arctic summer) and 6 in April (high-arctic winter), in Adventdalen, Svalbard (78° N).

Rumen pH. The rumen contents of each animal were mixed in situ within 15 min of death, and their pH values were determined immediately with a calibrated portable pH meter (KM 7001; Kane-May Ltd., Welwyn Garden City, Hertfordshire, United Kingdom).

Sampling. The rumen contents were subsequently removed from each animal and mixed thoroughly again, and a representative 2-liter sample was placed in prewarmed (39°C) Dewar flasks. Samples of digesta were fixed in 2% glutaraldehyde and in 0.1 M iodine (9). The remaining digesta was filtered through two layers of muslin within 30 min of death of the animals. Samples of the filtrate were fixed in 2% glutaraldehyde and in 0.1 M iodine.

Enumeration of bacteria. The total population density of bacteria in the fixed, filtered rumen liquor was determined by phase-contrast (light) microscopy (direct count) by the method of Warner (51) with a hemacytometer chamber for estimating the large bacteria and a Helber chamber (1) for estimating the small bacteria. At least 2,000 cells were counted for each estimate. The species of large bacteria were identified by their morphology (35).

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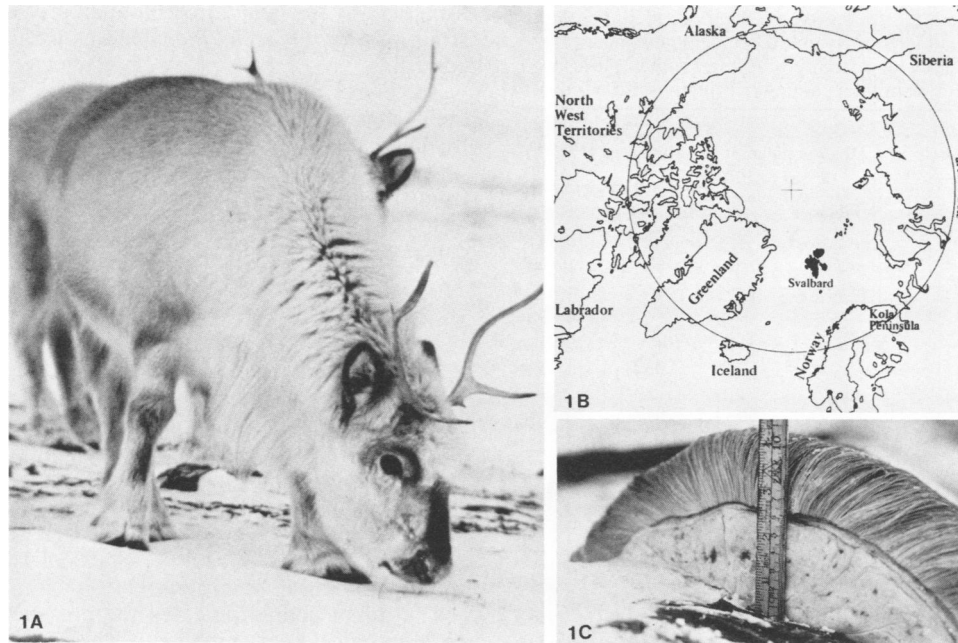


FIG. 1. The Svalbard reindeer (*Rangifer tarandus platyrhynchus*) (A) survive under the most austere nutritional conditions on the high-arctic archipelago of Svalbard (77 to 81° N) (B). The rumen microbes of this species are unusually effective at utilizing the poor-quality forage which is available during winter as well as the high-quality forage which is available during the short high-arctic summer. During summer enormous fat stores are deposited, particularly on the rump (C), for use in times of need in winter. However, despite these fat stores, 70 to 90% of daily energy expenditure during the dark part of winter must be provided by fermentation of plant material.

Colony counts of viable cells present in dilutions of rumen liquor of 10^7 to 10^{10} were made by the techniques of Hungate (21) with the habitat-stimulating medium of Henning and Van Der Walt (17). The dilutions were made in the same medium without carbohydrates (OC medium). The following groups of bacteria were also enumerated: cellulolytic bacteria were enumerated by the dilution method (30) with strips of Whatman filter paper plus 0.01% cellobiose as carbon sources in OC medium; lactate-utilizing bacteria were enumerated by colony counts in dilutions of OC medium supplemented with 1.4% sodium lactate and 2% agar; spirochetes were enumerated with the selective medium of Stanton and Canale-Parola (48); and methanogens were enumerated with the liquid medium of Edwards and McBride (12) supplemented with 0.04 M sodium formate and 0.04 M sodium acetate, with titanium (III) citrate as reductant (24). The presence of methanogens was determined by detection of methane in the gas phase after incubation for up to 1 month. Methane was determined with a Katharometer attached to a Pye 104 gas chromatograph fitted with a column of molecular sieve (5A, 60 to 80 mesh; Guild Corp., Bethel Park, Pa.) operated at ambient temperature. All incubations were performed in Hungate-type anaerobic culture tubes fitted with a screw cap and butyl rubber septum, incubated at 39°C. The media (excluding vitamins) were made up in bulk and dispensed into each tube under CO_2 (or 50% H_2 -50% CO_2 for the methanogens); the tubes were then sealed and autoclaved at 115°C for 20 min. Vitamins (43) were added by syringe after the tubes of medium had cooled to about 60°C. Each determination was made in quadruplicate for each animal, and the mean for each group of animals was calculated. The values for the total viable population represent the summation of the population densities of the viable count, the lactate utilizers, and the spirochetes.

Isolation of bacteria. Bacterial species were isolated by

picking colonies at random from tubes used in the viable count containing 10 to 50 colonies per tube. This occurred principally at dilutions of 10^9 in summer and 10^8 in winter; 50 colonies were picked to represent the bacterial population of each animal. The bacteria were isolated by streaking onto agar-containing habitat-simulating medium in petri dishes prepared in an anaerobic chamber (Coy Manufacturing Co., Ann Arbor, Mich.) under an atmosphere of 95% N_2 -5% H_2 . The petri dishes were incubated under CO_2 , and the bacterial strains were purified by repeated streaking from single colonies onto petri dishes of fresh habitat-simulating medium at 48-h intervals. Usually only two such purifications were necessary, but with some isolates four transfers were required to isolate the bacteria in pure culture. Lactate-utilizing species were isolated by the same procedure, with the OC medium supplemented with 1.4% sodium lactate.

Identification of bacteria. Bacterial isolates were identified by the methods described in Holdeman and Moore (18) and other standard texts. Fermentation products were determined as follows: fatty acids were determined by gas-liquid chromatography (39), lactate was determined by lactic dehydrogenase (2), and formate was determined by formic dehydrogenase (3). Substrate utilization patterns were determined by replica plating anaerobically with a maximum of 40 isolates per petri dish with the OC medium containing 0.2% glucose, maltose, and cellobiose as the master plate; replicas were formed on OC medium (control) and OC medium containing the substrate under examination at a concentration of 0.5%. Cellulolytic bacteria other than ruminococci and *Bacteroides succinogenes* were presumptively identified by plating on OC medium containing 0.2% cellobiose followed by replica plating on the same medium with the addition of 0.1% low-viscosity carboxymethyl cellulose (DP 7-9). After incubation for 24 to 48 h at 39°C, the carboxymethyl cellulose-containing plates were stained with Congo

TABLE 1. Population densities of some bacteria, including large bacteria, from the rumens of Svalbard reindeer, estimated by viable counts or direct microscopy of diluted rumen fluid, in September (high-arctic summer) and April (high-arctic winter)

Counting method and bacteria	Mean population density \pm SD ($\times 10^8$) in:		April populations as % of September populations
	September	April	
Total viable count	209 \pm 126	36.0 \pm 28.7	17.2
Lactate utilizers	26.2 \pm 30.1	0.39 \pm 0.19	1.5
Spirochetes	1.9 \pm 2.6	0.25 \pm 0.30	13.0
Viable count	181 \pm 124	35 \pm 29	19.6
Direct count	550 \pm 251	110 \pm 104	29.9
<i>Oscillospira guilliermondii</i>	0.07 \pm 0.03	0.008 \pm 0.032	11.4
<i>Magnovum eadii</i>	0.25 \pm 0.08	0.064 \pm 0.018	25.6
Quin's Oval	0.94 \pm 0.21	0.082 \pm 0.021	8.7
Large <i>Selenomonas</i> sp.	3.75 \pm 0.72	0.280 \pm 0.047	7.4
Other large bacteria	0.96 \pm 0.14	0.109 \pm 0.033	11.4
Total large bacteria	5.97 \pm 1.53	0.54 \pm 0.13	9.0

red (49). Presumptively cellulolytic isolates showed a zone of clearing, unstained by Congo red, around or immediately under the colony. All isolates giving a positive result were tested for their ability to grow on phosphoric acid-treated filter-paper cellulose (52). Strains positive in the latter test were regarded as cellulolytic. Fiber digesters were defined as isolates capable of growth on cellulose or hemicellulose. The xylan used was from oats; the pectin was from citrus fruits.

Proteolytic isolates were identified by their ability to hydrolyze azocasein (8) as modified by Hazlewood et al. (16). Representative isolates of each species hydrolyzing azocasein were tested for growth on glucose plates with plant fraction 1 protein (ribulosebiphosphate carboxylase; EC 4.1.1.39) as the major nitrogen source in a low-ammonium medium (16), by replica plating from OC medium supplemented with 0.2% glucose, maltose, and cellobiose. Ureolytic isolates were identified by ammonia production

from urea by the qualitative spot test of Wozny et al. (53). A strain of *Streptococcus faecium* was included as a positive control.

Zoospores of anaerobic rumen fungi were identified by their characteristic morphology, refractivity, motility in fresh preparations, and pale brown staining with iodine (36).

Statistics. Results are given as mean \pm standard deviation from the mean. The percentages were calculated from the absolute figures and corrected to one decimal place. Significance was calculated by the Student *t* test.

RESULTS

Direct count, viable count, and species distribution. All of the recognized species of large bacteria, i.e., *Oscillospira guilliermondii*, *Magnovum eadii* (37), Quin's Oval, and large strains of *Selenomonas* sp. were present during both seasons (Table 1). The winter population density of large bacteria showed a greater decrease when compared with the summer value than did that of the small bacteria. Differential counts of the large bacteria revealed a greater decrease in the population density of Quin's Oval and large *Selenomonas* sp. strains than in other species.

The direct counts revealed the presence of at least four different species of spirochete. Three of these grew in the spirochete enumeration medium. Three different colony types were evident: large, diffuse colonies (up to about 10 mm in diameter), small, diffuse colonies (up to 4 mm in diameter), and intermediate colonies (up to 8 mm in diameter with a distinct colony boundary).

Table 2 presents the species distribution and their percent occurrence in the population in both summer and winter. The population density of total viable bacteria decreased in winter to about 17% of the summer value. This decrease was not reflected by a similar decrease in all bacterial species identified, as is shown when the winter population density of each is expressed as a percentage of the summer population density (Table 2).

The viable population was dominated in the summer by *Butyrivibrio fibrisolvens* and *Streptococcus bovis*, which

TABLE 2. Culturable rumen bacteria of Svalbard reindeer: species composition and population densities in absolute numbers and percentage of total in September (high-arctic summer) and in April (high-arctic winter) as well as winter population densities as percentage of summer values

Bacteria	Mean population density \pm SD ($\times 10^7$) (%) in:		April population as % of September population
	September	April	
<i>B. fibrisolvens</i>	45.1 \pm 1.2 (22)	10.9 \pm 2.6 (30)	24
<i>B. fibrisolvens</i> ^a	20.1 \pm 14.1 (10)	6.6 \pm 2.8 (18)	33
<i>Selenomonas ruminantium</i>	24.3 \pm 8.5 (12)	5.0 \pm 2.3 (14)	21
<i>Selenomonas ruminantium</i> subsp. <i>lactilytica</i>	9.1 \pm 7.6 (4)	0.6 \pm 0.5 (2)	7
<i>Lachnospira multiparus</i>	4.1 \pm 3.5 (2)	ND ^b	
<i>Lactobacillus ruminis</i>	16.9 \pm 3.9 (8)	1.2 \pm 0.7 (3)	7
<i>Megasphaera elsdenii</i>	1.4 \pm 2.1 (1)	0.4 \pm 0.5 (1)	29
<i>Bacteroides ruminicola</i>	7.6 \pm 5.2 (4)	3.7 \pm 1.9 (10)	49
<i>Bacteroides amylophilus</i>	20.0 \pm 7.2 (10)	1.1 \pm 0.6 (3)	6
<i>Bacteroides succinogenes</i>	2.7 \pm 3.1 (1)	2.9 \pm 1.9 (8)	107
<i>Ruminococcus albus</i>	6.2 \pm 2.3 (3)	2.4 \pm 1.2 (7)	39
<i>Ruminococcus flavefaciens</i>	1.4 \pm 1.1 (1)	0.8 \pm 0.4 (2)	57
<i>Succinivibrio dextrinosolvens</i>	6.2 \pm 8.4 (3)	0.3 \pm 0.5 (1)	5
<i>S. bovis</i>	35.0 \pm 8.8 (17)	1.6 \pm 1.1 (4)	5
<i>Eubacterium ruminantium</i>	4.3 \pm 2.7 (2)	0.3 \pm 0.3 (1)	7
Others	22.0 \pm 6.2 (10)	5.0 \pm 1.2 (14)	23

^a Cellulolytic strains.

^b ND, Not detected.

together represented 39% of the culturable bacteria. The winter population was dominated by *B. fibrisolvens*, which had increased from 22% in summer to 30% of the population in winter. *S. bovis* was present in winter at 4% of the viable population. Among the other species, an increase in the differential population of all the cellulolytic species, including *B. fibrisolvens*, was recorded in winter, as was the case with *Bacteroides ruminicola*. Dilution counts of cellulolytic bacteria showed the population to be about 100-fold greater in summer than in winter. *Lachnospira multiparus*, a pectin digester, was present at 2% of the bacterial population in summer but was not detected in winter. The population of *Selenomonas ruminantium* subsp. *lactilytica*, a lactate utilizer, showed a large decrease in winter, to less than 7% of the summer population. Methanogens were present at 10^4 cells ml^{-1} in the summer and 10^7 cells ml^{-1} in the winter.

Figure 2 shows the population densities of rumen bacteria occupying specific ecological niches. It appears that the summer population consisted largely of starch-utilizing strains (68%), with a high population (13%) of lactate-utilizing species. Many of the starch-utilizing *B. fibrisolvens* strains were able to utilize xylan, cellulose, or both, as carbon sources (Fig. 3).

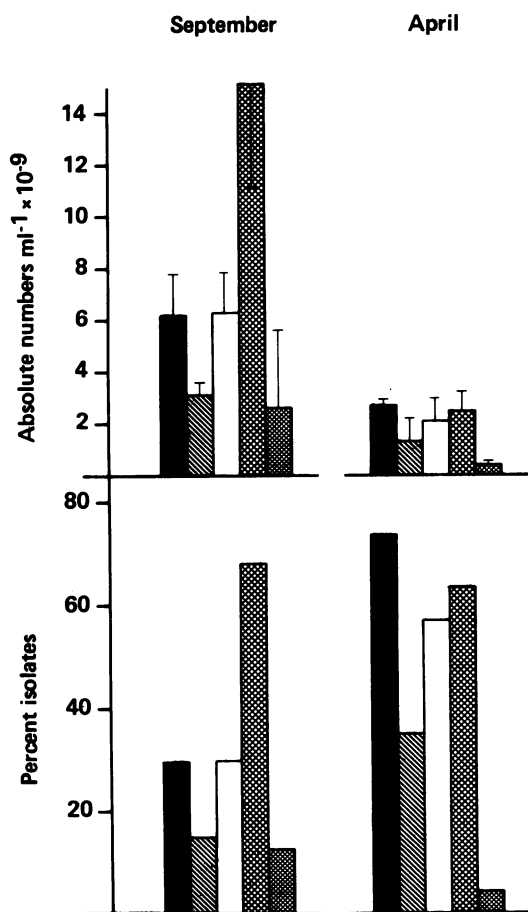


FIG. 2. Population densities of bacteria occupying fiber, cellulose, xylan, starch, and lactate-fermenting niches in the rumen of Svalbard reindeer in September (high-arctic summer) and April (high-arctic winter). Symbols: ■, fiber digesters; ▨, cellulose fermenters; □, xylan fermenters; ▩, starch fermenters; ▤, lactate fermenters.

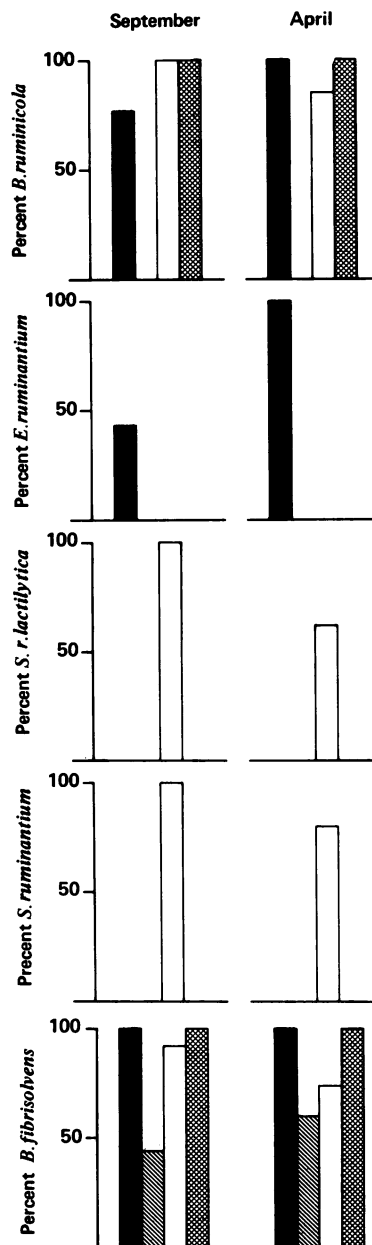


FIG. 3. Utilization of polysaccharides by some rumen bacteria from Svalbard reindeer. Values are expressed as the percentage of isolates of each species which grew on the different carbohydrates indicated. Symbols: ■, xylan; ▨, cellulose; □, starch; ▤, pectin.

Starch digestion. There was little difference in the proportion of the bacterial population able to utilize starch in summer and in winter, but the population density of starch-digesting bacteria in winter was only 16.6% of the summer population density. The dominant starch-fermenting bacterium during winter was *B. fibrisolvens*, whereas *S. bovis* dominated during summer. In winter, the proportion of *S. bovis* in the population decreased to 5% of the summer value, while *Bacteroides ruminicola* made up a larger proportion of the population, increasing from 4% in summer to 10% in winter. Of the *Bacteroides ruminicola* strains, 85% digested starch in winter compared with 100% in summer. Similar decreases in the starch-digesting ability of the winter

TABLE 3. Population densities of some cellulolytic bacteria in the rumen of Svalbard reindeer in September (high-arctic summer) and April (high-arctic winter)

Bacteria	Population densities ^a in:	
	September	April
<i>Ruminococcus albus</i>	3.0 ± 1.1	6.7 ± 3.3
<i>Ruminococcus flavefaciens</i>	0.7 ± 0.5	2.2 ± 1.0
<i>Bacteroides succinogenes</i>	1.3 ± 1.5	8.0 ± 5.2
<i>B. fibrisolvens</i>	9.5 ± 7.1	18.2 ± 7.7
Total viable cellulolytic species as % of total viable population	14.6 ± 5.4	35.0 ± 8.7
Cellulolytic <i>B. fibrisolvens</i> as % of total <i>B. fibrisolvens</i>	44.6	60.1

^a Values are expressed as percent viable bacterial population densities.

population as compared with the summer population were recorded for *Selenomonas ruminantium*, *Selenomonas ruminantium* subsp. *lactilytica*, and *B. fibrisolvens* (Fig. 3). The *Bacteroides succinogenes* isolates did not digest starch.

Xylan digestion. All of the cellulolytic isolates from summer and winter populations except *Bacteroides succinogenes* digested xylan. Of the *Bacteroides ruminicola* strains isolated in summer, only 78% would grow on xylan compared with 100% of those isolated in winter. All the *Eubacterium ruminantium* strains isolated in the winter digested xylan, compared with only 43% of those isolated in summer. The proportion of the population digesting xylan in winter (58%) was nearly double that in summer (30%).

Cellulose digestion. The cellulolytic species *Ruminococcus albus*, *R. flavefaciens*, and *Bacteroides succinogenes* were present both in winter and in summer. Cellulolytic *B. fibrisolvens* strains represented 45% of the total *B. fibrisolvens* in summer, increasing to nearly 60% in winter. This change was concomitant with a decrease in the proportion of *B. fibrisolvens* strains capable of digesting starch. All of the cellulolytic species made up a greater proportion of the population in winter than in summer (Table 3).

Fiber digestion. The combined population density of fiber-digesting species, those digesting cellulose and xylan, was $(6.2 \pm 1.56) \times 10^9$ cells ml⁻¹ in summer and $(2.7 \pm 0.21) \times 10^9$ cells ml⁻¹ in winter. These values represent 31 and 74% of the total population density of cultured bacteria in summer and winter, respectively. The winter population density was 44% of the summer population density.

Proteolysis. Proteolysis, detected by azocasein hydrolysis, was found in several species of bacteria, representing 51% of the summer and 28% of the winter population (Fig. 4). All the strains of *S. bovis*, 100 and 67% of *Bacteroides amylophilus*, 64 and 38% of *B. fibrisolvens*, and 80 and 43% of *Selenomonas ruminantium* showed some proteolytic activity in summer and winter, respectively. In the winter, 4% of the population, all unidentified gram-negative rods, were also proteolytic. With most isolates, the extent of proteolysis was small: only 5 to 8% (mean, 7%) of the available azocasein (5 mg ml⁻¹) was hydrolyzed in 24 h. Strains of *Bacteroides amylophilus*, however, hydrolyzed 14 to 20% (mean, 16%) during the same period. Replica plating of representative isolates grown on habitat-simulating medium with F1 protein as the major nitrogen source resulted in growth of 6 of 6 *Butyrivibrio fibrisolvens*, 5 of 6 *S. bovis*, 0 of 6 *Selenomonas ruminantium*, and 6 of 6 *Bacteroides amylophilus* strains. With the exception of *S. bovis*, the proportion of proteolytic isolates of each species was con-

siderably greater in summer than in winter. The absolute numbers of proteolytic isolates decreased from $(9.2 \pm 3.2) \times 10^9$ ml⁻¹ in summer to $(1.0 \pm 0.09) \times 10^9$ ml⁻¹ in winter, a decrease of about 85% (Fig. 4). F1 protein-utilizing isolates were estimated to make up 32 and 18% of the total population in summer and winter, respectively.

Ureolysis. Weak urease activity was found in a wide range of isolates both in summer and in winter. The proportion of the population showing this activity increased from about 40% in summer to 54% in winter, although in absolute numbers the winter ureolytic population was only about 23% of the summer value (Fig. 4). All of the isolates of *Bacteroides ruminicola* and *Succinivibrio dextrinosolvens* made in both summer and winter were ureolytic. Of the other species, the proportions of the cultured population isolated in summer and winter, respectively, that showed urease activity were as follows: *S. bovis*, 59 and 100%; *Selenomonas ruminantium*, 50 and 71%; *B. fibrisolvens*, 53 and 70%; unidentified, 40 and 57%. The urease activity of all these isolates was very low compared with that of *S. faecium*.

Other isolates. In both winter and summer a small proportion of the total isolates was not identified. These isolates were mostly gram-negative rods which we could not equate with known rumen bacteria. In one of the samples obtained in winter, 3×10^8 *Serratia marcescens* cells ml⁻¹ of rumen fluid were found. These formed brick-red colonies, unlike the more abundant rumen anaerobes, which formed white or cream-colored colonies in agar-containing medium.

Anaerobic fungi. Multiflagellated zoospores of *Neocallimastix frontalis* were observed at low population densities in summer and winter. Singly flagellated zoospores of *Piromonas communis* or *Sphaeromonas communis* were not

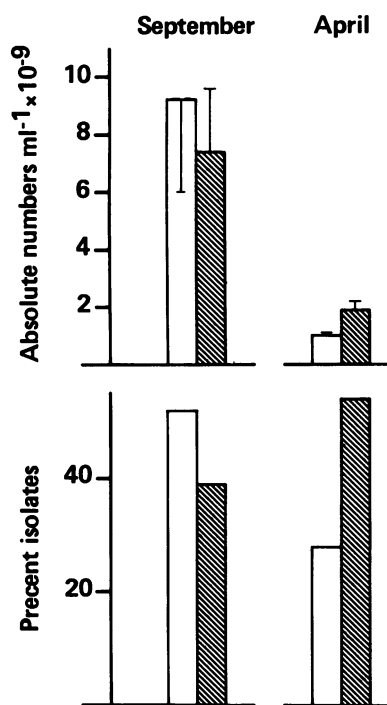


FIG. 4. Seasonal changes in absolute values and percent abundance of the proteolytic and ureolytic rumen bacteria of Svalbard reindeer. Symbols: □, proteolytic isolates; ■, ureolytic isolates.

observed in any animals. No parasitic fungi, such as *Sagittospora caemroni* found in caribou (27), were observed within the cells of the entodiniomorphid ciliates.

pH. The pH of the rumen liquor, measured 15 min after the death of each animal, was 6.19 ± 0.16 ($n = 6$) in summer and 6.75 ± 0.18 ($n = 6$) in winter. These values are significantly different ($P < 0.05$).

DISCUSSION

Most species of bacteria isolated from the rumen of Svalbard reindeer were the same as those known to occur in domestic ruminants. In summer, the population density of the rumen bacteria, measured by viable counting techniques, was similar to that found in domestic ruminants consuming a high-concentrate diet (5, 26). In the winter the population density had decreased to about one-fifth of the summer population density. The limited data on the chemical composition of reindeer forage plants collected in Adventdalen (47) shows that the crude protein levels are much higher in the summer than in the winter, particularly in the graminæ but not in the Bryophyta. In the summer, the level of nonstructural carbohydrates in the plant tissue would also be greater than in the winter. In September, when the summer experiments were conducted, many plants carried seed heads and consequently the animals were eating a high-starch and high-protein diet. This may explain the high population density of viable bacteria as well as the high proportion of starch-utilizing and proteolytic isolates in summer and the relatively low populations of these bacteria in winter. Even then, considering the poor quality and availability of food, the bacterial population density was higher than one would expect. In fact, it was equivalent to that found in domestic ruminants fed a high-roughage diet (26).

An important feature of the rumen bacterial population of the Svalbard reindeer compared with that of domestic ruminants was the presence of a high number of cellulolytic *B. fibrisolvans* cells when the animals were eating a high-quality diet in summer. We recognized that there is some diversity in the biochemical capabilities of different strains of *Butyrivibrio* spp., but in the absence of a very detailed genetic and taxonomic analysis of the isolates found by us and by other workers, we have classified all small gram-negative curved rods producing *n*-butyrate from carbohydrates as *B. fibrisolvans*. *B. fibrisolvans* is known to be a common rumen bacterium that is capable of fermenting a wide range of plant carbohydrates (18, 22). Because of this, its presence in Svalbard reindeer in high numbers is not surprising, but it is noteworthy that the proportion of cellulolytic *B. fibrisolvans* strains was high even when compared with the 5 to 8% previously found in sheep and cattle fed hay and concentrated diets (C. G. Orpin, unpublished data). Margherita and Hungate (31) found that *B. fibrisolvans* was the most abundant cellulolytic bacterium in semi-starved zebu cattle in Kenya. *B. fibrisolvans* strains may be more resilient, perhaps because of genetic versatility, than other cellulolytic rumen bacteria and so survive cycles of nutritional abundance and starvation of the host animal better than do other cellulolytic species. Dehority (11) found that 38% of the cellulolytic bacteria isolated from the rumens of Alaskan reindeer were *B. fibrisolvans*. Unlike the Svalbard reindeer, these animals were semidomestic and were fed lichen and cattle food; this value may therefore be unrepresentative of wild animals. In the present study, *B. fibrisolvans* represented about 65% of the cellulolytic bacte-

ria in summer and nearly 52% in winter. It would appear that the greater value in summer could be due to the ability of *B. fibrisolvans* to utilize other carbohydrates, such as starch, and the inability of other cellulolytic species to do so. Our finding of high numbers of cellulolytic bacteria in summer, when the animals were eating a high-quality diet was surprising, for when the diet of cattle is changed from high fiber to a high starch, the proportion of cellulolytic bacteria in the population often decreases dramatically (25). This may indicate that the population of *B. fibrisolvans* in the Svalbard reindeer is genetically unusually stable with regard to cellulase production or that the more easily metabolized plant constituents are digested very rapidly by the large microbial population.

During the short summer, the Svalbard reindeer not only have to meet the energy requirements for maintenance and lactation but must also deposit large quantities of fat and protein for subsequent use during winter. It has been suggested (42) that fat is crucial for the survival of this species during the winter. Using the data of Nilssen et al. (33, 34), however, we calculated that only 10 to 30% of the daily energy expenditure during the dark part of winter can be covered by mobilization of fat, depending on activity. It follows that even though fat is important, optimal utilization of the feed at all seasons is more likely to be crucial for the survival of the Svalbard reindeer. We suggest that the presence of very high numbers of fiber-digesting rumen bacteria during both summer and winter meets this need.

S. bovis produces lactate as a major fermentation product from starch. The high population density of *S. bovis* cells in the summer would generate much lactate and result in the stabilization of a high population density of lactate fermenters. *B. fibrisolvans* also produces some lactate, and this would contribute to the lactate pool. In the winter, both *S. bovis* and *B. fibrisolvans* were less abundant than in summer, and so both rumen lactate production and the numbers of lactate utilizers were much diminished.

The lower pH (6.2) of the rumen contents in summer than in winter was probably due to greater acid production from the higher level of readily metabolizable carbohydrates in the summer diet. In domestic ruminants fed a high-quality diet, the rumen pH is usually 5.5 to 6.7 (22). *B. fibrisolvans* does not usually form a significant proportion of the cellulolytic population in domestic ruminants (22) but is the major cellulolytic species in the Svalbard reindeer. *B. fibrisolvans* has a high growth rate at pH 6.2 (50), higher than those of the other cellulolytic species (45), and cellulolysis due to *B. fibrisolvans* should therefore be significant in Svalbard reindeer in the summer. This is supported by high *in vitro* digestibility of poor-quality forages during the summer (S. D. Mathiesen, unpublished results).

Isolates with some degree of proteolytic activity, measured by azocasein hydrolysis, were common in both summer and winter. Azocasein is not always a good substrate for protease (29), and some isolates may hydrolyze the diazo-protein bond without hydrolyzing the protein. The results obtained with F1 protein may more closely approximate the major proteolytic activity *in vitro*, since it is the most abundant protein in plants (28). No isolates could be regarded as highly proteolytic when compared, for example, with some isolates of *B. fibrisolvans* or *Bacteroides rumenicola* from sheep (14, 16).

The summer population density of proteolytic bacteria in Svalbard reindeer was higher than previously recorded from any ruminant. Fulghum and Moore (13) recorded up to 46% of proteolytic bacteria in cattle, but more frequently the

proteolytic population is only a small percentage of the total viable population (7).

Some strains of all the proteolytic species of bacteria presently found in Svalbard reindeer have previously been classified as proteolytic (4, 13). It was recently suggested that *S. bovis* was of significance in proteolysis in cattle (44), and this also appears to be the case in Svalbard reindeer in summer. All of the *S. bovis* strains isolated from Svalbard reindeer were proteolytic, in contrast to only about 30% in cattle that were fed lucerne (16). The lower proteolytic population in winter than in summer coincides with a lower level of protein in the winter diet.

The high population density of proteolytic bacteria would decrease the flow of dietary protein to the abomasum, but the large microbial population would to some extent offset this by the synthesis of higher quantities of microbial protein. Moreover, owing to the continuous daylight during summer, the animals eat nearly continuously, and the flow rate of digesta may therefore be great enough for some dietary protein to escape ruminal degradation.

The proportion of ureolytic isolates from the Svalbard reindeer was very high (32, 46, 53) at both seasons. These isolates, however, only showed weak urease activity. Nitrogen recycling via urea may be of some importance in maintaining the nitrogen balance in the animals in winter when food quality and availability are low (20). Our discovery that a large proportion of the population is ureolytic supports this suggestion and is in agreement with the work of Jones et al. (23), who estimated that the ureolytic bacteria form about 35% of the bacterial population in steers fed a diet containing urea. This value may be an overestimate, since total viable bacterial numbers were unusually low.

Rumen ureases are strongly inhibited by ammonia (10, 54). Thus, ureolysis is likely to be of little significance in the summer when dietary protein is high and ammonia levels in the rumen probably are also high. In winter, the protein intake decreases and a corresponding decrease in ruminal ammonia production is likely to occur. Urea flux across the rumen epithelium would be greater in the winter during periods of starvation, when dietary protein is very low and protein reserves are recycled (19, 20).

High ammonia production in the rumen is necessary to optimize the use of dietary carbohydrates by optimizing bacterial growth (22). In summer, ammonia derived from dietary protein probably would be sufficient to support a high level of fiber digestion, and in the winter ureolysis would provide much of the ammonia required.

Ammonia can be used by many, but not all, rumen bacteria as a nitrogen source (22). Some species require peptides or amino acids (5, 6, 15, 40). During periods of starvation, ammonia generated by the hydrolysis of urea, which enters the rumen in the saliva or by diffusion across the rumen epithelium from the blood, would satisfy the nitrogen requirements of the ammonia-utilizing species. It is probable that the large proteolytic population is necessary to maintain sufficient peptides and amino acids by hydrolysis of proteins from the diet, dead rumen microorganisms, or sloughed rumen epithelial cells to satisfy the nitrogen requirements of these bacteria in winter. During this hydrolysis, the release of branched chain amino acids, which are subsequently deaminated to branched chain fatty acids, would aid in the maintenance of the bacterial population under conditions of semistarvation (5, 6).

The low population density of methanogens in the summer compared with the winter would ensure that additional energy is conserved from the diet in summer. In addition, the

high-concentrate summer diet would result in higher propionate levels (25) than in winter and lead to increased gluconeogenesis in the animal.

The finding of numbers of the soil bacterium *Serratia marcescens* in one of the winter samples is indicative of ingestion of soil. This would happen when the animals tear food from the earth and eat the roots and when eating moss contaminated with soil. All of the winter rumen samples had the smell of soil, contained sand grains, and were brown, suggesting that soil ingestion was in fact not infrequent during winter.

The discovery of a highly specialized rumen microflora in Svalbard reindeer, in addition to other physical and physiological adaptations, helps to explain the ability of these animals to survive on the extreme archipelago of Svalbard.

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