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Isolation and Identification of Adherent Epimural Bacteria During Succession in Young Lambs†

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Successive changes in aerobic and anaerobic bacterial counts and changes in the generic composition of the epimural community in lambs from 1 to 10 weeks were determined. Bacterial culture counts revealed a predominantly anaerobic community, with the mean anaerobic count being 1.4×10^7 CFU/cm^2 of tissue surface. The aerobic count was highest at 1 week of age and declined significantly thereafter to a mean of 1.8×10^4 CFU/cm^2, thus representing only 0.13% of the mean anaerobic count after week 1. Of the 345 strains isolated anaerobically at 1, 2, 4, 6, 8, and 10 weeks of age, 47, 32, 12, 32, 2, and 5% were capable of growth in a partially reduced medium, indicating a reduction in the number of facultative anaerobes with time. The majority of isolated strains were identified as belonging to genera commonly isolated from rumen contents. In some instances, however, strains did not correspond to previously described species, and some genera were present in proportions different from those expected in rumen fluid. At three of the sampling times, one genus was dominant, constituting 45 to 55% of the isolates. These dominant isolates were *Streptococcus bovis*, *Bacteroides* sp., and an anaerobic *Streptococcus* sp. for weeks 1, 2, and 10, respectively. During the transition period (weeks 4 to 8), two or more groups were codominant.

The initial incidental observation of a bacterial community on the surface of the rumen epithelium (22) raised the question of whether these epimural (upon the wall) bacteria are functionally and taxonomically different or similar to those in the rumen contents. The epimural bacteria obviously occupy a distinct habitat or physical space in the rumen ecosystem, i.e., the rumen epithelium. Cheng et al. (9) proposed several unique functions for the epimural bacteria, suggesting that they also occupy a specific niche within their epithelial habitat. Although the role of the epimural community needs to be further defined, the existence of these proposed functions (oxygen scavenging, tissue recycling, and urea metabolism) is now supported by various amounts of evidence (10, 12, 23).

Bacterial isolation studies in sheep and cattle have yielded somewhat different conclusions with regard to the taxonomic uniqueness of the epimural community. Of the epimural bacteria isolated from mature sheep by Mead and Jones (18), 95% were identified as belonging to previously described genera of functionally significant rumen bacteria. Dehority and Grubb (11) similarly isolated epimural bacteria from mature sheep and identified them as belonging to four genera commonly found in rumen contents. However, some of the strains did not appear to be similar to previously described species of these genera. In preliminary findings from the bovine rumen (8, 9), large numbers of gram-positive organisms taxonomically distinct from those normally found in rumen contents were isolated. The isolated strains were identified as species of *Micrococcus*, *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Lactobacillus*, *Fusobacterium*, and *Propionibacterium* along with additional strains of unidentified anaerobes. Further studies appear necessary to determine the taxonomic uniqueness of the epimural community. The present study of the succession of epimural bacteria was intended to contribute to our knowledge of the taxonomic uniqueness of this bacterial community. One of the objectives of the present study was to determine whether the pattern of succession in the epimural community differs markedly from that of rumen contents as previously reported by Bryant et al. (5). If the epimural community is taxonomically distinct from that of rumen contents, the succession patterns as well as the bacterial groups present would likely differ.

In addition, one of the distinguishing features of indigenous bacteria is that they colonize their habitat during succession (21). Differentiation of truly indigenous epimural bacterial populations from transient populations should be considered a prerequisite to studies directed toward the definition of specific niches in the epimural community. Thus, in an effort to describe the succession pattern and identify the indigenous members of the epimural community of the sheep rumen, an initial scanning electron microscopy (SEM) examination of the epimural community in a mature wether was conducted, and a combination of SEM and bacterial isolation was used to study the successive changes which occur in the epimural bacterial community from its establishment until it reaches climax. The results of the SEM examination, which describe successive morphological changes in the epimural community with time, were reported separately (19). The results of the isolation study, which describe the successive changes in generic composition of the epimural community, are presented here.

MATERIALS AND METHODS

Samples. Tissue samples were obtained from four sites in the rumens of two groups of six lambs each (groups 1 and 2) at 1, 2, 4, 6, 8, and 10 weeks of age as described previously (19). By using these sampling times, two samples were obtained during the preruminant stage, three during the transitional stage, and one during the ruminant stage; the stage of development was determined by measurements of relative wet tissue weights of stomach compartments. The four sites sampled were those found by previous workers to differ in bacterial density (2). Tissue disks were washed three times by vigorous agitation in successive 100-ml amounts of sterile anaerobic dilution solution (3) to remove digesta and

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nonadherent bacteria. They were then homogenized in a Waring blender in 100 ml of fresh anaerobic dilution solution for 2 min under a continuous flow of CO_2. Serial 10-fold dilutions were then made in anaerobic dilution solution for both aerobic and anaerobic colony counts. All procedures except for aerobic counts were carried out under CO_2 by the Hungate technique (14) as modified by Bryant and Burkey (3).

Colony counts. The CCA medium of Allison et al. (1) was used for anaerobic colony counts on samples from lambs in group 1. Due to some difficulty with swarming in roll tubes with the CCA medium, the RGCSA medium of Grubb and Dehority (13), which contained lower substrate levels (0.025% each of cellobiose and glucose and 0.05% soluble starch), was used for colony counts in group 2 lambs. The swarming in the CCA medium allowed enumeration but not isolation of bacteria from lambs in group 1. Media for aerobic colony counts were identical to the respective anaerobic media (CCA, group 1; RGCSA, group 2) except for deletion of resazurin, sodium carbonate, and cysteine. Colonies were counted in five replicates after either 5 (aerobic) or 7 (anaerobic) days of incubation at 37°C.

Bacterial isolation and characterization. Bacteria were isolated only from anaerobic roll tubes since culturable aerobic bacteria in lambs from group 1 represented less than 2% of the anaerobic bacteria after 2 weeks of age. A total of 345 strains were obtained by picking 15 colonies from the highest dilution in each of four sites within each lamb in group 2. The sample from week 8, site 3 was lost as a result of blender malfunction. The isolates were characterized by the methods of Holdeman and Moore (14). End products were determined in cultures grown in the medium of Grubb and Dehority (13) without agar, with added growth factors (medium 10) (7), and with energy sources (cellobiose, glucose, and starch) increased to the level of 0.3% each. Short-chain acids were determined as previously described (17). Aerotolerance of individual isolates was determined in partially reduced slants made from the RGCSA medium (13). This medium was placed in tubes under 100% CO_2 without sodium carbonate and cysteine and sealed with a rubber stopper until inoculated. After inoculation, tubes were incubated aerobically; tubes with visible growth in the area of resazurin oxidation were classified as aerotolerant. Strains without growth or capable of growth only in the reduced butt of the slant were classified as anaerobic.

Strains were grouped on the basis of gram stain, morphology, motility, aerotolerance, and end product formation; representative strains from each group were further characterized by inoculation into biochemical test media according to the scheme of Holdeman and Moore (14). Final assignment of strains to genera was based upon the classification schemes of Holdeman and Moore (14) and *Bergey's Manual of Determinative Bacteriology* (6).

RESULTS

Colony counts. In both groups of lambs the aerobic count was highest at 1 week of age (Table 1) and then declined significantly, with the mean aerobic count beyond 1 week (1.8×10^4 CFU/cm^2 of rumen wall) comprising only 0.13% of the mean anaerobic count (1.4×10^7 CFU/cm^2 of rumen wall). The unexpectedly high percentage of aerobes (66.9%) in the 2-week-old lamb from group 1 appeared to be a result of a low anaerobic count (compared with week 2, group 2) rather than a high aerobic count. The anaerobic count was highest at weeks 4 and 6 for groups 1 and 2, respectively, whereas the lowest anaerobic count in both groups was at 2 weeks.

TABLE 1. Aerobic and anaerobic colony counts

Sample	Aerobic (CFU/cm^2 of rumen wall) ($\times 10^3$)		Anaerobic (CFU/cm^2 of rumen wall) ($\times 10^5$)		% Aerobes	
	Group 1	Group 2	Group 1	Group 2	Group 1	Group 2
Age (wk)						
1	348.6^a	237.3^a	156.7^b	20.2^e	3.6^b	11.9^a
2	11.0^c	14.0^c	0.3^d	7.8^e	66.9^a	3.6^b
4	18.5^{b,c}	4.3^c	225.5^a	65.8^d	0.3^b	0.1^c
6	30.6^{b,c}	32.6^b	31.2d	472.8^a	1.3^b	0.1^c
8	33.7^b	6.8^c	96.2^b	134.4^c	1.3^b	0.1^c
10	18.6^{b,c}	14.3^c	181.7^{a,b}	275.4^b	0.2^b	0.1^c
Site in rumen[f]						
1	71.9^{a,b}	49.1^b	133.0^{a,b}	195.5^a	5.4^{a,b}	2.9^b
2	87.4^a	69.4^a	91.9^{b,c}	102.1^b	2.3^b	3.7^a
3	84.9^a	63.4^a	165.6^a	232.3^a	12.6^{a,b}	2.7^b
4	63.1^b	27.4^c	71.4^c	125.7^b	19.6^a	1.3^c

$^{a-e}$ Values in the same column with the same superscript and from the same type of sample (age or rumen site) are not significantly different ($P < 0.05$).

[f] Sampling sites: 1, roof of cranial rumen; 2, roof of dorsal rumen; 3, floor of caudodorsal blind sac; 4, floor of caudorentral blind sac. All sampling sites were on the midline.

When the colony count data was summarized by site within the rumen, there were significant differences in both aerobic and anaerobic counts and the percentage of aerobes between the sites sampled (Table 1). Aerobic counts were significantly higher at sites 2 and 3. Sites 1 and 3 tended to have higher bacterial densities based upon anaerobic counts. The percentage of aerobes was highest at sites 3 and 4 in group 1 and at sites 1 and 2 in group 2. These patterns were not consistent in all animals, however, since there were some significant animal by site interactions.

Bacterial characterization. The 345 strains isolated from anaerobic roll tubes were divided into 24 groups on the basis of gram stain, morphology, motility, aerotolerance, and end product formation (Table 2). These 24 groups were tentatively assigned to genera, and representative strains from each group were then inoculated into biochemical test media (Table 3) to verify the tentative identification. Organisms from groups VI and VIII did not grow upon transfer from freezer stocks and thus could not be further characterized. Group XXIII included five miscellaneous rods and they were also not further characterized. Erythritol, inositol, and cellulose were not utilized by any of the groups, and several substrates were utilized by only one group. Group XV produced weak acid in arabinose, group XII produced acid from glycerol, group X produced weak acid from melezitose, and groups X, XII, and XXIV reduced nitrate. Red blood cells were not hemolysed by any of the group representatives.

The number of different groups present at each of the six sampling times was 9, 4, 6, 10, 9, and 10 for weeks 1, 2, 4, 6, 8, and 10, respectively. At each week sampled, a different group was dominant or codominant, i.e., no one group continued to be dominant over an extended period of time. At weeks 1, 2, and 10 a single group was dominant, with 45 to 55% of the isolates. In contrast, during the transition period (weeks 4 to 8), two or more groups were codominant.

In the 1-week-old lamb, a facultative anaerobe identified as *Streptococcus bovis* was the dominant organism, comprising 50% of the isolates. An organism identified as *Bacte-*

TABLE 2. Summary of bacterial characterization

Group	Tentative identification	Morphology	End products[a]	Gram[b] reaction	Motility	Aerobic growth	% of isolates (no.) at wk: 1	2	4	6	8	10
I	*Stretptococcus bovis*	Coccus or coccobacillus	L	+	−	(+)	50 (30)	15 (9)		1.7 (1)		3.3 (2)
II	*Bacteroides fragilis*	Rod	A,P,S	−	−/+	−	20 (12)					
III	*Clostridium ramosum*	Rod	2,A,(1),(f)	−	−	−	15 (9)					
IV	*Bacteroides* sp.	Rod	(2),(a),L	−	+	−	6.7 (4)					
V	*Bacteroides* sp.	Rod	(2),(a),L,S	−[v]	+/−	−	1.7 (1)	55 (33)				
VI	Unidentified	Rod	L,p	−	−	−	1.7 (1)					
VII	Unidentified	Coccus or coccobacillus	2,A,L	+	−	+	1.7 (1)					
VIII	Unidentified	Rod	None	−	−	−	1.7 (1)		1.7 (1)			
IX	*Bacteroides ruminicola*	Rod	A,S,P,(M),(L)	−	+	−	1.7 (1)			25 (15)	13.3 (4)	5 (3)
X	*Butyrivibrio fibrisolvens*	Rod, curved	A,B,L	−	+	−		25 (15)	6.7 (4)	10 (6)	17.8 (8)	16.7 (10)
XI	Unidentified	Rod	a,L,S,M,f	−	+	−		5 (3)				
XII	*Selenomonas ruminantium*	Rod, curved	A,P,l,(s)	−	+	−			31.7 (19)	23.3 (14)	13.3 (6)	13.3 (8)
XIII	*Lactobacillus ruminus*	Rod	2,(a),L	+	−	−			46.7 (28)			
XIV	*Ruminococcus flavefaciens*	Coccus	A,S	+	−	−			10 (6)		2.2 (1)	5 (3)
XV	*Bifidobacterium* sp.	Rod	(2),A,L	+	−	−			3.3 (2)			
XVI	*Succinivibrio dextrinosolvens*	Spiral	A,S	−	+	−				5 (3)		1.7 (1)
XVII	*Streptococcus* sp.	Coccus	(2),(a),L	+	−	−				10 (6)	20 (9)	45 (27)
XVIII	*Ruminococcus albus*	Coccus	2,A,(1)	+	−	−				10 (6)	2.2 (1)	
XIX	*Bacteroides* sp.	Rod	(A),(L),(S),f,M	−	−	−				8.3 (5)	8.9 (4)	
XX	Unidentified	Coccus	(a),(p),(B),(L),(v)	+[v]	−	−				5 (3)		
XXI	*Acidaminococcus*	Coccus	A,B	−	−	−				1.7 (1)		
XXII	Unidentified	Coccus	No major a,l,b	+	−	−					17.8 (8)	3.3 (2)
XXIII	Unidentified	Rod	(2),(A),L,(S)	−	−	−					8.9 (4)	1.7 (1)
XXIV	*Veillonella*	Coccus	A,P	−	−	−						5 (3)
Total no. of isolates							60	60	60	60	45	60
No. of different groups per week							9	4	6	10	9	10
% Aerotolerant							47	32	12	32	2	5
% Gram positive							52	15	60	22	40	57

[a] Abbreviations: a, A, acetate; p, P, propionate; b, B, butyrate; l, L, lactate; s, S, succinate; m, M, malate; f, F, fumarate; 2, ethanol. Lower case, production of <10 μM/ml; upper case, production of >10 μM/ml; ( ), product not formed by all strains in group.

[b] v, Gram variable.

roides fragilis comprised 20% of the isolates, and a *Clostridium* sp. most like *Clostridium ramosum* comprised another 15% of the isolates. Group IV organisms (*Bacteroides* sp.) comprised 6.7% of the isolates, with the remaining isolates being evenly divided among five groups (1.7% each; Table 2).

By 2 weeks, *S. bovis* had declined to only 15% of the isolates and a *Bacteroides* sp. (group V) had become the dominant organism, with 55% of the isolates. This *Bacteroides* sp. was a gram-variable, pleomorphic rod 2 to 10 μm long that did not clearly match any of the reported species. Motility and aerotolerance were variable, with 8 of the 33 strains capable of growth in the oxidized portion of a slant. Another organism isolated at 2 weeks comprising 25% of the isolates was an organism identified as *Butyrivibrio fibrisolvens*. The only other organism isolated at 2 weeks was an unidentified gram-negative, motile, anaerobic rod, 1 to 3 by 0.8 μm (group XI). End products included succinate (9.6 ± 1.5 μmol/ml), lactate (9.4 ± 4.7 μmol/ml), and a compound that cochromatographed with phenylacetate and malate (31.4 ± 18.4 μmol/ml).

The *Bacteroides* sp. dominant at 2 weeks was not isolated at 4 weeks. Instead, a *Lactobacillus* sp. identified as *Lactobacillus ruminus* was dominant, representing 46.7% of the

TABLE 3. Summary of biochemical test media results

Substrate	Bacterial group[a]																				
	I	II	III	IV	V	VII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII	XXIV
Amygdalin	w	w	w	−	w	w	−	−	−	−	w	w	w	w	−	w	w	−	−	−	−
Cellobiose	a	a	a	w	a	a	a	a	a	a	a	a	a	w	−	w	a	w	−	−	a
Esculin pH	$-^{w}$	$-^{w}$	−	−	$-^{w}$	−	−	−	−	−	$-^{w}$	w	−	−	$-^{w}$	−	−	−	−	−	−
Esculin hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fructose	a	a	a	w	a	a	a	a	a	a	a	a	a	a	$-^{w}$	w	a	−	w	w	
Galactose	a^{w}	a	a	w	a	a	a	w	a	a	a	a	a	a	$-^{w}$	w	a	w	−	w	
Glucose	a	a	a	a	a	a	a	a	a	a	a	a	a	a	$-^{w}$	w	a	w	−	$-^{w}$	a
Glycogen	w	w	−	−	w	w	−	w	w	−	−	w	w	−	−	−	a	−	−	−	−
Lactose	a	a	a	w	a	a	a	w	a	a	a	a	a	−	−	w	a	−	−	−	−
Maltose	a	a	a	w	a	a	−	w	a	a	a	a	a	a	−	−	a	−	−	−	−
Mannitol	−	−	w	w	a	a	−	a	−	a	−	−	a	a	−	−	−	−	−	−	−
Mannose	a	a	a	a	a	a	a	a	a	a	a	a	−	a	−	w	a	−	−	−	a
Melibiose	w	w	w	−	w	w	w	w	−	w	w	w	−	−	−	−	−	−	w	−	−
Raffinose	a	a	a	−	a	a	a	a	w	a	a	a	a	−	−	−	−	−	−	−	−
Rhamnose	−	−	w	w	−	−	w	−	−	−	−	w	−	−	−	−	−	−	−	−	−
Ribose	−	−	w	w	w	w	−	−	w	−	−	−	w	w	−	w	−	−	−	−	w
Salicin	w	w	w	w	w	w	w	w	−	w	w	w	w	−	w	−	−	w	w^{a}	w	w
Starch pH	a	a	−	−	a	a	−	a	a	a	−	a	a	w	−	−	a	−	−	−	−
Starch hydrolysis	+	+	−	−	+	+	−	+	−	−	−	+	+	−	−	+	+	−	+	−	−
Sucrose	a	a	a	w	a	a	a	a	a	a	a	a	a	a	w	w	a	−	−	w	a
Trehalose	w	−	w	w	−	w	−	w	−	w	w	−	w	−	w	w	−	−	−	w	w
Xylose	−	a	−	−	−	−	a	−	−	a	−	a	a	a	−	−	a	−	w^{a}	−	−
Gelatin digestion	−	w	−	−	−	−	+	w	−	−	−	+	−	−	−	−	−	−	−	−	+
Indole	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Bile gr.	+	−	++	−	−	−	−	−	−	−	−	+++	−	++	−	+	−	−	−	−	−
Xylan	w	w	−	−	−	−	−	w	−	w	−	a	−	w	−	−	a^{w}	−	−	−	−
Lactate utilization	−	a,p	−	−	−	−	−	−	a,p	−	A,P	−	−	a	−	a	−	−	−	−	a,p

[a] Groups VI, VII, and XIII were not further characterized as indicated in the text. Symbols: a, strong acid (≤ pH 5.5); w, weak acid (pH 5.5 to 6.0); −, negative reaction; +, positive reaction; $-^{w}$, most strains negative; some strains weak; w^{a}, some strains weak, some strains acid, a^{w}, most strains strong acid, some strains weak. For lactate utilization: a, 1 meq/100 ml of acetic acid; A, 1 meq/100 ml of acetic acid; p, 1 meq/100 ml of propionic acid; P, 1 meq/100 ml of propionic acid.

isolates. *Selenomonas ruminantium* was also present in high numbers at 4 weeks, comprising 31.7% of the isolates. A gram-positive coccus (group XIV) which produced acetate and succinate comprised 10% of the isolates and was identified as a non-cellulolytic *Ruminococcus flavefaciens*. *B. fibrisolvens* was again present at week 4, but at lower levels than at 2 weeks. Other organisms present at low levels at 4 weeks included an anaerobic, gram-positive, nonmotile rod which produced acetate and lactate, identified as *Bifidobacterium* sp. and an unidentified short, thick, gram-negative, anaerobic rod with no detectable end products (group VIII).

At 6 weeks *L. ruminus* was not isolated. *S. ruminantium* was again codominant, representing 23.3% of the isolates, with *Bacteroides ruminicola* (group IX) comprising 25% of the isolates. Three organisms, *B. fibrisolvens*, *Ruminococcus albus*, and an anaerobic *Streptococcus* sp., were present, representing 10% of the isolates. A nonmotile, gram-negative, pleomorphic rod (2 to 7 by 0.8 μm) which produced a compound that cochromatographed with phenylacetate and malate, in combination with succinic, acetic, or fumaric acid comprised 8.3% of the isolates. It was identified as a *Bacteroides* sp. *Succinivibrio dextrinosolvens* and an unidentified anaerobic coccus, both of which produced butyrate and lactate, each represented 5% of the isolates. One strain each of *Acidaminococcus* sp. and *S. bovis* were also isolated at 6 weeks.

In the 8-week-old lamb, five groups (IX, X, XII, XVII, and XXII) were present with 13 to 20% of the isolates, with no group clearly dominant. The anaerobic *Streptococcus* first seen at 6 weeks, the *Bacteroides* sp. codominant at 6 weeks, *B. fibrisolvens*, and *S. ruminantium* comprised 20, 13.3, 17.8, and 13.3% of the isolates, respectively. An unidentified gram-positive coccus (1 μm in diameter) which formed chains of up to 10 cells (group XXII) was present as 17.8% of the isolates. No major end products were detected; minor end products varied and included acetate, lactate, and butyrate. Two groups (XIX and XXIII) were present as 8.9% of the isolates. The *Bacteroides* sp. (group XIX) was also present at week 6. Group XXIII included several miscellaneous unidentified gram-negative rods, 1 to 4 by 0.8 μm. One strain produced acetate (21.8 μmol/ml) and succinate (20.6 μmol/ml), a second strain produced lactate (17.5 μmol/ml) and succinate (14.2 μmol/ml), a third strain produced lactate (30.8 μmol/ml) and ethanol (4.9 μmol/ml), and a fourth strain produced lactate (23.3 μmol/ml), acetate (20.4 μmol/ml), and ethanol (20.1 μmol/ml). A fifth strain present at week 10 produced only lactate (27.3 μmol/ml). *R. albus* and *Ruminococcus flavefaciens* each represented 2.2% of the 8-week isolates.

The anaerobic *Streptococcus* present at both 6 and 8 weeks was the dominant isolate at 10 weeks, comprising 45% of the isolates. *B. fibrisolvens* and *S. ruminantium* were again present as 16.7 and 13.3% of the isolates, respectively. Several other organisms present at the 5% level were *Bacteroides* sp. (group IV), *R. flavefaciens*, and an anaerobic, gram-negative coccus which produced acetate and propionate and was identified as a *Veillonella* species. *S. bovis* and the unidentified coccus in group XXII were present at the 3.3% level. One strain of *S. dextrinosolvens* was also isolated from the 10-week-old lamb.

DISCUSSION

Results of colony counts in the present study indicate that the epimural community colonizes to a level similar to that of

the adult relatively early. In three determinations from the roof of the dorsal rumen (our site 2), Dehority and Grubb (11) found an average of 1.2×10^7 CFU/cm^2 of rumen wall. The average anaerobic culture count for samples from the dorsal rumen in our study was 9.7×10^6 CFU/cm^2 of rumen wall. The average anaerobic count over all samples in the present study was 1.4×10^7 CFU/cm^2 of rumen wall, with a range from a low of 3.0×10^4 to a high of 4.7×10^8. Wallace et al. (23) reported numbers of bacteria associated with the rumen epithelium of hay-fed sheep ranging from 4.4×10^7 to 2.2×10^8 per g of wet tissue weight. The difference in method of reporting (bacteria per gram of wet tissue weight versus bacteria per square centrimeter) makes comparisons with the present study difficult.

Bauchop et al. (2) examined the same four sites in mature sheep that were sampled in this study. Based upon density scores assigned during SEM examination, they concluded that sites 2 and 3 (roof of dorsal rumen and floor of caudodorsal blind sac) were more densely colonized than sites 1 and 4 (roof of cranial rumen and floor of caudoventral blind sac). We also found differences between sampling sites based upon anaerobic culture counts; however, sites 1 (roof of cranial rumen) and 3 (floor of caudodorsal blind sac) had the highest counts in the present study. The higher counts we found at these two sites could be attributed to the fact that the papillae were significantly longer at sites 1 and 3 than at sites 2 and 4 (19). Thus, 1 cm^2 of rumen wall tissue would have included a larger surface area at these sites.

The levels (percentages) of aerobic bacteria found in the epimural community of the lamb rumen are comparable to those reported by Bryant et al. (5) for the rumen fluid of dairy calves. They reported 17.1, 4.5, 3.6, 1.2, and 1.4% aerobes for weeks 1, 3, 6, 9, and 13, respectively (TS medium counts/RGCA medium counts). The percentage of aerobes (aerobic count/anaerobic count) in the present study was 7.8, 35.3, 0.2, 0.7, 0.7, and 0.2% for weeks 1, 2, 4, 6, 8, and 10, respectively. These findings indicate that the epimural community is similar to the other bacterial communities of the rumen in that it is composed primarily of anaerobes. Another measure of aerotolerance in the present study was the number of strains isolated anaerobically which were capable of growth in partially reduced media. These percentages were 47, 32, 12, 32, 2, and 5% of the strains for weeks 1, 2, 4, 6, 8, and 10, respectively. Both of these aerotolerance measures reflect a trend toward decreasing aerotolerance with time; however, the second measure indicates that the number of facultative anaerobes also decreases with time.

These findings differ from those of Wallace et al. (23) who reported that 73% of the epimural bacteria isolated anaerobically from infused lambs were capable of growth aerobically. It seems highly probable that the high numbers of facultative anaerobes reported by Wallace et al. were a result of the infusion regime that the lambs were on, since the Eh in rumen fluid in these lambs was -14 to -50 mV compared to a normal of -250 to -450 mV. Kennedy et al. (16) also isolated epimural bacteria from lambs by using an aerobic medium (TBY medium) and estimated that 39 to 62% of the aerobic isolates were capable of growth on anaerobic TBY medium. Interestingly, none of the aerobic isolates grew anaerobically on medium 98-5 (4). The findings of Kennedy et al. are difficult to interpret since no clear statement was made regarding the percentage of the epimural community composed of aerobes. It appears from the data given that the anaerobic counts were 100-fold higher than the aerobic counts, which would mean that these facultative bacteria represented less that 1% of the culturable bacteria. Thus, one could not conclude that the epimural community is largely comprised of facultative anaerobes based on their findings unless the aerobic count was as high or higher than the anaerobic count.

The results of bacterial characterization in the present study compare favorably with those of previous studies of the epimural community of mature sheep. Mead and Jones (18) concluded that the epimural community of mature sheep was not taxonomically distinct from that of rumen contents, since 95% of the isolates belonged to genera commonly isolated from the rumen. The isolates included *Butyrivibrio* sp. (31%), *Bacteroides* sp. (22.4%), *S. ruminantium* (9.9%), *S. dextrinosolvens* (8.7%), *S. bovis* (8.1%), *Propionibacterium* sp. (4.3%), *Treponema* sp. (3.1%), *Eubacterium* sp. (2.5%), *Lachnospira multiparus* (2.5%), and *R. flavefaciens* (2.5%). Seventeen percent of their isolates were gram-positive and 17% were aerotolerant. Dehority and Grubb (11) similarly isolated 95 strains of epimural bacteria from the dorsal rumen of adult sheep and identified them as follows: *B. fibrisolvens* atypical (31.6%), *Butyrivibrio* sp. (5.3%), *B. ruminicola* (23.2%), *Lactobacillus* sp. (1.1%), and unknown *Bacteroides* sp. (38.9%).

Most isolates in the present study were identified as belonging to genera common to the rumen ecosystem; however, some strains did not correspond to previously described species. Six of the genera present in lambs at 10 weeks of age were also present in epimural isolates from mature sheep as reported by Mead and Jones (18). They were *Streptococcus*, *Bacteroides*, *Butyrivibrio*, *Ruminococcus*, *Selenomonas*, and *Succinivibrio*. These genera are very likely indigenous members of the epimural community based on the criteria of Savage (21). They grew anaerobically, colonized their habitat during succession, persisted after initial colonization, and have been reported in the adult epimural community. In addition, Mead and Jones found *Propionibacterium*, *Treponema*, *Eubacterium*, and *Lachnospira*, whereas *Veillonella* was isolated at 10 weeks in this study. Further studies are needed to determine whether these latter organisms are indigenous members of the epimural community.

A number of similarities also exist between the succession of the nonepimural community of the calf rumen as reported by Bryant et al. (5) and that of the epimural community in lambs. They isolated a facultatively anaerobic *Streptococcus* (C5) from 1-, 3-, and 6-week-old calves, with 8, 5, and 1 strains isolated at those ages, respectively. We isolated *S. bovis* at 1, 2, 6, and 10 weeks, with the highest levels at 1 week (Table 2). They isolated a *Butyrivibrio* sp., with 3, 4, 12, 35, 14, and 32 strains present at 1, 3, 6, 9, and 13 weeks and adult, respectively. We isolated *B. fibrisolvens* at 2, 4, 6, 8, and 10 weeks, representing from 7 to 25% of the isolates. They reported a *Selenomonas* sp. at weeks 6 and 9 and in the adult. We also isolated *S. ruminantium* at weeks 4 to 10. They isolated a non-celluloytic *Ruminococcus* sp. (C4) at weeks 6, 9, and 13. We isolated a non-cellulolytic *R. albus* at weeks 6 and 8, and a non-cellulolytic *R. flavefaciens* at weeks 4, 8, and 10. These similarities strongly suggest that the succession of the epimural community does not differ markedly from that of the other communities in the rumen ecosystem.

Our results differ somewhat from those of Kennedy et al. (16) in the lamb and the characterization study on the epimural community of adult cattle by Cheng et al. (9). Kennedy et al. characterized only those organisms isolated aerobically which were ureolytic. The majority of these organisms were identified as *Staphylococcus* spp., with

some *Propionibacterium* spp. and *Corynebacterium* spp. also identified. Cheng et al. (9) concluded that the epimural bacterial community of adult cattle was taxonomically distinct from that of the rumen contents, with high numbers of gram-positive, facultative anaerobes. The isolated strains were identified as species of *Micrococcus*, *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Lactobacillus*, *Fusobacterium*, and *Propionibacterium* along with additional strains of unidentified anaerobes.

In isolation studies where an anaerobic medium was used to isolate epimural bacteria, investigators have concluded that the epimural community is not for the most part taxonomically distinct from that of rumen contents. This includes the present study as well as those of Mead and Jones (18) and Dehority and Grubb (11). In studies where the investigators have concluded that the epimural community is taxonomically unique and composed primarily of facultative anaerobes, the epimural bacteria were isolated either with aerobic media or with a combination of aerobic and anaerobic media. There is a need for further definitive studies to compare these two approaches within the same animals to determine whether the majority of the epimural bacteria are isolated by using anaerobic or aerobic media. The number of culturable anaerobes was generally 100-fold higher than the culturable aerobes in the present study. It is possible that the use of a different aerobic medium, such as the TBY medium of Kennedy et al. (16), would result in a higher aerobic count.

Results of the present bacterial isolation study correlated well with SEM observations (19) in the same animals. For instance, SEM revealed a curved rod which persisted from 1 to 10 weeks, being most dense at 2 weeks of age. *Butyrivibrio* sp. (curved rods) were isolated from samples at weeks 2 to 10, representing the greatest proportion of the isolates (25%) at 2 weeks of age. Variation in cell size observed within the curved-rod morphotype via SEM was sufficient to suggest that more than one organism was present. The isolation of *Selenomonas* sp. (a larger curved rod) at weeks 4 to 10 helps to explain this observed variation in cell size.

In addition, SEM revealed that a coccus was dominant on the sides and base of the papillae at week 1, was to a large extent replaced by a curved rod by week 2, and was again the dominant morphotype at weeks 8 and 10. The dominant isolate at week 1 was a facultative *Streptococcus* sp., which represented 50% of the isolates at 1 week and declined to 15% by week 2. A different anaerobic *Streptococcus* sp. constituted 10, 20, and 45% of the isolates at weeks 6, 8, and 10, respectively. A spiral morphotype was also observed via SEM to constitute a small proportion of the population from weeks 6 to 10. *Succinivibrio* sp. was isolated in low proportions at weeks 6 and 10.

In summary, this study has shown that the epimural bacterial community of the sheep rumen is established shortly after birth and soon reaches population levels similar to those of the adult. This community follows a characteristic succession, with significant changes occurring in the generic composition through the first 10 weeks. The epimural community does not appear to be markedly different taxonomically from the bacterial community of rumen contents, since most isolated strains could be placed into common rumen genera. However, some of these genera were present in different proportions in the epimural community than in rumen contents. It is possible that there are several unique species present in the epimural community, since several isolates did not correspond to previously described species within these genera. Further work is needed to more clearly determine whether the epimural community has a unique functional role in the rumen ecosystem.

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