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Variations in Post-Weaning Development of Ruminal Mucosa in Lambs.* (25142)

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Earlier workers(1-5) have suggested that diet has some effect on rumen development. This was not convincingly shown however, until reports of Warner and coworkers(6-8), which strongly indicated that chemical factors, probably in the form of active microbial fermentation products, act as stimulants for development of the ruminal papillae. The literature, however, appears devoid of reference to either qualitative or quantitative relationship of development of ruminal mucosa to post-weaning growth patterns in the animal. The stimulus for the present study developed from observations of rumina of sheep slaughtered at termination of an earlier feeding trial. Some lambs in the earlier trial had gained poorly, and a striking relationship existed between gain and ruminal development although all lambs were of an age and were receiving a diet which was expected to have conditioned full development of the rumen. The sheep which gained weight slowly had poor development of the rumen while more rapidly gaining ones had rumina which had thickened mucosae with well-developed papillae. As far as we were aware, such a relationship between structural development of a tissue and rate of gain of the animal had not been previously reported.

Procedure. Detailed observations were made on rumina from 42 feed-lot lambs upon slaughter at the end of a 114-day feeding period. The lambs weighed an average of 62.1 lb (range of 49-90 lb) at the beginning of the feeding trial, prior to which they had been maintained on a diet of mixed forages. Weight and gain data were available on all animals. The self-fed basal diet consisted of 25% oat straw, 24% beet pulp, 14% wheat, 16% oats, 18% linseed meal, and 4% sucrose. Stabilized Vit. A (Chas. Pfizer & Co., Inc.) was supplemented at the rate of 650,000 I.U. per ton of feed. Other than addition of 48 lb of K_2HPO_4 per ton of feed (added in an attempt to induce urinary calculi formation), the diet may be considered adequate. The sheep were fed in 5 groups, their diets differing only in antibiotic content (Table I). The antibiotics chlortetracycline and oxytetracycline were supplied as crude supplements (Aurofac and TM-10, respectively). As soon as possible after evisceration, rumen and reticulum were tied off, slit at the dorsal midline, emptied, and washed. Weights of the emptied whole ruminoreticulum were taken and gross observations made of color, length, and density of papillae of the various rumina. Sections of tissue were immediately removed from the anterioventral sac of the rumen and placed in 10% formalin. Formalin fixed sections from 28 of the animals were later separated into 2 sections: a "mucosal" layer, consisting of the epithelial lining, submucosa, muscularis mu-

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TABLE I. Effect of Antibiotic Supplementation on Gain and Rumen Characteristics.

Group	I			II			III			IV			V		
Antibiotic	None			CTC,* 10 mg/lb feed			CTC,* 20 mg/lb feed			None			OTC,* 10 mg/lb feed		
No. of lambs	8			8			10			8			8		
Avg initial wt, lb	59	± 17	†	61	± 17		64	± 19		64	± 18		64	± 19	
" daily gain, lb (114 days)	.35	± .02		.41	± .02		.43	± .10		.38	± .10		.37	± .05	
Idem (last 28 days)	.24	± .15		.25	± .09		.40	± .10‡		.21	± .20		.29	± .14	
Avg reticulorumen wt, g	773	±166		843	±153		839	±163		734	±153		818	±132	
" length papillae, cm	5.3	± .33		5.3	± .38		5.7	± .29		4.7	± .21		4.7	± .35	
" width index, papillae‖	6.8	± 1.9		6.0	± 1.1		6.5	± 1.2		6.5	± 1.4		6.6	± 1.5	
" density index, papillae‖	6.4	± 1.1		7.5	± .5		7.9	± .3		7.4	± 1.3		7.5	± 1.3	
" color index, mucosa‖	5.7	± 1.1		5.7	± .9		7.4	± .5 §		6.3	± 1.4		7.0	± 1.9	

* CTC = Chlortetracycline, OTC = Oxytetracycline. † Stand. dev. ‡ Significantly different from Groups I & IV at .05 level of probability.
§ Significantly different from Groups I & IV at .01 level of probability. ‖ Indexes based on arbitrary classification of 1 to 9 with increasing avg relative width, density and color. See text.

cosa, and mucosa proper, and a muscle layer consisting of the striated muscles of the rumen wall. The sections were air-dried and weighed. From these weights the percent mucosa was calculated. Mucosal percentages ranged from 51% to 75%. Much of the tabulated data were determined from photographs of the anterioventral sac. Color index, relative papillary density, and relative papillary width of each rumen were estimated by placing these photographs into 9 classes of color, density, or width gradations established upon study of the variations observed in the rumina of over 70 lambs. Each class was assessed a value of 1 through 9 in order of increasing color, density, or width and these values were used as the basis for estimation of the relationships. Fig. 1 shows approximate range in color in the present experiment and Fig. 2 presents variation in density. Sections of formalin fixed tissues were taken approximately 2 inches immediately cranial to the anterior pillar, embedded in paraffin, sectioned, and observed histologically. Papillae lengths also were estimated from these paraffin blocks and varied from 2.5 to 7.5 mm. The anterioventral sac was the focal area of study, not only as a convenient point of reference between our data and those in the literature, but also because it appeared that variations in development in this area reflected variations in the rumen as a whole more so than any other segment. The 114-day gains used in calculating relationships were those made during the entire feeding period. The terminal 28-day gains were evaluated in an effort to estimate the relationship of the more immediate gain to the various characteristics of the rumen.

Results. The data showing the effect of antibiotics on 114 day gains, terminal 28 day gains and mucosal characteristics are summarized in Table I. Such differences in the 114 day gains as may have been elicited by the antibiotic treatment were not significant statistically. The lambs receiving 20 mg of chlorotetracycline per lb of feed showed a significantly higher average gain ($p<0.05$) during the terminal 28 days of the experiment.

Weights of total tissue of the ruminoreticulum and relative papillary densities were

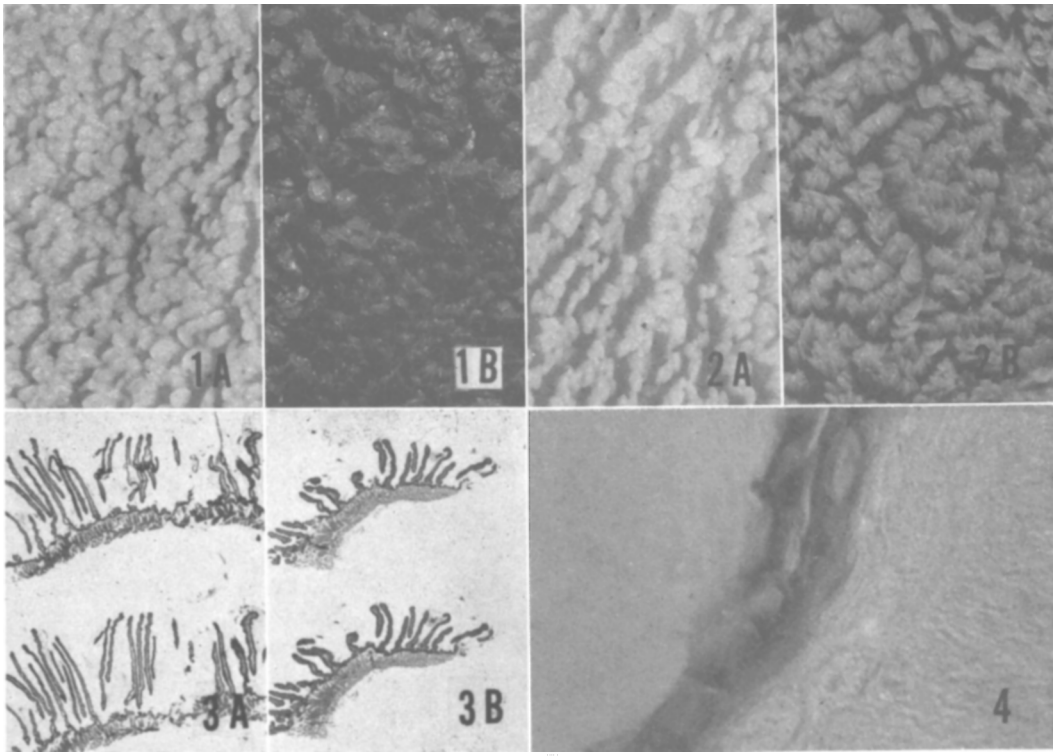

FIG. 1. Photograph of floor of anterioventral sac of rumen showing wide variation in color noted between individual lambs. A was assessed relative value of 4; B, value 9, maximum.

FIG. 2. Example of variation noted in density of papillae between individuals. A was assessed the relative value of 3 while B was classed as 9, a maximum density.

FIG. 3. Cross section of rumen wall showing variations in length between lambs. Papillae in A measured 7 mm and in B, 4 mm in length.

FIG. 4. Formalin fixed, frozen blade-sectioned, unstained rumen epithelium showing brownish-black keratinized layer. 970 ×.

slightly increased by the antibiotics but the differences were not statistically significant. Mucosal color, though not significantly affected by low levels of either antibiotic, was significantly intensified by 20 mg chlorotetracycline per lb of feed.

Coefficients of correlation were calculated to evaluate overall relationships between gains and various ruminal characteristics (Table II). A number of these coefficients were of sufficient magnitude and statistical significance to verify the working hypothesis that

TABLE II. Coefficients of Correlations among Gains, Initial and Final Weights, and Mucosal Characters.

	Gain, last 28 days	Initial wt	Final wt	Reticulorumen wt	% mucosa	Papillary length	Papillary width	Papillary density	Color index
Gain, 114 days	.51†	.34*	.67†	.60†	.21	.59†	.09	.31*	.12
" , last 28 days		.07	.27	.65†	.54†	.36*	.30	.40†	.39*
Initial wt			.93†	.64†	−.14	.20	−.15	.34*	.13
Final "				.74†	−.05	.40†	−.08	.39*	.12
Reticulorumen wt					−.06	.46†	.19	.48†	.20
% mucosa						.12	.30	.10	.25
Papillary length							.18	.32*	.04
" width								−.08	−.04
" density									.56†

* Significant at .05 level of probability. † Significant at .01 level of probability.

ruminal development is in part related to rate of gain of the animal. In addition, analyses of covariance within groups indicated that, with exception of the relation of mucosal color to terminal 28-day gains, statistically important relationships were not significantly affected nor explained by the effects of antibiotic treatment. The indication is that rumen development was largely an individual characteristic of physiologic consequence.

The highly significant correlation of total weight of ruminoreticulum to average daily gain (Table II) was as might be expected from the wide range of final body weights (69-150 lb). The weight of total ruminoreticulum tissue was largely proportional to overall size of the animal and feed-lot gain was also a determinative part of that size. Enhanced rates of gain, however, could have been due also to a number of factors including an increased functional capacity of the larger rumen.

Specific indications of a functional relationship between ruminoreticulum development and animal gain are noted upon closer inspection of the coefficients of correlation between total organ weight, final body weight, and terminating 28-day gain instead of total 114-day gain. Although correlation of the 114-day feed-lot gain to weight of the ruminoreticulum can be largely accounted for by the significant parallelism of both variables with final body weight, a similar conclusion cannot be stated for the relationship of ruminal tissue weights to more immediate changes in weight of the animal. The coefficient of correlation of terminal rates of gain to final weights was only 0.27. Significantly more of the variance in ruminoreticulum weights can be accounted for by the estimate of multiple correlation of tissue weight with terminal rate of gain and body weight ($R = 0.88$; $R^2 = 0.77$) than with either terminal gain ($r^2 = 0.42$) or body weight ($r^2 = 0.55$) alone.

Percentage of mucosa, which may represent functional development of the rumen(8), was likewise significantly correlated with gain during the terminal 28 days but to a smaller extent to gain during the entire period. Mucosal development, however, may not be independent of development of underlying muscle.

Histological evaluation of sections of the anterioventral sac suggested that papillary length is a definitive criterion for evaluating mucosal development (Fig. 3). The relationship of papillary length to rate of gain appears important but, in this instance, lengths of the papillae seemed to be more closely related to long-term rates of growth than to the more immediate gains. Only a relatively small part of the variance in length of papillae could be explained on the basis of rumen weight ($r^2 = 0.209$), initial weight ($r^2 = 0.042$), or final weight ($r^2 = 0.156$). Thus partial coefficients of correlation between 114-day gain and papillary length independent of rumen weight ($r_{gp.r} = 0.437$), independent of initial weight ($r_{gp.iw} = 0.560$), and independent of final weight ($r_{gp.fw} = 0.470$) were all highly significant statistically.

Papillary density, like papillary length, serves as an evaluation of surface area available for absorption or other functions that the rumen may perform. Though not so highly related as was length, estimated density was correlated significantly with gains in both periods. The multiple coefficient of correlation of 114-day gain with relative length of papillae and relative papillary density ($R = 0.60$), however, was only slightly greater than that with papillary length alone.

Widths of papillae were examined because of their great variation among different individuals, but although the relationships to gains were positive, they were small and not significant statistically.

The unexpected result of these studies was the extent to which mucosal color of the rumen was related to gain. The correlation was not high but was of statistical significance for the last 28 days of trial. Attempts were made to localize this color and apparently it is restricted to the cornified layer. Fig. 4 shows a section of formalin fixed, frozen blade-sectioned and unstained rumen epithelium. Where no organic solvents were used in the histological preparations, the cornified layer retained a dark brownish color which was like the coloration noted in gross examination of the rumen. An implication with respect to color is that it may represent products of microbial activity especially since the color appears to be affected by dietary antibiotics.

If so, color may provide additional means of evaluating bacterial action and thereby be also a means of evaluating the influence of dietary factors on rumen flora.

The coefficients of correlation obtained in this study are not high, but it should be noted that they are based on relatively unrefined descriptions of the rumen. From the data presented, however, it appears that variations in development of the ruminoreticulum in general and of the rumen mucosa specifically are a structural expression of rate of growth, and possibly of capacity for growth as it is affected by general nutritional state, inherent growth impulse of the animal, or capacity of individual sheep to adapt to a functional symbiosis.

Summary. Rumina of 42 sheep, fed an adequate diet, were examined for assumed characteristics of development: total weight of reticulorumen, percent mucosa, and papillary length, width, and density. The significant correlations of rumen weight, papillary length and papillary density to rate of gain present strong supportive evidence that growth and development of the animal as a whole is related to and may be dependent upon ruminal development. Evidence is presented indicating that dietary antibiotics, chlortetracycline and oxytetracycline, may have a slight effect on mucosal development. An unexplained relationship of mucosal color and gain is presented.

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Unstable Inhibitory Mechanism in Rheumatoid Sera.* (25143)

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Serial latex fixation tests in a large group of patients with rheumatic diseases revealed a pattern of agglutination with only the euglobulin fraction of serum and not with the whole serum. Repeat freezing and thawing of whole serum in the course of these studies appeared to convert a hitherto negative whole serum latex agglutination to a positive latex whole serum agglutination. These experiences suggested an active inhibitory mechanism that might be relatively unstable.

Materials and methods. The patients were from the outpatient and inpatient services of the Maimonides Hospital. The Fraction II latex particle tests were performed by the method of Singer and Plotz(1). Squibb's lyophilized fraction II was used throughout. Euglobulin fractionation was performed using Ziff's method(2) but modified so that the precipitate was redissolved in glycine saline buffer in order to utilize the latex system. Sheep cell agglutinations were also performed by the method of Ziff and the serum, as is customary, was inactivated by heating to 56° for ½ hour before absorption. Standing sera latex particle agglutinations were performed as follows: Immediately after being separated from whole blood, serum was allowed to stand for 48 hours at room temperature. Latex particle agglutination was then carried out in the usual manner. In other procedures, 2-fold and 4-fold additions of normal fresh serum from the same patient or from healthy donors were

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