SUMMARY

For optimal examination and evaluation of component sacs of the ruminoreticulum, a new dissection technique using the following steps was devised. First, manually detach serosal, fibrous and smooth muscle attachments in all grooves and then lay the ruminoreticulum on its right side and make three incisions. Start the first incision on the cranial surface of the reticulum and cut dorso-caudally in a sagittal plane to a point where an extension of the ruminoreticular groove would cross the dorsal greater curvature. Make a second incision from the cranial to caudal grooves, just dorsal to the left longitudinal groove and then join the end of the first incision with the cranial end of the second. Examine the mucosa and dissect the caudodorsal, caudoventral blind sacs and ventral sac free so that their mucosa may be examined carefully. Suggestions for photography of the ruminal mucosa and its preparation for light and scanning electron microscopy are given.

(Key Words: Rumen, Dissection, Scanning Electron Microscope.)

INTRODUCTION

Although numerous authors have described changes in the capacity, muscular development, and papillae of the rumen under the influence of different rations (Brownlee, 1956; Harrison et al., 1960; Stobo et al., 1966; Tamate et al., 1962) few have described, in detail, dissection techniques that allow careful evaluation of the mucosa of the ruminant forestomach. The standard necropsy technique of cutting along the greater curvature of a viscus is not optimal for exposure of the ruminal mucosa because the rumen is divided into smaller sacs. Also this incision divides the dorsal wall of the dorsal sac, making comparison of changes in that wall difficult. This paper describes a technique which allows orderly and consistent examination, photography, and preparation for scanning electron microscopy of samples of the mucosa of the different sacs of the ruminoreticulum.

Nomenclature of areas of the ruminoreticulum has been inconsistent in the literature. To avoid confusion the nomenclature of the Nomenclature of the Anatomia Veterinaria (NAV) (Int. Comm. on Vet. Anat. Nomenclature, 1973; Nickel et al., 1973) is used in this paper with the older terminology (Sisson and Grossman, 1938) in parentheses.

METHODS

Cut the esophagus several centimeters cranial to the cardia. Free the ruminoreticulum from the omasum and abomasum by cutting through the reticulo-omasal junction. Lay the ruminoreticulum first on its right side, then on its left and free the serosal, fibrous, and smooth muscle attachments around the cranial, caudal, left and right longitudinal, dorsal and ventral coronary and ruminoreticular grooves so that the caudodorsal and caudoventral blind sacs, recessus ruminis (cranial portion of the ventral sac), and cranial sac of the rumen (anterior dorsal blind sac), and reticulum are clearly delineated. Then lay the ruminoreticulum on its right side and make a knife incision on the cranial surface of the reticulum. Continue cutting dorso-caudally, in the sagittal plane, just to the left of the cardia until a point is reached where a dorsal extension of a hypothetical line through the ruminoreticular groove would cross the dorsal greater curvature (figure 1).

Make an incision into the dorsal sac at the caudal end of the cranial groove (just dorsal to the left longitudinal groove) and cut to the cranial end of the caudal groove thus leaving...
Figure 1. Left lateral view of the bovine ruminoreticulum. R = reticulum, CS = cranial sac, DS = dorsal sac, VS = ventral sac, RR = recessus ruminis, CDWS = caudodorsal blind sac, CVBS = caudoventral blind sac, rr gr = ruminoreticular groove, cr gr = cranial groove and cau gr = caudal groove. The initial incision (starred line) starts on the cranial surface of the reticulum and extends to a point where an extension of the ruminoreticular groove would cross the dorsal greater curvature. The second incision (dashed line) extends from the cranial to caudal grooves just dorsal to the left longitudinal groove. The final incision (dotted line) joins those two incisions.

the left longitudinal pillar attached to the ventral sac. Join the cranial end of the last cut with the caudal end of the first incision (figure 1). Open the ruminoreticulum and examine its contents and the mucosal surfaces of the different sacs. By judicious folding, much of the mucosa can be exposed for photography. However, for most careful examination, photography, and ease of fixation, the different ruminal sacs must be separated. Dissect the caudodorsal and caudoventral blind sacs free by cutting completely around their peripheries cranial to the dorsal and ventral coronary pillars, but the caudal pillar is left attached to the ventral sac. Because the coronary pillars are incomplete dorsally in the caudodorsal blind sac and ventrally in the caudoventral blind sac, the isolated sacs can still be oriented into their normal anatomic positions. Remove the ventral sac from the dorsal sac by cutting just dorsal to the right longitudinal and cranial pillars. Both the right and left longitudinal and cranial and caudal pillars will be attached to the ventral sac and allow orientation. The dorsal sac, cranial sac of the rumen (anterior dorsal blind sac), and the caudal pillar is left attached to the ventral sac. Join the cranial end of the last cut with the caudal end of the first incision (figure 1). Open the ruminoreticulum and examine its contents and the mucosal surfaces of the different sacs. By judicious folding, much of the mucosa can be exposed for photography. However, for most careful examination, photography, and ease of fixation, the different ruminal sacs must be separated. Dissect the caudodorsal and caudoventral blind sacs free by cutting completely around their peripheries cranial to the dorsal and ventral coronary pillars, but the caudal pillar is left attached to the ventral sac. Because the coronary pillars are incomplete dorsally in the caudodorsal blind sac and ventrally in the caudoventral blind sac, the isolated sacs can still be oriented into their normal anatomic positions. Remove the ventral sac from the dorsal sac by cutting just dorsal to the right longitudinal and cranial pillars. Both the right and left longitudinal and cranial and caudal pillars will be attached to the ventral sac and allow orientation. The dorsal sac, cranial sac of the rumen (anterior dorsal blind sac), and reticulum are still attached together. The cranial sac has already been separated from the dorsal sac on the left side (figure 1); make a similar cut on the right side to free it completely. Leave the reticulum attached to the cranial sac to allow easy orientation. Then all component sacs of the rumen are free and can be examined individually.

Photography. The ruminoreticulum is photographed by a 35-mm, single-lens reflex camera fitted with a 50-mm macro lens. Best illumination is supplied by a small halogen spotlight at 75° to the lens-subject axis and one photoflood as a fill-in lamp at 45°—60° to the lens-subject axis as described by Eastman Kodak (1966) (figure 2). Individual sacs of the rumen are photographed at half normal size (ratio of reproduction = ½) with same equipment. Usually only one spotlight is required to bring out the surface texture. Small sacs such as the caudodorsal blind sac can be supported over the bottom of a small bottle to allow the mucosa to lie flat (figure 3). Macrophotographs are taken with either a Zeiss Tessovar 2 or a Leitz Aristophot 3 camera. Light placement is critical to bring out texture and morphology of the papillae. Small spotlights are most successful (figure 4). Black backgrounds are easily obtained by suspending a 5 × 7.5 cm microslide over a box whose inside is painted black.

Fixation for Histopathology and Scanning Electron Microscopy (SEM). Each sac of the rumen is washed gently with cold tap water to remove ingesta and debris, then placed in 10% buffered neutral formalin (10% BNF) (Luna, 1968; Tamate and Kikuchi, 1971); at least 10 volumes of formalin to each volume of tissue. Individual containers for each ruminal sac have

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2 Carl Zeiss, Inc., 444 Fifth Avenue, New York, N.Y. 10018.
3 E. Leitz, Inc., Rockleigh, New Jersey.
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transitional fluid. Specimens are attached to metal stubs with conductive silver paste and double coated with carbon and gold palladium under vacuum on a rotating oscillating stage. They are examined under a scanning electron microscope at 5 KV at magnifications from 10–1,000×.

Discussion

Because the rumen is subdivided into component sacs, it is difficult to examine the whole of its mucosa easily. The most convenient method is to dissect each sac free, but if this is done, orientation of isolated sacs can be difficult. In our technique individual sacs can be oriented by coronary pillars attached to the caudodorsal and caudoventral blind sacs and the longitudinal pillars in the ventral sac. Another advantage is that the dorsal sac is removed in toto with its dorsal wall intact by incising just dorsal to the longitudinal grooves. This approach is similar to the one devised by Stobo et al. (1966). They incised from the reticulo-omasal orifice, along the reticular (esophageal) groove to the esophagus and down the right ruminal artery to separate the dorsal and ventral sacs of the rumen. In our technique the reticulum is incised in a sagittal plane and

proved most convenient. Selected pieces of ruminal wall 25 × 10 mm can be excised, placed on cork or polystyrene and fixed in 2.5% glutaraldehyde in .1 M phosphate buffer at pH 7.2 or in 10% buffered neutral formalin (10% BNF) for scanning electron microscopy. Pieces 8 × 3 mm are examined under a dissecting microscope to check the orientation of the papillae, dehydrated through graded ethanol, amyl acetate and dried in a critical point dryer4 using carbon dioxide as the

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4 Denton Vacuum Inc., Cherry Hill, New Jersey.

Figure 3. Mucosal surface of the caudoventral blind sac laid over the mouth of a small bottle to provide a flat field, X1.5.

Figure 4. Ruminal papillae, Zeiss Tessovar, X7.5.

Figure 5. Scanning electron micrograph of ruminal papillae, X18. Note that detail and depth of field are greater than in the macrophotograph (figure 4.).
the integrity of the reticular (esophageal) groove is preserved. Because the reticulum remains attached to the cranial sac, and the incision into the reticulum is sagittal, it is easy to identify ventral and lateral walls of the cranial sac. The ventral floor of the cranial sac has been the most frequently sampled site in most studies and thus it is important to be able to accurately locate the ventral floor of the cranial sac in different rumens.

Photographic results are good but, to bring out the texture of the papillae, low-placed spotlights are essential, even though arranging them is time consuming. Because resolution of a light optical system cannot compete with that of the SEM, macrophotographs taken with the Zeiss Tessovar or Leitz Aristophot are less detailed than S. E. micrographs (figures 4 and 5). SEM results are good but heavily keratinized papillae frequently need several coats of carbon and gold to reduce "charging". A similar problem was noted by Scott and Gardner (1973) and Bayer et al. (1974).

Preservation of delicate mucosa for SEM usually involves washing the surface with isotonic solutions followed by glutaraldehyde fixation. However, Tamate et al. (1971) could find no remarkable differences among tissues fixed in Palade's solution, 2.5% glutaraldehyde, 10% BNF, or neutral formalin. Therefore, routinely we wash the ruminal mucosa gently with cold water or 10% BNF to free it of debris and fix it in 10% BNF.

Because terminology used by different authors to describe sampling sites of the rumen has varied so widely, it has been very confusing. Most mucosa has been sampled from the cranial sac of the rumen (anterior dorsal blind sac), variously called "anterior dorsal blind sac" (Stobo et al., 1966), "anterior dorsal pouch" (Cody et al., 1972), "anterior dorsal blind sac of the rumen" (Brownlee, 1956; Tamate et al., 1962), and "dorsal cranial blind sac of the rumen" (Harrison et al., 1960). Unfortunately some descriptions leave doubt as to which sacs were sampled. For example, three papers (Haskins et al., 1969; Sinclair and Kunkel, 1959; Stobo et al., 1966) call the sampling site "ventral", but most likely refer to the cranial sac of the rumen. The standardized terminology of the NAV should be used to eliminate such confusion. Fortunately an excellently illustrated textbook in English (Nickel et al., 1973) utilizing the NAV has recently become available.

**LITERATURE CITED**


