DIET EFECT ON RUMINANT FORESTOMACH STRUCTURE[1]

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INTRODUCTION

A recent increase in number of condemned cattle forestomachs at abattoirs has focused attention on the need for more information about the structure of this organ.

A change in feedlot procedure to the use of high concentrate-low roughage fattening diets, some of which are pelleted, has paralleled the increased incidence of apparently abnormal rumens (first compartment of the ruminant forestomach). Because these modern diets produce faster gains and more efficient conversion of feed to meat, they are expected to be widely used. Information on the effects of various diets on forestomach structure can be of profound economic importance to the cattle and sheep feeder.

A review of literature on histology of the rumen wall has not been published; hence, one objective of this article is to cite all pertinent references to rumen structure. Another objective is to report the results of a study of rumen epithelium from sheep and cattle which were fed diets differing in chemical composition, texture and level of nutrition.

STRUCTURE OF THE RUMINANT FORESTOMACH

The forestomach of cattle and sheep is characterized by three compartments, the rumen (paunch), reticulum (honeycomb) and omasum (manyplies or book). Because ingested food may move freely btween the rumen and reticulum, the term ruminoreticulum is being increasingly used to designate these compartments. Figure 1 illustrates the nomenclature of the ruminoreticulum and the general nature of the strata or layers of ingested food within it.

In adult ruminants, the epithelium lining the interior of the rumen is arranged in leaf-shaped projections of the mucosa called papillae. These vary in size, shape and density (number per unit of area) in the different regions of the rumen and from animal to animal. In general, they tend to be flat,

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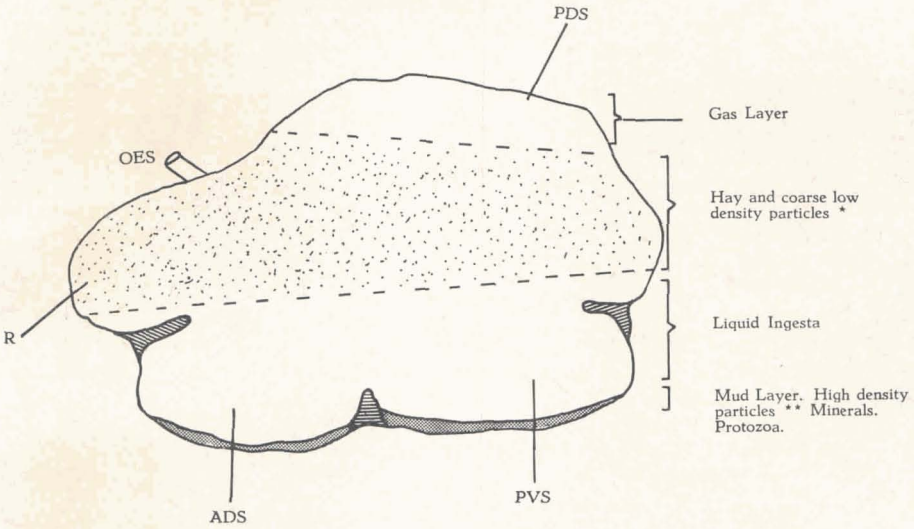

* Higher in T. N., N. P. N., NH3, V. F. A., Crude Fiber.

** Amount deposited will vary with physical character of diet.

Fig. 1. Rumen Strata.

oval or tongue-shaped and they are most dense in the lower region of the rumen which is occupied by the fluid ingesta. Papillae lining the dorsal or top part of the rumen are rudimentary without size or shape. Figure 2 shows that papillae have a lamina propria or central core which consists of collagen fibers interspersed by blood and lymphatic vessels which are in close contact with the basal layer (Dobsen *et al.* 1956). The central core is surrounded by non-glandular stratified epithelium. The closest stratum is a continous single layer of columnar cells called the stratum basale or basal layer. Numerous mitochondria have been described in the columnar cells in the stratum basale (Dobson *et al.* 1956). Three additional layers termed stratum spinosum, stratum granulosum and stratum corneum are not always present, separate and well-defined according to Dobson *et al.* (1956), Trautman and Fiebiger (1957), Wardrop (1961a) and Hendriksson and Habel (1961). The stratum basale and stratum spinosum are often termed the stratum germinativum because these layers germinate new cells which push out into the surface layers. A transitional zone, stratum transitionale, is described by Henriksson and Habel (1961) as a zone containing the stratum granulosum, if present, and adjacent cells

beneath the stratum corneum. Cells in this layer may contain keratohylin granules which stain deeply with hematoxylin. These are involved in the process by which soft keratin is formed (Bloom and Fawcett, 1962). The stratum corneum comprises the outermost layers of cells in the epithelium. These are mostly keratinized, flattened cells, part of which are continually solughing off into the rumen cavity.

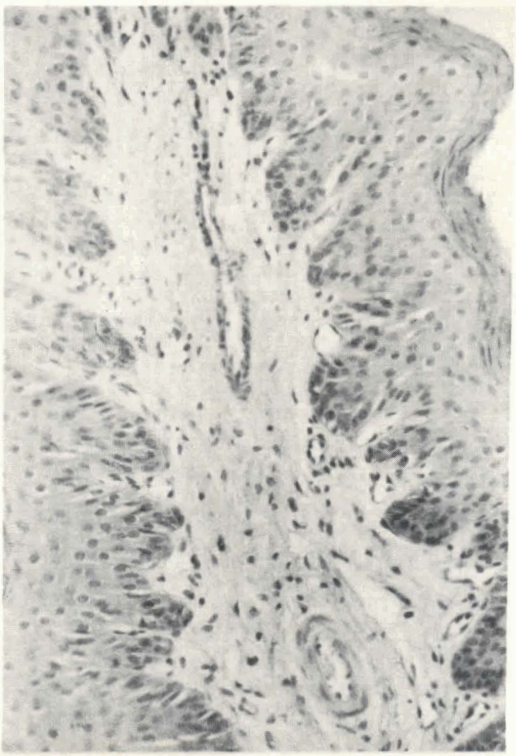

Fig. 2.—Strata of rumen papillae H + E × 20.

The largest blood and lymph vessels are found in the submucosa (Trautman and Fiebiger, 1957). The muscularis consists of two smooth muscle layers with muscle fibers laying at right angles to each other. The serosa contains nerves and blood vessels and may contain lipid deposits.

RELATION OF DIET TO RUMEN EPITHELIAL STRUCTURE

The role of the rumen mucosa in the absorption of nutrients has stimulated studies on how these tissues are developed to their maximum functional capacity.

That the diet exerted some effect on rumen development was not convincingly shown until the publication of Warner, *et al.* (1956). They postulated that the general histological development of the rumen wall was dependent largely on age but the detailed structure of this organ could be affected by the type and physical nature of the diet.

Wardrop (1961a) showed that the type of nutrition was more important than the plane of nutrition in determining growth rates of the fore-stomach of the lamb and that plant food was necessary for the full development of the anatomical and histological structure of this organ, particularly as far as the stratum corneum and stratum granulosum were concerned.

Harrison *et al.* (1960) observed greater papillary growth in calves receiving a 90 per cent concentrate ration than those receiving a reciprocal hay diet. A diet of milk and wood shavings produced considerable muscular development but no papillary growth. The latter finding suggests that the two tissues are influenced independently.

Brownlee (1956), who observed marked papillary growth of the rumen of calves receiving high energy feeds, has suggested that the amount of absorbable fraction of the diet was more critical in causing papillae to develop than was the texture of the feed.

Maximal rumen development has been associated with the development of a fermentation yielding high proportions of volatile fatty acids (VFA) Omar *et al.* (1962). Sutton *et al.* (1963) demonstrated that the ability of the (VFA) calf rumen to absorb VFA is low shortly after birth and does not change significantly during the first six months when the calf is fed milk only. Introduction of solid feed into the diet led to a marked increase in absorptive ability, the latter being closely related to the degree of papillary development and apparent physiological changes in the rumen mucosa. The nature of these physiological changes was described by Sanders *et al.* (1959). He proposed that an actively metabolizing rumen mucosa would stimulate the structural development and the absorptive ability of the mucosa.

Johns *et al.*, (1963) feeding sheep a mixture of rye grass and clover, observed a correlation of ruminal VFA production, especially butyric and propionic acids, with development of rumen epithelium.

Walker and Simmonds (1962) showed that rumen wall and epithelium development depended mainly on absorption of butyric and propionic acid. A preference for absorption of butytric over propionic or acetic acid by the ruminal mucosa followed closely by ketone body formation was observed. Pennington (1952) also demonstrated that the rumen mucosa metabolized butyric acid to a great extent and to a lesser extent acetic and propionic acids, with ketone bodies formation by butyric acid.

Tamate *et al.* (1962) indicated that VFA such as propionic and butyric can stimulate papillary growth if the amount is sufficient. In Sanders's *et al.* (1959) study 210 equivalents of propionic acid over a period of 11 weeks stimulated papillary growth. However, the minimum amount of equivalent of VFA to produce papillary growth has not been determined.

Rhodes and Woods (1960) found more butyric acid utilization by rumen epithelium in lambs receiving a pelleted concentrate ration than in those receiving a long hay ration. Rumen pH was lower for lambs on pelleted rations. In agreement with this data, Cullison *et al.* (1961) observed a significantly lower rumen pH in steers receiving a pelleted ration.

Sutton *et al.* (1963) reported that VFA absorption is slower at alkaline than at acid pH and that absorption of these acids decreased as their chain length decreased at acid pH values. Experiments by Pfander and Phillipson (1953) also showed than in an acid pH the specific absortion rates are in the order of butyric > propionic > acetic.

Dobson (1959) reported that increased fatty acid concentrations in the rumen could increase blood flow from the rumen. The relative rate of increase was butyric > propionic > acetic. The specific effects of an increased blood flow were associated with rapid tissue growth.

In usual roughage diets, acetate accounts for 65 to 70 molar per cent of the total, propionate for 15 to 20 per cent, butyrate for 10 per cent (Annison and Lewis, 1959). In general, the proportion of acetate decreases whereas that of propionate and butyrate increases as the proportion of concentrates increases in the diet (Bath and Rook, 1963; Elliot and Loosli, 1959; Shaw *et al.* 1960). Studies conducted by Rhodes and Woods (1962) and Woods and Luther (1962) revealed high total VFA and lower pH levels for lambs fed a pelleted high concentrate diet when compared to lambs fed a ground diet. Gray and Pilgrim (1951) reported that as the concentration of total VFA in the rumen increased, there was a decrease in the ratio of acetic to propionic acid. Popjak *et al.* (1951) found that 80 per cent radioactive carbon of acetate injected into a goat was oxidized to carbon dioxide in 6 hours.

A condition termed "rumen parakeratosis" characterized grossly by a hardened enlargement and capping of the papillae and microscopically by the accumulation of excessive layers of keratinized nucleated squamous cells on the papillae has been observed in rumens of cattle receiving high-concentrate and/or pelleted rations (Beardsley *et al.* 1959; Hinders and Owens, 1965; Hopkins *et al.*, 1960; McCroskey, 1961; Thompson *et al.*, 1958). However, such conditions were not present when long hay or oat straw were fed as part of the diet (Cullison et al., 1961; Alexander and Hentges, 1962; Hinders and Owens, 1965; Palmquist, 1963).

Jensen *et al.* (1958) observed that the conditions, which appear to favor ruminities were (1) feeding a ration in which the ratio of concentrate to roughage was high (3:1); and (2) making a rapid change from a ration of roughage to a ration high in concentrate.

Garret et al. (1961) reported an increase of VFA production on finely ground and pelleted alfalfa, coupled with less rumination and a likely decrease in the buffering capacity of the rumen. Similarly in Cullison's *et al.* (1961) experiment, animals fed ground or pelleted rations revealed less than normal rumination and cud chewing. In contrast, normal rumination occurred in cattle receiving long hay or straw. Harrison's *et al.* (1960) and Warner's et al. (1956) findings indicated that the additional weight contributed by inert materials stimulated rumen motility and, in turn, greater muscular development. A significant and common observation in cases of rumen parakeratosis is a matting of feed particles between the papillae. Probably this condition is a reflection of poor muscle tone of the rumen wall, and consecuently, poor mixing of the rumen contents and rumination. Hinders *et al.* (1961) observed that animals fed rations which produce parakeratosis consumed large amounts of dirt or gravel and consumed five to ten times the normal amount of salt and bonemeal. The hunger for fibrous feeds and the high consumption of minerals by cattle on pelleted and/or high concentrate rations may be a response to a demand to maintain rumen "fill" (Ward, 1962).

The low pH found in various experiments (Cullison, *et al.*, 1961; Rhodes and Woods, 1960; Sutton, *et al.* 1963) where high-concentrate pelleted diets were fed could be the result of slower neutralization of VFA by a decreased flow of salivary secretions. Low-hay, high-concentrate rations produced a decrease in the ration of acetic to propionic acid and a marked decrease in the time cows spent ruminating with a consequent decrease in the inflow of saliva to the rumen (Balch and Rowland, 1957). Dukes (1955) stated that one of the special functions of saliva in the rumen was the neutralization of organic acids produced by bacterial action, and that the normal stimulus for salivation in ruminants was roughage in the diet. Furthermore, parotid glands developed in response to the mechanical stimulation of the food of which hay provided the greatest stimulation (Wilson, 1963).

Sinclair and Kunkel (1959) reported pigmentation of the mucosa by feeding pelleted diets. Significant darkening of the epithelium was observed by feeding oxytetracycline and terephthalic acid in combination with unpelleted feeds. Packett and Butcher (1963) observed a decrease in pigmentation of the mucosa by feeding oxytetracycline with pelleted feed as compared to pelleted with or without sodium citrate. Tamate *et al.* (1962) localized a green pigment as present in the outermost layer of desquamating cells of the stratum corneum, which is in direct contact with the rumen contents. Existence of this pigment in the same locus of the rumen mucosa has been reported by Nicolai and Stewart (1963). In view of these observations, these authors have suggested that the pigment represents products of microbial activity, especially since it appears to be affected by dietary antibiotics.

MATERIALS AND METHODS

Tissue sections of tunica mucosa were removed from the anteriorventral part of the cranial sac of the rumen after exanguination of all subjects, and fixed in 10 per cent formalin or 80 per cent alcohol solution.

All tissues were embedded in paraffin and cut at 6 u. Staining methods included Delafied's hematoxylin and cosin, Von Kossas's silver nitrate and Pearse's alizarine red S for calcium, and Lillies's nile blue and ferric-ferricyanide for melanin pigments. Tissue samples for the periodic Acid-Schiff Reaction were fixed for 24 hours at 4° C. in 80 per cent alcohol containing 1.0 gm. of trichloroacetic acid per 100 ml. After being embedded in paraffin, they were stained according to the method of Pearse and Everson (1960).

EXPERIMENT I: Effect of Ground vs. Long Hay on Rumen Characteristics.

In a completely randomized design, twenty-four pregnant first-calf heifers were divided into three lots of eight animals each, with four Herefords and four Brahmans in each lot. Rations consisted of three Coastal Bermudagrass hays fertilized and processed as follows: 100 lb. nitrogen per acre, fed long; 200 lb. nitrogen per acre, fed long; and 200 lb. nitrogen per acre, fed finely ground. Each hay was offered *ad libitum* with trace mineralized salt and defluorinated phosphate offered free choice. The finely ground hay was processed in a hamermill with a 1/4 in. mesh screen and was fed in air-dry form in a self-feeder.

EXPERIMENT II: Effect of Low and High Protein Levels on Rumen Characteristics.

Sixty male Herford calves were divided into two dietary treatments, low and high protein groups. Forty of the sixty calves were then accordingly alloted to four sub-groups of ten each and castrated at the following days of age: 2-3, 60 $\pm$ 10, 210 $\pm$ 10, 255 $\pm$ 10 days. Twenty calves were left intact. The experimental period, 182 days, was divided into two phases of 98 and 84 days respectively. During phase I, half the animals received a high protein ration (15 per cent crude protein) and half recived a low protein ration (13 per cent crude protein). In phase II the protein ration was 13 per cent and the low protein ration 11 per cent crude protein. All groups recived an average of approximately two pounds of long grass hay per head per day.

EXPERIMENT III: Effect of Phosphorus Deficient Diets on Rumen Characteristics.

Three groups of 12 Florida native rams were randomly assigned to lots to receive two phosphorus-deficient diets differing only in source of dietary nitrogen, urea or Drackett protein, and one lot to receive a control diet. The diets were semi-purified with Solkafloc being used as a source of cellulose for bulk. The phosphorus content of the diets was 0.67%, 0.6% and 0.015% for the control, phosphorus-deficient (Drackett protein) and phosphorus-deficient (urea nitrogen) diets respectively. Later, these groups were subdivided with one-half of each being irradiated.

Irradiation was done in a cobalt 60 facility with the rams in wooden crates being subjected to whole body gamma irradiation at the rate of 3.27 roentgens per minute for a total dose of 300 roentgens.

EXPERIMENT IV: Effect of Dried Citrus Meal and Corn Meal on Rumen Characteristics.

Yearling Hereford and Angus steers were kept in open sandy lots and randomly assigned to five ration treatments. All subjects were allowed free access to self-feeders containing the pelleted ration described in Table 1. Low quality grass hay was offered *ad libitum* in a different feeder; later, the hay allowance was varied and controlled.

TABLE 1.—Ingredient Composition of Rations

TREATMENT	A	B	C	D	E
Varying ingredients					
Corn meal	72.0	54.0	36.0	18.0	0.0
Citrus meal	0.0	15.8	31.6	47.4	63.2
SBOM, 44%	2.5	3.5	4.5	5.5	6.5
CSOM, 41%	2.5	3.5	4.5	5.5	6.5
Defluorinated phosphate	0.0	0.2	0.4	0.6	0.8
Fixed ingredients					
Urea, 262%	1.0	1.0	1.0	1.0	1.0
Cane molasses	5.0	5.0	5.0	5.0	5.0
alfalfa meal	5.0	5.0	5.0	5.0	5.0
Mineral & vitamin mix[a]	2.0	2.0	2.0	2.0	2.0
Corn cobs, ground	10.0	10.0	10.0	10.0	10.0
Aurofac 10 Grass hay[b]	0.0375	0.0375	0.0375	0.0375	0.0375

a Mineral mix contained 18.9% calcium, 5.5% phosphorus, 30% sodium chloride, 1.4% iron, 0.108% copper, 0.01% cobalt, 0.48% manganese and 0.01% iodine. Vitamin mix contained 10,000 I. U. of Vitamin D2 per lb., and was adjusted to provide an average intake of 20,000 I. U. vitamin A per day.

b Varied uniformly among lots from none to two ponds per day after initially allowing **ad libitum** access for 14 days.

RESULTS AND DISCUSSION

Experiment I:

The rumen papillae were better developed in cows fed long hay than in those fed ground hay longer and wider. The stratum corneum of the stratified squamous rumen epithelium in cows fed ground hay was thicker. The cells of the stratum granulosum were more granular than in the corresponding epithelium of cows fed long hay (Figs. 3,4). The basal layer of the epithelium from

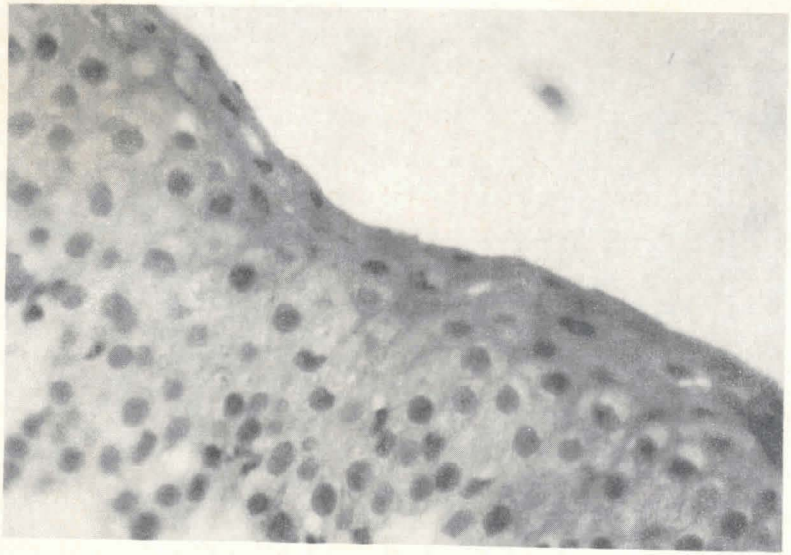

Fig. 3. Surface epithelium of papilla from rumen of a cow fed long hay. Stratum corneum is thinner and Stratum granulosum is less granular than in cattle fed ground hay.

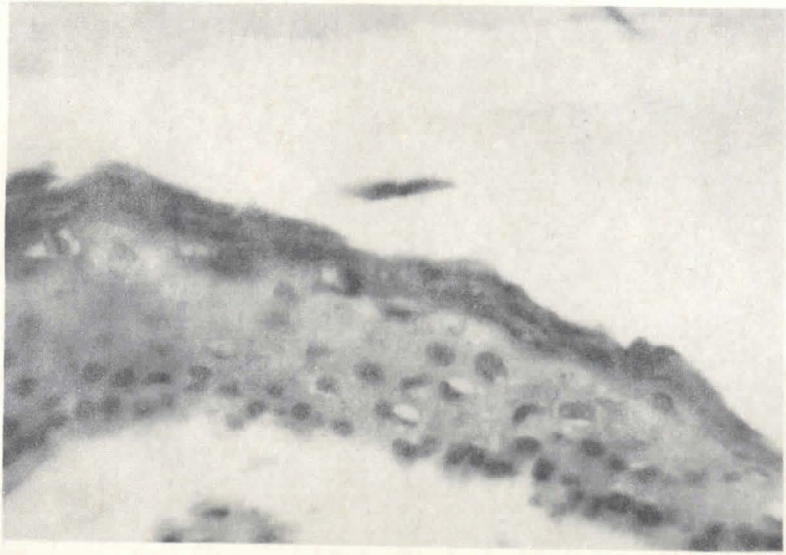

Fig. 4. Surface epithelium of papilla from rumen of a cow fed ground hay. Stratum corneum is thickened. H -|- E, X 105.

cows fed long hay was arranged in more obvious well-rounded folds. Papillary bodies between such folds were deeper in cattle fed long hay (Figs. 5, 6). Arterioles and lymphatics in the lamina propria were also better developed in the long hay-fed group. No differences in lipid infiltration of the epithelium were observed due to treatments.

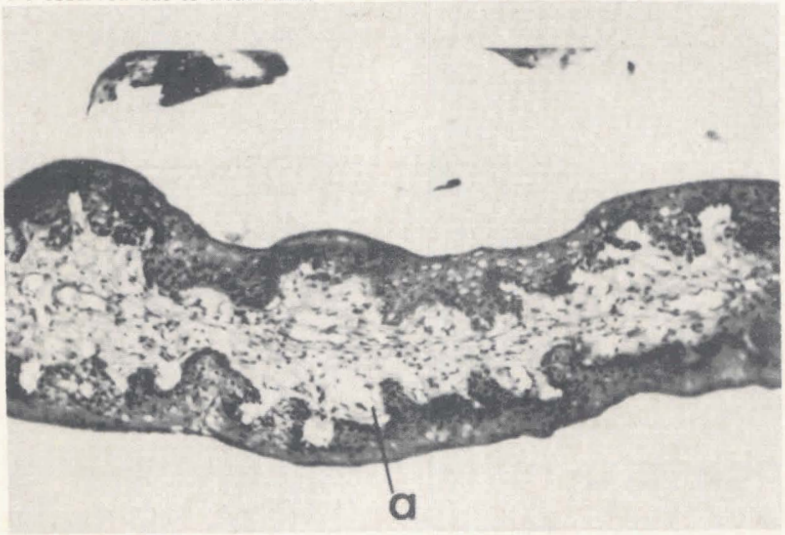

Fig. 5. Ruminal papilla from cow fed long hay. Note deep papillary bodies (a). H -|- E, X 90.

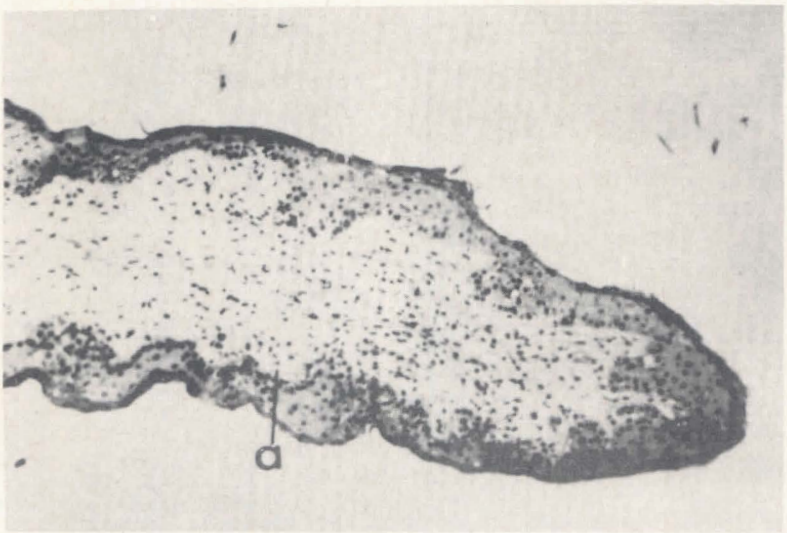

Fig. 6. Ruminal papilla from cow fed ground hay. Note poor development of papillary bodies (a). H -|- E, X 90.

Experiment II:

Animals receiving the low protein diet had extremely thin (0.8 cm.), long (5.0 cm.), brown colored papillae and there were areas on the rumen surface in which complete papillary regression had taken place. The lamina propria of these animals was poorly developed and contained a small network of collagenous fibers and small arteries. The stratum germinativum was not well defined and the papillary bodies were poorly developed (Fig. 7). In contrast,

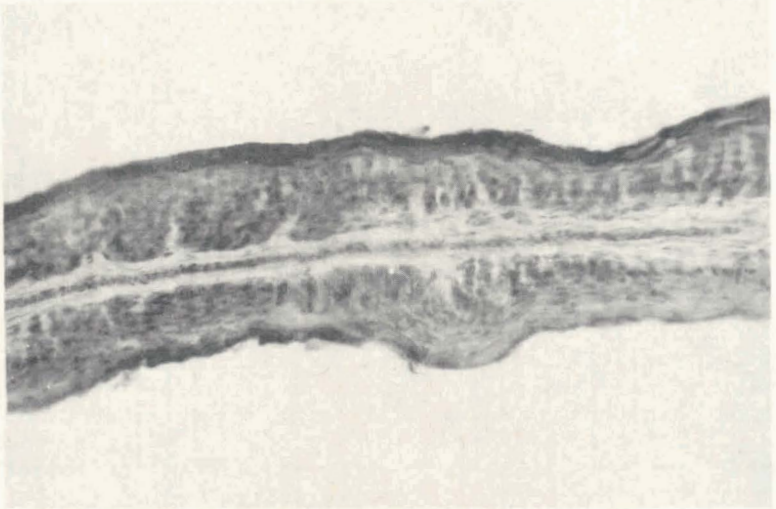

Fig. 7. Experiment II. Low protein diet papillae. Note poorly developed lamina propria and thin epithelium. Stratum corneum present. H -|- E, X 90.

sections from animals on the high protein diet showed more extensive development of the lamina propria and blood vessels. Cells in the basal layer were more numerous and stained darker with hematoxilin and eosin (Fig. 8). The stratum corneum was present in both groups as desquamating keratinized cells. Although no signs of foreign particle encrustation were observed in the stratum corneum, this layer appeared thicker than in sections from animals receiving only hay. Cullison *et. al.* (1961) reported rumen papillae with a thin stratum corneum when hay was fed at a rate of two pounds per head per day in contrast to a thicker stratum corneum with encrusted particles in a ration without hay. Wardrop (1961) stated that hay contains Opal-phytoliths which act as abrasive materials and tend to wear down the outermost layer of the rumen epithelium. Apparently feeding two pounds per head per day was enough to prevent deposition of particles in this experiment, but was not enough to erode the stratum corneum down to the extent of those papillae found in animals from experiment I. Still the question arises whether this is the true explanation for a thicker or thinner stratum corneum layer, or due to a more active metabolism coupled with cell division in the mucosa of those animals receiving higher volatile fatty acids producing diets.

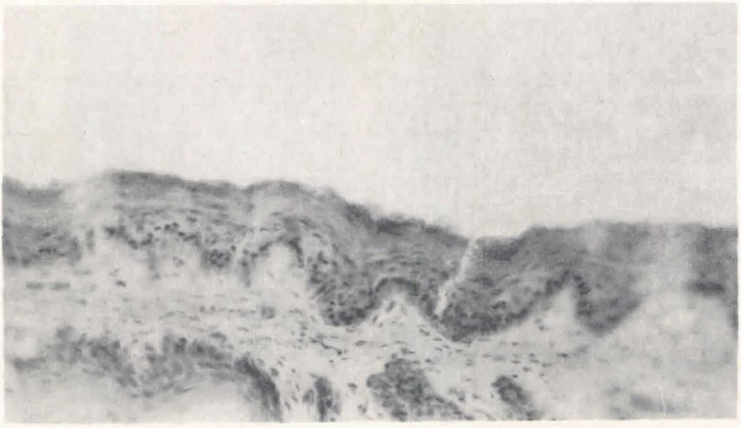

Fig. 8. Experiment II. High protein diet. Epithelium shows more stratification, thrown into deeper papillary bodies. H -|- E, X 90.

Experiment III:

Development of rumen papillae varied among treatments. Generally those animals receiving a diet without phosphorus showed complete repression of papillae. It was observed that coloration of rumen mucosa resembled that of the ingesta, varying from yellow to light gray in color. The nuclei of the columnar cells were lightly stained, in all treatments except the control group. The most striking difference in the urea + gelatin group was the poor development of the epithelium, and the distribution of blood vessels. In almost every section examined only a large blood vessel was present in the lamina propria (Fig. 9). Increased vesiculation accompanied with a thicker, better developed

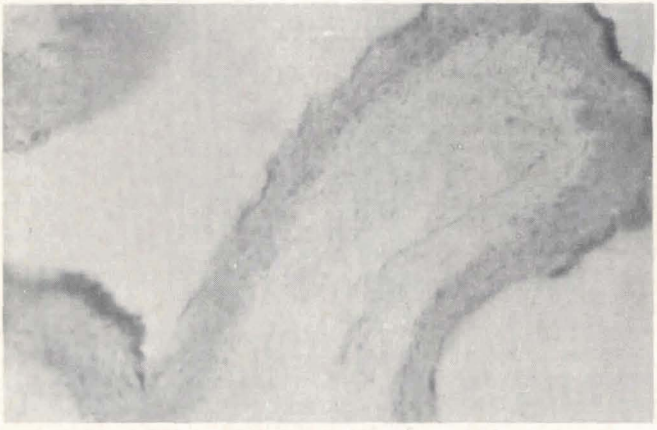

Fig. 9. Experiment III. Urea -|- gelatin. Absence of epithelial development. Undefined basal cell layer. Poor distribution of blood vessels. Cells of stratum corneum densely packed at tip of papillae. H -|- E, X 90.

epithelium was observed in the deficient return group (Fig. 10). In deficient and control irradiated groups a few elliptical or spherods crystals were seen. They consistently appeared close to a large blood vessel showing a dark yellow

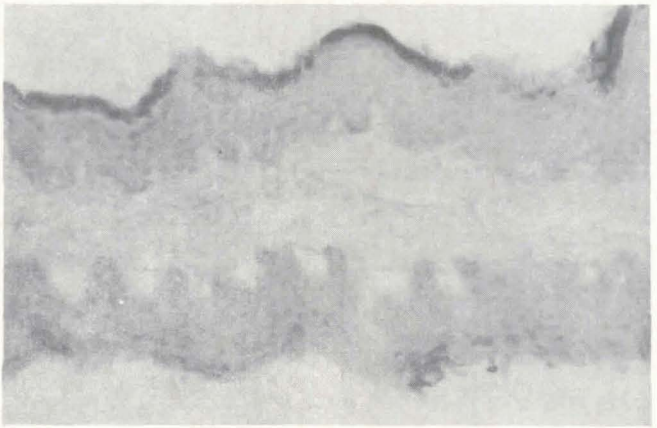

Fig. 10. Experiment III. Deficient return group. Increased vesiculation of lamina propria. Deeper papillary bodies. H -|- E, X 90.

color stained with hematoxylin and eosin. The cause for the presence of these crystals could not be determined. However, unusually large paranuclear vacuoles were observed frequently in the stratum granulosum layer. The vacuoles appeared to have caved in the nuclear membrane as they migrated toward the stratum corneum layer. The contents of the vacuoles were usually unstained and often occurred in large concentrations (Figs. 11, 12). Some of these

Fig. 11. Experiment III. Irradiated groups. Large paranuclear vacuoles present in stratum granulosum layer. H -|- E, X 90.

Fig. 12. Experiment III. Irradiated groups. Paranuclear vacuoles with visible compresed nucleus in stratum granulosum. H -|- E, X 400.

vacuoles were seen to contain, besides their compressed nucleus, the tiny crystals. Since this tendency was to appear in the lamina propria, always close to a blood vessel, fine capillaries were examined and seen to contain these crystals. Presumably these foreign bodies were able to come across the outermost layer of cells which were then transported by the finer blood vessels and cause their accumulation in the lamina propria (Fig. 13).

Fig. 13. Experiment III. Irradiated groups. Crystal accumulation near blood vessel. H -|- E, X 1000.

Experiment IV:

Papillae were characterized by being extremely long (6.5 cm.) and thick (3.4 cm.), leathery and dark in color with persistent firm attachment of particles forming clumps. The size and incidence of these clumps varied among treatments, being more prevalent in animals from treatment C and D (Fig. 14).

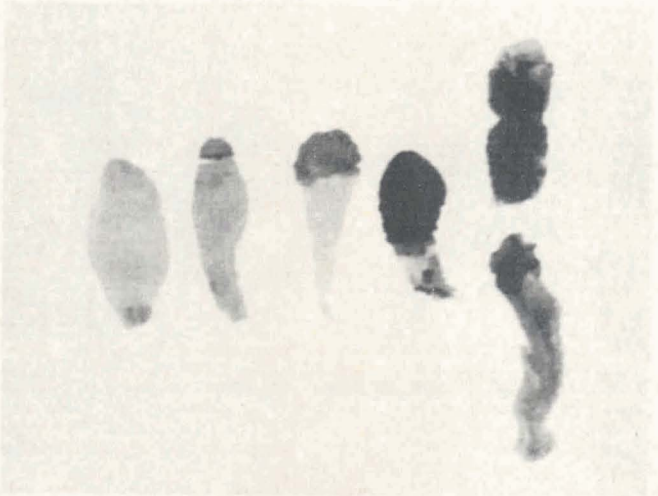

Fig. 14. Experiment IV. From left to right treatments A - E. Observe size of clumps on tip of papillae. Upper right papillae from treatment E shows covering of "mud".

A rich blood and lymphatic supply in close contact with the basal layer (Stratum germinativum) of the epithelium can be observed in figures 15 and 16. Capillary networks were seen to extend as far as the stratum granulosum layer and generally, more numerous and developed blood vessels were observed in samples from this experiment than in those from experiment I. This is an indication of a higher absorption of butyric acid and metabolic activity of rumen mucosa (Dobson, 1959; Rhodes and Woods, 1960; Walker and Simmonds, 1962) since higher production of this acid was reported in experiment IV (Hentges *et al.* 1964) than in experiment I (Alexander and Hentges, 1962). The basal layer of the epithelium, consisting of columnar cells, is well defined and stained darker than others (Fig. 15). Next to this layer a more flattened squamous-type of cell layer which is not always continued is observed. Cells in this layer compound to the stratum spinosum possess intercellular bridges extending from one cell to the other (Habel, 1963). Clearly visible are the cells corresponding to the stratum granulosum lying just outside of the stratum spinosum. The deepest cells of this layer resemble the cells of the stratum spinosum except for their granules that stain deeply with hematoxylin. These granules are probably keratohyalin granules. In skin these granules are concerned in the process by which soft keratin is formed (Bloom and Fawcett, 1962). Beyond this layer nearest the rumen contents are the cells corresponding to the stratum corneum.

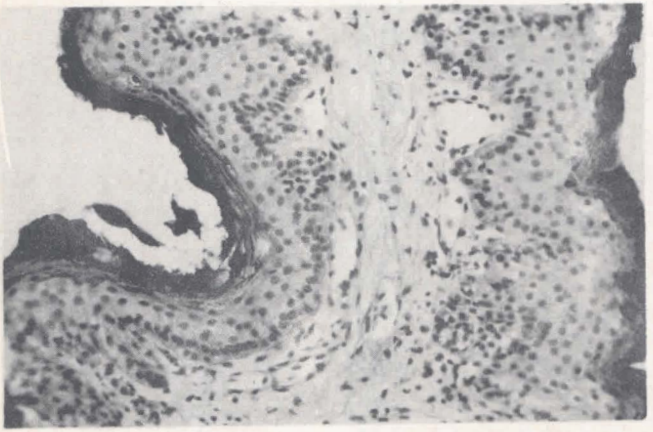

Fig. 15. Experiment IV. Treatment A. Layers of epithelium are clearly visible. Note desintegrated nuclei appearing in stratum granulosum layer. Network of blood vessels seen close to the basal epithelial layer. H -|- E, X 90.

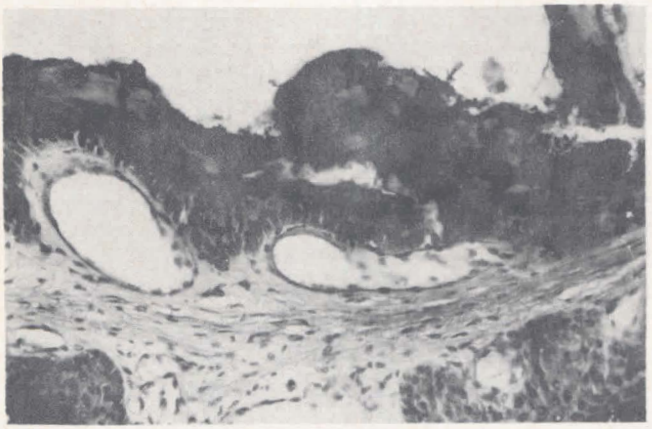

Fig. 16. Experiment IV. Treatment D. Note size of blood vessels beneath stratum granulosum layer. H -|- E, X 90.

Some deposition of feed particles close to the stratum corneum is shown in figure 17, which corresponds to animals in treatment A. The rumen epithelium from animals in treatment B was characterized by the increase in the

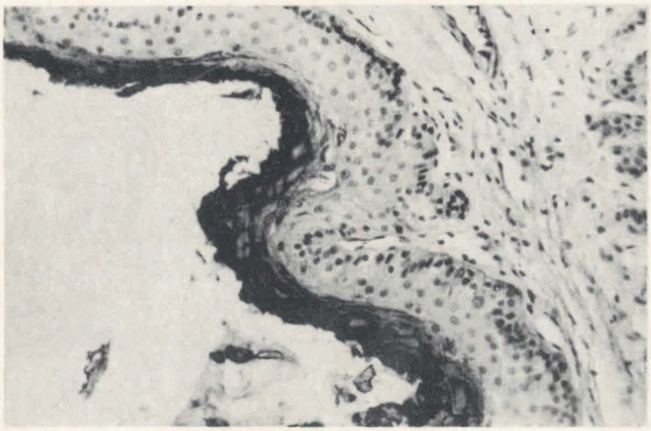

Fig. 17. Experiment IV. Treatment A. Deposition of feed particles present at outermost layer of stratum corneum. H -|- E, X 90.

thickness of the lamina propria as well as the stratum granulosum and corneum as shown in figure 18. Also the layer of particles added to the stratum cor-

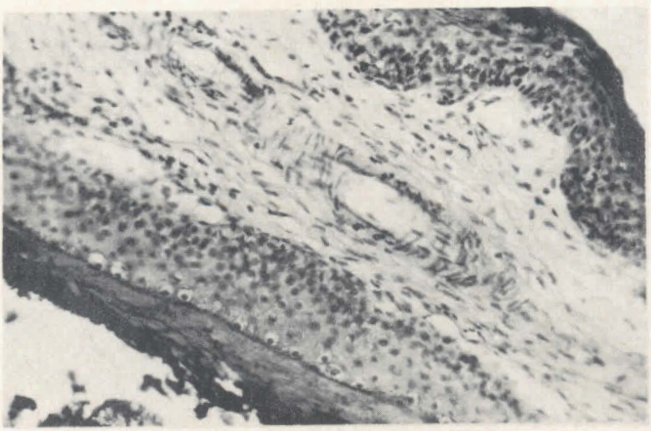

Fig. 18. Experiment IV. Treatment B. Increased vascularity and fibrosis of lamina propria. Note thickness of stratum corneum. H -|- E, X 90.

neum increased in size incidence. Figure 19 shows, closest to the rumen, the layer of particles adhered to the stratum corneum layer. Hyperaemia, fibrosis, and a marked infiltration with neutrophills were characteristic features of samples taken from animals in treatment C. These were accompanied with an increase in the size of the two layers of cells closest to the rumen,

Fig. 19. Experiment IV. Treatment B. Cells in the stratum granulosum are observed on extreme *left side. Densely packed stratum* corneum appear as clear homogenous layer. Particles adhered stained darker. H -|- E, X 400.

as well as deposition of particles (Fig. 20). Presumably the large amount of particles deposited coupled with an increase in the size of the stratum corneum acted as a barrier to absorption of VFA, as previously demonstrated (Hinders

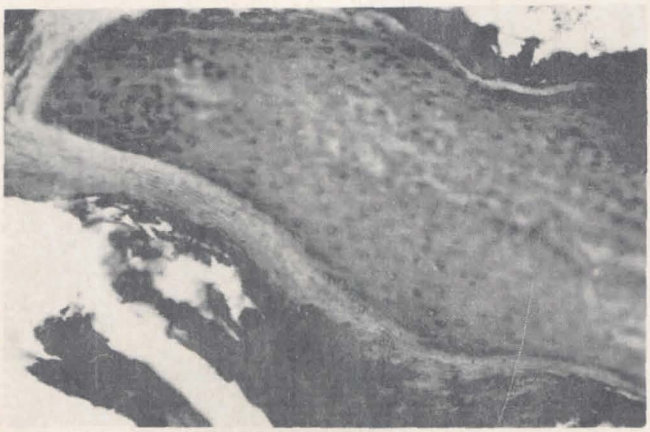

Fig. 20. Experiment IV. Treatment C. Longitudinal section of tip of papillae. Increased stratum corneum layer with larger particle deposition. H -|- E, X 90.

and Owen, 1965). Figure 21 shows the tip of a papillae which was surrounded by adhered particles causing complete degeneration to adjacent cells. Such areas of incrusted particles after prolonged periods of time become dry and probably cause an abstraction of fluid from cells and blood, causing severe dehydration which causes, in turn, a reduction in plasma volume, haemoconcentration and circulatory collapse. Similar results were obtained in treatments

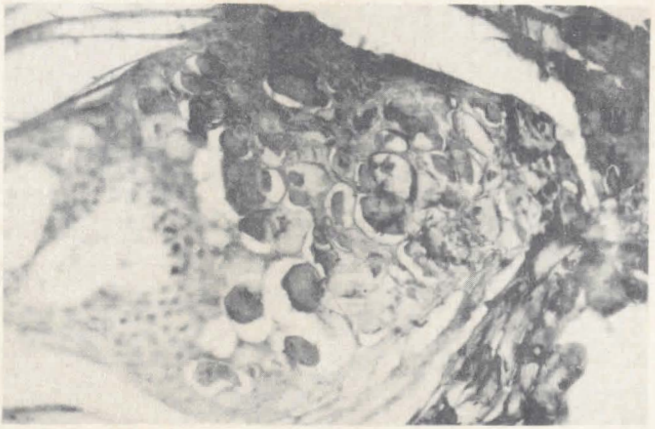

Fig. 21. Experiment IV. Treatment C. Note thick layer of particles forming a clump surrounding tip of papillae. Complete degeneration of cells is observed. H -|- E, X 90.

D and E as shown in figure 22. Papillae in treatment E were characterized by the presence of multiple irregular patches and horny growths. Although hyperaemia and subepithelial fibrosis prevailed in this group, the incidence for clumpness decreased.

Fig. 22. Experiment IV. Treatment D. Particle deposition adhered to stratum cornuem layer. H -|- E, X 90.

Of interest is the formation and histochemistry of the stratum corneum layer of the ruminant forestomach. In skin, the layer closest to the stratum corneum, the stratum lucidum consists of eleidin, which is a transformation product of the keratohyalin observed in the stratum granulosum. In the stratum corneum the eleidin of the stratum lucidum tranformed into keratin (Bloom and Fawcett, 1962). Ellenberger (1911) stated that the epithelium showed the same stratification as the epidermis, except that a stratum lucidum was seldom demonstrable. He described the stratum corneum as "relatively very thick". Dobson *et al.* (1956) was unable to confirm the presence of a stratum lucidum by means of the Buzzi stain for eleidin, however, keratin was present in the stratum corneum layer as shown by the birefringence in polarized light. According to Montagna (1956) and O'Flaherty *et al.* (1956), substantial quantities of fatty acids and their esters are produced by the transformation of cellular phospholipids during the process of skin keratinization. Habel (1959) by means of Sudan black B stain was able to support this hypothesis and identified coarse lipid droplets as triglycarides present in the stratum corneum. In the present study periodic acid-Schiff tests were conducted in Experiment I and IV only. A zone of lipid granules was present in the lamina propria on the surface of the collagen fibers below the stratum germinativum but none in the stratum corneum. Although no apparent differences were observed among treatments, lipid granules were more numerous in rumen samples from Experiment IV than I. The stratum corneum appears as a bright homogenous line that stained orange-red with hematoxylin and eosin, unstained but distinct with alizarine red S, and dark brown to black with silver nitrate.

Microscopic observations of particles from the clumps attached to the papillae, showed great similarity in the texture and coloration of feed particles from the ration. Such clumps were then carefully detached from the epithelium of the papillae and ashed (Snell and Snell, 1949) for determination of calcium (Welcher, 1957) and phosphorus (Boltz and Melton, 1948).

Table 2 shows the percentages of calcium and phosphorus recorded from these analysis. Since determination of plasma levels or feces content of calcium and phosphorus were not part of the procedure of experiment IV, the mechanism or factor responsible for the deposition of calcium and phosphorus con only be postulated.

Parthasarathy and Phillpson (1953) have shown that the rumen epithelium is permeable to a number of electrolytes. The principal sites of calcium absorption in the alimentary tract of the ruminant are apparntly unknown. Storry (1961) reported considerable proportions of calcium and magnesium in a non-ultrafilterable form in all organs of the sheep except the abomasum.

He postulated that the concentration of ultrafilterable calcium and magnesium in rumen fluid are insufficient for these elements to be absorbed as freely diffusing ions. Schachter and Rosen (1959) using surviving everted duodenal sacs, have shown that Ca^{45} can be transported against a concentration grandient from mucosal to the seresal side of the gut membranes, that this process is inhibited by metabolic inhibitors (NaCN, NaN_3 NaF, $HqCl_2$ 2,4-dinitrophenol) and also inhibited by magnesium and cobalt ion, and that vitamin was essential for the operation of the transport system. A variety of anions which precipitate, or complex ionic calcium such as oxalate, philate, phosphate or possibly sulfate, may interfere with calcium absorption if present in excess (Nicolaysen *et al.*, 1953). It has been demonstrated in a variety of animals (Hansard and Crowder, 1957) that, when diets high in calcium are ingested hypercalciuria is prevented, in adult animals, by rejection of calcium by the intestine so that the amount of calcium absorbed is in the range which can be excreted in the urine without precipitation. Intestinal preparations of rats given a low calcium diet for one week were able to transport Ca^{45} to a greater degree than rats maintained on high calcium diets (Wasserman, 1960).

Also, it has been a frecuent finding that animals on low calcium diets are more efficient absorbers of dietary calcium, than animals raised on high calcium diets (Gershoff *et al.*, 1958; Hansard and Plumlee, 1954). The fraction of ingested calcium that is absorbed in the rat has been shown to vary inversely with the calcium content of the diet using conventional balance techniques and also with Ca^{45} (Wasserman *et al.*, 1957). Similarly, net calcium absorption from the diet decreased with age in calves (Smith, 1961b).

The mechanism of phosphorus absorption by the gastrointestinal tract is not very well understood. The phosphate ion is readily absorbible and studies by McHardy and Parsons (1956) have shown that increasing levels of phosphate ions in the intestinal lumen linearly enhanced net absorption, a response typical of a diffusion-like process. The permeability of the rumen epithelium to phosphate is very low (Parthasarathy and Phillipson, 1953).

Another possibility for calcium and phosphorus deposition could be the degenerative condition of the stratum corneum in rumen epithelium of animals from treatment B, C, D, and E. Fell (1964) stated "it cannot be said that absorption is either increased or reduced when intestinal epithelium is damaged, but there is good evidence that selectivity in absorption is lost".

In order to determine the loci of calcium deposits, paraffin sections from all animals were treated with silver nitrate and alizarine red S stains. Positive reactions for calcium were obtained in tissues from steers fed dietary treatments B, C, D and E (diets containing dried citrus pulp) but none was obtainned from dietary treatment A (corn diet). The stain was mainly concentrated

in the particles adhered to the stratum corneum and extended to the upper part of this layer (Fig. 23). In areas of strong reaction, the adhered particles were stained to total opacity. Although the reaction in the superficial stratum

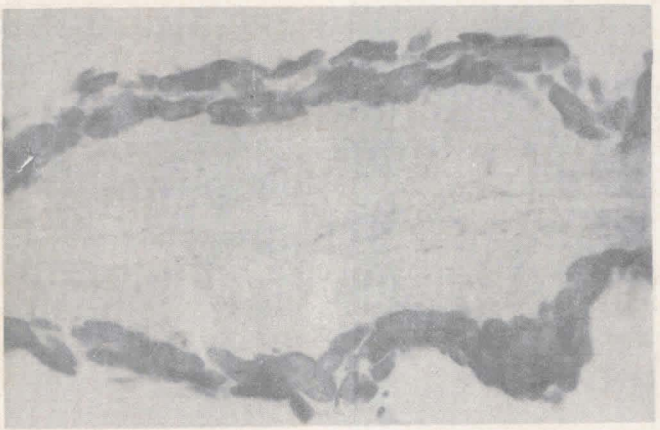

Fig. 23. Experiment IV. Particles adhered to stratum corneum showing intense calcium reaction. Alizarine Red S. X 90.

corneum was constant, that in the deep layers varied from strong to negative. No visible reaction was obtained in other cell layers of the epithelium or in the lamina propria. Figure 24 shows a thick layer of the stratum corneum

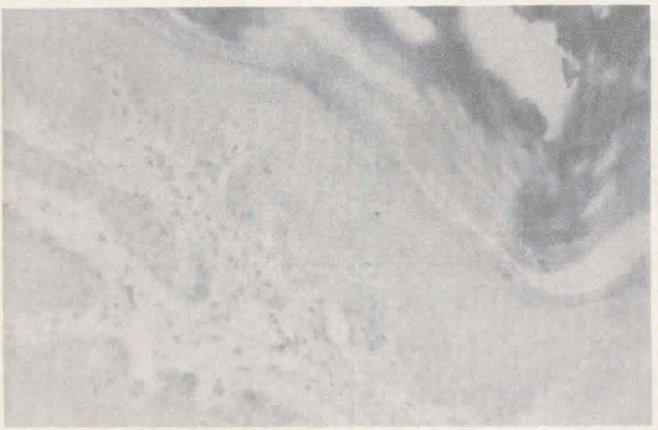

Fig. 24. Experiment IV. Conventration of calcium deposits in the stratum corneum layer. Alizarine Red S. X 90.

with adhered feed particles and calcium depositions. This is a probable indication that the layers of the stratum corneum provide sites for adherence of feed particles and calcium deposits. Excessive coloration of the rumen epithelium is one of the changes that occur as a result of diverse feeding practices. Some authors (Sinclair and Hunkel, 1959; Packett and Butcher, 1963) have indicated that coloration of the mucosa represents products of microbial activity, since it appears to be affected by dietary antibiotics. Tamate *et al.* (1962) and Nicolai and Stewart (1963) observed a green pigment in the outermost layer of desquamating cells of the stratum corneum. In skin, melanin granules are partly responsible for color variations (William and Fawcett, 1962).

When rumen tissue samples were stained with hematoxylin and eosin, pigment-like granules were observed in the stratum corneum. They were identified as keratohyalin granules since they showed color only when stained. In view of these findings, rumen papillae collected from animals in four different experiments were fixed in 10 per cent formalin and stained with Nile Blue and ferric-ferricyanide methods for detection of melanin pigment. Small green granules accumulated particularly at the basal layer of the stratum germinativum. Although some migration of these granules toward the stratum spinosum was present, none were found in the stratum corneum.

TABLE 2.—Calcium and Phosphorus Content[1] of Adherences

	Percent[2]	
Treatment	Calcium	Phosphorus
A	0.78	0.50
B	0.94	0.75
C	1.17	0.80
D	1.66	0.75
E	1.86	0.90

1 All figures are averages of ten samples.

2 Air dry matter.

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