LIGHT AND ELECTRON MICROSCOPIC STUDIES ON THE SUBMUCOSAL GLANDS OF RESPIRATORY NASAL MUCOSA IN CALVES EXPERIMENTALLY INFECTED WITH INFECTIOUS BOVINE RHINOTRACHEITIS VIRUS

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INTRODUCTION

Infectious bovine rhinotracheitis (IBR) was first recognized in 1950 as an acute upper respiratory infection of feedlot cattle (Miller, 1955). Since then, the causative virus has been isolated (Madin et al., 1956), and shown to produce a multiplicity of varied clinical syndromes. In addition to upper respiratory disease, syndromes commonly produced by the IBR virus include vulvovaginitis (Gillespie et al., 1959), keratoconjunctivitis (Abinanti and Plumer, 1961), meningoencephalitis (French, 1962), and abortion (Chow et al., 1964).

The pathology of the respiratory form of IBR has been studied. The gross and light microscopic lesions are well-documented (McKercher, 1959; Jubb and Kennedy, 1970). Electron microscopic techniques have been employed to study IBR virus in tissue culture (Armstrong et al., 1961; Cruickshank and Berry, 1965; Watrach and Bahnemann, 1966), but electron microscopic studies of tissues from infected animals have not been made.

Shroyer and Easterday (1968) reported that IBR virus produced no light microscopic changes in the submucosal glands of organ cultures of bovine nasal tissue. The purpose of this study was to determine if IBR virus produced electron microscopic changes in the submucosal glands of respiratory nasal mucous membranes of experimentally infected calves.

The normal ultrastructural morphology of submucosal glands has been described in man (Laudadio and Puxeddu, 1966; Merker, 1967; Terrahe, 1968) and rat (Burian and Stockinger, 1962). This study also reports the normal ultrastructure of bovine respiratory submucosal glands.

Paper I: Light and Electron Microscopic Studies on the Submucosal Glands of Respiratory Nasal Mucosa in Calves Experimentally Infected with Infectious Bovine Rhinotracheitis Virus

The pathology of the respiratory form of infectious bovine rhinotracheitis (IBR) has been reported previously. The lesions have been described in mucous membranes of the upper respiratory tract in both naturally occurring[6,8,10] and experimentally produced cases.[3,9,12,15,17] Lesions of submucosal glands of the nasal mucous membranes have not been reported in cattle infected with IBR. Shroyer and Easterday[14] reported that in IBR-infected organ cultures of bovine nasal and tracheal mucosa, immunofluorescent staining and histopathologic changes occurred only in epithelial cells. We used light and electron microscopy to study submucosal glands of nasal respiratory mucous membranes of experimentally infected cattle.

MATERIALS AND METHODS

Ten 4- to 6-month-old calves purchased from a herd known to be free of IBR were used. Serum samples submitted for IBR virus neutralization tests[a] were negative. The Colorado strain of IBR virus, maintained in Madin-Darby bovine kidney cells, was used.[b] The virus titer was $10^{5.5}$ median tissue culture infectious doses. Two calves were used as controls; the other 8

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[a]By Dr. J. D. Todd, Jensen-Salsbery Laboratories, Kansas City, Missouri.

[b]Furnished by Dr. W. P. Heuschele, Department of Infectious Diseases, Kansas State University, Manhattan, Kansas. Current address: Jensen-Salsbery Laboratories, Kansas City, Missouri.

were inoculated by swabbing the nasal mucosa of each nostril with 1 ml. of virus suspension.

Two infected calves were euthanized[c] and necropsied on each of post-inoculation days 2, 3, 4, and 5. The controls were euthanized and necropsied after 10 days. Transverse sections of the central portion of the right and left dorsal turbinates and the adjacent area of the nasal septum from each calf were taken at necropsy for light and electron microscopy. Tissues for light microscopy were fixed in buffered neutral 10% formalin (BNF) and Zenker's fluid with 5% glacial acetic acid (ZF). Sections were cut at 6 μ and were stained with hematoxylin and eosin.

For electron microscopy, tissues were fixed in cacodylate-buffered 2% glutaraldehyde (pH 7.2) and postfixed in cacodylate-buffered 1% osmium tetroxide. Dehydration was carried out through increasing concentrations of ethanol, and tissues were transferred to propylene oxide and embedded in epoxy resin.[d] Thirty blocks of tissue were processed from each calf, sectioned at 1 μ, and stained with 1% toluidine blue in 1% sodium borate for preliminary observation. Three to 5 blocks were selected from each calf, further trimmed, cut to thin sections and mounted

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[c]Barb-Euthol, Haver-Lockhart Laboratories, Kansas City, Missouri.

[d]Epon 812, Ladd Research Industries, Inc., Burlington, Vermont.

on bare, 300-mesh, copper grids, then stained with uranyl acetate and lead citrate, and examined in an electron microscope[e] at 75 kv.

Nasal swabs were collected from each calf immediately before euthanasia, and inoculated into coverslip cell cultures of bovine turbinate cells. Cultures were observed for cytopathic effect, then, 48 hours post-inoculation, were harvested for fluorescent antibody testing.

RESULTS

Gross Pathologic Changes. Lesions in all calves were minimal, and confined to the nasal cavities. Hyperemia of the nasal mucous membranes was uniform in all infected calves. Nasal passages of all infected calves contained a slight seromucinous exudate. A few scattered circular erosions, 1 to 3 mm. in diameter, were seen in the epithelium of the turbinates in calves necropsied on days 3, 4, and 5 post-inoculation. No significant changes were observed in other organs or tissues of the infected calves. Control calves were grossly normal.

Light Microscopic Observations. Changes seen in sections of the turbinates and nasal septa are presented in Table I. Inclusion bodies were not seen in either BNF- or ZF-fixed nasal epithelium. No lesions were observed in the submucosal glands

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[e]Hitachi Hu-11, Hitachi, Ltd., Tokyo, Japan.

of the nasal septa and turbinates of either infected or control calves.

Electron Microscopic Observations. Ultrastructural morphology of the submucosal glands of the nasal respiratory mucous membranes were similar in infected and uninfected calves. Swelling or dilation of mitochondria with vesiculation and loss of the cristae was the most frequently observed alteration in the serous cells (figures 1-3). The rough endoplasmic reticulum of the serous cells in 1 calf necropsied 3 days post-inoculation (figure 1) was markedly dilated, and dilated to a lesser degree in calves necropsied on days 4 and 5 post-inoculation. In calves killed 4 and 5 days post-inoculation, the Golgi networks were dilated and enlarged in the serous cells, but the same changes were present in serous cells of the control calves (figure 3). In no case were alterations in cytoplasmic organelles described above present in all serous cells in any single gland or section.

Mitochondrial vesiculation with loss of cristae was the only alteration seen in the mucous cells in the glands.

Results of the virus isolation and fluorescent antibody procedures are tabulated (Table II).

DISCUSSION

Light and electron microscopic studies demonstrated no significant ultrastructural pathology in the nasal submucosal

glands of IBR-infected calves. Mitochondrial swelling with loss of cristae seen in serous cells of infected calves was not considered a significant finding, because osmium fixatives characteristically produce swelling and vesiculation of mitochondria.[16] Mitochondria change their volume and structure with different respiratory states.[2]

The rough endoplasmic reticulum of serous cells of bovine submucosal respiratory glands typically assumes a more or less parallel, closely-packed, lamellar arrangement.[1] Dilation of endoplasmic reticulum we observed in the serous cells was similar to variations from the typical morphology of endoplasmic reticulum seen in rat hepatocytoes and bat pancreatic acinar cells during different stages of their normal activity.[5] Hruban et al.[4] suggested dilation of the endoplasmic reticulum was a sign of impending cell death in exocrine cells of the rat pancreas, but that has not been substantiated in the dog pancreas.[13]

The Golgi apparatus varies in configuration in cells of the canine exocrine pancreas,[13] and is dilated in actively secreting goblet cells.[11] As the serous cells of the submucosal glands of the bovine respiratory nasal mucous membranes are morphologically similar to pancreatic exocrine cells,[1] the morphologic alterations we observed likely result from fixation or represent functional alterations in the cytoplasmic organelles.

Mucous cells we observed in the glands were so densely packed with mucigen droplets that critical evaluation of

mitochondria, endoplasmic reticulum, and other organelles was difficult, however, vesiculation of mitochondria with loss of cristae was seen in several mucous cells.

Fluorescent antibody test results on cell cultures inoculated with nasal exudate were positive in 7 of the 8 inoculated calves. Failure to demonstrate viral particles in any of the glands examined by electron microscopy and lack of clearly demonstrable ultrastructural pathology substantiate morphologically the previously published report[14] that IBR virus does not infect bovine nasal submucosal glands.

SUMMARY

Light and electron microscopic examination of submucosal glands of respiratory, nasal mucous membranes of calves experimentally infected with infectious bovine rhinotracheitis virus revealed no pathologic changes. Viral particles were not demonstrated in the submucosal nasal glands of the infected calves.

TABLE I

Light microscopic changes in respiratory nasal mucous membranes of IBR-infected and control calves

Group	Calf No.	Hrs. p.i.*	Type of nasal exudate	Epithelial changes	Submucosal changes
I	3	48	Catarrhal	Migrating eosinophils	Lymphocyte, eosinophil infiltration
I	8	48	Catarrhal	Necrosis, ballooning degeneration	None
II	4	72	Catarrhal	Goblet cell hyperplasia	Lymphocyte infiltration
II	12	72	Catarrhal	Goblet cell hyperplasia	None
III	2	96	Mucopurulent	Ballooning degeneration, vesiculation, migrating neutrophils	Neutrophil infiltration
III	5	96	Catarrhal	Ballooning degeneration, vesiculation	None
IV	1	120	Mucopurulent	Necrosis, vesiculation, migrating neutrophils	Lymphocyte, neutrophil infiltration
IV	9	120	Fibrinopurulent	Migrating neutrophils	Lymphocyte infiltration
Control	11	120	None	None	None
Control	11	120	None	None	None

*Post-inoculation

TABLE II

Viral isolation and fluorescent antibody results on nasal exudate of experimentally infected and control calves

Calf No.	Cytopathic effect 24 hrs. p.i.*	Cytopathic effect 48 hrs. p.i.	Conjugate used	Result
3	++	++++	IBR	+
8	+	++++	IBR	+
4**	....	....	....	....
12	++	++++	IBR	+
2	++	++++	IBR	+
5	++	++++	IBR	+
1	+++	++++	IBR	+
9	++	++++	IBR	+
10#	Neg.	Neg.	IBR	Neg.
11#	Neg.	Neg.	IBR	Neg.
Positive control	++	++++	IBR	+
Positive control	++	++++	BVD##	Neg.
Negative control	Neg.	Neg.	IBR	Neg.
Negative control	Neg.	Neg.	BVD	Neg.

*Post-inoculation.

**Not determined due to mycoplasmal contamination of cultures.

#Control calves.

##Bovine virus diarrhea.

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FIGURE 1

Submucosal glands of nasal septum of calf 3 days post-infection. Lumen (L) of serous gland with microvilli is evident. Note mitochondrial vesiculation (M) and marked dilation of endoplasmic reticulum (ER). X10,300

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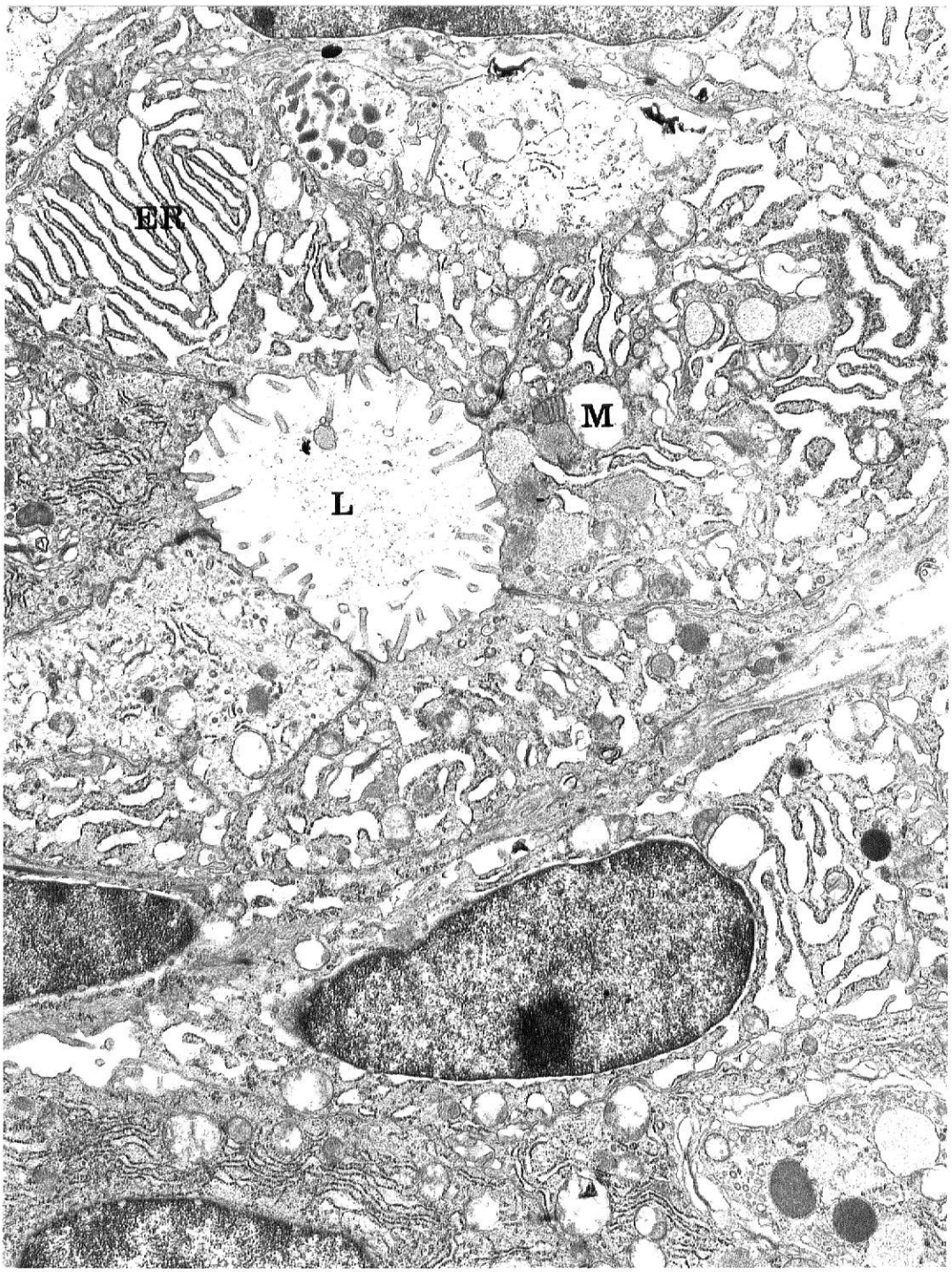

ER
M
L

FIGURE 2

Portion of mixed glands from nasal septum of calf 1 day post-inoculation. Vesiculated mitochondria (M), dilated Golgi apparatus (G) and endoplasmic reticulum (ER) are present. Note mucus (Mu) being released into lumen, centriole (C) and portions of 2 myoepithelial cells (My). X9,600.

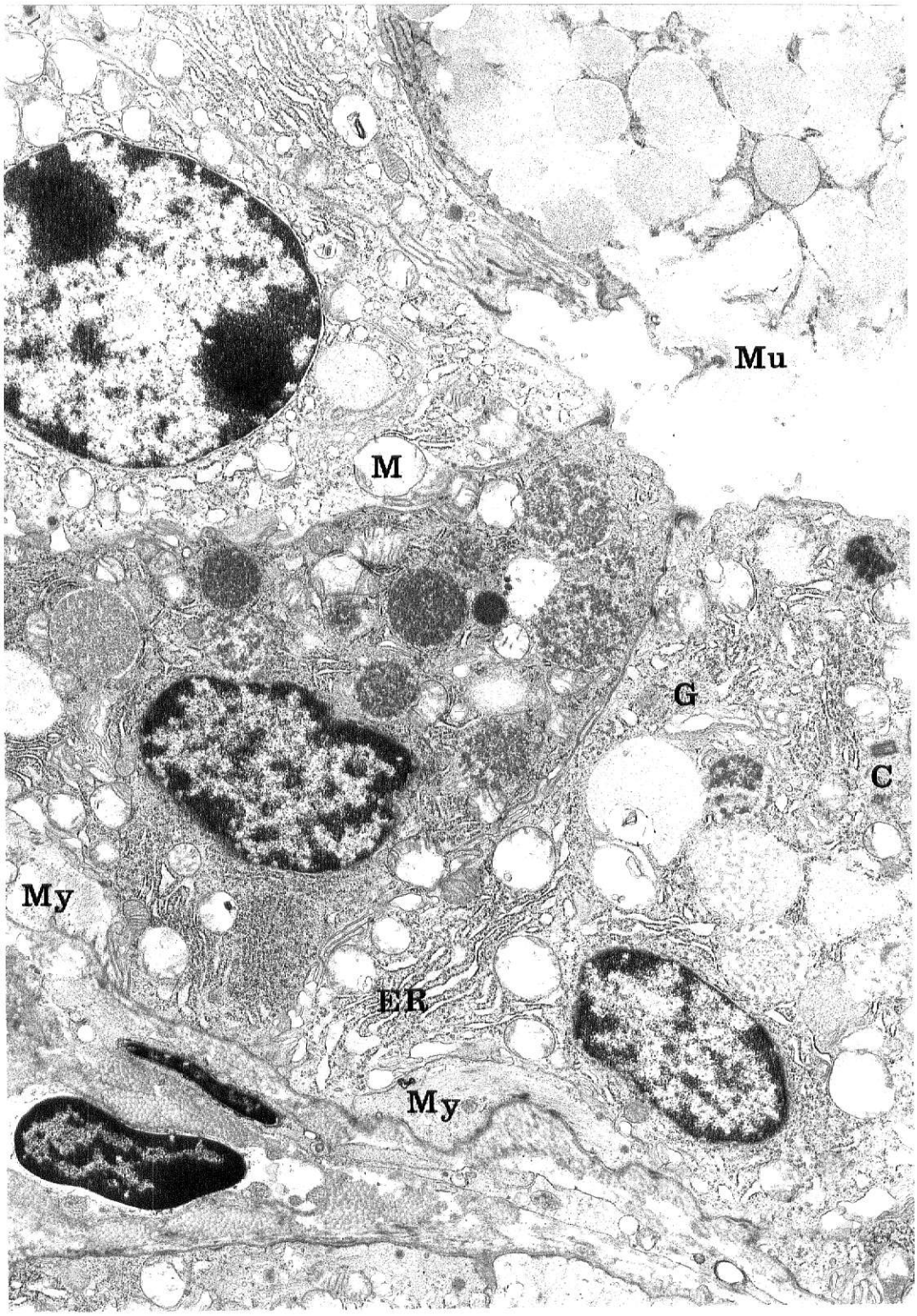
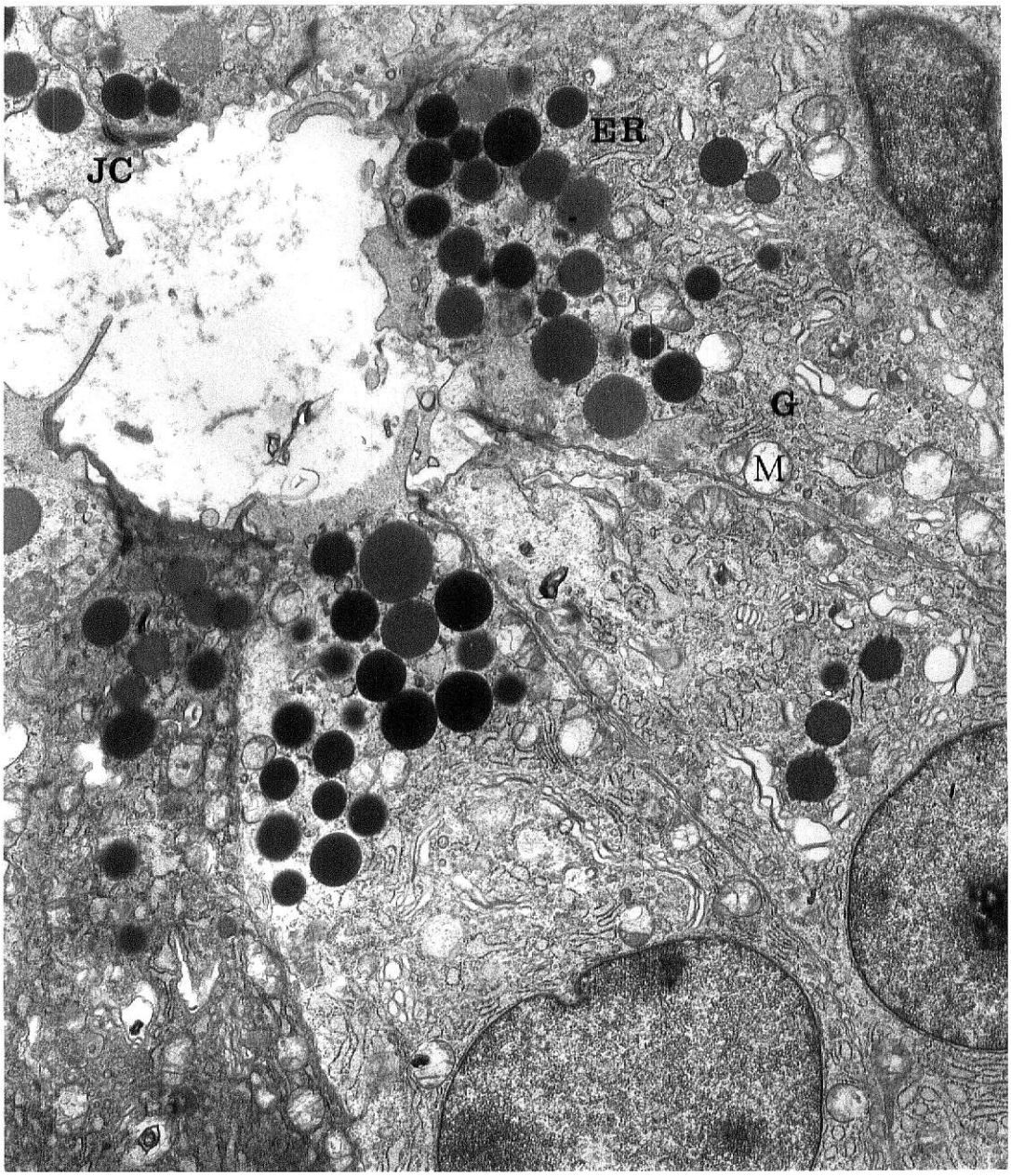

Mu
M
G
C
My
ER
My

FIGURE 3

Portion of serous gland from turbinate of uninfected control. Changes consist of dilated endoplasmic reticulum (ER) and Golgi apparatus (G), and loss of mitochondrial cristae (M). Junctional complexes (JC) are prominent. X13,500


JC
ER
G
M

Paper II: Ultrastructural Characteristics of the Submucosal Glands of Normal Bovine Respiratory Nasal Mucosa

The histology of the nasal respiratory mucosa of cattle,[20] other domestic species,[1,31] and man[3,4,10,11,13,14] has been described, and the ultrastructure has been studied in the dog,[19] the rat,[5-8,26] and man.[16,17,30] This paper describes the ultrastructure of the submucosal glands of the normal bovine nasal respiratory submucosa.

MATERIALS AND METHODS

Transverse sections of the central portion of the right and left dorsal turbinates and the adjacent area of the nasal septum from 2 healthy, 6-month-old Angus calves were taken at necropsy.

For light microscopy, tissues were fixed in neutral buffered 10% formalin; sections, cut at 6 μ, were stained with hematoxylin and eosin.

For electron microscopy, 30 blocks of tissues, were fixed in cacodylate-buffered 2% glutaraldehyde (pH 7.2) and postfixed in cacodylate-buffered 1% osmium tetroxide. Using increasing concentrations of ethanol, we dehydrated the tissues then transferred them to propylene oxide and embedded them in epoxy resin.[a] The 30 blocks of tissue from each calf were sectioned at 1 μ and stained with 1% toluidine blue in 1% sodium borate for preliminary observation. Three to 5 blocks were selected

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[a]Epon 812, Ladd Research Industries, Inc., Burlington, Vermont.

from each calf, and, after further trimming, cut to thin sections and mounted on bare, 300-mesh copper grids, stained with uranyl acetate and lead citrate, and examined in an electron microscope[b] at 75 kv.

RESULTS

Light microscopy. The glands, tubuloalveolar of the mixed type, contained both mucous and serous elements. The acini varied in cellular composition; serous cells, occasionally interspersed by groups of mucous cells, predominated. Most of the acini appeared to be entirely serous. The ducts were composed of slightly larger cuboidal to low columnar cells which closely resembled the serous cells but had more centrally located nuclei and cytoplasm stained more eosinophilic. The ducts opened directly into the nasal cavity. Typical myoepithelial cells were associated with the glandular acini.

The lamina propria was composed of fibroblasts, collagen, elastic and reticular fibers. Lymphocytes and plasma cells, scattered diffusely through the superficial layers, occasionally formed small clumps. In the interacinar glandular spaces, plasma cells were especially numerous, with a few neutrophilic and eosinophilic leukocytes and mast cells present.

In the toluidine blue-stained, epoxy-embedded thick sections, moderate numbers of deeply-stained secretory granules

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[b]Hitachi HU-11, Hitachi, Ltd., Tokyo, Japan.

were seen in the serous cells (figure 1). The granules tended to concentrate in the apical one-third of the acinar cells. The nuclei were round, contained 1 to 3 prominent round or oval nucleoli, and were situated in the basal portions of the cells. The acinar lumina were prominent. Occasional myoepithelial cells were identified at the periphery of the acini.

The mucous cells were roughly cuboidal and filled with purple-staining mucigen droplets. Nuclei were oval and appeared to be compressed against the basal membrane.

Between the acini were vascular and connective tissue elements, and the ducts. The duct cells were cuboidal, larger than the acinar cells, and occasionally contained a few serous granules.

Electron microscopy. The serous acinar cells contained few to moderate numbers of secretory granules, primarily in the apical one-third of the cells (figure 3). They were finely granular, enclosed in a membrane, and essentially homogenous. Their density varied; their diameters ranged from 0.2 to 1.4 u. Condensing vacuoles of lesser electron density were frequently present. Abundant granular endoplasmic reticulum was present throughout the cell, randomly arranged in the apical portions and distributed in parallel arrays in the basal areas. Mitochondria were numerous and randomly distributed throughout the cytoplasm; many were vacuolated (apparently due to artifact). Golgi complexes were not prominent, but were observed regularly

adjacent to the lateral or apical margins of the nuclei. Lysosomes, rarely seen, appeared to be autophagic vacuoles. The nuclei were large and basally situated, of moderate electron density, and contained 1 to 2 prominent nucleoli. Each acinus contained a central lumen and occasional intercellular canals. The apical surfaces of the cells exhibited microvilli. Junctional complexes between adjacent cells were prominent and well-defined at the apical perimeter. Occasional desmosomes and interlocking cytoplasmic processes were present on lateral surfaces of the cells.

The apical three-fourths of the mucous acinar cells were packed with mucigen droplets (figure 4), which were relatively electron-translucent, somewhat variable, and highly granular. The surrounding membrane was incomplete in many cases, indicating fusion of adjacent granules. The droplets ranged from 1.5 to 5.5 μ. Mucigen droplets of several cells contained electron-dense spherical bodies (figure 5).

Junctional complexes identical to those between serous and acinar cells were between adjacent mucous cells, and between mucous and serous cells at their apical borders. Occasional desmosomes and interlocking cytoplasmic processes likewise were present, similar to those between adjacent serous cells. Granular endoplasmic reticulum was concentrated around the nuclei, but it and the mitochondria were distorted and compressed by the tightly packed mucigen droplets. The Golgi complexes were

prominent, and usually near the apical side of the nuclei. Their vesicles were dilated, indicating active formation of mucous droplets. The apical surface of the mucous cells exhibited fewer microvilli than did serous cells.

Occasional cells were encountered that contained both serous granules and mucigen droplets but were otherwise identical with serous cells.

The cells of the ducts were characterized by pale cytoplasm, paucity of rough endoplasmic reticulum, few dense secretory granules .14 to .50 μ in diameter (figure 6), and prominent complex infoldings of basal plasma membrane (figure 7). The nuclei were round, located subapically, and were relatively pale-staining and homogenous. Mitochondria were large, numerous, and randomly distributed throughout the cytoplasm. They tended to align themselves parallel to the invaginations of the basal plasmalemma. Dense bodies resembling serous secretory granules were between the infoldings of the basal plasmalemma (figure 7). A few microvilli were on the apical surface. Basal lamina did not follow the plasma membrane in its basal invagination. The invaginations displayed desmosomal attachments (figure 7). Junctional complexes similar to those of the serous acinar cells were on the lateral surfaces of adjacent cells at the apical border. Occasional desmosomes and cytoplasmic folds were on the lateral surfaces.

The myoepithelial cells were in depressions of the acinar surface between the basal membrane and cells of the glands (figure 2). They had moderately dense oval nuclei, and the cytoplasm was relatively free of organelles. Small Golgi complexes, rough endoplasmic reticulum, free ribosomes, and a few mitochondria were seen concentrated in the juxtanuclear portion of the cell. Filaments resembling the myofilaments of smooth muscle cells were equally distributed throughout the cytoplasm. Occasionally, desmosomal attachments between myoepithelial cells and serous cells were seen. Myoepithelial cells were not seen in association with the glandular ducts.

DISCUSSION

Ultrastructural characteristics of the submucosal glands of the nasal respiratory mucous membranes of Angus calves were similar to those described for man[16,17,30] and rat.[6] The glands were composed of mixed serous and mucous elements, as described for man[11] and cat,[1] and not the strictly serous glands reported for the dog.[1] The predominance of purely serous glands agrees with earlier reports.[31] In man, mucous cells outnumber serous cells in normal, healthy individuals.[14] Morphology of the glands was remarkably akin to serous glands of other organs and species examined. The secretory granules of the bovine serous cells were morphologically indistinguishable from those described in the rat salivary gland,[2,15] rat lacrimal gland,[24] von Ebner's gland of the rat,[12] and the zymogen granules of the

exocrine pancreas,[22,23] as well as nasal glands of the rat[6] and man.[30] No discernible differences between bovine and other species were noted in other cellular components. The junctional complexes were as described in other glandular epithelia.[9]

The mucous cells were likewise similar to the mucous cells of sublingual salivary glands of the rat[24] and the nasal glands of the rat[6] and man,[16,17] and the process of mucous droplet formation by the Golgi apparatus was analogous to the formation of mucous droplets described in goblet cells.[18] Cells of nasal mucous glands of man have been reported to be identical to human respiratory epithelial goblet cells.[17] The dense bodies within individual mucous droplets (figure 5) were like those previously described in the nasal glands of man.[16,17] The presence or absence of these dense bodies has recently been shown to vary with fixation procedures,[29] but their precise nature remains uncertain. The intermediate cell types (those containing both serous and mucous granules) have previously been described in man.[17]

Cells of the ducts exhibited the characteristic infoldings of the basal portion of the plasma membranes seen in secretory ducts of rat salivary glands[24] and in proximal tubule cells of mouse kidney.[25] The infoldings are regarded as specialization of cells involved in high levels of water transport.[21]

Myoepithelial cells were observed with moderate frequency in the acini, but were not seen along ducts. The myoepithelial

cells of the bovine nasal glands presented no features differing from descriptions of myoepithelial cells of human or rat submaxillary salivary glands.[27,28]

The lamina propria was similar to that of other mucous membranes. Many plasma cells in the interstitial spaces of glandular foci were a consistent feature. Mast cells (figure 1) were also frequently seen; they are considered normal components of the nasal submucosa in man.[14]

SUMMARY

The submucosal glands of the respiratory nasal mucosa of 2 normal 6-month-old Angus calves were studied by electron microscopy to determine their ultrastructural morphology. They were similar to submucosal glands of the respiratory nasal mucosa of man and rat. Similarities were observed between serous cells of bovine respiratory glands and serous cells of salivary, lacrimal, and von Ebner's glands of the rat. Mucous cells of bovine respiratory glands were morphologically identical to mucous cells of rat salivary glands.

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FIGURE 1

Thick section of epoxy-embedded normal bovine turbinate lamina propria. Typical appearance of glands. Serous granules (SG), mucous cells (MC), plasma cells (PC), mast cell (Ma), and capillary (C). Toluidine blue; X400.

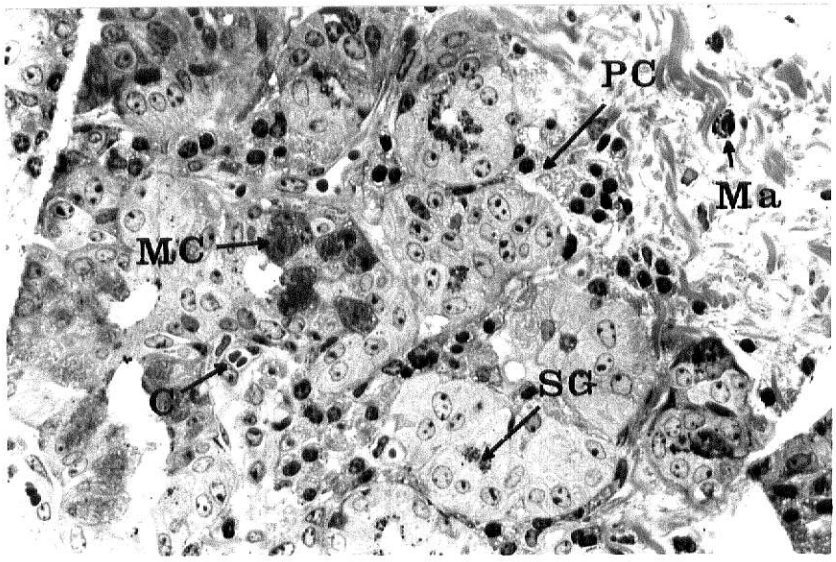

PC
Ma
MC
C
SG

FIGURE 2

Portion of glandular acinus. Lumen (L), serous cells (S), mucous cells (Mc), and myoepithelial cell (My). Note desmosomes (D), as well as portions of plasma cell (PC) and fibroblast (F) in interstitial tissue. X12,000.


D
L
Mc
S
S
F
My
PC

FIGURE 3

Portion of glandular acinus. Note variation in size and density of secretory granules (SG) and condensing vacuoles (CV). Rough endoplasmic reticulum (RER), mitochondria (M), Golgi complexes (G) and junctional complex (JC) are prominent. A portion of a fibroblast (F) is present in the interstitial space. X9,950.

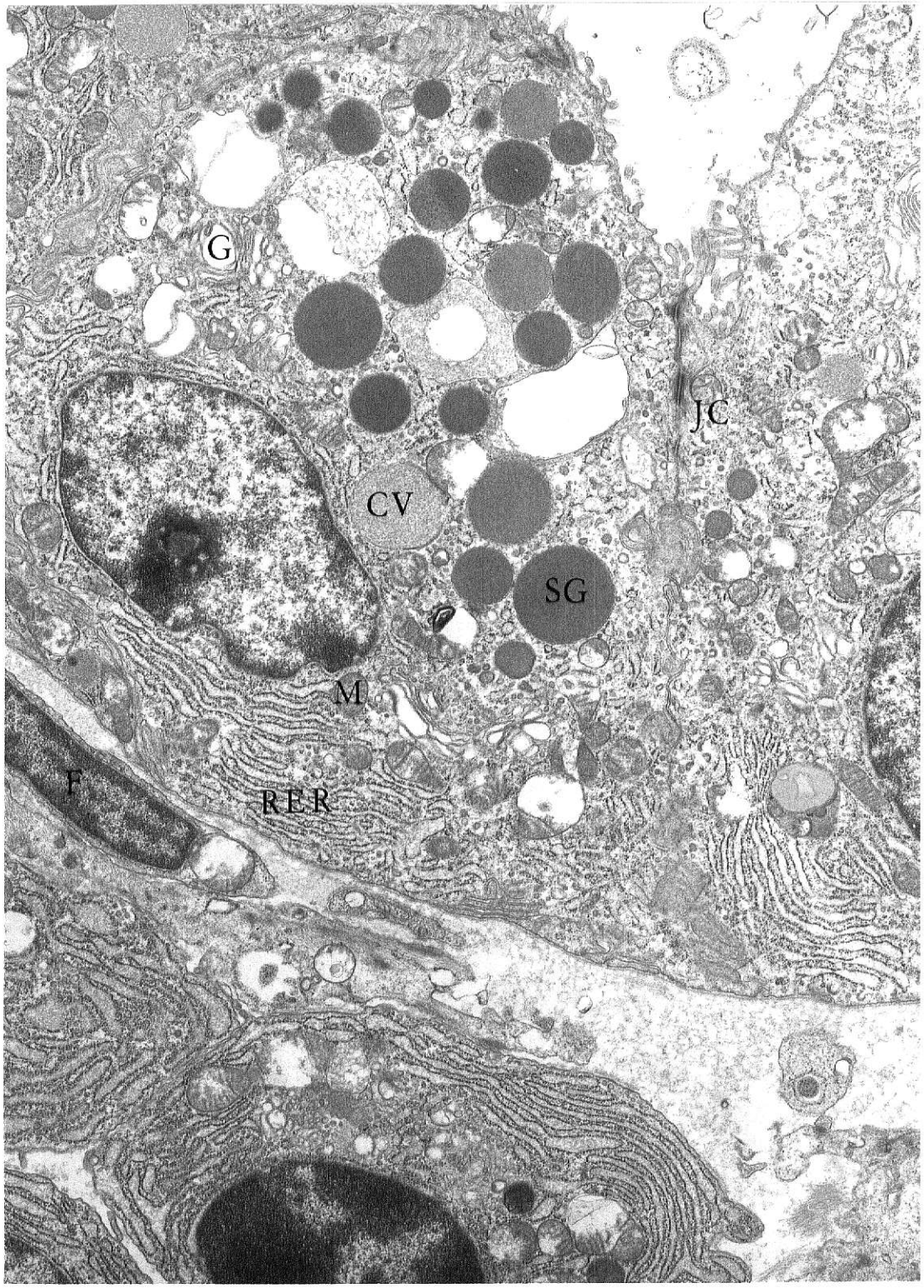

G
JC
CV
SG
M
F
RER

FIGURE 4

Apical portions of mucous cells. Lumen (L) and microvilli (Mv) are evident. Note loss of membrane and fusion of mucigen droplets, as well as compression of mitochondria (M) and rough endoplasmic reticulum (RER). X12,000.

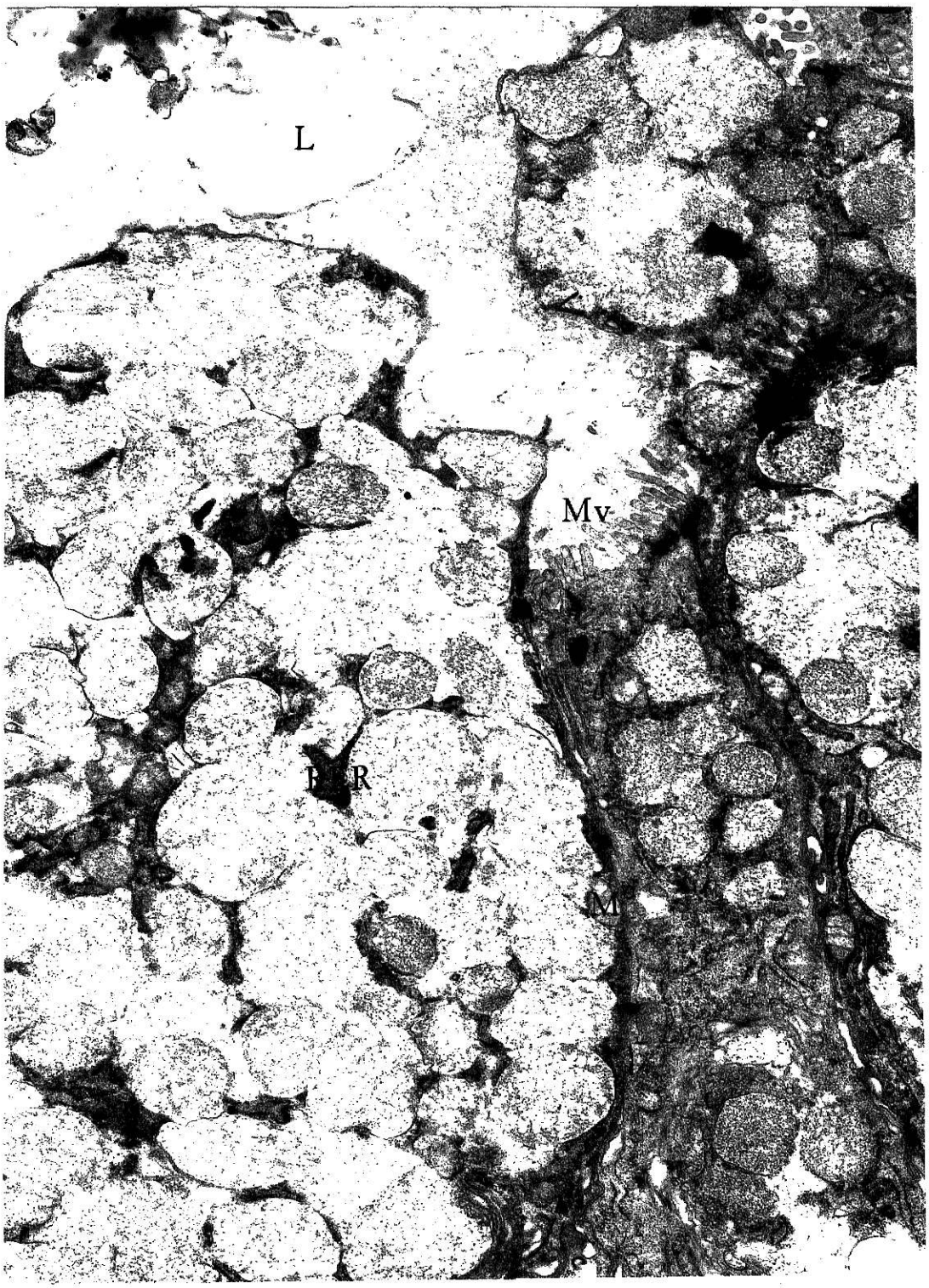

L
Mv
M

FIGURE 5

Mucigen droplets. Note fusion of droplets and dense spherical bodies (SB). X25,800.

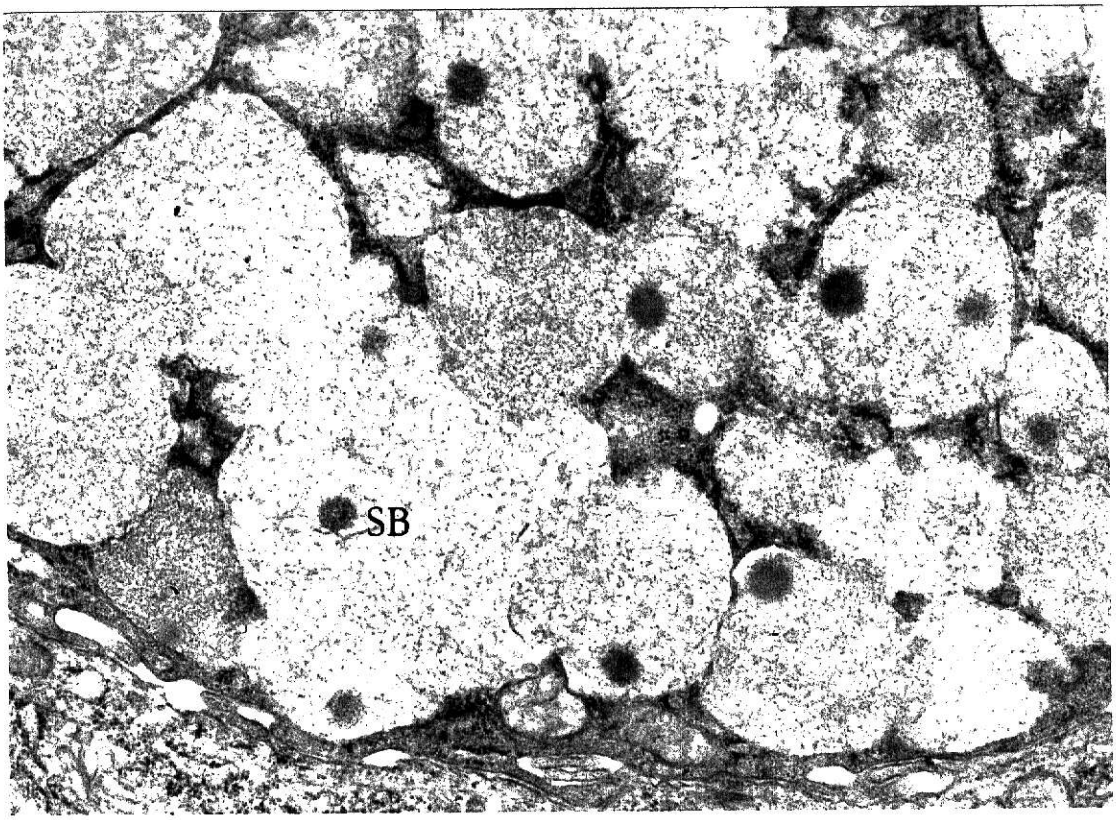

SB

FIGURE 6

Portion of secretory duct. Note short, stubby microvilli, free polyribosomes (R), and numberous desmosomes (D) between adjacent cells. X7,950.

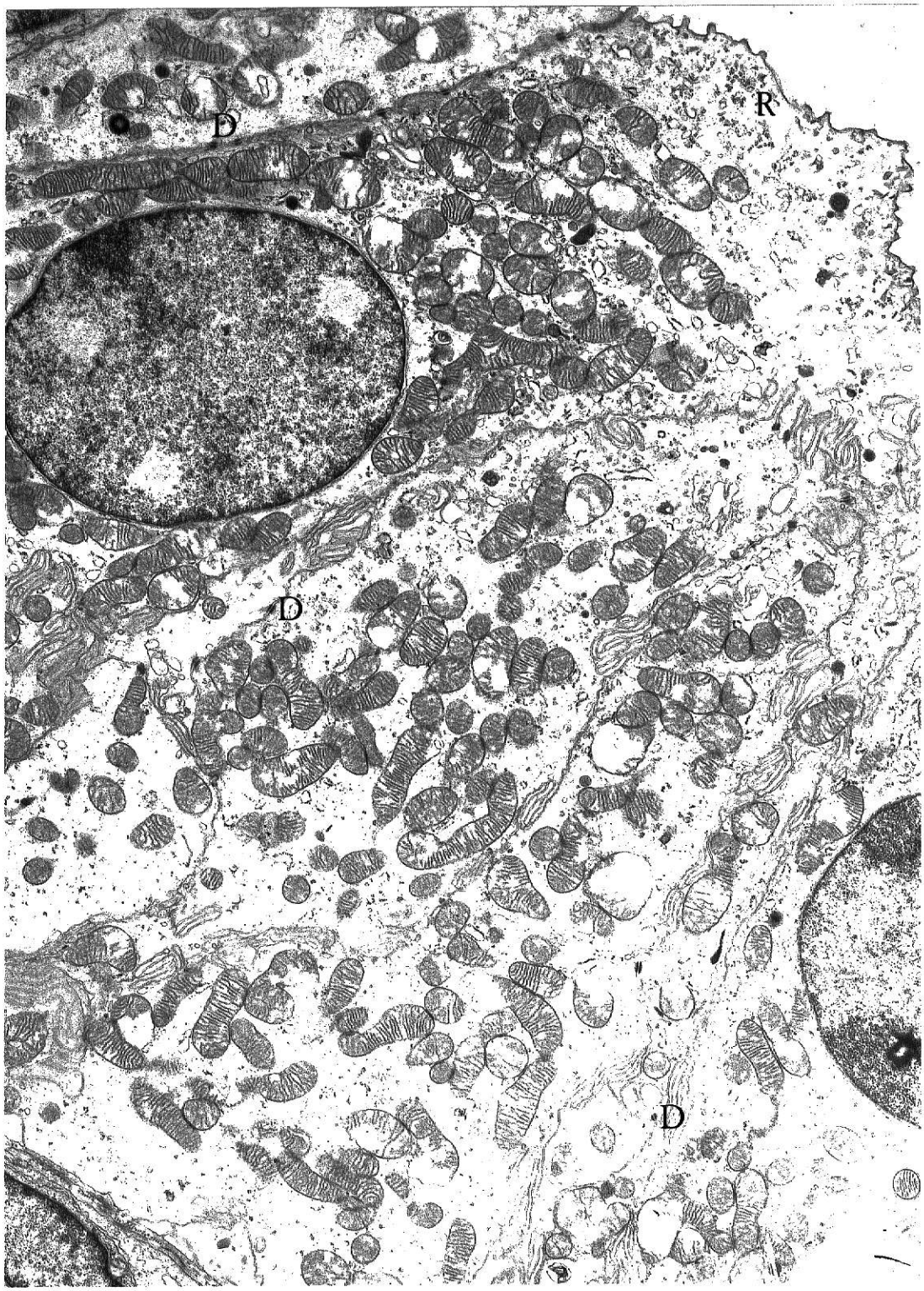

D
R
D
D

FIGURE 7

Basal portion of duct cell showing intricate infoldings of plasma membrane. Basal lamina does not follow invagination of plasmalemma. Note dense bodies (DB) which resemble secretory granules of serous cells. X13,500.


DB

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APPENDIX

SELECTIVE REVIEW OF LITERATURE ON INFECTIOUS BOVINE RHINOTRACHEITIS

First reports of the appearance in the United States of an apparently new transmissible upper respiratory disease of cattle were published in 1954 by McKercher et al. They stated that in 1953, an acute febrile condition of cattle characterized clinically by influenza-like symptoms appeared near Los Angeles, California, and soon reached epizootic proportions in a three-county area. The disease also occurred at about this same time in central California. Both dairy and beef cattle were affected; the incidence was higher in mature cattle, although calves as young as 4 months were also affected. These outbreaks were confined to areas of dense cattle populations, or to large herds in areas of lesser density. The disease appeared to be highly contagious, as it spread rapidly from herd to herd. Sporadic outbreaks also occurred in herds widely separated from affected herds, and in which no direct or indirect contacts were known to have occurred with either actively affected or recovered cattle.

The first symptoms seen in these herds were profuse salivation and a serous nasal discharge which progressed to a copious mucopurulent exudate. Agalactia occurred abruptly in milking cows. The initial temperature was 105°F. to 108°F. Dyspnea and congestion of the nasal mucosa were constant signs. Thirty-six to 48 hours following the initial rise, the temperature subsided and remained near 103°F. for a variable period

of time. Posterior lameness and conjunctivitis were seen in some cases. Ulceration of nasal and oral mucosae was not observed, diarrhea was rare, and the white blood count remained within normal limits. Most of the affected animals made rapid, uneventful recoveries in 7 to 10 days with minimal weight loss. A small percentage became progressively debilitated, showed marked weight losses, and a pronounced expiratory dyspnea. Most of these animals died or were sent to slaughter. The morbidity varied from 5 to 50 percent or more, with a mortality rate of 5 to 10 percent of the affected animals.

The principal lesions were limited to the upper respiratory tract. The less severe cases exhibited reddened respiratory mucous membranes with scattered petechiation. Seromucinous exudate was frequently present in the trachea. Microscopically, these cases showed excessive activity of the goblet cells and edema in the respiratory tract, with neutrophilic emigration through the epithelium and abscessation of lymphoid follicles. The respiratory mucosa of severely affected cases was swollen, congested, hemorrhagic, and covered with croupous or pseudomembranous exudate. Histologically, these membranes were fibrinopurulent in nature. Dense mononuclear cell infiltrates, hyperemia, and necrosis were present in the mucosa and submucosa.

Transmission studies were undertaken with negative results; although a temperature response was elicited in one case, the animals remained clinically normal. Numerous attempts to isolate a causative agent also were uniformly negative;

although many bacteria and pleuropneumonia-like organisms were recovered, none were consistently present.

Schroeder and Moys (1954) reported on the same outbreak as McKercher et al. (1954), with essentially identical observations.

McIntyre (1954) performed calf inoculation studies to determine if the disease could be experimentally reproduced. Lesions essentially the same as those occurring in the natural outbreak resulted in calves inoculated with blood, with sputum and nasal exudate, with chicken egg embryos from a third blind passage of sputum and nasal exudate, and an uninoculated calf held in the same pen with inoculated calves.

Miller (1955) reported the occurrence of a disease which affected the upper respiratory tracts of cattle in Colorado. This condition, which he named infectious necrotic rhinotracheitis, was first recognized in 1950 in a feedlot in Weld County, Colorado, where it occurred sporadically. In 1951, it became epizootic in this same feedlot during the spring and again in the fall, and it continued to occur in this feedlot through 1954. During the fall of 1951, the disease occurred sporadically in other feedlots in this area, and in 1952 it became epizootic in several of them. Prior to late 1954, infectious necrotic rhinotracheitis was almost exclusively a disease of mature beef-type feedlot cattle. During that year, it appeared in epizootic form in calves 3 weeks of age, as well as mature cattle, and in dairy herds and pastured beef cattle.

Gross pathologic changes seen in these cattle consisted of necrosis and diphtheritic inflammation of the mucosa of the nasal passages, pharynx, larynx, trachea and bronchi, and suppurative pneumonia, alveolar emphysema, focal caseonecrotic sinusitis, abomasal ulceration, and enteritis. Pulmonary and hepatic abscesses were seen in chronically affected animals. Miller (1955) presumed the cause to be viral.

Jensen et al. (1955) reported the symptomatology of several hundred natural cases and the pathology of 48 natural cases from Colorado feedlots. They suggested that the acute upper respiratory infection described by Schroeder and Moys (1954) in California and the infectious necrotic rhinotracheitis of Miller (1955) in Colorado were identical, and proposed the name "infectious rhinotracheitis."

Chow et al. (1955) stated that infectious rhinotracheitis had been identified in 3 additional areas: Arizona, Idaho, and Wyoming. They also reported experimental transmission of the disease to 13 of 15 cattle with nasal and tracheal exudates and sera from field cases, as well as with bacteria-free inocula, indicating the causative agent was possibly viral.

McKercher et al. (1955) also transmitted the disease experimentally; they infected calves with bacteria-free nasal washings from natural cases in both California and Colorado. They concluded that the California and Colorado diseases were identical, and proposed the name infectious bovine rhinotracheitis (IBR). This name was accepted at the 1955 meeting of

the United States Livestock Sanitary Association (York and Schwarz, 1956). Madin et al. (1956) isolated the IBR virus from nasal washings of experimentally infected calves in bovine embryonic kidney tissue cultures. They were able to reinfect susceptible cattle with the virus after several passages in tissue culture, and also failed to demonstrate any antigenic differences between the California and Colorado virus isolates. Attempts to recover pleuropneumonia-like organisms from infected tissue culture fluids were negative. York et al. (1957) also isolated the virus in tissue culture, and York and Schwarz (1956) conducted serum neutralization tests and cross protection tests in cattle which demonstrated that the isolate was indeed the cause of IBR. They were unable to identify any immunologic differences in the various strains of IBR virus which had been isolated at that time. Of great economic significance was their successful modification of the virus in tissue culture to produce an effective live virus vaccine.

It soon became evident that, in addition to respiratory disease, the IBR virus was involved in numerous other clinical syndromes. Among these were vulvovaginitis (Gillespie et al., 1959; Kendrick et al., 1968; McKercher, 1963), balanoposthitis (Hellig, 1965; Kendrick et al., 1968), keratoconjunctivitis (Abinanti and Plumer, 1961; Quin, 1961), meningoencephalitis (French, 1962a, b; Barenfuss et al., 1963), and abortion (Ormsbee, 1963; Chow et al., 1964; Kennedy and Richards, 1964; McKercher and Wada, 1964; Owen et al., 1964). Less frequently, IBR virus

was incriminated in several other syndromes, including mastitis (Baker et al., 1960), orchitis (Hellig, 1965; Studdert et al., 1964) and infertility Maré and van Rensburg, 1961; Saxegaard, 1970). McKercher (1963) demonstrated that Blaschenausschlag, or coital vesicular exanthema, was caused by a virus identical to the IBR virus and postulated the evolvement of the respiratory form of IBR from this venereal disease of European cattle. IBR virus has been recently suggested as a possible etiologic factor in the bovine "cancer eye" syndrome (Taylor and Hanks, 1969), and of nasal granuloma in Australian cattle (Snowdon, 1964).

By 1956, IBR had been identified in 15 states (Fincher et al., 1956) and, 5 years later, a serological survey of 31 states found sera positive for IBR-neutralizing antibodies in 28 of them (Newberne et al., 1961).

The pathology of the respiratory form of IBR has been well-described. Reporting on naturally-infected animals, McKercher et al. (1954) reported that gross lesions were limited to the upper respiratory tract, and ended abruptly in the major bronchi. Considerable variation in the severity of the lesions was noted. Less severely infected animals exhibited reddened, petechiated respiratory tracts, with seromucinous froth in the tracheal lumina. Severe cases had swollen, congested, petechiated respiratory mucous membranes covered with croupous or diphtheritic membranes. Nasal and epiglottal nodules of submucosal lymphoid follicular hyperplasia were seen in a few animals. Lungs were

grossly normal. Lymph nodes of the respiratory system were swollen, reddened and edematous.

Schroeder and Moys (1954) found hemorrhage, varying from petechiation and ecchymosis to diffuse hemorrhagic inflammation, of the nasal, laryngeal, tracheal and bronchial mucous membranes to be the most characteristic lesion in dairy cattle in California. Necrosis of the laryngeal and pharyngeal mucosa were frequently seen. Secondary alveolar emphysema, pneumonia, and enteritis were also noted.

Miller (1955), describing the Colorado feedlot outbreaks of 1950 to 1954, reported that necrotic areas, covered by diphtheritic membranes, in the nasal cavity, pharynx, larynx, trachea and bronchi, were the most common, consistent and characteristic lesions. Other lesions included pneumonia, alveolar emphysema, caseonecrotic sinusitis, abomasal ulceration, and enteritis. Lung and liver abscesses were frequently observed.

McKercher et al. (1957) reported that experimentally-infected cattle showed lesions essentially the same as naturally-infected ones, although the rhinitis usually was more severe and the tracheitis less severe in experimental cases.

The microscopic lesions of respiratory IBR infection are in general non-specific, with mild cases exhibiting edema of the mucosa with neutrophilic emigration through the epithelium and infiltration of lymphocytes, macrophages and plasma cells into the submucosa (McKercher et al., 1957). Abscessation and

eruption of solitary lymphoid follicles may occur (McKercher et al., 1954; 1957).

Shroyer and Easterday (1968) reported loss of ciliated epithelium, infiltration of the epithelium and submucosa with neutrophils and eosinophils, and diffuse and nodular accumulations of mononuclear cells in the epithelium of experimentally infected calves. Thickening of alveolar walls, intralobular septal edema, and mononuclear cell peribronchiolar cuffing were observed in the lungs. The tonsils contained large numbers of eosinophils.

McKercher et al. (1955) reported the early stages of infection were characterized by active mucus secretion and neutrophil emigration in the nasal epithelium, and mononuclear cell accumulation in the lamina propria. Erosion of the nasal epithelium was observed, followed by exudation of plasma which spread over the eroded surface where it coagulated, trapping necrotic epithelium and neutrophils to form a pseudomembrane. Laryngeal and tracheal mucous membranes showed similar hyperplasia and rupture in some animals. The retropharyngeal lymph nodes exhibited diffuse lymphoid hyperplasia. Reticulum cell hyperplasia and enlargement of the Malpighian corpuscles were observed in the spleens. Bronchopneumonia was present, but it was considered to have been present before the calves were infected, or to have been due to aspiration of exudates from the upper respiratory tract.

Jensen et al. (1955) found in naturally-infected cattle that hemorrhage and mucopurulent inflammation of the paranasal, palatine and maxillary sinuses were common, in addition to the changes reported by others.

Webster and Manktelow (1959) reported similar respiratory tract lesions in cattle experimentally infected with IBR virus isolated from a naturally-occurring case. In addition, edema and sinus catarrh, severe capsular and trabecular inflammatory reactions and lymphoid hyperplasia were seen in the prescapular lymph nodes, and extensive focal hyaline necrosis of the adrenal cortices was reported.

Currently, the lesions of the respiratory form are considered to be limited to the upper respiratory tract, with secondary bronchopneumonias being a frequent occurrence, and the usual cause of death (McKercher, 1959; Jubb and Kennedy, 1970). Fulminating forms have been reported in which death resulted from direct broncho-pulmonary involvement (Jubb and Kennedy, 1970). In this form, a severe necrotizing bronchitis and bronchiolitis, with cellular debris and exudate occluding the respiratory passages, produced a massive outflow of protein-rich edema fluid. The course was very short, and typical inclusions were seen in vesicular nuclear remnants of the bronchial epithelial cells.

Cheatham and Crandell (1957) were the first to report the occurrence of inclusion bodies in tissue cultures of IBR

virus, and in tissue sections of infected calves. In tissue culture, they observed cytopathic effects (CPE) beginning at 18 to 24 hours post-inoculation as small foci of degeneration which slowly increased in size. New or secondary foci of degeneration began to appear in 24 hours. The degeneration involved the entire monolayer of cells in about 72 hours. A constant feature of the CPE was the occurrence (in stained preparations) of intranuclear inclusions in a large proportion of the degenerating cells. They began to appear at about the time degenerative changes were first noticeable in unstained cells. The earliest change in the nucleus consisted of a single small irregular aggregate of amphophilic to eosinophilic, finely granular material among the nuclear chromatin. The size of the particles responsible for the granularity approached the resolution limits of the light microscope, so in some cases the inclusions appeared homogenous. The inclusions slowly increased in size until they occupied a major portion of the nucleus. Margination of the chromatin was evident. The nuclear membrane remained intact, and was separated from the inclusion by a clear halo. Intense cytoplasmic eosinophilia accompanied the neclear changes, but no cytoplasmic inclusions were observed.

Similar inclusions were demonstrated in tissues fixed with Zenker's and Bouin's fluids which were removed at necropsy from infected calves killed during the early acute stages of the disease. These inclusions were demonstrated in the

epithelial cells of the upper respiratory tract, and were detectable from 36 through 60 hours post-infection (Crandell et al., 1959).

Crandell et al. (1959) reported that in experimentally infected calves, the stratified squamous and pseudostratified columnar respiratory epithelial cells showed cytoplasmic vacuolation which progressed to ballooning degeneration, then granularity and loss of peripheral outline. Nuclear changes began as small, irregular aggregates of pale, acidophilic material dispersed among the nuclear chromatin. Simultaneously, the chromatin became marginated and the nucleolus fragmented and disappeared, or contracted and remained at the nuclear margin as a small basophilic body. The acidophilic aggregates became more homogenous and filled the major portion of the nucleus. Later this material contracted, creating a clear halo surrounding a distinct, acidophilic, intranuclear inclusion body. The inclusions were well-formed in the epithelium of the nasal septum at 36 hours post-infection, appeared in the turbinate and bronchial epithelium at 48 and 60 hours, and persisted through 60 hours, but had disappeared by 71 hours post-infection. Bouin's fluid and Zenker's acetic fixative were found to be superior to formalin for demonstration of inclusions with hematoxylin and eosin staining (Crandell et al., 1959; Stevens and Chow, 1959). Inclusions cannot be demonstrated in osmium-fixed infected cells (Grinyer et al., 1963).

In addition to upper respiratory epithelium, inclusions have been reported to occur in ruminal epithelium (Curtis et al., 1966; Baker et al., 1960; Van Kruiningen and Bartholomew, 1964), in esophageal epithelium, hepatocytes and renal tubular epithelium (Baker et al., 1960), in squamous cell carcinomas of bovine eyes (Russell et al., 1969), and in bronchiolar epithelium (Jubb and Kennedy, 1970). They are also present in genital epithelium in infectious pustular vulvovaginitis and balanoposthitis (Kendrick et al., 1958; Studdert et al., 1964) and were described by Hall et al. (1966) in neurons and astrocytes in calves affected with the meningoencephalitic form of IBR. In aborted fetuses studied by Kendrick et al. (1967) they were seen in cells of the adrenal cortex and liver.

Although inclusion bodies have been demonstrated to be a consistent feature of the disease, their short duration in naturally infected cases make them unreliable as pathognomonic changes (Crandell et al., 1959), and the necrosis and autolysis frequently present obscures them in many cases (Owen et al., 1964).

The IBR virus was first isolated in tissue culture by Madin et al. (1956) from nasal washings collected from experimentally infected cattle. They were able to reinfect susceptible cattle with the virus after several passages in tissue culture, and were unable to demonstrate any antigenic differences between California and Colorado virus isolates. York et al. (1957) also

isolated the virus in tissue culture, and York and Schwarz (1956) conducted serum neutralization tests and cross protection tests in cattle which confirmed that these isolates were the cause of IBR. York and Schwarz (1956) were unable to demonstrate any immunologic or serologic differences in the various strains of IBR virus available to them.

The successful modification of the IBR virus in tissue culture to produce an effective live-virus vaccine (York and Schwarz, 1956) was of tremendous economic importance.

Armstrong et al. (1961) reported that electron microscopic examination of bovine kidney cells inoculated with IBR virus demonstrated cytopathic features virtually identical with those reported for several Herpesviruses. The size, morphology, apparent mode of formation, and predilection for various tissues of IBR virus were all compatible with those features found in other members of the Herpesvirus group. Cruickshank and Berry (1965) confirmed these findings with the electron microscope by negative staining techniques. Watrach and Bahnemann (1966) conducted similar studies, and reported the essential components of the virus particles to be the core, the capsid, and the envelope. The core had a diameter of 945 Å, the capsid 1085 Å, and the envelope 2000 Å. The findings of Cruickshank and Berry (1965) were identical except they reported the particles to be between 900 Å and 950 Å in diameter.

IBR virus infects a wide range of species, although neither clinical signs nor lesions have been reported with any frequency. The presence of antibodies to the IBR virus has been reported in water buffaloes, horses, dogs, man (Afshar and Tadjbakhsh, 1970), wildebeests (Rweyemamu, 1970), hippopotami (Kaminjolo and Paulsen, 1970), sheep (McKercher et al., 1958), swine (Nelson et al., 1972), and deer (Chow and Davis, 1964); the virus has been recovered from naturally infected goats (Mohanty et al., 1972) and swine (Saxegaard and Onstad, 1967). Natural infection with IBR virus has produced lesions in swine (Saxegaard and Onstad, 1967) and goats (Nelson et al., 1972). Experimental infection of swine has also produced minor pathologic changes (Woods et al., 1968).

MATERIALS AND METHODS

Experimental Animals. Ten 4- to 6-month old Angus calves were purchased from a herd in which IBR had never been diagnosed. Serum samples were collected from each calf prior to purchase; results of virus neutralization tests for IBR[a] were negative. After the calves were placed in the experimental units, they were allowed a 9-day adjustment period. During this time, their body temperatures were recorded twice daily, and they were closely observed for signs of illness. Total and dif-

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[a]Tested by Dr. J. D. Todd, Jensen-Salsbery Laboratories, Kansas City, Mo.

ferential leukocyte counts were made on the 4th to 9th days to further insure their normalcy.

Virus. The Colorado strain of IBR virus,[b] maintained in Madin Darby bovine kidney cells, was used. The virus titer was $10^{5.5}$ median tissue culture infectious doses ($TCID_{50}$).

Calf Inoculation. Two calves were arbitrarily selected as controls, and the other 8 calves were inoculated by rubbing the nasal mucosa of each nostril with a stiff-bristled brush, and thoroughly swabbing the irritated areas with cotton-tipped applicators saturated with 1 ml. of tissue culture fluid containing the virus. The 2 control animals were swabbed in an identical manner with sterile tissue culture fluid.

Post-Inoculation Procedures. After inoculation, the calves were closely observed for clinical signs of illness. Rectal temperatures were recorded twice daily (Table 1).

Necropsy Procedures. Two calves were arbitrarily selected for necropsy on each of the 2nd, 3rd, 4th and 5th days post-inoculation. Immediately prior to euthanasia, nasal swabs were collected from each calf for viral isolation. The calves were euthanized intravenously[c] and necropsied. For light microscopy,

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[b]Furnished by Dr. W. P. Heuschele, Department of Infectious Diseases, Kansas State University, Manhattan, Kan. Current address: Jensen-Salsbery Laboratories, Kansas City, Mo.

[c]Barb-Euthol, Haver-Lockhart Laboratories, Kansas City, Mo.

3 to 5 mm. thick sections of each dorsal turbinate and the adjacent area of the nasal septum posterior to the inoculation site were collected. Sections of the submucosa were collected from these same sites for electron microscopy, and diced to 1 to 2 mm. cubes under cacodylate-buffered 2% glutaraldehyde (pH 7.2) on ice.

Additional sections of bronchial, suprapharyngeal and mesenteric lymph nodes, tonsil, lung, trachea, adrenal gland, kidney, liver, spleen and brain were collected for light microscopy.

Processing of Tissues. For light microscopy, tissues were immediately placed in 10% buffered neutral formalin (BNF) and Zenker's fluid with 5% glacial acetic acid (ZF). All sections were processed by a standard procedure, cut at 6 μ, and stained with hematoxylin and eosin.

Tissues for electron microscopy were further diced into approximately 0.5 mm. cubes and fixed in cacodylate-buffered 2% glutaraldehyde (pH 7.2) for 2 hours and post-fixed in cacodylate-buffered 1% osmium tetroxide (pH 7.2) for 1.25 hours. Dehydration was carried out through increasing concentrations of ethanol and transferred to propylene oxide per the following schedule.

50% Ethanol	5 minutes
70% Ethanol	5 minutes
90% Ethanol	10 minutes
95% Ethanol	15 minutes
95% Ethanol	15 minutes

100% Ethanol.....15 minutes
100% Ethanol.....15 minutes
100% Propylene Oxide.....15 minutes
100% Propylene Oxide.....15 minutes
50% Propylene Oxide-50% Epoxy Resin[d]..120 minutes

Tissues were then embedded in epoxy resin and allowed to polymerize at 60°C. for 48 hours.

Ten blocks were processed from each of the 3 sites in each calf. Blocks were sectioned at 1 μ and stained with 1% toluidine blue in 1% sodium borate for preliminary observation. Three to 5 blocks were selected from each site, and, after further trimming, thin sections were cut at 140 nm. and mounted on bare 300-mesh copper grids, stained with uranyl acetate and lead citrate, and examined in an electron microscope[e] at 75 kilovolts.

Viral Isolation. Nasal swabs collected immediately prior to necropsy were placed in 5 ml. of Hanks' balanced salt solution (BSS) containing 200 units of penicillin and 200 μgm. of streptomycin per ml. The BSS was centrifuged for 20 minutes at 3,000 RPM, and the supernatant used for inoculation of cell cultures.

Coverslip cell cultures[f] of bovine turbinate cells maintained in Eagle's Minimum Essential Medium with 10% fetal bovine

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[d]Epon 812, Ladd Research Industries, Inc., Burlington, Vt.

[e]Hitachi HU-11, Hitachi, Ltd., Tokyo, Japan.

[f]Prepared by Dr. W. P. Heuschele, Department of Infectious Diseases, Kansas State University, Manhattan, Kan.

serum were utilized. The medium was discarded, and 1.0 ml. of the supernate was added to each culture. Two cultures were inoculated for each sample. The cultures were then incubated for 1 hour at 37°C. After incubation the specimen material was poured off and the coverslip cultures were washed 3 times with phosphate-buffered saline (PBS) (pH 7.2). Fresh medium was then added to each culture, and they were incubated at 37°C. Cultures were observed for cytopathic effect at 24 and 48 hours post-inoculation.

Fluorescent Antibody Procedure. Coverslips were harvested 48 hours post-inoculation and washed with 0.01 molar PBS. After drying for 30 minutes at room temperature, they were fixed in acetone for 10 minutes, and again allowed to dry. Fluorescent antibody conjugates[g] were layered over the coverslips, and they were incubated at 37°C. for 30 minutes in a moist chamber. Following incubation, the coverslips were rinsed with 3 changes of PBS, dried at room temperature, and mounted cell side down on glass microscope slides using a solution of equal parts glycerine and PBS. Positive and negative controls were processed along with experimental specimens. Stained specimens were placed on the microscope[h] and examined for fluorescence.

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[g]IBR and bovine virus diarrhea conjugates obtained from Diagnostic Services, Animal Health Division, National Animal Disease Laboratory, Ames, Iowa.

[h]Leitz Ortholux with BG-12 and OG-1 filters.

RESULTS

Clinical Signs. Temperature responses occurred 12 to 24 hours post-inoculation in all but 1 of the infected calves. Calf 2 did not show a significant temperature change until 48 hours post-inoculation. Clinical signs observed in the infected calves were mild anorexia and depression. These signs correlated with the temperature responses in all calves; the higher the fever, the more pronounced were the anorexia and depression. The temperature response of each calf is tabulated in Table 1.

Gross Pathologic Findings. The gross lesions are presented in Table 2.

Light Microscopic Findings. Changes seen in sections of the turbinates and nasal septa are presented in Table 3. Inclusion bodies were not seen in either BNF- or ZF-fixed tissues. No lesions were observed in other tissues.

Electron Microscopic Findings. Group I--The glands of Calf 3 had marked loss of mitochondrial cristae in both glandular and myoepithelial cells (figure 1). Distention of the Golgi apparatus was also apparent in the glandular cells. The glands of Calf 8 did not differ from those of Calf 3.

Group II--Calf 4 had the same mitochondrial and Golgi changes as Calves 3 and 8. In addition, dilation of the endoplasmic reticulum was a consistent finding in the serous cells

of many glands (figure 2). Calf 12 exhibited glandular morphology identical to that seen in Group I.

Group III--Dilation of the Golgi networks and loss of mitochondrial cristae characterized the serous cells of the glands of Calves 2 (figure 3) and 5.

Group IV--Calves 1 and 9 exhibited the same alterations in the mitochondria, Golgi networks and endoplasmic reticulum seen in the other infected calves (figures 4 and 5).

Controls--All of the changes described in the infected calves were present in the control animals (figures 6 and 7).

Viral Isolation and Fluorescent Antibody Results. Results of viral isolation attempts and fluorescent antibody examination of nasal washings and swabs collected at necropsy are presented in Table 4.

TABLE I

Temperature responses in °F. of IBR-infected and control calves

Calf No.	Hours post-inoculation										
	0	12	24	36	48	60	72	84	96	108	120
3	101.2	101.8	102.6	102.6	102.4	---*	---	---	---	---	---
8	101.6	102.3	102.3	102.5	102.6	---	---	---	---	---	---
4	101.5	102.1	102.0	102.4	102.5	103.4	103.6	---	---	---	---
12	101.5	102.0	102.4	102.5	102.6	103.6	103.8	---	---	---	---
2	101.4	101.6	101.9	101.6	102.1	103.5	103.7	103.8	103.7	---	---
5	101.4	102.0	102.1	103.0	103.6	103.5	103.6	103.6	103.6	---	---
1	101.3	102.2	102.0	102.7	102.9	103.2	103.6	101.8	102.0	101.5	102.6
9	101.2	102.2	102.5	102.4	102.6	103.5	103.6	101.4	101.7	105.6	105.2
10**	101.2	101.3	101.3	101.4	101.5	101.4	101.4	101.6	101.5	101.3	101.3
11**	101.3	101.3	101.4	101.3	101.5	101.3	101.5	101.4	101.4	101.4	101.3

*Indicates previously sacrificed calf.

**Control calves.

TABLE 2

Gross pathologic changes in IBR-infected and control calves

Group	Calf no.	Hrs. p.i.*	Nasal exudate	Epithelial erosions	Other lesions
I	3	48	Slight	None	None
I	8	48	Slight	None	None
II	4	72	Slight	Present	None
II	12	72	Slight	Present	None
III	2	96	Moderate	Present	None
III	5	96	Moderate	Present	None
IV	1	120	Marked	Present	None
IV	9	120	Marked	Present	None
Control	10	120	None	None	None
Control	11	120	None	None	None

*Post-inoculation.

TABLE 3

Light microscopic change in respiratory nasal mucous membranes of IBR-infected and control calves

Group	Calf no.	Hrs. p.i.*	Type of nasal exudate	Epithelial changes	Submucosal changes
I	3	48	Catarrhal	Migrating eosinophils	Lymphocyte, eosinophil infiltration
I	8	48	Catarrhal	Necrosis, ballooning degeneration	None
II	4	72	Catarrhal	Goblet cell hyperplasia	Lymphocyte infiltration
II	12	72	Catarrhal	Goblet cell hyperplasia	None
III	2	96	Mucopurulent	Ballooning degeneration, vesiculation, migrating neutrophils	Neutrophil infiltration
III	5	96	Catarrhal	Ballooning degeneration, vesiculation	None
IV	1	120	Mucopurulent	Necrosis, vesiculation, migrating neutrophils	Lymphocyte, neutrophil infiltration
IV	9	120	Fibrinopurulent	Migrating neutrophils	Lymphocyte infiltration
Control	10	120	N.A.**	None	None
Control	11	120	N.A.**	None	None

*Post-inoculation.

**Not applicable.

TABLE 4

Viral isolation and fluorescent antibody results on nasal washings of IBR-infected and control calves

Calf no.	Cytopathic effect 24 hrs. p.i.*	Cytopathic effect 48 hrs. p.i.	Conjugate used	Result
3	++	++++	IBR	+
8	+	++++	IBR	+
4**	...	...	...	...
12	++	++++	IBR	+
2	++	++++	IBR	+
5	++	++++	IBR	+
1	+++	++++	IBR	+
9	++	++++	IBR	+
10#	Neg.	Neg.	IBR	Neg.
11#	Neg.	Neg.	IBR	Neg.
Positive control	++	++++	IBR	+
Positive control	++	++++	BVD##	Neg.
Negative control	Neg.	Neg.	IBR	Neg.
Negative control	Neg.	Neg.	BVD	Neg.

*Post-inoculation.

**Not determined due to mycoplasmal contamination of cultures.

#Control calves.

##Bovine virus diarrhea.

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FIGURE 1

Portions of 2 serous glands from nasal septum of Calf 3. Notice loss of mitochondrial cristae (M) and distended Golgi apparatus (G). Portions of 2 myoepithelial cells are present (My). X9,600.

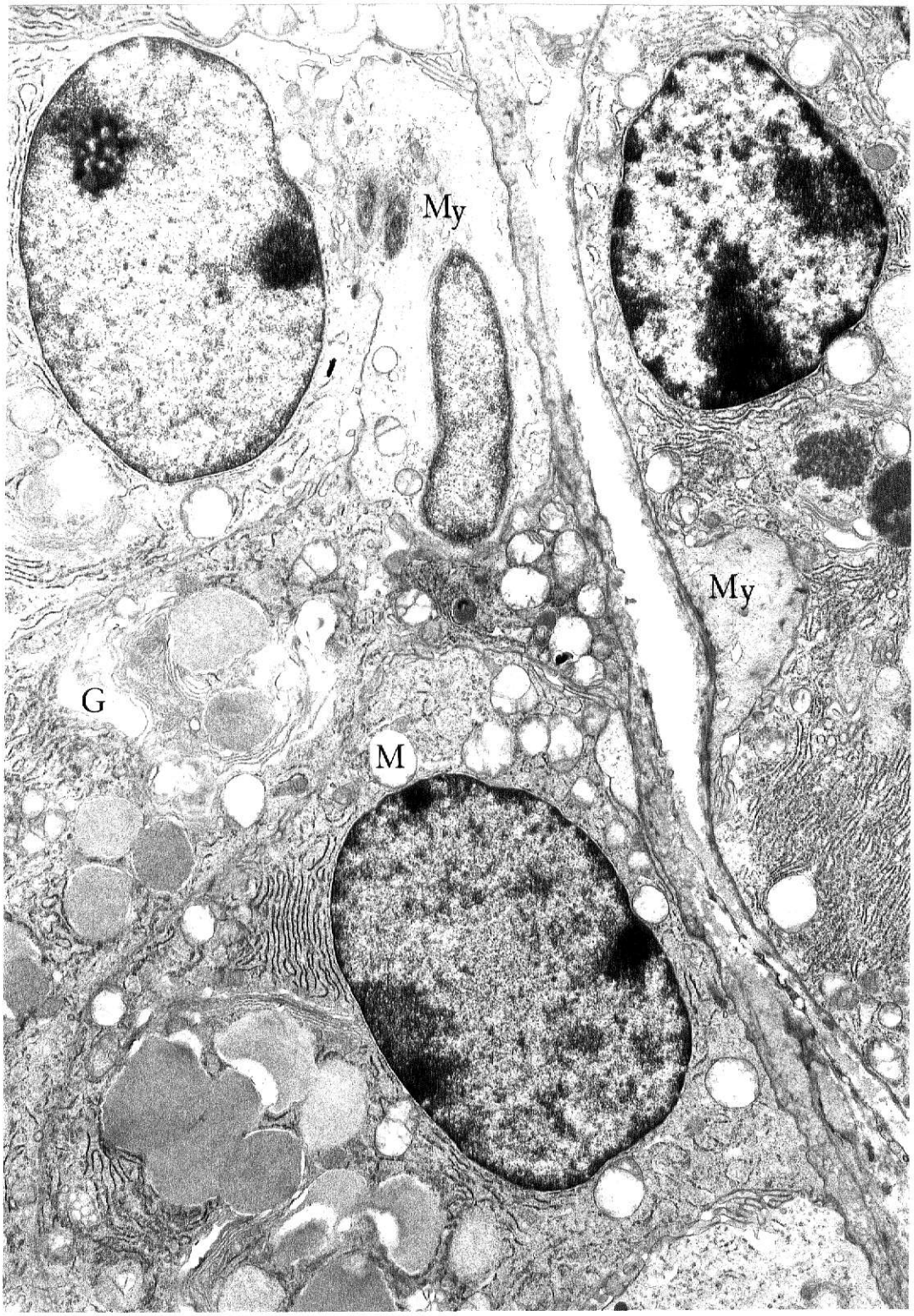

My
My
G
M

FIGURE 2

Central portion of serous gland from turbinate of Calf 4. The mitochondria (M) have lost their cristae, and the Golgi apparatuses (G) and rough endoplasmic reticulum (RER) are dilated. The lumen contains the secretory products (S). X10,900.

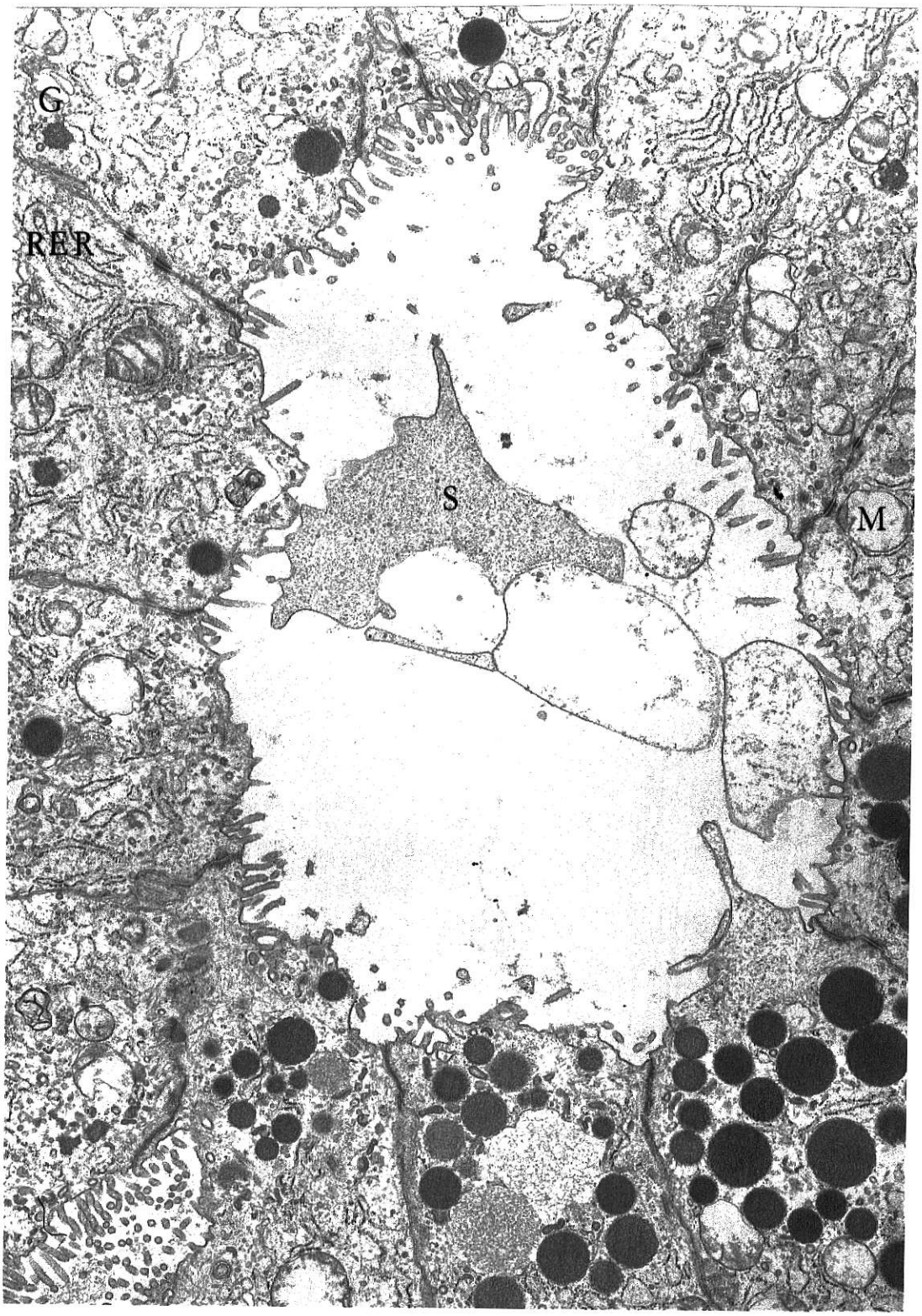
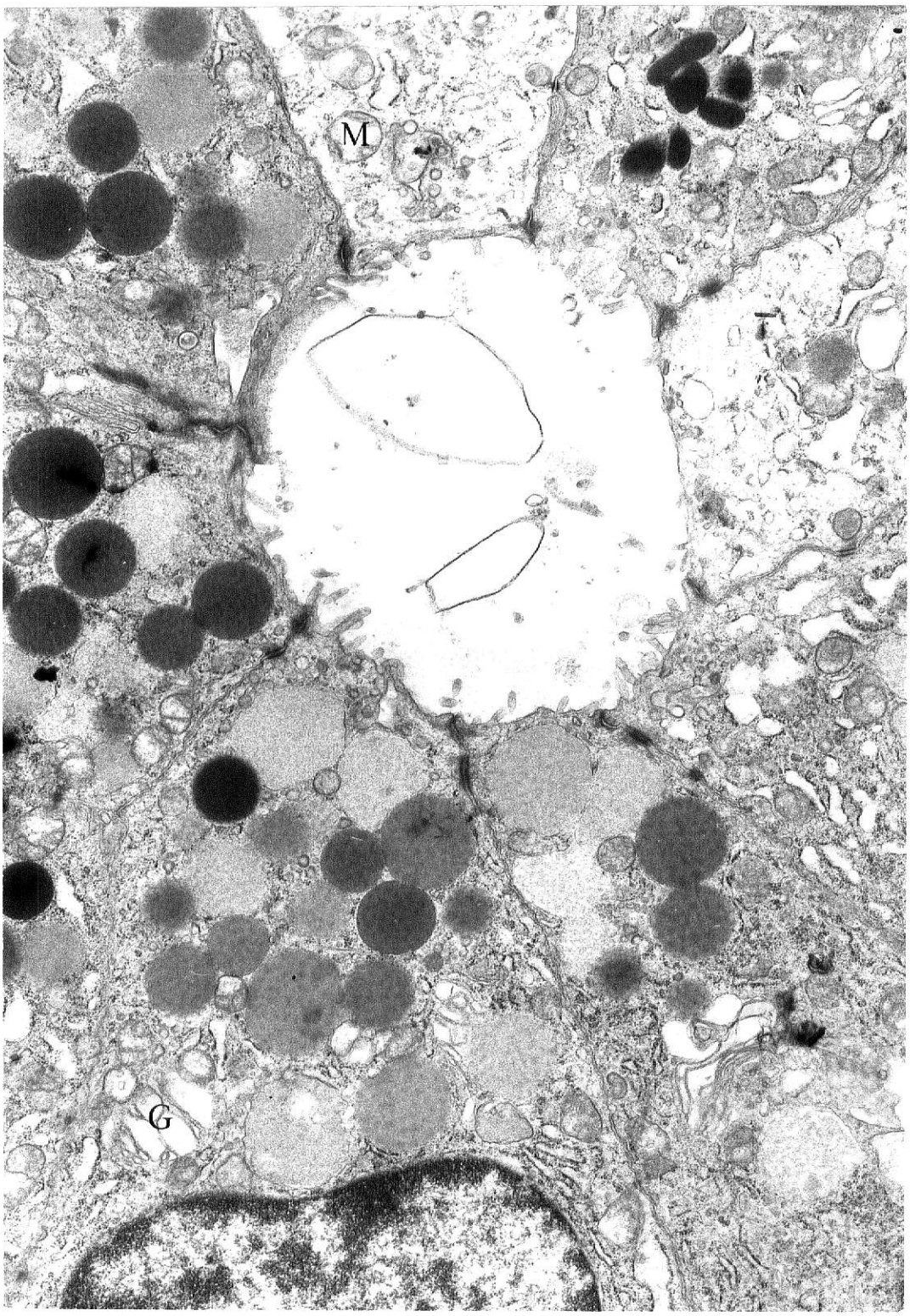

G
RER
S
M

FIGURE 3

Central portion of serous gland from turbinate of Calf 2. Dilated Golgi apparatuses (G) and loss of mitochondrial cristae (M) are again apparent. X14,700.


M
G

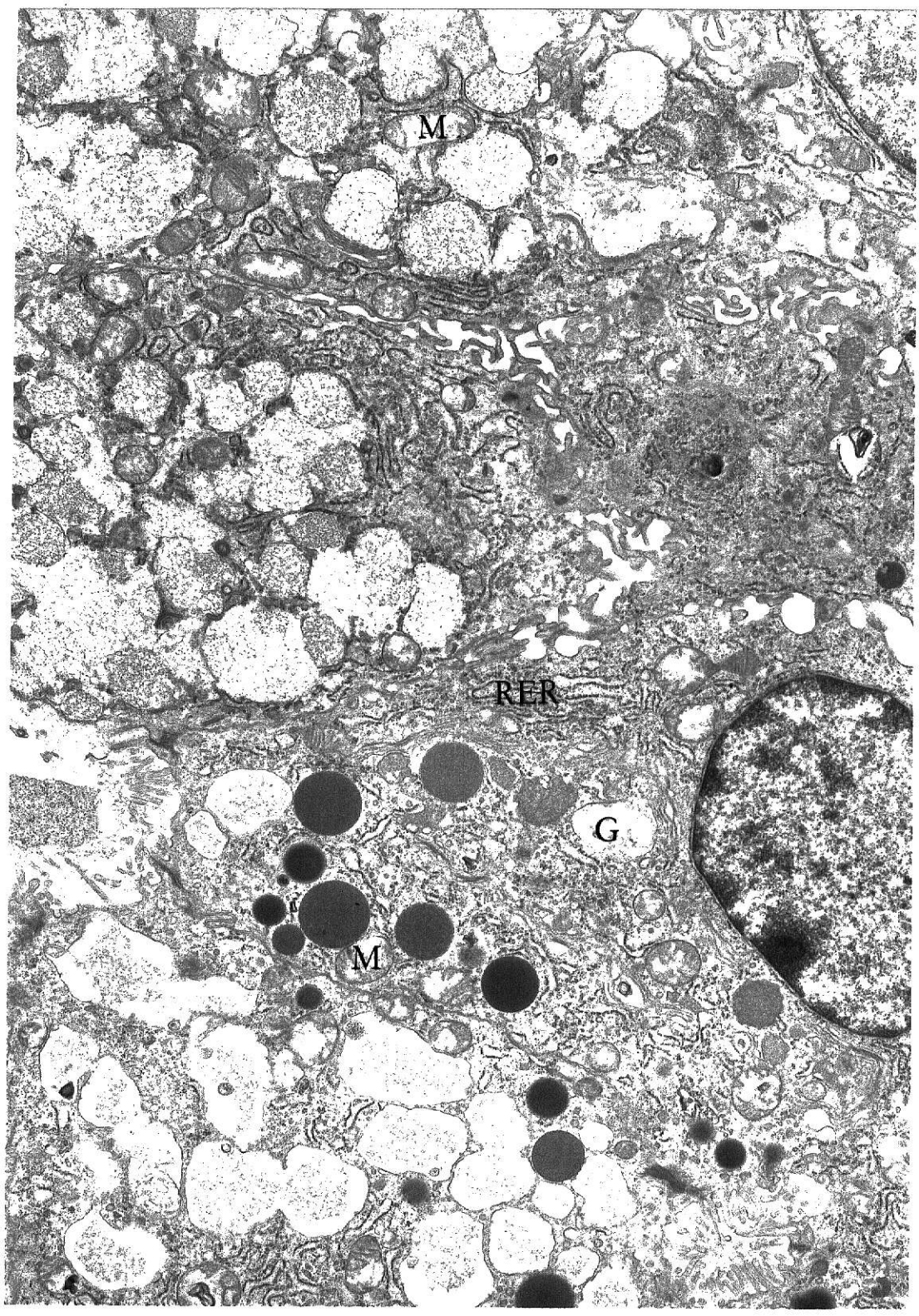

M
RER
G
M

FIGURE 5

Portion of serous gland from turbinate of Calf 1. Mitochondrial cristae (M) are lost or distorted, and the rough endoplasmic reticulum (RER) is slightly distended. X16,500.

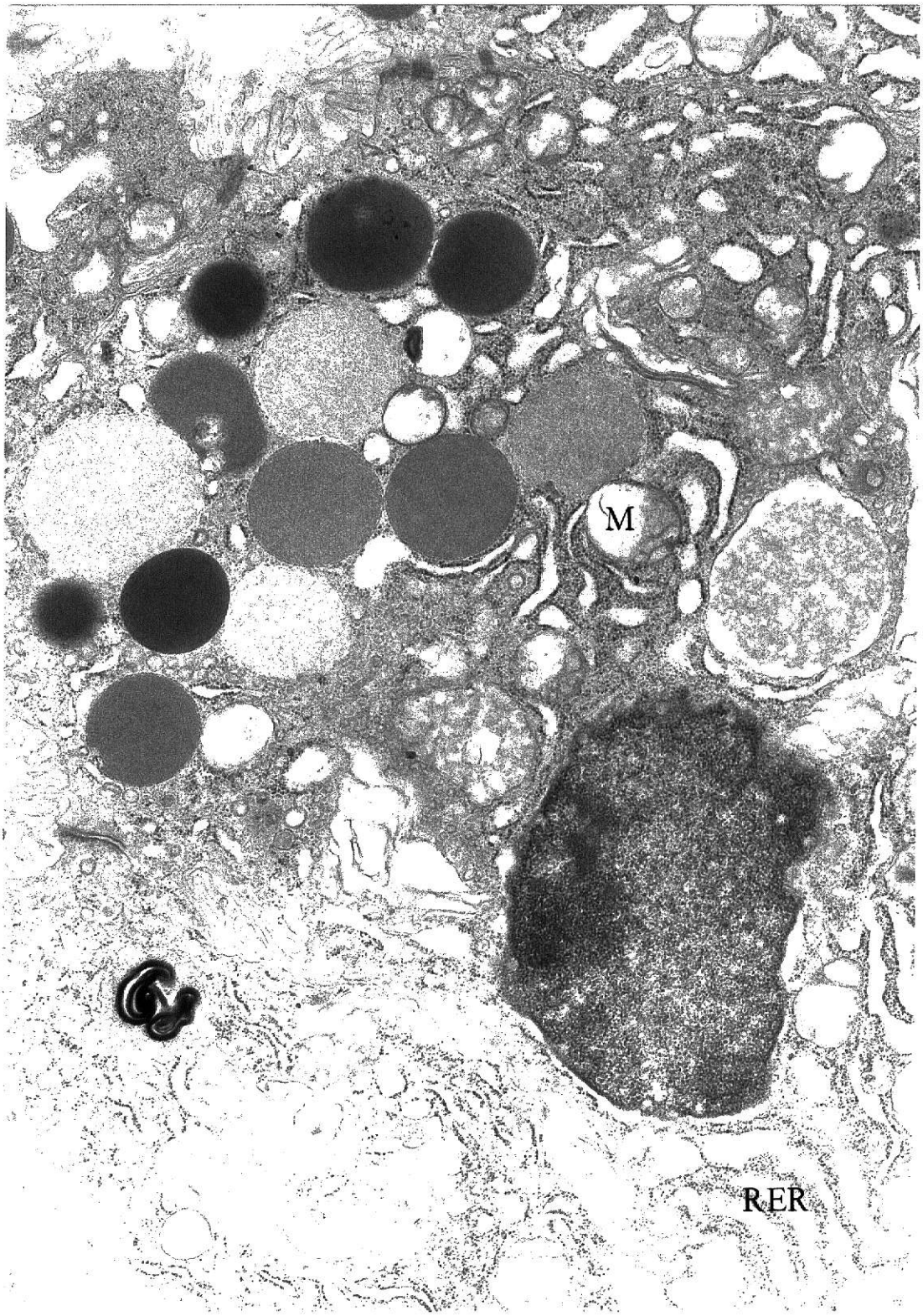

M
RER

FIGURE 6

Portion of serous gland from nasal septum of Calf 10 (uninfected control). Damage to mitochondrial cristae (M), distention of the Golgi apparatuses (G), and swelling of the rough endoplasmic (RER) are present. These changes are similar to those in infected calves. X10,800.

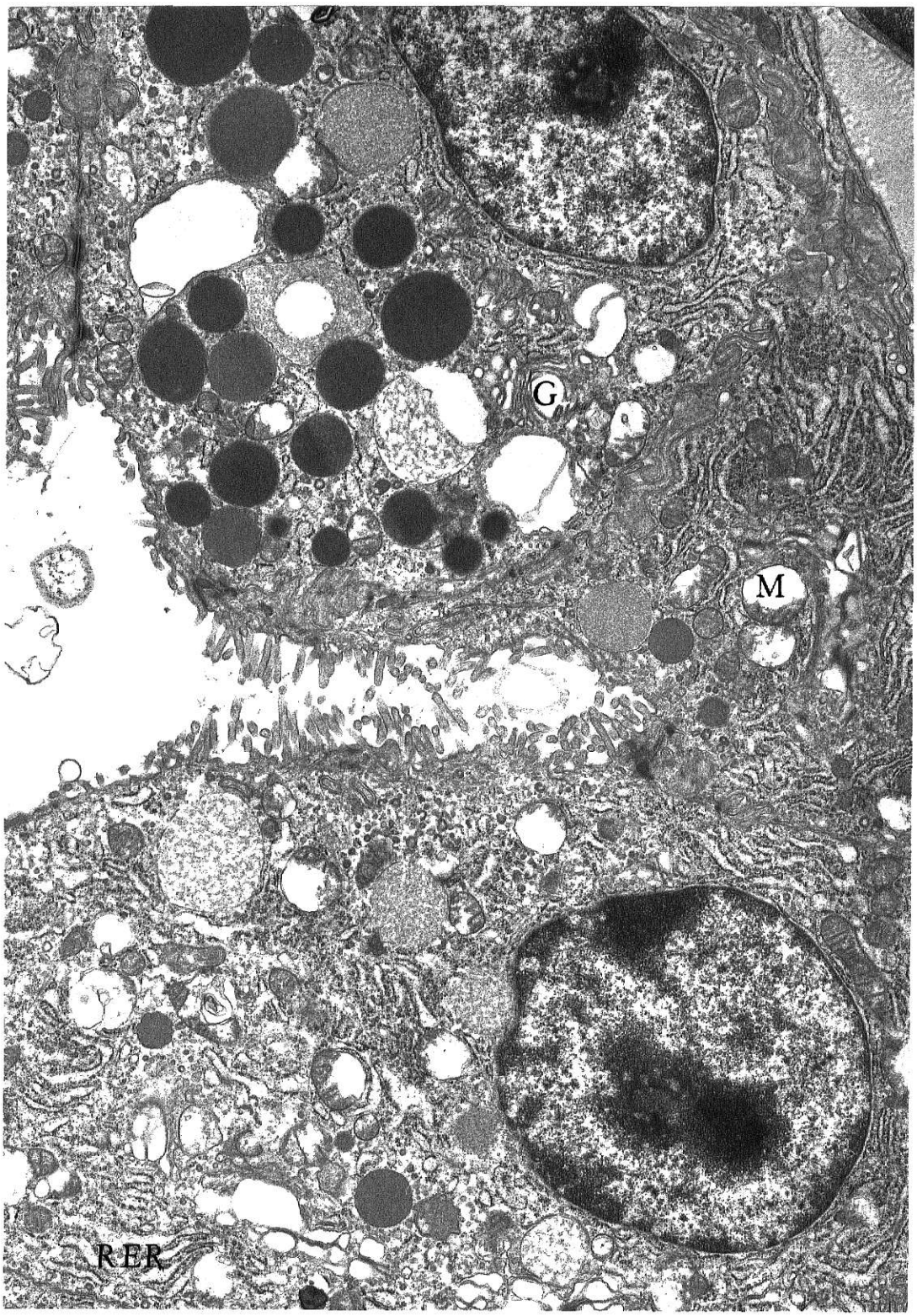
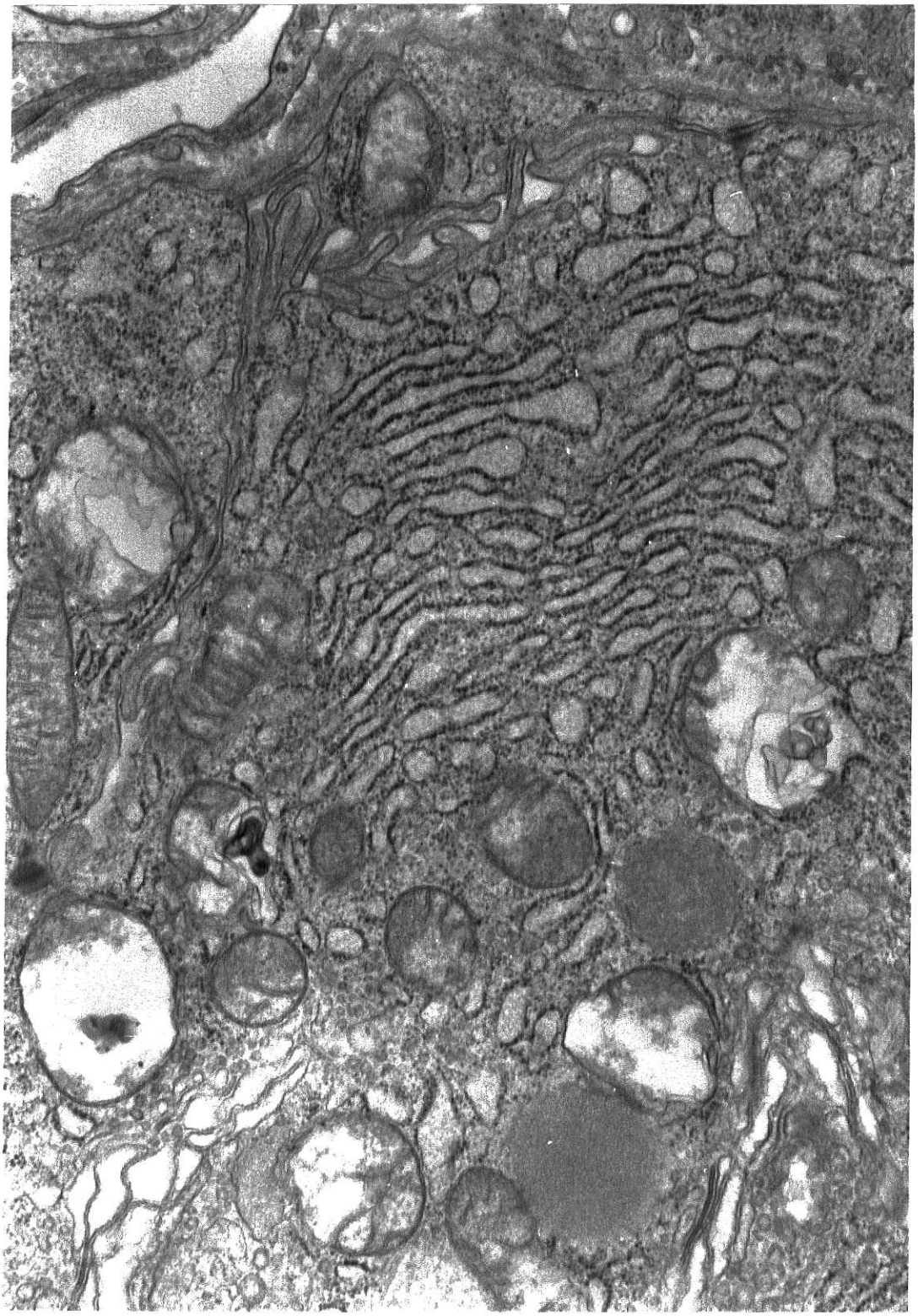

G
M
RER

FIGURE 7

Portion of serous cell from turbinate of Calf 11 (uninfected control). Extent of mitochondrial damage is clearly visible. X60,000.



LIGHT AND ELECTRON MICROSCOPIC STUDIES ON THE SUBMUCOSAL GLANDS OF RESPIRATORY NASAL MUCOSA IN CALVES EXPERIMENTALLY INFECTED WITH INFECTIOUS BOVINE RHINOTRACHEITIS

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AN ABSTRACT OF A MASTER'S THESIS

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A study was made to determine the light and electron microscopic changes in the submucosal glands of the nasal mucous membrane in calves experimentally infected with infectious bovine rhinotracheitis (IBR) virus. Ten calves were used in this study. Test calves were inoculated intranasally with 1 ml. of innoculum which contained $10^{5.5}$ median tissue culture infective doses of Colorado strain IBR virus. Control calves were given an equal amount of sterile tissue culture medium.

Two calves were euthanized and necropsied on each of the 2nd, 3rd, 4th, and 5th days post-inoculation. Tissues were collected for light and electron microscopy, and nasal washings were collected for fluorescent antibody testing.

Gross and light microscopic changes were not seen in the submucosal glands. Electron micrographs revealed no pathologic alterations, and IBR virions were not detected in the submucosal glands. Fluorescent antibody tests on nasal washings collected at necropsy were positive for 7 of 8 calves which received virus. It was concluded from these studies that IBR virus does not infect the submucosal glands of bovine respiratory nasal mucous membranes.

Detailed study was made of the ultrastructure of the nasal submucosal glands of 2 uninfected control calves, and these findings were compared to the reported normal ultrastructure of nasal submucosal glands of man and rat. Normal

bovine nasal submucosal glands were morphologically similar to those of man and rat, and numerous similarities were observed between serous and mucus cells of bovine nasal submucosal glands and serous and mucus cells of other organs and species.