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THE HISTOLOGICAL STUDY OF THE EFFECTS OF CERTAIN VIRUSES ON THE LACTATING BOVINE MAMMARY GLAND

by

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INTRODUCTION

Bacteria are usually considered to be the cause of bovine mastitis although the species involved may vary from case to case. Bacteriological examination of milk from mastitic cows does not, however, always reveal the etiological agent and the possibility of a viral mastitis must not be overlooked. The purpose of the present study is to compare the histopathology produced in the lactating bovine mammary gland by a virus of host range with that causedby a virus not of host range. A virus of host range is interpreted as meaning a virus which normally infects the bovine species. Such a study might yield results or observations which would aid in the diagnosis of viral mastitis. Furthermore if the cells in which virus multiplication takes place can be identified, the resultant information might prove to be of value in tissue culture work.

Veterinarians in general have shown little interest in the effects of viruses on the bovine mammary gland. Mastitis is reported as part of the syndrome in foot and mouth disease and in vesicular stomatitis but it still remains to be shown whether this mastitis is a primary result of virus infection of mammary tissues or is a secondary bacterial infection following vesicular formation on the teat. In malignant catarrhal fever tissue changes have been described

in the lactating and nonlactating mammary gland but in other virus diseases of the bovine the udder has received scant attention.

Certain studies have had as their objectives the propagation of virus in the bovine mammary gland and the measurement of the resulting antibodies. In other instances viruses were introduced into the mammary tissues solely in an effort to produce mastitis. However histological studies on the effects of the viruses introduced for any of the previously mentioned reasons have apparently not been reported. It would seem that information resulting from work conducted in this particular field should be both timely and pertinent.

LITERATURE REVIEW

HISTOLOGY OF THE BOVINE MAMMARY GLAND

Pathology might be defined as a science which attempts to correlate specific effects to definite causes. In order to recognize these specific effects one must have a thorough knowledge of the normal structure. For example, the bovine mammary gland has been reported to undergo various histological changes in relation to various phases of the reproductive cycle (Hammond 1927, Espe 1946, Turner 1933, Emmerson 1946) and changes were also noted in recently-milked glands as compared to milk-distended glands (Turner 1933). As such changes might be considered of pathological significance the normal structure of the udder must be considered.

The bovine udder is divided into two halves by the median suspensory ligament. Each half in turn is divided into two quarters by a thin connective tissue septum which is not obvious grossly. The gross features of a rear quarter are illustrated by a photograph of a sagittal section through the teat (Fig. 1).

Numerous histological studies have been conducted on the bovine mammary gland and many excellent reviews are to be found in the literature. Notable among these are the works of Helmboldt, Jungherr and Plastridge (1953), Turner (1933) and Hammond (1927). McFadyean (1930), Holm (1937), Kwong (1940), Venzke (1940), Emmerson (1946) and Yamazwa, Ono, Vemura and Ida (1958) contribute much to the present knowledge of the histology of this organ. The following description was compiled from a review of the above mentioned references.

The mammary gland consists of a system of ducts which show marked ramification and branching. The terminal ductules enlarge forming alveoli or acini (Fig. 2). The ducts are chiefly lined by two rows of cells (Fig. 3) except for the finest ductules (intercalary ducts) which are lined by a single row of cells (Fig. 2). Generally, in an alveolus there is only one epithelial cell layer (Figs. 2 & 3) but it is not uncommon to find alveoli possessing a multilayered epithelium in a portion of their circumference. While this difference is considered to be due to hyperplasia no pathological significance is attributed to it.

In the fully distended (milk-laden) epithelial cell one notes fat droplets and a clear homogeneous cytoplasm. The nuclei are usually pushed toward one end of the cell by the fat droplets; sometimes being near the free border but usually at the base of the cell. As milk

Fig. 1.

Fig. 1. Sagittal section through rear quarter of a lactating bovine mammary gland.

1. Streak canal
2. Teat cistern
3. Gland cistern
4. Ducts
5. Udder parenchyma
6. Supramammary lymph node

accumulates in the lumen of the alveolus there are changes in the epithelium. The lobules shrink as a whole when the gland is emptied by milking and the interlobular septa are broadened to the extent that they are visible to the naked eye, the alveolar epithelium thickens, its cells become columnar in shape and project irregularly into the lumen (Fig. 2). The alveolar walls are often wrinkled, particularily in the large alveoli which become elongated with cleft-like lumina. The smaller alveoli tend to remain spherical. The ducts are much narrower than in the distended gland but the lumina remain open and continuity of lumen between the ducts and cisterns is maintained. The walls of the contracted ducts are more thickened and folded.

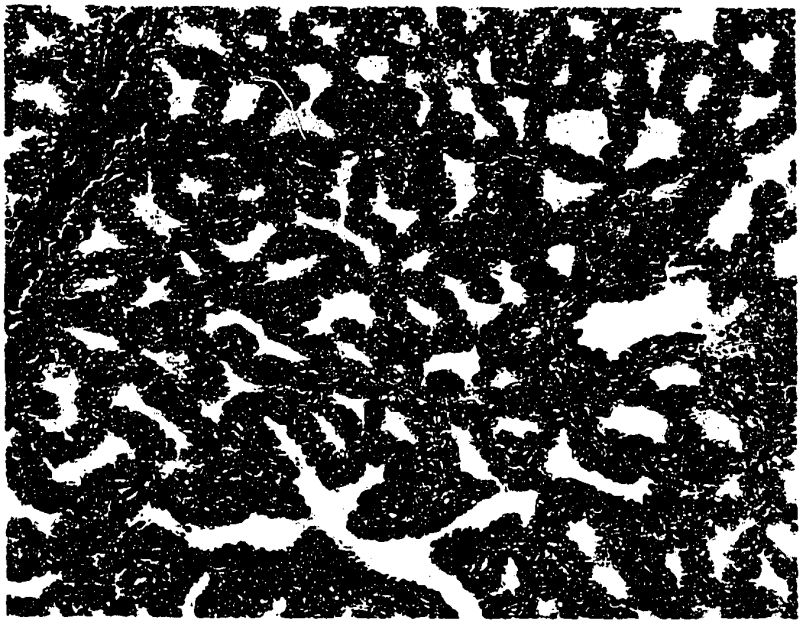
In a full udder the alveoli are widely distended, the cells markedly flattened and the nuclei widely separated. Mitotic figures are sometimes but not frequently noted in the secretory cells. The close relationship between the terminal ducts and the alveoli is indicated by the relatively cellular, non-fibrous character of the stroma surrounding the ducts. This suggests a more active metabolism in the terminal duct epithelium similar to the alveolar epithelium. There is a gradation of structure and function from the more peripheral excretory ducts to the central terminal ducts and alveoli.

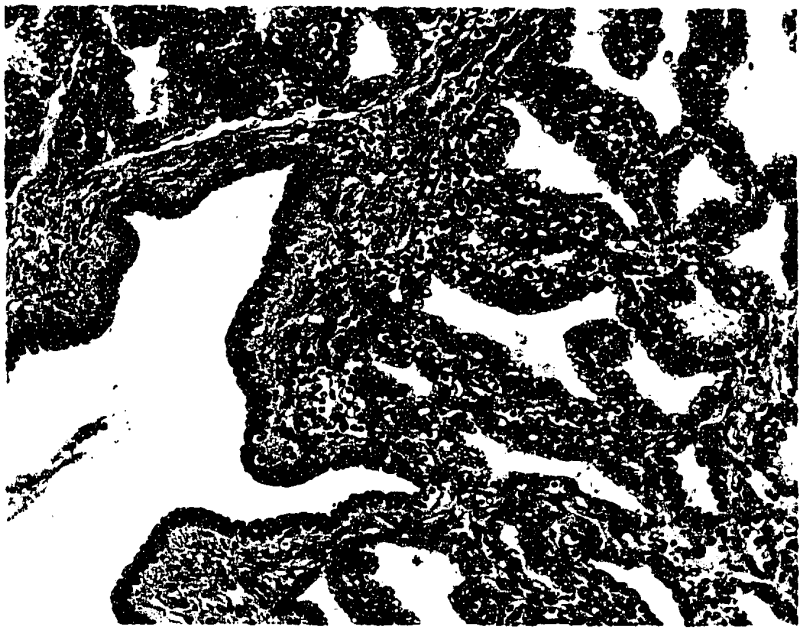
The parenchyma of the gland is divided by connective tissue septa into divisions of increasing size which are connected by a system of ducts. The alveoli are rarely separated by fibrous connective tissue. Phase microscopy has shown that the alveolar epithelium rests on a distinct membrana propria or basement membrane. Outside this basement

Figs. 2 and 3.

Fig. 2. Section of normal lactating bovine mammary gland (immediately after milking) showing alveolar epithelium, intercalary ducts and interlobular septa. H & E X 108.

Fig. 3. Section of normal lactating bovine mammary gland showing duct lined with two rows of cells, aggregates of lymphocytes and alveoli. H & E X 180.





membrane is a basketwork formed by myoepithelial cells. This network is particularily abundant about the ducts. These cells, variously named korbzellen, basket or myoepithelial cells consist of an elongated fibril which may extend half way around a large alveolus or medium sized duct and is associated with a small, flattened, moderately dense and sometimes wavy spindle-shaped nucleus.

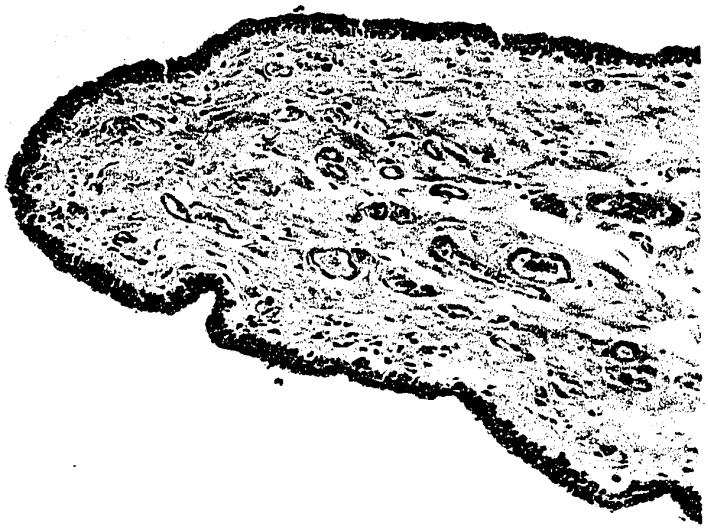
A group of alveoli form the lobule. The lobule is surrounded by a distinct fibrous connective tissue septum (Fig. 2). The intercalary duct (ductule) of each alveolus unites to form small intralobular ducts which drain into a central intralobular milk-collecting space. At the periphery of the lobule the intralobular collecting space narrows and passes through the connective tissue septum and becomes an interlobular duct. These are the ducts which are found in the broader septa of connective tissue between the lobules (Fig. 3). A group of lobules are united by broader septa into a lobe. The interlobular ducts unite in a dilated interlobular milk-collecting space. This space narrows at the periphery of the lobe into a single duct and passes out of the lobe to become an interlobar duct. These interlobar ducts in turn unite to form a dilated milk-collecting space, which in turn empties into the gland cistern. The ventral third of the udder tends to exhibit less evidence of secretion than the more dorsal portions. The region of the large ducts entering the gland cistern is surrounded by dense connective tissue. Caution should be employed in the histologic interpretation of the ventral levels since the supportive tissue might easily be mistaken for replacement fibrosis.

The lining of the gland cistern (sinus lactiferi or receptaculum lactis) is quite similar to that of the larger ducts (Fig. 4). Accessory glands lined by simple cuboidal epithelium and frequently surrounded by lymphocytic infiltrations are located in the tunica propria of the gland cistern. The gland cistern is contiguous with the teat cistern (ductus papillosus or ductus lactiferi). The lining of the teat cistern is similar to that of the gland cistern and accessory glands are usually found throughout the length of the teat. The accessory glands of the bovine udder appear to be analogous to the glands of Montgomery seen in the areola of man. The double layered epithelium of the teat cistern changes abruptly to stratified squamous epithelium which lines the teat canal (streak canal) and is contiguous with the stratified squamous epithelium covering the teat itself.

In most cows there is an increase in the rate of secretion for approximately one month following parturition after which time there is a gradual decline in this rate. Whether these changes are the result of the gradual decline in activity of individual alveolar cells or whether there is a gradual cessation of the most peripheral and least nourished parts is not known. As soon as the cells stop secreting milk the alveoli and the ducts with the one layer of epithelium involute until in the dry udder only the collecting ducts and interstitial tissue remain. As involution takes place the lymphocytes (Fig. 3) increase in number in the interalveolar tissue. They proliferate and form diffuse aggregates often simulating a picture of so-called diffuse interstitial mastitis. Care should be exercised in the interpretation of the pathologic significance of this reaction solely on the basis of finding lymphocytes.

Fig. 4.

Fig. 4. Section of gland cistern from normal lactating bovine mammary gland showing epithelial lining with two rows of cells.
H & E X 160.



Corpora amylacea are normally found in the bovine mammary gland. These concretions vary considerably in size usually consisting of stratified layers and are resistant to acids, alkalis and fat solvents. These bodies are found in the alveolar lumen, alveolar, duct and cistern epithelium and in the interstitial tissue. They occur in two forms. The commonest is a darkly staining solid concretion often smaller than the nucleus of the alveolar cells. The other type is a large nucleated body consisting of material precipitated around the alveolar epithelial cells. Occasionally the corpora amylacea become imbedded in the interstitial tissue by having an alveolus atrophy around them. The number of corpora amylacea is unrelated to bacterial infection or the length of lactation.

CLASSIFICATION OF BOVINE MASTITIS

Bovine mastitis is caused by a large number of infectious agents which gain entrance into the udder through wounds or are introduced through the teat canal or are carried there by the blood stream. Of these agents bacteria are by far the most important and most common causes of mastitis. There are however cases of mastitis from which no etiological agent can be demonstrated. Because of the great variety of bacterial and fungal agents involved in the etiology of bovine mastitis it has become a common practice to classify mastitis according to etiological rather than histological criteria. We thus have brucella mastitis, leptospiral mastitis, streptococcal mastitis, staphylococcal mastitis, cryptococcal mastitis, mycobacterial mastitis, etc. Histologically most cases of mastitis are almost solely an acute inflammation. Chronic mastitis if defined on the basis of histopathology is very uncommon and exists chiefly as a clinical entity.

Helmboldt, Jungherr and Plastridge (1953) reviewed the classification of bovine mastitis and presented a classification of their own based on histological criteria. It was felt that since their classification was relatively simple, yet workable, that it would serve our present needs and is summarized here:

Acute Mastitis

Grade I or Slight

Grade II or Moderate

Grade III or Marked

Chronic Mastitis

Acute mastitis

Grade I.

Histological changes are slight and confined to alveolar and ductal epithelium and rarely involve the cisternal regions. The alveolar cells appear hypertrophied and are perhaps unduly vacuolated. Free epithelial cells are seen in the area of the lesion but caution is necessary in interpreting small numbers of them as pathologically significant since such cells are seen in normal udders. These cells do not exhibit evidence of phagocytic activity nor do they form giant cells. The secretion tends to be clotted and remains within the alveolus, often attaching itself to the walls by pseudopodia-like processes. Under most circumstances normal milk is washed out of the tissue during processing, leaving patent lumina. Neutrophils and a few eosinophils are seen in the periductal tissue but these appear to be migrating to the affected area. Bacteria are not seen in the tissue but appear in some instances with the debris in the lumen.

Grade II.

This form is an extension of grade 1. The reaction is more intense and extends over a larger area, usually more than a lobule. Alveolar epithelial changes are similar to those of grade 1 but atrophic changes are seen. Areas where the alveolar epithelium has sloughed are rare but loss must be considerable judging by the number of free epithelial cells. Neutrophils predominate with small numbers of eosinophils, plasma cells, macrophages and lymphocytes. In this grade the granulocytic elements are usually seen within the lumen as well as in the periductal tissue. In coliform mastitis there is a surprising shift in the leucocytic type to a predominance of agranulocytes. The secretion is clotted and contains leucocytes, epithelial cells, and rarely, bacteria.

The interstitial tissue is also affected and in addition to the leucocytic infiltration, oedema and vascular engorgement are noted. The periductal tissue reacts by proliferation resembling involution. The lamina propria of the cisternal membranes is infiltrated with leucocytes, usually agranulocytes, and the normal double-layered epithelium may be hypertrophied, hyperplastic or both. In some instances metaplasia and desquamation are seen. This condition is often associated with varying degrees of involution.

Grade III.

The lesions are massive, usually there is necrosis with subsequent loss of architecture and infiltration with neutrophils and macrophages. Ducts are filled with cellular debris and exudate. The whole process

is essentially the same as grades I and II except that the lesions extend over a larger area and is therefore more destructive.

Chronic mastitis

Grade III mastitis sometimes results in a stalemate between infecting organism and the defense mechanism of the udder. The infecting organism becomes localized and the fibrous elements proliferate in an attempt to contain it. Lymphocytes replace the neutrophils in the infiltration.

REACTION OF THE BOVINE TO VIRAL INFECTION

A definition of the term virus might be of value before attempting to deal with the reaction of the bovine to virus infection. There appear to be several schools of thought on the subject of "What is a virus?" There are those who look upon viruses as micro-organisms, there are those that look upon viruses as molecules and there are those like Lwoff (1957) who say viruses are viruses.

Syverton (1960) in defining the term virus first defines a cell as a natural device for carrying out certain biochemical syntheses and for building replicas of itself. To permit this accomplishment it incorporates a built-in code of instructions, the genic-chromosomal apparatus. The virus likewise has a built in code of instructions, in the form of nucleic acid wrapped as a package to facilitate entry into a susceptible host cell.

This concept of virus involves two points: (i) foreignness to the cell, implied by the fact that the nucleic-protein code includes instructions for specific packaging and (ii) complete dependence of the

virus on the cell for physiologic activity. The virus is a relatively passive partner in a peculiar host-parasite relationship. It is a packaged blueprint that causes a cell to divert its energy partly or wholly to the production of more virus. Incidentally, but as a result, the cell is physiologically impaired or destroyed producing cytopathic effects.

Lwoff (1957) defines a virus more precisely as a strictly intracellular and potentially pathogenic entity with an infectious phase and (i) possessing only one type of nucleic acid, (ii) multiplying in the form of its genetic material, (iii) unable to grow nor to undergo binary fission and (iv), devoid of a Lipmann system. He qualifies the above by stating that any one of the characters (i) to (iv) is sufficient to identify an entity possessing an infectious nuclear-proteinic phase as a virus.

Syverton (1960) believes cellular impairment resulting in cytopathic effect might be attributed to (a) deleterious toxic influence of newly replicated virus particles as they accumulate in the infected cell; (b) a simple inadequacy of the cell to bear the combined load of cell maintenance and virus manufacture, and/or (c) the diversion of essential metabolic processes from cell maintenance to virus production.

Saunders (1957), however, does not feel that damage of animal cells is correlated with the amount of new virus produced as would seem to be the case with bacteriophage where the number of virus particles required to produce lysis is of the same order as the burst size. It would appear that animal viruses generally are able to escape with ease from cells almost as soon as they are formed. In the case of the

viruses of influenza, western equine encephalomyelitis and poliomyelitis there is a period after the infecting virus has penetrated the cells during which no infectivity is recoverable, followed by a period during which new virus is released in an exponential manner.

Rubin, Baluda and Hotchin (1955) have shown that western equine encephalomyelitis virus growing in chick fibroblasts produces in the order of 1,000 plaque-forming units per cell. The release of new virus begins about two hours after infection and continues for up to 12 hours. Released virus appears in tissue culture fluids about the same time as in the cells. These workers showed that only 4 to 10 viruses were present within the cell at any given time out of a total of 100 to 1,000 produced by the cell. It would thus seem unlikely that cytopathic changes are brought about by the accumulation of virus particles within the cell as would seem to be the case with bacteriophages. Eagle (1955) has shown that the ommission of an essential vitamin, amino acid, growth factor or glucose from the tissue culture medium brings about cytopathic effects. It is quite possible then that the cytopathic effect of virus infection may actually result from a deficiency of an essential nutrient brought about by virus multiplication.

When dealing with virus infected tissues of the bovine we see the results of a process in which at least two elements can be distinguished. Firstly we see the result caused by the direct attack of the virus upon the cells and secondly we see the response of the animal to the primary cellular damage by means of an inflammatory reaction.

The bovine reacts to viral infection in a number of ways. Infection may result in the proliferation of cells, the degeneration and necrosis of cells or a combination of the two.

The virus of bovine cutaneous papillomatosis is the best example of a bovine virus eliciting an almost purely proliferative response. Infection with this virus results in sessile or pedunculated papillomas which project above the surface of the skin as cauliflower-like growths. Cheville and Olsen (1964) have shown that following a morphologically dormant phase, the first reaction is that of fibroblastic proliferation. This is followed by epidermal hyperplasia resulting in the formation of a mature papilloma.

Bovine viruses eliciting both proliferation and necrotizing responses are represented by bovine papular stomatitis, bovine epizootic abortion, bovine malignant catarrhal fever, lumpy skin disease, cow pox and sporadic bovine encephalomyelitis. These are listed in order of a decreasing proliferative response and increasing necrotizing effect.

The earliest changes detected histologically in bovine papular stomatitis (Griessemer and Cole, 1961) were focal hydropic degeneration and epithelial hyperplasia of the mucosa and epidermis. The mucosa was thickened in nearly all instances and was often twice normal thickness with long slender rete pegs. The foci of hydroptic degeneration are about 200-300 u in diameter and are located in the stratum spinosum. The nuclei are shrunken, distorted and sometimes fragmented. The cytoplasm appears empty except for a network of very fine fibrils and

granules. As the disease progresses the foci of degeneration move closer to the surface because of the normal proliferation of basilar epithelium. Plowright and Ferris (1959) describe the presence of intracytoplasmic inclusion bodies in the affected cells. These are large granular bodies which show a more or less clear differentiation into an inner, paranuclear matrix which is eosinophilic and an outer arc or loop of more basophilic material. The nuclei show irregular shrinkage and folding of the nuclear membrane with margination of chromatin. The intercellular bridges disappear and vacuoles containing nuclear debris appear between the cells. In the underlying lamina propria of the dermal papillae there is capillary congestion, a mild increase in histiocytes and infrequently infiltrations of small numbers of lymphocytes. Bacteria were found only in the lesions with disorganized surface layers. In nearly half the lesions, most often at the margins of the nostrils and in the rumen, reticulum and omasum there is infiltration of the degenerative focus by neutrophils. This, according to Greisemer and Cole (1951) is not associated with bacteria. Occasionally the basilar epithelium was disrupted by migrating neutrophils. As the lesions progress neutrophils persist in the degenerative foci and in nearly healed lesions neutrophils are present in small clusters in the stratum corneum.

In bovine epizootic abortion (Kennedy, Olander and Howarth, 1960) the response is a diffuse or focal reticuloendothelial hyperplasia which involves all organs. The effects of the virus are not entirely proliferative and small necrotic foci may develop. Irregular areas of necrosis are often noted about dilated central veins and sinusoids in

the liver but a more specific granulomatous reaction develops in the hepatic capsule around the portal triads and in the adventitia of the central veins. This process varies from small foci of proteinaceous exudate accompanied by neutrophil and macrophage infiltration to larger areas of epithelioid cells surrounded by fibrous tissue. In lymphatic tissues such as the spleen, thymus and lymph nodes, there is hyperplasia of reticuloendothelial elements, infiltration of neutrophils in the sinusoids and the formation of multinucleated giant cells.

In bovine malignant catarrhal fever (Berkman, Barner, Morrill and Longham, 1960) the process in the stratified squamous epithelium begins as a multiplication of cells in the stratum germinativum accompanied by an increase of fibrous connective tissue cells and an infiltration of lymphocytes in the dermal papillae. This produces clubbing of the rete pegs. Increased cellular activity is apparent around blood vessels which are congested. Ballooning cells with condensed acidophilic cytoplasm were found in the central areas of the rete pegs. The basal layer becomes indistinct due to loss of nuclear polarity by proliferating cells. Thickened keratin layers often maintain the external continuity of the tissue. In more severe lesions large clumps of ballooning cells undergo reticulating colliquation, resulting in vesicular formation in the upper epithelium. These vesicles contain cellular debris, proteinaceous fluid and occasionally abundant neutrophils. The lesion pattern is consistent in all systems and consists of proliferation, necrosis and infiltration. Proliferation of connective tissue, endothelial and epithelial cells is widely

distributed in epithelial tissues and blood vessels. Infiltrations consisting of monocytes, fibroblasts, lymphocytes, polymorphonuclear leucocytes and plasma cells are generalized, affecting the laminae propriae of many structures as well as the periportal areas of the liver and blood vessels. The necrosis which occurs particularly in the squamous epithelium may be attributed to the ballooning degeneration, vesiculation and loss of epithelium which exposes the tissue to secondary invaders. The involvement of the vasculature in proliferative and infiltrative processes which obstruct the lumen or disrupt the architecture of the vessel wall affect the circulation to the tissues. This latter process would appear to be most important in the abomasum and intestine. The increase in perivascular cellular elements would also seem to produce a pressure which may affect cells directly and shut off lymphatic and vascular channels.

Burdin (1959) describes the early changes in lumpy skin disease as a ballooning degeneration of individual cells in the more basal layers of the epidermis. This results in swelling of the epidermis and marked enlargement of the rete pegs. When several adjacent cells are affected it gives the impression of a multilocular cyst but in no instance was vesicular formation evident grossly. Other nonvacuolated cells are also swollen, more acidophilic than normal and the intercellular spaces are indistinct or obliterated. Intracellular inclusion bodies are observed in the early nodules. These are usually round or ovoid and vary in size from that of a nucleolus to considerably larger than their accompanying nucleus. Most inclusion bodies tend to stain

basophilic. The majority of inclusion bodies are contained within vacuoles. A large proportion of nuclei of cells containing intracytoplasmic inclusions and a smaller number of those without, show degenerative changes and are generally smaller than normal. Margination of chromatin was the most striking feature, the nucleoli being visible against the nuclear membrane. In cells with advanced changes the nuclear membrane is collapsed and the nucleus pyknotic. The dermal papillae are markedly oedematous and are infiltrated with mononuclear cells, most of which are in varying stages of necrobiosis. In some cases the pressure of the exudate causes detachment of the overlying epidermis. The stratum reticulare of the corium is less oedematous but infiltrating mononuclear cells are present, especially about small blood vessels. In the deepest parts of the corium and in the subcutis cellular accumulations are most abundant about larger venules and arterioles. These infiltrations consist chiefly of macrophages, small and large lymphocytes, fibroblasts and a few plasma cells. Intracytoplasmic inclusion bodies are also noted in the infiltrating cells, especially the macrophages and lymphocytes. Venous thrombosis is also a part of the pattern, probably accounting for much of the overlying oedema. In the mature lesion or sitfast, areas of coagulation necrosis had occurred in the surface layers. In the most advanced lesions, a wedge of necrotic tissue is present with its base at the epidermis and extending down into the subcutis. Massive accumulations of infiltrating cells are present about blood vessels and fibroblastic proliferation is prominent.

In cowpox (Jubb and Kennedy, 1963) the lesions are typical pocks, beginning as small hyperaemic papules which are transformed into vesicles, and then pustules which rupture, dry up and scab. The vesicles are small and multiloculate so that they do not protrude but instead the surface appears indented as the margins are raised by rapid proliferation of the epithelium at the edge. Cytoplasmic inclusion bodies form in the epithelial cells. This is a vesiculating infection, the vesicles resulting from epidermal spongiosis with ballooning and reticular degeneration. In the pustular stage, the reticular network undergoes lysis so that the pustules are not umbilicate but instead are flat or protrude.

The microscopic findings in sporadic bovine encephalomyelitis (Menges, Marshfield and Wenner, 1953) are those of a fibrinous serositis involving the peritoneal, pleural and pericardial sacs as well as a diffuse and marked meningo-encephalomyelitis. There is an acute and subacute inflammatory exudate with organization of the exudate. Changes observed in the central nervous system consist of a diffuse inflammatory response producing a meningitis, encephalitis, myelitis and vasculitis. Cellular damage, particularly to ganglion cells in the brain and neurons of the spinal cord is pronounced. The endothelium of the blood vessels shows proliferative changes, vessel walls being infiltrated with mononuclear cells but occasionally polymorphonuclear cells predominate. Considering the diffuse vascular damage, it would appear that vasculitis was primary and the neuronal damage was secondary. Minute elementary bodies have been found in mononuclear and polymorphonuclear cells in the exudates in the central nervous system and of the fibrinous serositis.

Bovine viruses producing an almost purely necrotizing effect on the cell are infectious bovine rhinotracheitis, Aujeszky's virus, rabies, foot and mouth, vesicular stomatitis, virus diarrhoea and rinderpest.

In infectious bovine rhinotracheitis, the salient cytological features occur in the mucosa of the upper respiratory tract (Crandell, Cheatham and Maurer, 1959), vulva and vagina (Kendrick, Gillespie and McEntee, 1958), penis and prepuce (Studdert, Barker and Savan, 1964), the eye (Abinanti and Plumer, 1961), brain (French 1962) or foetus (Owen, Chow and Molello, 1964) depending upon the syndrome presented. Changes appear to be similar in both stratified squamous and pseudo-stratified columnar epithelial cells of the upper respiratory tract. The cytoplasm of affected cells was pale and vacuolated. As ballooning degeneration took place the cytoplasm became granular and the cell lost its outline. Nuclear changes were noted in cells with cytoplasmic alterations. Small, irregular aggregates of pale, acidophilic material were first noticed scattered about the nuclear chromatin. As the nuclear chromatin marginated the nucleolus either disappeared or contracted and remained as a small basophilic body at the nuclear margin. The acidophilic material tended to become more homogeneous and filled the major portion of the nucleus. This material then contracted and left a clear halo surrounding a distinct, acidophilic and often irregular inclusion body. Well-formed inclusion bodies were not found after 72 hours. When the above-mentioned changes resulted in disruption of the surface an acute inflammatory infiltration and secondary bacterial infection developed.

Changes in the genital tracts of males and females were quite similar to those described above for the upper respiratory tract with the exception that an intense inflammatory reaction took place before any disruption of the continuity of the tissue. This infiltration consisted largely of neutrophils and lymphocytes and was focal in the epithelium and diffuse in the subepithelial tissue. The neutrophils rapidly filled the area of destruction while large numbers of lymphocytes infiltrated the surrounding connective tissue. In the vulva, lesions tended to occur over the lymphatic follicles and necrosis of the connective tissue over the follicle took place. Lymphocytes accumulated around the follicle in such numbers that it was impossible to recognise the normally sharp boundary. Again inclusion bodies were few in number after three days.

In aborted foeti foci of coagulation necrosis were noted in the liver and spleen. In the liver these foci were distributed throughout the lobules but were more numerous in the central and paracentral areas. In the spleen the lesions were almost entirely confined to the red pulp. A few necrotic foci were found in the kidney. In first trimester foeti the necrotic foci did not attract any inflammatory response while a mononuclear cell response was elicited in third trimester aborted and dead foeti. In the latter foeti the infiltrating cells were concentrated in the portal triads, especially about the bile ducts. The predominating cell appeared to be lymphocytic in nature but macrophages and plasma cells occurred in small numbers.

Encephalomyelitis was reported in calves (French, 1962) but detailed histological studies are not reported in the literature.

The encephalitis of Aujeszky's disease (Shahan, Knudson, Siebold and Dale, 1947) is characterized by moderate cuffing with lymphocytes and occasional eosinophils. There is also an infiltration of the brain substance in the form of small foci. Neurons in the inflamed areas show definite degenerative changes, characterized by the disappearance of the nucleolus, margination of the nuclear chromatin and an accumulation of a pink or slate-coloured material replacing the normal nuclear structures. These formations resemble the inclusion bodies reported by others in other species of animals.

In rabies (Jubb and Kennedy, 1963) the disease process is usually characterized by a severe focal polioencephalomyelitis. There are perivascular infiltrations in the Virchow-Robin spaces consisting solely of lymphocytes, numerous glial and glio-mesenchymal nodules (the latter are referred to as Babes' nodules), focal invasions by amoeboid glia of neural tissue near affected vessels, and diffuse glial proliferations. Nerve cells show regressive changes, which in the bovine, are often out of proportion to the extent of the inflammatory reaction. Negri bodies, the intracytoplasmic inclusion bodies of rabies are round or oval structures usually about 2 to 8 u in diameter and have a visible internal structure, an acidophilic matrix containing a number of minute bluish granules of 0.2 to 0.5 u in diameter.

Foot and mouth disease and vesicular stomatitis are referred to clinically as vesicular diseases. The pathological changes in foot and mouth disease (Seibold, 1963) are quite similar to those of vesicular stomatitis and the changes seen in the latter will only be described here. Ribelin (1958) states that the first cytopathic effects are de-

tected in the deeper layers of the stratum spinosum. Simultaneously there is an increased prominence of intercellular spaces and a stretching of the intercellular bridges accomplished by a concomitant reduction of the cytoplasmic volume of affected cells. The internal morphology of the cells is not altered as the cytoplasmic volume decreases. The homogeneous finely granular character of the cytoplasm persists, but occasionally in the midst of epithelial cells showing early cytoplasmic shrinkage a cell would be found which had a ballooned, brightly eosinophilic cytoplasm. The intercellular spaces become wider and the connecting processes more extended until they finally give way. A state of epithelial spongiosis then exists which is rapidly followed by cytoplasmic contraction about the nuclei until only a narrow rim of cytoplasm remains. The cells then resemble lymphoblasts. At this time, the affected cells have lost all attachment and are floating freely in the enlarging vesicle surrounded only by a thin spongy reticulum of cytoplasmic remnants. The amount of fluid is greater than can be accounted for by the fluid component of degenerating cytoplasm and must be primarily an inflammatory exudate. At no time is swelling and cytoplasmic droplet formation that characterizes hydropic change in epithelial cells seen in affected cells. Up to this stage the nucleus has maintained its integrity and no detectable changes are seen in face of this obvious cytoplasmic disintegration. The nucleus then begins to contract with its rim of cytoplasm to form a small, granular, free-floating mass which is difficult to distinguish from the neutrophils which simultaneously enter the area. The fluid content of the vesicle increases

as does the number of neutrophils until a large, fully developed vesicle exists. The process generally involves the stratum germinativum and extends up to the lower surface of the stratum corneum. Oedema of the dermis is common and inflammatory changes such as hyperaemia, oedema and moderate neutrophil infiltration are visible in the musculature immediately beneath the dermis. The contents of the vesicle are never grossly purulent prior to rupture.

In virus diarrhoea (Carlson, Pritchard and Doyle, 1957) where the virus has an affinity for the epithelium of the digestive tract early lesions likewise occur in the stratum spinosum. The cells have pyknotic or fragmented nuclei and homogeneously pink-stained or vacuolated cytoplasm. The few infiltrating cells are polymorphonuclear leucocytes. Necrosis and sloughing occur, leaving well defined erosions. In the intestine, the reaction was that of acute catarrhal enteritis. The glandular crypts were filled with mucus, necrotic desquamated epithelial cells and a variable number of leucocytes. The marked distention of these glands results in pressure atrophy of the glandular epithelium and, in some cases, its complete destruction. Lesions are consistently found in Peyer's patches. These lesions vary from complete disappearance of densely packed aggregations of lymphocytes, leaving behind the spongelike stroma, to varying degrees of necrosis of the lymphoid tissue.

In rinderpest, (Maurer, Jones, Easterday and DeTray, 1955), the first cytological changes are noted just above the stratum germinativum in the stratum spinosum. The cells in a small focus swell and become necrotic. This process extends toward the mucosal surface before it spreads laterally. As a result of the pressure produced by the swollen

cells, small elevations appear above the normal surface. When necrosis has progressed sufficiently these elevations slough, leaving a well-like pit bounded by more or less normal cells. The extension of the necrotic process to the mucosal surface is by vesiculation. The rinderpest virus has a particular affinity for lymphoid tissue where it produces necrosis. The destruction of lymphocytes is first evidenced by the fragmentation of nuclei in the germinal centres. This is followed by the disappearance of most of the mature lymphocytes. The destruction of lymphocytes is often accompanied by an increase in plasma cells, macrophages and occasionally neutrophils. In addition, Thiery (1956) and Khera (1958) describe multinucleated giant cells or syncytia containing eosinophilic intracytoplasmic inclusions in lymphoid tissues and in the stratum spinosum of the stratified squamous epithelia of the upper alimentary tract. They also observed intranuclear inclusions.

The foregoing descriptions of the reaction of the bovine to infection with various viral agents is in no way a complete coverage of the subject. It is however an attempt to arrange viruses affecting the bovine in an order commencing with those that elicit an almost purely proliferative response through those which bring about both proliferation and necrosis to those that cause pure necrosis. Only those conditions for which a definite viral etiology has been established and for which adequate descriptions were available are included.

BOVINE VIRAL MASTITIS

References concerning a viral etiology for bovine mastitis are not numerous. Mastitis may occur as a sympton of a systemic virus disease.

Hutyra, Marek and Manninger (1946) describe a catarrh of the lactiferous ducts following the eruption of vesicles on the teats in foot and mouth disease. In cases where this happens milk is reduced in quantity, has a colostrum-like consistency, yellowish colour, rancid taste, acid reaction and a tendency to clot. They also state that it may contain streaks of blood and is difficult to make into butter and cheese.

The same authors state that parenchymatous inflammation of the udder may follow the formation of fissures on the skin of the udder in malignant catarrhal fever. Beckman, Barner, Morrill and Langham (1960) describe both proliferative and inflammatory changes in the udders of both lactating and dry cows in malignant catarrhal fever. Endothelial cells of the small capillaries in the interstitial tissue were enlarged, rounded and hyperplastic. Blood vessels were markedly congested. They describe loss of nuclear polarity and a piling up of pleomorphic alveolar cells in a manner which caused them to blend with the activated endothelial cells in the interstitium. The lumina of the glands were sometimes obliterated with masses of proliferated cells. Monocytes, lymphocytes and some plasma cells and eosinophils were present in the lamina propria which in some areas was poorly demarcated from the epithelium. Hyperplasia was noted in and about blood vessels and on occasion blood vessels were occluded by masses of hyperplastic endothelial cells which projected into the lumen. Perivascular cuffs of adventitial cells, lymphocytes and occasional monocytes, eosinophils, plasma cells and polymorphonuclear leucocytes were present. Fibrinoid degeneration and accumulations of proliferating cells were observed in the media.

McNutt (1956) states that the virus of vesicular stomatitis (VSV) can produce a primary mastitis and is especially apt to enter the mammary gland if a vesicle is located near the teat orifice. The affected gland becomes firm and hot and is subject to secondary infection. Milk production is reduced as the affected glands become fibrotic. Other investigators (Heiny, 1945, Brandly, Hansen and Chow, 1951, Stronzi and Ramos-Sacco, 1953) report teat lesions and associated mastitis occur in vesicular stomatitis. These authors however considered the mastitis to be of secondary bacterial origin. Easterday, Hansen and Simon (1959) inoculated the mammary glands of eight cows, five lactating and three nonlactating, with VSV via the streak canal. The mammary glands of these animals were free of Streptococcus agalactiae and coagulase-positive staphylococci and the blood serum had no significant VSV neutralizing ability prior to inoculation with VSV. A pre-inoculation milk sample was capable of neutralizing up to 5,000 embryo I.D. 50 of VSV. No abnormalities were observed or palpated in any of these cows. The virus was recovered from inoculated quarters at 24 hours but not at 48 hours after inoculation. Attempts to demonstrate a viraemia failed. Leucocyte counts in the milk from the inoculated animals were similar to those in the control animals. Using similar techniques the same workers produced udder swelling as well as increased leucocytic counts in milk with the viruses of Newcastle disease and vaccinia. No histological examinations were conducted in connection with these studies. The interesting fact in this study was that normal milk apparently had an inactivating effect on VSV.

Peterson, Hastings and Hadley (1938) postulated a viral etiology for what they termed non-specific mastitis. This condition was characterized histologically by an apparent hyperplasia of the epithelial cells of the alveoli. The authors were not sure whether this increase was apparent or real, whether it was due to actual cell multiplication or whether it was due to the contraction of the alveoli resulting in piling up and distortion of the cells. The interstitial tissue became highly cellular. These reactions were accompanied by a lymphocytic infiltration which varied in intensity from slight to severe. They noted eosinophils on occasion but polymorphonuclear leucocytes were almost completely absent. A viral etiology was not established in these idiopathic cases.

Broadhurst, Cameron and MacLean (1939) suggest that the acceptance of a bacterial etiology in mastitis may have hindered the search for other etiological agents. These authors claim to have isolated a filterable virus from mastitic cows and tested their hypothesis by two procedures; (i), a careful and meticulous microscopic study of the cells present in fresh mastitis milk, searching specifically for inclusion bodies; and (ii), tissue culture inoculations with filtrates of the blood and the milk of mastitic cows. They found inclusion bodies in cells of the mastitic cows, in their tissue cultures and in tissues of mice injected with the filterable agent. From the illustrations presented the inclusion bodies would appear to be approximately 1.0 u in size and one can see numerous particles of approximately the same size and density extracellularily in the same preparations. These workers used a Jena glass filter with an average pore diameter of 0.85 u which is large enough to allow the passage of Mycoplasma and their experiments do not preclude the presence of these minute bacteria.

Recent work by Hale, Helmboldt, Plastridge and Stula (1962) has shown that a species of Mycoplasma which they tentatively name Mycoplasma agalactiae var. bovis was responsible for a severe outbreak of mastitis in a dairy herd. These organisms were less than 0.5 u in diameter and would almost certainly have passed through the filters used by Broadhurst et al (1939). The histological picture of the mycoplasma mastitis of Hale et al (1962) was characterized by acute to chronic purulent mastitis terminating in a granulomatous formation considered pathognomonic. Stuart, Davidson, Slavin, Edgson and Howell (1963) report very similar findings except their Mycoplasma would not pass a 0.5 u gradacol membrane and they make no mention of a granulomatous tissue reaction.

Carter and Greig (1963) describe the recovery of diphtheroids from L-type organisms contaminating various cell line tissue cultures. They noted the presence of inclusion bodies in the tissue cultures and considered them as colonies or groups of the organism. It therefore seems possible that Broadhurst et al (1939) could have been dealing with a Mycoplasma or L-type organism rather than a viral agent.

Mitchell, Walker and Bannister (1953 a,b, 1954, 1956) showed that the virus of Newcastle disease, influenza A (PR8) and an unknown "duck virus" would propagate in the lactating bovine and caprine mammary gland. Mitchell, Norland and Walker (1958) showed that the mumps virus would propagate in the lactating bovine mammary gland. These experiments were designed to study the propagation of virus as well as the appearance and disappearance of antibody in the milk and serum of the inoculated animals. No mention was made of any clinical or pathological response observed in the inoculated animals.

Bannister, Boulanger and Rice (1959) found that the introduction of the virus of enzootic abortion of ewes (EAE), a member of the psittacosis-lymphogranuloma group, into the lactating bovine mammary gland produced marked gross alteration in the milk for a period of eight days. They were able to establish that the virus propagated within the gland. Matumoto, Omori, Morimoto, Harada, Inaba and Ishii (1955) produced a severe mastitis by the inoculation of their bovine P-L virus, a psittacosis-lymphogranuloma group virus, directly into the mammary gland of the cow.

With the exception of the work of Easterday et al (1959) no attempt was made by workers experimentally introducing viral agents into the mammary gland to obtain animals free of bacterial mastitis, and therefore their results may or may not have been affected by the presence of a bacterial infection in the mammary gland. With the exception of bovine malignant catarrhal fever no histological studies of the bovine mammary gland have been reported during the course of known viral infections. One worker (Bushnell, 1942) was unable to demonstrate the presence of a viral agent in milk from cows with typical clinical mastitis by the inoculation of 10 to 12 day chicken embryos on the chorioallantoic membrane.

There would appear to be only one convincing report of an authentic viral mastitis in the literature. Langer and McEntee (1961) isolated a virus (56R) from all four quarters of a cow with an acute bacteria-free case of mastitis. This agent apparently belonged to the myxovirus group being characterized by its sensitivity to ether and chloroform, comparable size, haemadsorption of erythrocytes, growth in the embryonating chicken egg, presence of a heat labile inhibitor in normal serum, and stability at -65°C and less stable at higher temperatures. The virus when inoculated

into the udders of cows reproduced the clinical picture present in the animal from which it was isolated. No histological studies were reported.

MATERIALS AND METHODS

VIRUSES

(a) Lapinized Rinderpest Virus. This virus was obtained through the courtesy of Dr. G.L. Bannister, Animal Diseases Research Institute, Hull, Quebec. The virus material consisted of frozen rabbit spleen R668. This rabbit had been inoculated intravenously with one ml. of rabbit spleen #31. The second post-inoculation day the temperature rose to 107.0° at which time the spleen was harvested. The virus is a sub-passage from a rabbit adapted Japanese strain (Nakamura III) of rinderpest virus received from Daubney from stock on hand in Cairo, Egypt (Boulanger, 1957). This strain has undergone eight rabbit passages since 1958. In addition to rabbit spleen R668 lapinized rinderpest rabbit spleens R200, 716, 717, 719, 720, 722 and 796 were obtained for use as challenge material and additional viral studies.

(b) Influenza A Virus (PR8). The PR8 strain of influenza A was obtained through the courtesy of Dr. F.P. Magler, Virus Laboratory, Laboratory of Hygiene, Department of National Health and Welfare, Ottawa. No history was available with this virus. The freeze dried material was reconstituted and passaged in embryonated eggs. The harvested egg fluids with a haemagglutination (HA) titre of 1:640 were employed in these studies.

(c) Infectious Bovine Rhinotracheitis Virus (IBR). The strain of this virus used was originally isolated from a naturally occurring outbreak of coital exanthema and was passaged through cows before being employed in laboratory studies. The virus was obtained through the kindness of Dr. A.S. Greig, (Greig, Bannister, Mitchell and Barker, 1958), Animal Disease Research Institute, Hull, Quebec. The virus material consisted of tissue culture fluids from the 20th passage of bovine embryo kidney cortex with a titre of 10^{-6} TCID 50.

(d) Enzootic Abortion in Ewes Virus (EAE). The strain of EAE virus employed was originally isolated by Dr. J.T. Stamp (Stamp, Watt and Cockburn, 1952), Moredun Institute, Edinburgh, Scotland and came to this laboratory in 1957 as the 22nd egg passage. Since that time the virus has been passaged in the yolk sac of embryonated eggs and was received as a yolk sac suspension from the 19th passage.

EXPERIMENTAL ANIMALS

All cattle used in these experiments were first calf heifers which had freshened six to twelve weeks prior to use. Cattle of both beef and dairy breeds were employed. All animals were tested serologically for the presence of antibodies against Leptospira pomona and Brucella abortus and found negative. The sera of animals used in the influenza A PR8 experiment exhibited no inhibition in a haemagglutination inhibition (HI) test and sera from animals used in the IBR experiment contained no neutralizing antibody titre in a serum virus neutralization test conducted in tissue culture. Sera from animals used in the EAE experiment showed no reaction in a complement-fixation test using EAE antigen.

The milk from each quarter of each animal used was examined bacteriologically at least twice and found free of bacteria. The udders of all animals were examined clinically and found free of any abnormality.

Rabbits used in the lapinized rinderpest experiment were young male white rabbits from the colony of the Animal Diseases Research Institute.

EXPERIMENTAL PROCEDURES

Experiment I. Lapinized rinderpest virus.

The three heifers selected for use in this experiment were designated as A, B and C. Daily milk production and temperature records for these animals were kept for five days prior to shipment to Grosse Ile Experimental Station (GIES) where the experiment was conducted. Upon arrival at GIES the animals were placed in isolation cubicles and given two days to adjust to their new quarters. Due to the isolation conditions the animals were deprived of hay during the course of the experiment.

Two solutions were prepared. Solution N (normal) was prepared as a control inoculum by grinding the spleen of an apparently normal rabbit in a Tenbrook grinder in 10 ml. of normal egg fluid and 5.0 ml. of penicillin, streptomycin solution. The normal egg fluid consisted of fluids harvested from normal ten day old embryonated eggs. The penicillin, streptomycin solution contained 250,000 IU of penicillin G procaine and 0.5 gms. of streptomycin in physiological saline solution. Solution LR (lapinized rinderpest) was prepared in a similar manner with the exception that lapinized rinderpest rabbit spleen R668 was used.

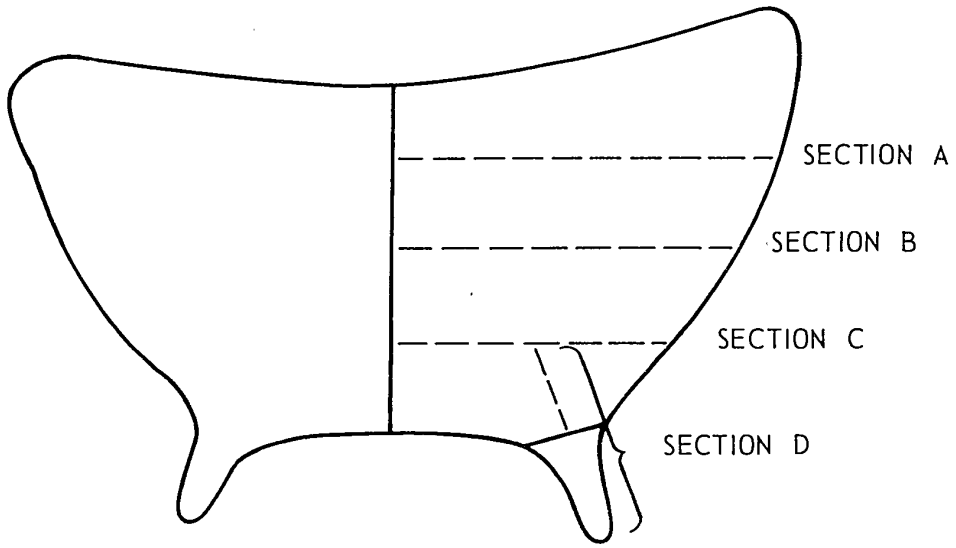
The experiment was commenced at an evening milking when all udders were washed with warm ETU15 disinfectant and each teat was swabbed with 70 per cent alcohol and allowed to dry. Preinoculation milk samples of approximately 30 ml. were collected in sterile tubes from each quarter. The cows were then milked out. Each teat was again swabbed with 70 per cent alcohol. Using a syringe and udder infusion cannula 2.0 ml. of solution R was infused into the teat cisterns of the left quarters of each animal. In a similar manner the right quarters were inoculated with 2.0 ml. of solution LR. All quarters were then massaged. Following infusion daily production and temperature records were maintained. Morning and evening milking was carried out. Prior to the evening milking, samples of approximately 30 ml. were collected in sterile tubes and placed immediately in dry ice and frozen.

Animal C was killed after three days, animal B after six days and animal A after nine days. Immediately after death both front quarters were removed, labeled and placed on dry ice. The rear quarters were infused via the teat canal with 200 ml. of 10 per cent formalin in physiological saline and massaged. The entire quarters were then placed in a five gallon can of 10 per cent formalin solution. All tissues and milk samples collected on dry ice were maintained in the frozen state by daily additions of dry ice until they reached the Animal Diseases Research Institute where they were stored in a deep freeze at -35°C.

Thin slices were cut through the entire fixed quarters as indicated (Fig. 5). Section A was taken as representing the upper third, section B as representing the middle third and section C as the lower third. Section B consists of a slice taken through the gland cistern and the complete teat.

Fig. 5

DIAGRAM OF UDDER TO ILLUSTRATE SELECTION OF TISSUES FROM EACH REAR QUARTER FOR HISTOLOGICAL EXAMINATION



EACH DOTTED LINE REPRESENTS A SLICE OF TISSUE APPROXIMATELY 1/8" THICK. TO COMPLETE SECTION D THE WHOLE TEAT WAS BLOCKED.

Each slice was placed on a piece of paper and a tracing of the outline was made. As the slice was cut into blocks they were marked on the tracing and numbered. Histological sections were cut at four to five microns and stained with haematoxylin and eosin. The letter designating the animal was used to prefix the section number. Tracings of the right rear quarters of all animals examined histologically are to be found in Appendix 1.

Experiment IA.

An attempt was made to show that the lapinized rinderpest virus infused into the mammary glands in experiment I had propagated there. The milk collected from the right rear quarter of cow A was used in this experiment. For each ml. of preinoculation milk a 0.3 ml. amount of a penicillin-streptomycin mixture containing 7,500 IU of penicillin G procaine and 15 mg. of streptomycin was added. Two rabbits were injected intravenously with 1.0 ml. of the above mixture. This same procedure was employed using the nine daily milk samples collected from the same cow and same quarter. Two rabbits were kept as an uninoculated control group. After inoculation the rabbits were held in individual Horsfall units and daily temperature records were maintained. Eighteen days following inoculation all surviving rabbits were challenged with lapinized rinderpest virus. One ml. of an approximately 10 per cent suspension of rabbit spleen R200 in physiological saline solution was administered intravenously. One ml. of an antibiotic solution containing 25,000 IU of penicillin G procaine and 50 mg. of streptomycin was added to each 10 ml. of inoculum. Temperatures were recorded daily. All animals were killed on the fourth post-challenge day and portions of spleen, lymph node and intestinal tract were collected in 10 per cent formalin in physiological saline for histological examination.

Experiment IB.

This experiment was designed to determine if the virus of lapinized rinderpest was inhibited by some factor present in fresh milk or by the freezing in fresh milk. To 100 ml. of freshly drawn cow's milk 10 ml. of antibiotic solution containing 50,000 IU of penicillin G procaine and 0.1 gm. of streptomycin per ml. was added. Lapinized rinderpest rabbit spleens R722 and R796 were removed from storage at -35°C, minced with scissors and mixed. A one gm. amount was ground in a glass tissue grinder with 9.0 ml. of the above mentioned milk mixture to give a 10^{-1} dilution and further dilutions were made to 10^{-8}. Another 0.5 gm. of splenic tissue was ground in a glass tissue grinder with 4.5 ml. of physiological saline to give a 10^{-1} dilution of virus in saline. Amounts of each dilution of the virus material in milk together with amounts of the milk mixture and virus in saline dilution were frozen and stored at -35°C.

Ten groups of two rabbits each were prepared. One group of rabbits was inoculated with each of the following: fresh virus in milk at dilutions of 10^{-1} to 10^{-8} inclusive, milk mixture and virus in saline. Each rabbit received 1.0 ml. intravenously. Each group of rabbits was held in an individual Horsfall unit and daily temperature records were maintained. All animals living after 19 days were challenged with 1.0 ml. intravenously of a 10 per cent suspension of lapinized rinderpest virus (rabbit spleens R716 and R717) in physiological saline.

A second series of rabbits similar to that mentioned above was prepared and treated similarily with the milk mixture, virus in saline mixture and the various virus in milk dilutions after storage of the materials at -35°C for 27 days. Daily temperatures were recorded and animals

dying were necropsied. All rabbits living after 20 days were challenged intravenously with 1.0 ml. of 10 per cent suspension of lapinized rinderpest virus (rabbits R719 and R720) in saline. The experiment was terminated four days later.

Experiment II. Influenza A Virus PR8.

Three heifers, designated D, E and F, were selected and prepared as in experiment I. Prior to commencement of the experiment preinoculation samples of milk were collected in sterile tubes from each quarter of each animal. After milking the cows out 2.0 ml. amounts of normal embryonating egg fluids with antibiotic solution (similar to that used in experiment I) added was used to infuse the left quarters of each animal. The right quarters of each animal received 2.0 ml. amounts of influenza A PR8 infected egg fluids. Daily temperature and production records were maintained. Morning and evening milkings were carried out with daily sampling being done prior to the evening milking. These samples were frozen and held at -35°C. Cow D was killed three days after inoculation and Cow E six days after inoculation. Tissues were harvested as in experiment 1. The collected milk samples were thawed and the whey separated by adding a drop of rennet and incubating at 37°C for one hour. HA tests were conducted using the whey of a number of the collected samples.

Serum from Cow F was tested for HI titre. Thirty-four days following initial exposure both left quarters of cow F were inoculated with 2.0 ml. of virus infected egg fluids and 2.0 ml. of a 72 hour broth culture of a coagulase positive staphylococcus. HA tests were conducted on a number of whey samples collected from the left quarters of this animal.

An additional three heifers designated K, L and M, were prepared and the experiment repeated as described above with the identical materials.

Experiment III. Infectious Bovine Rhinotracheitis Virus (IBR).

Three heifers, G, H and J, were selected and prepared as in the previous experiments. Cows G, H and J were inoculated in both left quarters with 2.0 ml. of uninoculated tissue culture fluids from cultures of bovine embryo kidney cortex. The right quarters were inoculated with 2.0 ml. of tissue culture fluids from the 20th passage of IBR virus on bovine embryo kidney cortex with a titre of 10^{-6} TCID 50. Daily production and temperature records were maintained. As in previous experiments morning and evening milking were carried out with the entire mornings milk being discarded and an approximately 30 ml. sample was collected in a sterile tube prior to the evening milking. These samples were frozen in a deep freeze and stored at -35°C. Cow G was killed after three days, Cow H after six days and Cow J after nine days. Tissues were collected and prepared as in experiment I. In addition to haematoxylin and eosin, some sections were stained by the Feulgen technique.

The collected milk samples were thawed and seeded in tissue cultures of bovine embryo kidney cortex in a dilution of 10^{-1}. If a cytopathic effect was noted at this dilution the sample was titrated. Two tubes were inoculated with each dilution. The growth medium contained a mixture of Hank's balanced salt solution; lactalbumin hydrolysate (enzymatic) (0.5%), proteose peptone No. 3 (Difco) (0.1%), cysteine HCl (0.001%) and calf serum (10%). A maintenance medium was used which

consisted of Hank's (based) Medium 199 supplemented with 0.5% lactalbumin hydrolysate (enzymatic) and 1.0% sodium bicarbonate.

Experiment IV. Enzootic Abortion in Ewes Virus (EAE).

Three first calf heifers, P, Q and R, were prepared as in the previous experiments. Following the collection of preinoculation milk samples the left quarters of each animal were inoculated with 2.0 ml. of a yolk sac suspension prepared from normal nine day old embryonated eggs as a medium control. The right quarters of each animal received 2.0 ml. of yolk sac suspension prepared from eggs inoculated with EAE virus in the yolk sac and allowed to die. No antibiotic solutions were added. Morning and evening milkings were carried out as in previous experiments with duplicate daily milk samples being collected in sterile tubes prior to the evening milking. One sample was used to prepare smears which were stained by Machiavello's technique and examined microscopically. The other sample was collected for virus isolation. The samples for virus isolation of the first three days were frozen and held at -35°C. whereas those collected in the succeeding days were inoculated directly into the yolk sac of eight day old embryonating eggs. Upon the death of the embryo impression smears were prepared from the yolk sac, stained according to Machiavello's technique and examined microscopically. The frozen milk samples were thawed and inoculated into embryonating eggs as previously described. Virus isolation in embryonating eggs was attempted from milk samples of the left and right rear quarters of all animals for each day of the experiment. Daily temperature and production records were maintained. Cow P was killed after three days, Cow Q after six days and

Cow N after nine days. Tissues were harvested as in previous experiments. Paraffin sections from all blocks were stained with hematoxylin and eosin and additional sections from selected blocks were stained with Giemsa, Feulgen and Machiavello's stains.

OBSERVATIONS AND RESULTS

Experiment I. Lapinized Rinderpest Virus.

Table I shows the daily milk production and temperature of the cows A, B and C for five days prior to their being shipped to Grosse Ile Experimental Station.

Throughout the course of the experiment the animals remained clinically normal as did the physical qualities of the milk. The temperature regulator in the cubicle housing cow C went out of control and the room temperature rose to 95° on the afternoon of the third day. Milk yield for this animal in the evening milking was only 250 ml. as compared to 1300 ml. for the previous evenings' milking. Temperatures and milk production of cows A, B and C during the course of the experiment are recorded in Table II. It would appear that the inoculation of lapinized rinderpest virus into the lactating bovine udder produced little or no clinical effect.

A total of 90 histological sections were examined representing all levels of left and right rear quarters of all animals. No lesions considered of pathological significance were noted.

Experiment IA.

For the most part temperatures remained within the normal range of 101.5 to 104.2 (Dukes 1955). Difficulty was experienced in that rectal perforation occurred in a number of rabbits due to careless handling on

Table I

Milk production in ml. and temperatures of Cows, A, B and C for five days prior to shipment to C.I.E.S.

Day	1		2		3		4		5	
	Prod.	Temp.	Prod.	Temp.	Prod.	Temp.	Prod.	Temp.	Prod.	Temp.
Cow A	7300	102.2	7100	101.9	7500	102.2	7150	102.0	6800	102.0
Cow B	8600	102.7	8100	102.2	8300	102.6	8250	102.2	7500	102.0
Cow C	3400	101.8	3460	101.8	3400	101.8	3350	102.6	3100	102.0

Table II

Milk production in ml. temperatures of Cows A, B and C during the course of the experiment.

	Cow A		Cow B		Cow C	
Day	Prod.	Temp.	Prod.	Temp.	Prod.	Temp.
1	5750	102.0	6200	102.0	2700	102.2
2	3100*	101.6	7550	101.4	3000	101.8
3	8700	102.1	4600*	101.9	2200	101.8
4	8350	102.4	8520	101.8		
5	7700	102.0	7250	101.3		
6	8790	102.2	7160	101.6		
7	8850	101.8				
8	8150	102.4				
9	8220	102.3				

*cow kicked and milk lost

the part of the attendent. As a result some rabbits died of peritonitis. Following challenge there was a rise in temperature which usually reached its peak in two days and then dropped. Post mortem examinations were conducted on all animals dying following challenge with the live virus or which were killed on the fourth post-challenge day. Lesions similar to those noted by Fukusho and Nakamura (1940) were noted in all animals. These changes were characterized by markedly swollen Peyer's patches in the small intestine and caecum. Areas of necrosis were evident and were visible from the serosal side as numerous greyish-white pin point foci. When these areas were expressed greyish-white pus-like material was expelled into the lumen of the gut. In the odd rabbit the caecum was filled with thick mucus. The mesenteric lymph nodes were markedly swollen in a number of cases and greyish-white foci of necrosis were visible. Histological examination revealed marked necrosis of lymphocytes of the lymph nodes and Peyer's patches. This necrosis was characterized by pyknosis and karyorrhexis (Figs. 6 & 7).

Experiment IB

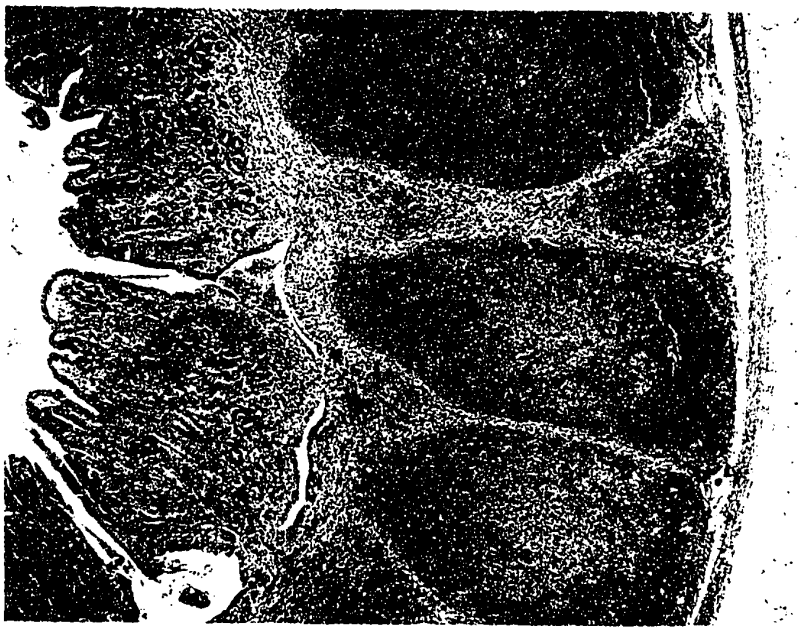
The milk mixture (fresh milk plus antibiotic solution) alone had little effect when injected intravenously and the rabbits responded in a typical manner with a temperature response when challenged 19 days later. The virus in saline mixture as well as titrations of the virus in milk to 10^{-8} produced a typical temperature response in all cases and death in three instances. All surviving animals were challenged and showed no temperature response indicating that they were immune to challenge.

Animals inoculated with milk mixture which had been held frozen for 27 days responded similarly to those which were inoculated with fresh

Figs. 6 and 7.

Fig. 6. Mesenteric lymph node. Necrosis of lymphatic tissue.
H & E X 108.

Fig. 7. Caecum. Necrosis of lymphatic tissue of Peyer's patches.
H & E X 36.



milk mixture. Rabbits inoculated with virus in saline mixture and the various titrations of virus in milk which was held frozen for 27 days in general responded in a similar manner to those which were inoculated with the unfrozen material. The uninoculated control animal and the two animals inoculated with milk mixture gave typical temperature responses when challenged, whereas those surviving inoculation of virus in saline and virus in milk withstood challenge.

Experiment II. Influenza A Virus PR8.

Haemagglutination titres above 1:10 were not obtained in the whey samples collected from cows D, E and F indicating that virus propagation had not taken place. In addition the serum sample from cow F showed no HI four weeks after inoculation. Whey samples prepared from cow F following inoculation of virus and staphylococcus showed no significant HA titre but abnormal milk was present for several days.

Haemagglutination was not demonstrated when the experiment was repeated in cows K, L and M and efforts to propagate this virus in the lactating bovine mammary gland were abandoned.

Experiment III. Infectious Bovine Rhinotracheitis Virus (IBR).

Table III illustrates the daily temperature and milk production of cows G, H and J for the two days immediately preceeding the inoculation of virus and throughout the course of the experiment. A few small fibrin clots were noted in the evening milk on the third post-inoculation day. The right quarters of all cows were tender and they tended to kick while being milked. Slight subcutaneous oedema was noted when skinning the right quarters of cow G and the lymphatics were prominent, being distended with lymph. On the fourth post-inoculation day the

Table III

Milk production in ml. and temperatures of cows G, H and J for two days prior to inoculation and throughout the course of the experiment.

	Cow G		Cow H		Cow J	
Day	Prod.	Temp.	Prod.	Temp.	Prod.	Temp.
-2	11,500	102.4	12,700	102.0	13,000	101.6
-1	13,700	101.6	12,900	102.2	12,900	101.2
1	13,800	101.4	12,800	101.4	13,200	101.8
2	10,800	106.0	11,600	104.0	10,500	102.4
3	10,600	105.2	11,100	106.6	10,000	105.4
4			10,500	106.8	9,300	106.0
5			10,000	103.6	9,300	103.6
6			9,500	105.2	8,900	105.0
8					7,250	101.4
7					8,000	102.4
9					8,700	101.6

right quarters of cows H and J were somewhat hot and swollen and difficulty was experienced in milking. Both cows ate well despite their high temperatures. On the following day the right quarters were definitely hot, red and swollen. Cow J was more severely affected. Small white clots were present in the milk but in bulk the milk appeared relatively normal. The sixth post-inoculation day the right quarters remained hot, red and swollen. Cow J appeared dull, with drooping ears and refused to eat. Cow H was killed and subcutaneous oedema one quarter to one half inch in thickness was noted in the pendent portions of the right quarters and marked oedema was noted in the subcutaneous tissues anterior to the right front quarter. On the seventh post-inoculation day cow J was still dull and ate very little. The affected quarters were still hot, red and swollen and extremely tender to the touch. Cow J showed much improvement on the eighth post-inoculation day, affected quarters were not as swollen or tender. The ninth post-inoculation day the swelling of the quarters continued to subside, the redness had disappeared and she allowed milking without kicking. Her appetite was good. Subcutaneous oedema while present over the right quarters was not as severe as in cow H.

Table IV illustrates the titrations at which cytopathogenic effects were obtained when the milk samples collected from cows G, H and J were seeded on tissue cultures of bovine embryonic kidney. Uninoculated tissue culture of bovine embryonic kidney is illustrated in Fig. 8 while the cytopathic effects of IBR virus are illustrated in Fig. 9. The cytopathic effects were characterized by a gradual shrinkage of the cytoplasm, pyknosis, karyorrhexis and lysis.

Table IV

Titration of milk samples collected from cows G, H and J during the course of the experiment.

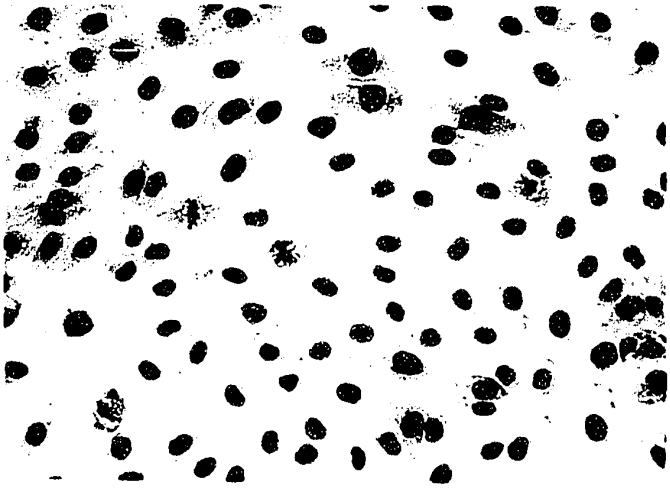
	Day	Quarter			
		RF	RR	LF	LR
Cow G	preinoculation	-	-	-	-
	1	2	-	-	-
	2	3	-	-	-
	3	6	4	-	-
Cow H	preinoculation	-	-	-	-
	1	-	-	-	-
	2	1	-	-	-
	3	2	2	-	-
	4	2	2	-	-
	5	3	3	-	-
	6	2	3	-	-
Cow J	preinoculation	-	-	-	-
	1	-	-	-	-
	2	1	1	-	-
	3	3	2	-	-
	4	3	4	-	-
	5	3	2	-	-
	6	3	6	-	-
	7	3	3	-	-
	8	2	4	-	-
	9	2	4	-	-

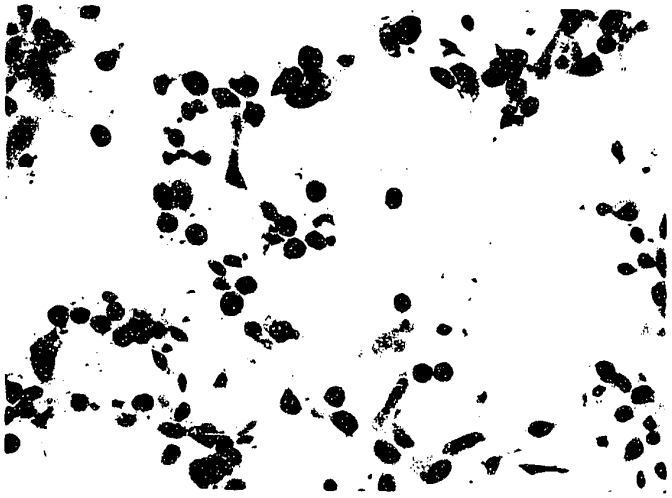
Figures represent the log-10 dilution at which cytopathogenic effect was still noted.

Figs. 8 and 9.

Fig. 8. Uninoculated culture of bovine embryonic kidney cells. May-Grunwald-Giemsa. X 180.

Fig. 9. Inoculated culture of bovine embryonic kidney cells showing cytopathic effects. May-Grunwald-Giemsa. X 180.





Histological examination was carried out on all blocks in all levels of both left and right rear quarters of cows G, H and J. Tracings of each large section indicating and identifying each tissue section for the right rear quarters are included in Appendix I.

Cow G Right Rear Quarter (inoculated with IBR virus and killed at three days.)

Section A

Section A was made up of 22 histological sections one of which was supramammary lymph node (to be dealt with later) and 21 of udder parenchyma. Nothing of significance histologically was found in 10 sections of the parenchyma. Changes noted in the other sections were relatively minor. In some, the odd alveolus was filled with milk which often contained a few polymorphonuclear leucocytes. The occasional focus of mature lymphocytes was noted in the interstitial tissue of some sections. In others these foci appeared to be hyperplastic. In some instances one or two epithelial cells of the alveolar lining rounded up, became dissociated from their fellows and had homogeneous densely staining eosinophilic cytoplasm and a densely staining nucleus. In section G9 foci of up to 12 alveoli were involved in an interstitial reaction where mononuclear cells (lymphocytes, macrophages and the odd plasma cell) together with the occasional polymorphonuclear leucocyte was infiltrating the alveolar walls. Capillaries in the interstitial tissue were congested. The alveoli appeared small and their lumina were filled with macrophages and desquamated alveolar epithelial cells, the nuclei of which were pyknotic but karyorrhexis was not noted. Nuclei of alveolar cells adjacent to some foci appeared swollen, pale

and vesicular, but no inclusion bodies were noted. In section G14 many foci were noted where the nuclei of the alveolar cells appeared very vesicular with margination of chromatin. Definite inclusion bodies were not found.

Section B.

Section B consisted of histological sections from G23 through to G46. Eleven histological sections contained relatively normal tissue. Many of the other sections contained foci of necrosis in which epithelial cells lining ducts or alveoli were undergoing necrosis. This was characterized by pyknosis and karyorrhexis with sloughing of cellular debris into the lumina. This reaction was accompanied by infiltration of polymorphonuclear leucocytes as well as mononuclear cells which were mainly lymphocytes and plasma cells. At the edges of some of these foci of necrosis alveolar cells or duct cells with margination of nuclear chromatin were noted. In some instances minute eosinophilic granules possibly representing the formation of early inclusion bodies were noted. In areas surrounding these foci marked swelling of the endothelial cells of blood vessels was noted. In one instance hemorrhage into a necrotic focus was noted.

Section C.

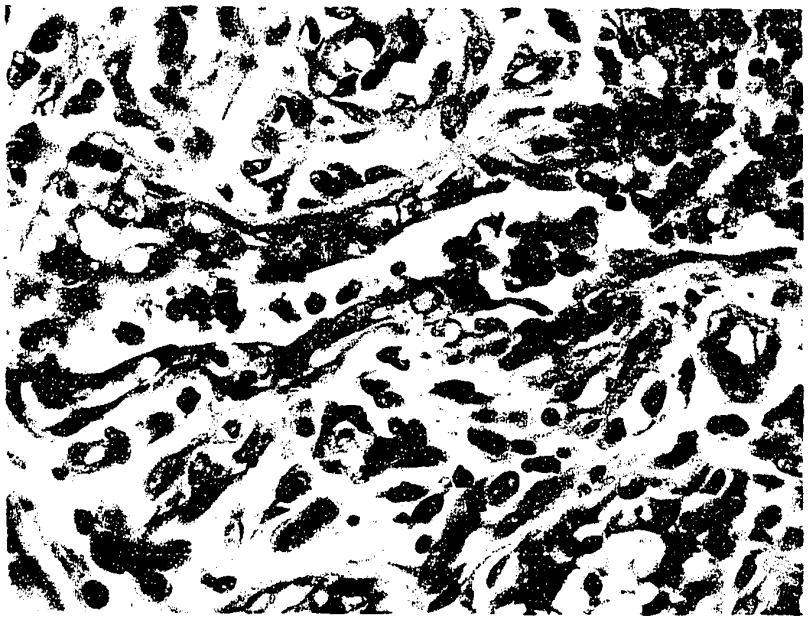
Section C consists of histological sections G47 through to G63. Only section G52 appeared relatively normal. Necrotic foci as noted previously in section B were found throughout the remainder of the sections. These foci were often found in duct epithelium and consisted of necrotic epithelial cells accompanied by an inflammatory reaction. Toward the periphery of these necrotic foci epithelial cells exhibiting

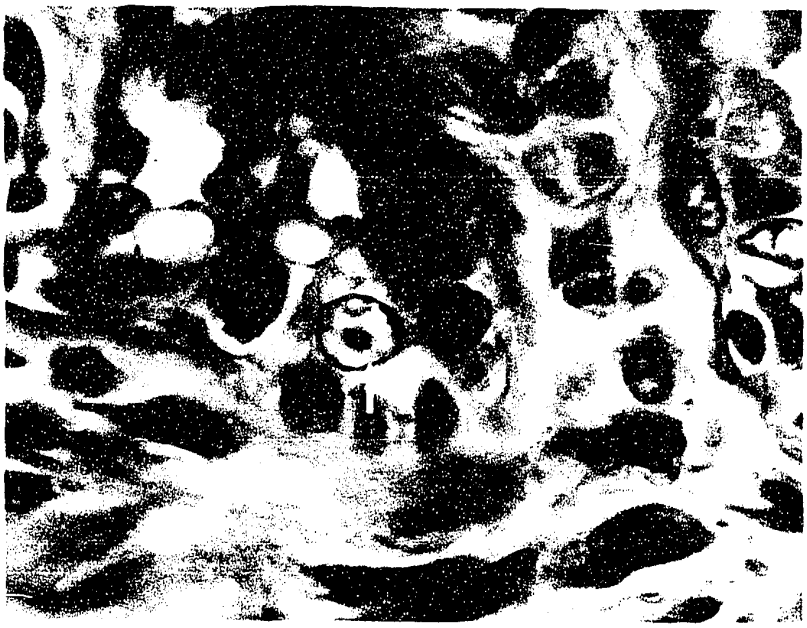
margination of nuclear chromatin were evident. In some instances, eosinophilic intranuclear inclusion bodies were present. Fig. 10 illustrates an area from section G48 where necrosis of duct epithelium has resulted in plugging of the lumen. Duct epithelial cells with margination of chromatin are evident together with one containing an intranuclear eosinophilic inclusion. In some instances intranuclear eosinophilic inclusion bodies were noted in the epithelium of ducts or alveoli where foci of necrosis were not yet evident. Such an inclusion body is illustrated in Fig. 11 from section G57. It will be noted that margination of nuclear chromatin is pronounced. Fig. 12 from section G48 illustrates a typical necrotic focus characterized by the presence of pyknotic nuclei in a fibrinous matrix. It will be noted that the surrounding capillaries are congested and the infiltrating cells are mainly plasma cells and a few polymorphonuclear leucocytes. Diapedesis of polymorphonuclear leucocytes is noted in Fig. 13 from section G51. Necrosis of alveolar epithelium is evident resulting in the obliteration of the lumen with fibrin and cellular debris. Necrotic foci in the duct epithelium result in the desquamation of epithelial cells along with the exudation of polymorphonuclear leucocytes and fibrin into the lumen of the duct. This exudation results in the plugging of ducts which in turn causes the damming back of secretion in higher levels. This exudation into the duct lumen from necrotic foci is illustrated in Fig. 14 from section G54. In sections of the cistern epithelium numerous foci of hyperplasia of the epithelium was noted. The epithelium was thrown up into numerous villi and judging by the number of vacuoles in these cells secretory

Figs. 10 and 11.

Fig. 10. 046 Duct. Necrosis of duct epithelium (top right) with resultant plugging of lumen. Top arrow indicates eosinophilic intranuclear inclusion, bottom arrows indicate margination of chromatin. H & E X 600.

Fig. 11. 057 Alveolus. Eosinophilic intranuclear inclusion body with margination of chromatin in nucleus of epithelial cell lining alveolus. H & E X 1450.

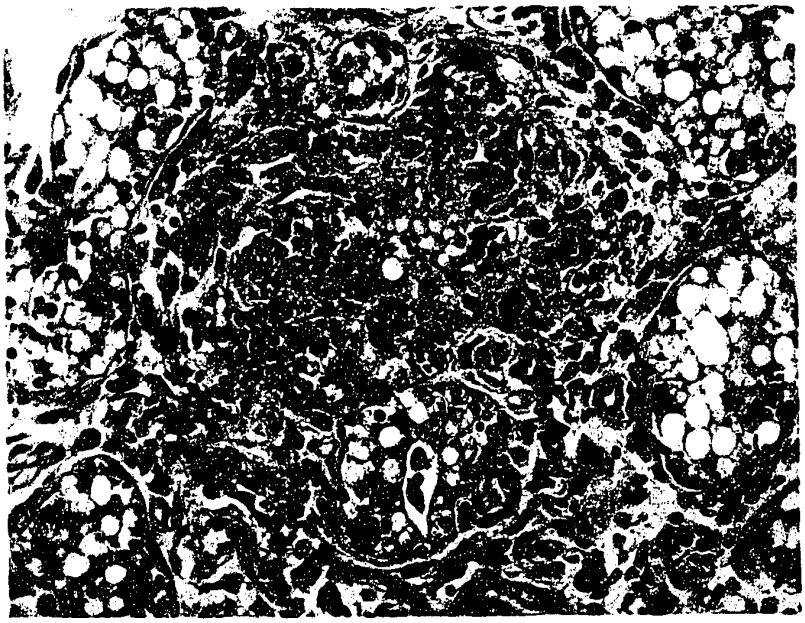


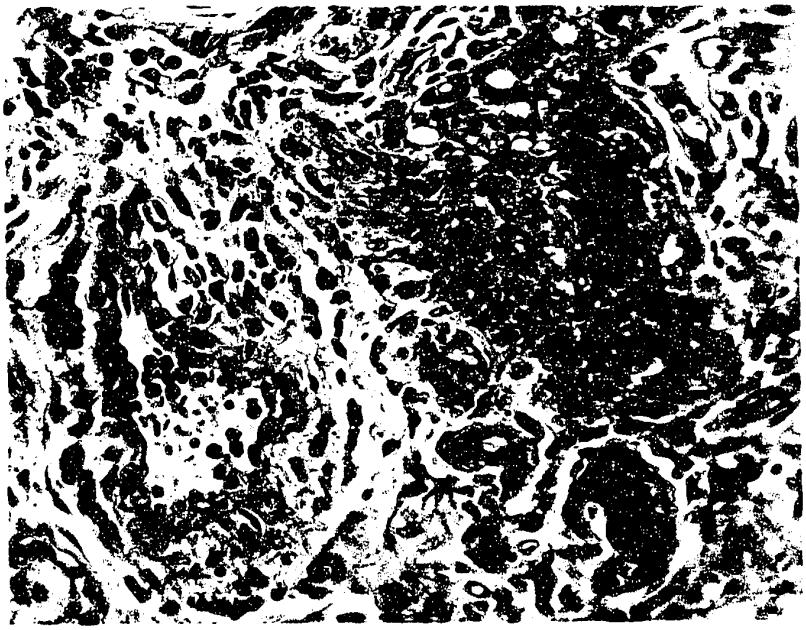


Figs. 12 and 13.

Fig. 12. G48 Udder parenchyma. Necrotic focus characterized by presence of pyknotic nuclei in fibrin matrix. Surrounding capillaries are congested and infiltrating cells are mainly plasma cells and a few polymorphonuclear cells. H & E X 360.

Fig. 13. G51 Udder parenchyma. Necrosis of alveolar epithelium (right) with obliteration of lumen with fibrin and cellular debris. Diapedesis of polymorphonuclear leucocytes from vessel at left. H & E X 360.





activity was taking place. In other areas the cistern epithelium was flattened with loss of nuclear polarity. Numerous ducts were seen completely plugged with inflammatory exudate. Areas immediately surrounding the foci of necrosis in the udder parenchyma and in the periductal tissues contained numerous neo-capillaries and an increase in fibrous connective tissue elements.

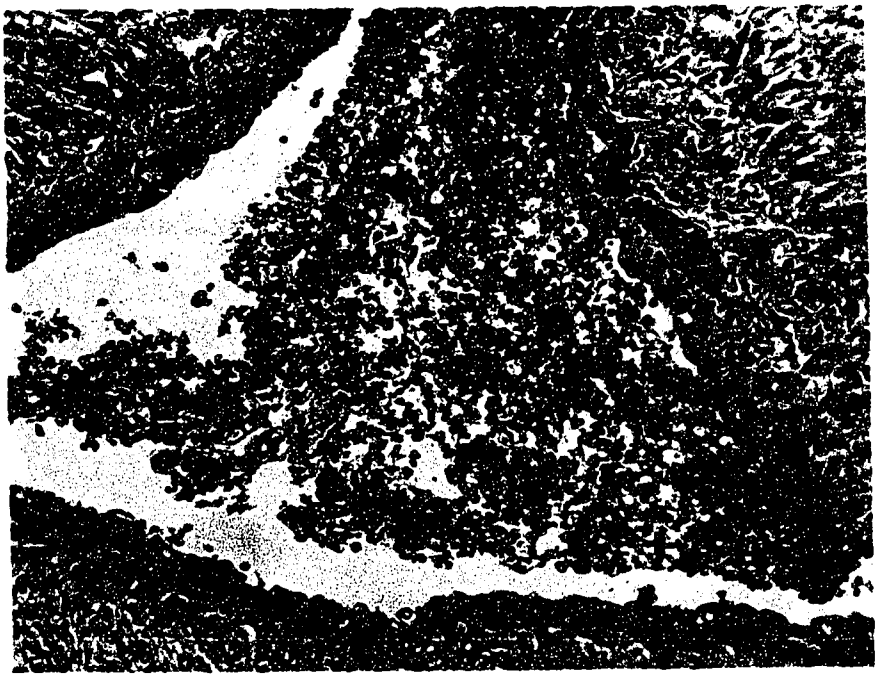
Section D.

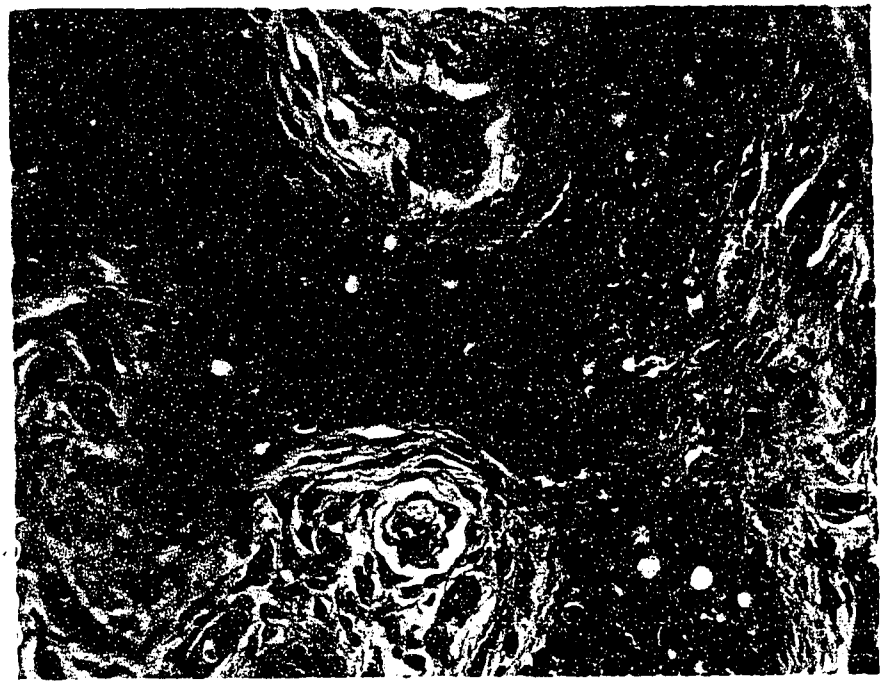
Section D consists of section G64 to G68 and G77 to G89. All sections with the exception of G87, 88 and 89 which are taken through the teat at the level of the streak canal showed pathological changes. Most sections contained numerous foci of necrosis as described in other sections above. These foci of necrosis were found both within the ducts and in the alveoli. In some instances whole lobules were involved in the necrotic process. Diapedesis of polymorphonuclear leucocytes was evident throughout the entire area. Small granular eosinophilic intranuclear inclusions were fairly common at the periphery of necrotic foci. Hyperplasia of duct epithelium was evident in some sections. Interlobular inflammatory oedema was also evident throughout many sections. In general the necrosis was more severe in this level than in higher levels. Fig. 15 illustrating an area from section G66 shows almost complete destruction of the alveolar epithelium with obliteration of the lumen by fibrin, polymorphonuclear leucocytes, and cellular debris. The epithelial cells remaining, either exhibit marked margination of nuclear chromatin or contained intranuclear eosinophilic inclusion bodies. Blood vessels appeared prominent and there was an increase in fibrous connective tissue elements. The inflammatory reaction in the

Figs. 14 and 15.

Fig. 14. G54 Duct. Desquamated epithelial cells, polymorphonuclear leucocytes and fibrin spewing forth from two areas of necrosis in the duct epithelium and plugging the lumen. H & E X 180.

Fig. 15. G66 Udder parenchyma. Almost complete destruction of alveolar epithelium and obliteration of lumen with fibrin, polymorphonuclear leucocytes and cellular debris. Blood vessels appear prominent and there is an increase in fibrous connective tissue. H & E X 360.





interstitial tissue consisted primarily of lymphocytes and plasma cells and seemed moderate in proportion to the amount of necrosis present. In the teat cistern there were numerous areas of necrosis of the epithelium, sloughing of cells was generalized, margination of nuclear chromatin was seen but definite intranuclear inclusion bodies were not noted in epithelium of the teat cistern. Both polymorphonuclear and mononuclear cells accompanied necrotic foci in the accessory glands. Fig. 16 represents an area from section G82 and illustrates the complete loss of epithelium from the teat cistern leaving a bare basement membrane. Where this complete loss had taken place the infiltration was primarily with polymorphonuclear leucocytes. Fibrinoid necrosis of vessel walls in the lamina propria of the teat cistern was a common finding. This is also illustrated in Fig. 16.

Supramammary Lymph Node

This node was covered by sections G1 and G69 through to G76. Polymorphonuclear infiltration of the cortical and paratrabecular sinuses was evident. This is illustrated in Fig. 17 from section G71. Oedema of the cortical sinus was also evident. Phagocytosis of cellular debris and polymorphonuclear leucocytes by large macrophages was evident in both the cortical and paratrabecular sinuses. Polymorphonuclear infiltration was evident within the trabeculi themselves.

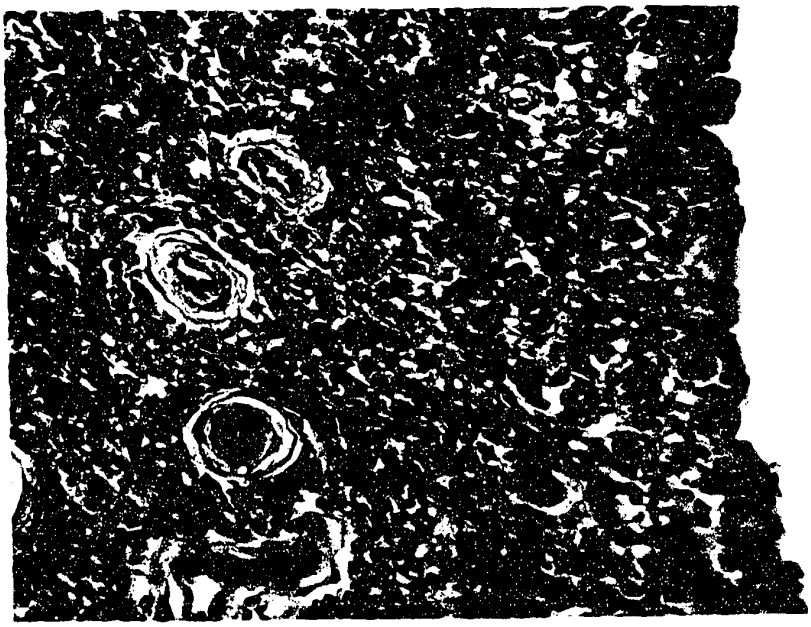
Cow G Left Rear Quarter (control quarter)

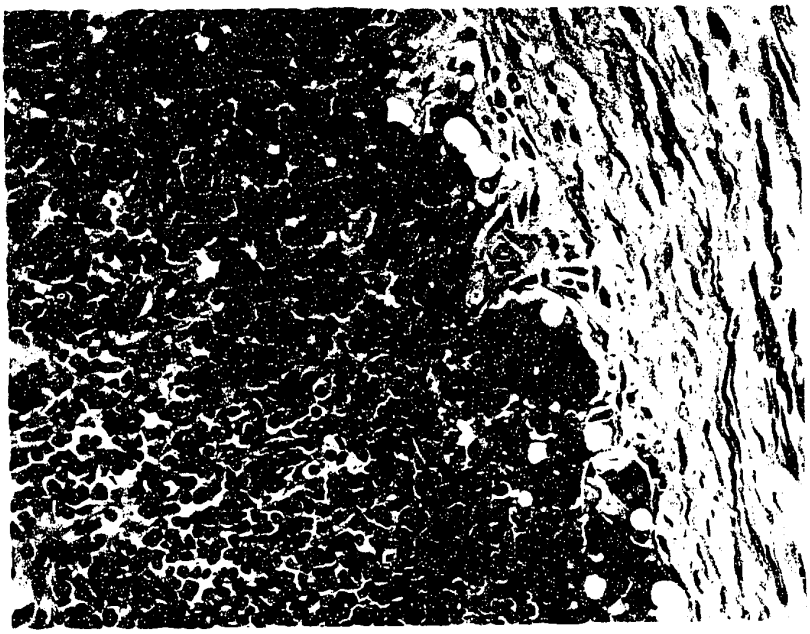
Histological examination of the supramammary lymph node from the left rear quarter revealed numerous macrophages in the paratrabecular sinuses. Small blood vessels in the cortex were stuffed with polymorphonuclear leucocytes. Tissue sections G118 and G190 contained nothing of significance and were considered relatively normal.

Figs. 16 and 17.

Fig. 16. G82 Teat cistern. Complete loss of epithelium leaving the bare basement membrane. Lamina propria is heavily infiltrated with polymorphonuclear leucocytes and blood vessels are prominent with swollen endothelial cells. Fibrinoid necrosis is evident in wall of vessel (2nd from bottom) H & E X 360.

Fig. 17. G71 Supramammary lymph node. Oedema and polymorphonuclear infiltration of cortical sinus. H & E X 360.





Cow H Right Rear Quarter (inoculated with IBR virus and killed at six days.)

Section A.

This section consisted of histological sections G3 - G5 and G8 - G37. Changes considered of pathological significance were found in only four sections. These consisted of minute areas of necrosis of alveolar epithelium accompanied by little in the way of an inflammatory reaction. In one instance, marked proliferation of ductal epithelium was noted.

Section B.

This section consisted of histological sections H46 through to H86. Ten sections contained relatively normal tissue. Throughout this section small groups of alveoli were found filled with milk, cellular debris, polymorphonuclear leucocytes and desquamated epithelial cells. Where this type of lesion was found often no interstitial inflammatory response was evident but on occasion a moderate lymphocyte infiltration was present. In some areas a mild polymorphonuclear leucocytic infiltration was evident in the interstitial tissues. Some sections showed foci of necrosis similar to those noted in cow G with necrosis of epithelial cells and an interstitial reaction primarily with mononuclear cell types. Whole lobules were noted where the alveoli were filled with secretion and leucocytes (mainly polymorphonuclear but also a few macrophages, and monocytes) with little or no necrosis of epithelium. Small intranuclear, eosinophilic granular inclusion bodies were noted in the epithelial cells along with margination of chromatin. Demarkation between affected and unaffected tissue in some instances were quite marked. Fig. 18 represents an area of udder parenchyma from

section H79 showing an interstitial septum of fibrous connective tissue separating affected and unaffected lobules. In general the interstitial reaction varied from a few polymorphonuclear leucocytes to almost purely mononuclear in type. In some instances it was possible to see where the plugging of a duct as the result of exudation from necrotic foci had dammed back secretion resulting in distention of alveoli with milk.

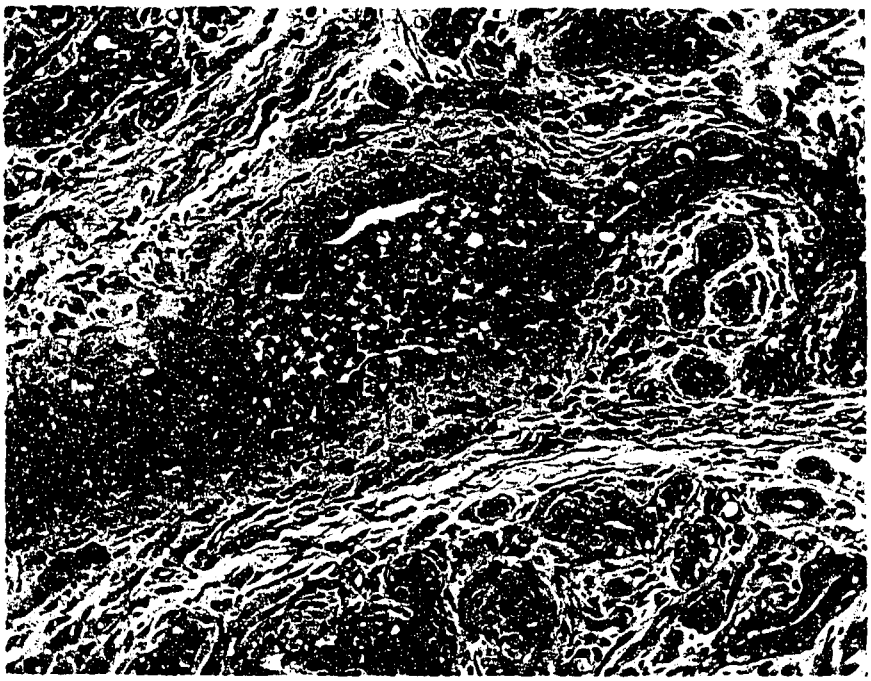
Section C.

Section C consisted of histological sections H87 through to H118. Changes considered of pathological significance were found in all sections. Marked oedema was noted in the subcutaneous tissue with diapedesis of polymorphonuclear leucocytes. Changes observed throughout section C were characterized by marked necrosis of duct epithelium with resultant plugging. The type of reaction noted is illustrated in Fig. 19 from section H95. The left side of the picture shows a duct with complete destruction of its epithelium, its lumen is plugged by desquamated epithelial cells and fibrinous exudate. In the centre of the photomicrograph the duct epithelium is intact but its lumen is plugged with desquamated epithelium cells and polymorphonuclear leucocytes. In the extreme right we see atrophy of the alveoli of the involved lobule and an increase in fibrous connective tissue and capillaries. In Fig. 20 which illustrates a portion of section H110 marked interlobular inflammatory oedema is seen along with necrosis of alveolar and duct epithelium and fibrinoid necrosis of fibrous connective tissue elements both within the interstitial septum and within the lobule itself. Fig. 21 represents a portion of interlobular septum of section H89.

Figs. 18 and 19.

Fig. 18. H79 Udder parenchyma. Interlobular septum separating affected and unaffected lobules. H & E X 180.

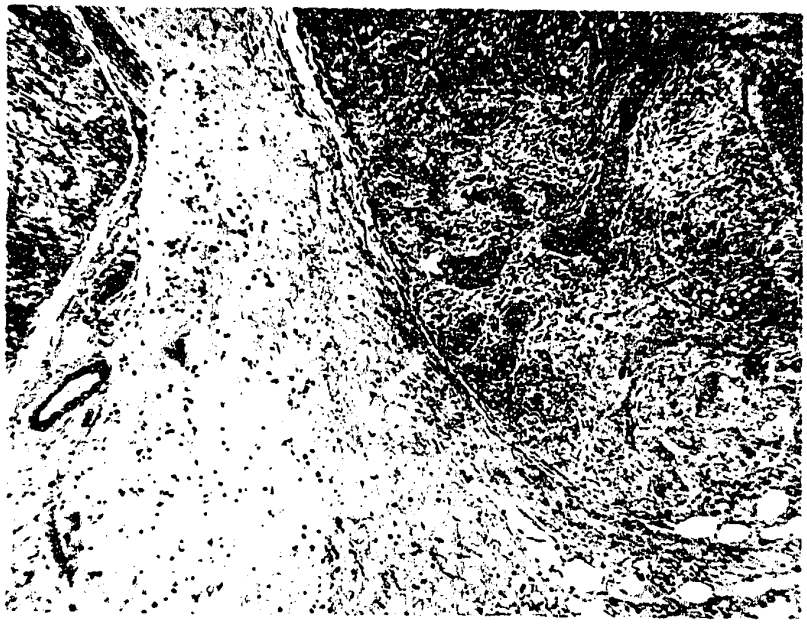
Fig. 19. H95 Udder parenchyma. Complete distruction of duct epithelium (left) with exudation and occluaion of lumen. Duct plugged mainly with desquamated epithelial cells (centre). Atrophy of alveoli and increase in interstitial fibrous connective tissue and capillaries. H & E X 180.

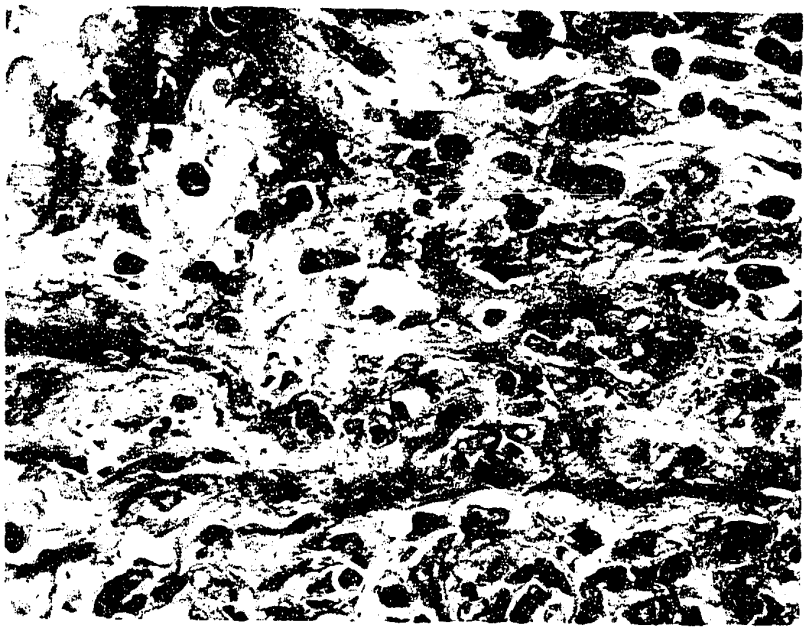


Figs. 20 and 21.

Fig. 20. H110 Udder parenchyma. Marked necrosis of alveolar and duct epithelium, marked oedema of interlobular septum with some fibrinoid necrosis (arrow) of fibrous connective tissue elements. Infiltrating cells are both polymorphonuclear and mononuclear. H & E X 90.

Fig. 21. H89 Interlobular septum. Fibrinoid necrosis of fibrous connective tissue elements with infiltration of polymorphonuclear cells and macrophages. H & E X 600.





Marked fibrinoid necrosis of the fibrous connective tissue elements with an infiltration of polymorphonuclear cells and macrophages is evident. Fibrinoid necrosis was also evident in capillary walls in the interstitial tissues. Hyaline droplet degeneration of the alveolar epithelium was found in several lobules. Inclusion bodies were found in a number of sections. These were often minute or made up of several small eosinophilic staining granules. In summary, it may be said that the most significant finding in section C was the marked necrosis of duct epithelium with resultant duct-plugging and damming back of secretion.

Section D.

Section D consists of histological sections H119 through to H138. With the exception of section H138 which was taken through the streak canal, all other sections showed pathological changes. Changes noted in the udder parenchyma were essentially similar to those noted in section C above. This was again characterised by the marked destruction of duct and alveolar epithelium, plugging of ducts and obliteration of alveolar lumina. Inclusion bodies were also found although they were small in size and relatively few in number. Mononuclear perivascular cuffing was noted in the wall of the teat. In many sections there was a complete loss of the epithelium of the teat cistern leaving a bare basement membrane. The alveolar epithelium of the accessory glands in most instances was completely necrotic and was characterized by karyorrhexis and sloughing of the epithelial cells into the lumen and accompanied by an inflammatory reaction largely made up of mononuclear cells but a few polymorphonuclear cells were also present.

The epithelium of some accessory glands however appeared relatively normal. Fig. 22 represents a photomicrograph taken through the teat cistern of section H137. There was complete destruction of the epithelium with the formation of a pseudomembrane composed of fibrin and cellular debris. The lamina propria was infiltrated with polymorphonuclear leucocytes, blood vessels were congested and the lymphatics as indicated by the arrows were thrombosed with fibrin plugs.

Supramammary Lymph Node

The supramammary lymph node was represented in sections H1 and 2, H6 - H8, H38 - H45. The cortical and paratrabecular sinuses were stuffed with large numbers of macrophages, lymphocytes, plasma cells, polymorphonuclear leucocytes and oedema fluid. Large numbers of plasma cells were found throughout the cortex adjacent to the sinuses and mitotic figures were often evident in these cells. A few extremely large cells, considered to be reticulum cells with extremely large nucleoli were found. The first impression was that these large eosinophilic nucleoli were inclusion bodies but they gave a negative Feulgen reaction. It is felt however, these reticulum or stem cells resemble the so called Reed-Sternberg cells. Fig. 23 taken from section H8 shows a cortical sinus descended with macrophages, plasma cells, lymphocytes and the odd polymorphonuclear leucocyte. The arrow indicates one of the large stem cells with a large eosinophilic nucleolus resembling a Reed-Sternberg cell.

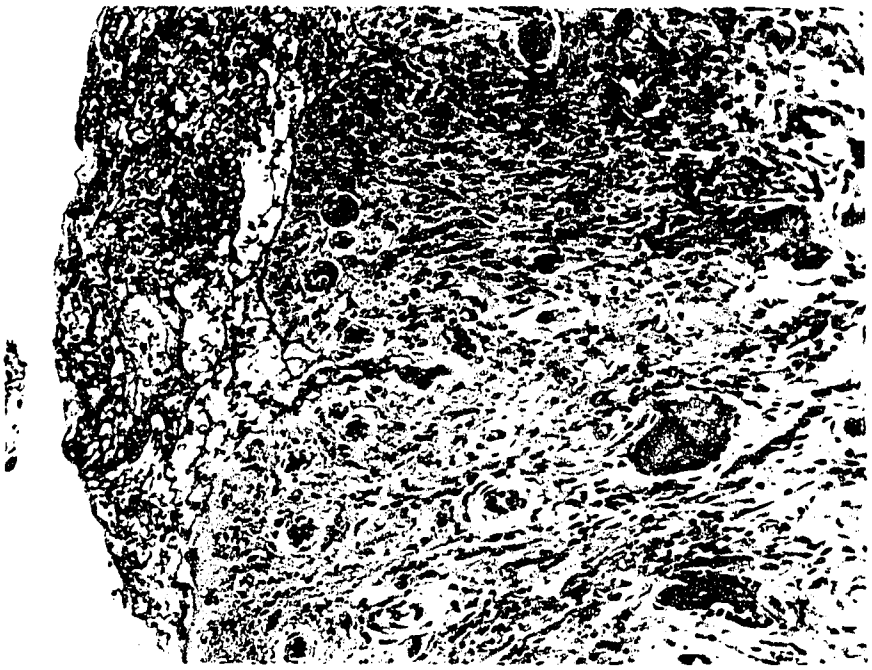
Cow H Left Rear Quarter (control quarter)

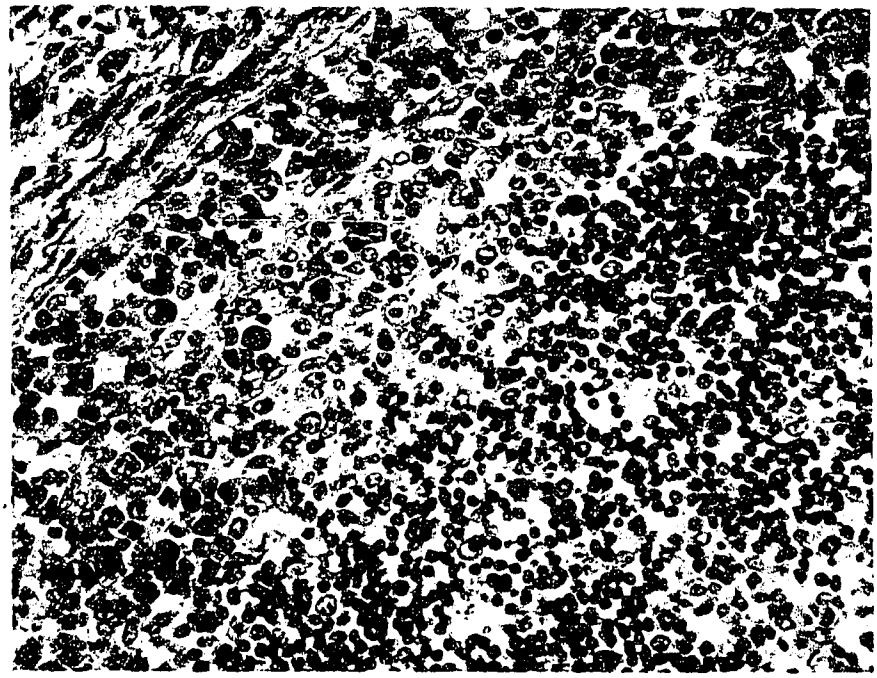
Generally speaking the tissues throughout all levels of the left rear quarter were essentially normal. In some areas, however, a number

Figs. 22 and 23.

Fig. 22. H137 Teat cistern. Complete destruction of epithelium with formation of a pseudomembrane composed of fibrin and cellular debris. Lamina propria is infiltrated with polymorphonuclear leucocytes, blood vessels are congested and lymphatics thrombosed (arrows). H & E X 180.

Fig. 23. H8 Supramammary lymph node. Cortical sinus distended with polymorphonuclear cells, lymphocytes, plasma cells and macrophages. Reed-Sternberg-like cell indicated by arrow. H & E X 360.





of the alveoli contained milk and a small number of polymorphonuclear leucocytes. In no instance however, was there any abnormality noted in the duct or alveolar epithelium. The cortical and paratrabecular sinuses of the supramammary lymph node contained small numbers of polymorphonuclear leucocytes.

Cow J Right Rear Quarter (inoculated with IBR virus and killed at nine days).

Section A.

Section A consisted of sections J7 - J12. Changes in this section were relatively mild and consisted of moderate lymphocytic infiltration in the interstitial tissues. One small necrotic focus was noted in alveolar epithelium surrounded by mononuclear cell reaction.

Section B.

This section consisted of histological sections J13 through to J40. Thirteen of these sections consisted of relatively normal tissue. In other sections many alveoli were greatly distended with milk. Areas of hyperplasia of duct epithelium accompanied by mononuclear cell infiltration of the lamina propria were also evident. The areas of hyperplasia often contained many vaculated cells indicating that these cells were carrying out a secretory role. Many of the lobules where the alveoli were distended with milk exhibited a moderate interstitial reaction of lymphocytes, plasma cells and a few polymorphonuclear leucocytes. A few polymorphonuclear leucocytes were seen in the distended alveoli. Several large ducts were noted which were plugged with cellular debris. Scattered intermittently throughout the interstitial tissue of the parenchyma were foci of lymphocytic hyperplasia. Within these foci the large blast-

type or reticulum-type cells as previously described in the supra-mammary lymph node from cow E were evident. Fig. 24 from section J32 illustrates one such focus. The arrow indicates the blast-like cell or cell resembling a Reed-Sternberg cell. Some of these foci of lymphocytic hyperplasia seem to spill over and through the alveolar epithelium into the lumen. Numerous mitotic figures were noted in the lymphocytes of these foci. Generally, in this section the alveoli were distended with milk and the ducts were plugged with cellular debris. Very little necrosis if any was noted in the alveolar or duct epithelium. The plugging seemed to be due to an accumulation of cells pushed up from below.

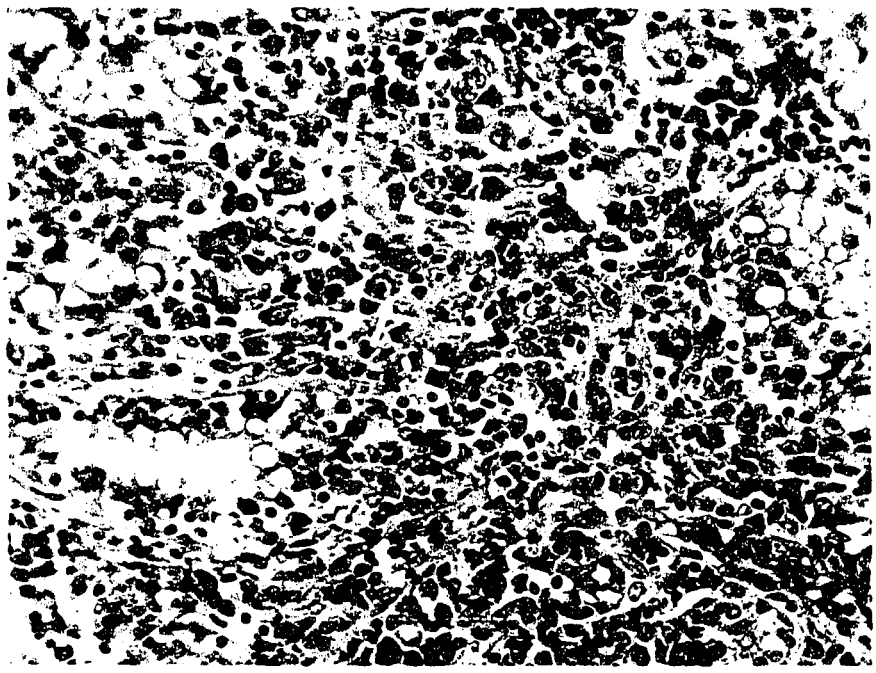
Section C.

Section C consists of histological sections J41 through to J63. Pathological changes were noted in all sections. In many sections it was often difficult to discern the normal architecture of the individual lobule. Mononuclear cell infiltration in these instances completely obscurred the architecture of the alveoli. The individual alveoli could often be identified within these zones of reaction and appeared collapsed and atrophic. Interstitial reaction was predominantly mononuclear and mitotic figures were fairly frequent. Blast-like cells or Reed-Sternberg type cells were found in these zones of reaction. Lobules surrounding these areas often contained alveoli distended with milk which contained the odd polymorphonuclear leucocyte. A number of relatively normal lobules also showed an increase in mononuclear cells in the interstitial tissue. Fig. 25 illustrates a lobule from section J49 where the normal architecture has been completely destroyed by the necrotic and

Figs. 24 and 25.

Fig. 24. J32 Udder parenchyma. Focus of lymphoid hyperplasia. Arrow indicates Reed-Sternberg-like cell. H & E X 360.

Fig. 25. J49 Udder parenchyma. Epithelium completely destroyed. Alveolar lumina obliterated with macrophages and polymorphonuclear cells stuffed with cellular debris. Interstitial tissue heavily infiltrated with lymphocytes and plasma cells. Fibrinoid necrosis of fibrous connective tissue elements in interstitial tissue. H & E X 180.

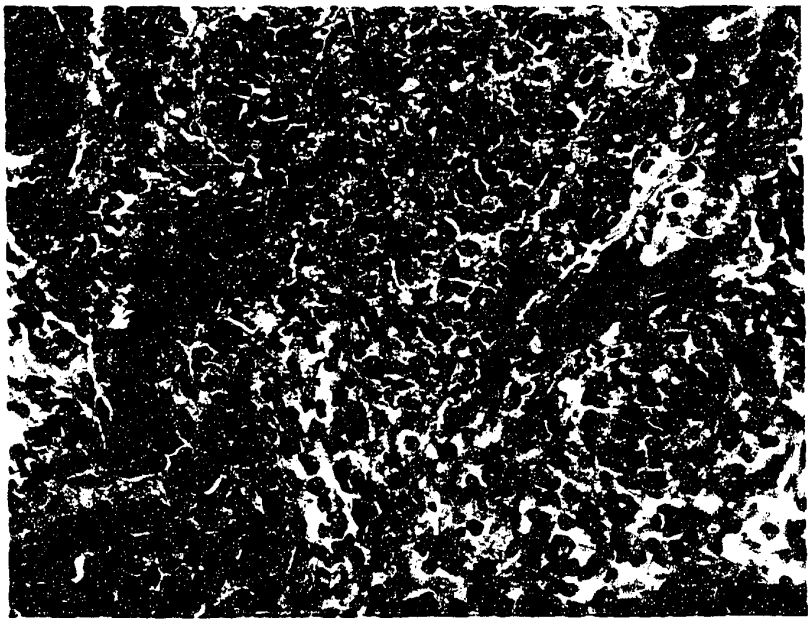


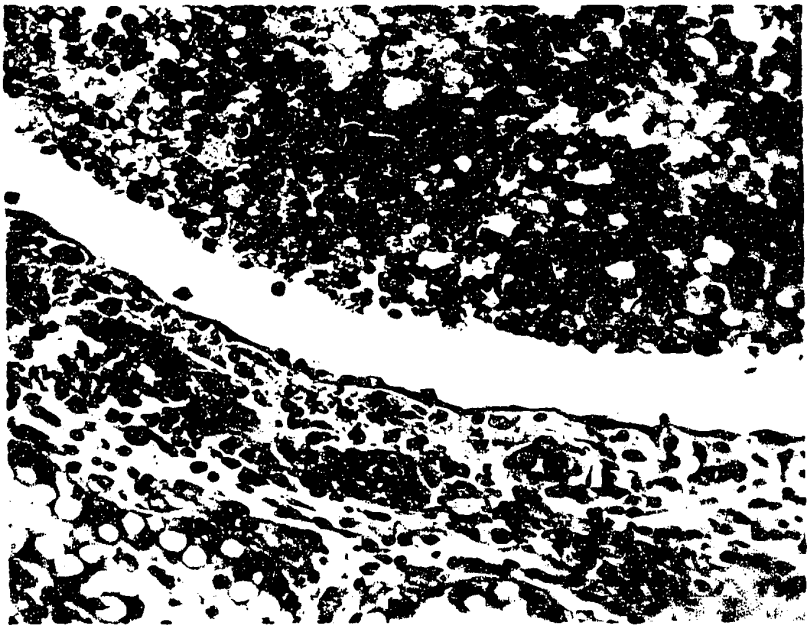
inflammatory process. The alveolar lumina are completely obliterated with macrophages and polymorphonuclear leucocytes which are stuffed with cellular debris. The interstitial tissue is heavily infiltrated with lymphocytes and plasma cells. Fibrinoid necrosis of the connective tissue elements was also seen in the interstitial tissue. This fibrinoid type of necrosis is illustrated in Fig. 26 taken from the same section as Fig. 25. Complete necrosis of alveolar walls and fibrinoid necrosis of the connective tissue elements is evident. In many instances it was possible to identify individual alveoli by the presence of the thin basement membrane. In many sections almost all ducts were plugged with necrotic cellular debris. Fig. 27 illustrates a duct from section J55. The lumen is filled with desquamated epithelial cells, polymorphonuclear leucocytes and debris-stuffed macrophages. Re-epithelization of the duct wall has taken place with a single layer of flat, simple squamous epithelium. The duct wall is moderately infiltrated with mononuclear type cells. Fig. 28 shows a duct from section J56. Squamous metaplasia ranging from simple to stratified has taken place. The lumen of the duct is occupied by a core made up primarily of fibrin with a few epithelial cells with pyknotic and karyorrhectic nuclei. The duct wall is infiltrated with a small number of mononuclear cells. It is also evident that some alveoli in the surrounding lobules contained milk and polymorphonuclear leucocytes and their epithelium is relatively normal and intact. Fig. 29 illustrates a duct from section J59. Marked fibrinous exudation from the duct wall which is completely devoid of epithelium is noted. The infiltrating cells are both polymorphonuclear leucocytes and lymphocytes.

Figs. 26 and 27.

Fig. 26. J49 Udder parenchyma. Complete necrosis of alveolar walls and fibrinoid necrosis of fibrous connective tissue elements. H & E X 360.

Fig. 27. J55 Duct. Re-epithelisation of duct with single layer of flat simple squamous epithelium. H & E X 360.

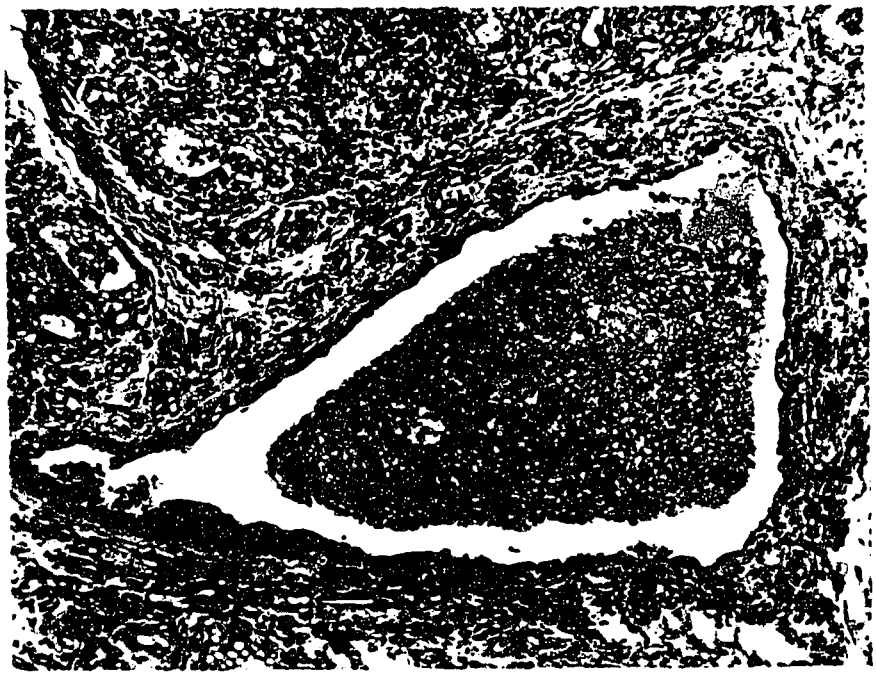


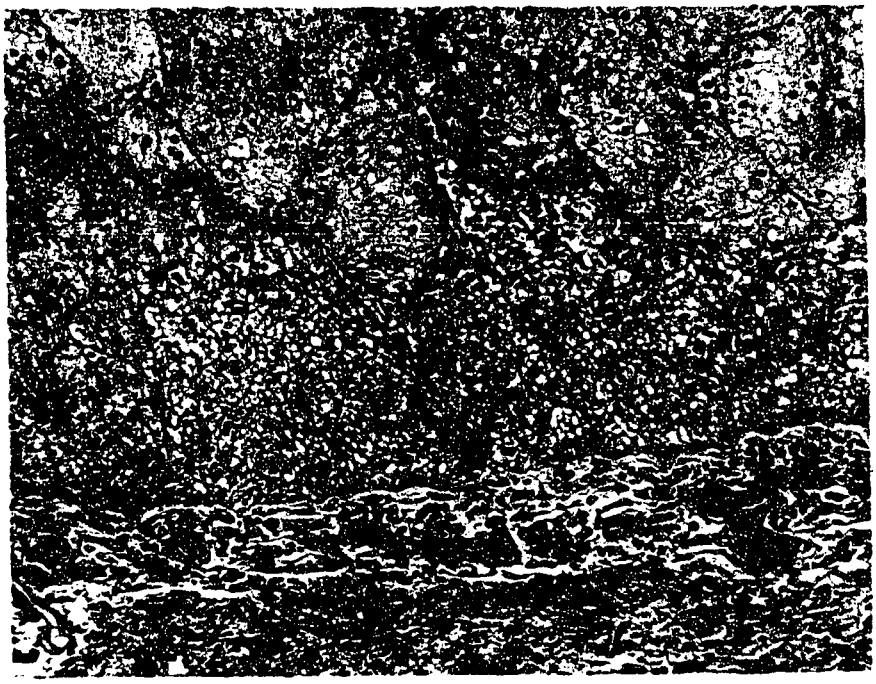


Figs. 28 and 29.

Fig. 28. J56 Duct. Squamous metaplasia ranging from simple to stratified in plugged duct. H & E X 150.

Fig. 29. J59 Duct. Marked fibrinous exudation from duct wall completely devoid of epithelium. Infiltrating cells are polymorphonuclear and lymphocytes. H & E X 180.





The capillaries in the lamina propria of the duct wall are prominent with swollen endothelial cells. To summarize section C, scattered throughout this level are lobules the architecture of which has been completely obliterated by the inflammatory process. In general the ducts are plugged and either have healing epithelium or exudation still continuing while in others squamous metaplasia is evident. Subcutaneous oedema accompanied by a mild mononuclear cell reaction of blood vessels was noted in this section.

Section D.

This section consists of histological sections J64 through to J84. Pathological changes were noted in all sections with the exception of J82 - J84 which were taken through the lower portion of the teat canal. Changes noted in sections J64 through to J73 were essentially similar to those noted above in section C. Subcutaneous oedema accompanied by mild mononuclear perivascular cuffing was present. Lobules were present where the architecture was obliterated by massive mononuclear cell infiltration consisting primarily of lymphocytes and plasma cells. In other areas the architecture of the lobules was relatively normal. In these areas however, most of the alveoli were distended with stagnant milk infiltrated by a few polymorphonuclear leucocytes. Interstitial tissue exhibited areas of lymphoid hyperplasia. Plugging of ducts which exhibited various stages of squamous metaplasia was general throughout this level. In the lamina propria of ducts and the gland cistern a mononuclear cell infiltration was evident and capillaries with swollen endothelial cells appeared prominent. Hyaline degeneration with the formation of droplets was evident both in the alveolar and ductal epithelium. In the teat there was complete destruction of the epithelial

lining of the cistern leaving a basement membrane covered with fibrin and infiltrated with mononuclear cells and polymorphonuclear leucocytes resulting in the formation of a pseudomembrane. In the lamina propria the capillaries were prominent with swollen endothelial cells. Accessory glands as such were not recognizable, complete destruction having taken place along with massive infiltrations of mononuclear cells with an occasional polymorphonuclear leucocyte present.

Supramammary Lymph Node.

This lymph node was represented in sections J1 - J6. The cortical and paratrabecular sinuses contained many plasma cells and macrophages but few polymorphonuclear leucocytes. Large blast-type cells resembling Reed-Sternberg cells were commonly encountered.

Cow J Left Rear Quarter (control quarter).

Little beyond a few alveoli containing milk and the odd polymorphonuclear leucocyte was noted throughout this gland. Small numbers of polymorphonuclear leucocytes were present in the cortical and paratrabecular sinuses of the left supramammary lymph node. Polymorphonuclear leucocytes were also present in the small blood vessels in the cortex of this node.

Experiment IV. Enzootic Abortion in Ewes Virus (EAE).

Table V illustrates the daily milk production and temperature records for cows P, Q and N for two days prior to inoculation and throughout the course of the experiment.

The first post-inoculation day the milk from the infected quarters appeared normal and all animals ate well. Decreased milk production especially in cows P and Q coincided with the elevation in temperature.

Table V

Daily milk production in ml. and temperatures of cows P, Q and N for two days prior to inoculation and throughout the course of the experiment.

	Cow P		Cow Q		Cow N	
Day	Milk Prod.	Temp.	Milk Prod.	Temp.	Milk Prod.	Temp.
-2	6300	101.2	2000	102.0	9600	101.2
-1	5900	101.0	1950	101.4	9150	101.2
1	2050	105.8	1425	104.8	8000	101.8
2	1200	107.2	200	107.0	2750	106.4
3	150	104.2	100	103.4	1350	103.0
4			100	102.4	4100	101.6
5			250	102.2	4300	101.4
6			400	101.4	6100	101.8
7					7100	101.4
8					6850	101.3
9					7400	101.2

The second post-inoculation day all animals were off feed. Marked swelling and heat extended down into the teats which were turgid. Milk from the left quarters was normal in gross appearance while that from the right quarters was urine-like and contained white fibrinous clots which on occasion plugged the streak canal. Cow Q appeared the most severely affected, was recumbent and very reluctant to stand. On the third post-inoculation day all cows were still off feed. All right quarters were hot and swollen and difficulty was experienced in milking because of blockage of the streak canal with fibrin clots. Milk from the inoculated quarters contained a large number of clots and was urine coloured like that seen the previous day. Cow P was killed because of a teat canal blockage. Marked subcutaneous oedema was noted over the right quarters. Total weight of the right quarters was 5,000 gms compared with only 2,600 gms for the left quarters. The fourth post-inoculation day the right quarters of cows Q and N were still quite hot and swollen although both animals appeared alert and were eating. The milk from the virus-inoculated quarters contained large numbers of clots and was still urine like in colour. The fifth post-inoculation day the animals showed continued improvement with the swelling in the inoculated quarters continuing to subside. The milk from the right front quarter of cow N contained a few clots but was more normal in appearance. The milk from the right rear quarter of cow N and both right quarters of cow Q was much the same as the previous day. The sixth post-inoculation day a few clots were noted from both right quarters of cow N but the milk was near normal in colour. The milk from both right quarters of cow Q was much the same as previously observed and

a fibrin clot formed in the sample collected from the right rear quarter within minutes of collecting. Marked subcutaneous oedema varying between one and two inches in thickness was present over the right quarters of cow Q. This oedema extended five to six inches along the abdominal wall anterior to the right front quarter. The right quarters weighed 5900 gms. as compared to 1950 gms. for the left quarters. The seventh post-inoculation day the milk from the virus inoculated quarters of cow N still contained a number of small clots but the secretion was normal in appearance. Swelling was reduced from the previous day but the right quarters were still quite hard. The following day very few clots were present in the milk from the affected quarters and swelling was continuing to subside and the glands were more pliable. The ninth post-inoculation day the milk from the affected quarters appeared relatively normal. No subcutaneous oedema was noted over the right quarters but the right half of the udder weighed 5750 gms. as compared to 3400 gms. for the left half.

Virus isolation was attempted from milk samples collected from both the left rear and right rear quarters of all cows throughout the course of the experiment. Success was not achieved in isolating virus from milk samples collected during the first three days of the experiment. These were the samples which were frozen and thawed prior to their inoculation into the yolk sac of eight-day-old embryonating eggs. Samples collected from the right rear quarters of cows N and Q on the fourth, fifth and sixth post-inoculation days resulted in death of the embryos in eight to ten days, whereas samples collected from the left rear quarters of these animals on the same days did not result in

embryonic death. Samples collected from the right rear quarter of cow N on post-inoculation days seven, eight and nine resulted in embryonic death while those collected from the left rear quarter did not. Virus particles were demonstrated in impression smears prepared from dead embryos when stained by Machiavello's technique. Fig. 30 illustrates a cluster of virus particles as seen in an impression prepared from yolk sac of a dead embryo.

Clusters of virus particles were seen in smears prepared from milk collected from the right quarters of all cows on post-inoculation day three. Smears prepared from the milk collected from these quarters on post-inoculation day one and two failed to show definite clusters. These clusters appeared similar to that which is illustrated in Fig. 30. Similar clusters were demonstrated from the virus inoculated quarters of cows Q and N in samples collected in post-inoculation days four, five and six and from cow N on days seven, eight and nine. At no time were clusters demonstrated in smears prepared from milk samples collected from the left quarters of all cows.

Histological examination was carried out on both rear quarters of cows P, Q and N.

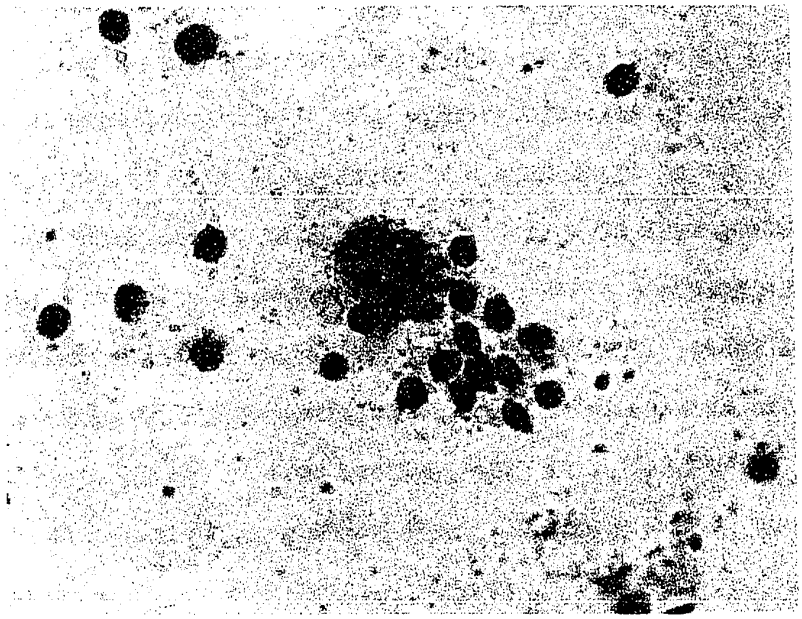
Cow P Right Rear Quarter (inoculated with EAE virus and killed at three days.)

Section A.

Section A consists of histological sections P1 through to P28. Six of these sections contained relatively normal tissue. Throughout the remainder of the sections many alveoli were seen which were filled with milk containing polymorphonuclear leucocytes and desquamated

Fig. 30.

Fig. 30. Yolk Sac. Cluster of virus particles seen in impression prepared from dead embryo. Machiavello's. X1450.

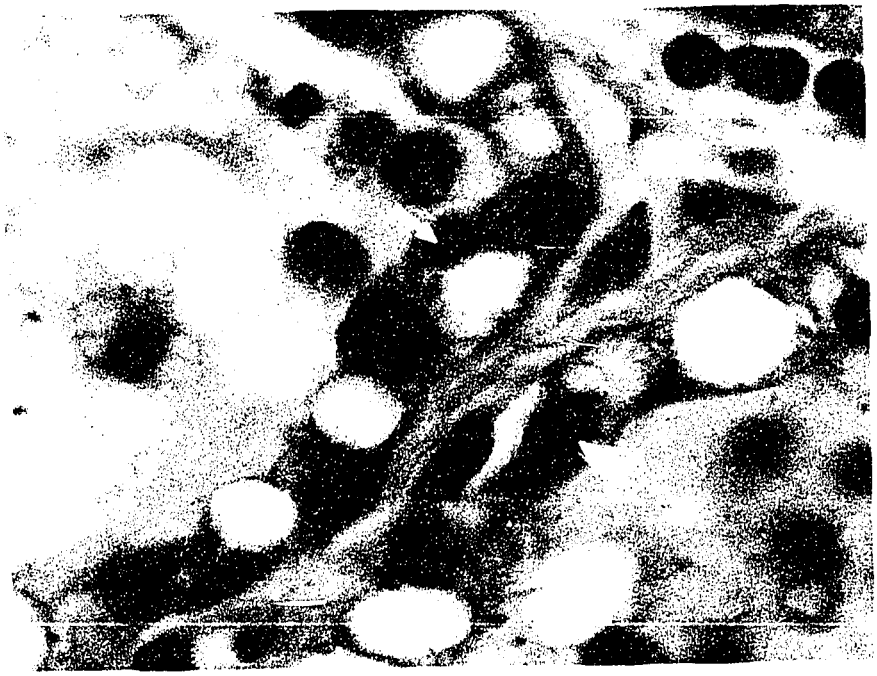


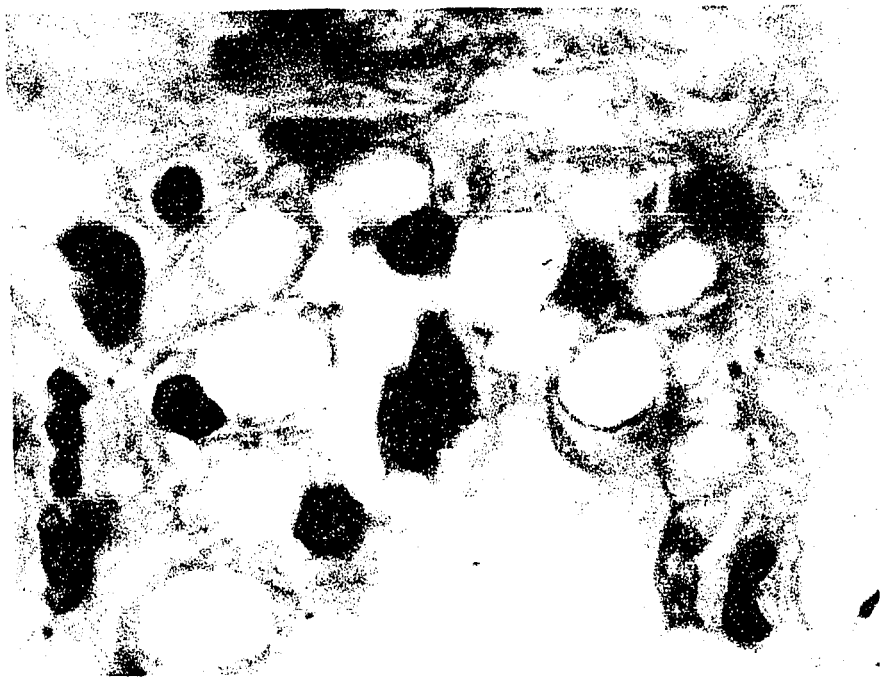
epithelial cells. No necrosis of duct or alveolar epithelial cells was noted and interstitial infiltrations were not evident. Clusters of elementary bodies were seen in sections P6, 11, 16 and 21. These clusters were usually found in alveoli which were filled with milk and were usually located at the distal end of the cell towards the alveolar or duct lumen. These clusters were found in various stages of development. The earliest stage was characterized by a dense staining matrix in which no individual elementary bodies or virus particles could be distinguished. This type of cluster referred to as a plaque is illustrated in Fig. 31 from section P89. The plaque indicated by the left hand arrow is typical of this early form. The next stage in the maturation of the virus colony is illustrated in Fig. 32 from section P97. This type of cluster was characterized by a dense staining matrix in which fine virus particles could just be distinguished. Other forms in which larger virus particles could be made out in the matrix are illustrated in Figs. 33 and 34 from section P43. It will be noted that the lumina of the alveoli in which these elementary bodies are found are filled with milk, infiltrating leucocytes and desquamated epithelial cells. Some clusters were noted which were relatively small in size and contained large virus particles. Such a cluster is illustrated in Fig. 35 also taken from section P43. While both Giemsa and Machiavello stains were utilized in attempting to demonstrate these elementary bodies H and E stain proved as useful for this purpose as the others. Clusters were found within fat vacuoles as illustrated in Fig. 36 from section P77. It is also noted that this particular cluster

Figs. 31 and 32.

Fig. 31. P89 Alveolus. Plaque formation characterized by dense matrix in which no individual virus particles can be distinguished. Arrows indicate plaques. The plaque on the left best exemplifys this. H & E X 1450.

Fig. 32. P97 Alveolus. Plaque in which fine virus particles can be distinguished within dense matrix. H & E X 1450.

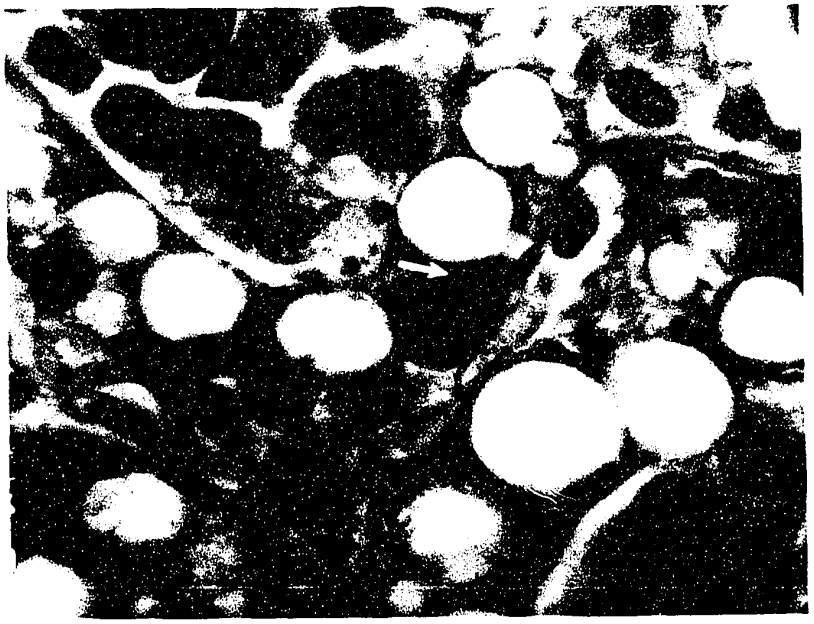


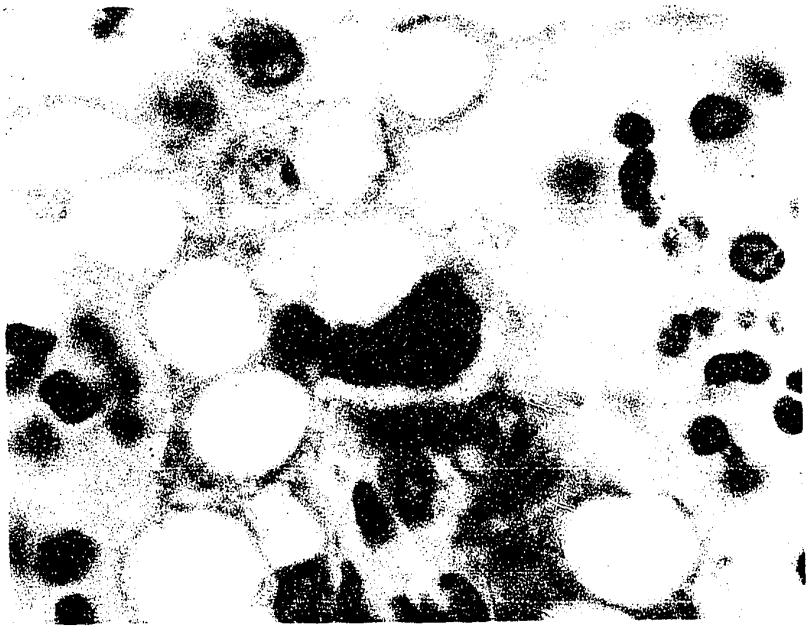


Figs. 33 and 34.

Fig. 33. P43 Alveolus. Cluster (arrow) showing large virus particles in the cytoplasm of alveolar epithelial cell. Alveolar lumen is plugged with desquamated epithelial cells and leucocytes. H & E X 1450.

Fig. 34. P43 Alveolus. A cluster of virus particles in epithelial cell. Alveolar lumen filled with milk and leucocytes. Giemsa X 1450.

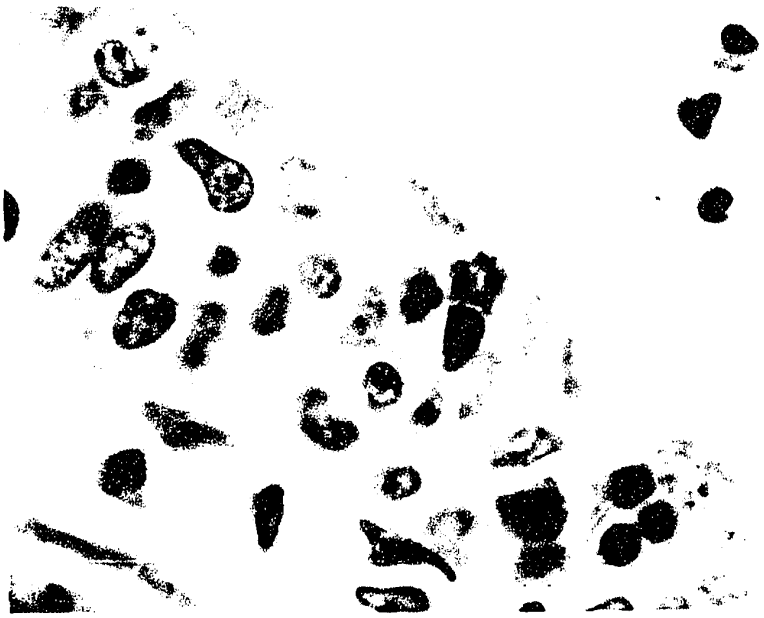


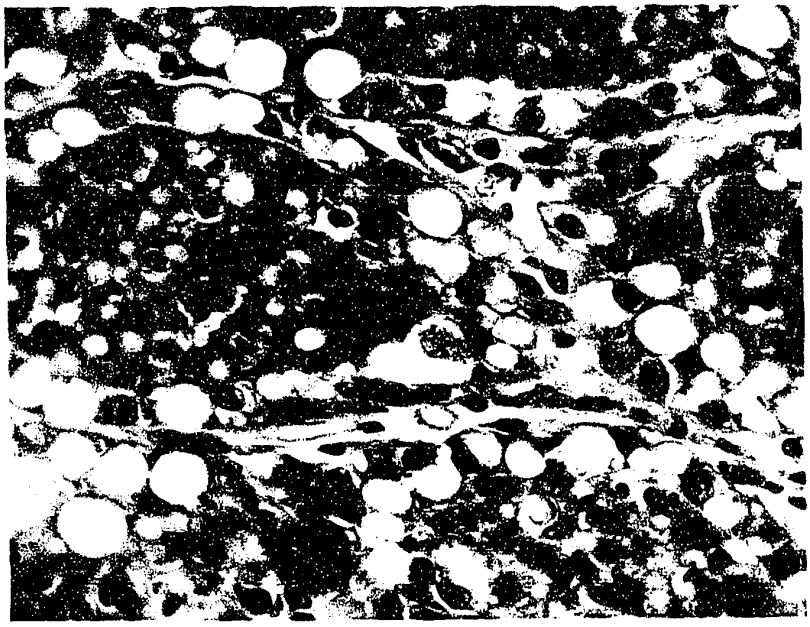


Figs. 35 and 36.

Fig. 35. P43 Duct. Small cluster with relatively large virus particles. Giemsa X 1450.

Fig. 36. P77 Alveolus. Cluster of small virus particles developing in a very pale matrix in the cytoplasm of an alveolar epithelial cell. Alveolar lumen is filled with milk, polymorphonuclear leucocytes and desquamated epithelial cells. H & E X 600.





had very pale staining matrix. Fig. 37 taken from section P43 illustrates a duct the lumen of which is plugged with mononuclear and polymorphonuclear leucocytes. The arrows indicate clusters of elementary bodies in the cytoplasm of duct epithelial cells. The mature type of cluster is illustrated in Fig. 38 from section P97. A number of clusters as indicated by arrows, of fine virus particles suspended in a pale staining matrix are present. It is noted that no evidence of necrosis was found in alveolar or duct epithelium where clusters of virus particles were found.

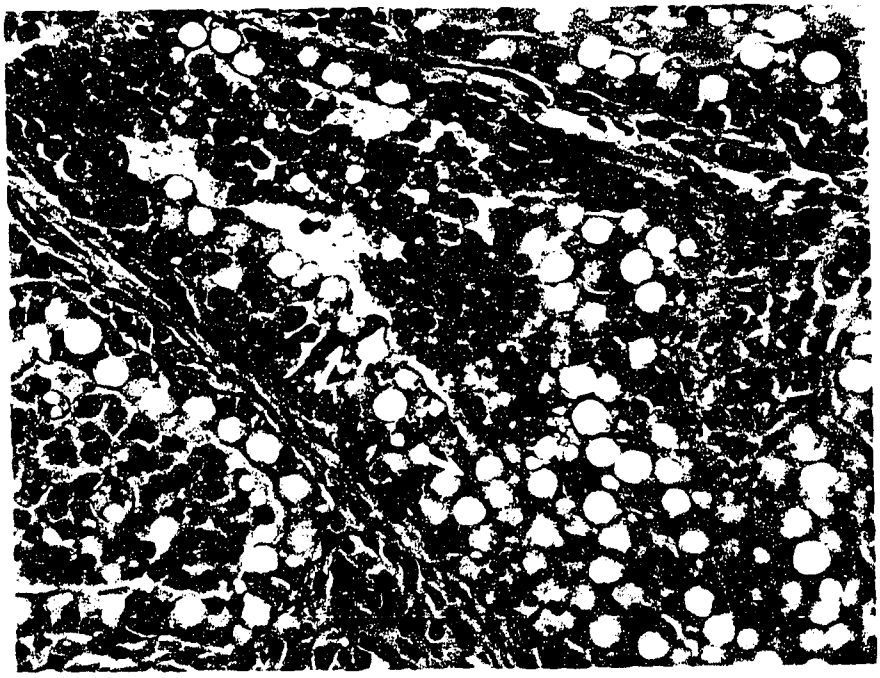
Section B.

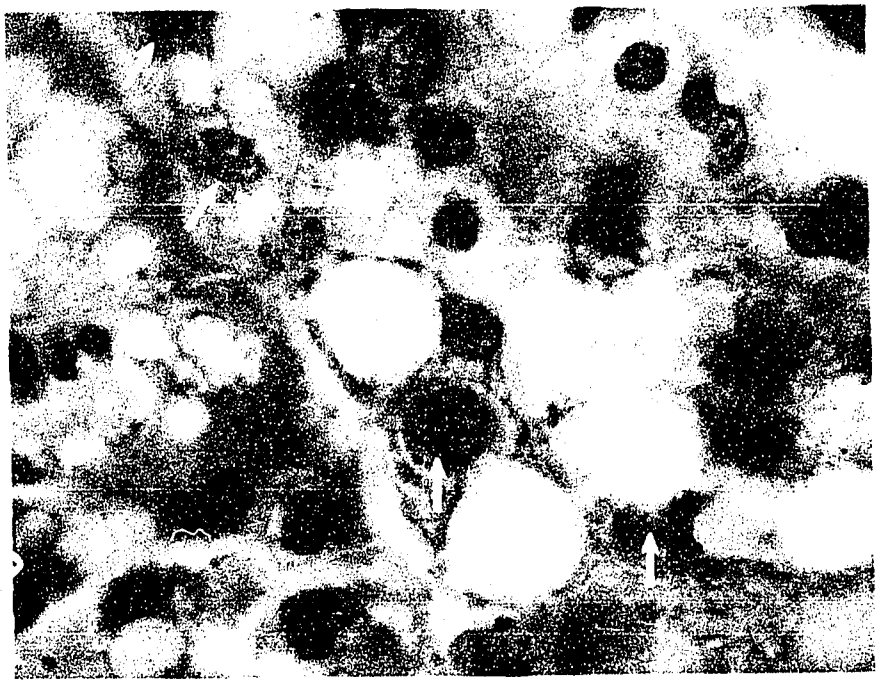
Section B consists of histological sections P37 through to P76. Section P37 was the only one in which pathological changes were not found. Clusters of elementary bodies were found in 12 sections distributed throughout section B. These clusters were of different forms as described above and illustrated in Figs. 31 through to 38. Necrosis was not found in intimate association with clusters of elementary bodies. The first indication of necrosis was pyknosis of the nuclei followed by karyorrhexis. These changes were often accompanied by swelling of the endothelial cells of blood vessels in the interstitial tissue and the appearance of mononuclear cells both lymphocytes and plasma cells in the same area. These necrotic cells then sloughed from the basement membrane into the lumen followed by a marked outpouring of fibrin from the interstitial tissue. This type of reaction is illustrated in Fig. 40 from section P45. The alveolar epithelial cells in the area between the arrows have become necrotic and sloughed off. A volcanic outpouring of fibrin from the interstitial tissue is forcing the lumen

Figs. 37 and 38.

Fig. 37. P43 Duct. Duct lumen plugged with mononuclear and polymorphonuclear leucocytes. Arrows indicate clusters of virus particles. H & E X 360.

Fig. 38. P97 Alveolus. A number of clusters (arrows) of fine virus particles in pale matrix. This represents the mature vesicle. H & E X 1450.





content of polymorphonuclear leucocytes and desquamated epithelial cells into a crescent-shaped mass at the left of the figure. It is noted that the remainder of the alveolar epithelium appears relatively unaffected. Fig. 39 from section P46 represents a more or less typical area where necrosis was found. The alveoli in the central portion of the figure contained fibrin, pyknotic and karyorrhectic nuclei and have discontinuities in their lining epithelium. The interstitial reaction is of both mononuclear and polymorphonuclear leucocytes. The surrounding alveoli are filled with milk containing polymorphonuclear leucocytes and desquamated epithelial cells. The epithelium of these alveoli was relatively normal. This latter type of reaction was found throughout section B.

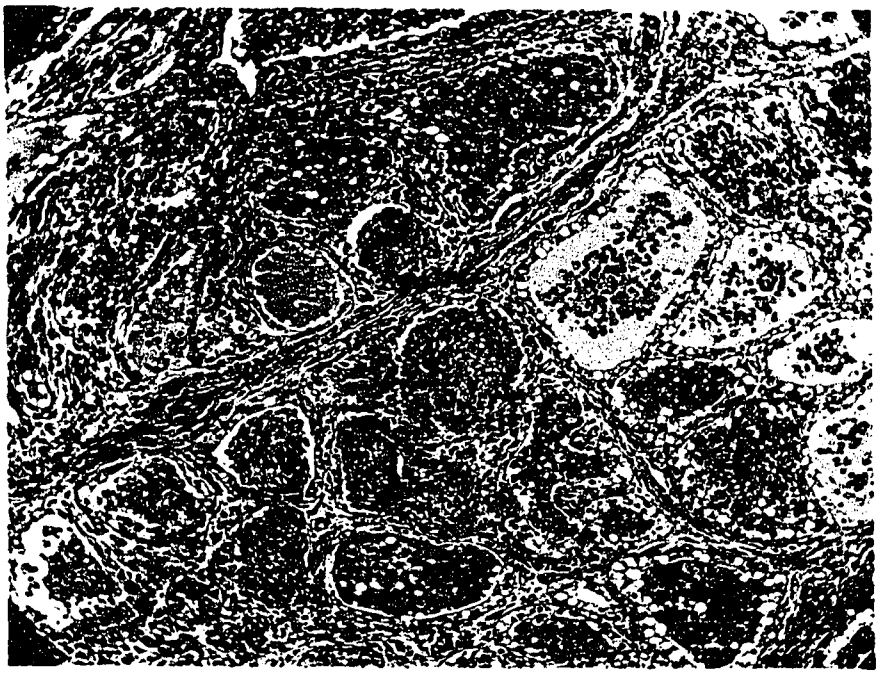
Section C.

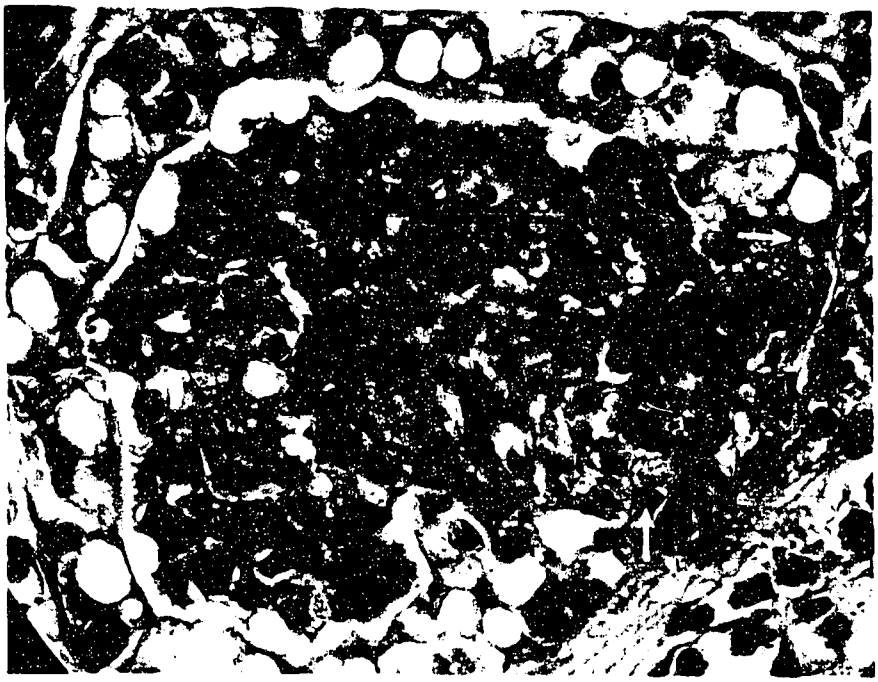
Section C was made up of histological sections P77 through to P101. Lesions considered of pathological significance were found in all sections. Clusters of elementary bodies were found in 16 sections and areas of necrosis of alveolar and duct epithelium were found in 15. The clusters of elementary bodies were similar to those described in section A but the areas of necrosis tended to be larger and in some lobules complete loss of architecture was noted. Moderate mononuclear cell infiltration consisting of lymphocytes and plasma cells was found in areas of necrosis. The plugging of ducts as a result of necrosis of their epithelium was much more prominent in this than in sections A and B. Fig. 41 illustrates such a duct from section P77. The duct is plugged with polymorphonuclear leucocytes and desquamated epithelial cells. The epithelium at the bottom of the duct has been completely

Figs. 39 and 40.

Fig. 39. P46 Udder parenchyma. Alveoli plugged with milk, polymorphonuclear leucocytes and desquamated epithelial cells. Central alveoli contain fibrin, pyknotic and karyorrhectic nuclei and have discontinuities in their lining epithelium. Interstitial reaction is both polymorphonuclear and mononuclear. H & E X 108.

Fig. 40. P45 Alveolus. Disruption (between arrows) in alveolar epithelium at lower right with outpouring of fibrin forcing lumen content of polymorphonuclear cells and desquamated epithelial cells into crescent shaped mass at left. H & E X 630.





destroyed and there has been a marked outpouring of fibrin and exudation of polymorphonuclear leucocytes. The arrow indicates intact epithelium at the top of the picture. This epithelium was undergoing hyaline droplet degeneration. It is noted that the surrounding alveoli are filled with milk and polymorphonuclear leucocytes while their epithelium is relatively normal and the interstitial infiltration minimal.

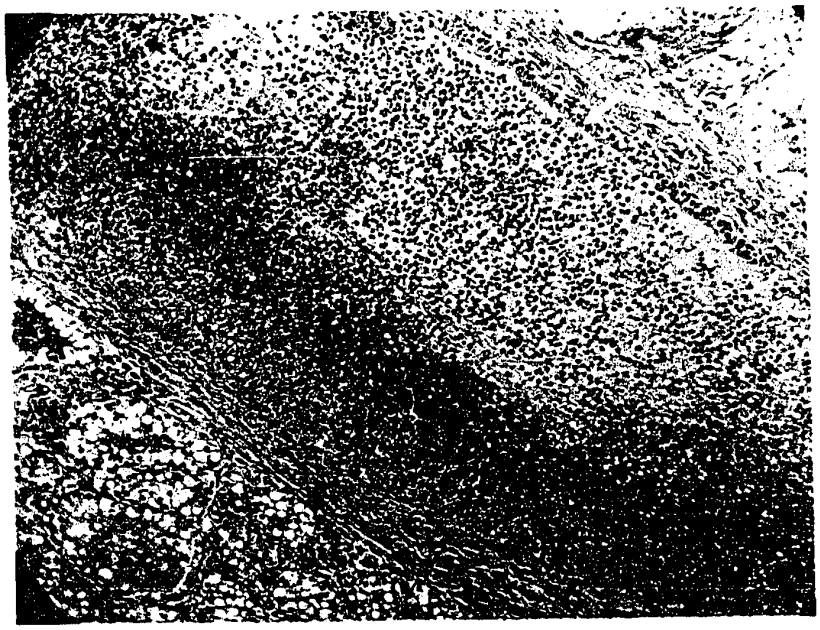
Section D.

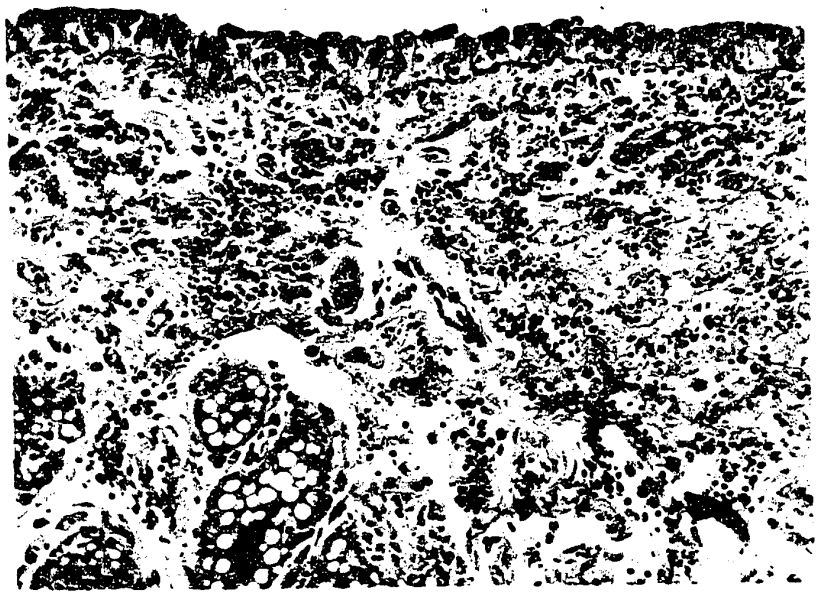
Section D consists of histological sections P102 through to P132. Changes considered of pathological significance were found in all these sections. Necrosis was general throughout the sections of udder parenchyma but clusters of elementary bodies were not found. As in section C the plugging of ducts with exudation and necrotic cellular debris was general throughout. Hyperplasia of lymphoid elements in the interstitial tissue was marked in some foci. Fig. 42 represents a section of gland cistern from P109. Oedema with infiltration of polymorphonuclear leucocytes and diapedesis of erythrocytes in the lamina propria is evident. A similar type of reaction was also evident in the lamina propria of many ducts throughout this section. Swelling of endothelial cells of capillaries and small blood vessels was sometimes a prominent feature in the periductal tissue. In some instances minute areas of fibrinoid necrosis of connective tissue elements was evident. Lymphatic vessels were often distended with lymph and contained numerous polymorphonuclear leucocytes. In some instances these leucocytes seem to occlude the vessel resulting in the formation of what might be termed a leucocytic thrombus. Inflammatory changes with necrosis

Figs. 41 and 42.

Fig. 41. P77 Duct. Duct plugged with polymorphonuclear leucocytes and desquamated epithelial cells. The epithelium of the duct has been completely destroyed at the bottom where there is an outpouring of fibrin. Arrow indicates intact epithelium at top. H & E X 108.

Fig. 42. P109 Gland cistern. Oedema with infiltration of polymorphonuclear leucocytes and diapedesis of erythrocytes in the lamina propria of the gland cistern. H & E X 180.





were also noted in the walls of lymphatic vessels. Sections through the teat showed marked necrosis of epithelium of the accessory glands. Fig. 43 from section P120 illustrates the marked oedema with moderate polymorphonuclear cell infiltration of the lamina propria of the teat cistern. In areas this sub-epithelial oedema was marked and extended below to the muscle layers. Swelling of endothelial cells of blood vessels in the areas was accompanied by infiltrations of polymorphonuclear leucocytes, macrophages, mononuclear cells and eosinophils. In some instances lymphatic vessels were plugged with fibrin thrombi. Fibrinoid degeneration of fibrous connective tissue elements as well as diapedesis of erythrocytes was evident in some foci. Sections taken through the streak canal appeared relatively normal. Subcutaneous tissues were oedematous and accompanied by mild polymorphonuclear cell cuffing of blood vessels together with the odd macrophage. In areas there was a marked vasculitis in the subcutaneous vessels with diapedesis of polymorphonuclear and mononuclear leucocytes. Sections P29 through to P36 represented the right supramammary lymph node. Fig. 44 from section P32 illustrates the oedema fluid which was found in the cortical and paratrabecular sinuses. In some areas this fluid was accompanied by polymorphonuclear leucocytes. This figure also illustrates a germinal centre which has been depleted and is undergoing a fibrinoid type of necrosis. Oedema of the perinodal tissues was also evident accompanied by swelling of endothelial cells of blood vessels and the diapedesis of leucocytes and erythrocytes.

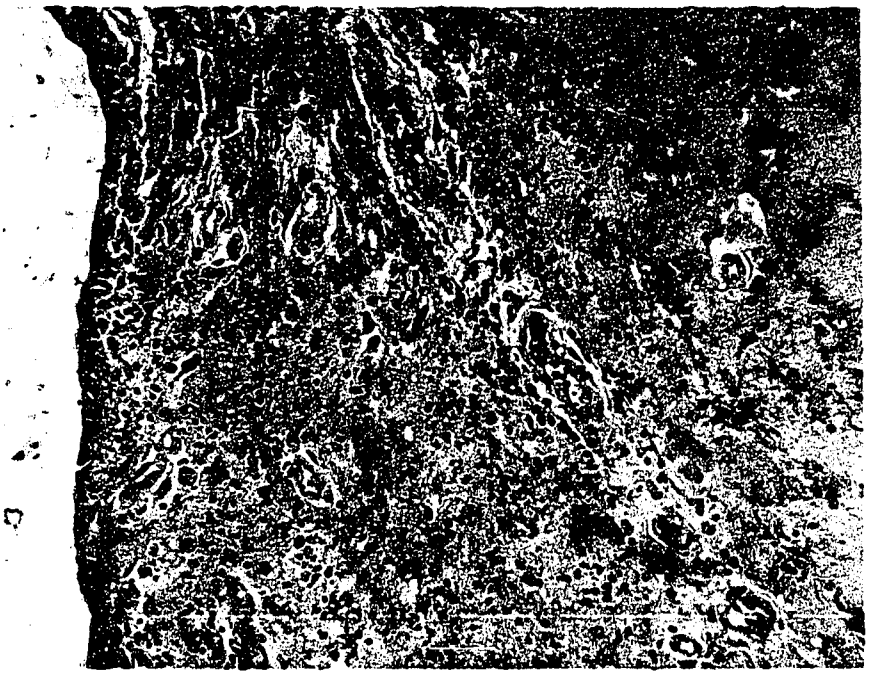
Cow P Left Rear Quarter (control quarter)

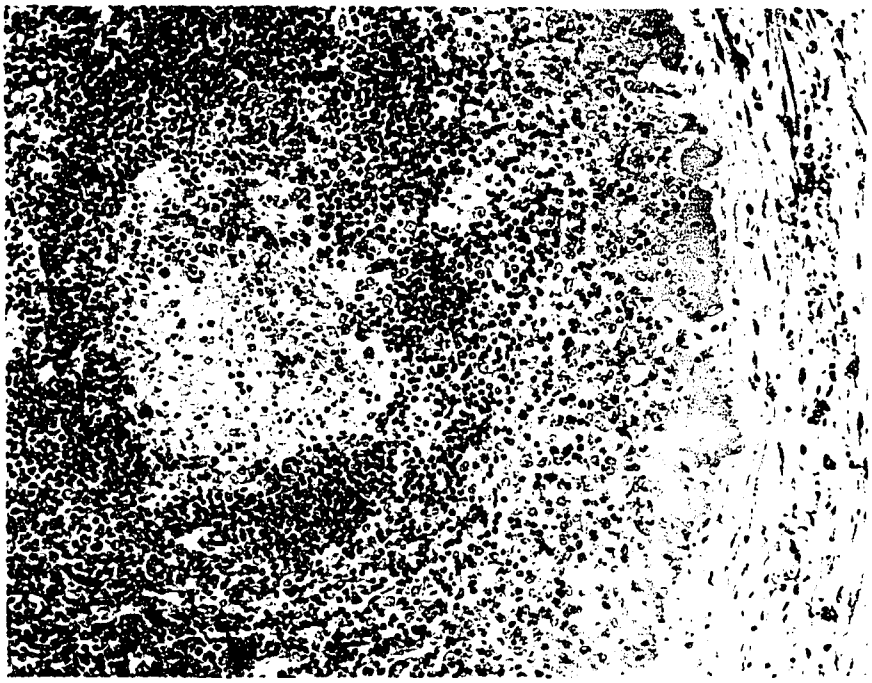
Small numbers of alveoli throughout all levels of this gland con-

Figs. 43 and 44.

Fig. 43. P120 Teat. Marked oedema with moderate polymorphonuclear cell infiltration of lamina propria of teat cistern. H & E X 180.

Fig. 44. P32 Supramammary lymph node. Oedema fluid in cortical sinus and depletion and necrosis of germinal centre. H & E X 180.





tained milk and small numbers of polymorphonuclear leucocytes. In no instance was any abnormality of duct or alveolar epithelium noted. Small numbers of polymorphonuclear leucocytes were found in the cortical and paratrabecular sinuses of the left supramammary lymph node.

Cow Q Right Rear Quarter (inoculated with EAE virus and killed after six days.)

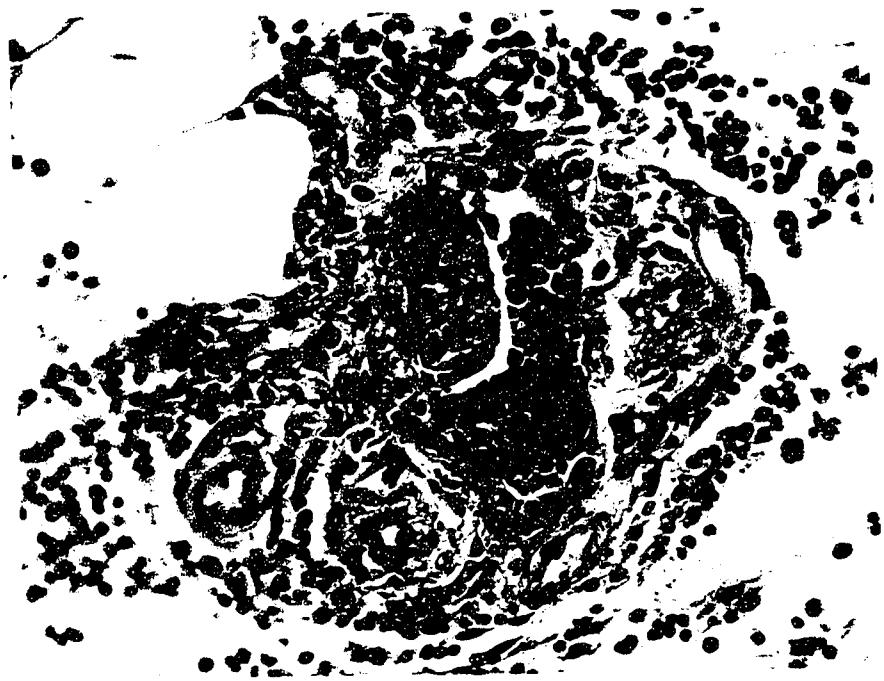
Section A.

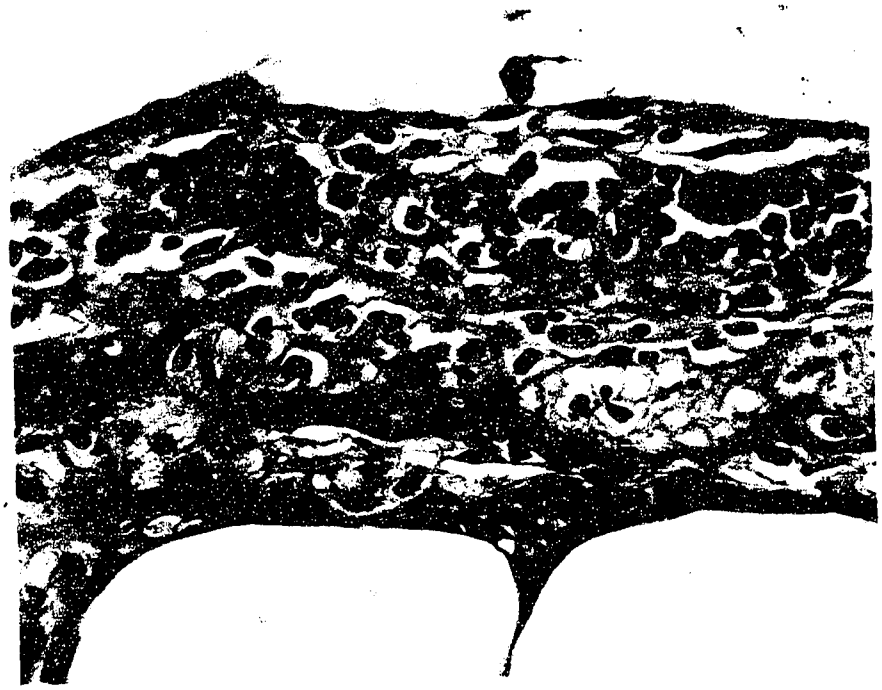
Section A consists of histological sections Q1 through to Q28. As illustrated in the tracing in Appendix 1 the area included within the inner solid line contains udder parenchyma whereas that outside of this area consists of periglandular and subcutaneous tissues. Throughout the periglandular and subcutaneous tissues capillaries with swollen endothelial cells and perivascular infiltrations, which varied from mild to severe and consisting of lymphocytes, macrophages and polymorphonuclear leucocytes were evident. Lymphatic vessels were distended with lymph and mononuclear leucocytes predominantly lymphocytes together with some polymorphonuclear leucocytes. Thrombosis of veins and lymphatic vessels was a prominent feature in these areas. Fig. 45 represents a portion of section Q110 but is typical also of changes seen in section A. A perivascular reaction characterized by lymphocytic and polymorphonuclear infiltration is noted about a group of small blood vessels and a lymphatic vessel with fibrinoid necrosis of its wall. Fig. 46 illustrates a part of a lymphatic vessel wall from section Q5. The wall is infiltrated by polymorphonuclear and mononuclear leucocytes and fibrinoid degeneration is evident in the vessel wall. A necrotic focus within a thrombus is illustrated in Fig. 47

Figs. 45 and 46.

Fig. 45. Q110 Periglandular tissue. Perivascular reaction characterized by lymphocytic and polymorphonuclear infiltration. Fibrinoid necrosis is evident in wall of lymphatic vessel. H & E X 360.

Fig. 46. Q5 Lymphatic vessel. Infiltration of polymorphonuclear and mononuclear cells with fibrinoid degeneration. Lumen is at top of photomicrograph. H & E X 600.



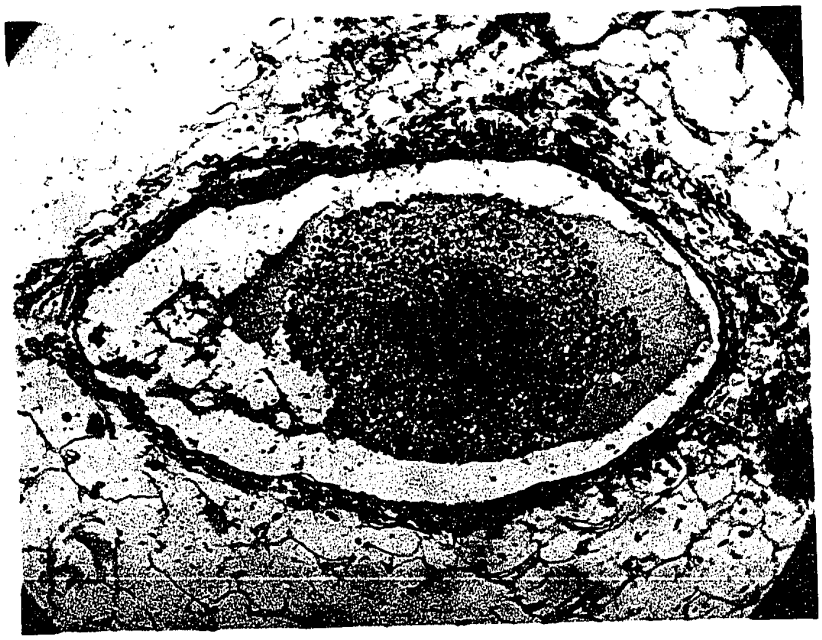


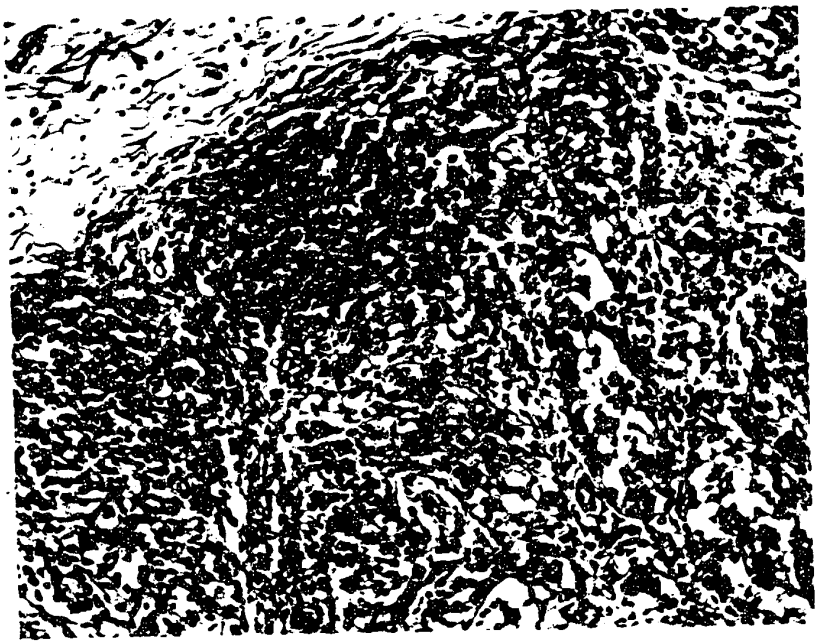
from section Q3. This focus was characterized by a deep pink staining matrix containing pyknotic and karyorrhectic nuclei. In the udder parenchyma itself reaction varied from alveoli distended with milk and polymorphonuclear leucocytes to areas where entire lobules were obliterated by an inflammatory reaction. In areas where the reaction was mild many of the alveolar cells contained fat vacuoles or were flattened. In some areas hyaline droplet degeneration of the alveolar epithelium was noted. In other areas an interstitial reaction consisting primarily of plasma cells caused separation of alveoli. Many of the plasma cells contained distinct hofs which contained one or two small orange staining granules. Mitotic figures were not uncommon in the infiltrating plasma cells. Capillaries and venules in these areas were prominent and their endothelial cells were enlarged. A few polymorphonuclear leucocytes were seen in the interstitial tissue but the interstitial infiltration was primarily of plasma cells whereas the alveolar lumina were obliterated with polymorphonuclear leucocytes and desquamated alveolar epithelial cells. The lumina of ducts also were plugged with polymorphonuclear leucocytes and epithelial cells and hyaline droplet degeneration was evident in the duct epithelium. Duct walls were infiltrated by polymorphonuclear leucocytes and plasma cells resulting in a loss of polarity of the epithelial cells. An increase in surrounding fibrous connective tissue was evident together with a marked vasculitis which on occasion was characterized by necrosis of adventitial cells with pyknosis and karyorrhexis of nuclei. Occasionally infiltrating mononuclear cells were numerous. On occasion the marked infiltration of plasma cells, lymphocytes and polymorphonuclear leucocytes

Figs. 47 and 48.

Fig. 47. ③ Lymphatic vessel. Necrotic focus in thrombus within a lymphatic vessel in periglandular fat.
H & E X 108.

Fig. 48. ②1 Udder parenchyma. Marked infiltration of lobule with plasma cells, lymphocytes and polymorphonuclear leucocytes resulting in complete loss of architecture.
H & E X 100.





resulted in the complete loss of the normal lobular architecture. Such an area is illustrated in Fig. 48 taken from section Q21. In other instances the normal lobular architecture was obscured by a reticular formation of epithelial cells infiltrated with polymorphonuclear leucocytes and plasma cells. Such a focus is illustrated in Fig. 49, a photomicrograph taken from section Q26.

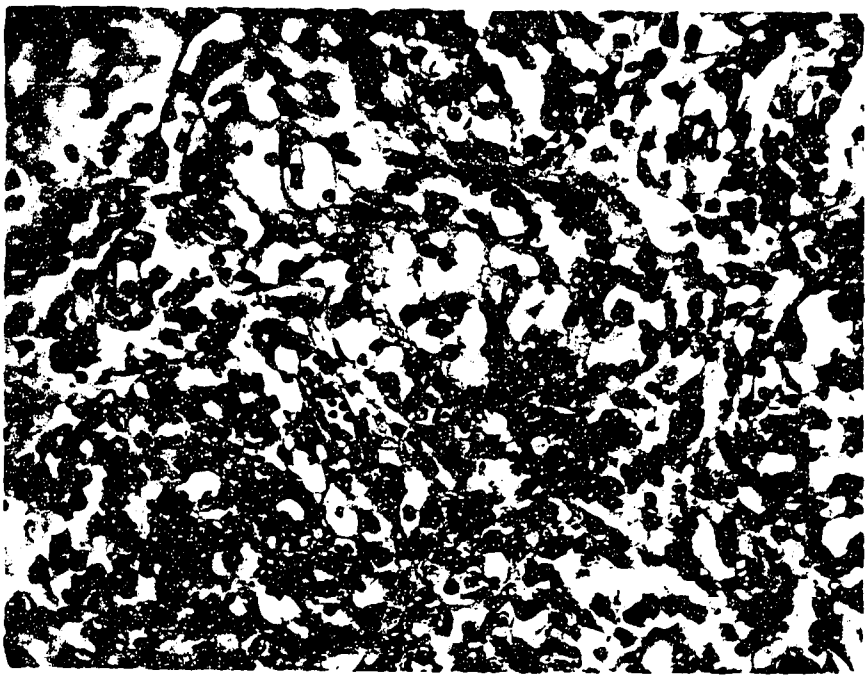
Section B.

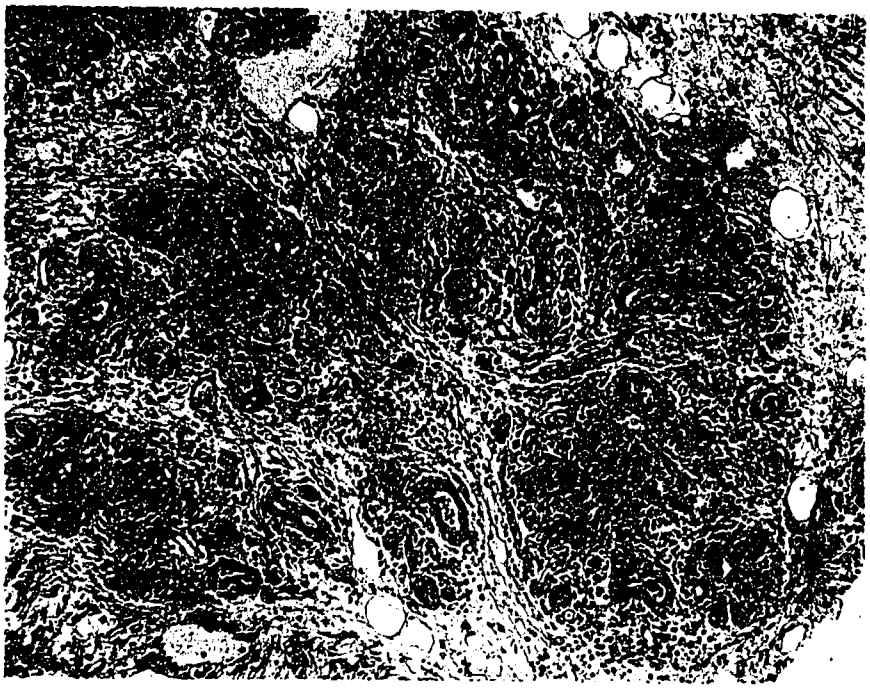
Section B consists of histological section Q29 through to Q77. Histological changes considered of pathological significance were noted in all sections. Perivascular cuffing and vasculitis similar to that noted in sections from section A was evident about the periphery of this section. Throughout the parenchyma changes varied from areas where alveoli were distended with stagnant milk containing polymorphonuclear leucocytes to areas where the predominantly mononuclear cell infiltration consisting of lymphocytes and plasma cells completely obscurred the entire lobular architecture. Plugging of ducts and alveoli with polymorphonuclear leucocytes and epithelial cells was also noted in many areas. The interstitial reaction was either predominantly lymphocytic or predominantly plasma cells depending on the area. In areas where marked destruction of the lobular architecture had taken place by the massive mononuclear cell infiltration the lymphatic vessels were distended and contained many lymphocytes. On occasion mitotic figures were noted in these cells. The lymphatics also contained macrophages which had phagocytized polymorphonuclear leucocytes. Many of the mononuclear cells had prominent eosinophilic nucleoli resembling Reed-Sternberg cells. One such cell is illustrated in Fig. 51 from

Figs. 49 and 50.

Fig. 49. Q26 Udder parenchyma. Architecture of lobule is obscured by reticular formation of epithelial cells infiltrated with polymorphonuclear cells and plasma cells. H & E X 360.

Fig. 50. Q137 Udder parenchyma. Atrophy of alveoli and predominantly mononuclear cell infiltration. H & E X 90.





section Q56. It is noted that the reaction surrounding the lymphatic vessel is predominantly lymphocytic. In some instances lymphatic vessels were thrombosed with fibrin thrombi which often contained pyknotic and karyorrhectic cells. In some foci squamous metaplasia of duct epithelium was evident. In areas where interstitial reaction was marked, mononuclear cells similar to Reed-Sternberg cells as illustrated in Fig. 51 were found in the interstitial tissue. Hyaline droplet degeneration was noted in the alveolar epithelium in areas where alveoli were plugged with milk. Interlobular oedema was also evident in many areas. Fibrinoid necrosis of the fibrous connective tissue elements was noted on occasion in the interstitial septa. In areas where there was marked loss of architecture on occasion fibrinoid necrosis of the adventitia of veins accompanied by a mononuclear cell infiltration or reaction was noted.

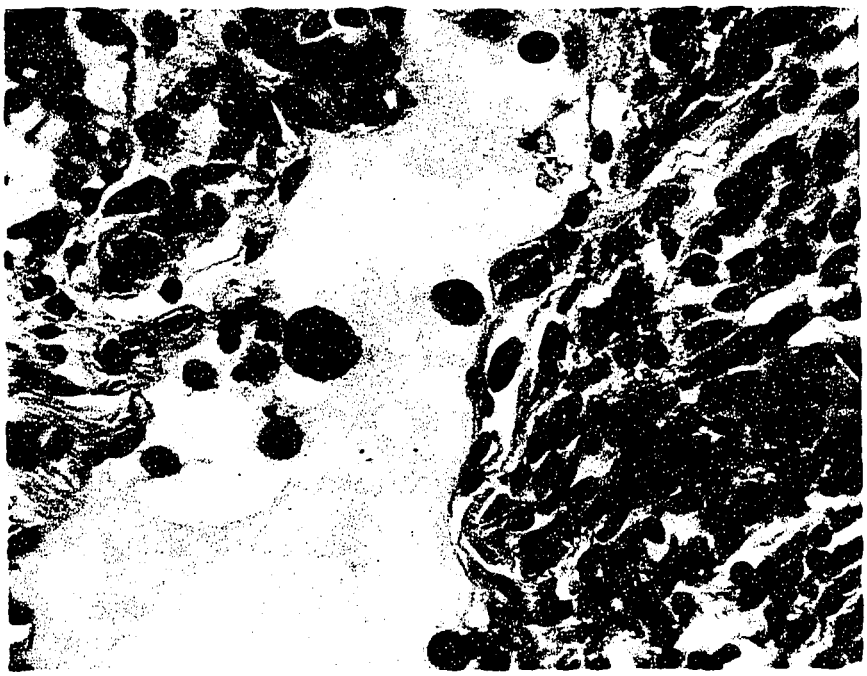
Section C.

Section C consisted of histological sections Q96 through to Q140. As noted in section A the tissue between the two solid lines on the right side of the tracing contained subcutaneous and periglandular tissues. Changes observed in the subcutaneous tissues were essentially similar to those described under section A above. Fig. 45 represents a typical reaction as noted in this section where the perivascular reaction was characterized by the infiltration of lymphocytes and polymorphonuclear leucocytes. Fibrinoid necrosis is evident in the wall of the lymphatic vessel. Subcutaneous oedema was marked. A number of vessels in the subcutaneous tissues and dermis were loosely cuffed with eosinophils. Many lobules consisted of alveoli distended with

Figs. 51 and 52.

Fig. 51. Q56 Lymphatic vessel. Reed-Sternberg-like cell in lymphatic vessel. Reaction surrounding vessel is mainly lymphocytic. H & E X 600.

Fig. 52. Q100 Udder parenchyma. Lymphatic thrombosis. H & E X 180.

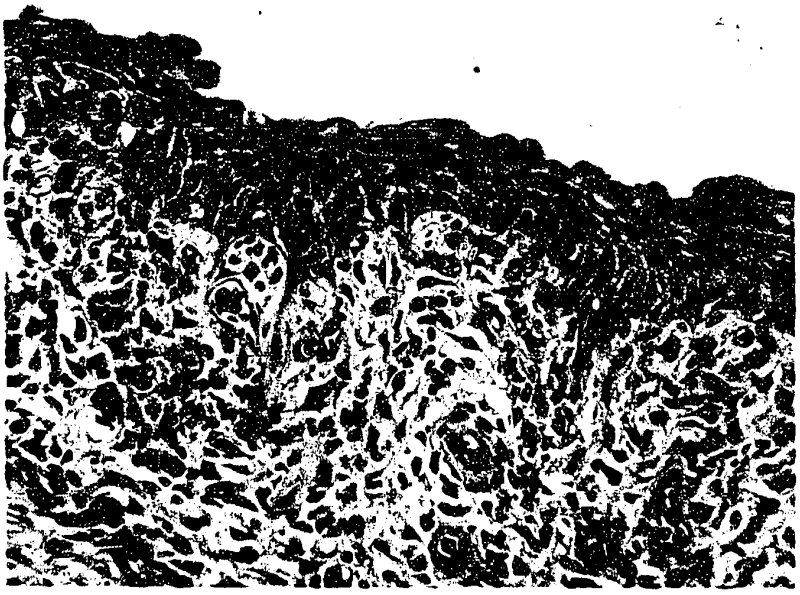


milk containing a few polymorphonuclear leucocytes and epithelial cells. In these lobules the interstitial reaction was minimal. Where interstitial reaction was present alveolar lumina were very small or non-existent. The alveolar cells in these instances formed a small nest surrounded by lymphocytes, plasma cells and a few polymorphonuclear leucocytes. There was an increase in interstitial fibrous connective tissue and neocapillaries with swollen endothelial cells were numerous. Hyperplasia of duct epithelium was noted on occasion. The hyperplastic duct epithelium formed small papilli and contained numerous vacuoles indicating a tendency to secrete. A typical lobule from section C is illustrated in Fig. 50 taken from section Q137. This photomicrograph illustrates a lobule showing marked atrophy of individual alveoli and a heavy mononuclear cell infiltration. Lymphatic thrombosis as illustrated in Fig. 52 from section Q100 was fairly general throughout section C. In some instances the thrombi were composed mainly of fibrin and small numbers of lymphocytes. The number of cells varied greatly however. Lymphatics were often distended with large numbers of lymphocytes and mononuclear cells resembling reticulum cells. Mitotic figures were frequently encountered in lymphatic vessels. Squamous metaplasia was evident in duct epithelium and ducts were plugged with fibrinous clots containing pyknotic and karyorrhectic mononuclear and epithelial cells. The duct epithelium was often heavily infiltrated with mononuclear and polymorphonuclear cells. Such an infiltration of the duct epithelium and lamina propria is illustrated in Fig. 53 from section Q119. There was an increase in fibrous connective tissue elements and neocapillaries in the lamina propria in such

Figs. 53 and 54.

Fig. 53. Q119 Duct. Heavy infiltration of duct epithelium and lamina propria with mononuclear and polymorphonuclear cells. H & E X 360.

Fig. 54. Q119 Gland cistern. Squamous metaplasia with mild polymorphonuclear cell infiltration of the lamina propria. H & E X 360.



instances. Squamous metaplasia also occurred in the epithelium lining the gland cistern. This was usually accompanied by a mild and moderate polymorphonuclear cell infiltration of the lamina propria. A section of the gland cistern is illustrated in the Fig. 54 from section Q119.

Section D.

Section D consists of histological sections Q141 through to Q169. Changes noted in the udder parenchyma of section D were essentially similar to those noted in section C. Thrombosis of lymphatics, plugging of ducts, squamous metaplasia of ducts and gland cistern, marked mononuclear cell infiltrations of individual lobules and atrophy of alveoli were the important findings. In the teat squamous metaplasia of the teat cistern was found to be similar to that seen in the gland cistern and is illustrated in Fig. 55 taken from the section Q161. It is noted that the tissues are infiltrated with both polymorphonuclear and mononuclear cells. The accessory glands of the teat were infiltrated with large numbers of lymphocytes and plasma cells as well as large numbers of blast-type cells. The glandular structure itself was completely masked by the massive infiltration and the architecture destroyed. Eosinophilic infiltration was noted in the subcutaneous tissues of the teat but the squamous epithelium in the streak canal was not affected.

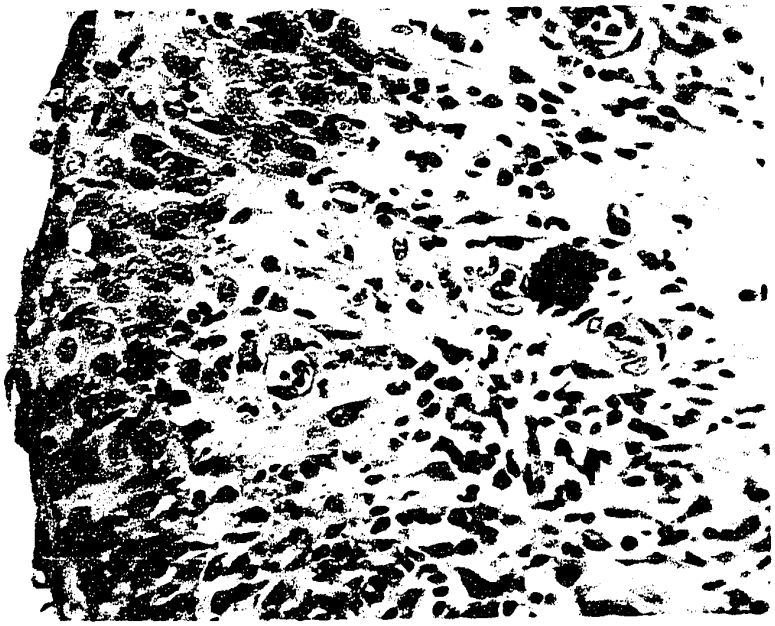
Supramammary Lymph Node

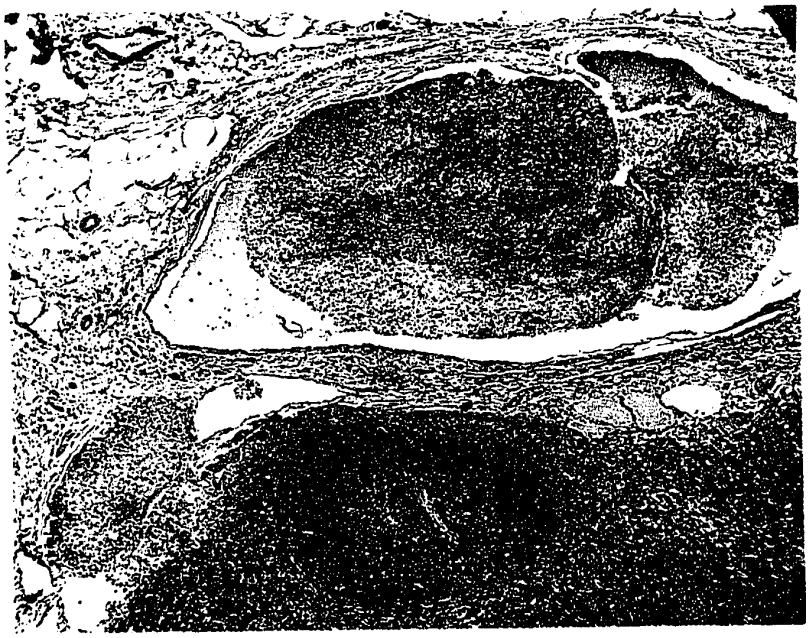
The supramammary lymph node was represented by sections Q78 through to Q95. A striking feature of the supramammary lymph node was the marked lymphatic thrombosis noted in the capsule. This thrombosis was similar to that seen in the parenchyma of the udder and is illustrated in Fig. 56 from section Q79. The cortical and paratrabecular sinuses were filled

Figs. 55 and 56.

Fig. 55. Q161 Teat. Squamous metaplasia of teat epithelium with infiltration by polymorphonuclear and mononuclear cells. H & E X 360.

Fig. 56. Q79 Capsule of supramammary lymph node. Lymphatic thrombosis. H & E X 54.





with large numbers of plasma cells and blast-type cells. Mitotic figures were observed fairly frequently. The odd polymorphonuclear leucocyte was also evident.

Cow Q Left Rear Quarter (control quarter)

Some lobules throughout this gland contained alveoli distended with milk and a few polymorphonuclear leucocytes. The same type of cell was also present in the cortical and paratrabecular sinuses of the left supramammary lymph node. Necrotic or inflammatory changes were not noted in the epithelium of alveoli or ducts.

Cow N Right Rear Quarter (inoculated with BAE virus and killed at nine days).

Section A.

Section A consists of histological sections N1 through to and including N54. Four sections contained relatively normal tissue. A number of lobules throughout this level had an interstitial infiltration of both polymorphonuclear leucocytes and mononuclear cells while the epithelium of both alveoli and ducts appeared normal. In some foci interstitial reaction was marked with large numbers of lymphocytes and plasma cells being present. Necrosis of alveolar epithelium was noted in only one area. Retention of milk was evident in some areas of interstitial reaction but was not a prominent feature. Hyperplasia of lymph follicles was noted on occasion.

Section B.

Section B consists of histological sections N70 through to and including section N132. No changes considered of pathological significance were found in 11 sections. In general changes noted throughout

this section were similar to those noted in section A with a mild to heavy interstitial infiltration of lymphocytes, plasma cells and polymorphonuclear cells. Heavy infiltration of duct walls with lymphocytes, plasma cells and a few polymorphonuclear leucocytes as illustrated in Fig. 57 from section N105 was a frequent finding. It is noted that the epithelium is intact and relatively normal in appearance. Foci of necrosis were seen in only three sections.

Section C.

Section C consists of histological sections C133 to C175. Changes considered of pathological significance were found in all sections. Heavy infiltrations were again seen in the walls of ducts. Squamous metaplasia of duct epithelium was commonly encountered. Fig. 58 illustrates a duct wall where a portion of epithelium which has undergone squamous metaplasia is sloughing and being replaced by a more normal type of epithelium. Interstitial infiltrations as noted in section B were general throughout this level and on the whole tended to be more severe. Fig. 59 illustrates a lobule from section N168 showing almost complete atrophy of the alveoli and with only the ducts remaining. The interstitial reaction is both polymorphonuclear and mononuclear in type. Atrophy of the alveoli is not as marked in Fig. 60 where a lobule from section N143 is illustrated. Here the infiltrating cells are predominantly plasma cells.

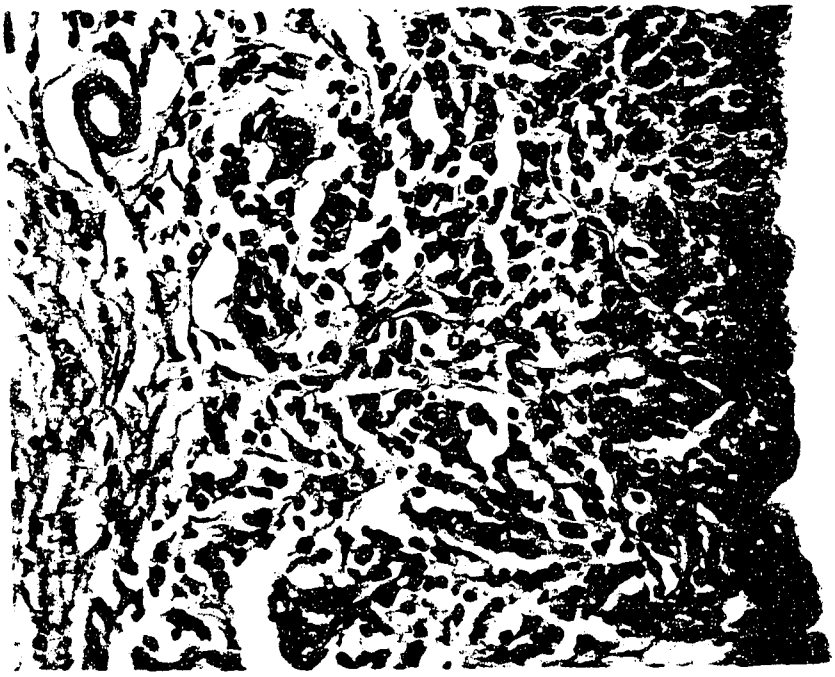
Section D.

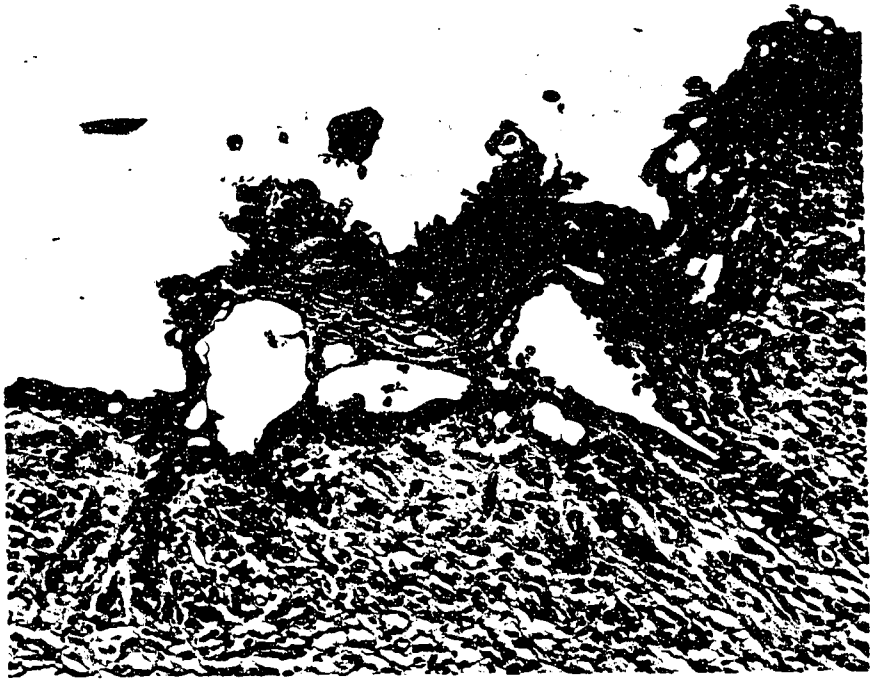
Section D consists of histological sections N176 through to N204. Changes of pathological significance were observed in all sections with the exception of N203 and N204 which were taken through the streak canal

Figs. 57 and 58.

Fig. 57. N105 Duct. Infiltration of lamina propria of duct with lymphocytes, plasma cells and a few polymorphonuclear cells. H & E X 360.

Fig. 58. N156 Duct. Sloughing of squamous epithelium and replacement with more normal type of epithelium. H & E X 180.

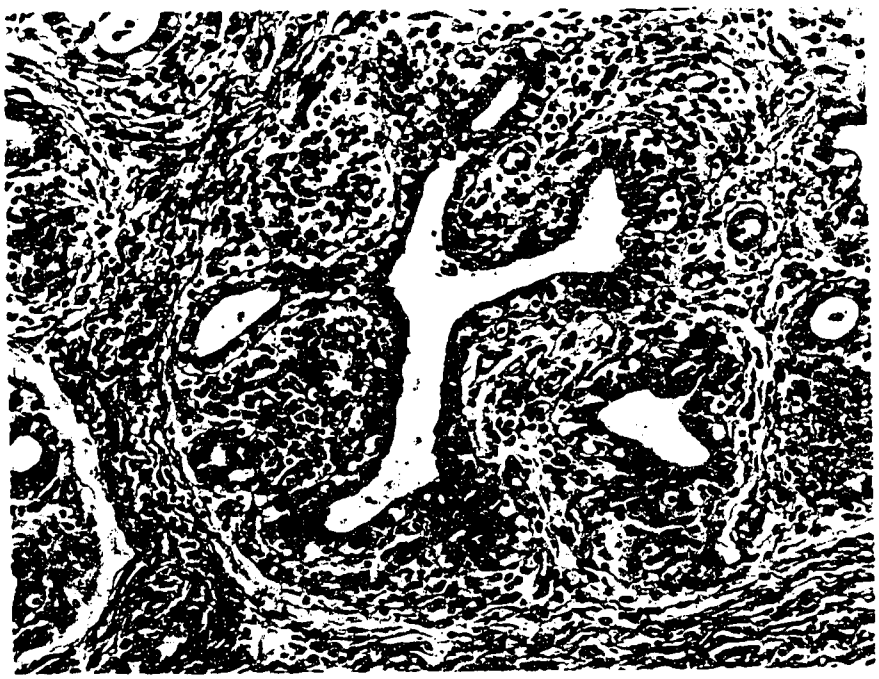


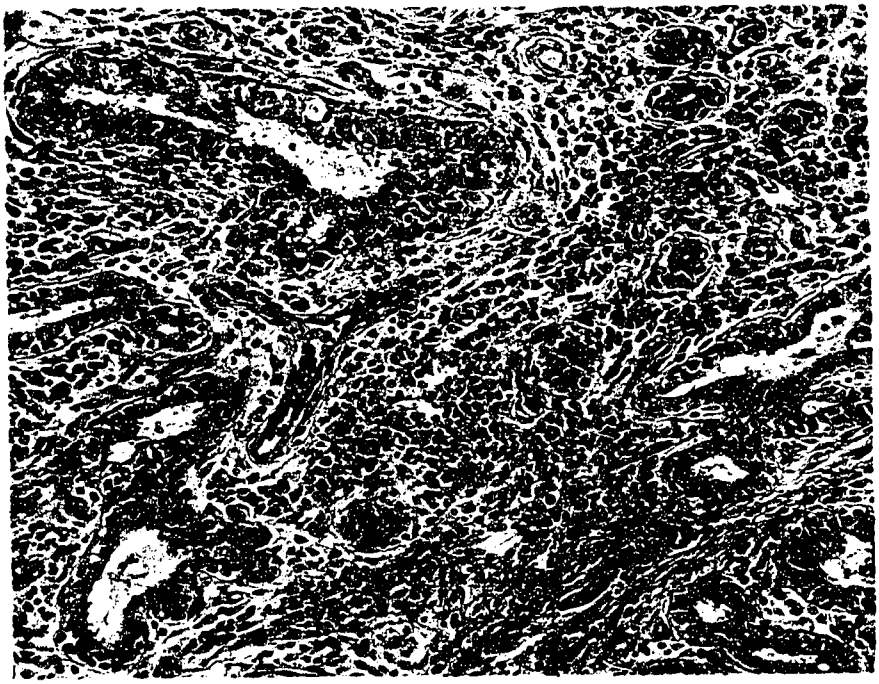


Figs. 59 and 60.

Fig. 59. M168 Udder parenchyma. Almost complete atrophy of alveoli with ducts remaining. Polymorphonuclear and mononuclear cell infiltration. H & E X 180.

Fig. 60. M143. Udder parenchyma. Atrophy of alveoli and interstitial reaction consisting mainly of plasma cells. H & E X 180.





of the teat. Changes noted in this level were essentially similar to those noted in section C with the interstitial infiltration of lymphocytes, plasma cells and polymorphonuclear leucocytes and atrophy of alveoli being the most characteristic changes. Heavy infiltration of the lamina propria of ducts and the gland cistern was present. The heavy infiltration of the gland cistern is illustrated in Fig. 61 from section N190. The cells are both lymphocytes and polymorphonuclear cells. In instances, sloughing of hyperplastic duct epithelium and replacement with a more normal type was seen. Eosinophilic infiltration was evident in the subcutaneous tissues but oedema as observed in cows P and Q was absent. Mononuclear cell infiltration of the lamina propria of the teat cistern was marked. The normal architecture of the accessory glands was completely obscured by heavy infiltrations of lymphocytes, plasma cells and polymorphonuclear cells. Squamous metaplasia of the teat cistern as illustrated in Fig. 62 was marked and often extended into the ducts of accessory glands. In many instances this epithelium was keratinized.

Right Supramammary Lymph Node.

The right supramammary lymph node was represented in histological sections N55 through to N69. The cortical and paratrabecular sinuses contained numerous plasma cells, macrophages, and large mononuclear blast-like cells. Numerous mitotic figures were noted in the geminal centres.

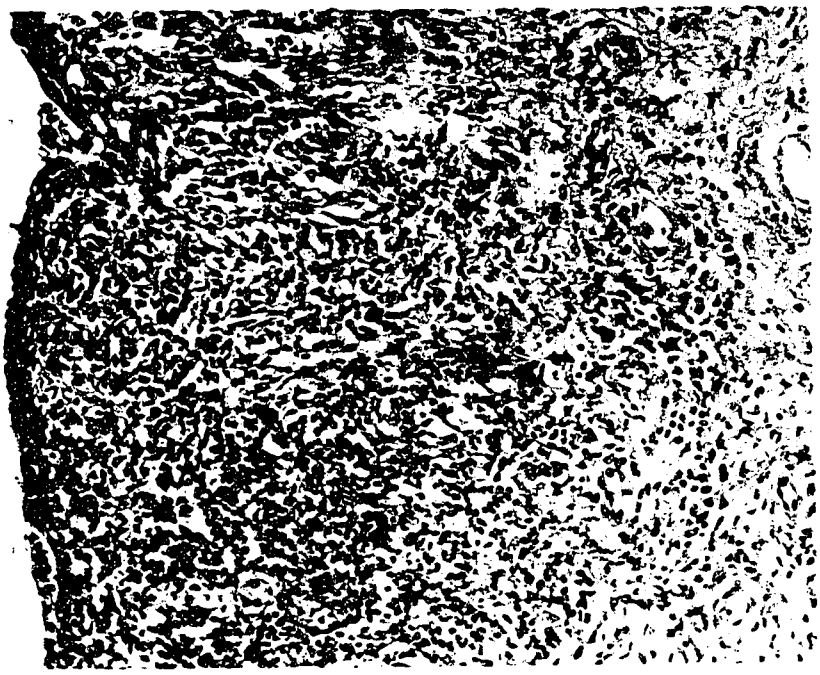
Cow N Left Rear Quarter (control quarter)

Histological examination of all levels of the left rear quarter failed to reveal lesions of pathological significance.

Figs. 61 and 62.

Fig. 61. M190 Gland cistern. Heavy lymphocytic and polymorphonuclear infiltration of lamina propria. H & E X 180.

Fig. 62. M196 Teat. Squamous metaplasia of epithelium of teat cistern extending into duct of accessory gland. H & E X 90.



DISCUSSION

The results obtained in experiments 1, 1A and 1B indicate that the virus of lapinized rinderpest failed to propagate in the lactating bovine mammary gland in spite of the fact that it was not affected by any inhibitory factor in fresh cows' milk nor was it adversely affected by being frozen in cows' milk. The lapinized rinderpest virus used in these studies was adapted to the rabbit by Nakamura, Wagatuma and Fukusho (1938) by the subcutaneous inoculation of 0.5 ml. of virulent bovine blood per 1.5 to 2.0 kilograms of rabbit weight. These workers did not observe the typical thermal reaction nor the necrotic lesions produced in the lymphatic tissues upon the initial injection of bovine rinderpest virus. They found however, that these features became more marked and definite with each successive rabbit passage. In addition to the increasing pathogenicity of the rinderpest virus for the rabbit during lapinization they noticed a decrease in virulence for cattle. At the present time this strain of virus produces nothing more than a slight temperature rise in cattle. It is possible that this particular strain of virus has become so rabbit-adapted that it is unable to infect cattle via the mammary gland. The use of cows late in their lactation period, giving a relatively small quantity of milk and milking only once a day conceivably might produce different results as the dilution of the inoculum would not be nearly as great and the time of contact with susceptible cells would be prolonged. It is suggested that this experiment be repeated using cows late in lactation.

In experiment II, three attempts to propagate influenza A PR8 virus in the lactating bovine mammary gland met with failure. Mitchell, Walker and Bannister (1953 a and b) reported on the propagation of influenza A PR8 virus in the bovine mammary gland. These workers however, used an animal late in lactation, producing a relatively small amount of milk and milking was carried out only once a day. It may well be that in selecting animals producing a large volume of milk in early lactation and milking twice daily the inoculated virus is diluted too much or flushed-out. Cook, Francis and Kendrick (1961) reported on the interaction between the influenza virus and staphylococci in the chick embryo. In their studies the virus alone produced 35 per cent embryo mortality, staphylococci alone produced 5 per cent mortality while a combination of the two produced 76 per cent mortality. With this in mind, cow P was inoculated with a combination of staphylococci and the influenza virus in an attempt to establish infection in the udder. It was not successful.

In experiment III the virus of infectious bovine rhinotracheitis, a virus of host range, propagated and produced mastitis in the lactating bovine mammary gland. In general the pathology produced by this virus in the mammary gland was similar to that produced in other tissues by the same agent. The basic lesion observed in the gland resulted from the necrotizing effect of the virus upon the epithelial cells of the ducts and alveoli accompanied by polymorphonuclear cell infiltration of the necrotic focus and followed by the accumulation of mononuclear cells. The lower sections of the mammary gland bore the brunt of the onslaught. The more dorsal the section or the farther away from the

site of inoculation, the less pathology was noted. In the dorsal portions of the udder the alveoli were distended with milk and infiltrated with polymorphonuclear leucocytes as a result of the plugging of ducts at lower levels with necrotic cellular debris.

In cow G (experiment III) which was killed three days following inoculation, eosinophilic intranuclear inclusion bodies were found in sections C and D and indefinite inclusion bodies were noted in section B. Small numbers of inclusion bodies were found in sections C and D of cow H. This would indicate that the infection was still spreading within the gland at six days. Crandell, Cheatham and Maurer (1959) using three strains of IBR virus were able to demonstrate inclusion bodies only between 36 and 60 hours post-infection in the upper respiratory tract of calves while Jubb and Kennedy (1963) state that the inclusion bodies of IBR are a transitory phenomenon and may not be present after the second or third day of infection. Kendrick, Gillespie and McEntee (1958) found small numbers of IBR inclusions present at the periphery of the lesions in the vagina at three days. Cows H and J showed a biphasic temperature response, the first peak being reached at four days, dropping on the fifth day and showing a second peak on the sixth day. This biphasic temperature curve is a common feature of many virus diseases and as the temperature peaks coincide with increased virus propagation it is perhaps logical that the inclusion bodies were evident in cow H at six days. It would seem therefore that the finding of these inclusion bodies at six days post-infection is somewhat unusual and may result from the fact that the virus was trapped in the ducts and alveoli as a result of the plugging of ducts with necrotic

cellular debris. The plugging of ducts reached its peak in cow H. Necrosis, while present in cow G at three days, was much more marked and extensive in cow H at six days. Resolution was evident in cow J at nine days. One may conclude that virus multiplication had taken place in the alveolar and duct epithelial cells especially in the lower one third of the mammary gland. The infection probably existed for a short period of time as a thelitis, progressed to a galactophoritis and quickly spread to the alveoli producing a full blown mastitis which could be classified according to Halmboldt, Jungherr and Plastridge (1953) as acute mastitis grade III. If specimens from field cases of mastitis showed similar lesions one would be led to the diagnosis of a viral mastitis in cows G and H by the presence of inclusion bodies and the absence of bacteria in the sections. In cow J it would be rather difficult to come to a diagnosis of viral mastitis because inclusion bodies were not demonstrated. The large number of mononuclear cells present might lead one to suspect a coliform etiology because of the shift to a predominance of agranulocytes often associated with this type of mastitis.

Of particular interest is the occurrence of the large reactive cell found in the supramammary lymph node of cow H and in the supramammary lymph node and udder tissue of cow J. This blast-type cell resembles the Reed-Sternberg cell described in Hodgkin's disease of man and to the writers knowledge it has been described only once in the bovine by Smith (1965) who reported on the rare occurrence of multinucleated Reed-Sternberg cells in lymphosarcoma. None of the cells observed in this study were multinucleated but their large size, large

vesicular nucleus and large nucleous and abundant cytoplasm make them a very distinctive cell.

In experiment IV the virus of enzootic abortion of ewes, a virus not of host range, propagated and produced a mastitis in the lactating bovine mammary gland. The mastitis produced by this virus was essentially similar to that produced in experiment III by IBR virus. The basic lesion in each instance was the necrosis of alveolar and duct epithelial cells. The mastitis produced by EAE virus was however, much more severe and would be classified as acute mastitis grade III. In the IBR mastitis milk production gradually declined reaching its lowest point at about eight days post-infection whereas with the EAE mastitis the drop in milk production was precipitous reaching a low at three days post-infection. The physical changes in the milk were more marked in the EAE mastitis. The demonstration of clusters of virus particles in section A of cow P and the fact that nearly all areas of section B showed pathological changes illustrated the extensive spread of the infection throughout the udder in only three days. Changes such as the swelling of endothelial cells of blood vessels, fibrinoid necrosis of vessel walls and connective tissue elements together with the marked outpouring of fibrin from areas of necrosis were much more severe in the EAE mastitis. While virus was demonstrated in the milk, by microscopic examination of the milk and chick embryo inoculation, evidence of its propagation in the tissues was not demonstrated in cows Q and N. In cow Q the necrosis and reaction was severe in all levels of the udder. The necrosis, inflammatory infiltration and oedema were more severe than in cow K with IBR mastitis. The thrombosis of lymphatic vessels was marked and extended into the capsule of the supramammary lymph node.

Blast-type cells resembling Reed-Sternberg cells and similar to those found in cows E and J were present in the udder and lymph node of cow Q. Similar cells were found in the lymph node from cow N. Urman, Underdahl, Aiken, Stair and Young (1962) described the presence of intranuclear inclusion bodies in macrophages of the reticuloendothelial system produced by the virus of hog cholera between five and nine days post-infection yet the staining of similar tissues with fluorescent antibody failed to reveal the presence of nuclear fluorescence. This, together with the fact that they were Feulgen negative, would seem to indicate that the type of inclusion described by Urman et al is not specific. The blast-type cells seen in cows E, J, Q and N resemble the cells which were described by Urman et al in hog cholera. It may be that virus infections in general might elicit the development of such a cell. This would make an interesting project for future investigation.

The inflammatory response in cow Q was predominantly mononuclear and here again one would conceivably have difficulty in distinguishing the pathology produced by EAE virus in this cow and in cow N from that produced in coliform mastitis. The finding of clusters similar to those in cow P would lead one to think of a virus infection caused by a member of the psittacosis-lymphogranuloma group. The examination of Machiavello stained smears of milk sediment would be of value if such an infection was suspected.

From the results obtained in experiments III and IV it is suggested that the epithelial cells derived from the bovine udder might be useful in the tissue culture propagation of certain viruses.

SUMMARY

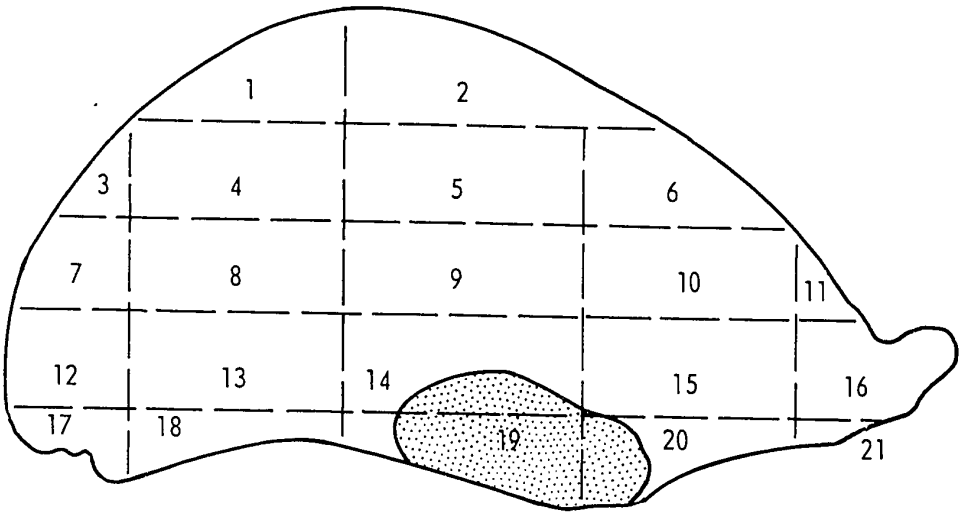
Unsuccessful attempts to produce mastitis and to propagate the virus of lapinized rinderpest and influenza A PR8 in the lactating bovine mammary gland are described. The viruses of IBR, a virus of host range, and of EAE, a virus not of host range, propagated and produced mastitis in the lactating bovine mammary gland. The basic lesion of the mastitis produced by both of these viruses resulted from the necrotizing effect of the virus upon the epithelial cells of the alveoli and ducts. The mastitis produced by EAE virus was more severe than that produced by IBR. In certain stages the lesions produced by both IBR and EAE viruses in the udder could be confused with those produced by coliform bacteria. Of interest was the appearance of a large reactive cell, resembling a Reed-Sternberg cell, in the udders and supramammary lymph nodes of cows killed at three and six days post-infection with both IBR and EAE viruses.

APPENDIX I

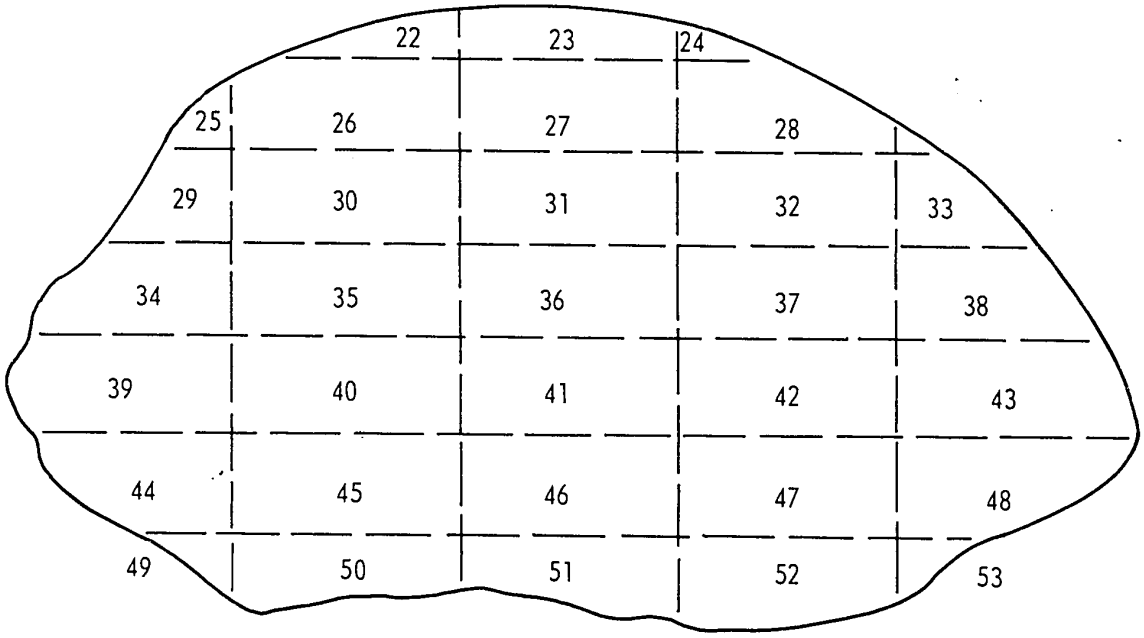
This appendix contains tracings of whole cross sections A, B, C, and D of the right rear quarters of cows A, B, C, G, H, J, P, Q and N. Not included are similar tracings from the left rear (control) quarters of the same animals. Consecutive numbering was used to identify sections from these control quarters as well. A total of 90 sections were examined from cows A, B and C, 555 from cows G, H and J and 820 from cows, P, Q and N, making a total of 1465 sections. Additional sections were cut and stained using different staining techniques as noted in the text.

COW A RIGHT REAR QUARTER

SECTION A

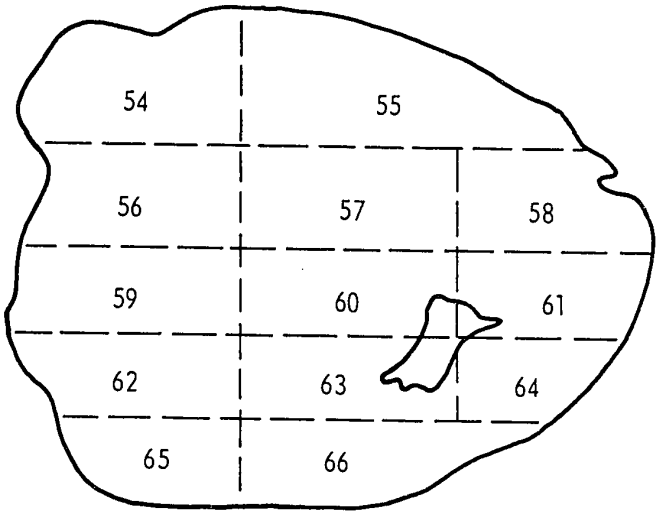


SECTION B

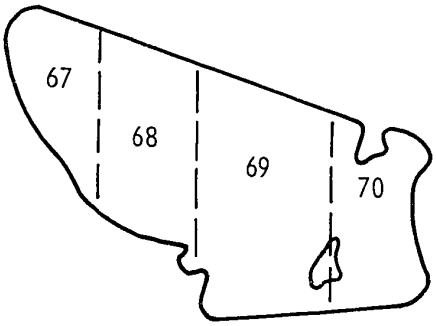


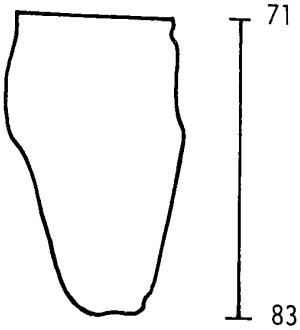
COW A RIGHT REAR QUARTER

SECTION C



SECTION D





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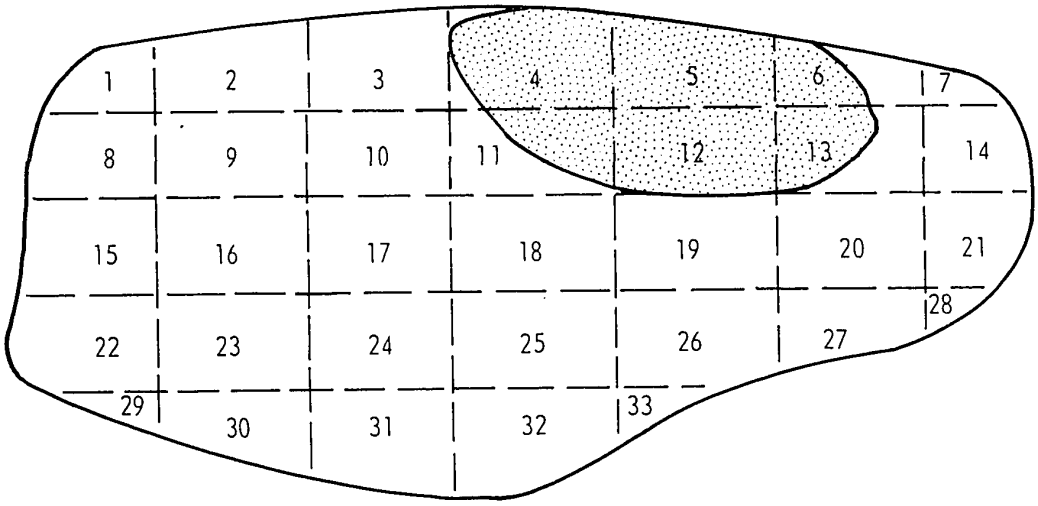

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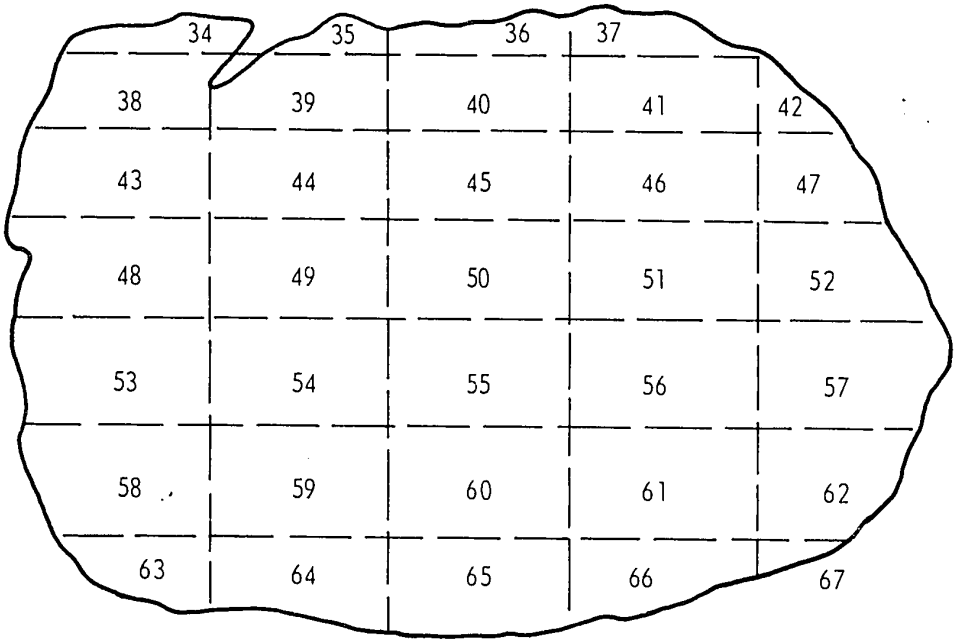

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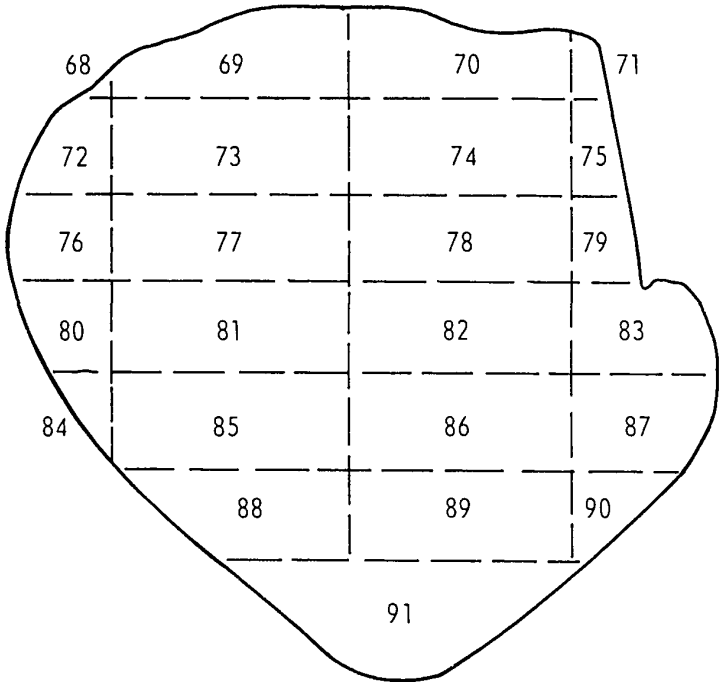

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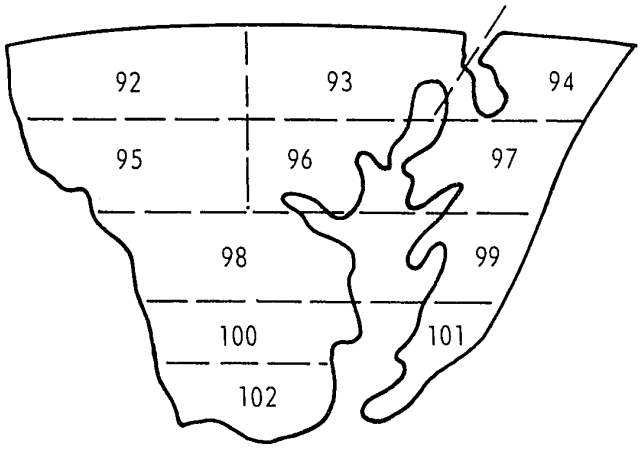

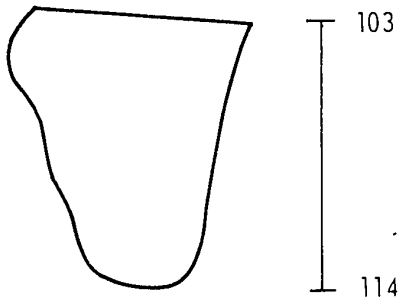

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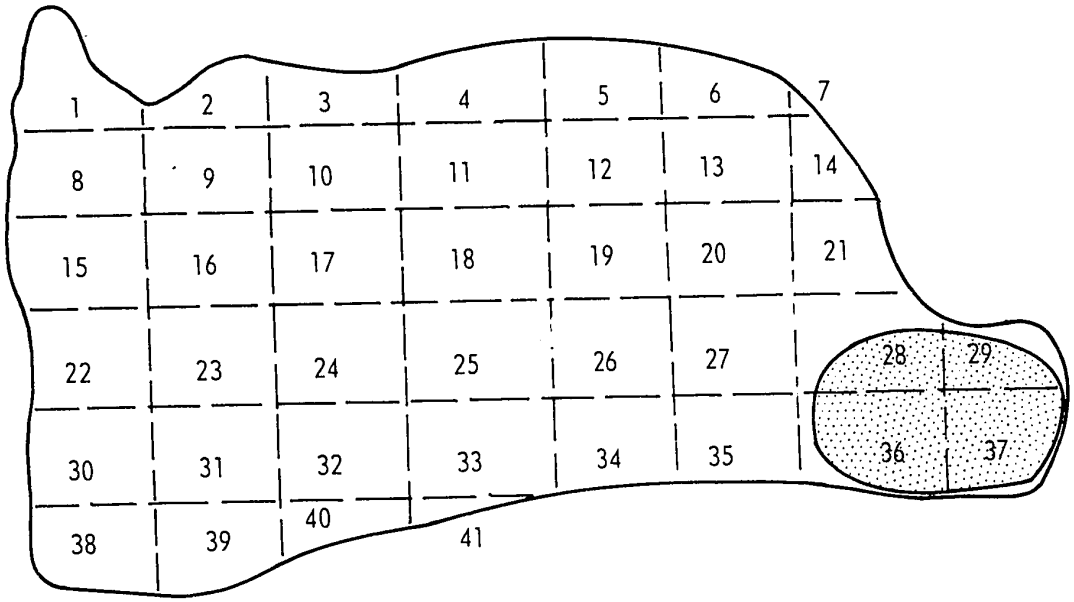

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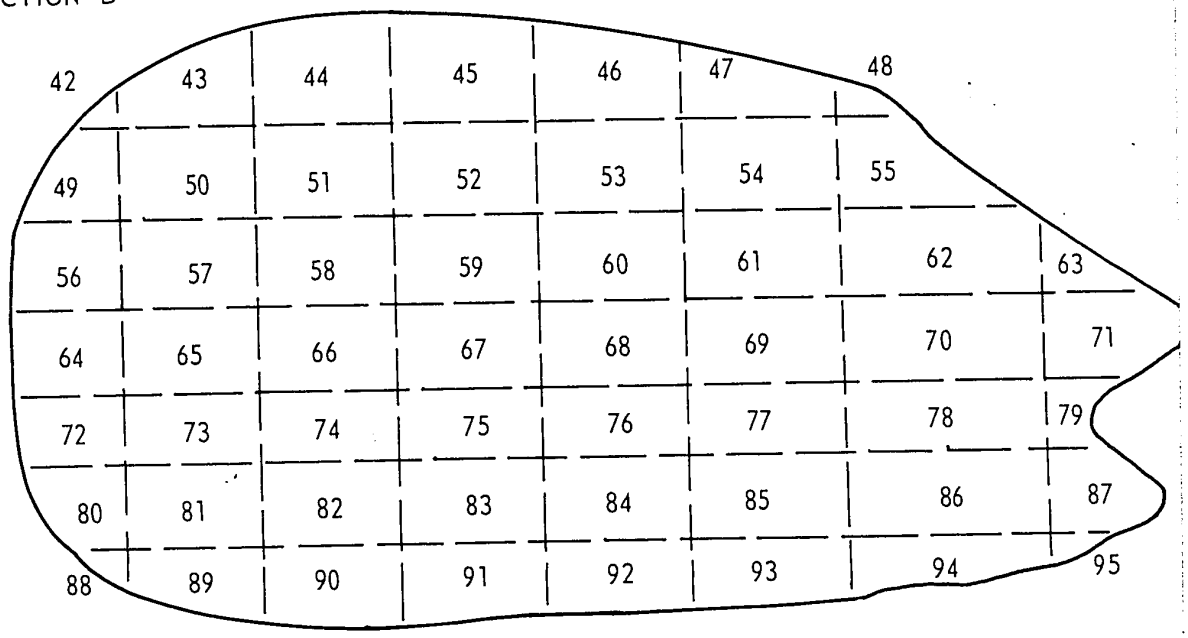

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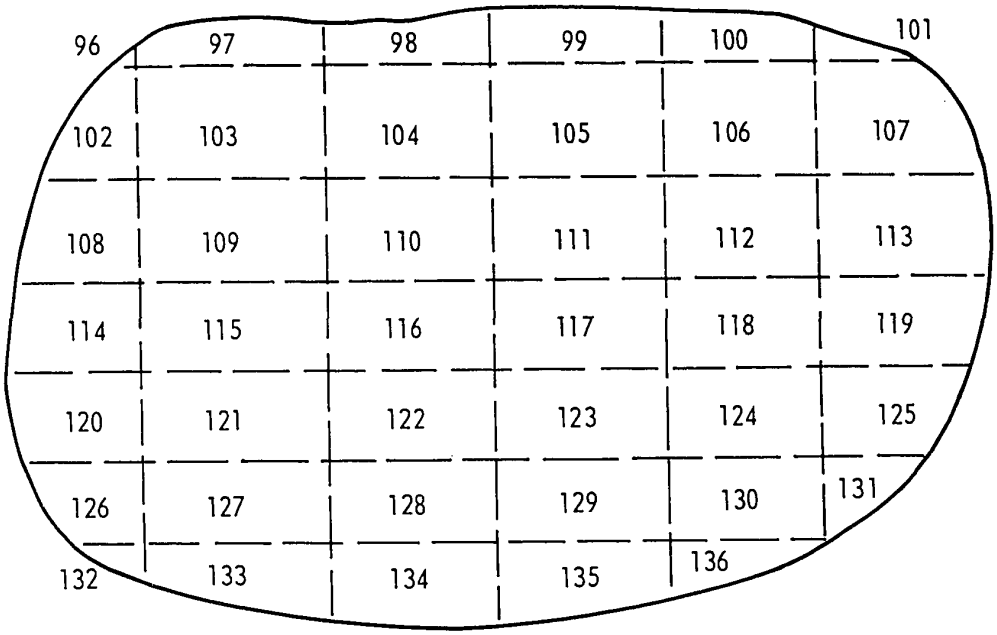

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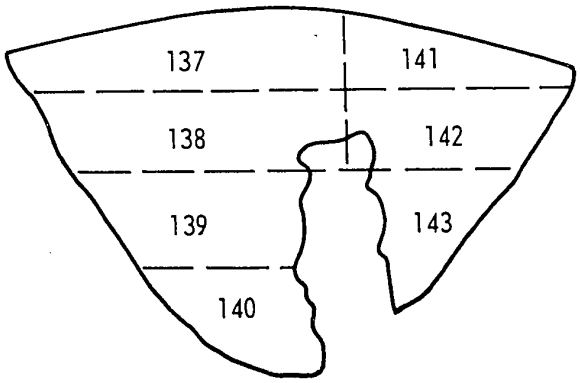

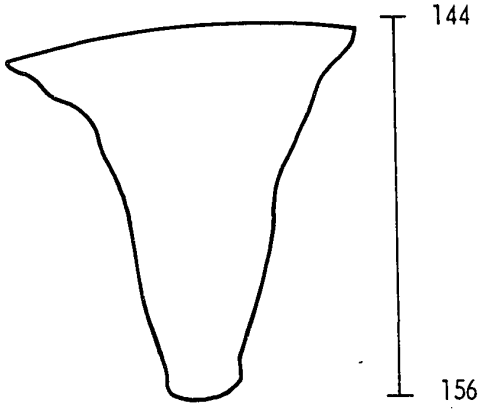

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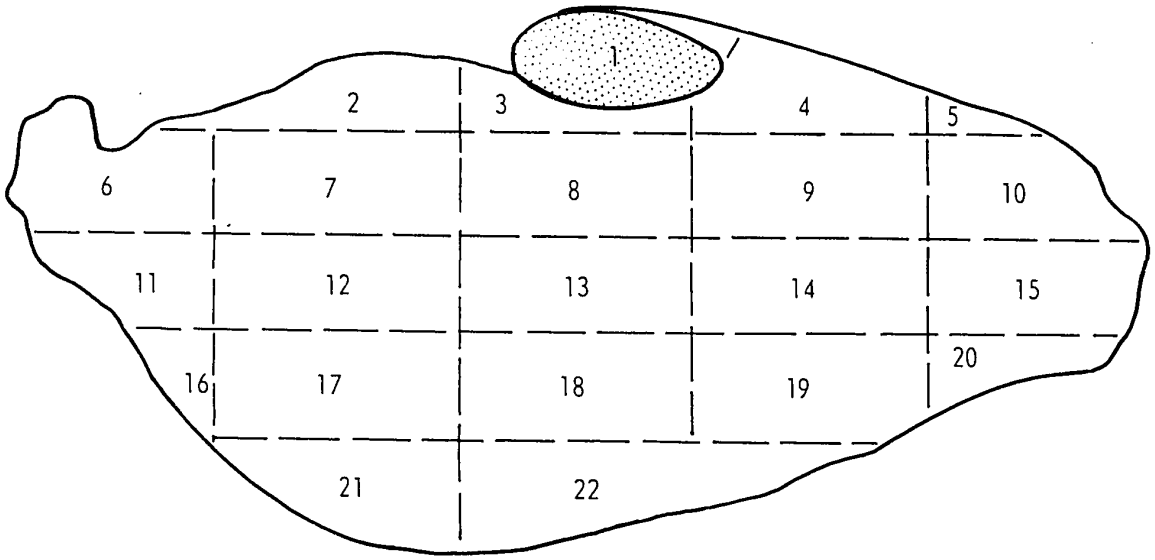

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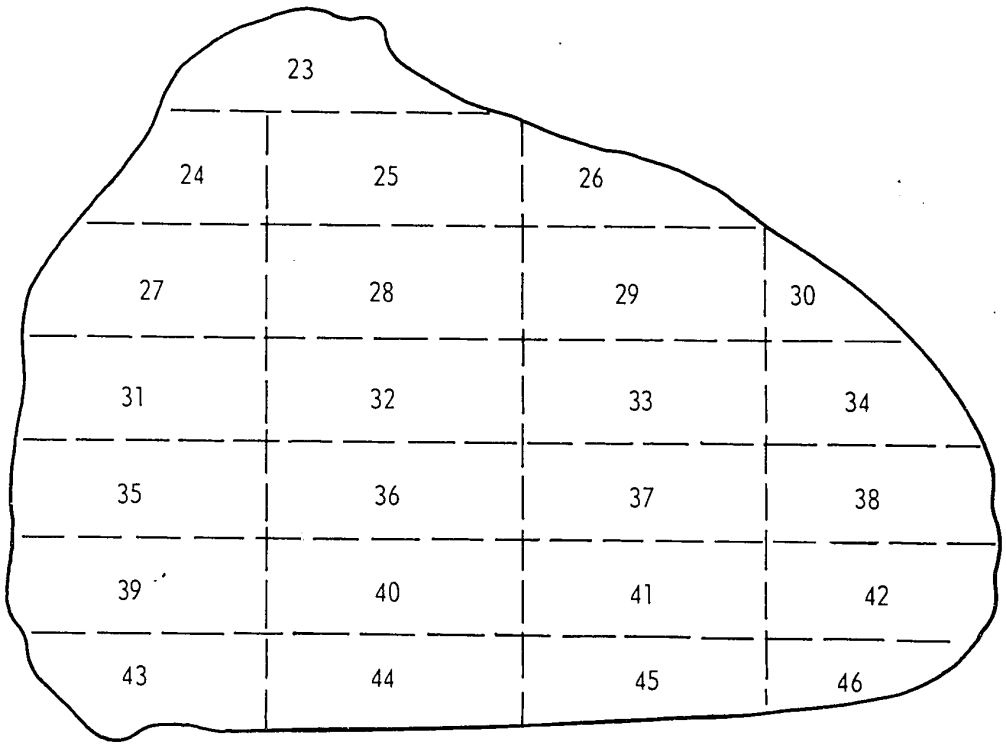

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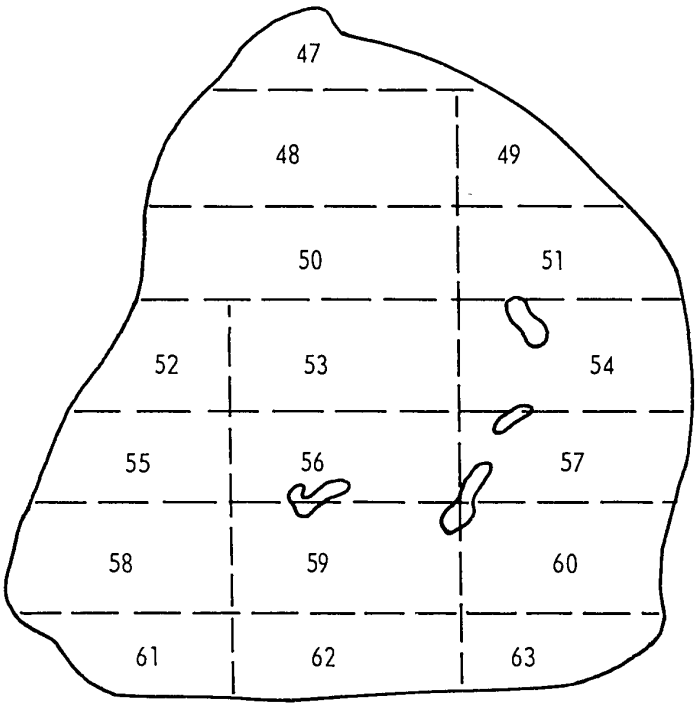

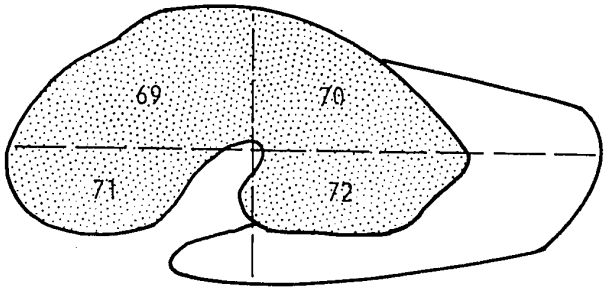

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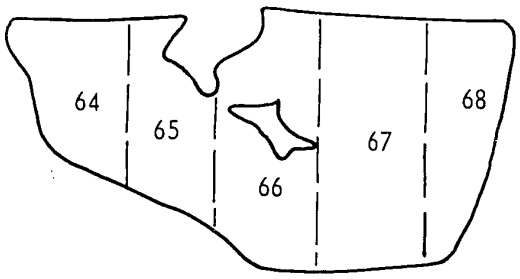

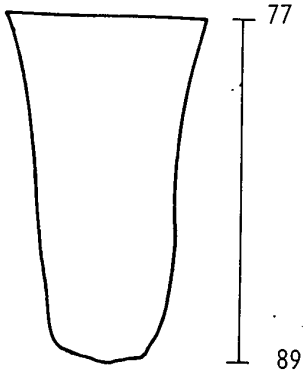

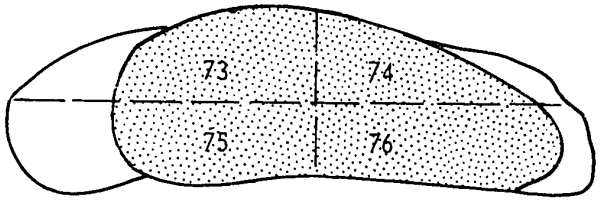

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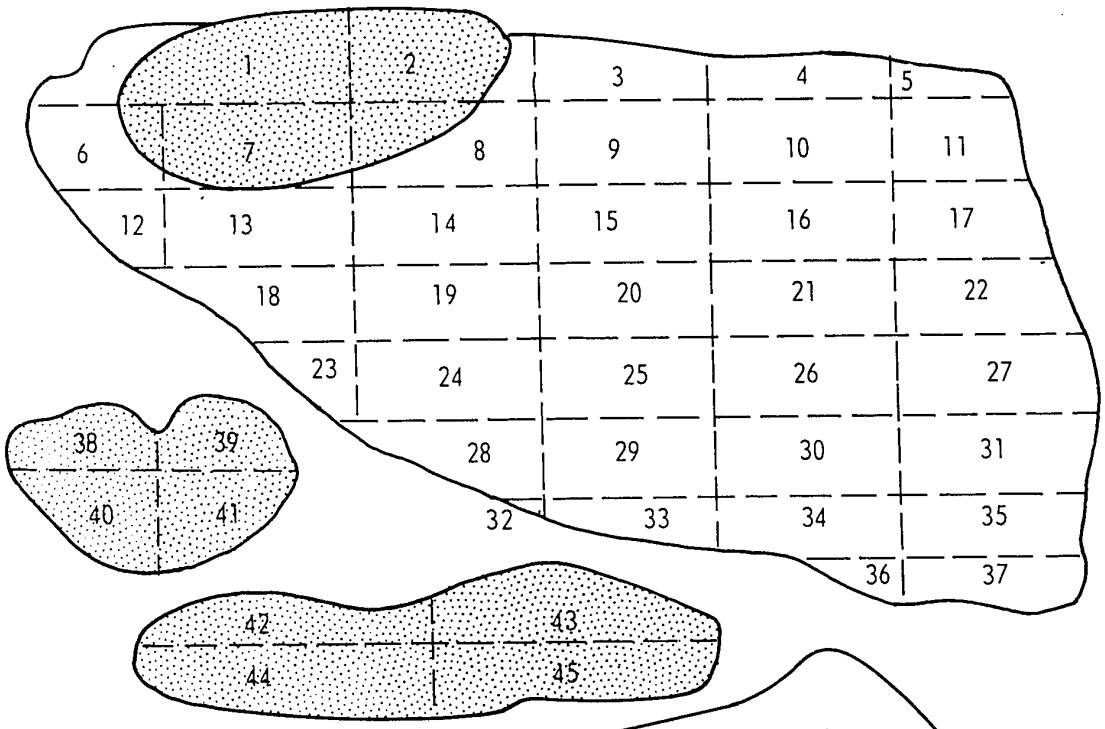

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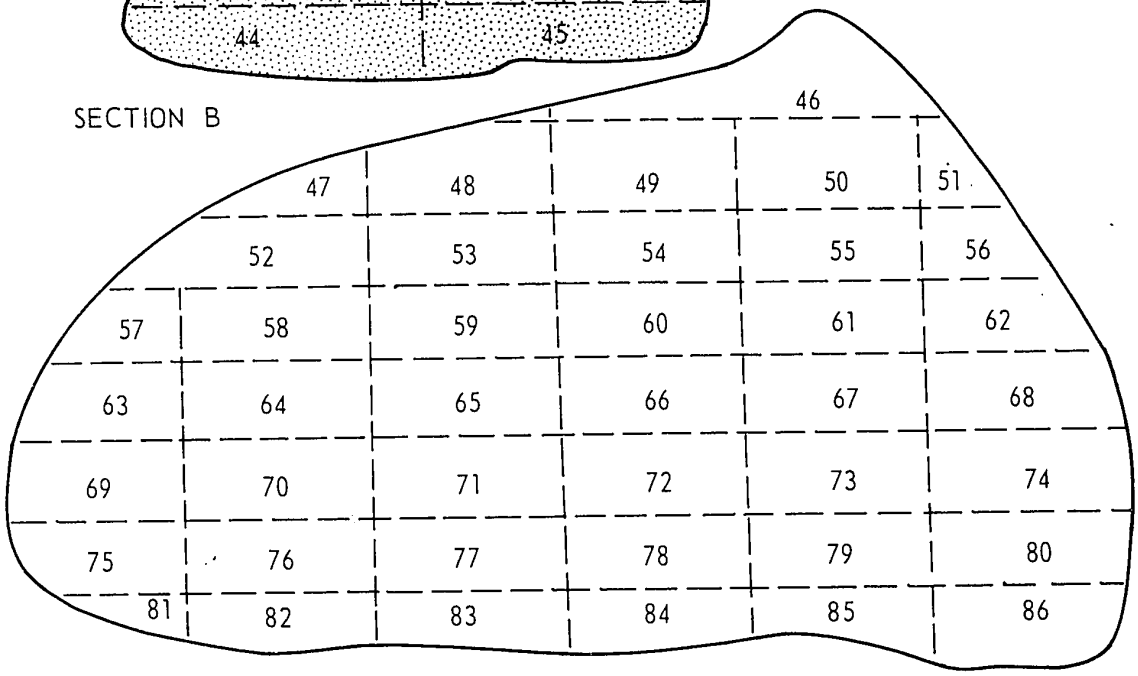

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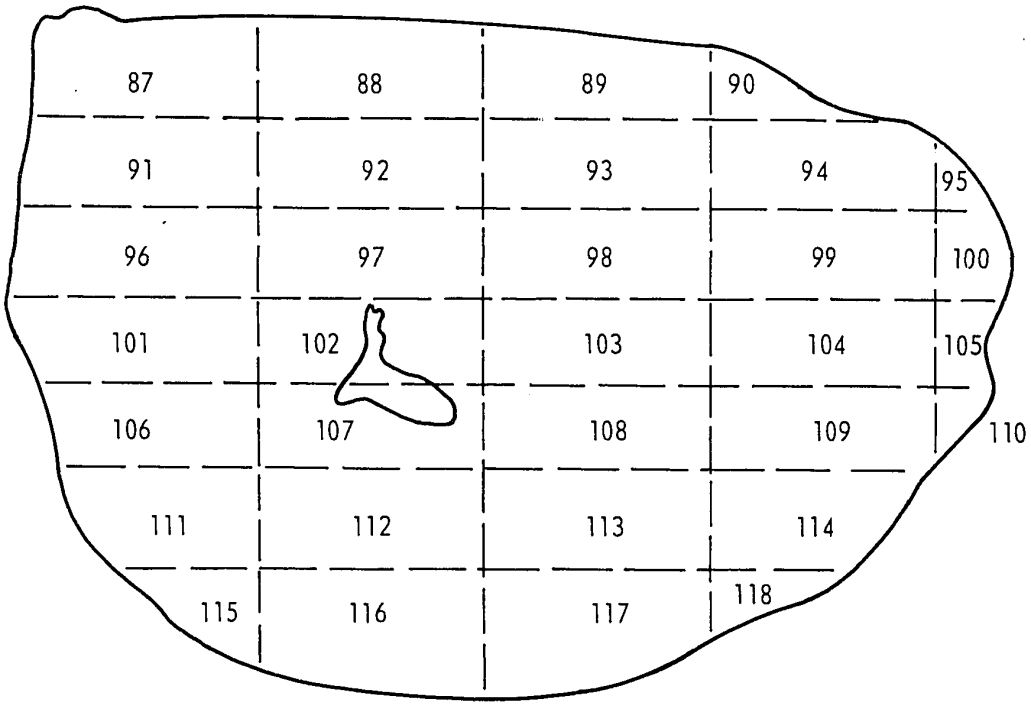

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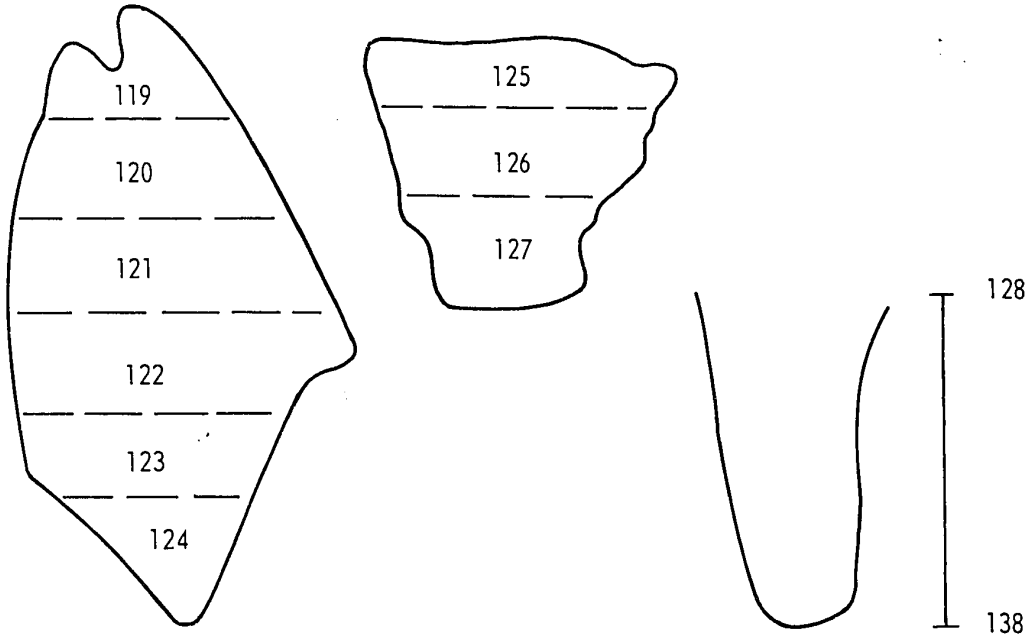

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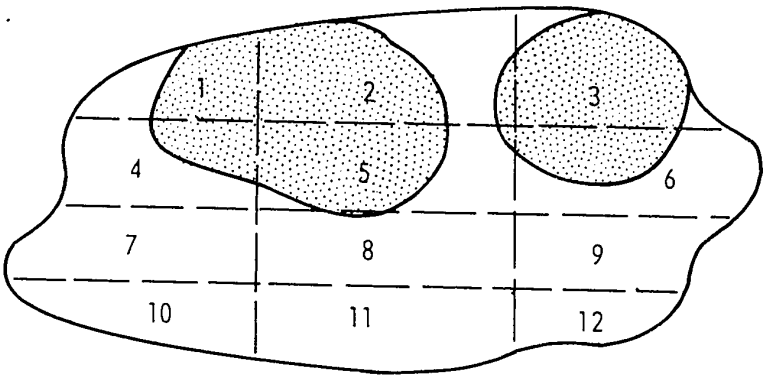

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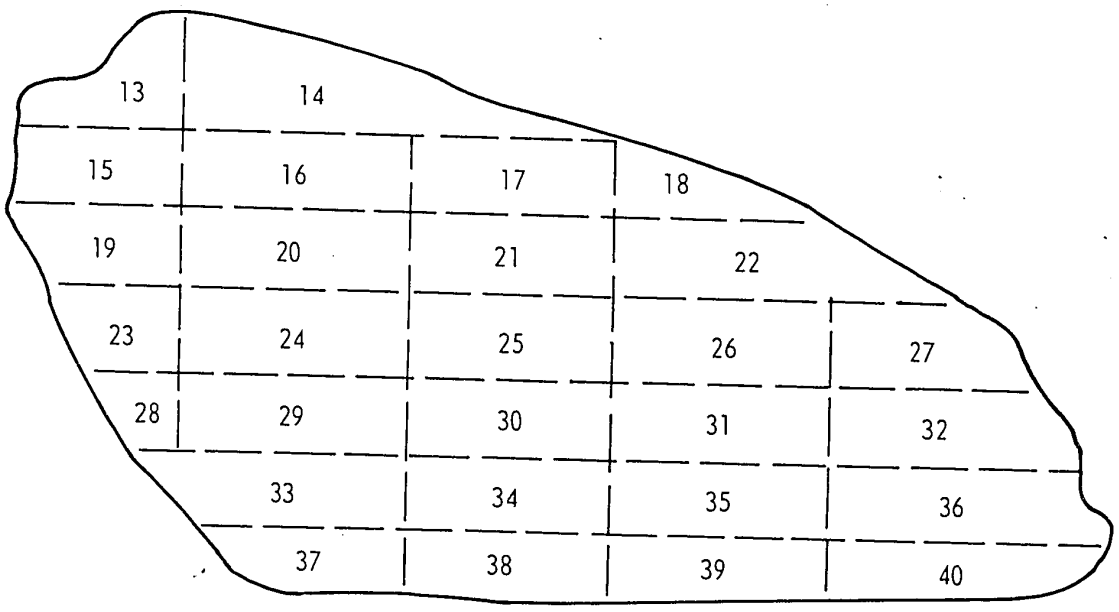

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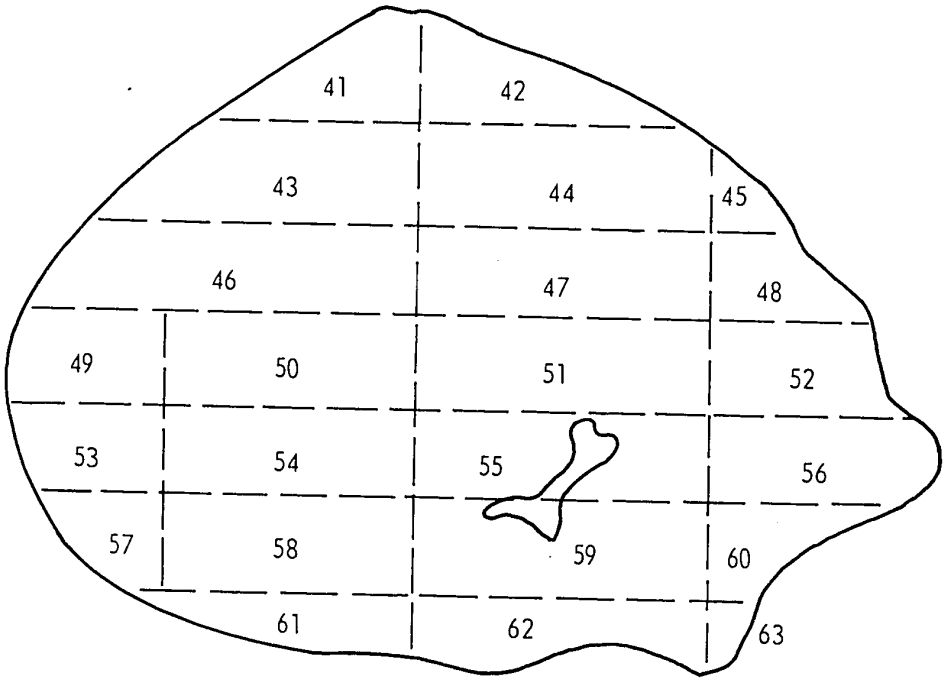

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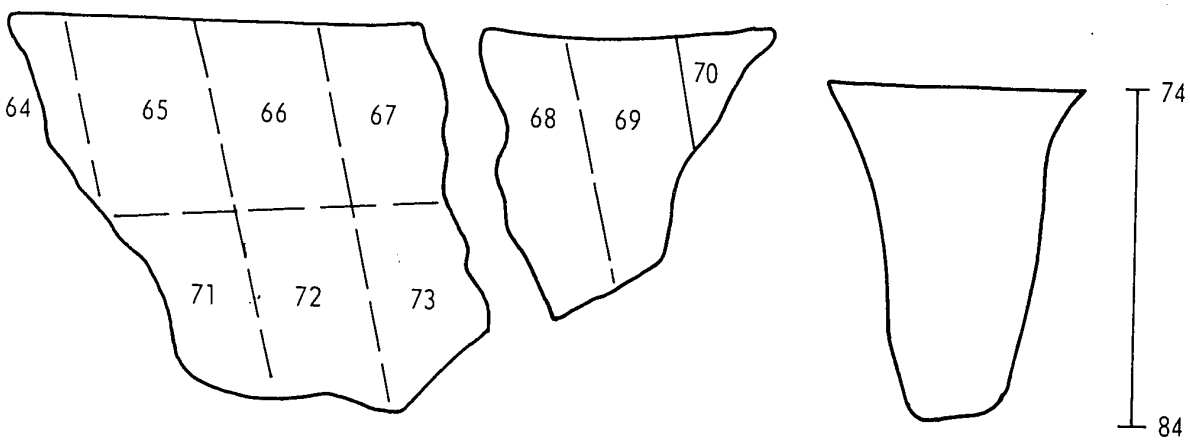

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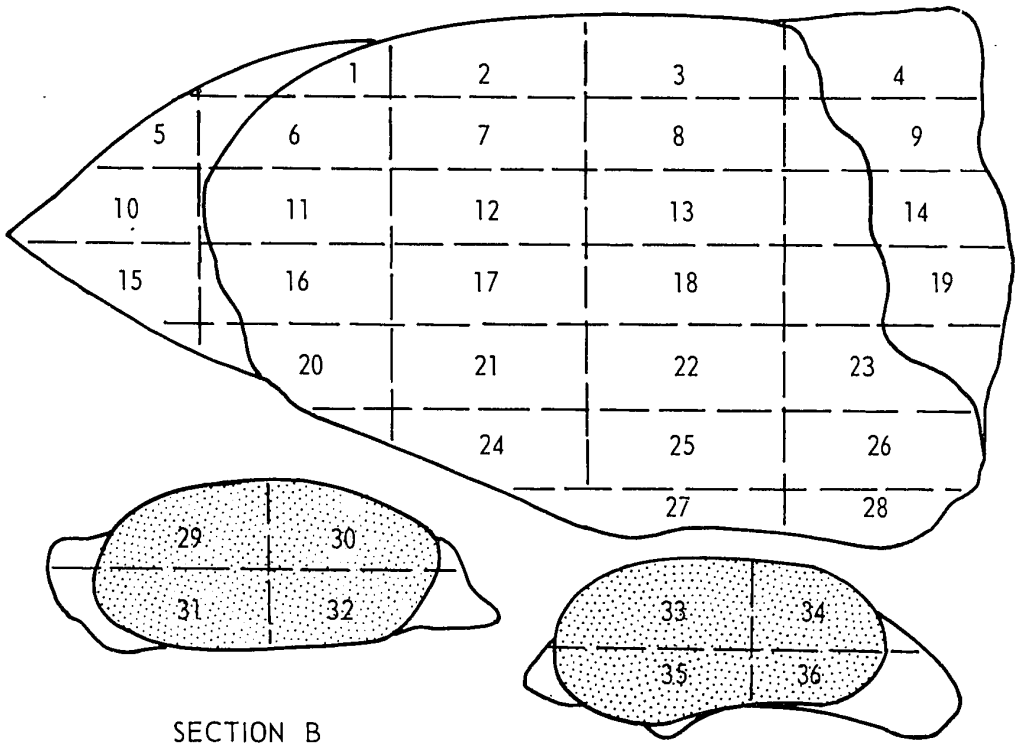

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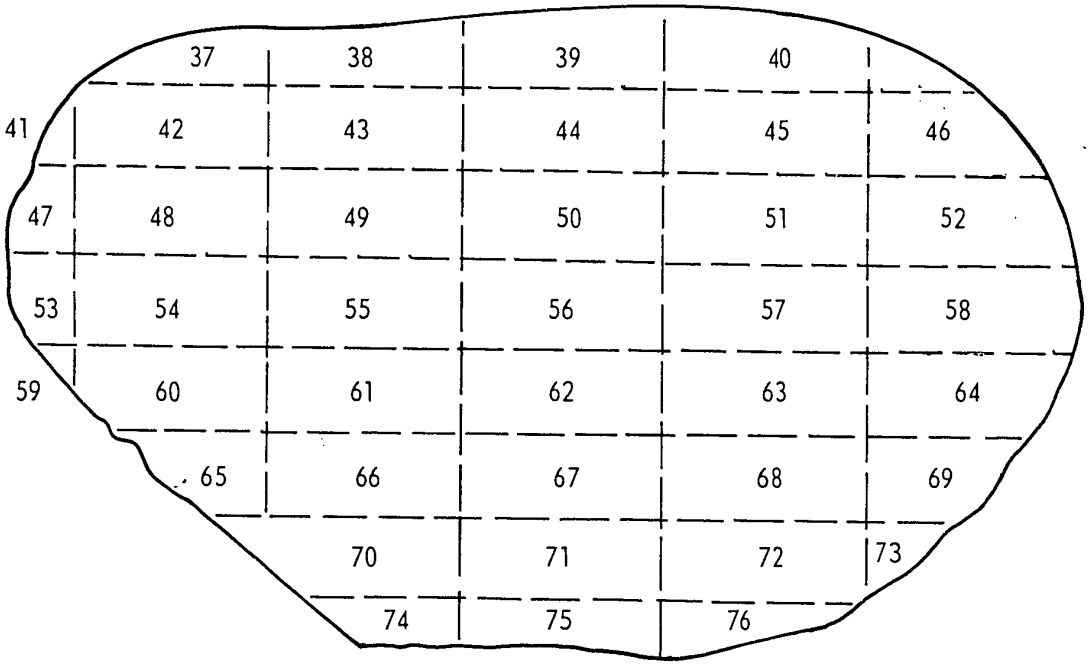

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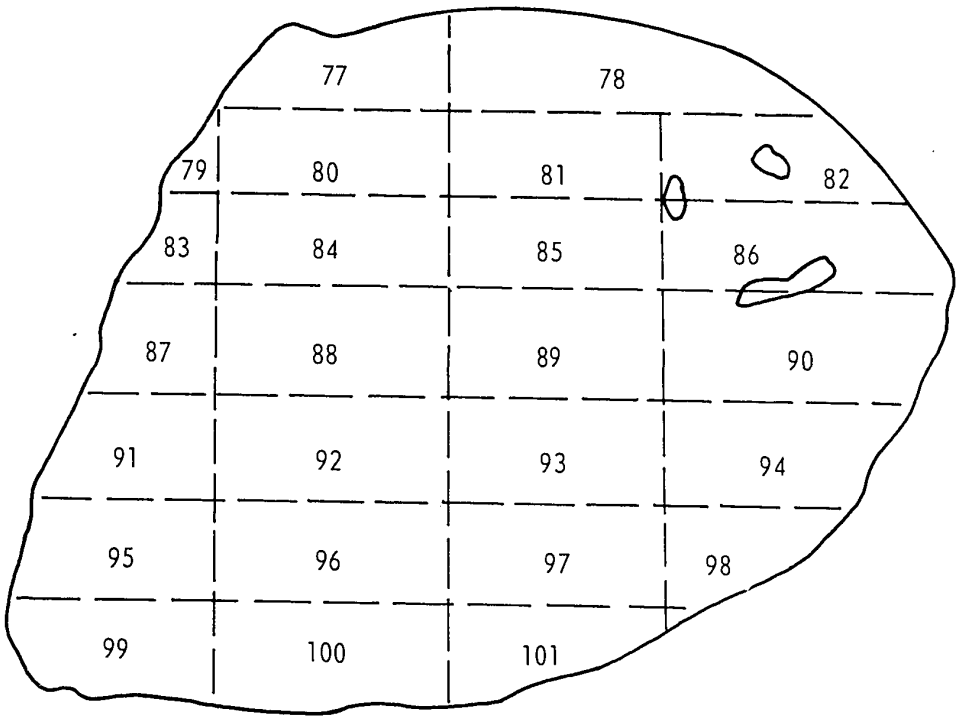

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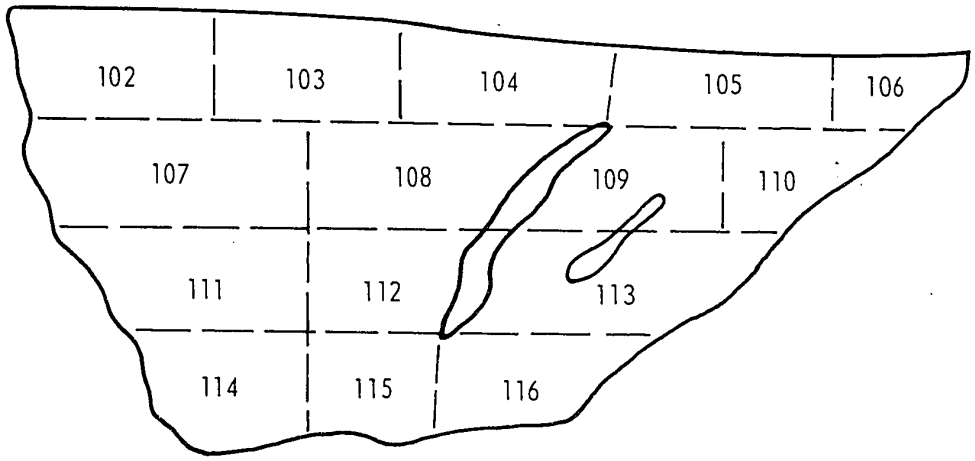

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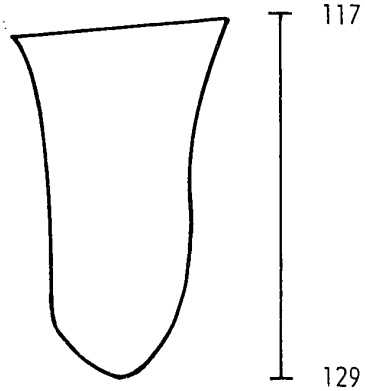

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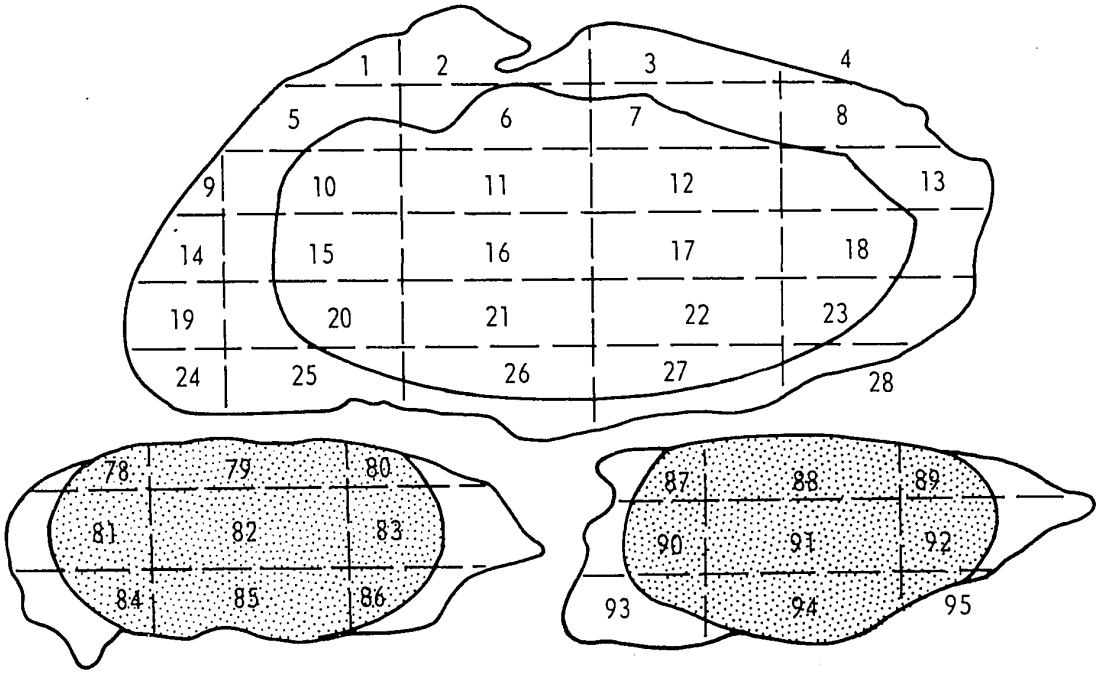

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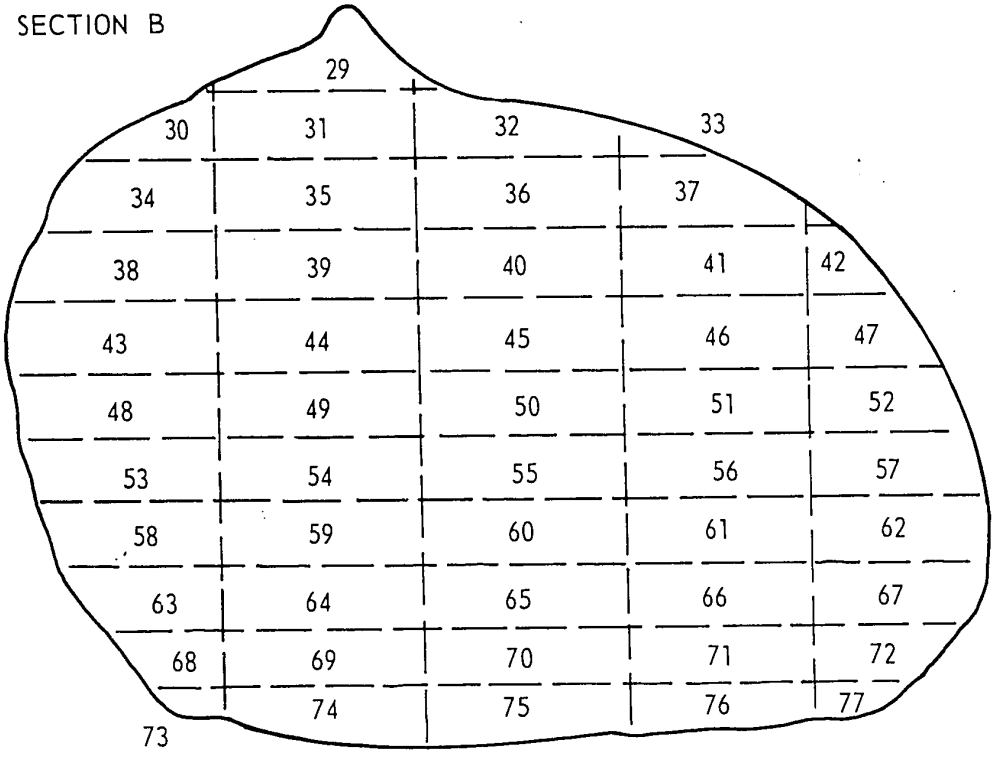

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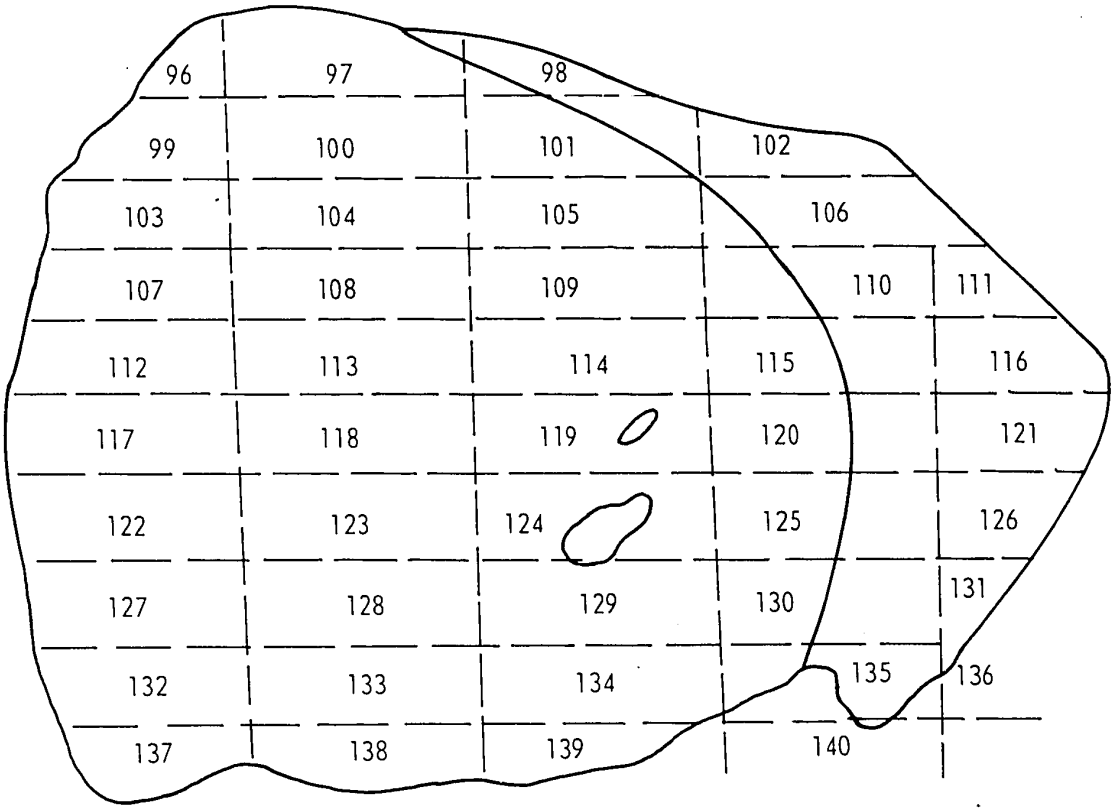

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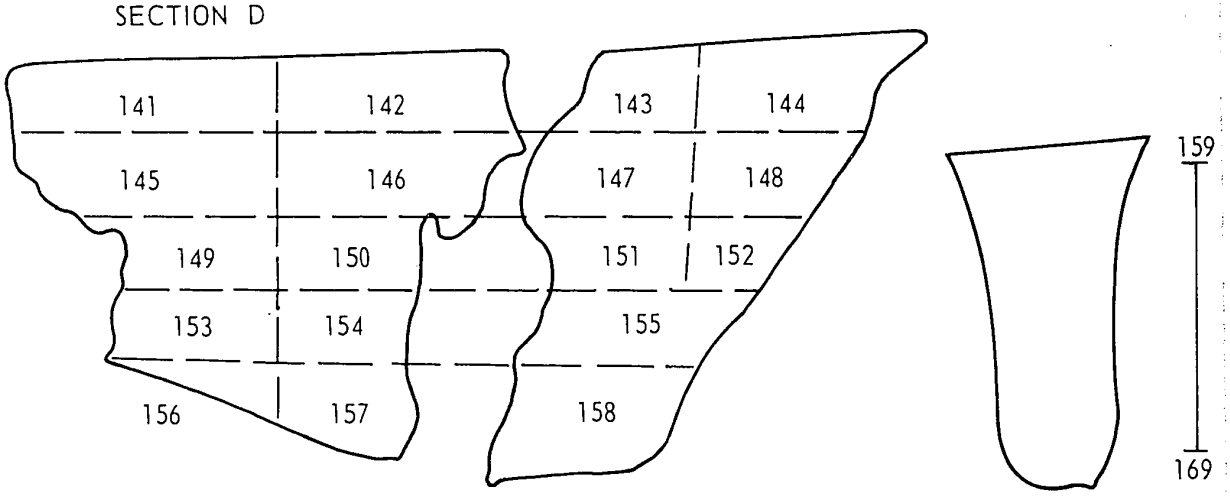

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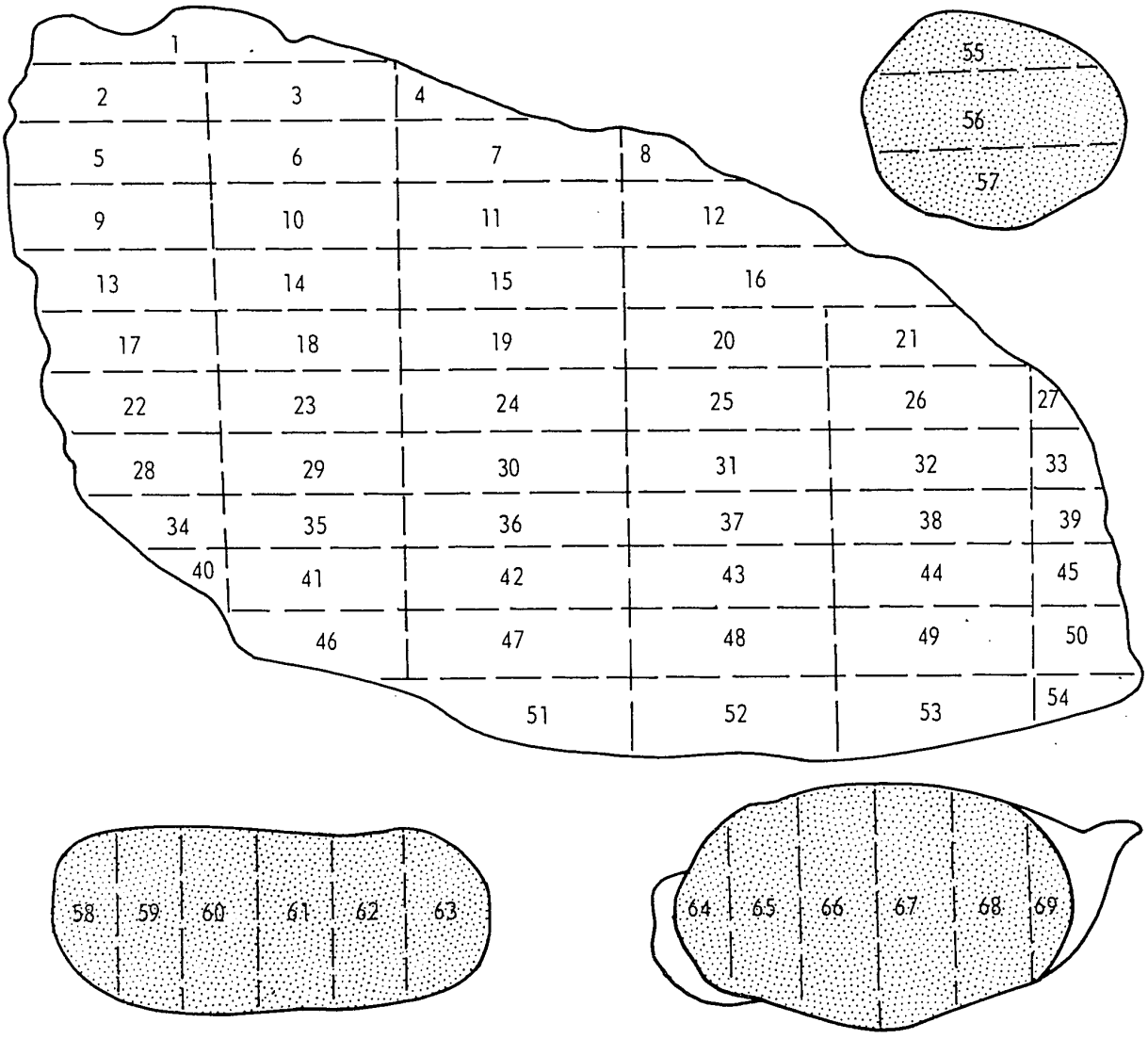

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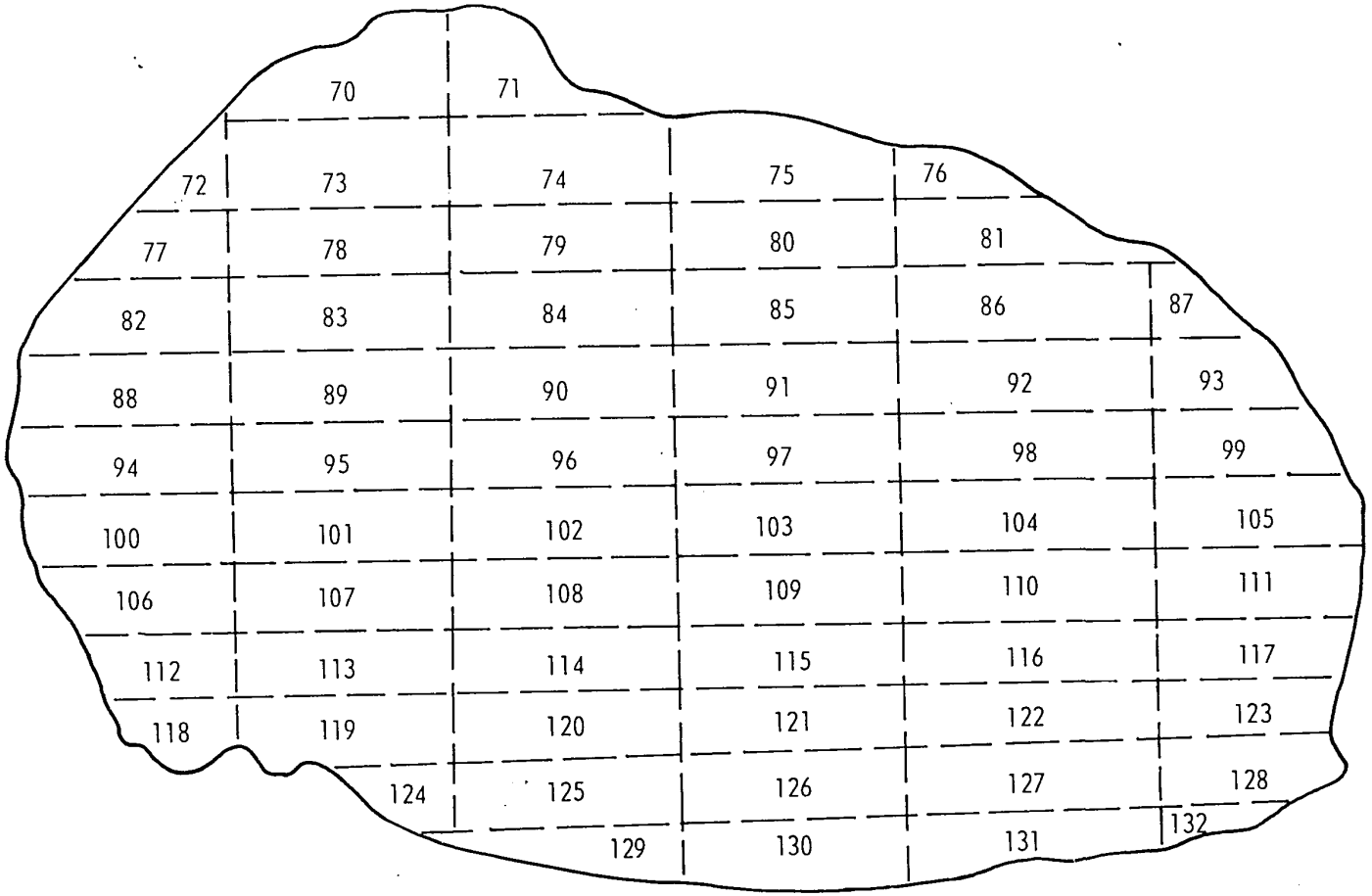

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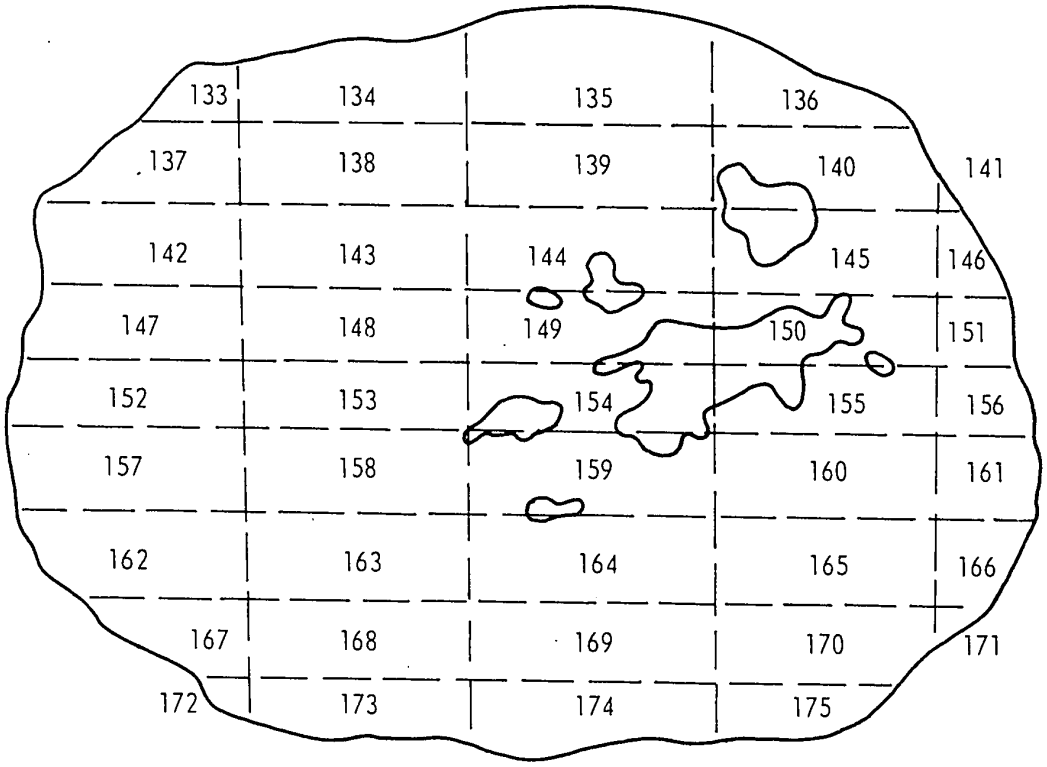

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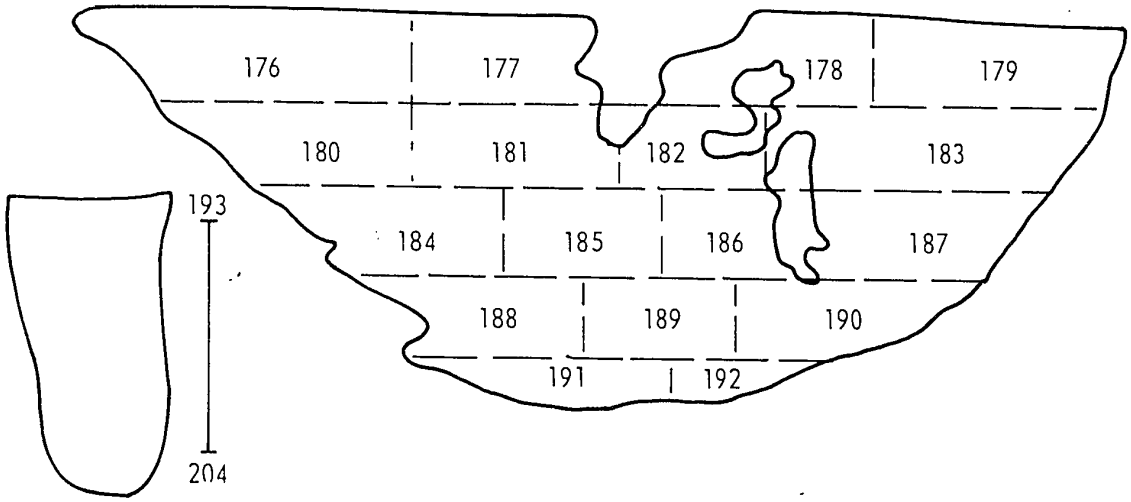

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I am indebted to Drs. A.S. Greig and G.L. Bannister of the Virology Section at the Animal Diseases Research Institute for their encouragement and guidance.

The author is thankful to Dr. P.J.G. Plummer, Director and to Dr. J.F. Frank, Assistant Director, of the Animal Diseases Research Institute for provision of facilities to carry out this work.

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