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A STUDY OF THE PATHOLOGY OF LUMPY SKIN DISEASE IN CATTLE

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ABSTRACT

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Microscopic lesions in cattle infected with the virus of the Neethling form of lumpy skin disease comprised a granulomatous reaction in the dermis and hypodermis which extended to the surrounding tissue. During the early stages of the lesions a vasculitis and lymphangitis with concomitant thrombosis and infarction resulted in necrosis and oedema. A hallmark of the acute to subacute stages of the lesions was the presence of intracytoplasmic eosinophilic inclusions in various cell types. The inclusions consisted of the viroplasm which was identified as aggregates of electron-dense, finely granular to fibrillar deposits in which membrane-enclosed virions and occasional groups of tubular structures were observed. Various cytopathogenic changes were observed in cells exhibiting viral proliferation. The morphogenesis of the virions is discussed in relation to the cytopathogenic changes.

INTRODUCTION

Lumpy skin disease (LSD) is an acute, subacute or in apparent viral disease of cattle, characterized by fever and the presence of firm, circumscribed skin nodules.

Similar lesions may be present in the skeletal muscles and the mucosa of the digestive and respiratory tracts. A subcutaneous oedema of the limbs and ventral parts of the body and a generalized lymphadenopathy are also characteristic of the disease (Weiss, 1968).

It is known in the Republic of South Africa that under field conditions at least 2 viruses are responsible for LSD, namely, the Neethling virus, which is a member of the poxvirus group, and the Allerton virus, which has many characteristics in common with the herpesvirus group (Weiss, 1968). Capstick (1959) studied the pathogenicity of the various cytopathogenic agents associated with LSD in cattle.

The clinical symptoms and histopathological lesions of the Neethling form of LSD have already been described (Thomas & Maré, 1945; De Boom, 1948; Burdin, 1959). The purpose of this study is to highlight the most important macroscopic and light microscopic as well as ultrastructural features of lesions in bovines inoculated with the virus of the Neethling form of LSD. Our findings will be compared with those observed in animals inoculated with other members of the pox group of viruses.

MATERIALS AND METHODS

Source of virus inoculum and virus isolation

The virus isolate used was cultured from a field case of LSD in a cow. Viral material was passaged twice in foetal lamb kidney (FLK) cell cultures, freeze-dried, and stored at 4 °C. Before inoculation of the animals the freeze-dried material was passaged once only in FLK cell cultures. The titre of the virus suspension varied between 4,5–6,0 log $TCID_{50}/m\ell$.

Blood and tissues from various organs (Table 1) were collected from the autopsied animals. The tissues were macerated and diluted $^1/_{10}$ in Eagle's medium containing 500 international units of penicillin and 500 micrograms of streptomycin/mℓ. Foetal lamb kidney cell cultures in roller tubes were inoculated and rolled for at least 8 days at 37 °C before they were passaged. All cultures were passaged twice during which time they were inspected for cytopathogenic changes. Virus neutralization tests and serum neutralization tests on FLK cell cultures in roller tubes were used to identify Neethling LSD virus as the cause of the cytopathogenic changes.

Experimental animals and procedures

Seven susceptible bovines of various breeds, ranging in age from 6–14 months, were inoculated intradermally and subcutaneously with 0,2 mℓ of the virus suspension at multiple sites along the lateral aspects of the neck midway between the shoulder joint and the angle of the mandible. A total of 2,0 mℓ of infective material was used for each animal.

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All the animals were clinically examined and their temperatures taken twice daily.

Macro- and microscopic pathology

Autopsies were carried out on the animals 1–4 days after the skin lesions were observed. A wide range of tissues was fixed in 10% buffered formalin. Suitable blocks were embedded in paraffin wax, sectioned at 3–5 μm thickness and stained with haematoxylin and eosin.

Electron microscopy

Small blocks of skin lesions and lesions in the upper respiratory tract and buccal cavity were fixed in 2,5% sodium cacodylate buffered glutaraldehyde (pH 7,3–7,4), rinsed in sodium cacodylate and post-fixed in sodium cacodylate-buffered osmium tetroxide (pH 7,3–7,4) with 4% sucrose for 1 h. Specimens were then dehydrated in a graded ethanol series (50–100%), passed through propylene oxide as the intermediate solvent and embedded in Epon 812. Survey sections, 1–2 μm thick, were cut and stained with Toluidine blue. Thin sections from selected tissue blocks were stained with aqeuous uranyl acetate and Reynold's lead citrate (Kay, 1965) at room temperature.

RESULTS

Clinical signs and macroscopic pathology

Within 4–7 days of inoculation all the animals developed a well-circumscribed, firm swelling 2–10 cm in diameter at the site of inoculation. This was accompanied by a lymphadenopathy of the regional lymph node. Generalized eruption of skin nodules with a predilection for the legs, ventral areas of the trunk and the muzzle (Fig. 1 & 2) were noticed in 3 of the bovines 2–3 weeks after inoculation. These lesions were associated with a marked oedema as well as lymphadenopathy of the superficial lymph nodes, lachrymation, salivation (Fig. 3 & 4), nasal discharge and inappetence. A febrile reaction was present in the animals that reacted systemically 3–9 days after inoculation and lasted for up to 12 days.

Skin nodules in animals with generalized and local lesions had a firm and whitish-grey appearance on cut section. These involved the entire skin, adjacent subcutaneous tissue and occasionally also the skeletal muscle (Fig. 5). Multifocal necrotic lesions were present in the buccal cavity and respiratory tract of animals with generalized skin lesions.

Microscopic pathology

The microscopic lesions corresponded to a large extent with those reported by Thomas & Maré (1945) and Burdin (1959). The main features will be highlighted.

Skin and adnexa

During the acute stage of the lesions a vasculitis and lymphangitis with concomitant thrombosis and infarction resulted in oedema and necrosis (Fig. 7–9). Necrotic

areas were infiltrated by neutrophils, macrophages and, occasionally, eosinophils. As the lesions progressed, these cells were gradually replaced by round cells (lymphoblasts, lymphocytes, plasma cells and macrophages) and by fibroblasts. The late stage of the lesions was characterized by fibroplasia and the presence of conspicuous perivascular round cell cuffs (Fig. 10).

Acanthosis, parakeratosis and hyperkeratosis were seen in the epidermis. The keratinocytes were swollen and had a spongy appearance. Lysis of necrotic cells terminated in intercellular oedema and vesicle formation (Fig. 11).

Intracytoplasmic homogeneous, occasionally granular, eosinophilic inclusions (Fig. 12) were present in macrophages, endothelial cells, pericytes, keratinocytes, mucus and serous ductal and acinar epithelial cells, skeletal muscle and fibroblasts. The size of the inclusions varied from approximately 1 μm to the size of the nucleus of the containing cell. Inclusions were most abundant during the acute and subacute stages of the lesions.

Other organs

The lesions in the mouth, pharynx, nostrils, larynx and trachea were characterized by erosions and ulcerations. The inflammatory response accompanying these lesions was similar to those in the skin. Hyperplasia and hypersecretion of mucus and serous glands were noted in the buccal cavity and upper respiratory tract. The lymph nodes draining affected areas were oedematous and also showed lymphoid hyperplasia in the follicular and interfollicular zones. The sinuses contained numerous lymphoblasts, lymphocytes and macrophages, while the reticuloendothelial cells were hypertrophic and hyperplastic.

In 2 of the animals with generalized skin lesions a non-purulent interstitial pneumonia was present. One of these animals had a mild, non-purulent meningoencephalitis.

Ultrastructural pathology

Virions in various stages of development were observed in inclusion-containing cells *(vide supra)* as well as in peripheral nerves (Fig. 13). The cytopathogenic changes observed in the various cell types were very similar. The viruses developed in areas within the cytoplasm which are termed viroplasms (Conroy & Meyer, 1971). These viroplasms appeared as well-delineated areas of finely granular to fibrillar deposits (viral matrix) in which membrane-enclosed, developing virions (MEDV) and, occasionally, groups of tubular structures were observed (Fig. 14–16). Mature viral particles occurred in and around the periphery of these viroplasms or were randomly distributed in the cytoplasm. Homogeneous material with a medium electron density were occasionally seen around dilated endoplasmic reticulum (ER) in or adjacent to these viroplasms.

Membrane-enclosed, developing virions were observed in different stages of development within the viroplasm (Fig. 17). In some MEDV the membranes were not completely developed and their contents appeared to be confluent with the viroplasm. Some MEDV were empty, while others had developed to nearly complete viral particles. A high percentage of developing virions were enclosed in a membrane which, in some cases, was ill-defined, while in others they consisted of granular endoplasmic reticulum (GER) (Fig. 17).

Mature virions appeared as oval to rectangular particles with rounded corners. A small electron-dense granule could often be seen within the core (nucleus). The virion envelope had a multi-layered appearance.

Apart from central chromatolysis, margination of chromatin and occasional myelin figures, no other changes were observed in the nucleus.

Mitochondrial alterations included swelling and hypertrophy with disruption of the internal structure and shape (Fig. 21 & 22). The internal structure was transformed into wavy fibrillar material which made identification of mitochondria difficult.

There was dilatation of the ER, the lumen of which contained either virus particles (Fig. 21) or electron-lucent or electron-dense granular material. The dilated ER was frequently encircled by a homogeneous layer of varying thickness and medium electron-density, often with virus particles adhering to the latter (Fig. 23 & 24). Invaginations of this dilated ER resulted in the formation of multi-laminated structures lying in the cytoplasm (Fig. 25 & 27). Within these structures one could observe either viral matrix, developing virions or even what appeared to be mature viral particles (Fig. 18 & 27).

The pericytes contained viral particles more frequently than endothelial cells in small blood and lymph vessels (Fig. 19 & 20). Virus-infected macrophages which showed cytopathogenic changes were occasionally seen in these vessels. A few thrombi were associated with necrotic endothelial cells, some of which contained viral particles (Fig. 26). Virions were also seen free in the vessel lumens.

Virions were commonly observed in fibroblasts within the endo-, peri- and epimysium of peripheral nerves. Virions with the associated cytopathogenic changes as described were also observed in Schwann's cells (Fig. 28). Although myelinated axiscylinders were devoid of virions, they were frequently observed in unmyelinated axis-cylinders (Fig. 28).

Virology

The virological results are summarized in Table 1. The virus was isolated from various organs from animals with generalized and local lesions (Table 1).

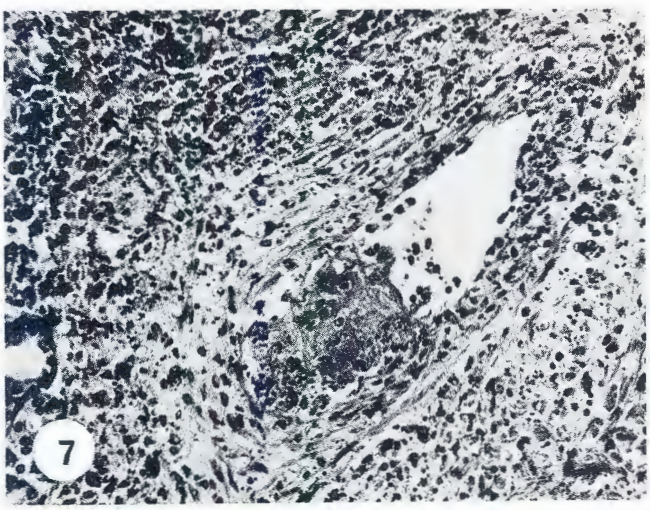
Discussion

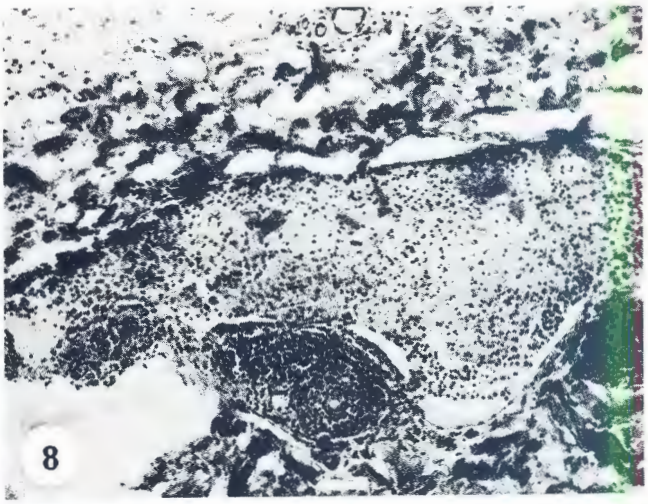
Light and ultrastructural studies showed a vasculitis and thrombosis to be central in the pathogenesis of the lesions in bovines infected with the Neethling form of LSD. Although replicating viral particles were regularly seen in the endothelial cells and pericytes of these vessels, a few necrotic endocytes were devoid of virus. As a rule the vascular changes were associated with multifocal areas of necrosis. These necrotizing lesions were followed by proliferation of various cell types. Joklik (1966) suggested that, apart from the cytopathogenic changes in pox virus-infected cells, the virus can also have a proliferative effect on non-infected cells.

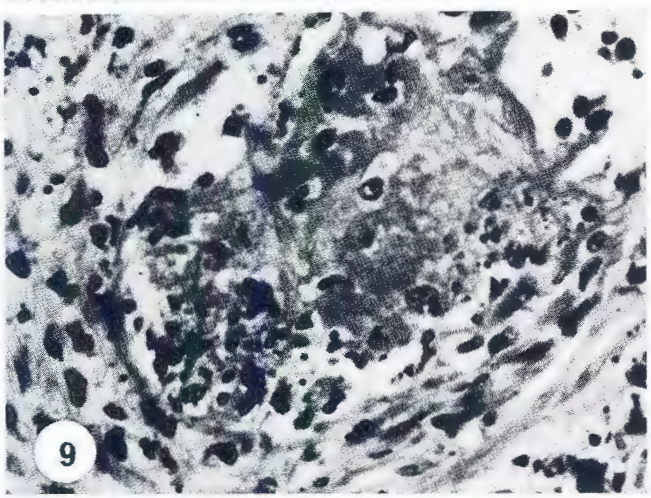
The light microscopic and ultrastructural changes in both animals with localized and generalized skin lesions were identical. Four of the animals reacted only locally at the site of inoculation. This natural resistance to infection confirms the findings of Weiss (1968), who showed that 50% or more of cattle exposed to natural and experimental infection either did not show any lesions or reacted only at the site of inoculation.

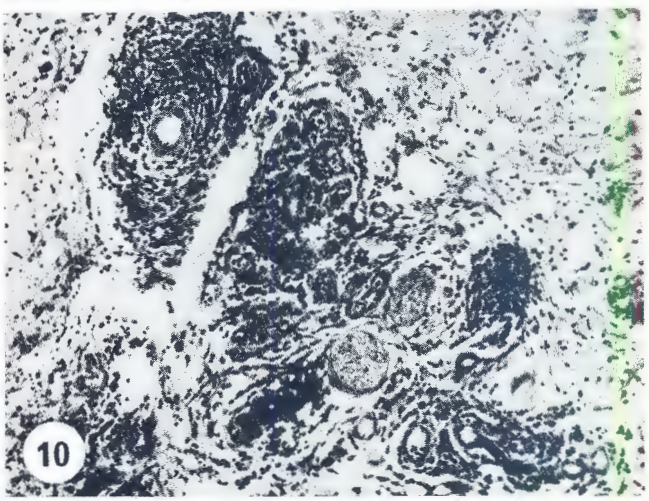
Inclusions consisted of a granular to fibrillar viral matrix in which MEDV in various stages of development were observed. Mature virus particles were present in and around the viroplasm or were free in the cytoplasm.

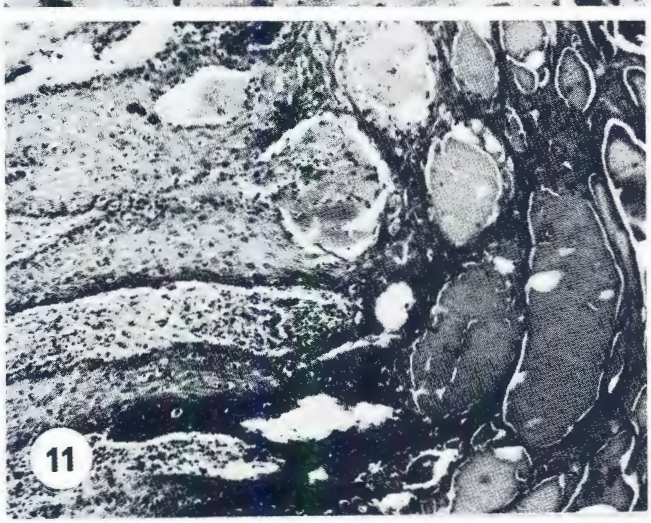
Viroplasms were not always well demarcated, and cells often contained more than one of them. In and around the viroplasms dilated ER was often present, surrounded by a homogeneous layer of varying thickness.











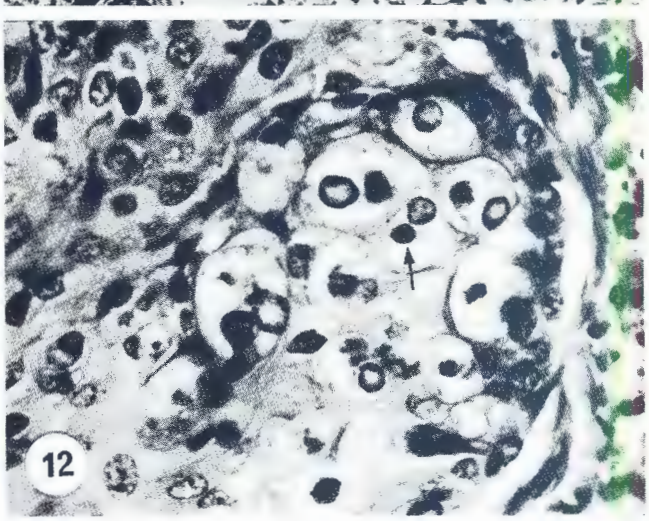
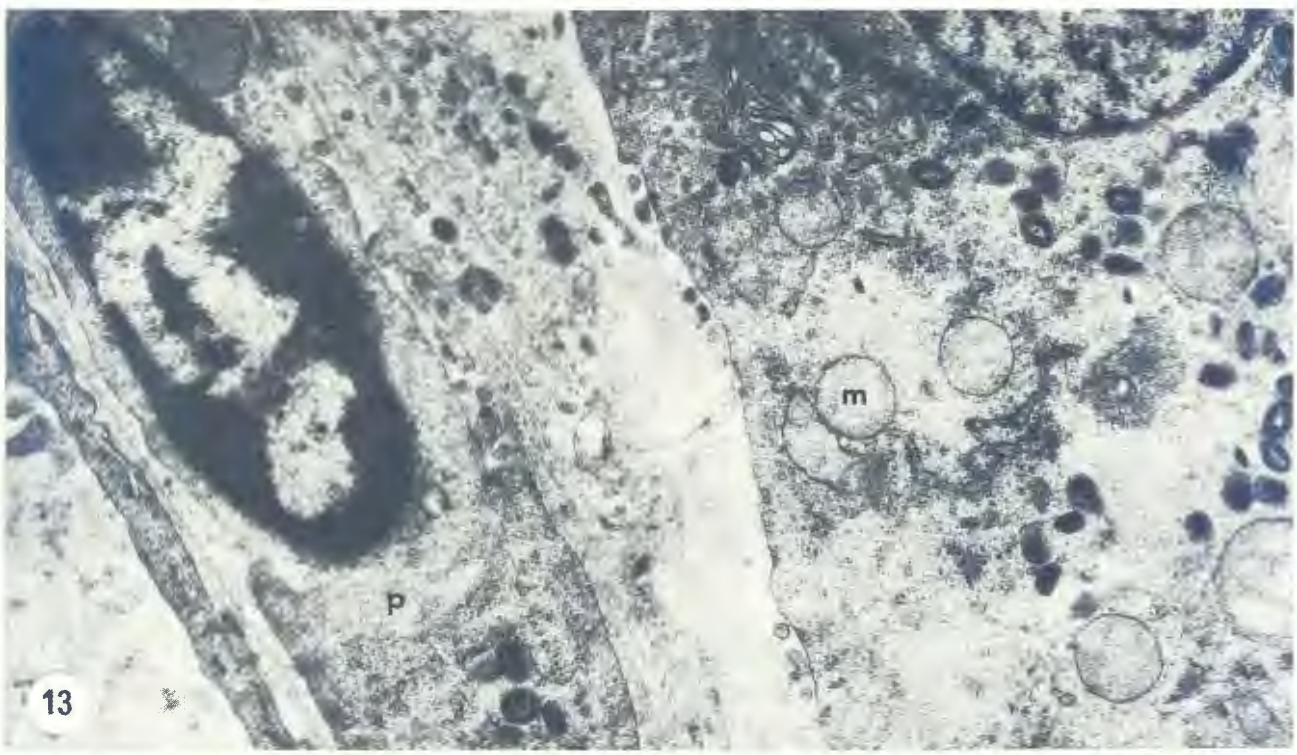


FIG. 7 Thrombosis of a blood vessel. Note the prominent perivascular necrosis: HE × 65
FIG. 8 Thrombosis of a lymph vessel: HE × 160
FIG. 9 Vasculitis and thrombosis of a blood vessel in the subcutaneous tissue: HE × 400
FIG. 10 Perivascular cuffing in the subcutaneous tissue: HE × 160
FIG. 11 Numerous intra-epidermal cysts: HE × 25
FIG. 12 Intracytoplasmic inclusions (arrow) in the epidermis: HE × 1 000



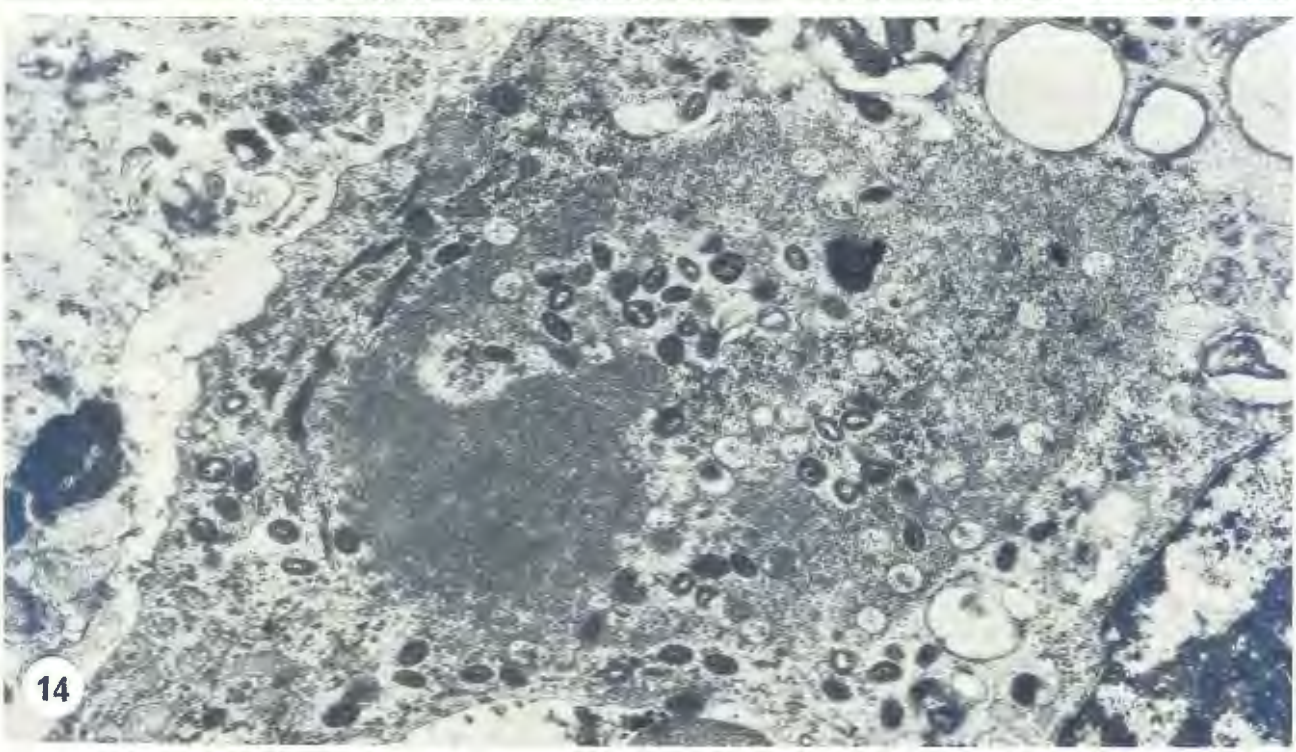


FIG. 13 Virions in the cytoplasm of a macrophage and pericyte (P). Note the swollen mitochondria (M): × 17 000
FIG. 14 Viroplasm in a keratinocyte with virions in various stages of development: × 14 000

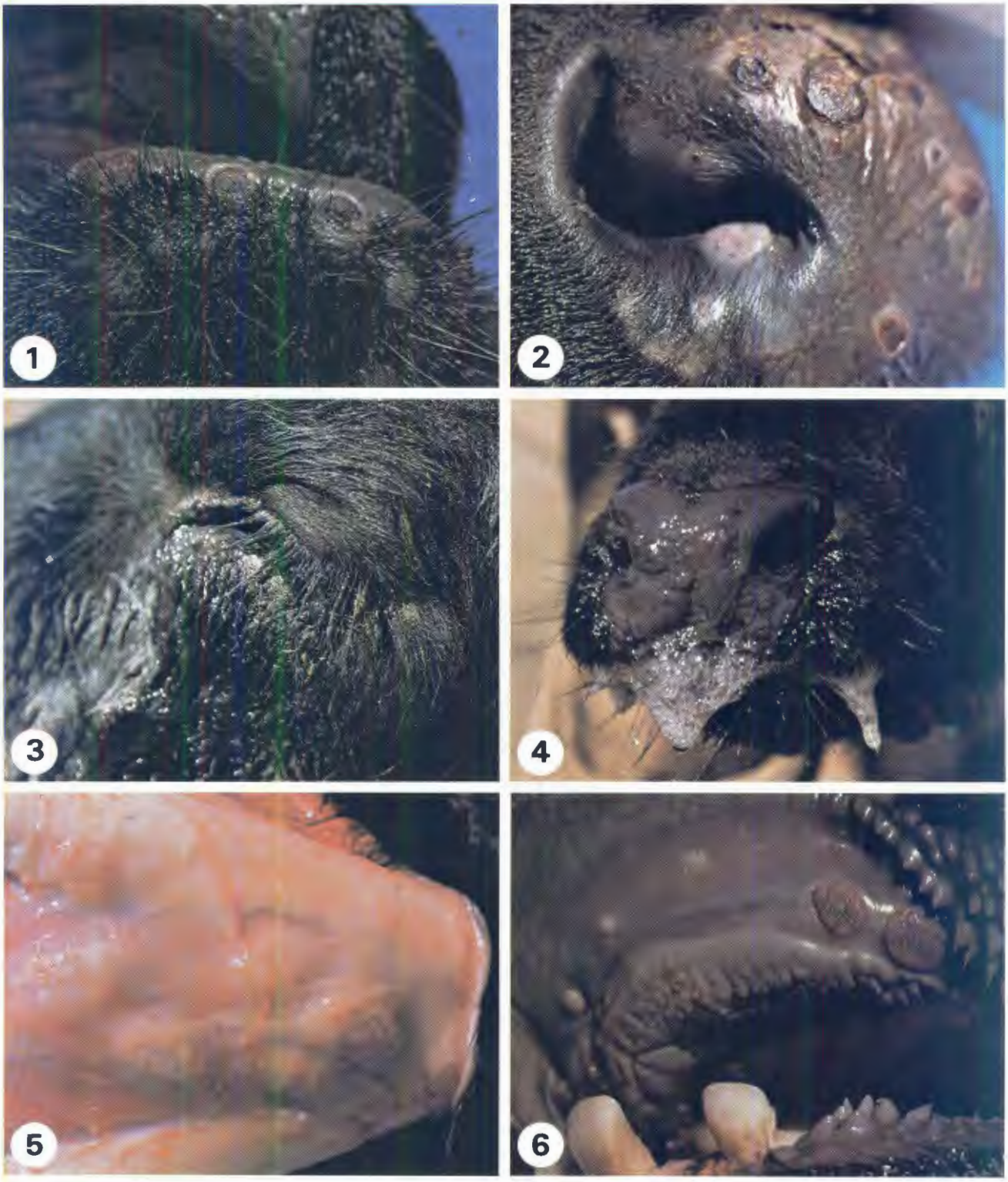
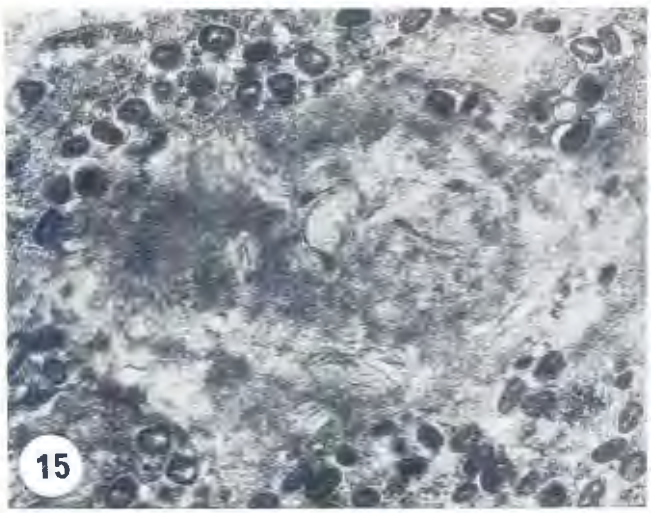
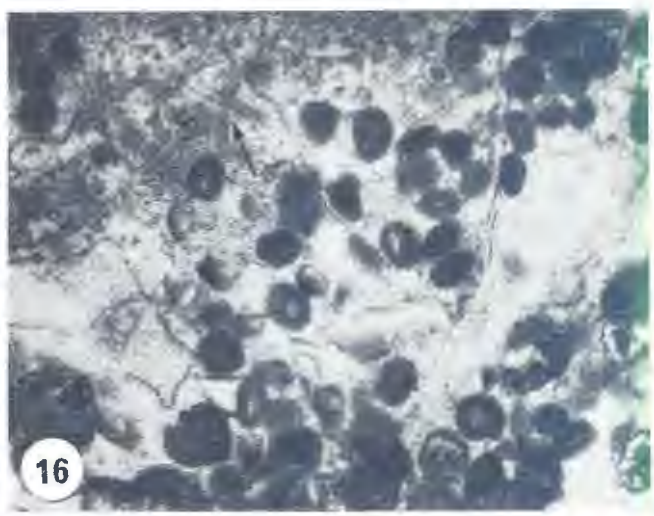
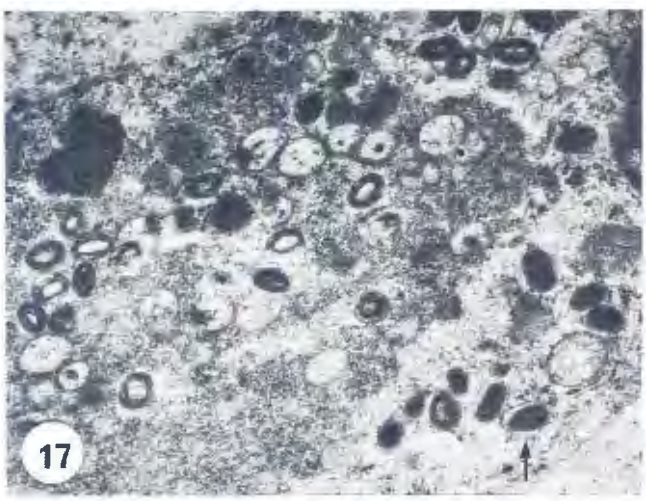
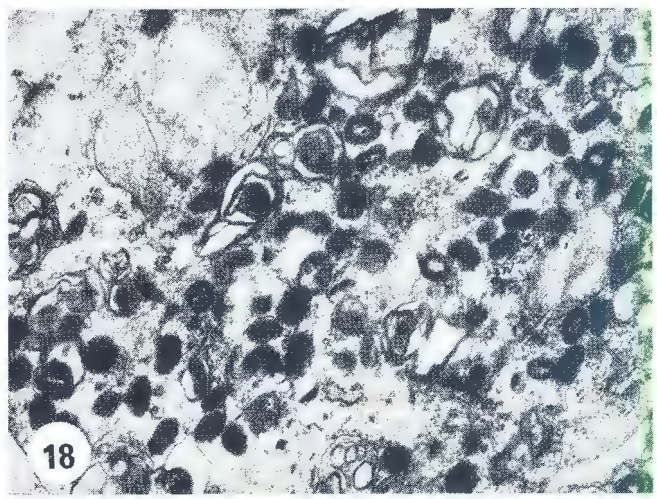


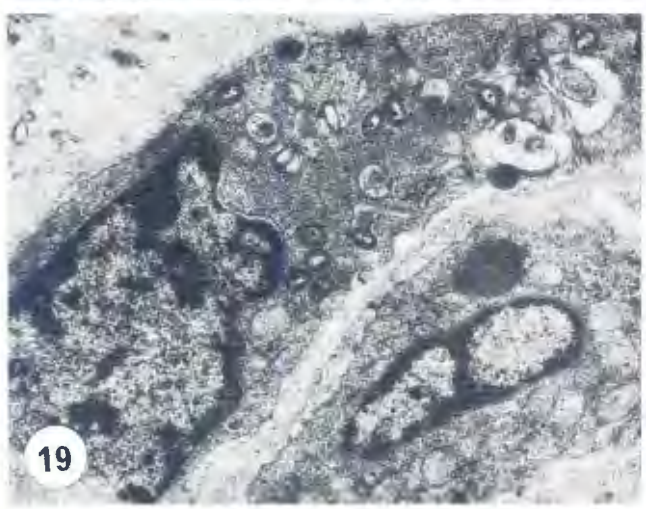
FIG. 1 Generalized skin lesions in a bovine
FIG. 2 Lesions in the nostril and on the muzzle
FIG. 3 & 4 Lacrimation and salivation in a bovine with LSD
FIG. 5 Prominent lesions in the skeletal muscle
FIG. 6 Well circumscribed lesions in the buccal cavity











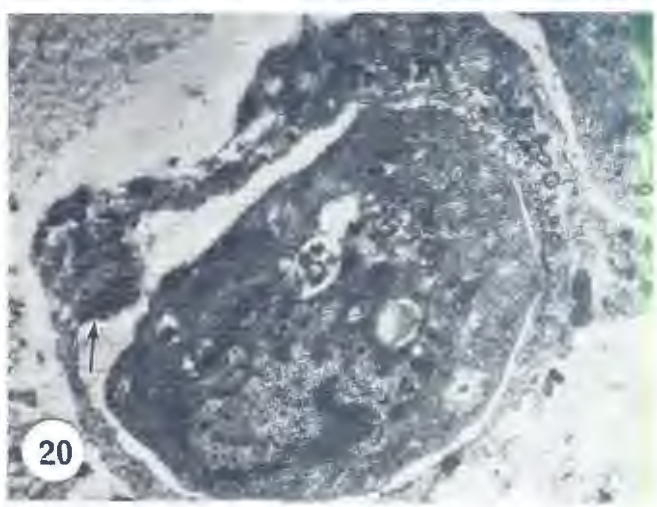


FIG. 15 Fibrillar material in a viroplasm: × 14 000

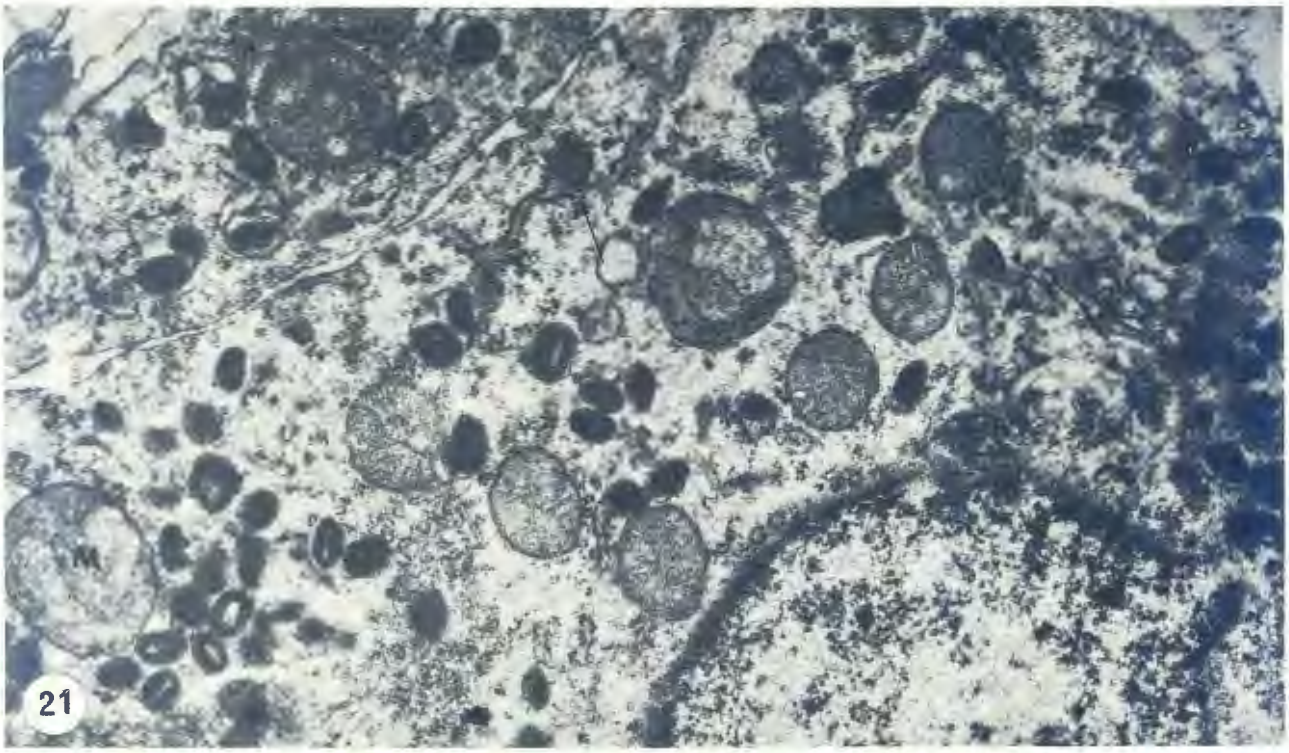
FIG. 16 Tubular structures in a viroplasm (arrow): × 20 000

FIG. 17 Membrane-enclosed, developing virions in various stages of development. Note the membrane around some of the virus particles (arrow): × 18 000

FIG. 18 Multi-laminated structures in the cytoplasm of a macrophage: × 16 000

FIG. 19 Developing virions in the cytoplasm of a pericyte: × 14 000

FIG. 20 Virions in a capillary endothelial cell. Note the fibrin deposits (arrow): × 6 000



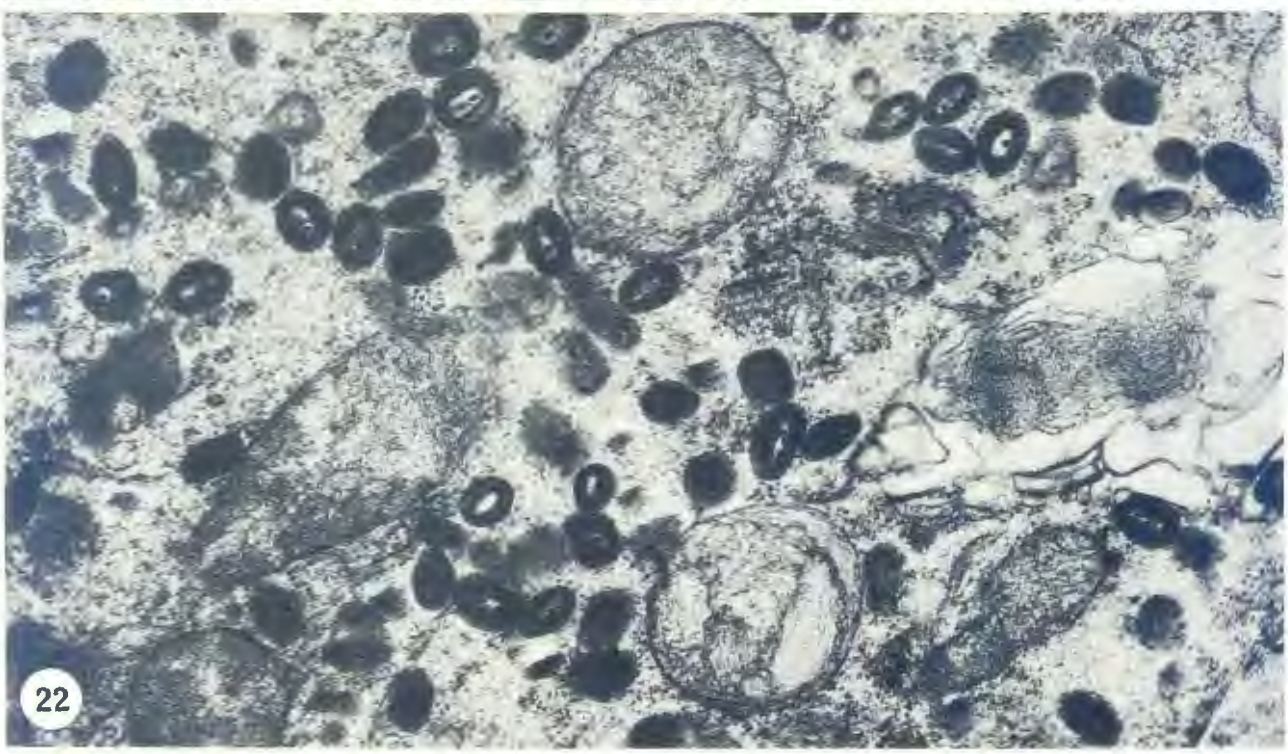


FIG. 21 Dilatation of the endoplasmic reticulum, some of which contains virus particles (arrow). Note the transformed internal structure in some of the swollen mitochondria (M): × 22 500

FIG. 22 Swollen mitochondria containing wavy fibrillar material: × 34 000

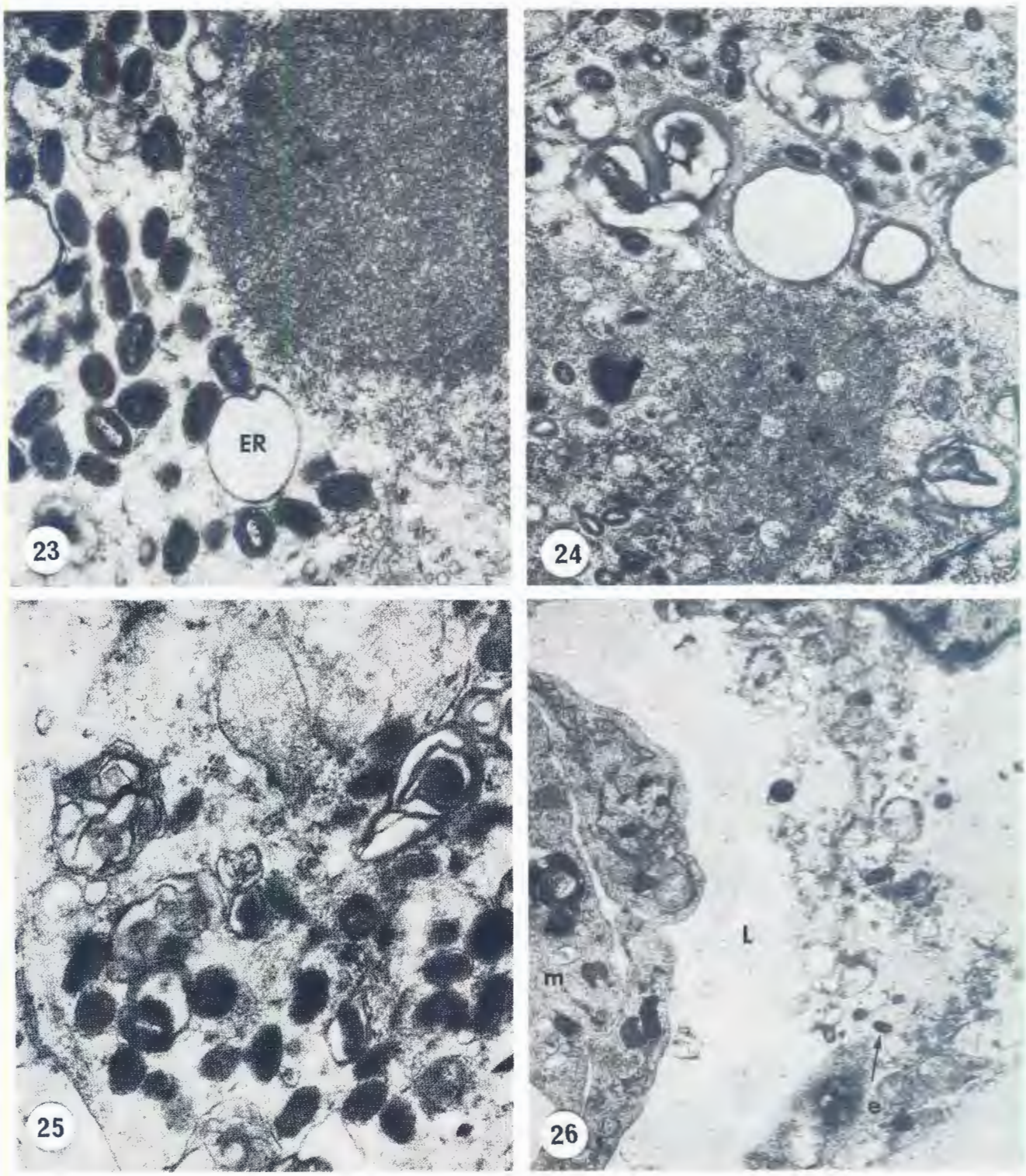
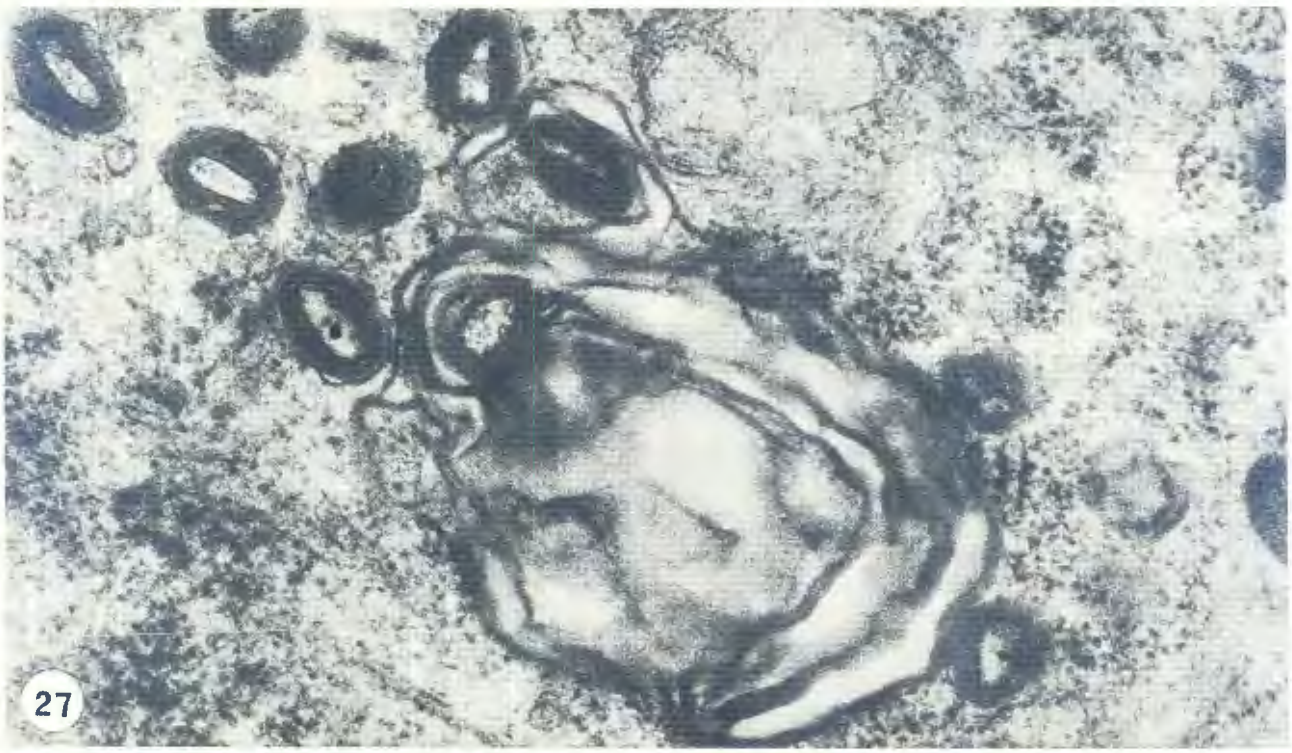


FIG. 23 Virus particles adhering to the dilated endoplasmic reticulum: × 27 000
FIG. 24 Dilated endoplasmic reticulum surrounded by a homogeneous layer of varying thickness: × 16 500
FIG. 25 Multi-laminated structures in some of which developing virions are discernible: × 23 300
FIG. 26 Endothelial necrosis (e) in a small blood vessel. Note the virus particles (arrow) and macrophage (m) in lumen (L): × 10 500



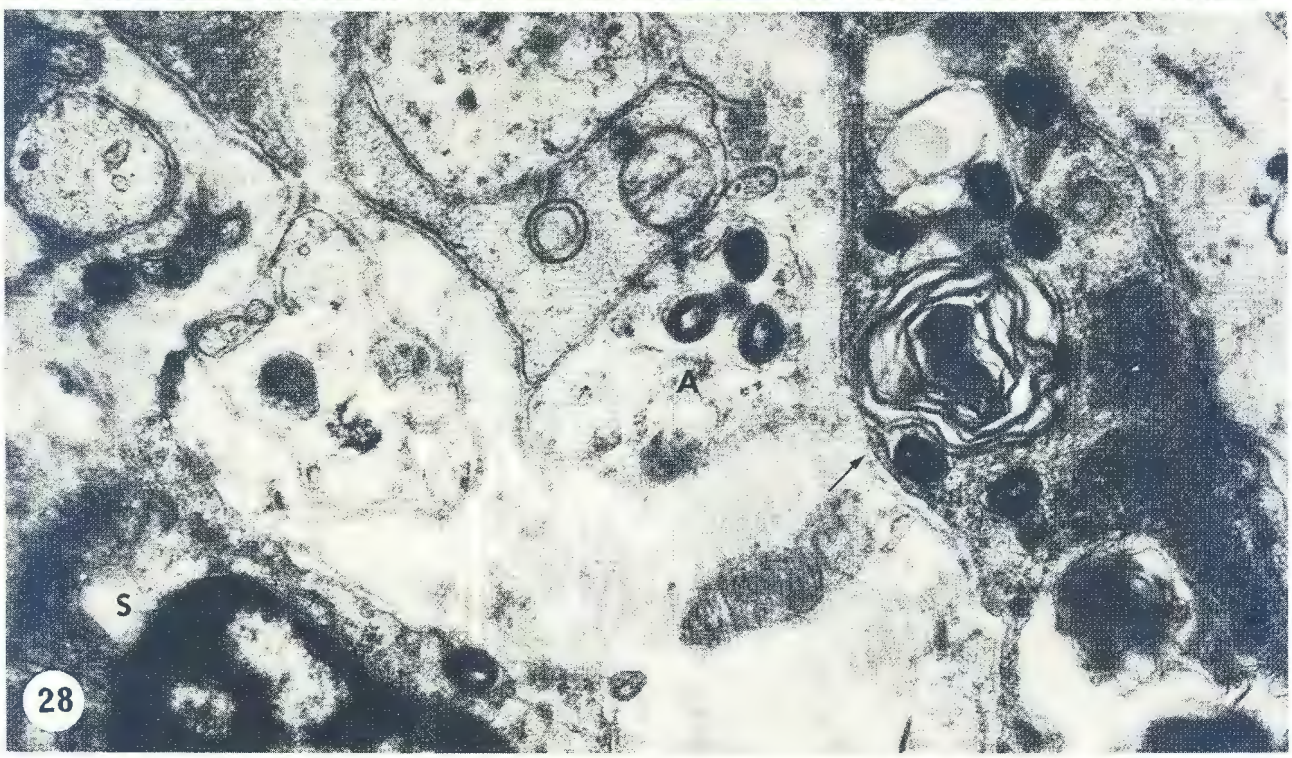


FIG. 27 Developing viral particles are dispersed between the laminae of a multi-laminated structure: × 55 000

FIG. 28 Virions in an unmyelinated axis cylinder (A). Note the virion in close contact to a Schwann cell (S) and multi-laminated structures in a fibroblast (arrow): × 23 000.

Table 1 Lumpy skin disease virus isolation

Bovine No.	Lesion distribution		Virus isolation						
	Localized	Generalized	Skin	Spleen	Prescapular lymph node	Liver	Lung	Kidney	Blood
1		+	+		+				+
2		+	+	+	+	+	+	+	
3		+	+		+		+		+
4	+		+						
5	+		+	+		+	+	+	
6	+		+	+		+	+	+	
7	+			+					

+ = Positive

Multi-laminated structures were observed either associated with the dilated ER or free within the viroplasm or cytoplasm of the cell. It is suggested that the multi-laminar structures originate from more than a single source. They may be myelin figures which are lipid membrane residues from necrotic organelles or they may be formed by intracisternal sequestration which denotes laminar profiles of ER lying within dilated ER. This would explain the presence of portions of viroplasm and developing virions among the membranes. Furthermore, the homogeneous material which occasionally surrounded dilated ER was very similar in appearance to some of the laminae of the multi-laminated bodies. The resemblance of the outer membrane of mature virions to some of the laminae in multi-laminar structures indicates that the latter could represent viral precursor material.

Marked swelling of mitochondria and associated disruption of the internal structure was a common sequel of cells in which viral replication occurred. Crystalloid deposits, observed in mitochondria of pig epidermal cells infected with swine pox (Conroy & Meyer, 1971), were not seen.

Apart from central chromatolysis and the occasional presence of myelin figures in nuclei of cells in which viral replication occurred, no other noteworthy nuclear changes were observed. Conroy & Meyer (1971) reported the presence of a finely filamentous network in the nuclei of epidermal cells of pigs and in swine kidney cell cultures infected with swine pox. They ascribed this to an altered metabolic function and not to a direct product of viral replication.

Cytopathogenic changes observed in Schwann cells were comparable to those observed in other cells invaded by viral particles. Apparently the myelin sheath serves as a barrier for viral penetration, as no virions were observed in myelinated axons, although they were present in non-myelinated axons. As far as we can ascertain, this is the first report of LSD virus in neural tissue. The importance of this phenomenon is at present unassessed.

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