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Visceral Target Organs in Systemic St. Louis Encephalitis Virus Infection of Hamsters

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Ultrastructural aspects of St. Louis encephalitis virus infection of the major extraneutral organs and tissues of suckling hamsters were examined. In the pancreas, both the exocrine and endocrine portions were equally affected by the virus. A feature apparently unique to flaviviruses was the accumulation of virus particles in all types of secretory granules in this organ. Virus particles were seen within myocardial fibers and within the smooth muscle cells and endothelial cells of small blood vessels of the heart. In the intestines, the lamina propria was the most severely infected, with virus particles accumulated in all cell types.

INTRODUCTION

St. Louis encephalitis (SLE) is the most important arthropod-borne viral disease affecting humans in the United States. It was first identified as a specific entity in 1933 when the virus was isolated from brain tissue of deceased patients in St. Louis, Missouri. Approximately 10,000 clinical infections and 1000 deaths have been attributed to the virus in the many outbreaks which have occurred since that initial epidemic (Chamberlain, 1980). Recent epidemics have been centered in the Mississippi and Ohio river valleys, the mid-South region, and along the Gulf Coast (Morbidity and Mortality Weekly Report, 1980).

The clinical spectrum of overt SLE virus infection ranges from a mild influenza-like syndrome to life-threatening, acute central nervous system (CNS) disease (Brinker and Monath, 1980). Deaths during this early clinical course are caused by direct damage to the CNS by the virus. The interval between onset of illness and death is rather consistent; 50% of the fatalities occur within the first week and 80% within 2 weeks of onset of clinical signs. A variety of complications have been reported which contribute to the 20% of deaths which followed a more protracted illness. Some of these were only secondarily related to the primary SLE infection, but others may be the consequence of extraneural organ and tissue damage caused by the virus infection. The complications most often reported have been lobar pneumonia, gastrointestinal hemorrhage, swelling and congestion of the kidney, coronary artery disease, and congestive heart failure (Brinker and Monath, 1980). To call attention to the possibility that some of these complicating processes in the human disease might be specific consequences of extraneural virus infection, the present study was designed to determine whether such infection sites are important in the course of the lethal disease caused by the virus in suckling hamsters.

SLE virus causes a widespread infection in suckling mice and hamsters when it is inoculated by a peripheral route and involves numerous organs and tissues in addition to the CNS, the principal target when the virus is injected intracerebrally. The CNS involvement of suckling mice was the subject of an earlier paper (Mur-

phy *et al.*, 1968). In the present report we describe the changes in major extraneural organs and tissues of suckling hamsters.

MATERIALS AND METHODS

The strain of SLE virus used was TBH-28, a standard laboratory strain that had previously been passaged seven times intracerebrally in suckling mice; it had an infectivity titer of 10^9 LD_{50}/ml when titrated in suckling mice by intracerebral inoculation.

Eight litters of 4-day-old Syrian hamsters (LAK-LVG, Lakeview Hamster Colony, Charles River Co., Newfield, N.J.) were inoculated subcutaneously in the hip with 10^3 LD_{50} of virus. Animals were anesthetized on the fifth day after inoculation when first signs of illness appeared or on the sixth day when they were moribund, and organs and tissues were excised and prepared for examination by immunofluorescent, light, and electron microscopy.

Light microscopic observations were of little help in identifying major extraneural target organs and tissues in the clinically ill and moribund hamsters, but frozen-section immunofluorescence of all organ systems indicated significant virus growth in the pancreas, adrenal gland, small intestine, heart, skeletal muscle, and kidney. Specimens from these organs/tissues were, therefore, selected for electron microscopic examination. Blocks, approximately 1 mm³ in size, were taken from each organ/tissue specimen and fixed in 2.5% phosphate-buffered gluteraldehyde for 1 hr. The tissues were then postfixed in 1% osmium tetroxide, dehydrated in graded concentrations of alcohol, and embedded in a mixture of Epon and Araldite (Mollenhauer, 1964). Sections were stained with uranyl acetate and lead citrate and were examined in Philips 200 and 300 electron microscopes.

RESULTS

Despite the widespread and consistent presence of virus specific immunofluorescence in all layers of the adrenal cortex and the tubular epithelium of the kidneys of all animals examined, we found little evidence of ultrastructural cytopathology. Therefore, in this study we focused on the ultrastructural pathologic changes in pancreas, heart, small intestine, and skeletal muscle. In each of these organs and tissues, virus particles indistinguishable from those described previously (spherical particles, 38–39 nm in diameter, with a dense central core and lucent envelope layer) were associated with the round and cylindrical membranous intracisternal structures characteristic of all flavivirus infections (Murphy, 1980).

Pancreas

Sections for ultrastructural examination were chosen by light microscopic examination of plastic-embedded 0.5-μm sections. In these selected areas, basic acinar structure was retained, but there was some interstitial edema and there were numerous mononuclear inflammatory cells, mostly in perivascular spaces.

By electron microscopy, it was evident that there was more variation in the extent and progression of necrotic changes from one lobule to another than was visible by light microscopy. In some cases only single cells were involved, whereas in others, entire acini were in various stages of necrotic change. These changes in acinar epithelial cells consisted of vacuolization–dilatation of the rough endoplasmic reticulum and rarefaction of the cytoplasmic matrix, giving
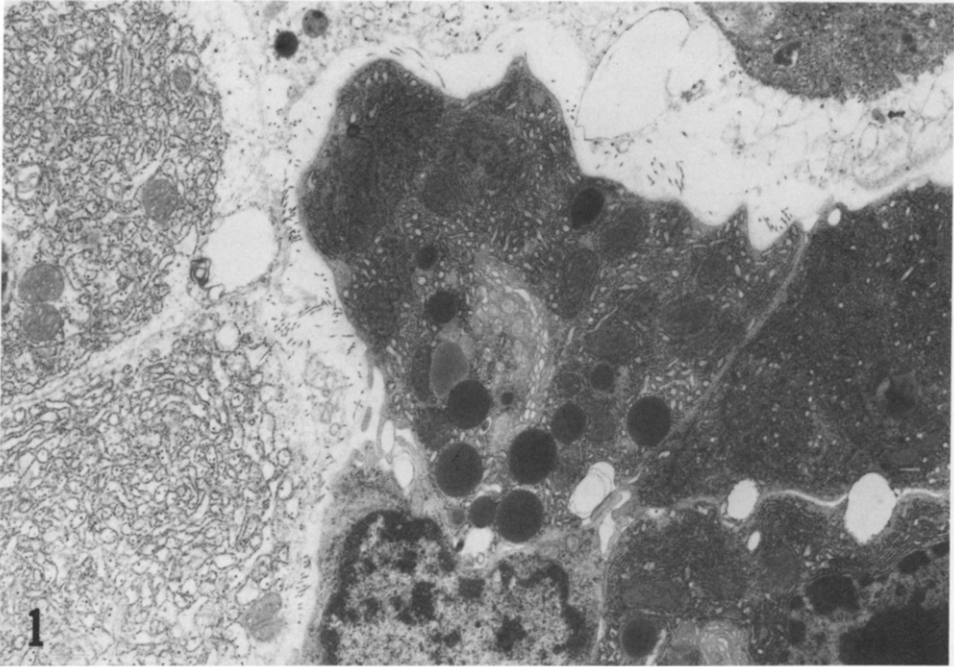
FIG. 1. Two types of necrotic change are seen in acinar cells of the pancreas: one, a vacuolization–dilatation of the rough endoplasmic reticulum with rarefaction of the cytoplasmic matrix, giving the cells a pale, lacy appearance; the other, a severe condensation of the cytoplasm, making the cells appear shrunken and electron dense. ×8050.
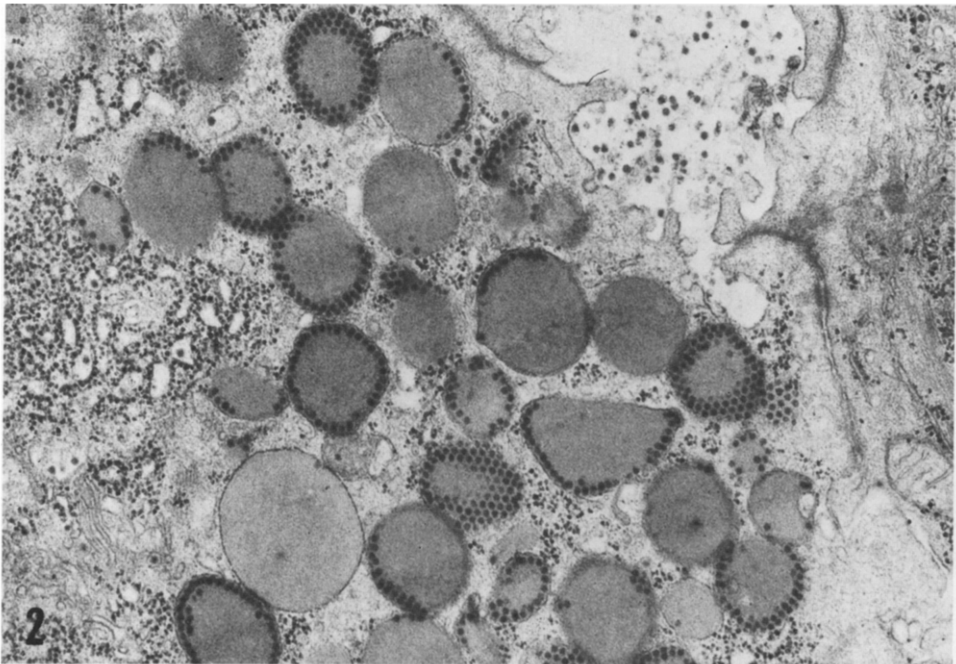

FIG. 2. Virus particles are in cisternae of the rough endoplasmic reticulum, in the Golgi complex, and in the zymogen granules of an acinar cell where they frequently assume a paracrystalline array. ×30,000.

cells a pale, lacy appearance. At the opposite extreme, the cytoplasm of some cells showed severe condensation; such cells appeared shrunken and very electron dense (Fig. 1).

Virus particles were found in cells in either state of degeneration as well as in cells otherwise normal in structure. They accumulated in cisternae of the endoplasmic reticulum and the Golgi, and also in zymogen granules where frequently they assumed paracrystalline arrays (Fig. 2). Virus particles were released into acinar lumina by a process of exocytosis, sometimes as individual particles and sometimes enclosed in membrane-bound remnants of cellular organelles. Centroacinar cells and duct cells were infected as well (Fig. 3). Myelin-like figures were present both in infected cells and in extracellular spaces. Macrophages were abundant, and many contained large accumulations of phagocytized infected cellular debris.

Nonspecific changes, i.e., edema, vacuolization, and monocytic infiltration, were seen in the islets of Langerhans of most of the animals examined. In three of the animals, however, severe, virus-specific cellular changes were also present. All cell types were affected—alpha, beta, and delta cells, as well as the endothelial cells of the capillaries and the invading macrophages. Virus particles and related tubular structures accumulated in cisternae of the endoplasmic reticulum and were concentrated in some of the islet cell granules (Figs. 4a–c). Virus particles were released from these cells, just as they were from the exocrine cells, by a process of exocytosis, either as individual particles or in clusters enveloped by membranes.

Heart

Hearts from three animals were examined by electron microscopy. The heart of one animal contained numerous focal areas of infection involving groups of adjacent myocardial fibers. The infection in the hearts of the other two animals was more severe and widespread, with multifocal to diffuse myocardial degeneration and necrosis (Fig. 5). The affected myocytes contained large lipid inclusions, swollen and vesiculated mitochondria, and widely dilated sarcoplasmic reticulum
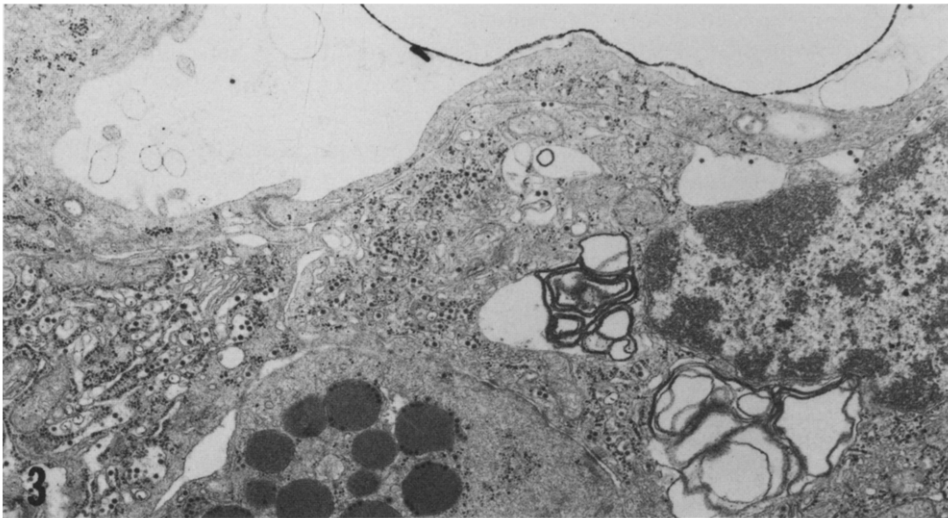

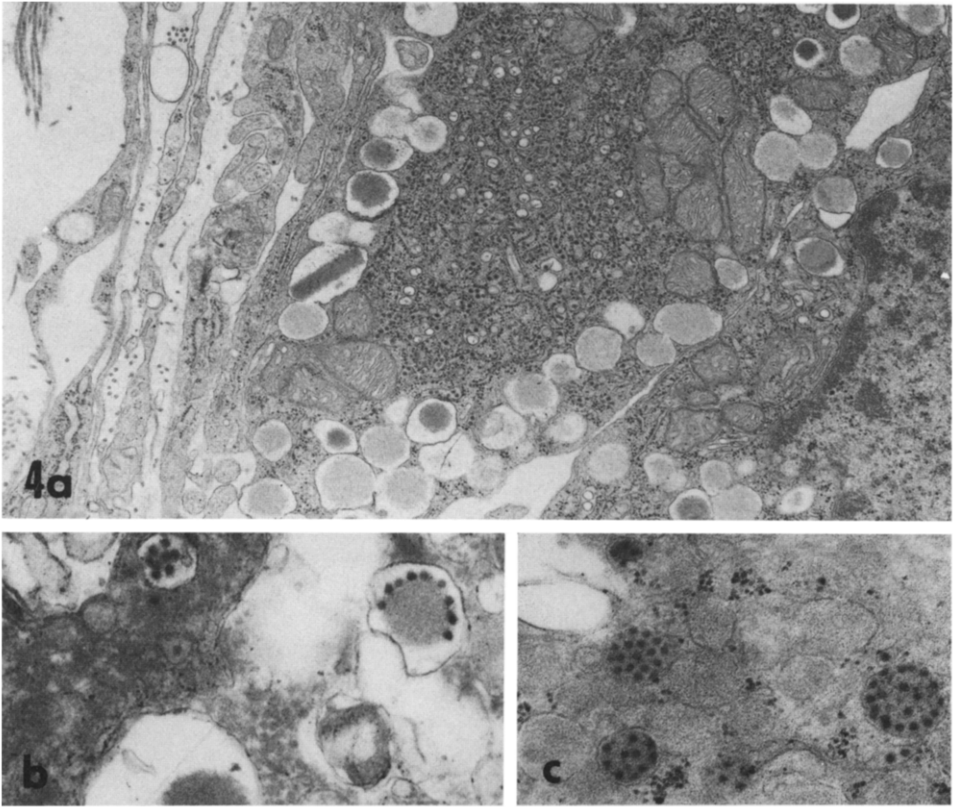FIG. 3. Centroacinar and duct cells are also infected and often contain myelin-like figures. ×16,630.

FIG. 4. Severe, virus-specific changes are evident in all cell types in the islets. (a) Viral accumulations in a beta cell have relegated the granules to a peripheral location. ×15,350. (b) Viral particles are clustered around the secretory product of an alpha cell granule. ×38,240. (c) Viral particles accumulate within delta cell granules in a manner similar to that seen in zymogen granules. ×37,600.

in which numerous virus particles and related inclusions accumulated (Fig. 6). These changes tended to push the myofibrils to the margins of the myocytes (Fig. 7). Occasionally such myofibrils retained some semblance of normal striations, but more frequently they were badly disrupted and were randomly scattered. Virus particles were also found within the Golgi cisternae and between the lamellae of the nuclear envelope of myocytes.

Macrophage activity was noted in sites of myocardial necrosis; many infiltrating macrophages were filled with phagocytized cellular debris (Fig. 8). In some vessels located near sites of myocardial damage, there was endothelial infection marked by cellular swelling and extension into the vascular lumen. Smooth muscle layers of the vessels were infected focally, and such sites were associated with perivascular mononuclear inflammatory infiltration.

Small Intestine

Lesions were similar in all three areas of the small intestine in each of the animals examined. Membrane-bound, complex structures (phagosomes) were common within the intestinal epithelium (Fig. 9), and occasional infected absorptive cells were observed (Fig. 10). No infected goblet cells were seen.

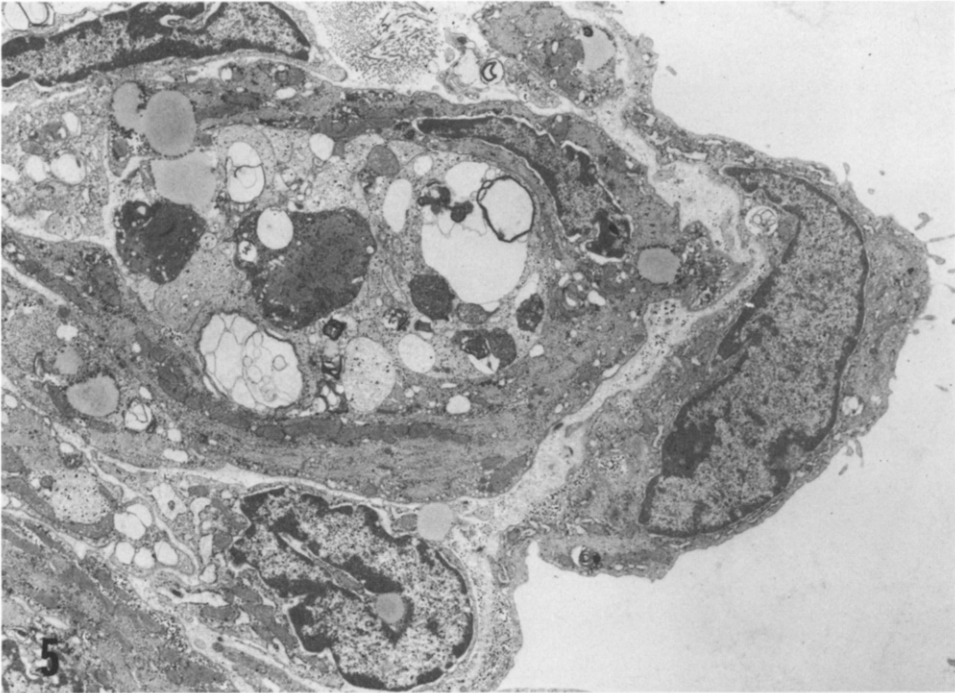Lesions were most pronounced in the intestinal lamina propria, where nearly

FIG. 5. Myocardial degeneration and necrosis is severe, with vacuolization, myelin-like inclusions, and large lipid droplets. ×5400.
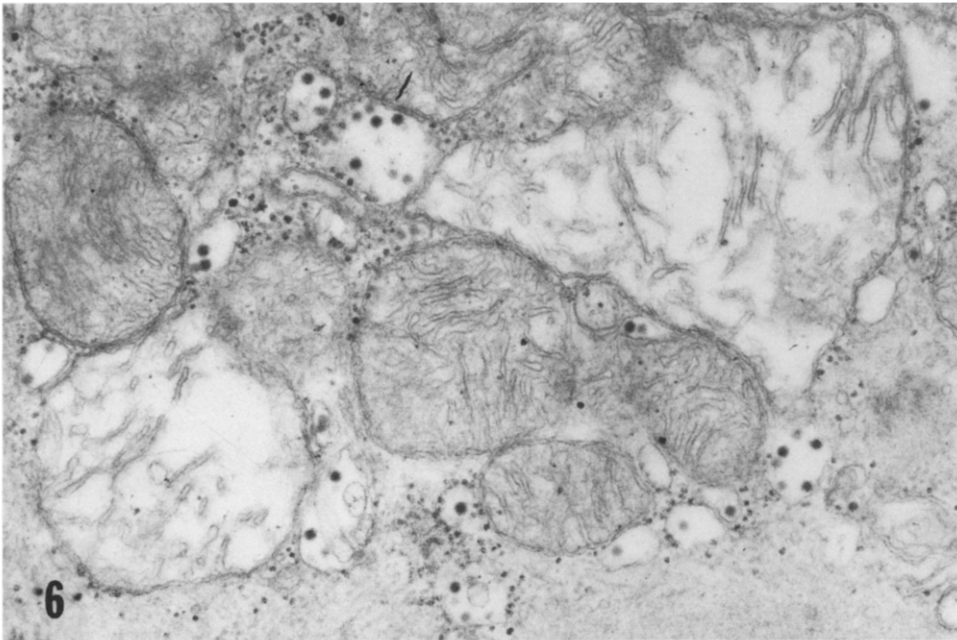

FIG. 6. A higher magnification of a portion of an infected myocyte shows the characteristic pale, swollen, vesiculated mitochondria and virus particles within cisternae of the sarcoplasmic reticulum. ×40,500.
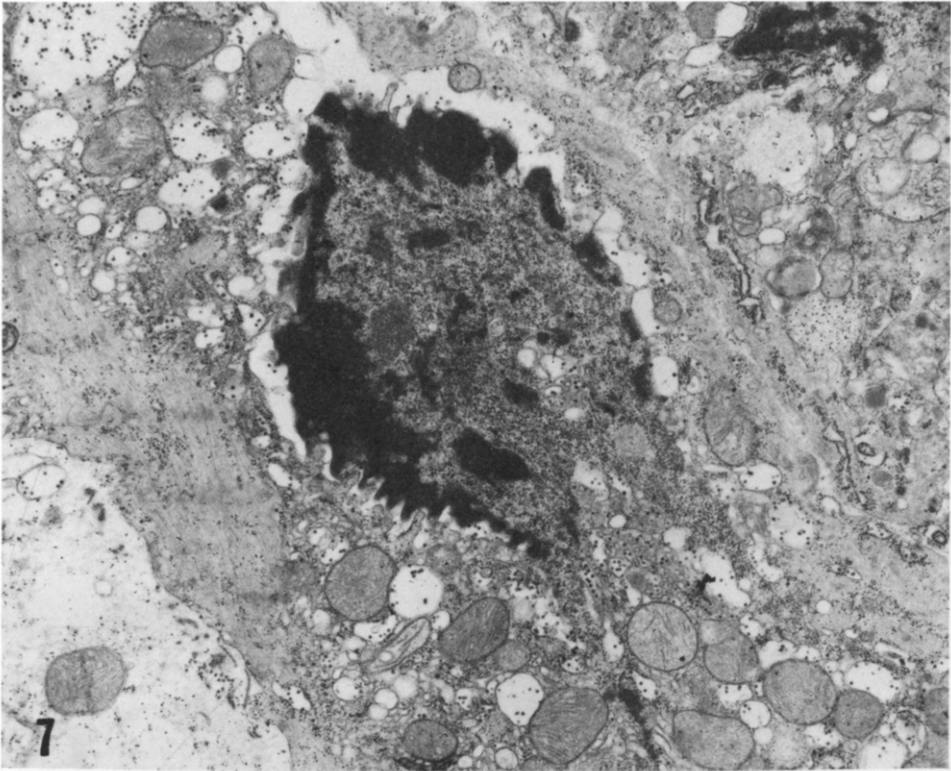
FIG. 7. The myofibrils are pushed to the margins of the myocyte by the accumulations of virus particles, but they still retain a faint semblance of striation. ×11,260.
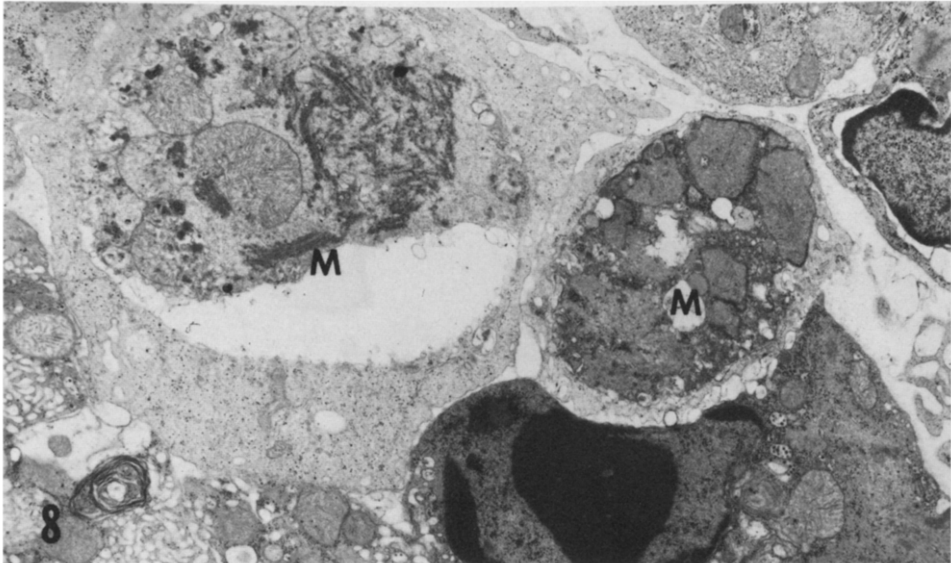

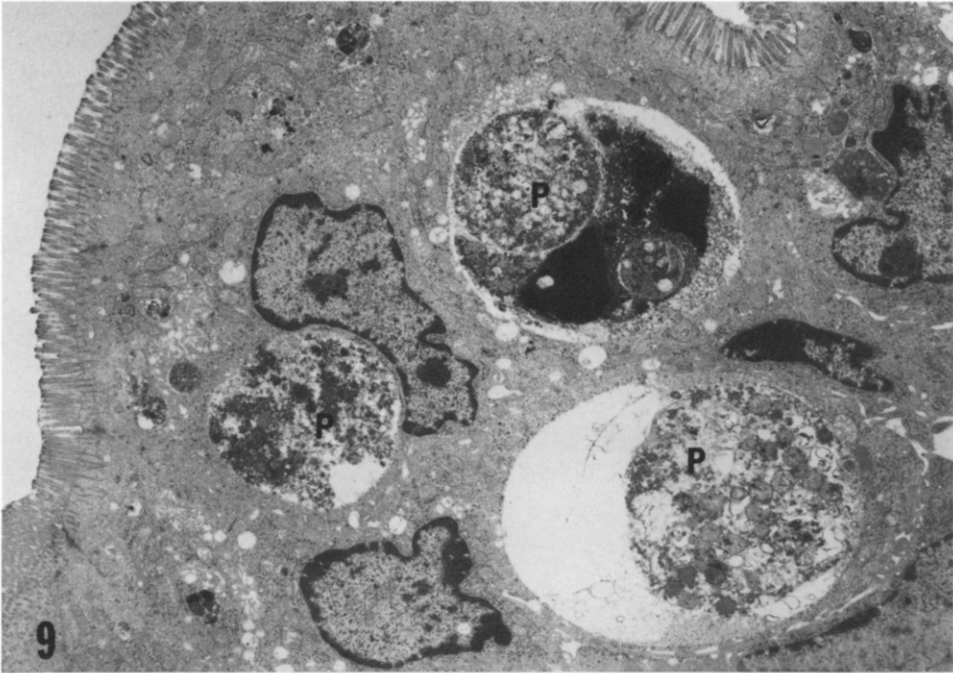FIG. 8. Phagocytized cellular debris nearly fills two macrophages (M) seen near some infected myocytes. ×9125.

FIG. 9. Complex membrane-bound structures, or phagosomes (P), are common in the absorptive cells of the small intestine. ×4800.
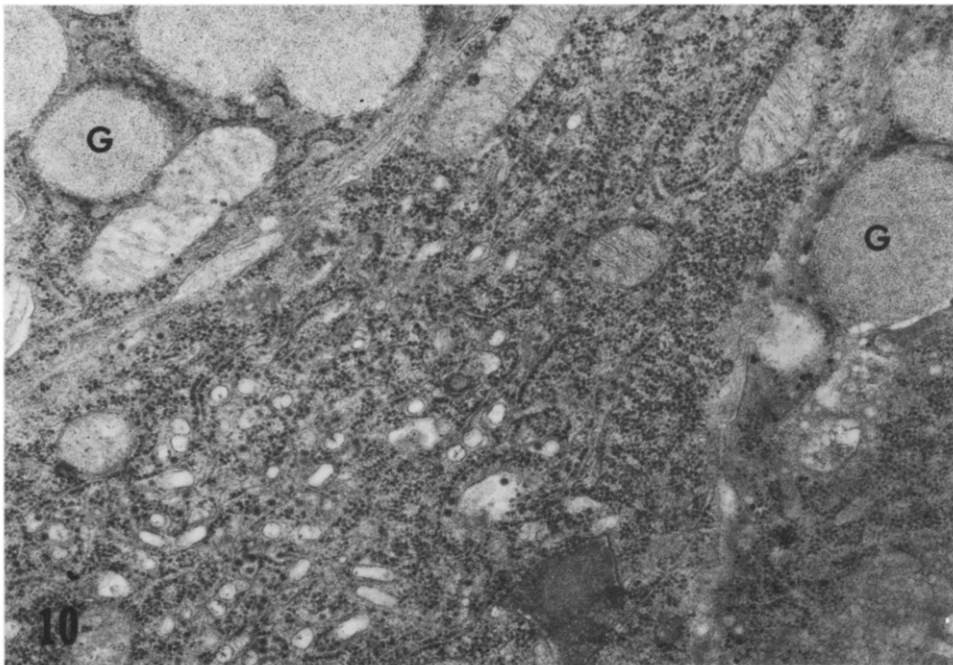

FIG. 10. A rare infected absorptive cell is located between two apparently normal goblet cells (G). ×20,070.

every cell type contained virus. Destruction was so severe in some areas as to make it virtually impossible to identify with certainty many of the cells involved. The endothelial cells of some of the blood vessels were infected (Fig. 11), as were scattered monocytes, macrophages, and fibroblasts (Fig. 12). Nerve endings throughout the lamina propria contained virus particles, and all parasympathetic ganglion cells observed were heavily infected. The muscularis mucosae were heavily infected as well. The centers of many of these myocytes were filled with proliferated sarcoplasmic reticulum containing virus particles and associated membranous structures. Within such myocytes, myofilaments were fragmented and disorganized, mitochondria were pale and swollen, nuclear chromatin was clumped and marginated, and large vacuoles and myelin-like figures were prominent.

Skeletal Muscle

Pathology in skeletal muscles was similar to that seen in cardiac and smooth muscles. Proliferation of sarcoplasmic reticulum and virus accumulations were most pronounced at neuromuscular junctions.

DISCUSSION

Nearly 50 years have passed since St. Louis encephalitis was first described. In spite of the numerous investigations of the natural history of SLE virus—its structure and chemistry, the pathology and pathogenesis of the infection in humans and experimental animals, the ecology of its natural transmission cycle, and the epidemiology of its impact on populations—the disease continues to be a major problem in many areas of North America (Chamberlain, 1980).

Most reports on the pathology of SLE in humans and in experimental animals have dealt only with the CNS, and because most fatal human cases have been in the older age groups, extraneural changes have been attributed to underlying preexisting conditions rather than to specific consequences of the virus infection per se. For example, even though acute changes were seen in the kidneys of one-third of the fatal cases in the initial outbreak in St. Louis, Missouri (swelling and intense congestion of the blood vessels with petechial hemorrhages in the pelvic mucosa) (Cumming *et al.*, 1935), little has been done to determine the basis of these changes. In the 1962 SLE epidemic in Florida, cerebral vascular lesions were found in most of the 43 fatal cases, but only 3 patients had known preexisting hypertension, 2 others were diabetics, and one was a chronic alcoholic. There was no demonstrable underlying illness in the other 37 cases (Quick *et al.*, 1965), but again, a mechanistic relationship to the SLE virus infection was not determined.

The ability of other flaviviruses to infect organs and tissues in addition to, or instead of, the CNS has been well documented. Yellow fever virus is best known for its severe necrotizing effect on the liver and kidneys. However, degenerative changes in the heart have also been reported (Clarke and Casals, 1965). Chief target organs of Kyasanur Forest disease virus were the gastrointestinal tract (Work, 1958) and bronchioles (Webb and Rao, 1961). Japanese encephalitis virus, known chiefly for its severe damage to the CNS, has involved the heart, adrenal glands, and gastrointestinal mucosa of some patients (Grascenkov, 1964; Miyake, 1964). Fatal human cases of dengue hemorrhagic fever had some common aspects of pathology, in particular: affection of the walls of blood vessels, causing conges-
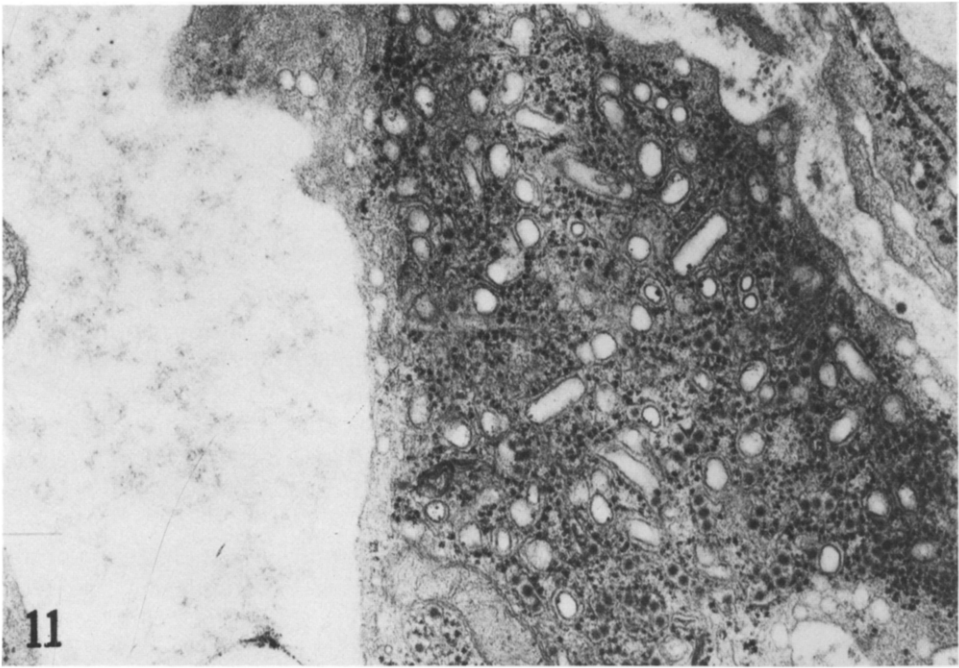
FIG. 11. Portion of an endothelial cell of a small blood vessel is filled with virus particles and lucent vesicles. ×33,400.
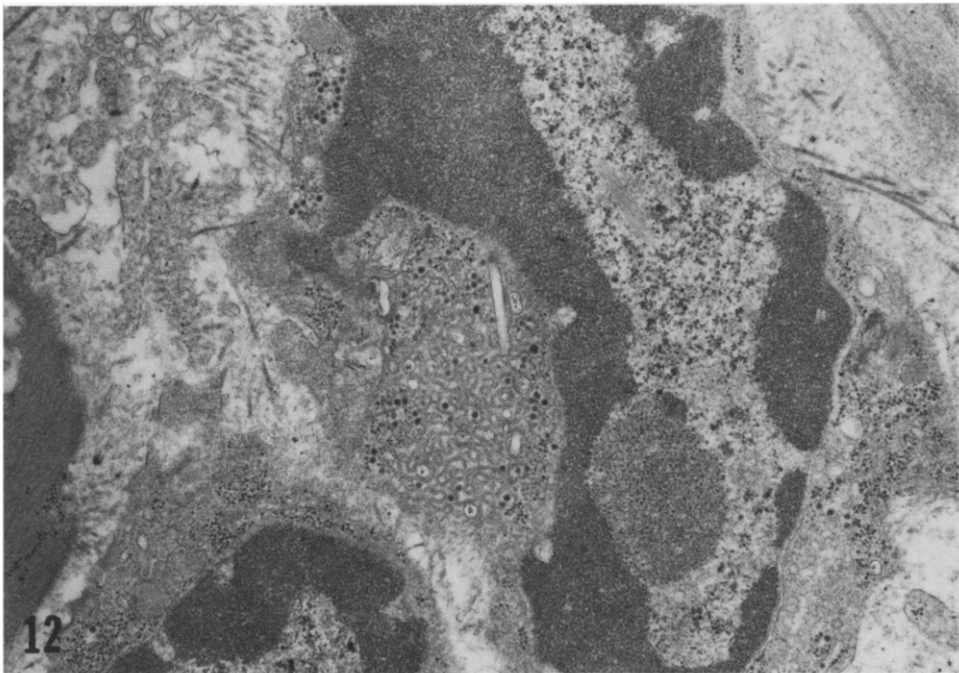

FIG. 12. Portions of two monocytes in the lamina propria of the ileum contain virus particles within cisternae of the ER. ×22,100.

tion, hemorrhage, and edema; and degeneration of parenchymatous cells, especially of the liver (Hotta, 1969).

Immunofluorescence studies of mice experimentally infected with tick-borne encephalitis virus indicated that, in addition to the CNS, the peripheral nervous system, the special sensory nerve cells of the retina and olfactory mucosa, the secretory glands, the tubular epithelium of the kidney, and both striated and smooth muscle tissue were affected (Albrecht, 1960). Immunofluorescence studies of West Nile virus infection in experimentally inoculated suckling mice produced similar results (Kundin, 1963).

A recently described flavivirus, Rocio virus, which was the etiologic agent of an epidemic of human encephalitis in Sao Paulo State, Brazil (Lopes et al., 1978), caused severe pancreatic and myocardial necrosis in addition to severe CNS damage in neonatal mice and hamsters (Harrison et al., 1980). In the present study, the pattern of SLE virus infection of the pancreas of hamsters was similar to that found in Rocio virus infection, differing in severity only in the islets of Langerhans where all cell types were involved, whereas Rocio virus affected only the beta cells.

SLE virus utilizes the cell's pathway of protein synthesis in its infection of the pancreas. Virus particles were first seen in cisternae of the endoplasmic reticulum and then in the Golgi complex where they were packaged with the cell's secretory products and were seen in the granules of both the endocrine and exocrine portions of the pancreas. Such a phenomenon seems to be unique to the flaviviruses. Earlier studies of pancreatic lesions in mice infected with Coxsackie B3 virus (Harrison et al., 1972) or with Venezuelan equine encephalomyelitis (VEE) virus showed no such association of virus with secretory granules (unpublished observations).

Reviews of the role of viruses in the pathogenesis of pancreatic disease and diabetes mellitus implicate many other viruses in addition to Coxsackie B viruses and VEE virus: they include foot-and-mouth disease viruses, encephalomyocarditis (EMC) virus, reoviruses, mumps virus, rubella virus, oncornaviruses, murine hepatitis virus, cytomegalovirus (CMV), EB virus, and varicella virus (Craighead, 1972, 1975; Notkins, 1977; Rayfield and Seto, 1978).

The role of viral infections in human myocarditis has received considerable attention in recent years (Abellman, 1971; Overall, 1972; Lerner and Wilson, 1973; Lansdown, 1978). Coxsackie viruses and, to a lesser extent, rubella virus are significant causes of myocarditis in adults and, even more important, in young children. In experimental animals, CMV, Coxsackie B3 and 4, reovirus type 1, murine adenovirus, vaccinia (Rabin and Jenson, 1967; Lansdown, 1978), VEE (Garcia-Tamayo, 1973), and Rocio virus (Harrison et al., 1980) all have been shown to produce myocardial infection. SLE virus appears to be particularly cardiotropic, being able to replicate in nearly all cell types of this organ.

In most reports about viral infections of the intestine, investigators have emphasized cytopathic changes in the epithelial cells, which result in diarrhea (adenoviruses: Yunis et al., 1975; rotaviruses: Gray et al., 1973; Holmes et al., 1975; Suzuki and Komo, 1975; Snodgrass et al., 1977; Pearson and McNulty, 1978; Rodriguez-Toro, 1980; and coronavirus: Doughri et al., 1976; Takeuchi et al., 1976). In the present study, SLE virus was found to primarily infect the lamina propria of the intestine, where it replicated in blood, endothelial, nerve, and muscle cells, and only rarely involved the intestinal epithelium.

Unlike other flaviviruses such as yellow fever, tick-borne encephalitis, dengue, and West Nile viruses which are regularly isolated from serum during the first week of onset of symptoms, SLE virus is seldom recovered from the blood because of the extremely short period of viremia (Hammon and Sather, 1969; Calisher and Poland, 1980). Attempts to recover SLE virus from the cerebrospinal fluid of patients with CNS symptoms have also failed, raising questions concerning its continued mode of spread within the body.

Immunofluorescence antibody studies have indicated that some flaviviruses may replicate in blood vessels and thus grow across the vascular wall (Albrecht, 1960; Kundin, 1962). Our electron microscopic studies of both Rocio and SLE viruses show that with these two viruses this is indeed the case. The nervous system may also be involved in their spread, and this possibility remains to be explored further.

ACKNOWLEDGMENTS

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