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Encephalomyocarditis (EMC) Virus Infection of the Femoral Artery of Newborn Mice[1] (38271)

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The encephalomyocarditis (EMC) virus is highly infective in newborn mice. It has been shown with light and electron microscopy to damage readily the myocardium (1–4), cardiac valves (5), endocardium (6), aorta (7), kidney (8), pancreas (9–11), and liver (12). The damage observed ultrastructurally consists of vesiculation and vacuolization of cytoplasmic membranes, with the formation of membrane–vesicle complexes, dilatation of rough endoplasmic reticulum, margination of nuclear chromatin and/or pyknosis of nuclei in damaged cells, and inflammation.

Because of our observation that EMC virus will infect the aorta of newborn mice (7), we were interested in learning if smaller arteries were similarly affected by infection with EMC virus. Consequently, we extended our studies to include the femoral artery of the newborn mouse. We believe that verification of infectivity of the femoral arteries with EMC virus is important since viral infections of arteries may lead with healing to scar formation of various types. The importance of this concept to the development of arteriosclerosis in man has already been noted (13).

Methods and Materials. A total of six newborn mice of a random HaM/ICR strain was used. Five of the mice were inoculated intraperitoneally with 0.05 ml EMC virus culture fluid with a titer of 10^{-6} $TCID_{50}$, and one mouse was inoculated with virus-free culture fluid to serve as a control. All mice were killed 24 hr after inoculation. After spinal dislocation, the skin and muscle of the leg were dissected away to expose the femoral vessels to the fixative. The portion of the leg with the femoral artery and vein intact was placed in cold 3% phosphate-buffered glutaraldehyde for 2 hr. Following buffer rinses, excess tissues and bone around the femoral vessels were removed using a dissecting microscope. The exposed femoral vessels were placed in cold 1% osmium tetroxide in phosphate buffer for 1.5 hr. Dehydration was accomplished with a series of increasing concentrations of cold methanol, beginning with 50% methanol and ending with three rinses of absolute methanol at room temperature. Embedment was in epoxy resin in flat molds so as to achieve cross-sectional orientation. Thick epoxy sections were cut and stained with toluidine blue to ascertain tissue orientation. Thin sections were cut from selected trimmed areas which contained portions of the femoral artery. Serial thick sections following thin sectioning verified which vessels were contained in sections viewed with the electron microscope and aided in recognition of the orientation of the vessels under the electron microscope. Thin sections were stained with uranyl acetate and lead citrate and examined with a Siemens Elmiskop I electron microscope.

Results. Control study. The wall of the femoral artery of the control mouse consisted of a single endothelial layer, a tunica media composed of 2–4 layers of smooth muscle cells, and a loosely constructed tunica adventitia. A well-formed, circularly arranged internal elastic lamina separated the endothelium from the tunica media. The internal elastic lamina was usually convoluted, with projections from the endothelial cells and smooth muscle cells interdigitating into the convolutions. The smooth muscle cells overlapped and interdigitated at

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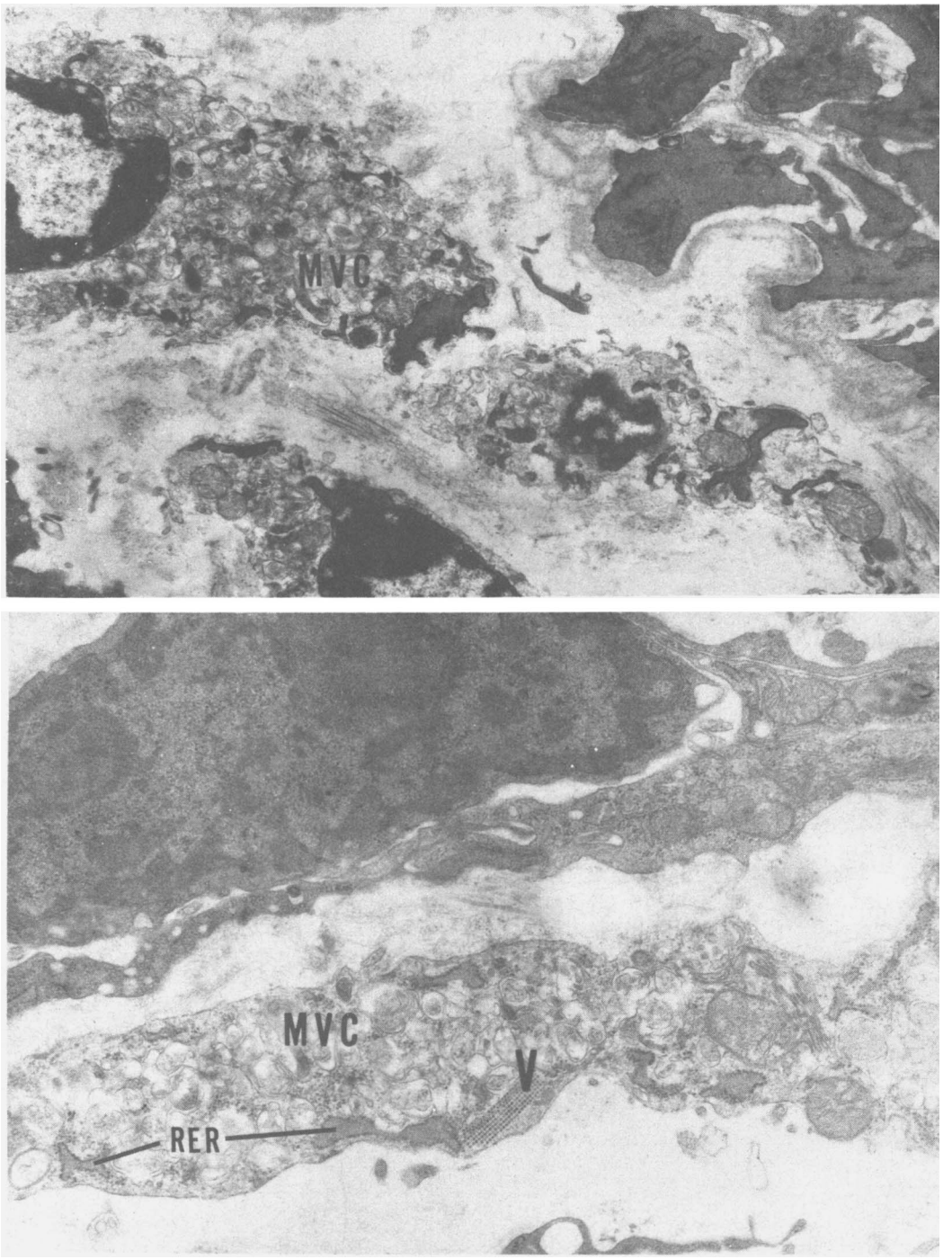

FIG. 1. Electron micrographs of the tunica adventitial layer of the femoral arteries of newborn mice infected with encephalomyocarditis virus and killed 1 day later. (A) Margination of nuclear chromatin with early pyknotic nuclear changes in fibroblasts is seen. The cellular architecture is markedly disrupted and cytonecrosis (MVC) is evident. × 11,400. (B) A viral crystalline aggregate (V) in an adventitial fibroblast is associated with numerous membrane–vesicle complexes (MVC). The rough endoplasmic reticulum (RER) is fragmented and dilated, with electron dense material within the cisternae. × 20,000.

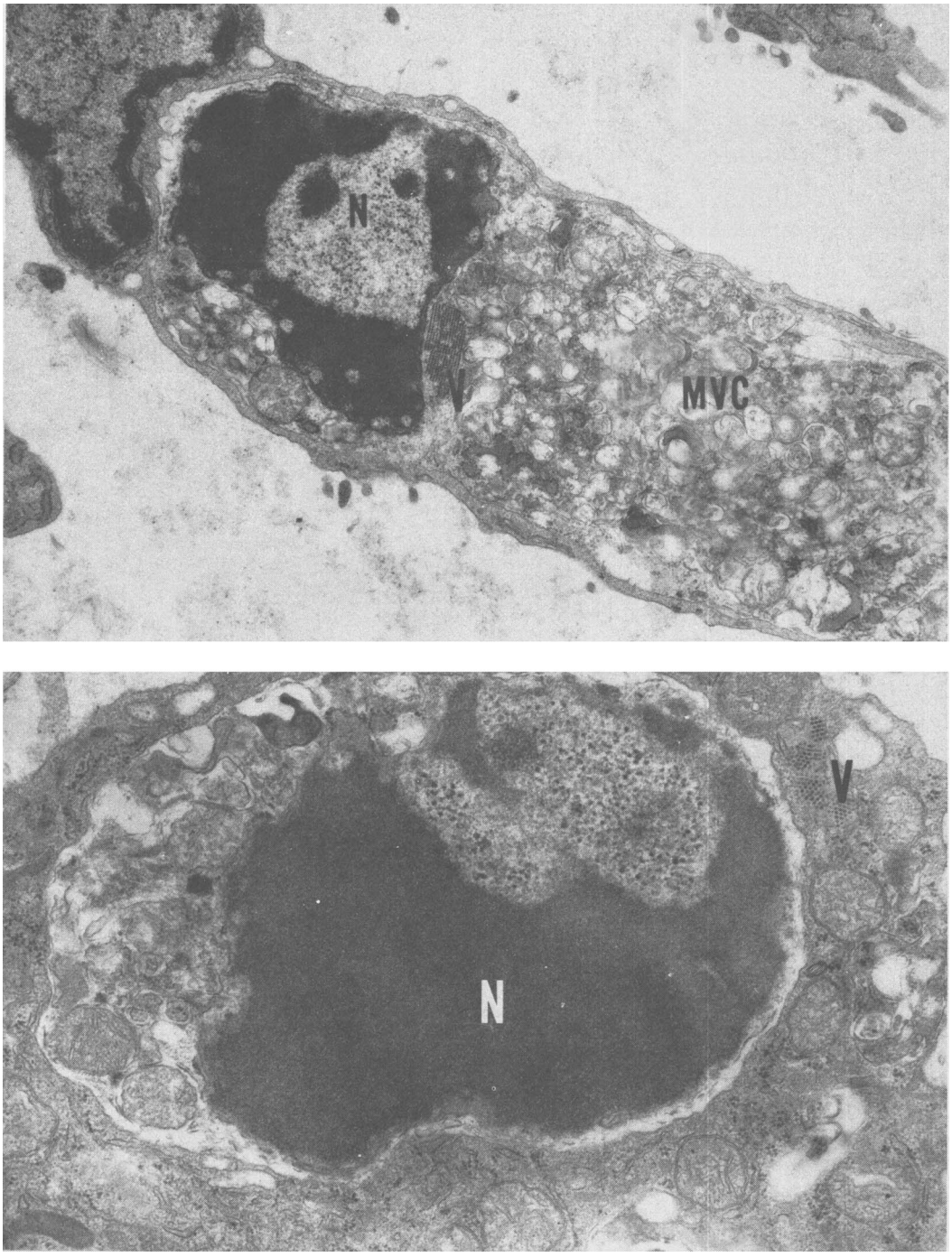

FIG. 2. Electron micrographs of macrophages observed in the adventitia of the femoral artery of an EMC virus infected newborn mouse 1 day after inoculation. (A) A degenerative cell is enclosed in a large autophagic vacuole of this macrophage. The nucleus (N) of the engulfed cell is pyknotic. A perinuclear virus crystal (V) is present. There are numerous membrane–vesicle complexes (MVC) within the degenerative cytoplasm. × 15,200. (B) Autophagic vacuole of a macrophage is shown with a pyknotic nucleus (N) and degenerative cytoplasm of the engulfed cell. Within the cytoplasm of the macrophage are crystalline aggregates of virus (V). × 28,000.

their ends with other smooth muscle cells. An external elastic lamina separated the tunica media from the tunica adventitia. This lamina was thinner than the internal one and did not appear as a continuous band around the circumference of the artery, as did the internal elastic lamina.

Viral study. As observed electron microscopically, the nuclear chromatin of medial smooth muscle cells was frequently marginated in the femoral arteries of mice infected with EMC virus. Also, collagen fibers were observed between the endothelial lining and the internal elastic lamina and surrounding the external elastic lamina. Fibroblasts of the adventitial tunic were commonly degenerative. Frequently, there was marked margination of nuclear chromatin and nuclear pyknosis of adventitial fibroblasts. In such cases, cellular disruption was common (Fig. 1A). In four of the animals studied, viral crystals were observed (Fig. 1B) within adventitial and loose connective tissue fibroblasts in which picornaviral cytonecrosis was apparent. The most prominent feature of such cytonecrosis was the abundance of membrane–vesicle complexes containing cytoplasmic material (Fig. 1B). The rough endoplasmic reticulum was fragmented and occasionally dilated. Macrophages were occasionally observed in the adventitia and loose connective tissue surrounding the femoral artery (Fig. 2A, 2B). In some cases, small to large portions of degenerative cells with pyknotic nuclei were observed within autophagic vacuoles of macrophages. Characteristic picornaviral cytonecrosis, consisting of abundant membrane–vesicle complexes, was associated with a perinuclear viral crystal in one engulfed cell (Fig. 2A). Viral crystalline aggregates were also observed within the macrophage cytoplasm (Fig. 2B).

Discussion. Cytonecrosis consistent with the cytopathic effects seen in infections with other picornaviruses (14–17) were observed in adventitial and loose connective tissue fibroblasts of the femoral artery of newborn mice infected with encephalomyocarditis virus. Viral infection of medial smooth muscle cells, as observed in the aorta of EMC virus infected mice (7), was not observed in the femoral artery of the animals studied here. The cytonecrosis consisted of apparent membrane proliferation with the formation of numerous membrane–vesicle complexes, fragmentation and dilatation of rough endoplasmic reticulum, margination of nuclear chromatin in smooth muscle cells and margination and/or pyknosis of chromatin in adventitial and loose connective tissue fibroblasts, and involvement of macrophages in the adventitial space. Viral crystalline aggregates were observed in the adventitial fibroblasts of 4 of the 5 animals inoculated with EMC virus, as well as in a macrophage of one of these animals. These findings verify that infection with encephalomyocarditis virus occurred in the wall structure of the femoral arteries.

The increase in collagen fibers around the internal elastic lamina and the presence of phagocytosing macrophages around injured arteries may suggest an irritation or inflammation of the arterial wall. Such early inflammation, which may indicate a toxic reaction, appears to be initiated in the adventitia of the vessel. Because adventitial cells are shown to be infected with EMC virus, it can be speculated that the virus was the cause of the injury observed.

Summary. The femoral arteries of newborn mice which were experimentally infected with encephalomyocarditis (EMC) virus were studied electron microscopically. Viral crystalline aggregates were observed within the cytoplasm of adventitial fibroblasts in 4 of 5 animals studied and within a macrophage of one animal. The nuclear chromatin of medial smooth muscle cells was frequently marginated, and margination or pyknosis of nuclear material was observed in fibroblasts of the tunica adventitia.

Cytonecrosis typical of infection with picornavirus was observed in adventitial fibroblasts. The most prominent feature of this cytonecrosis was the abundance of membrane–vesicle complexes. The rough endoplasmic reticulum was occasionally dilated and fragmented. Macrophages were present in the adventitial and loose connective tissue surrounding the artery. There was an increase of collagen fibers around the internal elastic lamina, which may suggest an irritation or inflammation of the arterial wall. Such early inflammation, which may indicate a toxic reaction, may be the result of viral injury.

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