

Coxsackie B₄ Virus Crystal Formation in Mouse Pancreas¹ (38921)

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The Coxsackie B₄ virus is a highly infectious agent for man. It is known to cause damage to many organs of the body (1-5). However, the actual presence of Coxsackie B₄ virus crystals within any of these organ tissues has never been demonstrated previously. This report shows for the first time crystalline configurations of Coxsackie B₄ virus within animal tissue.

Materials and Methods. Twenty-three newborn mice were inoculated intraperitoneally with 0.01 ml Coxsackie B₄ virus culture fluid with a high titer of 10^{-8.5}TCID₅₀. The mice were killed 1 day later and the pancreases, as well as other organs, were removed for study. The pancreatic tissues were prepared for ultrastructural study as previously described (6) and examined with a Siemens Elmiskop I electron microscope.

Results. Coxsackie B₄ virus crystals were located in the pancreas of three of the 23 newborn mice studied (Fig. 1). In addition, intracellular necrosis (7) characterized by membrane-vesicle complexes and dilated rough endoplasmic reticulum (Fig. 1) was observed in association with the viral crystals.

Discussion. For the first time crystalline configurations of Coxsackie B₄ virus were found in experimental animal tissue. These crystals have never been observed or reported previously in tissues of any kind in any animal. Unlike other Coxsackie viruses (6, 8, 9) and similar picornaviruses (10-13), Coxsackie B₄ virus was never shown to form crystalline aggregates. Without crystal formation it is very difficult to prove that viral agents invade and damage the tissues of man and other animals. Heretofore, we have had

to rely on immunofluorescent antibody staining techniques to verify the presence of Coxsackie B₄ virus in tissues (14-16) and to correlate tissue cytopathology with viral infections. This finding of Coxsackie B₄ viral crystals in the pancreas of experimentally infected mice is important, therefore, because it proves without doubt that this virus, which is highly infectious for man, does directly invade tissues, at least the pancreas of mice.

It has already been shown that Coxsackie B₄ virus (4), as well as other picornaviruses (6, 17-19), causes damage to the pancreas. Virus induced pancreatitis in experimental animals, coupled with recent evidence of hyperglycemic state in mice (unpublished data), supports the contention that viruses are important etiologic agents in pancreatic disease in man and even possibly a cause of human diabetes mellitus. The observation of Coxsackie B₄ virus crystals in the pancreas of mice in the study reported here is additional support for that contention. Furthermore, the intracellular cytopathology observed in association with these crystals is characteristic of damage produced by viruses, at least by those of the picornavirus group (7, 8, 17, 20).

The crystalline pattern demonstrated here for Coxsackie B₄ virus is similar morphologically to crystals previously demonstrated for Coxsackie group A (8, 9) and B (6, 17) viruses and for encephalomyocarditis virus (10, 20). The individual viral particles of the crystal, however, are slightly smaller in diameter (approximately 170 Å) than those of other picornaviruses.

The finding of viral crystals in tissues of experimentally infected animals makes it possible to correlate ultrastructural cytopathology with viral infections. This, of course, is true also for Coxsackie B₄ virus, which is extremely infectious for man. There is a need, therefore, to learn the "condi-

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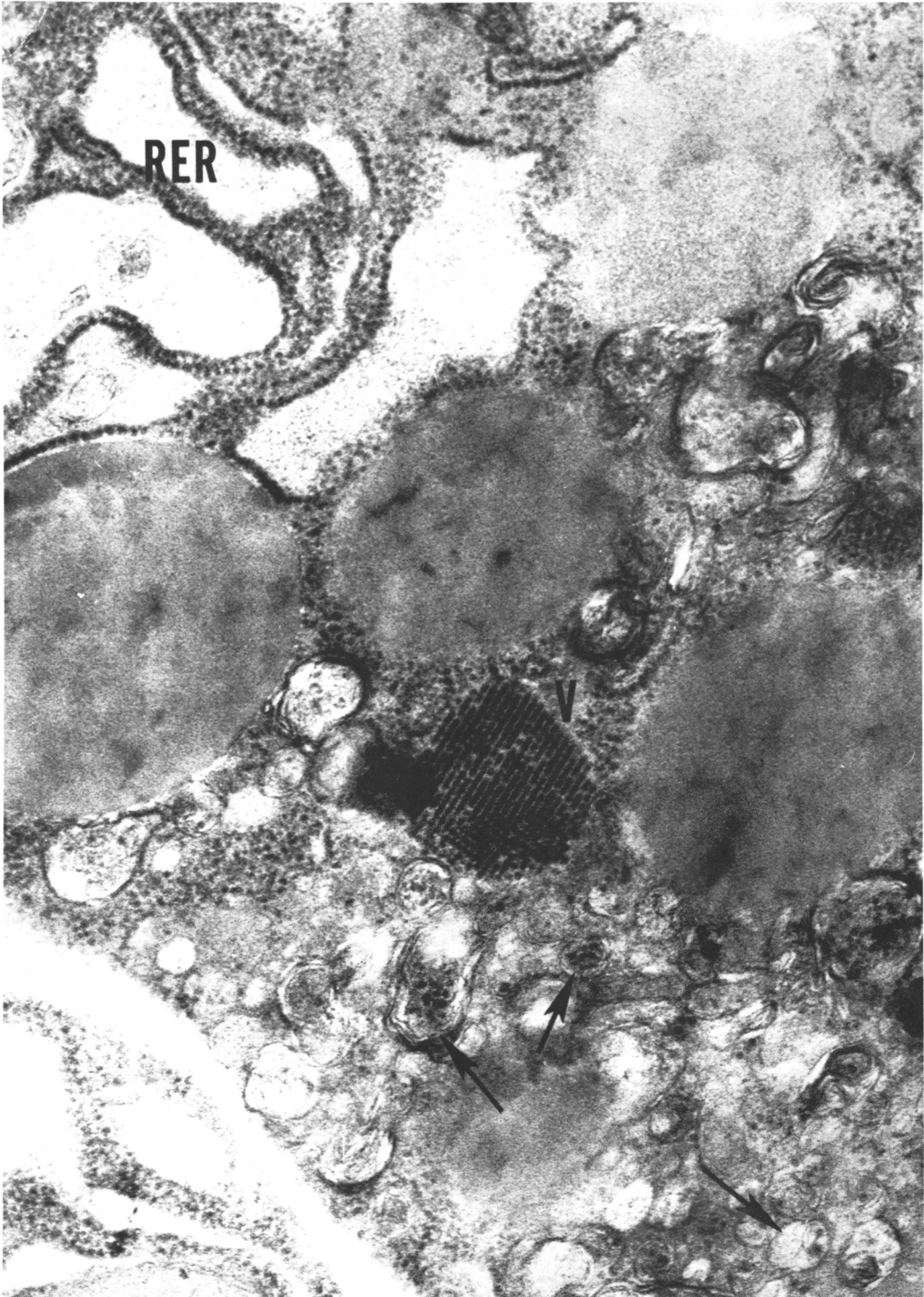


FIG. 1. Portion of a pancreatic acinar cell of a newborn mouse infected with Coxsackie B₄ virus culture fluid and killed 24 hr later. A crystal of Coxsackie B₄ viral particles (V) is located within a necrotic area which is characterized by the formation of membrane-vesicle complexes (arrows) and dilated rough endoplasmic reticulum (RER). Magnification $\times 61,500$.

tioning" factors responsible for crystal formation of this virus.

Summary. For the first time, Coxsackie B₄ virus crystals are demonstrated in tissues of animals inoculated with the virus. Until this report, Coxsackie B₄ virus had never been found to form crystalline aggregates in tissues of any kind. The viral crystals which were located in the pancreas of newborn mice were associated with ultrastructural damage to the pancreas not observed in control animals, thereby indicating that viruses directly invade and damage these tissues. Thus, this virus must be considered among etiologic factors in pancreatic disease.

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