Coxsackievirus Infection in Skeletal Muscles of Mice
An Electron Microscopic Study

II. Appearance and Fate of Virus Progeny

By

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With 10 Figures

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Summary

Different arrangements of coxsackievirus A1 in striated muscle of new born mice display different fates of the virus progeny:

1. Two-dimensional crystals and rows of virus particles between membranes represent the mechanism, by which virus is continuously released from the cell. Such formations are always found at the periphery of the cell, virions being thereby transported more or less directly from a nuclear pore to the sarcolemma. By this way, infection is spread from one cell to another.

2. Larger or smaller virus crystals in vacuoles are non-released virions, which have been accidentally trapped by autophagic vacuoles. Later on they are converted into phosphatase positive autolysosomes.

The possible sites of virus synthesis as well as some aspects of cellular defense are discussed.

1. Introduction

As shown previously (K. Bienz, G. Bienz, Weiss and Loeffler, 1969) coxsackievirus is found in different arrangements in infected striated muscle. In the present paper these different manifestations of the viruses, above all (a) virus particles in rows between membranes and (b) virus particles enclosed into vacuoles will be brought into relation with the previously described cellular alterations (see our first publication of this series). Thus, the "functional aspects" of the mentioned virus arrangements, especially the synthesis and release of virus as well as the cellular defense will be elucidated.

2. Materials and Methods

The same virus strain, animals and inoculation procedures as well as electron microscopic techniques were used as previously described (K. Bienz, G. Bienz, Weiss and Loeffler, 1969).
The demonstration of acid phosphatase was done, after fixation of the tissue in 2.5% glutaraldehyde, by the method of Gomori (1952) at a pH of 5.0, omitting the conversion of lead phosphate into lead sulfide. Sections of tissue, treated in such a way, were not stained with lead citrate in order to avoid a confusion of phosphatase reaction product with lead deposits of the stain.

3. Results

3.1. Appearance and Release of Virus Progeny

One possible aspect of coxsackievirus in infected muscle cells, as mentioned earlier, consists of a row or a two-dimensional crystal of virions on or between membranes. These configurations are always found at the periphery of a cell near a nucleus. As shown by Fig. 1, such membranes may lead from the nucleus directly to the sarcolemm. By this way the virus particles between these membranes are released from the cell. Careful examination of the membranes in this region shows, that the viral particles are lying in the cytoplasm and not inside the ER, being therefore in direct connection with the nuclear pore. A similar situation is found in Fig. 2, showing two rows of particles reaching from close proximity of a nuclear pore to the sarcolemm where they are released. In this case no direct connection of the membranes with the nuclear membrane is noted.

We must emphasize here, that virions have never been found inside the nucleus, but only in nuclear pores as shown by Fig. 3. This section is the first of a series, which also shows that virions are not only released from the cell through straight channels but also by complicated membrane formations such as double-walled tubes. Figure 4 shows the fifth section of this series with the virus containing tube in the middle between the nucleus and the sarcolemm. In Fig. 5 the same tube, six sections further, has reached the cell membrane. In releasing the virus particles, the virus containing membranes apparently break and re-fuse with the sarcolemm, forming thus an orifice.

The release of virus progeny begins about 10 hours after infection and lasts rather long, i.e. about another 10 hours until complete cell destruction. If one individual virus releasing membrane formation is indeed active during the whole time, can of course not be decided.

3.2. Cellular Defense

Very characteristic in enterovirus infected cells is a strongly vacuolated cytoplasm. These vacuoles, originated from the ER, are also found in coxsackievirus infected muscles. These formations are of three different types:

1. primary lysosomes;
2. autophagic vacuoles;
3. autolysosomes, resulting from fusion of 1. and 2.

Of course, the existence of other types of vacuoles with other functions can not be ruled out.

Figure 6 shows the first type of vacuoles, namely primary lysosomes. They contain among other enzymes, acid phosphatase as demonstrated by the electron-dense contents produced by the Gomori reaction.

Figure 8 shows the second type: autophagic vacuoles. They are phosphatase negative and are apparently homologous with the so-called small bodies (Dales,