The basic tenet of the membrane theory is contradicted in that, in an intact living cell, the Na^+, which has substituted for K^+, is not free but adsorbed. Thus, in agreement with the association-induction hypothesis (*3–5*), most of the intracellular K^+ is adsorbed. When K^+ is low in the external medium, the cell loses its intracellular K^+. The adsorption sites thus vacated are occupied by Na^+ (*5*) which may be expected to be NMR-invisible (*14*); and it was.

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Poliovirus Crystals within the Endoplasmic Reticulum of Endothelial and Mononuclear Cells in the Monkey Spinal Cord

Abstract. *The lumbar motor columns of a cynomolgus monkey that had become tetraplegic after experimental infection with a highly virulent strain of type 3 poliovirus were examined by electron microscopy. Crystalline aggregates of poliovirus occurred within the endoplasmic reticulum of endothelial cells as well as of mononuclear inflammatory cells. This finding suggests that the endoplasmic reticulum might be much more involved in poliovirus multiplication than has been previously supposed.*

The findings described here were obtained in an adult cynomolgus monkey that had developed a severe tetraplegia following an intramuscular injection with 2×10^7 TCD_{50} (tissue culture dose, 50 percent effective) of a highly virulent strain of type 3 poliovirus (*1*). Six days after the infection the anterior columns of the lumbar spinal cord were examined by electron microscopy (Zeiss EM 9A), for which conventional fixation and embedding methods (glutaraldehyde, osmium tetroxide, and Epon 812) were used. The remainder of the spinal cord was examined by routine histological methods and by a fluorescent antibody technique.

Histological sections of the lumbar spinal cord showed an almost total loss of motor neurons and a focal-to-diffuse infiltration of the severely damaged anterior horns by inflammatory cells, the predominating types of which were mononuclear elements, including mature macrophages. By the aid of immunohistochemical examinations, the presence of poliovirus antigen could be clearly demonstrated within the walls of intraspinal blood vessels, as well as within the cytoplasm of many mononuclear cells. Electron microscopic study of this process offered interesting additional information. Within the cytoplasm of many endothelial cells, monocytes, histiocytes, and macrophages there occurred aggregates of dense particles of uniform size. These particles appeared spherical or polyhedral in shape, measured about 270 Å in diameter, and were frequently arranged in ordered lattices, the three-dimensional character of which became obvious by their repeated presence in serial sections. Within these ordered lattices the individual particles were usually hexagonally packed but, in some instances, square arrays were also encountered (Fig. 1, a–c).

The size of the individual particles approximates that previously determined for mature polioviruses by different electron microscopic methods (*2–5*). Furthermore, the lattice pattern of the particle aggregates closely resembles that of poliovirus crystals seen in infected tissue culture cells (*5–7*), in cells of infected animal hosts (*8*), and in virus preparations of highest purity (*2, 4*). Finally, the cells containing the crystalline arrays were of the same type as those in which poliovirus antigen was demonstrated by means of the fluorescent antibody technique. Accordingly, there is convincing evidence that the observed particles were indeed poliovirus.

The poliovirus crystals, especially the larger ones, frequently lacked a limiting membrane and hence were embedded in the ground cytoplasm proper. However, smaller crystalline virus aggregates could be found sporadically within membrane-bounded cysts. The limiting membranes of the latter were habitually very delicate and, as a rule, appeared to be blurred or interrupted in many places (Fig. 1, a–c). In some instances, cysts enclosing poliovirus crystals exhibited direct continuity with tubular channels, along the outer surfaces of which typical ribosomes (Fig. 1, b and c) were observed. These tubules, which evidently represented a part of the rough-surfaced endoplasmic reticulum, often converged radially to the virus-containing cysts, thus forming starlike figures (Fig. 1c). They usually included a homogeneous substance of moderate density, but no particles with the appropriate size and shape for mature poliovirus could be detected within them.

At first glance one might be tempted to believe that the poliovirus crystals within endothelial and mononuclear cells were due to endocytosis of virus from the blood or from necrotic neurons, or from both. However, there are two facts incompatible with such an assumption. On the one hand, the described crystalline formations were

never found within organelles resembling typical phagosomes or residual bodies. On the other hand, phagocytosed material is usually not deposited in the ground cytoplasm proper or segregated into dilated cisternae of the endoplasmic reticulum as are the virus particles described in this report. It seems most likely, therefore, that the observed poliovirus crystals in nonneuronal cell types really reflect proliferative phases, that is, are aggregates of newly synthesized progeny particles.

The occasional occurrence of poliovirus crystals within the endoplasmic reticulum suggests that this cell constituent might be much more involved in poliovirus multiplication than has been previously supposed (*7*). On the basis of our present findings, it seems conceivable that poliovirus precursors or subunits (viral RNA and coat protein) can be segregated into tubules of the rough-surfaced endoplasmic reticulum and subsequently accumulated into smooth-surfaced cisternae in which assembly of complete progeny particles and formation of small virus crystals take place. After the virus crystals have increased by accretion to a certain magnitude the limiting membranes of the cisternae probably dissolve, thus releasing the former into the cytoplasmic matrix. Such a membrane disintegration would explain why only a relatively small proportion of the crystalline virus aggregates was found inside the endoplasmic reticulum. This interpretation of our findings is admittedly rather speculative and requires confirmation by further investigation. We wish to emphasize here, however, that density gradient centrifugation studies of cytoplasmic extracts from poliovirus-infected HeLa cells have revealed particulate structures apparently polyribosomal in nature and held together by membranes. Viral RNA and protein synthesis as well as virus assembly seemed to be closely connected with these structures (*9*), corresponding, at least in parts, to the endoplasmic reticulum. It is worth mentioning, moreover, that in some other picornaviruses template sites for virus replication have also been shown to be intimately associated with the endoplasmic reticulum (*10*).

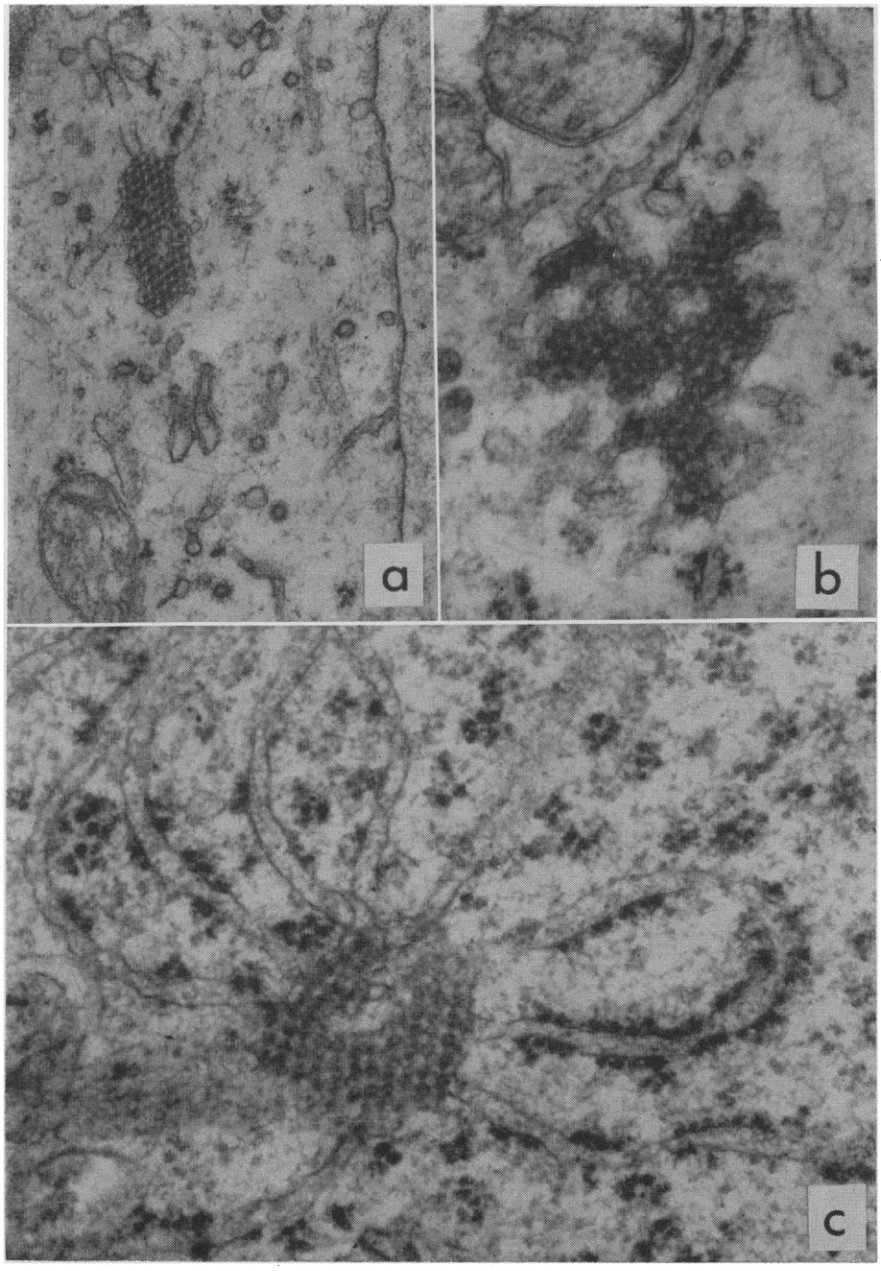

Fig. 1. Crystalline arrays of poliovirus particles lie within membrane-bounded cysts. In (b) and (c) these cysts are confluent with tubules of the rough-surfaced endoplasmic reticulum. (a) Part of an endothelial cell (intraspinal vien) (× 36,000). (b) Part of a macrophage (× 54,000). (c) Part of an endothelial cell (intraspinal vein) (× 72,000).

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