calcium sensitivity in this case was lower at 20 °C than for fish myofibrils at 0 °C.

It is now clear that the speed of contraction of a muscle is not necessarily related to redness of the fibres, though there does appear to be a good correlation between the speed of contraction and myosin ATPase activity[13]. Preparations of myosins from either red or white muscles of different species of fish have given widely differing values for the $Ca^{2+}$-activated ATPase[14–18].

In many cases low activities have been attributed to the apparent instability of fish myosins[14,16,18–20]. Variation in the stability of myosin preparations could obscure any intrinsic differences in enzymic activity. However, the results obtained in this study using myofibril preparations clearly show that the ATPase activity under these conditions is stable and differs considerably between red and white muscle fibres. It therefore seems from this work that the white muscle has the biochemical properties of fast muscle whereas the red muscle has the biochemical properties of slow muscle.

It has been shown that the slow twitch muscles of mammals are efficient in maintaining tension, while the fast muscles are efficient when contracting isotonically and doing external work[21–23]. It is, however, difficult to understand what advantage it is to the fish, from the efficiency point of view, to have slow muscles for swimming, as swimming requires isotonic contractions rather than the maintenance of tension. If the red muscle is used for slow cruising it may be that this is best achieved by a slow muscle, rather than a heavily loaded fast muscle or antagonistic fast muscles. The division of labour between these different types of muscle at different swimming speeds requires further study.

*Zusammenfassung.* Die $Ca^{++}$- und nicht-$Ca^{++}$-abhängige ATPase-Aktivität von isolierten Myofibrillen der roten und weissen Muskeln von drei Meerfischen wurde gemessen und festgestellt, dass die weissen Muskeln eine höhere Aktivität besitzen.

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## Initiation and Spreading of Coxsackievirus $A_1$ Infection in Muscles of Newborn Mice[1]

Histopathological changes induced by coxsackievirus in muscles of the newborn mouse are characterized by the so-called 'segmental involvement' of individual muscle fibers[2,3]. The term 'segmental involvement' is used to describe the observation that in coxsackievirus infected muscles one or several distinct parts of the muscle cell seem to be altered and other parts remain intact. This is due to an asynchrone cytopathic effect[4] within one muscle cell. The present paper deals with this phenomenon of asynchrony and elucidates the mechanisms by which the infection spreads in vivo from one cell to another. Moreover, the results reported earlier on mechanisms of virus release from an infected cell[5] and on the localization of viral RNA synthesis[6] are confirmed.

*Materials and methods.* Newborn mice between 12 and 24 h after birth received an injection of 0.1 ml ($10^6$ to $10^7$ $LD_{50}$) coxsackievirus $A_1$ suspension in one foreleg. To demonstrate viral RNA synthesis the animals were injected with 2.5 $\gamma$ actinomycin D (Merck, Sharp and Dohme) to stop cellular RNA synthesis before administration of 100 $\mu$Ci uridine-$^3$H (The Radiochemical Centre, Amersham, England). Uninfected control animals were treated in exactly the same manner. After fixation, the muscles were embedded in the usual way in Epon 812. Light microscopic (LM) and electron microscopic (EM) autoradiographs were made from the same blocks. We used essentially the autoradiographic techniques given by STEVENS[7]. For technical details of our autoradiographic methods see[6].

*Results and conclusions.* Up to 8 to 10 h p.i., viral RNA is synthesized to a very small extent within or in close proximity to the nuclei of infected muscle cells[6]. As soon as typical small bodies[8] are formed, uridine is also incorporated in these regions. Figure 1 shows an EM autoradiograph of an actinomycin and uridine-$^3$H treated, coxsackievirus infected muscle about 5 h p.i. The nucleus is lobed and its chromatin is condensed. These are the first signs of a coxackievirus infection[9]. In the zone around the nucleus, the first small bodies are seen. As indicated by the silver grains, viral RNA synthesis takes place in the

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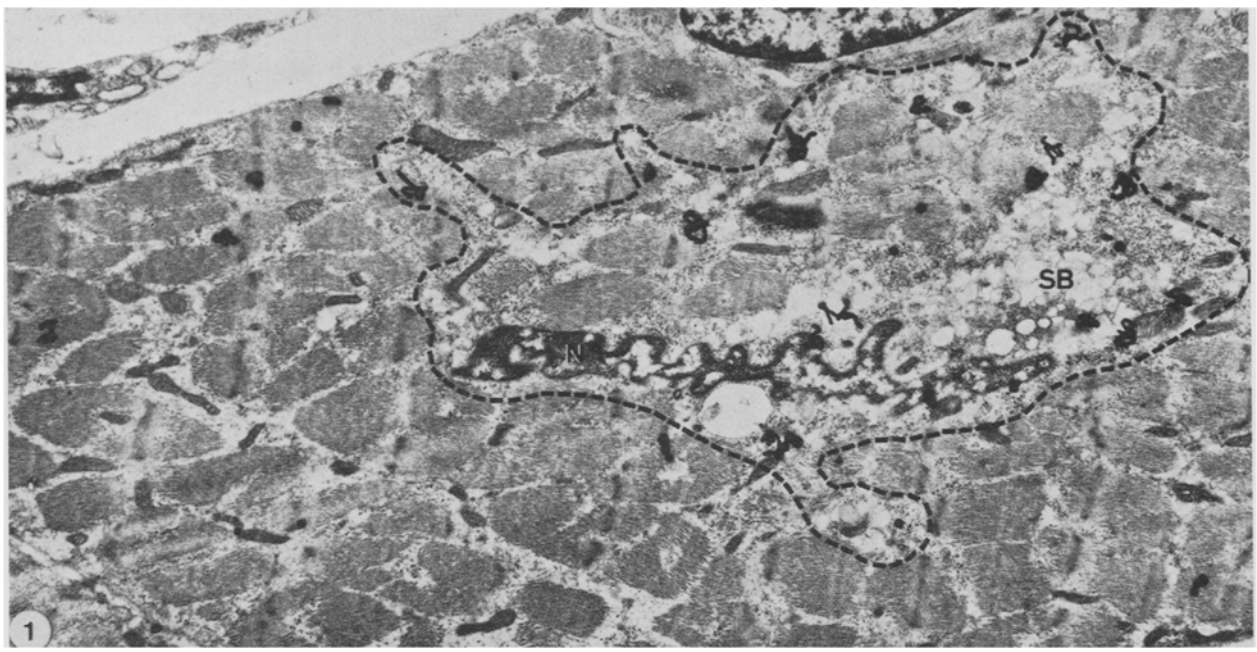


Fig. 1. EM autoradiograph of a coxsackievirus infected, actinomycin D treated muscle cell. Viral RNA synthesis is found in the region around the nucleus (N). It is the same region (surrounded by a dotted line) which shows morphological signs of a coxsackievirus infection (SB = small bodies), whereas the remainder of the cell is unaltered. ×7700.

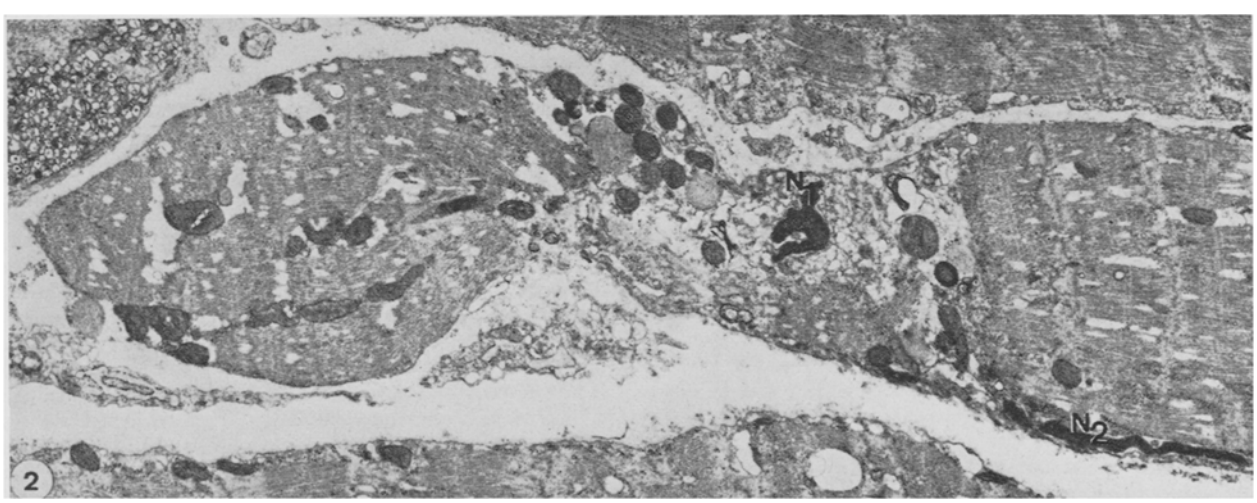


Fig. 2. Electronmicrograph, showing a fiber with the typical 'segmental involvement'. Around nucleus 1 ($N_1$), the cytoplasm is strongly destroyed (about 12 h p.i.). Nucleus 2 ($N_2$) lies in a part of the fiber which must have been infected about 7 h later than the part around nucleus 1. ×7500.

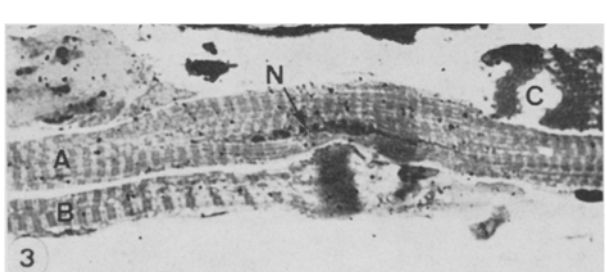


Fig. 3. The LM autoradiograph shows the spreading of infection from one fiber to another. The fiber A in the centre is freshly infected, presumably by the adjacent, partially destroyed fibers (B+C). The silver grains around the nucleus (N) of fiber A indicate viral RNA synthesis. ×780.

same zone. The remainder of the cytoplasm, outside the dotted line, is essentially unaltered. We conclude from this and similar pictures that the virus infection in muscle cells starts in and/or around the nucleus.

Obviously, if one or several regions around the nuclei in the same fiber are infected almost at the same time, the resulting local cytopathic effects give rise to the picture of a 'segmental involvement'. This phenomenon, known from LM studies[2–4], is shown in Figure 2 in an EM micrograph without autoradiography. Apparently, the fiber in the centre of the picture has been infected at the site of the nucleus 1 in the centre of the cell. The cytoplasm around this nucleus is destroyed and represents a stage of infection of about 12 h p.i. The adjacent part at the right, apparently belonging to nucleus 2, shows alterations

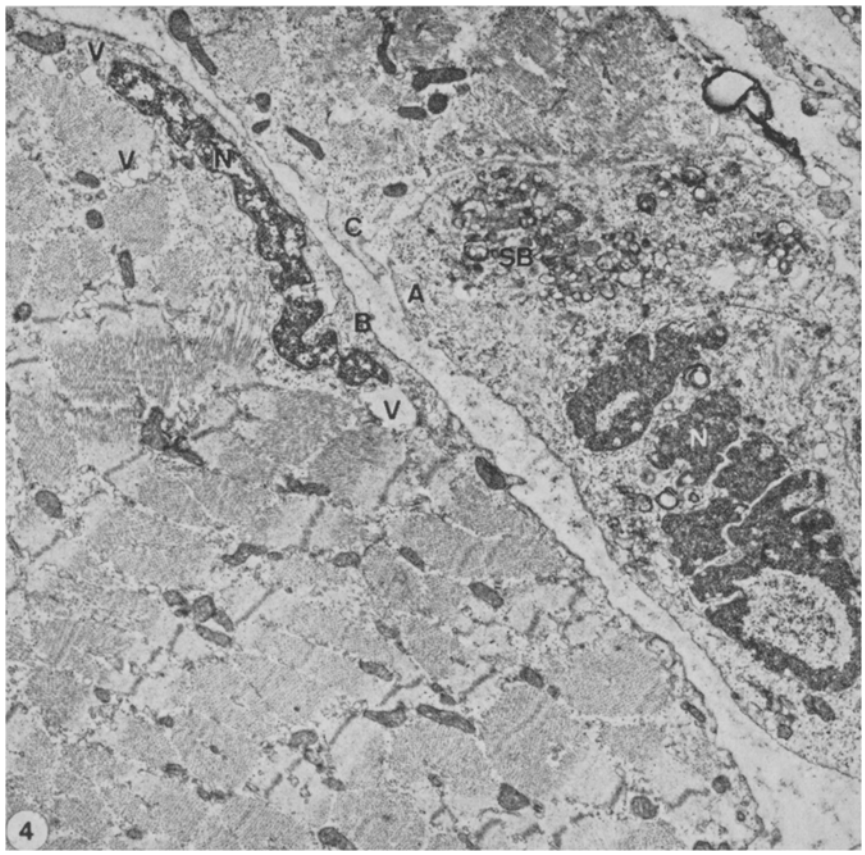


Fig. 4. Electron micrograph, showing fiber A with strongly altered nucleus (N) and cytoplasm (SB = small bodies), about 12 h p.i. Infection spread to fiber B. which shows a stage of infection of about 2 to 3 h p.i. with some vacuoles (V) around the condensed nucleus (N). Above fiber A lies another fiber (C). ×7500.

corresponding to a time p.i. of about 5 h[6] and the same holds true for the left part of the fiber.

From such pictures it is concluded that the virus synthesis in muscles is initiated by a nucleus and after that the cytopathic effect remains restricted to a rather small area of cytoplasm around this nucleus. The reason for this local restriction is not clear, as we do not know mechanisms in the nucleus or in the cytoplasm which can restrict an infection to limited areas within a cell.

Further areas in the same fiber can be infected by other virions. Such virions may have been released from parts of the same or other fibers in a later stage of infection[5]. Figure 3 is a LM autoradiograph, which shows such a spreading of infection from one cell to another. In the middle of the picture, there is a fiber (fiber A) in an early stage of infection with radioactivity, thus viral RNA synthesis, around the nucleus. The cell shows a stage of 3 to 5 h p.i. It has apparently been infected by one or both adjacent cells (fibers B + C), both being in a late stage of infection. Especially fiber B shows itself also the 'segmental involvement'. Its most severely affected part lies in close proximity to the newly infected area of fiber A.

In Figure 4 an early stage of the spreading of infection is shown in an EM micrograph. A destroyed fiber (fiber A) with numerous small bodies and a shrunken and lobed nucleus (approx. 12 h p.i.) is lying next to a freshly infected cell (fiber B). The cytoplasm of the latter cell is not altered, except for some vacuoles in a small area around the nucleus. The nucleus itself shows clearly the first signs of a virus infection such as condensation of chromatin, invagination of the nuclear membrane and beginning of swelling of the perinuclear space[9]. These alterations correspond to a stage of about 2 to 3 h p.i. Therefore, fiber B was infected when fiber A was in a stage of 9 to 10 h p.i. This is the time when the first newly synthesized viruses are released from an infected muscle cell[5].

The data presented lead us to the general conclusion that in vivo the spreading of coxsackievirus infection in muscles is like a 'jumping' from one nucleus to another. This may happen within the same fiber or from one muscle cell to another. Such a spreading is possible because, over a considerable period of time (between 8 and 15 h p.i.), newly synthesized virions are continuously released from infected cells through membraneous channels[5].

The view that a nucleus is the starting point for the infection is also supported by previously reported observations: the first signs of an infection are always seen in the nucleus[5] and in early stages of infection there is a weak, but consistently found viral RNA synthesis in the nuclei[6].

*Zusammenfassung.* Mittels licht- und elektronenmikroskopischer Autoradiographie wird die Ausbreitung der Coxsackievirusinfektion in vivo gezeigt: Die Viren befallen einzelne Abschnitte von Muskelfasern, wobei sich die Kerne als Ausgangspunkte der Infektion erweisen. Im zugehörigen Cytoplasmaabschnitt werden neue Viren synthetisiert, welche fortlaufend ausgeschleust werden und ihrerseits neue Abschnitte der gleichen oder anderer Muskelfasern befallen. Damit erklärt sich das bekannte Bild des asynchronen, segmentalen Zerfalls der infizierten Muskelzellen.

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