Susceptibility of chick neural retina to viral multiplication in vitro during embryonic development

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Summary. Decrease in the susceptibility of embryonic chick neural retina cultures to the multiplication of various viruses was observed with increasing age of the embryo. In contrast the retinal cells supported the multiplication of Sindbis virus irrespective of the age when they were infected with the viral RNA. These results suggest that the restricted multiplication of the viruses observed is due to the modulated inability of the cell to process the adsorbed viruses for subsequent replication.

Much work has been done concerning the interactions of virus with cell originated from nervous tissues. However, little information is available as to the events relating them in neural retina (NR). We report here the susceptibility of chick NR to viral multiplication in vitro in the course of embryonic development. Neural retina was selected for these experiments since it consists of a well-defined avascular population of neutral cells derived from brain and is capable of facile experimental control in vitro[1-3].

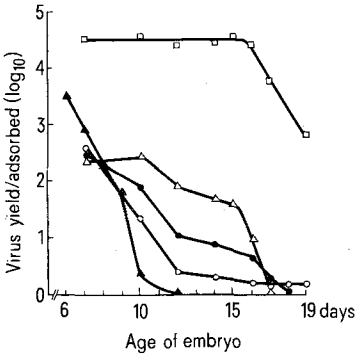

Fig. 1. Temporal change of the susceptibility of chick NR to viral multiplication in vitro depending on the embryonic age. □, Newcastle disease, Miyadera strain; △, Sindbis, obtained from Dr S. Baron[9] and propagated on BHK-21 cells; ○, vesicular stomatitis, Indiana strain; ●, mouse encephalomyelitis, GD VII strain; ▲, measles virus, TYCSA strain.

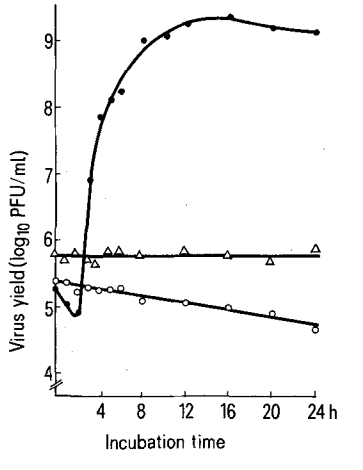

Fig. 2. Time-course of the changes in the infectivity of Sindbis virus inoculated to the NR-cells. ●, 7-day NR; ○, 19-day NR; △, control without NR. Total cell and media were homogenized at intervals after inoculation and assayed for the virus infectivity.

Suspensions of dissociated NR-cells obtained by dispersing briskly through a capillary pipette in Eagle minimum essential medium (MEM) were distributed into 30-ml Erlenmeyer flasks and inoculated with either mouse encephalomyelitis, vesicular stomatitis, Sindbis, Newcastle disease or measles virus, respectively.

Incubations were carried out at 37 °C with gyratory shaking (70 rpm) at multiplicity of infection (MOI) around 0.1 to 0.01 plaque-forming units (PFU)/cell for 1 h, subsequently cells were washed 4 times with MEM in small centrifuge tubes. In case of measles virus infection, cells were treated with acidic MEM (pH 2.0 with HCl) for 20 sec to facilitate the inactivation of the unadsorbed virus. Portions (about 5×10^6) of infected cells were seeded in 35 mm diameter Falcon plastic plates containing 2 ml of MEM supplemented with 10% fetal bovine serum (FBS) and antibiotics, incubated at 37 °C under humidified atmosphere of 5% CO_2 in air. Total homogenates of cells and media (by Kontes Microsonicator) were stored at −70 °C immediately after adsorption and after incubation for 24 h. The titration of the virus infectivity was carried out in multi-dish trays (16 mm diameter, Linbro) according to the plaque methods described for each virus[4-7].

The susceptibility of NR to viral replication is presented in figure 1. Since no detectable change of the adsorption rate of the individual virus to the NR was observed, irrespective of the age of the embryo, the susceptibility was expressed as the number of plaques after incubation for 24 h divided by the number after inoculation. Loss of its susceptibility was demonstrated to the multiplication of various viruses tested, although it retained the capability of supporting the multiplication of Newcastle disease virus still in the later stage of embryonic development. In case of measles virus infection, the virus growth was detectable only when infected cells were treated with acidic MEM to inactivate the unadsorbed virus, suggesting that the viral production was largely restricted. Cultures consisted mainly of small, round cells with an average diameter of approximately 5 μm and no cytopathic change was evidenced.

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To characterize the cell-virus interactions at different ages of the embryo, 7-day and 19-day NR were infected with Sindbis virus. In 7-day NR, the virus production was first detected at 2–3 h and increased rapidly until 12 h after adsorption. In contrast, slight reduction of the virus infectivity was observed in 19-day NR. No significant loss of infectivity of the virus incubated with MEM and 10% FBS was detected during the incubation period (figure 2).

In a parallel experiment, NR-cells were infected with Sindbis virus RNA employed as a probe to examine the cellular capability of the viral translation. Sindbis virus purified according to the method of David[8] was suspended in 0.01 M Tris-HCl buffer (pH 7.4) containing 0.1 M NaCl and 1 mM EDTA, extracted for its RNA with phenol containing 1% SDS. NR cells were washed three times with phosphate-buffered saline (PBS) and incubated with the sample of RNA (equivalent to MOI about 100) in diluted PBS (×4) containing 1 mg/ml of DEAE-dextran at room temperature for 20 min. The virus yield determined after 10 h incubation was 9.7×10^8 and 1.7×10^8 PFU/ml in 7-day and 19-day NR, respectively. This difference might be attributed to the reduced level of the cellular protein synthesis of the 19-day NR which is about 50% of that of 7-day NR (unpublished observation).

It may therefore be concluded that NR from 19-day-old embryo still retained the cellular capability to support viral translation and maturation. The restricted multiplication of the virus observed is thus due to the modulated inability of the cell to process the adsorbed virus for subsequent replication. To understand the nature of biochemical events associated with the restricted cycle of virus infection in the cell, further analyses of the pathways of uncoating and initiation of replication will be necessary.

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Effects of the spinal cord section and of subsequent denervation on the mechanical properties of fast and slow muscles

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Summary. The Soleus muscle of the rat, 3–6 months old, becomes significantly faster than in the controls, if the spinal cord is cut at birth. Mechanical properties of Extensor Digitorum Longus (EDL) muscle are not altered by spinal cord section. In cordotomized animals Soleus muscle always remains slower than EDL muscle. Denervation, performed 3–6 months after birth, has the same slowing effects in the Soleus and EDL muscles, both in cordotomized and in the control animals.

The contraction speed of striated muscles in mammals is at birth uniformly slow. During the 1st few weeks after birth, the contractions become faster, and more so in the muscles subjected to a phasic activity than in the muscles with a tonic activity, with the consequent differentiation into the fast and the slow types[1–2].

The process of muscle differentiation appears to be altered, if normal motor activity at birth is either reduced by spinal cord section or completely abolished by nerve section. In the 1st case, slow muscles in the cat are reported to become nearly as fast as normal fast muscles[1]; in the other case, the differentiation of the slow muscle seems to be unaffected, while the speeding of the fast muscle does not occur, so that after 3 weeks the contraction times of the 2 muscles are reported to be, in the rat and in the rabbit, much about the same[3].

It must be observed that denervation, when performed in adult animals, does not abolish the mechanical differences between fast and slow muscles[4], although the differences are reduced in the rabbit[5]. Thus, it seems possible that muscle differentiation affects the changes following denervation. In the present work, we repeated in the rat the experiments of spinal cord section at birth, partially confirming the results obtained by Buller et al.[1] in the cat; subsequently, with a view to investigate whether the degree of muscle differentiation is of some importance for the effects of denervation on the dynamic properties of muscles, we compared the effects of nerve section in the cordotomized and in the control animals.

Methods. Spinal cord section was carried out at the mid-thoracic level, under ether anaesthesia, in albino rats 2 days old. After 3–6 months, the sciatic nerve was cut unilaterally, always under ether anaesthesia, near the trochanter. In a group of normal animals of the same age, the sciatic nerve was also cut unilaterally, as a control. 3 weeks after denervation, contractile properties of Soleus and EDL muscles were examined in vitro. The muscles, immersed at 37 °C in the Krebs solution (pH 7.2–7.4) aerated with a mixture of 95% O_2 and 5% CO_2, were connected to an isometric transducer and stimulated through platinum electrodes, with the massive stimulation method[6]. The stimuli were supramaximal, of 0.2 msec duration. Mechanical responses were recorded by a storage oscilloscope (Tektronic 5103 N/D11), and contraction time (CT), half relaxation time of the twitch (1/2RT), tetanic fusion frequency (TFF), Tetanus: twitch ratio (T:t ratio) and maximum rate of rise of tetanus were measured. The muscles were weighed at the end of the experiments.

Results. Soleus muscle (table 1). In Soleus muscles from the animals with spinal cord section, CT and T:t ratio were significantly smaller ($p < 0.05$), TFF and the rate of rise of tetanus significantly greater than in the control muscles. When denervated after spinal cord section,

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