Studies on the Enhancement of Myotropism in Col-SK Virus by Muscle-to-Muscle Passage in Mice

By

T. Kuwata

With 1 Figure

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Since viruses of the encephalomyocarditis group have the capacity to multiply in the skeletal and heart muscle as well as in the brain\(^1\)\(^-\)\(^3\), it is possible to pass these viruses using these tissues other than the brain in mice. Thus, Rustigian and Pappenheimer made ten serial passages of Col-SK virus from muscle to muscle in mice\(^1\); Chambers et al. made 13 serial passages of MM virus from foot pad to foot pad in the hamster\(^4\). In these passages no changes in the character of the viruses were observed. On the other hand, when Col-SK virus was passed from spleen to spleen in mice, the viscerotropic character of the virus was enhanced and the virus also caused a more widespread necrosis of muscles than did the brain-passed strain\(^5\). To test the plasticity of this virus tropism, an attempt was made to follow any changes in the character of the virus when passed from muscle to muscle in mice\(^6\). In the present paper, the character of the muscle-passed virus is described in greater detail and the pattern of its variation is analyzed.

Materials and Methods

Virus. The Col-SK strain was used as representative of the encephalomyocarditis group viruses. The strain was made available to us through the courtesy of Dr. Takemori of the National Institute of Health of Japan. Infected mouse brain in 50% glycerin-saline was kept in the refrigerator and before the beginning of each experiment the virus was passed 3 times in mouse brain.

Infectivity titration. Tissues to be tested were aseptically removed, ground in a mortar with glass powder and then made up to 10% suspen-
sions with normal saline. After 10-minute centrifugation at 2000 r. p. m., supernates were removed and serial dilutions were made with beef infusion broth. Each dilution was injected into a group of 4 mice, either intracerebrally (0.03 ml) or intramuscularly (0.1 ml) into the left thigh. The mice were observed for 3 weeks and the LD₅₀ of the virus was calculated by the method of Reed and Muench.

**Muscle-to-muscle passages of the virus.** When mice showed paralysis of one or both lower extremities following intramuscular inoculation, 2 moribund mice were killed and their thigh muscles removed. From these a 10% suspension was prepared as described above. In turn, a further 10-fold dilution of this suspension was made and 0.05 ml injected into the left thigh muscle of 5 mice. In this fashion, serial muscle-to-muscle passages of the virus were made every 3 or 4 days.

**Neutralization tests.** Equal volumes of immune serum and 10-fold dilutions of the virus were mixed and kept at room temperature for 2 hours. Thereafter, an aliquot of each dilution mixture was injected into the brain (0.03 ml) or into the thigh (0.1 ml), or into the peritoneal cavity (0.1 ml). The mice were observed for 3 weeks and the LD₅₀ of the virus was then calculated. The neutralizing power of the sera was expressed as the difference between the log titer of normal and immune sera.

**Mice.** Hybrid albino mice were supplied from a local dealer. When the virus was injected by peripheral routes, the age of the mice was found to exert considerable influence on the virus titer achieved, as described later. Therefore, special attention was paid to the age of the mice used. In most experiments mice of about 25–30 days old were used, weighing from 8–10 grams. For certain experiments, older mice, weighing from 15–20 grams, were used.

**Results**

**Muscle-to-muscle passage of Col-SK virus.** As reported previously, the Col-SK virus was easily passed from muscle to muscle in young mice. One line, begun by injecting a 1:100 suspension of infected mouse brain, was carried for 120 passages in the course of 15 months, and the virus titer in the muscles followed from time to time by either an intracerebral or an intramuscular infectivity titration (Table 1). The original SK virus constantly titered between 0.5 and 1.5 log higher by the intracerebral than intramuscular infectivity assay. The reverse relationship of titers was observed to obtain for the virus of the 23rd muscle passage. In addition, the fact that the mice showed paresis and ataxia, rather than flaccid paralysis of the hind legs, also suggests that the principal sites of virus multiplication and the cause of death of the mice shifted from the central nervous system to other tissues, presumably to the muscle.