PERSISTENCE OF THE VIRUS OF ST. LOUIS ENCEPHALITIS IN THE CENTRAL NERVOUS SYSTEM OF MICE FOR OVER FIVE MONTHS

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Many demonstrations of persistence of viruses in the tissues of various animal hosts have been recorded (Olitsky and Long, 1929). As a rule, the presence of these infectious agents has been a sequel to clinically manifest disease in the host concerned. The prolonged incubation periods which may follow the peripheral injection of rabies (street) virus or the intracerebral inoculation of the virus of Borna disease are special instances of an apparently different nature. Evidence offered herewith seems to show that under certain conditions the virus of St. Louis encephalitis may invade the central nervous system (CNS) of susceptible mice and remain dormant there for considerable periods of time.

Relatively few instances of recovery of neurotropic viruses from the CNS after extended periods subsequent to an initial infection have come to the present writer's attention. Da Fano and Perdrau (1927), and Perdrau (1938) claimed to have detected herpes virus in the brains of rabbits up to several months after experimentally induced infections. The specific nature of the recovered agent was not demonstrated, however, and the evidence that herpes virus had actually been retrieved rested upon the clinical syndrome and incubation period in passage animals. Olitsky, Rhoads and Long (1929), using the technic of cataphoresis to concentrate virus from a filtrate of spinal cord, succeeded in demonstrating poliomyelitis virus in a monkey 26 days after intracerebral inoculation, at a time when the animal had almost completely recovered from the resulting paralysis. Webster and Clow (1936a), working with St. Louis encephalitis virus in mice, state that from a single animal of a resistant strain they were able to recover virus 4 weeks after it had been dropped into the nares. Theiler (1937) has been able to show that the virus of an enzootic encephalomyelitis of mice is present in the brain and spinal cord over a year after recovery from the clinical disease.

It has been possible to demonstrate that mice, passively protected against the virus of St. Louis encephalitis, may continue to harbor the virus for several months after it has been dropped into the nares. Mice passively immune to various viruses may be obtained by breeding them of mothers that have been actively immunized. The antibody transferred from mother to young persists for several weeks after birth. In the course of experiments designed to test the protection thus afforded the progeny of immune mothers against St. Louis encephalitis virus instilled intranasally when the mice were 2 weeks of age (Slavin, Hale, and Berry, to be published), a certain proportion of the animals survived a lethal dose of virus. On the other hand, control animals died regularly within

8 days after receiving a similar inoculum. It was noted that a few of the passively-protected survivors developed encephalomyelitis after prolonged periods of incubation—from 14 to 33 days—at a time when antibody acquired from the mother was in all probability rapidly diminishing. This observation made it seem reasonable to inquire whether, of the survivors who remained well, a few might continue to harbor the virus.

To test the point, 28 mice were available. All had been born of mothers hyperimmunized to St. Louis encephalitis virus (Freeman strain), and had received intranasally 100 minimal cerebral lethal doses of the same strain when they were 2 weeks of age. Frequent inspection had disclosed no clinical evidence of disease of the nervous system. At irregular intervals 2 of the mice were killed by exsanguination from the heart under ether anesthesia. The brains and spinal cords of the pair were triturated as a pool, and the supernatant fluid obtained by low-speed centrifugation was used to inject from 6 to 15 stock mice intracerebrally. Serial passage was not done. Of the 14 pairs passed in this fashion, the CNS of 3 pairs proved to be infective, producing the typical syndrome of encephalomyelitis. Ten-per cent suspensions of the brains of the infected passage animals were used for intracerebral inoculation of both stock mice and mice that had, as a result of immunization, previously displayed a solid cerebral immunity to the virus of St. Louis encephalitis. Without exception, second passages resulted in the death of stock mice from encephalomyelitis within 4 or 5 days, the time usually required for a 10-per cent suspension of mouse brain antigen of the Freeman strain to kill the mice after inoculation by the intracerebral route. Immune mice, on the other hand, remained well indefinitely. This fact serves to establish the identity of the virus. The 3 successful demonstrations of the presence of St. Louis encephalitis virus in the CNS were accomplished 71 days, 106 days, and 162 days after intranasal inoculation. In the 11 failures, the lapse of time between intranasal inoculation and passage varied from 55 to 162 days. Of the animals in which the CNS was shown to contain virus, the spleens and nasal mucous membranes of 2 pairs were also tested for virus activity, but with only negative results. The lungs, spleens, and nasal mucous membranes of 2 pairs whose central nervous systems proved non-infective were also passed by intracerebral injection into susceptible mice, again with negative results.

All the mice used in the present experiments were from an inbred stock of the Swiss strain among which spontaneous encephalitis has been singularly absent. Blind passages of the CNS of stock mice have been carried out on 2 occasions, on each of which passage was performed at weekly intervals through 6 brain-to-brain transmissions. No infectious agent was obtained from the brains and spinal cords of stock mice.

DISCUSSION

The apparent latency of infection of the CNS of mice of a susceptible strain by the virus of St. Louis encephalitis finds an analogy in some experiences of Bedson (1929) with psittacosis virus. From the spleens of mice inoculated with apparently neutralized mixtures of virus and antiserum, or from the spleens of those

inoculated with a small dose of active virus after previous immunization with formalinized virus, Bedson was able to obtain the infectious agent after a lapse of several months, although the infected animals remained well throughout that period. In his experiments, as in that herein reported, it appears that virus initially restrained by the presence of immune bodies ultimately achieves a nice balance with inhibiting factors of the host, is able to survive, and in all probability to multiply to some extent.

The carriage of St. Louis encephalitis virus by arthropod vectors may be important in the transmission of the disease to man. Recently, Hammon (1943) has shown *Culex tarsalis* to be a naturally infected host of the virus, and has demonstrated that this, as well as other species of mosquitoes, are capable of transmitting the St. Louis infection in the laboratory. It is suggested by Hammon that, since *Culex tarsalis* winters as an adult, it may serve to carry the virus over from one season to another. Although this author also found that the serums of a variety of vertebrates present in an endemic focus of the disease contain neutralizing antibodies against the St. Louis virus, the agent has not thus far been recovered from any other naturally infected vertebrate host than man.

The demonstration herein recorded of the persistence of St. Louis encephalitis virus in the CNS of a mammalian host suggests the possibility that vertebrate reservoirs may exist. Continued harborage of the virus in tissues other than the CNS has not been demonstrated, but Webster and Clow (1936b) have shown that the virus is capable of multiplication in the spleen of the mouse, and Lennette and Smith (1940) have propagated it in mouse testicle. Whether or not the virus harbored for a long period of time within or outside the CNS is capable eventually of reaching the circulation where it would be available to an arthropod seeking a blood meal, and whether in doing so it may exist in a state in which it is able to endure within an invertebrate vector are not known.

SUMMARY

Under certain conditions, the virus of St. Louis encephalitis occasionally produces subclinical infection of Swiss mice. The virus has been recovered from the central nervous system of such mice as late as 162 days after instillation into the nares.

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