# IN THE LABORATORY

## A Simple Technic for Passage of Certain Viruses[1]

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In making a passage of a virus, the usual technic is to triturate an organ or tissue of an infected animal using a mortar, pestle, and abrasive. The ground tissue is suspended in a suitable diluent, centrifuged, and the supernatant used to subinoculate other animals. This procedure requires considerable sterile equipment and is time consuming. The purpose of this report is to describe a simplified method which has proved to be of value in subinoculating the viruses of St. Louis and Japanese encephalitis from the brains of experimentally infected mice and hamsters. The method is similar to that used to obtain specimens for culture of bacteriologically infected tissues, e.g. aspiration of buboes, and for microscopic examination of infected tissues, e.g. examination of biopsied brain tissue for exoerythrocytic stages of malarial parasites (*1*).

The technic consists merely of inserting a hypodermic needle, attached to a small syringe, through the disinfected skin, calveria, and into the cerebrum of the animal from which one desires to make the transfer, applying moderate suction with the attached syringe, and then inserting the contaminated needle intracerebrally in the same manner into a recipient mouse. The only equipment needed is the sterile syringe and needle. When more than one animal is subinoculated, the needle is reinserted into the "donor" cerebrum before each subinoculation.

Since the brains of mice infected with either of the viruses we have used (Hubbard strain of St. Louis encephalitis; Nakayama strain of Japanese encephalitis) are consistently infectious for mice in dilutions of $10^{-7}$ to $10^{-8}$ or higher, the technic described probably carries over many infectious units from an animal with a full-blown infection. In order to test the method a hypodermic needle was contaminated with St. Louis encephalitis virus by dipping it into a $10^{-1}$ dilution of infected brain emulsion. The needle was then inserted into the cerebrum of a mouse and withdrawn. Eight mice were inoculated in this way. All died with typical signs of St. Louis encephalitis.

Seven other mice were inoculated intracerebrally with a needle which had been contaminated with Japanese encephalitis virus by inserting it into the cerebrum of a mouse which had just died of an infection with that virus. All died with typical symptoms.

The success of the method is probably, in great part, dependent upon the presence of a high concentration of the infectious agent in the tissue to be subinoculated and a high degree of susceptibility of the animals used. We have tried the method with the Lansing mouse-adapted strain of poliomyelitis virus with little success. Subinoculations to 3 mice were made by this method from each of 9 mice paralyzed after intracerebral inoculation. The incubation period in the donor mice was 5 days or less, during which period the virus is present in the brain in the greatest concentration (*2*). In only two instances did the subinoculated mice develop poliomyelitis (one of three mice in each case) in a 21-day observation period. The failure of the method here was probably due to the relatively low concentration of virus present in the donor brains.

This technic may be used either with living or dead mice, but we have found its greatest use to be in the situation where a mouse is found dead without previously having shown signs of illness, or having shown indefinite signs. In attempts to isolate the encephalitis viruses from a variety of neural and extraneural tissues of infected hamsters (*3*), mice were injected intracerebrally with tissue emulsions in the usual manner. The tissue specimens were occasionally contaminated with bacteria or contained minimal amounts of virus, or both. Many mice died without showing typical signs of viral encephalitis, with obvious bacterial encephalitis, or after prolonged incubation periods. A passage from such mice was desirable in order to assist in the interpretation of the result, and the technic described proved to be advantageous in making such passages.

To date, a total of 171 successful passages of virus have been made with this technic from the brains of mice dying after intracerebral inoculation with various hamster tissue emulsions and from those of hamsters following intracerebral or extraneural inoculation. In 87 of the 171 instances the donor animal died without typical signs of viral encephalitis having been seen, although observations were made at least once a day. In these 87 instances it would not have been possible to establish the cause of death without passage. In 13 of the successful passages the subinoculations were done from animals showing signs of bacterial infection of the brain, resulting from a bacterially contaminated inoculum. The usual method of emulsifying the brain tissue mixes any bacteria present into the whole preparation and renders a bacteria-free passage inoculum impossible without careful centrifugation, filtration, or some other manipulation. The method described is particularly useful in such cases because one can avoid the usual localized abscess in the cerebrum by inserting the transfer needle into the opposite hemisphere from the original inoculation.

The technic described here has the advantage of simplicity and speed, and has proven of value in the application mentioned. It should be applicable to other viruses, or other infectious agents, that occur in tissues in high concentration.

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### References

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