Dr. John Dempsher  
Department of Biophysics  
Johns Hopkins University  
Baltimore 15, Maryland  

Dear Dr. Dempsher:

This is in reply to your letter of October 16, 1951 in which you posed some questions about the effect of pseudorabies virus on the superior cervical sympathetic ganglia. The answers which I am giving you here are based on experiments which were carried out in 15-day old mice, using a 10% suspension of infected rabbit brain as the source of virus.

1) After 0.01 cc is injected into the vitreous of one eye of such mice, they invariably begin to scratch the inoculated eye 4 to 6 hours after inoculation. Mice that are sacrificed within a few hours after the beginning of this scratching sign show the specific intranuclear inclusions in the superior cervical sympathetic ganglia of the same side. I have no precise data to indicate when the virus may have reached those ganglia, but a safe guess would be that in the case of pseudorabies virus it is probably about 12 hours before the appearance of signs. All mice which are inoculated with this dose of virus by the intra-ocular route die within 5 to 10 hours after the beginning of scratching.

2) The intranasal route, using 0.03 cc as the dose of virus, is much less effective since in one test only 3 of 8 mice developed signs and died. The interval between nasal instillation of the virus and the beginning of scratching over the nose is somewhat longer than after intra-ocular injection and was found to be 6 to 8 hours. I have observed unilateral lesions after nasal instillation of the virus, but I have not studied enough animals to be able to say that it may not also be bilateral occasionally because the virus after all is instilled in both nostrils.

3) If you wish to obtain superior cervical sympathetic ganglia infected with pseudorabies virus, I would suggest that you inoculate 15-day old mice in the vitreous of one eye with 0.01 cc of rabbit brain virus. (I don’t know how much familiarity you have with virus techniques. I would like to stress that the infected rabbit brain should be prepared as a 10% suspension in 10% heated rabbit serum saline and centrifuged at 2,000 r.p.m. for 10 to 20 minutes.)
supernatant liquid
The/ should either be used fresh or be kept frozen in a sealed ampule in a dry ice chest.) You may sacrifice the mice as soon as they begin to scratch the inoculated eye or within a few hours thereafter. At that stage the neurons in the superior cervical sympathetic ganglion will have the appearance which you will see in the sections which I am sending you under separate cover. If the mouse is too small an animal for your purposes, I would suggest that you make some preliminary tests with rabbits. Although I have had no experience myself with intra-ocular injections of pseudorabies virus in rabbits, there is good reason to believe that they will respond in a manner similar to the mice.

Under separate cover I am sending you 8 sections taken from a series of serial sections on the brains of 3 different mice inoculated with pseudorabies virus. The sections are as follows:

Mouse A - inoculated into left eye; sacrificed 44 hours after inoculation and 4 hours after the beginning of scratching of the left eye and face. The lesions in this animal are on the left side,

Slide 11 - is to show the inoculated eye which shows some inflammatory reaction in the iris and several inclusion bodies.

Slide 24 - shows both Gasserian ganglia with intranuclear inclusions only in some of the cells of the left ganglion.

Slide 39 - shows both superior cervical sympathetic ganglia at the base of the skull imbedded in fat and muscle tissue and the intranuclear inclusions, as well as some associated degenerative changes in the cytoplasm of many of the neurons, are to be found only in the left ganglion.

Mouse inoculated into right eye; sacrificed 51 hours after inoculation and about 5 hours after the beginning of scratching the right eye.

Slide 4 - shows the inoculated eye.

Slide 14 - shows the Gasserian ganglia.
Slide 24 — shows the superior cervical sympathetic ganglia — the specific intranuclear inclusions and cellular changes all being on the right side.

Mouse inoculated intramuscularly into the calf muscles of the right leg; sacrificed 24 hours after inoculation when it was found to be biting the inoculated leg.

2 slides are being sent on this mouse to show that both Cассеrian ganglia and both superior cervical sympathetic ganglia are without lesions. After intramuscular inoculation the comparable lesions are in the spinal sensory and sympathetic ganglia.

These sections were prepared a little more than 14 years ago and the stains have slightly faded. Zenker-acetic was the fixative and Eosin-Methylene blue was used for the mouse inoculated in the left eye and a modified Giemsa stain was used for the other slides that I am sending you. If you have no experience with the recognition of intranuclear inclusions, you may have a little difficulty picking out specific cellular changes in these slides. Comparison with the unaffected side, as well as with the controls of the intramuscularly inoculated mouse should, however, make it easier for you to detect the specific changes in the nucleus. If you should encounter any difficulty, however, I am sure that either Dr. Bodian or Dr. Howe would be able to help you. You may keep these slides until such time as you may have prepared some of your own. Eventually, however, I would appreciate it if you could return them to me. I should be most interested to hear how you make out. Please do not hesitate to write to me again if you need any further help.

When Dr. Bronk was in Cincinnati earlier this year, we talked about the problems of the migration of viruses along specific neural pathways, and I think that he might be interested in seeing these particular sections. I would like to make it clear that the ganglia of the opposite side which show no intranuclear inclusions at the time the animal is sacrificed cannot be used as normal controls for physiological experiments because they are probably already invaded by virus even though enough time has not elapsed for them to develop the specific changes. The reason the lesions are seen only in the ganglia corresponding to the inoculated side is that the virus had gotten there first and affected those neurons before it became distributed elsewhere in the body.

With all good wishes,

Sincerely yours,

Albert Sabin, M.D.