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Dr. Mikhail P. Chumakov and  
Dr. Marina K. Voroshilova  
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Dear Professor Chumakov and Dr. Voroshilova:

I am sorry to have been so long in writing you concerning the progress we have made with the Type IV poliovirus. It has always been a matter of putting it off while waiting for further developments. Even though we have not completed our studies as yet, in view of Dr. Shannon's visit I am giving you the summary of our results to date.

As you recall, while you were still in this country we had been successful in passing the original cotton rat virus through suckling mice and in monkeys. We have not done very much in monkeys since that time. The original material did not bring down a rhesus monkey when inoculated intracerebrally except with fever and tremors but no paralysis, whereas the monkeys inoculated intramuscularly with the same material did develop paralysis. One attempt to pass the virus from the cord of the paralyzed monkey into 5 fresh monkeys inoculated intramuscularly was negative. The monkey originally inoculated IC was bled on the 30th day and the serum when tested in suckling mice had a neutralization titer at only 1:2 dilution against 1000 infectious mouse doses of virus. The monkey was then given a booster dose of suckling mouse carcass virus and was bled 10 days later. At that time the neutralization titer of the serum was 1:25 dilutions against 100 LD50 in mice. Mouse passage virus has been negative in adult mice by both the IC and IP routes and also negative in chick embryos.

The original virus was inoculated into HeLa, human fibroblasts, human skin and monkey kidney tissue culture. Only the monkey kidney was positive, after an incubation period of 9 - 10 days. Subsequent passage of this monkey kidney strain was very irregular for the first 7 passages, having an incubation period between 5 and 8 days and never involving the entire sheet of cells. However, starting with the 7th passage the incubation period became shorter and shorter and involvement of the cell sheet more extensive, and now in the 22nd passage of kidney the undiluted supernatant causes complete degeneration of all the cells in 24 hours. The 13th passage when titered in a monkey kidney tissue culture went to 10^{-4} and at that time was neutralizable in tissue culture by our and your monkey immune serum. This 13th monkey kidney tissue culture
passage virus was inoculated into suckling mice and into one monkey IC with completely negative results. All attempts to show relationship between Type IV virus and other known viral agents have been negative so far. The following tests have been done:

Immune sera tested against AB IV virus in suckling mice:

Coxsackie B1   EMC (Encephalomyocarditis)
  "          B2   LCM (Lymphocytic choriomeningitis)
  "          B3   Polio Type 1, 2, and 3, alone and combined
  "          B4   St. Louis
  "          B5
  "          A9
Mengo

AB IV immune serum against virus in adult mice:
  TO FA GD-VII
  Mouse encephalitis virus

AB IV immune serum against viruses in tissue culture:
  ECHO Types 1 -14
  Herpes
  Vaccinia

AB IV immune serum tested by Hemagglutination Inhibition against the following viruses:
  Dengue, Type 1 and 2
  Yellow fever
  Venezuela encephalitis
  Eastern equine encephalomyelitis
  Ilheus
  St. Louis
  West Nile

All of these above mentioned tests have been negative. The only positive test that we have obtained has been with 2 batches of American gamma globulin tested against AB IV virus in suckling mice, one batch of which had a titer of 1:5, the other of 1:25 against 100 ID of virus.

We have screened a total of 38 sera of American children in various age groups at a 1:4 dilution of serum against 100 ID of virus in suckling mice and have found:

6 out of 10 children under the age of 5 were positive,
7 out of 12 children, ages 5 - 10 were positive,
3 out of 7 children, ages 10 - 15 were positive,
1 out of 8 children, ages 15 - 20 were positive,
And 1 adult was positive.

From this it certainly appears that American children are having experience with this virus at a relatively early age since several of the positive of the under-5 group were only 2 years of age. The children under 10 were from the Washington area, whereas those over 10 were from Texas, which may explain the difference in the results in the different age groups.

A few tests on the characteristics of this virus have shown that it is ether resistant, inactivated by 60°C for 10 minutes, Seitz and Selas filtrable, and not influenced in mice by penicillin, streptomycin, or achromycin.

I almost forgot to give you the results of the histopathology done on the monkeys and mice infected with this virus. The pathology in monkeys, according to our pathologists who reviewed the slides, is entirely consistent with the picture of polio. We have not had very extensive investigation of pathology and we therefore sent the virus to Dr. David Bodian at Johns Hopkins University, whom I understand plans to do more extensive work in monkeys along this line. A few suckling mice that were examined histologically have shown practically nothing in the central nervous system but very extensive degenerative lesions in all muscles. The pancreas and subcutaneous fat did not appear to be involved.

I am currently carrying out two procedures with this virus. One is a series of passages at the ultimate dilution in suckling mice in an attempt to rule out an admixture of 2 viruses. After 5 such passages in suckling mice I will then attempt to infect monkeys. The other project involves an attempt to build up the tissue culture titer of this virus to the point where it can be used as a complement fixing antigen for serological tests. As soon as they are completed I shall probably write up our results for publication.

Again, I am sorry to be so long in giving you this information. I would be very interested in hearing of any further experimental results that you may have obtained with this agent.

Best wishes to you and your colleagues.

Sincerely yours,

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KH: dmb