14 January 1946

SUBJECT: Properties and Identity of an Alleged Mouse-Adapted Dengue Virus Obtained from Dr. N. Ishii in Japan, 18 October, 1945.

TO: Preventive Medicine Service, Office of The Surgeon General, U.S. Army, Room 2E, 275, Pentagon Building, Washington, D.C.

History of Material Obtained.—On 18 October, 1945, Lt. Colonel Murray Sanders obtained from Dr. N. Ishii in Japan, two strains of virus, each in the form of 3 mouse brains in 50 per cent glycerine-saline, designated as K-strain (293rd generation) and M-strain (286th generation) reported to represent specimens of mouse-adapted dengue virus. These specimens were brought by Colonel Bruce Webster and were said to have been kept on ice during most of the trip over, but were at room temperature when they were received by General Bayne-Jones in Washington, D.C., on 23 October, 1945. They were sent to Cincinnati by ordinary air mail on 24 October, and were received on 25 October.

The only information about these strains of virus which accompanied these specimens was: "Mice will usually be killed 40 hours after inoculation." To-date I have received no other information regarding the work of the Japanese investigators with these viruses, the circumstances of their isolation, the evidence for designating them as dengue virus. The institution with which Dr. Ishii is associated was also not indicated, nor whether these viruses are the same or different from those mentioned in the report of studies by Professor Yoneji Miyagawa and Staff of the Institute for Infectious Diseases of the Tokyo Imperial University, first transmitted by the Medical Intelligence Division to General Bayne-Jones on 26 October, 1945. The "Final Report of the Committee for the Technical and Scientific Investigation of Japanese Activities in Medical Sciences" (Medical Intelligence Document No. 19593) contains the same Miyagawa report on dengue, but no additional information. In the memorandum for General Bayne-Jones, dated 26 October, 1945, submitted by Lt. Colonel Gaylord W. Anderson, the Miyagawa report under "III, Dengue" states, "A sample of this virus is to be forwarded under separate cover." The nature of the viruses sent by Dr. Ishii is so different from that briefly mentioned by Prof. Miyagawa that I have reason to believe that they represent different agents and different groups of investigators.

Purpose of Present Investigation.—The primary purpose of the studies undertaken with these Japanese strains of alleged dengue virus was to determine whether or not they were related in any way to the strains of dengue virus that I have isolated. When no such relationship was found, the studies were extended to determine the identity of these viruses.
Work on "X" Strain

Passage of Japanese Material in Mice.--A 10 per cent suspension of the glycercinated mouse brains was injected intracerebrally into 10 mice. The mice began to show nervous signs and die 40 hours after inoculation and were all dead within 48 hours. The mice became jumpy and convulsive and would often be prostrate or dead 30 to 60 minutes after the first onset of signs. Although the original glycercinated brains were contaminated with bacteria, the brains of the passage mice were bacteria-free and served as the source of virus for subsequent passages and studies.

Properties of the "X" Virus in Mice

Intracerebral Inoculation.--The intracerebral titer of the virus has varied from $10^{-6}$ to $10^{-7}$ and was found to be identical in very young and old mice. Those inoculated with the $10^{-1}$ dilution (10% centrifuged suspension) appear well at 24 hours, but are all dead at 28 hours. At 28 to 30 hours, the $10^{-2}$, $10^{-3}$ and $10^{-4}$ mice exhibit CNS signs and die, while at about 44 hours the titration is usually complete.

Intraperitoneal Inoculation.--Quantitative titrations have revealed that mice are as susceptible to the virus introduced intraperitoneally as intracerebrally--a single intracerebral MLD being also lethal by the intraperitoneal route. Young and old mice were equally susceptible to the smallest dose of virus injected intraperitoneally. Furthermore, the mice exhibited signs and died as fast as, or faster, after intraperitoneal than after intracerebral inoculation when the same dose of virus was used; however, with the larger doses that can be injected intraperitoneally, such as 0.3 cc. of the 10% centrifuged suspension, young mice all died in 24 hours or less.

Subcutaneous Inoculation.--Young and old mice are equally susceptible and the titers obtained by this route are almost as high as those by the intracerebral or intraperitoneal routes. Death is somewhat delayed, but not by more than 24 to 48 hours.

Nasal Instillation.--Nasal instillation of the virus under ether anesthesia produced fatal infection, but the infectious titer was only about 1/50 of that by the intracerebral route. No pneumonia or other gross pathologic change was found in the lungs of the dead mice. The mice which received sub-lethal doses developed no immunity to subsequent inoculation by the intraperitoneal route.

Pathology.--No microscopic pathologic changes, beyond extreme congestion, were found in the central nervous system of mice succumbing after intracerebral or intraperitoneal inoculation. No inclusion bodies were seen. Marked congestion was present in the viscera after intraperitoneal inoculation, with perhaps an increased number of polymorphonuclear leukocytes in the sinusoids of the liver.
NOTE.--Most of the viruses which are pathogenic for mice do not possess the high infectiosity by the subcutaneous and intraperitoneal routes in old mice exhibited by the "K" virus. Those which share this property, like the viruses of Russian spring-summer encephalitis or Venezuelan encephalitis, and, perhaps also, the Semliki Forest virus, produce definite pathological changes in the central nervous system and possess other properties which differentiate them from the "K" virus.

**Host Range of the "K" Virus -- Pathogenicity for Various Species**

**Hamsters.**--Hamsters are as susceptible as mice and, perhaps, even more so to both intracerebral and intraperitoneal inoculation, and die faster. Four hamsters (weighing 44 to 55 Gm. each) were dead within 22 hours after intracerebral injection of 0.05 cc. of the 10% centrifuged suspension of mouse brain virus. An intraperitoneal titration in full grown hamsters yielded no endpoint at 10^-7.5 with the hamsters dying faster than the mice inoculated simultaneously.

**Cotton Rats.**--Cotton rats inoculated intracerebrally or intraperitoneally with 10% suspension, died as fast as mice. No titrations were carried out.

**Rabbits.**--Intracerebral, intracutaneous, intratesticular or corneal inoculation of fully potent mouse virus into rabbits produced no distinct clinical evidence of infection. 16 days later, however, all rabbits possessed neutralizing antibodies for the "K" virus.

**Guinea Pigs.**--No clinical signs of infection following intracerebral or intracutaneous and subcutaneous inoculation, but neutralizing antibodies present 16 days later in each of 4 guinea pigs tested.

**Rhesus Monkeys.**--Two young rhesus monkeys (weighing about 3 Kg.) each received approximately 10 million mouse LD50 doses of virus intracerebrally. Both monkeys were afebrile for a period of 15 days and have remained well, but while neither of them had any antibodies for the virus before inoculation, each possessed potent antibodies 15 days after inoculation.

**Chickens.**--Two full-grown chickens were inoculated intramuscularly with approximately 12 million and 20 million mouse intraperitoneal LD50 doses respectively. Both remained well and neither of them possessed any neutralizing antibodies for the virus either before or 18 days after inoculation.

**SUMMARY.**--Fatality infection . . . . . . . . . . . . Mice, hamsters, cotton rats.

No apparent infection but antibodies develop . . . Rabbits, guinea pigs, monkeys

No apparent infection and no antibodies develop . . Chickens
Size of the "K" Virus

The size of the "K" virus was determined by filtration through Gradocel membranes, using the same technique and the same lots of membranes which I recently used for establishing the size of dengue, sandfly fever, and Japanese B viruses and for checking the size of St. Louis encephalitis virus. Two separate tests yielded filtration endpoints indicating that the probable size of the virus is in the range of 40 to 60 μm. This is to be contrasted with an estimated size of 15 to 22 μm or at most 17 to 25 μm for the dengue virus. The only known viruses with an estimated size of 40 to 60 μm are lymphocytic choriomeningitis and sandfly fever, while fowl plague (60-90 μm) may be close to it. It is noteworthy that the various spontaneous mouse encephalomyelitis viruses isolated by Theiler all have a size of 8 to 12 μm.

Neutralization Tests with the "K" Virus

The sera of rabbits and guinea pigs previously inoculated with the "K" virus proved to contain neutralizing antibodies of high titer, particularly when the tests were done by the intraperitoneal route. Thus in simultaneous tests carried out by the intracerebral and intraperitoneal routes, pooled rabbit serum had a neutralization index of 250 in the intracerebral test and 300,000+ in the intraperitoneal test. The intraperitoneal technique was, therefore, adopted as the method of choice for all tests with the "K" virus. The following antiviral sera, tested by this most sensitive technique failed to neutralize the "K" virus:

- Dengue—Hawaii strain
- Dengue—New Guinea "B" strain
- Dengue—New Guinea "C" strain
- Dengue—New Guinea "D" strain
- Sandfly Fever—Middle East and Sicilian strains
- Sandfly Fever ? --Naples, Italian strain convalescent serum
- Yellow Fever
- Lymphocytic Choriomeningitis
- Russian Spring-Summer Encephalitis
- Venezuelan Equine Encephalitis
- Eastern Equine Encephalitis
- Western Equine Encephalitis
- Japanese B Encephalitis
- St. Louis Encephalitis
- West Nile Encephalitis

Work on "M" Strain

Since there was no indication whether or not the "M" strain was identical with or different from the "K" strain, the following work was done with it. A suspension of the 3 original mouse brains in 50 per cent glycerine-saline was injected intracerebrally into 10 mice. One was found dead on the 5th day and another on the 7th day while the eight remaining mice all survived. However,
passage of the brain from the mouse found dead on the 5th day, resulted in
death of all mice in less than 42 hours. The "M" virus so obtained was com-
pletely neutralized by the anti-"K" virus serum and can, therefore, be assumed
to be identical with the "K" virus.

Summary and Conclusions

1. The mouse brain viruses, strains K and M, submitted by Dr. N.
Ishii of Japan, as specimens of dengue virus, were found to have a set of prop-
erties possessed by no other known virus.

2. The K virus (immunologically identical with the M virus) is highly
pathogenic for mice (killing old mice in the smallest effective doses by both the
intraperitoneal and subcutaneous routes), hamsters and cotton rats; rabbits,
guinea pigs and rhesus monkeys survive after inoculation with the largest doses
of virus without exhibiting clinical signs of infection, but develop neutralizing
antibodies; chickens exhibit no signs of disease and develop no antibodies. No
significant pathological changes, beyond marked congestion, have been found in
mice killed by the virus. The virus has a size in the range of 40 to 60 μμ.

3. Dengue virus has a size of 15 to 22 μμ or at most 17 to 25 μμ.
The mouse-adapted virus undergoing passage in my laboratory and proved beyond doubt
to be a dengue virus is pathogenic for mice only by the intracerebral route, and
is not at all pathogenic for hamsters and cotton rats. Sera, shown to possess
antibodies against four different strains of dengue virus, failed to neutralize
the smallest amounts of the "K" virus. While it is conceivable that a totally
distinct immunologic type of dengue virus could also possess a different host
range and different pathogenic properties, it is inconceivable that it should
have a different size.

4. Since the size of the "K" virus is in the same range as that of
sandfly fever, lymphocytic choriomeningitis, and possibly fowl plague, it is
noteworthy that convalescent sera of human beings experimentally infected with
the two immunological types of sandfly fever virus as well as potent anti-
lymphocytic choriomeningitis serum failed to neutralize the "K" virus. The com-
plete lack of susceptibility of chickens to the "K" virus eliminates the fowl
plague virus.

5. Japanese investigators have reported that some of their laboratory
mice have been found to be spontaneously infected with the viruses of lympho-
cytic choriomeningitis, Japanese B encephalitis, and the Theiler variety of
spontaneous encephalomyelitis. The "K" virus does not correspond to any of these.

6. It remains to be established whether or not the "K" virus is a
spontaneous mouse virus, or is the cause of human disease. If Dr. Ishii possesses
the human progenitor of the "K" virus or convalescent sera from human beings
experimentally or naturally infected with it, it should be possible to establish
the relationship of this virus to human disease.
Albert B. Sabin,
Lt. Colonel, M.C.

cc.: Dr. Francis G. Blake
     Dr. John E. Paul.