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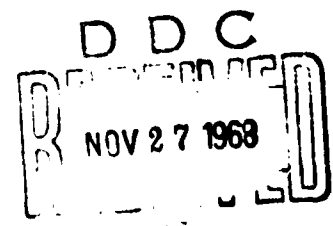
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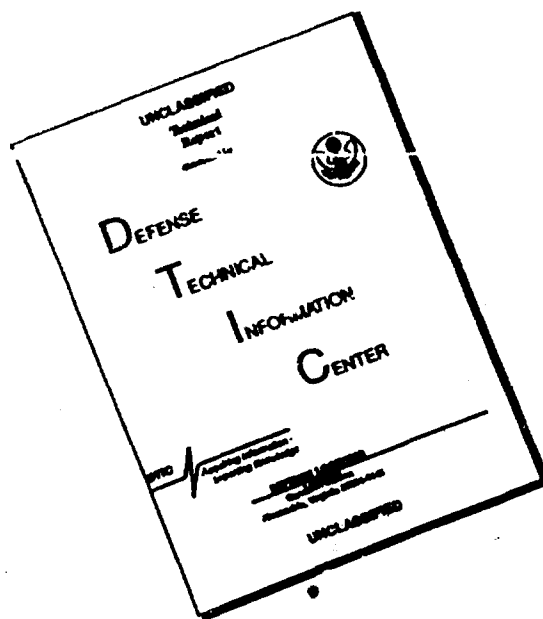
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EXPERIMENTAL TRANSMISSION OF PHLEBOTOMUS FEVER

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Epidemiological data indicate that Phlebotomus papatasi is the transmitter of phlebotomus fever in epidemic foci. There are foci of the disease in the Crimea, Uzbekistan, Tajikistan, and Turkmenistan, where P. papatasi is widespread. Other representatives of the insects may also play a role. Ye. I. Martainovskiy suspects the involvement of P. caucasicus (in the Caucasus). P. A. Petrishcheva and A. Ya. Alymov regard this species as a possible vector in Osha (Kirghizia) because it was prevalent during an outbreak of phlebotomus fever in Osha in 1935. P. perniciosus is suspected in Portugal, P. perfili in Macedonia. We wish to stress, however, that there are no experimental data confirming the role of the above species in the epidemiology of phlebotomus fever.

Experiments on the transmission of the virus by P. papatasi bites were performed on human volunteers. The lack of susceptible animals and the complications inherent in experiments on humans have obviously held up research on the role of various species of sand flies in the transmission of phlebotomus fever.

Soviet investigators were the first to adapt the virus to laboratory animals. N. I. Khodukin, M. N. Soshnikova, Ye. Ya. Shterngol'd, and M. P. Mevzos in 1940 using the method of subcutaneous inoculation succeeded in adapting the virus to rabbit and dog brain tissue.

The availability of laboratory animals susceptible to the virus and producing clinical symptoms of the disease were the necessary prerequisite of this work. Our main purpose was to

study the possibility of infecting the suspected transmitter of P. caucasicus with phlebotomus fever virus, ascertain the survival time of the virus in the sand fly, and determine whether the virus can be transmitted by bites of this insect. To permit an objective evaluation of the findings, we ran experiments at the same time on the well-known vector P. papatasi.

We used "wild" sand flies caught in Tashkent (known to be free from phlebotomus fever) in apartments and outhouses along Bekhterev Street (Leninsk Rayon). We raised some of the insects in the laboratory by A. I. Shurenkova's method.

The flies were infected with phlebotomus fever virus isolated from a sick person and adapted to suckling mice by M. N. Sosnina in 1951 (strain 6). The virus was passaged in suckling mice following inoculation into the brain.

The specificity of the virus was systematically checked by the neutralization test both with the serum of convalescent persons and with the serum of hyperimmune animals (guinea pigs).

The strain was passaged 35 times and had a titer of 10^{-5} to 10^{-6} . The incubation period in the mice was 4 days. The disease invariably presented the characteristic symptoms.

To infect the sand flies, we used A. I. Lisova's method (1950), which she worked out in experiments involving the inoculation of mosquitoes with a leishmania culture. The insects were allowed to feed on the skins of mouse tails filled with a mixture of mouse blood and suspension of virus-containing mouse brain. The mixture contained 10% brain tissue suspended in physiological saline and mouse blood.

At various times after feeding (from 3 to 12 days), the sand flies were ground up and suspended in physiological saline in the proportion of 10 flies to 1 ml of solution. After treatment with penicillin and streptomycin, the suspension was injected into the brains of 1- to 2-day-old suckling mice. The volume of the infectious dose was 0.01 ml. The number of days the virus survived in the insects was then determined.

In experiments involving infection of the mice by bites, the sand flies at various times after feeding on the sucklings (from day 3 to day 12) were again placed on the animals. The infected animals were kept under observation. In the positive experiments, usually on day 4 after infection by bites or with a suspension of ground flies, the mice became highly excited when

touched. By the end of day 6 the mice lay on their sides suffering from spasms of the extremities and opisthotonos.

The material used for inoculation was carefully checked for sterility. Virus isolated from the sand flies was checked for specificity in the neutralization test as was the case with the original strain. The method commonly used for neutrotropic viruses was followed in the neutralization test (in a volume of 0.3 cm²). The serum was taken in a constant amount, with the dilutions of virus decreasing from 10⁻³ to 10⁻⁶.

In the experiments in which sand flies were allowed to feed on mouse tails, 3385 insects of two species were used -- P. papatasi and P. caucasicus. Only 1380 individuals ingested the virus-containing suspension. Of these, 691 (381 P. papatasi and 310 P. caucasicus) survived until the start of the experiment.

A total of 381 male P. papatasi were examined 3, 4, 5, 6, 7, 8, 10, and 12 days after feeding on the mice. From 5 to 26 flies were examined in the individual experiments. Some 860 suckling mice were infected in the primary and passage experiments and in the experiments involving the typing and neutralization of virus isolated from the sand flies. The presence of active virus was detected in the insects 4, 5, 6, and 8 days after feeding on the mice.

A total of 310 mosquitoes was used in parallel experiments with female P. caucasicus (32 experiments). In the primary and passage experiments to determine the survival of the virus and in titration and neutralization tests, 1000 suckling mice were inoculated with a suspension of ground flies. The presence of active virus was detected 5, 6, and 8 days after the insects fed on the mice.

The virus remained active in P. papatasi and P. caucasicus about 7 days. Consequently, the conditions for survival and accumulation of phlebotomus fever virus are identical in the two species of sand flies studied.

In experiments on virus transmission by P. papatasi bites, the phlebotomi were allowed to feed on suckling mice 3, 4, 5, 6, 8, 10, and 12 days after they were infected. A total of 104 flies were used in 21 experiments. In the primary experiments, 26 suckling mice were used; in the passage experiments, 436. It was found that the virus was transmitted by P. papatasi bites 5 to 8 days after feeding on the mice. The recipient mice developed a latent infection.

The virus was transmitted by P. caucasicus bites at the same intervals after the flies fed on the mice. A total of 152 flies were used in 30 experiments. In the primary experiments, 45 suckling mice were used; in the passage experiments, 630. Transmission of the virus by P. caucasicus bites was demonstrated in one of 8 experiments run on day 7 after the flies fed on the mice. Virus identical to the original was isolated from the mice on which the infected sand flies were allowed to feed.

Conclusions

1. With suckling mice used as an experimental model, P. caucasicus was infected with phlebotomus fever virus.
2. P. papatasi and P. caucasicus preserved the virus in an active state for 7 days and transmitted the infection by bites when allowed to feed on normal suckling mice. This confirms the epidemiological data on the role of P. caucasicus in the spread of phlebotomus fever.

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