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METHODS OF ISOLATION OF WEAK VIRUS CULTURES OF PASTEURELLA PESTIS

[Article by L. A. Timofeeva; Irkutsk Antiplague Research Institute of Siberia and the Far East, L.A. Laboratorio Delo, Russian, 1966, p 112]

(Annotation)

The isolation of the causative agent in rodents killed by acute plague is not especially difficult since the concentration of virus cells is very great in these animals.

This work presents a discussion of the isolation of weak virus strains of the plague virus from native rodents by the following methods:

1) The conventional method of inoculation -- organ imprints. Sections of liver, spleen, lungs and heart were touched to an agar surface and the imprint material was spread by a loop uniformly on the culture medium surface.

2) Sections of these same organs and of two lymph nodes (inguinal and para-aortic) were carefully rubbed into the agar surface.

3) Part of the organs and of the lymph nodes from each animal were placed in a porcelain mortar and triturated with pre-washed sterile sand. Then, after addition of 2-3 ml of physiological solution, the organs and lymph nodes were once more triturated. A drop of the suspension, remaining on the pestle after trituration, was placed on the agar surface at the edge of the Petri dish. Part of this drop was scattered by a loop for partial streaking in an area equalling approximately one third of the agar, the loop was then plunged into the same drop of suspension and a second one third of the agar surface was streaked. The remaining area of the agar was treated in the same way.

Parallel cultures were made from each animal by all the methods described above on Khottinger agar (pH 7.2) with the addition of 0.01 percent of hemolysed blood. We investigated only animals which survived infection by plague virus cultures. These animals were sacrificed daily by chloroforming at periods ranging from two to ten days after infection.

Plague virus cultures were isolated from 103 of 249 experimental animals. Inoculation by organ imprints resulted in the isolation of cultures from the liver of only two animals and from the spleen of ten, while no cultures were isolated from the lungs and blood from the heart. Inoculation by embrocation resulted in the isolation of the causative agent of plague from the liver of 14 animals, from the spleen of 37 animals, from the lungs of four animals and from the blood of three animals. We isolated cultures from the para-aortic lymph nodes of 78 animals and from the inguinal lymph nodes of 25 animals.

The causative agent of plague is isolated most regularly and most frequently by means of inoculation of suspensions of the organs and of the lymph nodes. We isolated plague virus cultures by this method from 93 animals (90.3 percent of all animals from which cultures were isolated).

The generally accepted model for a biological assay is the guinea pig. However, plague virus cultures isolated in recent years often were weakly virulent or virulent for white mice and correspondingly avirulent or weakly virulent for guinea pigs. Our material also shows that white mice are more sensitive than guinea pigs: of 120 guinea pigs infected by strains of the plague virus in doses of 10^8 and 10^9 virus cells only one, infected by a 10^9 dose died from plague while only 15 of 214 white mice infected by these same cultures in lower doses (10^5 and 10^6 virus cells) died. This suggests the benefits from using white mice for the biological assay.

The sensitivity of white mice can be increased with the help of various preparations (cortisone, chicken egg yolk, novembichin, histamine, sodium glycocholate and others). The most effective, available and convenient for preservation and transportation (of these preparations) is chicken egg yolk, dried by sublimation in ampules.

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