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# ANNOTATION

## EXPERIMENTAL MODEL IMMUNIZATION OF SWINE AGAINST TESCHEN DISEASE WITH A FORMALIN-KILLED VIRUS FROM TISSUE CULTURES

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The authors made preliminary experiments with the propagation of the Encephalomyelitis enzootica suum virus on homologous embryo tissues in 1954, and since 1955 they have studied it systematically, following standard techniques. Results of their first experiments, along with a description of the virus's cytopathogenic effect, have been published in ČsEMI, VI, 3 (I & II), 1957.

During 1957 a greater amount of the virus, cultivated on homologous tissues, was accumulated in order to prepare a formalinized vaccine for an experimental model of active immunization.

Results of the titration are given in the following table.

| Virus concentration | TC $10^{-2}$ | TC $10^{-3}$ | TC $10^{-4}$ | TC $10^{-5}$ | TC $10^{-6}$ |
|---|---|---|---|---|---|
| Number of infected pigs | 3 | 3 | 3 | 3 | 3 |
| Symptoms present in | 3 | 2 | 2 | 1 | 0 |

The virus from tissue cultures, used throughout the experiments, was stored in closed containers at $25^0$ C below zero.

In the first stage of the experiments the virus from tissue cultures was titrated on hogs inoculated intrathalamically with 1 ml. of virus in ten fold dilutions, after determining preliminarily that the concentrated virus diluted $10^{-1}$ produces lethal paralysis on intracerebral administration following a very short incubation period of 5, and 7 days respectively.

Calculating after Reed & Muench, it was determined that *the titre of the virus from tissue cultures, tested intracerebrally, is* $LD_{50} = 10^{-4,25}$.

In view of the fact that the $LD_{50}$ of the suspension of infected spinal cord used to inoculate tissue cultures was $10^{-3,1}$ it is apparent that the propagation

of the virus in tissues is by one order greater. Therefore it can be assumed that an antigen from tissue cultures will be substantially more effective in active immunization.

A trial comparison of the extent of the cytopathogenic effect with the above determined infectious titre demonstrated the cytopathogenic effect to be a more sensitive indicator of very small amounts of virus, because it was still apparent (though not complete) in concentrations of $10^{-5}$.

In a series of three experiments the infectivity of the titrated virus on intranasal inoculation was studied. 200 (intracerebral) $LD_{50}$, applied intranasally, produced symptoms of lethal paralysis with an incubation period of 11 days; 500 $LD_{50}$ produced the same picture in 9 days in two animals.

Amounts of 50 ml. of virus suspension of the above mentioned titre were inactivated with formaldehyde at $37^0$ C for 12 days, following the technique used in the preparation of the human poliomyelitis vaccine.

After 12 days inactivation was interrupted and the remaining formaldehyde was blocked with bisulphite of sodium. Additionally, during inactivation samples were taken to determine the inactivation curve.

Safety tests were performed with the vaccine on homologous tissue cultures and on four animals. Two animals were inoculated with 1 ml. intrathalamically and two intranasally with 2 ml. The cytopathogenic effect was completely negative, and all four animals remained healthy during the observation period of 35 days,. proving the vaccine to be safely inactivated.

In the last stage of the experiments eight pigs were immunized with 2 ml. of the formalin-killed virus suspended in lipoid adjuvants (according to Freund) injected intramuscularly. After 21 days the animals received a booster-dose of 2 ml. of the vaccine without lipoid adjuvants. No apparent clinical symptoms were produced either on vaccination or on revaccination.

Six weeks following revaccination eight immunized animals and six control animals were simultaneously inoculated intranasally with approximately 400 $LD_{50}$ determined on intracerebral test of tissue cultures virus.

Typical symptoms of severe paralysis developed in two of the control animals on the tenth day following infection, and in four on the eleventh day; the disease was histologically confirmed.

In the immunized group of eight, two animals also developed symptoms of Teschen disease on the fifteenth day following infection, and two on the sixteenth. *The four remaining animals (50%) survived without any symptoms whatsoever during the whole of the observation period, i. e., longer than 40 days.*

In the opinion of the authors the reported experiment amply demonstrates the immunogenic capacity of the formalin-killed virus derived from tissue cultures.

The final evaluation of the antigenicity of the thus prepared vaccine and of its practical utilization in the field is hampered by the small number of animals employed in the experiments.

It is necessary to elaborate and modify the preparation of the vaccine, the period of its inactivation, dosage and vaccination scheme in further series of experiments.

The authors also keep in mind the fact that the amount of the virus employed in the challenge was many times higher than the highest amount of virus that can be encountered in natural infection. Since such a small number of animals was available in the experiments, the application of such a high dose was necessary to make sure that all the control animals would succumb.

In view of their earlier studies on the immunology and pathogenesis of hog paralysis, notwithstanding the just described successful experiment, the authors, nevertheless, suppose that complete immunity can be attained only with a live virus greatly attenuated, having retained its immunogenicity.

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