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ABOUT SOME BIOLOGICAL AND IMMUNOLOGICAL PROPERTIES OF HOG ENCEPHALOMYELITIS VIRUS (ENCEPHALOMYELITIS ENZOOTICA SUUM)

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The results of a series of experiments bearing on a detailed study of the biological and immunological properties of the virus of Encephalomyelitis enzootica suis, are the following facts, partly newly ascertained, partly older, newly proved by us:

At the time of the first symptoms of the disease, the largest amount of the virus is present in the CNS of the diseased animal. Of this, the spinal cord contains by far the largest amount of the virus; a much smaller amount is found in the cerebellum and brain stem with the bulbus olfactorius. In the further course of the disease, the amount of virus in the spinal cord decreases rapidly so that on the 5th to 7th day after the beginning of the paralytic phase it cannot, experimentally, be proved at all.

We have determined the approximate size of the Tesín disease virus by ultrafiltration through collodion membranes as being about 25 nu/ in the range of 20-28 nu/, on the assumption of its having a more or less spherical shape.

The virus is inactivated in a 10 p.c. cord suspension at a temperature of 60° C after 20 minutes. On partial purification by protamine sulphate, it is destroyed even at a temperature of 50° C after the same period. The same purified virus is inactivated by 0.5 p.c. chloramine in 5 minutes. The same concentration of this antiseptic destroys the virus in a 10 p.c. cord suspension only after 15 to 30 minutes.

The Tesín disease virus is active at a very wide pH range, namely from pH 2.5 up to pH 13, when acted upon for two hours previous to inoculation. It seems, judging by the length of the incubation period, that alkaline pH/ from pH 8 to 11/ rather increases the activity of the virus.

The virus from cord suspension can be partly purified either by acid precipitation, or methanol, or acetone, at a temperature below 0°C; the purificates, however, are less active than the original virus suspension.

In an attempt at purifying the virus by clupeinsulphate, we have found that approximately equal amounts of the virus both remain in the clear supernatant and pass into the sediment. Since clupeinsulphate considerably reduces the pH of the suspension and, consequently, causes besides adsorption or, perhaps, chemical bond,

also acid precipitation of the virus, we have modified the method of the American authors in the following way: on adding clupeinsulphate, pH was immediately raised to 7.2 and only then the sediment was centrifuged. Even then, however, the virus remained divided into the clear supernatant and the sediment.

We have elaborated a new method of purification of the Encephalomyelitis enzootica suum virus which consists in direct digestion of the cord suspension with papain at pH 5.5, followed by delipidation of the virus. The final preparation is practically stripped of all free lipoids of the CNS and contains approximately 200 times less proteins than the suspension it has been prepared from. The virus purificate prepared in this way is only about 10 times less active than the original suspension.

We succeeded to demonstrate twice in several experiments the virus in feces of orally infected animals, using the tannin precipitation of centrifuged stool specimens (once pooled stools from different stadiums of incubation period and then eight days after virus feeding). It seems therefore, that the virus is excreted by feces in extremely low quantities.

We have worked out the methods of active immunisation of pigs by active virus with lipoid adjuvants and that, on the whole, according to the original Freund prescription: 2 parts of lanolin were homogenized with 2 parts of 10 % infectious cord suspension, and 1.5 part of paraffin oil. The aim was to achieve the quickest possible hyperimmunisation of animals for the purpose of cross immunity tests with strains from different parts of the country on the one hand, and of obtaining hyperimmunesera on the other. We have decided in favor on the intramuscular administration of active virus with adjuvants with respect to the low infective titer /$10^{-3.1}$, PD_{50}/ of the virus and the relative innocuity of subcutaneous or intramuscular injections of living virus with lipoid adjuvants.

On the conclusion of preliminary laboratory experiments, this method has been adapted to the field requirements for the purpose of active immunization. The immunization scheme consists of 2 intramuscular injections administered at one month's interval and one 0.5 to 3.0 ccm dose of the suspension depending on the size of the pigs. The efficacy of the method has been tested in 3 field experiments carried out since the summer of 1950 on more than 3000 animals. The immunization resulted in paralytic disease in a small number of animals and that only after the first injection. Part of these complications partly of transitory character may be ascribed to local morbidity of the districts in question. Several animals immunized by active virus (with as well as without paralytic complications) have been subjected to investigation as to possible excretion of virus feces. Though a combined cerebral intranasal and intraperitoneal inoculation scheme was applied, excretion of virus could not be found in any of these cases.

Antigenic difference, if any, of 4 field strains from different parts of the state has been examined by the cross immunity test in pigs hyperimmunized by the labo-

ratory strain. So far no antigenic difference between 5 strains of different origin could be demonstrated.

The presence of small quantities of neutralizing antibodies has been proved in hyperimmunized animals. The serial dilution of serum and constant virus dose (15 PD_{50}) was used. The titer of neutralizing antibodies of sera from pigs hyperimmunized by as much as 9 intramuscular injections of active virus with lipoid adjuvants, has been found to amount to 1:16 at the most 1:32 of serum dilution though the hyperimmunized animals were solidly immune against intracerebral infection of massive doses of virus. This low neutralizing titer is likely to be connected on the one hand with the poor reaction of swine to this virus, on the other hand with the weak antigenicity of the hog paralysis virus.

Antigenic relationship of the hog paralysis virus and the poliovirus of the Lansing type was examined by means of neutralizing antibodies. In the main no profounder antigenic similarity could be found.

Immune gamma globulin has been produced from hyperimmune sera by methods using a) ethanol, b) ammonium sulphate precipitation and c) Horejsí's Rivanol method. The preparation was four times concentrated as compared with the serum and possessed about a fourfold neutralizing capacity. In examining the prophylactic efficacy of hyperimmune gamma globuline it could be shown however that it contains despite the immunizing properties a certain amount of neurotoxic antibodies.

Immunity of new-born suckling pigs born of hyperimmune sows could not be proved by the authors so far in orientation experiments not even against about 10 PD_{50} of virus inoculated intracerebrally.

Electron microscopy disclosed the presence of spherical bodies in preparations of highly purified cord suspensions from experimentally infected hogs. These formations measured 30-50 mμ, and may be identified with the hog paralysis virus, since they are absent in parallel preparations of cord suspensions from healthy animals.

Patocka, Fr., Kubelka, Vl., Zácek, K.: Pokus o cástecnou purifikaci viru Encephalomyelitis enzootica suum. Biol. listy, Vol. XXXI, No. 1, 1950.

Patocka, Fr., Kubelka, Vl., Slavík, K.: O nékterych biologickych vlastnostech viru Encephalomyelitis enzootica suis (Tésínská choroba). Véstník Csl. akad. zem., Vol. XXV, 10, 1951 - October 1951.

Patocka, Fr., Kubelka, Vl., Bohác, J.: Príspévek k immunologii nakazlivé obrny vepru. HEM, No. 1, 1953.

Kubelka, Vl., Patocka, Fr.: Ein Purifikat des Virus der Schweinelähme im Elektronenmikroskop. Archiv f. experimentelle Tierheilkunde, 1954.