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ZONAL ELECTROPHORESIS OF PSEUDORABIES VIRUS IN SUCROSE DENSITY GRADIENT

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Summary. — Electrophoretic properties of several strains of pseudorabies (Aujeszky's disease) virus with different virulence were investigated by zonal electrophoresis in sucrose density gradient. Electrophoretic homogeneity was proved with three virus populations propagated in chick embryo cells (CEC) (viz. the virulent Rača-CEC-5 strain, and the avirulent cold variant Buc-CEC-300 and the attenuated Buc-CEC-200 line, both derived from Bucuresti strain). On the other hand, the population of the strongly attenuated Buc-CEC-900 line equally derived from the Bucuresti strain appeared electrophoretically heterogeneous The electrophoretic mobilities of Buc-CEC-200 and Rača-CEC-5 viruses did not significantly differ, but that of Buc-CEC-300 virus was significantly higher. The propagation of Buc-CEC-200 and Rača-CEC-5 viruses in stable lines of calf kidney cells (CKC) and pig kidney cells (PKC) resulted in changed electrophoretic properties. The possible causes of electrophoretic mobility differences depending on virus properties and the kind of tissue used for virus propagation are discussed.

Introduction

The wide possibility of using zonal electrophoresis in virology was outlined by Cramer and Svensson (1961). On the basis of different electrophoretic mobility of several enteroviruses, Polson and Deeks (1962) tried to classify them biologically. The zonal electrophoresis was also used for purification purposes (van Regenmortel, 1964).

In our work we employed zonal electrophoresis for the study of several strains of pseudorabies virus possessing different virulence. The influence of host cell on the electrophoretic properties of virus was investigated, too.

Materials and Methods

Cell cultures and media. Monolayer cultures of CEC prepared from 11-days old chick embryos and cultures of PKC and CKC stable lines (Somogyiová, 1962) were used. The growth medium for CEC cultures consisted of Earle's solution with 0.3% lactalbumin hydrolysate (LAH), 2% calf and 1% chick sera and that for PKC and CKC cultures of Earle's solution plus 10% calf serum. Maintenance medium was Earle's solution containing 0.3% LAH. For titration purposes, tubes were seeded with 200,000 CEC per 1 ml of Parker's medium (Institute of Sera and Vaccines, Prague; Slonim *et al.*, 1960) containing 5% of calf serum. The cells were incubated for 48 hours at 37° C and then infected without change of medium. All media contained 100 I.U. of penicillin, 100 μg streptomycin, and 100 μg neomycin per ml.

Viruses. The Rača strain, virulent for all species of domestic and laboratory animals, was subjected to sixfold plaque purification after five previous passages in CEC cultures. Virus lines Buc-CEC-200 and Buc-CEC-900 were derived by threefold plaque cloning from Bucuresti cultures (Žuffa, 1963, 1966) serially passed in CEC cultures at 37° C, at the level of the 200th and 900 th CEC passage, respectively. The Buc-CEC-300 line, i.e. the cold variant of Bucuresti strain, was isolated by threefold cloning by the plaque method after the strain had undergone 200 passages in CEC at 37° C and 100 passages at 30° C (Žuffa and Žuffová, unpublished). The individual lines of the Bucuresti strain showed marked differences in virulence. The Buc-CEC-200 line was avirulent for pigs when applied paraneurally but caused death of 4 weeks old or younger pigs after intracerebral inoculation of more than 10^6 $TCID_{50}$ of virus. The Buc-CEC-900 line was avirulent for pigs even after intracerebral inoculation of large virus doses both for cattle and sheep but it killed guinea pigs and rabbits, while the Buc-CEC-300 line was not harmful for the species of laboratory animals mentioned.

Cultivation and purification of viruses. Continuous monolayers of CEC, PKC and CKC cultures grown in Roux bottles containing approx. 25 million cells in 100 ml medium were infected with 2 ml of virus suspension containing 10^7-10^8 $TCID_{50}$/ml. After 2 hours adsorption at room temperature, sucking off the inoculum and threefold washing with preheated Earle's solution, the cultures infected with Rača-CEC-5, Buc-CEC-200 and Buc-CEC-900 viruses were incubated for 48 hours at 37° C, while cultures infected with Buc-CEC-300 virus were incubated for 72 to 96 hours at 30° C. For electrophoretic investigations we used both non-purified and purified viruses. In the first case the infectious culture medium was clarified by centrifuging for 20 minutes at 3000 rev/min at 10° C (Janetzki K 60 centrifuge) and dialysed for 24 hours against electrophoretic buffer. In purifying the virus, the infected medium in amounts of at least 500 ml was subjected to two cycles of differential centrifugation (20 minutes at 3000 rev/min at 10° C and 45 minutes at 27000 rev/min at 5° C; Janetzki centrifuge VAC 60, 6×100 ml rotor). The pellets were resuspended in 0.02 M tris (hydroxymethyl) aminomethane-HCl (Tris-HCl), pH 7.8, in half of the original volume after the first cycle and in 0.5 ml after the second cycle. After clarifying the suspension by low speed centrifugation, the virus was further purified by sucrose density gradient rate centrifugation. The gradients prepared by overlaying equal volumes of 10—60% (w/v) sucrose solutions were kept for 24 hours at 5° C. Then 0.1—0.15 ml of the concentrated virus suspension (10^9-10^{10} $TCID_{50}$/ml) was placed on the gradient. Centrifugation lasted for 20 minutes at 21000 rev/min and at 10° C (Janetzki VAC 60, swing-out rotor 3×5 ml). The virus band was collected by puncturing the bottom of the tube, diluted with 0.02 M Tris-HCl and the virus sedimented again by high speed centrifugation. The pellet was resuspended in 0.02 M Tris-HCl or borate buffer (see below) and the suspension, clarified by low speed centrifugation, was used for electrophoretic investigation.

Zonal electrophoresis. The LKB 3340 apparatus was used. It was sterilized for 5 hours with beta-propiolactone (Fluka AG., Switzerland) diluted 1 : 1000 with twice distilled water and then washed three times with sterile twice distilled water. A 0—35% (w/v) linear sucrose gradient in 0.02 M Tris-HCl, pH 7.8 (15 cm height, 3 cm diameter) served as stabilization medium. The gradient base was formed with 45% (w/v) sucrose solution in Tris-HCl. Equal volumes of virus material with the addition of a small quantity of phenol red and 70% (w/v) sucrose solution in Tris-HCl were mixed and 3.5 ml of the mixture was applied to the bottom of the gradient where it formed a well visible band 0.5 cm high. The bottom electrode was the cathode and the upper one the anode. Phenol red, showing a marked mobility in the electric field, served as an indicator for the accomplishment of the electrophoretic process. Electrophoresis was over when the indicator reached the top of the gradient which lasted about 8 hours at 400 v and 8 ma. All electrophoretic investigations were performed at room temperature. Fractions of 3.5 ml each were collected, corresponding to a 0.5 cm decrease in the gradient height per fraction.

Clarck-Lubs borate buffer (0.025 M; pH 8.6) was used in some experiments. Sucrose reacts with borate ions and forms complexes, which results in a decrease of pH (Polson and Deeks, 1962). The base for the 0—50% (w/v) sucrose gradient (30 cm height) formed a 70% (w/v) sucrose solution. In this case the virus suspension placed on the bottom of the gradient contained 50% (w/v) sucrose.

Virus titration. Tube cultures of CEC were inoculated with 0.1 ml of serial tenfold virus dilutions prepared in Parker's medium using 4 tubes per dilution. The cultures were incubated at 36° C for 7 days, when the cytopathic effect was read; the $TCID_{50}$ titres were calculated according to Reed and Muench.

Results

Comparison of electrophoretic mobilities of various pseudorabies virus strains

In preliminary experiments we found no differences in electrophoretic mobility of purified and non-purified virus materials. Losses on infectious virus after electrophoresis ranged from 30—50%.

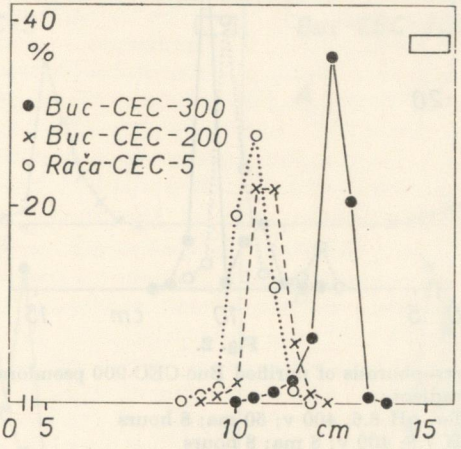

Fig. 1.

Zonal electrophoresis of three purified pseudorabies virus strains
Abscissa: distance of migration; ordinate: per cent of infectivity recovered
The quadrangle in the upper right corner indicates the distance of phenol red migration.
0 — 35% w/v sucrose gradient in 0.02 M Tris-HCl, pH 7.8; 400 v; 8 ma; 8 hours.

The Rača-CEC-5 strain and two lines of Bucuresti strain, i.e. Buc-CEC-200 and Buc-CEC-300, appeared electrophoretically homogeneous (Fig. 1). The virulent Rača-CEC-5 strain showed a nearly equal electrophoretic mobility as the attenuated line Buc-CEC-200. A significantly higher migration in the electric field was observed with the cold variant Buc-CEC-300. On the other hand, Buc-CEC-900 virus exhibited two infectious peaks (Fig. 2). The electrophoretic heterogeneity of the population of this strain was checked by the following investigations. Fractions corresponding to the respective infectious peak were pooled, mixed with a twofold volume of 0.02 M Tris-HCl, pH 7.8 and the virus was sedimented by centrifugation. The pellets were resuspended in 4 ml of Tris-HCl buffer and again subjected to electrophoretic investigation. No change was established in the electrophoretic mobility as compared with the results of the first investigation. Two infectious peaks were confirmed on electrophoresis in both Tris-HCl and borate buffer (Fig. 2). A more marked separation of the two infectious peaks was reached in 0 to 50% (w/v) sucrose gradient (Fig. 3).

Influence of the host cell on the electrophoretic mobility of pseudorabies virus

Suspensions of the Rača-CEC-5 and Buc-CEC-200 viruses were investigated to determine the host cell influence on the electrophoretic mobility of

virus. Both were obtained from PKC and CKC cultures infected either with viruses propagated in CEC cultures or with viruses which had undergone

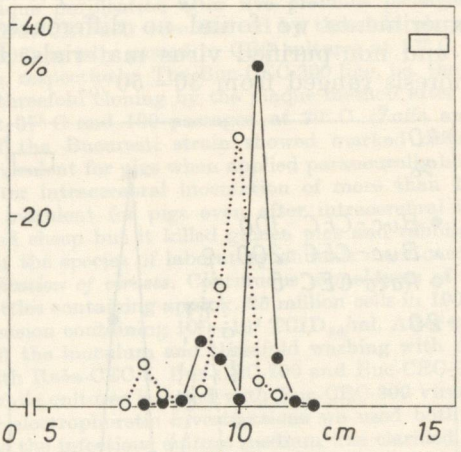

Fig. 2.

Zonal electrophoresis of purified Buc-CEC-900 pseudorabies virus
0 — 35% w/v sucrose gradient
○ 0.025 M borate buffer, pH 8.6; 400 v; 50 ma; 8 hours
● 0.02 M Tris-HCl, pH 7.8; 400 v; 8 ma; 8 hours
Other explanations as in Fig. 1.

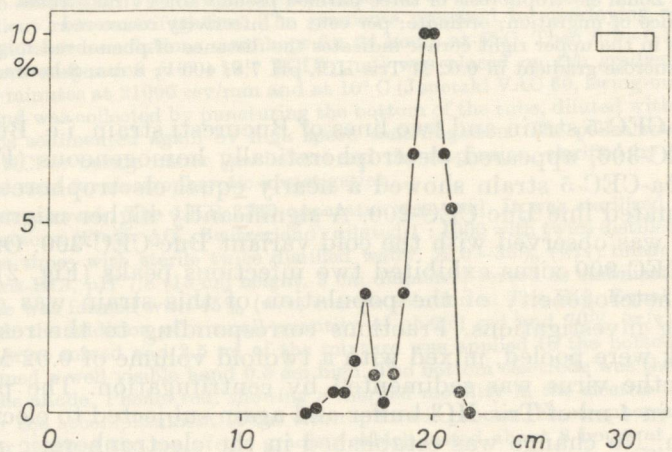

Fig. 3.

Zonal electrophoresis of purified Buc-CEC-900 pseudorabies virus
0 — 50% w/v sucrose gradient in 0.02 M Tris-HCl, pH 7.8; 600 v; 10 ma; 17 hours

10 succesive passages in the respective cells (PKC or CKC). The results of these experiments are shown in Fig. 4.

Both viruses passed once in stable PKC or CKC presented two infectious peaks,

indicating inhomogeneity of the virus populations. On the contrary, the virus suspensions obtained from PKC and CKC cultures infected with viruses which had undergone 10 passages in the respective cells proved to be electrophoretically homogeneous, showing only one infectious peak. A comparison of electrophoretic mobilities of viruses propagated in CEC and PKC

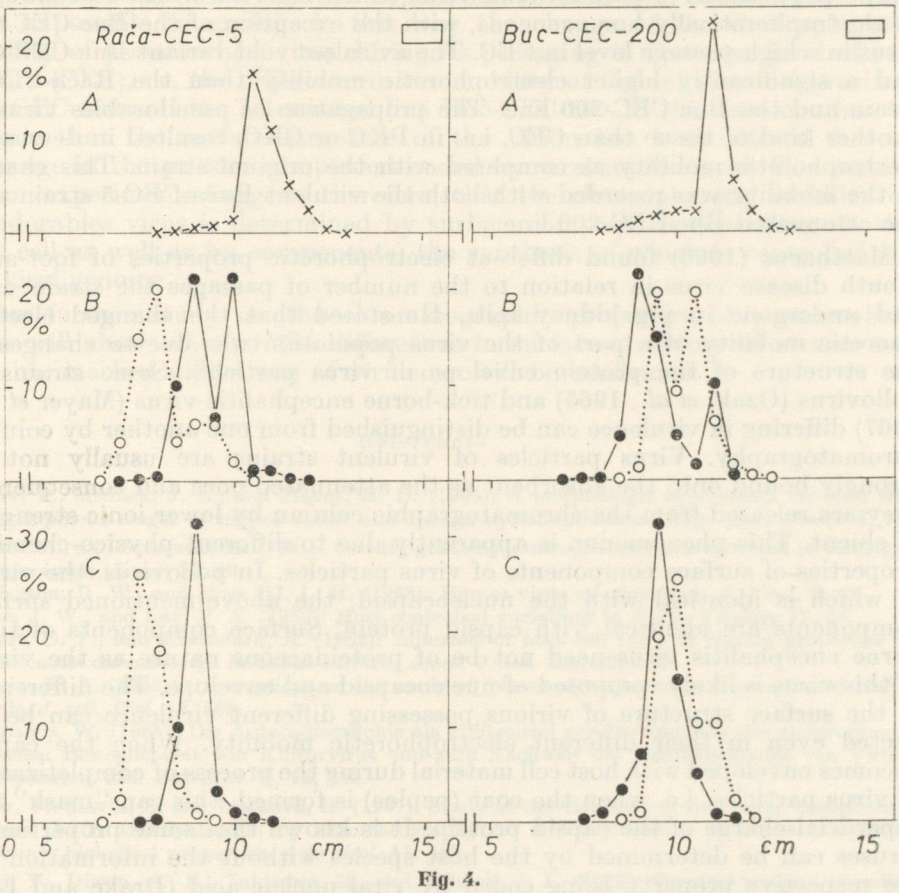

Fig. 4.

Electrophoretic mobilities of purified Rača-CEC-5 and Buc-CEC-200 pseudorabies virus strains after different number of passages in PKC and CKC cultures

A: virus propagated in CEC
B: after one passage in PKC (●) or CKC (○)
C: as in B, but after the 10th passage
For other explanations see Fig. 1.

or CKC cultures showed that viruses propagated in PKC and CKC displayed a lower electrophoretic mobility than the same viruses propagated in CEC. This decrease was more marked for the viruses propagated in CKC than those propagated in PKC.

Discussion

The results of electrophoretic investigations showed that pseudorabies virus is characterized by a great mobility in the electric field. This property seems to be typical of viruses of the herpes group (Polson and Kipps, 1967).

The populations of pseudorabies virus tested behaved in the electric field as electrophoretically homogeneous, with the exception of the Buc-CEC-900 virus in a high passage level in CEC. The avirulent cold variant Buc-CEC-300 had a significantly higher electrophoretic mobility than the Rača-CEC-5 strain and the Buc-CEC-200 line. The propagation of pseudorabies virus in another kind of tissue than CEC, i.e. in PKC or CKC, resulted in decreased electrophoretic mobility as compared with the original strain. This change in the mobility was recorded with both the virulent Rača-CEC-5 strain and the attenuated Buc-CEC-200 line.

Matthaeus (1966) found different electrophoretic properties of foot-and-mouth disease virus in relation to the number of passages the strain used had undergone in pig kidney cells. He stated that the changed electrophoretic mobility of a part of the virus population was due to changes in the structure of the protein envelope of virus particles. Some strains of poliovirus (Ozaki *et al.*, 1965) and tick-borne encephalitis virus (Mayer *et al.*, 1967) differing in virulence can be distinguished from one another by column chromatography. Virus particles of virulent strains are usually not so strongly bound onto the adsorbent as the attenuated ones and consequently they are released from the chromatographic column by lower ionic strengths of eluent. This phenomenon is apparently due to different physico-chemical properties of surface components of virus particles. In poliovirus, the virion of which is identical with the nucleocapsid, the above-mentioned surface components are identical with capsid protein. Surface components of tick-borne encephalitis virus need not be of proteinaceous nature as the virion of this virus is likely composed of nucleocapsid and envelope. The differences in the surface structure of virions possessing different virulence can be reflected even in their different electrophoretic mobility. When the capsid becomes enveloped with host cell material during the process of completization of virus particles, i.e. when the coat (peplos) is formed, this can "mask" the superficial charge of the capsid protein. It is known that some properties of viruses can be determined by the host species without the information for the respective property being coded by viral nucleic acid (Drake and Lay, 1962; Durant and Eisenstark, 1962).

It is assumed that the process of maturation and envelopment is accomplished by herpesviruses on the nuclear membrane of the host cell (Becker *et al.*, 1965; Darlington and Moss III, 1968) or that the envelope of the virus particles is formed of cytoplasmic cell elements (Epstein, 1962). Watson and Wildy (1963) proved by serological reactions that the envelope of herpes simplex virus consisted almost exclusively of host cell material probably modified by viral genome.

The change in electrophoretic mobility of pseudorabies virus propagation in a different kind of tissue, i.e. in PKC or CKC instead of CEC, can be

explained by the incorporation of other cell-specific elements into the surface structure of the virion when the virus is propagated in another host cell system. Pseudorabies virus, the virion of which contains besides the nucleocapsid also an envelope, differs from enteroviruses having no envelope, since these migrate in the electric field with the same speed irrespective of the kind of tissue used for their replication (Polson and Deeks, 1962). But it is impossible to explain in this way the change of the electrophoretic mobility of pseudorabies virus during its passaging in the same kind of cell culture (CEC), i.e. the difference in the mobility of the avirulent cold variant Buc-CEC-300 and the attenuated line Buc-CEC-200. In this case one has to assume that the surface structure of the virion has been altered due to a change in the protein component coded by viral nucleic acid. The results of our experiments suggest, therefore, that the electrophoretic mobility of pseudorabies virus is determined by surface components depending on the host cell as well as by components, the synthesis of which is controlled by the viral genome.

Our further study on the chromatographic properties of pseudorabies virus will show whether the differences in surface structure of strains exhibiting marked differences of virulence will be confirmed.

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