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TYPE OF PLAQUES AS A GENETIC CHARACTER OF POX GROUP VIRUSES

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Recently there appeared a greater number of works in which it is being shown that the size and type of plaques formed by viruses in the tissue culture under agar constitute a genetic character that permits us to differentiate individual strains of viruses as well as their mutants inside the strain. With respect to pox group viruses the possibility of obtaining plaques under agar was described by Royes [4] and Younger [5], and an improved technique was proposed by Porterfield and Allison [7]. A number of authors have detected differences in the size of plaques formed by pox group viruses [1, 5, 7] as well as by mutants inside the strain [2], but no detailed study of plaques as a genetic character of pox group viruses was carried out.

The aim of the present investigation was to study the capacity of the production of plaques at various temperatures, their size and type, in pox group viruses and subgroups smallpox - smallpox vaccine, which includes according to Ferrer's classification [3] viruses of natural smallpox, smallpox vaccine, cowpox, rabbit pox and mouse pox.

Materials and Methods

There were utilized: virus of smallpox vaccine - strains used in the preparation of dermal smallpox vaccine in the Soviet Union (21, 25A, 26), England (Lister Institute), USA, Poland and Hungary, as well as a neuropathogenic variant of dermavaccine; virus of cowpox - original strain isolated from a cow (H+), and a white mutant (H); virus of rabbit pox - strain Viroch; virus of mouse pox (ectromelia) - strain isolated from a mouse; virus of natural smallpox - strain isolated from a patient, which underwent

passages in chick embryos: virus of alastrim - strain "Bogdan", isolated from a patient and subjected to one passage in chick embryos and one passage in tissue culture. Strains of cowpox vaccine and cowpox were serially subinoculated in calf skin. A strain of cowpox vaccine was subinoculated in a rabbit's brain, a strain of rabbit pox - in rabbit's skin, and a strain of ectromelia - in mice through a foot infection. From the material obtained from a single passage there was prepared 10% suspension which was diluted 1:10 using McIlwain's buffer (pH 7.2), poured into 1-ml ampoules, and stored at -15°C .

In each experiment there were infected with each individual strain not less than 6 Povitskaya-type matrasses, measuring 0.5 l and containing chicken fibroblast tissue culture. The virus was introduced in a dose of 30-50 plaque-forming units (PFU), in a volume of 0.2 ml, with a subsequent adsorption at 37°C during 3 hours. Nutrient agar was prepared according to the recipe of Porterfield and Allison [7]. Cultures embedded in agar were incubated in thermostats at temperatures which varied within the limits of 30.1°C . Recordings were made on the 7th day. In all, with strains studied there were carried out not less than 4 comparative simultaneous experiments. The mean dimensions of plaques, and their types, presented in the Table are a result of the study of not less than 400 plaques of each strain.

Results

Capacity to form plaques at 36°C . All the studied strains of smallpox vaccine, cowpox, and rabbit and mouse pox, constantly produced plaques in contrast to the studied strains of the viruses of natural smallpox and alastrim, although in experiments on chick embryos the viruses of natural smallpox and alastrim had titers of 10^3 - 10^4 PFU/g-ml. The results obtained coincide with the data of Mike and Pirsch [5], which showed that 13 strains of natural smallpox virus and alastrim strain, studied by them, did not form plaques in the chicken fibroblast tissue culture at 36°C .

Capacity to form plaques at elevated temperatures. At 39°C , plaques were formed by all strains of smallpox vaccine, and cow- and rabbit pox, although the number of plaques was somewhat less than at 36°C . The virus of mouse pox (ectromelia), as in experiments of Porterfield and Allison [7], did not produce plaques. The viruses of natural smallpox and alastrim also did not form plaques. Upon holding the infected cultures for 18 hours at 40°C and their subsequent transfer to a thermostat with a temperature of 36°C ($40^{\circ}\text{C} \rightarrow 36^{\circ}\text{C}$; the character proposed by Camp and Konrowski [2]) there were obtained identical results as at 39°C . At 40°C none of the obtained strains formed any plaques despite the fact that the layer of cells preserved its viability during 7 days of observation. It must be noted that upon titration of pox viruses in a monkey kidney tissue culture, as well as in that of chicken fibroblasts

Table

Comparison of Various Strains of the Rabbits
 Rabbits - Rabbits Vaccine

1) Name	19) (in ml)	20) (in ml)	21) (in ml)	19) (in ml)	20) (in ml)	21) (in ml)	22) (in ml)	23) (in ml)	24) (in ml)	25) (in ml)	26) (in ml)	27) (in ml)
2)												
3)												
4)												
5)												
6)												
7)												
8)												
9)												
10)												
11)												
12)												
13)												
14)												
15)												
16)												
17)												

Key: 1--Virus; 2--Herpesvirus, strains; 3--Lister; 4--HAI; 5--Poland; 6--Hungary; 7--TL; 8--Tal; 9--Ker; 10. Neurovariant of Herpesvirus; 11--Rabbit pox, strain Utrecht; 12--Coxsack; 13--original strain; 14--white mutant; 15--mouse pox (ectromelia); 16--Natural rabbit pox; 17--Alastria; 18--Incubation temperature; 19--titer (in 10^6 /ml); 20-- Size of plaques (in ml); 21--Type of plaques; 22--large, transparent; 23--same; 24--large, clouded; 25--small, transparent; 26--does not form plaques; 27--Formation of plaques; 28--Does not form

with amniotic fluid nutrient medium, the viruses of natural smallpox, alastrim and ectromelia reproduced at 37° but did not reproduce at 40°, whereas viruses of smallpox vaccine and those of rabbit pox and cowpox reproduced at 40° as well.

Size of plaques. All the strains studied, with the exception of ectromelia virus, formed plaques of rather large dimensions (2-4 mm). Strains of ectromelia virus always formed small plaques measuring 1 mm.

Types of plaques. Two clearly distinguishable plaque types were ascertained - transparent and clouded ones. Strains of revaccinia, rabbit pox and strain H₄ of cowpox formed only clouded-type plaques with uneven edges, inside which there was preserved a network of undestroyed cells. The majority of smallpox vaccine strains and the white mutant of cowpox formed only transparent-type plaques with a smooth edge. Two strains of smallpox vaccine (V₁ and V₂) formed plaques of both types - transparent ones (has'c was 2-10%) and clouded ones. It is interesting to note that strains which formed only clouded-type plaques displayed a marked neurotoxicogenicity for laboratory animals.

Clones isolated from plaques distinct in size or type did not serve this character in the process of serial passage in the tissue culture, chick embryos and animals, which is indicative of a genetic nature of this character. It must be noted that the observed differences in types of plaques (transparent and clouded) were so distinct that they made it possible to obtain pure viral lines providing plaques of transparent or clouded type not only from artificial mixtures but also from naturally mixed strains (V₁ and V₂).

Conclusions

1. In the studied strains of pox group viruses there were found two types of plaques - transparent ones with smooth edges and clouded ones with uneven edges. The latter type of plaques was formed by strains with a marked neurotoxicogenicity for laboratory animals.

2. The strain of ectromelia virus, in contrast to strains of smallpox vaccine, and cow- and rabbit pox, forms small-size plaques and is not capable of producing plaques at 38°. Strains of the virus of natural smallpox and alastrim were found to be incapable of forming plaques in the chicken fibroblast tissue culture at 36 and 38°. None of the studied strains of pox group viruses did form plaques at 40°.

3. Differences in the capacity of the studied strains of pox group viruses to form plaques, as well as differences in size

in which the virus is present in the cytoplasm of the cells, which is in the form of a granular material of high electron density.

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