

A COMPARISON OF THE PROPERTIES OF VACCINIA VIRUS STRAINS  
USED FOR PRODUCTION IN VARIOUS COUNTRIES

by

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Introduction

It has been shown (Fenner, 1958; Krag-Andersen, unpublished data; Marennikova et al., 1962) that the properties of the smallpox vaccines issued in different countries also differ. These differences may be due to the properties of the strains used as well as to special features of the production process. In view of this, it was decided to make a comparative study of the production strains of vaccinia virus used in different countries.

Materials and Methods

The following strains were selected for study:

- The S strain - a third-generation lapinized vaccine of the Institute for Research on Viral Preparations, Moscow;
- the R strain of the Lister Institute, Elstree, England;
- the D strain of the State Serum Institute, Copenhagen, Denmark;
- the E and I strains from countries with a tropical climate (Ecuador and Indonesia); and
- the C strain used in the Chinese Peoples Republic.\*

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\* The strains used for the production of vaccine in Denmark, Ecuador and England were kindly supplied by Dr S. Krag-Andersen and Dr P. Krag, and the Indonesian strain by Professor Sumiajtno.

For comparison, use was made in these experiments of the cowpox virus in the form of a strain obtained from Professor Gispen (No. 58) and of two mutants isolated from the Brighton strain and supplied by Professor Downie.

Dried smallpox vaccines (in the form of the commercial product) or seed lymph obtained from the countries and institutes listed above, served as material for isolation of the strain. The strains were investigated after one passage in chick embryos. This made it possible to discount the effect of a number of side factors (the presence of various preservatives or accidental attenuation) and thus to place all strains on an equal footing before the beginning of the experiments.

Chick embryos were infected in the chorio-allantoic membrane (CAM) by the method of Westwood et al (1957), and incubated for 48 hours at 39°C. The nature and size of the pocks were studied in membranes with between 5 and 20 pocks on them. To determine the pathogenicity of the viruses for chick embryos, the embryos were infected on the CAM and in the allantoic cavity and incubated for three to seven days at 35°C.

Pathogenicity for rabbits was tested in animals of the Chinchilla breed, weighing 2.0 - 2.5 kg, by injection of the virus into the brain or the testes, by intradermal injection using the Groth method, and by application to the scarified skin. White mice weighing six to seven grams were infected intracerebrally and intravenously and suckling mice intranasally. In comparative assays on rabbits, white mice and chick embryos, practically equal doses of virus were used ( $10^5$  and  $10^7$  pock-forming units per 0.1 ml). In every case in which an animal died, the specificity of the process was confirmed by isolation of the virus from the organs and its identification.

The heat-stability of the materials examined was tested by keeping liquid chorio-allantoic cultures of the virus at temperatures of 37°C and 41°C. The activity of the heated cultures was determined by subsequent titration in a monolayer tissue culture of chick fibroblasts. The haemagglutination test was performed with a volume of 1 ml containing four agglutinating units of the virus and a one per cent suspension of chick erythrocytes highly sensitive to vaccinia virus.

The nature of the cytopathic effect was studied in a monolayer culture of human embryo myodermal tissue, using identical doses (1000 TCID<sub>50</sub>) of the viruses. The cytological investigations were based on the plate method (Marennikova et al, 1959). Plaque-forming capacity was tested in a culture of chick fibroblasts with agar overlay prepared by the technique of Porterfield & Allison (1960).

### Results

It was established that all the test strains of vaccinia virus when applied to the CAM caused a generalized infection of the chick embryo, leading to its death on the third or fifth day after inoculation. A similar picture was observed when various strains of vaccinia virus were introduced into the allantoic cavity. When the cowpox virus was introduced into the allantoic cavity it caused specific changes, mainly at the site of inoculation, but as a rule did not lead to the death of the embryo. Of the three strains investigated, one was completely nonpathogenic and the other two caused the death of less than 50 per cent of the infected embryos.

The nature of the lesions on the CAM were studied 48 hours after infection. Examination showed noteworthy differences between the strains, consisting, in the first place, of differences in the nature of the pocks that were formed. Altogether, at least three basic types of lesion can be identified in vaccinia virus strains: continuous white lesions, diffuse surface lesions and an intermediate type.

In this respect, the strains examined can be divided into two groups:

- (1) strains leading to the formation of practically only one type of pock;
- (2) strains that cause the formation of two or three types of pock - the white, surface and intermediate types.

The quantitative relationships between the various types of pock and their dimensions in the case of the different strains studied are given in Table 1.

Investigations of haemagglutinating activity (Fig. 1) revealed that the vaccinia virus strains possess differing degrees of ability to form haemagglutinins. The highest indices were given by strains S, I and C. In one of the cowpox strains no ability to agglutinate chick erythrocytes was found, while the two other strains possess only slight haemagglutinating capacity.

The results of testing the pathogenicity of these strains for white mice and rabbits when administered by different routes are given in Tables 2 and 3.

As will be seen, pathogenicity for these species of animals varies considerably in the different strains. In particular, there are strains that are pathogenic for rabbits and white mice when administered intracerebrally and others that are non-pathogenic by this route. The strains differ also in the nature of the reaction following intradermal administration, their pathogenicity after intranasal administration to suckling mice and in other particulars.

In the nature of their cytopathic effect (CPE), all strains except the I strain showed a quite similar picture. The cowpox virus differed in the nature of its CPE. All strains of the vaccinia virus caused the formation of homogeneous and granular cytoplasmic inclusions (Guarnieri bodies), produced intranuclear inclusions in small numbers and failed to produce the eosinophil inclusions typical of cowpox (Table 4).

When plaque-forming capacity was studied (Table 4), it was established that strains R, E and D, which proved genetically homogeneous when tested in chick embryos, formed only transparent plaques ("negative colonies" with a marked lytic centre), while strains C and S produced a mixture of transparent and reticulated plaques (in which the lytic centre was practically absent), and the I strain produced only reticulated plaques. The cowpox virus - Strain 58 and the red mutant - produced plaques of rather peculiar appearance, which differed considerably from the plaques formed by the white mutant of the cowpox virus and by the various strains of vaccinia virus. The reticulated zone in these plaques occupied a central position with the zone of lysis forming a narrow border round it. No essential differences in the size of the plaques formed by vaccinia virus were discovered, except in the case of the I strain, which produced smaller plaques, with a maximum diameter of 3 to 3.5 mm.

Investigation of heat-resistance showed differences in this respect also (Figs 2 and 3). Thus, it was found that among the strains of vaccinia virus studied strains E and I possessed the greatest heat-resistance. It is an interesting fact that both the highly heat-resistant strains come from tropical countries.

When antigenic structure was examined by Gispén's method of double diffusion into agar gel with vaccinia antiserum (1955), it was found that all the strains form at least three precipitation zones, of which two are major. The number of minor zones varied in the different strains from one to three. The largest number of precipitation zones was found in strains S and E.

A feature of the cowpox virus was the absence of one of the main precipitation zones when it was tested with vaccinia antiserum. This agrees with Gispén's findings.

When the capacity to form antihæmagglutinins was tested it was found that all strains of the vaccinia virus (and the cowpox virus) caused the formation of antihæmagglutinins in the sera of immunized rabbits. The intensity of antihæmagglutinin formation differed (Table 5).

The immunogenicity of the strains was tested in rabbits by the method proposed by Solov'ev and Mastjukova in 1956: intradermal immunization of rabbits with various dilutions ( $10^{-1}$  to  $10^{-10}$ ) of virus-containing material and subsequent intracerebral challenge with neurovaccinia virus. In addition, a modification of this method proposed by us was used; instead of being given an intradermal inoculation, the animals were immunized on the skin, previously scarified with seven lengthwise and seven crosswise scarifications on an area of  $2.5 \text{ cm}^2$ , with an identical dose ( $3.5 \times 10^5$  pock-forming units).

The figures in Table 5 show that all the strains examined possess marked immunogenicity.

In view of the fact that the stability of the main biological properties of the virus is of essential importance when the value of a production strain is to be assessed, this feature was compared in all the strains of vaccinia virus examined. For this purpose, one of the techniques used was serial passages in chick embryos. After six consecutive passages the main genetic characters of the strains were again checked. It was found that strains S, D, I and C had kept their properties more or less unchanged, except for a certain increase in pathogenicity for rabbits after intradermal administration and an increase in hæmagglutinating activity, obviously due to the growth of infective titre during these passages. It is noteworthy that one of these

to rabbits, remained so despite the increase in infective titre due to passaging. The Lister Institute strain acquired, as a result of the passages, the property of causing fatal encephalitis when administered intracerebrally to rabbits and mice. In contrast to this, the E strain, which is very similar to the R strain, retained its non-pathogenicity for both species of animal when administered intracerebrally after these passages. Its pathogenicity on intranasal administration to suckling mice showed an inconsiderable increase.

#### Conclusions

The findings from these tests suggest that the question of the criteria for selecting production strains must be raised. The results of our experiments show that the properties of strains may be associated in different combinations and that in the long run the selection of a strain must be based on a combination of optimum characters.

According to our data, the E strain is of the greatest interest in its combination of properties. Indeed, it combines genetic homogeneity, low pathogenicity for animals when administered by different routes, high antigenic and immunogenic potency, stability of genetic characters and, finally, heat-resistance, in which it is only slightly inferior to the Indonesian strain, while surpassing all the rest. The R strain is close to it in a number of properties, although it is inferior in the first. The D strain, the last of the genetically homogeneous strains, is reminiscent in its properties of the white mutant of the Soviet vaccinia virus.

The research carried out also showed the existence of clear-cut differences between all the vaccinia virus strains studied and the cowpox virus.

ENGLISH KEY TO FIGURES 1, 2 AND 3

Figure 1

Title: HAEMAGGLUTININATING ACTIVITY OF VARIOUS STRAINS OF VACCELLIA VIRUS

Vertical axis: Titre in haemagglutination test

Key, reading from top to bottom: S; I; E; C; H; cowpox; D

Figure 2

Title: HEAT-RESISTANCE OF VARIOUS STRAINS OF VACCELLIA AND COWPOX VIRUSES

Temperature: 37°C

Vertical axis: Relative TCD<sub>50</sub> in percent

Horizontal axis: Day of observation

Key, reading from top to bottom: I; E; cowpox; R; C

Figure 3

Title: HEAT-RESISTANCE OF VARIOUS STRAINS OF VACCELLIA AND COWPOX VIRUSES

Temperature: 41°C

Vertical axis: Relative TCD<sub>50</sub> in per cent

Horizontal axis: Day of observation

Key, reading from top to bottom: I; E; S; H; cowpox; C

TABLE 1. NATURE OF LESIONS IN THE CHORIO-ALLANTOIC MEMBRANE OF THE CHICK EMBRYO

Virus	Strain	Type of Pocks	Proportion (per cent) of			Diameter of pocks in mm *		
			white pocks	surface pocks	intermediate pocks	white pocks	surface pocks	intermediate pocks
Influenza	S strain, 3rd generation lapinized vaccine	white, surface, intermediate	65	23	12	1.7	1.1	1.7
	R	white, intermediate	98.9	none	1.1	1.5	-	1.55
	E	white, intermediate	98.8	none	1.2	1.6	-	1.55
	D	white, intermediate	99.2	none	0.8	1.5	-	1.3
	C	white, surface	37	63	none	0.68	0.49	-
	I	white, surface	6	94	none	0.79	0.45	-
Cowpox	No. 58	white, red	1	red 99	none	1.7	red 1.46	-
	"Brighton" (red mutant)	white, red	1	99	none	1.7	1.46	-
	"Brighton" (white mutant)	white	100	none	none	1.7	-	-

\*The tests with cowpox virus were carried out 72 hours after inoculation



TABLE 2. PATHOGENICITY FOR WHITE MICE

Virus	Strain	Infective potency	Results of administration	
			Intracerebrally	Intranasally (to suckling mice)
Vaccinia	S	$2.8 \times 10^7$ PoFU/ml	10/10	12/12 LD <sub>50</sub> - $10^{3.74}$
	R	$2.0 \times 10^7$ PoFU/ml	0/10	9/12 LD <sub>50</sub> - $10^{2.5}$
	E	$5.2 \times 10^7$ PoFU/ml	0/10	3/12 LD <sub>50</sub> - $10^{1.0}$
	D	$3.6 \times 10^7$ PoFU/ml	10/10	12/12 LD <sub>50</sub> - $10^{3.0}$
	I	$0.8 \times 10^6$ PoFU/ml	10/10	8/12 LD <sub>50</sub> - $10^{2.75}$
	C	$1.2 \times 10^5$ PoFU/ml	0/10	12/12 LD <sub>50</sub> - $10^{3.0}$
Complex	No. 58	$1.8 \times 10^7$ PoFU/ml	10/10	12/12

Key: Numerator - number of animals that died of the specific infection

Denominator - total number of animals in the experiment

TABLE 3. PATHOGENICITY FOR RABBITS

Virus	Strain	Results of Administration				
		Intradermal		Cutaneous	In the testes	Intracerebral
		Necrosis present	Nature of reactions			
Vaccinia	S	+	Marked infiltration	Typical lesions developing in characteristic fashion	Orchitis	4/4
	R	-	Less continuous infiltration	"	"	0/4
	E	-	Less continuous infiltration, reabsorbed more quickly	"	"	0/4
	D	+	Marked infiltration	"	"	2/2
	G	+	"	"	"	4/4
	I	+	"	"	"	0/4
Cowpox	No. 58		Areas of infiltration with clearcut haemorrhages	Lesions with haemorrhage	Marked orchitis with generalisation of the process	4/4
	"Brighton" (red variant)	+	"	"	"	4/4
	"Brighton" (white variant)	+	"	"	"	4/4

Key: As for Table 2

TABLE 4. BEHAVIOUR IN TISSUE CULTURE

Virus	Strain	Type of CPE	Presence of inclusions		Nature of plaques under agar overlay
			Guarnieri bodies	Eosinophil inclusions of hyaline type	
Vaccinia	S	Characteristic of vaccinia virus	+	-	Transparent and reticulated
	R	"	+	-	Transparent
	E	"	+	-	Transparent
	D	"	+	-	Transparent
	C	"	+	-	Transparent and reticulated
	I	Somewhat atypical	+	-	Reticulated and smaller
Cowpox	No. 58	Characteristic of cowpox virus	+*	+	Reticulated, with a zone of lysis on the periphery and a small number of transparent plaques of smaller size
	"Brighton" (red mutant)	"	+*	+	Reticulated with a zone of lysis on the periphery
	"Brighton" (white mutant)	Reminiscent of the CPE of vaccinia virus	+	+	Transparent, of smaller size

\* Present in small numbers

TABLE 5. RESULTS OF STUDY OF ANTIGENIC AND IMMUNOLOGIC POTENCY

Strain	Method of immunization	Antinaemagglutina titre in rabbit serum 30 days after immunization	Resistance to 1000 <sub>LD</sub> of neurovaccinia virus
S	On scarified skin	200	0/4
	Intradermal	120	0/4
R	On scarified skin	96.5	0/4
	Intradermal	25	1/4
E	On scarified skin	225	0/4
D	Intradermal	83	0/4
C	On scarified skin	163	0/4
I	Intradermal	165	0/4
	"	44	0/4
Control	Non-immunized rabbits	0	3/3

Key: As for previous tables

Note: The mean data for 8 rabbits are given in each group

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