



Title	Inclusion Markers of Cowpox Virus and Alastrim Virus
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Inclusion Markers of Cowpox Virus and Alastrim Virus

Two types of inclusions ("A" and "B") have been reported in cells infected with cowpox virus LB red and LB white strains (Kato *et al.*, 1959a, 1959b). The "A" type inclusions (Downie type) of these two strains were free from any virus particles. Morphological studies of the infected cells were extended to five other strains of cowpox virus and alastrim virus.

Five strains of cowpox virus (Amsterdam, 53, 58, 60 and 61) which had been identified as cowpox virus by their serological reactions (Gispen, 1955), were kindly given by Dr. Gispen (NIH, Netherlands), and the Juba strain of alastrim virus was kindly given by Dr. Tagaya (NIH, Japan). About 3×10^5 FL cells were dispersed into a Leighton tube containing a 11×45 mm coverslip. The medium was Earle's balanced salt solution containing 0.5 per cent lactalbumin hydrolyzate, and 10 per cent calf serum. The virus titre of each virus samples was adjusted to about 10^6 pock forming units per ml. Cultures were inoculated with 0.5 ml of virus material 24 hours after cell dispersion. After a period of an hour to allow viral adsorption, the medium was replaced by maintenance medium. Twelve and twenty four hours after viral inoculation, the coverslips were taken out and divided into three pieces. One of the pieces was fixed with methanol and stained with Giemsa solution and another was fixed with Bouin's fluid and stained with hematoxylin-eosin. The third piece was observed by phase contrast microscopy without fixation.

All strains of poxvirus tested were found to produce typical "B" type inclusions. No morphological differences could be seen between them. Besides "B" type inclusions, all five strains of cowpox virus produce "A" type inclusions. It is interesting that the "A" type inclusions of four strains of cowpox virus (53, 58, 60, 61) definitely contained a number of elementary bodies (Fig. 1, 2, 3, 4). The morphological appearances of these "A" type inclusions closely resembles those of the "A" type inclusions of ectromelia virus. The "A" type inclusions of the Amsterdam strain of cowpox virus were free from elementary bodies like those of the LB red and white strains of cowpox virus. These characteristics of the "A" type inclusions were stable though more than 5 viral passages on chorioallantoic membrane.

Thus the strains which produce "A" type inclusions with elementary bodies were designated as *cowpox virus "A"v⁺ strains* and the strains which produce "A" type inclusions without elementary bodies were designated as *cowpox virus "A"v⁻ strains*.

Because of the close resemblance of these cowpox virus strains to ectromelia virus, three other markers (the characteristics of the pocks formed on the chorioallantoic membrane, the susceptibility of mice to the virus and the appearance of the

Table 1.

Virus	Tissue culture FL cells			CAM		Animal experiments	
	Inclusions		Giant cell formation	Color of pock	Inclusions	Mortality of mice	Lesions of rabbit skin
	"B"	"A" Elementary bodies					
Cowpox LB red	"B"	"A" EB (—)	±	red	"B" "A"	—	+
Cowpox LB white	"B"	"A" EB (—)	±	white	"B" "A"	—	+
Cowpox Amsterdam	"B"	"A" EB (—)	±	red	"B" "A"	—	+
Cowpox 53	"B"	"A" EB (+)	±	red	"B" "A"	—	+
Cowpox 58	"B"	"A" EB (+)	±	red	"B" "A"	—	+
Cowpox 60	"B"	"A" EB (+)	±	red	"B" "A"	—	+
Cowpox 61	"B"	"A" EB (+)	±	red	"B" "A"	—	+
Ectromelia Hampstead	"B"	"A" EB (+)	††	white (small)	"B" "A"	+	—
Alastrim Juba	"B"	—	±	white	"B"	—	+

lesions on rabbit skin) were examined on these poxviruses. The results are shown in Table 1. All strains of cowpox virus except the white variant of the LB strain produced red pocks on chorioallantoic membrane. The LB white strains of cowpox virus and alastrim virus produced large white pocks, while ectromelia virus produced small white pocks. Intraperitoneal inoculation of 0.2 ml of ectromelia virus (10^6 plaque forming units on L cells per ml) always killed adult mice, while the same titre of cowpox virus or alastrim virus did not. It is not necessary to mean that mice are not susceptible to cowpox virus. As a matter of fact, cowpox virus can multiply both in Ehrlich ascites tumor cells and in abdominal monocytes in mice. However the virus rarely kill adult mice. Intradermal inoculation of ectromelia virus never caused any lesions in rabbit skin while the other viruses tested caused reddish swollen indurations.

We therefore concluded that the "A" type inclusion markers could be used as a simple indicator of differentiating between cowpox virus and vaccinia virus derived from variola virus and alastrim virus based on this point of distinction.

Summary; The inclusion markers of five strains of cowpox virus (53, 58, 60, 61 and Amsterdam) and the Juba strain of alastrim virus were studied. No difference could be seen in their "B" type inclusions. "A" type inclusion formation was found to be a stable marker for all cowpox virus strains. Furthermore "A" type inclusions of four strains of cowpox virus (53, 58, 60, 61) were always filled with a number of elementary bodies unlike the "A" type inclusions of the LB red and white strains and the Amsterdam strain of cowpox virus. The former strains

were designated as *cowpox virus* “A”^{v+} strains and the latter strains as *cowpox virus* “A”^{v-} strains.

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EXPLANATION OF FIGURES

- Fig. 1. “A” type inclusions of FL cells infected with cowpox virus 53, observed by phase contrast microscopy. The “A” type inclusions are filled with a number of elementary bodies.
- Fig. 2. A similar picture of cowpox virus 58.
- Fig. 3. A similar picture of cowpox virus 60.
- Fig. 4. A similar picture of cowpox virus 61.

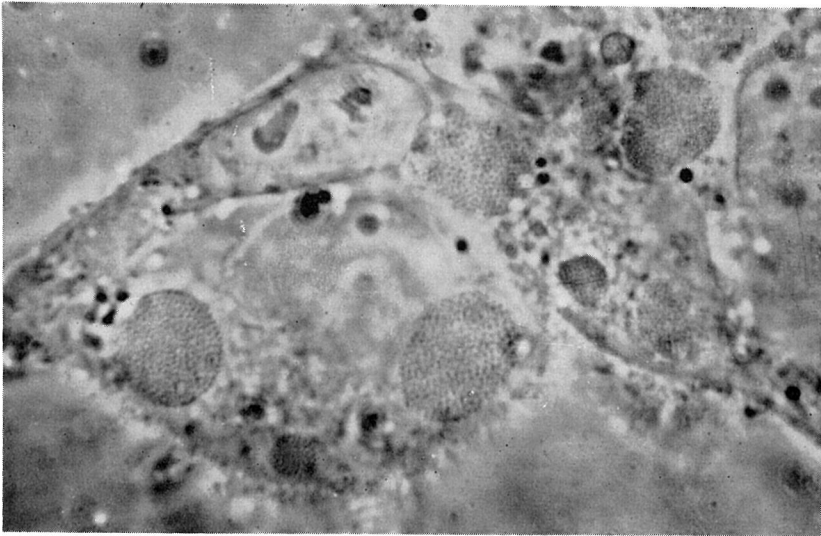


Fig. 1

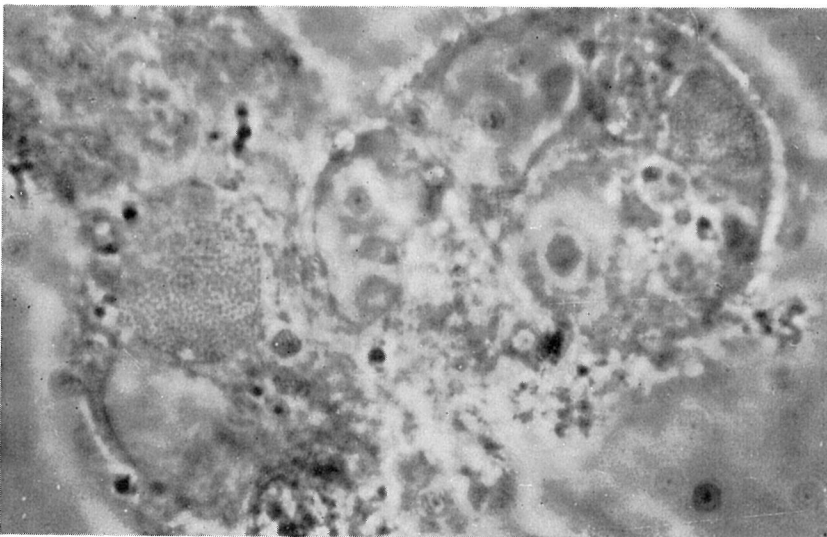


Fig. 2

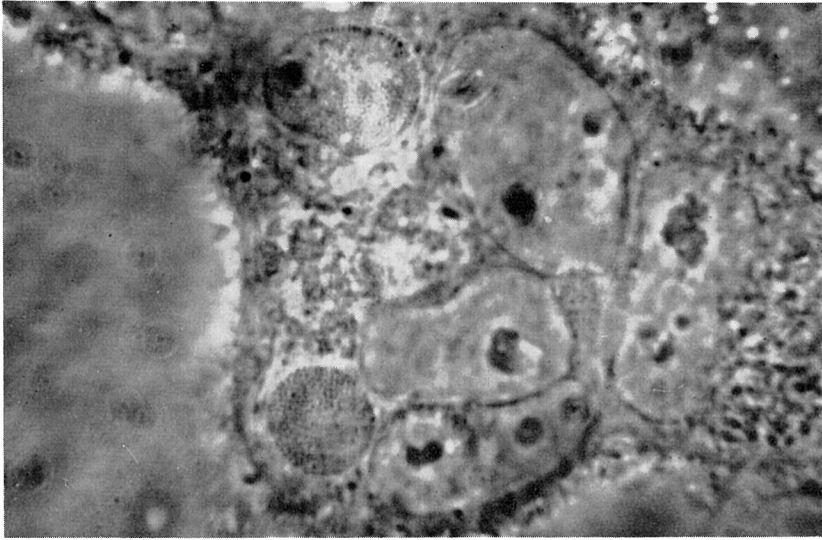


Fig. 3

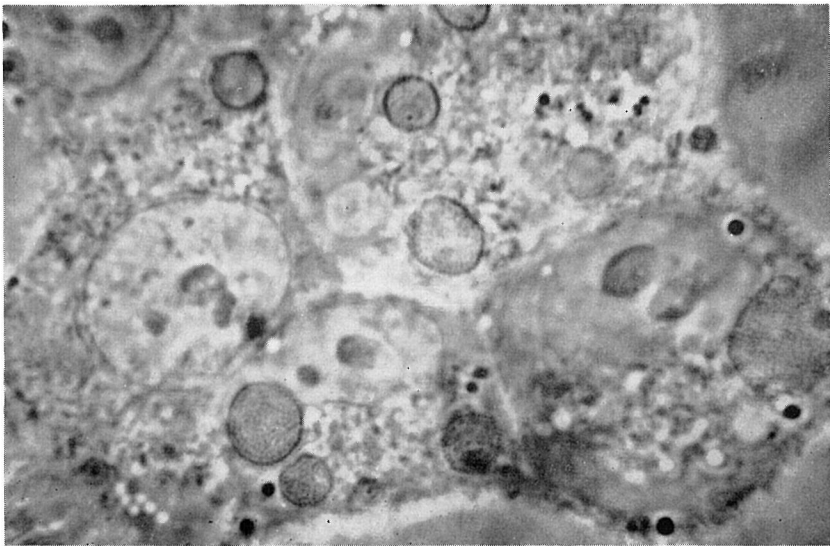


Fig. 4