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A Study of New Inclusion Bodies of Cowpox Virus

The first description of inclusion bodies of cowpox virus was made by Downie (1947). He described large sharply defined homogeneous round inclusions which were eosinophilic with hematoxylin-eosin dye (H-E). He also noticed the difference in appearances between the inclusions of cowpox and vaccinia virus, the latter being irregular granular masses in the cytoplasm of infected cells. We have already reported that there were two types of inclusion bodies designated "A" and "B" type bodies, in various pox virus infections. The "B" type bodies are found always in all pox virus infections so far studied. (Kato *et al.* 1955; Kato 1955; Kamahora *et al.* 1955; Kamahora *et al.* 1958; Kato and Cutting 1958, 1959; Kato *et al.* 1959a). Recently almost all "B" type bodies have been found to be sites of virus antigen (Takahashi *et al.* 1958; Takahashi 1959; Takahashi *et al.* 1959; Kato *et al.* 1959b). There is a fair likelihood that there are "B" type inclusions in cowpox virus. The inclusions Downie described might be classified as "A" type bodies. This

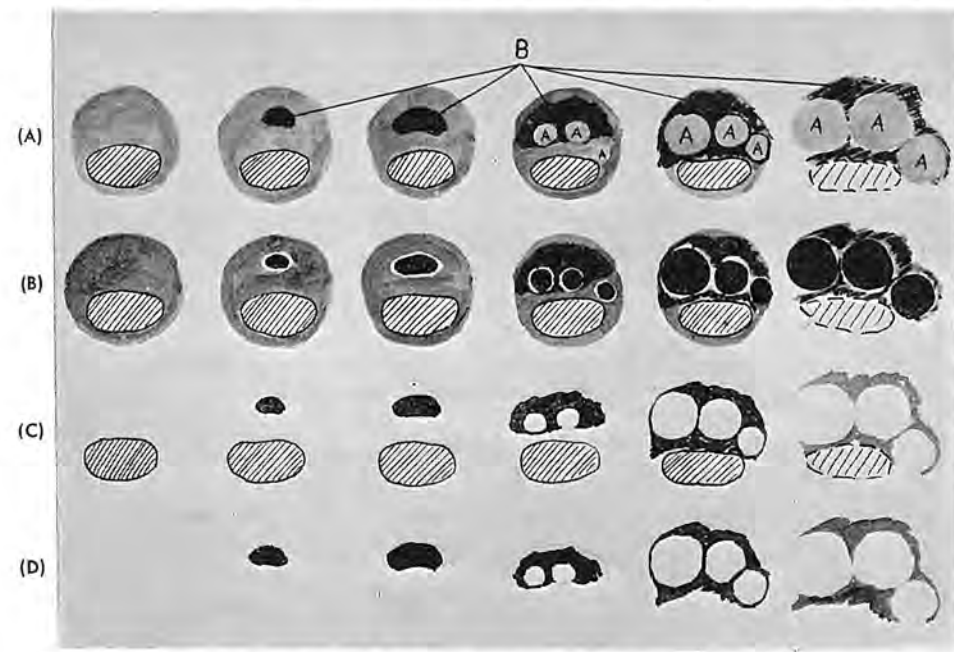

Fig. 1. Development of two kinds of inclusion bodies and comparison of several histochemical and histoimmunological reactions of the infected cells.
(A) Giemsa, fixed with methanol. "B" stains reddish purple, "A" stains pale blue.
(B) Hematoxylin-eosin, fixed with Bouin's fluid. "B" stains with hematoxylin with a halo, "A" takes on only a red tinge with a halo.
(C) Feulgen reaction. "B" absorbs fuchsin red, "A" is not stained.
(D) Fluorescein-isothiocyanate couplied antibody staining. "B" shows yellow green fluorescence and neither the nucleus nor "A" is demonstrated.

letter describes two kinds of inclusion bodies of cowpox virus which are entirely different in appearance. The cowpox virus (CP LB red strain) was kindly given by Dr. Tagaya of the National Institute of Health, Japan. HeLa cells, FL cells and L cells in tissue culture and rabbit skin, rabbit cornea, CAM of embryonated eggs and mouse Ehrlich ascites tumor cells were very susceptible to cowpox virus. Virus was inoculated into these host cells by the usual technique. Both sections and smear preparations of infected tissues were made at intervals after infection. As shown in Fig. 2 and 4, as Downie described, many sharply defined round strongly eosinophilic inclusions were found in the H-E stained preparations of rabbit skin, cornea and CAM. Furthermore careful observation revealed that there are many cells which contained inclusions having irregular form and hematoxylin tinge which look like typical vaccinia inclusions (Fig. 3). The relationship between these two types of inclusions is easily seen when tissue culture cells are used as hosts. For instance, when 10^6 PFU virus was inoculated into 1×10^5 FL cells, an inclusion appears in the cytoplasm about 4 hours after inoculation, which is reddish purple with Giemsa stain and hematoxylinophilic with H-E stain. About 8 hours after virus inoculation, there appear in the cells which have already primary inclusion bodies, clearly outlined round inclusions which are pale blue with Giemsa stain and red with H-E stain (Fig. 5,6). The secondary bodies do not contain any elementary bodies. No transitional form was found between the two forms. Secondary bodies never appear independently in the cell, but are always accompanied with primary inclusions. The primary inclusion is Feulgen positive and is a site of virus antigen, as shown by the fluorescein isothiocyanate coupled antibody technique. The secondary inclnsions are Feulgen negative and do not show any antigenicity (Fig. 7, 8, 11, 12). The primary bodies, first described here, have exactly the same characteristics as the "B" type bodies of other pox viruses. The secondary bodies should be classified as "A" type and the histochemical and histo-immunological nature of the "A" type bodies is very similar

Table 1. Comparison of histochemical and histoimmunological characteristics of "A" and "B" type inclusions of cowpox virus:

Method \ Inclusion	B	A (Downie type)
Giemsa	Reddish purple	Pale blue
Hematoxylin	Hematoxylinophilic	—
Hematoxylin-eosin	Hematoxylinophilic and slightly eosinophilic	Red
Feulgen reaction	+	—
Lipid	—	—
Glycogen Polysacchalide	—	—
Phasecontrast (supravital)	Not demonstrable	Round sharply defined
Viral antigen (Fluorescein antibody)	+	—

Table 2. Comparison of attitude of various pox group viruses to tissue culture cells

Virus	Host cell range	Inclusion		Giant cell formation	Carrier culture
		B	A		
Ectromelia	FL HeLa L	+	+	remarkable	−
Vaccinia (derm. Biken)	FL HeLa	+	−		−
Vaccinia (neuro. IHD)	FL HeLa L	+	−	remarkable	−
Cowpox	FL HeLa L	+	+		−
Rabbit myxoma	FL HeLa	+	−		+
Rabbit fibroma	FL HeLa	+	−		+

Table 3. New and classical names of pox virus inclusions

Virus \ Inclusion	B-type body (primary)	A-type body (secondary)
Ectromelia	B (Kato et al)	Marchal body (Marchal)
Fowlpox	B (Kamahora et al)	Bollinger body (Bollinger)
Canarypox	B (Kato et al)	Eosinophilic body (Burnet)
Cowpox	B (Kato et al)	Eosinophilic body (Downie)
Vaccinia	Guarnieri body (Guarnieri, in section Ewing, in smear)	Bluish body (rare) (Ewing, in smear)
Myxoma	Eosinophilic body (Splendore)	?
Fibroma	Eosinophilic body (Shope)	?

to the "A" type bodies of the ectromelia virus (H strain) (Hagiwara 1957). The inclusions Downie described correspond to "A" type bodies. Development of the two types of inclusion, comparison of their characters, comparison with other pox virus inclusions and new nomenclature for the inclusions are shown respectively in Fig. 1 and Tables 1,2 and 3.

Details of the results and further discussion will be published in Biken's Journal and Virus.

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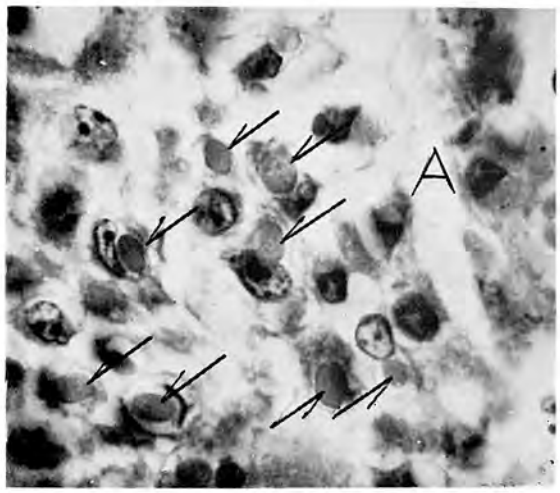

Fig. 2. Section of CAM of embryonated egg infected with cowpox virus, fixed with Bouin's fluid, stained with H-E. 48 hours after virus inoculation. Many "A" type bodies (Downie type) taking on a red eosin tinge can be found in the proliferated ectodermal layer. "B" type bodies are indistinguishable from cytoplasm in this part, because the bodies become diffuse and are pushed aside by "A" bodies.

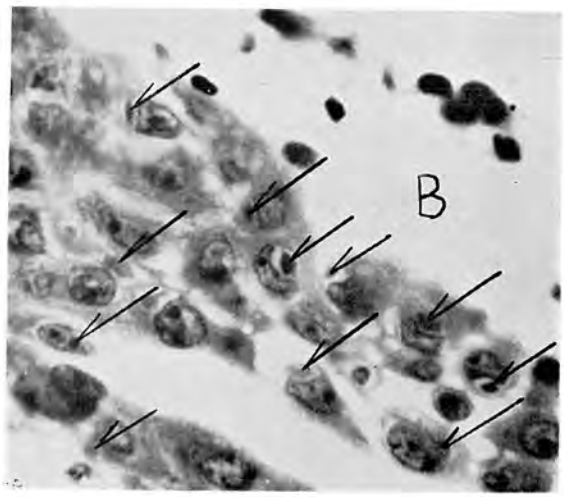

Fig. 3. The adjacent area to Fig. 1. Many "B" type bodies (Guarnieri type) staining with hematoxylin can be found.

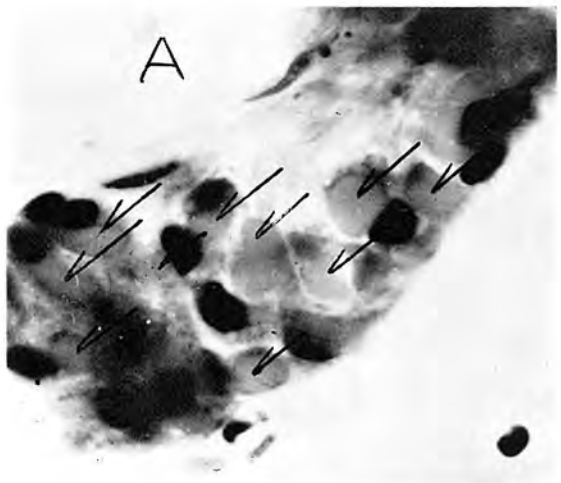

Fig. 4. Section of rabbit skin infected with cowpox virus, fixed with Bouin's fluid, stained with H-E. 48 hours after virus inoculation. Many "A" type bodies stainnig eosin red can be found in the epithelial layer of the hair follicle.

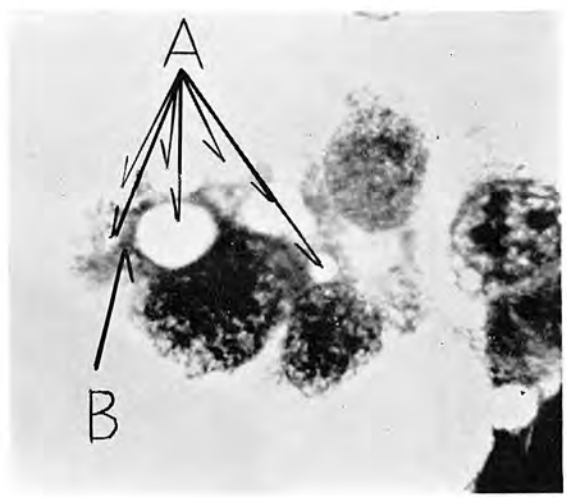

Fig. 5. Cultured FL cells infected with cowpox virus, fixed with methanol, stained with Giemsa solution. "A" type bodies staining pale blue and "B" type bodies staining reddish purple can be seen.

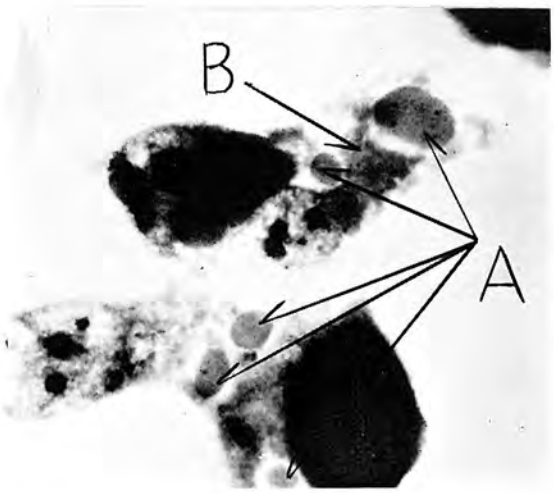

Fig. 6. Cultured FL cells infected with cowpox virus, fixed with Bouin's fluid, stained with H-E. "A" type bodies staining eosin red. "B" type bodies are hematoxylinophilic. Diffuse "B" bodies are often indistinguishable from the cytoplasm.

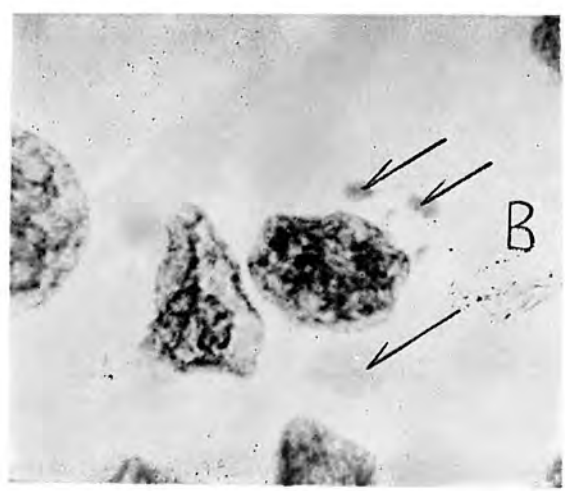

Fig. 7. Showing positive Feulgen reaction of "B" type bodies in FL cells

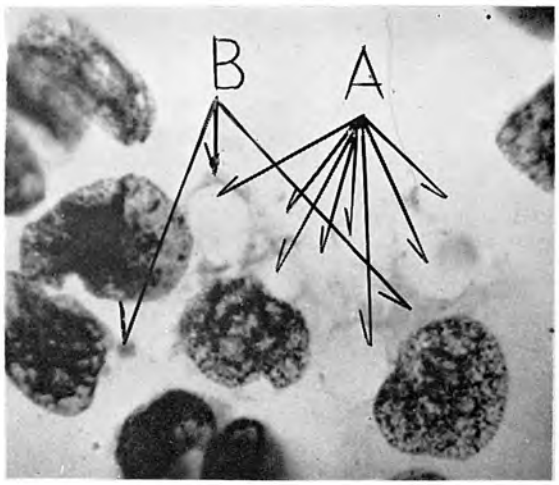

Fig. 8. Showing positive Feulgen reaction of "B" type bodies in FL cells. "A" type bodies always give a negative reaction.

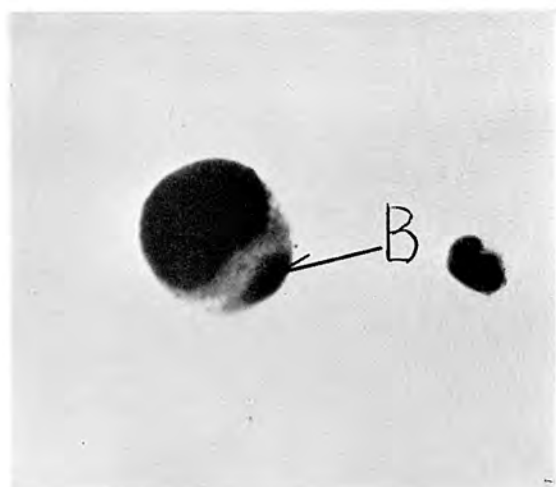

Fig. 9. Single "B" type body (reddish purple) of a mouse Ehrlich ascites tumor cell infected with cowpox virus, fixed with methanol, stained with Giemsa solution.

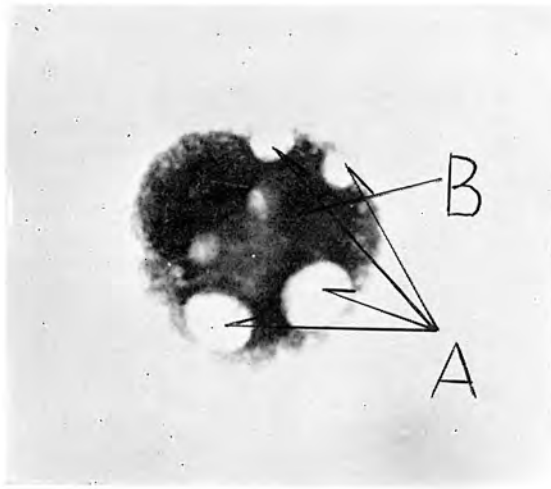

Fig. 10. An Ehrlich ascites tumor cell with several pale blue "A" bodies and a diffused reddish purple "B" body, stained with Giemsa solution.

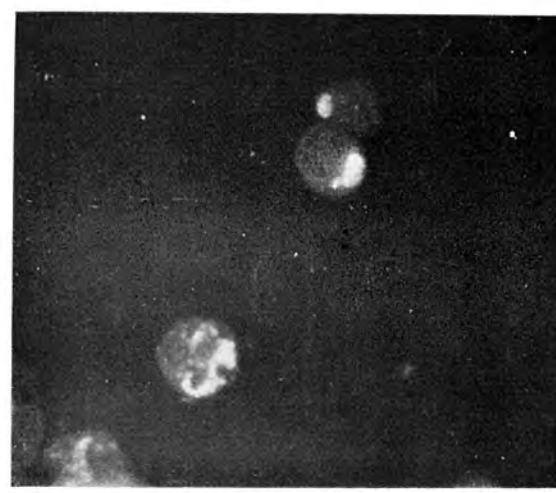

Fig. 11. Yellow green fluorescent areas in the infected Ehrlich ascites tumor cells stained with fluorescein isothiocyanted coupled cowpox virus antibody, which correspond to "B" type bodies.

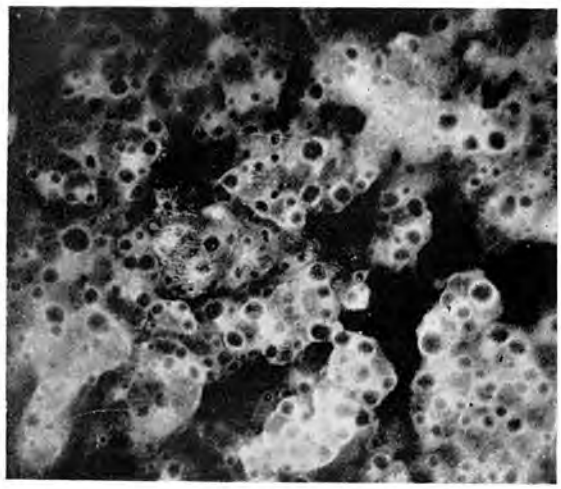

Fig. 12. Yellow green areas in the infected FL cells stained with fluorescein-isothiocyanate coupled cowpox antibody, which correspond to "B" type bodies. "A" type bodies do not become fluorescent.