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A Study on the Morphological and Cyto-immunological Relationship between the Inclusions of Variola, Cowpox, Rabbitpox, Vaccinia (Variola origin) and Vaccinia IHD and a Consideration of the Term "Guarnieri body"

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SUMMARY

The inclusions of variola, cowpox, rabbitpox, vaccinia (variola origin) and vaccinia IHD were studied. All these viruses produce the same type of inclusions designated "B" type inclusions. These "B" type inclusions have been proved to contain a great deal of virus antigen as well as Feulgen positive material. Besides the "B" type inclusions, cowpox virus produces "A" type inclusions which correspond to the inclusion Downie described. The "A" type inclusions of two strains of cowpox virus (LBR and LBW) were Feulgen negative and did not show any antigenicity by Coons' fluorescent antibody technique. The term "Guarnieri body" and "Vaccinia virus" are discussed. The "Guarnieri body" Guarnieri described, was found to correspond to the "B" type inclusions.

INTRODUCTION

Our previous studies have shown that all pox viruses so far studid, *i.e.* ectromelia (Kato *et al.*, 1955; Kato, 1955), fowlpox (Kamahora *et al.*, 1955), canarypox (Kato and Cutting, 1958), vaccinia (Kamahora *et al.*, 1958), cowpox (Kato *et al.*, 1959), rabbit myxoma and rabbit fibroma virus (Kato and Cutting, 1959) produce "B" type inclusions which are considered to be the sites of virus multiplication in the infected cells. Morphologically, these "B" type inclusions are indistinguishable from each other. Histochemically these bodies show a positive Feulgen reaction. Cyto-immunological studies have proved that they contain a great deal of virus antigen (Takahashi *et al.*, 1958; Takahashi *et al.*, 1959a; Takahashi *et al.*, 1959b; Kato *et al.*, 1959; Kameyama *et al.*, 1959). The antigenicity of the inclusions of fowlpox and canarypox viruses have not been studied yet. Among these viruses, ectromelia, fowlpox, canarypox, cowpox and vaccinia viruses produce "A" type inclusions besides the "B" type inclusions, although the "A" type inclusions of vaccinia were rarely if ever seen. Cyto-immunologically Noyes and Watson (1955) reported the occurrence of fluorescent area in the cultured human cells infected with vaccine virus.

As to the inclusions of variola, rabbitpox and the so-called vaccinia, innumerable papers have been published. However, no comparative studies have been made

on the two types of inclusions in regard to their morphology, histochemistry and cyto-immunology. The present study is concerned with these respects and the terms "Guarnieri body" and "vaccinia virus" are clarified.

MATERIALS AND METHODS

1. *Viruses*

Variola virus (Yamamoto strain passed through chorioallantoic membranes of embryonated eggs 47 times after isolation from a variola patient), cowpox virus (LB White and LB Red strains) and rabbitpox (Utrecht strain) were kindly given by Dr. Tagaya of the National Institute of Health of Japan. Vaccnia virus (Biken's dermotropic strain) which is now widely used for vacciniation in Japan, was established by passing it through rabbits and cows after isolation from a variola patient in 1937. Vaccinia IHD strain, origin of which is not yet certain, was kindly given from Dr. Kanazawa (Takeda Pharmaceutical Comp.). Ectromelia virus ("G" strain) isolated by Hagiwara, which forms the "A" type inclusions with many elementary bodies, were also used for comparison (Hagiwara, 1959). Each of these viruses was inoculated into the chorioallantoic membrane of embryonated eggs and 2 days later the infected area of the membrane was removed and ground with alundum. The supernatant was used to obtain virus material after centrifugation at 3,000 rpm for 15 minutes.

2 *Tissue culture*

HeLa cells (clonal strain), FL cells (clonal strain) were kindly given by the Tissue Culture Center in our Institute. About 5×10^5 cells were dispersed into a Porter culture tube containing a 5×40 mm coverslip. The medium was Earl's balanced salt solution containing 0.5 per cent lactalbumin hydrolyzate, 0.1 per cent yeast extract and calf serum which was adjusted to be 10 per cent.

3. *Histological technique*

Chorioallantoic membranes of 10 day old eggs were inoculated with these viruses by Burnet's method. Forty eight hours after virus inoculation, the membranes were fixed with Bouin's fluid. They were then sectioned by ordinary histological technique and stained by hematoxylin-eosin (H-E).

4. *Fluorescein antibody technique*

Fluorescein isothiocyanate was kindly given by Dr. Saiwald of San Francisco University. Cowpox virus (LB Red strain), vaccinia (Biken's strain, variola origin) and vaccinia (IHD strain) were inoculated intradermally into rabbits. After the development of a reddish induration and complete disappearance of the lesion, the diluted virus material was inoculated intravenously and blood was collected from the rabbits 1 week later. The titers of these three kinds of immunesera were about 1:320 by the complement fixing reaction. The globulin fraction obtained by precipitation with half saturated ammonium sulphate was conjugated with fluorescein isothiocyanate after Marshall's method (Marshall *et al.* 1958) in the same way as reported in our previous papers for myxoma virus (Takahashi *et al.*, 1958; Takahashi et al., 1959). The specificity of these conjugates was confirmed in the following way. 1) Uninfected culture cells did not stain with fluorescent antibody. 2) Pretreatment of infected culture cells with non-conjugated rabbit antiserum inhibited staining by fluorescent antiserum, whereas pretreatment with normal rabbit serum did not inhibit the staining.

RESULTS

1. *Morphology of the inclusions appearing in FL cell or HeLa cell cultures*

The coverslips of each kind of viruses were first examined 6 hours after virus

inoculation. The coverslips taken from the test tubes were fixed with methanol and stained with Giemsa solution. In these preparations, similar types of cytoplasmic inclusion were recognized as reddish purple bodies. Successive observation revealed that their nature and development from round small compact bodies to large diffuse granular networks was exactly the same as those of the "B" type inclusions of several other pox viruses such as ectromelia virus, which we have reported. Among the viruses two strains of cowpox virus produced another kind of inclusion which stained pale blue, besides the "B" type inclusions. This corresponds to the "A" type inclusion. "A" type inclusions were more common in cowpox virus than in ectromelia virus. The LBR strain of cowpox virus especially produced "A" type inclusions in almost all infected cells. On the contrary, variola, rabbitpox, vaccinia IHD and vaccinia (variola origin) usually did not produce "A" type inclusions under these tissue culture conditions. The inner structure of the "A" type inclusion of cowpox virus appeared rather homogeneous unlike the "A" type inclusion of "G" strain of ectromelia virus which was filled with minute elementary bodies. The appearance of Giemsa stained preparations are shown in Fig. 4-11.

The response of these tissue culture cells to the viruses varied. Variola virus forms a rather syncitial type of giant cell in a HeLa cell culture, while the vaccinia IHD strain forms a round multinucleated giant cell similar to that of the ectromelia virus. Vaccinia virus (variola origin), cowpox virus (LBR and LBW strain) and rabbitpox also seemed to form giant cells to some extent, not so much. Generally speaking, the cells in the test tubes inoculated with the viruses showed total degeneration by the end of the observation period without any tendency to carrier culture as in the case of the FL cell-rabbit fibroma or rabbit myxoma virus system (Kato *et al.*, 1959).

When the coverslips were fixed with *Bouin's* fluid instead of methanol and stained with hematoxylin-eosin, the morphological aspect of both "A" and "B" type inclusions changed entirely. Thus the "A" type inclusions of cowpox virus appeared bright red and were surrounded by halos, probably due to some shrinkage of their contents. The "B" type inclusions took on a hematoxylin combined with eosin tinge. The "B" type inclusions in an early stage were also accompanied by some shrinkage with halo formation. However, the border of the large diffuse "B" type inclusions was dim and blurred into the hematoxylin colour of cytoplasm. (Fig. 12-17).

The inclusion bearing cells were studied supravitally with a phase contrast micrscope. The "A" type inclusions were easily recognizable as sharply defined round glittering spheres. However, elementary bodies could not be demonstrated in most of them contrary to the case of the "A" type inclusions of the "G" strain of ectromelia virus (Fig. 46, 47). Isolated single "A" bodies seemingly free from any cytoplasmic granular covering especially showed a homogeneous structure. The structure of the "A" type inclusions of cowpox virus might be similar to those of ectromelia virus, and probably depends upon the kind of strain of virus used. An electronmicroscopic study of thin sections of "A" type inclusions will clarify this to some extent. We could not recognize the exact location of "B" type inclusions in the cells of any of these pox viruses by supravital examination.

The best way to recognize both types of inclusions in the same preparation was therefore Giemsa staining after methanol fixation, although "A" type inclusions were very clear by hematoxylin-eosin staining after Bouin's fixation.

No essential difference in inclusion formation of these viruses between FL and HeLa cells was noticed.

Three types of development of the inclusions of pox viruses are shown in the scheme in Fig. 1.

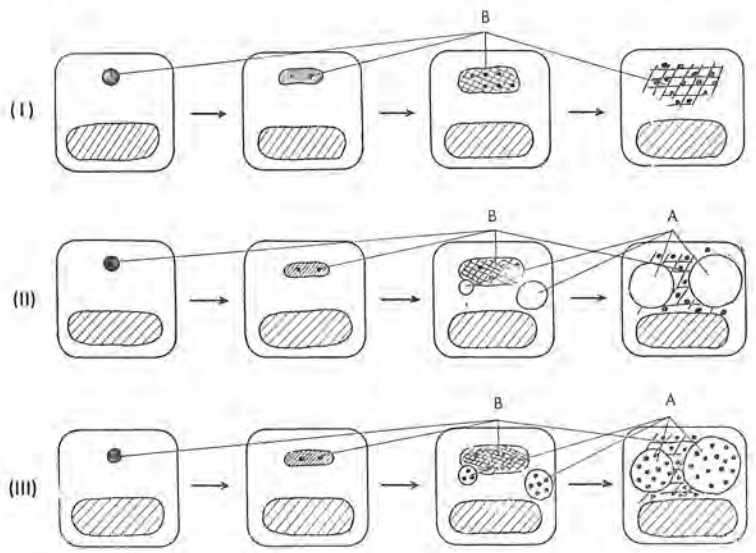


Fig. 1. Scheme of development of three different types of inclusions.
(I): Variola, vaccinia (variola origin), vaccinia (IHD), rabbitpox, rabbit myxoma and rabbit fibroma viruses.
(II): Cowpox (LBR and LBW) and ectromelia(H) viruses.
(III): Ectromelia(G), fowlpox and canarypox viruses.

2. *Histochemical study of the inclusions*

All these "B" type inclusions gave a Feulgen positive reaction, whereas the "A" type inclusions of neither strain of cowpox virus did. (Fig. 18-23.) Neither fat nor polysaccharide was demontrated in either type of inclusion. Probably the "A" type inclusions of the cowpox virus have a protein nature like the "A" type inclusions of the ectromelia virus.

3. *Cyto-immunological studies of the infected cells*

Using the fluorescein-isothiocyanate coupled antibody of cowpox virus, of vaccinia virus (variola origin) and of vaccina virus (I H D strain) the cross experiments shown in Table 1. were made. By using the method of restaining with Giemsa solution published before, the yellow green fluorescent spots were all found to coincide with the site of "B" type inclusions in each virus examined, although the fluorescent area seemed to be larger. The "A" type inclusions of

Table. 1 Cyto-immunological cross reaction among poxgroup viruses.

"B" type inclusion	Virus	Strain	Fluorescein coupled antibody: Vaccinia virus (Variola origin)	Cowpox virus (LB Red)	Vaccinia virus (I H D)
	Variola	Yamamoto	+	+	+
	Vaccinia	Biken (Variola origin)	+	+	+
	Vaccinia	I H D	+	+	+
	Rabbitpox	Utrecht	+	+	+
	Cowpox	LB Red	+	+	+
	Cowpox	LB White	+	+	+
	Ectromelia	G	+	+	+
	Ectromelia	H	+	+	+

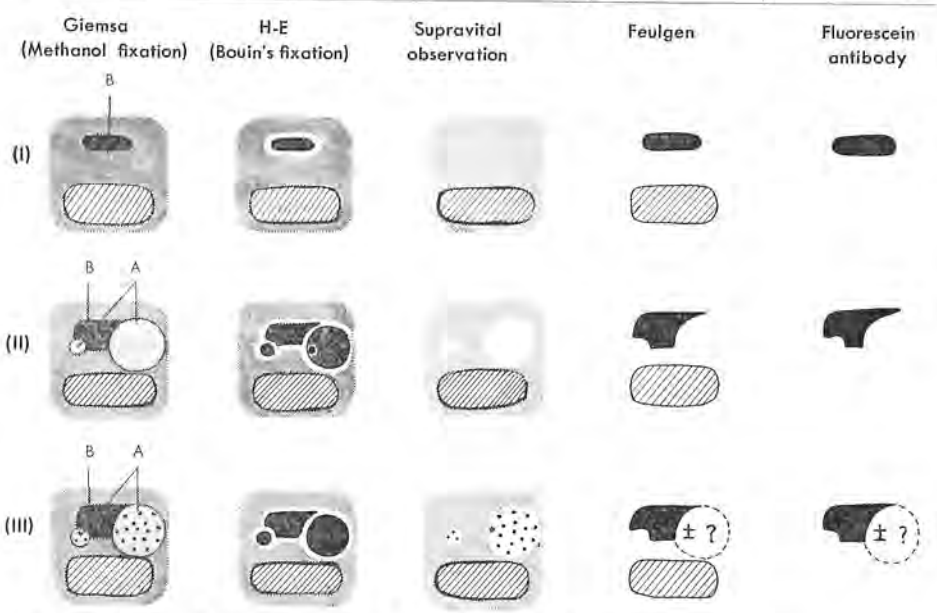


Fig. 2. Scheme of the appearance of the "A" and "B" type inclusions in various cytological procedures.

(I) : Variola, vaccinia (variola origin), vaccinia IHD, rabbitpox, rabbit myxoma and rabbit fibroma viruses.
(II) : Cowpox (LBR and LBW) and ectromelia (H strain) viruses.
(III) : Ectromelia (G strain) virus.
Giemsa : "B" stains reddish purple. "A" of (II) stains pale blue. "A" of (III) stains faint reddish granular on a pale blue back ground.
H-E : "B" stains hematoxylinophilic with combined eosin tinge, surrounded by a halo. "A" of (II) and (III) stains red, surrounded by a halo.
Supravital observation : "B" is invisible. "A" of (II) is sharply defined round and empty. "A" of (III) is a sharply defined round body containing many minute particles.
Feulgen reaction : Nucleus and "B" take fuchsin red.
Fluorescein antibody: Only "B" shows definite fluorescence.

cowpox virus did not show any fluorescence. A careful observation was made of the localization of the fluorescence in the nuclear area, since intranuclear inclusions of vaccinia virus have been reported. However, no definite fluorescent spots could be demonstrated in the nuclei, although the peripheral part of some nuclei had a faint fluorescence probably due to diffuse "B" type inclusions covering them. (Fig.32-45).

Any of three kinds of fluorescein isothiocyanate coupled antibody (anti-cowpox (LBR), anti-vaccinia (variola origin) and anti-vaccinia I H D) could stain any "B" type inclusions of these pox viruses studied.

The characters of all "A" and "B" type inclusions of pox viruses are shown schematically in Fig. 2.

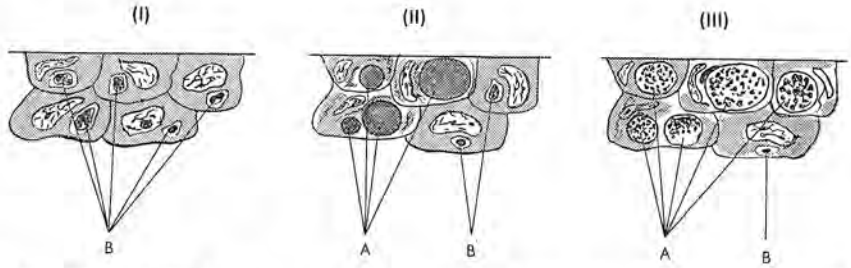
4. *A study of the inclusions in hematoxylin-eosin stained sections of chorioallantoic membrane infected with the viruses*

Many reports have been published of studies in which the virus inclusions were studied in sections, probably because sections are the commonest and most routine method for pathological study. To clarify the appearance of "A" and "B" type inclusions in section preparations, such experiments were undertaken. Ectromelia virus was used for the comparison with these viruses. In the sections of two strains (LBR and LBW) of cowpox virus, many round, sharply defined bodies were seen in proliferated ectodermal layers, as described by Downie 1947. They also look like the "Marchal bodies" in ectromelia virus infection (Marchal, 1935). From their stainability in tissue culture cells, it is reasonable to regard them as "A" type inclusions. In the areas adjacent to these inclusions, some rather irregular hematoxylinophilic and eosinophilic bodies were noticed. These usually have halos. These must be the "B" type inclusions seen in section. As mentioned before, the diffuse "B" type bodies were almost impossible to distinguish from the cytoplasm and other hematoxylinophilic material. In fact, in the infected area of the CAM, many small hematoxylinophilic granules could be seen. These were very probably cell debris and made it difficult to identify "B" type inclusions exactly. In the sections of CAM infected with variola, vaccinia I H D, vaccinia (variola origin), and rabbitpox no area containing grouped round, reds, sharply defined inclusions was seen. Some irregular bodies which took up both hematoxylin and eosin and were usually surrounded by a halo, were found in the cytoplasm of cells in the proliferating ectodermal layer, which were consistent with the description of Guarnieri. Thus all these pox viruses were shown to produce "B" type inclusions in infected CAM. However there was difficulty in identification of "B" type inclusions. On very rare occasions, a few small, round, red bodies were noticed in preparations infected with variola and vaccinia (variola origin). The identification of these must await a specific reaction for "A" type inclusions, although we once regarded them as "A" type inclusions of vaccinia virus (Kamahora *et al.*, 1958). The appearance of H-E stained sections is shown in Fig. 24-31.

Thus the inclusion bodies were demontrated in H-E stained sections of CAM infected with these viruses. However, it is not advisable to use section prepara-

otins for study of inclusions. The difficulties in identification we encountered often cause misunderstanding or confusion about the morphology of the inclusions.

The appearance of the "A" and "B" type inclusions in the H-E stained sections is shown schematically in Fig 3.



B: Irregular, hematoxylinophilic combined with eosin tinge.

A: Round, homogeneous, red, probably because of the protein nature of the ground substance.

B: irregular, hematoxylinophilic combined with eosin tinge.

A: Round granular red. The lipid constituting the ground substance is eliminated and mainly elementary bodies are left.

B: Irregular, hematoxylinophilic combined with eosin tinge.

Fig. 3 Scheme of the appearance of the "A" and "B" type inclusions in H-E stained sections.

(I): Variola, vaccinia (variola origin), vaccinia IHD, rabbitpox, rabbit myxoma and rabbit fibroma viruses.

(II): Ectromelia (G and H strains), cowpox (LBR and LBW strains) viruses.

(III): Fowlpox and canarypox viruses.

DISCUSSION

1. *Inclusion formation of these viruses*

All the viruses studied produce at least one type of inclusion "B" in common which is reddish purple with Giemsa stain and hematoxylino-eosinophilic with H-E stain, histochemically Feulgen positive and has been shown to be the site of virus antigen.

Furthermore the "B" type inclusion of the pox viruses studied, could be stained well with any of three kinds of fluorescein isothiocyanate coupled antibody (anti-cowpox(LBR), anti-vaccinia (variola origin) and anti-vaccinia I H D).

The "B" type inclusions of these viruses closely resemble each other. The descriptions of the inclusions of variola virus by Guarnieri himself and those of rabbitpox virus (Rosahn *et al.*, 1936) are considered to indicate these "B" type inclusions. Two strains (LBR and LBW) of cowpox virus produce "A" type inclusions. "A" type inclusion formation is always subsequent to "B" type formation in infected cells. "A" type incluclsion formation never occurs without "B" type inclusion formation. It is vrey interesting to note that the nature of these "A" type inclusions is different in different pox viruses and even in the strains of a single virus.

Our previous report (Kamahora *et al.*, 1958) showed the existance of "A"

and "B" type inclusion in vaccinia virus infection in CAM, rabbit cornea or rabbit skin, although the appearance of "A" type inclusion is very rare. "A" type inclusion of vaccinia virus in tissue culture was almost none (Kato and Cutting, 1958). Further investigation will be required to know whether a certain factor existing in host cell or in surrounding media or in virus itself, might give an influence upon "A" type inclusion formtion.

All our results suggest that "B" type inclusion must be a site for pox virus multiplication and "A" type inclusion seems to play no essential role for virus multiplication.

2. *Vaccinia virus*

Our results indicate that variola virus and vaccinia virus (variola origin) as a rule, produce only one type of inclusion, the "B" type, while cowpox virus definitely forms two types of inclusions, both "B" and "A". Thus there are two modes of inclusion formation.

Before we discuss the term "Guarnieri body", the terminology of vaccinia, variola and cowpox virus, must be discussed, for it seems that their histories are somewhat complicated. In fact, in the text book "Viral and Rickettsial Infections of Man" (*second* edition 1952), Smadel considers cowpox as a synonym of vaccinia. In the third edition of the same book, Downie (1959) wrote under the heading of "Vaccinia" as follows. "The term vaccinia is used here to in dicate the infection which is caused by the virus propagated in laboratories and used for prophylactic vacciniation against smallpox. As indicated above *the history and the origin of many of the strains used for this purpose are obscure. While certain strains are alleged to have been derived from smallpox, it seems to the writer that the properties and the characters of most strains now in use suggest that they have been derived from cowpox virus, i.e., the virus responsible for the pox disease of cows.*" Thus "Vaccinia" does not seem to denote a definite virus, but it is used conventionally in the laboratory because its origin is obsure. There seems to be three kinds of vaccinia virus in the laboratories in the world as shown in Table 2. The same situation is found in the paper written

Table 2.

Vaccinia virus		
Variola origin	Cowpox origin	Unknown origin

by Fenner and Burnet (1957) on the interpretation of the nomenclature of the poxvirus group. The name Poxvirus variolae, Poxvirus bovis and Poxvirus officinale may well correspond to variola virus, cowpox virus and vaccinia virus respectively. The new term Poxvirus officinale, however, can not add anything essential to the interpretation of vaccinia. Apart from medical convenience, it is desirable, if possible, to call the vaccina virus originating from variola virus simply vaccinia virus. Or the origin should be mentioned each time, as far as it is known, as vaccinia virus (cowpox origin) or vaccinia virus (variola origin). Incidently there seems to be no similar case in which the name of the virus has been changed simply because of a variation in its pathogenicity, made in the laboratory. In the virological sense of the word, "VARIOLA VIRUS vaccinia strain" or "POXVIRUS

VARIOLAE vaccinia strain" and "COWPOX VIRUS vaccinia strain" or simply "COWPOX VIRUS" or "POXVIRUS BOVIS" would be ideal for academic usage. To a so-called vaccinia virus whose origin is unknown, the name "VACCINIA VIRUS (unknown origin) or "POXVIRUS OFFICINALE" might be given.

3. *"Guarnieri body"*

A vague concept that inclusion bodies of poxgroup viruses are eosinophilic or acidophilic has prevailed until the establishment of the two inclusion body theory. Careful analysis of the papers published shows that there are many discrepancies in the descriptions of the nature of the poxgroup inclusions. Guarnieri himself reported (1893) "basophil, hematoxylinophil" inclusions in variola and vaccinia virus infection. Since then many investigators who studied the variola and vaccinia virus inclusions using section or smear preparations stained with various dyes, have used various expressions for the nature of the inclusions, i. e. "acidophilic", "basophilic", "eosinophilic", "hematoxylinophilic", "red", "reddish purple" etc. James Ewing observed a bluish body as well as an eosinophilic body in the cell infected with vaccinia virus. A question may be raised as to the entity of inclusion they obseved and the origin of the vaccinia virus they used. Furthermore, electronmicroscopic study of the so-called vaccinia infected cells revealed the existance of two entities differing in electron density and morphology (Gaylord and Melnick, 1953). They designated them *"matrix"* and *"inclusion (Guarnieri body)"*. Their description about *"matrix"* reminds us of the "B" type inclusions (Guarnieri body) of vaccinia virus (variola origin). We do not know whether the *"inclusion (Guarnieri body)"* they described, is an "A" type inclusion or not. However, the answer to this problem will probably depend upon the origin and strain of vaccinia virus they used.

There seems to be some confusion with regard to the terminology and the expression "Guarnieri body". There have been more than a hundred papers dealing with vaccinia inclusions. We are not in a positions to know the origin of the vaccinia virus they used. Nor it is our purpose to classify them exactly. As long as a vaccinia virus of uncertain origin is used in section preparation for a study of inclusions, without knowledge about the two inclusions, discussion will never cease about the stainability and the morphology of the "Guarnieri body".

Now we would like to give a definition of the "Guarnieri body". A "Guarnieri body" is an inclusion found in variola virus infections. The "Guarnieri body" is now shown to stain reddish purple with Giemsa stain and hematoxylinophilic with hematoxylin stain and to contain Feulgen positive material, and also viral antigen. It is advisable not to use any vague expression such as "red", "acidophilic", "eosinophilic", "basophilic" etc., because the "Guarnieri body" contains both basophilic and acidophilic material. Probably the basophilic material is DNA and the eosinophilic material, protein. It is also proper to call the "B" type inclusion of vaccinia virus(variola origin) the "Guarnieri body". If the vaccinia in use is of unknown origin, we can not help calling the "B" type inclusion the "Guarnieri body" according to historical usage, although it is not ideal. The usage of the term "Guarnieri body" should give place to "B" type inclusion in all pox viruses other than the viruses mentioned above.

The classification of the inclusions of poxviruses are shown in Table 3.

Table 3. The classification of the name of poxvirus group.

Inclusion / Virus	"B" type	"A" type
Variola	Guarnieri body	
Vaccinia (Variola origin)	Guarnieri body	very rare (Kamahora et al.)
Vaccinia (I H D)	Guarnieri body	
Rabbitpox	B	
Cowpox	B	A (Downie)
Ectromelia	B	Marchal body
Fowlpox	B	Bollinger body
Canarypox	B	A (Burnet)
Rabbit myxoma	B	
Rabbit fibroma	B	

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Fig. 4-11. FL cells infected with various poxviruses, fixed with methanol and stained with Giemsa solution "B" type inclusions stain reddish purple.

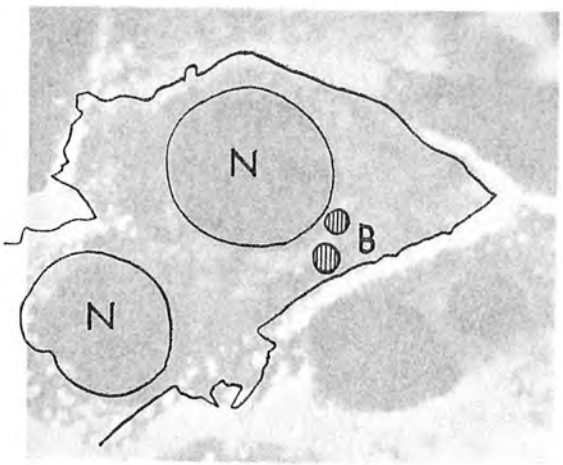

Fig. 4. Variola virus infection. Small compact homogeneous "B" type inclusions.

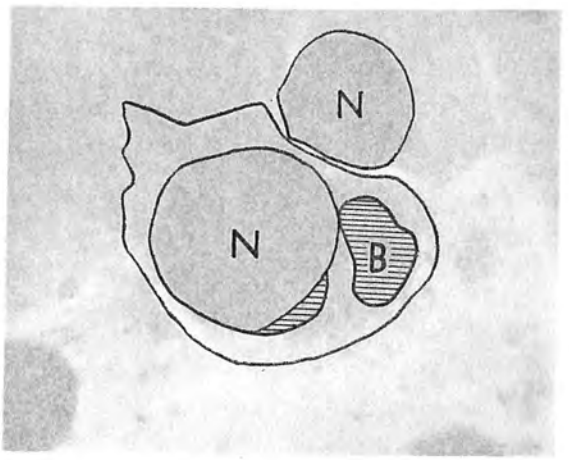

Fig. 5. Variola virus infection. Diffused granular network "B" type inclusions containing many minute elementary bodies.

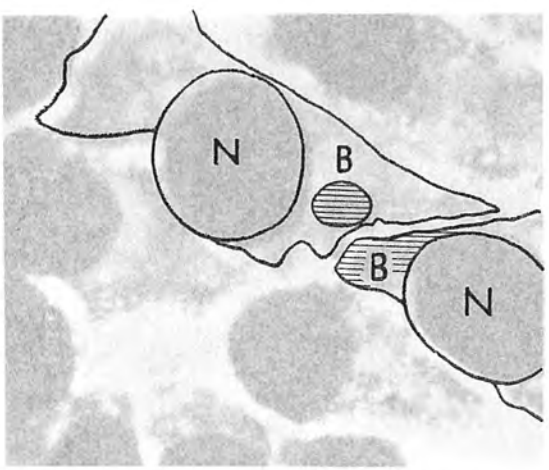

Fig. 6. Vaccinia virus (variola origin) infection. "B" type inclusions.

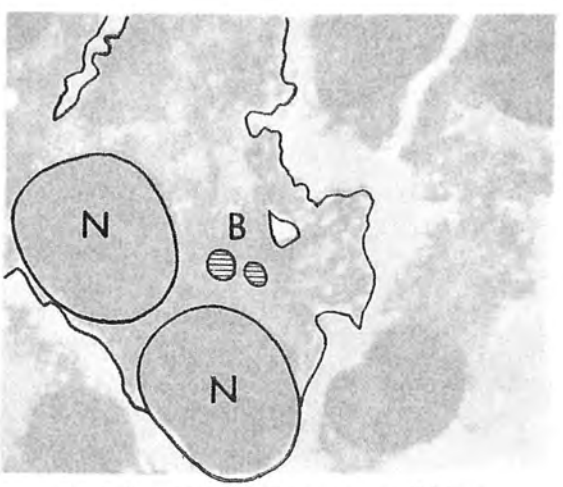

Fig. 7. Vaccinia virus (IHD) infection. "B" type inclusions.

Fig. 4-11. FL cells infected with various poxviruses, fixed with methanol and stained with Giemsa solution "B" type inclusions stain reddish purple.

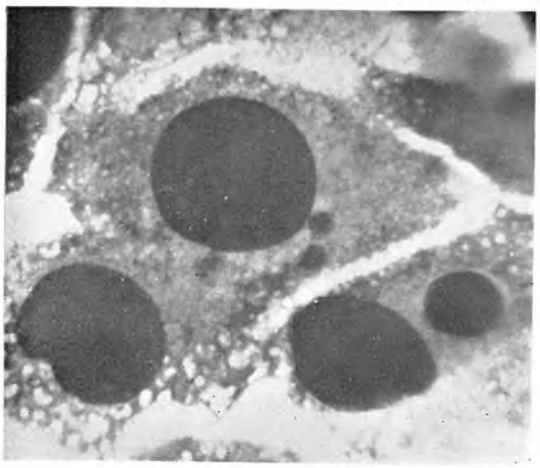

Fig. 4. Variola virus infection. Small compact homogeneous "B" type inclusions.

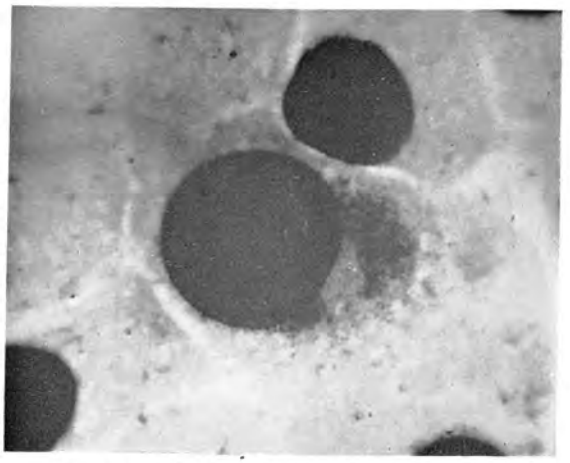

Fig. 5. Variola virus infection. Diffused granular network "B" type inclusions containing many minute elementary bodies.

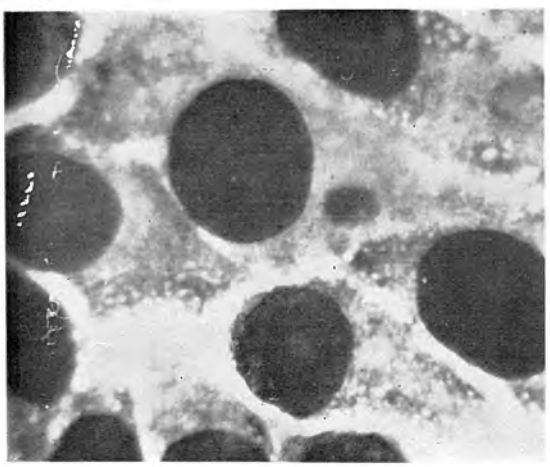

Fig. 6. Vaccinia virus (variola origin) infection. "B" type luclusions.

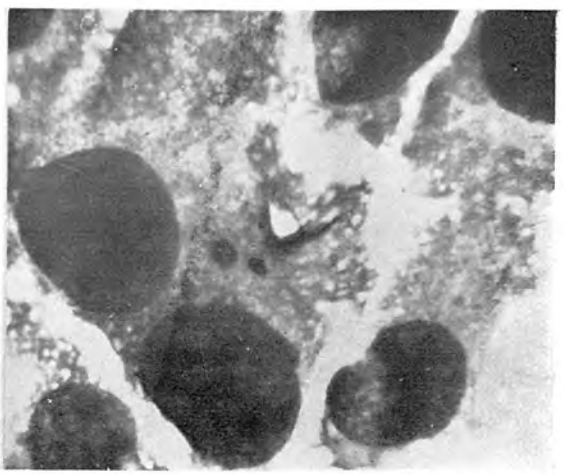

Fig. 7. Vacccinia virus (IHD) infection. "B" type inclusions.

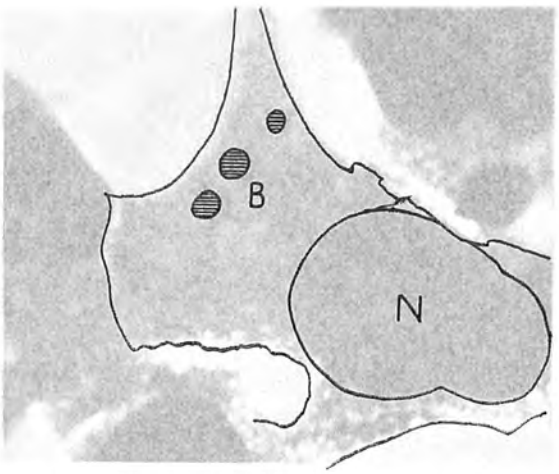

Fig. 8. Rabbitpox virus (Utrecht) infection. "B" type inclusions.

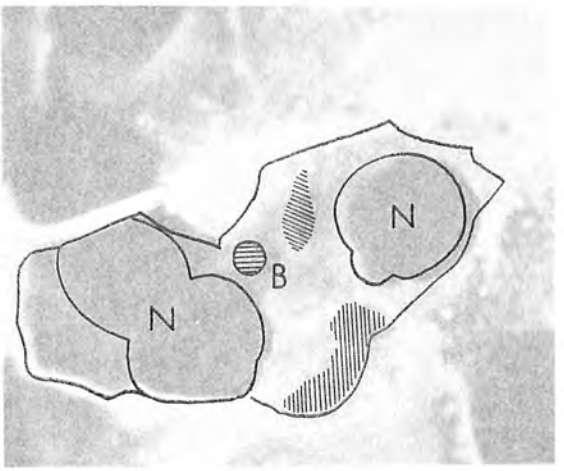

Fig. 9. Cowpox virus (LBW) infections. "B" type inclusions.

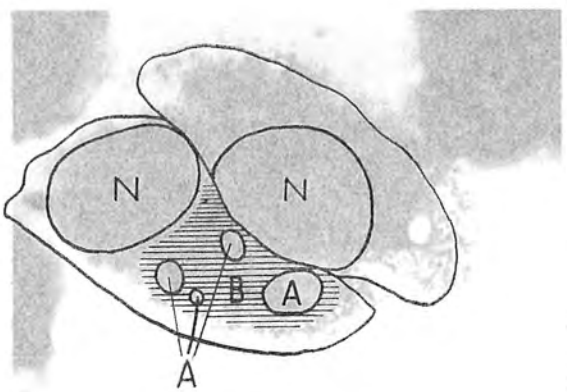

Fig. 10. Cowpox virus (LBW) infection. "A" type inclusions stain pale blue. Diffuse "B" type inclusions spread in cytoplasm.

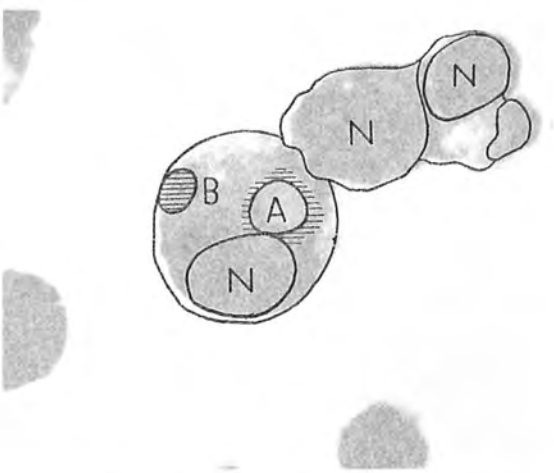

Fig. 11. Ectromelia virus (G) infection. "A" type inclusions contain many minute red elementary bodies.

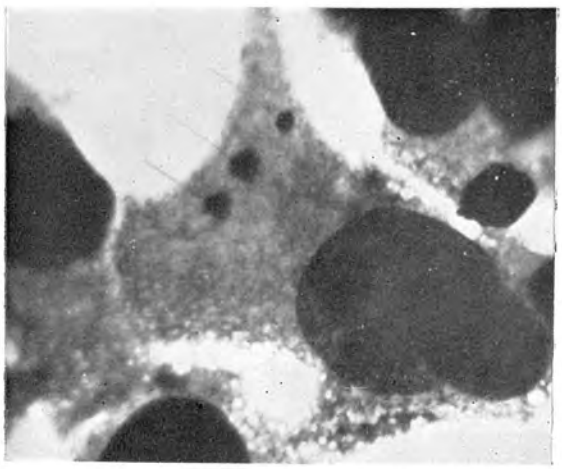

Fig. 8. Rabbitpox virus (Utrecht) infection. "B" type inclusions.

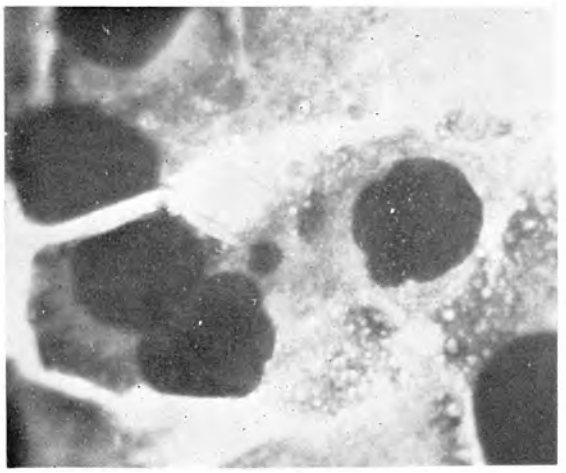

Fig. 9. Cowpox virus (LBW) infections. "B" type inclusions.

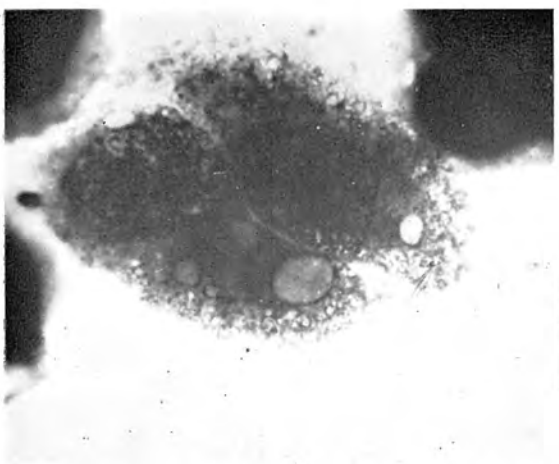

Fig. 10. Cowpox virus (LBW) infection. "A" type inclusions stain pale blue. Diffuse "B" type inclusions spread in cytoplasm.

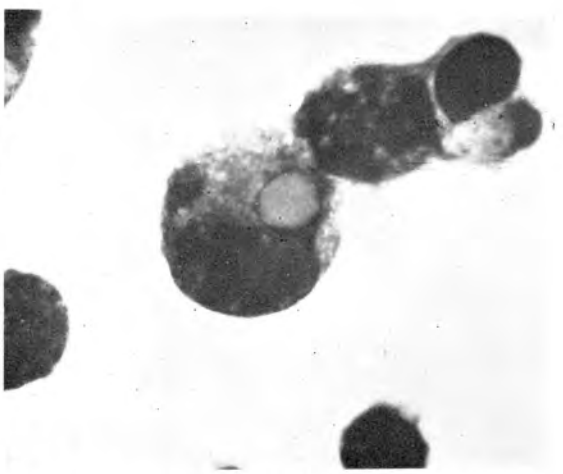

Fig. 11. Ectromelia virus (G) infection. "A" type inclusions contain many minute red elementary bodies.

Fig. 12-17. FL cells infected with various poxviruses, fixed with Bouin's fluid and stained with hematoxylin-eosin. "B" type inclusions are usually surrounded by halos and take on both hematoxylin and eosin tinge.

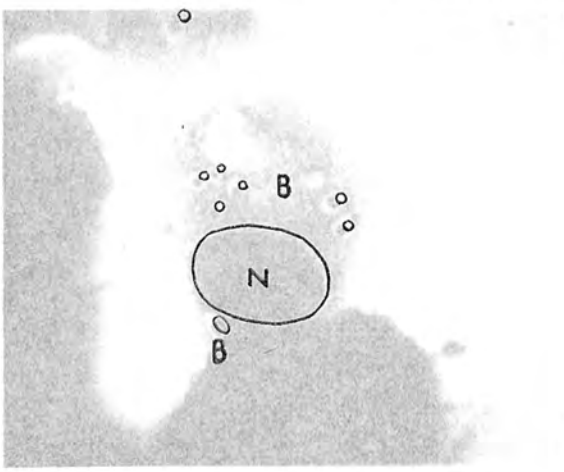

Fig. 12. Variola infection. "B" type inclusions.

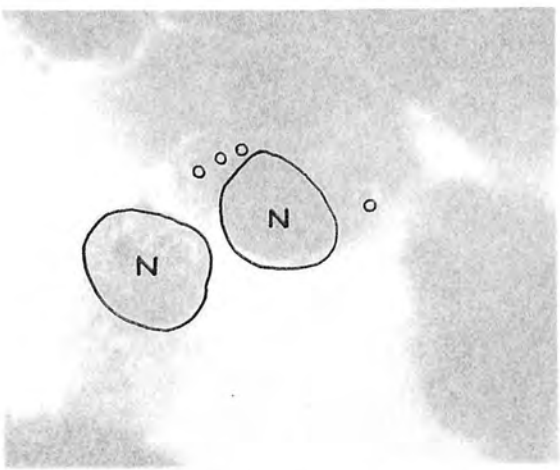

Fig. 13. Vaccinia virus (variola origin) infection. "B" type inclusions.

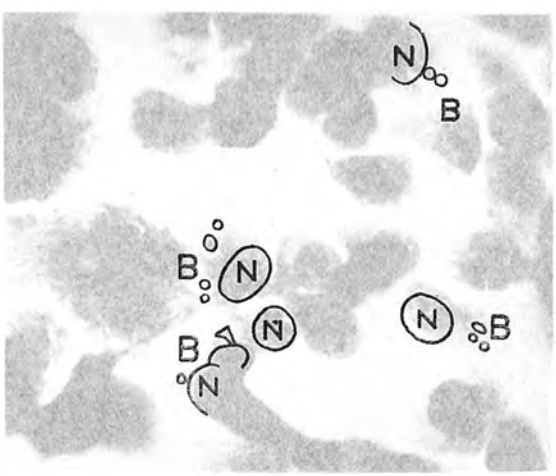

Fig. 14. Vaccinia virus (IHD) infection. "B" type inclusions.

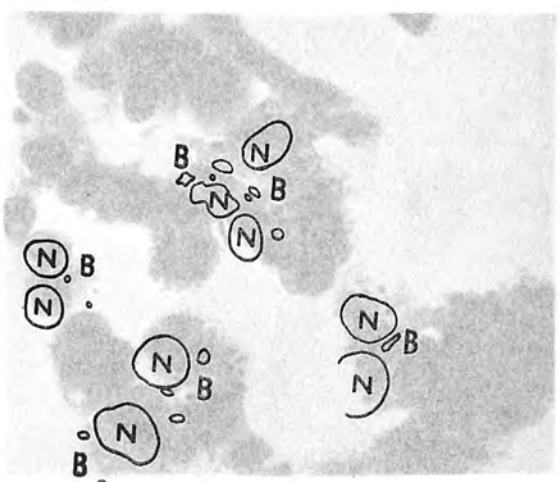

Fig. 15. Rabbitpox virus (Utrecht) infection. "B" type inclusions.

Fig. 12-17. FL cells infected with various poxviruses, fixed with Bouin's fluid and stained with hemotoxylin-eosin. "B" type inclusions are usually surrounded by halos and take on both hematoxylin and eosin tinge.

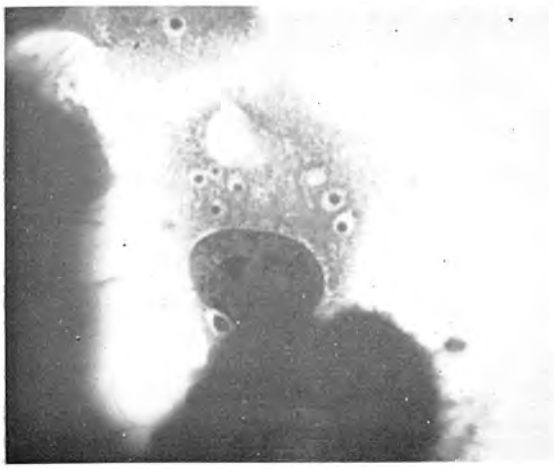

Fig. 12. Variola infection. "B" type inclusions.

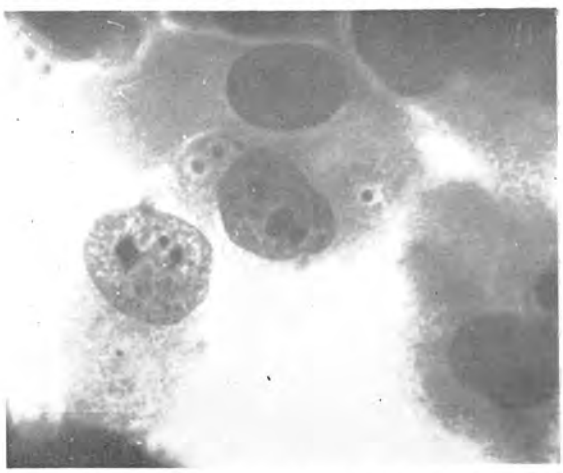

Fig. 13. Vaccinia virus (variola origin) infection. "B" type inclusions.

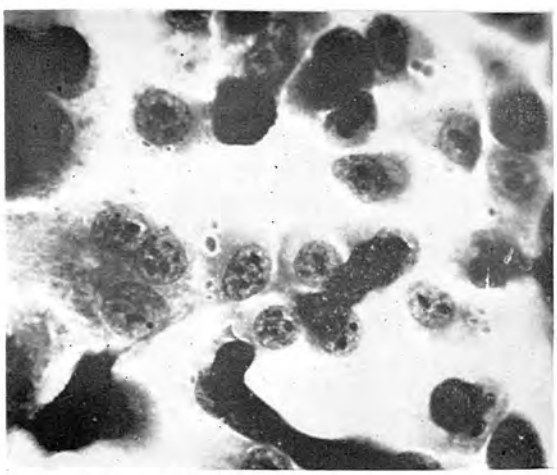

Fig. 14. Vaccinia virus (IHD) infection. "B" type inclusions.

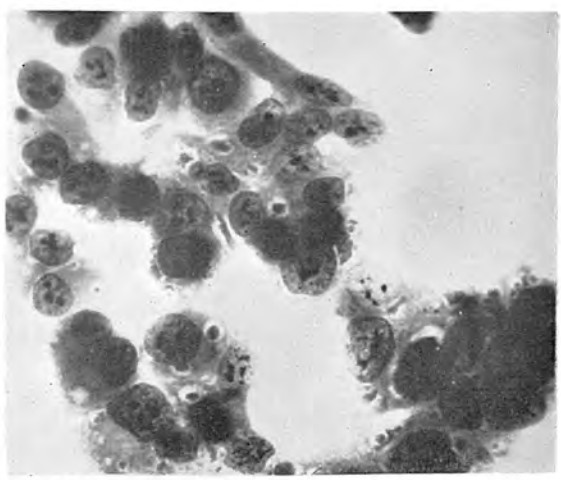

Fig. 15. Rabbitpox virus (Utrecht) infection. "B" type inclusions.

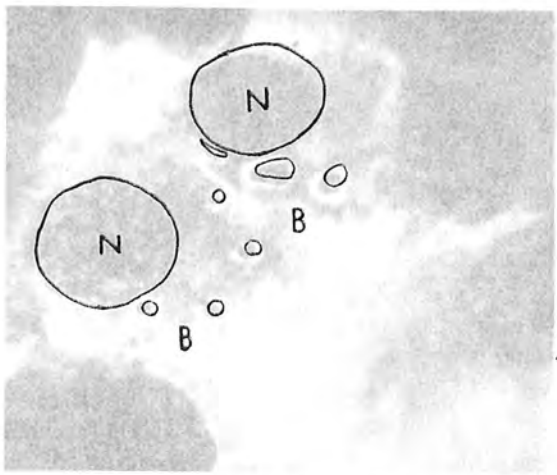

Fig. 16. Cowpox virus (LBW) infection. "B" type inclusions.

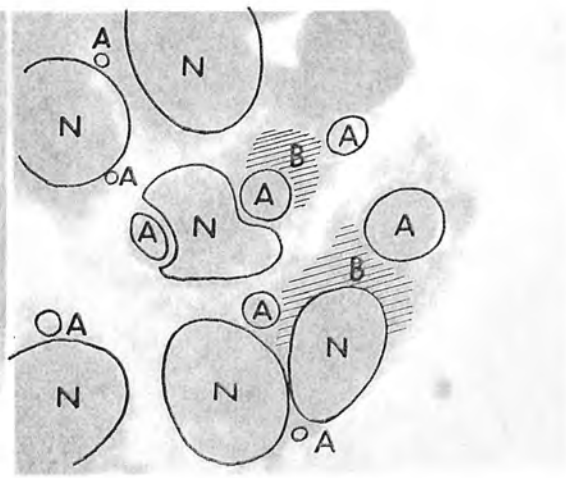

Fig. 17. Cowpox virus (LBW) infection. "A" type inclusions surrounded by halos, stain bright red.

Fig. 18-23. Feulgen reaction of FL cells infected with various pox viruses. All "B" type inclusions and nuclei were positive.

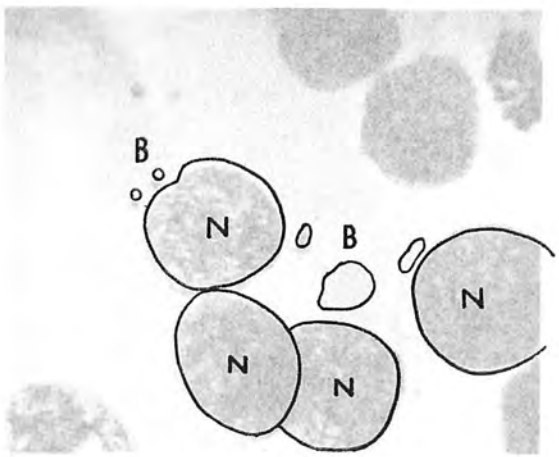

Fig. 18. Variola virus infection. "B" type inclusions.

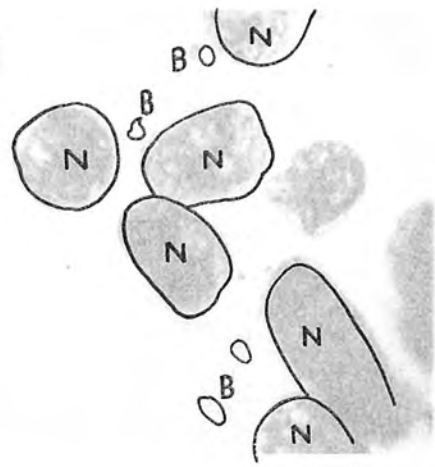

Fig. 19. Vaccinia virus (variola origin) infection. "B" type inclusions.

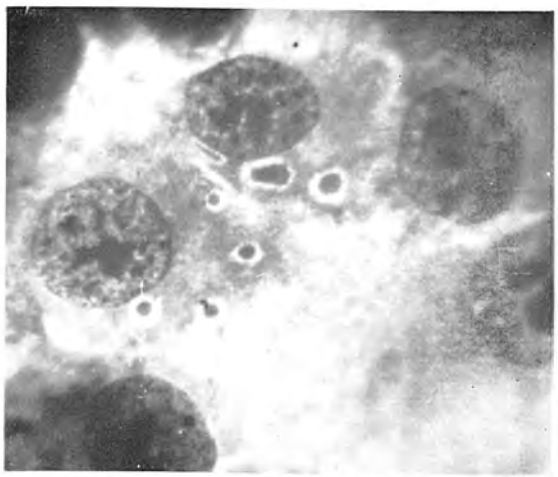

Fig. 16. Cowpox virus (LBW) infection. "B" type inclusions.

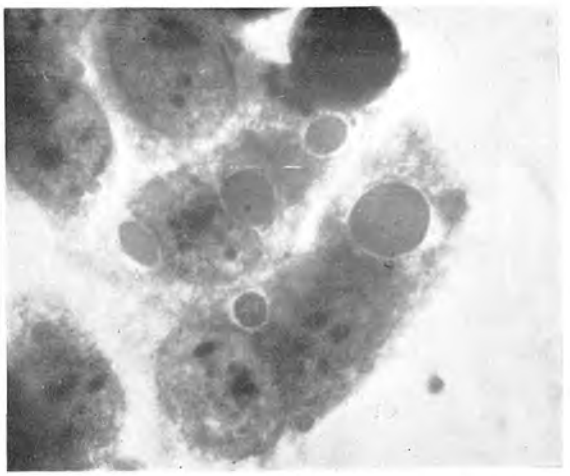

Fig. 17. Cowpox virus (LBW) infection. "A" type inclusions surrounded by halos, stain bright red.

Fig. 18-23. Feulgen reaction of FL cells infected with various pox viruses. All "B" type inclusions and nuclei were positive.

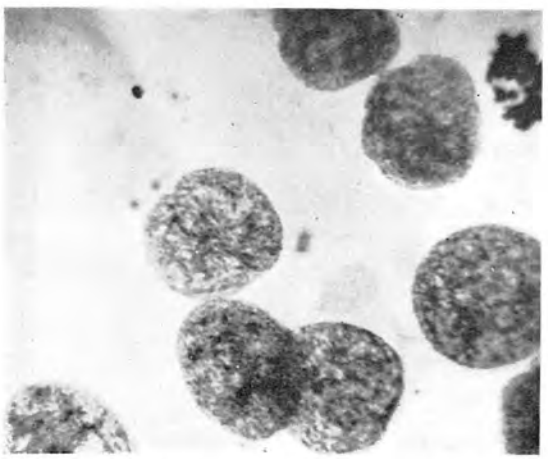

Fig. 18. Variola virus infection. "B" type inclusions.

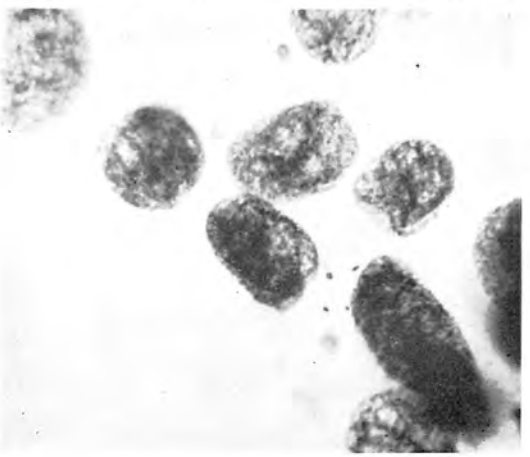

Fig. 19. Vaccinia virus (variola origin) infection. "B" type inclusions.

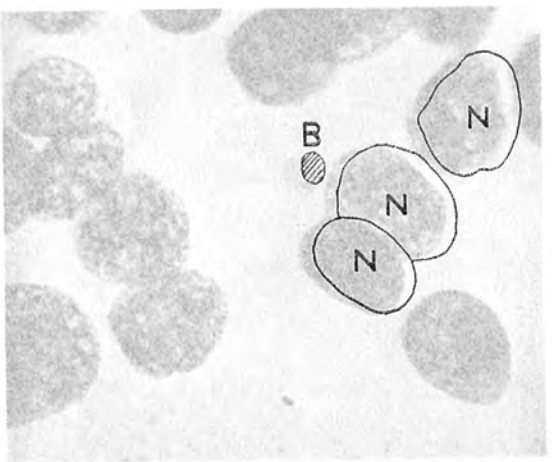

Fig. 20. Vaccinia virus (IHD) infection. "B" type inclusions.

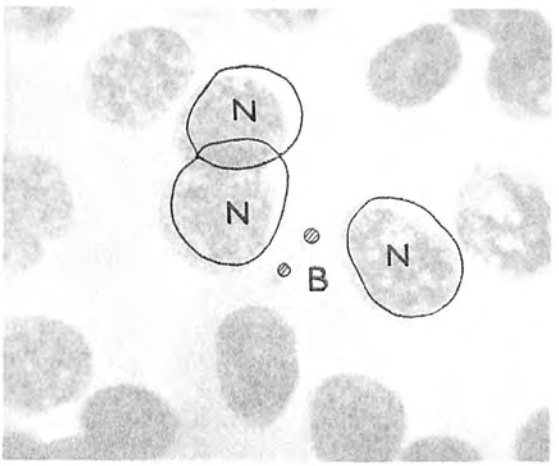

Fig. 21. Rabbitpox virus (Utrecht) infection. "B" type inclusions.

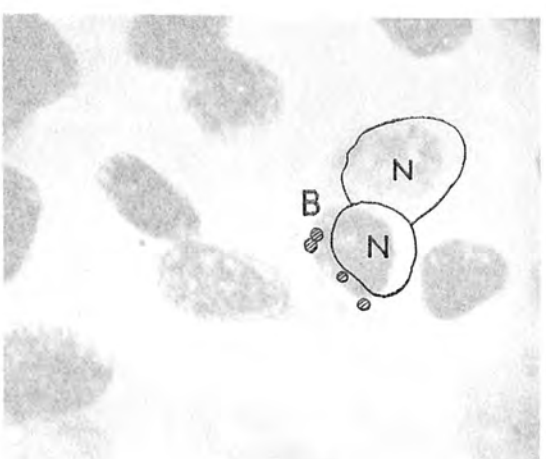

Fig. 22. Cowpox virus (LBW) infection. "B" type inclusions.

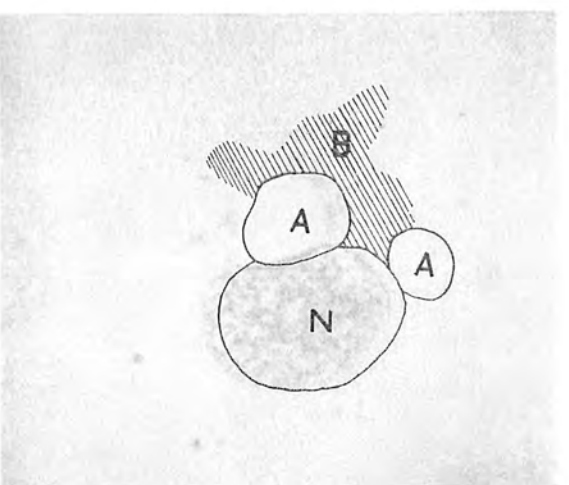

Fig. 23. Cowpox virus (LBW) infection. "B" type inclusion showed positive reaction, while "A" type inclusions were negative.

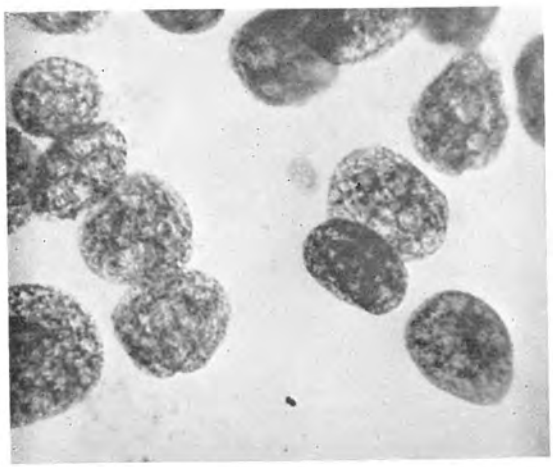

Fig. 20. Vaccinia virus (IHD) infection. "B" type inclusions.

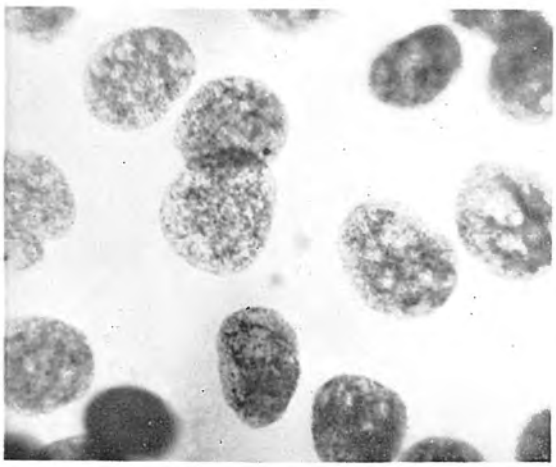

Fig. 21. Rabbitpox virus (Utrecht) infection. "B" type inclusions.

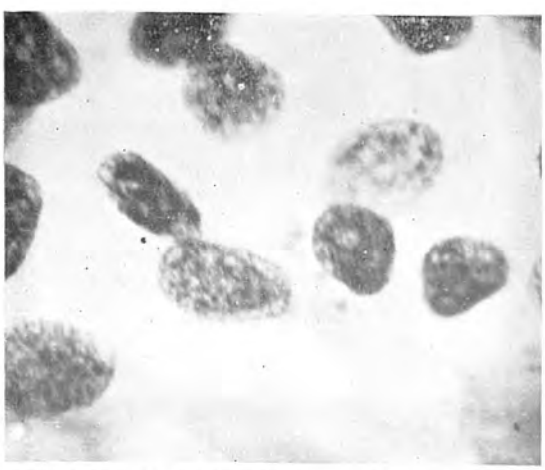

Fig. 22. Cowpox virus (LBW) infection. "B" type inclusions.

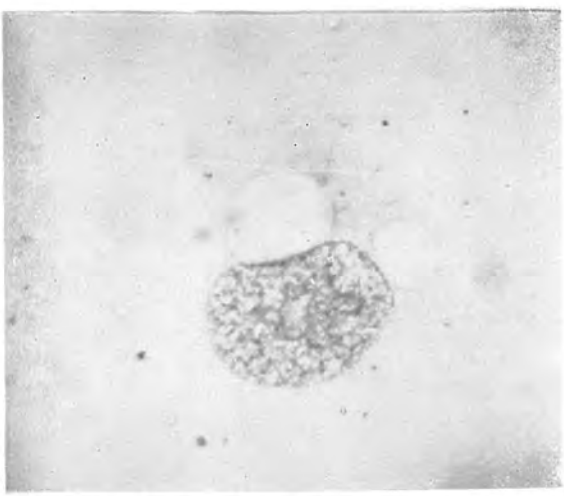

Fig. 23. Cowpox virus (LBW) infection. "B" type inclusion showed positive reaction, while "A" type inclusions were negative.

Fig. 24-31. Section preparations of the chorioallantoic membranes infected with various poxviruses, fixed with Bouin and stained with hematoxylin and eosin. "B" type inclusions were surrounded by halos and took on both hematoxylin and eosin tinge.

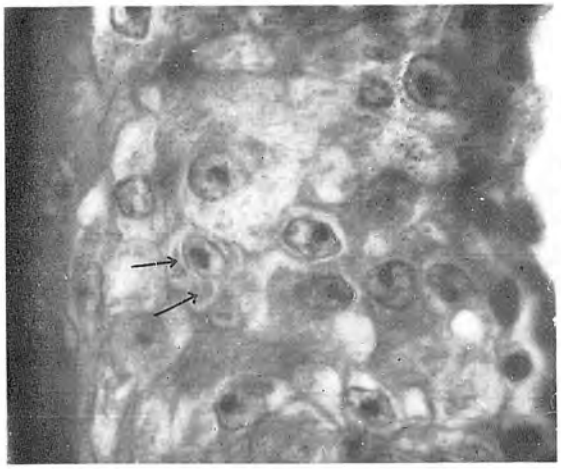

Fig. 24. Variola virus infection. "B" type inclusions.

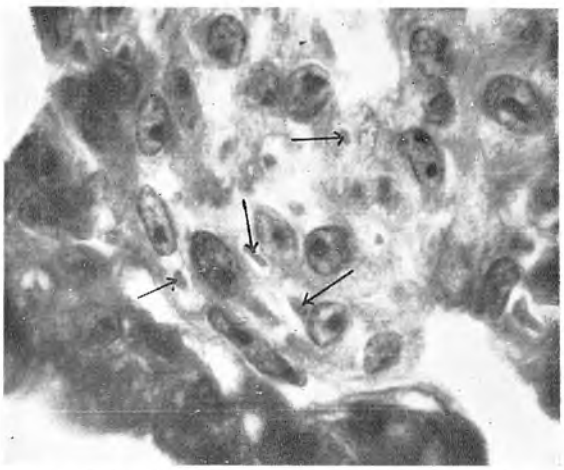

Fig. 25. Vaccinia virus (variola origin) infection. "B" type inclusions.

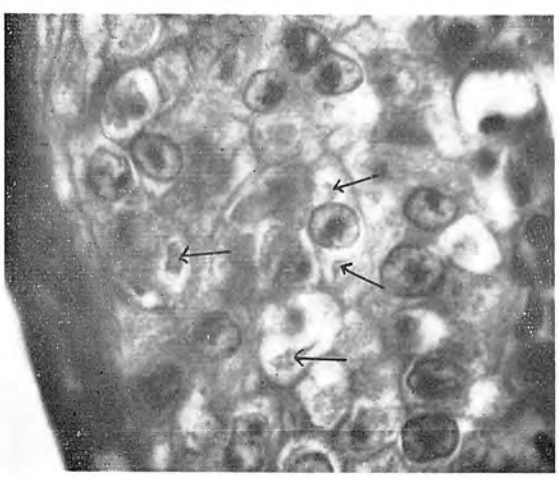

Fig. 26. Vaccinia virus (IHD) infection. "B" type inclusions.

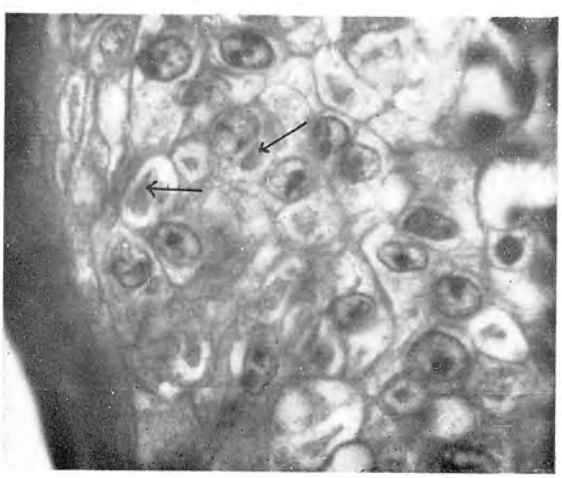

Fig. 27. Rabbitpox virus (Utrecht) infection. "B" type inclusions.

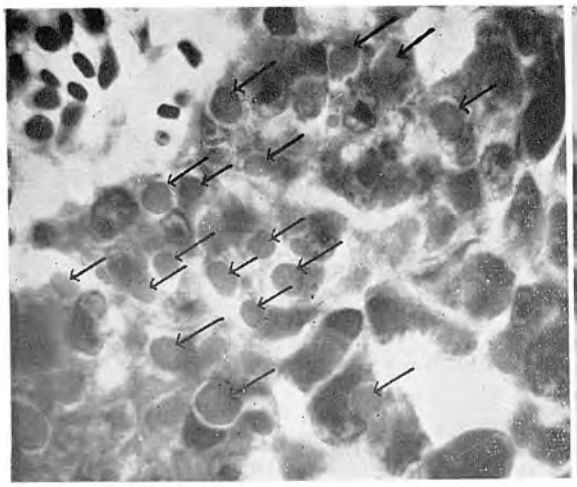

Fig. 28. Cowpox virus (LBW) infection. "A" type inclusions surrounded by halos, stain bright red. "B" type inclusions are indistinguishable from cytoplasm in this part because the bodies become diffuse and are pushed aside by "A" bodies.

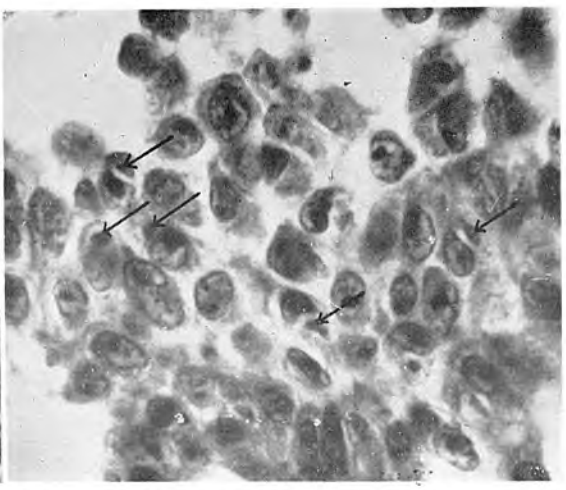

Fig. 29. Cowpox virus (LBW) infection. "B" type inclusions.

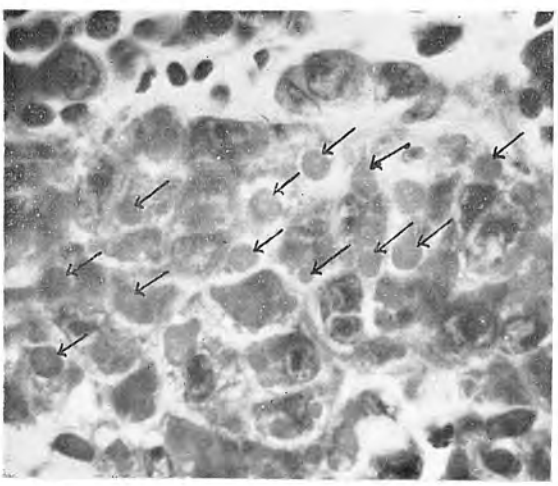

Fig. 30. Ectromelia virus (G) infection. "A" type inclusions surrounded by halos, stain bright red. "B" type inclusions are indistinguishable in this part.

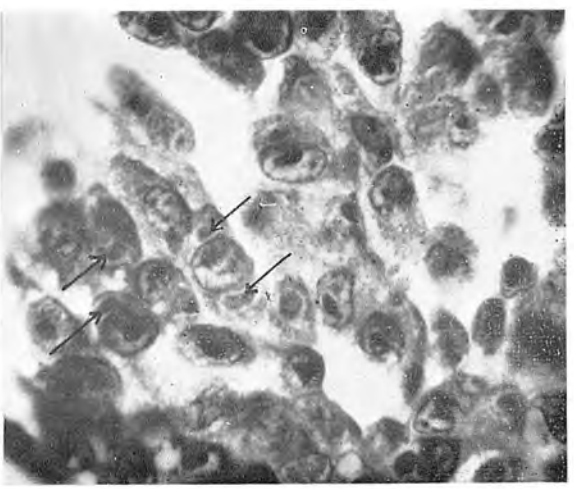

Fig. 31. Ectromelia virus (G) infection. "B" type inclusions.

Fig. 32, 33, 34, 36, 38, 40, 42 and 44. FL cells infected with various pox viruses. Fluorescein isothiocyanate coupled antibody technique. All yellow green fluorescent areas were found to be the sites of "B" type inclusions.

Fig. 35, 37, 39, 41, 43 and 45. The same area as shown in the left corresponding fluorescent photograph, restained with Giemsa solution. Most of the RNA in cytoplasm disappeared probably due to long exposure to ultraviolet rays.

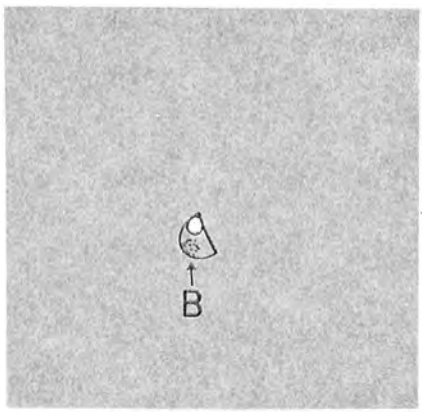

Fig. 32. Variola virus infection, stained with antivaccinia (variola origin) fluorescent antibody. A compact "B" type inclusion.

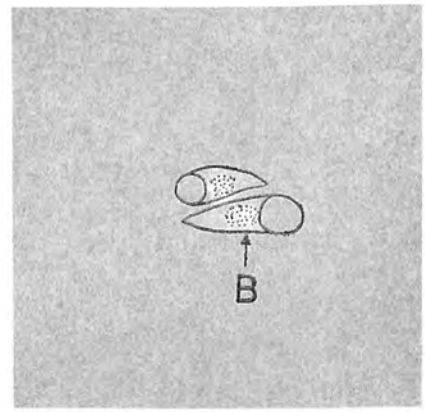

Fig. 33. Vaccinia virus (variola origin) infection, stained with antivaccinia (variola origin) fluorescent antibody. "B" type inclusion.

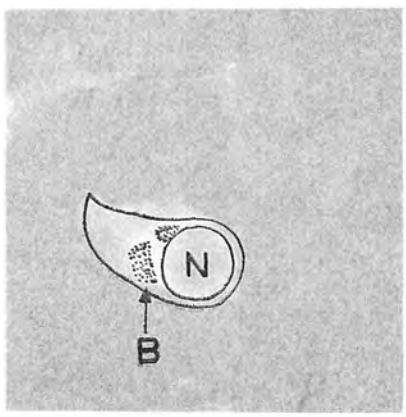

Fig. 34. Variola virus infection, staind with antivaccinia (variola origin) fluorescent antibody. A diffuse "B" type inclusion.

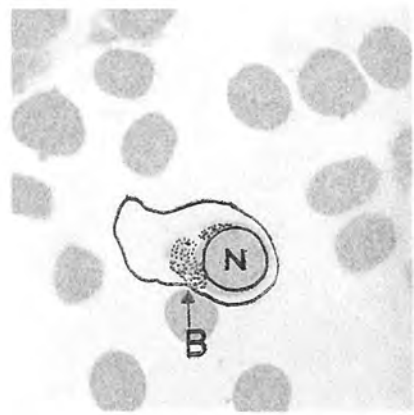

Fig. 35. The same field as Fig. 34.

Fig. 32, 33, 34, 36, 38, 40, 42 and 44. FL cells infected with various pox viruses. Fluorescein isothiocyanate coupled antibody technique. All yellow green fluorescent areas were found to be the sites of "B" type inclusions.

Fig. 35, 37, 39, 41, 43 and 45. The same area as shown in the left corresponding fluorescent photograph, restained with Giemsa solution. Most of the RNA in cytoplasm disappeared probably due to long exposure to ultraviolet rays.

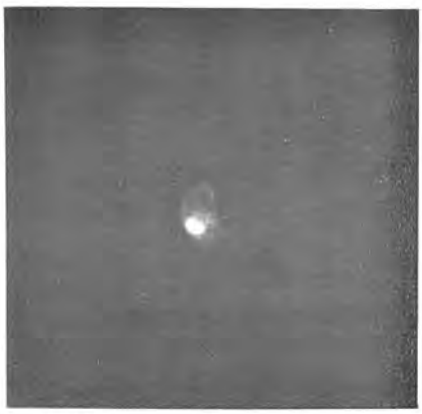

Fig. 32. Variola virus infection, stained with antivaccinia (variola origin) fluorescent antibody. A compact "B" type inclusion.

Fig. 33. Vaccinia virus (variola origin) infection, stained with antivaccinia (variola origin) fluorescent antibody. "B" type inclusion.

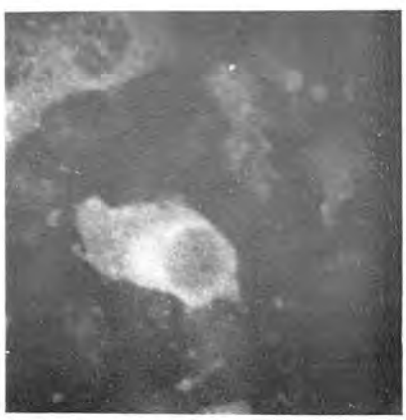

Fig. 34. Variola virus infection, staind with antivaccinia (variola origin) fluorescent antibody. A diffuse "B" type inclusion.

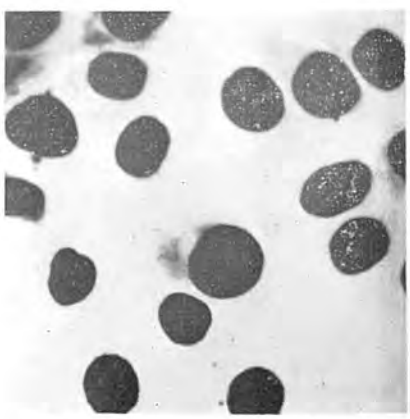

Fig. 35. The same field as Fig. 34.

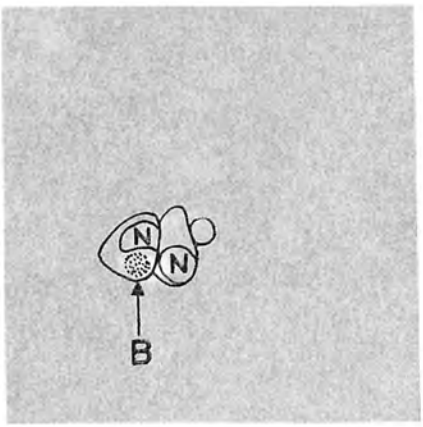

Fig. 36. Vaccinia virus (variola origin) infection, stained with antivaccinia virus (variola origin) fluorescent antibody. "B" type inclusions.

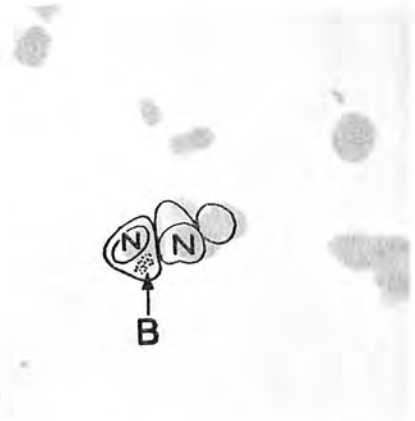

Fig. 37. The same field as Fig. 36.

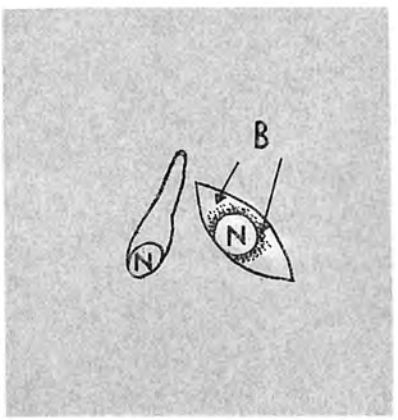

Fig. 38. Vaccinia virus (IHD) infection, stained with antivaccinia virus (variola origin) fluorescent antibody. "B" type inclusion.

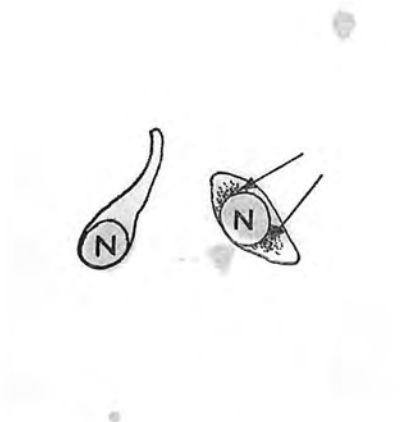

Fig. 39. The same field as Fig. 35.

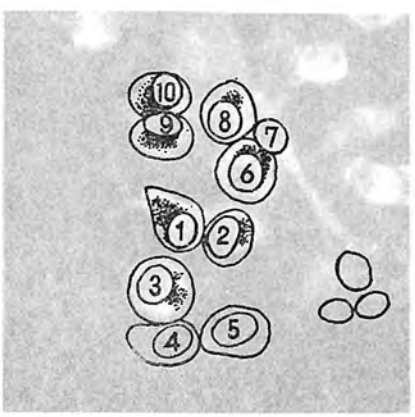

Fig. 40. Rabbitpox virus (Utrecht) infection, stained with antivaccinia (IHD) fluorescent antibody. "B" type inclusions.

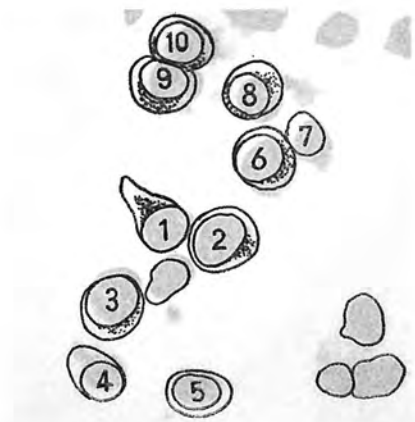

Fig. 41. The same field as Fig. 40.

Fig. 36. Vaccinia virus (variola origin) infection, stained with antivaccinia virus (variola origin) fluorescent antibody. "B" type inclusions.

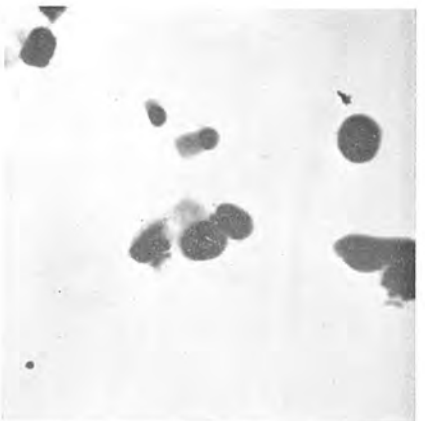

Fig. 37. The same field as Fig. 36.

Fig. 38. Vaccinia virus (IHD) infection, stained with antivaccinia virus (variola origin) fluorescent antibody. "B" type inclusion.

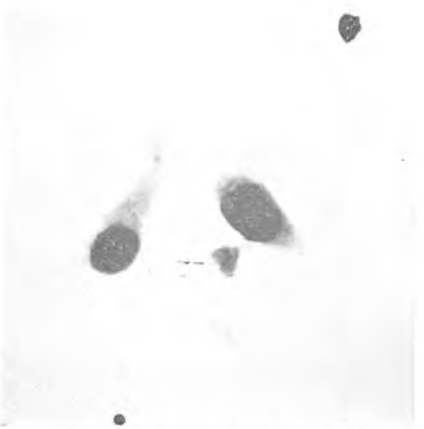

Fig. 39. The same field as Fig. 35.

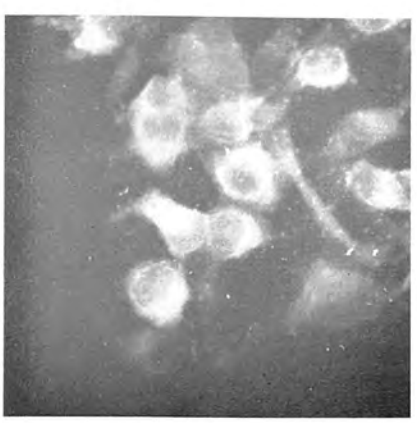

Fig. 40. Rabbitpox virus (Utrecht) infection, stained with antivaccinia (IHD) fluorescent antibody. "B" type inclusions.

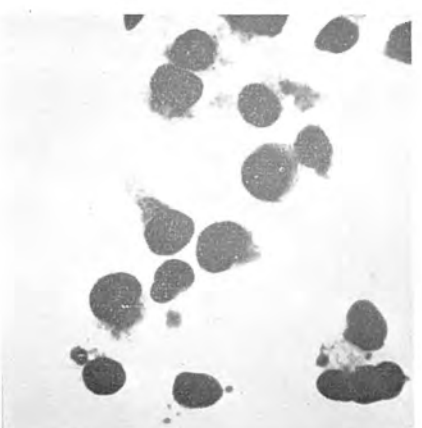

Fig. 41. The same field as Fig. 40.

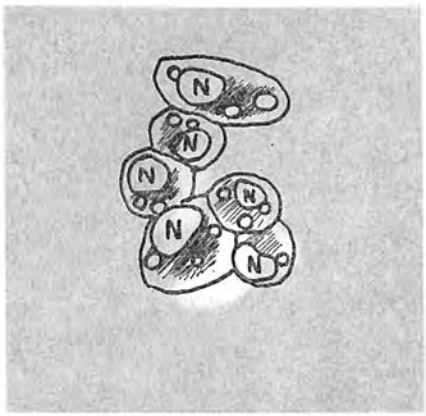

Fig. 42. Cowpox virus (LBW) infection, stained with anticowpox (LBR) fluorescent antibody. "A" type inclusions do not show any fluorescence.

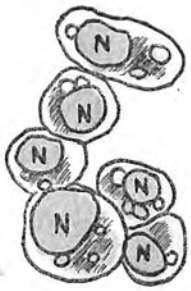

Fig. 43. The same field as Fig. 42.

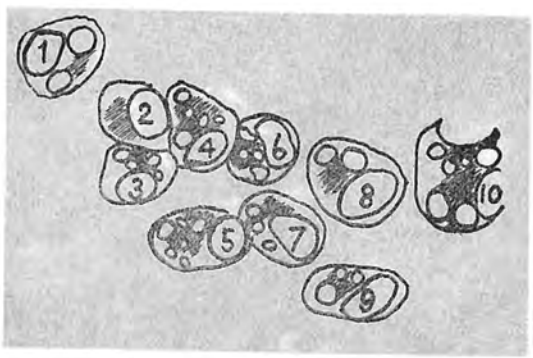

Fig. 44. Cowpox virus (LBW) infection, stained with anticowpox (LBR) fluorescent antibody. "A" type inclusions do not show any fluorescence.

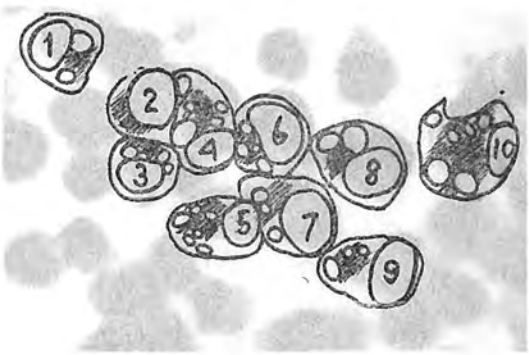

Fig. 45. The same field as Fig. 44.

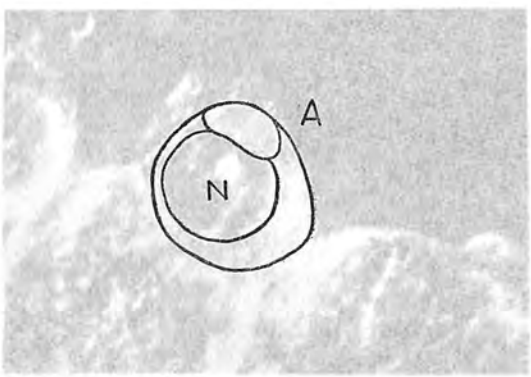

Fig. 46. Supravital observation of a FL cell having an ample "A" type inclusion of cowpox (LBR). Phase contrast microscope.

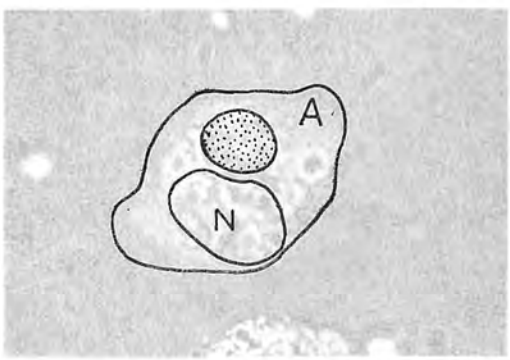

Fig. 47. Supravital observation of an Ehrlich tumor cell having an "A" type inclusion which is filled with many minute elementrary bodies in extremelic virus (G strain) infection. Ordinary microscope with the condensor lowered.

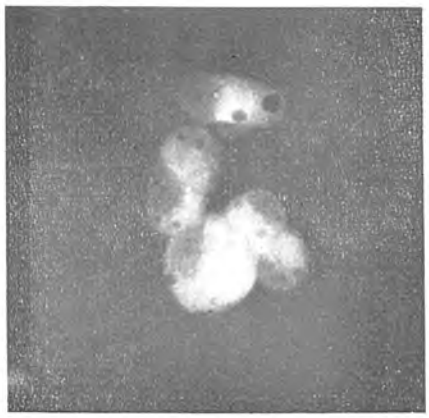

Fig. 42. Cowpox virus (LBW) infection, stained with anticowpox (LBR) fluorescent antibody. "A" type inclusions do not show any fluorescence.

Fig. 43. The same field as Fig. 42.

Fig. 44. Cowpox virus (LBW) infection, stained with antivaccinia (IHD) fluorescent antibody. "A" type inclusions do not show any fluorescence.

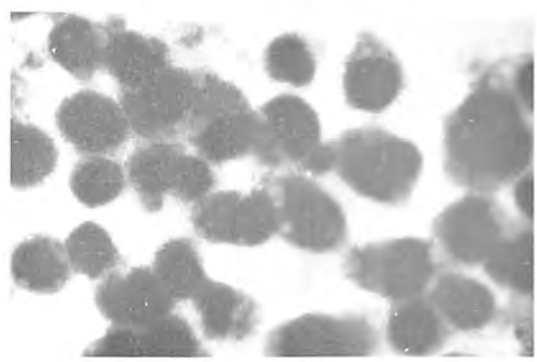

Fig. 45. The same field as Fig. 44.

Fig. 46. Supravital observation of a FL cell having an empty "A" type inclusion of cowpox (LBR). Phase contrast microscope.

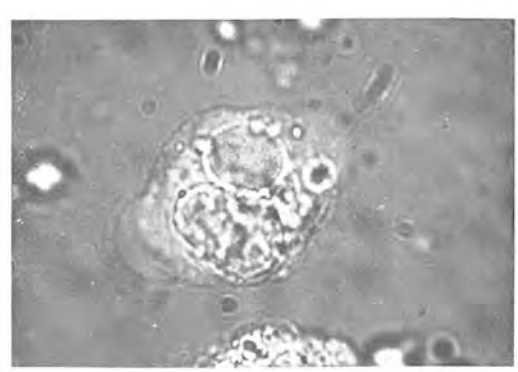

Fig. 47. Supravital observation of an Ehrlich tumor cell having an "A" type inclusion which is filled with many minute elementrary bodies in ectromelia virus (G strain) infection. Ordinary microscope with the condensor lowered.