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Plaque Formation and Some Cytopathogenic Characteristics of Ectromelia Virus (Hampstead Strain) and Vaccinia virus (IHD Strain) on Monolayers

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SUMMARY

1. Ectromelia virus forms small plaques on L cell monolayers. They begin to appear on the 4th or 5th day and reach a maximum diameter of about 1 mm on the 7th or 8th day. The plaques of strain IHD of vaccinia appear on the 2nd or 3rd day and reach a maximum diameter of about 1 mm on the 5th day.

2. There is a linear relationship between the number of plaques and virus concentration after tenfold dilution.

3. The titer of ectromelia virus as determined by counting plaques is about the same or a little lower than the titer using MLD_{50}.

4. Both viruses produce focal degeneration on L monolayers. The number of foci of ectromelia virus on the 5th or 6th day is proportional to the virus concentration and is used as a simple titration method.

5. Both ectromelia and vaccinia (IHD) viruses form large fused giant cells on L cell monolayers which are characterized by their nuclear arrangement.

INTRODUCTION

There are many reports on the effects of viruses on cells cultivated *in vitro* (Sanders and Lagnoff, 1953, Lynn and Morgan, 1954, Ross and Syverton, 1947).

Attempts have been made to compare and classify the cytopathogenic effects of many viruses morphologically (Enders, 1954) (Syverton, 1957). Since Dulbecco (1952) succeeded in the plaque formation with Western equine encephalitis virus on a monolayer of chicken embryo fibroblasts grown *in vitro* and proved the possibility of studying an animal virus with a technic comparable to the plaque method of assay for bacteriophage, the ability to produce plaques with several other animal viruses has been demonstrated (Dulbecco and Vogt, 1953, Black and Melnick, 1955, Granoff, 1955, Takemori *et al.*, 1955, Cooper, 1955, Kaplan, 1957).

Among poxviruses, plaque formation of vaccinia was reported by Noyes (Noyes, 1953).

We studied the growth mechanism of the ectromelia virus propagated in

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Ehrlich ascites tumor cells for several years (Kato *et al.*, 1955, Hagiwara *et al.*, 1956, Hagiwara and Kamahora, 1956).

Ectromelia virus destroyed Earle's L cells and formed two kinds of inclusion bodies as in Ehrlich ascites tumor cells *in vivo* (Furusawa *et al.*, 1958).

This paper is on the production of plaques and some characteristics of the cytopathogenic effects on monolayer cultures with ectromelia virus. Similar effects with vaccinia strain IHD are described.

MATERIAL AND METHODS

Virus:

Ectromelia virus: The Hampstead strain of ectromalia virus isolated in this Institute was used throughout the present work. Viruses used in many experiments were obtained on and after the 21st passage through Earle's L cell cultures. The culture fluids were collected when the destructive effect of ectromelia virus on L cells became severe, and centrifuged at 2,500 rpm for 10 minutes. The supernatants were used. In experiments to compare the titers as determined by counting plaques with those obtained by measuring infectivity in mice, the livers of the infected mice were submitted to the following treatment: The whole liver of the infected mouse was removed just before death, ground in a glass mortar with 3–4 ml of PBS (Dulbecco, 1954) or Hanks' BSS, and a 10–20 per cent liver emulsion was prepared which was then centrifuged for 10 minutes at 2,500 rpm. The supernatant was used. Virus dilutions were made in PBS or Hanks' BSS or in La-Ye solution.

Vaccinia virus: We received the IHD strain of vaccinia virus through the courtesy of Dr. Kanazawa, who had recognized plaque formation with this virus on Earle's L-strain monolayers. We made the mouse passage of this virus in Ehrlich ascites tumor cells. The tumor cells of the 10th passage were removed and emulsified in PBS. We transferred the supernatant to an L monolayer and made the subsequent passages in L cells. Culture fluids of the infected cells were removed and centrifuged to remove cellular debris. This supernatant was used as a direct source of virus in our experiments.

Cells:

Cultures of mouse fibroblasts cells (Earle's L strain) (Sanford *et al.*, 1948) and human cervical carcinoma cells (Strain HeLa were employed. Both are maintained in this laboratory. L cells were detached from the glass wall of the culture bottles by spraying vigorously the wall with 5–6 ml of fresh culture medium with a bent tip pipette. The L cell suspensions were made by pipetting the liquid back and forth. Suspensions of HeLa cells were made by trypsinization. Cell suspensions were diluted with fresh media. Both strains were subcultured in 200 ml bottles.

Preparation of monolayer cultures for plaque experiments:

5 ml aliquots of L cell suspension diluted with fresh medium were introduced into 50 mm Carrel flasks or 50 ml bottles and 8 ml aliquots were put into 200 ml bottles. After 3 to 4 days, continuous cell sheets were formed. The cell counts in the 50 mm Carrel flasks or 50 ml bottles were about 1×10^6 and those in the 200 ml bottles about 3×10^6.

Culture media.

L cells were cultivated in the following medium:

La-Ye Solution—95 parts Hanks' BSS containing 0.5 per cent lactalbumin hydrolyzate and 0.1 per cent Yeast Extract).

Bovine serum inactivated at 56°C for 30 minutes—5 parts.

Penicillin (100 units per ml) and Streptomycin (100 μg per ml) were also added. HeLa cells were cultivated in the following medium:

La-Ye Solution	85 parts
Bovine serum	15 parts

Composition of agar overlayer:

The agar overlayer was composed of the following ingredients.

1) 10 ml of double strength La-Ye Solution containing 200 units of Penicillin per ml and 200 μg of Streptomycin per ml.

2) 10 ml of distilled water containing 200—220 mg of Difco agar melted and sterilized by autoclaving at 10 lb for 15 minutes and then cooled to 43°C.

3) 2—4 ml of bovine serum inactivated at 56°C for 30 minutes.

Plating of virus:

The nutrient media were removed from the bottles. L monolayers were washed twice with 2 ml of prewarmed PBS or Hanks' BSS and then 0.15 or 0.2 ml of the virus samples were added to the cells. Two or three drops of nutrient medium containing 10–20 per cent bovine serum were also added to preserve the monolayers during the adsorption period.

Bottles were incubated at 37°C to permit adsorption of the virus onto the cells and agitated intermittently to prevent drying of the cells. Just before overlaying, ingredients (1), (2) and (3) were prewarmed at 43°C. Equal volumes of (1) and (2) were mixed, and then (3) was added.

The cell sheets were covered with the melted agar immediately.

After the agar had coagulated, rubber stoppers were replaced by sterile cotton stoppers and incubated at 37°C in a CO_2 chamber.

The inflow of CO_2 gas was controlled to keep the pH of the nutrient agar at about 7.4 as judged by the color of phenol red in the agar. To see the plaques clearly, neutral red in distilled water was added to the nutrient agar at a final concentration of 1:5,000. The neutral red solution had previously been sterilized by autoclaving.

Morphological observations:

The following methods were used to see the morphology of the infected cells.

(a) Culture cells were observed under the microscope through the wall of the glass bottle and were photographed after removal of the culture fluid. Neutral red was sometimes added to the culture fluid at a final concentration of 1:5,000. After incubation at 37°C for half to 1 hour, cells were stained supravitally and photographed.

(b) Cells grown on coverglasses were sometimes used for staining to see whether cytoplasmic inclusion bodies were produced in L cells infected with ectromelia virus.

(c) Sometimes the following method was also used to stain the monolayer cells on the glass wall of the bottle for direct observation (Cameron, 1950).

1) Fix in 10 per cent formalin for 1/2 to 1 hour.

2) Wash in running water for 1 hour.

3) Dehydrate in 60 per cent, 70 per cent alcohol (each for 10 minutes) and 80 per cent, 95 per cent alcohol (each for 20 minutes), and finally in a mixture of alcohol and ether (1:1) for 20 minutes.

4) Embed in collodion (one part of collodion and one part of the above mentioned alcohol-ether mixture).

5) Discard the collodion, dry the glass wall with the cells while revolving the bottle.

6) Pour cold water into the bottle and then strip the collodion membrane with the culture cells carefully off with forceps. Lay it on a coverglass.

7) Put the coverglass in the alcohol-ether mixture to remove the collodion.

8) H-E staining.

RESULTS

Formation of plaques:

Plaques of ectromelia virus appeared as small, round, white, opaque areas of destroyed cells on the 4th or 5th day. These continued to increase in size and number and reached a maximum diameter of about 1 mm on the 7th or 8th day. Mostly they ranged from 0.5 mm to 1 mm and rarely exceeded 1.5 mm in dia-

meter. Even in unstained preparations, white areas were clearly seen. To count them exactly, however, it was necessary to stain the living cells pink with neutral red solution. This was done on the 8th to 10th day. (Fig. 1, 2)

The IHD strain of vaccinia virus was also inoculated and overlaid to compare plaque formation with that of the ectromelia virus.

Plaques of vaccinia appeared on the 2nd or 3rd day after plating of virus and increased in size more rapidly than those of ectromelia virus. They reached a maximum diameter of about 1 mm on the 5th day. (Fig. 3, 4).

Table I. Components of agar overlay and plating of virus

Ingredients of agar overlay							Virus		
No. of experiment	Vessel used	2 fold La-Ye soluton	Distilled water	Difco-agar	Bovine serum	Amount of melted agar overlaid	Diluent	Amount of virus sample	Media added with virus
1,2	c (i) b (ii)	10 ml	10 ml	220 mg	4 ml	5 ml	Hanks' BSS▲ 90%, B. S.* 10%	0.15 ml	2 drops of La-Ye 80%, B.S.* 20% (with a pipette)
3	B (iii)	〃	〃	〃	〃	10 ml	PBS	〃	〃
〃	c	〃	〃	〃	〃	5 ml	〃	〃	〃
4	c,b	〃	〃	〃	〃	2.5 ml	Hanks' BSS	〃	2 drops of La-Ye 90%, B. S.*10%
〃	B	〃	〃	〃	〃	5 ml	〃	0.2 ml	3 drops 〃
5	c,b	〃	〃	〃	〃	3 ml	〃	0.1 ml	〃 〃
6	b	〃	〃	〃	2 ml	3 ml	〃	0.15 ml	〃 〃
〃	B	〃	〃	〃	2 ml	6 ml	〃	0.20 ml	〃 〃

(i) Carrel flask (ii) Culture bottle containing 50 ml (iii) Culture bottle containing 200 ml

▲ Hanks' balanced salt solution * Bovine serum

L monolayers were washed twice with 2 ml of prewarmed PBS or Hanks' BSS and then virus samples in 0.15 or 0.2 ml were pipetted onto the cells and two or three drops of nutrient medium containing 10–20% bovine serum were also added with a pipette with care of keeping monolayers in a good state during adsorption hours.

Test for the proportionality between the numbers of plaques and the virus concentration: Experimental conditions and results were shown in Table 1, 2, 3 and 4. Counting of plaques was done on the 7th and 10th days in Exp. No. 1. They were counted on the 7th day in unstained preparations. The count on the 10th day was performed after adding neutral red. The increase in the number on the 10th day was due to plaques which appeared after the 7th day and to those which were missed on counting unstained preparations.

Table II. Proportionality between the number of plaques and the concentration of virus

No. of experiment	Vessel used	Adsorption hours	Concentration of virus after dilution					Day on which plaques are counted
			10^{0}	10^{-1}	10^{-2}	10^{-3}	10^{-4}	
1	c (i)	5 ½		120	10	1	0	7
				172	20	2	0	10
2	b (ii)	3		111	12	0		〃
3	B (iii)	4 ½			61			10
〃	c	4		Confluent	86	12		〃
4	C	3		309	22	3		9
〃	b	〃			24			〃
〃	B	〃			54	4		〃

(i) Carrel flask (ii) bottle containing 50 ml
(iii) bottle containig 200 ml
Different viral sample was used in each experiment.

Teble III. Experimental procedure and results of Exp. No. 5.

Adsorption hours incubation at 37°C	$b_{(i)}$	b	b	$c_{(ii)}$	c	c
		↓			↓	
40 min.		agitation			agitation	
1 hr. 50 min.		↓			↓	
2 hrs. 50 min.		agitation			overlay	
3 hrs. 30 min.		↓				
4 hrs.		agitation				
5 hrs.		↓				
		overlay				
No. of plaques	420	33	3	250	26	1
Virus dilution	10^{0}	10^{-1}	10^{-2}	10^{0}	10^{-1}	10^{-2}

(i) bottle containing 50 ml (ii) Carrel flask
In this experiment 3 bottles and 3 Carrel flasks were used.

Table IV. Experimental procedure and results of Exp. No. 6.

Time and procedure	b (i)	b b b b	b b b b	B (ii) B B	B B B
incubation at 37°C	10^{0}	10^{-1}	10^{-2}	10^{-1}	10^{-2}
1 hr. overlay		109	5	302	37
1 hr. 5 min. agitation					
2 hrs. overlay		128	7	356	32
2 hrs. 5 min. agitation					
3 hrs. overlay		186	11	440	49
3 hrs. 5 min. agitation					
4 hrs. overlay	confluent	146	23		

(i) bottle containing 50 ml (ii) bottle containing 200 ml
In this experiment 9 small bottles and 6 large bottles were used.
The numbers indicate the plaque counts overlaid after each adsorption period.

Experiments No. 5 and No. 6 were performed to see the time required for adsorption. A series of monolayers were inoculated with virus and incubated at 37°C. At hourly intervals, one of a series of bottle or Carrel flasks was removed from the incubator and overlaid.

Thus, the supernatants of culture fluids of infected L cells contained about 10^{4}-10^{5} PFU/ml.

The same experiment with strain IHD of vaccinia is indicated in Table 5.

Comparison of the titer of ectromelia virus by plaque counts and by mouse infectivity:

A mouse liver emulsion was used for this purpose. The data are summarized in Table 6. Both titers were nearly the same.

Table V. Plaque experiment with strain IHD of vaccinia

Concentration of virus after dilution	10^{-2}	10^{-3}	10^{-4}
Counts of Exp. 1	Confluent	125, 131	15
Counts of Exp. 2	137	19	1
Counts of Exp. 3	Confluent	75	5
Counts of Exp. 4	83	11	

50 ml bottles were used. Each bottle was inoculated with 0.2 ml of virus sample. Overlay was performed after 3 hours adsorption. Different virus sample was used in each experiment.

The details of the results are shown in Table 7. In Exp. No. 1 and No. 3, the titers of MLD_{50} were a little higher than those of plaques. In Exp. No. 2 the mice died at irregular times, so an exact comparison could not be made. A little

Table VI. Comparison of virus titers on monolayers and in mice

Exp. 1	0.8×10^7 PFU/ml	1.0×10^7 LD_{50}/ml
Exp. 2	2.0×10^7 PFU/ml 1.9×10^7 PFU/ml	0.5×10^7 LD_{50}/ml
Exp. 3	2.5×10^7 PFU/ml	7.4×10^7 LD_{50}/ml

Fifty per cent lethal doses (LD_{50}) were calculated by the Reed and Muench method from the number of deaths occurring within a 10 day observation period.
In Exp. No. 2, the mice died at irregular intervals and so an exact comparison could not be made.
Adsorption time taken for this series of experiments was 2–2½ hours.

Table VII. MLD_{50} and plaque

	Virus concentration after dilution	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	
Exp. 1	Number of plaques	Confluent	162	12	1	0		0.8×10^7 PFU/ml (0.2 ml inoculated per bottle)
	Death of mice		●	●	○	○		1.0×10^7 MLD_{50}/ml (0.2 ml inoculated intraperitoneally)
			●	●	●	○		
			●	●	●	○		
			●	●	●	○		
Exp. 2	Number of plaques	Confluent	343	33	2			2.0×10^7 PFU/ml (0.2 ml inoculated per large bottle)
		Confluent	401	45	6			
	〃		311	36	2			1.9×10^7 PFU/ml (0.15 ml inoculated per small bottle)
	〃		268	26				
	Death of mice	●	●	○	○	○		? (0.2 ml inoculated intraperitoneally)
		●	●	●	○	●		
		●	●	●	○	●		
		●	●	●	●	●		
Exp. 3	Number of plaques		453	39	4			2.4×10^7 PFU/ml (0.2 ml inoculated per bottle)
			538					
	Death of mice		●	●	●	●	○	7.4×10^7 MLD_{50}/ml (0.2 ml inoculated intraperitoneally)
			●	●	●	●	○	
			●	●	●	●	○	
			●	●	○	○	○	

lower titer obtained by plaque counting may be the results of a shorter adsorption period after virus plating.

Morphological changes:

A. Focus formatoin:

During the passages of ectromelia virus in L cells, we often found many

degenerative foci formed in L monolayer. This was so only when a small inoculum of virus was employed.

The appearance of such foci depended on the conditions of L cell culture. In most cases it occurred on the 2nd or 3rd day after inoculation with the virus. Microscopically it could be detected within 24 hours. But when monolayer cells growing on top of each other, the appearance was delayed until the 5th or 6th day.

When cellular debris in the degenerative foci failed to be detached from the glass wall, they appeared as numerous white disseminated areas which looked like real plaques under the nutrient agar. They gradually increased in size and number, with variable diameter never exceeding 2 mm. An example of this is shown in Table 8. At the later stage of culture they fused. (Fig. 5, Fig. 6)

Table VIII. Counts of foci.

Date	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	8	9
VII B_1*	○				4	7	10	10	11	16	20	30		40						about 100				×							
VII B_2	○				2	6	12	12	12	13	20	24		37×																	
VIII B_1											○		4	4	5	5		5	5	5	5	7	7	7	×						
VIII B_2											○				1			1		1		3		14	19						24

The 7th passage was made on the 9th of Nov. (1957)
The 8th passage from the 7th was made on the 19th of Nov. (1957)
Each was inoculated with 5 drops of culture fluid by pipette.
Thereafter the foci were counted every day.
○ On the day passage was made. × Culturing discontinued.
* Bottle No. 1 of the 7th passage.

The foci mentioned above were considered identical with the plaques.

Their counts on the 5th or 6th day were almost proportional to virus concentration and they were recognized as primary foci (Black and Melnick, 1955).

Table IX. Relationship between the numder of faci and the virus concentration

Concentration of virus	10^{-1}	10^{-2}	10^{-3}	10^{-4}
Counts of Exp. 1	Confluent	96	9, 10	
〃 Exp. 2		143, 121	8, 12	
〃 Exp. 3			28	4, 2
〃 Exp. 4				18, 12
〃 Exp. 5		87	9	0, 1

Some of the data in this table shows the relationship between the number of foci and the virus concentration.
Foci were counted macroscopically and microscopically on the 5th or 6th day after plating the virus.
Different virus sample was used in each experiment.

This method of titration is now tested. Some results were indicated in Table 9. It will be used as a simpler plaque technique.

Strain IHD of vaccinia virus also formed similar foci.

However their appearance and increase in size and number occurred earlier than those of the ectromelia virus. (Fig. 7)

Microscopically at the advancing front of the focus the infected cells seemed to form a conspicuous assemblage, in striking contrast with the apparently intact cell layer. Inclusion bodies were demonstrated in these cells at the advancing border of the focus and also in the free cells remaining in the vacant area behind.

B. Giant cell formation:

When a small quantity of liver emulsion of the infected mice (containing 10^7-10^8 MLD_{50} per ml.) was introduced onto L cells or HeLa cells, much giant cell formation was regularly observed.

Fig. 8 represents a small giant cell which contains four nuclei, two large typical "A" bodies (so-called Marshal bodies) and a diffuse "B" body. Such giant cell formation was also seen at the margin of the focus. (Fig. 9)

Even when a focus was very scarce in giant cells, these cells increase in number as the infection proceeds if the culture is maintained and only the fluid is changed.

There are two foci in Fig. 9. One focus in the center has four distinct giant cells around its vacant area. In the giant cells, the marginal portion is composed of a large number of nuclei which stain strongly with Giemsa solution.

However if the cells are stained supravitally with neutral red, the central portion of the cell becomes dark, while the marginal portion including the nuclei loses its dark colour. (Fig. 10)

In such giant cells, clumping or agglutination of nuclei progressed further and there is contraction of the central area, which stained strongly with neutral red.

Then ballooning of the large cytoplasmic membrane took place, so that it was finally destroyed.

Such large giant cells were also seen in strain IHD of vaccinia (Fig. 11, Fig. 12). A magnification of a part of a plaque under nutrient agar is shown in Fig. 13. Cellular debris and a number of large giant cells are seen here and there.

DISCUSSION

There are many reports on direct propagation of virus in cell to cell infection.

Weller (1953) described serial propagation *in vitro* of agents producing inclusion bodies derived from varicella and herpes zoster.

Using herpes simplex virus, Wildy (1954) observed that pocks appeared on the chorioallantoic membrane of hatching egg and increased in size even in the presence of the specific antiserum.

Black and Melnick (1955) studied the microepidemiology of the poliomyelitis virus and of herpes B virus in tissue culture. They found that, when the inoculum of the latter was small, degenerative cells were localized as plaques for the first 2 or 3 days even though they were bathed in a fluid medium. This was also the case in the presence of the specific antiserum. They suggested that herpes B virus

might migrate from one to a neighbouring cell.

Youngner (1956) came to almost the same conclusion using the agar overlay method with herpes B and vaccinia viruses.

Ectromelia virus seems to be like the virus mentioned above. It also forms foci on monolayers. When the inoculum of virus was small, many days were required before these became confluent. Their increase in size is somewhat dependent on the condition of the monolayer cells. If the culture fluids are not changed for many days and their pH is kept low, the focus size is apt to be restricted or they grow very slowly, if at all. In these cases infection often seemed to be arrested. Only rarely do the cells at the margin of the foci to proliferate and cover the vacant area of foci. It is unknown whether the proliferating cells are really infected or not. But when the culture fluid is replaced by fresh medium, a cytopathogenic effect appears and the proliferating cells vanish. Ectromelia virus caused a slower increase in size and number of foci than does strain IHD of vaccinia. This difference may be the effect of their different rates of propagation. The virus titer of ectromelia in culture fluids from L-cell monolayer passage was 10^4-10^5 PFU per ml., while that of vaccinia was 10^6 PFU per ml.

Ryden and Randall (1957) using vaccina virus observed that degenerative changes occurred during a period of 36 hours in cultures with or without immune serum, whereas they ceased after 36 hours in the presence of antiserum. These authors hinted at the possibility of the entrance of antibodies into the cells. Inhibition of focus formation with antibody has been attempted in our laboratory, but the results were ambiguous.

Sherer and Syverton (1954) described focal formation in HeLa cells with vaccinia, herpes simplex and pseudorabies viruses. Syverton (1957) classified cytopathic changes into several groups. Herpes B and pseudorabies may belong to one of these groups in which viruses slowly produce a focal degeneration with centrifugally progressive development. Ectromelia virus falls into this classification. Viruses belonging to the former group usually produce intranuclear inclusion bodies, while ectromelia virus produces two kinds of inclusion bodies in the cytoplasm. At the margin of a focus many cells having two or three "B" type bodies could be seen. The develpmental feature of "B" type inclusion bodies had been studied much in this laboratory (Kato, 1955, Hagiwara, 1956), and it was suggested that one virus particle is capable of producing one "B" body. Multiple infections are suggested in the focal area by the appearance of cells containing many compact "B" type bodies.

In some instances a number of cells remained apparently normal in the center of the focal area. (Fig. 14) However, these cells were not resistant to infection. When such monolayer cultures were passaged further they were also affected with cytopathogenic effects and finally disappeared.

In application of these plaques of ectromelia virus to virus titration, great care should be taken over the following points. If monolayer cells are overgrowing or the CO_2 chamber is not humid or the pH of the media is kept lower, the growth of plaques is apt to be restricted and they may not be counted because they are small size. Moreover the counts of foci on the 5th or 6th day are more

convenient than those of typical plaques.

Ectromelia virus and the IHD strain of vaccinia belong to a group of viruses which produce giant cells containing a number of nuclei. They are formed by fusion and the arrangement of their nuclei is quite characteristic.

But the Biken strain of vaccinia (dermotropic strain) failed to produce giant cells.

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Fig. 1. Carrel flask (D-5) preparation showing L monolayer and plaques of ectromelia virus. In the ieft and lower parts of the flask, plaques have fused.
Exp. No. 4, Virus dilution : 10^{-1}

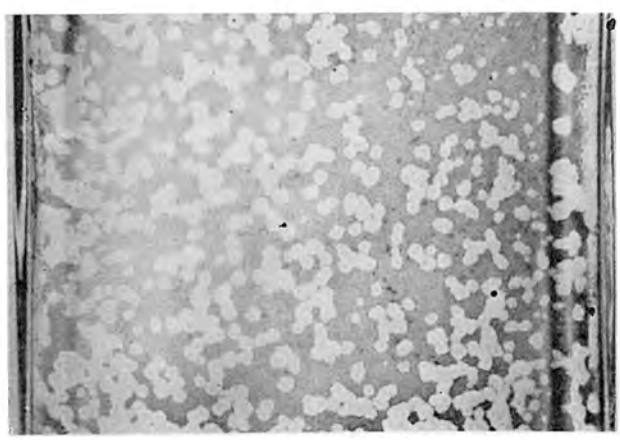


Fig. 2. Fused plaques of ectromelia virus on an L monolayer in a 50 ml bottle.
Exp. No. 6, Virus dilution : 10^{0}

Fig. 3.

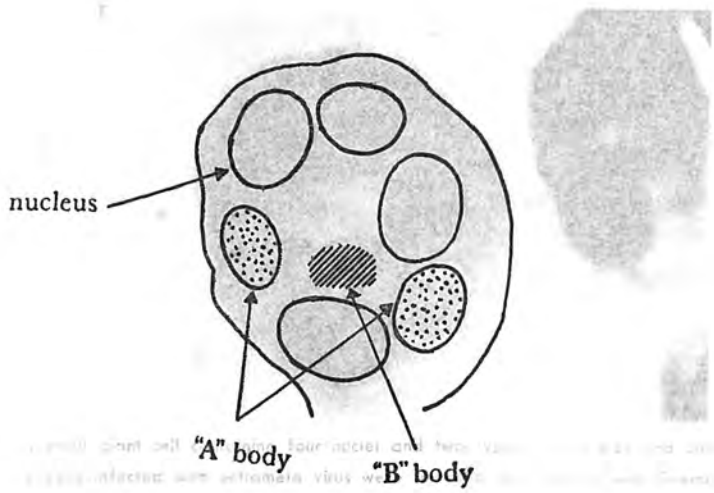
Fig. 4.

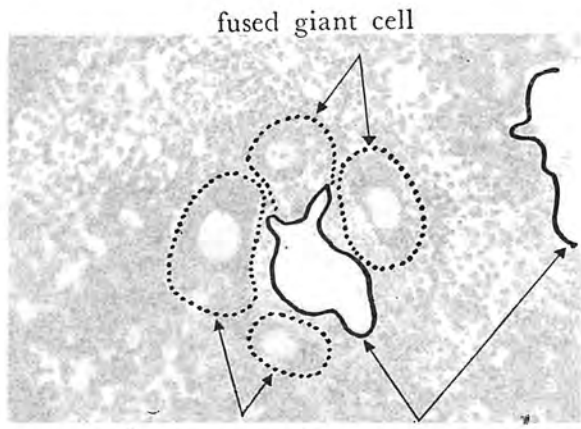
Plaques of strain IHD of vaccinia on an L monolayer in a 50 ml bottle.

Fig. 5. A great numbr of foci of ectromelia virus are seen on an L monolayer 200 ml bottle preparation.

Fig. 6. Foci of ectromelia virus on an L monolayer in a 50 ml bottle preparation.

Fig. 7. 50 ml bottle preparation on an L monolayer showing comparison of foci of ectromelia and vaccinia viruses (IHD) (ectromelia : right, vaccinia : left).
The lower two bottles were inoculated with twice as much virus as the upper bottles.
On the 3rd day after plating the virus, these bottles were stained with Giemsa solution and photographed.


nucleus
"A" body
"B" body


fused giant cell
fused giant cell
vacant area

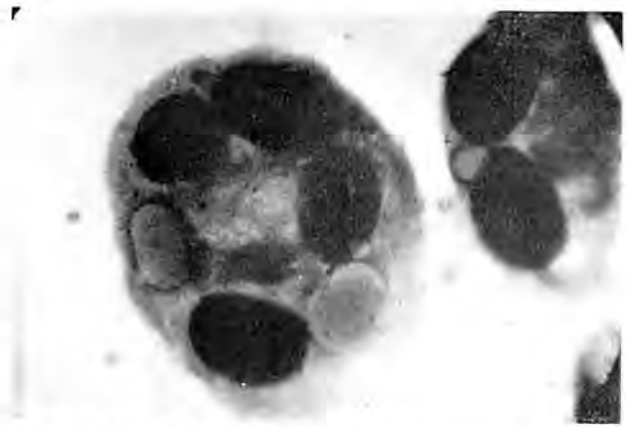


Fig. 8. A small giant cell containing four nuclei and two typical "A" bodies and one diffuse "B" body. L cells infected with ectromeia virus were smeared and stained with Giemsa solution. 450 x

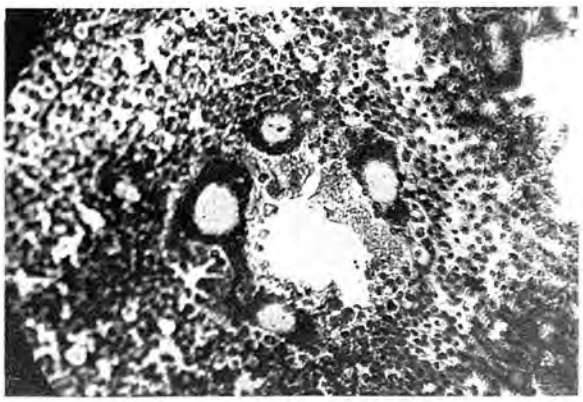


Fig. 9. Giant cell formation at the edge of the cell sheets. Two foci are shown. One focus is in the center and another in the upper right corner of the plate. L monolayer in a Carrel flask is infected with small inoculum of virus. After 2 days, the culture fluid was discarded and fixed and stained with Giemsa solution. A monolayer photographed through the glass of the flask. 50 x

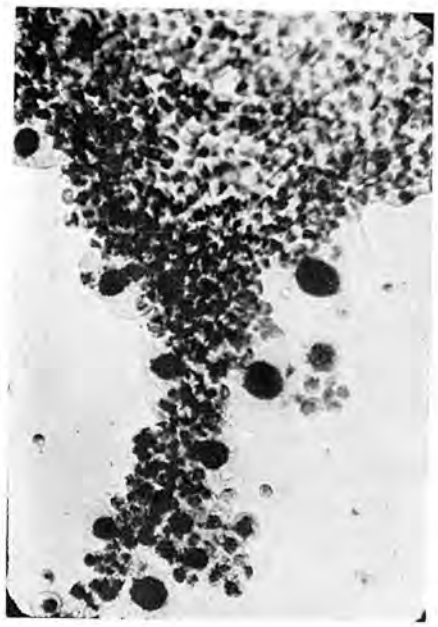


Fig. 10. The giant cells on the advancing border are stained supravitally with neutral red and photographed.
In the plate are two neighbouring foci.
The central area of each giant cell is stained strongly dense. 50x (Ectromelia virus-L cell)

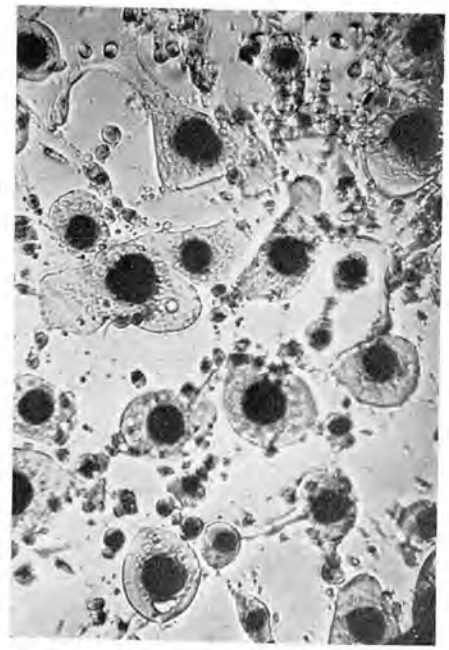


Fig. 11. Giant cell formation of vaccinia virus (IHD) in an L monolayer with neutral red staining. 50 x

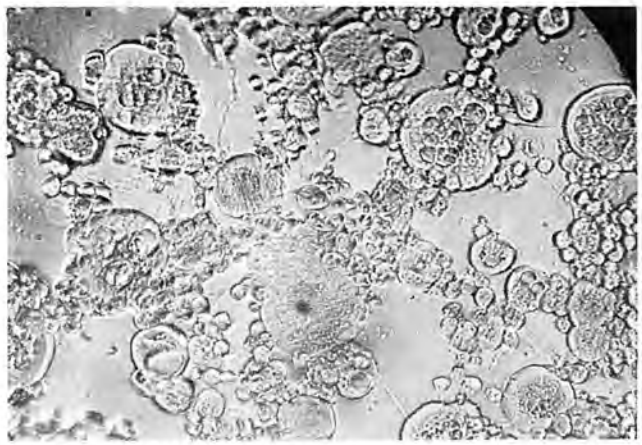


Fig. 12. Giant cell formation of vaccinia virus (IHD) in a HeLa monolayer with no staining. 50 x

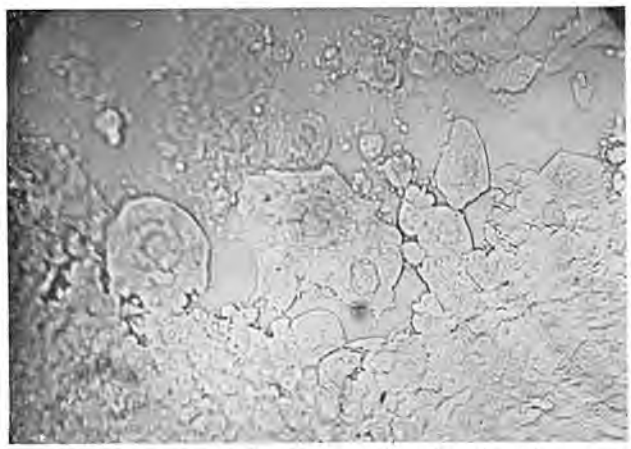


Fig. 13. A plaque of vaccinia virus (IHD) is photographed through nutrient agar.
Cytolysis of the infected cells occurred and cell debris seen in the upper part of the plate.
In the center of the plate large giant cells are seen.
The lower part is an advancing border.

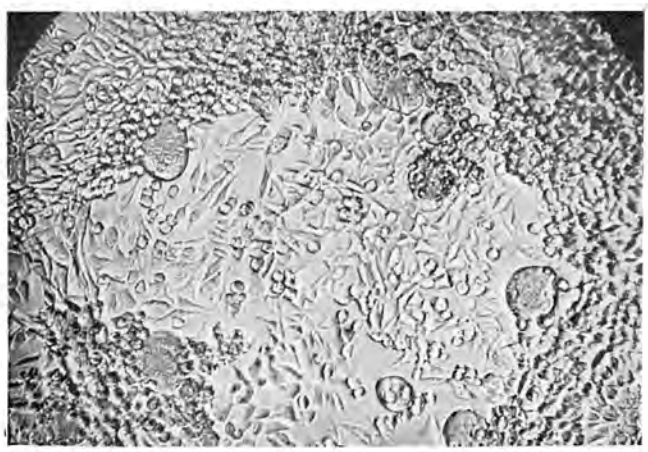


Fig. 14. In the focus many cells remain intact or are not destroyed.
They look normal.
On the advancing border giant cells are seen.