Arch. Inst. Razi, 1971, 23, 93 - 105

Comparative Adaptation of some pox viruses in two cell systems (*)

by

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Introduction

A camel kidney cell strain (ckcs) was recently established in our laboratory with a wide spectra of capacities to support growth of several DNA and RNA viruses (Mirchamsy et al, 1971). This cell was highly susceptible to vaccinia virus, showing a cell alteration at the first passage of virus in less than 24hr. The present study was undertaken in order to compare the relative susceptibility and the growth characteristics of some pox viruses in ckcs and in green monkey (Cercopithecus aetiops) kidney cell line (Vero), also known to be susceptible to vaccinia virus (MacFarlane and Somerville, 1969, Rhim et al, 1969).

MATERIALS AND METHODS

Viruses. Two strains B and E of Variola were kindly supplied by Dr. R. Pournaki, Pasteur Institute-Tehran. These viruses were isolated from vesicules of two smallpox infected patient in primary human foetal kidney cell and were stored frozen at -70°C.

The vaccinia virus was derived from the calf lymph vaccine routinely produced by Pasteur Institute-Tehran. Camelpox was recently isolated from sick camels and together with goatpox, sheeppox and contagious pustular dermatitis (Orf) were kindly supplied by Dr. H. Ramyar, department of ovine viral disease, Razi Institute. Finally fowlpox was received from Dr. M. Baharsefat, department of avian disease of this Institute.

CELLS. All studies were conducted either with ckcs at its 90th-95th serial passage or with Vero cell. Details about growth and maintenance of these cells have been given in previous reports (Mirchamsy et al, 1971, Mirchamsy and Rapp, 1969). Both cells were cultivated in ordinary test tubes and on coverslips in Leighten tubes. The former received one ml and the latter 1.5 ml of maintenance medium containing 2% heat inactivated calf serum. All cultures were incubated at 36°C.

Virus assay. For virus titration 0.1 ml of each dilution of viruses were inoculated into four tubes of cells. After an adsorption time of 90 minutes at 36°C. cultures were washed with warm phosphate buffer saline (PBS), pH 7.2 one ml of maintenance medium containing 2% heat inactivated calf serum was added

(*) An Abstract of this work was presented to the Second International Congress of Virology, 27 June-3 July 1971, Budapest.

to each tube and cultures were incubated at 36°C. Observations for cytopathic effect (CPE) of viruses were made for seven days following incubation.

Plaque assays were done according to the method described by Postlethwaite (1960) using vero or ckcs in tubes. Cultures were then treated as above. Plaques without overlay could be seen in vero cells as early as the second day after incubation and after 3 days in ckcs but were best counted on the 4th to 5th day. Before counting cultures were stained with Ziehl-Neelsen stain. The 50% end point was calculated by the Reed-Muench method (1938).

Histological technique. Coverslips of infected and control cells were washed with PBS, fixed with Bouin fixative and stained with Haematoxyline-Eosin.

RESULTS

The data presented in table 1 show that ckcs supported the multiplication of vaccinia, variola and camelpox viruses. In vero cell, on the other hand, beside the above mentioned viruses, fowlpox virus was propagated. The infectious titer of vaccinia and variola viruses in these host cells is similar to that observed in the other mammalian cells (Bernkopf et al., 1959, Pirsch et al., 1963, Ryden and randall, 1957 and Sureau and Brygoo, 1959). At the 8th serial passage in each cell system the inoculated cultures were examined daily for evidence of cellular changes which would indicate viral proliferation. Infection of ckcs and vero cells with undiluted vaccinia virus caused the first cellular change in less than 12hr. Rounding, granulation and clumping of cells were present in all parts of the cell sheet 24hr. after infections (figs 1& 2). The cellular changes increased rapidly until the appearance of cell lysis, which occurred on the second to third day. In stained preparations the nucleus was mostly pycnotic and seemed to have been pushed to the periphery of the cell. Syncytia with randomely arranged nuclei and vacuoles (fig. 3) were also seen. The cytoplasmic alterations were mainly the appearence of inclusions with or without detectable halo. Most cells were detached from the glass on the 3rd to fourth day after incubation. Both cell systems inoculated with undiluted variola strain B and E showed CPE on the second day. In unstained cultures of three to five days old foci of rounded and clumped cells together with syncytia (fig. 4 & 5) were frequent. In stained preparations, granulation and pycnosis of cells were observed. Also cytoplasmic inclusions with irregular shapes were noticed (fig. 6).

Similar CPE were produced in both cell strains infected with camelpox. The appearence of cell alteration was however faster in ckcs than in vero cell. On the third day after infection multinucleated giant cells as well as pycnosis and intracytoplasmic inclusions were present in both cell systems (figs. 7,8,9 & 10). At later stage of infection a characteristic observation on ckcs was the phagocytosis of rounded cells by other cells. In some preparations a rounded cell having an excentric nucleus contained another cell phagocyted inside the cytoplasma or the victim cell was engulfed into the cytoplasm of a giant cell (fig. 10). This phenomenon was also observed in both cultures infected with vaccinia virus (fig. 11).

The main characteristics of CPE of fowlpox virus in vero cell was the appearence of foci of rounded cells distributed all over the whole cell sheet (fig. 12), formation of syncytia and rare intracytoplasmic inclusion bodies.

Plaques, 1-3 mm in diameter, could be seen by the naked eye in vero cell infected with vaccinia virus, two days after incubation. On the third day primary plaques of vaccinia or fowlpox viruses were surrounded by secondary plaques (fig. 13). Microscopic examination revealed that the central area of vaccinia, camelpox and fowlpox plaques contained some remenants linking degenerate or packed cells (fig. 14). Degeneration and lysis of cells were more progressed in plaques of variola virus which were seen as a large hole on the cell sheet (fig. 15). Plaques developped in ckcs were smaller in size (fig. 16) comparing with those observed in vero cell. While vaccinia plaques had an average size of 1-2 mm, those of variola or camelpox were of less than 1 mm.

DISCUSSION

Propagation of vaccinia, variola and camelpox viruses was demonstrated in a camel kidney cell strain developped in this laboratory and in green monkey kidney cell line "vero". Only the latter cell system supports growth of fowlpox and both cells failed to show any cellular alterations after serial passages of sheeppox, goatpox and pustular dermatitis virus (orf). In case of pox viruses showing cytopathic changes, sufficient number of serial passages was conducted in both cells so that the infectious titers of the original inocula were exceeded widely. Camel or vero cell infected with vaccinia virus underwent rapid degeneration, clumping and pycnosis, similar to the alterations reported for the other cells. Inclusion bodies, irregular in shape and size and with or without halo were more frequent in infected camel cell than in vero cell. In cultures inoculated with variola or camelpox after four serial passages the first cellular changes were recorded on the second day post infection. Rounded and clumped cells and foci of degeneration forming plaques were scattered over the whole cell layer. Large syncytia were formed in both cells by the latter two viruses. Inclusion bodies were however more frequent in camel cell than in vero. The degeneration of vero cell by fowlpox virus after four serial passages was evident on the second day post infection. The changes were similar to those reported for vaccinia virus. Although growth of fowlpox virus in chicken embryo fibroblasts has been reported as described by Chang and Jasty (1970) but to our knowledge its adaptation to mammalian cells has not been reported. The growth pattern of camelpox virus in cell cultures also has not been reported.

A characteristic observation in the present study was the phagocytosis of pycnotic ckcs cells by other cells infected with camelpox. This phenomenon was also observed in vero cell infected with vaccinia or camelpox. This finding is similar to that observed by Bernkopf et al. (1959) in human amnion cell infected with vaccinia virus.

The ability of vaccinia, variola, camelpox and fowlpox to develop plaques in cultures not overlaid is of interest. The present study showed that these viruses were able to form plaques in the most easy way without overlay and therefore the quantitation of these viruses is simple.

We should like to make one more point. The camel kidney cell strain is a new mammalian cell system which supports cultivation of many of animal viruses. This may be a good alternative to avoid balancing of hazards of oncogenic or latent viruses when other mammalian cells are to be used as substrate for virus vaccine production.

SUMMARY

The comparative susceptiblity of a camel kidney cell strain and of Vero cell line to Vaccinia, Variola, Camelpox, Fowlpox, Sheeppox, Goatpox and of orf viruses was studied. Both cell system support growth of vaccinia, variola, and camelpox viruses. Fowlpox virus was only propagated in Vero cell. Both cell systems failed to support growth of sheeppox, goatpox and of orf viruses. Pox viruses already adapted to these cells showed plaques of different sizes when proper dilution of viruses were inoculated into monolayers of cells and cultured without adding any overlay.

Acknowledgement

Acknowledgements are due to Dr. R. Pournaki of Pasteur Institute, Dr. H. Ramyar and Dr. M. Baharsefat of Razi Institute for providing required virus strains. We wish to thank Dr. M. Kaveh, Director of Razi Institute for his support and encouragements.

REFERENCES

1 — Bernkopf, H., Nishmi, M. and Rosin, A. (1959). Effect of active and inactive vaccine virus preparation on human amnion cell culture. J. Immuno, **83**, 635.

2 — Chang, P.W. and Jasty, V. (1970). Multiplication of Fowl pox virus in chicken embryo fibroblastic cell cultures. Am. J. Vet. Res., **31**, 1463.

3 — MacFarlane, D.E. and Sommerville, R.G. (1969). Vero cell (Cercopithecus aetiops) Growth characteristics and viral susceptibility for use in diagnostic Virology Arch. ges. Virusforsch., **27**, 379.

4 — Mirchamsy, H. and Rapp, F. (1969). Role of interferon in replication of virulent and attenuated strains of measles virus. J. gen. Virol., **4**, 513.

5 — Mirchamsy, H., Bahrami, S., Amighi, M. and Shafyi, A., (1970). Development of a camel kidney cell strain and its use in virology. Appl. microb., **20**, 276.

6 — Pirsch, J.B., Mika, L.A. and Purlson, E.H. (1963). Growth characteristics of variola virus in tissue culture. J. Inf. Dis., **113**, 170.

7 — Postlethwaite, R. (1960). A plaque technique for the titration of vaccinia virus in chick embryo cells and some featuers of vaccinia infection in this system. Virology, **10**, 466.

8 — Reed, L.J. and Muench, H. (1938). A simple method of estimating fifty per cent endpoints. Am. J. Hyg., **27**, 493.

9 — Rhim, J.S. Shell, K. Creasy, B. and Case, W. (1969). Biological characteristic and viral susceptibility of an African green monkey kidney cell line Vero. Proc. Soc. exp. Biol. Med., **132**, 670.

10 — Ryden, F.W. and Randall, C.C. (1957). A study of three strains of vaccinia virus in stable cell strains. L., LLc-M1 and Hela Amer. J. Path., **33**, 293.

11 — Sureau, P. et Brygoo, E.R. (1959). Culture du virus de la vaccine 11. Culture du virus de la vaccine sur cellules epitheliales de rein du veau couche monocellueire. Ann. Inst. Past., **96**, 343.

Table 1_ Susceptibility of „Vero„ and camel kidney cells to some pox Viruses.

Virus (strain)	CPE (days post inoculation)		TCID50/ml		PFU/ml	
	Vero	CKCS	Vero	CKCS	Vero	CKCS
Vaccinia	++++(3)	++++(3)	6.5	6.0	7.2	6.5
Variola (B)	++++(5)	++++(5)	6.5	5.5	7.0	6.4
Variola (E)	++++(5)	++++(5)	6.5	5.25	7.1	6.2
Camel pox	++++(5)	++++(4)	5.25	5.5	6.0	5.3
Fowl pox	++++(4)	–	7.0	–	7.6	–
Goat pox	–	–				
Sheep pox	–	–				
Orf	–	–				

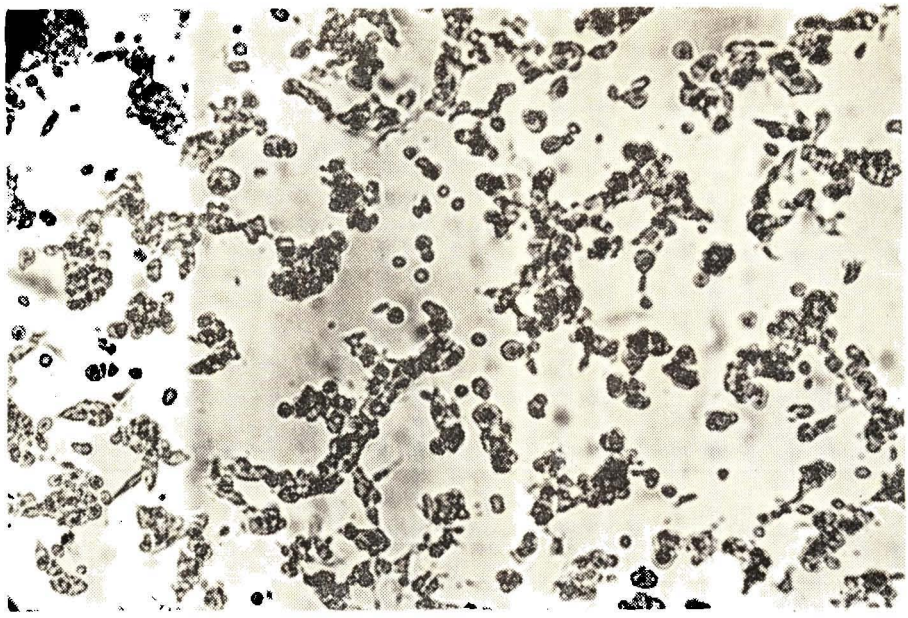


Fig. 1 — Vaccinia virus in vero cell, 48 hours after inoculation. Scattered, rounded and degenerated cells remain on the glass X125.

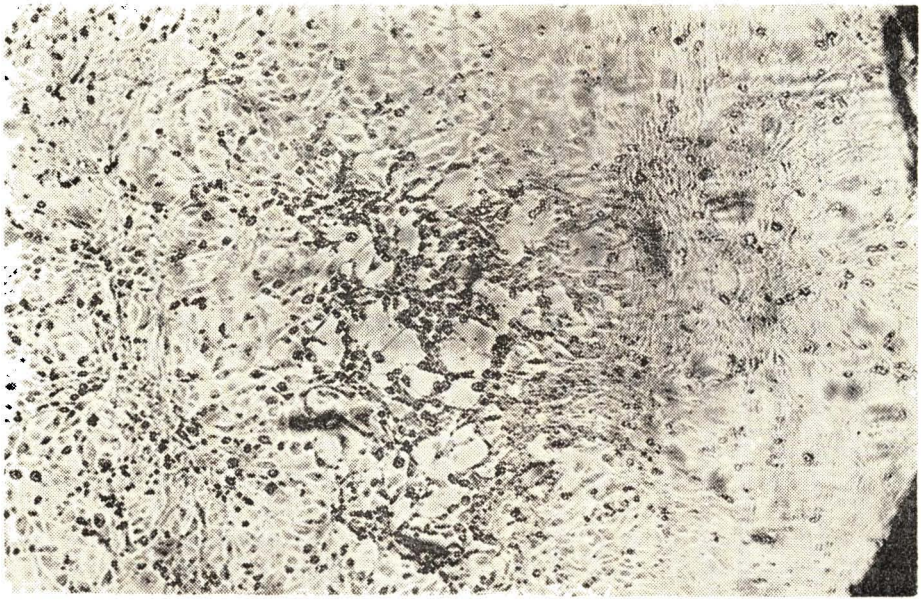


Fig. 2 — Vaccinia virus in camel kidney cell, 48 hours after inoculation. An area of separation and rounding of cells is seen X125.

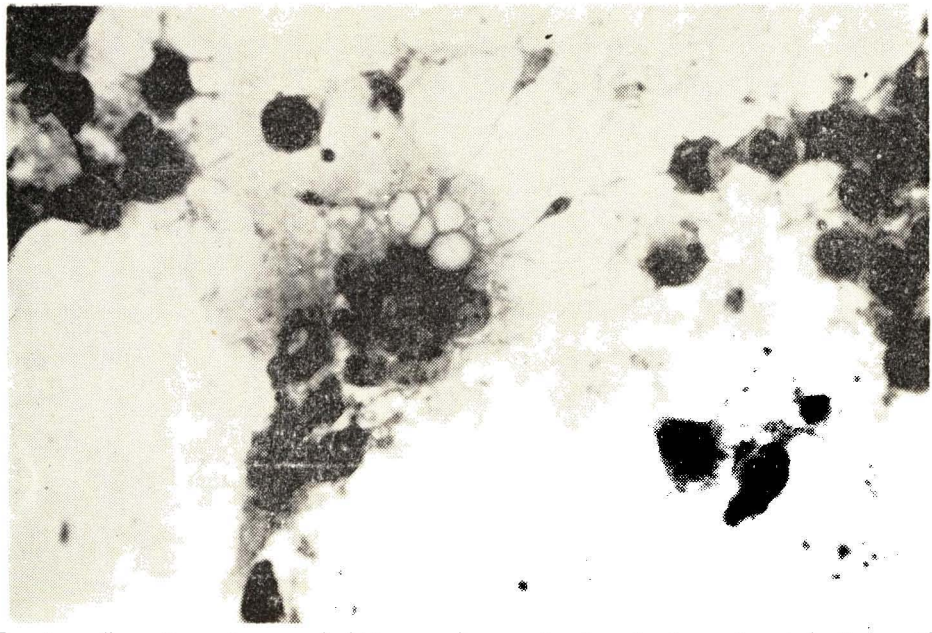


Fig. 3 — Syncytium in camel kidney cell on the fourth day after infection with vaccinia virus X450.

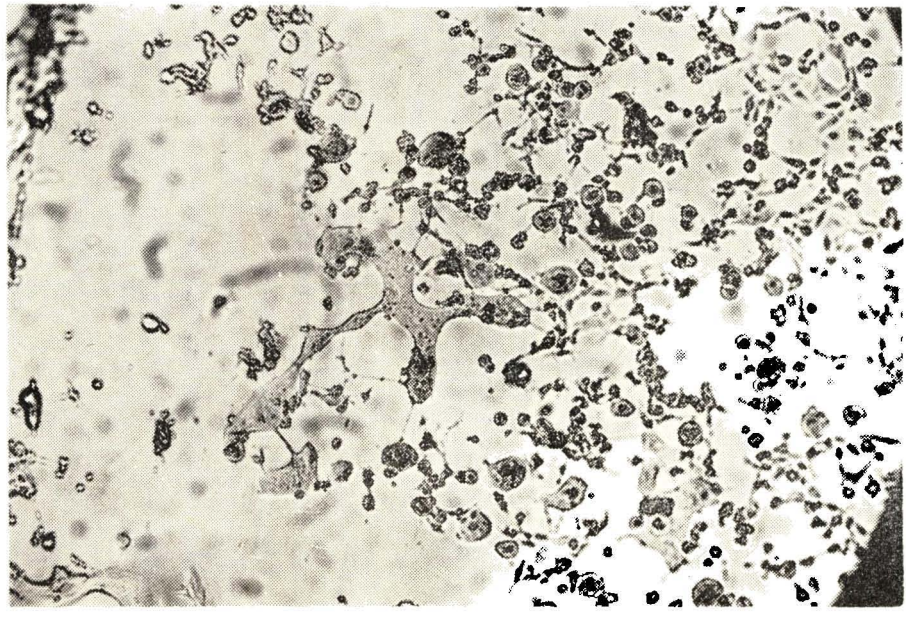


Fig. 4 — Variola virus, strain E in vero, fourth day after inoculation. There are syncytia and most of cells show signs of degeneration X125.

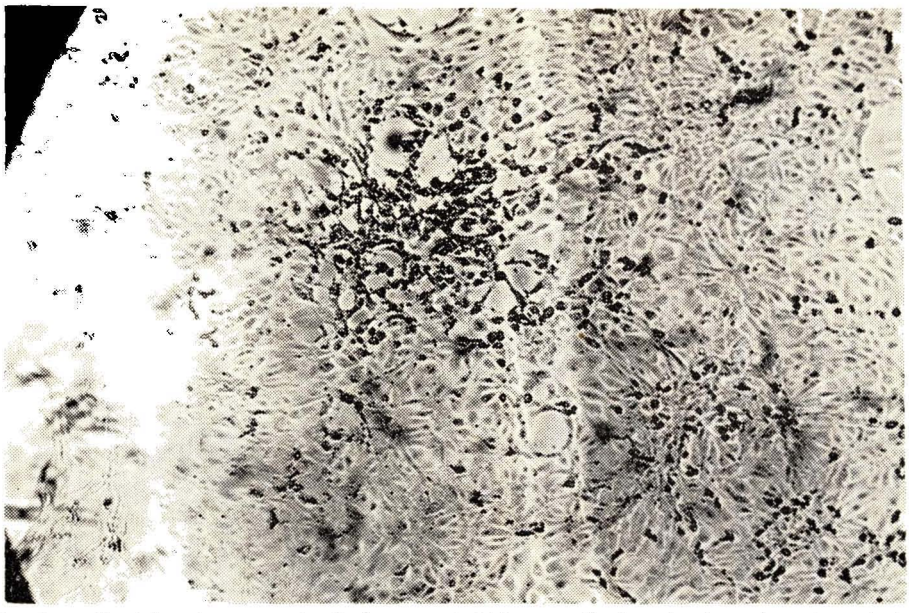


Fig. 5 — Variola virus, strain E in camel kidney cell, fourth day after inoculation. Notice the separation and rounding of cells X125.

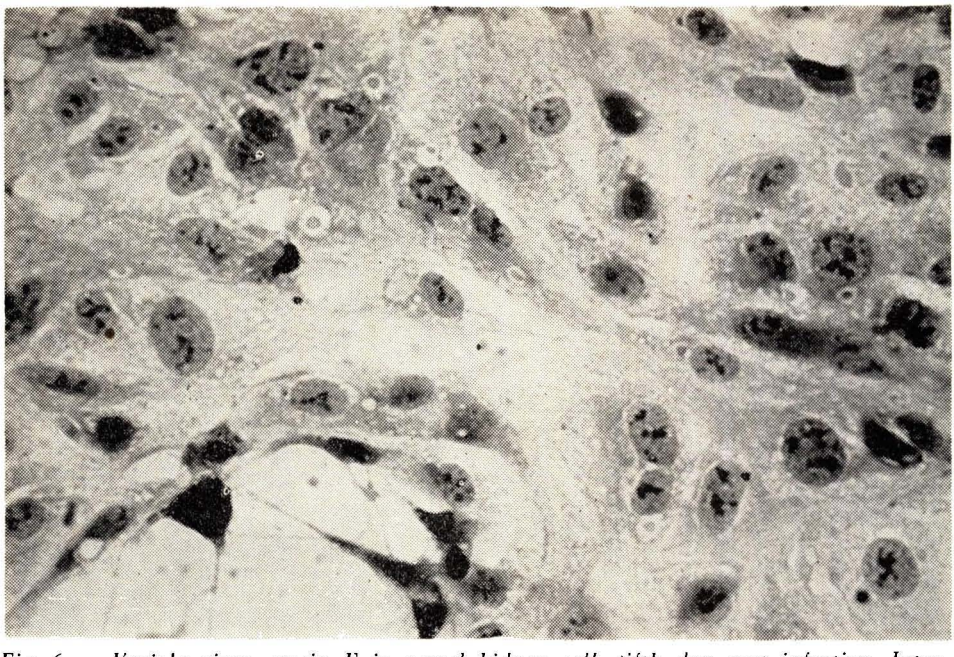
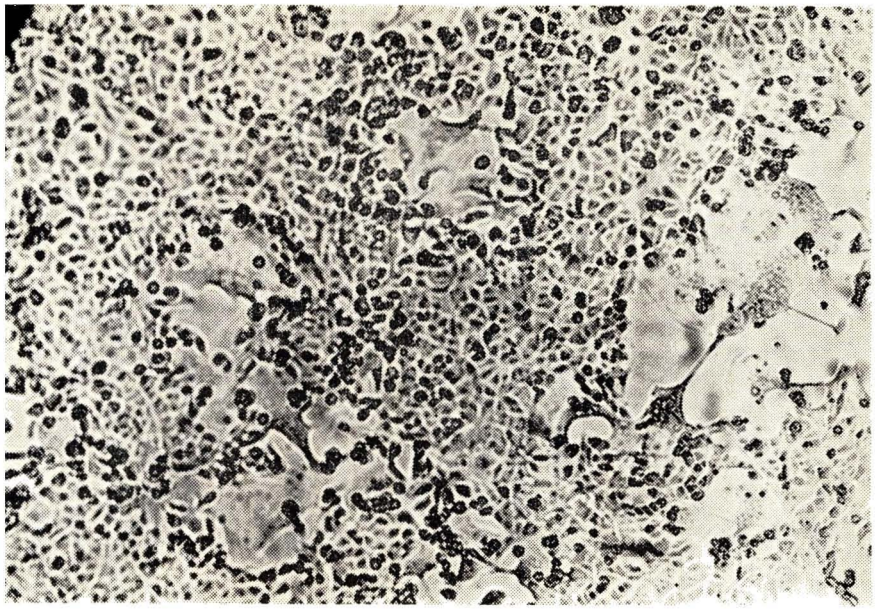
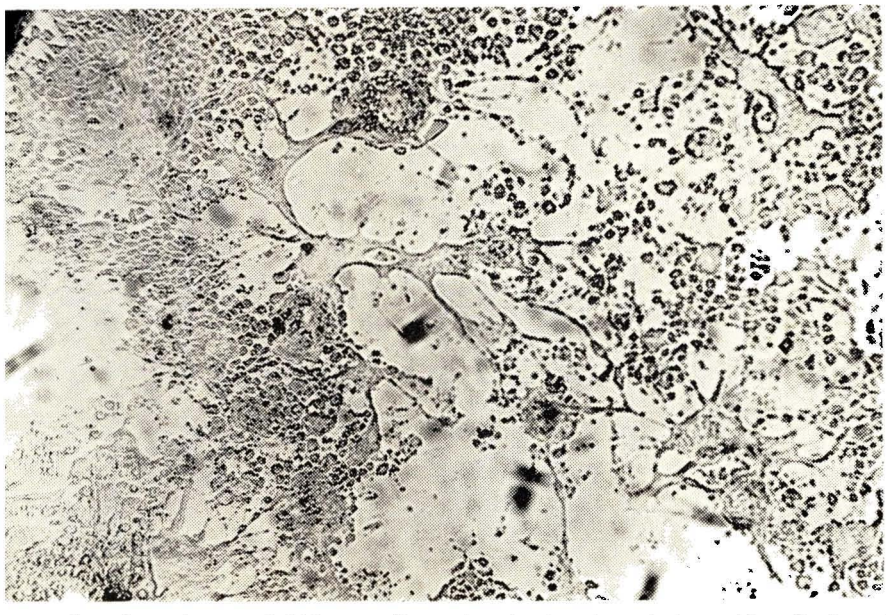


Fig. 6 — Variola virus, strain E in camel kidney cell, fifth day post infection. Intracytoplasmic inclusions are frequent X540.



— *Camelpox in vero cell, 48 hours after inoculation. Area of degeneration and syncytia are present X125.*



8 — Camelpox in camel kidney cell on day 3 after inoculation. All cell sheet is involved with degeneration, rounding and formation of syncytia X125.

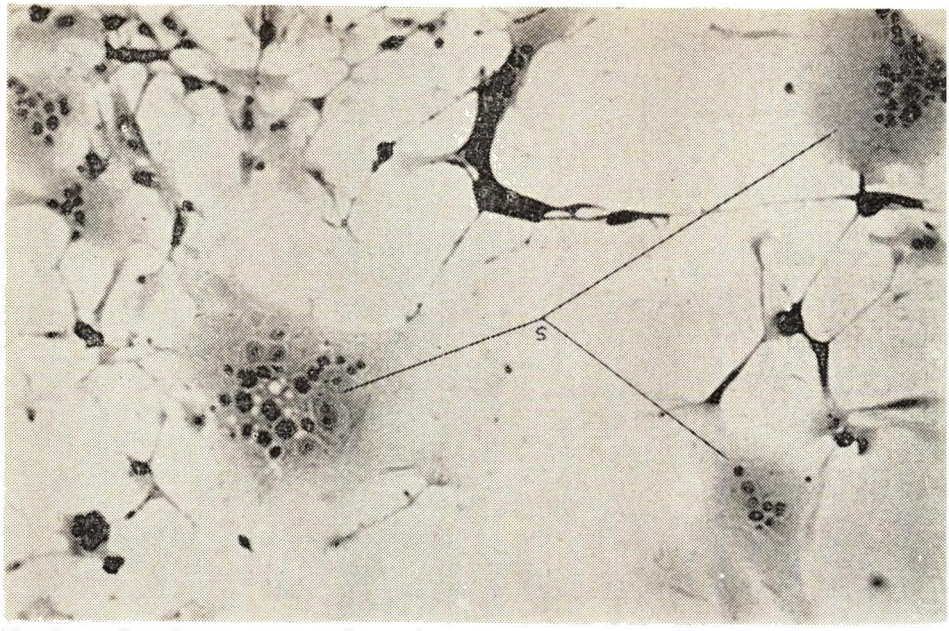


Fig. 9 — Camelpox in vero cell on day 3 post infection. Syncytia (s), pycnotic cells and advanced degeneration of the cell layer X540.

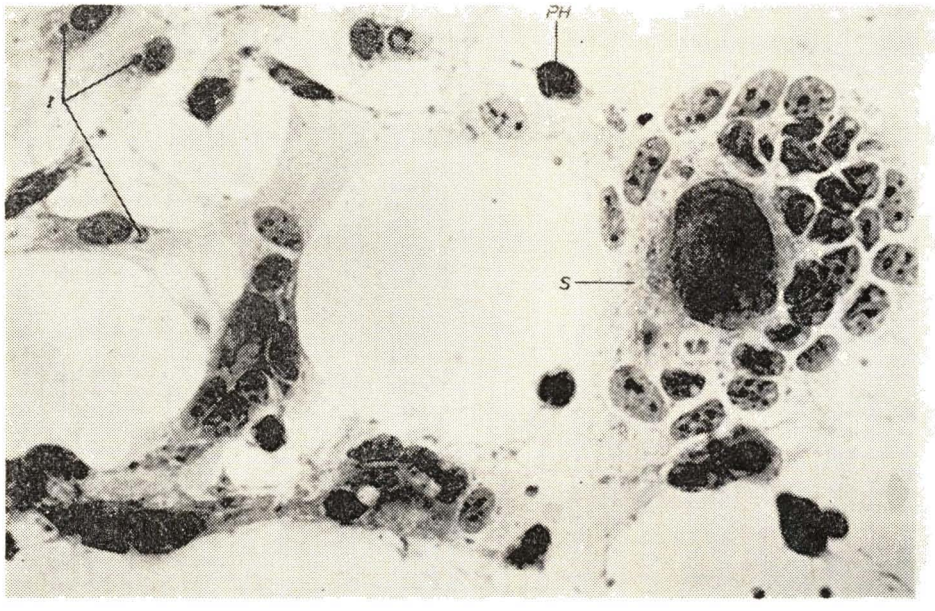


Fig. 10 — Camelpox in camel kidney cell. Notice inclusions, (I), syncytium (S) and cell phagocytosis (PH). X540.

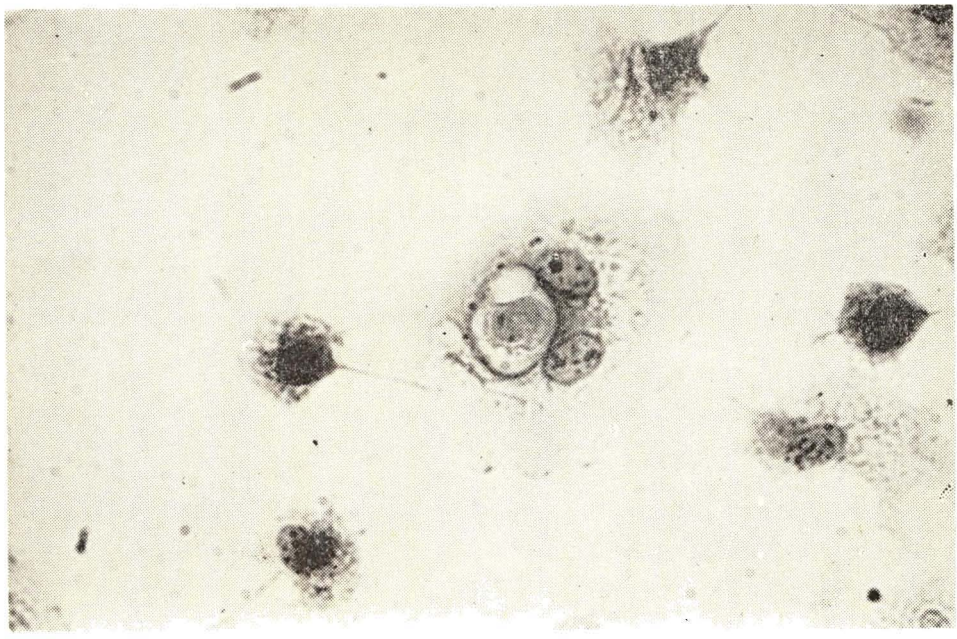


Fig. 11 — Vaccinia virus in camel kidney cell. Phagocytosis of one cell is seen X540.

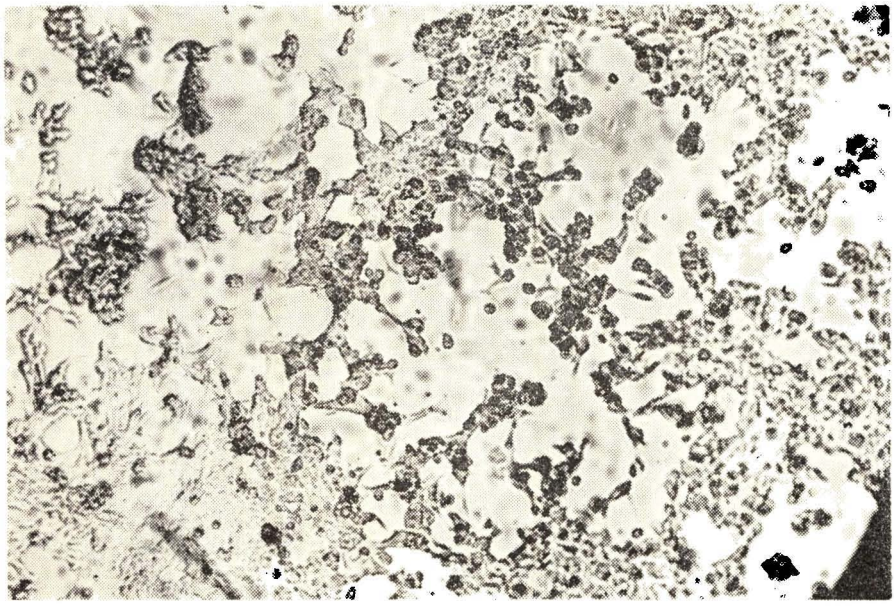


Fig. 12 — Fowlpox in vero cell, 48 hours after inoculation. Cells are rounded and clumped. X125.

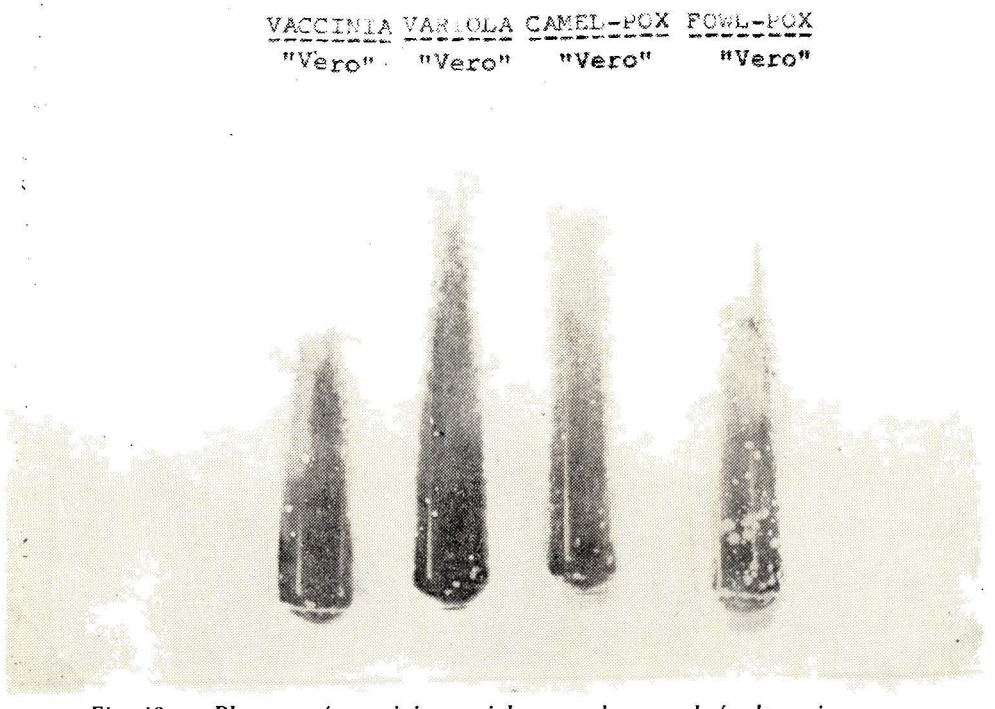


Fig. 13 — Plaques of vaccinia, variola, camelpox and foulpox in vero cell without overlay.

Fig. 14 — Microscopic examination of a plaque of vaccinia virus in vero cell Degenerated and rounded cells are present in central and peripheral zone of plaque.

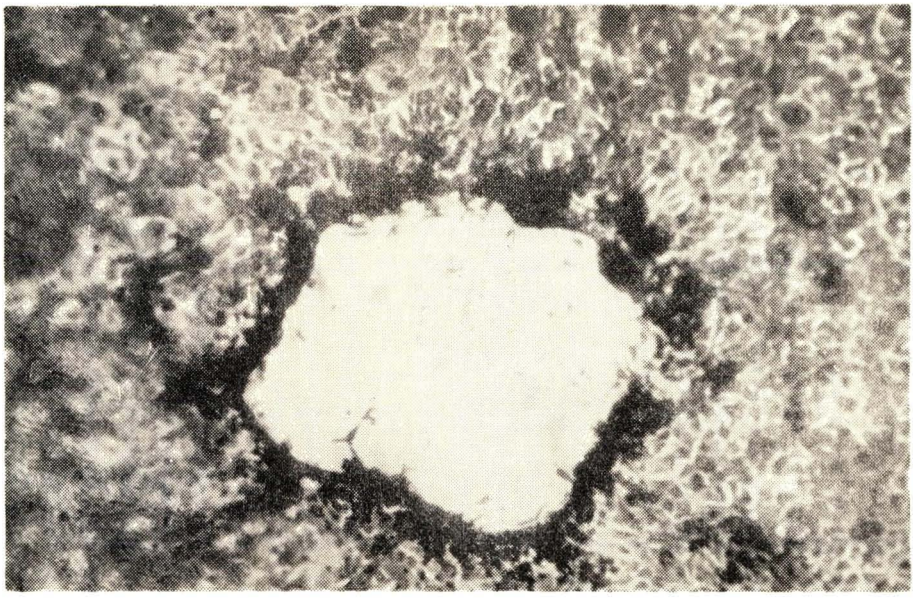


Fig. 15 — Microscopic examination of plaque of variola virus. E strain in vero cell. Total lysis of cells inside the plaque is evident.

Fig. 16 — Plaques of vaccinia, variola and camelpox viruses in camel kidney cell without overlay.