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DETECTION OF SWINE POX AND BUFFALO POX VIRUSES IN CELL CULTURE USING A PROTEIN A-HORSERADISH PEROXIDASE CONJUGATE

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Summary. — Buffalo pox virus antigen was detected in Vero cells and swine pox virus antigen in the cytoplasm and nucleus of PK-15 and IB-RS-2 cells as early as 6 hr post infection (p.i.) by indirect immunoperoxidase technique using a Protein A-horseradish peroxidase (HRP) conjugate. The viral antigens localized in the cytoplasm of infected cells were the most prominent after 24 hr p.i.

Key words: swine pox virus; buffalo pox virus; immunoperoxidase technique; detection in cell culture

Introduction

Poxviruses are believed to replicate only within the cytoplasm of infected cells. However, many of the poxviruses such as swine pox virus have been reported to produce nuclear inclusions and cause margination of chromatin in the nuclei of infected cells. In addition, swine pox virus antigen was detected in the cytoplasm of PK-15 cells (Kasza *et al.* 1960; Garg and Meyer, 1973). The immunoperoxidase technique (IPT) has been used for detection of buffalo pox virus-induced antigens in chick-embryo fibroblast cells infected with buffalo pox virus (Vaid *et al.*, 1978; Grover *et al.*, 1980). Here we present the results of immunoperoxidase staining in detection of buffalo pox and swine pox viruses in various cell cultures.

Materials and Methods

Viruses. Swine pox virus was isolated from the skin lesions during an outbreak at Izatnagar in 1981 in India (Verma, 1987). Buffalo pox virus (BPV) laboratory strain BP4 was propagated in Vero cells. A field strain of BPV designated BP 86 and recovered during and outbreak in buffaloes in India in 1986 was grown in Vero cells.

Cell lines. The PK-15, IB-RS-2 and Vero cells were grown in Minimum Essential Medium supplemented with 10 % calf serum, L-glutamine and antibiotics (Penicillin G 100 IU/ml, Streptomycin 100 µg/ml).

Preparation of hyperimmune serum. Immune serum against swine pox virus was raised in rabbits (1967). Briefly, 0.5 ml of the PEG-concentrated virus was injected by i.v. and intramuscular routes (the latter emulsified with an equal volume of complete Freund's adjuvant). After 14

days rabbits were inoculated by intramuscular route only; eight such injections were given and then the animals were bled one week after last injection. The immune serum against BPV strain BP4 was raised in rabbits as described by Baxby and Hill (1971).

Convalescent serum was collected from buffaloes affected with a natural outbreak of buffalo pox strain BP 86 was used.

Preparation of protein A — horseradish peroxidase (HRP) conjugate. Conjugation of protein A with HRP was done as described by Kurstak *et al.* (1984). Briefly, 10 mg of HRP (Sigma RZ 3.0) was dissolved in 1 ml of freshly prepared 0.1 mol/l sodium bicarbonate solution in a closed tube to activate the enzyme. 1 ml of 15 mmol/l sodium metaperiodate solution was added to this and left for 2 hr in the dark at 20 °C. Then 2.5 mg protein A (Pharmacia) dissolved in 2 ml of 0.1 mol/l sodium carbonate buffer (pH 9.2) was added to the above mixture and incubated at 25 °C for 3 hr to form the Schiff's base. After incubation, 0.2 ml of freshly prepared sodium borohydride solution (5 mg/ml $NaBH_4$ in 0.1 mmol/l NaOH solution) was added. After 30 min, 0.6 ml of another freshly prepared $NaBH_4$ solution was added to stabilize the Schiff's base. The conjugate was precipitated by the addition of equal volume of saturated ammonium sulphate solution by mixing for 1 hr. The precipitate (centrifugation at 2000 rev/min) was dissolved in 2 ml of acetate buffer supplemented with 1 mol/l NaCl, 1 mmol/l $CaCl_2$, 1 mmol/l $MgCl_2$ and 1 mmol/l $MnCl_2$ and stored at — 20 °C for further use.

Immunoperoxidase staining. Detection of viral antigen was done according to Kurstak (1971) and Rai (1985). Vero, PK-15 and IB-RS-2 cells were grown on coverslips in Leighton tubes. Monolayers were infected with buffalo pox and swine pox viruses and harvested at 6, 12, 18 and 24 hr post infection. After appropriate time the cells on coverslips were rinsed with PBS (pH 7.2), fixed with acetone for 10 min, and incubated with a few drops of immune serum for 1 hr at 37 °C. In some experiments BPV convalescent sera were used. After washing, a few drops of the conjugate were incubated with the coverslips for 1 hr. The coverslips were rinsed three times in PBS and finally with distilled water. After drying, 2—3 drops of freshly prepared Nadi reagent (Kurstak, 1971) was placed on coverslips. After 2—3 min they were rinsed in PBS and dehydrated by treating with alcohols (70 %, 90 % and absolute alcohol) followed by a quick wash with xylene. The coverslips were mounted on microscopic slides with DPX mountant, microphotographs were taken using Olympus Photo and Cinemarographic system (Model PM-10AD automatic exposure system) with a built in microcomputer.

Results and Discussion

In both PK-15 and IB-RS-2 cells the swine pox virus antigen was detected as early as 6 hr p.i. but the reaction was most prominent at 24 hr p.i. In PK-15 cells, the virus antigens were detected in the cytoplasm. However, some cells also showed virus antigen in the nucleus (Figs. 1—3). Similarly, IB-RS-2 cells revealed viral antigen in cytoplasm and in some areas also in the nucleus. Intracytoplasmic inclusion bodies were observed in some cells (Figs. 4—5). It seems that certain stages of virus multiplication involve nucleus as well as cytoplasm; it seems that virus antigen might be present either in nucleus or in cytoplasm of infected cells depending on the stage of virus multiplication. Since IPT can detect minute amounts of virus antigens, we could find the viral antigen in both cytoplasm and nucleus. Garg and Meyer (1973) demonstrated the presence of virus antigens in the cytoplasm of swine pox infected PK-16 cells as well as multiple nuclear inclusions using fluorescent antibody technique. Our findings confirmed these observations.

The detection of buffalo pox virus strain BP4 in Vero cells by indirect IPT using BP4 anti-serum showed that 24 hr was the period when peroxidase stained granules in the cytoplasm reached maximum in the infected cells. There was a progressive staining reaction starting from 6 hr onwards (Fig. 6) which became more marked until a major CPE was observed at 12, 18 and

24 hr. Areas showing CPE could be seen as spots with brown granules. Buffalo pox virus BP86 stained with convalescent serum also showed positive reaction, when rabbit HRP conjugate was used reacting with buffalo Ig.

Sequential development of buffalo pox virus antigens was revealed in Vero cells. Viral antigens could be detected from 6 hr onwards, staining of the antigen filling the entire cytoplasm was seen more intensive at 12, 18 and 24 hr p.i. IPT has been used to detect BPV induced antigens in CEF cells (Vaid *et al.*, 1978; Grover *et al.*, 1980). Kaushik and Pandey (1981) used indirect fluorescent antibody assay for the detection of BPV antigens in CEF cells in good correlation with virus infectivity titration. Similarly, Ghildyal *et al.*, (1986) used IPT for the detection of BPV antigens in formalin-fixed and paraffin-embedded tissue sections and detected pale to dark brown stained inclusions. The indirect IPT was sensitive and efficient means for detection of viral antigens. The main advantage of the suitability of the technique was viewing of slides under the light microscope and their permanent storage. Thus, it could be an easy tool for detection of viral antigens. It is evident that IPT can be used more efficiently then FAT for rapid detection of pox virus replication as early as 6 hr p.i. in PK-15, IB-RS-2 and Vero cells. The protein A-HRP conjugate could be used as a master conjugate and the use of various immune sera against different viruses would make the diagnosis of viral diseases easy, rapid and reliable.

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Explanation to Figures (pages 294—296):

Fig. 1. Swine pox virus infected PK-15 cells, 24 hr p.i., IPT control without antiserum, magn. × 504.

Fig. 2. Swine pox virus infected PK-15 cells, 24 hr pi., showing presence of swinepox antigen in cytoplasm as well as in nucleus. IPT, protein A — HRP conjugate, magn. × 1260.

Fig. 3. Swine pox virus infected PK-15 cells, 24 hr p.i. showing viral antigen in the nucleus and cytoplasm, IPT, magn. × 1260.

Fig. 4. Swine pox virus infected IB-RS-2 cells, 24 hr p.i. IPT control without antiserum, magn. × 1260.

Fig. 5. Swine pox infected IB-RS-2 cells, 24 hr p.i. showing the presence of viral antigen in the cytoplasm (a), nucleus (b) and intracytoplasmic inclusions (c), IPT, magn. × 1260.

Fig. 6. Indirect IPT in Vero cells infected with BPV strain BP4, 6 hr p. i. showing intensive peroxidase staining, magn. × 300.

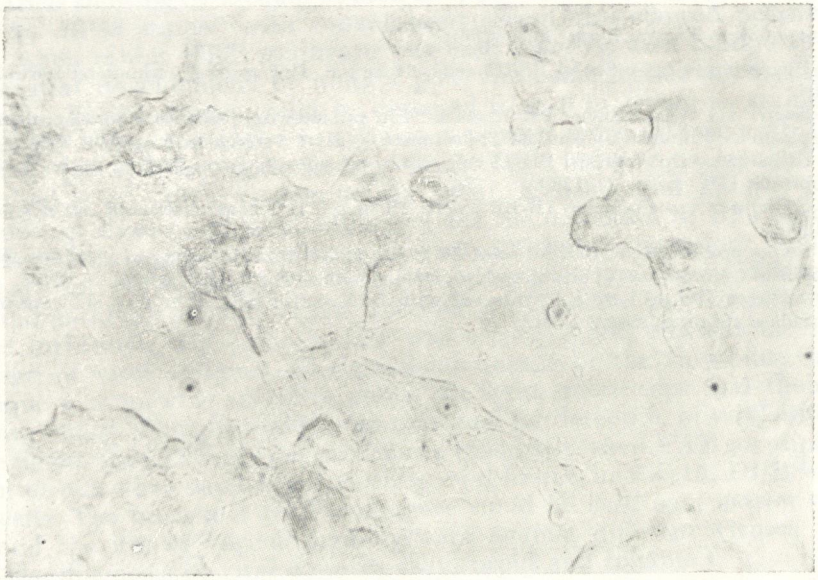

Fig. 1

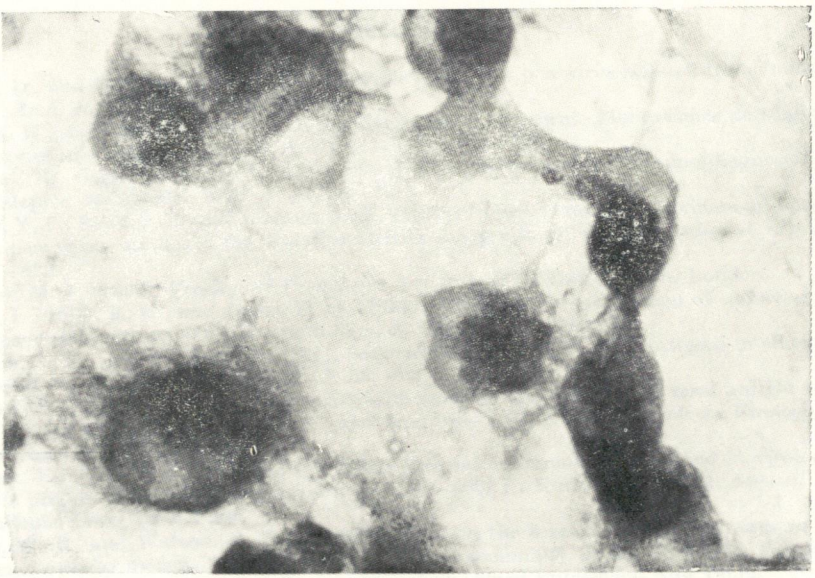

Fig. 2

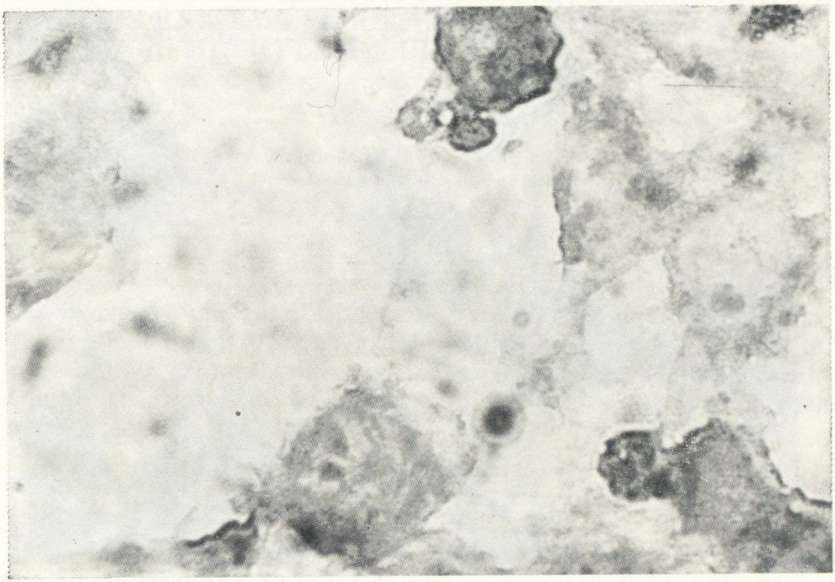

Fig. 3

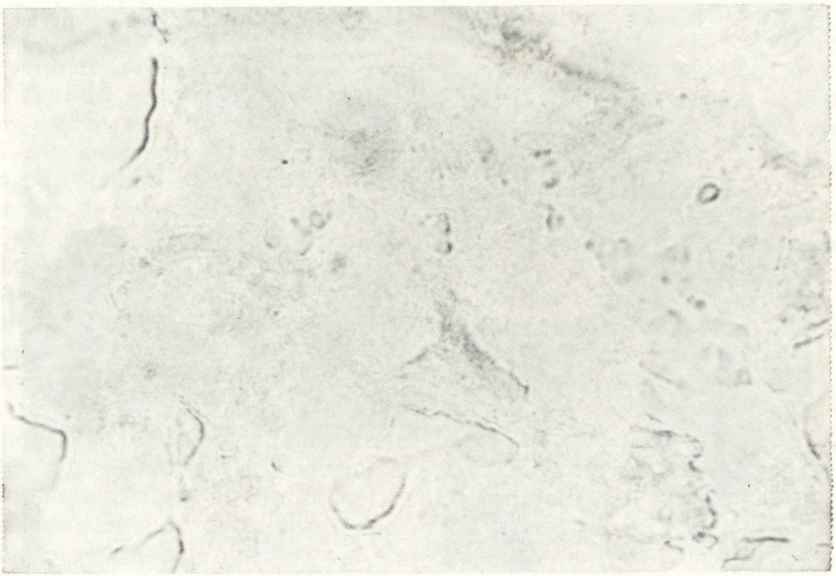

Fig. 4

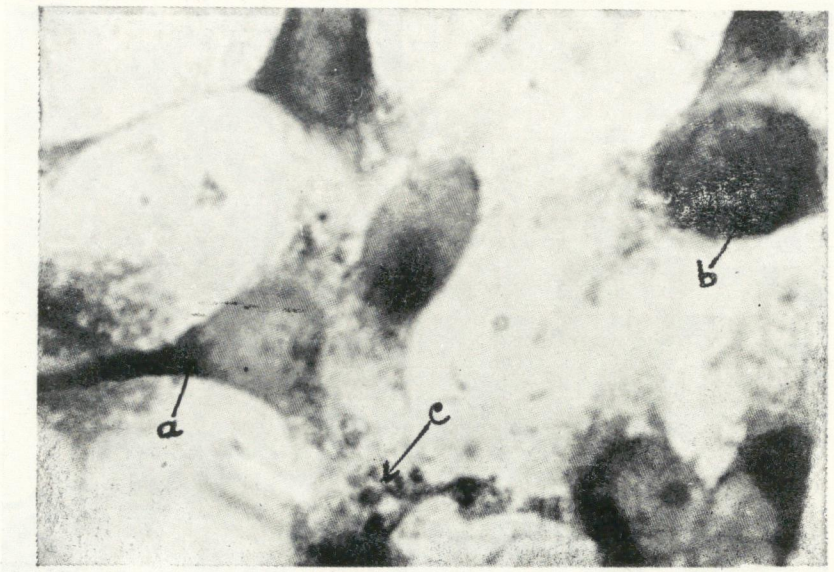

Fig. 5

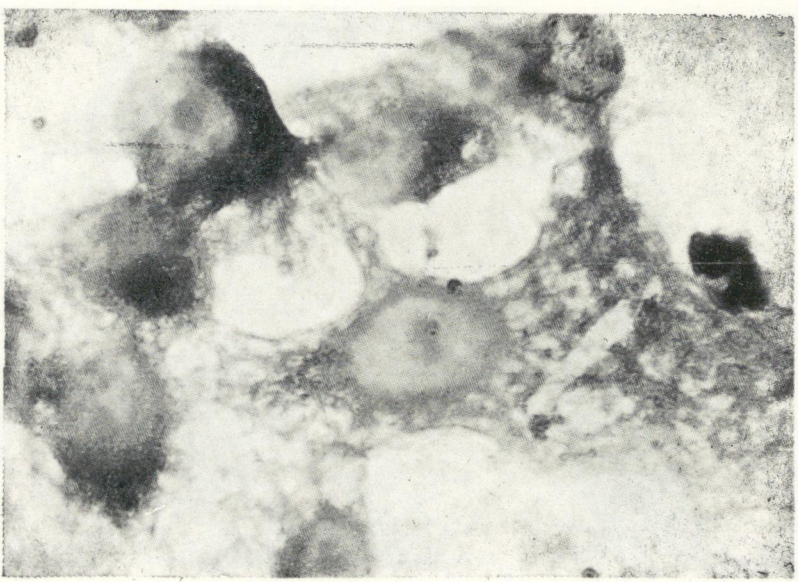

Fig. 6