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Isolation of Parapox Viruses from Man and Animals: Cultivation and Cellular Changes in Bovine Fetal Spleen Cells

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SUMMARY

Maculopapular, pock-like lesions were detected repeatedly on hands and arms of students having contact with sheep or cattle. Pustular lesions were seen on the mammary gland and vulva of a goat. Biopsy samples of the lesions from 3 students and the goat were cultured on bovine fetal spleen (BFS) cells. Viruses were isolated from all samples. The viral isolates induced similar cytopathic changes in BFS cells. Slowly spreading plaque-like changes consisting of large, round cells developed in BFS monolayers. In a growth curve experiment involving the Shoe strain and BFS cells, virus infectivity increased 8 hours after inoculation. The major portion of the infectivity remained associated with the cell fraction. Maximal titers were found 36 hours after infection. Eosinophilic inclusions of varying size were detected in the cytoplasm 8 to 18 hours after infection of BFS cells. Later these inclusions enlarged, coalesced, stained basophilically, and remained attached to the pycnotic nuclei. Vacuoles created a ballooning appearance and separated the plasma membrane from the nucleus-viral inclusion complex by 24 hours after infection when the cells detached. Negatively stained preparations of purified virus of the isolates contained ovoid virions covered with diagonally woven bands. The long axis of the virions measured 220 and 250 nm and the short axis was 120 to 140 nm. These features are characteristic of the parapox virus genus which includes members that induce orf.

INTRODUCTION

The virus of orf is also known as contagious ecthyma or contagious pustular dermatitis, and it infects sheep and goats as well as man. Based on the

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characteristic viral morphology it is a member of the parapox virus genus of the pox virus family (1,13). A virus of similar morphology causes teat-lesions in cows and milker's nodules in man which are virtually indistinguishable from human orf lesions (12). Man contracts these infections through handling infected animals or by contact with contaminated fomites. In rural population as well as in students enrolled in animal science or veterinary curricula, lesions with orf characteristics continue to occur (3,10). Diagnosis is usually based on epidemiologic history, clinical signs and the lesions present. An accurate and fast etiologic diagnosis should be based on isolation of the causative agent or electron microscopic demonstration of the characteristic virus in samples taken from the lesions. Cultivation of the orf virus has been reported (2,8,11,14) but numerous unsuccessful attempts to isolate this agent from clinical specimens also have been described (3,4,5,6,7). Techniques employed in the isolation and cultivation of parapox viruses from clinical specimens of man and animals using bovine fetal spleen (BFS) cells are described and the cytologic changes induced by these isolates are analyzed in this report.

MATERIALS AND METHODS

Collection and Treatment of Clinical Specimens

Viral isolation was attempted with specimens from three human patients and from a goat. A small biopsy sample and lesion exudate was collected from the finger lesions of a girl. This sample was homogenized in tissue culture fluid and centrifuged at 2,000 xg for 20 min. The supernatant fluid was used as inoculum for BFS celi cultures. Lesion fluids were aspirated from the other patients and the goat by inserting a 25-gauge needle attached to a syringe into the skin lesion. The needle contents were then washed into the medium of BFS monolayers as the inoculation step.

Cell Culture Propagation

The procedure to establish BFS cells was a modification of the method of Malmquist and coworkers (9) as described previously (15). Initially the cells were grown in Earle's minimum essential medium (MEM) with 10% lamb serum. The cell culture medium contained 500 ug/ml streptomycin and 500 units/ml penicillin. When it was desirable to maintain contact-inhibited BFS cells for longer periods, the cells were grown in Earle's medium enriched with lactalbumin and vitamins (Lavit). Inoculated cells were examined daily with an inverted microscope. The Lavit maintenance media of inoculated cell cultures contained 3% heat-inactivated fetal calf serum.

Storage of Virus Stock

Cell cultures of BFS that had developed more than 50% cytopathic changes were frozen and thawed once. The fluid and cell debris were then harvested, placed into 2 ml ampules which were sealed and stored at-20 C.

One-Step Growth Curve

Cells were plated in one-ounce prescription bottles at a concentration of 250,000 cells per ml. When confluent, these cells were inoculated with 1 ml of virus stock. Adsorption occurred at 37 C for 1 hr. The unadsorbed inoculum was removed and the monolayers were washed 5 times with 5 ml of warm MEM. The bottles were then given 5 ml maintenance medium and incubated at 37 C. At 0, 4, 8, 12, 18, 24, 30 and 36 hours after adsorption, bottles were taken for infectivity assays. The cells were scraped off with a rubber policeman. The resulting cell suspension was centrifuged for 10 min at 1,500 xg. The supernatant fluids and cell pellets were collected separately for infectivity titration. The cell pellet was suspended in 1 ml of MEM, and the cells were disrupted by 4 ultrasonic bursts of 15 seconds each at a setting of 75 watts with a Sonifier Cell Disruptor, Heat Systems Co., Melville, New York.

Titration of Viral Infectivity

A microtiter method was developed to assay the infectivity of the viral isolates. Cell culture microplates with 96 wells were seeded with 1.25×10^4 cells in 0.05 ml per well with growth medium. Tenfold serial dilutions of virus were prepared and 0.05 ml of the different dilutions were added to each of 4 wells. The plates were incubated for 4 to 6 days at 37 C in a 5% CO2 atmosphere. Cytopathic changes were then scored, and the 50% end-point (TCID50) was calculated by the method of Spearman-Kärber.

Analysis of Cytologic Changes

Monolayers of BFS grown on coverslips were inoculated as described above. At the intervals after inoculation mentioned, cover slips were removed, rinsed repeatedly in phosphate buffered saline (PBS) at 7.2, and treated with Bouin's fixative. The cells on the cover slips were then stained by the Giemsa method.

Preparation of Negative Stains and Electronmicroscopic Examination

Cells infected with the different isolates were sedimented at 3,000 xg for 30 min. A few drops of distilled water were added to lyse the cells. Centrifugation was repeated and a small drop of the supernatant fluid was placed on carbon-formvar coated grids. Excess fluid was removed with filter paper and a small drop of 1.5% sodium phosphotungstic acid (PTA) was applied (13). After 2

minutes the excess PTA was removed with the edge of a filter paper. The grids were dried overnight under vacuum at room temperature. The grids were then examined with an Hitachi HU-12 electron microscope at 75KV acceleration and a screen magnification of 40,000.

RESULTS

Epidemiologic Aspects of Infection

One of the patients (Till) had worked with dairy cows while another (Shoe) had handled young lambs and the third (Eric) could not identify a definitive source. He developed the lesions on his finger shortly after he returned from a trip to Australia where he had contact with sheep and cows. The goat had small papular and pustular lesions on the mammary gland and the vulva.

Isolation of Virus

All specimens induced cytopathic changes in the first passage in BFS cells. A simple needle aspirate with immediate washing of the needle in the cell culture fluid was successful in establishing infection in BFS cells. Initial changes were seen 3 to 7 days after inoculation. Plaque-like focal areas of large, highly refractile cells were detected which often detached to set up secondary foci of infection in the BFS monolayers. Cells aggregated in a grape-like pattern as the infection progressed.

Morphology of Virions of Different Isolates

Virions of all isolates were detected in negatively stained preparations of supernatant fluids of infected cells lysed by hypotonic shock. The virions were ovoid and were covered with characteristically woven, diagonal bands (Fig. 1). Other viral particles were not detected. The size ranged from 220 to 250 nm in length and 120 to 140 nm in width. This morphology distinguished all isolates as parapox viruses.

Growth Curve of Shoe Isolate in BFS Cells

Infectious virus was not completely removed through washing. Some infectivity remained associated with the cells. An increase in cell-associated virus was observed 8 hours after infection, and virus release into the medium was detected after 12 hours. The maximal infectivity titer reached 6×10^6 TCID 50 per 0.05 ml 36 hours after infection and remained mainly associated with the cellular fraction.

Cytologic Changes in Infected BFS Cells

Small eosinophilic cytoplasmic inclusions were detected in BFS cells 8

hours after infection with the Shoe isolate. Most infected cells had one such inclusion but some had 2,3 or 4 (Fig. 2). These eosinophilic inclusions increased in size, coalesced, and displaced the nucleus (Fig. 3). After 18 hours of infection vacuoles developed in peripheral cytoplasmic areas and subsequently enlarged (Fig. 4). By 30 hours the viral inclusions stained basophilically and remained attached to the pycnotic nuclei (Fig. 5). The cytoplasmic membranes were separated from the nucleus-viral inclusion complex by a wide irregular halo which was traversed by a few cytoplasmic strands (Fig. 6). These cells then detached and lysed. This time sequence of development was also seen with the goat isolate. The Till isolate induced identical changes, but they developed about 4 hours later than the changes in Shoe-infected cells.

DISCUSSION

The nature of these viral isolates was clearly characterized as a parapox virus by their unique morphology after negative staining. Although the isolates were made from a goat and human patients who had contacts with sheep or cattle, significant morphological differences between the isolates were not detected.

These parapox viral strains were readily isolated in BFS cells. Detection of viral infection was possible in the first cell culutre passage 3 to 7 days after inoculation. Nagington (11) reported that native sheep orf multiplied only in sheep cells while sheep virus from human lesions had lost this specificity. Parapox virus of bovine origin had also a broader host cell range. He differentiated parapox viral isolates of ovine and bovine origin through cytopathic differences and cytological changes in infected cells. Although the human isolate of bovine origin induced inclusions and other cytopathic changes at a slower rate than the other isolates, the nature of the cytopathic alterations induced by our isolates was identical.

Our isolates grew equally well and induced virtually identical cytopathic changes in BFS cells. The cytoplasmic inclusions progressed from initial small eosinophilic structures which enlarged, coalesced, and late in infection formed large basophilic inclusions that remained attached to the pycnotic nuclei. Vacuolization and ballooning separated the cytoplasmic membrane from the nucleus-viral inclusion complex.

Infections with parapox viral agents can be easily diagnosed if skilled clinicians are consulted and the proper procedures for laboratory diagnosis are employed. This infection is of public health significance, and it appears to occur more frequently than is commonly acknowledged.

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CAPTIONS TO FIGURES

Figure 1 Two viral particles of goat isolate of parapox virus. PTA stain, 120,000 X.

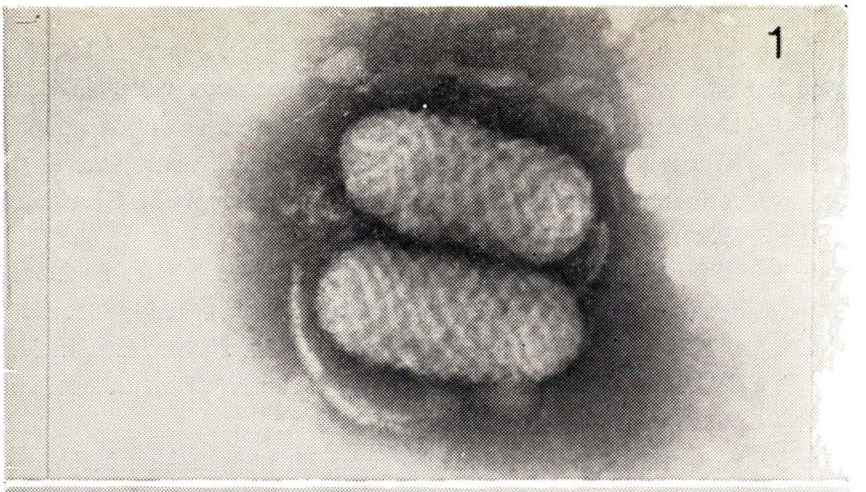
Figure 2 BFS cell 8 hours after infection with Shoe isolate. Several small eosinophilic inclusions (I) are visible in the cytoplasm. Giemsa stain, 1031 X.

Figure 3 BFS cell 12 hours after infection with Shoe isolate. Eosinophilic cytoplasmic inclusions (I) are enlarged. Giemsa stain, 1031 X.

Figure 4 BFS dikaryon 18 hours after infection with Shoe isolate. Inclusion (I) is associated with nucleus (N) and vacuoles (V) are present in peripheral cytoplasm. Giemsa stain, 1031 X.

Figure 5 BFS cells 24 hours after infection with Shoe isolate. Inclusion (I) remains attached to the nucleus (N) while peripheral cytoplasm separates. Giemsa stain, 1031 X.

Figure 6 BFS cells 30 hours after infection with Shoe isolate. Inclusion (I) remains attached to the pycnotic nucleus (N) and cytoplasmic membrane (M) forms ballooning contour. Giemsa stain, 1031 X.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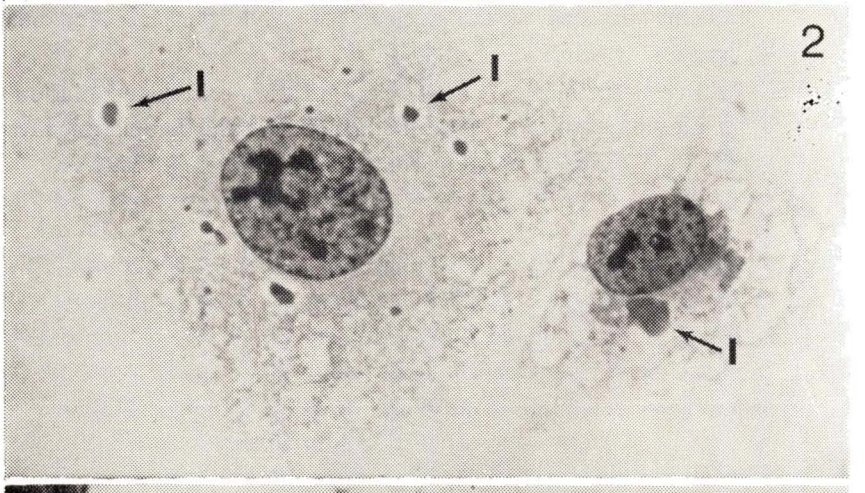
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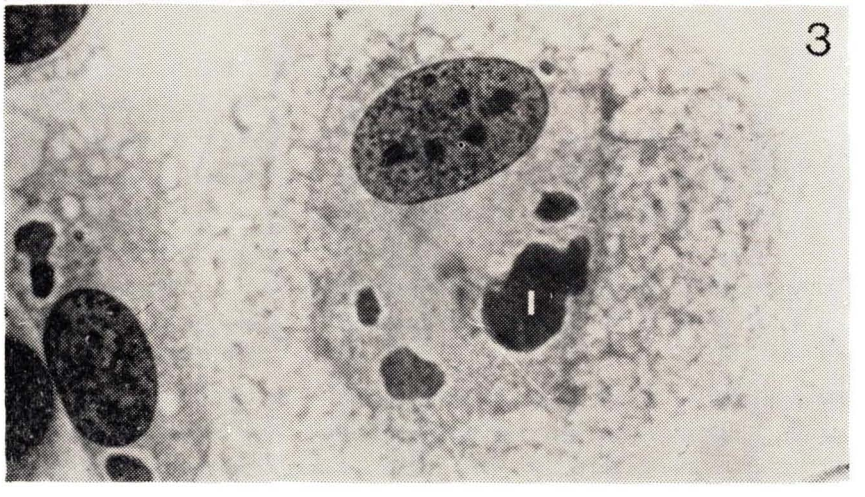
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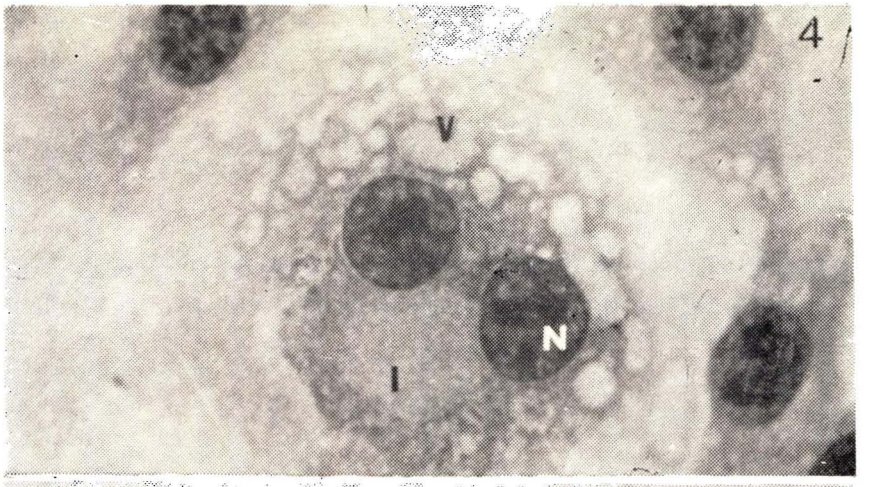
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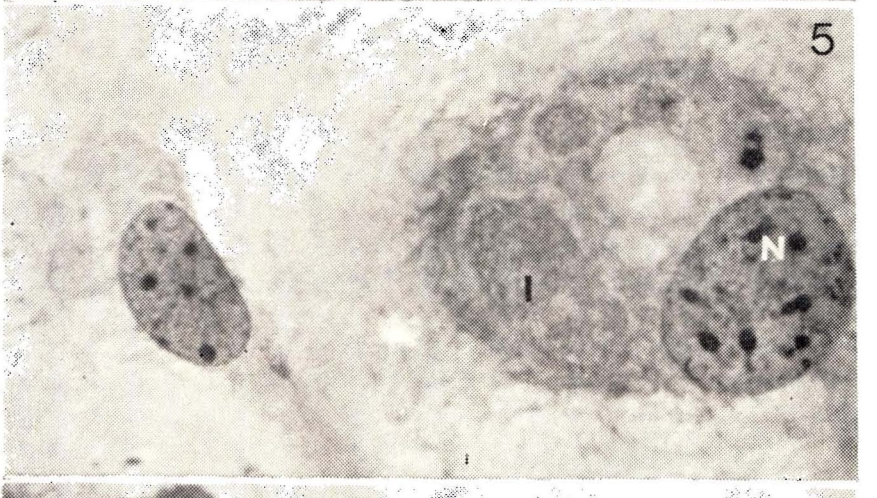
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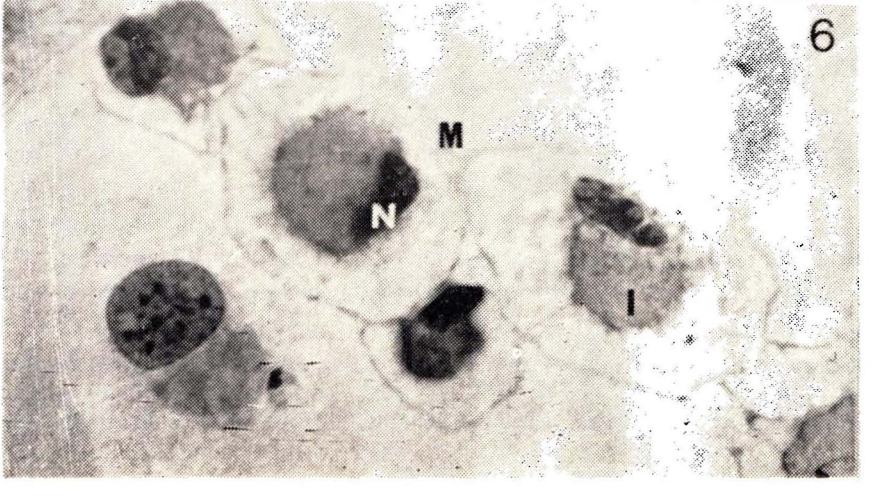
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