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# EXPERIMENTAL PATHOGENESIS OF BUFFALO POX VIRUS IN RABBITS: CLINICO-PATHOLOGICAL STUDIES

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*Summary.* — Buffalo pox virus produced typical pock lesions on the skin of rabbits at the site of primary inoculation following an incubation period of 48-72 hr. Gross lesions in internal organs, characterized by focal or diffuse necrotic areas on lung, liver and spleen were seen from day 5 post-inoculation (p. i.). Isolated lesions of approximately 2 mm diameter appeared in skin, stomach, intestine and uterus from day 7 p. i. Histopathological changes, i. e. intra-alveolar and intra-bronchial haemorrhages were seen in lungs and severe fatty changes were found in the liver. Multinuclear cells were detected in liver during recovery. Virus particles were demonstrated by electron microscopy in skin, lung, liver and spleen lesions.

*Key words*: *buffalo pox*; *pathogenesis*; *histology*; *electron microscopy*

## *Introduction*

The aetiologic agent of buffalo pox, a virus related to vaccinia and cowpox viruses, was established for the first time by Singh and Singh (1967) and later confirmed by Baxby and Hill (1971). Although the disease has been experimentally produced in buffalo calves, rabbits and guinea pigs (Singh and Singh, 1967; Srinivasappa, 1973) and infant mice (Dogra *et al.*, 1978), its pathogenesis has not been yet established. The present communication describes the sequential development of macro- and microscopic lesions in rabbits.

## *Materials and Methods*

*Buffalo pox virus (BPV).* The buffalo pox virus (BPV) strain $BP_4$ was originally obtained in freeze dried form through the courtesy of Dr. I. P. Singh, formerly Senior Professor and Head, Department of Bacteriology and Hygiene, H.A.U., Hissar, Haryana (Now Dean, College of Veterinary Sciences, Pantnagar). The virus was passaged on chorioallantoic membrane (CAM) of developing chicken embryos (CE) and finally maintained by regular passages in buffalo calf skin. The buffalo pox skin lesions were collected aseptically and homogenized with Hank's balanced salt solution (HBSS) to make a 20% suspension. The suspension was centrifuged at 2000 rev/min for 30 min and was used as a source of virus.

*Experimental procedures.* Fifty apparently healthy albino rabbits weighing 1—1.5 kg were inoculated intradermally into the abdominal area with 1 ml of virus suspension containing

6.2 log egg infective doses ($EID_{50}$)/ml. Each of the animals was inoculated on two points with 0.45 ml of virus suspension (a total of 0.9 ml) and 0.1 ml was applied on the scarified area. The rectal temperatures and clinical signs exhibited by the surviving animals were recorded daily. Two rabbits were sacrificed daily up to day 9 and then at 2—4 days intervals up to day 24 p. i. The whole blood was collected in equal vol of 3.8 per cent sodium citrate solution and stored at —10 °C until use. After post-mortem examination, samples of skin, lung, liver, spleen, kidney, stomach, intestine and uterus/testis were stored in 10 per cent formol-saline for histopathology.

*Virus titration in blood.* The blood was frozen and thawed three times and centrifuged at 3000 rev/min for 15 min. The virus infectivity was measured in the supernatant by inoculating 0.2 ml of 10-fold dilutions into the CAM of 12-day-old CE (3 eggs were used per dilution). The eggs were incubated at 37 °C for 12 hr. After 72 hr the CAM were harvested and $EID_{50}$/ml was calculated.

*Electron microscopy.* The papulopustular skin lesions and fresh tissues from lung, liver and spleen collected up to day 5 p. i. from sacrificed rabbits were trimmed to 1 mm cubes and processed according to the technique of Conroy and Meyer (1971). However, after dehydration in graded alcohols, the tissues were impregnated in epoxy resin as described by Luft (1961). Sections were cut and stained with 5 per cent uranyl acetate at 60 °C for 10 min and lead citrate at room temperature for 5 min and examined in a SEM 3-2 electron microscope (Veb Werk für Fernsehelektronik, Berlin).

## *Results*

Out of 50 infected rabbits 28 were sacrificed, 10 of these showed secondary skin lesions and 6 showed bilateral corneal opacity. Fourteen rabbits succumbed to infection. Eight rabbits survived the experiment and recovered completely by day 30 p. i.

### *Primary skin lesions*

Primary lesions at the inoculation site occurred at 48 hr p. i. as a slight inflammation which was followed at 72 hr p. i. by marked swelling. The involved area became reddish black with diffuse whitish mass on day 6 (Fig. 1). The scab completely peeled off between 21—30 days p. i. The scarified skin area showed small vesicles on day 4 which became whitish yellow pustules 2 mm in diameter with or without a central depression on day 6 p. i. The pustules turned into scabs by day 11 p. i.

**Table 1. Log $_{10}$ infectivity titres* of BPV in blood of infected rabbits and their mean rectal temperature in °C**

| Observations | Days p.i. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| Mean rectal temperature | 38.5 | 38.5 | 40.0 | 41.4 | 40.4 | 41.0 | 40.3 | 39.6 | 39.5 | 39.5 | 39.5 | 39.2 |
| ±S.D. | ±0.55 | ±0.50 | ±0.65 | ±0.60 | ±0.46 | ±0.32 | ±0.47 | ±0.62 | ±0.90 | ±0.66 | ±0.55 | ±0.37 |
| Virus titres in blood | — | + | 2.2 | 2.2 | 2.7 | 4.7 | 4.7 | 3.2 | — | ND | — | ND |

* Fifty per cent $EID_{50}$/ml. ND = Not done. S.D. = Standard deviation.

*Clinical signs and secondary external lesions*

The rise in mean rectal temperature was recorded from the third day onward with its peak on day 4 followed by a fall on day 5. A rise in temperature was recorded on day 6, then followed by a decline. The temperature was normal from day 12 p. i. Viraemia persisted from day 2 to 8 p. i. (Table 1). Secondary lesions appeared between days 7—10 p. i. on the lips, around the nostrils, on tongue, cornea, eyelid, neck, back and perineum. The vesicles turned into pustules on the following day. The eyes of six infected rabbits revealed severe inflammation on day 10 with a watery discharge which became purulent on day 12 resulting in bilateral corneal opacity and blindness. Rabbits showing the severe changes were debilitated; 5 of them developed diarrhoea. Before death, they exhibited dyspnoea and a frothy discharge from the nostrils.

*Gross lesions in internal organs*

The lungs did not reveal any significant gross change up to day 5 p. i. However, on days 6 and 7 lung parenchyma revealed small areas of haemorrhages and had a marbelled appearance (Fig. 2). Varying degree of consolidation was recorded on day 11 p. i. in both lungs. The liver was slightly enlarged with well marked randomly distributed necrotic foci on the surface on day 6; they became diffuse on day 7 p. i. By day 17 large depressed areas appeared on its surface. The spleen was invariably increased in size in all rabbits sacrificed between days 5—11 p. i. The splenic capsule revealed white, raised areas of 0.5 to 1 mm in diameter on days 6 and 7 (Fig. 3). The kidney was enlarged with focal or diffused white necrotic areas; the stomach, intestine and uterus showed few pustules and ulcers between day 7—14 p. i.

*Histopathological changes*

Hydropic degeneration of the epidermal prickle cells was seen on days 3 to 5 p. i., on the next day the affected cells of epidermis showed vacuolation, severe necrosis and massive infiltration with mononuclear cells (Fig. 4). Intracytoplasmic eosinophilic inclusion bodies could be rarely detected. In the dermis, massive haemorrhagic areas with widespread mononuclear infiltration and a few polymorphonuclear cells were seen on day 6 p. i. From day 11 macrophages and plasma cells were found in abundance and there was evidence of regeneration of epidermal cells and dermal connective tissue.

Lung tissue revealed massive intra-alveolar and intra-bronchial haemorrhages on day 6 p. i. Degenerative and necrotic changes along with complete denudation of epithelial (lining) cells in some bronchi were marked on days 7 and 8 p. i. The interalveolar septa revealed extensive infiltration of mononuclear cells resulting in consolidation and atelectasis. Plasma cells and a few macrophages were also seen on day 21 p. i.

Hepatic cells revealed hydropic degeneration and coagulation necrosis on day 7 p. i. Severe perilobular and centrilobular fatty degeneration was seen on day 8 p. i. in fatal cases (Fig. 5). These changes were subsequently

recorded and on day 17, extensive necrosis of hepatic cells was evident with large areas of heavy mononuclear infiltration resulting in loss of normal hepatic architecture. Multinuclear cells were seen in large numbers from day 17 onward.

Splenic red pulp showed initially marked congestion; large amounts of blood pigment was seen in macrophages as well as in the extracellular area. White pulp revealed mild rarefaction of lymphoid tissue. Lymphoid hyperplasia in a few follicles on day 5 increased in intensity on subsequent days. Patchy thickening of the capsule due to serosal hyperplasia was seen on day 8 p. i. in fatal cases.

Renal parenchyma revealed mild haemorrhages in some areas on day 8 p. i. Tubular epithelium showed degeneration, necrosis and desquamation. However, on day 9 and onward mild hyperplastic changes were recorded. Glomeruli were not affected but by day 15 they showed degenerative and atrophic changes.

Epithelial cells of intestinal vili showed degenerative changes and necrosis followed by occasional desquamation. Rabbits showing diarrhoea developed diphtheric membranes from fibrin, mononuclear cells and a few polymorphs attached to the luminal surface of vilous epithelium since day 7 p. i. In submucosa mild infiltration with mononuclear cells was observed. Changes in large intestine were similar to those in small intestine, but less severe.

The vilous epithelium of stomach revealed changes similar to those of the intestine. In the submucosa focal mononuclear infiltration was recorded on day 8 p. i.

The endometrial epithelial cells of the uterus showed degeneration, necrosis and desquamation. In some places hyperplasia of uterine glandular epithelium was recorded on days 9 and 11 p. i. Simultaneously, haemorrhages associated with infiltration of mononuclear cells and macrophages were also recorded. The changes in the testes were not severe; spermatogenesis was arrested only in some of the seminiferous tubules on days 8, 9 and 11 p. i.

*Electron microscopy*

Electron microscopy revealed complete and incomplete forms of virus particles in the cytoplasm of skin epidermal cells at 24 hr p. i. The proportion of mature particles relatively increased on days 3—5 p. i. (Fig. 6). Lung, liver and spleen also revealed the presence of mature and immature virus particles by day 5 p. i. Complete virus particles were seen as osmophilic complex structures. They were rectangular in shape with rounded corners situated free in the cytoplasm. No virus particles were found in the nucleus. Membrane bound virus particles were present in the majority of infected cells since day 3.

## *Discussion*

The appearance of primary lesions at the site of skin inoculation and the course of disease were very similar to that described by Srinivasappa and Garg (1977). However following primary viraemia on day 2 p. i., the first

macroscopic lesions characterized by focal or diffuse white necrotic area in lungs, liver and spleen were observed on days 5—8 p. i. The gross lesions in the spleen were not always found and in some rabbits only slight splenomegaly could be observed. The early development of gross lesions characterized by white necrotic areas in the liver and spleen have also been reported in ectromelia of mice (Fenner, 1949) and in lungs and liver in buffalo pox infection of infant mice (Dogra *et al.*, 1978). However, Srinivasappa (1973) reported small necrotic spots on the liver only. In the absence of inclusion bodies, the specificity of lesions was confirmed by electron microscopic examination of skin, lung, liver and spleen. The microscopic changes were very similar to ectromelia in mice (Fenner, 1949, 1981; Allen *et al.*, 1981) except of severe haemorrhages in the lungs appearing due to the damage of capillary endothelium. Multinuclear cells in the liver during the recovery process were also described by Fenner (1949). In contrast, Allen *et al.* (1981) described that hepatic lesions in mouse pox were short lived and underwent rapid repair.

At following secondary viraemia from day 5 p. i., the skin showed generalized eruptions between days 7—10 in 20 per cent of rabbits. There was no such report on experimental infection of rabbits, but in natural outbreaks of buffalo pox generalized skin lesions have been reported in buffaloes by many workers (Ramkrishnan and Ananthapadmanabham, 1957; Maqsood, 1958). In experimental infections with other pox viruses such as ectromelia (Fenner, 1948*a*) and rabbit pox (Westwood *et al.*, 1966), generalized skin lesions were observed during secondary viraemia. Because pox viruses are reported to be associated with leukocytes (Gresser and Lang, 1966), the passage of infected leukocytes across capillary endothelium by diapedesis may be a way by which pox viruses initiate the secondary skin lesions.

The lesions characterized by focal pustules and ulcers or focal and diffuse necrotic spots were also seen in kidney, stomach, intestine and gonads from day 7 p. i., as has also been described in mouse pox by Fenner (1948*a*) and Allen *et al.* (1981). This is indicative of the fact that these organs were infected during secondary viraemia (Fenner, 1948*b*). Similar observations have also been reported in rabbit pox (Westwood *et al.*, 1966). The diphtheric membrane observed in the intestine of some rabbits did not seem to be in correlation with the virus but with secondary bacterial infection.

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*Explanation to Figures* (Plates XLIII–XLV):

*Fig. 1.* Primary lesions of BPV at the site of inoculation (6 days p. i.).
*Fig. 2.* White necrotic areas on the liver and lungs of rabbit infected with BPV (7 days p. i.).
*Fig. 3.* Spleen of rabbit infected with BPV showing white raised areas on its surface (8 days p. i.).
*Fig. 4.* Skin of rabbit infected with BPV showing degenerative and infiltratory changes in the epidermis (8 days p. i.). HE, ×400.
*Fig. 5.* Liver of rabbit infected with BPV showing severe fatty degeneration (8 days p. i.). HE, ×100.
*Fig. 6.* Electron micrograph of skin of rabbit infected with BPV showing numerous mature BPV particles in the cytoplasm of epidermal cells (5 days p. i.). ×12,000.