Cytopathic Changes in Lesions of a Pox Disease in Monkeys

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Several recent outbreaks of an apparently new pox disease of monkeys have been reported in the United States. The disease occurred in rhesus *(Macaca mulata)* monkeys in Oregon,[10] Texas,[3] and California,[7] all of which were Asian imports obtained from the same distributor. In Oregon, the disease spread to the following species: *Macaca fuscata, Macaca nemestrina,* and *Cynopithecus niger.* Five animal caretakers were infected through proliferative epidermal lesions.[14] This disease, as it occurs in rhesus monkeys, allegedly differs from monkeypox as follows: (1) clinically, it infects only primates and usually produces discrete proliferative epidermal lesions, (2) histopathologically, the lesions have very large cytoplasmic and nuclear inclusions, and (3) serologically, it is closely related to Yaba virus but not to vaccinia. Vaccination (with vaccinia) does not protect against this disease and the virus fails to grow on the chicken chorioallantoic membrane.

The disease known as monkeypox was superficial skin wounds and they developed first reported by von Magnus *et al.* in 1959

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TABLE 1. SUMMARY OF RESULTS FROM PREVIOUSLY REPORTED SPONTANEOUS OUTBREAKS OF POX DISEASE IN PRIMATES

Author, year	Monkey species developing lesions	Number affected and number exposed	Protection or cross reaction with vaccinia
von Magnus *et al.*, 1959	*Macaca* (cynomolgus)		+
Sauer *et al.*, 1960	*Macaca philippinesis*†		+
	Macaca mulata (rhesus)		
McConnell *et al.*, 1962	*Macaca mulata*	3/27	+
Peters, 1966	*Myrmecophaga tridactyla* (anteater)	2/2	+
	Simia satyrus (orangutan)*	10/10	
	Pan troglodytes (chimpanzee)		
	Gorilla gorilla (gorilla)		
Hall & McNulty, 1967	*Macaca mulata*	121/445	–
	Macaca fuscata	57/57	
	Macaca nemestrina	10/40	
	Cynopithecus niger	3/24	
	Homo sapien	5/?	
Casey *et al.*, 1967	*Macaca mulata*	15/50	?

†Death loss occurred.

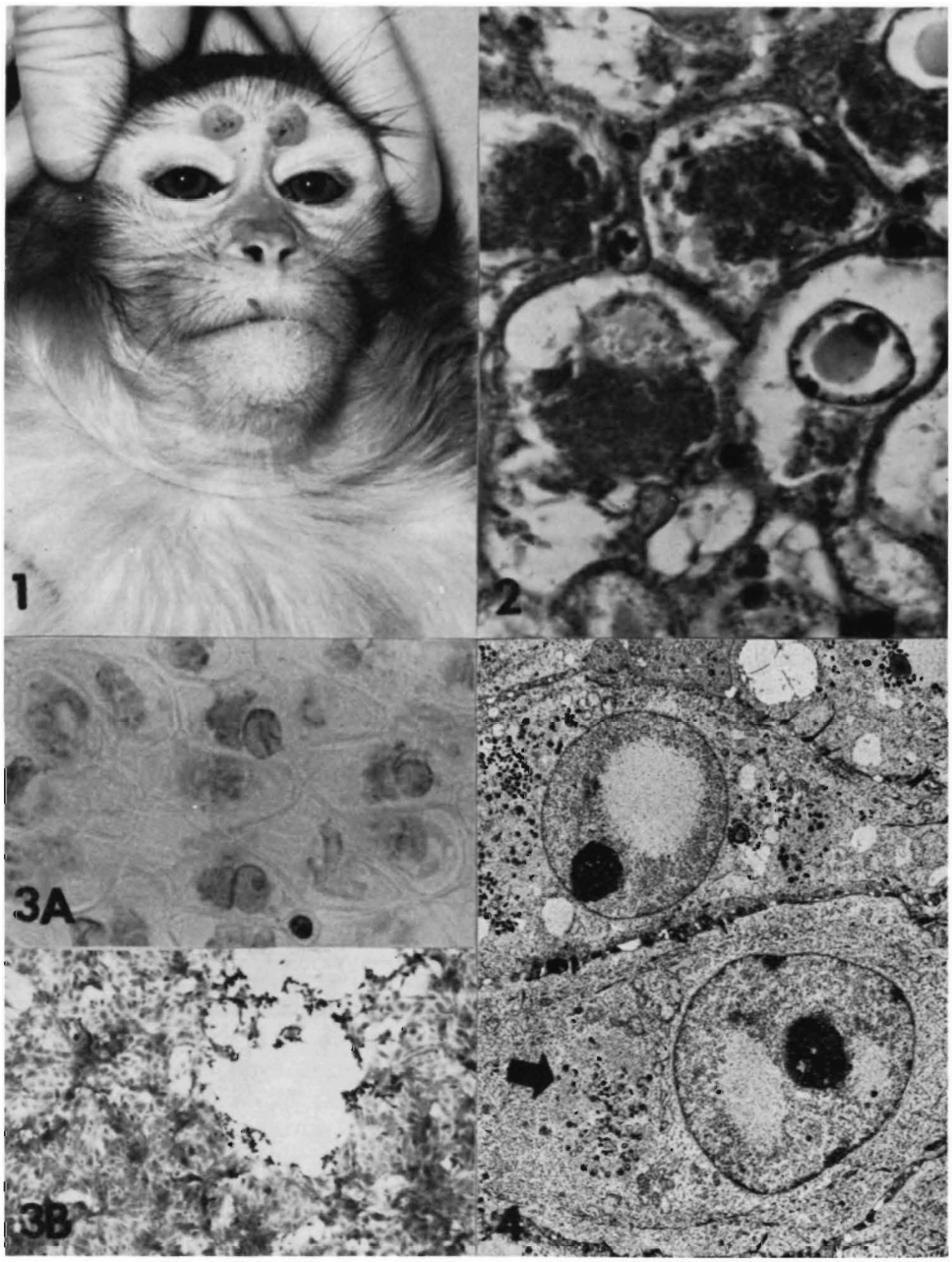

Figure 1. Experimentally produced monkey pox lesions on the forehead of a rhesus monkey.
Figure 2. Cytoplasmic and nuclear inclusions of monkey pox. The nucleolus is enlarged and located peripherally in the nucleus. Hematoxylin-eosin, Bouins fixation.
Figure 3. A—Feulgen reaction-cytoplasmic inclusion faintly positive. B—Cytopathic effect in PK-15 cell culture line.
Figure 4. Electron micrograph of two infected Keratinocytes from the skin. Large cytoplasmic "viral factories" are present (arrow), and are the ultra structural appearance of cytoplasmic inclusions. Nucleoli are dense and enlarged. The ultrastructure of the nuclear inclusion should be compared with that in Figure 2. Keratin is limited to the periphery of the cell.

in cynomolgus monkeys with maculopapular rash and pustule formation.[12] Systemic disease was not apparent and no fatalities occurred. The viruses isolated produced: (1) encephalitis in mice upon intracerebral injection, (2) pocks on the chick embryo chorioallantoic membrane and rabbit cornea, (3) severe hemorrhagic lesions in adult rabbit skin, (4) fatal infection with visceral lesions in neonatal rabbits, and (5) cytoplasmic inclusion on primary monkey kidney cell cultures. The virus was related serologically to vaccinia. Subsequent reports have shown monkeypox viruses to be infective for many primates[3, 13, 14, 16, 17] (Table 1). Subclinical infection is common, as the number of monkeys having a positive hemagglutination inhibition (HI) titer far exceeds the number with lesions. In most outbreaks, stress such as tatooing, irradiation, or shipping was associated with the initial cases.

This report presents the morphologic changes occurring in rhesus monkeys inoculated with virus obtained from monkeys in the Oregon Regional Primate Laboratory outbreak. Since the cellular changes closely resembled those of swinepox,[4] pigs were also inoculated with virus. A 10% tissue suspension of lesion material was centrifuged at 2,500 x g for 30 minutes for bacterial clearance and the supernatant fluid was injected intradermally and applied to the scarified skin of two rhesus monkeys and four 3-month-old pigs. The pigs did not develop local or systemic reactions following inoculation. The monkeyes, however, developed large proliferative lesions at the point of inoculation (Fig. 1). These lesions were biopsied at postinoculation days 7, 10, and 14 and were processed for light and electron microscopy.

Skin sections contained swollen epidermal cells with large, pleomorphic granular cytoplasmic inclusions resembling those of swinepox. Nuclei were swollen and had enlarged eccentric nucleoli. Pale, eosinophilic inclusions occurred in nuclei and were especially prominent with alcoholic or Bouin's fixatives (Fig. 2). Skin lesions were chiefly proliferative—differing from most pox infections in that vesiculation was not present. Histochemical procedures were also done on infected cell cultures. The results were similar to those of the skin sections except for oil red 0 which revealed a greater amount of neutral lipid in infected cell cultures.

Electron microscopic examination of skin lesions (Figs. 4 and 5) revealed the following cytologic changes in the cytoplasm: foci of viroplasm and pox viruses, dense accumulations of keratin precursors against the cell wall, lipid droplets, enlarged vesicular mitochondria and protein fibrils. In cells with large inclusions, myelin figures and lipoprotein lattices (Fig. 6) were usually an integral part of the inclusion body. In this respect, this disease closely parallels swinepox.

Nuclear changes included peripheralization of chromatin leaving a round area which was void of all structure except for uniform, small particles. The association of this area with the nuclear inclusion is unknown.

In the dermis, endothelial cell necrosis, thrombosis, and perivascular lymphocyte cell infiltration were present. Necrotic endothelial cells also contained virus particles (Fig. 7). This observation provides a logical basis for the presence of viremia in monkeypox.

The morphologic changes in this pox disease of monkeys (Oregon isolate) do not resemble the changes usually present in the vaccinia subgroup of pox viruses[5] (vac-

TABLE 2. HISTOCHEMICAL EXAMINATION OF NUCLEAR AND CYTOPLASMIC INCLUSION BODIES

Stain	Nuclear inclusions	Cytoplasmic inclusions
Feulgen	–	Pale pink*
Acridine Orange	–	Green
Oil Red O	–	–
Periodic Acid-Schiff	–	–

* Positive only after prolonged hydrolysis (50 minutes).

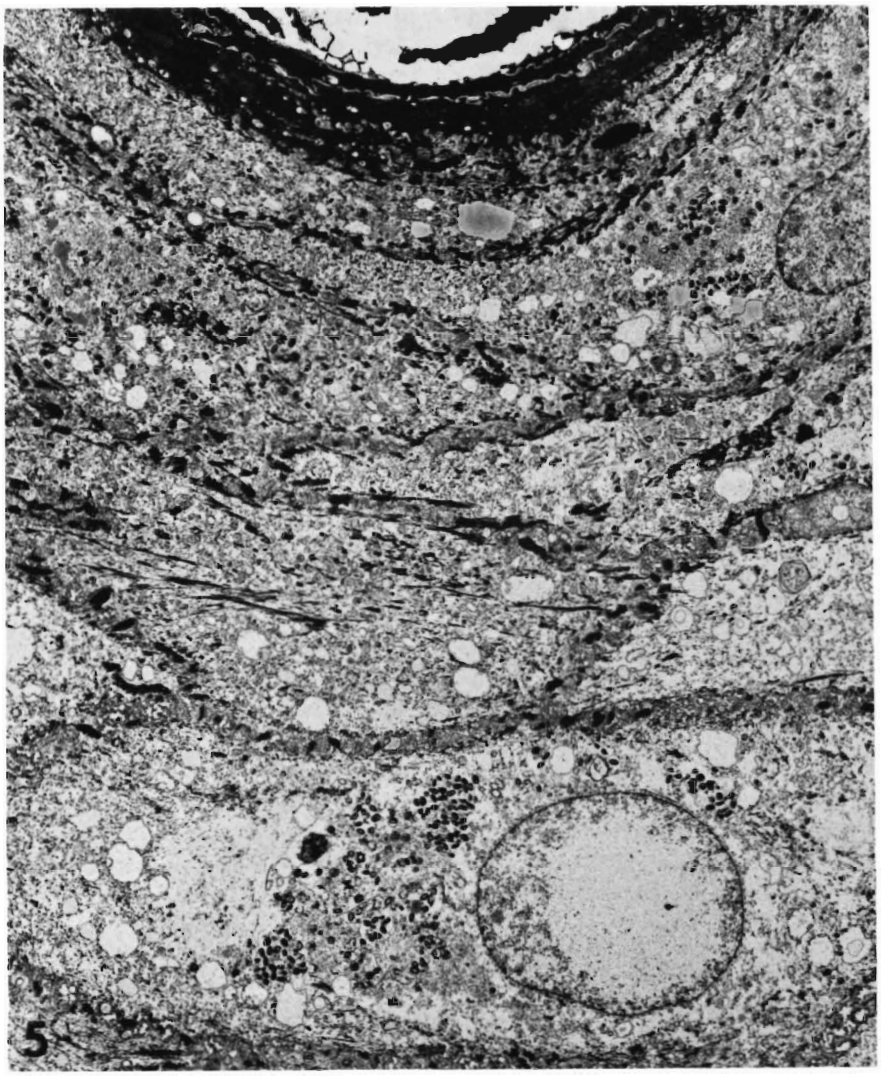

Figure 5. Cross section of epidermis showing degenerative infected keratinocytes. The stratum corneum is at top.

cinia, cowpox, variola, rabbit pox, horsepox, ectromelia and pseudoswinepox), of which monkeypox is reported to be a member. Furthermore, vaccination did not alter the transmission of the disease as it has been reported to do in monkeypox outbreaks. Since the ultrastructural and histologic findings indicate a striking morphologic relationship with the epidermal changes in swinepox, further studies on this pox disease are indicated. Although Yaba virus is closely related serologically, Yaba lesions[1, 9, 11] are histiocytic proliferations limited to the dermis. Molluscum contagiosum has been reported in chimpanzees but the lesion is characteristic and should not be confused with other simian pox disease. Two early reports of variola-like skin diseases[2, 6] of monkeys cannot be confirmed as monkeypox.

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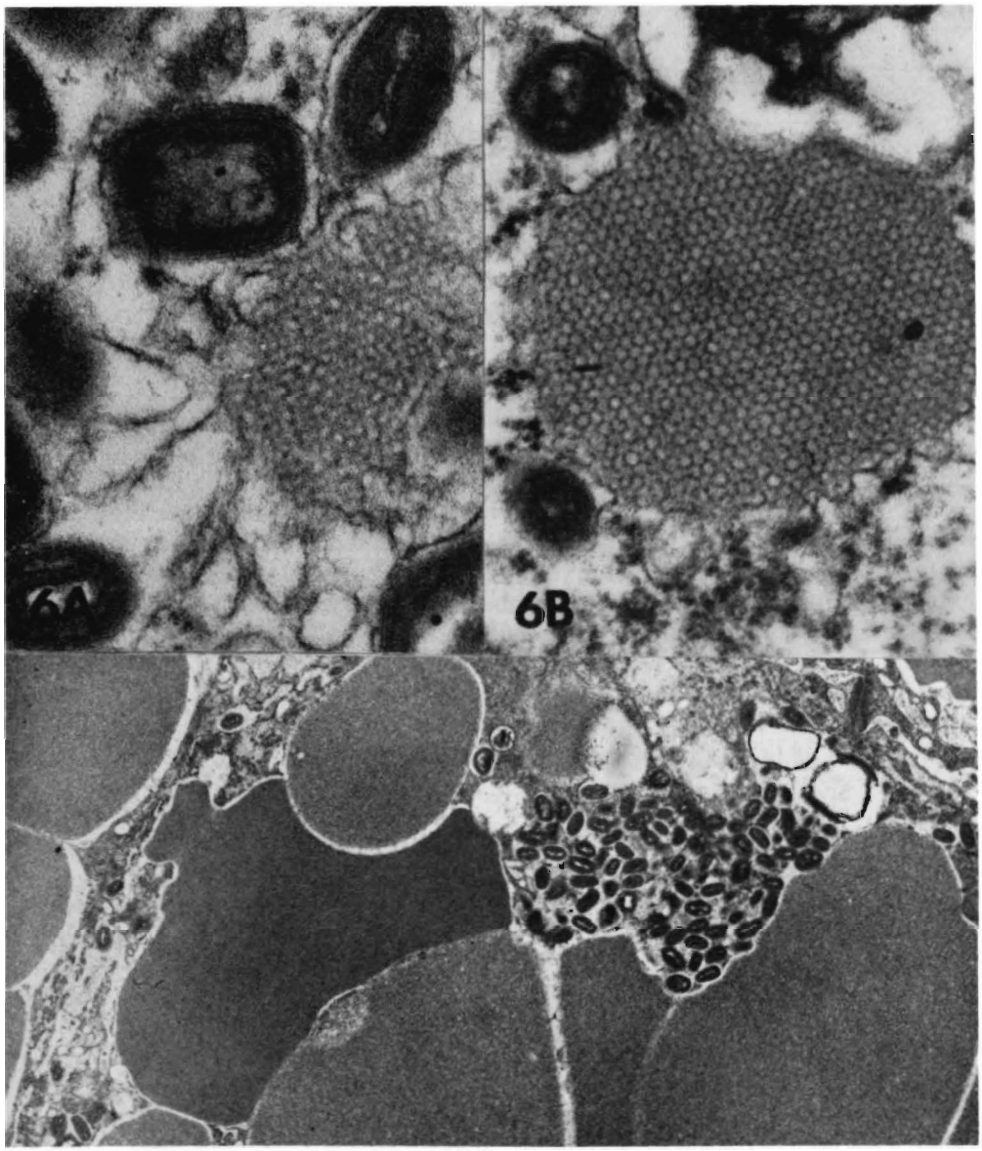

Figure 6. Lipoprotein lattice structures characteristic of the center of monkey pox cytoplasmic inclusions. Their relationship with the virion remains to be determined.
Figure 7. Necrotic dermal endothelial cell with large numbers of pox virions. Note red blood cells at lower part of picture.

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