NOTE

The Fine Structure of Cytoplasmic and Intranuclear Inclusions of Seal Pox

Kōsuke OKADA*, and Yutaka FUJIMOTO

Department of Comparative Pathology, Faculty of Veterinary Medicine, Hokkaido University, Kita-ku, Sapporo 060, Japan

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In 1975, we encountered a case of seal pox at an aquarium in Hokkaido. Electron microscopy was performed on cytoplasmic and intranuclear inclusion bodies in the prickle cells. The basophilic cytoplasmic inclusions consisted of electron opaque matrix with immature and mature virions, while eosinophilic ones contained no virions. The mature virions were of typical parapox viruses 300 by 140 nm in size. The intranuclear inclusion consisted of a mass of filamentous structures and may be a secondary product due to viral replication.—*Key words:* Parapox virus, Sea lion.

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Seal pox is an epizootic proliferative dermatitis caused by a parapoxvirus [11]. There have been only a few reports of seal pox in California sea lions (*Zalophus californianus*), harbor seals (*Phoca vitulina*) and South American sea lions (*Otaria byronia*) in North and South America [9–13], but no reports in other countries. We encountered seal pox at an aquarium in Hokkaido. The purpose of the present report is to describe the fine structure of cytoplasmic and intranuclear inclusions of seal pox.

In September, 1975, three South American sea lions (two female and one male), which were obtained from Peru, each weighing about 30 kg and approximately one year of age, were found to have many cutaneous nodules over the entire body surface. The nodules were firm, approximately 0.5 to 1.7 cm in diameter and sometimes were found to be hairless, and having erosions and hemorrhages.

Histopathological observations were performed on 3 skin samples obtained by biopsy

from one sea lion. Skin biopsies were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (HE).

Nodular lesions were diagnosed as subacute papular necrotic dermatitis. The epidermis was involved with acanthosis and ulcer. There were hemorrhages, necrosis and bacterial colonies on the surface of the ulcerous lesions. Neutrophilic accumulation was observed from the dermal surface to the deeper parts of the dermis along the hair follicles. There were prominent proliferation of histiocytic cells, infiltration of lymphocytes and neutrophils, and vascularization in the corium. Hyperemia and hemorrhages were evident.

Hydropic degeneration was observed in the prickle cells of the epidermis and epithelial cells of the hair follicles. These cells frequently contained large basophilic or neutrophilic cytoplasmic inclusions of various shapes (Fig. 1). The inclusions showed sometimes fine granules of network appearance. The cytoplasm occasionally contained small spherical or fine granular eosinophilic inclusions. Two types of cytoplasmic inclu-

*Present address: *Department of Veterinary Pathology, Faculty of Agriculture, Iwate University, Morioka 020, Japan.*

Fig. 1. Epithelial cells of the hair follicle. There are basophilic (B) and eosinophilic granular (E) cytoplasmic inclusions as well as intranuclear inclusions (N). HE stain. $\times 700$.

sions were tentatively called "basophilic" and "eosinophilic", respectively. The nucleus was swollen and the nuclear wall tended to be hyperchromatic. The nucleus occasionally contained a fine granular or homogeneous basophilic inclusion (Fig. 1).

The basophilic cytoplasmic, eosinophilic cytoplasmic, and intranuclear inclusions were reddish purple, pinkish and reddish purple with Giemsa stain, respectively. The basophilic cytoplasmic inclusions were faintly positive for Feulgen reaction, negative for periodic acid Schiff (PAS) reaction and yellowish fluorescent with acridine-orange (AO) stain. The eosinophilic cytoplasmic and basophilic intranuclear inclusions were negative for both Feulgen and PAS reactions and greenish fluorescent with AO stain.

Formalin fixed specimens corresponding to

Fig. 2. A cytoplasmic inclusion consists of electron opaque matrix, immature and mature particles, and may correspond to the basophilic inclusion. Numerous mature virus particles are seen around it. $\times 7,800$.

the lesions containing light microscopic cytoplasmic inclusions were refixed in 1% osmium tetroxide and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined under an electron microscope type HU12 A.

Numerous mature virus particles were observed in the cytoplasm of the prickle cells (Fig. 2). Cytoplasmic organelles of some of the prickle cells decreased or disappeared. Basophilic cytoplasmic inclusions consisted of electron opaque matrix with immature and mature particles (Fig. 2). Furthermore, there were clumps of electron opaque granular matrix in the cytoplasm (Fig. 3), which did not contain virus particles and was considered to represent the eosinophilic inclusions in the sections stained with H-E. On the other hand, intranuclear inclusion consisted of a

Fig. 3. There are filamentous structures (F) in the nucleus, and a granular matrix (M) in the cytoplasm. They may correspond to the intranuclear and eosinophilic cytoplasmic inclusions, respectively. Many virus particles are also seen in upper right corner. $\times 18,500$.

mass of filamentous structures (Fig. 3). The outline of the nucleus was irregular in shape and the chromatin was margined. Mature virus particles were cylindrical or ovoid in shape with a central core, and measured 300 by 140 nm (Fig. 4). Immature virus particles were spherical in shape and 250 to 300 nm in diameter.

The basophilic and eosinophilic cytoplasmic inclusions recognized in the present study may correspond to the B- and A-type inclusions described by Kato *et al.* [4], respectively. The virions observed in the present study were typical to the parapoxvirus described by Peters *et al.* [7]. Immature virus particles were mainly located in the light microscopic basophilic cytoplasmic inclusions, which corresponded to viroplasm reported in bovine papular stomatitis (BPS)

Fig. 4. Mature and immature (arrows) virus particles in the cytoplasm. $\times 65,000$.

[6] and human orf [14]. Previous authors [11–13] have not commented on clumps of electron opaque granular matrix without virus particles which may correspond to A-type inclusion. Intranuclear filamentous structures which have not been described in the previous reports on seal pox [11–13] were occasionally detected in the present case. Similar structures have been observed in BPS [3] and milker's nodules [2], and may represent to nuclear change characteristic of infection with various pox group viruses *in vivo* and *in vitro* [1, 5, 6, 8]. These intranuclear structures may be a secondary product due to viral replication [8].

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要 約

Seal pox の細胞質ならびに核内封入体の超微形態(短報): 岡田幸助・藤本 胖(北海道大学獣医学部比較病理学講座)——1975年に北海道の水族館のオタリア (*Otaria byronia*, *Otariidea*) に発生した皮膚 seal pox の3種類の封入体を電顕的に検索した。好塩基性細胞質封入体は多数の未熟ならびに成熟ウイルス粒子を含む電子密度の高い物質からなり、成熟粒子は $140 \times 300 \text{ nm}$ でパラボックスウイルスの特徴をそなえていた。好酸性細胞質封入体は電子密度の高い顆粒状物の塊りでウイルス粒子を含まなかった。核内封入体は細線維の束よりなり、ウイルス複製の二次的産物とみなされた。