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Authors: WILSON, T. M., and SWEENEY, P. R.

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# Morphological Studies of Seal Poxvirus

T. M. WILSON\* and P. R. SWEENEY

*Department of Pathology, Ontario Veterinary College,  
University of Guelph, Guelph, Ontario, Canada*

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## Abstract

The fine structure of the M and C forms of seal poxvirus as observed in negative stained preparations in the electron microscope are described. The M form has a beaded surface and a serrated outline and particles averaged 312.3 m $\mu$  in length and 193.8 $\mu$  in width. The C form was not beaded, had a serrated periphery and averaged 352.8 m $\mu$  in length and 196 m $\mu$  in width. A short discussion of the morphology of seal poxvirus in relation to other poxviruses is presented.

Seal pox was reported in captive California sea lions (*Zalophus californianus*) in 1969 and described as a diffuse proliferative skin disease in which the characteristic dumbbell shaped pox virions were observed in cells throughout the lesion during electronmicroscopic

examination.<sup>10</sup> An international survey of aquariums and veterinarians working primarily with marine mammals has revealed that seal pox is rare in captive pinnipeds and that pox does occur in wild California sea lions.<sup>9</sup>

## Materials and Methods

A 10% suspension was prepared by grinding frozen skin nodules with saline in a Tenbroeck grinder. This suspension was centrifuged for clarification (20 minutes at 800 x g), for bacterial clearance (20 minutes at 2,000 x g), and for

virus sedimentation (60 minutes at 60,000 x g). The resulting pellet was resuspended with five drops of distilled water and was then negatively stained with 2% phosphotungstic acid at pH 4.3 and viewed in a Philips 200 electron microscope.

## Results

Negative stained preparations revealed two recognizable mature poxvirus particles, the beaded mulberry form, M, and the capsular form, C, the former being more abundant in our preparations.

All M forms observed were morphologically similar and were oval or cylindrical with convex ends. The surface of

the M form appeared beaded and the periphery scalloped (Fig. 1). Measurement of eleven apparently undistorted M particles indicated a mean length of 312.3 m $\mu$  (range 284.6 - 349.8 m $\mu$ ) and a mean width of 193.8 m $\mu$  (range 182.4-217.8 m $\mu$ ). An axial ratio of 1.61 was determined for the M particle.

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FIGURE 1. *M* form showing surface filaments and marginal scalloping. Phosphotungstic acid stain, pH 4.3 X 185,000



FIGURE 2. *M* form illustrating orderly pattern of surface filaments. Phosphotungstic acid stain, pH 7.0 X 240,000.



FIGURE 3. *C* form showing capsule with serrated margins enclosing inner body. Phosphotungstic acid stain, pH 4.3. X 121,400.

The surface structure of the M form was apparently due to the appearance of filamentous structures irregularly crisscrossing the particle. Occasionally these filaments were seen to be parallel to each other and in an orderly pattern (Fig. 2). Examination of these surface filaments, which was possible for only short distances, indicated their structure to be composed of two peripheral lines and a faint central line which could be interpreted to represent a tubular structure. Measurement of twenty-five filaments indicated an average width of 10.9  $m\mu$ .

C particles appeared slightly larger, did not have a beaded surface, were less electron dense and more rectangular than the M form (Fig. 3). Measurement of several undistorted particles indicated a mean length of 352.8  $m\mu$  and a mean width of 196  $m\mu$ . The outer capsule was approximately 22.5  $m\mu$  wide and its periphery was scalloped. The capsule could be divided into inner and outer portions which surrounded an inner body of granular appearance. Radial partitions of the inner portion of the capsule as described for vaccinia virus<sup>6</sup> and other poxviruses were not observed.<sup>7</sup>

### Discussion

The morphological characteristics of this seal poxvirus as revealed in our studies indicated length and width values comparable to figures reported for other poxviruses.<sup>1</sup> One particular feature of this seal poxvirus was the narrow width in relationship to length.

The presence of M and C forms in our preparations correspond to reports of similar particles in other poxvirus morphological studies.<sup>4</sup> The relationship of these two forms is not precisely known, however, Westwood et al.,<sup>6</sup> in a morphological study of vaccinia virus, demonstrated M to C conversion using biochemical procedures.

Surface beading of the M form is a constant structural pattern evident in a variety of negative stained poxviruses<sup>2,3,4,5,7,8</sup>. No information is available as to

the exact morphological and biochemical character of these surface structures.

The first report of seal pox<sup>10</sup> and the results of this investigation indicate the presence of a poxvirus in proliferative epithelial lesions of California sea lions. A precise classification of this seal poxvirus into one of the poxvirus subgroups on morphological evidence gained through negative staining is presently not possible. However the oval-cylindrical shape, axial ratio of 1.61 and the occasional orderly pattern of the surface filaments resembles Orf (Contagious Pustular Dermatitis) and Bovine Papular Stomatitis poxviruses<sup>2,3,6</sup> which are grouped in the paravaccinia subgroup. Other techniques, in addition to negative staining, must be employed for subgroup classification.

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