electrophysiological recording[11,12]. In the neuroendocrine hypothalamus, for example, cultured neurones from the area of the supraoptic nucleus have been injected in order to study their dendritic and axonal processes[13]. Andrew et al.[14] showed that dye-transfer amongst magnocellular hypothalamic neurones occurred frequently in a slice preparation, an observation which we failed to confirm. Other studies, aimed at describing the morphology and the electrophysiological characteristics of neurones located in the arcuate-ventromedial region of hypothalamic slices, have used a different fluorescent dye, i.e. Procion Yellow[14,15].

Many workers have recently used the hypothalamic slice preparation to study the bioelectrical and pharmacological properties of endocrine neurones located in the supraoptic[17–19] and paraventricular nuclei[4,14,20–22], including studies by intracellular recording[14,19,21]. We therefore investigated whether it might be possible to apply the methods pioneered by Reaves and Hayward[2,3] to the rat hypothalamus in vitro and thereby to characterize its magnocellular neurones by the use of a combination of electrophysiology, dye injection and immunocytochemistry. The results thus far obtained are encouraging, since they showed the persistence of peptide hormone immunoreactivity in hypothalamic slices kept in vitro for several hours. The existence of a differential sensitivity of presumptive oxytocinergic neurones ('milk-ejection cells')[23] and vasopressinergic neurones (i.e. cells not accelerated at milk ejection) to transmitter substances has been suggested[24–26]. The method described here should allow a direct test of this conjecture.

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Cytomegalovirus-like particles in the red-backed vole submandibular gland

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Summary. Cytomegalovirus-like particles were observed in the acinar cells and in the intralobular ducts of the submandibular gland of the red-backed vole. The particles were target-shaped, had a glycoprotein-positive, electron-dense central core, and the core was surrounded by a protein-rich layer and glycoprotein-positive outer layer.

During a study of ultracytochemistry[1], the author observed a virus infection in the submandibular glands of the red-backed vole (*Clethrionomys rufocanus bedfordiae,* Thomas). The ultrastructural morphology of cells infected with cytomegalovirus, a Herpes group virus, has been previously reported[2–6]; the target-shaped virus particles observed in the present study were very similar to those of the cytomegalovirus in their morphological features. This report describes the ultracytochemistry of the mature form of the cytomegalovirus-like particles.

Materials and methods. 25 red-backed voles (9 adult male and 16 female voles) were used in this study. Trapped in Sapporo, Hokkaido, all animals appeared healthy and free of desease. They were killed shortly after capture. The submandibular glands were fixed in buffered 10% formalin for 6 h and processed for paraffin sectioning. Sections (5–6 μm) were cut serially and stained with hematoxylin-eosin, Millon reaction for tyrosine, the p-DMAB method for tryptophan, 0.05% toluidine blue (McIlvaine, pH 2.5, 4.1 and 7.0) for acid and neutral mucopolysaccharides, or the PAS reaction for neutral mucopolysaccharides. For the ultrastructural investigations, small tissue blocks were dissected from the submandibular glands of 18 voles, and fixed after continuous shaking in a cold 5% formalin fixative (pH 7.4, phosphate buffer) for 3 h or in a cold 50% Karnovsky fixative (the same buffer) for 3 h. The blocks were rinsed and shaken in the same buffer for 6–12 h, after which half of the blocks were dehydrated with ethanol and embedded in Epon. The rest of the blocks were post-fixed in a cold 1% OsO_4 solution (pH 7.4, phosphate buffer), dehydrated, and embedded in Epon. Ultrathin sections were cut by a LKB 8800 ultramicrotome and observed under a Hitachi HU-11DS electron microscope. To determine the presence of glycoprotein, the PA-TSC-SP staining method[7] was used for the ultrathin sections. The presence of protein was determined by pronase digestion[8] (Kaken

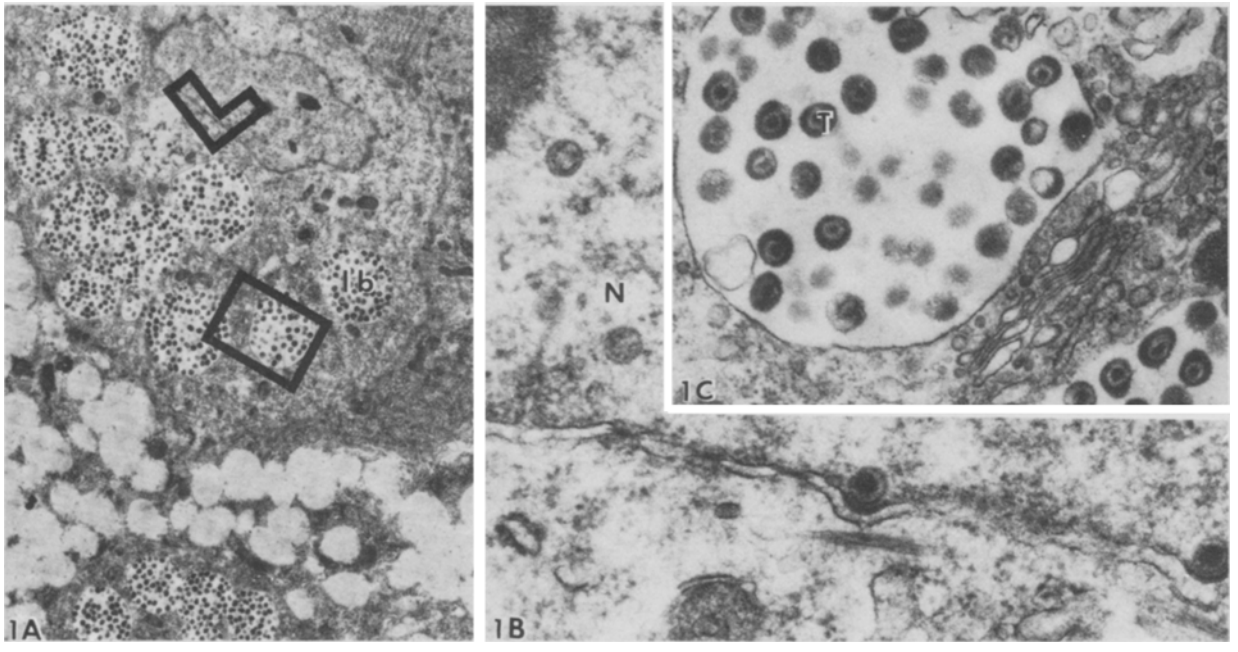

Figure 1. *A* Many spherical inclusion bodies (Ib) are observed in 2 acinar cells. ×4400. *B* Enlarged figure of figure 1A. Various stages of viruses were observed in the nucleus (N). ×53,200. *C* Enlarged figure of figure 1A. Many target-shaped viruses (T) were observed in the 2 inclusion bodies. ×28,000. 5% buffered formalin and 1% OsO_4 solution fixation.

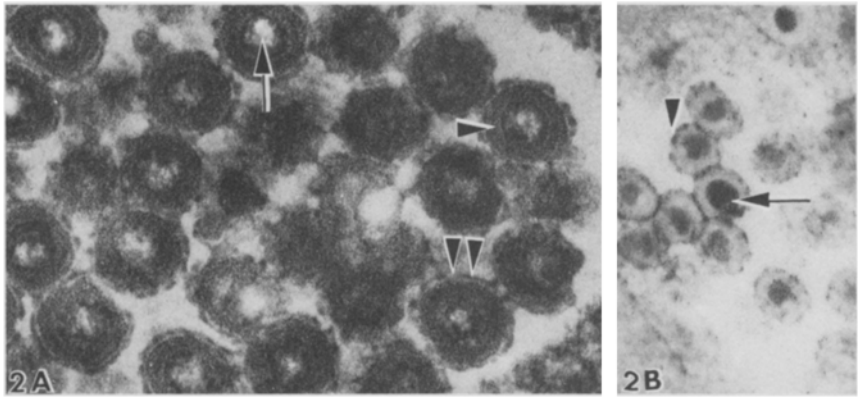

Figure 2. *A* High power view of viruses. The electron-lucent core (arrow) was surrounded by an inner concentric electron-dense layer (arrowhead) and an outer electron-dense layer (double arrowheads). ×82,000. *B* PA-TSC-SP technique revealed a glycoprotein-positive core (arrow) and concentric outer layer (arrowhead) in an inclusion body. ×43,400. 5% buffered formalin fixation.

Chemicals, Tokyo, Type E, 7000 p.u.k./g), which was performed for 4 h after periodic acid oxidization, and then treatment with thiosemicarbazide and silver proteinate was done.

Results and discussion. Histological findings for the submandibular glands of the 25 voles studied revealed the virus infection in only 2 male voles, and their infected cells were recognized as enlarged cells (giant cells) with a diameter of 10 to 12 µm. Situated within the acinar cells and the intralobular ducts, the cells contained inclusion bodies, which showed eosinophilia, toluidine blue-metachromasia (pH 2.5, 4.1 and 7.0) and were Millon reaction negative, tryptophan negative and PAS positive. Electron microscopic findings of the cells revealed the presence of small groups of target-shaped cytoplasmic particles and large vacuoles containing 60–130 viruses with diameters of 150–160 nm in cross section (fig. 1, A and C). A high power view of these mature form viruses demonstrated an electron-lucent (fig. 2A, arrow), glycoprotein-positive core (fig. 2B, arrow) and a concentric, electron-dense layer (fig. 2A, arrowhead) and another glycoprotein-positive layer (fig. 2B, arrowhead). The inner electron-dense layers decreased in density by pronase digestion. The viruses located within the degenerating nuclei were smaller than those of the cytoplasmic particles, ranging from 70 to 82 nm in diameter. Hypertrophy of the inner nuclear membrane and duplication of the outer nuclear membrane were observed (fig. 1B). Filamentous structures from the virus scattered near the inner membrane of the nucleus were divergent to the tubular structure of the cytoplasm. The mature form of those viruses was also observed within the intercellular canaliculi of acinar cells and within the cytoplasms and nuclei of the intralobular ducts. The pathogenicity of these virus particles and their role within the salivary glands is unknown.

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