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**UNIQUE CYTOPLASMIC MEMBRANES IN ROUS SARCOMA  
VIRUS-INDUCED TUMORS OF A SUBHUMAN PRIMATE**

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Although the natural host of the Rous sarcoma virus (RSV) is the chicken, many strains of the virus have induced tumors in various mammals. Recently, a New World monkey, the marmoset (*Saguinus sp.*), was added to the list of animals susceptible to RSV oncogenesis (1). This report deals with a unique membranous cytoplasmic structure found in cells from two intracerebral and four subcutaneous tumors which developed following neonatal inoculation with the Schmidt-Ruppini strain of RSV.

**MATERIALS AND METHODS**

Biopsies from subcutaneous sarcomas of four marmosets were obtained on the 22nd–100th days following neonatal injection of RSV at the site of subsequent tumor development. Samples of cerebral tumor were taken from two other animals on the 24th and 29th days following intracerebral inoculation. Tissues were fixed in cacodylate-buffered 5% glutaraldehyde at pH 7.2 and then in Dalton's chrome-osmium tetroxide (2). Additional blocks of tumor from one intracerebral and one subcutaneous tumor were fixed only in the chrome-osmium tetroxide. All specimens were embedded in Epon 812 following dehydration in graded alcohols. Ultrathin sections cut with diamond or glass knives on a Porter-Blum microtome were stained on the grid with lead citrate and uranyl acetate and examined in an RCA-3H or Hitachi HS-11 electron microscope. At least 300 cells from each tumor were examined; most were examined as single profiles and few as semiserial sections.

**RESULTS**

In 2–7% of the tumor cells from all of the six animals, a complex of membranes and associated

particles was found within the cytoplasm (Fig. 1). Each complex had a remarkably regular, repeating pattern which formed circles (Fig. 2), cisternae (Fig. 3), or loops, or a combination of these configurations (Fig. 4). Circles measured from 70 to 120 m $\mu$  in inside diameter and were 60–110 m $\mu$  apart. The circular portion of loops was of similar dimension. The walls of the cisternae were 80–100 m $\mu$  apart, and the cisternae showed alternating constrictions and expansions (Fig. 3), resulting in a wavy structure with a fixed modulus and frequency.

Graphic reconstruction of the tridimensional appearance of the membrane complex was carried out through analysis of combined and intermediate forms, i.e. circles and loops, and of a few semiserial sections. Although there may be other possibilities, the various patterns can all be ascribed to different planes of sections of a multilamellar structure, with each wavy plate containing evenly spaced evaginations forming spherical, membrane-lined surface projections (Figs. 5 and 6).

The walls of the complex measured 20 m $\mu$  in thickness and were composed of three layers, two electron-opaque layers separated by an electron-lucent zone. High resolution micrographs of cells fixed only in osmium tetroxide further revealed a bilaminar substructure in each dense layer (Fig. 7).

At the periphery of the complex, the membranes were continuous with and appeared to be derived from granular endoplasmic reticulum (Fig. 8). At the point of transition into membranes of the complex, two apposing membranes of the endoplasmic reticulum abruptly converged to

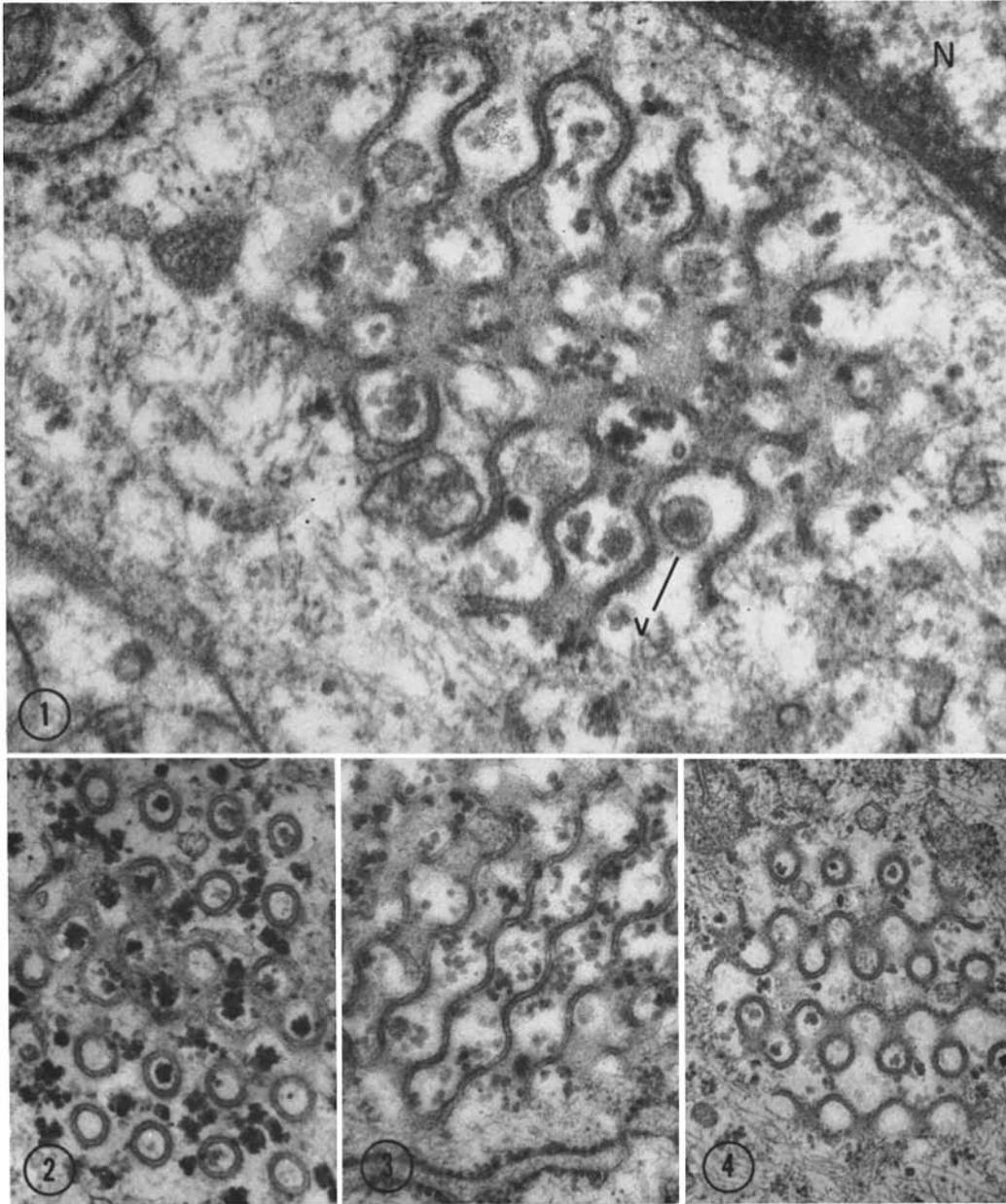
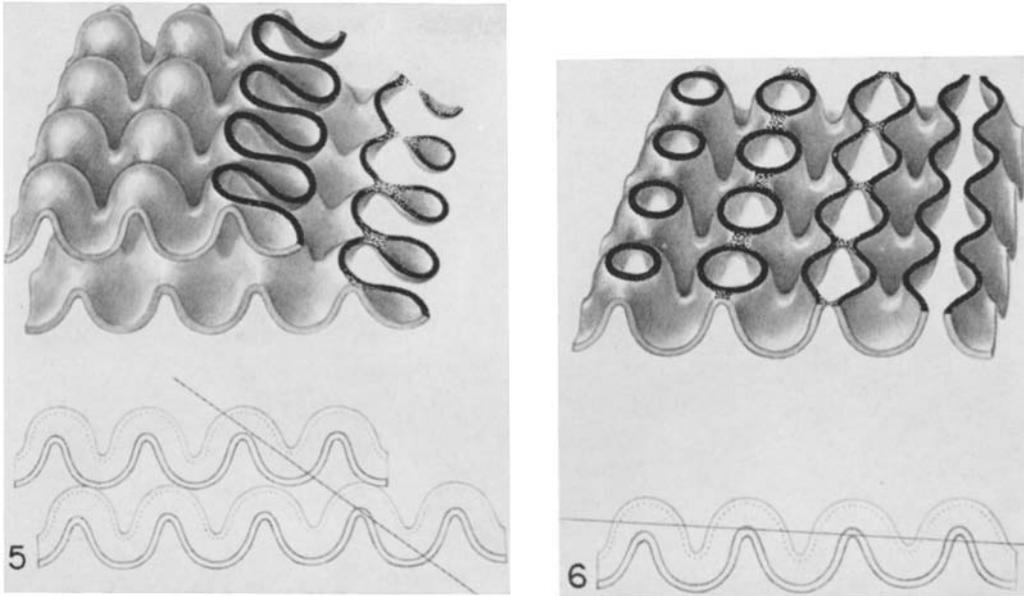


FIGURE 1 Electron micrograph of a Rous sarcoma-induced marmoset tumor cell showing part of the nucleus (*N*) and a cytoplasmic complex of membranes and associated virus-like particles (*v*).  $\times 68,000$ .

FIGURES 2-4 Electron micrographs of membranous cytoplasmic complexes in three different tumor cells. Either circles, cisternae, loops, or a combination of these configurations produce a repeating pattern in all of the complexes. Figs. 2 and 3,  $\times 44,400$ ; Figure 4,  $\times 36,500$ .



FIGURES 5-6 Graphic reconstructions of the tridimensional appearance of the membrane complex. The various patterns can all be ascribed to different planes of section of a multilamellar structure, with each plate having surface evaginations.

form a three-layered, two-membrane plate lining the circles, loops, and cisternae.

Two types of particles were observed within the complex and were associated with the membranes. Particles measuring 20-30  $m\mu$  and aggregating in clumps and rosettes were probably glycogen since they stained with lead but not uranyl acetate alone. However, similarly appearing particles attached to the membrane surfaces did occasionally take the uranium salt and may be of ribosomal nature. The second type of particle found in about 10% of the complexes was spherical, measured 75  $m\mu$  in outside diameter, and contained a central electron-opaque core that measured 40  $m\mu$  (Fig. 1). About one-half of these particles had only a single membrane and no core. Budding of particles within the complexes was not observed, nor were virus-like particles found at other intracellular locations or at the cell surface.

Additional marmoset tissues taken from non-tumor-bearing areas of the same animals were obtained as controls and examined in a similar fashion. No membrane complexes or virus-like particles were found in any of the cells of control tissue.

#### DISCUSSION

The membranous structures observed in this study to our knowledge, have not been previously described. Ultrastructural studies of other RSV-induced mammalian tumors have revealed aggregates of cytoplasmic crystalline material in tumors of the rhesus monkey (3) and hamster (4) and budding virus particles in intracerebral tumors of the dog (5) but no cytoplasmic virus particles or unusual changes in cytoplasmic membranes. Chicken fibroblasts infected by defective RSV of the Bryan strain contained parallel arrays of endoplasmic reticulum associated with particles thought to be glycogen (6, 7), but similar arrays of hypertrophied endoplasmic reticulum have been reported in tumor cells of diverse origin (8, 9).

The walls of the cytoplasmic membrane complex in the marmoset tumor cells appeared to be formed by fusion of two apposing membranes of the endoplasmic reticulum. Each dense layer of the wall was individually continuous with one membrane of the endoplasmic reticulum and exhibited a bilaminar substructure consistent with the unit membrane concept (10). Since bridging of these closely applied unit membranes was never seen and since the walls did not appear as

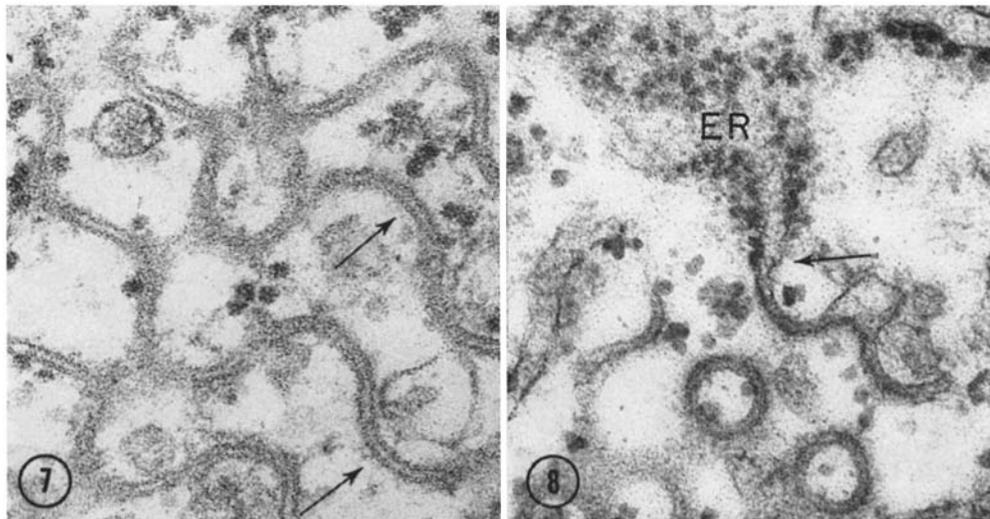


FIGURE 7 Electron micrograph of a membranous cytoplasmic complex. Each of the two dense layers of a cisternal wall contains a bilaminar substructure (arrows), in keeping with the unit membrane concept.  $\times 135,000$ .

FIGURE 8 Electron micrograph of the periphery of a membranous cytoplasmic complex. The walls of the complex are continuous with granular endoplasmic reticulum (*ER*), and at the point of transition (arrow) the two apposing membranes of the *ER* abruptly converge.  $\times 78,000$ .

hollow tubes in cross-section, the intermediate clear zone must be instrumental in holding the two membranes together to form a plate rather than a microtubule.

The spatial symmetry of the membranes was remarkably constant in each of the marmoset tumors. Although the graphic tridimensional reconstruction presented in this study is largely theoretical, it is noteworthy that a similar structural pattern can be reproduced by compression of a stiff wire mesh first in one axis, resulting in corrugations, and then in an axis at right angles to the first one, resulting in equally spaced surface projections. It is conceivable that the symmetrical cytoplasmic membranes were derived in a similar fashion from specialized membranous lamellae of the endoplasmic reticulum.

The virus-like particles associated with the cytoplasmic membranes were similar in structure and dimension to particles described in chicken cells infected by the defective Bryan strain of RSV (6, 7, 11). However, unlike the chicken cells, the marmoset tumors showed no typical budding

virus particles or extracellular virus despite the recovery of infectious RSV from these tumor cells following cocultivations of the cells in tissue culture with chick embryo fibroblasts (12). Thus, the recovery of virus from the marmoset tumor cells focuses attention on the apparently incomplete cytoplasmic virus-like particles and associated membranes which may be identified with the virus carrier state. However, a definite association of membranes with RSV is yet to be established.

This work was supported by the United States Public Health Service, contract No. PH 43-62-179 from the National Cancer Institute within the special virus-leukemia program.

Grateful acknowledgment is made to Mr. Robert F. Parshall of the Department of Medical Illustration for aid in the graphic reconstruction. The authors wish also to thank Miss Lynn Verhoven for her skilled technical assistance.

*Received for publication 29 March 1967, and in revised form 1 February 1968.*

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