Observations of Apparent C-Type Particles in Baboon (Papio cynocephalus) Placentas

Abstract. *Ultrastructural investigations have revealed the presence of apparent endogenously derived C-type particles in the placental villi of each of 13 baboons studied. Both budding and mature forms were observed in the syncytiotrophoblast of these animals at various stages of pregnancy.*

Recent biomedical literature has abounded with reports of the association of C-type virus particles with solid tumors and leukemias in a variety of animal species. Neoplastic tissues derived from reptiles, birds, and mammals have yielded structures morphologically similar to RNA viruses (*1*). Moreover, a series of experiments describing stimulation of C-particle production in presumably "normal" cultures (*2*) has strongly reinforced the theories of the vertically transmitted virogene (*3*) and the more recently advanced protovirus (*4*). This preliminary report, we believe, introduces a most significant parameter to the field of cancer virology and, indeed, to the above hypotheses.

During electron-microscopic examination of the terminal placental villi of experimentally infected and normal baboons, evidence of endogenous rather than exogenous viral associated structures was observed. These particles, all morphologically similar to budding and mature C-type particles, were seen in trophoblast cells of each of several herpesvirus- or poxvirus-infected animals studied. In contrast, structures indicative of herpes- or poxvirus infections were conspicuously absent. When similar materials obtained from normal animals were examined, each specimen revealed numerous structures of identical configuration—all bearing morphological resemblance to C-type viruses (Fig. 1).

Six experimental and seven control

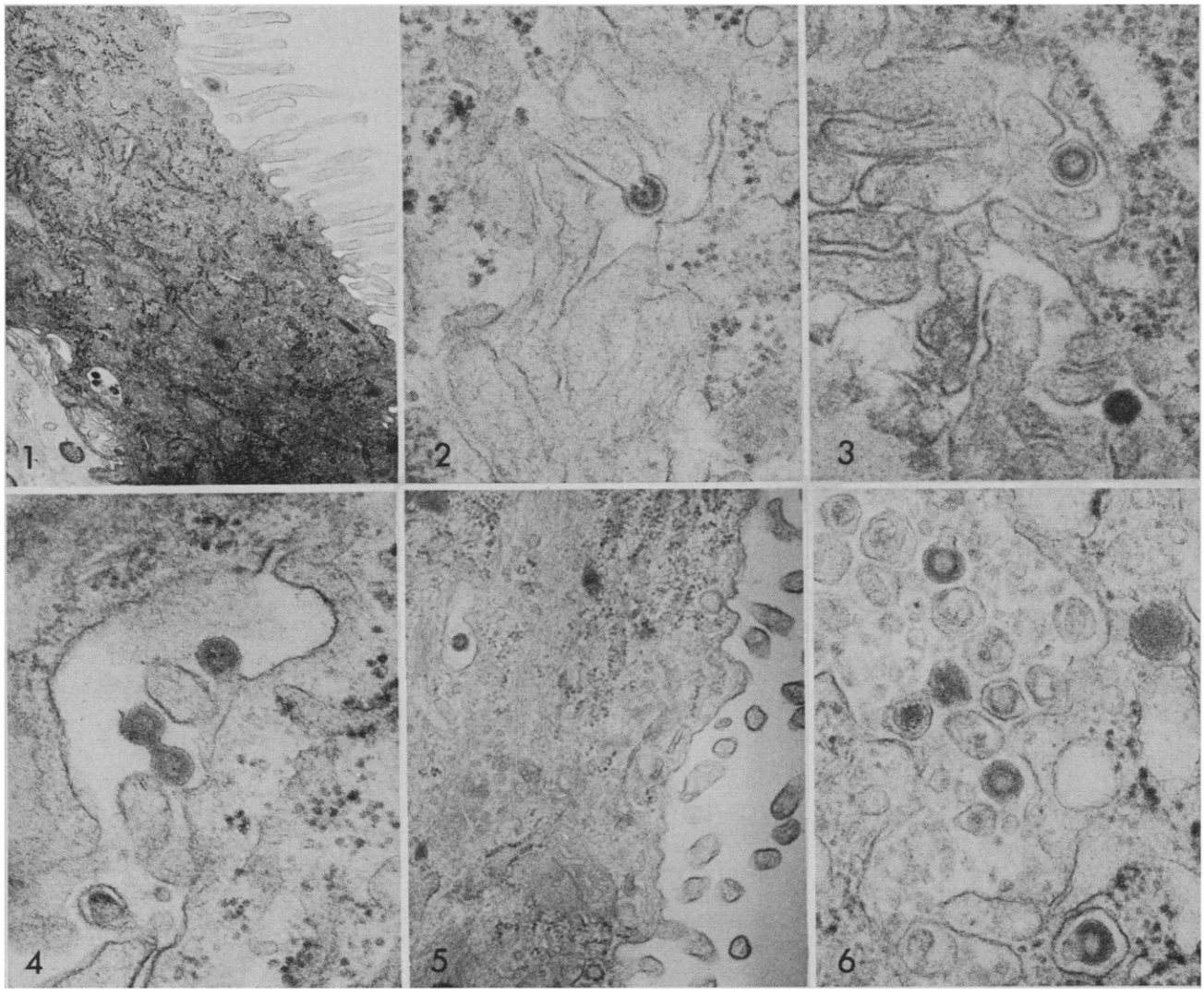

Fig. 1. Electron micrograph showing complete particle and double budding structure in the syncytiotrophoblast cell. Virus particles were consistently seen at sites opposite the microvilli, usually in areas of convoluted plasma membrane (× 13,000). Fig. 2. Budding structure at convoluted plasma membrane (× 78,000). Fig. 3. Complete particle among podocyttic processes of plasma membrane (× 78,000). Fig. 4. Single budding structure and doublet particle at plasma membrane (× 78,000). Fig. 5. Single particle budding into an intracytoplasmic cisterna (× 26,000) Fig. 6. Several C-type particles and various pleomorphic structures within an intracytoplasmic vesicle (× 78,000). All preparations were fixed in glutaraldehyde, and then in osmium tetroxide; sections were stained with uranyl acetate and lead citrate.

animals have been studied thus far, with the duration of pregnancy at time of cesarean delivery of placentas ranging from 27 to 170 days. In every instance particles could be located within 30 minutes while scanning thin sections at a magnification of × 20,000. Although the preparations varied in quantity and ease of recognition of particles, all yielded several typical budding and complete structures.

Sites of budding particles were usually confined to the "inner" surface of the syncytiotrophoblast, that is, the side opposite the villus surface (Fig. 1). Most frequently, buds appeared to emerge from cytoplasmic processes formed by convoluted plasma membranes at the junction between syncytial and cytotrophoblast layers (Figs. 2 to 4). Where cytotrophoblast cells were no longer present, budding occurred from the podocytic processes of the syncytiotrophoblast into the basal lamina. Occasionally, buds were observed in intracytoplasmic cisternae (Figs. 5 and 6). Average particle diameter was determined to be 100 nm; both complete and budding forms usually possessed electron-lucent cores of approximately 50 nm surrounded by three layers. Albeit superficial, description of these structures as "C type" was further based on the absence of surface spikes, eccentric nucleoids, and intracytoplasmic, 70-nm "A-type" particles. Therefore, the criteria for classification as a C-type virus as described previously (*5*) have been met.

These observations of C-type structures in "normal" primate placentas at various stages of pregnancy would appear to contribute substantial support to current views on viral etiology of neoplasia and differentiation. Furthermore, it is most intriguing that these structures are so prevalent in trophoblast tissue, which is both highly invasive and immunologically unique. Obviously, caution must be exercised in interpreting these observations, particularly because of the polymorphic nature of the ultrastructural aspects of the trophoblast. Chandra *et al.* (*6*) have reported the finding of C-type virus particles in normal human embryonic muscle and liver tissue. This observation would tend to support the findings reported here on the presence of C-type particles in the placenta, another fetal tissue. From more extensive electron-microscopic studies of other primate, especially human, placentas and embryonic tissues, we have evidence of the presence of these particles in rhesus monkey and human placentas. In vitro studies are necessary to isolate and grow these putative viral agents for characterization with respect to group-specific antigens, RNA-directed DNA polymerase, and other criteria.

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Cloning of Rat Kangaroo (PTK_2) Cells Following Laser Microirradiation of Selected Mitotic Chromosomes

Abstract. *Mitotic chromosomes of rat kangaroo cells were irradiated with a green argon laser microbeam without prior dye sensitization. Deoxyribonucleic acid–negative chromosome paling was observed. The irradiated cells were isolated and cloned into viable populations.*

The deletion of chromosome segments (0.5 to 2 μm in length) by focusing a coherent laser microbeam onto specific mitotic chromosomes of tissue culture cells could be a powerful genetic mapping tool. This technique is already being used to determine the precise location of the nucleolar (ribosomal) genes of the salamander (*Taricha*) (*1*) and the marsupial (*Potorous*) (*2*). In these studies, location of the rDNA (the DNA that is complementary to ribosomal RNA) relies upon the formation of a nucleolus in late telophase and early G1 phase of the irradiated postmitotic cell.

The more general application of this technique to somatic cell genetics requires cloning and eventual establishment of cell populations from single irradiated cells. In a previous report it was demonstrated that laser microirradiated cells of the rat kangaroo (*Potorous tridactylis*, PTK_1 cell line) were capable of undergoing at least one postlaser mitosis (*3*). These cells had one of their chromosomes irradiated with the laser microbeam after 5 minutes of incubation in the vital dye acridine orange (0.001 μg/ml), which selectively sensitized the chromosomal DNA to the green laser light (*4*). Rat kangaroo cells are used in these studies because of their low chromosome number ($2n = 12$ to 14), large chromosomes, and ability to remain flat during the entire mitotic process.

Attempts to establish that acridine orange–sensitized irradiated cells could undergo more than one additional postlaser mitosis, however, were fruitless. It was felt that perhaps the acridine orange was in some way affecting the viability of the cells, since this dye is known to be a mutagen in higher concentrations. In addition, its intercalation between the base pairs of the DNA double helix distorts the molecule. Consequently, studies were initiated to determine if DNA-negative lesions could be produced without the use of an exogenous sensitizing agent.

We now report that DNA Feulgen-negative chromosome lesions can be produced without dye sensitization by firing the laser at least six times in rapid succession (within about a 2- to 3-second period). Typical chromosome "paling" is detected within 5 seconds of the irradiation. The cells continue through mitosis and appear to have normal nuclear and cytoplasmic morphology. These cells have been isolated, cloned, and established into apparently viable populations. A description of the isolation and cloning procedure for two male rat kangaroo (PTK_2-ATCC. classification CCL56) cells follows.