The Occurrence and Frequency of Type C Virus-Like Particles in Placentas From Patients With Systemic Lupus Erythematosus and From Normal Subjects

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Type C RNA virus-like particles were found by electron microscopy in term placentas from 3 patients with systemic lupus erythematosus (SLE), 1 with probable SLE, and 2 normal patients. The virus-like particles were mainly of a budding or immature type located at or near the cell membrane of syncytiotrophoblasts in chorionic villi. Type C virus-like particles were not observed in the term placenta from a patient with chronic discoid LE, nor in early gestation specimens from 1 normal patient and 4 patients with SLE. The frequency of the Type C particles varied greatly: They were readily found in the patient with probable SLE, and only here were groups of budding particles observed. Type C particles were less numerous in one normal placenta and rare in the other positive placentas, both SLE and normal. Heretofore undescribed crystalline inclusions were found in the cytoplasm of chorionic villous endothelial cells from 3 patients with SLE and 1 with discoid LE. Tubuloreticular structures were observed in the maternal endometrium of 1 patient with SLE. (Am J Pathol 83:383–394, 1976)

THE CAUSE OF HUMAN systemic lupus erythematosus (SLE) remains an enigma, but it may involve a chronic virus infections.[1,2] Although no specific virus has been implicated,[3,4] recent studies suggest that Type C virus antigen expression is enhanced in SLE patients.[5-7] This would be a further parallel with New Zealand mouse disease which, because of its many clinical and immunologic similarities, is the most compelling animal model for human SLE.[8-10] An endogenous murine Type C virus is intimately involved in the pathogenesis of New Zealand mouse disease,[11,12] and Type C virus antigen expression is greatly increased.[13]

Recent observations, pioneered by Kalter and co-workers, of Type C particles in normal primate and human placentas,[14-21] and the isolation of an endogenous Type C virus from a baboon placenta [22] suggest these viruses may be preferentially expressed in placental tissues. There is much current interest in finding evidence of Type C virus expression in human tissues, both in health and diseases such as neoplasia and SLE. We

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thus undertook an electron microscopic study of human placentas, particularly from patients with SLE, for the presence of Type C virus-like particles. In the course of this work, we also found a new intracellular inclusion in SLE placentas.

Materials and Methods

Placentas at a gestational age of 8 to 9.5 months were obtained from 3 patients with SLE, 1 with probable SLE (idiopathic thrombocytopenic purpura with antinuclear and anti-native DNA antibodies), 1 with chronic discoid LE, and 2 normal subjects (Table 1). In addition, placental, decidual, and endometrial tissues were obtained at a gestational age of 2 to 5 months from 3 patients with SLE, 1 with probable SLE, and 1 normal subject (Table 1). The diagnosis of SLE conformed with the American Rheumatism Association criteria.[23] All specimens were derived as a consequence of customary therapeutic procedures carried out by the clinical staffs, independently of the authors, and were obtained for study with the informed consent of the patients involved.

Portions of placenta were placed either in fixative as soon as available after delivery, or in Hanks' balanced salt solution at 4 C and brought to the laboratory within a few hours of delivery, where dissection of the villous tree and fixation in 1.5% phosphate-buffered glutaraldehyde (pH 7.3) were carried out. The specimens were postfixed in 1% osmic acid, dehydrated in graded ethanol and propylene oxide, embedded in Epon 812, and sectioned on a MT2-B Porter Blum ultramicrotome. The thin sections were double-stained with uranyl acetate and lead citrate and examined with a Philips 201 electron microscope at 80 kV. Each tissue sample was extensively examined at a magnification of 24,500 for Type C virus-like particles and photographed at a magnification of 3,450 to 28,500.

Results

Of the seven term or near-term placentas examined by electron microscopy, Type C virus-like particles were found in all except one (Case V, discoid LE). Thus all four definite and probable SLE patients and both normal subjects were positive for Type C virus-like particles (Table 1). Of the five specimens obtained during earlier gestation, two contained only maternal tissue (Cases X and XI); none of the five had Type C virus-like particles (Table 1).

The Type C particles were seen both free and budding from cells (Figures 1 to 3 and 5 to 7), and were morphologically identical whether in SLE or normal placentas. The outer diameter of the budding particles was approximately 110 to 120 nm. The particles generally had no lucent space between the envelope and the lucent inner component (Figures 1 and 6). Extracellular free particles were approximately 100 nm in diameter, somewhat smaller than that of the budding particles. They generally had a wide electron-dense outer layer, a denser inner layer, and a lucent inner component or core of 50 nm (Figure 2). Particles with a less dense outer layer and a dense core were seen rarely (Figure 3).

Type C particles were usually located at or near the junction of the syncytiotrophoblasts and the underlying basement membrane (Figures 1

Table 1—Ultrastructural Findings in Gestational Tissues From Patients With SLE and From Normal Subjects

	Case	Diagnosis	Gestation period (mons)	Tissue	Type C particles	Other features
I	(R74-261)	SLE	8	Placenta CS	+	Crystalline inclusions
II	(R75-203)	SLE	9	Placenta SD	+	Crystalline inclusions
III	(R75-207)	SLE	8	Placenta SD	+	
IV	(R75-179)	Probable SLE	9.5	Placenta CS	+ + +	Crystalline inclusions
V	(R74-175)	Discoid LE	9	Placenta SD		Crystalline inclusions
VI	(R74-128)	Normal	9	Placenta CS	+ +	
VII	(R74-143)	Normal	9	Placenta CS	+	
VIII	(R75-204)	SLE	4-5	Placenta TA		
IX	(R75-210)	Probable SLE	3	Placenta TA		
X	(R74-114)	SLE	2	Endometrium TA		Tubuloreticular structure
XI	(R74-193)	SLE	2	Decidua TA		
XII	(R74-135)	Normal	3	Placenta TA		

SLE systemic lupus erythematosus, discoid LE discoid lupus erythematosus, CS cesarian section SD spontaneous delivery, TA therapeutic abortion, + + + readily found, + + less readily found, + found after exhaustive searching, not found.

to 3). They were not found at the brush border or outer surface of the chorionic villi. Type C particles were also observed in the intercellular space between syncytiotrophoblasts (Figures 5 and 6) and within cytoplasmic vacuoles of these cells (Figure 7). Dense particles of varying appearance with a similar diameter to the Type C particles were occasionally seen among the massive fibrin fibers around the chorionic villi or at the basement membrane located between syncytiotrophoblasts and villous mesenchyme (Figure 4). These were not classified as virus-like because they lacked the characteristic inner structure of Type C particles.

There was considerable variation in the frequency of Type C virus–like particles in placentas both from SLE and normal patients (Table 1). The particles were readily found within a few minutes of searching in only 1 case (Case IV, probable SLE), and only here were groups of budding particles observed (Figures 1, 5, and 6). Type C particles were less readily found in Case VI (normal), where 10 to 20 minutes of searching was required. In the remaining placentas from 3 patients with SLE and 1 normal individual, exhaustive search of several thin sections was necessary to find particles.

Peculiar crystalline inclusions were occasionally found in the cytoplasm of chorionic villous endothelial cells from placentas of the 3 patients with SLE and the patient with discoid LE (Table 1). They were not observed in normal term placentas nor in the earlier gestation specimens. They were electron dense, 0.7 to 1.0 μ in diameter and located near the nucleus (Figures 8 to 10). They had a periodic lattice structure, and no limiting membrane around them was identified.

Tubuloreticular structures were found only in an earlier gestation specimen from a SLE patient (Case X). They were located in capillary endothelial cells of the maternal endometrium, and had the typical branched tubular configuration 22 to 30 nm in diameter (Figure 11).

Discussion

This study extends previous observations of Type C virus–like particles in normal human placentas.[15,17,24] The majority of term placentas, both those from SLE-affected patients and normal patients, contained Type C virus–like particles, both budding and free, but their frequency varied greatly. They were most frequent in a patient with probable SLE (Case IV), where the many groups of budding particles suggested active virus replication. Although less readily found, a normal placenta (Case VI) also contained frequent particles. These 2 cases contrasted with the other positive cases (three SLE and one normal) and with previously reported normal human placentas, where the particles were described as "seen

after extensive scanning"[15] or as "extremely rare."[17] In one term and three earlier gestation placentas we did not find Type C particles even after exhaustive searching, as others have also reported.[15-18] The significance of such quantitative variations in human placentas is uncertain, but similar differences have also been described in chimpanzee placentas.[19] Although numerous Type C particles were found in Case IV of the present study, the significance of this remains to be determined. It appears from this study that Type C virus–like particles are not regularly increased in the placentas of patients with SLE.

The Type C virus-like particles found here were 100 to 120 nm in diameter, with budding particles usually larger than free ones. Like those seen by others in primate and human placentas,[17,21,24] the budding particles lacked a lucent intermediate layer between envelope and core. They thus differ from the budding particles of oncogenic Type C viruses of mice and other animals.[25] Others differ on whether mature Type C particles are present in human placentas.[15,17] We found only one possibly mature particle, with a lucent space between envelope and denser core (Figure 3). Mature Type C particles, if present, are extremely rare in human placentas. Most of the particles found in the present study appeared to be immature, suggesting that the viral genome was not fully expressed. This interpretation is compatible with the failure at isolating Type C viruses from normal human placenta and from SLE tissues, the latter including some of the tissues studied here.[26] In New Zealand mouse disease, an endogenous Type C virus is clearly involved,[11-13] and the markedly enhanced production of a viral envelope glycoprotein antigen seems not to be totally related to mature Type C virus formation.[13] It is possible that such a discordant or partial expression of Type C viral genes may also take place in human SLE.

The peculiar crystalline inclusions occasionally found in the chorionic villous endothelial cells of both SLE and discoid LE patients are probably protein in nature from the ultrastructural findings, but their biologic significance remains to be determined. Such inclusions were not seen in any normal placentas in this study and have not been described in previous ultrastructural studies on normal human placenta.[27-30] They did not resemble the membrane-bounded endothelial specific granules which in size and ultrastructure are similar to microbodies.[31] Further study of more normal cases would be necessary before postulating a relationship between the inclusion and lupus erythematosus.

Tubuloreticular structures have been frequently found in the endothelial cells of various tissues and in peripheral blood lymphocytes from patients with SLE and related diseases.[2,32,33] They are presently not

thought to be actual viral components.[34] Here the structures were found only in the endothelial cells of the endometrium from one SLE patient. They were not found in the placental capillary vessels, which are known to be of fetal origin.[29,30] Thus, although Klippel *et al.*[35] found tubuloreticular structures in the umbilical cord lymphocytes from infants of mothers with SLE, our findings suggest that they may not be found in the endothelial cells of such infants.

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[*Illustrations follow*]

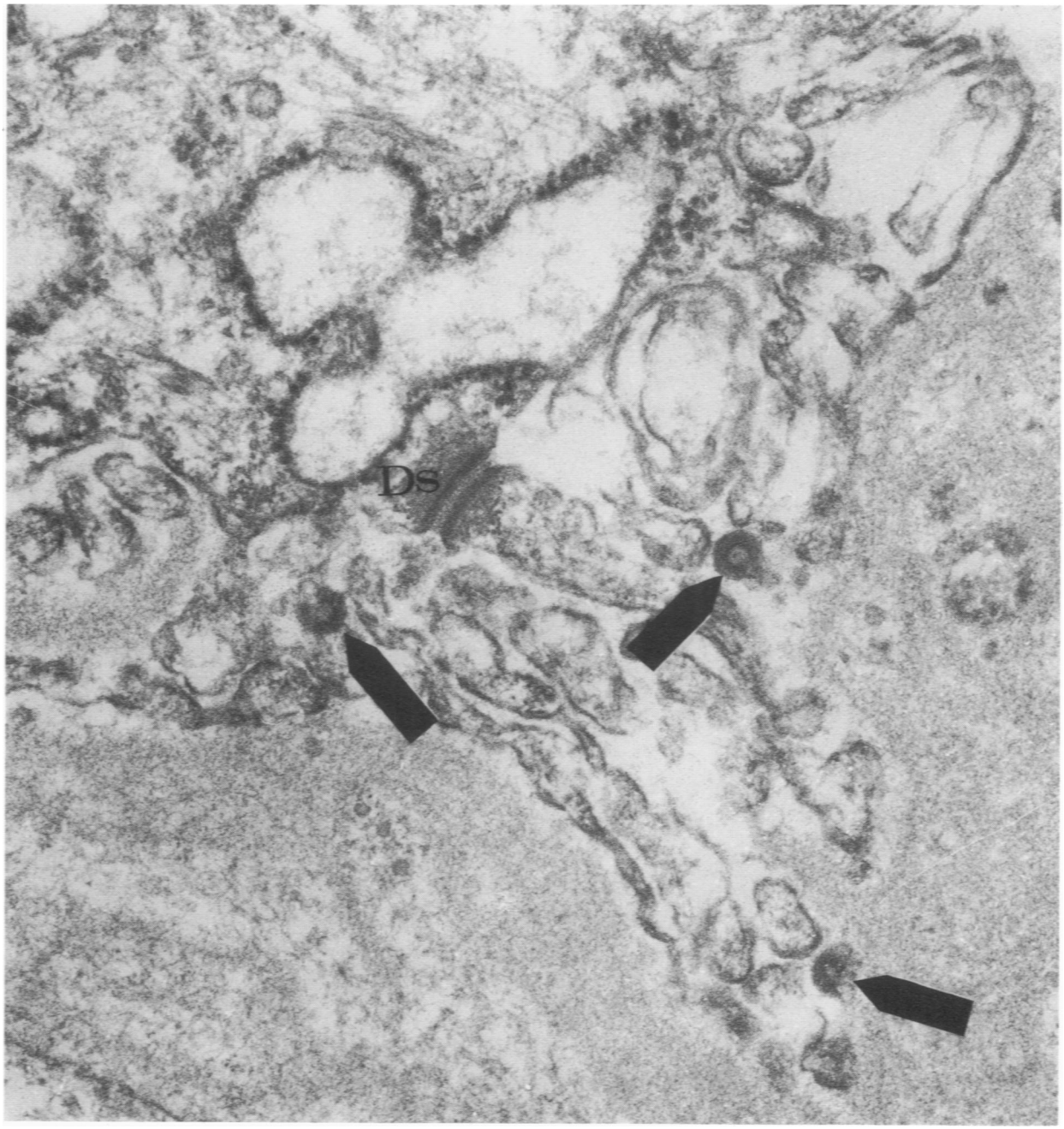

Figure 1—A group of budding Type C virus-like particles (*arrows*) is seen at basal portion of the syncytiotrophoblasts. *Ds* = desmosome; Case IV. (× 76,000)

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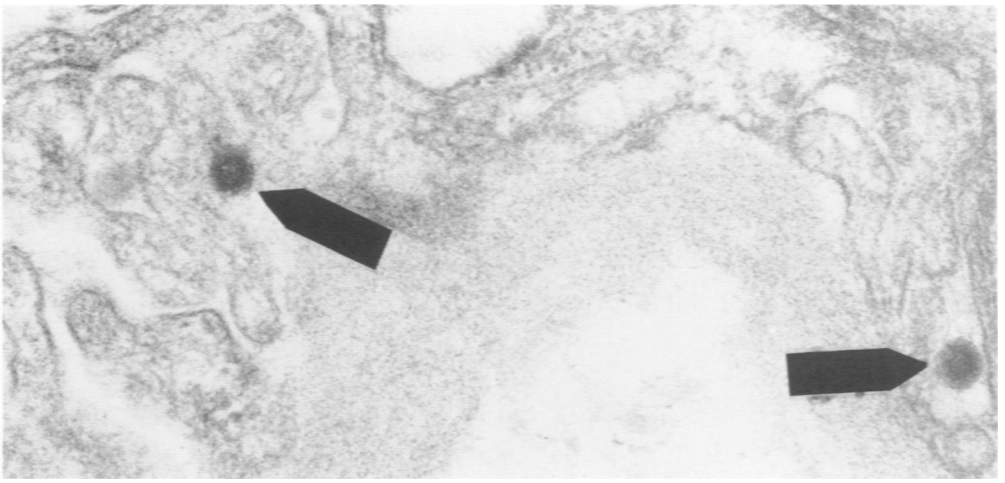

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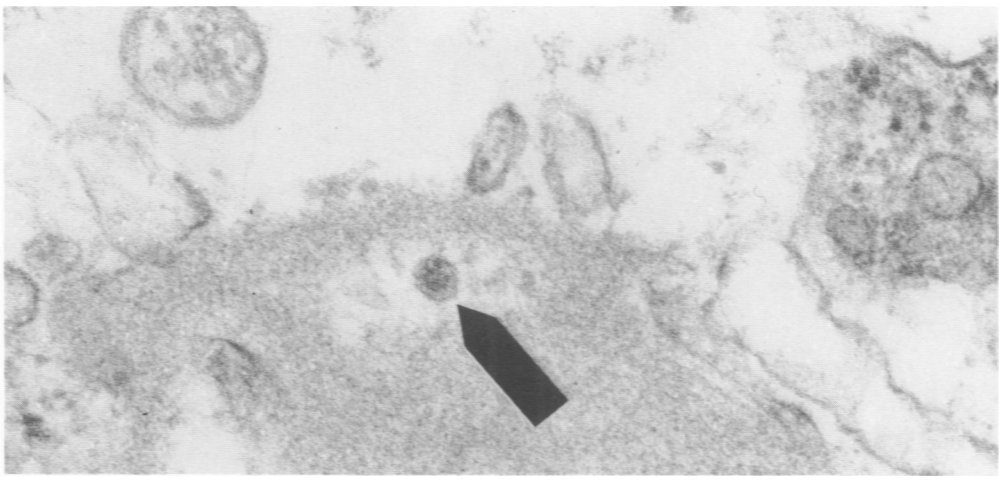

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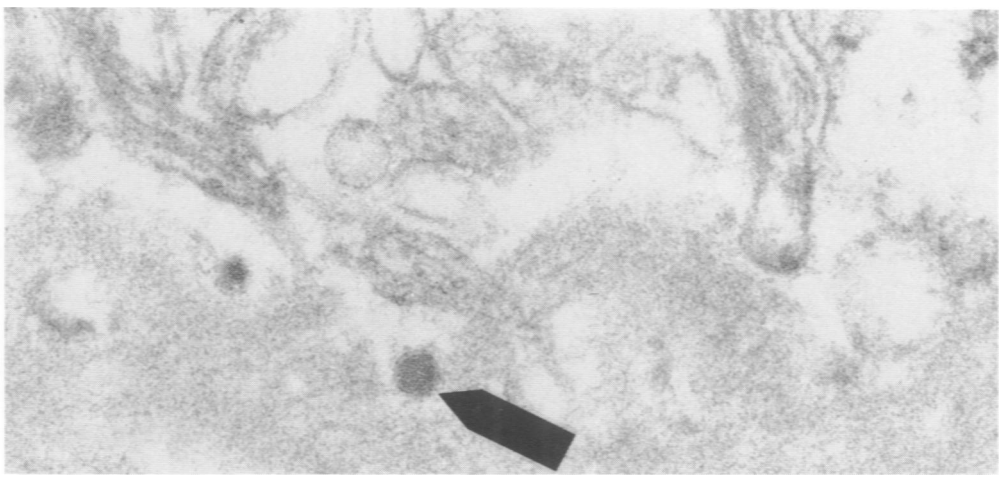
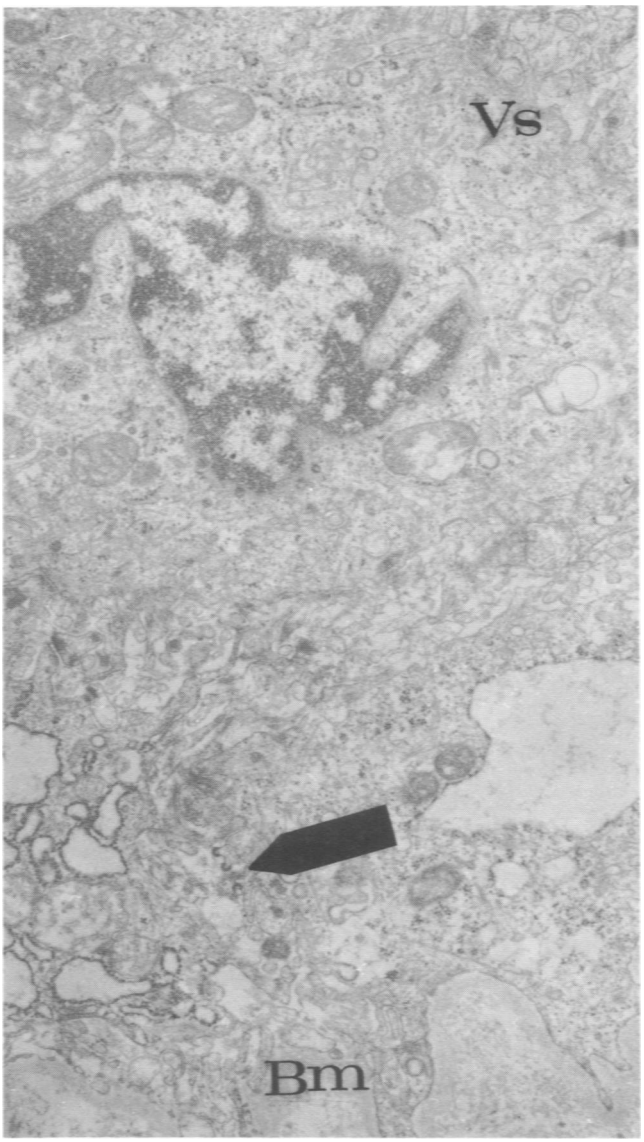
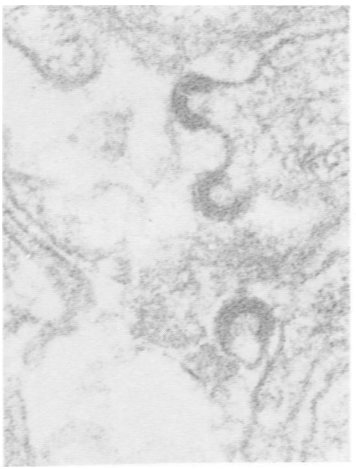


Figure 2—Type C virus-like particles (*arrows*) with a wide dense outer layer (envelope) and a less dense inner component (core) are seen in the space between a syncytiotrophoblast and the basement membrane. Case IV. (× 76,000) **Figure 3**—Type C virus-like particle (*arrow*) with a lucent space between the envelope and the dense inner core is seen at the basement membrane under the syncytiotrophoblast. Case III. (× 76,000) **Figure 4**—An ambiguous dense particle (*arrow*) is seen in the space between a syncytiotrophoblast and the basement membrane. Case II. (× 76,000).





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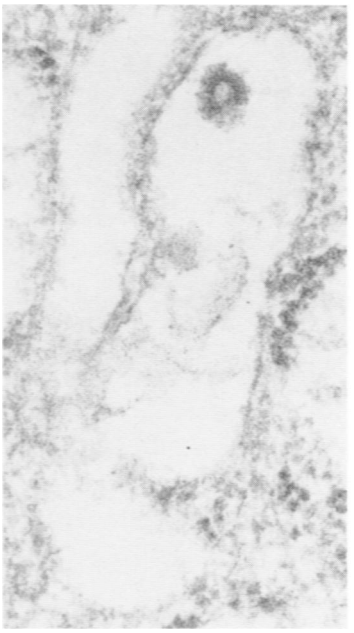

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Figure 5—Placental chorionic villous with a group of budding Type C virus-like particles (*arrow*) in a space between syncytiotrophoblasts. *Vs* = villous surface, *Bm* = basement membrane; Case IV. (× 16,000) **Figure 6**—High power view of budding particles from Figure 5 (× 76,000). **Figure 7**—Type C virus-like particle is seen within a vacuole in the syncytiotrophoblast. Case II. (× 76,000)

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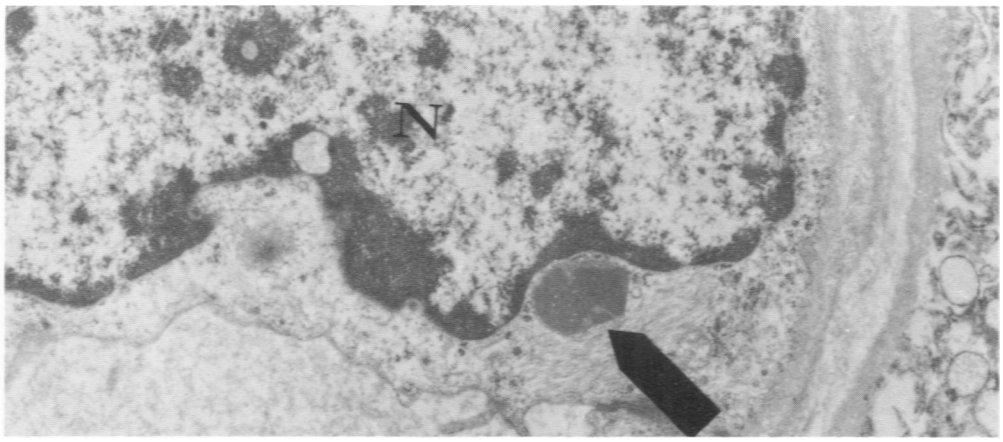

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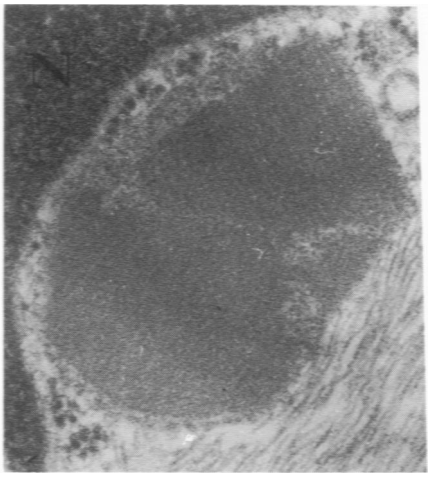

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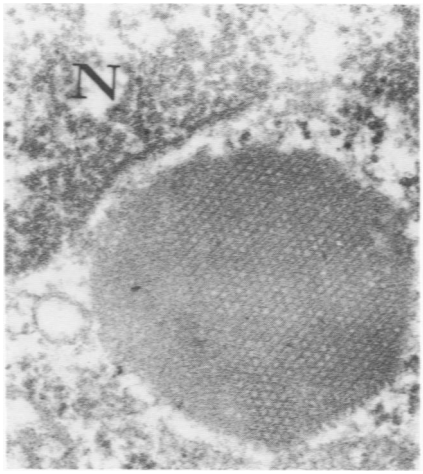

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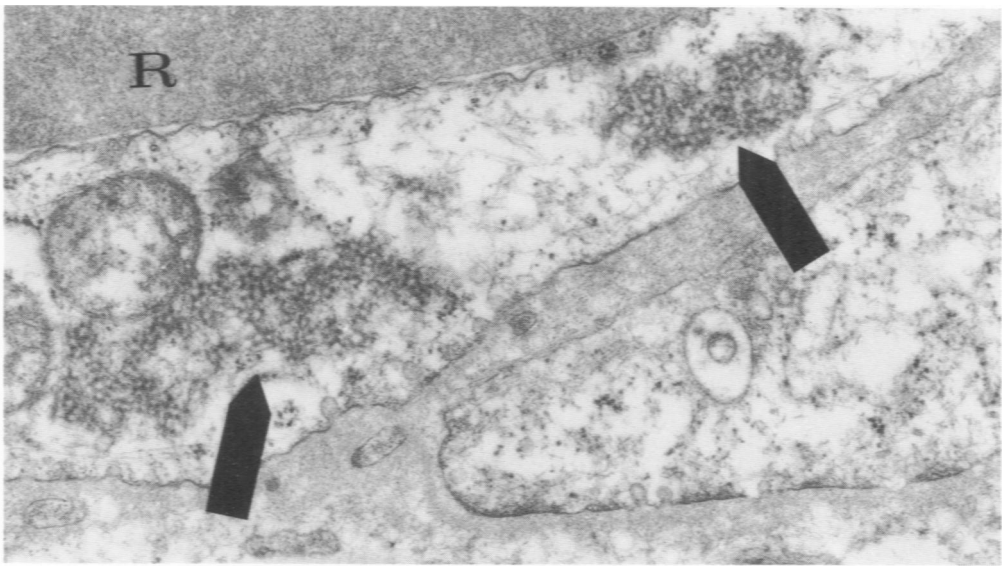

Figure 8—A peculiar crystalline inclusion (*arrow*) is seen near the nucleus (*N*) of villous endothelial cell. Case II. (× 16,000) **Figure 9**—High power view of the crystalline inclusion from Figure 8 (× 57,000). **Figure 10**—Another peculiar crystalline inclusion. *N* = nucleus of villous endothelial cell; Case I. (× 57,000) **Figure 11**—Tubuloreticular structures (*arrows*) are seen in the cytoplasm of endothelial cell in endometrium. *R* = red cell; Case X. (× 27,000)