Ultrastructural pathologic changes of rat extraembryonic visceral endodermal cells exposed to teratogenic antibodies *in vivo*

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Summary. It has been well established that certain heterologous tissue antibodies may induce abnormal embryonic development when injected into pregnant rodents during the organogenetic period. It has been postulated that these antibodies indirectly cause embryopathy by interfering with the normal functions of the yolk-sac placenta. The exact mechanism whereby these antibodies may induce placental pathology is not known.

Specific teratogenic antibodies against a homogeneous rat kidney glycoprotein or a visceral yolk-sac glycoprotein antigen were injected intraperitoneally into 9th day pregnant rats. Electron microscopic examinations of the extraembryonic visceral endodermal cells of the egg cylinder were performed at 4, 6, 9, and 24 hours after the administration of the teratogenic antibodies. Control animals were injected with normal rabbit serum proteins. Extraembryonic visceral endodermal cells were similarly processed and examined as the experimental groups. The results seemed to indicate that the teratogenic antibodies induced increased autophagocytosis and morphologic changes associated with the phagolysosomes (secondary lysosomes) within the extraembryonic visceral endodermal cells at 9 hours following antibody administration. After 24 hours there was an apparent reduction or a complete disappearance of the supranuclear phagolysosome-like and lysosome-like structures, and the appearance of many large and small electron lucent vacuoles containing finely granular materials. Similar ultrastructural pathology was not observed in the 4 and 6 hour experimental and all of the control groups of animals. No other obvious intracellular or intercellular changes were observed in all of the experimental groups. Although the exact mechanism whereby the teratogenic antibodies may induce pathologic changes in the extraembryonic visceral endodermal cells remains to be

determined, the present ultrastructural study demonstrated, for the first time, that teratogenic antibodies induced abnormal pathology in the extraembryonic visceral endodermal cells during the critical period of organogenesis.

Key words: Endodermal cells - Teratogenic antibodies

Introduction

The experimental model of inducing abnormal embryonic development by using heterologous tissue antiserum was first introduced by Brent and his colleagues (Brent et al., 1961). These authors reported that rabbit antiserum against rat kidney homogenate caused embryonic death and abnormalities in the offspring when the antiserum was injected into pregnant rat during the organogenetic period. Following this interesting discovery, many workers have confirmed and extended Brent's findings (Barrows and Taylor, 1971; Bragonier et al., 1970; Gebhardt et al., 1970; Leung et al., 1974; Vaillancourt and Callion, 1972; Yamamoto et al., 1981). It was further documented that rabbit antiserum directed against rat visceral yolk-sac endodermal cells was a potent teratogen (Leung, 1983).

Our laboratory has recently reported the isolation of the responsible antigen from both rat kidney homogenate (Leung, 1982) and from visceral yolk-sac endodermal extract (Leung et al., 1985). The antigen appears to be associated with the microvilli of the renal proximal tubules and the visceral yolk-sac endoderm. It appears to be a glycoprotein of variable molecular species depending upon the tissue from which it was isolated and also upon the purification procedures. Using specific teratogenic antibodies against a homogeneous antigen, we may better delineate the pathologic mechanism without the presence of other tissue antibodies which may simultaneously exert some other biological effect or mask the effects of the

responsible antibodies. Since our previous immunofluorescent studies demonstrated that the teratogenic antibodies localized *in vivo* in the extraembryonic visceral endoderm, we are now reporting our findings on the ultrastructural changes of these cells after the administration of a teratogenic dose of specific antibodies.

Materials and methods

Random-bred Wistar rats were used. Females were placed with males overnight. Vaginal smears were examined for sperms the next morning. Females that had been inseminated were considered to be at the 1st day of pregnancy. Rats were housed in stainless steel cages and given food (Purina Mouse Chow) and water *ad libitum*.

On the morning of the 9th day of pregnancy, rats were intraperitoneally injected with 5 mg of lyophilized rabbit immunoglobulins G (IgG) directed against the homogeneous kidney antigen (Leung, 1983) or IgG against the visceral yolk-sac antigen (Leung, 1985). These two populations of IgG cross-reacted with each other and formed a common single immunoprecipitin band with crude homogenates (or extracts) of adult kidney or visceral yolk-sac of 14th to 21st day of gestation. The IgG were purified from whole rabbit antisera by ammonium sulfate precipitation followed by diethylaminoethyl (Whatman DE52) cellulose chromatography as described previously (Leung, 1977). It was determined by biological assay on pregnant rats that injection of 5 mg IgG would result in (1) bilateral or unilateral anophthalmia in all of the surviving fetuses, and (2) resorption in about 20% of the implanted embryos. Control pregnant rats were injected with 5 mg of normal rabbit serum proteins.

Both the experimental and control rats were perfused with 0.1 M phosphate buffer, pH 7.4 containing 3% glutaraldehyde at the following time-points following injection: 4, 6, 9, and 24 hours. The conceptuses were quickly dissected *in toto* with the deciduoma from the uterus under a dissecting microscope and maintained in the same fixative for another 3 hours at room temperature. Tissues were postfixed in Sorenson's buffer containing 1% OsO_4, dehydrated through propylene oxide and embedded in Maraglas 655. For orientation of the conceptus, thick sections were cut and stained with a 2% toluidine blue solution and examined by light microscopy. Thin sections were cut, stained with uranyl acetate and lead citrate and examined with a RCA M4 electron microscope.

Four conceptuses from each of the four control animals were removed for each time-point. For the experimental groups, 8 conceptuses were removed from 2 injected mothers for each of the four time-points. Attempts were made to select conceptuses of bigger sizes under a dissecting microscope in order to avoid using possibly dying embryos.

Results

At the 9th-10th day of gestation, that is within the 4

time-points, the egg cylinder is lined by Reichert's membrane, extraembryonic parietal and visceral endodermal cells, and embryonic endoderm (Fig. 1). Only those extraembryonic visceral endodermal cells located between the open arrows on the egg cyclinder were examined by electron microscopy. As shown in Fig. 2A, these columnar epithelial cells have numerous microvilli extending into the yolk cavity. A zonula occludens is present between the two epithelial cells. Beneath the base of the microvilli, there is a zone of cytoplasm which is primarily occupied by structures involved in endocytosis. These structures consist of intermicrovillous coated invaginations, condensing smooth membrane canaliculi, small vesicles and vacuoles. Further down in the cytoplasm, there are membrane-bound storage vesicles or vacuoles of different sizes and shapes; some of these vacuoles are filled with granular, medium electron density or darkly stained materials (see Fig. 2A). The high or medium electron density vacuoles are the phagolysosomes, or secondary lysosomes, which are the predominant vacuolar structures. The cells nucleus is basally located. There are abundant free ribosomes and mitochondria (elongated and oval) distributed throughout the cell. Rough endoplasmic reticulum is rather well-developed. Occasionally primary lysosomes can be observed near the Golgi complex.

The observations for the experimental groups appeared to be the same for both populations (anti-kidney glycoprotein and anti-visceral yolk-sac antigen) of antibodies utilized. Therefore the following described morphologic changes applies to the effects of both antibodies. A total of more than eleven hundred extraembryonic visceral endodermal cells were examined. At 4 and 6 hours after the administration of the teratogenic antibodies, the ultrastructural morphology of the extraembryonic visceral endodermal cells appeared to be the same as that observed in the control animals injected with normal rabbit serum proteins. However, at 9 hours after antibody injection, it appeared that there was a reduction or complete absence of the small to large vacuoles containing materials of high to medium electron density. Most of the vacuoles of similar sizes and shapes appeared to be electron lucent containing granular materials (Fig. 2B). Some of the large vacuoles contained membranous whorls (MW) as demonstrated in Fig. 2C. Occasionally some MW could be observed in the cytoplasm without being surrounded by the limiting membrane of vacuoles. No recognizable cellular organelles, such as mitochondria, were present with the MW in the vacuoles examined. Otherwise, there were few apparent ultrastructural changes. The number of microvilli, endocytic vesicles, free ribosomes, endoplasmic reticula, and mitochondria appeared to be the same. The normal morphology of the extraembryonic visceral endoderm cells at 24 hours after injection of rabbit serum proteins is shown in Fig. 3A. The morphology of the extraembryonic visceral endodermal cells at 24 hours after exposure to antibodies appeared to be the same as that described for the experimental 9-hour time-
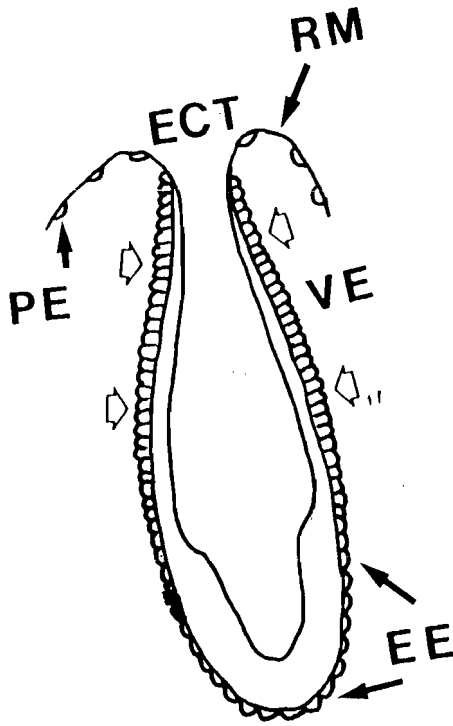
Fig. 1. A diagram illustrating the rat egg cylinder at the 9th day of gestation. Extraembryonic visceral endodermal (VE) cells located between the two open arrows were examined by electron microscopy. These cells were columnar in shape whereas the embryonic endodermal cells (EE) overlying the embryonic portion of the egg cylinder were flat. The parietal endodermal cells (PE) are associated with Reichert's membrane (RM). ECT, Ectoplacental cone.

Fig. 2. Electron micrographs of rat extraembryonic visceral endodermal cells at 9 hours after teratogenic antibody or rabbit serum protein injection. **A.** Two endodermal cells exposed to rabbit serum proteins, × 12,140. **B.** Several endodermal cells from an embryo whose mother was injected with teratogenic antibodies. Most of the vacuoles were electron lucent containing granular materials, × 9,190. **C.** Two adjacent endodermal cells with three membrane - limiting vacuoles containing membranous whorls, × 15,450. **G,** Golgi apparatus; **m,** mitochondrion; **mv,** microvilli; **V,** vacuole; **yc,** yolk cavity; **zo,** zonula occludens.
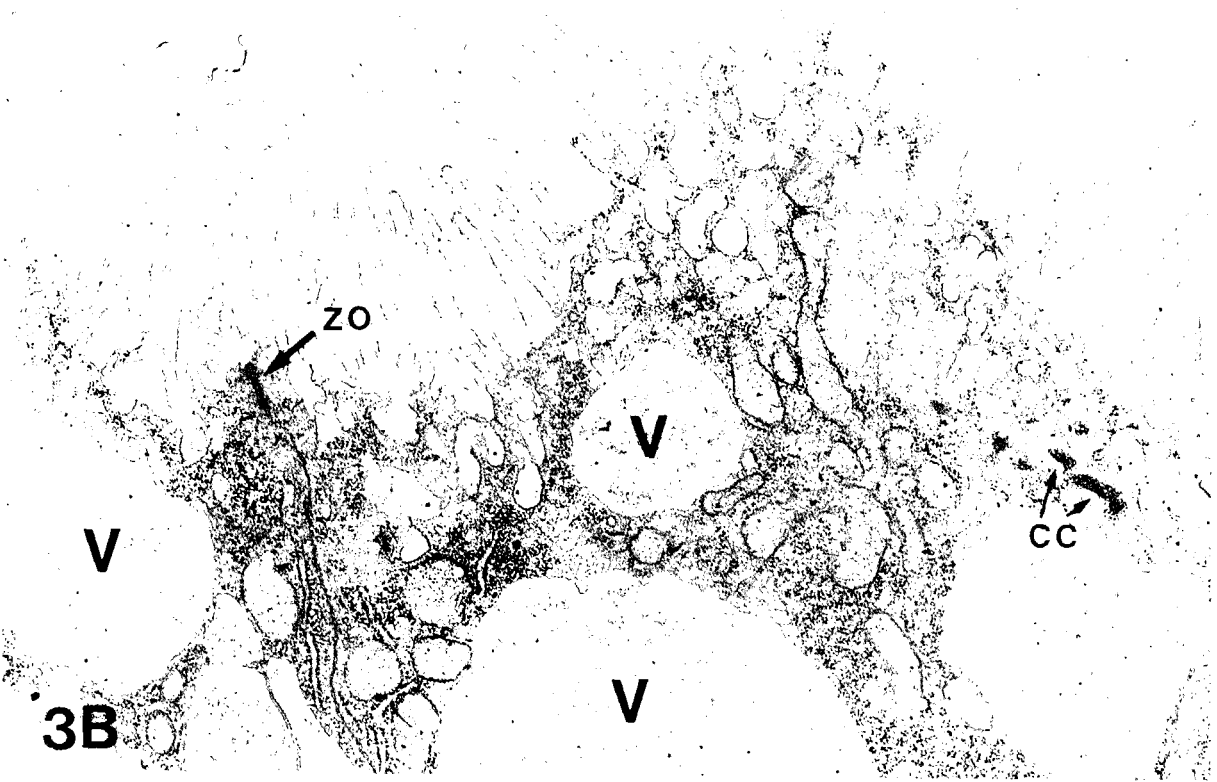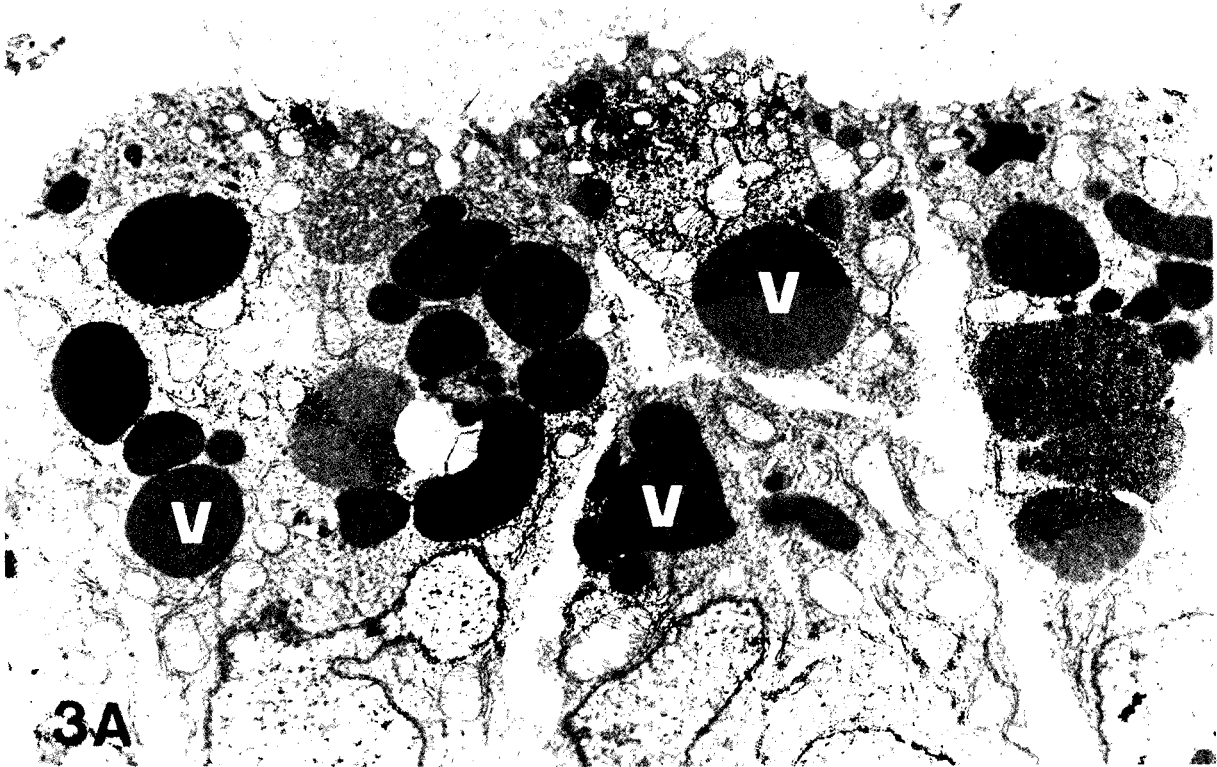
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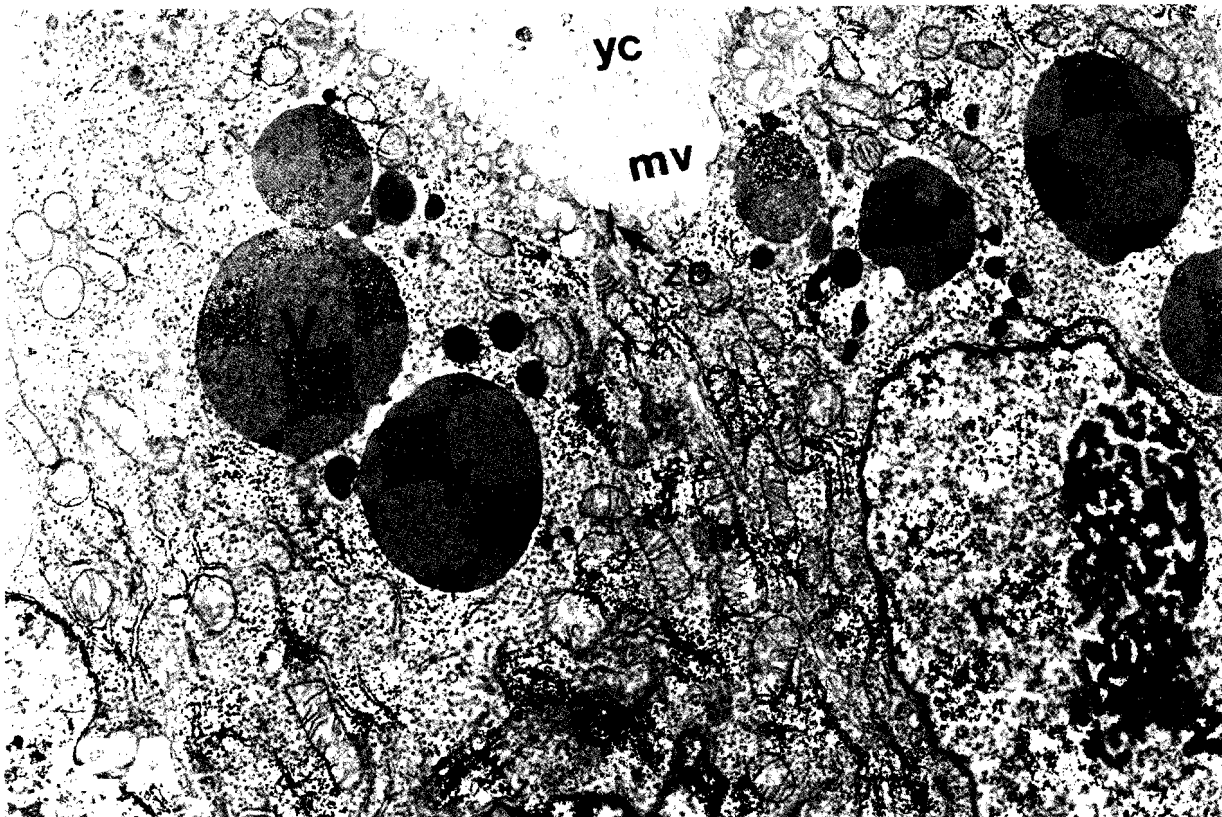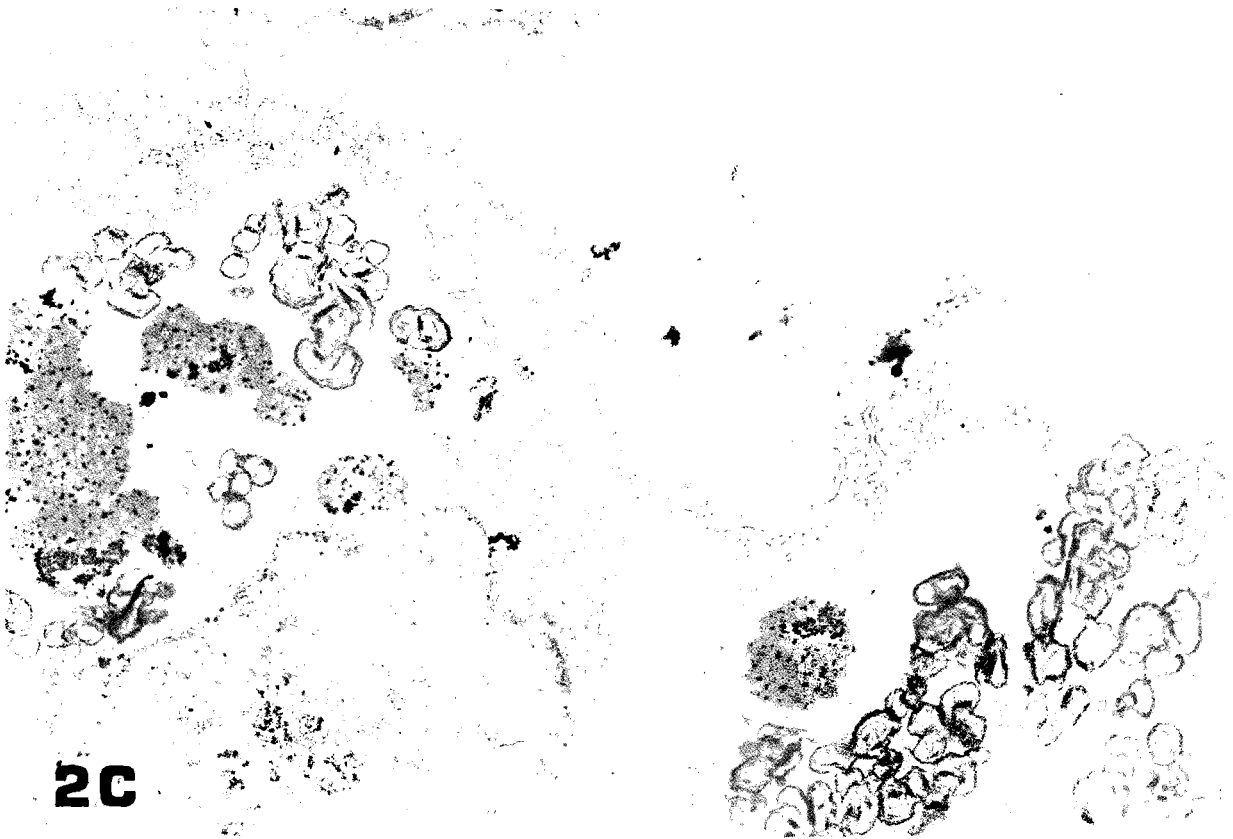
Fig. 3. Electron micrographs of rat extraembryonic visceral endodermal cells at 24 hours after exposure to either rabbit serum proteins or teratogenic antibodies. **A.** Several endodermal cells from an embryo whose mother was injected with normal rabbit serum proteins, × 9,350. **B.** Two endodermal cells from an embryo whose mother was injected with teratogenic antibodies. Note the difference in the vacuoles when compared with the control in 3A, × 17,790

point except that (1) the electron lucent vacuoles generally appeared to be bigger and containing much less granular materials (Fig. 3B), and (2) the frequency of the presence of MW in the vacuoles had greatly decreased. It is interesting to note that there were still numerous endocytic vesicles and condensing canaliculi still present.

Discussion

Since the original discovery of Brent and his colleagues (Brent et al., 1961) that rabbit antiserum against rat kidney homogenate could induce congenital defects in the offspring of rats injected with the antiserum during the period of organogenesis, much progress has been made in identifying the antigen and its cross-reactivity among the kidney, extraembryonic visceral endoderm and yolk-sac placenta tissues. Recent investigations have focused on the possibility that teratogenic antibodies may induce yolk-sac placenta dysfunction, thereby causing birth defects. Using rabbit antiserum agains whole rat yolk-sac placenta, Freeman et al., (1982) presented biochemical evidence to support the view that teratogenesis may have resulted from decrease in pinocytic uptake of proteins by the visceral yolk-sac endoderm. Using light microscopy, Brent and his colleagues (1983) documented histopathologic changes in the rat embryo 48 hours after the administration of rabbit or sheep antiserum against whole rat visceral yolk-sac placenta. This latter light microscopic study indicated that the initial abnormal histology of the conceptus was observed in the embryo rather than the yolk-sac placenta. Our present study examined the pathologic changes of the extraembryonic visceral endoderm at the ultrastructural level following the administration of specific teratogenic antibodies. To study the changes in the extraembryonic visceral endoderm at the time of organogenesis is more appropiate than to study changes occurred in the visceral yolk-sac endoderm, which is derived from the former, later in development. Using two populations of better defined antibodies (against a homogeneous kidney or visceral yolk-sac antigen), this current study presented ultrastructural evidence to support the view that both teratogenic kidney and visceral yolk-sac antibodies affect the lysosomal-vacuolar system of the extraembryonic visceral endodermal cells during the organogenetic period. Because our teratogenic antibodies have been shown to be specific against an antigen associated with the microvilli and coated pits of the visceral yolk-sac endoderm (Leung et al., 1985) and because Freeman et al. (1982) showed that teratogenic antiserum against whole yolk-sac placenta decreased pinocytic uptake of proteins by the visceral yolk-sac endoderm. it was

somewhat surprising to note in our present investigation that the predominant morphologic change was associated with the lysosomal system rather than the endocytotic region of the cell. Furthermore, the finding that many vacuoles contained MW may be of considerable significance. It is possible that these vacuoles were autophagic in nature. By combining with their cell surface antigen and through some still unknown mechanism, the teratogenic antibodies might have induced sublethal injury to the extraembryonic visceral endodermal cells during organogenetic period. Increased autophagocytosis implicating sublethal cell injury has been reported in several experimental conditions (Fedorko et al., 1968; Fedorko et al., 1968; Ericsson et al., 1969; Tulkens et al., 1970; Dingle et al., 1973). Similarly, increased autophagy has been well documented in perfused rat hepatocytes deprived of amino acids (Schworer et al., 1981; Poso et al., 1982). In this respect, our ultrastructural findings of presumably autophagy may result from a decrease in intracellular amino acid pool due to decreased pinocytic uptake of proteins by the endodermal cells.

Studying the effects of goat or rabbit antiserum to rat chorioallantoic placenta on rat visceral yolk-sac, Franke and his colleagues (1973, 1975) observed ultrastructural changes in the yolk-sac relative to the microvilli and pinocytic vesicles as well as phagolysosomes following the administration of the antiserum. These investigators also observed the appearance of large electron-lucent vacuoles. It is possible that our antibodies, when given in a higher dose, may induce similar changes on the microvilli and endosomes as reported by these authors. Nevertheless, it is difficult to correlate our present results with the morphologic findings of Franke and his co-workers, because (1) these authors utilized antiserum to a homogenate of chorioallantoic placenta cotaining a multitude of antigens while our antibodies were directed against a glycoprotein antigen, and (2) the biologic effect of the antiserum to chorioallantoic placenta on embryonic development was not reported by these authors. Our present report has provided, for the first time, ultrastructural evidence to suggest that as early as 9 hours after injection of specific teratogenic antibodies, the extraembryonic visceral endoderm begins to manifest morphologic changes. Although our present study has focused on the extraembryonic visceral endoderm and has not examined the embryo itself, it is very likely that the embryonic changes occurred 48 hours after antiserum injection, as reported by Brent and his colleagues (1983), is secondary to the initial sublethal injury of the extraembryonic visceral endoderm during the period of organogenesis.

The control ultrastructural morphology of the extraembryonic visceral endodermal cells at 4, 6, 9, and 24 hours time-points appeared to be the same as that first described by Lambson (1966) for rat visceral endodermal cells at the 10th day of gestation. The temporal appearance of abnormal morphology 9 hours after antibody injection, as demonstrated in this report, has to be interpreted together with the dosage of antibody

injected. With higher antibody dosage, it is conceivable that earlier and more severe morphologic changes may occur.

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