Morphologic Analysis of Spontaneous Teratocarcinogenesis in Developing Testes of Strain 129/Sv-ter Mice

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Spontaneous teratocarcinogenesis in the mouse testis begins during the early stages of gonad differentiation. Using inbred strain 129/Sv-ter mice which are highly susceptible to these tumors, the authors have examined the morphologic features of the testis during the gestational period defined from Day 13 through birth. Normal inbred mice (129/J) and random bred mice (Swiss Webster, SW) were used as control groups. Serially sectioned gonads were evaluated at the light- and electron-microscopic levels for histologic changes. In agreement with studies by other workers, embryonal carcinoma cells (ECCs) were observed in tumor-susceptible mice. Cellular arrangements varied from vesicular to nodular. Cell death within advanced tumors was labeled "apoptosis" (shrinkage necrosis). Also encountered were syncytial arrangements of gonocytes (atypical gonocytes), which were present in all animal groups. The significance of atypical gonocytes in relation to degeneration and preneoplasia is addressed. (Am J Pathol 1986, 124:263–280)

TERATOCARCINOMAS belong to a group of testicular cancers that accounted for the majority of cancer deaths in men between the ages of 25 and 29 in 1981.[1] These tumors are thought to develop prenatally from primordial germ cells,[2–4] although their etiology is not well understood. Studies of their development before birth are not feasible in humans, making early diagnosis and treatment all the more difficult. Spontaneous tumors in inbred strain 129 mice, however, provide a model for studying the incipient stages of the disease (reviewed by Jewett[5]).

Teratocarcinogenesis in murine testes begins during the early stages of gonadal differentiation, which is evident beginning on Day 13 of gestation.[6] Spontaneous tumors have been positively identified in embryonic testes as early as Day 15 of gestation,[7] and tumors have been induced in 12–13-day urogenital ridges by grafting them to adult testes.[2] From these studies it has been postulated that teratocarcinogenesis begins at about Day 12 of gestation in the mouse.

The present study examines developing testes in a subline of Strain 129 mice, 129/Sv-ter, which is highly susceptible to teratocarcinogenesis.[8] We have used recent morphologic methods to ask these questions: 1) Is spontaneous teratocarcinogenesis morphologically evident earlier than Day 15? 2) What histologic changes are occurring during this period of testicular development in tumor-susceptible animals?

Materials and Methods

Animals

The incidence of spontaneous testicular teratocarcinomas in mice varies among sublines of Strain 129. Strain 129/Sv-ter was chosen as the experimental group in this study because the incidence of congenital tumors is high, 10–30%.[9] Animals for control groups were selected from two populations: 1) another subline (129/J) of the inbred Strain 129, in which the tumor incidence is less than 1%, and 2) random bred Swiss Webster (SW) mice, which have not been reported to bear testicular teratomas.

Strain 129 mice were obtained from The Jackson Laboratory, Bar Harbor, Maine. Strain 129/Sv-ter mice were a gift from Dr. Leroy Stevens. Mice from the *J* subline were supplied by the Production Department at The Jackson Laboratory. Swiss Webster mice were purchased from Bio-Lab Corporation, St. Paul, Minnesota. The mice were kept in the animal quarters of the Department of Cell Biology and Neuroanatomy and were given Purina Mouse Chow and water *ad libitum*. One male

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was housed with four females for breeding purposes. Females were examined daily for vaginal plugs, with the day on which a plug was found being considered Day 0 of gestation. Embryos were obtained at each day of gestation from Day 13, when sexual dimorphism of the gonards becomes evident, through birth (Day 20).

Tissue Manipulation

Pregnant females were killed by cervical dislocation, and their uteri were exposed. Embryos were dissected from their extraembryonic membranes before being immersed in fixative. Removal of embryonic testes was carried out in primary fixative with watchmaker's forceps under a dissecting microscope. The embryos were anchored at the head, neck, and pelvis by pins, ventral side up, on Sudan-black-stained paraffin. Gestational age was verified, and the embryos were staged by developmental features such as umbilical herniation, whiskers, and hair follicles. and foot plate indentations.[10] The ventral body wall, gut, and liver were removed, and the urogenital system was thus exposed. Urogenital ridges from 13- and 14-day male embryos were processed intact, and testes from older embryos were separated from their epididymides before processing.

Altogether, 48 litters consisting of 6 litters (6 different mothers) from each of the 8 days of gestation were obtained. Testes from all male embryos were processed for morphologic studies.

Tissue Processing

Urogenital ridges and whole testes were immersed in a primary fixative of 5% glutaraldehyde buffered in 0.16 M collidine at pH 7.4. Fixation was from 2 to 2½ hours at 4 C. After postfixation in 1% osmium tetroxide/0.15 M collidine buffer for 2 hours at 4 C, tissues were dehydrated in ethanol and embedded in Epon.

Morphology

Plastic sections 1.0–1.5 μ thick were cut with glass knives, heat-fixed in distilled water onto glass microscope slides, and stained with alkaline toluidine blue. Serial sections of entire urogenital ridges from 13- and 14-day embryos were examined microscopically for teratomas. For testes of embryos 15 days old and older, serial sections from areas 20–30 μ at a time were examined, with areas of 15–20 μ in between being discarded. The smallest tumors would measure 20–40 μ in diameter[7] and should not be overlooked. When evidence of any histologic change was encountered, that testis was not sectioned further. The morphologic features of these changes were evaluated at both the light- and electron-microscopic levels. From blocks selected for ultrastructural studies, thin sections were cut with a diamond knife, mounted on uncoated 200-mesh copper grids, and double-stained with aqueous uranyl acetate and lead citrate.

Preparations were viewed in either a Philips 201 Transmission Electron Microscope or a JEOL 100-CX TemScan Electron Microscope at accelerating voltages of 40 or 60 kV.

Results

Evidence for Early Tumors in Strain 129/Sv-ter Animals

From 130 male embryos obtained, a total of 254 testes were recovered and processed. Examination revealed histologic changes in about 40% (101) of the testes.

In the present study, "atypical" gonocytes were first visible on Day 13 of gestation. They were recognizable as cell nests confined principally to central regions of the testis cords. These cells have been called embryonal carcinoma cells (ECCs) in earlier studies,[4,7] but they closely resemble gonocytes, which are undergoing morphologic changes and will thus be referred to as "atypical" gonocytes in this study. Atypical gonocytes lose their overall round contours and become swollen and distorted as they form an apparent syncytium (Figure 1). Electron microscopy confirms and extends observations made with the light microscope, as seen in Figure 2, where loss of cell borders, dispersal of cytoplasm, and aggregation of nuclei in the center of a common cytoplasm are characteristic. Except for dissolution of cell membranes between adjacent cells, the gonocyte cytoplasm retains its typical morphologic features. There was no evidence of necrosis within these syncytial masses. Several foci of atypical gonocytes were normally identified among different testis cords throughout the testis but were most prevalent in those cords connected to the rete tubules. In one case the basement membrane of the testis cord appeared to be ruptured, with atypical gonocyte aggregates and normal gonocytes spilling into the interstitium (Figure 3). Atypical gonocytes were identifiable in all stages of testicular differentiation studied and were often present in testicular tissue which contained more advanced stages of tumor formation.

The smallest, clearly identified tumors were found on day 14 of gestation (Figure 4). Tumor cells (ECCs) formed a vesicle in contact with the basement membrane of the testis cord. The ECCs were pleomorphic cells with a high nucleocytoplasmic ratio, an indented nucleus, and large dense nucleoli. The undifferentiated

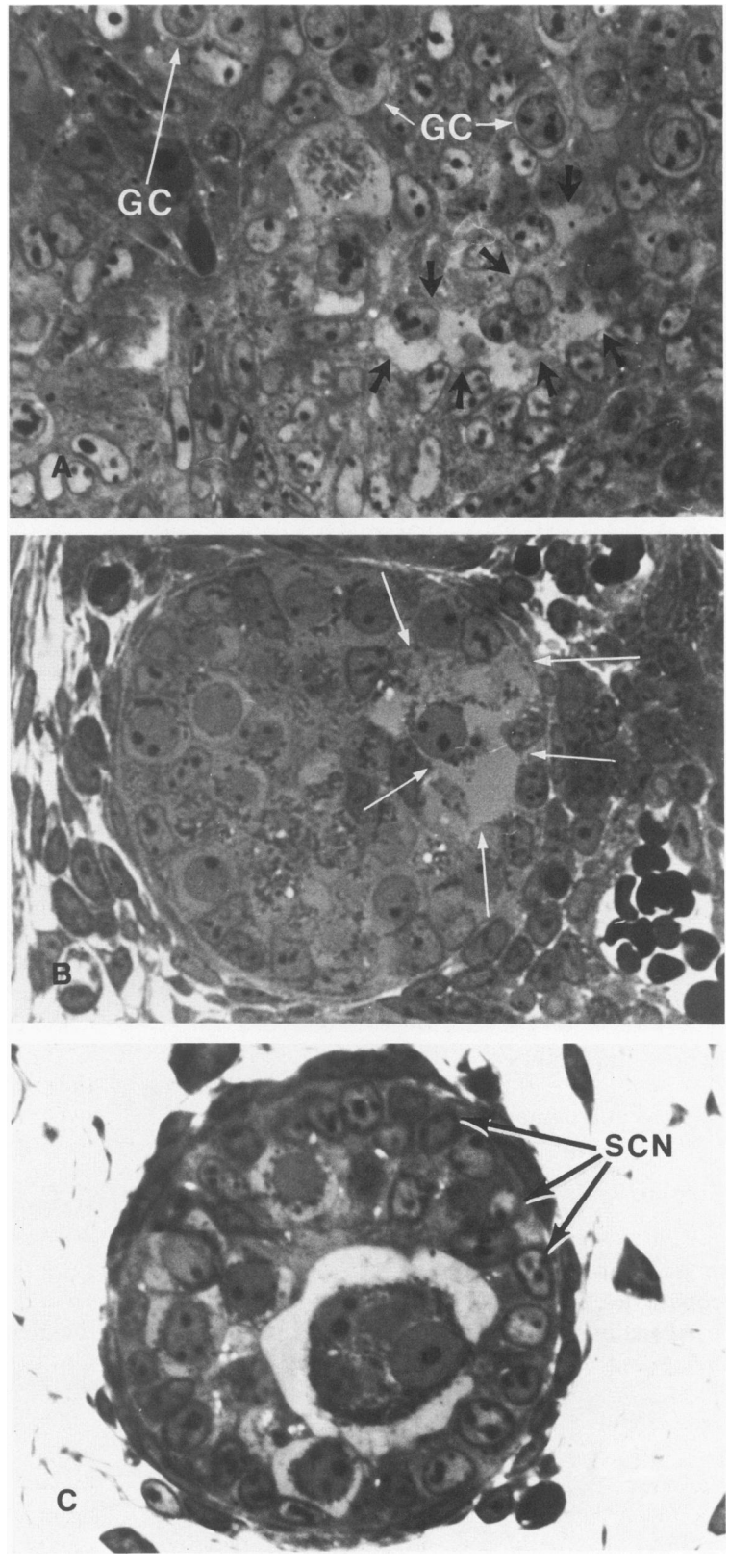

Figure 1—Light micrographs of embryonic testes show atypical gonocytes contained within the testis cords. **A**—An atypical gonocyte aggregate is indicated by *black arrows*. The borders of individual gonocytes are lost, and nuclei gather in the center of a common cytoplasm. Note normal gonocytes (*GC*), with their round contour and large spherical nucleus. Genital ridge at 13 days of gestation. (×60) **B**—Cross-section of a testis cord. *Arrows* indicate an atypical gonocyte with marked swelling and dispersal of cytoplasm and a nucleus of normal size. Testis at 14 days of gestation. (×35) **C**—Cross-section of a testis cord. A group of atypical gonocytes is situated centrally. Their nuclei and mitochondria occupy the center of the gonocyte syncytium. Small, darkly stained Sertoli cell nuclei (*SCN*) are aligned along the basement membrane. Testis at 19 days of gestation. (×40)

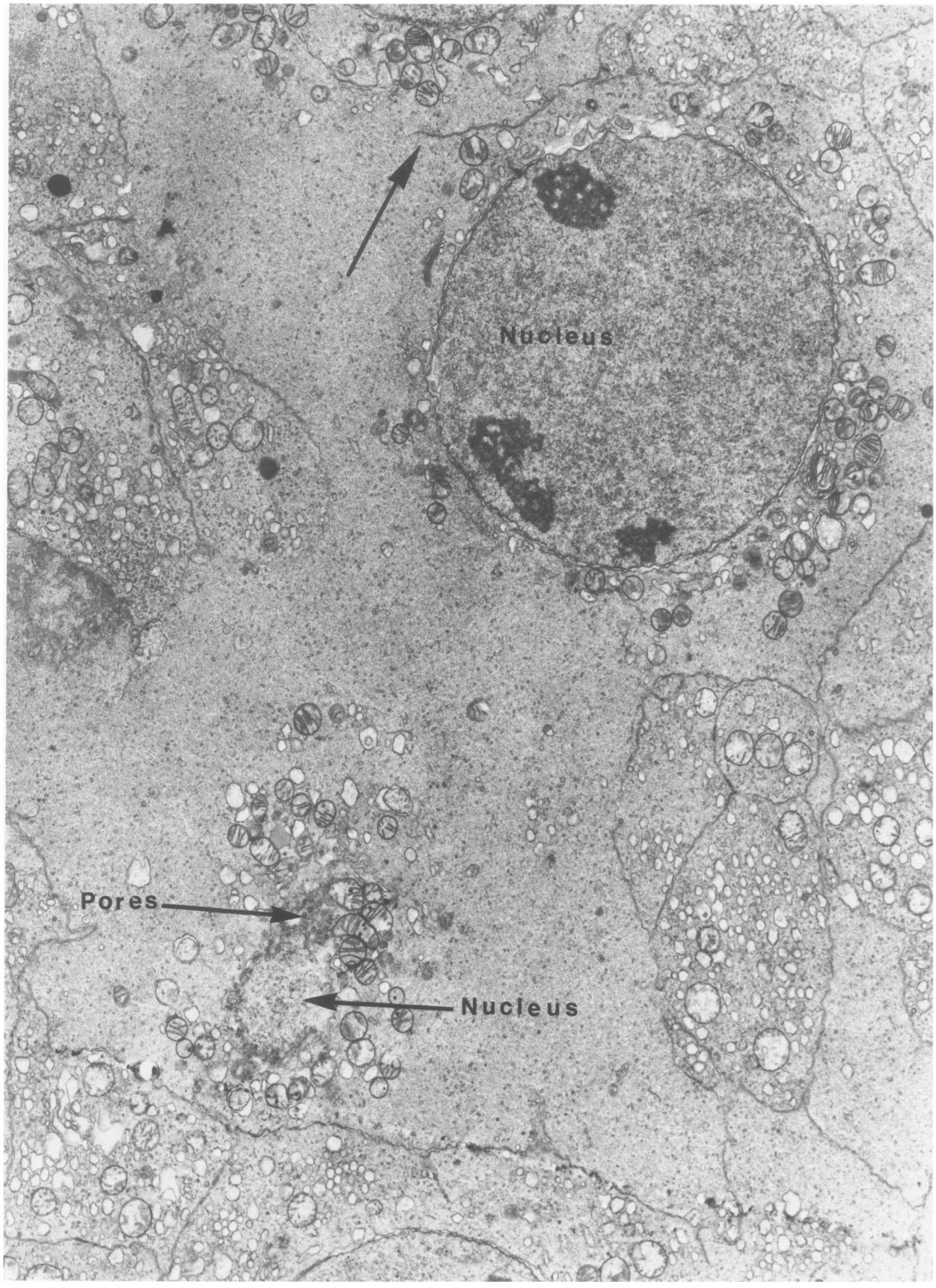

Figure 2—Electron micrograph of an atypical gonocyte aggregate within a testis cord at 17 days of gestation. Two gonocyte nuclei sharing a common cytoplasm are evident. The cell membrane adjacent to one of these nuclei is partially broken down (*arrow*). The abundant cytoplasm contains free ribosomes, vesicles, and lipid droplets. Mitochondria remain grouped around gonocyte nuclei. (×1100)

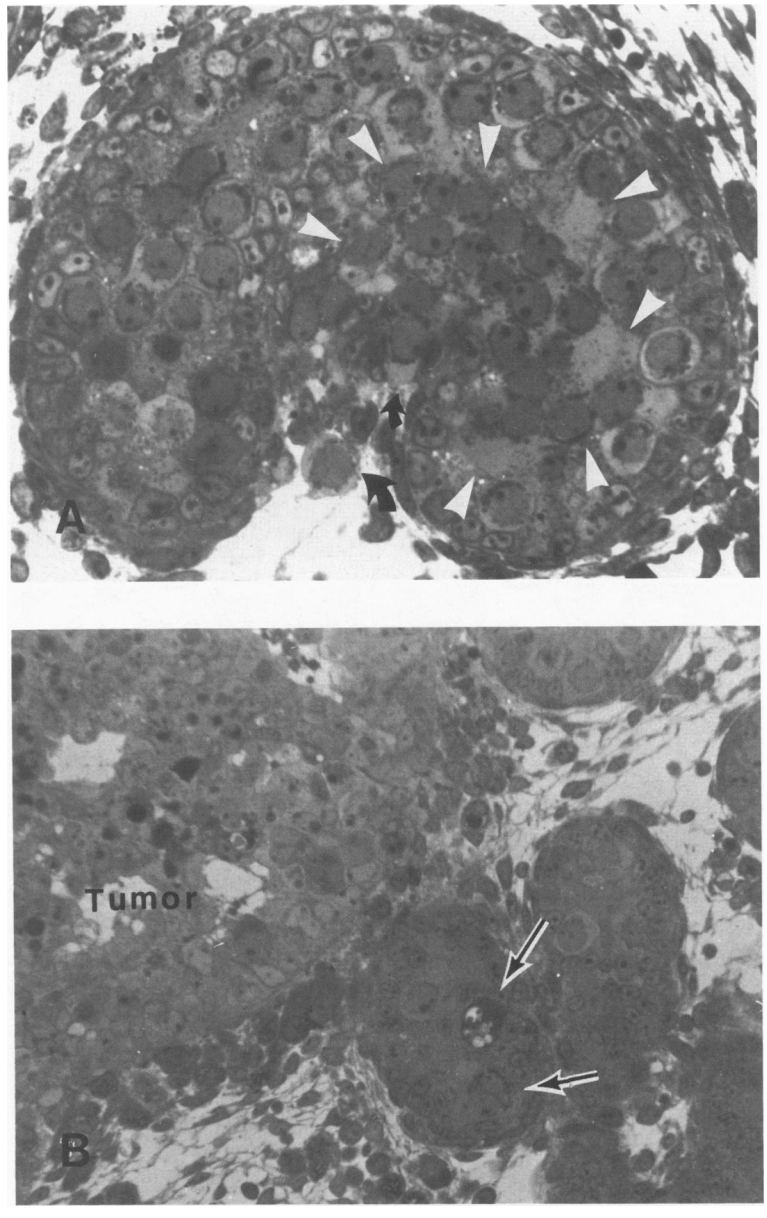

Figure 3A—Light micrograph of the testis of a 16-day-old embryo. The central region of a testis cord contains dispersed gonocytes with swollen cytoplasm and loss of cell borders (*white arrows*). Note the ruptured basement membrane, with atypical and normal gonocytes (*black arrows*) spilling into the interstitium. (×70) **B**—Light micrograph of the testis of a newborn animal. An advanced tumor (*left*) may grow simultaneously with testis cords containing necrotic cells and atypical gonocytes (*arrows*). (×40)

cytoplasm contained abundant free ribosomes, sparse endoplasmic reticulum, and scattered mitochondria (Figure 5).

Advanced Tumors in Strain 129/Sv-ter Animals

The more advanced tumors occupied interstitial areas of the embryonic testis and were frequently continuous with the seminiferous epithelium, indicating invasive rupture of the testis cord basement membrane (Figure 6). Solid nodules of ECC often contained apparently normal gonocytes interspersed (Figures 4 and 6). Tumors were not encapsulated, and cellular arrangements within tumor masses varied (Figure 7). Some cells showed evidence of polarity around a lumen, while other cells in the same tumor formed a disorganized mass. Cells circumscribing central lumens were better differentiated than cells in solid areas (Figure 8). Some luminal cells had irregular microvilli on their free surfaces. Terminal bars and profiles of endoplasmic reticulum were also present. These signs are believed to be the first steps in the differentiation of ECCs.[4]

In testes of neonates, extensive tumor development presented masses of ECCs aligned around extravasated blood and cell debris (Figures 7–10). Necrosis among tumor cells was evident, and ultrastructural features were consistent with a type of physiologic cell death termed "apoptosis" or "shrinkage necrosis."[11] Apoptosis takes place in two discrete stages. The first is formation of apoptotic bodies, involving nuclear conden-

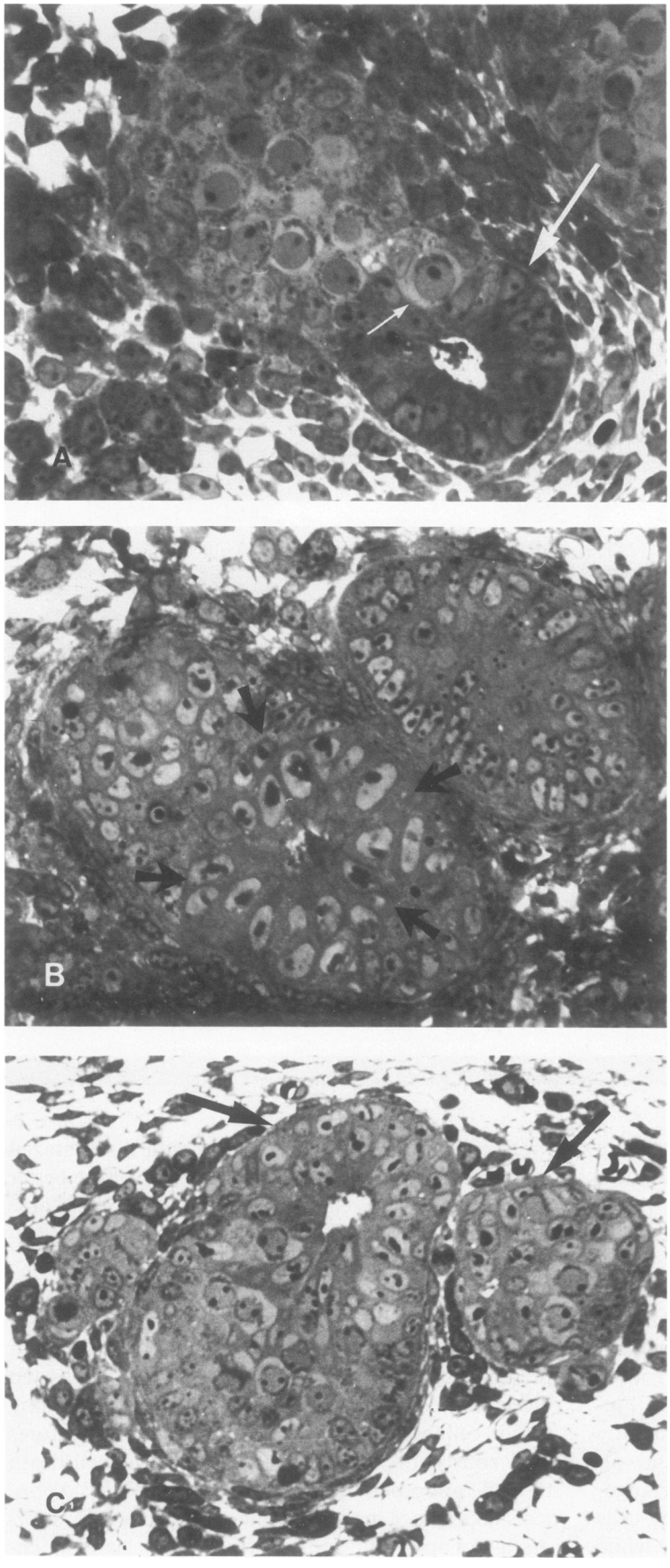

Figure 4—Light micrographs of embryonic testes illustrating small tumors, consisting of ECCs, forming vesicles within the testis cords. **A**—The vesicular structure of this tumor (*large arrow*) has begun to distort the testis cord. An apparently normal gonocyte (*small arrow*) appears to be part of the vesicle. Genital ridge at 14 days of gestation. (×60) **B**—A tumor (*arrows*) nearly fills the testis cord in a virtually sterile animal. Note the large, dense nucleoli of the ECCs. An adjacent testis cord contains only Sertoli cells. Testis at 18 days of gestation. (×60) **C**—ECCs (*arrows*) in adjacent testis cords, which become confluent. Testis at 19 days of gestation (×15)

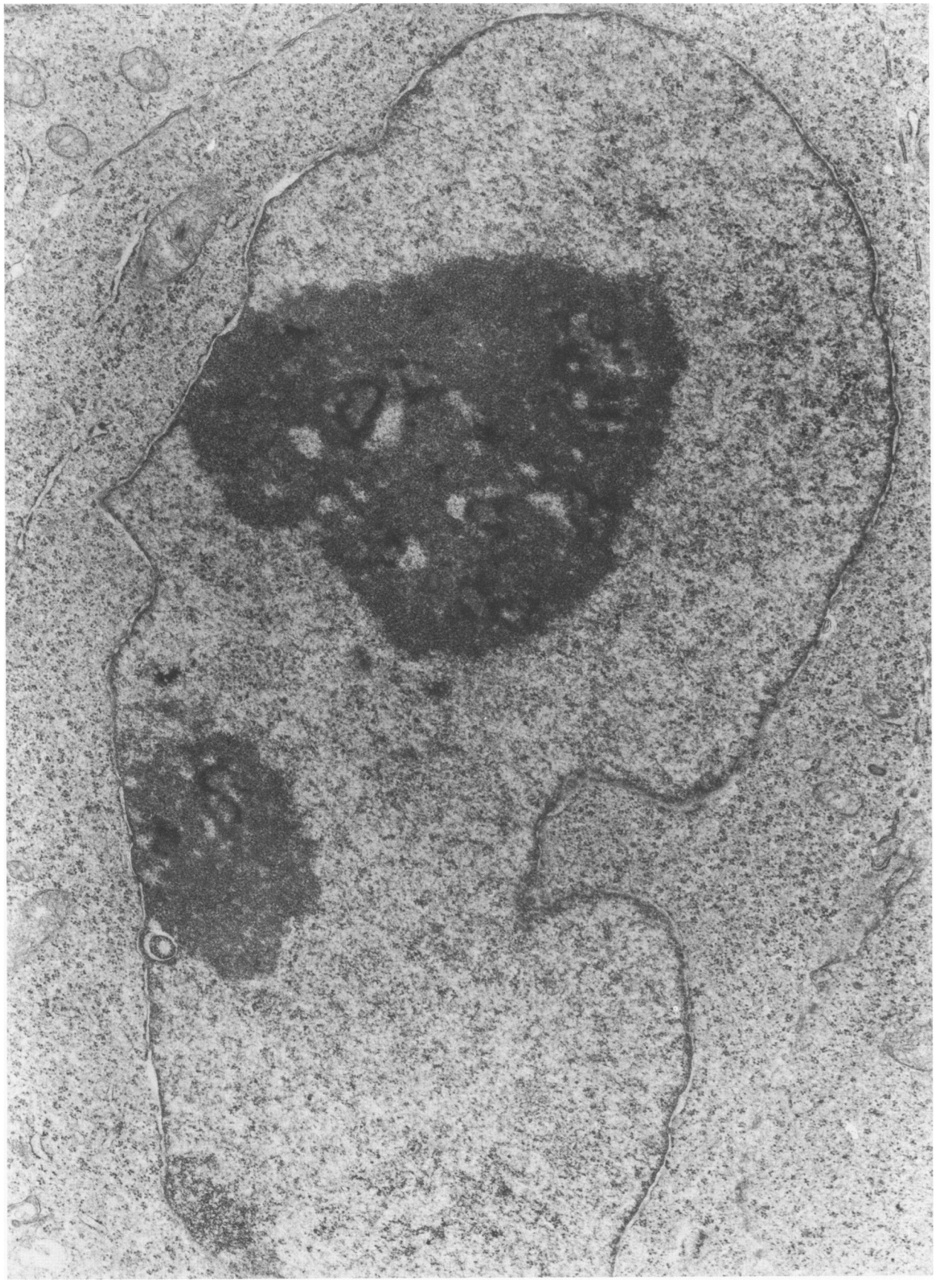

Figure 5—Electron micrograph of an ECC from a tumor within a testis at 17 days of gestation. The large nucleus is often indented and is characterized by very dense, persistent nucleoli. The cytoplasm is undifferentiated, as evidenced by the paucity of organelles, save free ribosomes. (×2600)

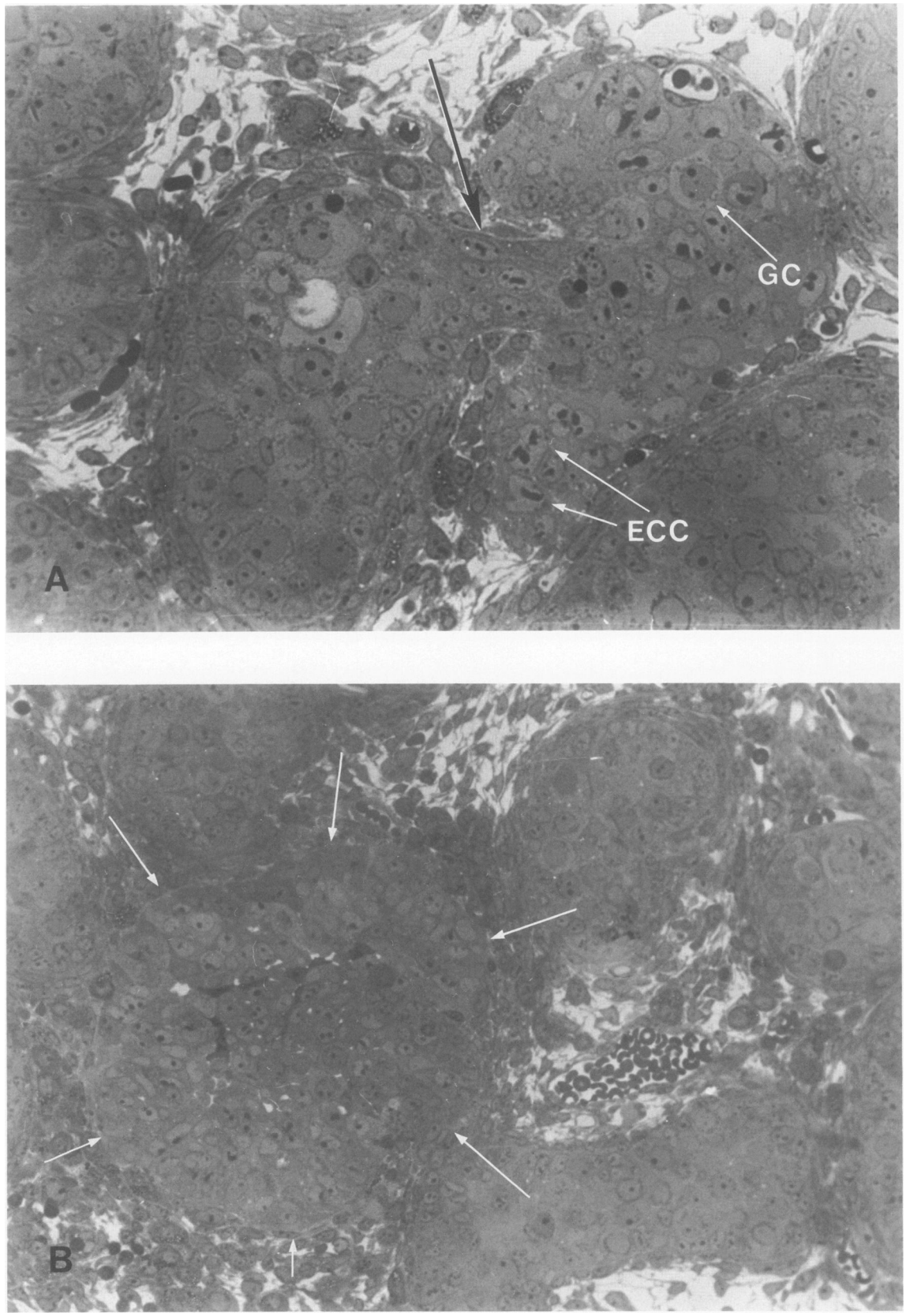

Figure 6—Light micrographs of advanced tumors expanding their growth into the interstitial areas of the embryonic testis. **A**—This tumor contains normal gonocytes (*GC*) interspersed with tumor cells (*ECC*) and can be seen "mushrooming" out of the testis cord (*arrow*). Testis at 17 days of gestation. (×40) **B**—This portion (*arrows*) of an advanced tumor consists of a solid mass of pleomorphic cells filling the interstitium between several normal testis cords. Testis at 17 days of gestation. (×50)

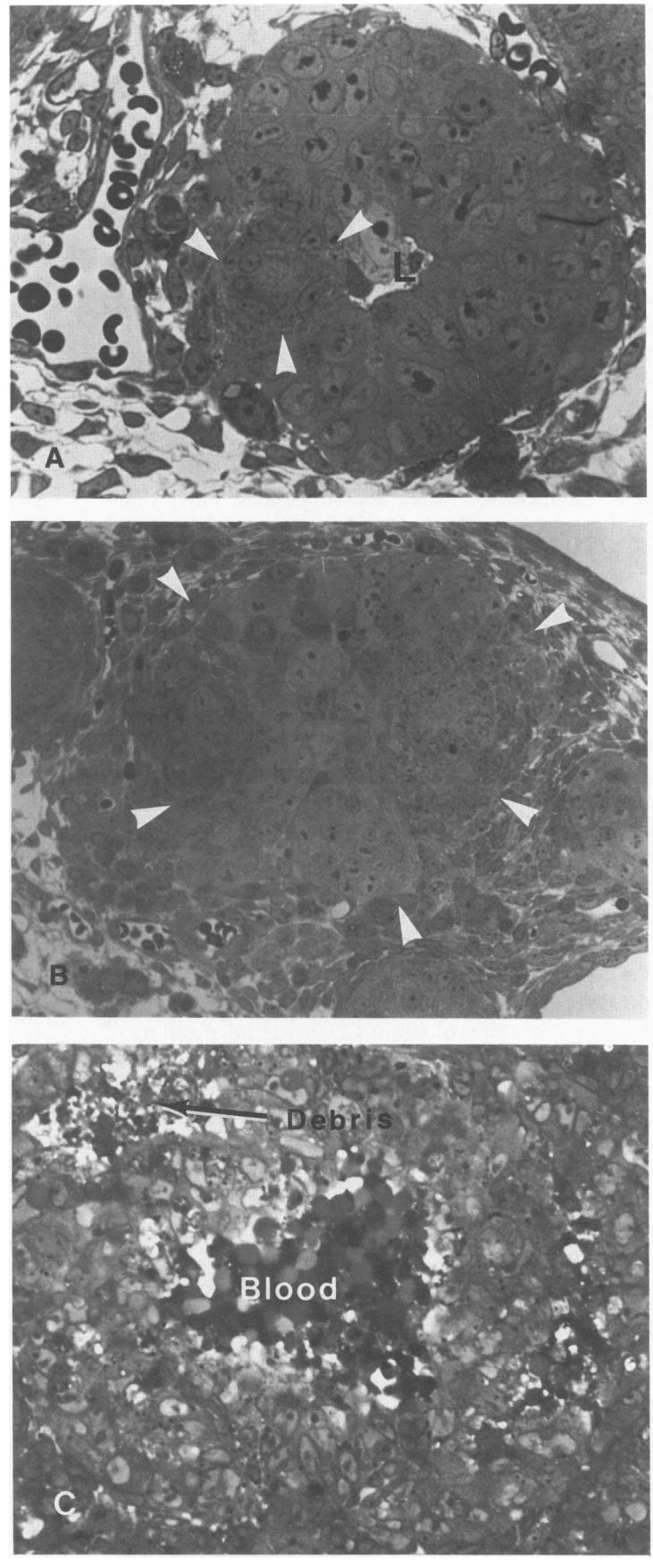

Figure 7—Light micrographs of advanced tumors illustrating the various cellular arrangements. **A**—The majority of ECCs are organized around a central lumen (*L*) in this tumor, although a few cells form a discrete nodule (*arrowheads*) within the main tumor mass. Testis at 17 days of gestation (×45) **B**—The tumor mass (*arrowheads*) is nonencapsulated and consists of several compact nodules of ECCs. Testis at 17 days of gestation. (×60) **C**—This extensive tumor presents areas of extravasated blood and cellular debris. Testis from a newborn animal (×55)

sation and fragmenatation (karyorrhexis) and cytoplasmic condensation. Cell remnants protrude from the cell surface and are shed in membrane-enclosed compartments whose contents continue to condense. Rapid phagocytosis of apoptotic bodies by adjacent cells marks the second stage. Apoptotic bodies become enclosed in a second plasma membrane as phagosomes undergo autolysis and final degradation by lysosomal enzymes to produce residual bodies.

Early stages of apoptosis occurring within the tumors are shown in Figures 9 and 10. Tumor cells begin to condense and separate from their neighbors. Debris in the lumens consists of apoptotic bodies shed from condensing cells. Evidence of phagocytosis of these bodies by neighboring tumor cells is shown in Figure 11. Contents of apoptotic bodies (within phagosomes) include dense masses of nuclear material, well-preserved mitochondria, fragmenting nuclei, and ribosomes. Early signs of degradation (Figure 12) are evidenced by swollen mitochondria containing flocculent material, breakdown of membranes, and the presence of lysosomes.

Control Animals

In a recent morphologic study of neonatal rabbit testes by Gondos and Byskov,[12] a description of degenerating germ cells was presented similar to our description of atypical gonocytes. For determination of whether this early germ cell phenomenon was unique to the tumor-bearing mice, a small sample of three testes from each of the 8 days of gestation was likewise examined microscopically for each of the mouse control groups: inbred strain 129/J and random-bred SW. Atypical gonocytes were found in testes of these mice as well, and the morphologic features were identical to those described for testes of strain 129/Sv-ter mice and neonatal rabbits. No ECC nests of progressively developing tumors were evident in the control animals.

Discussion

Atypical Gonocytes

The first morphologic study of testicular teratomas in embryonic mice was undertaken by Stevens in 1962.[7] Tissues were processed by conventional methods, with the use of an ethanolic Bouin's fixative, paraffin embedding, and section thicknesses of 6–8 μ. The youngest embryos examined were 15 days old. Tumors in 15-day testes were "completely enclosed by walls of the seminiferous tubules"[7] and consisted of "neoplastic cells forming discrete nodules that could be easily distinguished from adjacent normal testicular tissue."[7] These nodules were usually sharply demarcated from normal cells by a space, which Stevens attributed to processing artifacts. The description of the tumor cells could apply to either gonocytes or ECCs: nuclei approximated the size of germ cell nuclei, and the nucleoli were larger than those of the germ cells. No details of the tumor cell cytoplasm were given, however, except to say that these cells were undifferentiated. Stevens has routinely used the same histologic preparations to continue his work with teratomas in Strain 129 mice. The youngest gonads thus far examined were taken from 14-day embryos, strain 129/Sv-ter. In this subline he was unable to recognize tumors in gonads from embryos younger than 16 days of gestation.[8]

We decided to reexamine developing embryonic testes in strain 129/Sv-ter mice to obtain a clearer understanding of the early stages of teratocarcinogenesis and the histologic appearance of the testis during this period. Our approach differed from that of Stevens in two ways: 1) we included gonads from 13-day embryos, whose gonocytes should be most susceptible to teratocarcinogenesis; and 2) we improved tissue preservation and resolution using a method which included glutaraldehyde fixation, plastic embedding, a maximum section thickness of 1.5 μ, and ultrastructural studies.

The earliest histologic changes that we noted in testes of strain 129/Sv-ter embryos were atypical gonocyte aggregates. These may be identical to the neoplastic nodules described by Stevens, with our method providing additional details: nuclei within the nodules could be gonocyte nuclei, and his artifactual space could be represented by the swollen, dispersed gonocyte cytoplasm. Several factors, however, led us to interpret our findings cautiously before attempting to correlate this phenomenon to early tumorigenesis. First, we encountered identical morphologic changes in gonocytes in our control animals as well. To our knowledge, such an association of primitive germ cells has not been described in mammalian embryos. Second, gonocytes appeared in these configurations throughout the embryonic period of testicular differentiation—the phenomenon was not restricted to gonocytes in the susceptible phase of teratocarcinogenesis (Day 13). Third, our account of gonocyte kinetics in mouse embryos concurs exactly with special features of prespermatogenic germ cells in neonatal rabbits.[12] In both animals this germ cell behavior follows peaks of mitotic activity. Perhaps the prolonged postnatal prespermatogenic period in rabbits[13] allows for a requisite step in germ cell kinetics that occurs prenatally in mice. Whereas these authors classify such gonocytes as degenerating, we hesitate to agree. Germ cell degeneration in embryonic and neonatal testes has been repeatedly described as involving nuclear breakdown, cytoplasmic degradation, and phagocytosis by supporting cells.[14–18] If these atypical germ cells are connected with degeneration, then they

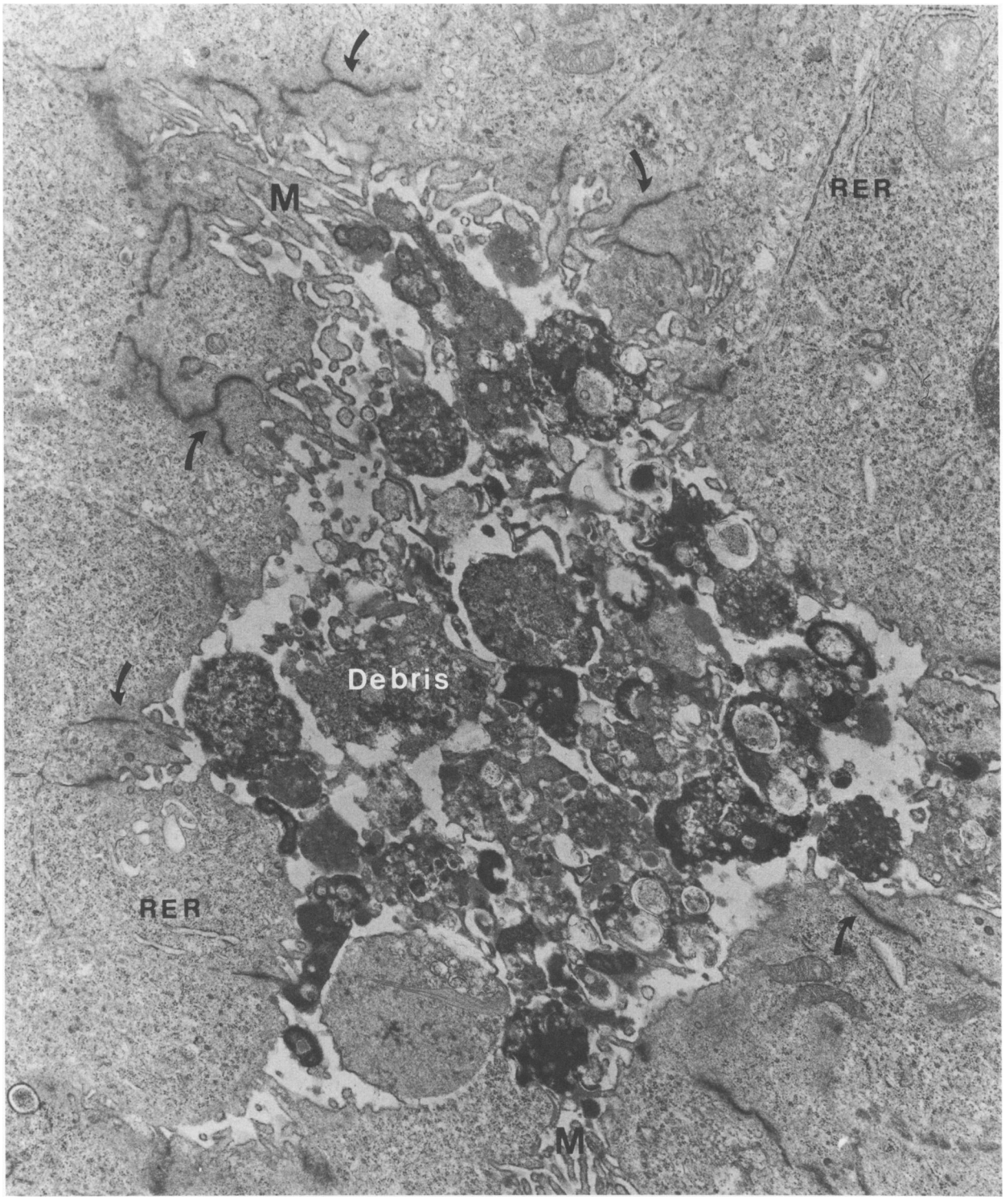

Figure 8—Electron micrograph of portions of ECCs circumscribing a debris-filled lumen. There are signs of epithelial differentiation evident: profiles of rough endoplasmic reticulum (*RER*), microvilli (*M*) on the free surface of the plasma membrane, and terminal bars (*arrows*) near the luminal border of the cell. Tumor within a testis at 17 days of gestation. (×1500)

might represent a stage immediately prior to the more obvious signs of degradation.

A fourth consideration was to compare our findings with descriptions of atypical germ cells present in human testicular biopsy material. Skakkebaek first detected abnormal germ cells in seminiferous tubules of infertile men who later developed embryonal carcinoma.[19,20] These atypical germ cells satisfied some of the criteria of malignancy: irregular mitoses, increased DNA content, enlarged nucleus and nucleoli, higher

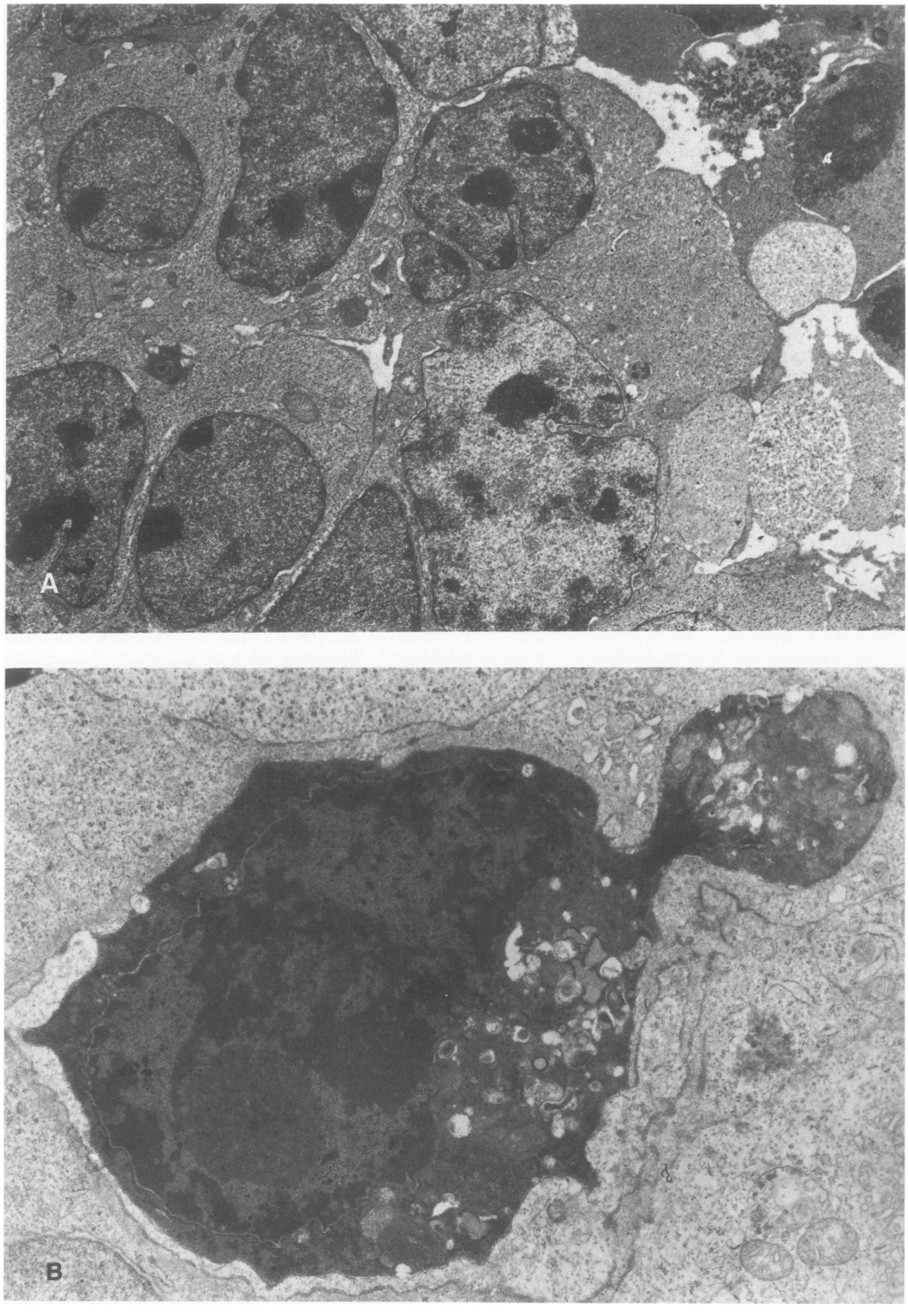

Figure 9—Electron micrographs of the initial stages of apoptosis occurring within advanced tumors in neonatal animals. **A**—ECCs border a lumen in which debris (*upper right*) consists of clusters of apoptotic bodies. (×1200) **B**—The formation of apoptotic bodies begins with nuclear condensation, followed by cytoplasmic condensation. This condensing tumor cell takes on an ameboid form as it begins fragmenting. (×4000)

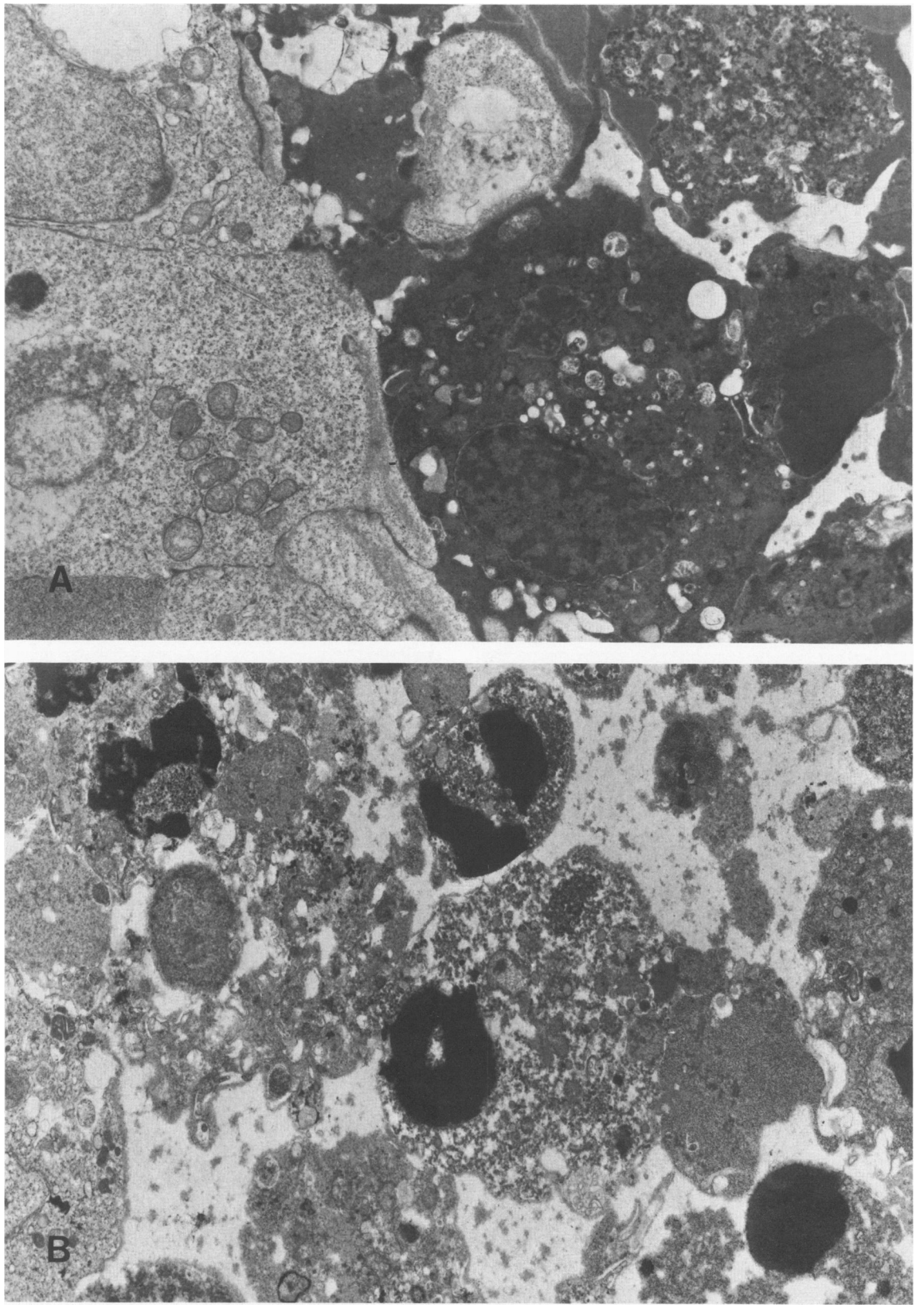

Figure 10—Electron micrographs of the initial stages of apoptosis occurring within advanced tumors in neonatal animals. **A**—Condensing tumor cells (*left*) border a lumen which contains newly shed apoptotic bodies (*right*). The apoptotic bodies (cell remnants) continue condensing and will soon be phagocytosed by neighboring tumor cells. (×2600) **B**—Lumen of an advanced, necrotic tumor filled with extracellular apoptotic bodies. Dense nuclear remnants are present in some bodies but not in others. Note the autolytic changes in cytoplasmic remnants. (×2800)

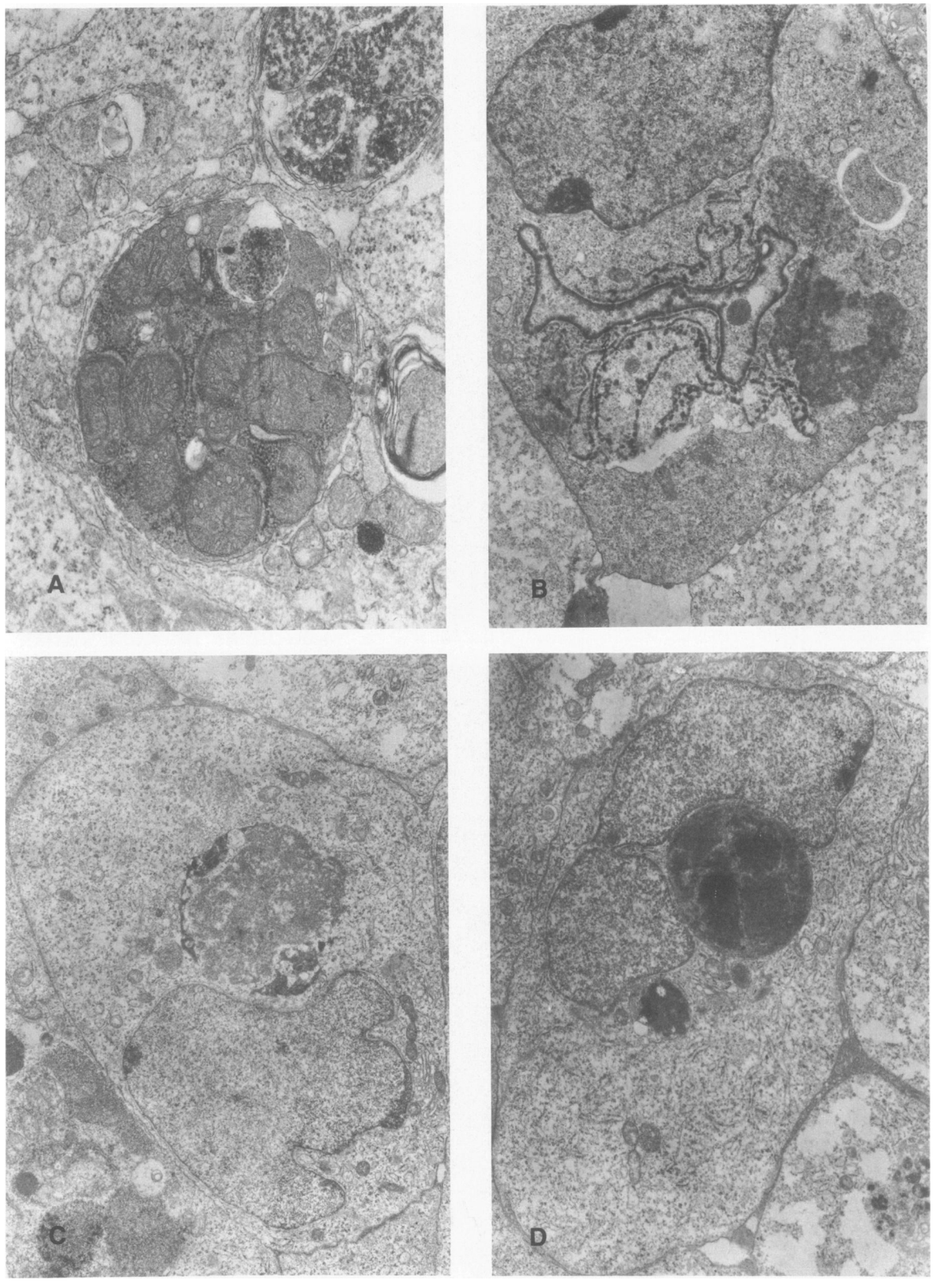

Figure 11—Electron micrographs of the later stages of apoptosis occurring within advanced tumors of neonatal animals. Apoptotic bodies within membrane-bound phagosomes fill the cytoplasm of tumor cells which ingested them. **A**—An apoptotic body contains well-preserved mitochondria. (×2500). **B**—A tumor cell has ingested nuclear fragments exhibiting condensed, marginated chromatin and condensed nucleoli. (×2250) **C** and **D**—Adjacent to the viable nuclei of these tumor cells lie apoptotic bodies containing pyknotic nuclear remnants. (**C** ×3200; **D**, ×2900)

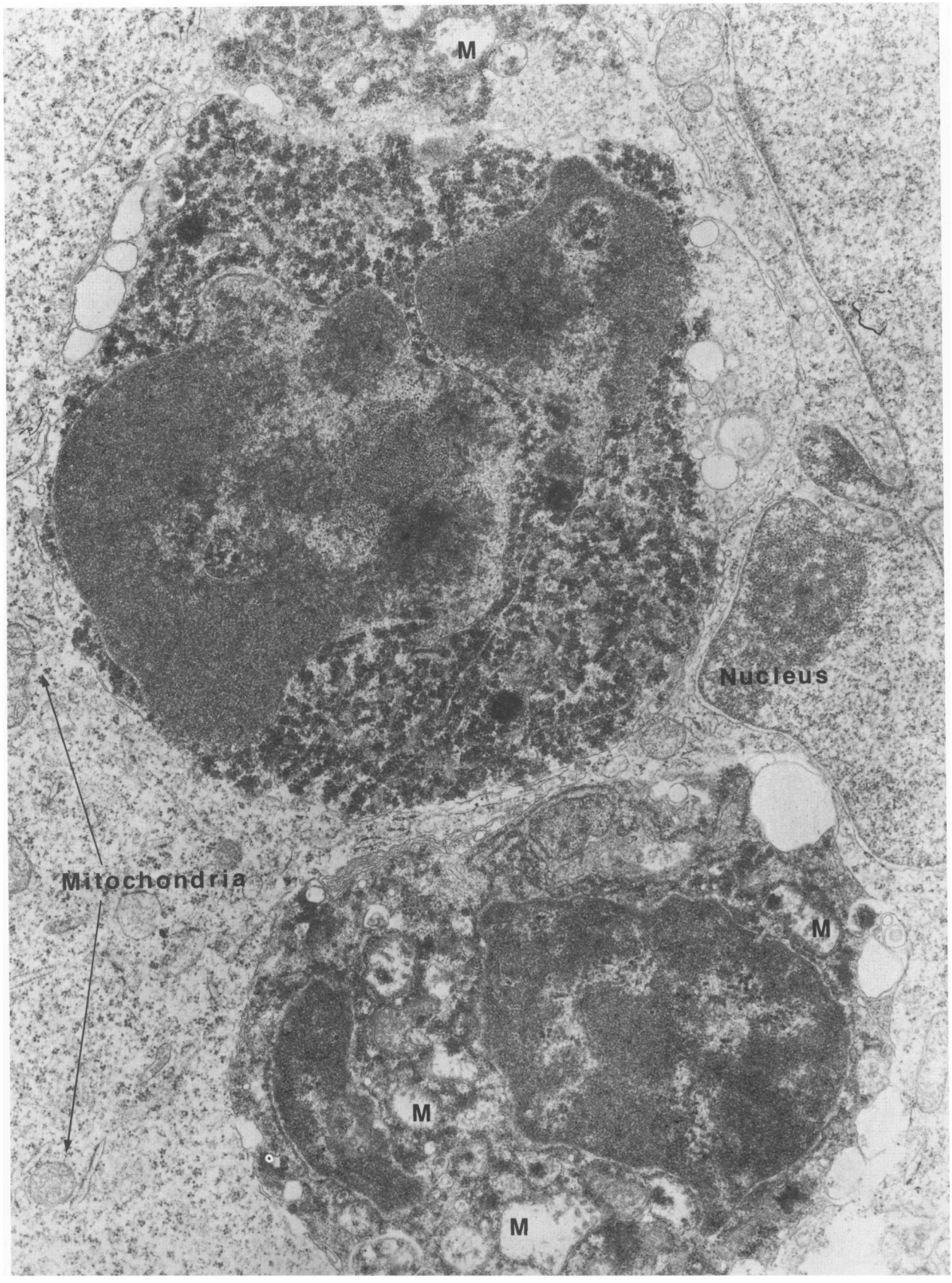

Figure 12—Electron micrograph of the later stages of apoptosis occurring within an advanced tumor of a neonatal animal. A tumor cell has phagocytosed apoptotic bodies containing condensed nuclear remnants, endoplasmic reticulum, ribosomes, glycogen, and swollen mitochondria (*M*) containing flocculent material. Parts of the membranes that originally surrounded the bodies are intact; most of them have begun to disintegrate (×1700)

density of free ribosomes and rough endoplasmic reticulum, and invasion of the interstitial tissue. It was suggested that the abnormal germ cells represented carcinoma *in situ*, a malignant stage of germ cells preceding the development of all types of germ cell tumors.[21] The morphologic characteristics of our atypical gonocytes are comparable to those seen in human tissue except for the formation of syncytial masses. Furthermore, we observed atypical gonocytes in normal mice, whereas atypical germ cells are normally not found in the human testis.[22]

The arrangement of mouse gonocytes in a multinucleated cytoplasm merits further discussion. Two mechanisms could account for the formation of a syncytium. Dividing cells are more vulnerable to disruption than resting cells,[23] and it is possible that a syncytium could form as the result of incomplete cytokinesis of dividing gonocytes. The fact that mouse germ cells cease dividing from about Day 15 of gestation until birth[6] and the fact that primordial germ cell settling into the genital ridges at the end of their migration (Day 12) are mostly independent of one another[24] make it improbable that gonocyte syncytia are the result of interruptions in the cell cycle. The alternate mechanism involves a coalescence of gonocytes by breakdown of cell boundaries. Our ultrastructural examination of the syncytium indeed revealed degenerating cell boundaries between adjacent cells (Figure 2). The significance of germ cell syncytia remains obscure. Formation of a syncytium may be a normal phase of germ cell maturation that permits passage of numerous cells through development or degeneration in a coordinated fashion.

Another important observation made here, which was also made in the neonatal rabbit, concerns the location of atypical gonocytes near the rete tubules. Gondos and Byskov,[12] who believed these germ cells to be degenerating, suggested that the rete network provides a route for expulsion of lysed cells. The rete testis/ovarii is believed to derive from the mesonephros,[25] and its embryonic functions are still being investigated. In cocultures of fetal rabbit testis with mesonephros, Grinsted et al[26] have shown that mesonephric tissue lowers the amounts of testosterone originally released into the medium by the testis, probably by accumulation of the steroid. The human rete ovarii is known to be influenced by hormones,[27] and the possibility cannot be excluded that rete tubules may act as mediators between systemic hormones and germ cells. We pose the possibility that the rete may be involved with the variants of germ cell development: the effect on cells located near the rete could be direct, through a localized concentration of testosterone, or indirect, through mediation of systemic hormones.

Interstitial germ cells observed in our study corroborated observations of atypical germ cells in human testicular biopsies[20] and gonocytes in late fetal rabbit testes.[28] Extratubular germ cells may represent 1) primordial germ cells detained en route during their migratory phase which failed to become enclosed within the testis cords or 2) a second migration of germ cells out of the testis cords. We interpret our observation as a second migration, involving basement membrane penetration. The significance of the extratubular migration could be to decrease gonocyte numbers in the developing testis cords or to become potential foci of extragonadal teratomas.

Teratocarcinomas

The undifferentiated stem cells (ECCs) of teratomas were easily recognized in our embryonic testis preparations. These cells were characteristic of ECCs previously described[4,29] and were only found in testes of Strain 129/Sv-ter mice. The most distinctive morphologic feature which set ECCs apart from other cells within the testis cords was the large, dense nucleolus. This observation has been corroborated in studies with ECC cultures. Sheldon et al[30] studied a series of ECC lines and found them to exhibit nucleolar persistence: nucleoli were retained through metaphase and anaphase instead of disaggregating upon dissolution of the nuclear membrane during prophase. These nucleoli maintained a low level of ribosomal RNA transcriptional activity throughout mitosis. When these cells differentiated, either spontaneously or upon chemical induction, the level of nucleolar persistence dropped significantly.

Early teratomas are regularly accompanied by cytolyzed material and hemorrhage into the testis.[31,32] Spontaneous loss of cells is an important parameter in neoplastic growth as well as in the cell turnover of healthy adult tissues, normal embryogenesis, and atrophy/involution of tissues. It is agreed that cell death accounts for most of the loss, although little is known about the mechanisms involved. The ultrastructural signs of degeneration described in our tumor material were consistent with a type of necrosis termed apoptosis.[11] Unlike coagulative necrosis, which appears to be invariably caused by noxious stimuli and involves intracellular autolysis and inflammation, apoptosis occurs spontaneously or in response to physiologic stimuli and involves phagocytosis by neighboring cells without an inflammatory response. Its significance in tissue kinetics lies in its mechanism for controlled cell deletion, being of equal importance to mitosis in the control of cell populations. Growing malignant neoplasms progressively increase their cell numbers; yet continuous cell death is also an inherent property in these growths. Our belief that apoptosis is active in the growth of teratomas can

be supported by detailed investigations of the associated morphology in other tumors: mouse ascites tumors,[33] human basal cell carcinomas,[34] rectal adenocarcinomas,[11] and carcinomas of the uterus[35] and lung.[36] Pierce et al[4] reported membrane-bound, necrotic inclusions within ECCs of induced murine teratocarcinomas, and they inferred that tumor cells engulfed other cells. Little is known about the factors that initiate apoptosis or of the cellular mechanisms activated prior to the morphologic changes. This phenomenon probably involves an interaction between inherent cell programs and environmental cues such as diffusable substances. Apoptosis in response to steroid hormone administration or withdrawal is common in mammalian tissues: rat prostate,[37] hamster uterine epithelium,[38] rat adrenal cortex.[39] The possible effects of fetal androgens and/or maternal steroids on regulating apoptosis in both normal and malignant embryonic tissues awaits further study. Besides balancing the rapid rate of cell proliferation occurring within tumors, cellular degeneration may be a factor in the genesis of spontaneous[31] and metal-induced[40,41] teratomas in fowl and mouse testes.

A relatively unexplored factor in teratocarcinogenesis and germ cell kinetics concerns the fetal endocrine milieu. Leydig cells are functional soon after testicular differentiation — Day 13 in the mouse[42] — and may very well be a contributing factor in tumorigenesis and/or formation of atypical gonocytes. Data concerning the role of hormones in teratocarcinogenesis have relied on experimental induction of tumors and are not wholly conclusive. Prior to the discovery of spontaneous teratomas in mice, such tumors were chemically induced in adult fowl testes by administration of metal salts.[40,43] Fowl testes were most susceptible to treatment in the spring, when testicular hormones increased spermatogenesis. Similar experiments combining chemical trauma with a background of hormonal stimulation were successful in mice.[41] Stevens[44] addressed the hormone problem in his studies of tumor induction by genital ridge grafting in mice. He concluded that in male genital ridges grafted to adult sites (scrotal testes, epididymides of intact or castrate males, ovarian fat pads plus testosterone injections), teratocarcinogenesis could occur in the absence of host testicular hormones. Stevens, however, did not consider the fetal Leydig cells contained in genital ridge grafts which might develop normally within the grafts and contribute necessary androgen stimulation.

Two experimental approaches would allow further studies of the behavior and interactions of primordial germ cells. Mouse primordial germ cells can be isolated and cultured *in vitro*.[45] Grafting experiments could be repeated with the use of isolated primordial germ cells instead of intact genital ridges. Direct endocrine effects on primordial germ cells could be simulated by various hormones *in vitro*. A different avenue of research involving incubations of embryonic testes would allow comparisons of androgen synthesis and metabolism between normal animals and those genetically susceptible to cancer.

While the present study clearly illustrates the morphologic changes involving gonocytes and spontaneously occurring tumor cells in embryonic testes of Strain 129/Sv-ter mice, there is no evidence to support any implication that atypical gonocytes are precursors to tumor cells. We did not observe a spectrum of morphologic abnormalities or cells intermediate between atypical gonocytes and ECCs. Atypical gonocytes may simply be a variant of normal gonocyte development.

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