Light- and Electron Microscopic Studies of the Renal Carcinoma Induced in Syrian Hamsters by Diethylstilbestrol

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LIGHT- and electron-microscopic studies were made on the renal carcinoma of golden Syrian hamster which was induced by diethylstilbestrol (DES) and some characteristic features such as clusters of fibrillar material, cilia-formation and intranuclear bodies were noted in addition to various changes of cytoplasmic organizations.

A presence of the fibrillar material is probably representing ciliary development which indicates a process of dis-differentiation during carcinogenesis. The occurrence of intranuclear bodies was considered to reflect a nuclear hyperactivity of the entire kindney tissues that was induced by DES rather than a change which is directly associated with the carcinogenesis. Anaplastic cells which had intracytoplasmic lumen suggested that the neoplasm preserves a property of forming glandular structure. Biological and morphological characteristics of this tumor are in agreement with the reports by other investigators.

INTRODUCTION

Numerous chemical agents (nitrosoamine, aromatic amines, afratoxin -B, lead acetate, cycasin, estrogen, etc.) are capable of inducing renal tumors in experimental animals.[1)-7)] The tumors of various histologic types, adenoma, carcinoma, benign non-epithelial tumor and sarcoma can be induced. However, it is interesting to note that there is a correlation between carcinogen and histological structures of the induced neoplasms. Diethylstilbestrol (DES), for example, is known to produce tumor which preserves some morphological characteristics of the proximal convoluted tubular epithelium[5),7)]. Although there are many reports on histological study of DES-induced renal carcinoma, ultrastructural studies are limited and, to the knowledge of the present author, there is only one paper by Mannweiler & Bernhard[7)]. They described the presence in the

tumor of brush borders, microvilli, intercellular digitation, desmosome and cilia.

The present report deals with light-and electron-microscopic investigation of DES-induced renal carcinoma of male golden hamsters. In addition to cellular pleomorphism and other findings described by Mannweiler & Bernhard, some other features that appeared rather specific to this tumor were noted such as clusters of fibrillar material, intranuclear bodies, and intracytoplasmic lumens.

Although carcinogenetic action of estrogens is now well known and extensive investigations have been undertaken to clarify their molecular interaction with cellular constituents, there remains much to be studied about morphological expression of estrogen action[8,9]. Not all of the findings to be described in this report reflects essential process leading to tumorigenesis, but some may simply be coincient changes. However, the significance of these changes will be discussed in relation to dis-differentiation of neoplastic cells.

MATERIALS AND METHODS

Male golden hamsters of indeterminate ancestry, bred in this laboratory, were used in this experiment. Fifteen hamsters, all approximately 6 to 7 weeks of age, were taken at random and each were given subcutaneous injection of 0.6 mg DES solved in 0.2 ml of sesam oil every other day. The hamsters were killed on the 200th, 250th, 300th day and 350th day of DES-treatment and their kidney specimens were processed for electron microscopy.

Two methods of fixation were employed. In one experiment the animals were killed by decapitation. Small pieces of neoplastic kidney were fixed in buffered 2% glutaraldehyde for 2 hr and then postfixed in buffered osmium tetroxide. In another experiment animals were anesthetized with ether. The kidney was perfused by way of the renal artery in situ with 2% glutaraldehyde in cacodylate buffer. The kidney were then removed carefully and well-fixed areas were cut into 1 mm cubes. These cubes were fixed again in buffered 2% glutaraldehyde and postfixed in buffered osmium tetroxide.

Each of the fixed specimens was rinsed in phosphate buffer and dehydrated in rising series of 45, 70, 90 and 100 per cent ethylalcohol. The total time for dehydration was approximately 120 min. Finally, the specimens were passed through propylene oxide. The samples were immersed first in a propylene oxide-Epon mixture for 4 hr and then in

fresh Epon for 2 hr. Epon was polymerized at 35°C, 45°C and 60°C total time of polymerization being 48 hr. Thin sections were cut on a Porter-Blum MT-2 Type ultramicrotome with a JEM 100-Type electron microscope.

In addition, semithin sections from the blocks for electron microscopy were examined with light microscope after toludine blue staining. Routine histological studies of the same tumor were also performed in parallel.

RESULTS

Changes of the renal cortex were noted with light microscope from 150th day of DES-treatment. There were scattered areas of tubular epithelium which showed increased basophilism the cytoplasm and contrasted with the adjacent normal proximal tubules. After this period, some tubular epithelium in the cortex exhibited a proliferative activity with slight cellular atypism. They projected into the lumen, though not penetrated the tubular basement membrane. After 200th days of treatment, solitary or multiple well-demarcated nodular lesions were visible in the kidney. The majority of these were small, white, and solid masses measuring from 0.5 to 2 mm in diameter. Histologically, they consisted of anaplastic cuboidal or columnar cells arranged in sheet, trabecular or tubular fashion (Figs. 1, 2, 3). After about 300th to 350th days, the neoplastic nodules were markedly increased in size and in number. The tumor showed a tendency of de-differentiation and their microscopic features varied greatly in different parts of the same tumor. In some tumors there were areas of anaplastic spindle-shaped cells which resembled sarcoma (Fig. 4). In larger tumors, the central portion was often necrotic, hemorrhagic areas or cysts were found frequently. The nuclei were hyperchromatic but mitoses were infrequent (Fig. 3). The metastatic lesions were occasionally seen in the splenic hilum, parietal peritoneum and liver parenchyma. For the details, see to the previous report.

Ultrastructural Findings The DES-induced renal tumor was composed of atypical cells growing in coherent sheets, tubular or acinar fashions. They were cuboidal or polygonal and exhibited a large nucleo-cytoplasmic ratio (Figs. 5, 6). The ultrastructural characteristics varied greatly from moderately well-differentiated to apparently anaplastic types and cellular polarity was lost completely. The nuclei were frequently lobed or irregular with infolding of the nuclear membrane and showed pleomorphism like the cells themselves (Fig. 7). Nucleoli were smaller and less than those of non-neoplastic counterpart although their morphological abnormality was unremarkable. The cytoplasm was abundant and

organellae were relatively sparse (Fig. 7). Infoldings of basal laminae of normal tubular epithelium were lost, Mitochondria were reduced in number and their morphology was markedly variable, some being bizarre-shaped (Fig. 10). Their features were rather characteristic in that they were swollen with a paucity of cristae and a decrease in the density of mitochondrial matrix (Fig. 10). Like in other primitive cells endoplasmic reticulum was unorganized and free ribosomes were increased (Fig. 7). Golgi apparatus was well-developed in most tumor cells (Fig. 11). The tumor cells existed in close apposition and were inter-locked with cytoplasmic invaginations and protrusions (Fig. 7). Interspersed among these cells there were sometimes encountered dark cells whose cytoplasm as well as the nucleus showed higher electron density with osmic tetroxide staining (Fig. 5). On the average they were smaller than other tumor cells. Complex cellular interdigitations and desmosomes were distributed at variable intervals along cellular margin (Fig. 7). On the lateral surface of cells or in the intercellular space there were observed irregular microvilli-like projections in which fine filamentous structures were enclosed (Figs. 6, 8). Some anaplastic tumor cells had intracytoplasmic lumen which contained a considerable number of cilia and microvilli as described above (Fig. 8). Secrectory granules or pinocytotic vesicle could scarcely be seen. However, a presence of lipid or glycogen droplets was frequent. Noteworthy features in this tumor were 1) intracytoplasmic cluster of fibrillar material, 2) cilia-formation and 3) peculiar intranuclear inclusions.

Intracytoplasmic clusters of fibrillar material. In some tumor cells cluster of fine fibrils measuring up to 35 A° in diameter was seen in the cytoplasm close to the nuclear membrane (Figs. 11, 13). The fibrillar material was arranged in whorl and did not show a regular architecture. The fibrils differed from amyloid or myogenic fibers. Punctate density in the cluster could be interpreted as representing cross sections of the fibrils. The fibrils were not enclosed in limiting membrane and merged inperceptibly with the surrounding cytoplasmic matrix (Fig. 12). In the circumference of the cluster free ribosome were found increased and centrioles and cilia were observed occasionally (Fig. 11). However, they were otherwise unremarkable.

Cilia-formation. Cilia were found in the cytoplasm singly or in group. The fascicles of cilia were enveloped by membrane (Fig. 9). On cross section the cilia showed characteristics structure with two central and nine peripheral filaments surrounded by stalk-membrane (Fig. 9). However, typical structure of basal plate, basal body or rootlet fiber which extends from the basal body could hardly be identified. Sorokin reported that in the early stage of ciliary-genesis a daughter centrioles (basal body)

are formed by the extension of fibrillar material from centriole, the a vesicle is formed at the distal end of a basal body and a ciliary bud appeared beneath it (Fig. 10). In the present investigation, however, no evidence was afforded which is particularly in favor of his thesis.

Intranuclear inclusion. Spherical bodies measuring from 0.4 to 2μ in diameter were frequently observed within the nuclei of tumor cells. They existed singly or in group and were scattered at random (Figs. 14, 15). Despite of a considerable morphological variation, they had central core and outer capsule. The central core was made up of finely fibrillar materials arranged in whorl with or without dense granules but sometimes it appeared homogeneous (Fig. 14). The granules were similar to chromatin granules. The outer capsule was composed of microfibrils and granules which resembled ribosome. The surface was covered by a zone of fluffy substance. Surrounding the capsule or in between the central core and the capsule, an electron lucent halo was frequently noted. The intranuclear inclusions were common in the neoplastic cells but they were occasionally found in the mesenchymal or endothelial cells as well (Fig. 16). Neither virus particles nor any other change suggestive of viral infection were found both in neoplastic or in non-neoplastic tissues.

DISCUSSION

Basic histological pictures of this tumor were common with those described previously[5,7]. The tumors were composed of atypical polygonal cells arranged in coherent sheets, trabecular or tubular fashions. Although this tumor exhibited a wide variety of histological appearances, it can be regarded as an expression of anaplasia rather than a constillation of different origins. The early changed (about 20 weeks after administration) were basophilization and proliferation of the proximal convoluted tubular epithelium. The lesions of similar nature have been reported frequently[5,7,8]. It is not apparent, however, whether it is simply hyperplasia or precancerous change. After the basophilization and proliferation occured, small cellular clumps consisting of atypical polygonal cells appeared in the interstitium of the renal cortex, although the evidence that the clumps were derived from the proliferative tubular epithelium was lacking. With advance of time tumors were increased in size and they became less differentiated showing sufficient pleomorphism to be diagnosed as malignant. Some investigators are in favor of the theory that tubular cyst is a necessary precursor in the natural history[10]. In the present investigations, however, there was no renal cyst.

Before processing for electron microscopy, semithin sections were

checked with light microscope in order to rule out a possible error of observing a non-neoplastic portion. In spite of many years of electron microscopic research of cancer cells, no specific and universally acceptable structural changes have ever been detected. Bernhard had stated that the ultrastructural features of malignant cells in early stages of its transformation are not strikingly different from those of the homologous normal cells[11]. Although some molecular abnormality may be present from the early stage of tumorigenesis, it will be only after a certain number of cell divisions that it is manifested morphologically. Nevertheless, it is well known that characteristic morphological changes common to many different kinds of tumors are an increased clumping of chromatin, swelling of the mitochondria and endoplasmic reticulum, irregular cisternae of membranous organellae, appearance of lipid, intracytoplasmic and intranuclear inclusion, or viral particles. Nuclear membrane will have some bearings with a regulatory mechanism of transport and its irregular invagination may facilitate nucleo-cytoplasmic exchange of substance. Perichromatin granules were increased in number but were uniform in size. They are considered to contain RNA and protein, although their function is still unknown [12]. In contrast to many other malignant tumors, nucleoli of this tumor were decreased in number and in size. Swelling of mitochondrial matrix, accompanies by pallor and loss of granules, seems to be the result of increased water uptake and is expected to counterbalance a difference of osmotic pressures between the mitochondrial matrix and the cytoplasm [13]. Dense granules are thought to be binding sites for cation especially for Mg[14]. It is known that the mitochondria are more fragile and more liable to structural alterations in neoplastic cells than non-neoplastic counterparst. Morphological changes of tumor cell mitochondria are considered to reflect biochemical abnomalities. In the present investigation, swelling of mitochondria and paucity of cristae were observed and various forms of abnormal mitochondria were encountered. Endoplasmic reticulum was generally sparse and primitive in appearance. Golgi apparatus was well developed in spite of marked cellular atypism. Lysosomes, pigmented droplets, glycogen granules, lipid droplets or intracytoplasmic inclusions were occasionally seen. There were many anaplastic cells having intracytoplasmic lumen. A considerable number of cilia and microvilli projected into the lumen from the free surface of neoplastic cells but it was rare to see secrectory granules or pinocytotic vesicles of the luminal surface. Similar structure have been described in gastric cancer[15], breast cancer[16], adenoma of the thyroid gland[15] or Morris hapatoma[17] and have been considered significant in increasing the surface area of cells and in facilitating absorption or secretion. As far as the present author is aware, the

intracytoplasmic lumen with cilia and microvilli, has never been repored in experimental renal carcinoma.

Numerous accumulation of fine fibrils, measuring up to 35 A° in diameter, appeared near nuclear membrane of many tumor cells. The fibrils were arranged at random. A presence of similar fibrillar structure have been reported in some cases of monocytic leukemia[18], beta-cell tumor of the pancreatic islets[19] and breast cancer[16,20]. The width of the fibrils is comparable to that of myofibrils although the former does not exhibit Z band-like structure. Some tumor cells which contain the fibrils have also irregular desmosome between cytoplasmic membrane of adjacent cells. It was also reported that some epithelial tumor cells in vitro can produce basement membrane-like material[20]. Active protein synthesis that underlies the formation of fibrils is suggested by the facts that the fibrils are located near the cisternae of endoplasmic reticulum and that ribosomes are increased around clusters of fibrils.

Newly formed cilia of the tumor show typical ultrastructure of normal cilia. It is known that cilia may be formed in tumor cells originating from ciliated and non-ciliated cells. Although Kirkman & Mannweiler found cilia in DES-induced renal carcinoma, no such structure had been reported. Renal tubular epithelium of Syrian hamster has no cilia except under pathological condition. There are many reports in the electron microscopic studies on the ciliarygenesis[21]. Although the findings in the present investigation have much in common with the reported observations such as existence of fibrillar cluster, a variety of punctate dense granules within the fibrillar clusters, increase of centriole at the peripheral areas of the clusters or appearance of complete cilia within the same cytoplasm with the cluster, we could not find the intermediary stages to cilia-formation. It is of interest to note that such a specialized organella as cilia is present in a anaplastic tumor. Neoplastic transformation may be accompanied by the derangement of organ-specific or tissue specific functions or structure. In fact, there are reports that some tumor cells produce abnormal protein[22], enzyme[23] or hormone[24]. Clever's experiment was interpreted that only a few genes of an organism is transcribed and greater part of chromosomal DNA is masked with non-specific substance[25]. Therefore, it is not surprising that non-specific phenotypes are manifested by the tumor cells through unmasking of the genes.

Intranuclear bodies showing a considerable morphological variation were observed in the tumor cells. Since first described by dé the many investigators reported its presence in normal and pathological cells[26,27]. But they were observed more frequently in such pathological conditions as viral infection, inflammatory lesion, drug-intoxication and neoplasm.

Bouteille classified them into five morphological types[27]. According to his criteria, the majority of intranuclear inclusions which were found in the present investigation belongs to Type 1 and Type 2. Beaded-Type (type 5) was extremely rare in our case. Dupuy-Coin considered that the simple nuclear bodies (Type 1 and Type 2) are constant nuclear organellae which under certain conditions are increased in number, and then transformed to be granular intranuclear bodies (Type 3 and Type 4)[28]. It has been shown by cytochemical studies that the simple nuclear bodies consist mainly of protein without DNA or RNA, whereas granular nuclear bodies contain ribonucleoproteins in the central granules[29]. Furthermore it was demonstrated in the present study that some of the latters contained chromatin clumps which resemble perinucleolar chromatin in its density and grain size. Their molecular structure and function are unknown. The presence of intranuclear bodies in experimental renal carcinomas has never been described by other investigator including Mannweiler & Bernhard. Weber and his associates advanced the hypothesis that nuclear bodies is hormone receptor in the adrenal of calf[26]. However, it seems to have no direct relation to carcinogenetic process of the DES-treated kidney because the simple nuclear bodies are also observed in non-neoplastic epithelium or endothelium. Rather, the intranuclear inclusion may be a morphological expression of cellular hyperactivity induced by DES-administration. Some investigators consider that the dense granules in the center of the intranuclear bodies are virus particles because of their presence in virus infected cells.

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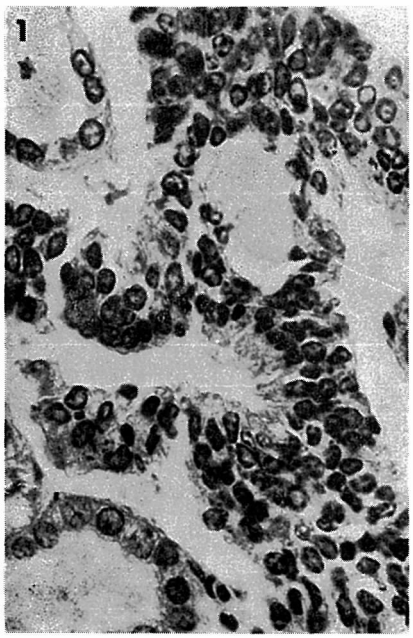
EXPLANATION OF PLATES

Fig. 1. Proximal convoluted tubular epithelium showing slight atypism. ×400.
Fig. 2. Proliferation of atypical epithelial cells in the proximal convolution in early stage of DES-treatment. ×250.
Fig. 3. Mitotic figures in precancerous lesion. ×1,000.
Fig. 4. Areas of anaplastic spindle-cells which resembled sarcoma. ×250.
Fig. 5. A dark cell interspersed among clear cells. ×5,000.
Fig. 6. Atypical polygonal cells growing in coherent sheet. ×3,000.
Fig. 7. Cellular interdigitations and desmosomes distributed at variable intervals along tumor cell margin. ×10,000.
Fig. 8. Anaplastic tumor cells having intracytoplasmic lumen which contained a number of cilia and microvilli. ×4,500.
Fig. 9. The fescicles of cilia enveloped by membrane. ×15,000.
Fig. 10. High power view of cilia in longitudinal section. ×12,000.
Fig. 11. Cluster of fibrils accompanies by solitary cilia, dense granules and centriloes. ×8,000.
Fig. 12. Higher magnification of fibrils. ×45,000.
Fig. 13. Cluster of fibrils near nuclear membrane. ×10,000.
Fig. 14. Multiple simple intranuclear bodies within tumor cells. ×7,000.
Fig. 15. Higher magnification of granular body in tumor cell. ×25,000.
Fig. 16. Endothelial cell of DES-treated non-neoplastic Kidney tissue contains intranuclear bodies. ×8,000.



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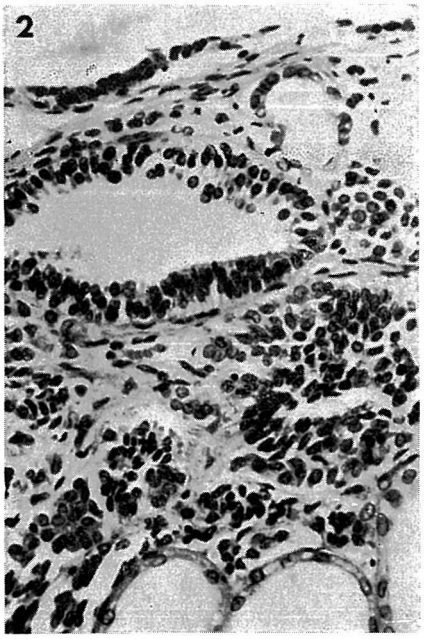

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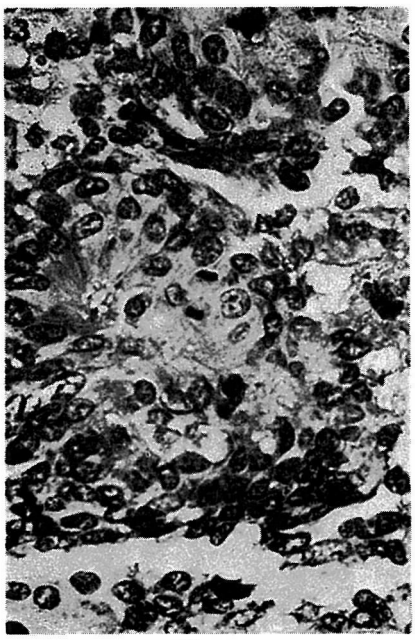

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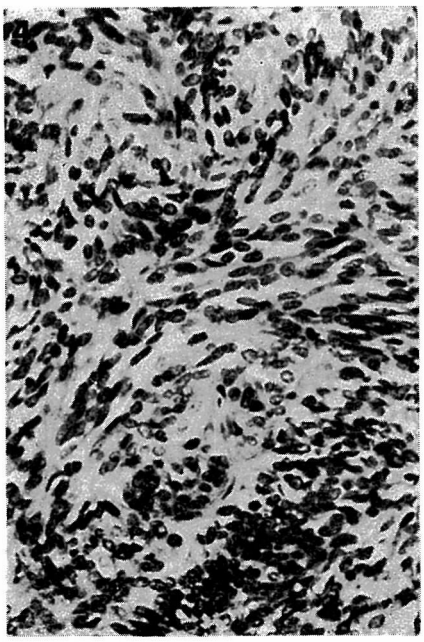

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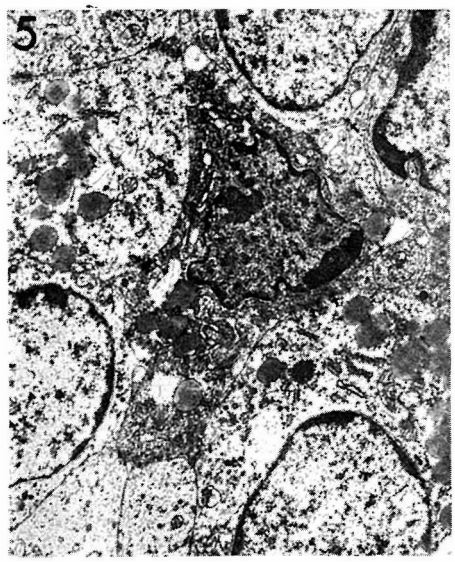

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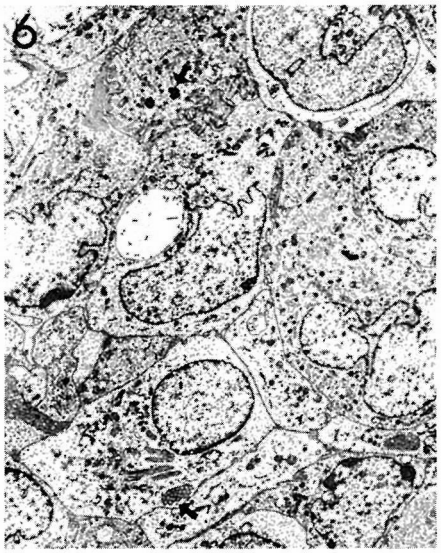

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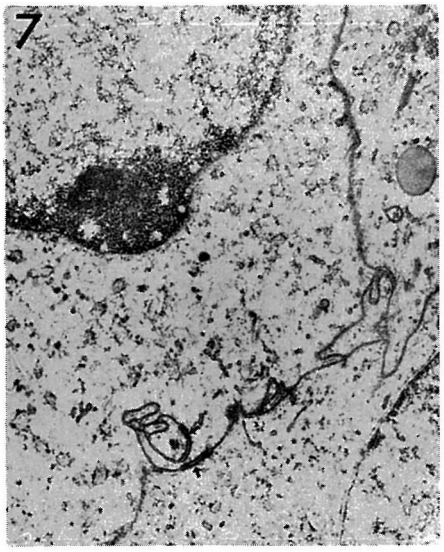

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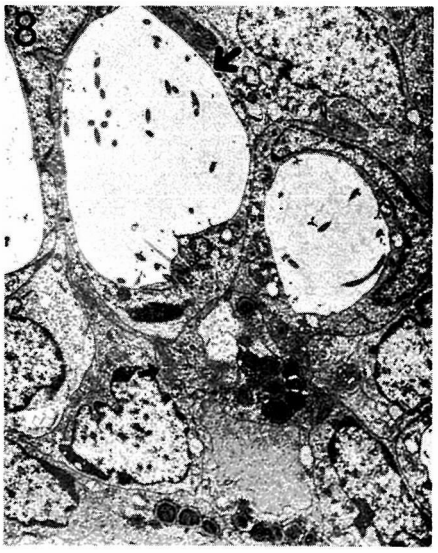

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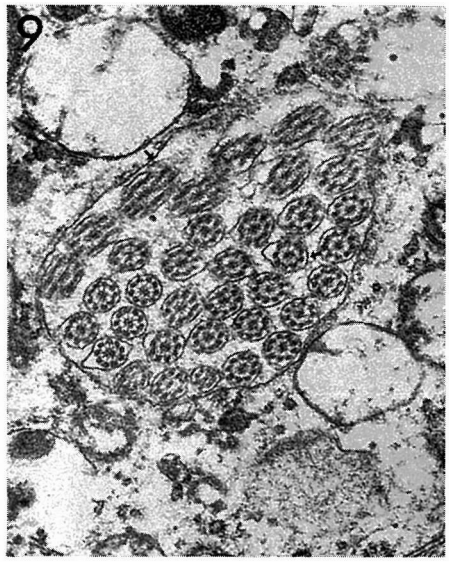

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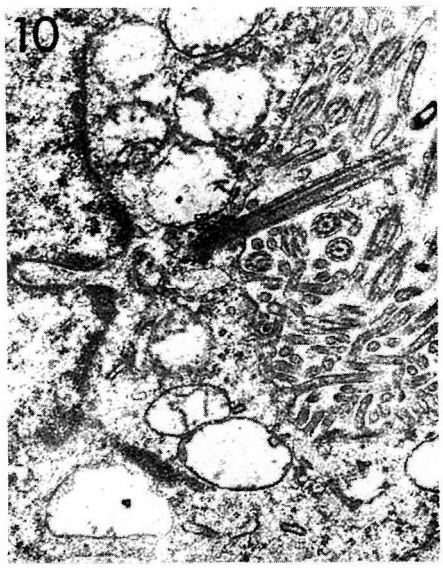

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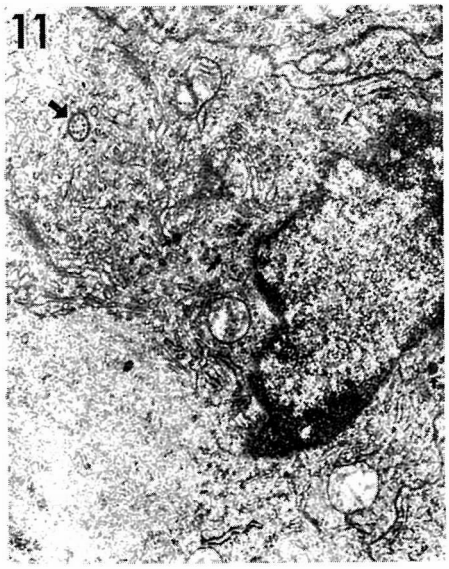

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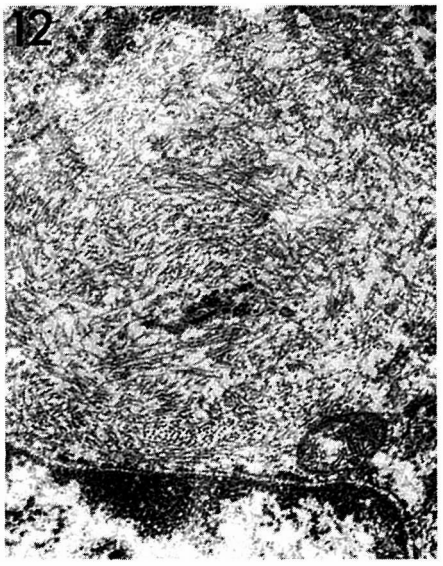

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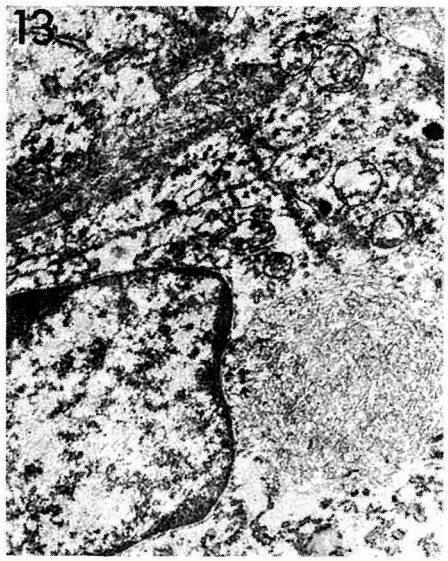

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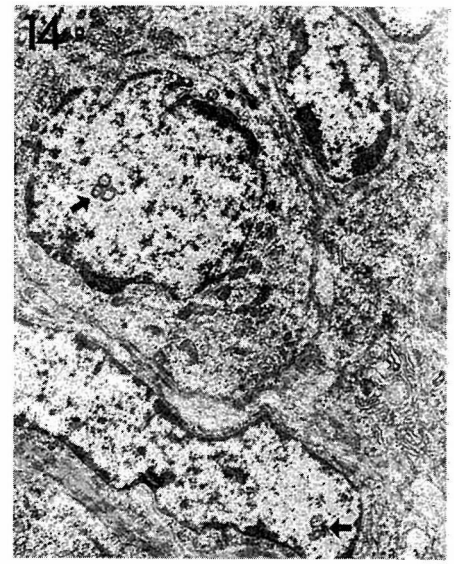

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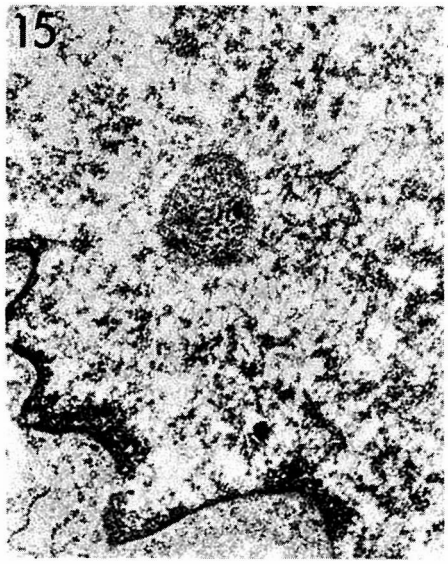

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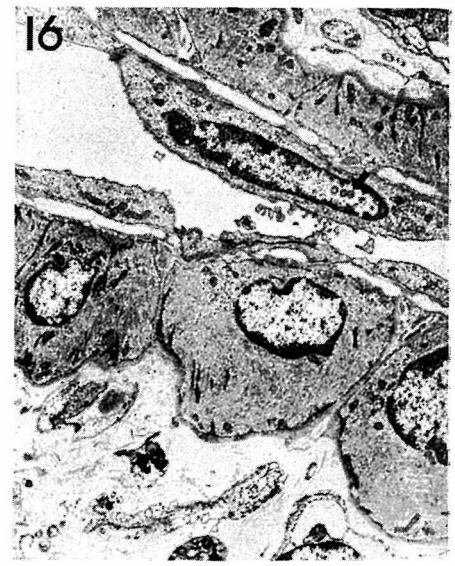

Fig. 16.