Virchows Arch. Abt. A Path. Anat. 355, 29–40 (1972)

Mesonephroma of the Uterine Cervix

Submicroscopical Study and Comparison with Fine Structure of Endocervical Adenocarcinoma

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Received July 21, 1971

Summary. The fine structure of the so-called mesonephroma of the uterine cervix was compared with that of endocervical adenocarcinoma.

Mesonephroma tubules have a peculiar type of basement membrane composed of numerous stratified basal laminae. The lining of the tubules shows a sinuous outline of the lumen between individual cells of the epithelium. In contrast, the tubules of endocervical adenocarcinoma have a double-layered basement membrane and their outlines are regular.

The outlines of the neoplastic tubules and the structure of the basement membranes are the main ultrastructural features distinguishing these two varieties of cervical tumour.

The histogenesis of mesonephroma has been under discussion for many years and continues to be controversial (Fawcett *et al.*, 1966; Novak *et al.*, 1954). A number of authors share the view of Schiller (1939), that it originates from mesonephric structures; some, however, are of the opinion that the tumour, like many other neoplasms of the female sex organs, is of Müllerian nature (Czernobilsky *et al.*, 1970; Herbst, Scully, 1970; Long, Taylor, 1964; Scully, Barlow, 1967; Tarride, Kingsley, 1968). A group of tumours has been separated from the neoplasms originally defined by Schiller (1939), as mesonephroma: these are the mesoblastomas (endodermal sinus tumours) (Teilum, 1965; Teilum, 1959). The group mesonephroma has been expanded to include clear-cell tumours described by Saphir and Lackner (1944), the clear-cell areas in these tumours occur in association with structures considered typical of mesonephroma (Novak *et al.*, 1954; Suprun and Soferman, 1960).

The typical microscopical appearance of the mesonephroma comprises tubules and fine follicles, lined by cells of hobnail shape, together with structures resembling primitive glomeruli and areas of clear-cells. This picture is distinctive and differs from that of other neoplasms arising in the female sex organs.

However, mesonephromas sometimes fail to display this typical structure. In less typical instances there may be difficulty in distinguishing these ovarian tumours from certain serous cystadenomas, cystadenocarcinomas and endometrioid carcinomas. Problems may also arise when a mesonephroma of the uterine cervix or vagina has to be distinguished from adenocarcinomas of local origin (Fawcett *et al.*, 1966).

In addition, tumours have been described in which structures of mesonephroma-type are associated with those characteristic of neoplasms of Müllerian

origin, such as cystadenocarcinoma and endometrioid carcinoma (Czernobilsky *et al.*, 1970; Scully, Barlow, 1967).

The view that the tumours known as mesonephroma are of distinctive histogenesis seems thus unfounded, and it may be suspected that these tumours, like other neoplasms of the female genital tract, are of Müllerian origin. Moreover, histochemical studies are not a reliable basis for the differential dagnosis of a mesonephroma: (Fawcett *et al.*, 1966) their results are not conclusive in relation to the problem of the histogenesis of these tumours.

It is likely that in the future ultrastructural features of tumours of the female genital tract will be considered as a basis for their differential diagnosis. It is less probable that these studies might clarify the problems relating to their histogenesis.

This paper records a cervical mesonephroma of the Schiller type, studied with both the light microscope and the electron microscope. To our knowledge, such a tumour has not been previously investigated with the latter. We have also compared the electron microscopical picture of this tumour with that of a non-mucigenic adenccarcinoma of the endocervical mucosa.

Materials and Methods

Mesonephroma. A 7-years-old girl was admitted because of increasingly heavy vaginal bleeding of one month's duration. There had been vaginal discharge during the preceding year. Gynaecological examination showed a tumour of the cervix uteri, with a cauliflower-like surface that bled readily. The tumour replaced the cervix and filled the upper part of the vagina. Biopsy showed it to be an adenocarcinoma of mesonephroma type. Hysterectomy and bilateral salpingo-oophorectomy were performed, and followed by X-irradiation (3500 r). Six months afterwards there were no signs of recurrence. Examination of the hysterectomy specimen revealed that the tumour occupied the whole cervix and infiltrated parts of the vaginal fornix as well as the inferior part of the uterine corpus.

Non-Mucigenic Endocervical Adenocarcinoma. The biopsy material was obtained in the case of a 64-years-old woman.

Methods. Samples of both tumours were minced into minute blocks and immediately fixed in 1% osmium tetroxide solution as well as in 1% glutaraldehyde, post-fixed in 1% osmium tetroxide solution, dehydrated in a graded series of acetones and embedded in Vestopal. Ultrathin sections double-stained with lead citrate and uranyl acetate were examined with a UEMW-100 electron microscope. The remaining portions of the tumours were fixed in 70% alcohol and in 10% formalin and processed by routine histological methods. Sections were stained by haematoxylin and eosin, mucicarmine and periodic-acid/Schiff (PAS) methods.

Results

Mesonephroma

Light Microscopy. The neoplasm consists of tubules and minute follicles, most of them lined by one layer of relatively low cells with large nucleus, distinct nucleolus and relatively scanty cytoplasm (Fig. 1A). In some cells the cytoplasm is very scanty, the nucleus projecting into the lumen of the tubule: these cells are quite often at a distance from one another, so that there are cleft-like spaces between them. It is these appearances that give the cells their supposed likeness to hobnails. Papillary structures, formed of scanty stroma covered by cells resembling those of the lining, protrude into many of the follicles (Fig. 1A). Fine papillae also protrude into many of the tubules, producing an appearance resembling primitive glomeruli (Fig. 1A). In some areas more solid collections of

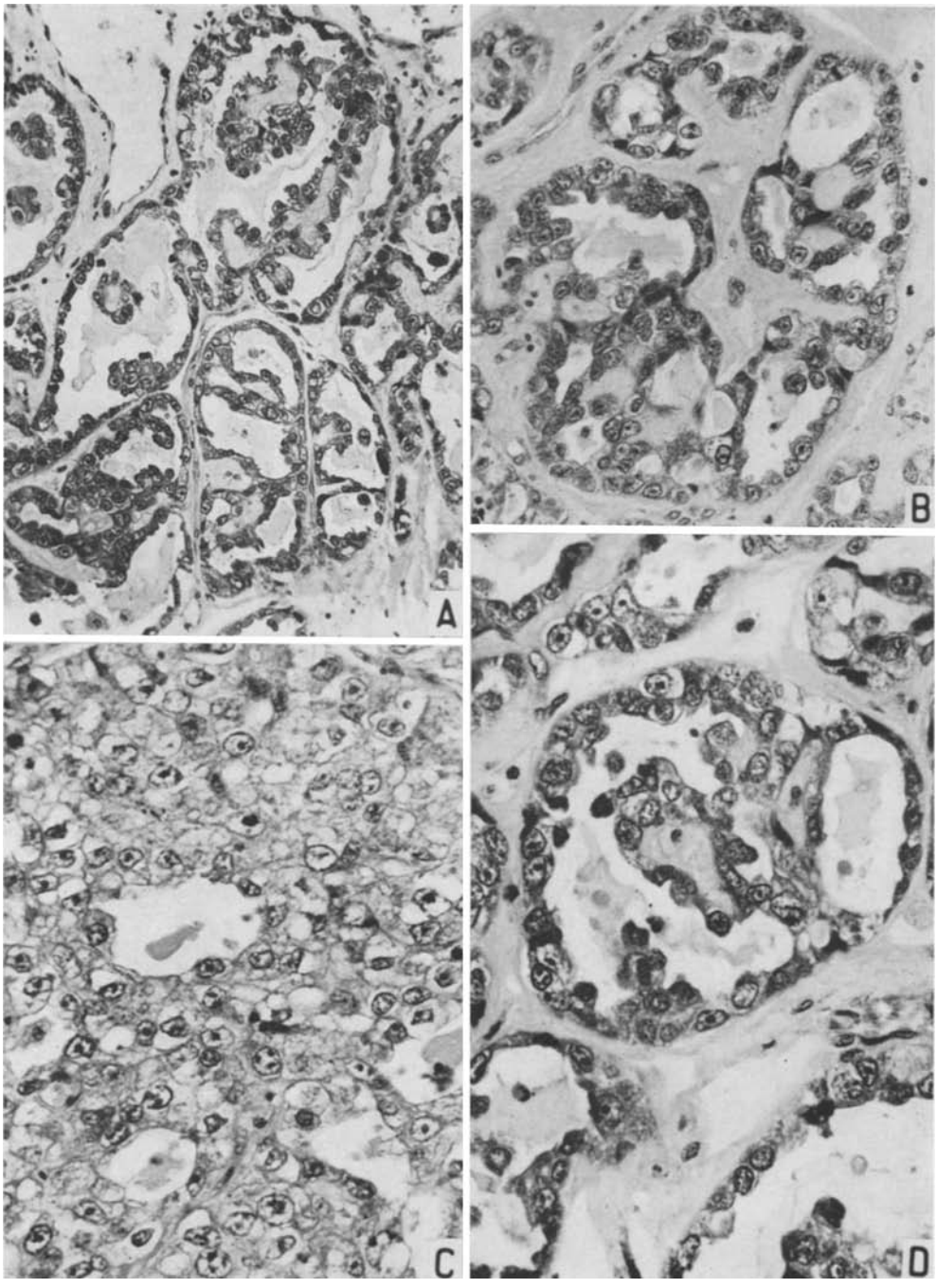

Fig. 1 A–D. Mesonephroma. A Tubules lined by hobnail cells, with papillae protruding into their lumen. (HE. ×170). B Invagination of loose stroma into nest of neoplastic cells. Empty spaces, constituting anlage of the tubules, can be seen among the cells. (HE. ×270). C Clear-cell component of the tumour. (HE. ×370). D Glomerulus-like structure with intraluminal papillar projection. (HE. ×350). (A–D: for reproduction reduced to $^{9}/_{10}$)

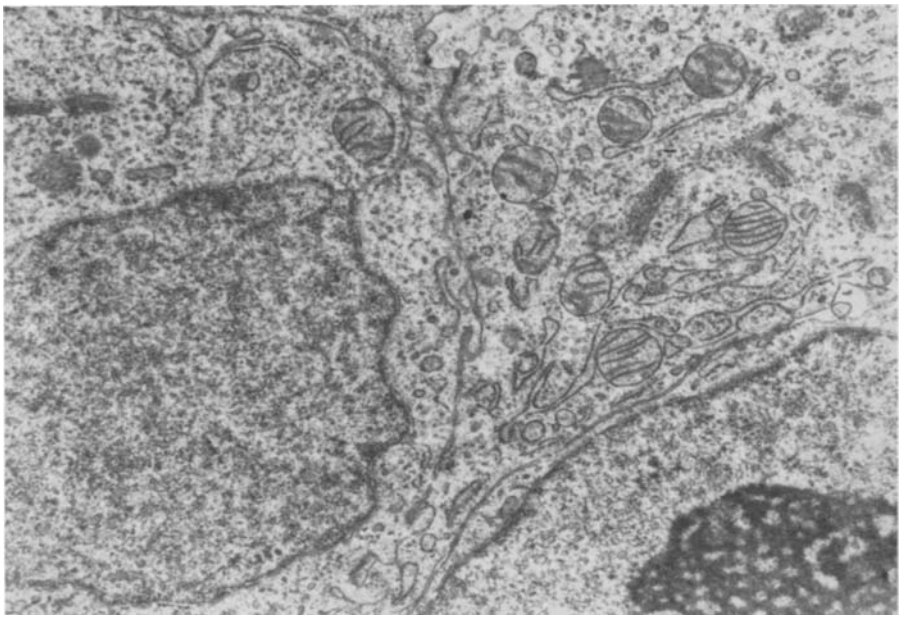

Fig. 2. Mesonephroma. Fragments of two cells in close proximity. Small intercellular space with microvilli in the upper part of the picture. Slightly dilated profiles of rough endoplasmic reticulum, several Golgi zones and part of a large nucleolus in cell at right of field. The cytoplasm of the cell at left of field contains numerous polysomes and few organelles. (×20700; for reproduction reduced to $^4/_5$)

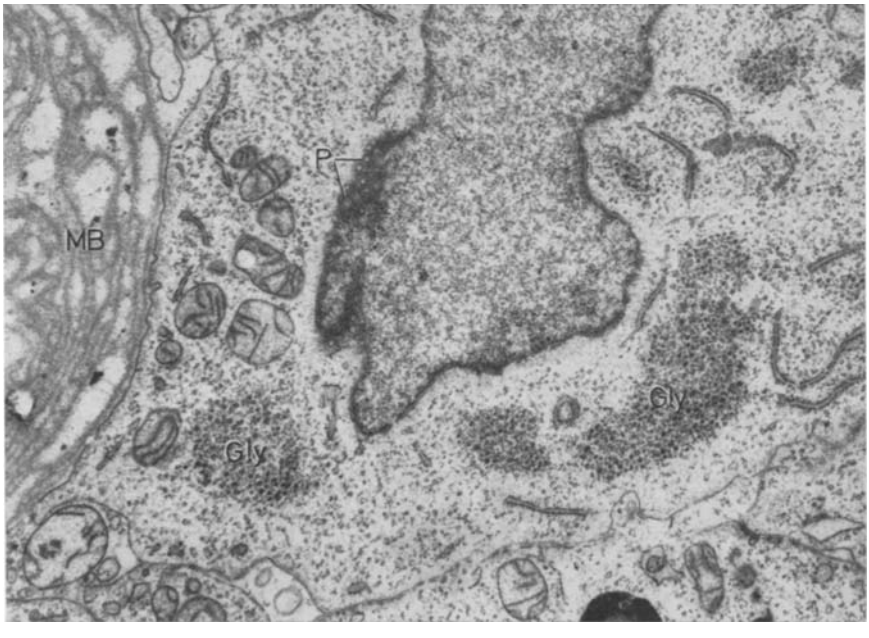

Fig. 3. Mesonephroma. Neoplastic cell with adhering broad basement membrane of multilayered structure (*MB*). Mitochondria, crowded in one area of cytoplasm, and large amounts of glycogen (*Gly*) are seen. Tangentially sectioned nuclear membrane shows prominent nuclear pores (*P*). (×18270; for reproduction reduced to $^4/_5$)

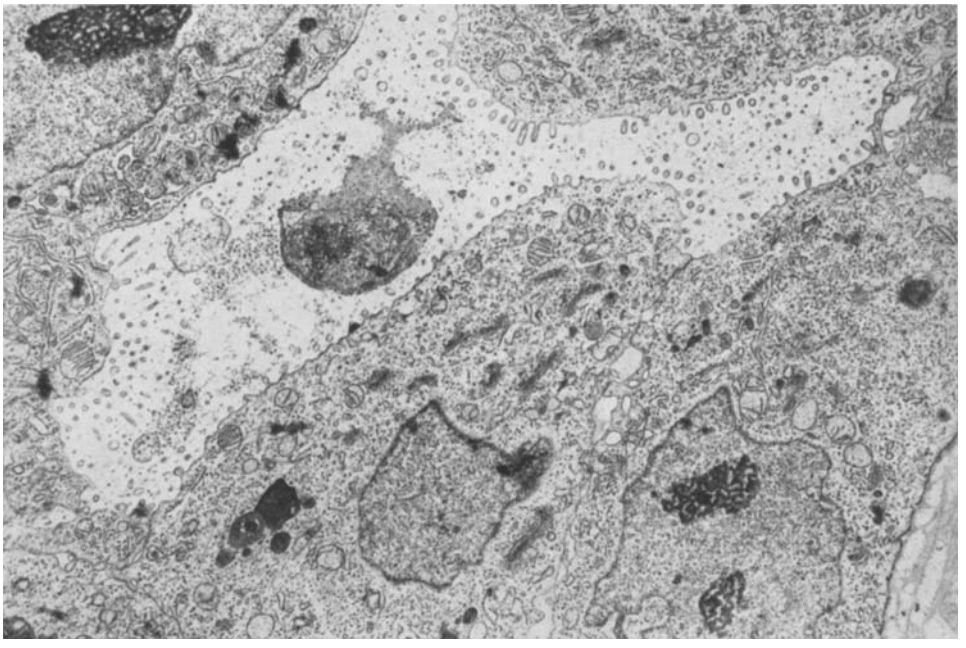

Fig. 4. Mesonephroma. Fragment of necrotic neoplastic cell in lumen of tubule of irregularly sinuous contour. The tubule-lining cells protrude in dome-like manner into lumen. Numerous microvilli on the luminal aspect. Several well-developed Golgi fields in cytoplasm of cells of tubular lining. Part of the multilayered basement membrane can be seen at the periphery of the tubule (lower right). (×6900; for reproduction reduced to $^4/_5$)

neoplastic cells are seen. The cells in contact with the surrounding and 'invaginating' stroma are well-preserved; the others are necrotic (Fig. 1B). We believe the glomerulus-like bodies and papillae to be formed by this process of stromal ingrowth and tumour cell necrosis. The stroma that penetrates the epithelial nests and that of the 'glomeruli' is loose, slightly acidophilic and devoid of blood vessels.

The matrix of the invaginating and surrounding stroma is PAS-positive, particularly in the immediate neighbourhood of tubules and epithelial nests. The scanty contents of tubules and follicles are also PAS-positive. A trace of PAS-positive diastase-resistant substance is present in the cytoplasm of certain cells. In some places mucicarmine staining gives a faintly positive reaction, confined to the surface of the lining cells.

Quite numerous, often sinusoidal, capillaries are seen in the stroma between the cell nests and the tubules (Fig. 1A).

Examination of many sections of the tumour in the hysterectomy specimen disclosed very occasional areas composed of small clear cells: these areas were either solid or contained small, rounded, empty spaces (Fig. 1C).

Electron Microscopy. The ultrastructure of the neoplastic cells is practically uniform. Its characteristic feature is a nucleus with large nucleolus and moderately abundant cytoplasm.

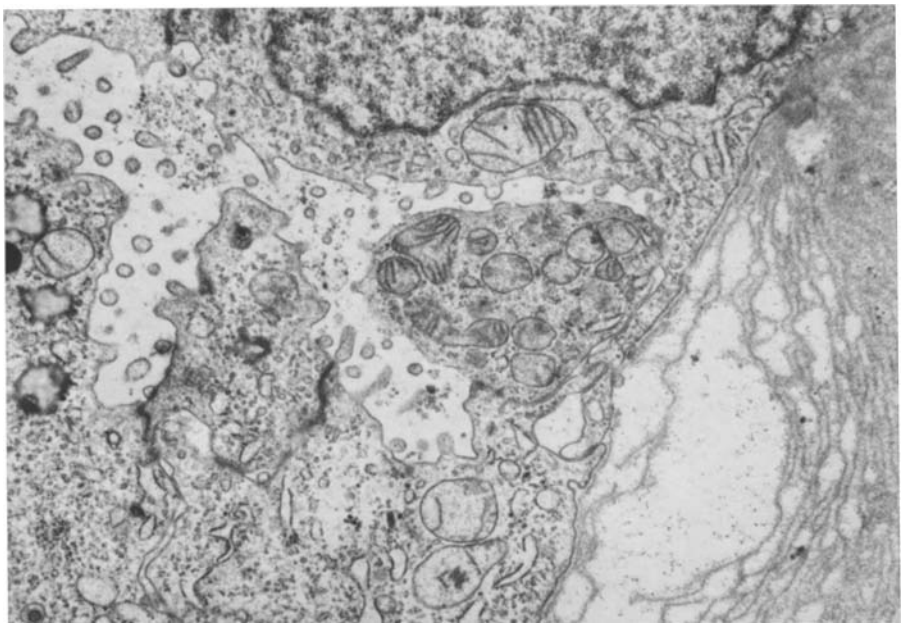

Fig. 5. Mesonephroma. Fissured extension of tubular lumen between lining cells. Irregularly sinuous shape of fissure, with numerous microvilli, is clearly visible. Some mitochondria in the lining cells are enlarged and of low electron density. Broad, multilayered basement membrane at periphery. (×23650; for reproduction reduced to $^4/_5$)

The nucleoli are very large, and have well-defined nucleolonemas. They are usually single (Figs. 2, 6). Nuclear outlines are irregularly undulated. Each is surrounded by a double membrane in which distinct pores are visible in tangential sections (Fig. 3). Nuclear chromatin is evenly distributed, with a narrow rim of marginal condensation.

The moderately abundant cytoplasm contains a large number of free ribosomes and polyribosomes (Figs. 3, 4). Mitochondria, which are not numerous, are rounded or oval and of medium size, with cristae running in various directions. Sometimes, mitochondria are grouped in a particular area of cytoplasm (Fig. 3). Single, large mitochondria with matrix of low electron density are also present (Fig. 5). Rough endoplasmic reticulum, relatively poorly developed, is encountered in the form of single, quite often long, narrow streaks (Fig. 3). Slightly dilated cisternae of rough endoplasmic reticulum can also be found in some cells (Fig. 2). A well-developed Golgi apparatus is evident in almost every section of each cell (Figs. 2, 4, 6). In many cells there are several small Golgi zones made up of flat parallel tubules and minute vesicles. Larger areas of glycogen (Fig. 3) as well as single small droplets of lipids are present in the cytoplasm of some cells.

In the more solid cellular areas the cells adhere tightly one to another, with interdigitation of delicate cytoplasmic projections. Occasionally, there are minute free spaces between neighbouring cells, with microvilli protruding into them (Fig. 2)—these may be interpreted as rudimentary tubules. In other places, the

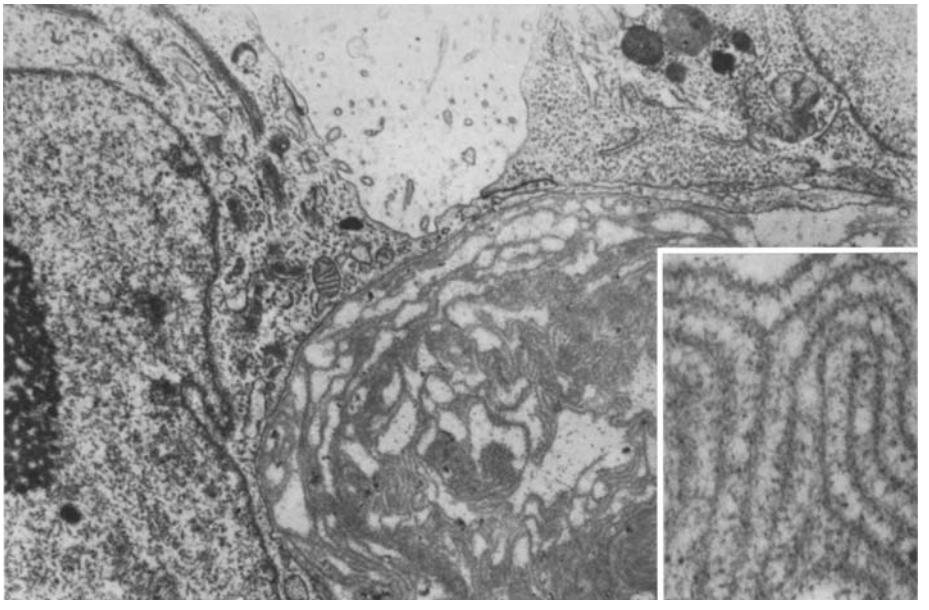

Fig. 6. Mesonephroma. Neoplastic tubule deeply invaginated by stroma composed of numerous basement membranes. The basal laminae are partly arranged in a labyrinthine manner. Only narrow cytoplasmic projections separate basement membrane from the lumen. (×14950; for reproduction reduced to $^4/_5$). Inset: Higher magnification of fragment of parallel-stratified basal laminae. (×64000; for reproduction reduced to $^4/_5$)

lumen of the tubules is better formed, though irregularly sinuous in outline because the cytoplasm of the cells lining them protrudes into it (Fig. 4). No tubules with regularly linear outlines are seen. The outline of the lumen of still larger tubules is regularly sinuous, often reaching between individual cells of the tubular lining (Fig. 5) to the proximity of the basement membrane. The lumen is then separated from the basement membrane merely by narrow cytoplasmic projections (Fig. 6). Detached, dead neoplastic cells or their fragments are often seen in the lumen (Fig. 4). Not infrequently it is possible to observe the disintegration of most of the cytoplasm of the cells lining a tubule, the naked cell nucleus then protruding into the lumen. Such nuclei often present a well-preserved internal structure. In many places, the necrotic cells have become separated from the basement membrane and a gap is then to be seen between the lining cells. It is likely that it is in this way that the sinuous intercellular bulging of the tubular lumen comes into being.

A most striking ultrastructural feature of the mesonephroma is the stroma immediately abutting on the tubules (Figs. 3, 5) and that invaginating the epithelial nests (Fig. 6). Usually, wide basement membranes are composed of numerous parallel basal laminae. The number of laminae may reach some scores. Each basal lamina is separated from its neighbours by a lamina lucida of variable thickness. Quite often there are irregular empty spaces between the basal laminae of the wide basement membrane (Figs. 5, 6). In some sections the stratified basal

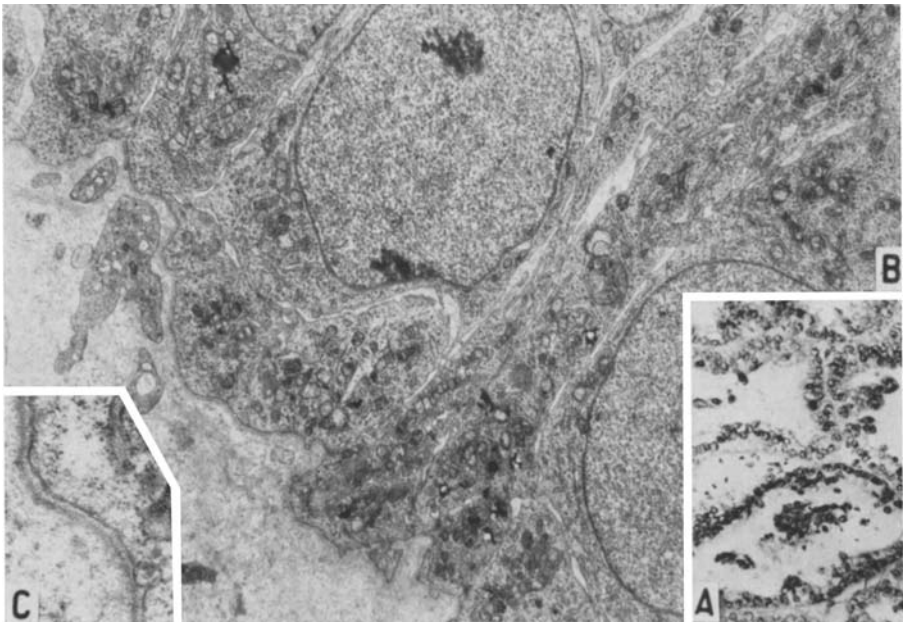

Fig. 7 A–C. Endocervical adenocarcinoma. A Photomicrograph showing well-preserved tubular structure. (HE. ×160). B Electron micrograph. Peripheral part of a neoplastic tubule. The lining cells are slender and most of them touch the basement membrane. Relatively numerous mitochondria and narrow profiles of rough endoplasmic reticulum visible in their cytoplasm. Narrow basement membrane composed of basal lamina and lamina lucida is seen. Fragments of fibroblast cytoplasm are present within loose stroma. (×5250). C Higher magnification of part of basement membrane. (×30500). (A–C: for reproduction reduced to $^4/_5$)

laminae form a labyrinthine system (Fig. 6). The blood vessels in the stroma are separated from the broad layer of the basement membrane that surrounds the neoplastic tubules by a loose-textured zone of microfibrils and solitary collagen fibres. These vessels have their own narrow, single-layer basement membrane.

Adenocarcinoma

Light Microscopy. For electron microscopical comparison with mesonephroma we have chosen an endocervical adenocarcinoma of tubular structure. The cells lining its neoplastic tubules are columnar or cuboidal and are arranged in one or several layers, sometimes with fine papillae bulging towards the lumen (Fig. 7 A). These cells do not form mucus (we recognized that a mucigenic adenocarcinoma would show essential differences in comparison with the structure of a mesonephroma).

Electron Microscopy. When compared with the cells of a mesonephroma, those of the endocervical adenocarcinoma differ noticeably in structure and arrangement. They have smaller nucleoli and their cytoplasm is richer in organelles (Fig. 7 B). Mitochondria, often irregularly elongated, are relatively numerous, and abundant rough endoplasmic reticulum can be seen. Long narrow strands of

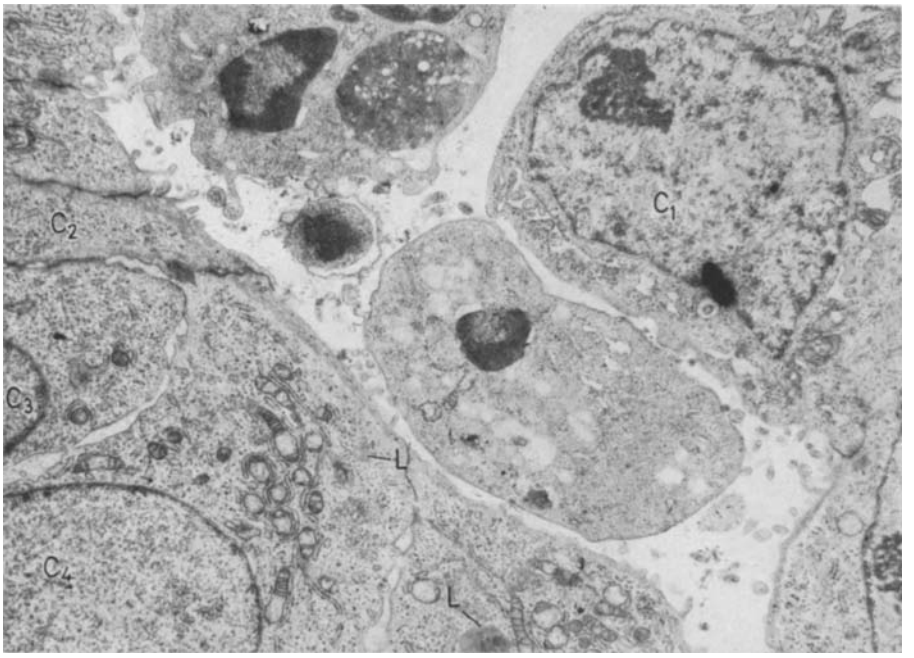

Fig. 8. Endocervical adenocarcinoma. Central part of neoplastic tubule. In the lumen of the tubule necrotic cells are visible. One lining cell (C_1) with decreased electron density of nucleoplasm and dilation of rough endoplasmic reticulum cisternae is in a stage of necrosis. The flat, free surface of the lining cells (C_2, C_3, C_4) shows microvilli. Relatively numerous mitochondria, entwined with long profiles of rough endoplasmic reticulum are seen in cytoplasm of these cells. In close proximity to the lumen the cytoplasm is slightly condensed. In the cytoplasm of some cells single, round bodies of low electron density are seen (L). ($\times 6750$; for reproduction reduced to $^4/_5$)

rough endoplasmic reticulum often surround mitochondria (Figs. 7B, 8). Free ribosomes and polysomes are as abundant as in mesonephroma cells. The cytoplasm of some cells contains single, rounded, homogeneous bodies of low electron density, surrounded by a single membrane (Figs. 7B, 8). It has been impossible to determine whether they are lysosomes or secretory granules.

Microvilli are seen on the tubular aspect of the cell, just as in mesonephroma. The outlines of the tubules are much more regular than in mesonephroma, since the lumen surface of the cells which line the tubule is flattened, and the cells are mutually adjusted (Fig. 8). The cells that constitute the lining of the neoplastic tubules are separated by narrow intercellular spaces of irregular width. There are no wide clefts between them.

As in mesonephroma, free-lying, necrotic cells and their fragments are seen in the tubular lumen. Necrotic cells with nuclear chromatin of low electron density and dilated cisternae of endoplasmic reticulum which have not yet separated are also seen. The necrotic cells are close to the tubular lumen but never close to the basement membrane. This finding distinguishes the adenocarcinoma from the mesonephroma. The structure of that basement membrane is different, too. It is

formed of a singular layer of basal lamina running parallel to the basilar portion of the cells lining the tubule. The basal lamina being separated from the plasma membrane by a lamina lucida of uniform width is of rather constant thickness, ranging from 400 to 800 Å (Fig. 7B, C).

Discussion

The basement membranes which surround the tubules and invaginate the groups of neoplastic cells constitute the most striking ultrastructural feature of the Schiller-type mesonephroma. It is likely that they correspond to the PAS-positive layers revealed by the light microscope. The electron microscopical studies of a mesonephroma of Saphir's hypernephroid type recorded by Okagaki and Richart (1970), characterize the tumour as having a distinct, double-layered basement membrane in relation to all tubular structures. The multistratified basal laminae in our mesonephroma show a wide band-like arrangement which separates the neoplastic tubules from the blood vessels deeper within the stroma. It is possible that the thick layer of the basement membranes plays some role in the transport of nutritional substances from the vessels to the neoplastic cells and that this may influence the morphology of the tumour.

It is believed that the prognosis of mesonephroma of the uterine cervix is more favourable than that of endocervical adenocarcinoma (Fawcett *et al.*, 1966; Hameed, 1968). It is possible that the degree of malignancy of mesonephroma depends to some extent upon the structure of its basement membranes.

The adenocarcinoma of the endocervix that we used for comparative studies has a regular double-layered basement membrane composed of lamina lucida and basal lamina as in most epithelia. A similar double-layered basement membrane, but varying in width, was found by Roberts and his associates (1970), in ovarian papillary serous cystadenocarcinoma. As far as we know the band-like basement membrane of the mesonephroma has not been previously described in carcinomas of the female genital tract. However, stratified basement membranes composed of a few irregular layers have been observed in some other carcinomas (Frithiof, 1969). Multiple basement membranes have also been observed in certain non-epithelial neoplasms (neurilemmoma) (Wechsler and Hossmann, 1965). A picture of a multilayer basement membrane, somewhat similar to that found in our case of mesonephroma, is included by Tarin (1969), in a paper on the fine structure of some chemically induced murine mammary tumours.

Another feature that distinguishes the ultrastructure of the mesonephroma studied from that of the endocervical adenocarcinoma is the peculiar sinuous extension of the tubular lumen between the lining cells and toward the basement membrane. It is likely that this feature is related to necrosis of single cells adjacent to the basement membranes. This phenomenon is responsible for the 'hobnail' character of the cells lining the tubules, as seen with the light microscope.

The fine structure of the mesonephroma cell and of the cell of the endocervical adenocarcinoma differs relatively little. We noted somewhat more numerous organelles in the cytoplasm of the adenocarcinoma cell and a larger nucleolus in the mesonephroma cell.

The ultrastructural studies which we have carried out in our case of mesonephroma do not permit conclusions about the histogenesis of this type of tumour. Such conclusions might be possible if we compared the submicroscopical structure of the mesonephroma and of various other tumours of the female sex organs with that of the human mesonephros in consecutive stages of its development. Such studies have not so far been undertaken.

Okagaki and Richart (1970), have compared the ultrastructure of the clear cell type of ovarian mesonephroma with published descriptions of the ultrastructure of various human tumours and suggest that the mesonephroma is of coelomic origin. Their view is not supported by embryological studies and therefore we feel that it cannot be regarded as acceptable.

It remains, of course, to be established to what extent the ultrastructural features that we have found in our case of mesonephroma are typical of these tumours. Further studies on this point are necessary so that the role of ultrastructural investigations in the differential diagnosis of mesonephroma in all parts of the female genital tract may be determined.

References

Czernobilsky, B., Silverman, B. B., Enterline, H.: Clear-cell adenocarcinoma of the ovary. A clinicopathologic analysis of pure and mixed forms and comparison with endometrioid carcinoma. Cancer (Philad.) **25**, 762–772 (1970).

Fawcett, K. J., Docherty, M. B., Hunt, A. B.: Mesonephric carcinoma of the cervix uteri. A clinical and pathologic study. Amer. J. Obstet. Gynec. **95**, 1068–1078 (1966).

Frithiof, L.: Ultrastructure of the basement membrane in normal and hyperplastic human oral epithelium compared with that in preinvasive and invasive carcinoma. Acta path. microbiol. scand., Suppl. **200**, 1–63 (1969).

Hameed, K.: Clear cell carcinoma of the cervix. Amer J. Obstet. Gynec. **101**, 954–958 (1968).

Herbst, A. L., Scully, E. R.: Adenocarcinoma of the vagina in adolescence. A report of 7 cases including 6 clear-cell carcinoma. (So-called mesonephromas.) Cancer (Philad.) **25**, 745–757 (1970).

Long, M. E., Taylor, H. C.: Endometrioid carcinoma of the ovary. Amer. J. Obstet. Gynec. **90**, 936–950 (1964).

Novak, E., Woodruff, J. D., Novak, E. R.: Probable mesonephric origin of certain female genital tumors. Amer. J. Obstet. Gynec. **68**, 1222–1239 (1954).

Okagaki, T., Richart, R.: Mesonephroma ovarii (hypernephroid carcinoma)—light microscopic and ultrastructural study of a case. Cancer (Philad.) **26**, 453–461 (1970).

Roberts, D. K., Marshall, R. B., Wharton, J. T.: Ultrastructure of ovarian tumors. I. Papillary serous cystadenocarcinoma. Cancer (Philad.) **25**, 947–958 (1970).

Saphir, O., Lackner, J. E.: Adenocarcinoma with clear cells (hypernephroid) of the ovary. Surg. Gynec. Obstet. **79**, 539–543 (1944).

Schiller, W.: Mesonephroma ovarii. Amer. J. Cancer **35**, 1–21 (1939).

Scully, R. E., Barlow, J. F.: "Mesonephroma" of ovary, Tumor of Müllerian nature related to the endometrioid carcinoma. Cancer (Philad.) **20**, 1405–1417 (1967).

Suprun, H., Soferman, N.: A mixed "clear cell" adenocarcinoma with mesonephroma of the ovary. Report of a case with special clinical aspects. Obstet. Gynec. **16**, 175–179 (1960).

Tarin, B.: Fine structure of murine mammary tumours: the relationship between epithelium and connective tissue in neoplasms induced by various agents. Brit. J. Cancer **23**, 417–425 (1969).

Tarride, J., Kingsley, W. B.: "Mesonephroma" of the ovary. A report of five cases. Cancer (Philad.) **22**, 1208–1214 (1968).

Teilum, G.: Endodermal sinus tumors of the ovary and testis. Comparative morphogenesis of so-called mesonephroma ovarii (Schiller) and extraembryonic (yolk sac-allantoic) structures of the rat's placenta. Cancer (Philad.) **12**, 1092–1105 (1959).

— Classifications of endodermal sinus tumour (Mesonephroma vitellinum) and so-called "embryonal carcinoma" of the ovary. Acta path. microbiol. scand. **64**, 407–429 (1965).

Wechsler, W., Hossmann, K. A.: Zur Feinstruktur menschlicher Acusticneurinome. Beitr. path. Anat. **132**, 319–343 (1965).

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