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Comparative light and electron microscopic studies of cystic and papillary tumors of the peritoneum

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Summary. Using a cystic lymphangioma of the greater omentum and a benign cystic mesothelioma as examples, the distinguishing characteristics of cystic peritoneal tumors are shown, using light microscopic and ultrastructural findings. A benign papillary mesothelioma is used for comparison. The cellular structures and growth rate of the mesotheliomas are contrasted with the tissue reactions which are typical for irritated serosa. The diffusely growing papillary mesothelioma is a very rare tumor, the cells of which are similar to normal serosa cells, but also show characteristics of other benign or malignant mesothelial tumors and of reactive proliferated mesothelial cells. The diffusely growing benign cystic mesothelioma has only been described in isolated cases and is characterized by cysts grouped in acini with mainly flat, localized cuboidal cell lining. The histochemical properties and cytological findings correspond closely to those of the papillary tumor or normal peritoneal lining cells. In contrast, the cystic lymphangioma probably represents a congenital defect with a slow growth rate. The structure is characterized by a sponge-like arrangement of smooth-walled cysts, in the walls of which smooth muscle cells and lymph follicles are embedded. The endothelium is also flat and ultrastructurally resembles that of lymph vessels.

Key words: Cystic mesothelioma – Papillary mesothelioma – Cystic lymphangioma – Comparative study-light- and electron microscopy

Cysts of the peritoneal cavity are a common finding in the female pelvis, especially on the surface of the tubes, in the mesosalpinx, in the ligamentum

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latum, and in adhesions. Occasionally they are also found in the coats of the testicles (v. Gierke 1926; Otto 1976). In the majority of cases these cysts are round pea-sized structures, which are thought to be either dilated rests of the Wolffian ducts (Ackerman 1954) or segments of peritoneal serosa (v. Gierke 1926; Blaustein 1977). Outside the pelvic peritoneum large unilocular or multilocular cysts are a rarity. Excluding the mainly single chambered enterocystomas, haemorrhagic cysts, cystically degenerated soft part tumors, and the occasionally reported cavities lined with mesothelium (Ford

Table 1. Cystic mesothelioma of the peritoneum and comparative observations

Author	Age	Sex	Localisation	Diagnosis	Remarks
Henke (1899)	50	m	Omentum majus, bursa omentalis diaphragm, spleen, liver, cecum	Multiple cystic lymphangioma-like tumor	Coincidental post-mortem findings, congenital malformation
Ziegler (1899)	–	f	Entire abdomen	–	Discussion remark to Henke
Ernst (1904)	65	m	Entire abdomen	Cystic lymphangio-endothelioma	Benign tumor
Kirchberg (1912)	51	f	Omentum majus and minus, minor pelvis, duodenum, colon	Cystic lymphangiectasis	Pleural diaphragm involvement
Hamdi (1927)	30	m	Entire abdomen	Cysto-coelothelioma	Benign, enterostenosis
Plaut (1928)	44	f	Appendix, ovary, tube	Benign cystic serous epithelioma	Probably congenital
Goldzieher (1929)	–	f	Entire abdomen	Implantation metastasis of ovary epithelium	Discussion remark to Plaut
Mills (1941)	47	f	Ileum, omentum majus, uterus retroperitoneum	Multiple lymphangiomas	Coincidental findings at herniotomy
Krieger (1952)	29	f	Omentum majus, uterus, bladder, small intestine, appendix	Multiple mesothelial cysts	Hamartoma
Martelli (1953)	29	m	Surface of intestines, capsule of spleen and liver	Cystic mesothelioma	
Jacobson (1974)	43	f	Adnexa, pelvic peritoneum omentum majus, sigmoid colon	Benign papillary cytosis	
Moore (1980)	73	m	Surfaces of appendix, small intestine and mesentery, parietal peritoneum	Benign cystic mesothelioma	Two recurrences, ultrastructural investigation

1960; Ackerman 1975), these lesions are usually designated as lymphangiomas or chyle cysts (see Moynihan 1897; Beller and Nach 1950; Krieger et al. 1952; Majnarich 1955; Ford 1960; Walker and Putnam 1973; Caropreso 1974). They are located in the mesentery of the small intestine or colon, retroperitoneally or in the greater omentum, they may become quite large multi-chambered complexes. By 1955, 700 such cases had been reported (Wernicke 1955). The fact that children are more often affected is seen to be an indication of a congenital origin (Wernicke 1955; Landing 1956). A clear-cut classification of the lesion as either a defective embryonic anlage with gradual size increase ("blastomatous dysplasia", Otto 1976) or as an autonomously growing tumor, is usually not possible (v. Gierke 1926; Landing 1956; Otto 1976). In addition to these localized forms, multilocular cysts have been described. These include parts of the visceral and parietal peritoneum, are usually attached to the peritoneum and in some cases fill the whole peritoneal cavity. Some authors see the mesothelium as their matrix, while others, especially in older reports, contend that the lymph vessel system forms this structure (see Table 1). As the histogenetic classification of intraabdominal cysts can be problematical (compare Otto 1976), we will try to point out the differences between cystic lymphangiomas and mesothelial cysts and comment on their biological value, using two of our own cases with ultrastructural findings. The very rare diffuse papillary mesothelioma of the peritoneum which has been included in the classification of serosal tumors (Ackermann 1954; WHO 1969), with its characteristic histological and cytological structures of proliferating mesothelium, can be compared with the cystic formations.

Case studies

Case 1 (E.Nr. 3616/81): A 74-year old woman was operated on for cholecystolithiasis. When the abdomen was opened, multiple bean-sized grayish-red foci were noticed on the surface of the greater omentum, and in the gastrocolic and hepatoduodenal ligaments. The intraoperative specimen for immediate microscopic examination of a small portion of the greater omentum showed a benign papillary mesothelioma. Following the report of these findings the cholecystectomy, a resection of the greater omentum and a liver biopsy were performed. The other abdominal organs, in particular the ovaries, showed no pathological findings; neither ascites nor adhesions were found. On the surface of the $55 \times 19 \times 3$ cm sized portion of the resected omentum there were multiple soft, grayish-red, papillary structures with a maximal size of $1.5 \times 1.2 \times 0.5$ cm (Fig. 1a).

Microscopically, fine or coarse papillary structures can be recognized.The papillae are branched and covered by partially flat but mostly cuboid or cylindrical cells, which turn into typical flat mesothelium at the edge of the folds (Fig. 1b). In the somewhat rounded apical portion, which shows a positive Alcian-blue reaction at the edges (at pH 1 and pH 2.5) (PAS reaction negative), there are brush-like cell-processes (Fig. 1b). The nuclei are usually uniform, oval to round in shape. The cells have clearly defined borders. There is no infiltrative growth into the loose oldematous or fibrous stroma. Several of the oedematous papillae are diffusely infiltrated by round cells and contain small haemorrhages and deposits of iron-containing pigment. Ultrastructurally the surface of the cuboidal or cylindrical-shaped cells is covered by numerous microvilli arranged partially in bunches. Occasionally there is a single cilium (Fig. 2). These cells lie on a continuous basement membrane. The tile-like overlapping flat cells show either a few processes only, or a totally smooth surface. Between the cells there are numerous desmosomal areas of attachment and sinus-like intercellular spaces. There

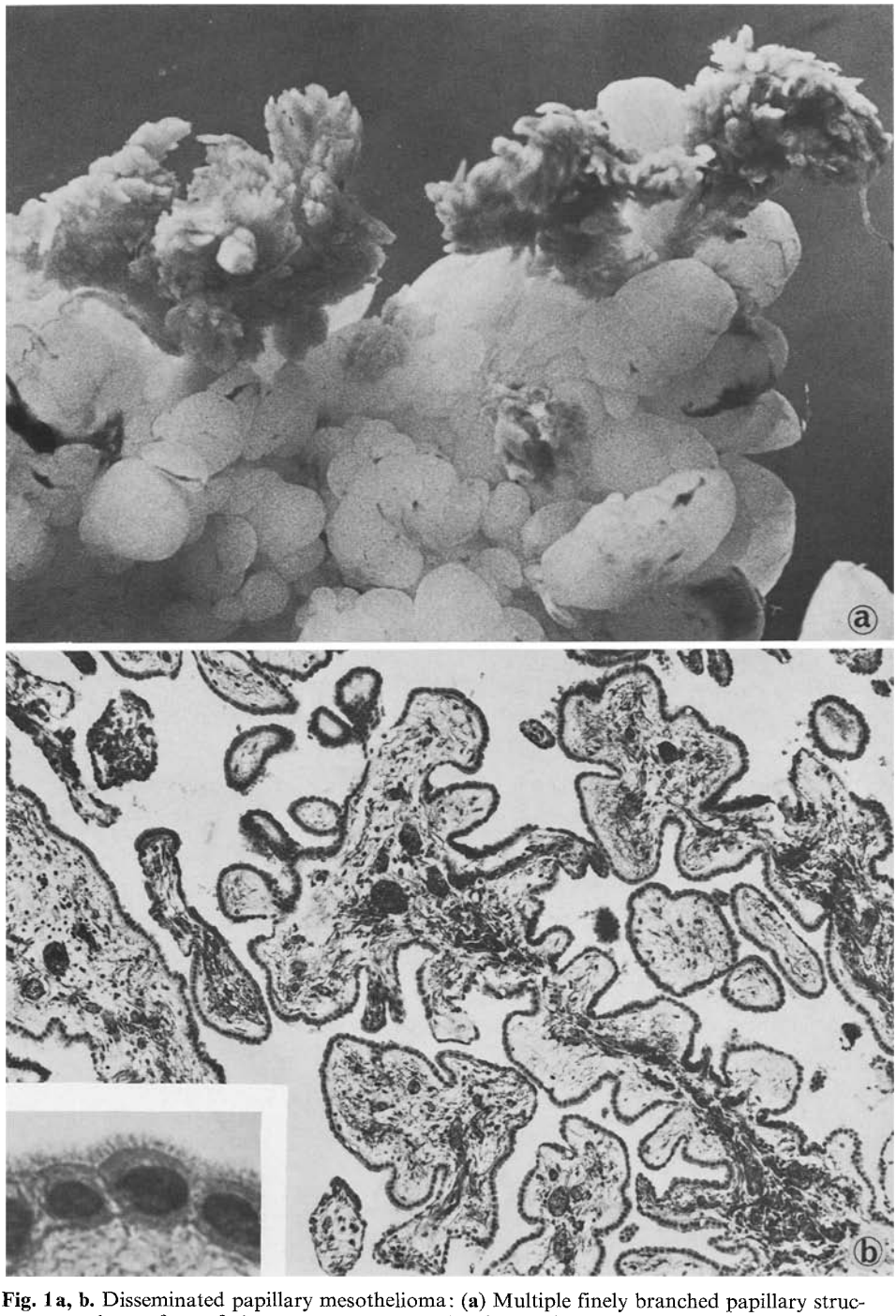


Fig. 1 a, b. Disseminated papillary mesothelioma: (**a**) Multiple finely branched papillary structures on the surface of the greater omentum (section). (**b**) Cuboidal mesothelial covering, minimal fibrosis and localized round cell infiltration of the papillary stroma. (van Gieson, Obj. 6.3 ×). *Inset:* Cuboidal mesothelial cells with microvilli (Haematoxylin-Eosin, Obj. 100 ×)

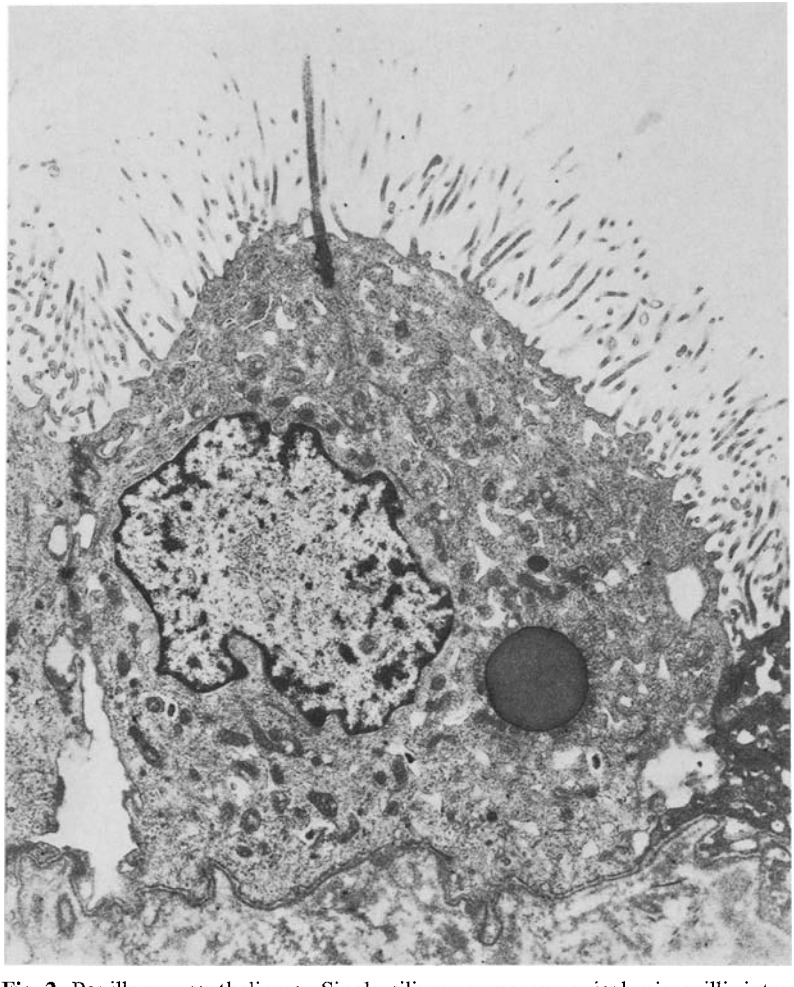
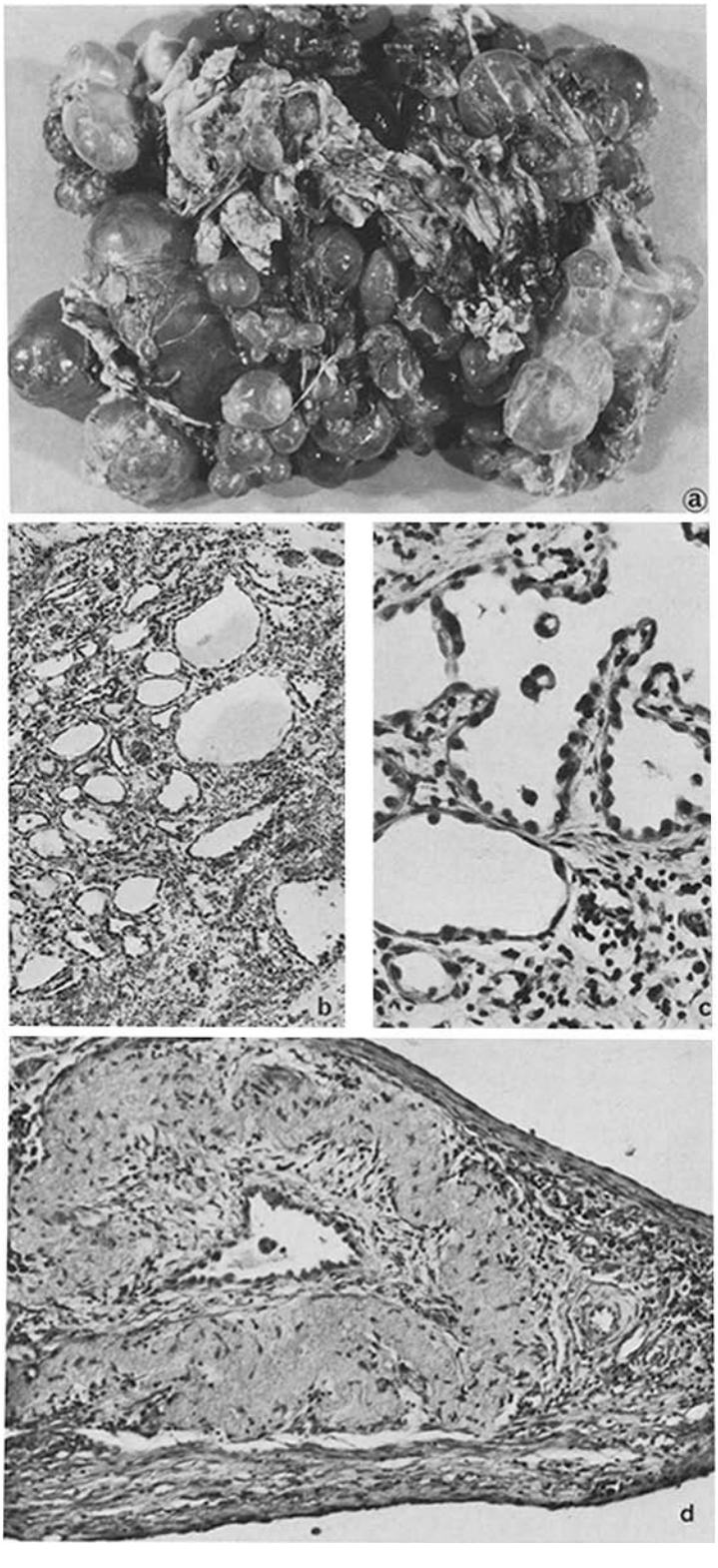


Fig. 2. Papillary mesothelioma: Single cilium, numerous apical microvilli, intracytoplasmatic fat droplets, intercellular desmosomal connections and spaces, continuous basement membrane (7,500 ×)

are many mitochondria, tonofilaments and single fat droplets in the cytoplasma. In the apical as well as in the portions near the basement membrane, one finds numerous pinocytotic vesicles in the flat cells. The nuclear membrane often contains many folds.

A diagnosis of disseminated (diffuse) benign papillary mesothelioma was made.

Case 2 (E.Nr. 4579/79 and 14896/81): A 46-year old Yugoslavian male was operated on because of a relapse of an inguinal hernia on the right side. After the indirect hernial sac was opened, multiple cysts with a transparent wall and ranging in size from a pin-head to a pea, were discovered. The consistency of the contents ranged from watery to thread-like. The intraoperative microscopic examination showed benign cysts lined with mesothelium or endothelium. An echinococcus cyst could be ruled out. On further preparation these structures could be seen to extend to the abdominal cavity. After the abdominal cavity was opened, it was seen to be filled with multiple barely visible cysts with a diameter of up to 7 cm. They were attached to all abdominal organs excluding the liver, and to the parietal peritoneum.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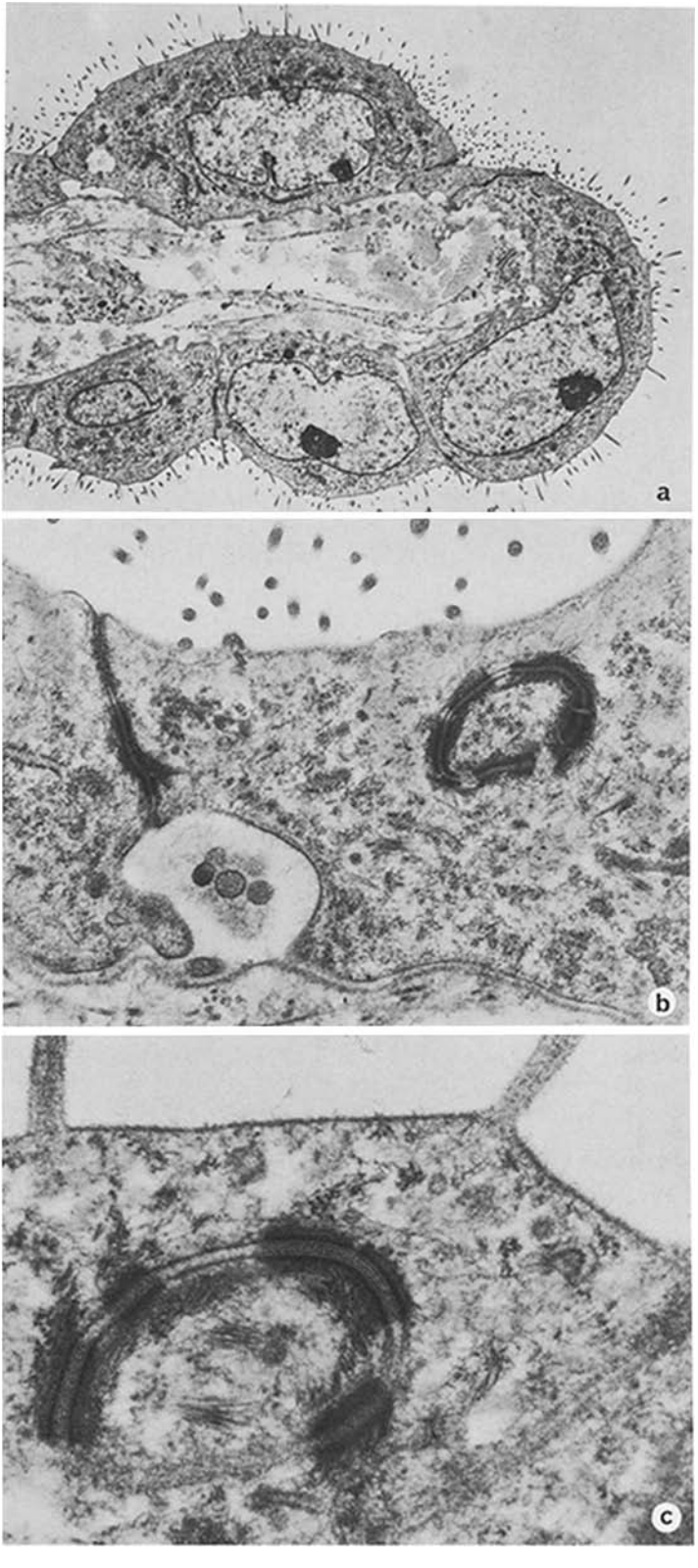
In the greater omentum they were integrated in to the fatty tissue. All in all 2,900 g of the cystic material (including the resected greater omentum) was collected and an appendectomy was performed (Fig. 3a). As complete removal was not possible and macroscopically multiple echinococcus cysts could not be ruled out, 6 g of cyclophosphamide were injected into the peritoneal cavity. Two and one-half years after the operation, the patient had to be reoperated because of a hernial sac on the left side. In the hernial sac, which was attached to a small bowel loop, the same cysts as in the first operation were found. These were up to 2 cm in size and attached to the parietal peritoneum.

Microscopically, the cavities and peritoneal serosa are often separated only by a very narrow layer of connective tissue. The lumen is optically empty or contain finely granulated, eosinophile, Alcian-blue positive (PAS-reaction negative) material. The lining of the cysts consists primarily of endothelial-like flat cells, in the small cysts some cuboidal cells are seen with long or round nuclei and small nucleoli (Fig. 3b). Apically brushlike cell processes can be seen. Rarely papillae or layered cell complexes which extend into the lumina of the cysts can be recognized (Fig. 3c). Particularly in the area of the greater omentum there are long, partly mesh-like groups of cells, directly connected to the lining of the cysts. Within these mainly solid strands some small cystic lumina are seen. Electron microscopic examination showed cuboidal cell forms with brush-like processes in addition to endothelium-like flat cells with few microvilli. The cells, which lie on a basement membrane are connected by desmosomes. The cytoplasm contains numerous filaments which, in part, are connected to the areas of attachment. Pinocytic vesicles are not seen. The nuclei, are often strongly twisted especially those of the flat cell forms (Fig. 4a–c). The relapse shows basically the same light and electron microscopic findings. Smaller lumina are sometimes grouped around loose connective tissue which contains blood vessels. The cysts are often also surrounded by a wide covering of connective tissue, where the cells of the inner lining are desquamated. Several round structures contain only fissure-like lumina or are completely solid. This phenomenon was sometimes seen in the first operative specimen but was a conspicuous finding here (Fig. 3d). Ultrastructurally we found closely packed connective tissue cells in these areas with Golgi fields and relatively abundant rough endoplasmatic reticulum. Sometimes they contain isolated intracytoplasmatic collagenous fibers with typical rhythmic transverse striation. Outside of the cell abundant fibrous material of the same type can be found. Formation of collagenous fibers within the cell itself cannot be demonstrated without doubt in this case; several authors however have pointed out the possibility of intracytoplasmic collagen fiber formation (Welsh 1966; Tannenbaum 1971; Allegra and Broderick 1973; Ghadially 1980).

A diagnosis of Benign cystic mesothelioma was made.

Case 3 (E.Nr. 8348/79): During a routine gynecological examination in a 24-year old female a cystic ovarian tumor about the size of a goose egg was found. Intraoperatively a polycystic structure about the size of a fist was found in the greater omentum, extending into parts of the gastrocolic ligament. A cyst the size of a chickens egg extended into the bursa omentalis; this cyst was torn in the process of resection and a clear liquid was drained. The operative specimen, which consisted of the greater omentum and parts of the gastrocolic ligament was $18 \times 8 \times 3$ cm in size. On the surface there were multiple transparent cysts of various sizes. The largest lumina were somewhat larger than a cherry; however, the majority were about the size of a pea. The contents were watery, the interior wall of the cyst was smooth. Between the lumina there were septa of fatty and connective tissue of various widths, giving the impression of a sponge on the cut section (Fig. 5a). There were no postoperative complications. At this point, 3 years after the operation there are no indications of a relapse. Microscopically

Fig. 3a–d. Cystic mesothelioma: (**a**) Greater omentum with thin-walled multiple cysts, maximal cyst diameter 7 cm, arranged in acini. (**b**) Multiple small cysts in the greater omentum with flat to cuboidal mesothelial lining (Haematoxylin-Eosin, Obj. $6.3\times$) (**c**) Isolated papillary structures near cysts of the greater omentum (Haematoxylin-Eosin, Obj. $16\times$) (**d**) Cystic mesothelioma (Recurrence): Widened cyst wall with narrow mesothelial-lined restlumen. (Haematoxylin-Eosin, Obj. $6.3\times$)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the cysts are optically empty or contain small amounts of finely granulated, weakly positive Alcian-blue, PAS-negative material and isolated lymphocytes. They are lined by a continuous layer of endothelium like flat cells with longitudinal to ovally shaped nuclei with weakly staining chromatin and a bright cytoplasm. The bordering septa are formed by patches of fatty tissue as well as loose connective tissue, in which there are numerous lymph follicles, some with a germinal center (Fig. 5b). Only in a few places can smooth muscle fibers be seen in the wall of the cyst. Ultrastructurally the lining of the cyst corresponds to the endothelium of the lymph vessels, often with tile-like overlapping of the cells. Desmosomes can not be seen. The surface contains only few microvilli. Sometimes a few tonofilaments can be found in the cytoplasm; however, no pinocytotic vesicles are present. The material of a basement membrane is present only in fragments.

A diagnosis of Benign cystic lymphangioma of the greater omentum was made.

Discussion

These tumors are rare findings. There are no obvious clinical symptoms; possibly the cystic mesothelioma causes an increase in intraabdominal pressure which predisposes to inguinal hernia. A causal relationship between the tumors and exposition to asbestos as with malignant mesotheliomas of the peritoneum too (Winslow and Taylor 1960; Roberts and Irvine 1970; Moertel 1972; Davis 1974; Kannerstein and Churg 1977; Foyle et al. 1981; Boon et al. 1981) does not exist. Benign papillary mesotheliomas in their diffuse form belong to the group of the rarest peritoneal tumors (Wells 1935; Yoshida 1973; Ackerman 1975; Foyle et al. 1981). Because of the characteristic surface with a continuous transition to the flat mesothelial lining of the peritoneal cavity the diagnosis was made by histological examination during operation. The tumor is made up of cuboidal or flat-cylindrical-shaped cells and shows no infiltrative growth (Ackerman 1954). Only one of the benign papillary mesotheliomas described by Foyle et al. (1981) contained papillary structures alone, as in our case; the others also showed tubular structures. Typical features of the epithelial type of mesothelioma are numerous desmosomes, intracytoplasmatic filaments, partially arranged in groups and long stalk-like microvilli, which could be recognized with the light microscope. A well-formed basement membrane is also noteworthy (Wang 1973; Ghadially 1980) together with numerous basal and apical pinocytotic vesicles in the flat cell forms. In addition, typical single cilia, characteristic of the embryonic coelom epithelium and described in malignant mesotheliomas (Ferenczy et al. 1972) were seen in this highly differentiated tumor. In this case an inflammatory process or mechanical irritation, which might precipitate a reactive papillary mesothelial proliferation, was not present, so that we assumed the existence of a neoplastic process. The

Fig. 4a–c. Cystic mesothelioma: (**a**) Cuboidal mesothelia with microvilli on the free surface, partially twisted nuclei, desmosomal connections, nearly continuous basement membrane (3,500 ×). (**b**) Flat mesothelial cells with intracytoplasmatic filament bundels, intercellular and apparently intracellularly positioned desmosomes, continuous basement membrane, wide intercellular space (24,000 ×). (**c**) Section of **b**: Ring-like arranged desmosomes, probably formed by processes of a neighboring cell. Intracytoplasmatic filament bundle. Single glycogen granule (90,000 ×)

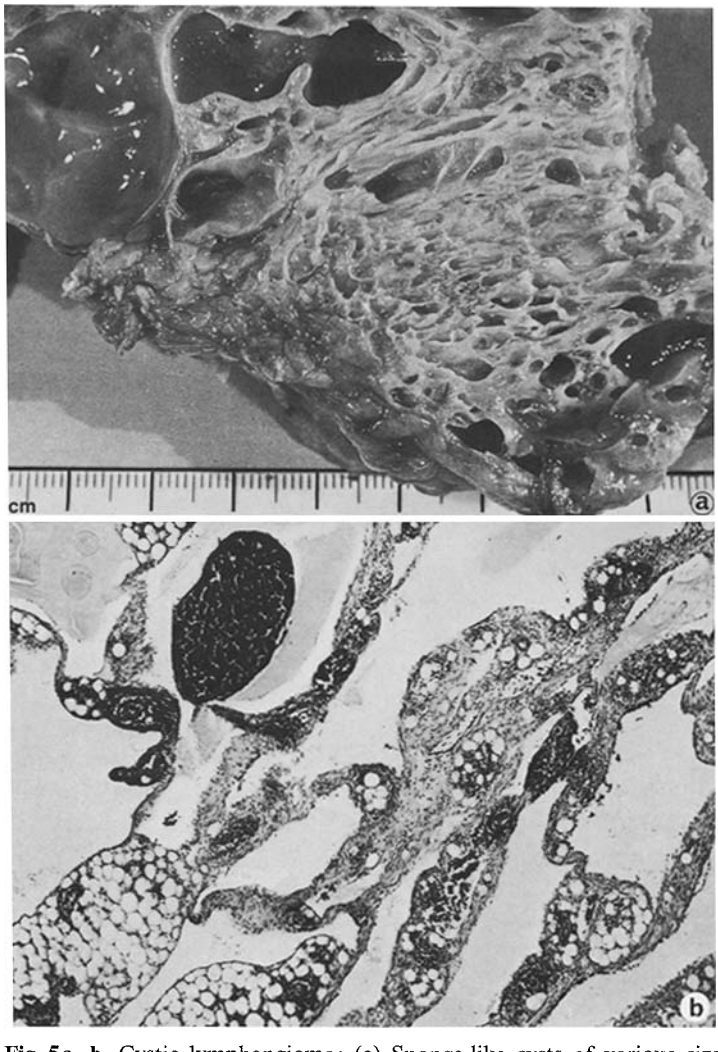

Fig. 5a, b. Cystic lymphangioma: (**a**) Sponge-like cysts of various sizes with smooth inner surface (section). (**b**) Overview with endothelially lined lumina. Much lymphatic and fatty tissue in the septa (Hämatoxylin-Eosin, Obj. 1 ×)

diagnosis of this diffusely growing, benign, monophasic mesothelial tumor was not difficult. The characteristic light microscopic and ultrastructural findings in the highly differentiated tumor cells seemed to be particularly suited for a comparison with the cystic tumors (Table 2).

After reviewing the literature, we found only a few cases (Table 1) which can be compared with cystic mesothelioma (case 2). The findings are almost identical, but only one (Moore et al. 1980) was examined with the electron microscope. The lumina were not, as in most cases of cystic lymphangiomas, limited to the greater omentum but extended to the bowel serosa and/or

Table 2. Differential diagnostic aspects of benign papillary mesotheliomas, benign cystic mesotheliomas and cystic lymphangiomas (literature and personal studies). All Tumors may show localized secondary inflammatory changes and are characterised by uniform nuclei

	Benign papillary mesothelioma	Benign cystic mesothelioma	Cystic lymphangioma
Macroscopically	Solitary or disseminated, foci, visceral and/or parietal peritoneum	Multiple cysts arranged in acini, subserous, visceral and/or parietal peritoneum	Cyst grouped in the subserous layer, mainly greater omentum or mesentery
Microscopically	Branched papillae, oedematous or fiber-rich stroma, flat to cuboidal cell covering	Flat to (rarely) low cylindrical cell-lining, solid cell strands and papillae may form	Flat cell lining, papillary formation possible, lymphatic-tissue, smooth muscle fibers
Histochemically	PAS−, Alcian-blue (pH 1)+	PAS−, Alcian-blue (pH 1)+	PAS−, Alcian-blue (pH 1)−
Ultrastructurally			
Microvilli	+++[a]	+++[a]	(+)
Basement membrane	++	++	(+)
Mitochondria	++	++	+
Desmosomes	++	++	−
Filament bundles	++	++	(+)
Pinocytotic vesicles	+[a]	−	−
Single cilia	+	−	−

[a] Localized findings

the parietal layer. The older authors in particular were of the opinion that these were lymph vessel tumors (Table 1); however, Henke (1899) indicated the possibility of cyst formation by segmentation of the mesothelium. To our knowledge, Hamdi et al. (1927) were the first authors to regard their case 1 (cysto-coelothelioma) as a tumor originating in the peritoneal serosa. The authors show isolated direct connections between the cysts and the serosal surface but in our cases 2 and 3 such connections were not present. The cysts were closed systems, even if they were directly beneath the serosa. The classification of these tumors either as lymphangiomas or mesotheliomas is made more complicated on the one hand by the close topographical relationship between peritoneum and subperitoneal lymph channels and on the other by the primarily endothelial-like flat cell lining of the cystic mesothelioma.

In the mesothelioma (our case 2) cytological and histological findings typical of reactive mesothelium were observed too, particularly in specimens of the greater omentum. In connection with mechanical or inflammatory irritation, cuboidal or cylindrical cells together with solid nodules and strands of cytoplasmrich cells are formed in the subserosal connective tissue (v. Gierke 1926; Rosai and Dehner 1975). Layered cell complexes and small cystic formations with chronic inflammatory processes are a common find-

ing (v. Gierke 1926; Blaustein 1977; Klima et al. 1977). After serosal defects cells which are rich in cytoplasm appear on the surface; these become flatter until they are ultrastructurally identical to the typical mesothelial cells (Ellis et al. 1965; Ryan et al. 1973). The origin of these cells is not fully explained. Possibly pluripotent subserous connective tissue cells, which can become serosal cells, take part in the repair of the defect.

The capacity of the mesothelium (Brandenburg 1953; Pendergrass 1954) to transform itself into epithelial and mesenchymal structures is shown by the presence of metaplasia to squamous epithelium and by the formation of connective tissue structures in the fibrous type of mesothelioma (Ackermann 1954). The connective tissue cells with intracytoplasmatic collagenous fibers which are seen especially in the recurrence of the cystic mesothelioma can be seen as a sort of mesenchymal differentiation. The possibilities of differentiation exhibited by the lymph vessel endothelium are relatively few. It can form cuboidal cell forms (Wernicke 1955) and papillary structures (Kuo and Gomez 1979), but cannot transform itself into epithelial-like cells with secretory properties. These were seen as narrow Alcian-blue positive mucus tops in the papillary and cystic mesotheliomas (Table 2). The secretion of acid mucin (primarily hyaluronic acid) is a characteristic of many mesotheliomas (Winslow and Taylor 1960; Wagner et al. 1962), but in our cases the hyaluronicdase-reaction was negative (see Wang 1973; Boon et al. 1981). Boon et al. (1981) mentioned the possibility of secretion of mucolipids using a malignant peritoneal mesothelioma as an example. The tumor cells contained numerous vacuoles and were characterized by a uniformity of the nuclei.

Those masses which are diagnosed as cystic lymphangiomas or lymphangiectasias by earlier authors and which are partially lined by tall cylindrical cells, are said to have the ability to secrete mucous substances. Ultrastructurally the lining of the cysts of the mesothelioma partly correspond to the normal serosal cells (Vogel 1957; Carr 1967; Simionescu and Simionescu 1977; Ghadially 1980) or to the tumor cells of the adenomatoid tumor (Marcus and Lynn 1970; Mackay et al. 1971; Ferenczy et al. 1972). It shows similarities to the epithelially differentiated malignant mesothelioma (Wang 1973; Klima et al. 1977; Ghadially 1980). Parts of cystic tumors sometimes are found in malignant serosal tumors (Rhind 1949; Godwin 1957) where they are lined by flat or cuboidal cells. In agreement with Moore et al. (1980) and the findings in normal serosal cells (Simionescu and Simionescu 1977) pinocytotic vesicles are not present in the cystic mesothelioma but are numerous in the papillary tumor. In both cases there are many desmosomes, a nearly continuous basement membrane, and microvilli of the same number and length.

As in our case 3 endothelial cells of the lymphangiomas are typically flat. Localized cuboidal cell forms are present (Winkler 1924; Wernicke 1955). Secretion of mucus is not described except in those lesions which are described as lymphangiomas in the table but are similar to our cystic mesothelioma. Smooth muscle fibers are usually embedded in the walls of the lymphatic cysts (Landing 1955); we were able to see only few such

fibers. Lymph follicles are typical and were numerous in our case. Electron microscopically the flat lymph vessel endothelial cells overlap in a tile-like fashion and are connected by maculae adhaerentes, sometimes by conulae occludentes. We were not able to recognize any desmosomes. They are not normally present in humans (Ghadially 1980) but are seen in mice now and then (Schipp 1965). On the endothelial surface only a few short cytoplasmic processes are present. A basement membrane is present only in small fragments in the lymph vessels (Borst 1969) (Table 2).

These macroscopic and microscopic observations and the development of the lesions suggest benign processes. Infiltrative growth, extending beyond the subserosal connective and fatty tissue, was not present in any case. Noteworthy nuclear or cellular polymorphism was not seen, nor were mitoses. In the cystic lymphangioma, which is composed of various cell types such as blood vessel endothelia and aggregates of lymphocytes and smooth muscle cells, we are probably dealing with a congenital anomaly (Landing 1956), which in the site described and (case 3) with a slow growth rate, manifested itself rather late. In spite of disseminating growth there is no evidence of malignancy in the papillary mesothelioma (case 1). Foyle et al. (1981) indicate the good prognosis of this tumor. The presence of hyperchromatic, enlarged nuclei and variant sizes of the mesothelium cells suggest transition to a malignant tumor (Klima et al. 1977). In the cystic mesothelioma (case 2), microscopic findings showing only localized secondary inflammation and the course of the illness, suggest a benign tumor. Since this tumor is rare there is only one report which enables the growth rate to be assessed. In the patient observed by Moore et al. over a period of 20 years, relapses and further growth were seen without any signs of malignancy. In the case of the cystic mesothelioma described here further relapses are to be expected.

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