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Comparative Light and Electron Microscopic Observations of the Cytoplasmic Matrix in Renal Carcinomas*

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"Clear" and "granular" cells are the predominant cell types in most renal carcinomas (ALLEN; LUCKÉ and SCHLUMBERGER; MELICOW). It is generally agreed that the clear cells contain abundant glycogen and fat in various proportions (ALLEN; HAMPERL; LINDLAR; LUBARSCH), while the granular cells appear to be devoid of these substances or only contain small amounts of them (BÖTTIGER; BÖTTIGER and IVEMARK). Although most investigators claim that the PAS-positive substance in the tumor cells is solely due to the presence of glycogen (ALLEN), it has been suggested that there may be an intracellular PAS-positive component which is diastase-resistant (BÖTTIGER and IVEMARK). Further unresolved problems are related to the question of whether translucent or clear appearance of cytoplasm may occur in cells which contain neither glycogen nor fat (ALLEN; BÖTTIGER and IVEMARK). The importance of elucidating these problems is reinforced by the suggestion that there may be a correlation between the clinical characteristics of renal carcinomas and their intracellular content of PAS-positive materials (BÖTTIGER).

The cytoplasmic matrix ("cell sap", "cytoplasmic ground substance") represents the continuous phase of cytoplasm, in which ribosomes, particulate glycogen, fat, and various filamentous, microtubular and unidentified particulate materials are suspended. The aim of the present study has been to clarify the structure of the cytoplasmic matrix in renal carcinomas, with particular attention to PAS-positive materials and fat and the relationship of these to cytoplasmic translucency. To this end, a correlated light and electron microscopic study of paraffin and resin embedded, osmium and aldehyde fixed renal carcinomas has been performed.

Materials and Methods

Eleven surgically removed renal tumors — all with a diameter exceeding 5 cm — were obtained from patients under general anesthesia. In general, the tumors were made available for preparation for the morphologic studies and determinations of glycogen within 2—3 min after the renal artery was clamped. The kidney with the tumor was rapidly bisected and portions of tumor tissue lacking gross signs of necrosis along with unaffected kidney were excised. Materials from at least three different areas of the tumor were investigated. In those cases where the tissues showed marked variation in the gross appearance of different areas, additional material was removed in order to obtain samples with representative morphology. In order to secure a strict correlation between the morphology and the content of glycogen in a given portion of the tumor, relatively small areas of tissue were trimmed out and were subsequently cut into pieces of suitable size for the different preparatory procedures.

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For electron microscopy, small cubes of tissue with a side not exceeding 1 mm were immersed in 2 per cent osmium tetroxide (OsO_4) buffered with *s*-collidine, or in 1 per cent phosphate-buffered OsO_4 (pH 7.4) at $+4^0$ C for a period of $1^1/_2$—2 hours. The tissues were dehydrated at $+4^0$ C in a series of ethanol solutions of increasing concentrations, starting at 70 per cent; they were immersed in propylene oxide at room temperature and were then embedded in Epon 812 (LUFT). Thin sections were prepared on LKB Ultrotomes supplied with glass knives and were studied in Siemens Elmiskop I, either unstained or stained with lead hydroxide, aqueous uranyl acetate, or a combination of these methods. Prior to thin sectioning, the fixation was controlled by light microscopic studies of $\sim 1\ \mu$ thick sections stained with alkaline toluidine blue. In two cases, slices of tumor tissue ($\sim 100\ \mu$ thick) were fixed in 3 per cent glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) for 6 hours and were subsequently treated with 0.5 per cent diastase for 8 hours at 37^0 C. The tissue was then washed in 0.1 M phosphate buffer containing 7.5 per cent sucrose, immersed in OsO_4, and processed for electron microscopy. Control tissues were incubated in a 0.9 per cent solution of NaCl for 8 hours at 37^0 C, and were subsequently processed as described above.

Light microscopic observations were made of $\sim 1\ \mu$ thick sections of osmium-fixed sections stained with alkaline toluidine blue, or the periodic acid-Schiff (PAS) method with or without prior digestion with diastase. In addition, light microscopic observations were performed on aldehyde-fixed tissues embedded in paraffin or Epon. These tissues (blocks 1 to 2 mm thick) were fixed in 3 per cent glutaraldehyd or 2 per cent paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) at $+4^0$ C for periods varying between 6 and 24 hours. Following fixation, the tissues were transferred to a 0.1 M tris-maleate buffer (pH 7.4) containing 7.5 per cent sucrose and were stored in the buffer at $+4^0$ C. Tissues washed in buffer were dehydrated and embedded in paraffin according to routine histological techniques. Sections cut at $3\ \mu$ were stained with hematoxylin and eosin (H and E), van Gieson's connective tissue stain, and the PAS technique with or without prior digestion with diastase. Sections of liver from protein-deficient dogs (ERICSSON, ORRENIUS, and HOLM) were run as controls for the diastase digestion. Digestion times ranged from 30 min to 4 hours.

For light microscopy, some aldehyde-fixed tissues were post-fixed in OsO_4 and were thereafter embedded in Epon (see above). Approximately $1\ \mu$ thick sections were stained with toluidine blue or the PAS technique as described previously.

For the demonstration of triglycerides, frozen sections of aldehyde-fixed tissues were stained with Oil Red 0.

For the quantitative determinations of glycogen, appropriate portions of tissue were homogenized in 0.15 M KCl. The amount of glycogen was determined in the homogenate (HASSID and ABRAHAM).

In order to compare the appearance of routinely formaldehyde-fixed renal carcinomas with that of the present, specially fixed and treated material, twenty consecutive cases from the files of the department were studied. In some of these cases, material processed for electron microscopy was also available. Sections of the routinely formaldehyde-fixed, paraffin-embedded tumors were stained with H and E and the PAS technique (with and without prior diastase digestion).

Results

I. Tissues Fixed for Fine Structural Studies

1. Light Microscopy. A summary of the light microscopic appearance and glycogen content of the tumors is given in the Table. Five of the tumors were of clear cell type (as revealed in paraffin sections of aldehyde-fixed tissues stained with H and E, see Fig. 1), while 3 were of granular cell variety (Fig. 2), and 3 were of mixed composition.

All of the clear cells contained abundant PAS-positive, diastase digestible material in their cytoplasm, as demonstrated in paraffin sections of aldehyde fixed tissues (Figs. 3 and 4). This material appeared to be diffusely distributed

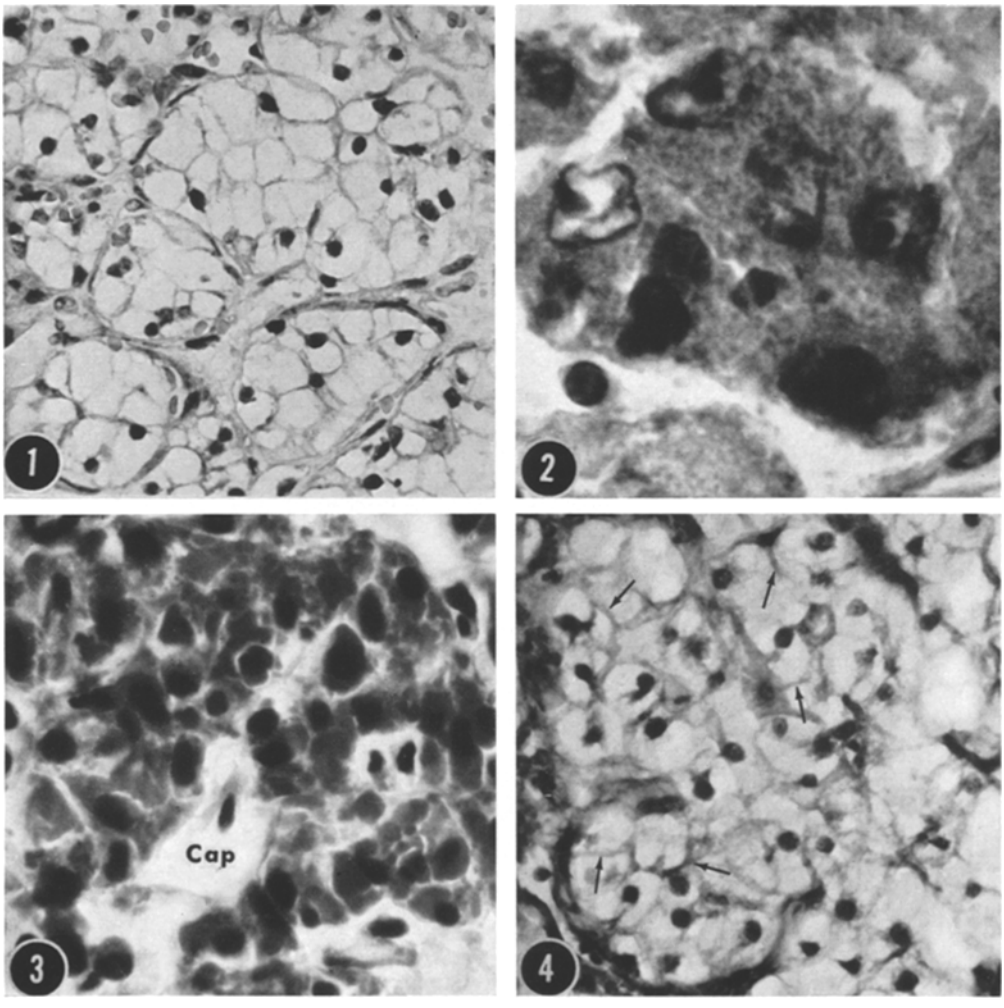


Fig. 1*. Case no. 6. Section of clear cell carcinoma fixed in glutaraldehyde, embedded in paraffin, and stained with H and E. Note translucency of the cytoplasm in all the cells. × 320

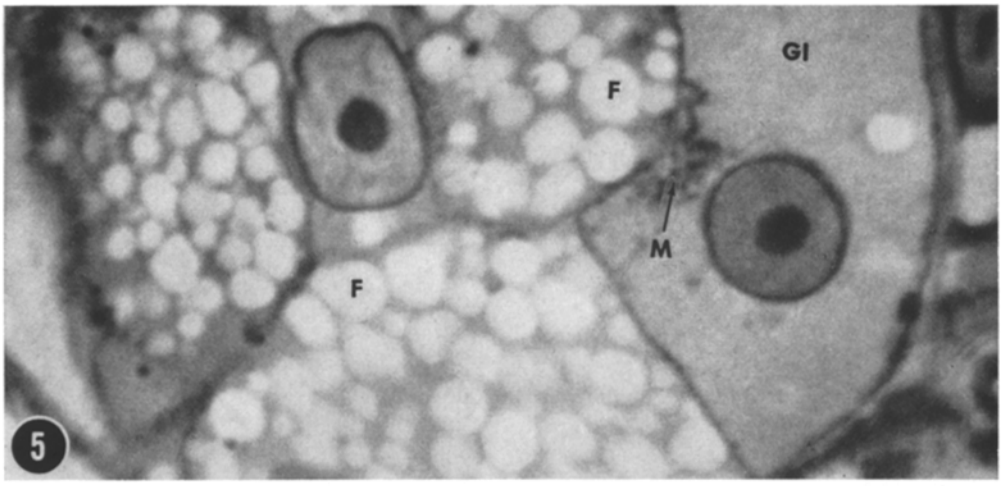
Fig. 2. Case no. 2. Granular cell carcinoma with rather marked pleomorphism. The cytoplasm is filled with numerous tightly packed granules. Specimen fixed in glutaraldehyde and embedded in paraffin; section stained with H and E. × 1,100

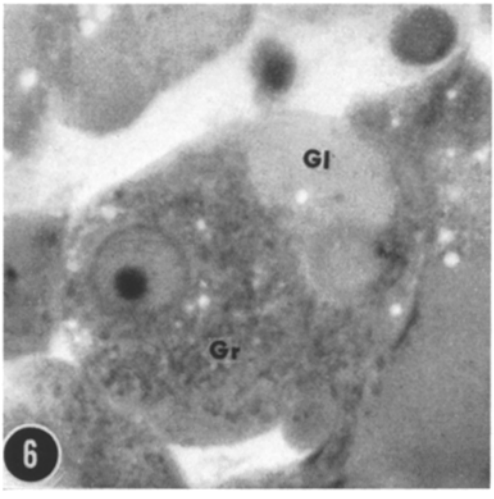
Fig. 3. Case no. 6. Section of clear cell carcinoma fixed in glutaraldehyde, embedded in paraffin, and stained with PAS. Abundant PAS-positive material in the cytoplasm of all the tumor cells. This picture should be compared with the ones in Figs. 1 and 4. *Cap*, capillary. × 320

Fig. 4. Case no. 6. Section of clear cell carcinoma fixed in glutaraldehyde, embedded in paraffin, and stained with PAS following digestion with diastase for 2 hours. The cytoplasm of all the cells is devoid of PAS-positive material. Note diastase-resistant, PAS-positive material in relation to cell walls and intercellular spaces (*arrows*). × 300

throughout the cytoplasm, while the nucleus was non-reactive. In some cells, clear, rounded areas completely surrounded by PAS-positive material were observed; some of the clear areas appeared to correspond to the droplets of triglyceride which were revealed in frozen sections stained with Oil Red 0 (see below).

* Figs 1—10 are light microscopic pictures of renal carcinomas, fixed, embedded, and stained as indicated in the legend to each figure.





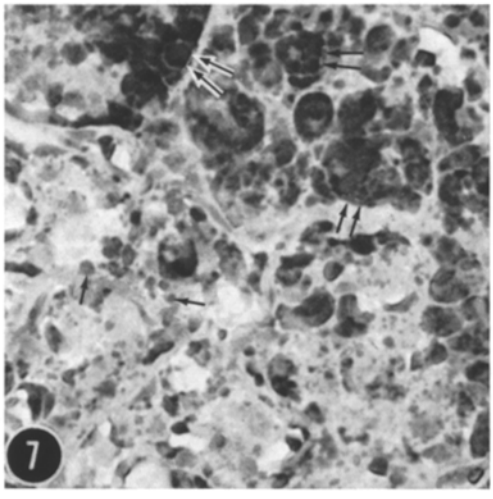


Fig. 5. Case no. 10. Section approximately 1 μ thick of clear cell carcinoma fixed in OsO_4, embedded in Epon, and stained with toluidine blue. Portions of 5 cells are shown. Of these, 3 contain numerous large, pale vacuoles (*F*) presumed to represent dissolved neutral fat (an Oil Red 0 stained frozen section of aldehyde-fixed tissue from this tumor is shown in Fig. 7). In one cell, the cytoplasm is homogeneous with occasional granules presumed to represent mitochondria (*M*). An adjacent section stained with PAS demonstrated that the homogeneous material (*Gl*) was PAS-positive and appeared to represent glycogen. × 2,900

Fig. 6. Case no. 11. Section of tumor with mixed population of cells treated as the one shown in Fig. 5. One cell contains numerous granules (*Gr*) in its cytoplasm. Electron microscopy showed that these mainly represented mitochondria. Another cell has a homogeneous cytoplasm presumed to contain glycogen (*Gl*) (cf. Fig. 5). × 1,200

Fig. 7. Case no. 10. Frozen section of glutaraldehyde-fixed clear cell carcinoma stained with Oil Red 0. Droplets of neutral fat of highly variable size are present in the cytoplasm. Small droplets are indicated by *single arrows*, large droplets almost filling entire cells are marked by *two arrows*. × 550

Although some intracellular PAS-positive material was present following digestion with diastase for 30 minutes to 1 hour — the times usually recommended in textbooks — this material could be completely digested away by prolonged incubation in diastase for 2—3 hours. Similarly, incomplete digestion of PAS-positive material was noted in control sections of tissues containing abundant glycogen (livers from protein deficient dogs) incubated for 1 hour. In thick blocks of

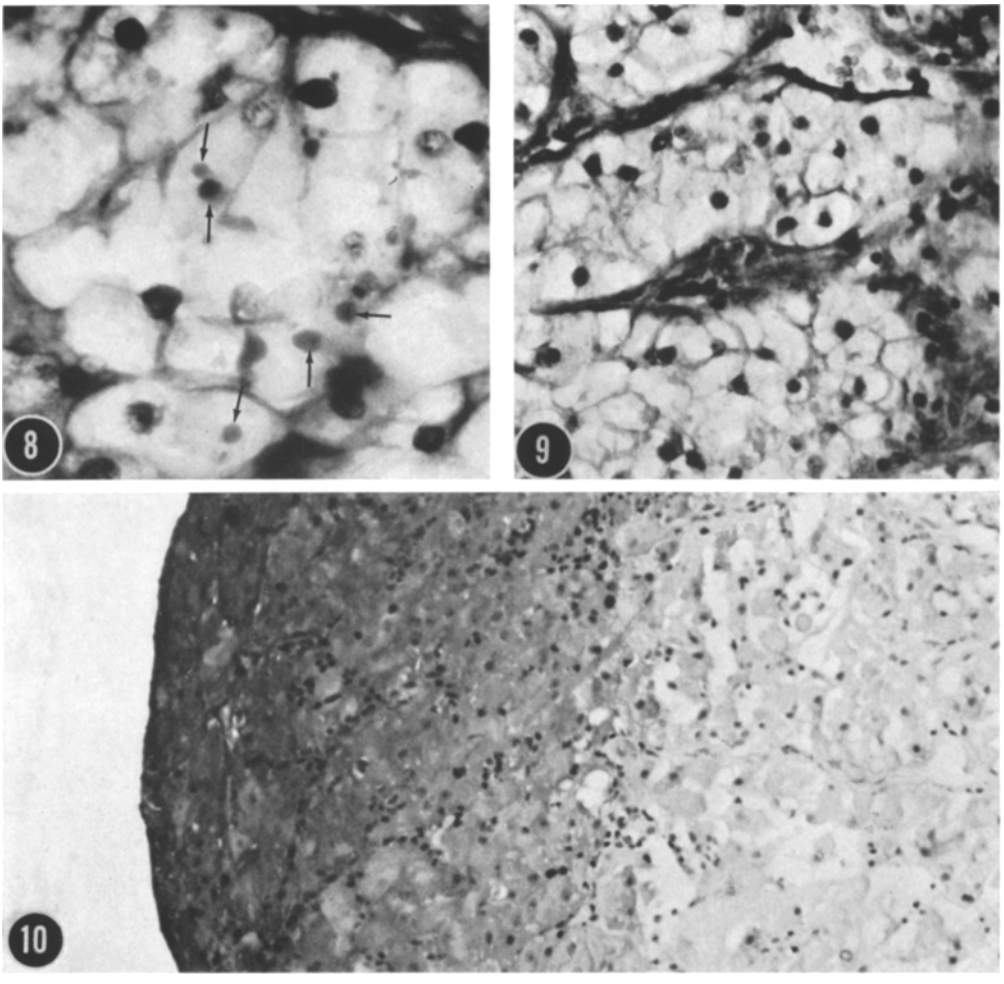


Fig. 8. Section of routinely fixed surgical specimen of clear cell carcinoma stained with PAS. There are occasional irregular PAS-positive areas (*arrows*) in the cytoplasm. Cell walls and basement membranes are also PAS-positive. (Neutral fat could not be demonstrated in this tumor). × 500

Fig. 9. From the same specimen as illustrated in Fig. 8. Section treated with diastase prior to staining with PAS. Only cell walls, basement membranes, and extracellular materials have retained a positive staining reaction. × 260

Fig. 10. Case no. 6. Section of clear cell carcinoma fixed for 24 hours in glutaraldehyde, embedded in paraffin, and stained PAS. This picture illustrates the narrow peripheral zone with good fixation and PAS-positive cells (left hand part of the picture). Deeper in the tissue (right hand part of the picture) preservation is poor, intercellular spaces show irregular widening, and the cells are PAS-negative. × 170

tissue (exceeding 2 mm in thickness), PAS-positive material was only seen in the well-fixed peripheral zone (see Fig. 10). As described previously (Seljelid and Ericsson), the tumor cells were often surrounded by bands or coatings of PAS-positive, diastase-resistant substance which appeared to be closely related to the plasma membrane (Figs. 4, 8, and 9). This material blended with the similarly staining basement membrane material surrounding groups of tumor cells growing as solid cords or papillary formations. Granular cells contained no or only traces of PAS-positive intracellular material but were often surrounded by a small amount of diastase resistant PAS-positive substance.

As shown in the Table, there was a good correlation between the amount of glycogen determined chemically in homogenates of tumor tissue and that appreciated by the observation of PAS-stained sections.

The amount of neutral fat varied greatly between different tumors and also between different areas of the same tumor. Although some clear cells contained large amounts of fat (Fig. 7), others contained no or only occasional droplets (cf. Table). Granular cells contained no or very small amounts of fat. The fat

Table

Case No.	Histological type (H and E)	Glycogen content mg/g tissue [1]	PAS-positive, diastase digestible material	Neutral fat
1	Clear cell		++	+
2	Granular cell		—	(+)
3	Clear cell	20.6	+++	—
4A	Mixed, with predominance of granular cells	4.0	+++ (in clear cells)	—
4B	Mixed, with predominance of granular cells	4.0	— (in granular cells)	+
5	Clear cell	14.0	++	—
6	Clear cell	32.0	+++	(+)
7A	Mixed		+++ (in clear cells)	
7B	Mixed		— (in granular cells)	
8A	Granular	3.7	—	—
8B	Granular (metast.)	2.9	—	—
9A	Mixed	17.3	+++ (in clear cells)	—
9B	Mixed	17.3	— (in granular cells)	—
10	Clear cell	41.2	+++	+++
11A [2]	Mixed		++ (in clear cells)	+
11B [2]	Mixed		— (in granular cells)	+

[1] The values for normal renal cortex ranged between 0.8 and 3.8 mg/g tissue.

[2] In this tumor, all transitions between granular, PAS-negative, and granular, PAS-positive cells with more or less clear cytoplasm were encountered.

appeared as intracellular droplets varying in size between 0.2 and 8 μ or more. In some instances, the major part of the cytoplasm was occupied by one large droplet. Although fat-containing cells were more numerous and contained larger amounts of fat in portions of tumor tissue located in the vicinity of necrotic areas, most of the tumor cells containing neutral fat did not appear to show degenerative alterations. Clear cells containing neither glycogen nor fat were not observed.

The light microscopic appearance of clear and granular cells in osmium-fixed tissues embedded in Epon is demonstrated in Figs. 5 and 6. Clear cells from

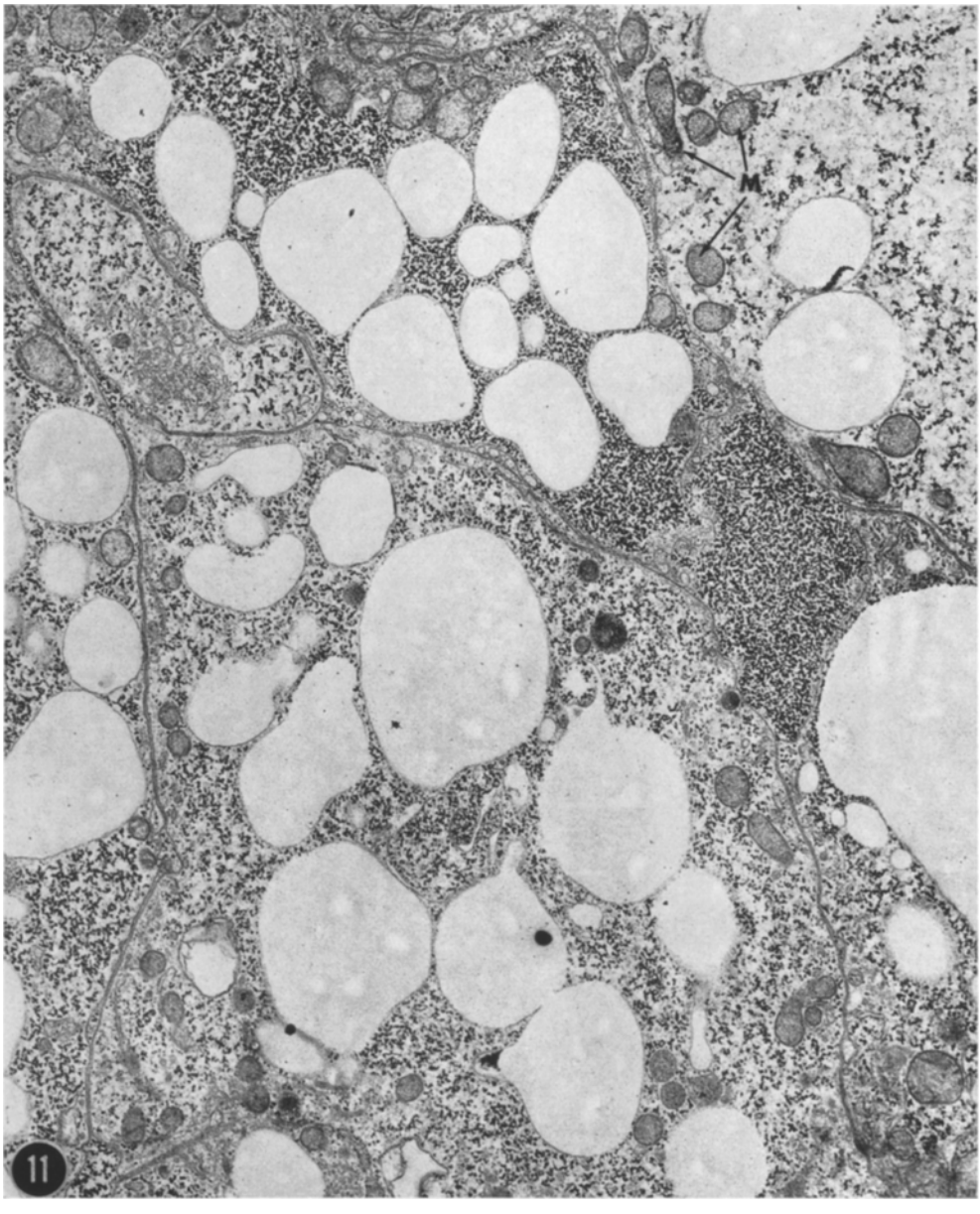


Fig. 11*. Case no. 10. Portions of several cells containing abundant particulate glycogen and numerous variously-sized vacuoles. Many of the latter appear to be surrounded by membrane-like condensations of cytoplasmic matrix or true membranes. Note sparsity of mitochondria (*M*) as well as other cytoplasmic organelles. × 9,300

tumors containing both glycogen and fat had abundant cytoplasm with homogeneous, pale blue matrix in sections stained with toluidine blue. The homo-

* Figs. 11—23 illustrate the electron microscopic appearance of thin sections of cells from renal carcinomas stained with lead hydroxide. The tissues shown in Figs. 11—13, and 16—23 were fixed in OsO_4 buffered with *s*-collidine, while the ones in Figs. 14 and 15 were fixed in glutaraldehyde and were post-fixed in OsO_4.

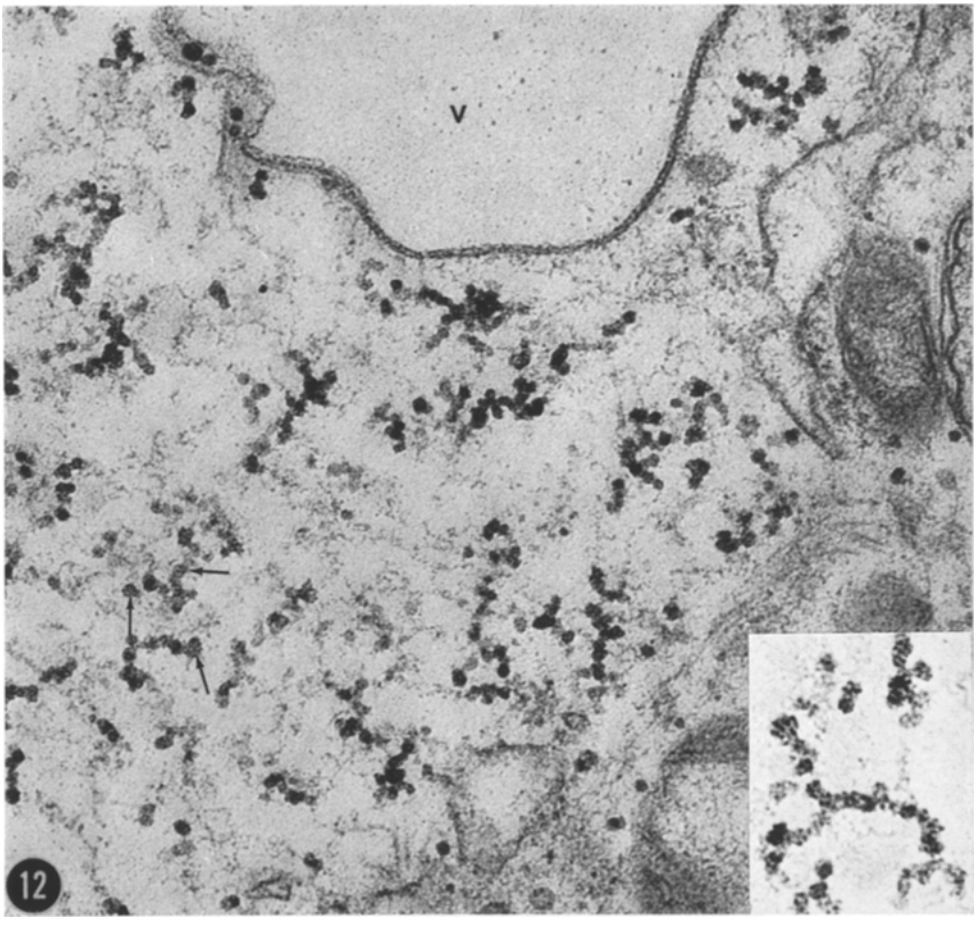


Fig. 12. Case no. 1. High magnification picture illustrating the appearance of particulate glycogen. Some of the particles seem to form chains of variable length. However, "rosettes" are not observed, and the general structure is that of "monoparticulate" glycogen. Substructures consisting of short rods or dots are revealed in some of the glycogen particles (*arrows* and inset). Note that the vacuole (*V*) is limited by a double-contoured membrane. × 55,000; inset × 80,000

geneous, blue-staining material appeared to correspond to the PAS-positive, intracellular material, as revealed in Epon sections stained with PAS. Within these areas, vacuoles of variable size were sometimes randomly distributed. Granular elements with a size corresponding to that of mitochondria and cytosomes were sparse. In the granular cells, on the other hand, such granules — with a size of 0.3 to 1.0 μ — were abundant and filled the cytoplasm. Occasional small vacuoles were observed in some of these cells.

2. Electron Microscopy. The fine structural appearance of clear cells is shown in Figs. 11—18. These cells appear to represent neoplastic proximal tubular cells, as reported previously (Seljelid and Ericsson). In sections stained with lead hydroxide, the cytoplasmic matrix contained large numbers of ~300 Å large granules with the appearance of particulate glycogen in mono-particulate form (Figs. 11, 12, 16, and 17). Similar, although much fewer, granules may occasionally be seen in normal proximal tubular cells (Fig. 22). In the tumor cell glycogen

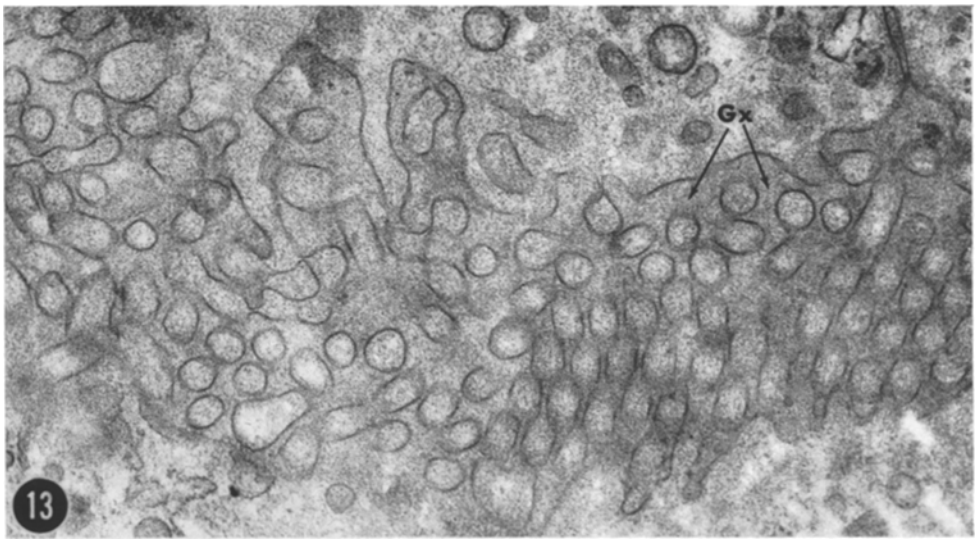


Fig. 13. Case no. 3. Portion of the surface of clear cell. The plasma membrane forms microvillus-like structures surrounded by a moderately electron dense, homogeneous, extracellular "coating" or "glycocalyx" (*Gx*) believed to represent the PAS-positive, diastase-resistant material seen in the light microscope along the cell borders (*cf.* Figs. 4, 8, and 9). × 35,000

particles, ~40 Å large substructures, possibly short rods — were revealed (Fig. 12). The glycogen occupied by far the largest portions of cytoplasm, and cytoplasmic organelles — such as mitochondria, cytosomes, endoplasmic reticulum, and Golgi apparatus — were sparse (Fig. 11). In blocks of tissue that had been incubated with diastase, the glycogen particles were absent, while cells incubated in control medium still contained the particles (Figs. 14 and 15). The extracellular "coatings" which were observed in osmium-fixed tissues (Fig. 13) were retained during diastase digestion.

In addition to the glycogen particles, clear "empty" vacuolar elements — varying in diameter between 0.2 and 8 μ — were observed in the cytoplasmic matrix of the clear cells. Although, in most instances, these structures occurred in cells which contained triglyceride, similar elements were occasionally observed in tissues lacking histochemical evidence of neutral fat (cf. Table). Their size and distribution corresponded to that of the fat droplets in tissues stainable with Oil Red 0. Some of the vacuoles (Figs. 14 and 16) were delimited from the cytoplasmic matrix by a single-contoured membrane, while others lacked a clearly identifiable membrane (Figs. 15, 17, and 18); still others had a double-layered envelope (Fig. 12). Mitochondria and smooth-surfaced endoplasmic reticulum were sometimes closely associated with the vacuoles (Figs. 16 and 17). Occasional cells contained structures with an appearance similar to that of fat droplets in other tissues with an irregular, peripheral rim of electron opaque material (Fig. 18). Empty-appearing areas of ground cytoplasm lacking glycogen, vacuoles and cytoplasmic organelles were never encountered. In some tumors, capillaries surrounding cells with abundant glycogen contained clearly identifiable glycogen particles in their lumens (Fig. 19).

In the granular cells, areas with predominant cell sap were small, while mitochondria — and in some instances cytosomes — were abundant (Fig. 20). In between these organelles occasional granules with the appearance of glycogen

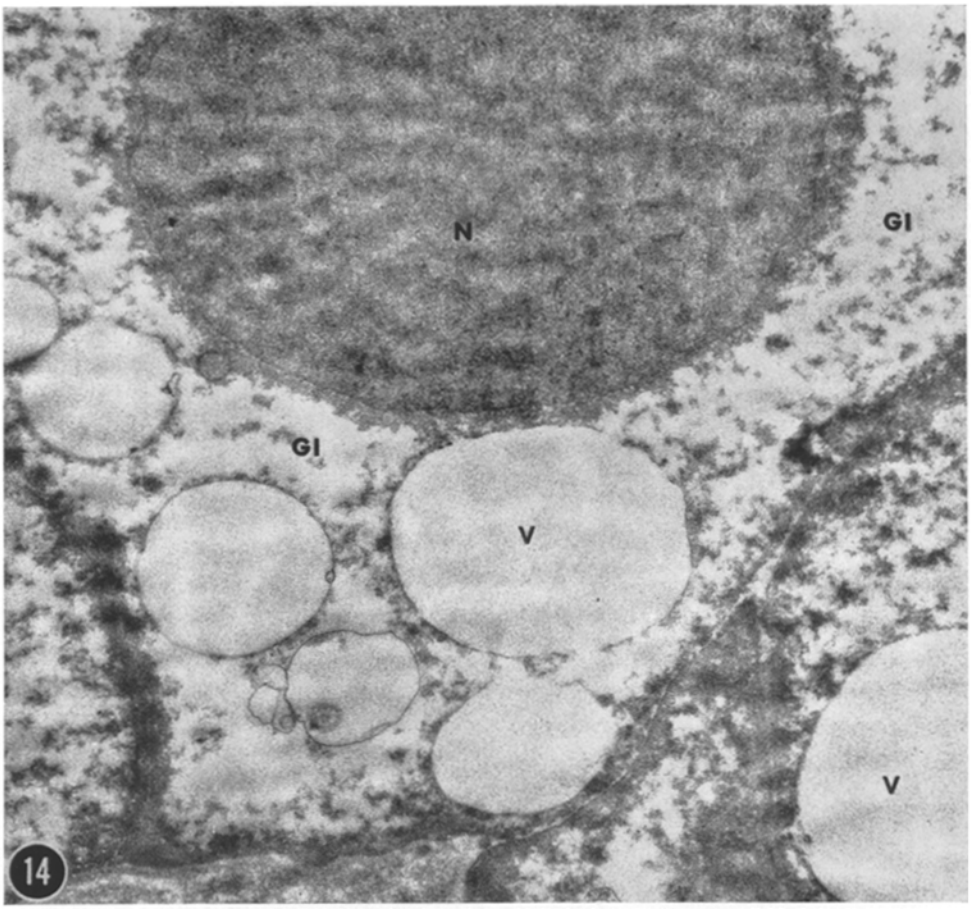


Fig. 14. Case no. 6. From tissue which was fixed in glutaraldehyde, incubated as a small block in diastase, and subsequently was postfixed in OsO_4. Empty areas of cytoplasmic matrix (*Gl*) appear to represent glycogen areas where the glycogen has been dissolved (*cf.* Fig. 15). *N*, nucleus; *V*, vacuoles, partially surrounded by membrane-like structures or condensations of ground cytoplasm. × 12,500

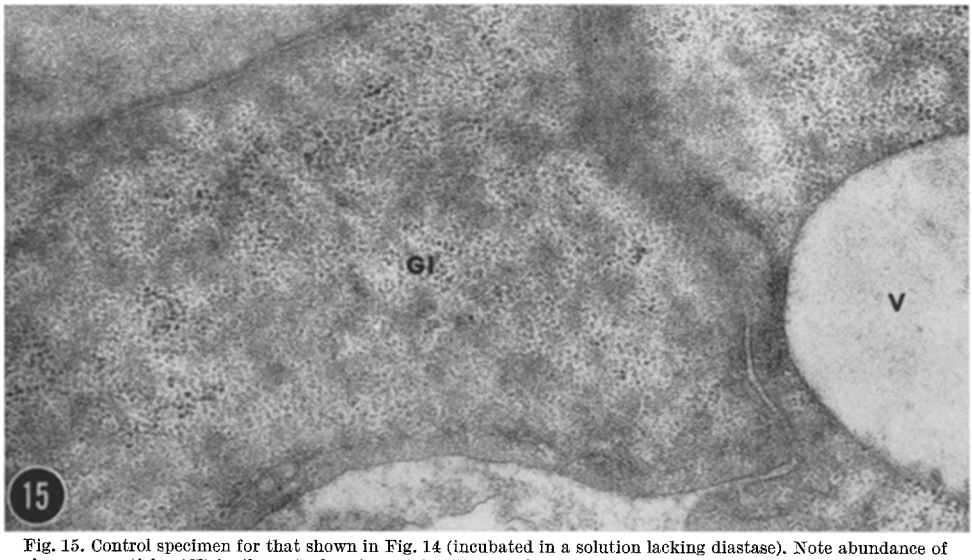


Fig. 15. Control specimen for that shown in Fig. 14 (incubated in a solution lacking diastase). Note abundance of glycogen particles (*Gl*) in the cytoplasmic matrix. *V*, vacuole, possibly surrounded by a membrane. × 25,000

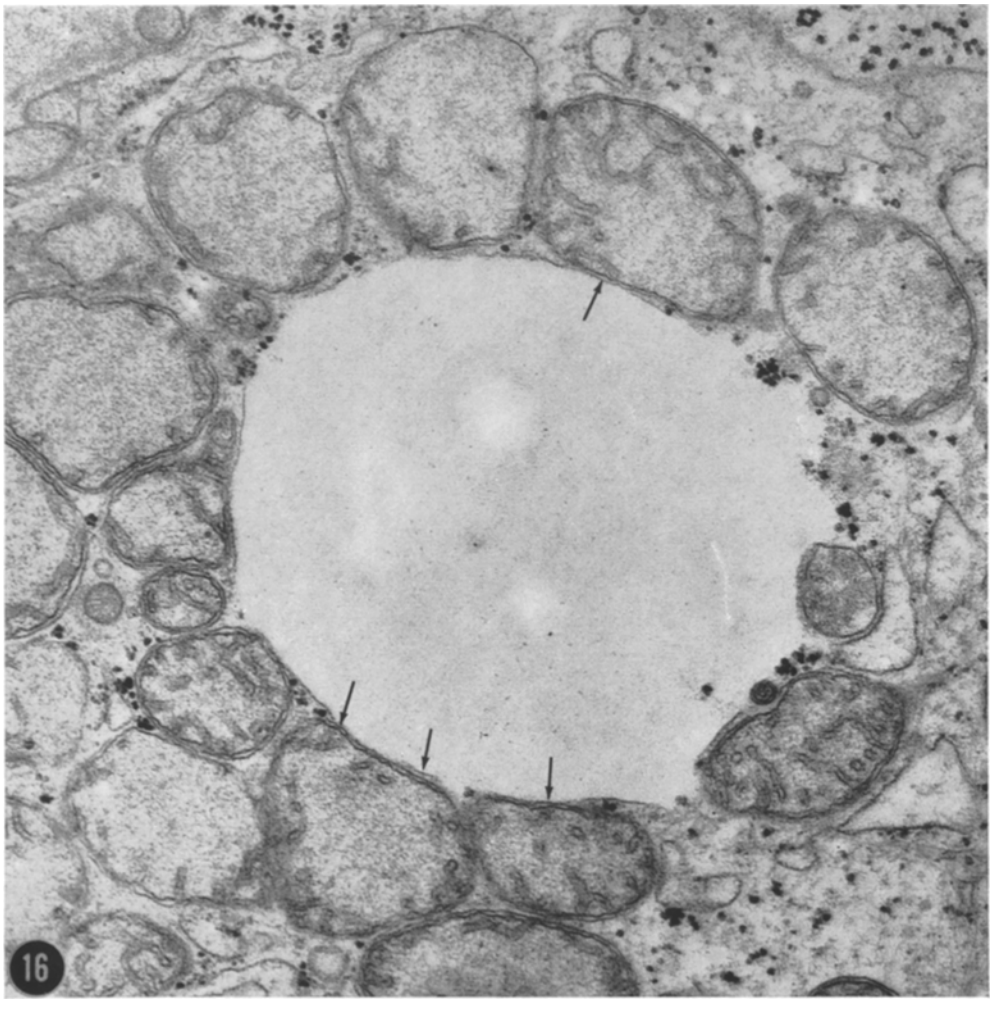
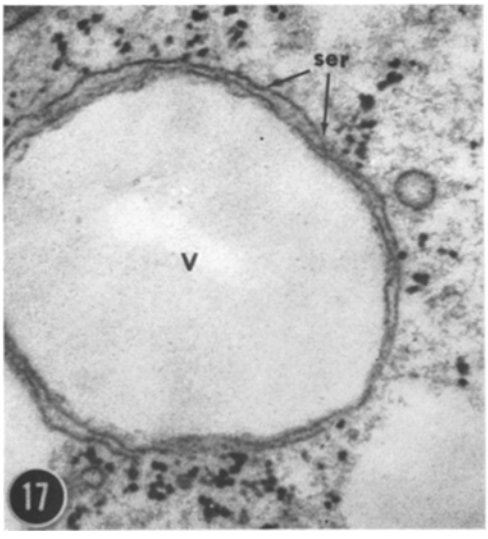


Fig. 16. Case no. 4 (mixed granular and clear cell type). A large vacuole is partially delimited by a membrane-like structure. It is closely surrounded by numerous mitochondria. Some of the latter appear to flatten out against the membrane (*arrows*). Suboptimal fixation is indicated by mitochondrial swelling and membrane abnormalities (disappearance of the outer leaflet in some mitochondria). × 32,000

particles were encountered (Fig. 21). Sometimes the cells also contained small vacuoles (Figs. 20 and 23); some of these were homogeneously electron dense and showed a structure similar to that of lipid droplets in other tissues. They were surrounded by condensed cytoplasmic matrix but usually did not show a clearly recognizable membrane.

Free ribosomes were very sparse in the clear cells. In the granular cells, ribosomes tended to be more numerous, particularly in those cells which had large, pleomorphic nuclei and showed evidence of a low degree of differentiation. In some tumors composed of a mixed population of granular and clear cells, all transitions between the two cell types were encountered.



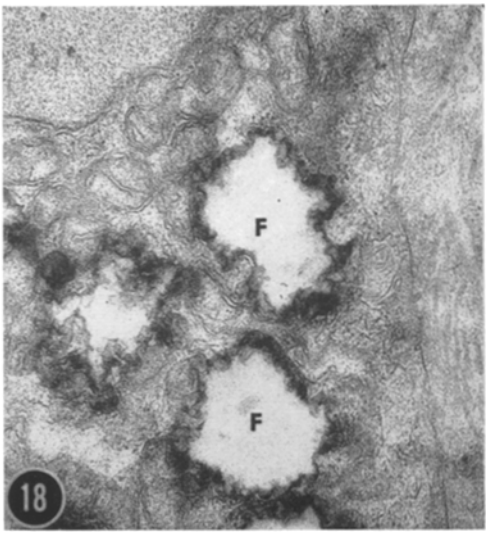


Fig. 17. Case no. 4 (mixed granular and clear cell type). A vacuole (*V*) is closely associated smooth-surfaced tubular elements (*ser*) presumed to represent smooth-surfaced endoplasmic reticulum. × 33,000

Fig. 18. Case no. 5 (clear cell carcinoma). Fat droplets (*F*) were identified in this case by the presence of a narrow electron opaque rim in the vacuole-like structures. × 27,000

Fibrils and microtubules were rarely encountered in the osmium-fixed tissues. In one case (No. 11) large, round, tightly packed aggregates of a filamentous-appearing or granular substance were observed (Fig. 23). These aggregates were clearly located in the cytoplasmic matrix and were not membrane-limited. They were up to 10 μ in diameter. Although some of the aggregates tended to surround fat vacuoles (Fig. 23), a definitive spatial relationship to cytoplasmic organelles was not evident. They appeared to correspond to slightly acidophilic, rounded cytoplasmic inclusions as seen in aldehyde-fixed tissues embedded in paraffin and stained with H and E. They did not seem to be PAS-positive.

II. Routinely Fixed and Processed Tissues

In general, the preservation of cytoplasmic details and intercellular relationships was much inferior to that in the specially fixed tissues. In tumors composed of clear cells, staining of the cytoplasm with the PAS technique was irregular and varied greatly among different areas of the blocks. Most of the clear cells contained no intracellular PAS-positive material at all. When present, PAS-positive substance tended to be limited to small rounded or irregular portions of the cytoplasm (Fig. 8). This intracellular substance was digestible with diastase (Fig. 9). On the other hand, the diastase-resistant, extracellular PAS-positive material in basement membranes and "coatings" appeared to be well preserved in the routinely fixed and processed tissues (Fig. 9). Similarly, droplets of neutral fat could be demonstrated in many of the specimens in frozen sections.

In tumors composed of granular cells, no PAS-positive, intracytoplasmic material was present. The cells contained a few or no fat droplets.

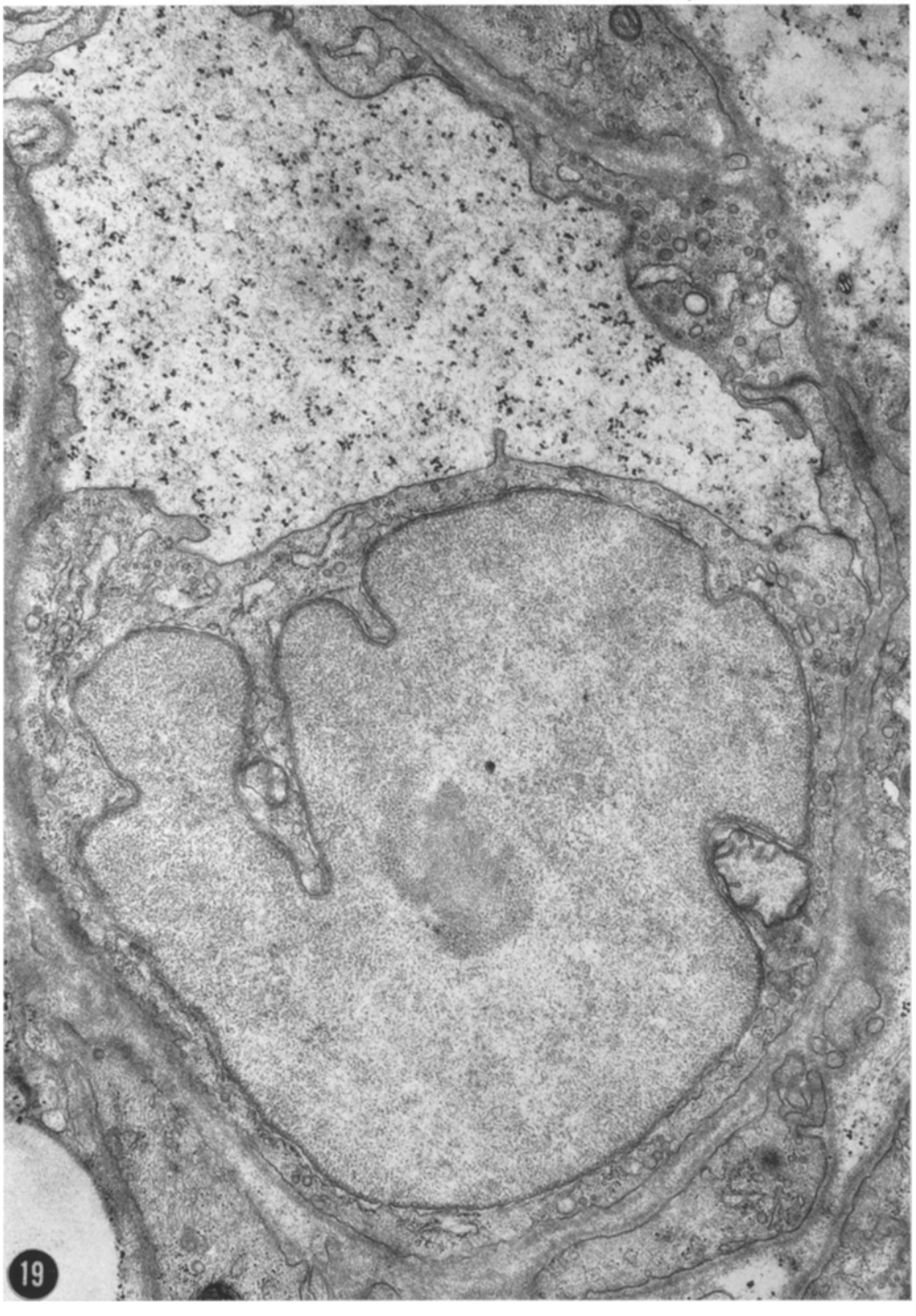


Fig. 19. Case no. 3 (clear cell carcinoma). Portion of a capillary containing numerous glycogen particles. Note that these particles are absent from the endothelial lining of the capillary but may be seen in extracellular spaces of the interstitial tissue (right upper and lower corner of the picture). × 16,000

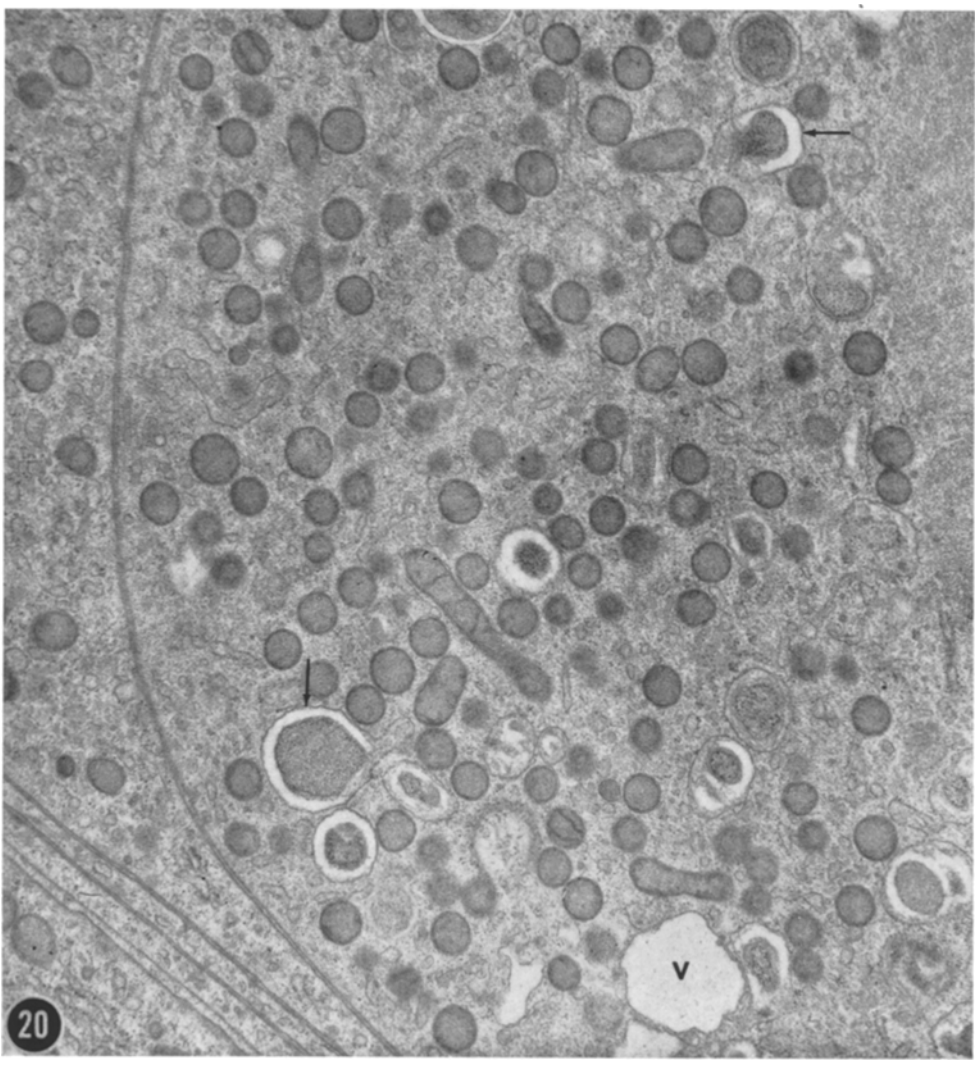


Fig. 20. Case no. 8B (metastasis from granular cell carcinoma). Abundant small mitochondria in the cytoplasm. In addition, there are occasional vacuoles (*V*), some with cytoplasmic organelles and matrix substance bulging into the cavity (*arrows*). × 13,000

Comment

The findings in the present study support the contention (HAMPERL; LUBARSCH) that translucency of the cytoplasm in the cells of renal clear cell carcinomas is due to the presence, in the cytoplasmic matrix, of glycogen, in some cases in combination with droplets of triglyceride. These substances occupy the major part of the cytoplasm while cellular organelles are sparse. Since clear cells containing neither glycogen nor fat have not been observed, there is nothing to suggest that the clarity is caused by pronounced increase in water content which is often noted in tumor cells (WINZLER). The presence of droplets of neutral fat in some clear cells is apparently related to a specialized metabolism of the particular cell and does not seem to represent a degenerative alteration (BUTENANDT and DANNENBERG).

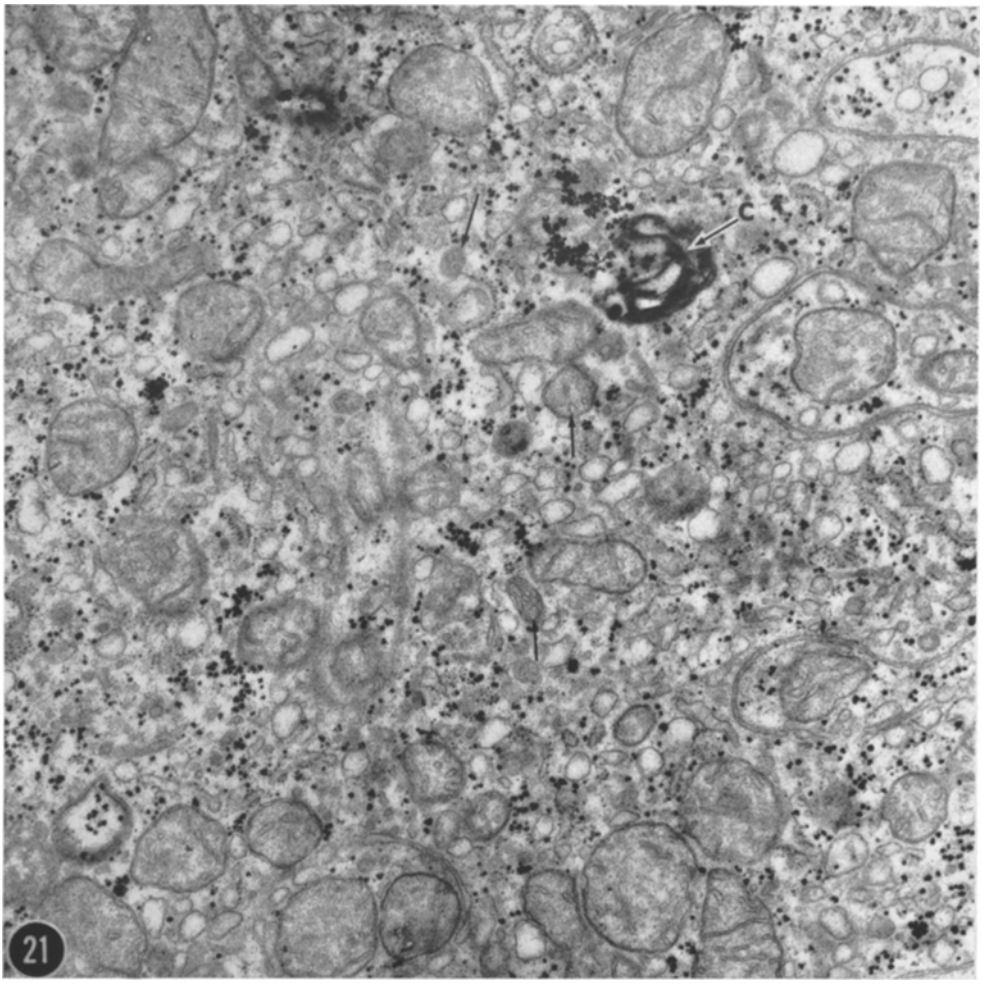


Fig. 21. Case no. 2 (granular cell carcinoma). Numerous mitochondria occupy the major part of the cytoplasm. The mitochondria show wide variations in size and some are extremely small (*arrows*). *C*, cytosome. × 20,000

Within granular cells, cytoplasmic organelles — especially mitochondria — are numerous, while glycogen and fat is lacking or is only present in small amounts. The existence of transitions between granular and clear cells in the same tumor, with cells containing mitochondria and glycogen particles in highly variable proportions, appears to indicate that in such tumors the two types of cells are of similar origin but differ with respect to their metabolism. The presence of glycogen particles in normal proximal tubule cells suggests that these cells too contain the enzymic set-up necessary for the metabolism of glycogen. The polysaccharide showed the same morphologic appearance — monoparticulate form — both in normal and neoplastic cells. The substructure of the glycogen particles appears similar to the one observed by Biava in human tissues. The finding confirms the observation by Drochmans of "unit filaments" ("γ-particles") in glycogen particles.

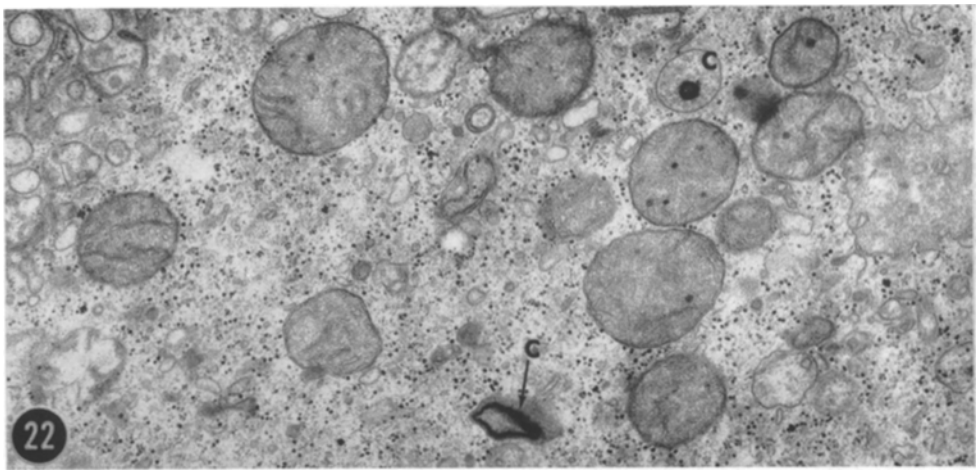


Fig. 22. Portion of a *normal proximal tubule cell* (concerning source of this material, see SELJELID and ERICSSON). There are rather numerous glycogen particles in the cytoplasmic matrix. *C*, cytosomes. × 27,000

Due to the fact that glycogen is readily soluble in water, it is generally suggested that fixation of tissues for demonstration of glycogen must be performed in alcohol-containing solutions (BARKA and ANDERSON). However, it seems that a relatively small amount of glycogen is solubilized following fixation in aldehyde or OsO_4 and that this does not interfere with the morphologic qualities of the specimens. Indeed, this mode of fixation has the advantage of offering a much better general preservation of the cytoplasm than does fixation in ethanol. It is pertinent in this connection to draw attention to the very narrow zone with optimal fixation in tissues fixed by immersion in aldehydes as well as in OsO_4. Our findings with renal carcinomas in the present investigation — as well as studies on other tissues (ERICSSON and BIBERFELD; ERICSSON, SALADINO, and TRUMP; TRUMP and ERICSSON) — suggest that not only OsO_4, as is well known, but also the conventionally utilized aldehydes do not penetrate to give optimal fixation in more than up to a 0.5 mm wide zone of tissue within a reasonable time after the application of the fixative. As postulated by BÖTTIGER and IVEMARK this may explain some of the variability of the results with regard to glycogen content in the population of cells in renal carcinomas. Thus, in our studies of routinely fixed tissue PAS-positive material was only rarely demonstrated, while there were regularly large amounts of glycogen in appropriately fixed tissues from the same tumor.

Another source of interpretative error related to the reported occurrence of different types of PAS-positive materials may be the digestion with diastase. With large amounts of glycogen in the cells, digestion in 0.5 per cent diastase for one hour at $+37^0$ C did not seem to be sufficient to remove all the glycogen. Any remaining glycogen would be falsely interpreted as representing non-glycogenic, diastase resistant material. Although the cytoplasmic organelles containing lysosomal enzymes ("lysosomes", "cytosomes", "dense bodies", etc) are usually PAS-positive and diastase resistant — probably due to their content of glycolipoproteins (NOVIKOFF) — these bodies are usually very small and sparse in the tumor cells. It appears then that at least the major portion of PAS-positive,

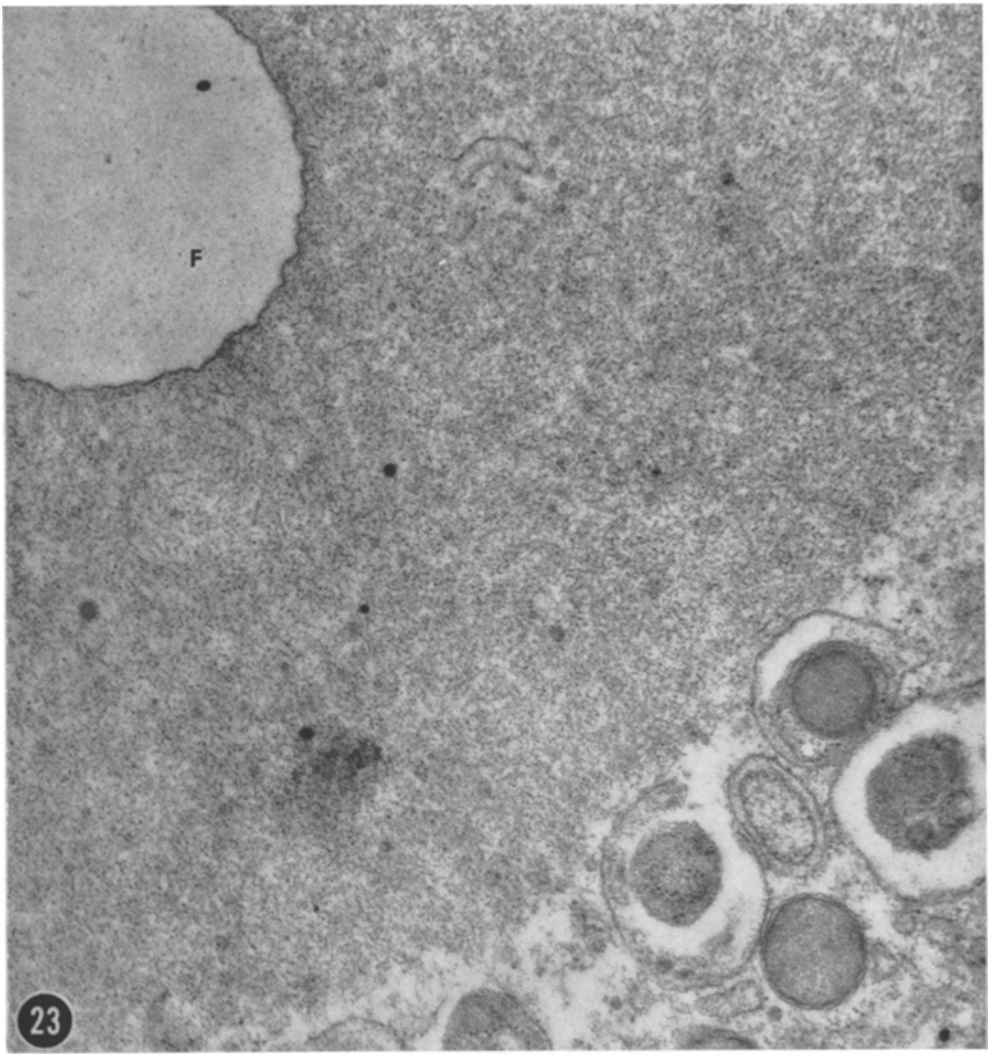


Fig. 23. Case no. 11 (mixed clear and granular cell carcinoma). Portion of a large area of cytoplasmic matrix containing tightly packed granular or filamentous material is illustrated. Note vacuole (*F*) in left upper corner with moderately opaque background matrix. The appearance of the background matrix suggests that this structure represents a droplet of neutral fat where dissolution of triglycerides in propylene oxide is not complete. × 29,000

diastase-resistant material in renal carcinomas is located extracellularly and corresponds to the "glycocalyx" of Bennett (Seljelid and Ericsson). The observation of particulate glycogen in the lumens of tumor capillaries is difficult to interprete. At the present time we favor the idea this is probably caused by rupture of tumor cells with release of glycogen to the capillaries.

Although cytoplasmic inclusions of triglycerides generally appear as homogeneous, moderately electron dense bodies surrounded by a membrane-like condensation of ground cytoplasm, preservation of the fat is difficult and unpredictable in tissues dehydrated in propylene oxide (Idelman). Comparisons of the

size and distribution of fat droplets in frozen sections of aldehyde-fixed tissues, with the vacuoles seen in tissues fixed in OsO_4 and processed for electron microscopy, indicated that many of the vacuoles represented dissolved neutral fat. It is not clear whether all the vacuoles seen in the tumor cells represent dissolved lipids. This is particularly questionable concerning those occasional vacuoles which were surrounded by a clearly identifiable double-layered membrane. Since apparently the clear cells are capable of endocytosis (SELJELID and ERICSSON), the membrane-bounded vacuoles may represent endocytosis vacuoles unrelated to the cytoplasmic matrix. In normal renal proximal tubule cells, a "unit membrane" structure similar to the one illustrated in Fig. 12 can be resolved in the "apical vacuoles" (ERICSSON and TRUMP) which apparently represent large pinocytosis vacuoles ((ERICSSON). In some instances, where dense material was retained at the periphery of tumor cell vacuoles (see Fig. 18), these structures undoubtedly represent neutral fat. As is also noted in hepatic parenchymal cells (ORRENIUS and ERICSSON; PALADE; SMUCKLER, ROSS and BENDITT; TRUMP, GOLDBLATT and STOWELL), smooth-surfaced endoplasmic reticulum and mitochondria tended to be closely associated with the vacuoles. This suggests that fat is actively metabolized by the tumor cells; as discussed by PALADE, the close association of mitochondria and fat droplets may be the morphologic expression for fatty acid oxidation by mitochondria.

The nature of the large matrix-accumulations of filamentous like or granular substance is not clear. They do not seem to represent PAS-positive, diastase-resistant material. Somewhat similar structures have been described in other tissues, *e.g.* in hepatic parenchymal cells, where they correspond to "Mallory alcoholic hyaline" (FLAX and TISDALE) and appear to represent "fibrillar degeneration of ergastoplasm" (BIAVA and MUKHLOVA-MONTIEL). It is interesting to note that the tumor cells in which the filamentous or granular aggregates occurred contained frequent whorl-like proliferates of endoplasmic reticulum (ERICSSON and SELJELID). However, transitions between these and the filamentous or granular aggregates have not been observed in tumor cells.

Summary

With the aim of elucidating the structure and composition of the cytoplasmic matrix in renal carcinomas, eleven tumors obtained during surgery were subjected to a comparative light and electron microscopic study, complemented with determinations of glycogen in homogenates of tumor tissue and normal renal cortex. The findings indicated that in carcinomas of clear cell type, translucency of cytoplasm is due to the presence of glycogen, in some instances in combination with occurrence of neutral fat. These substances occupy the major part of the cytoplasm while cytoplasmic organelles are sparse. The granular cells contain abundant mitochondria while glycogen and fat is lacking or is only present in small amounts. PAS-positive, non-glycogenic material could not be demonstrated within the cytoplasm of either cell type; such material is, however, forming extracellular "coatings" on both cell types and is probably of glycoprotein nature. The significance of the findings was discussed and reference was made to pitfalls in the technique for properly demonstrating different types of PAS-positive materials.

A comparison with the appearance of routinely fixed tissues indicated that reliable information concerning PAS-positive substances cannot be obtained from such material.

Vergleichende licht- und elektronenmikroskopische Untersuchungen der cytoplasmatischen Matrix in Nierencarcinomen

Zusammenfassung

Mit der Absicht, die Struktur und Zusammensetzung der cytoplasmatischen Matrix in Nierencarcinomen klarzustellen, wurden elf durch Operation erhaltene Tumoren einer vergleichenden licht- und elektronenmikroskopischen Untersuchung unterworfen und mit Bestimmungen von Glykogen in homogenisierten Tumorgewebe und in der normalen Nierenrinde ergänzt. Die Resultate zeigen, daß die Durchsichtigkeit des Cytoplasmas in klarzelligen Carcinomen auf dem Vorhandensein von Glykogen, zuweilen auch in Kombination mit Neutralfett beruht. Diese Substanzen nehmen den größten Teil des Cytoplasmas ein, während cytoplasmatische Organellen nur spärlich vorhanden sind. Die granulären Carcinomzellen enthalten viele Mitochondrien; Glykogen und Fett dagegen kommen gar nicht oder nur in geringen Mengen vor. PAS-positives, nichtglykogenes Material konnte im Cytoplasma keiner der beiden Zelltypen nachgewiesen werden. Dieses Material bildet extracelluläre „coatings" an beiden Zelltypen und ist vermutlich glykoproteidiger Natur. Die Bedeutung der Untersuchungsergebnisse wird diskutiert, und es wird auf technische Schwierigkeiten für die exakte Bestimmung der verschiedenen Typen PAS-positiven Material hingewiesen. Ein Vergleich mit der Struktur routinefixierten Materials zeigte, daß zuverlässige Informationen über das Vorkommen von PAS-positiven Substanzen in solchem Material nicht zu erhalten sind.

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