THE FINE STRUCTURE OF THE CELLS IN MOUSE SARCOMA 37 ASCITIC FLUIDS

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The many advantages presented by the ascites forms of transmissible animal tumours as a source of cellular material have lead to their increasing use, in recent years, in various fields of investigation.

The employment, in particular, of cells from mouse tumour ascitic fluids to support the growth of viruses (1–3) and in biochemical work involving homogenisation and the preparation of cell fractions (4, 5) made it appear that a study defining the fine structure of the cells in such mouse tumour exudates, would be of value.

The ascites form of mouse Sarcoma 37 was selected to start the work, since electron microscope observations had already been made on fresh, thinly spread whole cells from it (6). In addition, this ascites tumour, among other peculiarities, was known to produce a high percentage of reaction cells in its ascitic fluids (7, 8), many of them acidophilic peritoneal cells. Since the tumour cells were basophilic, an electron microscope investigation of the morphology of the cell population in Sarcoma 37 ascitic fluids using thin sectioning techniques, was considered to afford a valuable opportunity for comparing the fine structure underlying these two types of staining reaction. The present communication describes the results which have been obtained in the course of such an investigation.

Materials and Methods

Maintenance of Tumour.—Mouse ascites Sarcoma 37 was passed in series by intraperitoneal inoculation of 0.1 ml. volumes of whole ascitic fluid; the animals used were young adult albino mice obtained throughout from the same stock. Strict aseptic technique was employed and tests for the presence of contaminating organisms were negative.

Fixation.—Three to 4 days after inoculation a mouse was killed by cervical dislocation and, while its heart continued to beat, some of its pearly grey ascitic fluid was drawn off into a glass tuberculin syringe previously warmed to 37°C. About 0.25 ml. of this fluid was then gently squirted from the syringe through an intradermal needle into 2 ml. of 1 per cent iced OsO_4 buffered at pH 7.6 (9) and containing 4.9 per cent sucrose. The use of an intradermal needle ensured that the ascitic fluid containing the cells passed into the fixative in a fine stream and the best results were obtained when the tip of the needle was held just above the surface of the fixative. The time between killing the mouse and the moment of fixation was always less than 30 seconds.

Fixation was carried out in a 7 ml. lusteroid centrifuge tube (of the International Equipment Company, Boston, Massachusetts) and lasted between 10 and 14 minutes; during this time the cells were deposited as a pellet at the bottom of the fixative by centrifuging in an horizontal centrifuge for 7 minutes at 9000 *g*. When fixation was completed, the fixative was decanted from the pellet of cells, the fixing tube was cut open, and part of the pellet was then lifted out with a small spatula.

Dehydration and Embedding.—The sample of the pellet was transferred to a drop of 50 per cent ethyl alcohol on a sheet of dental wax, trimmed into cubes of about 1 mm., and transferred on filter paper strips to more 50 per cent alcohol in a stoppered bottle; the technique was a modification of one described by Palade and Siekevitz (10). The cubes were further treated by passage through 70 per cent alcohol, 90 per cent alcohol, three changes of 100 per cent alcohol, a mixture of equal parts of absolute alcohol and *n*-butyl methacrylate, and then three changes of *n*-butyl methacrylate containing 1 per cent by weight of benzoyl peroxide as catalyst. Each change lasted 5 minutes; this short period, together with the elimination of rinsing after fixation and the use of a relatively high percentage alcohol to start dehydration, greatly reduced extraction effects. For embedding, the cubes were finally placed in No. 00 gelatin capsules containing *n*-butyl methacrylate and 1 per cent catalyst which had already been prepolymerised for about 1 hour at 57°C. Polymerisation was carried out at 57°C. for 18 to 24 hours.

In some cases, part of the pellet was not cut into cubes but into brick-shaped blocks of similar size, so shaped that their small end faces contained the whole thickness of the pellet. These blocks were reorientated after embedding to allow sections to be cut from one of their end faces; such sections traversed the pellet from surface to base and showed the types of cells lying at each level.

Microtomy and Microscopy.—Sections were cut with glass knives on a microtome advanced mechanically (11), and those showing silver interference colours were selected for examination. They were examined, supported on collodion-coated copper grids and without removal of the embedding material, in a Philips electron microscope (EM-100) fitted with a high resolution objective lens. A 30 μ objective aperture gave best image contrast and this was further increased by the use of a prototype flat Wehnelt cylinder (kindly given to us by Mr. S. Newton of Philips Electrical Ltd., London). Electron micrographs were taken using an accelerating voltage of 60 kv.

Cell Counting.—The cells in the samples of ascitic fluid were counted in an improved Neubauer counting chamber after they had been diluted 1 in 20 in 1 per cent aqueous eosin with a white cell pipette. Differential counts of tumour cells and peritoneal cells were done on smears prepared in the manner of a blood film, fixed in Carnoy's fluid, and stained with haematoxylin and eosin; at least 1,000 cells were counted in each case.

OBSERVATIONS

The ascitic fluids, harvested as described 3 or 4 days after inoculation, were found to contain two distinct categories of cells, those having a diameter of about 5 to 10 μ and those having a diameter of 15 to 25 μ or, occasionally, even more.

The small cells consisted of lymphocytes and all three types of granulocytes in proportions which varied very widely from one fluid to another; the large cells were basophilic tumour cells or acidophilic peritoneal cells (Fig. 1), the latter forming up to as much as 20 per cent of the total large cell count. The tumour cells usually numbered something in the region of 50,000 per cu.mm. and constituted from about 50 to 70 per cent of the cell population.

Lymphocytes and Granulocytes:

The fine structure of the leucocytes examined was similar in all respects to the descriptions of such cells, either in general or with regard to particular structures, already given by other workers (12–17). In addition, however, it has been found in the present study that the characteristic granules of eosinophil granulocytes are bounded by two fine limiting membranes about 7 mμ apart and that the plasma membrane of such cells appears to be composed of more than one layer (Fig. 2).

Peritoneal Cells:

The peritoneal cells were of the same dimensions as all but the largest tumour cells (Fig. 1), usually having a diameter of about 15 to 25 μ; their nuclei were relatively small and rounded with a depression or even a branching cleft, in one area.

Endoplasmic Reticulum.—Throughout much of the cytoplasm the endoplasmic reticulum was represented by sparse circular or oval vesicles limited by a thin smooth membrane. Such elements were for the most part distributed haphazardly, but sometimes they appeared grouped near the cell surface. Where this occurred, flask-neck connections between the cell membrane and the vesicles of the reticulum were seen (Fig. 3).

In addition, two more highly organised forms of the endoplasmic reticulum were apparent in the cells. The first was found in the vicinity of the nuclear indentation (the centrosome region) and consisted of piles of parallel, smooth surfaced cisternae packed tightly together and associated with moderate numbers of small vesicles; there were about 4 to 6 cisternae in each pile and the piles radiated in a whorled stellate pattern from a central focus (Fig. 4). Occasionally U-shaped piles of cisternae were encountered. The second well organised feature of the endoplasmic reticulum, always situated in some other region of the cell, was made up of long cisternae measuring up to 7 or 8 μ in length, preferentially orientated, and having a limiting membrane covered on the outside by moderate numbers of small particles. These cisternae tended to be roughly parallel to one another and sometimes to the nuclear membrane as well (Fig. 5). In another common arrangement the cisternae were in concentric rings about large bodies, presumably lipoid (Fig. 6). Apart from the particles attached to the membranes of these cisternae, a few particles lay in small groups in the cytoplasm between the cisternae which were usually 100 to 200 mμ apart (Figs. 5 and 6).

Mitochondria.—Mitochondria, having a characteristic internal structure (18–20), were scattered sparsely in the cytoplasm with a tendency to lie near the organised regions of the endoplasmic reticulum. They were spherical or short stout rod-shaped bodies and bent or branched forms were not observed. The shelf-like cristae were parallel to one another, close together, and usually at right angles to the long axis of the organelle; the mitochondrial matrix was dense (Fig. 4). Occasionally villous forms of cristae were present and likewise a longitudinal orientation.

Other Cytoplasmic Features.—Apart from the structures already described, the

cytoplasm always contained a number of prominent electron-dense, presumably lipoid bodies of about 200 to 800 mμ diameter (Figs. 5 and 6) which did not possess a limiting membrane and which tended, in the thinner sections, to be distorted from their usual rounded shape by knife compression. In addition, less dense bodies having a diffuse granular appearance, a limiting membrane, and a diameter of about 200 to 600 mμ were observed (Figs. 5 and 6) and were considered to be phagocytic vacuoles. Similar sized vacuoles likewise limited by a membrane, but without recognisable content were sometimes also present in the cells.

The cytoplasmic matrix itself was remarkable for its empty structureless appearance (Figs. 3 to 5, and 14).

Nucleus.—The nucleus and nucleolus were composed of fine granules (Figs. 3 to 5), those in the latter (nucleolus) showing a greater degree of aggregation. The double nuclear membrane described by Hartmann (21) was very evident and showed frequent pores (Figs. 3 to 5); in some cases a fine line could be distinguished running across the opening of the pore (Figs. 4, 5, and 7), giving the appearance of a very thin membrane. The space between the two nuclear membranes was sometimes seen to communicate with the interior of elements of the endoplasmic reticulum (Fig. 7). Mitoses were never observed.

Tumour Cells:

The tumour cells measured between 15 and 30 μ in diameter, and were occasionally even larger. Their nuclei were large kidney-shaped structures filling much of the cell and having a depression on one face (Fig. 1). Multinucleate cells were not uncommon.

Endoplasmic Reticulum.—Simple round or oval smooth surfaced vesicles of the endoplasmic reticulum were absent from most of the cytoplasm. They were found sometimes, however, below the cell surface with which, on occasions, they were seen to communicate (Fig. 8). More highly organised forms of the endoplasmic reticulum of both smooth and rough surfaced type were also encountered.

Firstly, lying against the nuclear indentation and filling a considerable portion of the centre of the cell (the centrosome region), very large numbers of small round vesicles were observed associated with a few randomly distributed relatively poorly organised (*cf.* Fig. 4) piles of tightly packed, parallel, smooth surfaced cisternae (Fig. 9). Well organised, U-shaped piles of these cisternae were found only in association with and surrounding a rounded granular body of 225 mμ diameter with an electron-dense centre (Fig. 10); this body did not appear to be bounded by a definite membrane.

A second type of organisation in the endoplasmic reticulum was observed in the region of the preceding complex. It consisted of rows of parallel smooth surfaced cisternae which were not packed together, but about 150 mμ apart; the outsides of the smooth limiting membranes were covered with amorphous material (Fig. 12).

Lastly, elongated cisternae, whose limiting membranes were covered on the outside by many small particles, were also found. These, however, were relatively rare and were not arranged in ordered parallel arrays; they occurred singly or in small groups here and there in the cytoplasm (Fig. 9).

Mitochondria.—Mitochondria, though seen occasionally in any part of the cell, tended to be concentrated in profusion in a zone bounding the large centrosome region with its vesicles and piled cisternae (Fig. 9). Filamentous, rod-shaped, spherical, bent, and branching forms were all encountered. The organelles were slender, contained relatively few shelf-like cristae usually running cross-wise, and had little matrix (Figs. 9 and 10; *cf.* Fig. 4).

Other Cytoplasmic Features.—Electron-dense, presumably lipoid bodies of about ½ μ diameter and without a limiting membrane, were observed in the cytoplasm of the tumour cells. They were infrequent in the actively growing cells examined. Large vacuoles without detectable content and several μ in diameter were also seen occasionally in the cytoplasm; very rarely one or two huge vacuoles were encountered which almost filled the cell and compressed its normal structures into a narrow peripheral zone.

The cytoplasm itself presented two important features. In the region of the centrosome, fine threads of the order of 2 mμ in diameter crossed the cytoplasmic matrix in all directions between the vesicles and piled cisternae which formed a complex in the area (Fig. 12). Throughout the rest of the cell, very dense particles about 15 mμ in diameter were present in profusion (Figs. 13 and 14); they were scattered at random without order or pattern and gave the cytoplasm a grey hazy appearance when seen at low magnifications (Fig. 14).

Nucleus.—As in the case of the peritoneal cells already described, the nucleus and nucleolus were composed of fine granules (Fig. 9), those in the latter being more closely aggregated. A double nuclear membrane was present and was found to have frequent pores (Fig. 9). Continuity between the space separating the two nuclear membranes and cavities of the endoplasmic reticulum has been observed.

Frequent mitoses were encountered and the chromosomes were observed to be structureless apart from a fine granularity.

DISCUSSION

In preparing the ascites cells for electron microscopy it was found essential to fix them as soon after harvesting as possible. For this reason centrifugation, as a means of separating the cells from the ascitic fluid, was avoided until after fixation. Additional points against it lay in the fact that it has been found to be deleterious to cells grown *in vitro* when applied at anything above the slowest speeds (22), and preliminary unpublished studies undertaken in connection with a technique involving the power of cells to spread widely on flat surfaces *in vitro* (6) have also shown that even gentle centrifugation affected cells in some unfavourable way which impaired this power.

The high percentage of reaction cells in Sarcoma 37 ascitic fluids which has been observed in the present work and the great variation, from one fluid to the next, in the relative proportions of the different cell types taking part in the reaction, confirms previous observations (7, 8). However, the fact that a very considerable number of these reaction cells, in the case of Sarcoma 37, consist of large acidophilic peritoneal cells of the same dimensions as the

tumour cells (Fig. 1) does not seem to have been recorded; it would rule out the possibility of differentiating this type of tumour cell from the other cells in the fluids on a size basis alone, as has been done by Patt and Blackford (23) in the case of the Krebs ascites tumour.

With regard to the nature of the peritoneal cells, they would all appear to be of macrophage type. In the electron microscope they all possessed the same fine structure and it is considered impossible to form an opinion as to whether they were derived from the blood, the mesothelium, the tissues, or all three sources.

The description of the endoplasmic reticulum used here in presenting the electron microscope observations, is based on the interpretation of this cytoplasmic component worked out by Porter (24), Palade and Porter (25), and Palade (15). The whole subject has recently been elegantly reviewed and illustrated by Palade (26) who has discussed at the same time the contributions of other workers to this field and the differences in terminology which have arisen, particularly with regard to the membranous structures of the centrosome region. Apart from being described as smooth surfaced cisternae of the endoplastic reticulum (15, 26), these structures have been referred to as agranular reticulum (27) and have also been identified with the Golgi apparatus by various workers (28–30). Most of the present findings regarding cells in Sarcoma 37 ascites fit into this general framework of what is known of cellular fine structure, and a few of the points recorded about the tumour cells have recently been mentioned by Dalton and Felix (31). However, certain new observations have been made which call for comment.

The two fine limiting membranes around the granules of eosinophil granulocytes (Fig. 2) are of much smaller dimensions and much closer together than the limiting membranes of mitochondria. They thus do not suggest, morphologically, that these two types of organelles are related as has been claimed by Rinehart (16). The significance of these membranes is not known. Likewise, no explanation for the appearance of lamination in the plasma membrane of such cells (Fig. 2) can be put forward, but it should be noted that a somewhat similar triple-layered cell membrane has recently been reported by Zetterquist (32) in mouse jejunal epithelial cells.

The moderately electron-dense granular cytoplasmic bodies with a limiting membrane observed in the peritoneal cells (Figs. 5 and 6) appear to be identical with the bodies taken by Palade (26) to represent the terminal appearances of phagocytic vacuoles in splenic macrophages. In these latter cells, however, the vacuoles contained extremely dense granular material thought to be derived from haemoglobin. Such very dense material was absent from the vac uoles of the peritoneal cells which, of course, did not have access to blood pigments to the same extent as would cells in a splenic blood sinus.

The fact that the pores in the double nuclear membrane of the peritoneal

cells appeared, when normally sectioned, to have a fine membrane running across them (Figs. 4, 5, and 7) would bring the structure of such pores in mammalian cells into line with that proposed by Afzelius (33) for the pores in the nuclear membrane of certain echinoderms. Watson (34, 35) has described mammalian nuclear pores as containing a diffuse faintly particulate material, but did not observe a fine line across the opening such as that seen here and interpreted as a very thin membrane.

In the tumour cells, the body 225 mμ in diameter composed of fine granular material with a centre of greater electron density (Fig. 11) is not reminiscent of any previously described cytoplasmic structure; its nature is not known.

Another feature of some of the tumour cells which is considered unusual, is the presence in the centrosome area of rows of parallel cisternae having the characteristic wide spacing of rough surfaced elements of the endoplasmic reticulum (*cf.* Figs. 5 and 6), but limiting membranes entirely free of attached particles (Fig. 11); nothing but amorphous material was observed covering these structures. This form of local differentiation of the endoplasmic reticulum has only been previously reported in developing spermatids (15, 26) in which it has been considered a transient unique phenomenon specific to this type of cell. Sampling difficulties inherent in the examination of thin sections of cells make it impossible to say whether all Sarcoma 37 ascites tumour cells possess such a structure or whether it is only present in the cells at a particular stage of development.

The relative paucity of lipoid bodies in the tumour cells is not surprising when it is considered that the material examined was harvested 3 or 4 days after inoculation. It has been reported earlier that these bodies increase in number when the environment of the cells deteriorates in any way (6), as, for example, in the later stages of tumour growth. The occasional large vacuoles in the tumour cells were already known from the study of whole, thinly spread, Sarcoma 37 ascites cells (6); these rare vacuoles are similar to the vacuoles which regularly occur in profusion in Rous ascites tumour cells (36).

With regard to the fine filamentous threads in the cytoplasmic matrix of the tumour cell centrosome (Fig. 12), it is difficult to decide whether these should be viewed as a fixation artefact or whether they are a feature of the fine structure akin to the filaments already described, for example, in epidermal cells (37) and in neurones (27).

The abundant small electron-dense particles of about 15 mμ diameter which filled all regions of the tumour cell cytoplasm apart from the centrosome (Figs. 13 and 14) are the same as those first noted by Sjöstrand and Rhodin (38) in cells of renal tubule epithelium and by Palade (39). The latter has subsequently described them in a wide variety of cell types (40) and has found that they are present in the greatest concentration in rapidly proliferating cells (embryonic cells and certain epithelial cells) and in active glandular

cells (mammary, salivary, and exocrine of pancreas). Palade points out (40) that the particles can occur either predominantly attached to the rough surfaced cisternae of the endoplasmic reticulum as in the glandular cells, or both attached and lying free between the cisternae as in Nissl bodies (27), or yet again, evenly distributed throughout the cytoplasm as in embryonic cells, proliferating epithelial cells, and young lymphocytes in the spleen and lymph glands. The actively proliferating tumour cells described in the present work show this last type of distribution of the particles. Now these particles have been recently shown to consist of ribonucleoprotein (10, 41) which is known to be largely responsible for cytoplasmic basophilia. This staining property has sometimes been ascribed to the membranes of the rough surfaced cisternae (42), to which particles are, of course, attached, and in this context the observations reported here are considered of interest since they tend to confirm that it is the particles alone which are responsible for basophilia. For the basophil tumour cells contained large numbers of free particles and only very rare rough surfaced cisternae, whilst the peritoneal cells with well developed rough surfaced cisternae (Figs. 5 and 6) having relatively few attached particles and fewer free ones, were acidophilic.

Finally, in considering the present electron microscope findings, it is clear that both the peritoneal cells and the Sarcoma 37 ascites tumour cells show the same fundamental structure common to all other cells, with variations in detail such as can be found between one type of normal cell and another. It is true that the peritoneal cells give an impression of order and function, whilst the tumour cells appear in comparison to be but poorly differentiated, but, as Dalton (31) has pointed out, differences between such tumour cells and normal cells are not qualitative. However, the fact that the peritoneal cells and tumour cells differ in fine structure as much as has been found in the present investigation is considered of importance for future biochemical and virological work involving the large cells in the ascitic fluids of this tumour.

SUMMARY

The tumour cells and the reaction cells in Sarcoma 37 ascitic fluids have been studied in thin sections with the electron microscope. The reaction cells were either leucocytes or much larger acidophilic peritoneal cells of the same dimensions as the tumour cells; the peritoneal cells formed as much as 20 per cent of the large cell population.

The fine structure of the cells is described and some new observations recorded. It has been found that the cell membrane of eosinophil granulocytes has a laminated composition and the characteristic granules of these cells a double limiting membrane. The pores in the double nuclear membrane of the peritoneal cells have been observed to have a fine line running across them. In the tumour cells, a rounded granular body with a central dense area has

been found in the region of the centrosome; these cells were also seen to contain rows of parallel smooth surfaced cisternae lying 150 mμ apart similar to those hitherto only observed in spermatids. There was a feltwork of fine filaments in the cytoplasm of the centrosome region of the tumour cells.

The cytoplasmic fine structure underlying the basophilia of the tumour cells and the acidophilia of the peritoneal cells is compared and discussed.

BIBLIOGRAPHY

1. Ackermann, W. W., and Kurtz, H., *Proc. Soc. Exp. Biol. and Med.*, 1952, **81,** 421.
2. Flanagan, A. D., Love, R., and Tesar, W., *Proc. Soc. Exp. Biol. and Med.*, 1955, **90,** 82.
3. Moore, A. E., and Diamond, L. C., *J. Immunol.*, 1956, **77,** 81.
4. Kun, E., Talalay, P., and Williams-Ashman, H. G., *Cancer Research*, 1951, **11,** 855.
5. Lindberg, O., Ljunggren, M., Ernster, L., and Revesz, L., *Exp. Cell Research*, 1953, **4,** 243.
6. Epstein, M. A., *Exp. Cell Research*, 1955, **9,** 547.
7. Klein, G., *Exp. Cell Research*, 1951, **2,** 518.
8. Goldie, H., and Felix, M. D., *Cancer Research*, 1951, **11,** 73.
9. Palade, G. E., *J. Exp. Med.*, 1952, **95,** 285.
10. Palade, G. E., and Siekevitz, P., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 171.
11. Porter, K. R., and Bloom, J., *Anat. Rec.*, 1953, **117,** 685.
12. Watanabe, Y., *J. Electronmicroscopy (Japan)*, 1954, **2,** 34.
13. Sheldon, H., and Zetterquist, H., *Bull. Johns Hopkins Hosp.*, 1955, **96,** 135.
14. Policard, A., and Bessis, M., *Exp. Cell Research*, 1955, **8,** 583.
15. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 567.
16. Rinehart, J. F., *Am. J. Clin. Path.*, 1955, **25,** 605.
17. Pease, D. C., *Blood*, 1956, **11,** 501.
18. Palade, G. E., *Anat. Rec.*, 1952, **114,** 427.
19. Sjöstrand, F. S., *Nature*, 1953, **171,** 30.
20. Palade, G. E., *Henry Ford Hosp. Internat. Symp., Enzymes*, 1956, **4,** 185.
21. Hartmann, J. F., *J. Comp. Neurol.*, 1953, **99,** 201.
22. Puck, T. T., Marcus, P. I., and Cieciura, S. J., *J. Exp. Med.*, 1956, **103,** 273.
23. Patt, H. M., and Blackford, M. E., *Cancer Research*, 1954, **14,** 391.
24. Porter, K. R., *J. Exp. Med.*, 1953, **97,** 727.
25. Palade, G. E., and Porter, K. R., *J. Exp. Med.*, 1954, **100,** 641.
26. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** No. 4, suppl., 85.
27. Palay, S. L., and Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1,** 69.
28. Dalton, A. J., and Felix, M. D., *Am. J. Anat.*, 1954, **94,** 171.
29. Sjöstrand, F. S., and Hanzon, V., *Exp. Cell Research*, 1954, **7,** 415.
30. Lacey, D., and Challice, C. F., *J. Biophysic. and Biochem. Cytol.*, 1956, **2,** 395.
31. Dalton, A. J., and Felix, M. D., *Ann. New York Acad. Sc.*, 1956, **63,** 1117.
32. Zetterquist, H., The Ultrastructural Organization of the Columnar Absorbing Cells of the Mouse Jejunum, Thesis, Stockholm, 1956.

33. Afzelius, B. A., *Exp. Cell Research*, 1955, **8**, 147.
34. Watson, M. L., *Biochim. et Biophysica Acta*, 1954, **15**, 475.
35. Watson, M. L., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 257.
36. Epstein, M. A., *Brit. J. Cancer*, 1956, **10**, 33.
37. Weiss, P., and Ferris, W., *Exp. Cell Research*, 1954, **6**, 546.
38. Sjöstrand, F. S., and Rhodin, J., *Exp. Cell Research*, 1953, **4**, 426.
39. Palade, G. E., *J. Appl. Physics*, 1953, **24**, 1419.
40. Palade, G. E., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 59.
41. Palade, G. E., and Siekevitz, P., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 671.
42. Bernhard, W., Haguenau, F., Gautier, A., and Oberling, C., *Z. Zellforsch. u. mikr. Anat.*, 1952, **37**, 281.

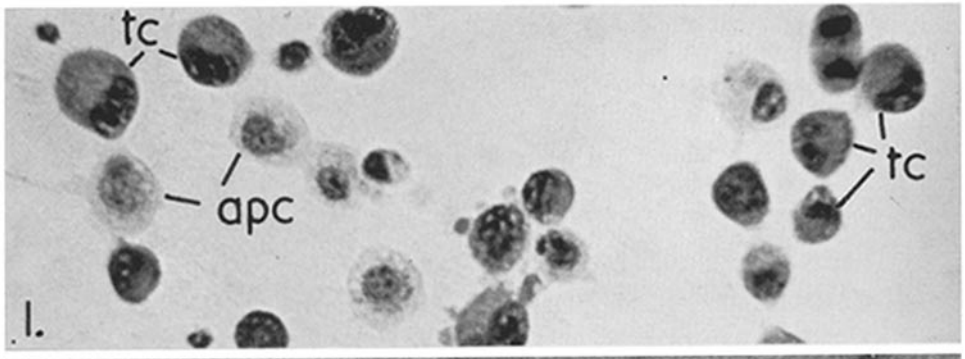
EXPLANATION OF PLATES

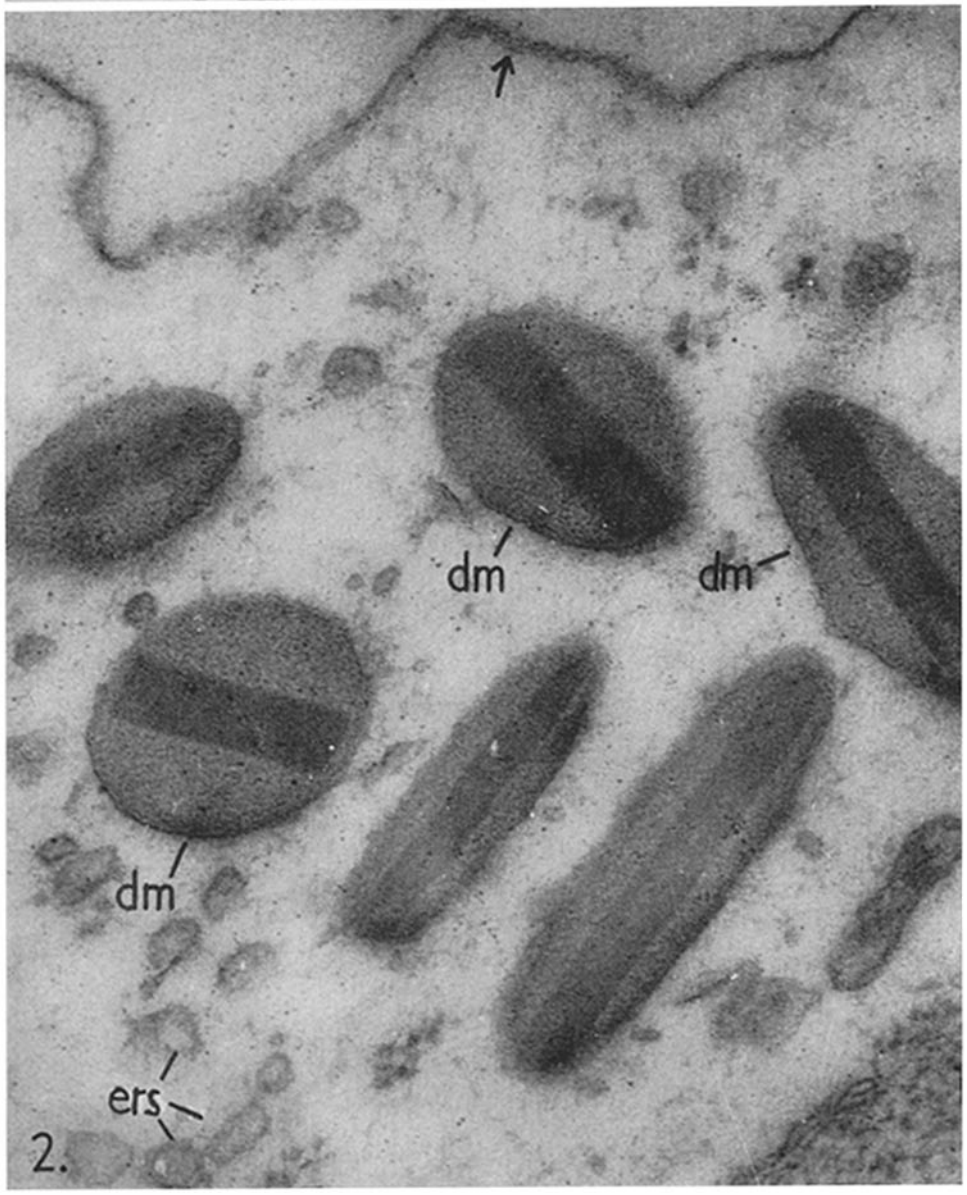
All the figures, apart from the first one, are electron micrographs.

PLATE 173

FIG. 1. Photomicrograph of a group of large cells in a smear of Sarcoma 37 ascitic fluid, fixed in Carnoy's fluid and stained with haematoxylin and eosin. Basophil tumour cells with large nuclei lie at *tc* and acidophilic peritoneal cells with smaller nuclei at *apc*. × 740.

FIG. 2. Small area of an eosinophil granulocyte. A portion of the granular nucleus lies in the bottom right hand corner of the field and the cell membrane runs across the top; when sectioned at an angle not quite normal to its surface this membrane appears to have a laminated structure (arrow). The cytoplasm contains a few round or oval smooth surfaced vesicles of the endoplasmic reticulum (*ers*) and six eosinophil granules cut at various angles. Where the surface of these granules has been sectioned normally, a double limiting membrane can be distinguished (*dm*). × 120,000.



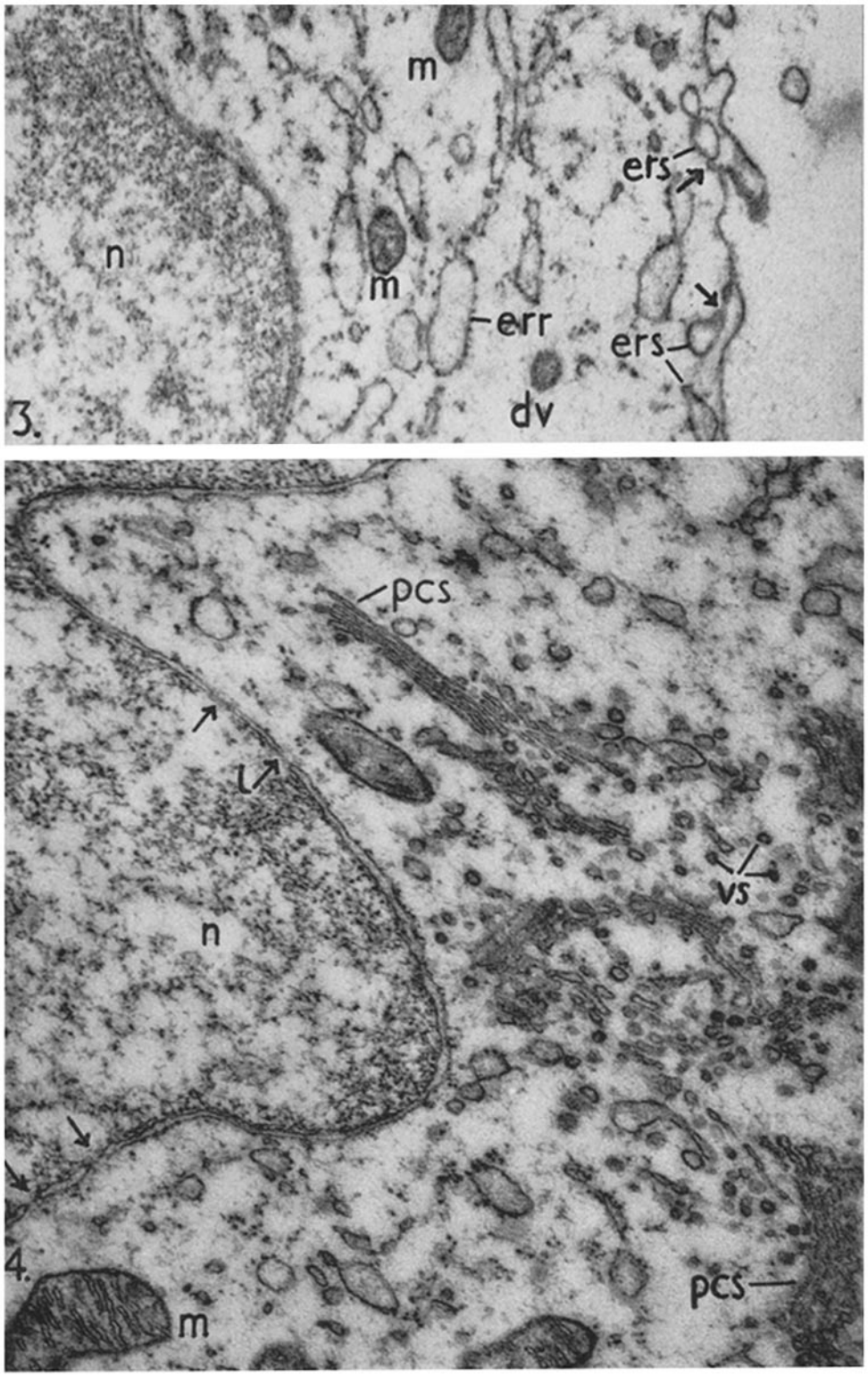


(Epstein: Cells in Sarcoma 37 ascitic fluids)

PLATE 174

FIG. 3. Peritoneal cell. The granular nucleus *n* lies on the left of the figure and is bounded by a double membrane. The cell membrane runs up and down on the right and rounded smooth surfaced vesicles of the endoplasmic reticulum (*ers*) lie in the cytoplasm beneath it; two of these communicate with the cell membrane through flask-neck connections (arrows). Mitochondria (*m*), rough surfaced elements of the endoplasmic reticulum (*err*) and a phagocytic vacuole (*dv*) are also present. The cytoplasmic matrix itself is empty and structureless. × 36,000.

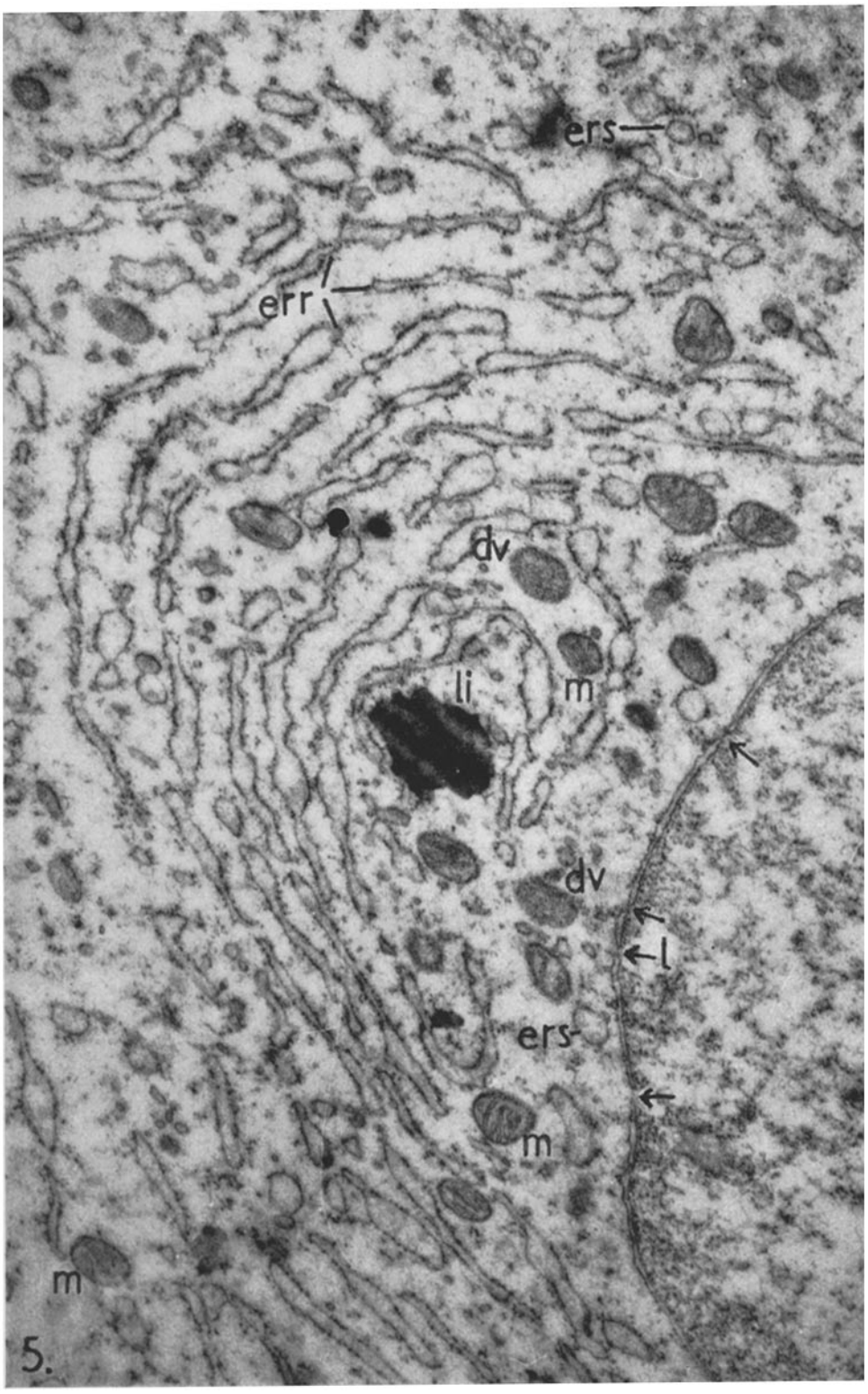
FIG. 4. Centrosome region of a peritoneal cell. The granular nucleus (*n*) lies on the left and the double nuclear membrane contains numerous pores (arrows), one of which appears to have a fine line running across it (arrow *l*). Near the nuclear indentation, the cytoplasm contains several well organised piles of packed smooth surfaced cisternae (*pcs*) associated with moderate numbers of small smooth surfaced vesicles (*vs*). A mitochondrion typical of this type of cell on account of its regular, closely spaced, parallel cristae and dense matrix, can be seen at *m*. The cytoplasm is otherwise empty and structureless. × 50,000.



(Epstein: Cells in Sarcoma 37 ascitic fluids)

PLATE 175

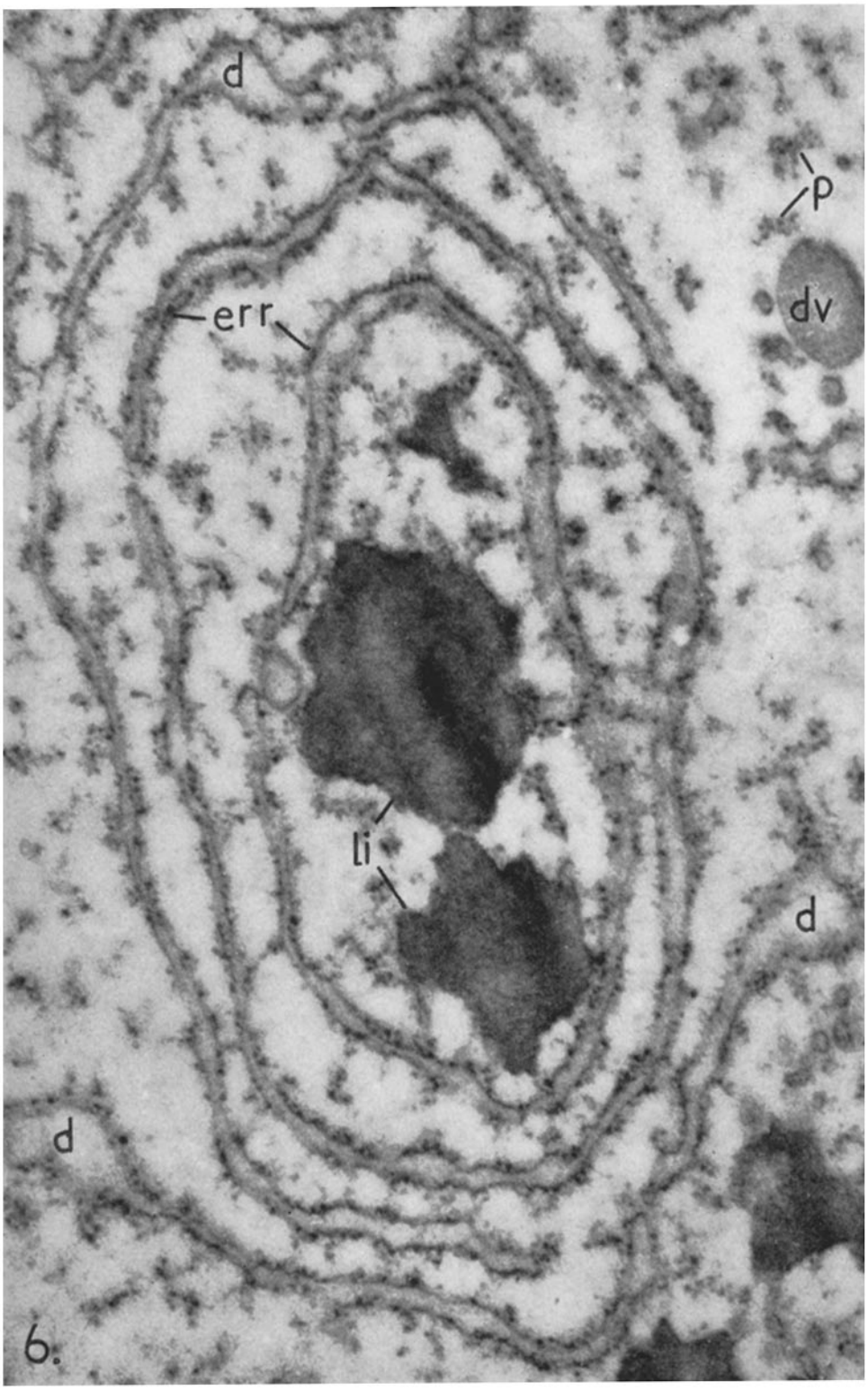
FIG. 5. Peritoneal cell. The nucleus lies at the bottom right hand side of the figure and its double membrane contains the usual pores (arrows), one of which shows a fine line running across it (arrow *l*). A few smooth surfaced vesicles are present (*ers*), but most of the endoplasmic reticulum in the area of the cell shown in the field is of the rough surfaced type (*err*) and consists of cisternal elements covered with moderate numbers of small particles, arranged roughly parallel to one another and to the nuclear membrane, and lying about 150 mμ apart. In addition, mitochondria (*m*), a lipoid body (*li*), and phagocytic vacuoles (*dv*), are also present. × 30,000.



(Epstein: Cells in Sarcoma 37 ascitic fluids)

PLATE 176

FIG. 6. Peritoneal cell. Rings of elongated rough surfaced cisternae of the endoplasmic reticulum (*err*) are arranged concentrically around two large lipoid bodies (*li*) lying in the cytoplasm; some of the cisternae end in dilatations (*d*). The small dense particles on the outer surface of the limiting membranes of the cisternae are not very profuse. Particles lying free in small groups between the cisternae as at *p*, are rare. A phagocytic vacuole (*dv*) is also present. × 60,000.



(Epstein: Cells in Sarcoma 37 ascitic fliuds)

PLATE 177

FIG. 7. Peritoneal cell; detail of nuclear membrane. The nucleoplasm lies in the upper part of the figure and the cytoplasm in the lower, separated by the double nuclear membrane which crosses the field. A pore in the membrane can be seen (arrow) and a fine line crosses its opening. The space between the two nuclear membranes communicates at *c* with a tubule of rough surfaced endoplasmic reticulum (*err*) which passes out of the plane of the section. ×70,000.

FIG. 8. Tumour cell; detail of cytoplasm beneath the cell wall. The cell wall (*cw*) crosses the field and rounded smooth surfaced vesicles of endoplasmic reticulum (*ers*) lie in the cytoplasm beneath it in a chain. One of the vesicles communicates with the cell membrane through a flask-neck connection (arrow). × 45,000.

FIG. 9. Part of the centrosome region of a tumour cell. The granular nucleus (*n*) lies at the top of the field bounded by a double nuclear membrane containing numerous pores (arrows). The cytoplasm is filled with large numbers of small round smooth surfaced vesicles (*vs*) associated with a few randomly distributed poorly organised piles of packed smooth surfaced cisternae (*pcs*). Typical mitochondria having relatively few widely spaced cristae and little matrix lie here and there in the field at m_1 and in a zone bounding the centrosome at m_2. Elements of rough surfaced endoplasmic reticulum (*err*) can also be seen as well as groups of free particles (*p*). × 44,300.

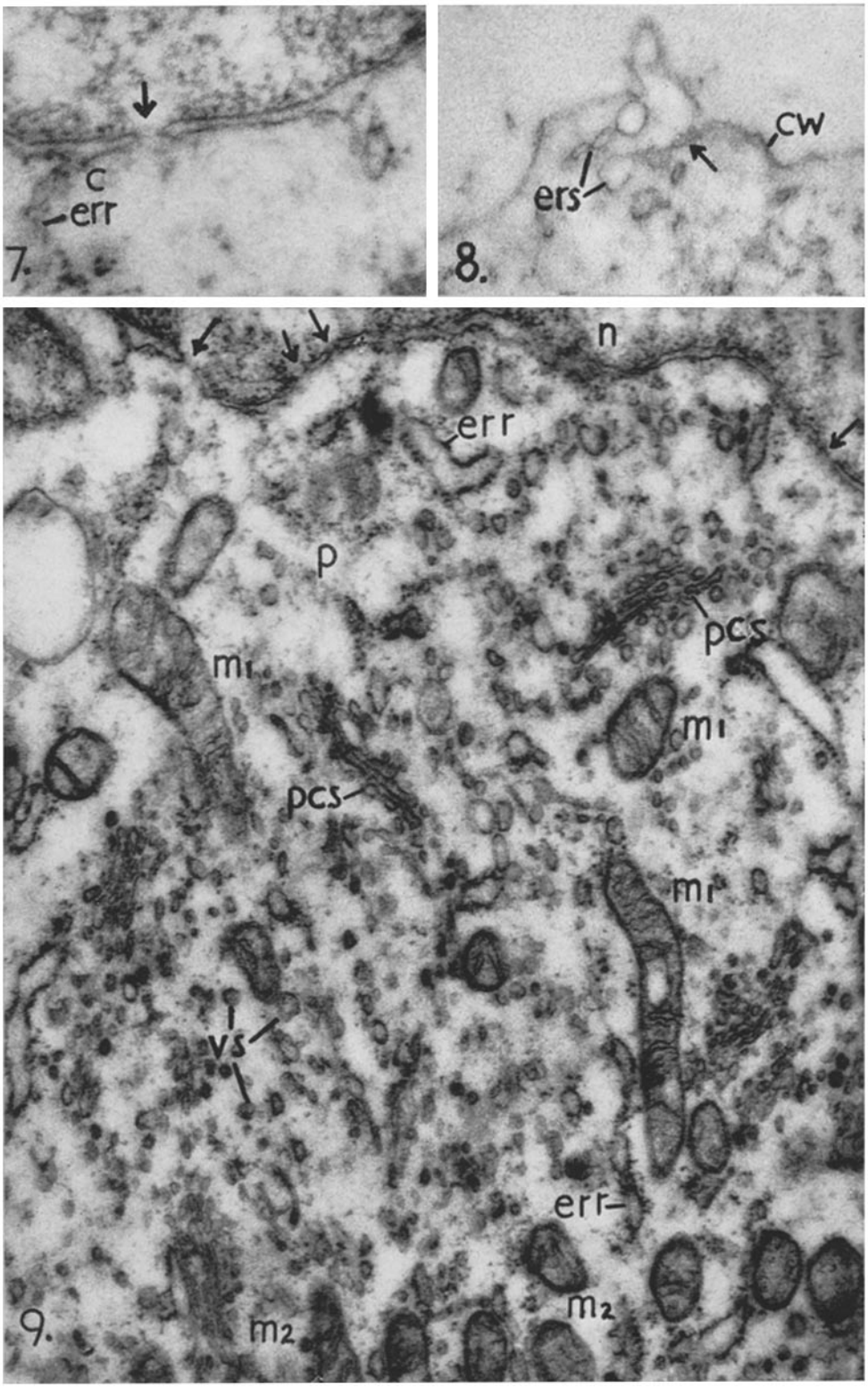


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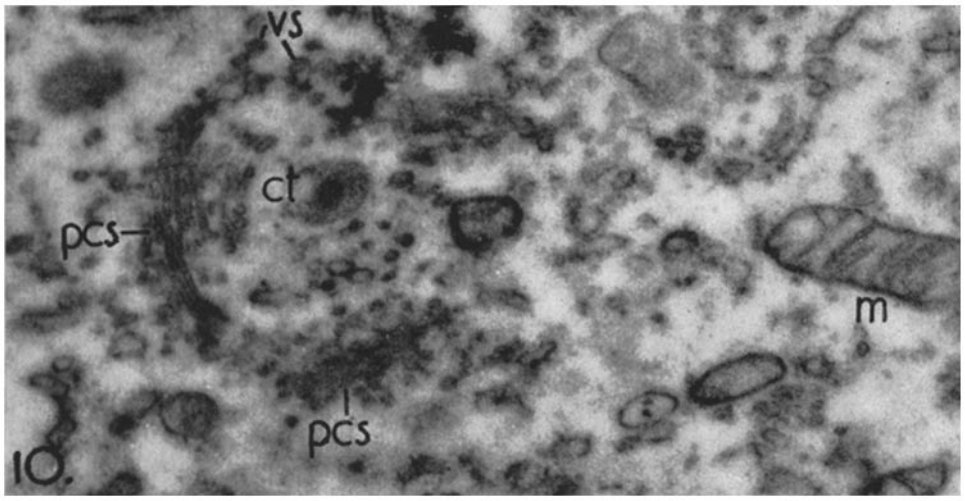
PLATE 178

FIG. 10. Part of the centrosome region of a tumour cell. A U-shaped group of well organised piles of packed smooth surfaced cisternae (*pcs*) associated with many small round smooth surfaced vesicles (*vs*), surround a round granular structure having an electron-dense centre (*ct*). A mitochondrion, typical of this type of cell on account of its sparse cristae and lack of dense matrix, lies on the right of the figure at *m*. × 43,700.

FIG. 11. Small area of the centrosome region of a tumour cell. An array of five roughly parallel smooth surfaced cisternae (*cs*) fills the field. These cisternae are not packed but lie about 150 mμ apart, a spacing characteristic of rough surfaced cisternae. However, no particles are attached to the membranes of the elements shown here, but a condensation of matrix is present instead. Some interruptions can be seen in the cisternae (arrows). × 100,000.



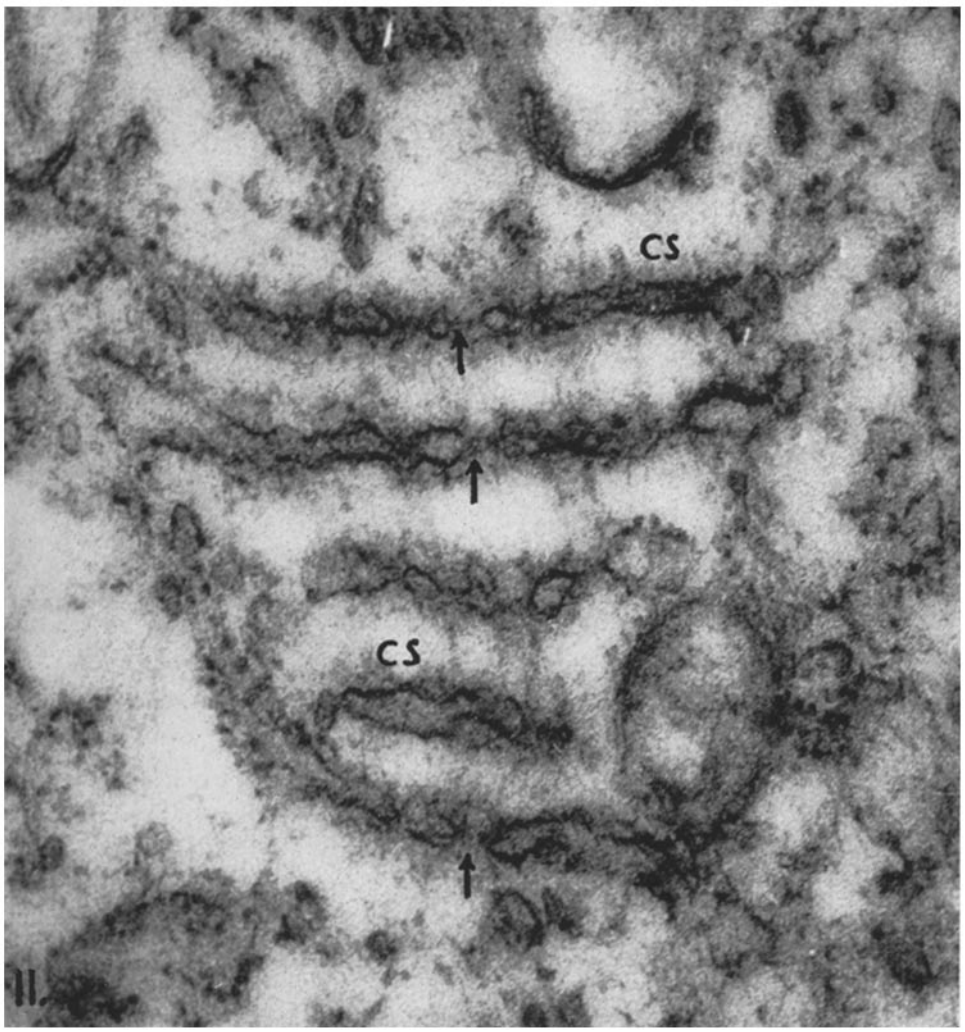


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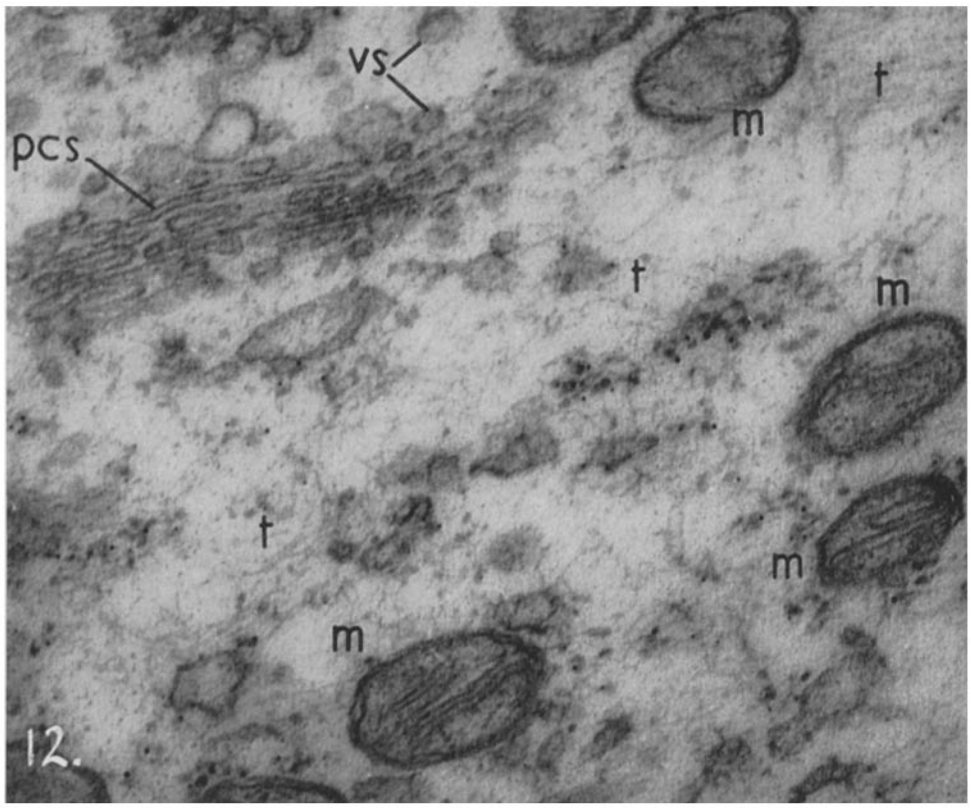
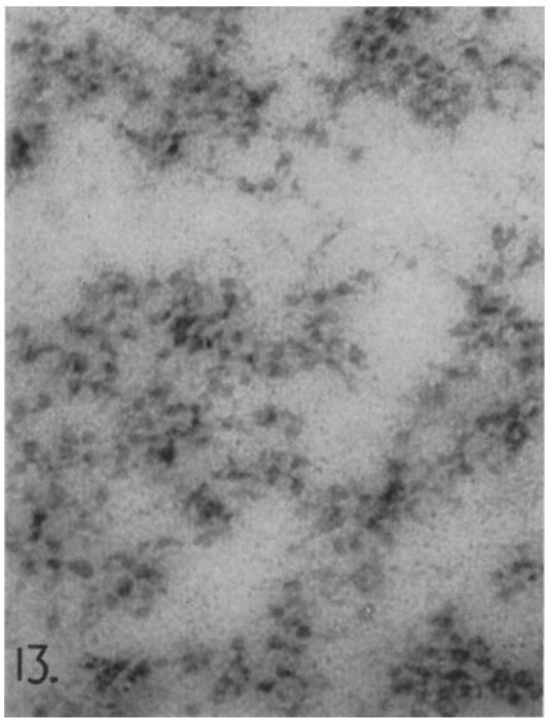
PLATE 179

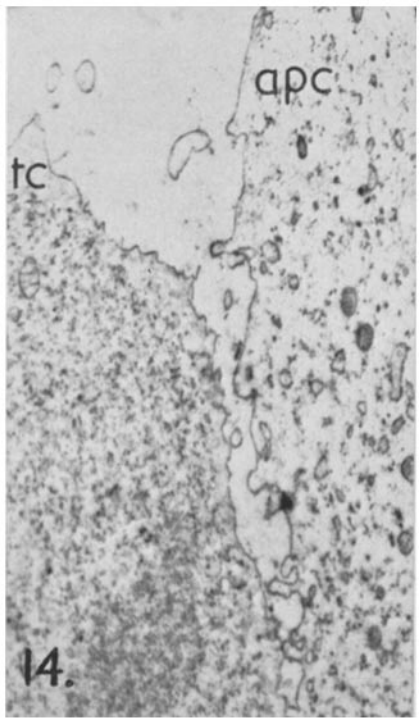
FIG. 12. Small area of the centrosome region of a tumour cell. A pile of packed smooth surfaced cisternae (*pcs*) lies in the top left hand part of the field associated with small round smooth surfaced vesicles (*vs*); mitochondria can be seen at *m*, and between these various structures fine threads (*t*) about 2 mμ in diameter cross the cytoplasmic matrix in all directions. × 100,000.

FIG. 13. Small area of cytoplasm of a tumour cell. The cytoplasm is filled with large numbers of particles about 15 mμ in diameter scattered at random without order or pattern. × 120,000.

FIG. 14. Peripheral cytoplasm of a tumour and a peritoneal cell lying side by side. The tumour cell (*tc*) occupies the left hand side of the field and its characteristic hazy granular appearance can be distinguished. The acidophilic peritoneal cell (*apc*) on the right has, in contrast, a structureless cytoplasmic matrix. × 12,000.







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