SOME UNUSUAL FEATURES OF FINE STRUCTURE OBSERVED IN HeLa CELLS

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ABSTRACT

HeLa cells from conventional culture media have been studied in thin sections with the electron microscope; in many cases cells were examined in sets of sections cut in series. The fine structure of the cells is described including three unusual features not hitherto reported. It has been found that numerous cells contained rows of parallel smooth surfaced cisternae spaced about 150 mμ apart and communicating with rough surfaced elements of the endoplasmic reticulum. These cisternae resembled "annulate lamellae" but did not contain regular arrays of pores. In many cells an area of juxtanuclear cytoplasm was occupied by a membranous structure composed of closely applied pairs of narrow cisternae either arranged in concentric rings or else extending in several directions in a haphazard manner. Sparse particles were present on the outer membranes of each pair of cisternae. Communications between the double cisternae and other membrane-bounded structures were not observed. A small number of cells contained areas of cytoplasm devoid of organelles and filled with amorphous fuzzy material. The observations recorded are discussed.

INTRODUCTION

Since HeLa cells were first established *in vitro* as a strain readily grown by serial subculture (1, 2) they have been exceptionally widely used for the propagation of viruses and for a variety of other purposes such, for example, as the study of cell adherence (3, 4) and mitosis (5), the assay of toxic substances (6), and the investigation of biosynthetic processes (7). However, despite the ubiquity of HeLa cells in many types of laboratory, relatively little has been reported concerning their fine structure under normal conditions of culture, the electron microscope having mainly been used to study them after infection with various viruses (8–13). Uninfected HeLa cells are known to contain dense cytoplasmic bodies (14), and the connection of these with ingestion by pinocytosis has been explored using particles of colloidal gold (15). In addition, brief mention has been made of the rarity of cytoplasmic membranes and particles in such cells, and of the presence of microvilli at the cell surface (8).

During the examination of "normal" HeLa cells with the electron microscope as a preliminary to experiments on virus infection, certain features of fine structure were observed which were both unrecorded in this type of cell and unusual in a more general context. It was accordingly decided to study ordinary cells of the HeLa strain in thin sections with the electron microscope so that these features might be investigated and the fine structure of the cells defined; the present communication describes the results which have been obtained.

MATERIALS AND METHODS

Maintenance of HeLa Cells: Cultures of HeLa cells were grown in flat 8 oz. medical bottles by the methods of Pereira and Kelly (16), modified as described elsewhere (17). When confluent sheets of cells developed, they were used as a source of fresh cultures or as material for electron microscopy, the

latter being taken from time to time over a period of 3 months.

Preparation of Cells for Electron Microscopy: The confluent cultures were harvested either directly from growth medium (medium I) (16, 17) or after about 48 hours in maintenance medium (medium III) (16, 17), or after the same period in a medium similar to the growth medium but having rabbit serum substituted for human serum.

When the cells were to be collected, the culture fluid was removed and replaced by 3 ml of a 0.25 per cent *w/v* solution of solid trypsin (British Drug Houses Ltd., Poole, Dorset, England) in Gey's balanced saline (18) free of calcium and magnesium. The trypsin solution was warmed before use to 37°C and was left in contact with the cell sheet at that temperature for 1 to 3 minutes as a thin covering film. The cells were then shaken into suspension in the trypsin solution, drawn up into a warmed syringe, and immediately fixed by methods already described (19), except that 1.5 ml of cell suspension was squirted into the fixative, fixation was continued for 15 to 20 minutes, and a 10-minute period of centrifugation was used in collecting the cells during fixation. After fixation, dehydration and embedding were likewise done as in earlier work (19), each reagent used, however, being changed after 15 minutes.

Sections were cut with glass knives on a Porter-Blum microtome and those showing silver interference colours were mounted on carbon-coated grids (20) for examination in an electron microscope, either a Philips type EM 100 or a Siemens Elmiskop I. Structures were studied in serial sections whenever possible, both with and without lead hydroxide staining (21, 22).

OBSERVATIONS

The cells were similar in all the cultures examined and showed a constant fine structure irrespective of the nutrient fluid in which they had been grown. Cultures from the maintenance medium, however, contained fewer cells in mitosis and more degenerating cells and debris than those from the growth media.

In shape, the HeLa cells were flattish and irregularly rounded, with large oval or kidney-shaped nuclei and prominent nucleoli. Microvilli, as has already been reported (8), were sometimes present at the cell surface (Fig. 1).

Endoplasmic Reticulum: Various-sized oval or rounded profiles of smooth surfaced vesicles of the endoplasmic reticulum were scattered in the cytoplasm, and in some cells chains of such vesicles extended inwards from the cell membrane at the base of microvilli (Fig. 1).

In addition, a large area of juxtanuclear cytoplasm, frequently adjoining the nuclear indentation, was found to be filled with swarms of small smooth vesicles associated with randomly disposed piles of smooth surfaced cisternae (Figs. 1 and 9). Such a wide dispersal of poorly developed elements of the Golgi component (23–25) has been noticed previously in other rapidly growing undifferentiated tumour cells (26, 19).

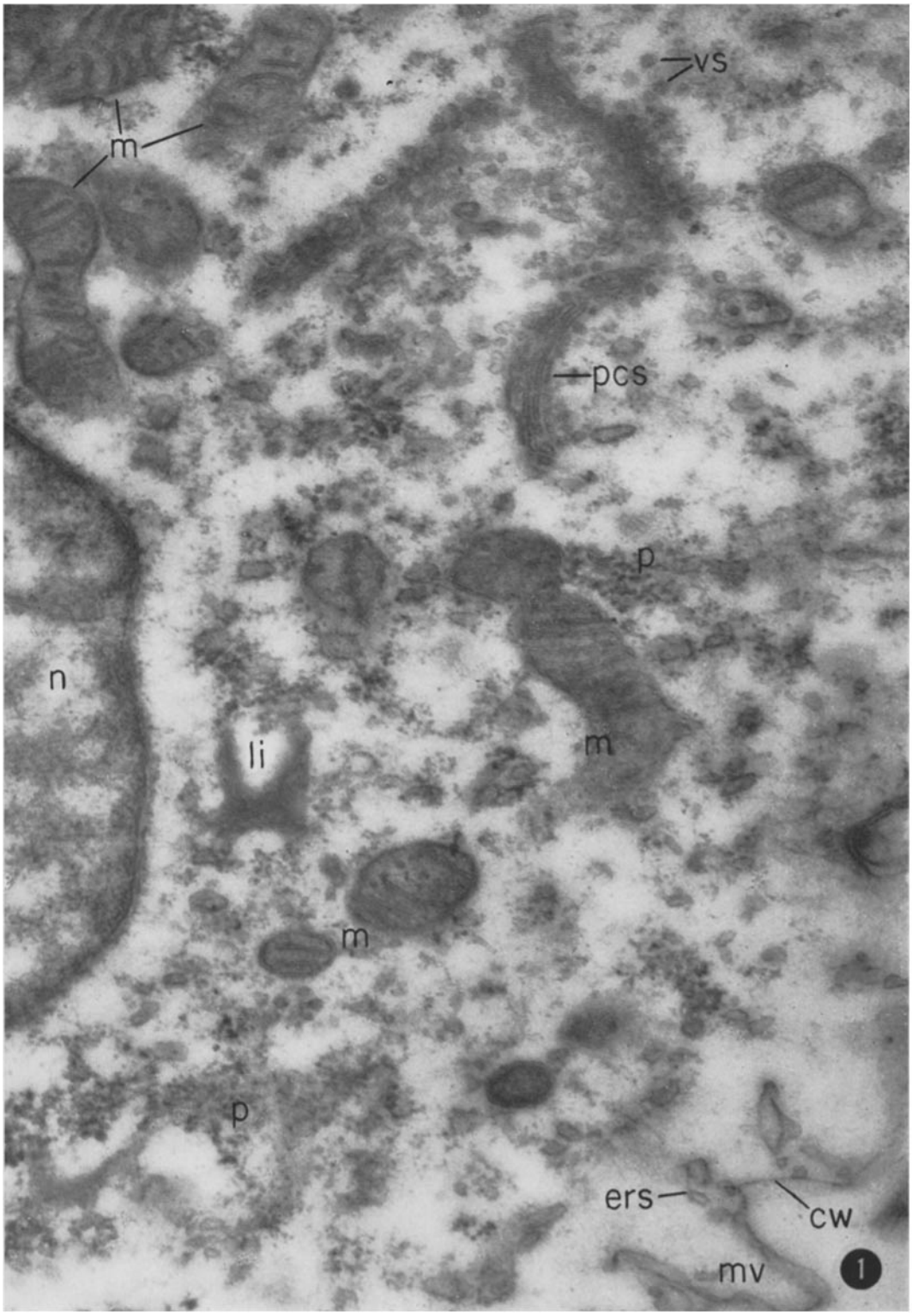
A third form of smooth endoplasmic reticulum was present in numerous cells in each preparation. It consisted of parallel rows of flattened membrane-bounded profiles spaced about 150 mμ apart (Fig. 2), and in serial sections it could be seen that these profiles belonged to regularly arranged fenestrated cisternae (Figs. 2 and 3). The limiting membranes of this structure were covered on the outside with a condensation of amorphous material and communicated in some places with particle-bearing elements of the endoplasmic reticulum (Figs. 2 and 3).

Rough surfaced cisternae of the endoplasmic reticulum were relatively infrequent; they were usually short, and ordered parallel orientation was rare (Figs. 5 and 9).

All the figures are electron micrographs of thin sections of HeLa cells grown in ordinary culture media.

FIGURE 1

Detail of cytoplasm. The nucleus (*n*), bounded by a double membrane, lies on the left of the field, and the cell wall (*cw*) in the lower right-hand corner; the cell wall includes a short microvillus (*mv*) in its course, from the base of which chains of smooth surfaced vesicles (*ers*) extend into the cell. The upper half of the field includes the edge of the large Golgi region with its swarms of small vesicles (*vs*) and associated piles of packed smooth cisternae (*pcs*), together with rod-shaped mitochondria (*m*). A lipoid body (*li*) and free particles (*p*) are also present. Lead hydroxide staining. × 50,000.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Mitochondria: Although rod-shaped mitochondria were scattered sparsely throughout the cytoplasm, they were concentrated as a rule in and around the large juxtanuclear Golgi zone (centrosome region) (Figs. 1 and 9).

Other Cytoplasmic Structures: The most striking additional cytoplasmic feature encountered was made up of four closely placed parallel membranes arranged either in concentric rings (Fig. 4) or extended over an irregular course (Fig. 5); where the latter was the case, the ends of adjoining pairs of membranes were found to be continuous (Fig. 5). The membranes were always about 20 mμ apart, and the study of serial sections indicated that they limited pairs of narrow cisternae (Figs. 6 and 7) rather than long tubules. In some cells this structure measured several microns in extent (Fig. 5); electron-opaque material was never observed enclosed within the membranes and only relatively few particles were attached to their outside, although free 15 mμ cytoplasmic particles (27–29) were often present close by (Figs. 4 to 7). No communications could be detected between the paired cisternae and the endoplasmic reticulum or the cell membrane, but in some instances the cisternae came into very close relation to the nuclear envelope; however, unequivocal junctions at such points could not be established.

Large, dense lipoid bodies (Figs. 1 to 4 and 9) and digestive vacuoles similar to those described by Harford and his coworkers (14) were sometimes seen. Some of the latter contained rod-shaped crystals up to about 250 mμ in length (Fig. 8). As has already been mentioned, scattered 15 mμ particles (27–29) were a feature of many areas of the cytoplasm (Figs. 1 and 5 to 7).

In addition, a small proportion of cells in each culture possessed considerable areas of cytoplasm devoid of organelles but occupied by uniform fuzzy material (Fig. 9) apparently composed of beaded strands (Fig. 10).

Nucleus: The nuclei and nucleoli were structureless apart from the usual fine granularity (Figs. 1, 4 to 7, and 9), the former being bounded by a double envelope containing pores (Figs. 1, 4 to 7, and 9). The chromosomes of cells in mitosis were likewise granular.

DISCUSSION

The general advantages of the fixation procedure used here have already been pointed out (19). In the present work the method had the additional advantage of inhibiting the trypsin used to free the cultures from the glass, as soon as its role was completed. For, by collecting the loosened cells in suspension in the trypsin solution and squirting this into fixative, both the enzyme and the cells were exposed to osmium tetroxide at the earliest possible moment, thus inactivating the former (Holt, unpublished results) whilst the latter were being fixed.

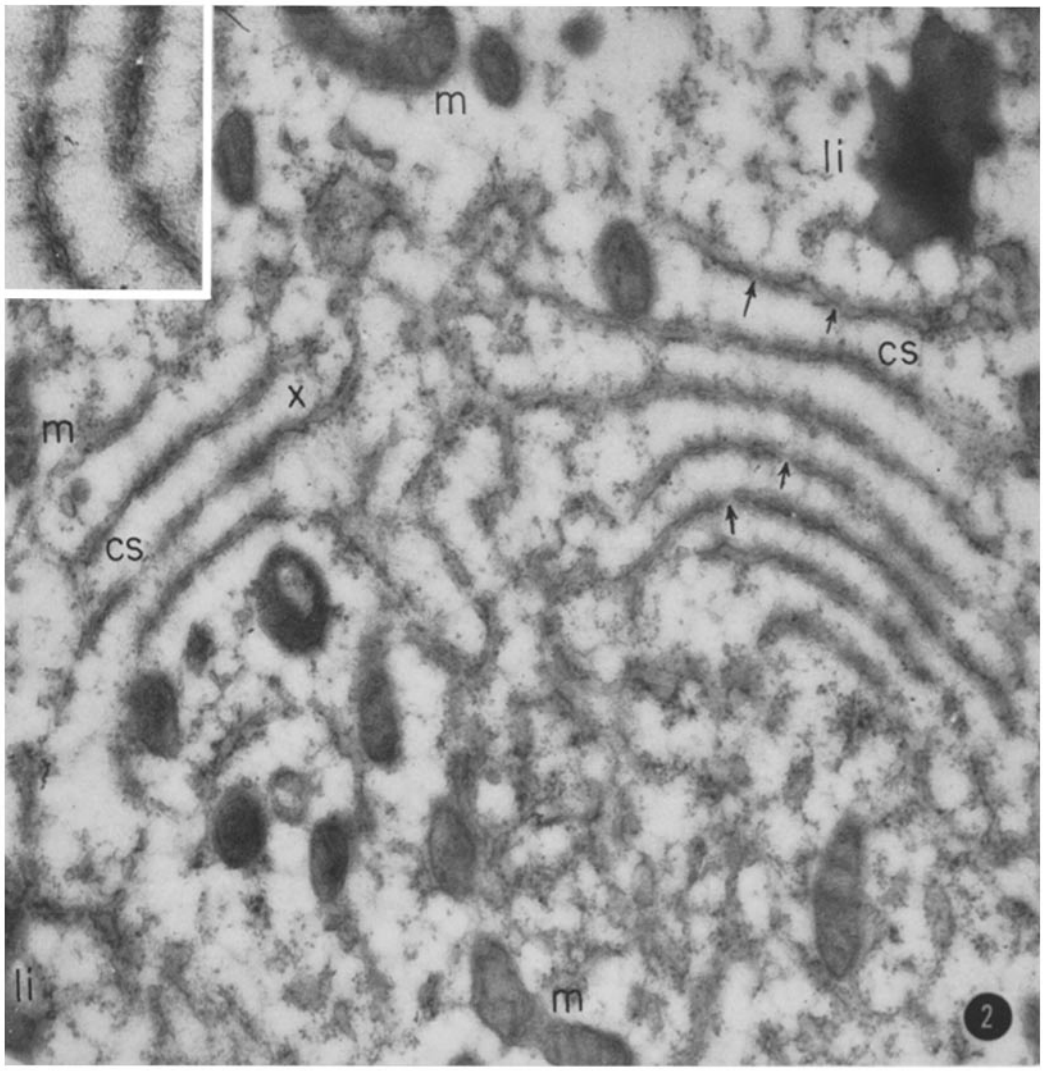
The over-all picture of fine structural organisation reported here for the HeLa cells is in accordance with that commonly present in undifferentiated malignant cells of various types (26, 19, 30, 31). However, three important and unusual features have been found and call for comment.

The rows of parallel, smooth surfaced, fenestrated cisternae having the characteristic 150 mμ spacing of rough endoplasmic reticulum (Figs. 2 and 3) closely resemble the structures of this type first described by Palade (32, 33) in developing rat spermatids and believed by him to be a transient local differentiation of the endoplasmic reticulum connected with the evolution of such cells. Very similar membranous elements have

FIGURES 2 AND 3

Small area of cytoplasm shown in two sections from a set cut in series. Arrays of four and seven roughly parallel smooth cisternae (*cs*) occupy, respectively, the left and right sides of the field; they lie about 150 mμ apart, which is the usual spacing for rough surfaced cisternae, and are covered with a condensation of amorphous cytoplasmic matrix. Interruptions are present in the cisternae (arrows) and comparison of the two sections shows these to be irregular fenestrations rather than regularly arranged pores or annuli. Towards the centre of the field the cisternae are clearly continuous with particle-covered rough elements of the endoplasmic reticulum as at *x*. Lipoid bodies (*li*) and mitochondria (*m*) can also be seen. Lead hydroxide staining. × 40,000.

Inset: cisternae at higher magnification (× 70,000) show the fenestrations and condensed amorphous matrix in greater detail.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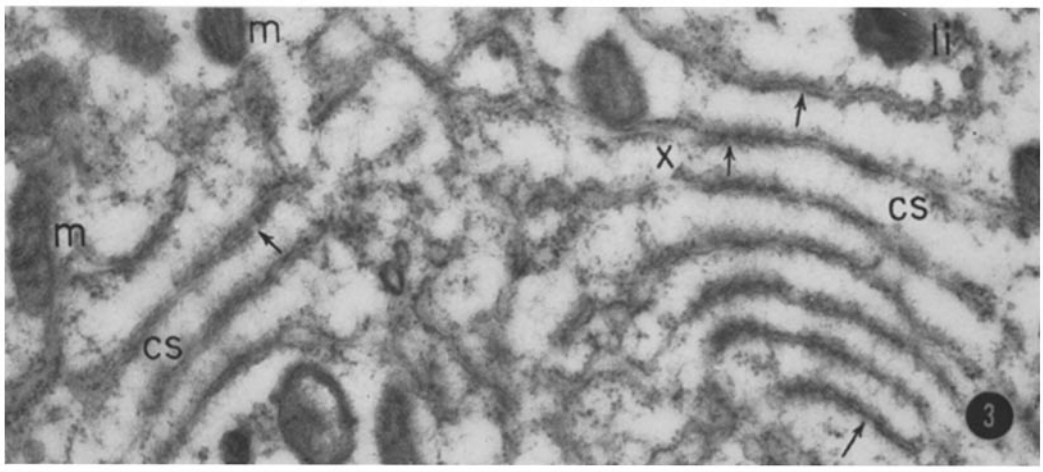
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also been reported in various oocytes (34, 35), where they have been designated "annulate lamellae" on account of their regularly arranged pores, as well as in the cells of a mouse ascites tumour (19). There is good evidence that these structures, although free of particles, contain a high concentration of ribonucleic acid (35, 36), and, as seen here in HeLa cells, that they can be continuous with rough surfaced membranes of the endoplasmic reticulum (Figs. 2 and 3), of which system they thus appear to form a special part. The frequent presence of the structure in rapidly growing anaplastic tumour cells such as HeLa cells and mouse sarcoma 37 ascites cells (19), together with its association with early germ cells from many sources (32–36), suggests that it might represent a primitive characteristic of the endoplasmic reticulum, or perhaps, as Barer has recently proposed (37), a specialised part of it concerned with storage of the nuclear envelope during cell division. Although the stacks of cisternae in the human cells studied here failed to show regular pores even when examined in serial sections (Figs. 2 and 3), the characteristic fenestrae known from other mammalian cells (32, 33, 19) were well seen (Figs. 2 and 3). It seems therefore that mammalian cells are probably without an analogue of the well defined pore-covered region of this structure present in the "annulate lamellae" of lower animals (34–36).

The second unusual finding of the present investigation concerns the pairs of double cytoplasmic membranes shown in Figs. 4 to 7. Short, fine double tubules having similar structural detail have been described in a rat endothelioma by Porter (38), who has reported that they seem to be involved in the spindle and aster structure of dividing cells and might have some connection, perhaps transitory, with the centriole. In addition, a double membranous element considerably more like those described here has been shown in a single illustration of a herpes-infected HeLa cell, where its presence has been ascribed to the effects of the virus (12). The serial and other sections of ordinary HeLa cells examined in the present study indicate that the membranes of this complex belong to closely applied cisternae (Figs. 4 to 7) and that these are a constant feature of the cells from "normal" cultures grown in conventional media; their relationship to the double tubular spindle filaments of Porter (38) is not known.

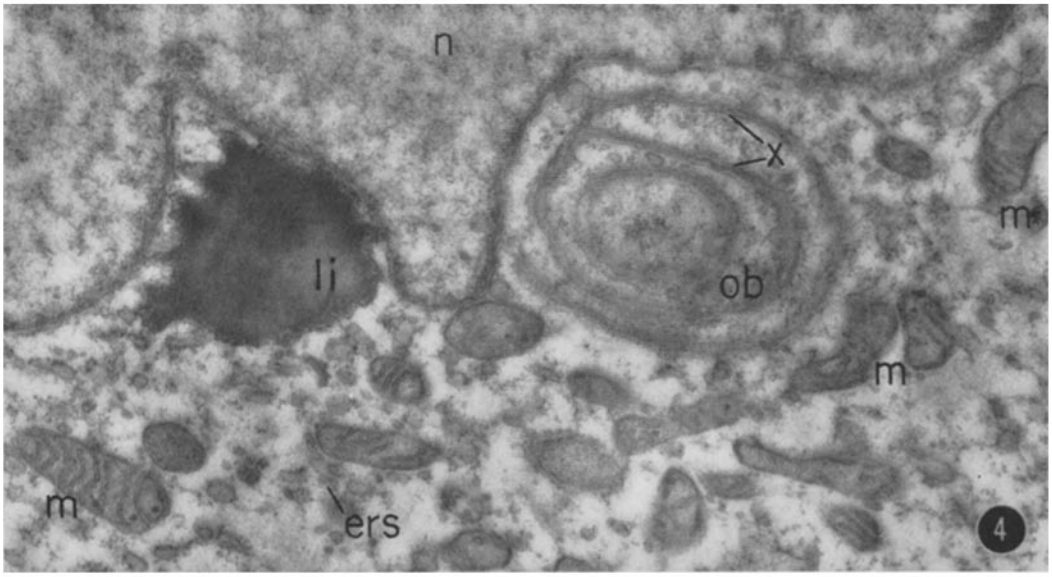
No junctions have been observed between the paired cytoplasmic cisternae and any of the membrane-bounded cavities of the endoplasmic reticulum, and although reduplication of the nuclear membranes is known to occur in HeLa cells infected with at least two varieties of virus (12, 39), the resulting lamellar arrays were very different from the paired cisternae in appearance and were confined to the nucleus or its envelope. Nor have any unequivocal connections been observed between this envelope and the paired

FIGURE 4

Portion of nucleus and neighbouring cytoplasm. The indented nucleus (*n*) occupies the top of the field and is bounded by the usual double membrane. Within the cytoplasm a large lipoid body (*li*) can be seen to the left of the centre, and a membranous structure arranged in concentric rings to the right. This structure is composed of two pairs of closely applied membranes which can be well seen at *x*, where the plane of the section has passed through them at right angles; in other parts the structure has been cut obliquely (*ob*). Mitochondria (*m*) and smooth vesicles of the endoplasmic reticulum (*ers*) are scattered through the cytoplasmic matrix. Unstained. × 30,000.

FIGURE 5

Part of the nucleus (*n*), above, and adjacent cytoplasm; the cell membrane (*cw*) crosses the lower right-hand corner of the field and there is a pore present in the nuclear membrane (arrow) in the upper right corner. A convoluted membranous structure extends through the cytoplasm in a haphazard manner. It is composed of two pairs of closely applied membranes, exactly similar to those shown in Fig. 4, and the ends of adjoining pairs can be seen to be continuous in various places (*x*). Mitochondria (*m*), smooth vesicles of the endoplasmic reticulum (*ers*), a rough surfaced cisterna (*err*), and many free particles (*p*) lie in the cytoplasm between the convolutions of the membranous structure; a few similar particles appear in close relation to the outermost membrane as at *y*. Unstained. × 40,000.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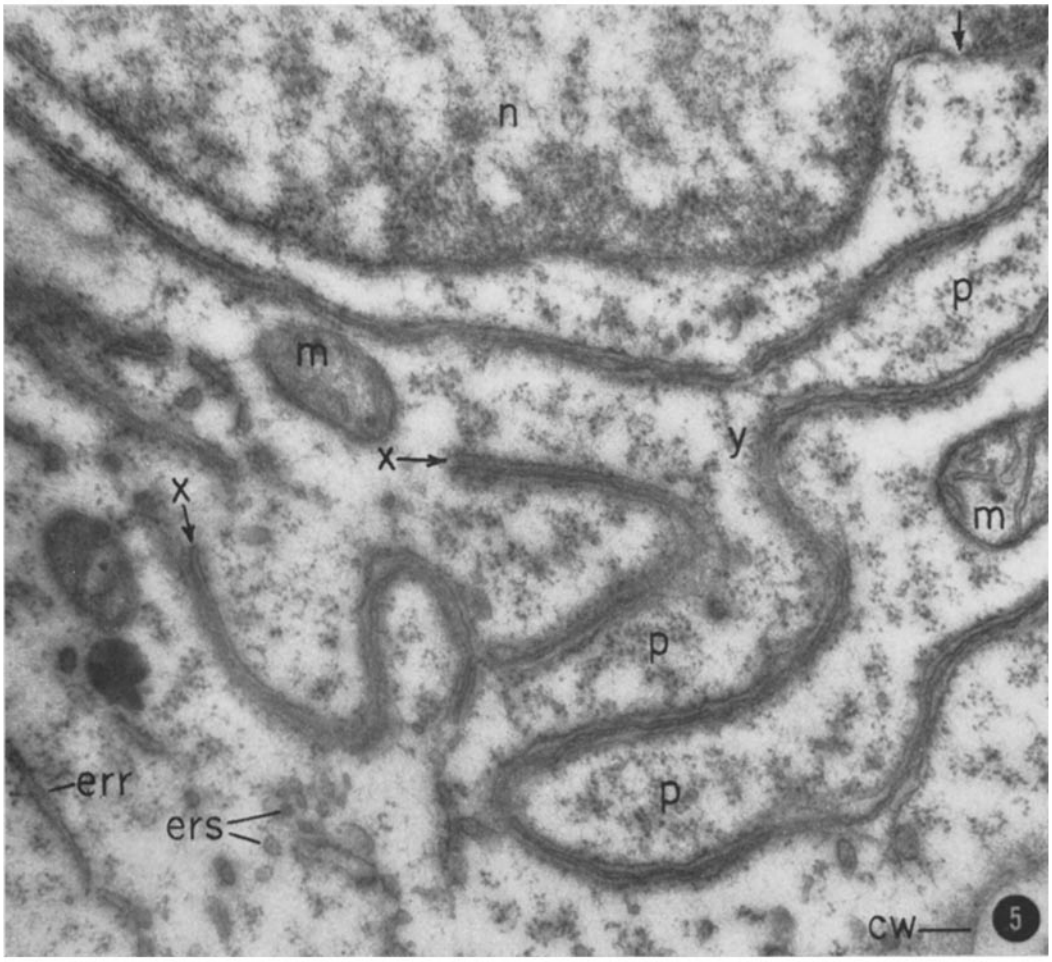
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cisternae, such as those well seen in the case of rough cisternae in Rous sarcoma cells (40). The particles on the outer membranes of the paired cisternae (Figs. 5 to 7) were much sparser than is usual for rough endoplasmic reticulum, and since the paired disternae were only seen in isolation and not connected to other membranous structures, their origin and nature remain obscure.

Although vacuoles containing crystals (Fig. 8) were not uncommon in the cells, it is not possible to say whether the crystals were present during life or whether they formed from the contents of the vacuoles as a result of some step in the procedure used to prepare the cells for electron microscopy.

The last point of interest relates to the fuzzy material found sometimes in the cytoplasm (Figs. 9 and 10). The fine structure of this substance is somewhat reminiscent of that reported elsewhere for extracellular polysaccharide (41, 42), and even though speculation based solely on morphological appearances is often fraught with danger, it is tempting to suggest that such material in the cytoplasm, perhaps in the form of glycogen, might be connected with the considerable versatility shown by HeLa cells in the metabolism of carbohydrates (43).

Thus, besides defining the fine structure of HeLa cells cultured in ordinary media, the present work has shown them to possess certain attributes of interest in the wider field of cell micromorphology as a whole (38, 44). For although HeLa cells are clearly both abnormal and altered, the fact that cells can exhibit such features is of intrinsic interest in itself. Furthermore, as has been pointed out in the case of Rous sarcoma cells (40), when considered from the point of view of survival and growth under a variety of adverse circumstances, the HeLa cell must be regarded as a very highly successful biological entity.

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FIGURES 6 AND 7

Two sections from a set cut in series showing a portion of the nucleus (*n*) on the right bounded by a double membrane containing pores (arrows), and a small area of adjoining cytoplasm filled by a membranous structure showing the closely applied paired membranes. It is evident that each pair of membranes encloses a narrow cisterna rather than a tubule, since the structure is present in both sections and could, indeed, be followed through many others in the series. Mitochondria (*m*) and smooth vesicles (*ers*) are also present together with scattered particles, some of which lie against the outer membranes of the paired cisternae. Unstained. × 37,500.

FIGURE 8

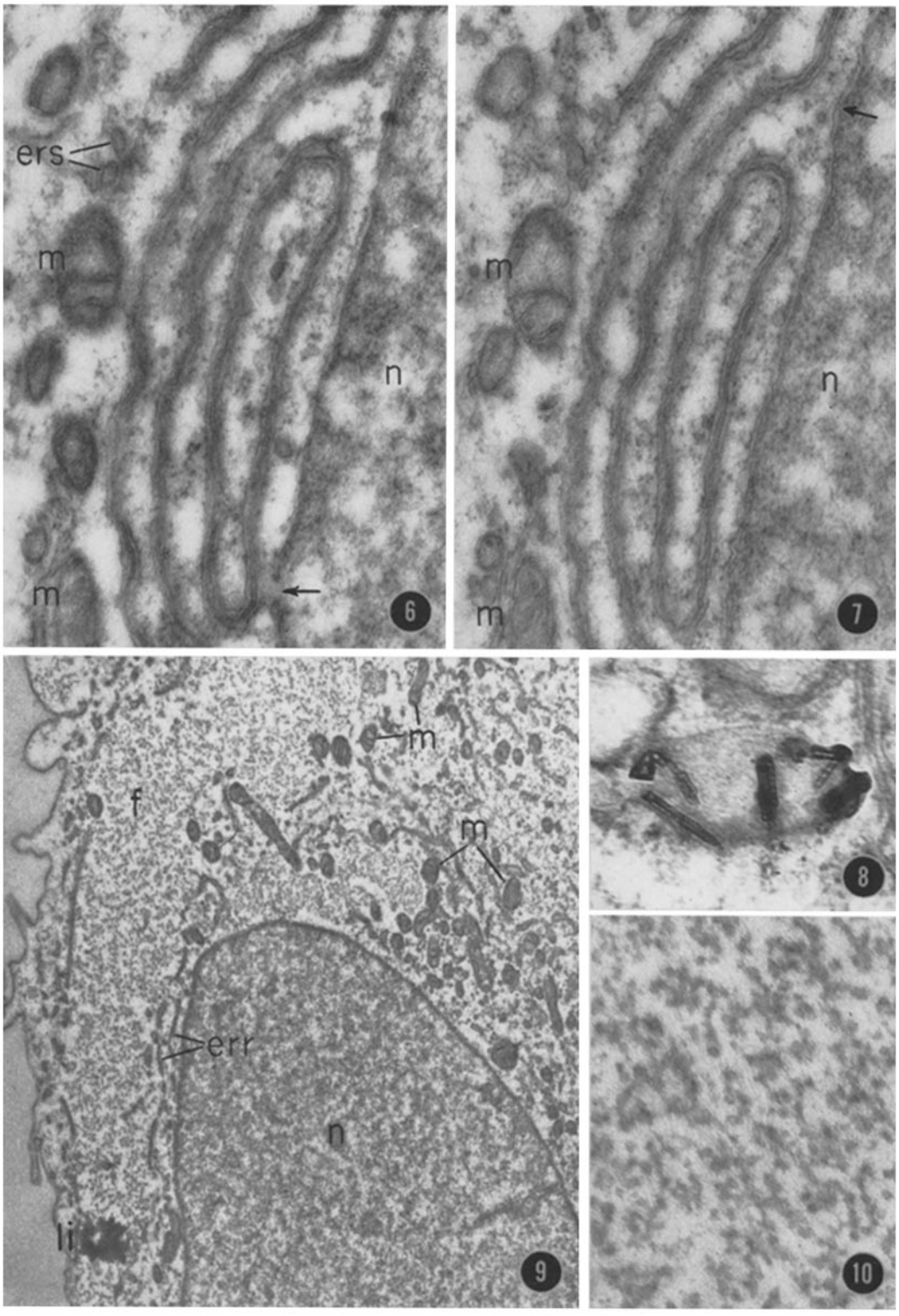
Detail of cytoplasm showing a small vacuole bounded by a fine membrane and having a dense content in which a number of crystals are embedded. Lead hydroxide staining. × 75,000.

FIGURE 9

Survey picture of about one-third of a cell. The nucleus lies at *n* bounded by its double membrane, whilst the cytoplasm in the upper right-hand part of the field contains a small part of the extensive juxtanuclear Golgi zone with many mitochondria (*m*) and the membranous Golgi components. The cytoplasm in the left half of the figure is occupied by a large area of uniform fuzzy material (*f*). Short rough cisternae of the endoplasmic reticulum (*err*) and a lipoid body (*li*) can also be seen. Unstained. × 10,000.

FIGURE 10

Detail of cytoplasm from an area filled with fuzzy material; this can be seen to consist of electron-opaque beaded chains. Unstained. × 44,000.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