THE FINE STRUCTURAL ORGANISATION OF ROUS TUMOUR CELLS

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The recent intracellular identification of the Rous virus (1) by a combined biological and electron microscope investigation of tumour cells from the ascites form of the Rous sarcoma (2), has been followed by reports describing the morphology of this virus (3, 4). However, the problem of how the Rous virus actually multiplies within the tumour cells remains unsolved, although various hypotheses have been suggested (3, 5). The elucidation of this problem calls for co-ordinated investigation in a number of fields, morphological study being one of the more important.

The histology of the Rous sarcoma is, of course, well known (6–11), but no detailed account of it as it appears in the electron microscope in thin sections has so far been presented. Gaylord (5) has illustrated pores in the nuclear membranes of Rous tumour cells and has noted both their cytoplasmic vacuoles and also their paucity of mitochondria, whilst the centriole of such cells has recently been shown to resemble that of other cells (12). In addition, some features of the cells have been mentioned briefly in passing by Bernhard and his collaborators (3), but have not been illustrated.

It was, therefore, considered essential that any morphological work on the mode of Rous virus multiplication should start by defining in detail the fine structure of Rous sarcoma cells. The present paper reports observations that have been made in a study of such cells with the electron microscope. The question of the relationship of the virus and its precursors to the cell constituents is not considered in this communication, which is solely concerned with the organisation of the sarcoma cells themselves.

Materials and Methods

Maintenance of Tumour.—The Rockefeller Institute strain of the Rous No. 1 fowl sarcoma which was used has been described elsewhere (1). It was passed in series by inoculation of 0.5 ml. volumes of tumour hash into the breast muscles of pedigreed susceptible Brown Leghorn fowl from the Poultry Research Centre, Edinburgh; the birds were between 7½ and 10 weeks old when inoculated, depending on the exigencies of supply. Strict aseptic technique was employed during the tumour passages and tests for the presence of contaminating organisms were negative.

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Preparation of Tumour for Electron Microscopy.—A tumour-bearing bird was killed by cervical dislocation 8 to 11 days after inoculation and, while its heart continued to beat, a fragment of soft, pale, friable, papillary growth was excised from its tumour and trimmed at once into 1 mm. cubes while in a drop of fixative, in the manner described by Palay and Palade (13). Iced 1 per cent OsO₄ buffered at pH 7.6 (14) and containing 4.9 per cent sucrose was used for the fixation which lasted 75 minutes. After fixation, dehydration and embedding were carried out by methods already described (15) except that in the present study each change of the reagents used lasted for 30 minutes; microtomy and microscopy were done as in previous work (15).

Observations

Numerous cells from each of five different Rous sarcomata have been examined in thin sections in the electron microscope; in some cases sets of serial sections were studied as well.

The tumours consisted chiefly of fusiform, fibroblast-like cells arranged either in bundles or, in some areas, in a loose meshwork having large intercellular spaces. In addition there was a small variable proportion of rounded, macrophage-like cells showing the fine structural features typical of this cell type (16, 15).

The fibroblast-like cells, which formed the principal element in the tumours, were remarkable for their uniformity of fine structure. This appeared as follows.

Endoplasmic Reticulum.—Simple round or oval smooth surfaced vesicles of the endoplasmic reticulum were rare, but could be met with in any region of the cytoplasm (Figs. 5 and 11); they were small, being only about 100 mμ in diameter, showed no tendency to lie grouped below the cell surface, and were not seen to communicate with the cell membrane. Deep infoldings of the cell membrane were, however, observed.

A more highly organised form of smooth surfaced endoplasmic reticulum occurred near the nucleus in the centrosome region of the cell; it consisted of well developed piles of parallel, tightly packed cisternae associated with numerous small vesicles (Fig. 1). These piles, besides sometimes presenting the unusual feature of occupying two areas of juxtanuclear cytoplasm in the same cell on opposite sides of the nucleus (Figs. 1 and 2), were often arranged in a characteristic quadrilateral pattern (Fig. 3).

Rough surfaced elements of the endoplasmic reticulum were also observed in the cells; they consisted of long cisternae, up to 3 or 4 μ in length, having limiting membranes covered on the outside with many small particles. The rough surfaced cisternae usually formed an important, well developed system filling much of the cytoplasm in several regions of the cell. The cisternae were either arranged in parallel array spaced 100 to 200 mμ apart (Fig. 4) or, more often, formed a convoluted maze on account of their having many branches and interconnections (Figs. 1, 6, 10, and 11). Dilatations of the cisternae were frequently present, and the cisternal contents were of uniform, slight to mod-
erate electron density (Figs. 1, 4, 6 to 8, 10, and 11), varying from cell to cell, much as has been reported in the case of pancreatic exocrine cells (30). The small particles attached to the limiting membranes of these rough surfaced elements of the endoplasmic reticulum were relatively profuse and arranged in ordered patterns; this was well seen where sections cut the cisternal membranes obliquely, thus revealing that the particles on them were lying in chains, rosettes, and spirals (Fig. 5). Rough and smooth surfaced elements of the endoplasmic reticulum were occasionally seen to communicate (Fig. 3).

Two other important characteristics of the rough surfaced cisternae were often observed. Firstly, many cisternae communicated with the space between the two nuclear membranes either by opening at one end directly into this space (Figs. 6 and 10) or, when orientated parallel to the surface of the nucleus, by short side branches; serial sections showed these branches to be tubes and not cisternae (Figs. 7 and 8). In both types of connection the limiting membrane of the cisterna was continuous with the outer nuclear membrane (Figs. 6, 7, and 10) which, with its attached particles, resembled it closely.

Secondly, rough surfaced cisternae have been found to open directly at the cell surface (Fig. 9), in which case their limiting membranes were continuous with the cell membrane. In some instances in which the cell membrane has been observed showing such an unbroken connection with the limiting membrane of a rough cisterna, the continuity has been traced further, right through to the point where the cisternal membrane has in turn joined, without interruption, the outer nuclear membrane (Fig. 10).

Mitochondria.—Typical mitochondria (17–19) were observed in the cytoplasm in unusually small numbers, a point which has already been commented upon (5, 12). The organelles were filamentous or rod-shaped, sometimes bent, and contained relatively few, usually transverse cristae and little matrix (Figs. 4, 5, and 11). They tended to be found in the neighbourhood of rough surfaced cisternae of the endoplasmic reticulum (Figs. 4, 5, 7, 8, and 11).

Other Cytoplasmic Features.—Electron-dense, presumably lipid bodies of about \( \frac{3}{2} \) \( \mu \) diameter and without a well defined limiting membrane were sometimes present in the cytoplasm (Figs. 4, 7, and 8). Large vacuoles up to several \( \mu \) in diameter and with a well marked limiting membrane were also encountered; 2 or 3 were occasionally present in a section of a single cell. The cytoplasmic matrix contained small electron-dense particles about 15 \( m \mu \) in diameter in addition to those attached to the rough surfaced elements of the endoplasmic reticulum; these free particles were arranged in clusters near the rough surfaced cisternae (Figs. 6, 10, and 11) and in some cases extended into neighbouring areas of cytoplasm which were otherwise free of structures.

Nucleus.—The nuclei were of the moderate size usual in cells of fibroblast type; they and their nucleoli were composed of fine granules (Figs. 1, 2, 4, and 6), those in the latter being both larger and more densely packed (Fig. 6).
The double nuclear membrane first seen in nerve cells by Hartmann (20) was well marked (Figs. 1, 2, 6 to 8, and 10) and, as reported by Gaylord (5), contained frequent pores (Figs. 1, 2, and 7).

In cells undergoing mitosis the chromosomes appeared structureless apart from a fine granularity.

**DISCUSSION**

The terminology used here in describing the cytoplasmic membranous structures of the Rous cells is that which has been worked out by Porter and Palade (21-23, 16). It should be noted that different descriptions have been used by other workers particularly for the juxtanuclear piles of packed, smooth surfaced cisternae which have sometimes been referred to as agranular reticulum (13) and also as the Golgi apparatus (24, 25).

Although the general arrangement of the cells described in the present paper conforms to the broad pattern of fine structure found in all cells, certain of the features recorded are unusual. Thus, the repeated finding of packed, smooth surfaced cisternae and associated vesicles in two widely separated areas of an individual cell (Figs. 1 and 2) is considered of interest since these membranous structures have hitherto always been found in the centrosome region alone.

The observations which have been made here on the rough surfaced elements of the endoplasmic reticulum are particularly significant. Watson (26) has suggested that the nuclear envelope is but a specialised form of rough surfaced cisterna and has shown that the space between the nuclear membranes is continuous with the interior of the endoplasmic reticulum in certain cells of the spleen. The perinuclear space has also been observed to communicate with smooth vesicles of the endoplasmic reticulum in lymphocytes (23), and with short rough surfaced cavities in granulocytes (23) and macrophages (15). The present work indicates that frequent communications between this space and well developed rough surfaced cisternae can also occur (Figs. 6 to 8); this assumes a special importance in the light of the further finding that at the other extremity of the cytoplasm the same rough cisternae communicate with the exterior of the cell (Fig. 9). Current ideas on the membrane-bounded cavities and spaces of the cytoplasm tend increasingly to regard them all as forming part of a single interconnected system, the endoplasmic reticulum, in which each distinct element—rough surfaced cisternae, packed piled smooth surfaced cisternae, simple vesicles, nuclear envelope—represents but a local differentiation (16, 19, 27). The finding that rough and smooth surfaced cavities communicate in the way described here (Fig. 3) and previously observed in neurons (13), granulocytes (23), and exocrine cells of the pancreas (30), lends support to this concept of a single interconnected system. When the work of Watson (26), discussed above, is taken in conjunction with what is known of
the manner in which elements of the endoplasmic reticulum communicate with the exterior of the cell (16, 33, 15), the continuity of this system right through from the cell surface to the perinuclear space has to be assumed. This has rested, so far, only on surmise; the observations reported here offer, for the first time, morphological evidence for the fact that the cell membrane can have an unbroken connection with the outer nuclear membrane through continuity with the limiting membranes of elements of the endoplasmic reticulum (Fig. 10). It must not be forgotten, of course, that Rous cells are abnormal cells; yet if they are regarded from the point of view of individual efficiency rather than from that of the diseased host organism harbouring them, they appear to be highly successful cells. Abnormal or not, at least they show that direct communication from outside the cell to the nucleus can and does occur.

With regard to the nature of the Rous cells, their general organisation has certain likenesses to that of macrophages (16, 15). At the same time, some of their features, apart from their fusiform shape and the unusual findings just discussed, render them distinct. For example, the rough surfaced elements of the endoplasmic reticulum have many more attached particles than do those of macrophages and the particles themselves are arranged in ordered patterns (Fig. 5). Free particles, as opposed to those attached to the rough endoplasmic reticulum, are also more profuse than in macrophages and are sometimes present in parts of the cytoplasm containing no other structures. Particles of this type (28) are now known to consist of the ribonucleoprotein (29, 30) responsible for cytoplasmic basophilia; the fact that the Rous cells have more of both the free and the attached particles than do macrophages would thus account for their "vesicular" type of staining when treated, for example, with haematoxylin and eosin, as distinct from the frank acidophilia of macrophages (15). In addition, the Rous cells usually have fewer vacuoles and lipoid bodies than do macrophages and are deficient in simple round and oval smooth surfaced vesicles, which are plentiful in macrophages where they frequently communicate with the cell exterior (16, 15).

Despite these differences, however, the general similarity in fine structure between the fibroblast-like Rous cells and macrophages is sufficient not to rule out the old idea (31, 32) that these two types of cell are but different forms of the same element.

Finally, the remarkable constancy which has been found in the fine structural organisation of the Rous cells, affords a fortunate starting point for the study of the mode of multiplication of the Rous virus within these cells and the consequent changes in their fine structure.

SUMMARY

The fibroblast-like tumour cells of Rous sarcomata have been studied in thin sections with the electron microscope.
A description is given of the fine structure of the cells which includes some features not hitherto recorded. The tightly packed piles of smooth cisternae usually found only in the centrosome region have been observed, in individual Rous cells, in two separate areas of cytoplasm at opposite poles of the nucleus. Continuity between the perinuclear space and the lumen of rough surfaced cisternae of the endoplasmic reticulum has frequently been found; a similar continuity between the cisternae and the exterior of the cell has also been seen. In some cases, the cell membrane has been shown to have an unbroken connection with the outer nuclear membrane through continuity with the limiting membranes of elements of the endoplasmic reticulum.

These findings are discussed.

BIBLIOGRAPHY

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All the figures are electron micrographs of fibroblast-like, Rous tumour cells.

Figs. 1 and 2. Two areas of juxtanuclear cytoplasm from the same cell; the regions shown lie at opposite poles of the nucleus. In each figure the granular nucleus (n) is bounded by a double membrane containing pores (arrows). Several well organised piles of packed, smooth surfaced cisternae (pcs) are present, associated with small smooth surfaced vesicles (vs). Rough surfaced cisternae of the endoplasmic reticulum (err) can also be seen and these show both branches (b) and dilatations (d). × 45,000.

Fig. 3. Juxtanuclear cytoplasm. Piles of packed, smooth surfaced cisternae (pcs) are arranged in a quadrilateral pattern; the usual associated small smooth surfaced vesicles (vs) are also present, as well as a mitochondrion (m). Some rough surfaced elements of the endoplasmic reticulum (err) are included in the field, and at x, one of these communicates with a smooth surfaced vesicle indicating that both types of element are part of a continuous system. × 80,000.
(Epstein: Fine structure of Rous cells)
FIG. 4. Relatively large field showing the cytoplasm from the nucleus almost to the cell membrane. The nucleus occupies the top left hand corner of the field, having been cut by the section almost at a tangent to its surface; the double nuclear membrane is, in consequence, only distinct for a short distance in the region of \( \text{nm} \). The rough surfaced cisternae of the endoplasmic reticulum (err) lie in roughly parallel array about 150 \( \text{nm} \) apart and show branchings (b), dilatations (d), and some convolutions (c). Some of the cisternae have been sectioned normally so that their limiting membranes with attached particles (mp) appear sharp; other cisternae have been sectioned obliquely (ob) and appear, therefore, less distinct. Mitochondria (m) and a lipoid body (li) are also present. \( \times 45,000 \).
(Epstein: Fine structure of Rous cells)
Fig. 5. Small area of cytoplasm. A fenestrated cisterna of rough surfaced type lies in a band across the middle of the field (err); it has been sectioned through most of its course at an oblique angle and lies therefore in, and nearly parallel to, the plane of section, thus affording an almost full faced view of much of its limiting membrane. It can be seen that the attached particles are arranged in spirals (s), chains (c), and rosettes (r). Normally sectioned limiting membrane of the cisterna is present at mp, fenestrae at f, and branchings at b. Mitochondria (m) and smooth surfaced vesicles of the endoplasmic reticulum (ers) are also present. × 60,000.

Fig. 6. Portion of nucleus and neighbouring cytoplasm. The area of granular nucleus included on the left of the field contains part of the nucleolus (ndl) composed of aggregated particles and is bounded by a double limiting membrane. At x, the perinuclear space is connected to the lumen of a rough surfaced cisterna of the endoplasmic reticulum (err, arrow), the outer nuclear membrane (nm) with attached particles being continuous with, and similar to, the cisternal membrane (mp). Other rough cisternae (err) can also be seen and these show branchings (b), convolutions (c), and dilatations (d). Free particles lie in the cytoplasmic matrix between the cisternae, as at p. × 80,000.
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Figs. 7 and 8. Part of the nucleus and adjacent cytoplasm shown in two sections from a set cut in series. The nucleus (n) in the top left hand corner of the field is bounded by a double membrane in which a pore can be seen (Fig. 7, arrow). At x in Fig. 7 the perinuclear space is connected to the lumen of a rough surfaced cisterna (err, arrow) lying parallel to the nucleus. That the connection is through a tubule can be seen from the fact that it is not present in the next section shown in Fig. 8. Other rough cisternae (err) are present in the field as well as mitochondria (m) and a lipoid body (li). × 40,000.

Fig. 9. Small area of peripheral cytoplasm. The cell membrane (cm) runs across the top of the field parallel to that of the next cell except on the extreme left, where there is an intercellular space (s). The terminal portion of a rough surfaced cisterna (err) crosses the field from top to bottom; this cisterna extended through the cytoplasm for 3.5 μ, forming part of a parallel array. At x the lumen of the cisterna opens to the exterior of the cell, the cell membrane being continuous with the cisternal limiting membrane. × 80,000.

Fig. 10. Part of the nucleus and adjacent cytoplasm. The granular nucleus (n) lies on the right of the figure and is bounded by a double membrane. The cell membrane (cm) runs up the left side of the field and at y becomes continuous with the limiting membrane of a convoluted rough surfaced cisterna of the endoplasmic reticulum (err). At x the outer nuclear membrane likewise shows an unbroken connection with the limiting membrane of this same cisterna, whose lumen opens there through a narrow neck into the perinuclear space. Many groups of free particles (p) lie in the cytoplasmic matrix near the cisterna. × 45,000.

Fig. 11. Small area of peripheral cytoplasm. Rough surfaced cisternae (err), showing dilatations (d) and branchings (b), are grouped round a mitochondrion (m). The latter is typical of this type of cell in that its cristae are sparse and its matrix lacking in density. Smooth surfaced vesicles of the endoplasmic reticulum (ers) can also be seen, together with groups of free particles (p) between the rough cisternae. × 60,000.
(Epstein: Fine structure of Rous cells)