LAMELLAE IN THE SPINDLE OF MITOTIC
CELLS OF WALKER 256 CARCINOMA

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ABSTRACT
In mitotic cells of Walker 256 carcinoma some four-layered lamellar structures were observed which had the appearance of paired cisternae of the ER. Two inner membranes were regular, smooth surfaced, and closely applied to each other. The two outer membranes were somewhat irregularly placed in relation to the inner pair; they showed attached RNP particles and connections with cisternae of the ER. The membranes often appeared to radiate from the region of the centrosphere towards the compact mass of chromosomes. Thus, they lay amid the spindle fibres and are referred to as "spindle lamellae." They approached the centrioles closely but were not observed to be continuous with them. They appeared to terminate in the pole of the spindle by joining smooth surfaced membranes in the centrosphere. Their equatorial termination was in relation to the chromosomes. At the surface of the chromosome mass they frequently split into two double membranes, which were closely applied to the chromosome substance. The most prominent and complicated membranes were seen in anaphase cells. An hypothesis is advanced which ascribes the development of the nuclear membrane to the spindle lamellae.

INTRODUCTION
In mitotic cells of different kinds of animal tissues Porter (9, 10) observed some double tubular structures which, because of their tendency to be associated with other spindle structures, he called "spindle filaments." They were thought to be equivalent to long, slender filaments observed earlier in whole cell mounts of cultured cells (12). A careful description was provided of the appearance of these structures in sections but no study of them seems to have been made by other workers. Chentsov (5) has independently and very briefly referred to some equivalent "five-layered" structures in cells of an induced rhabdomyoblastoma. Recently, the finding of the "spindle filaments" in animal cells was briefly reviewed by Porter and Machado (13) who compared the cytoplasmic structures of mitotic animal and plant cells. A component of the spindle in the latter was found to be certain membranes of the endoplasmic reticulum (ER) which were suspended, like drapes, among the other parts of the spindle. With regard to the double tubules of the animal cell the authors stated, "It is entirely possible that they are the animal cell equivalent of the ER elements noted here in the plant cell, but it must be admitted that the aspect of doubleness has not been seen with certainty in Allium cells fixed in either OsO₄ or KMnO₄."

My observations support their suggestion and indicate that these structures, like the ER of the plant cells, are important in the development of the nuclear membrane of certain mammalian cells.

MATERIALS AND METHODS
Most of the observations have been made on cells of the Walker 256 carcinoma after growth intramuscu-
larly in the leg of Sprague-Dawley rats for a period of 4 to 8 days. Tissues were fixed in 1 per cent buffered osmium tetroxide at pH 7.3 for 1 hour at 4°C, treated for another hour at the same temperature with 1 per cent aqueous uranyl acetate, dehydrated with ethanol-water up to 70 per cent ethanol, and then with acetone. They were embedded in Vestopal W (15), and sections mounted on uncoated grids were examined in an RCA EMU3D electron microscope. Sections of about 1 μ thickness stained with hot 1 per cent methylene blue were studied with the light microscope for the purpose of locating cells in mitosis and determining, whenever possible, the phase of the mitotic cycle and the orientation of the cell.

OBSERVATIONS

In mitotic cells of the Walker tumor, profiles were seen consisting of four dense narrow lines separated from each other by lighter material (Fig. 1). The profiles, when studied in serial sections, were observed to persist in the third dimension, sometimes for several microns (Figs. 2 and 3). Usually only relatively short profiles were found but occasionally they were tortuous, branching and several microns in length (Fig. 4). Since profiles showing branching, tortuosity, and considerable length would not likely be produced by the sectioning of tubular structures, they were interpreted as representing broad membranes or lamellae. The shorter profiles might represent either short lamellae or tongue-like extensions from the margins of larger ones.

These quadrilaminar membranes were composed of an inner pair, closely applied to each other, and an outer pair. The inner membranes measured about 70 Å in width and were separated from each other by a fairly uniform light space about 40 Å to 70 Å wide. Both surfaces of the inner membranes were smooth.

The outer pair of membranes, separated from the inner pair by a somewhat irregular space varying from 60 to 350 Å, frequently showed saccular or fusiform dilatations. In many places a continuity of the outer membrane with cisternae of the ER was seen (Fig. 4). The outer membranes often showed a small number of attached RNP particles, comparable in size and number to those seen on the cisternae of the ER in the interphase state of the Walker tumor cell.

By serial sectioning it was established that a particular quadrilaminar membrane was in continuity with other similar membranes. In fact, it is possible that the quadrilaminar membranes constitute a continuous system, although this would be very difficult to establish.

Each of the inner membranes was continuous with its homolateral outer membrane at the ends of the profile of a particular quadrilaminar membrane but not with the other inner membrane (Fig. 1). Thus the four layers were considered to
A cell, which is probably in metaphase, cut obliquely at two levels. A system of four-layered profiles is seen near the surface of the chromosome mass (CH) in both sections. The profiles are thought to represent lamellae having appreciable width. The tendency for the lamellae to radiate towards the surface of the chromosomal mass is also apparent. × 8,000.

represent an association between two very flat cysternae. The adjoining membranes of the cysternae were smooth, regular, and closely applied to each other. The outer membranes had the general character of the membranes of the ER, with which they were continuous.

A complete description of the distribution of the membranes within the dividing cell would be difficult to provide as they seemed to be found, in one section or another, in almost every conceivable site. The obvious reason for this is that one is not always aware of the plane of sectioning and relations are then difficult to establish. From the study of favourably sectioned material and from serial sections, it was established that the membranes were most frequently found in the region between the centrosphere and the mass of chromosome material. In many sections they appeared to fan out from the region of the centrosphere (Figs. 2, 3, 5). However, they were not observed extending all the way to the centrioles. No direct connection between the tubules of the centrioles and these membranes could be found, although in places they approached each other very closely (Fig. 6).

On the other hand, a continuity with some delicate, irregular, smooth membranes of the centrosphere was sometimes seen (Fig. 6). The latter membranes outlined an irregular network of saccular spaces between many small spherical vesicles of the centrosphere. They resembled the smooth surfaced component of the ER and were considered to represent the polar termination of the quadrilaminar membranes.

From this point the membranes ran towards the surface of the compact chromosome mass taking, at first, a relatively straight course between the spindle fibres. On approaching the chromosomes they became tortuous and branching. In some sections the four membranes appeared to pass into the chromosome mass, presumably between chromosomes. No unequivocal instances were found in the Walker tumor of the membranes passing from one chromosome set to the other. Usually, membranes appeared to extend over a part of the surface of the chromosomes. Sometimes all four layers were seen running parallel to and against the chromosomes. More often, however, the four-layered system became split at the chromosome surface into two separate double mem-

FIGURES 2 AND 3

A cell, which is probably in metaphase, cut obliquely at two levels. A system of four-layered profiles is seen near the surface of the chromosome mass (CH) in both sections. The profiles are thought to represent lamellae having appreciable width. The tendency for the lamellae to radiate towards the surface of the chromosomal mass is also apparent. × 8,000.

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briefly, the splitting taking place between the inner pair of membranes (Figs. 4 and 7). Frequent examples were found of profiles of short, and apparently separate, pieces of double membrane on the surface of the chromosomes, especially on the polar surface (Fig. 8). These images were interpreted as representing a progression of the process illustrated in Fig. 7.

Although the phase of the mitotic cycle in many sections could not be established with certainty, particularly when, as in the Walker tumor, multipolar mitoses occurred (Fig. 6), my opinion is that in anaphase the membranes reached their greatest degree of development. Short profiles were still seen in telophase (Fig. 9). They have not been found in interphase nor in prophase cells. Some of the cells showing the membranes were probably in metaphase, but on this point I am uncertain. However, examples have been observed in metaphase in another tumor (spontaneous lymphosarcoma in a Swiss mouse).

Even in anaphase it appeared that only some of the cells possessed the membranes, although conclusive proof for this statement would be difficult to provide. Certainly, their very extensive development was observed only occasionally.

The configuration and relations of the membranes are shown diagrammatically in Fig. 10.

DISCUSSION

Except for the fact that the double structures of the mitotic spindle were described as tubular, rather than membranous, Porter's (9, 10) earlier
account is confirmed in all important respects. Because they appeared to him as double tubules and because they formed part of the spindle, he investigated the possibility that they might join the double tubules of the centrioles. Porter was unable to find any conclusive evidence for this interesting possibility but the suggestion was put forward that they might, at any rate, act as centres or axes of spindle fibre organization. Their continuity with the cisternae of the ER and their association with RNP particles were seen.

If my interpretation is correct, that these structures represent profiles of flat cisternae and not tubules, then Porter’s term “spindle filaments” should properly be changed to “spindle lamellae.”

Except for their double nature the spindle lamellae are similar to parts of the ER described by Porter and Machado (13) in plant cells, and these authors, whom I quoted earlier, suggested that this might be the case.

The observations show that in Walker tumor cells in anaphase the spindle lamellae come into relation with the surface of the chromosomes and appear to be involved in the formation of the nuclear membrane. The studies of Watson (16, 17) clearly showed that in the interphase cell the nuclear envelope is a specialized part of the ER. It is, therefore, not surprising that recent studies of dividing cells have already demonstrated that, in the dissolution of the nuclear membrane, vesicles are formed which are finally indistinguishable from those of the ER, and during its

**Figure 5**

This polar view shows the radial arrangement of spindle lamellae from the centrosphere (centriole at X) toward the chromosomes (CH). In this location they run between the spindle fibres (SF) and some irregular, smooth surfaced membranes of the ER. X 33,400.
Spindle lamellae at the chromosome (CH) surface. The upper part of the field shows many vesicles and membranes of the centrosphere and spindle region. In some places the spindle lamellae are applied to the chromosome surface as a four-layered membrane. In other places the lamellae divide so that they are continued as two-layered membranes. × 43,000.

The membranes of the ER are involved (1-5, 8, 11, 13, 18). Multiple membranes (parafusorial lamellae) are seen in spermatogenic cells of Drosophila (6). It is thought that the nucleolus may be implicated in the formation of the inner layer of the nuclear membrane in Amoeba proteus (14). Although Bernhard (4) has suggested the possibility of the de novo origin of the nuclear membrane, Jones (7) appears to be alone in supporting it.

On the basis of the present observations the hypothesis is advanced that in the Walker tumor cells, at least, a membrane destined to become the nuclear membrane forms in the region of the
spindle during anaphase. This preformed nuclear membrane (the spindle lamellae) may become very extensive, in fact, more extensive than the membranes of the interphase cell except the plasma and nuclear membranes. It is suggested that the formation of the spindle lamellae begins by the apposition of cisternae of the ER and that the lamellae grow both interstitially and by the incorporation of more cisternae of the ER. In late anaphase the spindle lamellae begin to unfold and separate into double membranes which spread over the surface of the compact chromosome mass. The “smooth” inner and “rough” outer layers of the nuclear envelope are thus derived, respectively, from the “smooth” inner and “rough” outer membranes of the spindle lamellae. The process is not completed until late telophase, parts of the spindle lamellae retaining their integrity, yet connected to the nuclear membrane already laid down. How such a process might be accomplished is shown diagrammatically in Fig. 11.

The question of the prevalence of the spindle lamellae in different types of mitotic mammalian cells cannot be answered yet. Porter's observations, those of Chentsov (5), and my own have been made principally on tumor cells. Perhaps fewer normal somatic cells in mitosis have been studied. Yet, in collaboration with Mr. J. M. Tisdale, I examined several hundred mitotic cells in the...
Diagram to show the structure and relation of the spindle lamellae. A lamella consists of four layers of membrane, the outer ones being continuous with cisternae of the ER at various points on their surface and at their polar extremity. From the region of the centrosphere the lamellae radiate towards the chromosome surface, sometimes branching before reaching it. At the chromosome surface the lamellae are split into two double membranes.

Schema for the development of the nuclear membrane in Walker tumor cells. According to an hypothesis advanced in the text, the spindle lamellae develop by the close apposition of cisternae of the ER (1), which are part of a continuous membranous system (dotted lines). The lamellae grow during anaphase (2, 3). In late anaphase (4, 5) they begin to separate at the chromosome surface (shaded mass) and a double nuclear membrane is laid down (6) from the preformed spindle lamellae.

Ependyma of 10- to 12-day-old rat embryos without finding a single instance of spindle lamellae. On the other hand, profiles have been seen in the regenerating liver of rats 48 hours after partial hepatectomy. It is possible that although the spindle lamellae exist in normal mitotic cells, they are more transitory than in tumor cells.

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Daughter cells in telophase in which the nuclear membranes (NM) are almost reformed. At the level of this section the plasma membranes (PM) appear to separate the cells. Spindle lamellae are still found in the cytoplasm of both cells and in the cell on the right a part of the nuclear membrane resembles a spindle lamella. × 24,500.
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