Virchows Arch. A Path. Anat. and Histol. 363, 337—349 (1975)

Mixed Müllerian Tumors of the Uterus

Ultrastructural Studies on the Differentiation of Rhabdomyoblasts

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Received November 4, 1974

Summary. In the present study the differentiation of rhabdomyoblasts of two carcinosarcomas of the uterus is analysed electronmicroscopically. During the development of rhabdomyoblasts three cell types can be distinguished:

1. the undifferentiated mesenchymal cell with abundant ribosomes but few other cell organelles. Usually these cells are already associated to each other in strands of 2–4 cells. Occasionally cytoplasmic areas with numerous nonspecific filaments can be observed.

2. the light rhabdomyoblast which is characterized by its conspicuous nonspecific cytoplasmic filaments and its reduction in ribosomes. Specific myofilaments can be visualized only occasionally in this cell type.

3. more differentiated rhabdomyoblasts. These cells can be identified clearly by the presence of large numbers of myofibrils and myofibrillar fragments. Highly organized myofibrils as seen in skeletal muscle are seldom found. Transitional forms between these cell types are also observed. The functions of Z-bodies and of the sarcotubular system in the process of myofibrillar differentiation are discussed.

Key words: Carcinosarcoma — Rhabdomyoblast — Myofilaments — Uterus.

Zusammenfassung. Die Entwicklung von quergestreiften Rhabdomyoblasten in 2 gemischten Schleimhautsarkomen des Uterus konnte licht- und elektronenoptisch analysiert werden. Es lassen sich folgende 2 Differenzierungsphasen unterscheiden:

1. Die 1. Phase ist charakterisiert durch die Bildung unspezifischer cytoplasmatischer Filamente. Ausgangspunkt der Differenzierung ist eine primitive mesenchymale Zelle mit reichlichen Ribosomen und wenigen anderen Zellorganellen. Im Cytoplasma dieser Zellen findet man zunächst umschriebene Areale mit zahlreichen unspezifischen Filamenten. Durch erhebliche Proliferation dieser Filamente mit Ausdehnung über den gesamten Cytoplasmaleib sowie durch gleichzeitige zahlenmäßige Abnahme der übrigen Organellen entsteht der sogenannte helle Rhabdomyoblast.

2. In der 2. Phase werden die beiden spezifischen Myofilamentsysteme gebildet. Die ersten Myofilamente sind in Form parallel gelagerter Aktin- und Myosinfilamente erkennbar. Auf Querschnitten sind die dünnen Aktinfilamente hexonal um die dicken Myosinfilamente angeordnet. Die Filamentbündel nehmen an Zahl erheblich zu und liegen meist regellos im Cytoplasma. Nur in einzelnen Zellen sind die Filamentbündel in Myofibrillen sarkomerisch hintereinandergeschaltet. Diese hochorganisierten Myofibrillen besitzen eine Querstreifung, wie sie von quergestreiften Skelettmuskelfasern her bekannt ist. Die Rolle der Z-Bänder und des sarkotubulären Systems bei der Ausreifung der Myofibrillen wird diskutiert.

Introduction

Heterologeous tissue components like striated muscle, cartilage adipose tissue, and bone are often found in mixed Müllerian tumours (mixed mesodermal tumours, carcinosarcomas) of the uterus (Sternberg *et al.*, 1954). As prognosis seems to

depend in part on the type of heterologeous tissue components (Kempson and Bari, 1970; Norris and Taylor, 1966) a precise histopathological classification is necessary. Typical rhabdomyoblasts are easy to identify, but it may be difficult to distinguish less differentiated stages of rhabdomyoblasts from mesenchymal cells.

In the present study of two cases of mixed mesenchymal tumours at light microscopic level one is proven to contain rhabdomyoblasts whereas in the other they are suspected. The ultrastructural features of this cell type could be analysed electronoptically. The results of this investigation are discussed in the light of our present knowledge on the development of striated- (Ezerman and Ishikawa, 1967; Ishikawa, 1968; Ishikawa *et al.*, 1968; Kelly, 1969; Knappeis and Karlsen, 1968) and heart muscle (Rash *et al.*, 1970) with particular attention being paid to the different types of cytoplasmic filaments. With the exception of chondroplastic tissue components (Silverberg, 1971) there are to date no electron microscopic communications on the differentiation of heterologeous mesenchymal structures of uterine tumours.

Material and Methods

The two carcinosarcomas for study were found in an 62 and an 81 year old potmenopausal woman. Both lesions were diagnosed in curettage material. Paraffin sections of formalin fixed tissues were stained by the following methods for routine histology: H-E, PAS-reaction, Masson-Goldner. Semithin sections were stained with toluidine blue and examined for purpose of orientation. For electron microscopic investigation small pieces from the uterine curettage were immersed in 2.5% glutaraldehyde, buffered to pH 7.4 with 0.1 m phosphate buffer and fixed for two hours. The tissue was postfixed in aqueous 1% OsO_4, followed by alcohol dehydration, and embedded in either Westopal W or in Epon 812. Ultrathin sections were stained by uranyl acetate and lead citrate.

Results

Light Microscopy

The histologic picture of both tumours was characteristic of carcinosarcoma. The carcinomatous (endometrial, in part papillary adenocarcinoma) and sarcomatous elements were closely intermingled. In one of the cases (2018/70, Universitäts-Frauenklinik Hamburg) half of the tumour consisted of rhabdomyosarcoma with occasional cross striations of a few cells. Additionally small areas of lipoblastic differentiation could be seen. In the other case (9156/74, Pathologisches Institut der Universität Hamburg) rhabdomyoblasts, while lacking striation, were only suspected. Undifferentiated sarcomatous areas predominated.

The typical rhabdomyoblasts had abundant eosinophilic granular or fibrillar cytoplasm. Usually a single prominent nucleolus could be detected in the excentrically located nucleus. Cross striations were seen in only few of these cells.

In the semithin sections the following types of cellular arrangements of rhabdomyoblasts were encountered:

(1) single cells, whose shape varied from tadpole- to ribbon-like (found mainly in case 9156/74);

(2) clusters of rounded to elongated large cells usually closely related to each other in a pseudoepithelial formation. Often definite cross striation was easily

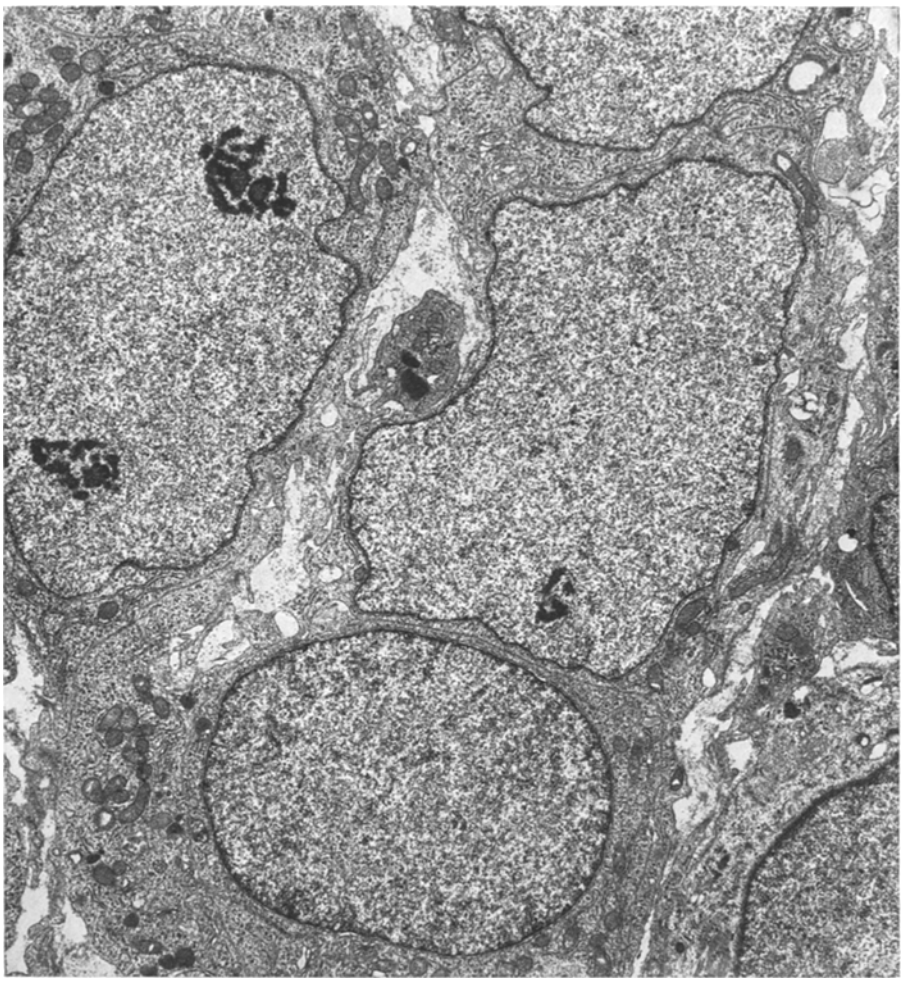

Fig. 1. Presumptive rhabdomyoblasts. Grouping of tumour cells with close apposition of plasma membranes. Poorly developed basement membrane at this stage of differentiation. Magn. × 7200

identified within the cytoplasm of these cells. The spaces surrounding the cell clusters contained few small mesenchymal cells, blood capillaries and collageneous fibrils.

Electron Microscopy

At ultrastructural level groups of 2–4 polygonal large cells predominated, often in close apposition to one another but without junctional attachments (Fig. 1). These cells were segregated from the surrounding mesenchymal tissue by the acquisition of a basal membrane which initially was present only in low concentration on the cell surfaces of presumptive rhabdomyoblasts. Usually cells in different stages of development could be seen even within a single cluster.

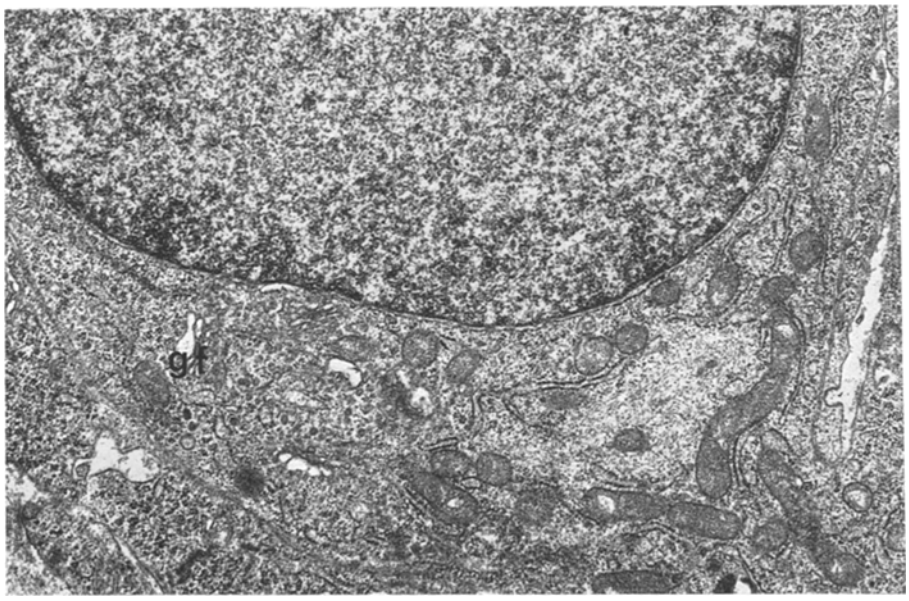

Fig. 2. Part of presumptive rhabdomyoblast. Focal appearance of nonspecific 100 A filaments. *gf* golgi field. Magn. × 13500

During differentiation cytoplasmic variations were found which were mainly due to type, number and arrangement of filaments. The plasma membranes of these cells were clearly defined and separated from each other by an about 120 A wide intercellular space. Occasionally however it was possible to observe zones of cytoplasmic bridges. Furthermore a few desmosome-like specializations could be visualized. In the process of differentiation the following three cell types could be distinguished:

(1) undifferentiated mesenchymal cell herein referred to as presumptive rhabdomyoblast;

(2) light rhabdomyoblast with abundant intermediate filaments;

(3) more differentiated rhabdomyoblasts with myofibrils.

Transitional forms between these cell types were also observed.

Presumptive Rhabdomyoblast. These cells were already associated with each other in strands of 2–4 cells (Fig. 1). Often a discontinuous ill defined basement membrane was present which seperated the cells from the surrounding mesenchyme. The cytoplasm contained abundant free ribosomes. Varying amounts of mitochondriae and a few lamellae of the rough surfaced endoplasmatic reticulum could be found. The Golgi apparatus was moderately developed but more prominantly than in better differentiated cells. It consisted mainly of cisternal and vesicular elements. The large euchromatic nucleus had one or two prominent nucleoli. Occasionally cytoplasmic areas with numerous aperiodic filaments of about 100 A thickness could be observed within these cells. Usually there was a striking reduction in electron density in these areas due to a lack of ribosomes and other cell organelles (Fig. 2).

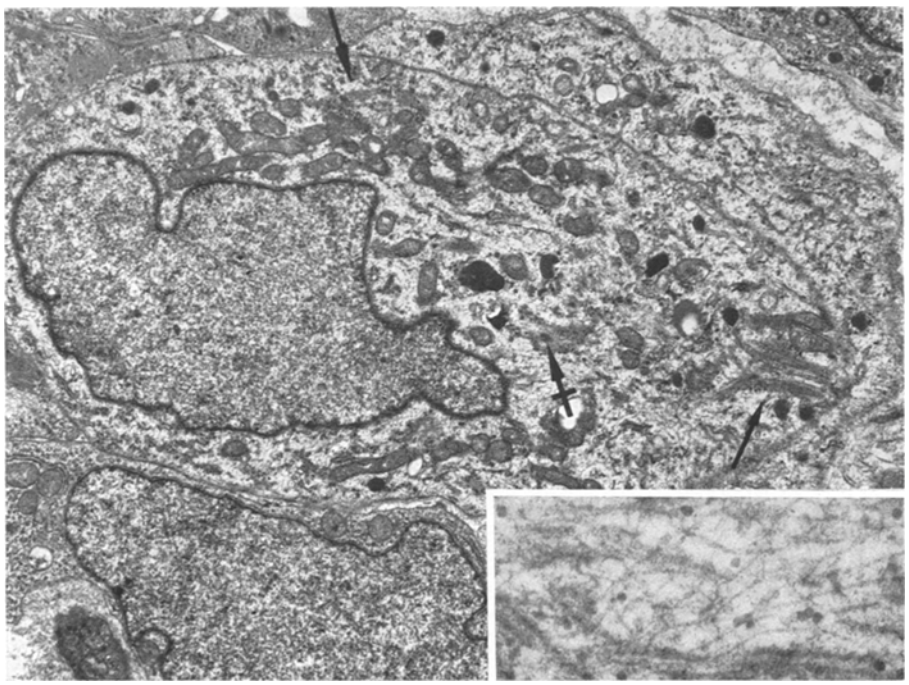

Fig. 3. Light rhabdomyoblast. Cytoplasm crowded with nonspecific 100 A filaments. Small bundles of specific myofilaments (arrows) are present. Note single Z-body (cross-barred arrow). Magn. ×8600. Inset: Intermediary nonspecific 100 A filaments. Magn. ×59000

Light Rhabdomyoblast. This cell was characterized by its conspicuous cytoplasmic (100 A) filaments and an occasional formation of bundles of thick (about 180 A) filaments (Fig. 3). The former filaments with an average diameter of 100 A were wavy and non-branching and course through the cytoplasm in various directions. The thick filaments (about 180 A in diameter) which are normally located randomly throughout the cytoplasm occured singly or in bundles. They had a fuzzy surface and tappered ends and did not exceed 1.5 u in length. Occasionally large polyribosomes could be found in the vicinity of these filaments (Fig. 4). Thin (70 A) filaments were poorly visualized in longitudinal sections of myofilament bundles.

A number of microtubules traversed the cytoplasm. Generally it was very small but varied greatly from cell to cell. The mitochondriae seemed to be increased in number when compared with the presumptive rhabdomyoblast. Some cells contained a few membrane bound lysosomes. Lamellae of rough and smooth endoplasmic reticulum as well as Golgi fields were rarely seen in this cell. Z-body-like densities were haphazardly distributed in the cytoplasm. These densities varied in width and thickness and were accompanied with intermediate, possibly also fine, filaments.

More Differentiated Rhabdomyoblast. Almost all of these tumour cells could be identified clearly by the presence of large numbers of myofibrils and myo-

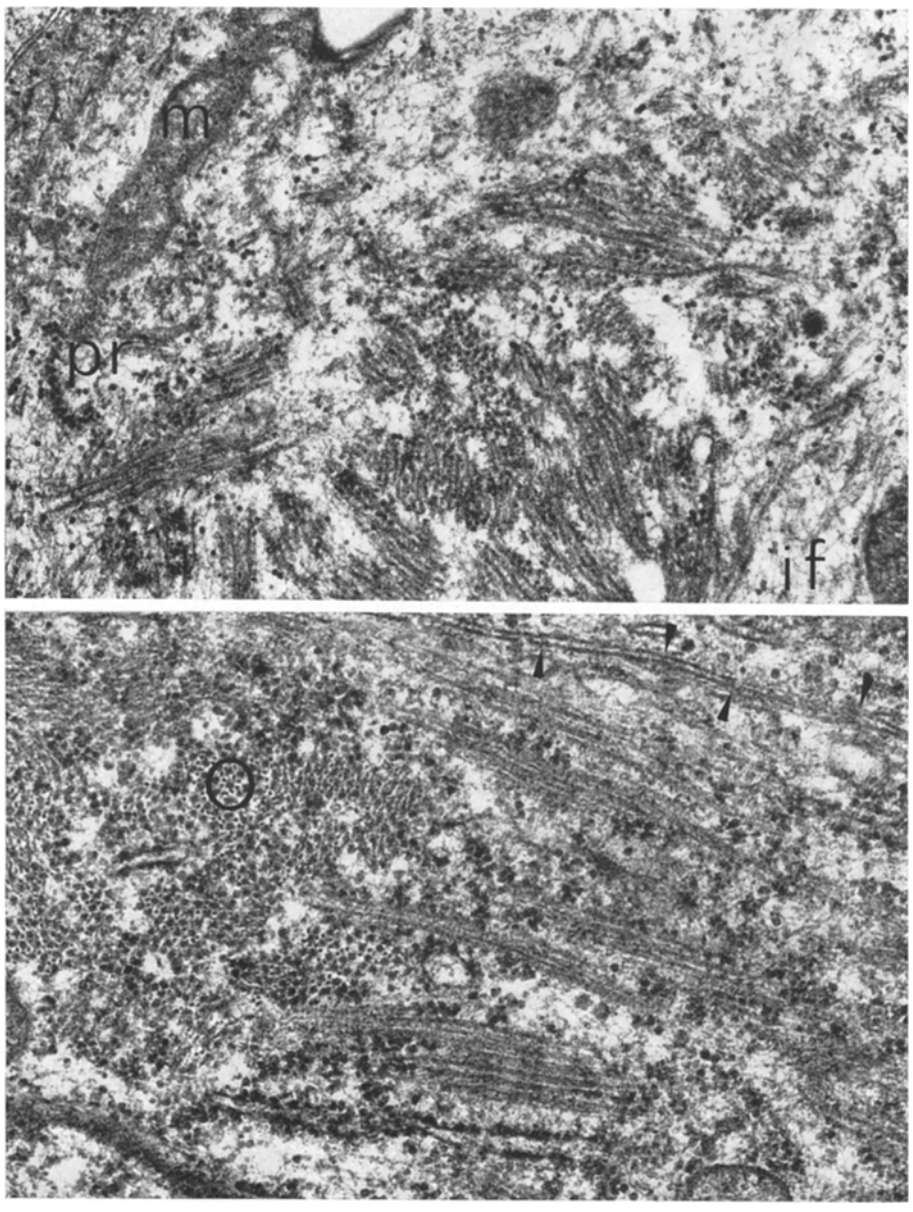

Fig. 4a and b. Myofibrillogenesis. (a) Bundles of thick filaments and ribosomes are randomly orientated. *pr* helically arranged polyribosome, *m* mitochondria, *if* intermediary filaments. Magn. × 35000. (b) More regularly arranged myofilaments. Circle with hexagonal array of thin filaments surrounding a thick filament. Plasma membranes of two opposed cells (arrows). Magn. × 59000

fibrillar fragments (Fig. 4). Bundles of thick filaments were observed. Only occasionally could thin (70 A) filaments be detected. The cytoplasm was usually crowded with tufts of thick filaments which are randomly orientated (Figs. 5

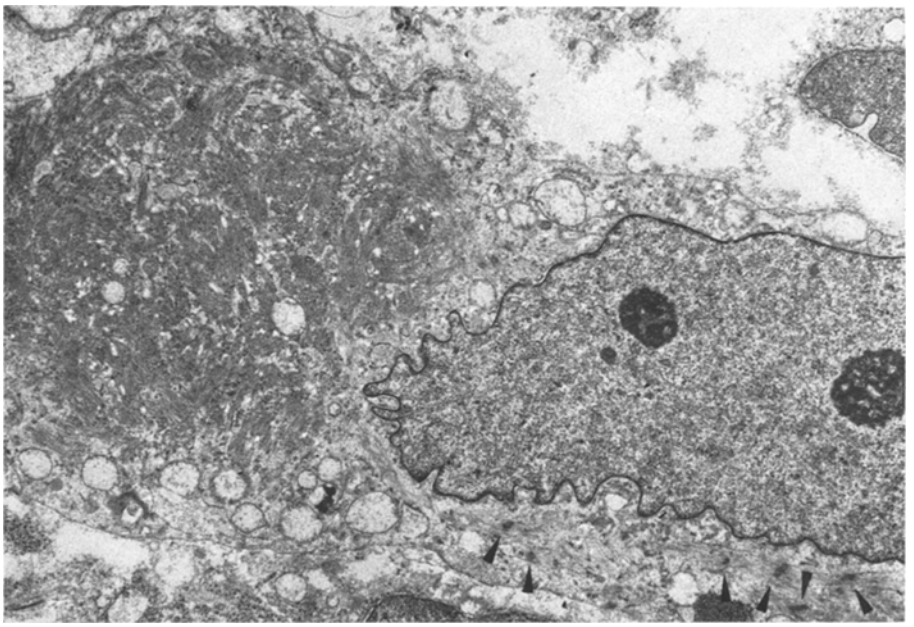

Fig. 5. Rhabdomyoblast. Randomly orientated poorly organized Myofibrils. Note Z-bodies (arrows). Magn. × 6100

and 7). Those cells which did not show a sarcomeric banding of their myofibrils usually lacked or have at least a reduced number of Z-like material. In addition there seemed to be a correlation between the presence of components of the sarcotubular system and the differentiation of myofibrils. Less frequently well differentiated myofibrils were observed with 5–7 continuous sarcomeres (Figs. 6 and 7). Such highly organized fibrils were undoubtedly the ultrastructural substrate of cross striation at light microscopic level.

These well organized fibrils showed the characteristic banding of striated muscle. Their width varied greatly (0.2–0.3 u), while the length of single sarcomeres is constantly about 1.7 u (A-zone: 1.5 u; I-zone: 0.2 u). Even the H-band (0.16–0.17 u) and the M-line (0.8 u) were clearly defined. The Z-line was about 500 A in thickness. In cross sections (Fig. 4) a regular hexagonal arrangement of thin filaments surrounding thick ones could be seen.

Even when the cytoplasm of rhabdomyoblasts was crowded with myofibrils, the intermediate type of filaments could still be detected. Usually they were located at the periphery of the cells and in a smaller amount between the fibrils.

In addition large elongated mitochondriae, small amounts of glycogen and elements of the sarcotubular system were present at this stage of differentiation. The euchromatic nuclei of these cells were usually excentric in position and contained a single prominent nucleulus. Contrary to the light microscopic appearance a peripheral condensation of chromatin could not be detected.

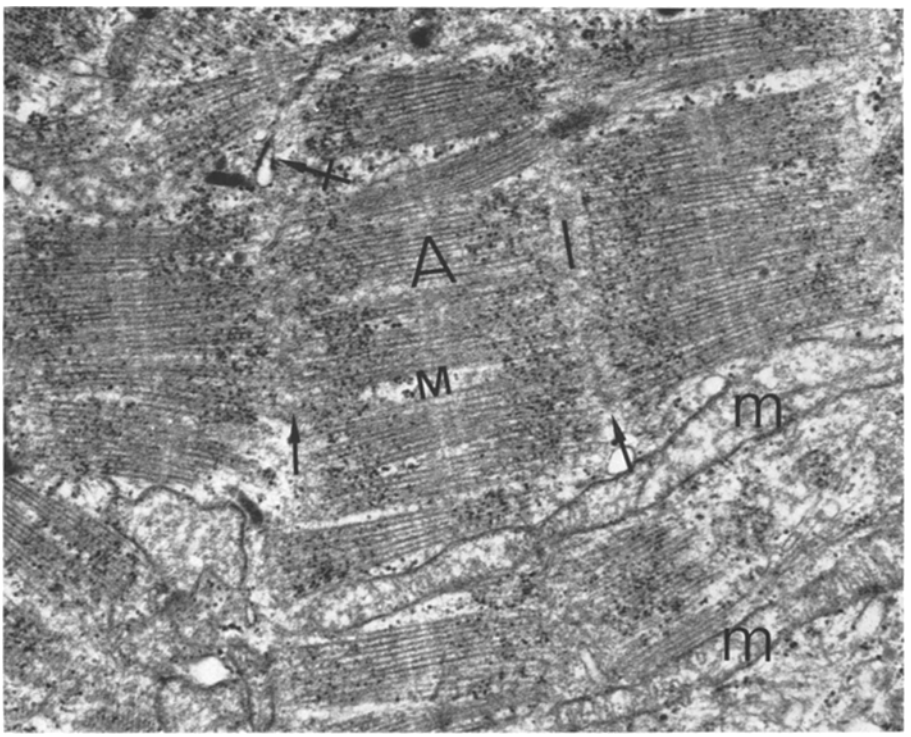

Fig. 6. Well organized myofibrils. Note regular sarcomeric pattern with formation of I- and A-zones. *I* I-zone, *A* A-zone, *M* M-band, *m* mitochondriae, Z-bands (arrows), sarcotubular system (cross barred arrows). Magn. × 24400

Discussion

General Considerations

Rhabdomyoblastic components are among the more common heterologeous tissue differentiations in mixed mesodermal tumours of the uterus. According to the WHO-classification of soft tissue tumours (1969) most of these rhabdomyoblastic areas belong to the embryonal type of rhabdomyosarcoma with sometimes myxomatous appearance and varying degrees of maturation. The rhabdomyoblasts in uterine sarcomas, like other heterologeous elements, are now widely believed to arise from multipotential cells of the Müllerian mesenchyme (Kempson and Bari, 1970; Norris *et al.*, 1966; Sternberg *et al.*, 1954). Nevertheless the triggering mechanism which leads to this heterologeous differentiation remains unknown.

From the present study on two cases of carcinosarcomas the impression was gained that the rhabdomyoblasts strikingly recapitulate the ontogenic development of striated muscle (lit. see above). Thus during the development of rhabdomyoblasts two phases can be distinguished: one with a tremendous proliferation of nonspecific intermediate filaments and the other with the production of specific myofilaments. Occasionally cells at different stages of differentiation are

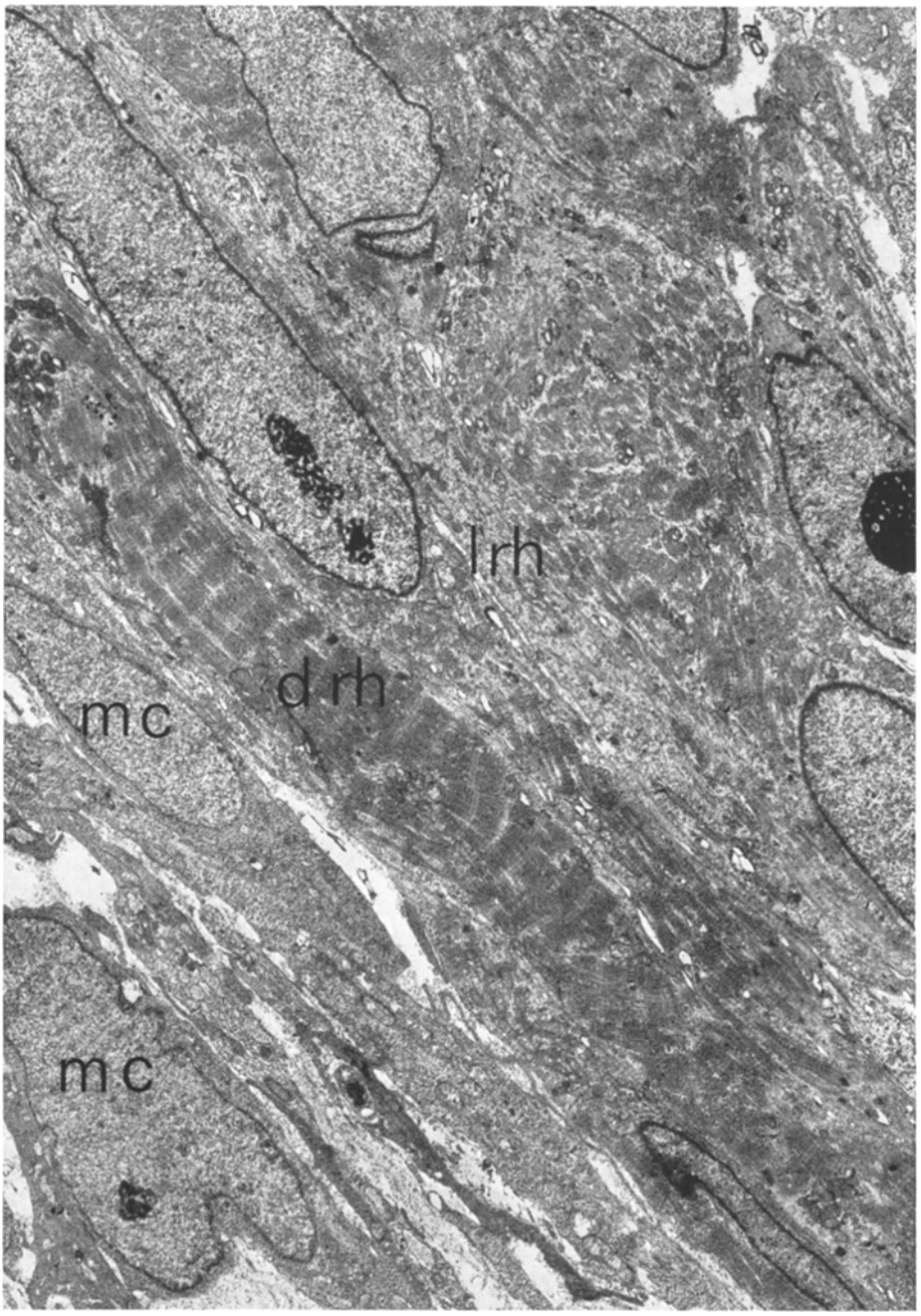

Fig. 7. Rhabdomyoblasts in different stages of development. Note rhabdomyoblast with highly organized myofibrils. *drh* differentiated rhabdomyoblast, *mc* mesenchymal cell, *lrh* light rhabdomyoblast. Magn. × 5800

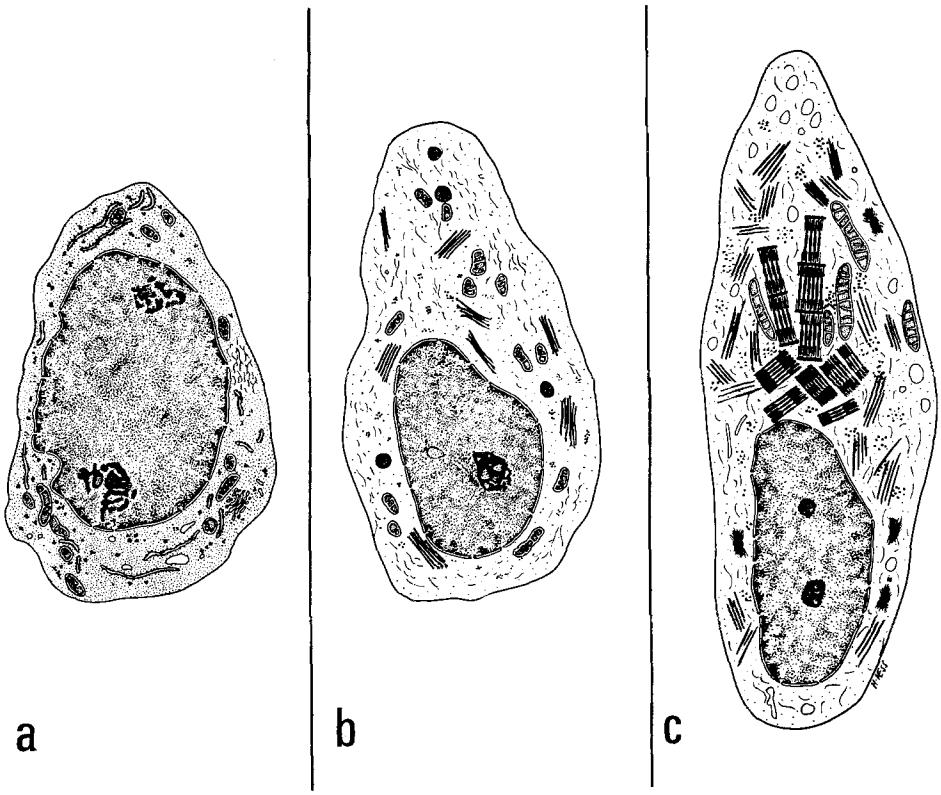

Fig. 8a—c. Schematic diagram proposed for the development of rhabdomyoblasts from mesenchymal cells. (a) Mesenchymal cell with abundant ribosomes but few other cell organelles. (b) Light rhabdomyoblast with predominance of intermediate type of filaments and occasional bundles of thick filaments. Reduction in electron density. (c) Differentiated rhabdomyoblast with well organized myofibrils

intimately connected by cytoplasmic bridges. Our findings suggest that these bridges seem very likely to represent a fusion of rhabdomyoblasts, analogeous to normal myogenesis and muscle regeneration. However the possibility that they are the result of incomplete mitotic divisions, as seen in oogenesis (Gondos, 1970; Stegner and Wartenberg, 1963), cannot be totally excluded. The specific function of Z-bands (Kelly, 1969) and of the sarcotubular system during sarcomeric organization of myofibrils still remains unknown.

Differentiation of Rhabdomyoblasts

Electron microscopic studies of myogenesis (Allen, 1973; Ezerman and Ishikawa, 1967; Ishikawa, 1968; Ishikawa *et al.*, 1968; Kelly, 1969; Knappeis and Karlsen, 1968; Shimada *et al.*, 1967; Rash *et al.*, 1970a and b; Reznik, 1973) suggest the following two stages in the differentiation of rhabdomyoblasts (see also Fig. 8): A first phase (1) with proliferation of nonspecific cytoplasmic filaments and a second stage (2) with production of the specific myofilaments.

(1) Undifferentiated mesenchymal cells become arranged in clusters or cords of 2–5 cells which gradually develop a more extensive cell contact with formation of a glycocalyx. These cells contain many ribosomes and polyribosomes but with

few other cell organelles. From their arrangement in the tissue, the presence of a basement membrane and the internal cell morphology, it is suggested that these cells are already developmentally determined and probably arise from multipotential stromal cells.

The cytoplasmic differentiation begins with the appearance of intermediate (100 A) filaments. These filaments have been found to exist with other types of filaments in cardiac (Rash *et al.*, 1970), skeletal (Ishikawa *et al.*, 1968; Kelly, 1969), smooth muscle (Campbell *et al.*, 1970; Nehara *et al.*, 1971) and in a variety of other cells (Ishikawa *et al.*, 1969; Ferrans and Roberts, 1974). They have been assumed by most authors to represent the as yet unincorporated actin filaments. Recent investigations on these filaments (see Rash *et al.*, 1970) suggest that they comprise a new class, intermediate between actin (50–70 A) and myosin (160–170 A). They are found scattered throughout the cytoplasm of various developmental stages and they form the vast majority of filaments only a short time prior to myofibril formation. The result is the light rhabdomyoblast with its abundance of 100 A filaments. There may be several reasons why these filaments have not been described in recent electron microscopic studies on rhabdomyosarcomas of various locations (Friedman *et al.*, 1965; Morales *et al.*, 1972; Overbeck, 1966; Sarkar *et al.*, 1973). One of the reasons is obviously the bad preservation and/or fixation of tumour tissue. On the other side more importance may have been attached in these studies to the detection of specific myofibrils.

Most authors hypothesize that the intermediate filaments make up a cytoskeleton and it may well be so that they provide a similar cytostructural function at this stage of rhabdomyoblast differentiation. At least these filaments are not involved with the formation of myofibrils (Kelly, 1969; Rash *et al.*, 1970).

(2) Up to this stage of development specific myofilaments are rarely observed and the organelle content alone does not provide sufficient basis for the identification of rhabdomyoblasts. The first detectable specific filaments, 180 A in diameter, are found scattered throughout the cytoplasm. In longitudinal sections they are arranged in parallel fashion, often forming filament bundles.

There has been much controversy about the question whether thin or thick filaments appear first in the developing muscle cells (Allen, 1973; Fischman, 1967; Ishikawa *et al.*, 1968; Kelly, 1969). In our tumour cells we could not identify thin (70 A) filaments prior to the appearance of thick ones. Rather the 70 A filaments could often only be detected on cross sections of more advanced myofibrils.

The sarcomeric pattern of myofibrils seldom comes up to the high degree of organization which is found in the striated muscle. More often filament bundles form sarcomeric-like, randomly orientated units, not linked to a myofibril by Z-bands. These our findings suggest that thin and thick filaments can aggregate in an hexagonal pattern without the presence of Z-band material while the sarcomeric spacing of sarcomere-like units to a myofibril seems to be regulated by Z-bands. This suggestion is further supported by the fact that Z-bodies seem to be reduced in number in those cells which do not show sarcomeric spacing of myofibrils. But besides these quantitative differences there may be qualitative alterations not detectable by morphological methods. In addition missing topo-

graphical relationships of filaments (randomly orientated in rhabdomyoblasts in contrast to peripheral orientation in developing striated muscle) with cytoplasmic membranes (plasma membrane, sarcotubular system) might be responsible for the failure of sarcomeric myofibril organization.

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