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ADULT HUMAN SKIN MAINTAINED IN ORGAN CULTURE
II. THE ULTRASTRUCTURE OF THE CELLULAR COMPARTMENT OF CONNECTIVE TISSUE

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Abstract. Dermal cells maintained in cultured human skin explants for up to 10 weeks were studied by electron microscopy. Common findings in the cell organelles were: 1) altered nuclear shapes and numerous nuclear bodies; 2) reduced rough endoplasmic reticulum; 3) a prominent Golgi complex; and 4) an increasing number of free ribosomes, mitochondria, glycogen granules, lysosomes and lipid droplets. The individual types of dermal cells showed various cytoplasmic changes according to duration of the culture. The *fibroblasts* displayed a moderately developed rough endoplasmic reticulum. The *macrophages* were filled up with lysosomes and residual bodies. The *mast cells* showed typical as well as atypical granules. The *smooth muscle cells* had a variable content of myofilaments. The *pericytes* and the smooth muscle cells, both surrounded by a basal lamina, became increasingly difficult to discern from each other as a consequence of the decreasing number of myofilaments in the latter. The *endothelial cells* were recognized because of the preserved blood vessel architecture. Besides the above-mentioned cell types, two other types appeared with the culture time. The *unidentifiable* cells were characterized by dense zones of the cell membrane and an adjacent extracellular basal lamina-like material. This cell type may be derived from the pericytes of the small blood vessels. The *primitive cells* had a large rounded nucleus and a cytoplasm with few organelles. This cell type is assumed to represent a precursor cell common to various cell types.

Key words: Organ culture, Connective tissue, Skin

The experimental studys of diseased skin has been restricted because of the lack of suitable laboratory models. The skin organ culture is proposed to be a valuable tool in experimental studies on human skin (26). Before introducing skin organ culture to pathological and experimental conditions, it is necessary to know the morphological and biochemical events associated with the *in vitro* condition of normal skin.

It has been claimed that the ultrastructural changes of cultured normal skin occur in the epidermis, while the cellular changes of dermis are hitherto unknown (26). Sarkany & Gaylarde (31) reported that human skin explants cultured for up to 3 days reveal no ultrastructural changes in the dermis. However, previous publications from this laboratory documented that long-term cultured normal human skin showed resorption of the acellular compartment of the connective tissue (19). Several dermal cells, i.e. fibroblasts, macrophages, unidentifiable cells and smooth muscle cells participated in this resorption process (20). Thus, evidence has been presented that the dermis of skin explants changes during culture. Furthermore, previous ultrastructural studies demonstrated age-associated changes occurring in cultures of isolated dermal cells (8, 27). Similar morphological findings may be expected in cells of cultured skin explants. Therefore, the purpose of this study was to follow the time sequence of changes appearing in dermal cells and their relation to duration of culture.

MATERIALS AND METHODS

The origin and preparation of skin, conditions of cultures and procedures of electron microscopy have previously been described (19). The skin explants were grown in Falcon® organ culture dishes in Eagle's MEM supplemented with 20% fetal calf serum, antibiotics and HEPES at 37°C in 5% CO_2 in atmospheric air.

The explants were fixed in 3% glutaraldehyde of cacodylate buffer, pH 7.4, with 7.5% sucrose for one day, and postfixed in 1% osmium tetroxide for one hour, dehydrated in ethanol and embedded in Epon 812. Thin sections were stained with lead citrate and uranyl acetate and studied with a Siemens electron microscope (Elmiskop 1 A).

RESULTS

Cultured dermal tissue showed disintegration of some cells in the first culture week. The ultrastructure of the cultured cells was not identical with that

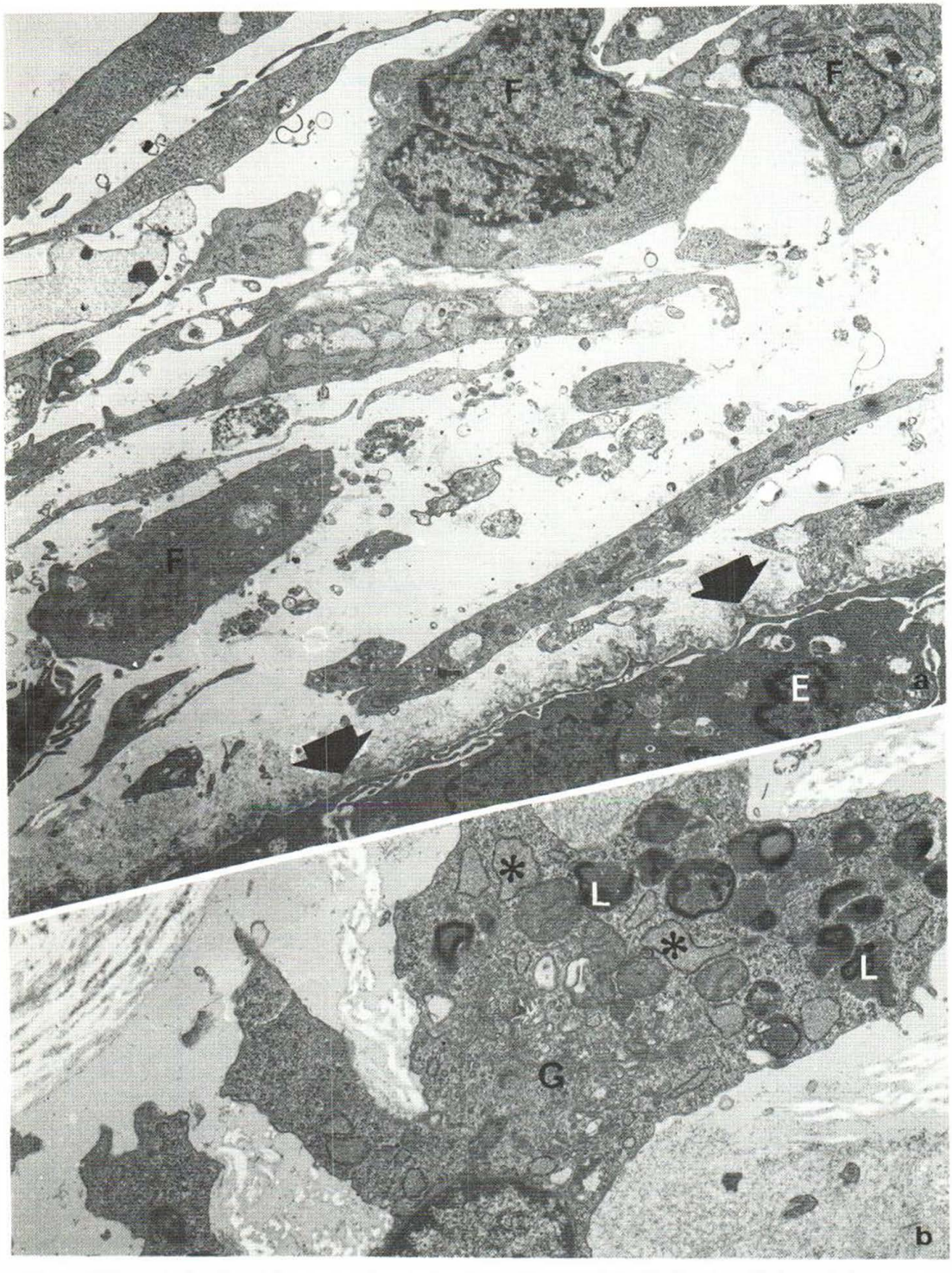

Fig. 1 a. Skin explant cultured for one week shows complete collagen fibril resorption. The epidermis (*E*), dermo-epidermal junction (arrows) and fibroblasts (*F*) are preserved. ×3 000.

Fig. 1 b. A fibroblast cultured for 3 weeks has a prominent Golgi complex (*G*), fairly well developed rough endoplasmic reticulum with dilated cisternae (asterisks) and some lysosomes (*L*). ×7 500.

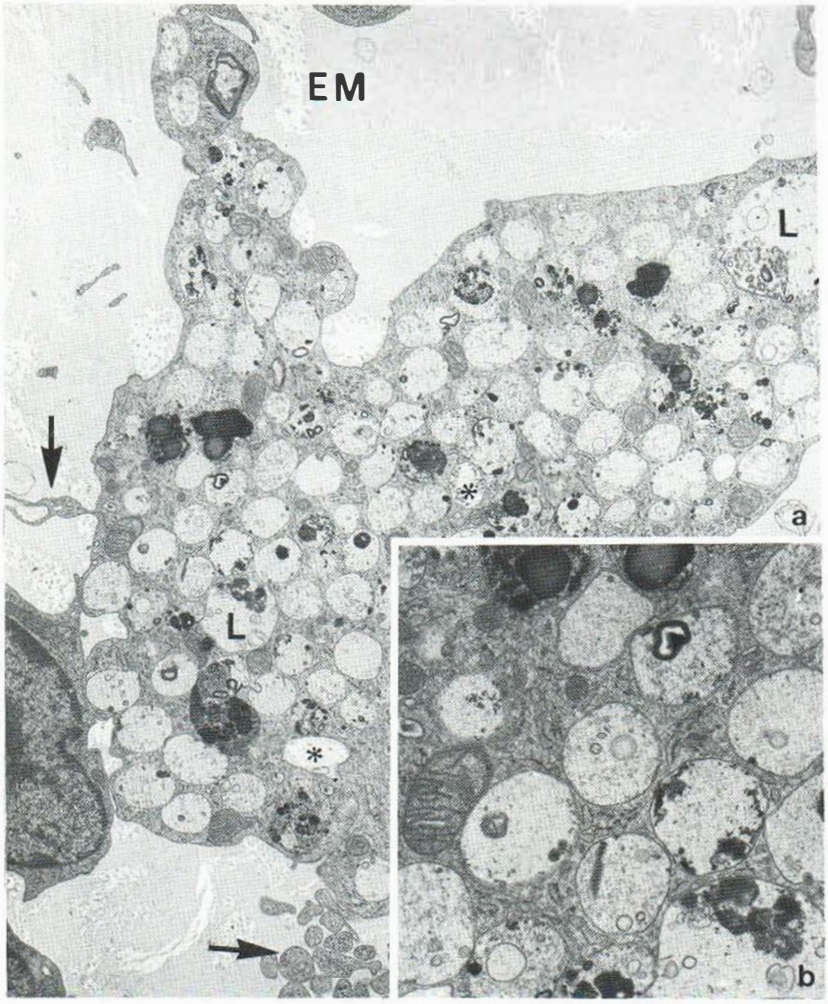

Fig. 2a. After 6 weeks of culture the connective tissue is without collagen fibrils, while the elastic matrix (*EM*) remains. The macrophage shows an abundance of secondary lysosomes (*L*) and a few cytoplasmic projections (arrows). Note the intracellular elastic matrix (asterisks). ×2 700.
Fig. 2b. The content of the secondary lysosomes varies considerably. ×27 000.

of cells *in vivo* although some of the characteristics of the different cell types were preserved. The onset of the changes occurred some 3–5 days after culture initiation. The changes became distinct during the second and third culture week, while further cultivation caused no additional findings. No differences were found between skin explants obtained from individuals of differing age and sex or taken from different anatomical regions.

A. *Common changes of cell organelles*

Nuclei were either irregularly shaped with deep infolds (Fig. 1*a*) or rounded (Fig. 4*c*). In the latter type, the chromatin granules were evenly scattered in the karyoplasm, while they conglomerated in the nuclear periphery. One or two prominent nucleoli were present. In the former type, the chromatin granules formed large conglomerates along the inner surface of the nuclear envelope as well as in the karyoplasm. After the third week of culture, the cells with rounded nuclei became frequent. Nuclear fibrous lamina appeared in most cells cultured for more than one week. An increased number of nuclear bodies (approx. 35 %) occurred in both types of nuclei during the culture period. No significant differences were found between numbers of nuclear bodies and shapes.

Ribosomes and endoplasmic reticulum. After 1–2 weeks of culture, the number of free ribosomes increased, while fewer ribosomes adhered to the endoplasmic reticulum (RER). The cisternae of the RER became widened with an amorphous content

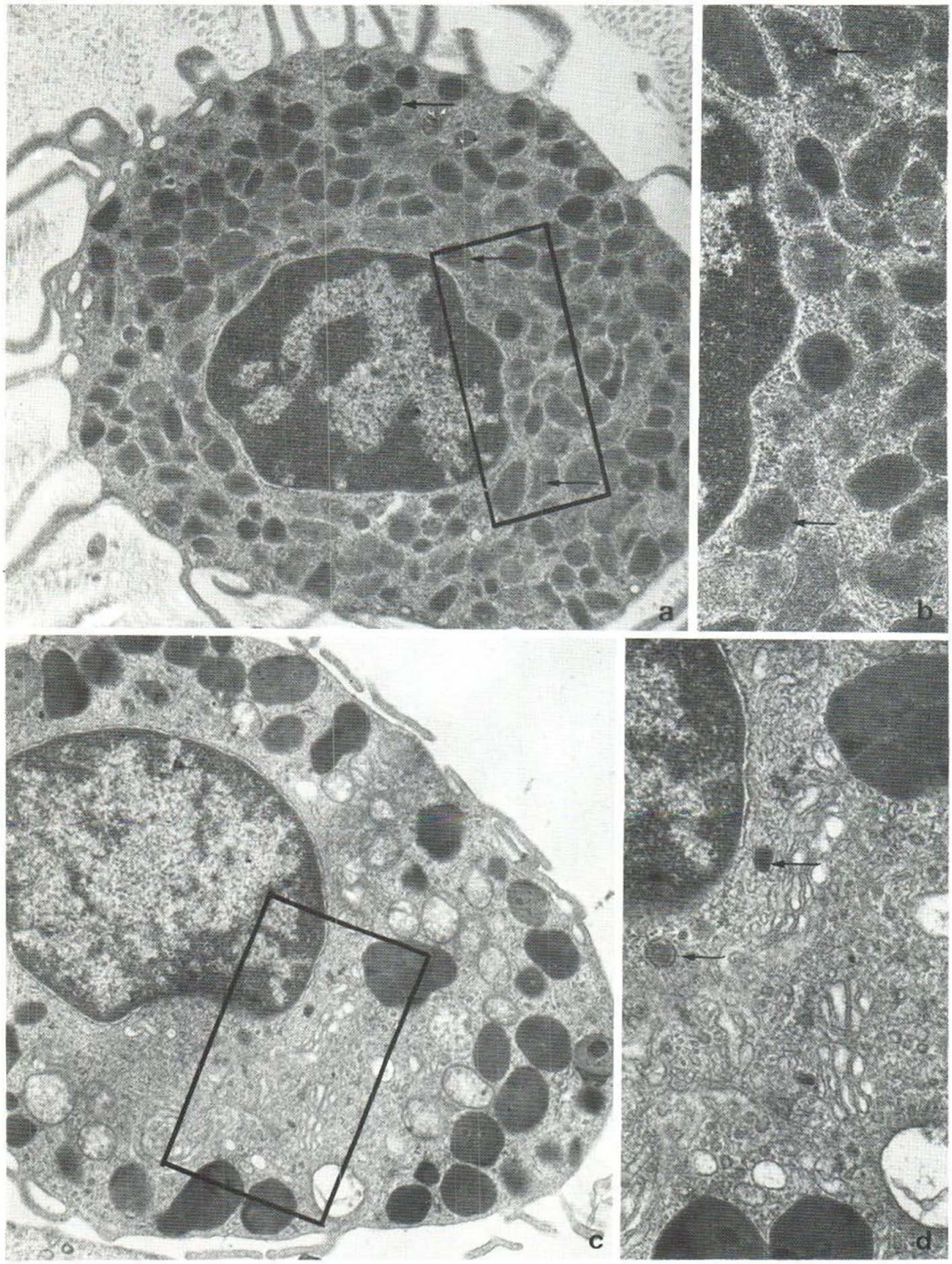

Fig. 3 a. A mast cell in an explant cultured for 3 days shows some atypical granules (arrows). ×14 250.

Fig. 3 b. Higher magnification of the framed area in Fig. 3 *a* demonstrates atypical granules with dense cores (arrows). ×28 500.

Fig. 3 c. After 6 weeks of culture a mast cell appears with few cytoplasmic villi and peripherally arranged granules. A prominent Golgi complex is seen (framed area). ×14 250.

Fig. 3 d. The Golgi complex of Fig. 3 *c* shows pro-granules (arrows) adjacent to the Golgi cisternae. ×28 500.

(Fig. 1*a*, 1*b*). The smooth surfaced endoplasmic reticulum was also present. No additional changes of either type of endoplasmic reticulum were noticed in the 3–10-week cultures.

Golgi complex. During the culture period, this cell organelle became prominent and consisted of numerous vesicles and flattened cisternae with an electron-lucent content (Figs. 1*b*, 3*c*). The Golgi complex occupied large areas of the juxtanuclear region.

Mitochondria of the cultured cells generally showed a slight increase in number.

Glycogen granules accumulated in the cytoplasm of some cells cultured for more than one week.

Lipid droplets were found in cells cultured for more than 3 weeks.

Lysosomes. The number of lysosomes increased during the culture period. The primary lysosomes were relatively less increased than the secondary lysosomes and the residual bodies. The number of lysosomes in various cell types showed considerable differences. The content of these pleomorphic structures was either a dense homogeneous and fine granular material, concentric lamellae, vesicles, or degraded collagen fibrils (Figs. 1*b*, 2*a*, 2*b*).

B. *Changes of specific cell types*

1. Dermal cells without surrounding basal lamina.

The *fibroblasts* were characterized by 1) an extensive RER which was frequently dilated and filled with an amorphous material, 2) the large number of ribosomes, and 3) the well-developed Golgi complex (Fig. 1*a*). This cell type was among the most frequent before cultivation, but it became less frequent as the culture proceeded. After 2–3 weeks of culture, typical fibroblasts were an exceptional finding. The majority of cultured fibroblasts appeared less active according to the moderately developed RER. Among the increased number of lysosomes, the secondary lysosomes and the residual bodies became progressively more frequent in cultured skin explants (Fig. 1*b*). Along the cell membrane, an increasing number of surface vesicles became evident. Occasionally, dense zones of the cell membrane of cultured fibroblasts appeared. Delicate filaments approx. 6–8 nm in diameter were present in some cells. These filaments appeared in parallel arrays along the cell membrane or adjacent to the nucleus.

The *macrophages* were characterized by 1) the sparse RER, 2) the well-developed Golgi complex, and 3) the large number of lysosomes (Fig. 2*a*, 2*b*).

During the culture period, the macrophages contained an increasing number of primary and secondary lysosomes and residual bodies. Some surface vesicles were seen along the cell membrane. The cells often engulfed collagen fibrils or elastic fibres. The cell membranes showed finger-like projections which decreased in number with the culture time.

The *mast cells* were characterized by their content of 1) specific granules with parallel-arranged lamellae and a fine granular material, and 2) cytoplasmic villi (Fig. 3*a*, 3*c*). During cultivation the cytoplasmic villi decreased in number and atypical granules appeared. Mast cells cultured for 3 days showed mature granules, while mast cells cultured longer than one week displayed various granules. Three types of granule were observed in the cultured mast cells, viz.: immature progranules, mature granules and disintegrating granules.

The small progranules, consisting of a fine granular material, enclosed by a single-layered membrane, were demonstrated only in cells cultured for more than one week and appeared close to the prominent Golgi complex. Cells with immature granules showed relatively few granules, which were arranged in the periphery of the cytoplasm (Fig. 3*c*).

The mature granules were electron-dense, with parallel-arranged lamellae, either flat, bent or scrolled, and enclosed by a single membrane of low density. Some granules contained a fine granular material with or without a faint crystalline structure. These granule types were found in all mast cells throughout the culture period.

Disintegrating granules consisted of a coarser granular material and had a dense central core of varying size (Fig. 3*b*). Some granules had a crescent-shaped electron-dense area, others remnants of parallel lamellae or crystalline structure. The enclosing membrane became more distinct in these granules. Granules releasing their content into the extracellular space appeared electron-lucent, with sparse amounts of subgranular material enclosed by a membrane. Fully degranulated mast cells were only occasionally observed and always in the first day or two of culture. The cytoplasm of these cells displayed a honeycomb appearance.

The *unidentifiable cells showed* 1) sparse RER, 2) few lysosomes, 3) numerous surface vesicles and in between these several dense zones of the cell mem-

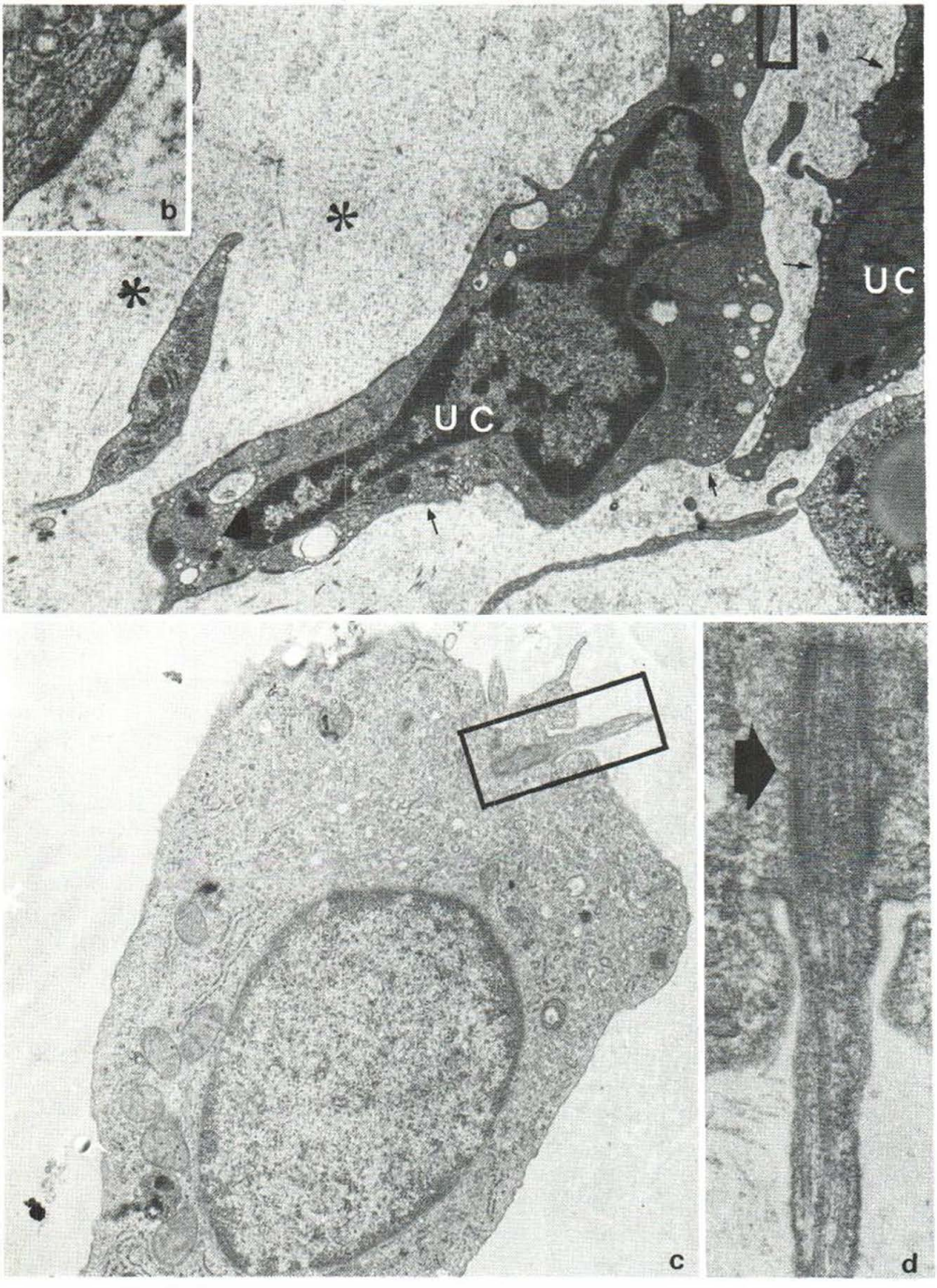

Fig. 4 a. Unidentifiable cells (*UC*) of a 6-week-old cultured explant. Numerous surface vesicles and dense zones (arrows) are seen. The collagen fibrils are replaced by cross-banded filamentous aggregations (asterisks) in the extracellular compartment of the connective tissue. ×13 500.

Fig. 4 b. A dense zone and several surface vesicles seen in the framed area in (*a*) are shown in higher magnification. ×54 000.

Fig. 4 c. A primitive cell of an explant cultured for 10 weeks. The cell is characterized by the large rounded nucleus and a cytoplasm containing a few organelles. Occasionally, single cilia (framed area) are present. ×13 500.

Fig. 4 d. The micrograph shows the cilia in higher magnification. A basal body is shown (arrow). ×54 000.

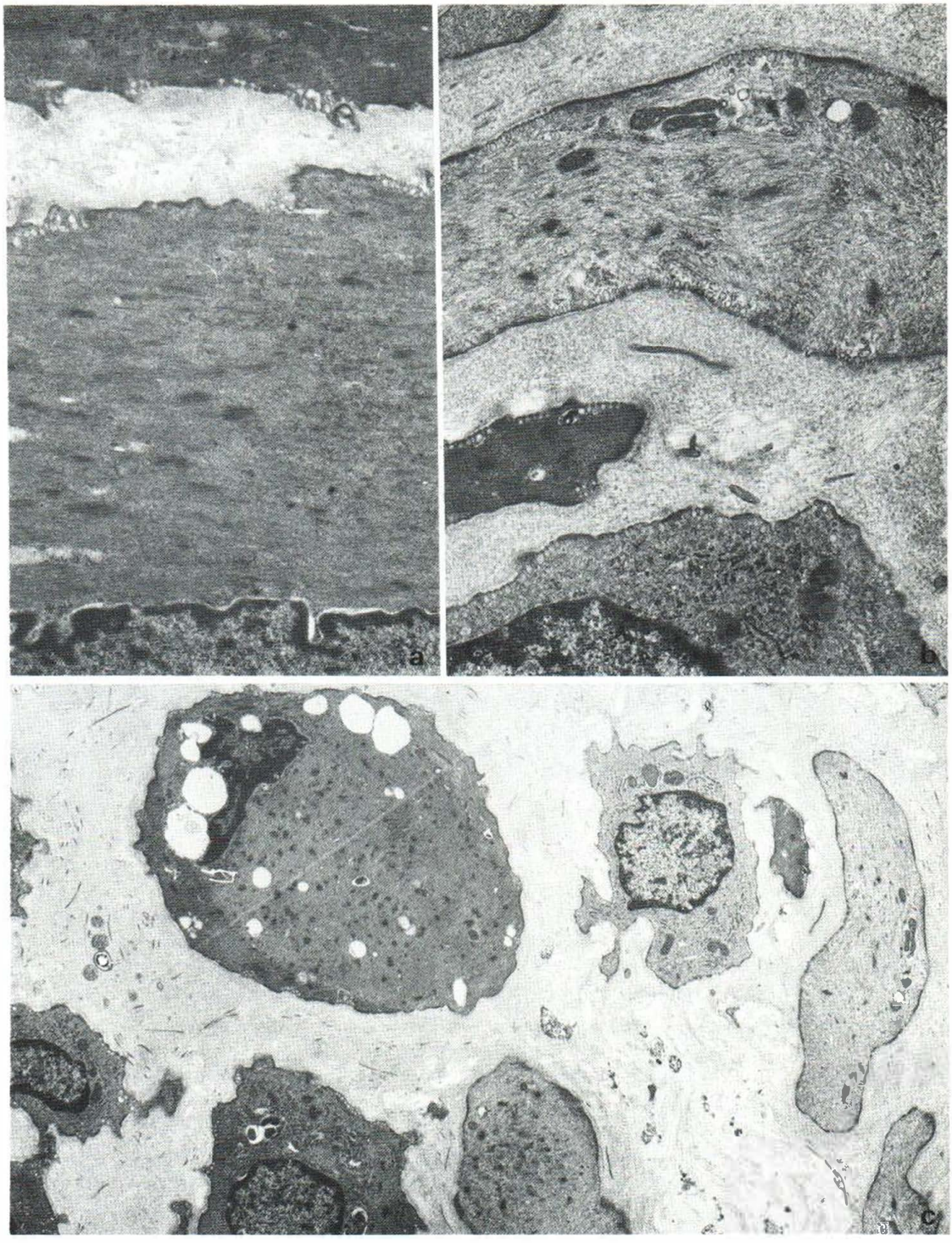

Fig. 5 a. The cytoplasm of smooth muscle cells cultured for one week is filled with dense myofilaments. ×15 000.

Fig. 5 b. The smooth muscle cells cultured for 2 weeks show a decreased number of myofilaments. Many surface vesicles and dense zones appear along the cell membrane. ×15 000.

Fig. 5 c. Explants cultured for 2 weeks contain smooth muscle cells with varying numbers of myofilaments. ×6 000.

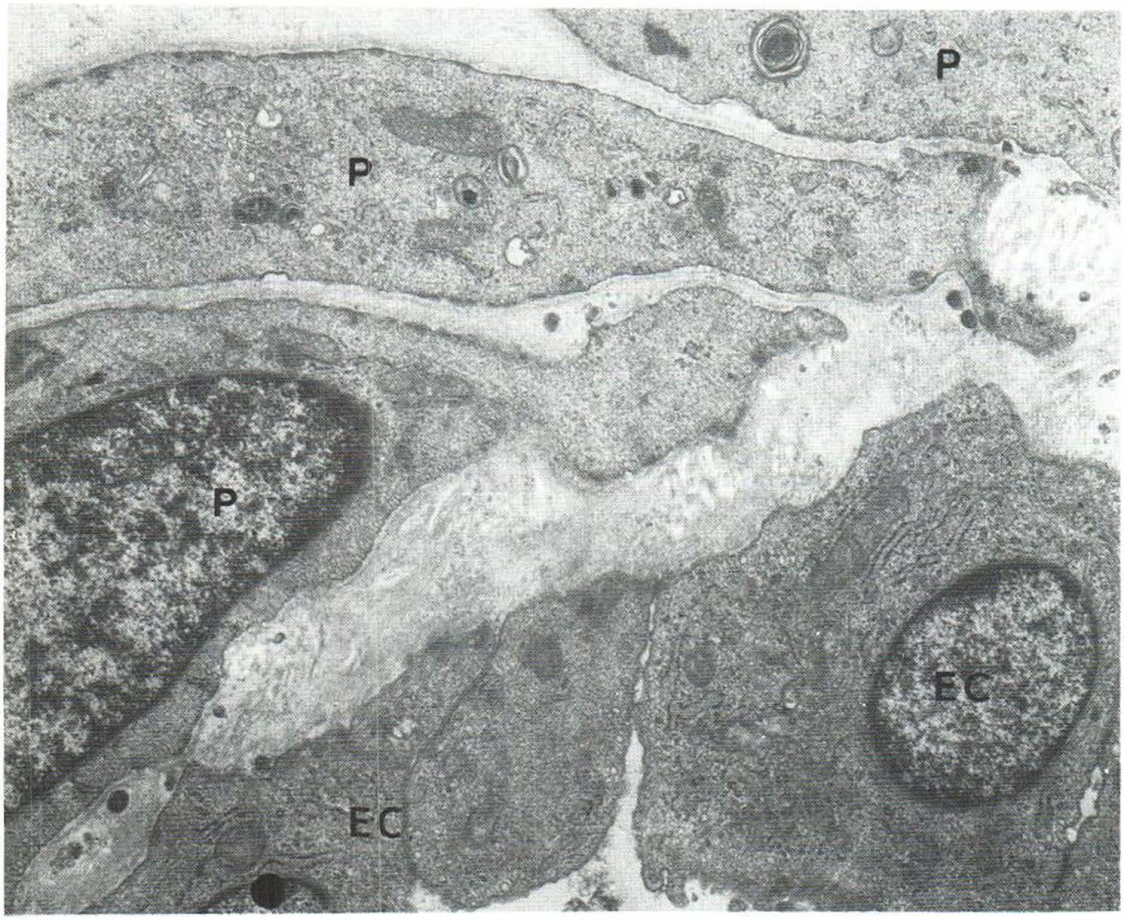

Fig. 6. A blood vessel of a 3-week-old cultured skin explant shows endothelial cells (*EC*) and pericytes (*P*). The latter have many dense zones along the cell membrane and are surrounded by a basal lamina. ×15 000.

brane (Fig. 4*a*). This cell type became prominent with time in culture. The cytoplasmic densities subjacent to the cell membrane appeared focally and were approx. 300–600 nm in length and 30–50 nm in thickness. At these sites a homogeneous layer of basal lamina-like material occurred in the extracellular space, which layer was separated from the cell membrane by a translucent layer (Fig. 4*b*). Cells which were close to each other occasionally formed desmosome-like structures. Each cell contained several of these cell membrane densities. Cytoplasmic filaments mimicking myofilaments were also found.

The *primitive cells* were characterized by few cell organelles—with the exception of the Golgi complex. This cell type had a large, rounded nucleus and relative sparse cytoplasm (Fig. 4*c*). Occasionally, the cells had single cilia. The cilia appeared as small solitary projections of the cytoplasm. At the base of the cilia, a characteristic basal body was seen (Fig. 4*d*). In one case we found two cilia with an angle of 90° between the shafts (Fig. 4*c*). We never had occasion to study a cross-section of the cilia.

2. Dermal cells surrounded by basal lamina. The *smooth muscle cells* were characterized by myofilaments which were arranged parallel in the sarcoplasm. Surface vesicles appeared along the cell membrane and in between the dense zones (Fig. 5*a*, 5*b*). The cells were surrounded by a basal lamina. The smooth muscle cells were well preserved during the first two weeks of culture, although the amount of myofilaments varied considerably (Fig. 5*b*, 5*c*). Cells with dense myofilaments contained a few common cell organelles such as mitochondria, RER, and lysosomes, while those with dispersed

myofilaments showed more. After 2 weeks of culture, the myofilaments decreased in number. The cultured smooth muscle cells were enclosed by a basal lamina which often became discontinuous. In explants cultured for 2–3 weeks, the distinction of smooth muscle cells from unidentifiable cells and pericytes was difficult.

The *endothelial cells* were identifiable as long as the small blood vessels of cultured skin explants preserved their integrity. During the culture period, the endothelial cells showed a large Golgi complex, increased number of microfilaments and some lysosomes and surface vesicles. The semidesmosomes, desmosomes and basal lamina were well preserved (Fig. 6).

The *pericytes* adjacent to the blood vessels were always surrounded by a basal lamina. This structure became discontinuous with culture duration (Fig. 6). The cytoplasmic structures of pericytes resembled those of cultured smooth muscle cells and unidentifiable cells. Hence, the distinction between these cells was often impossible.

The *perineural cells* resembled the pericytes both before and after culture.

The *Schwann cells* showed a decreased number of cell-organelles concomitant with culture time.

DISCUSSION

A. *Changes in organelles in cultured cells*

The present study demonstrated two types of nuclear shapes. The irregular type may represent the nucleus of a senescent cell as shown in ageing fibroblast cultures (3, 24). Cells with rounded nuclei and little chromatin condensation, which in the present study became frequent in primitive cells, have been correlated with immaturity (8). The nuclear bodies were frequently found both in normal and in cultured human skin (18). The function of nuclear bodies is unknown, although they are present in most tissues. The large number of nuclear bodies found in the present study may be associated with an increased metabolic activity of the cells (4).

By utilizing morphological criteria it is possible to differentiate between cells in logarithmic and resting stage of growth (3, 5, 8, 24, 27). The resting senescent cells are characterized by 1) an increased number of lysosomes, 2) an increased transformation of primary lysosomes into secondary lysosomes and residual bodies, 3) an increased amount of glycogen granules and free ribosomes, 4) a prominent Golgi complex, and 5) a moderately developed rough endoplasmic reticulum. The cytoplasmic findings in most dermal cells in the present study are fully in accordance with the above-mentioned reports indicating that most of the skin explant cells are maintained in a resting stage of growth during the culture period.

In cultured cells, the Golgi complex and the number of lysosomes increased concomitantly. The enlargement of the Golgi complex is stimulated by a high concentration of serum in the medium as used in the present study (6). The Golgi complex is associated with the secretory activities of various cells and may also play a role in the formation of primary lysosomes (25). The primary lysosomes may either transform into secondary lysosomes releasing their content of hydrolytic enzymes into the extracellular space, or they may fuse with phagocytosed material forming the pleomorphic secondary lysosomes (25). The residual bodies represent vacuoles with incompletely digested material which is too bulky to pass through the lysosomal membrane. The increased lysosomal activity of most cultured skin explant cells may be responsible for the connective tissue resorption reported in previous works (19, 20).

B. *Changes in specific cell types during culture*

The *fibroblasts* present in cultured skin explants showed the characteristics of resting cells (3, 5, 8, 16, 24, 27). Previous studies demonstrated that cultured fibroblasts had a moderately developed and dilated RER, which late in culture tended to be larger. The less extensive but dilated RER with few attached ribosomes displays a restricted collagen fibril synthesis of fibroblasts, whether grown in vitamin C deficient medium as used in the present study or in scorbutic wounds (28, 30).

The increased number of surface vesicles has been reported previously in cultured fibroblasts. The vesicles may be related to the process of pinocytosis and phagocytosis (16, 28).

The bundles of thin filaments occasionally seen beneath the cell membrane of fibroblasts are a common feature of cultured fibroblasts (16) and myofibroblasts (13). These filaments may play a role in the cell locomotion (33).

The changes in cultured *macrophages* in skin explants are reminiscent of those described for macrophages grown in cell culture (11). The macrophages were separated from fibroblasts on ac-

count of the scarce RER, the abundance of lysosomes and the cytoplasmic projections. However, it became increasingly difficult to distinguish macrophages and fibroblasts from each other as culture time went on, which suggested that the cells were less differentiated.

Separated *mast cells* can be maintained in culture, while the cells differentiate only in association with a fibroblast-feeding layer (7, 15). The mast cell granules are probably formed in the Golgi complex (21). The immature mast cells show a prominent Golgi complex associated with small, electron-dense progranules (7, 21). As the mast cells became more mature, their cytoplasm contained an increasing number of granules (7, 21). The cytoplasm of the mature cells was filled with granules, there were few mitochondria and a reduced Golgi complex (7). These stages of mast cell differentiation are substantiated by our findings.

The morphology of the developing granules was identical with that observed in normal skin (21). In diseased skin an increasing number of the mast cell granules become atypical, a phenomenon representing an abnormal process of granule formation (21). The progressive increase in atypical granules during the culture period suggests that the maturation process was disturbed. The fact that the honeycomb-like appearance of the mast cell cytoplasm was a rare finding indicates that degranulation was not induced by the culture conditions or mechanical preparation of the explants.

The origin of the *unidentifiable cells* is unknown. Parts of the cell surface were covered by a basal lamina-like material and in such regions the cells showed a dense zone reminiscent of the attachment sites of cultured smooth muscle cells (29) and endothelial cells (17). The pericytes are capable of differentiation into smooth muscle cells (34). Both pericytes and endothelial cells develop from a common precursor cell (1, 2). We propose that the unidentifiable cell may be derived from pericytes. The evidence for this suggestion is based on the morphological findings. The assumption is confirmed by previous work concluding that most cell lines derived from different organs had a similar morphology and represented pericytes and endothelial cells of small blood vessels (9, 10).

The *primitive cells* lacking type-specific characteristics may represent undifferentiated cells of a different origin. The single cilia of these cells are thought to be an evolutionary remnant, as they are relatively more frequent in tissues of lower animals. However, cells with single cilia have been demonstrated in most tissues (32). The function of this organelle is still obscure.

Cultured *smooth muscle cells* derived from blood vessels can be characterized by the presence of bundles of myofilaments (12, 14). The cells preserved this ultrastructural feature for up to 8 weeks of culture (29). Some investigators have reported that the smooth muscle cells in explants from swine aorta underwent a rapid dedifferentiation (12). After 4 days in culture, most smooth muscle cells showed few or no myofilaments, hence resembling fibroblasts (12). This observation was not confirmed by the present study, though we found a decreasing number of myofilaments. As a consequence of the scarcity of myofilaments we found that it became difficult to distinguish between cultured smooth muscle cells and pericytes. The pericytes and smooth muscle cells could be distinguished from fibroblasts by the surrounding basal lamina. The unidentifiable cells contained a cytoplasm with bundles of thin filaments and a cell surface partly covered with dense zones and a basal lamina-like material. Hence, these cell types resemble the cultured smooth muscle cells and pericytes. Under certain conditions, the fibroblasts can assume ultrastructural and functional characteristics similar to smooth muscle cells. These myofibroblasts are a common feature in granulation tissues and cell cultures and may represent a contractile fibroblast (13). On the other hand, there is also evidence that smooth muscle cells can assume fibroblastic features (12).

The *endothelial cells* were easily identified because of the preserved blood vessel architecture. However, the ultrastructural features of isolated endothelial cells may remind one of pericytes, smooth muscle cells and unidentifiable cells. The Weibel-Palade body, which is a specific marker for endothelial cells, was not present in the endothelium of our explants. These granules were regularly found in endothelial cell cultures derived from human umbilical cord veins by Haudenschild et al. (17). Moreover, we found that the ultrastructural changes in cultured endothelium of skin explants were similar to those reported from previous studies (17).

The ultrastructural studies of cultured skin have demonstrated that the dermal tissue loses its architecture because of collagen fibril resorption (19,

20). Although the individual cell types preserved most of their characteristics throughout the culture period, some new cell types were identified. In conclusion, it must be emphasized that cultured skin has a morphology essentially different from that of normal skin. This should be borne in mind, when conclusions are drawn from skin organ culture in experimental research.

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