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PALATAL MUCOSA OF THE ADULT CAT MAINTAINED AS ORGAN CULTURE IN CHEMICALLY DEFINED MEDIA

A LIGHT AND ELECTRON MICROSCOPE STUDY

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INTRODUCTION

The maintenance of adult human skin in vitro was first reported by *Ljunggren* in 1898. Since that time numerous investigations of different adult tissues in organ culture such as skin, liver, kidney have been performed. However, only few reports of adult oral mucosa in organ culture are available (*Hoorn*, 1966; *Powell*, 1967, 1969; *Imagawa & Ishikawa*, 1968). Embryonic oral mucosa in organ culture has been described by *Porter* (1960), *Chang & Maibach* (1967), *Flaxman, Lutzner & van Scott* (1967) and by *Melcher & Hodges* (1968). Most of these authors have used culture medium supplemented with serum. *Hoorn* (1966), *Chang & Maibach* (1967), and *Melcher & Hodges* (1968) have, however, succeeded in maintaining oral mucosa in chemically defined media.

Most cultured tissues have been studied under the light microscope. Only *Flaxman, Lutzner & van Scott* (1967) and *Jepsen & Theilade* (1967) have used electron microscopy in the investigation of oral mucosa. *Flaxman et al.* studied the epithelial outgrowth from human skin and oral mucosa taken from the inner surface of the lower lip and *Jepsen & Theilade* studied the epithelial-like outgrowth from palatal mucosa of the guineapig. Both groups used Eagle's medium supplemented with serum.

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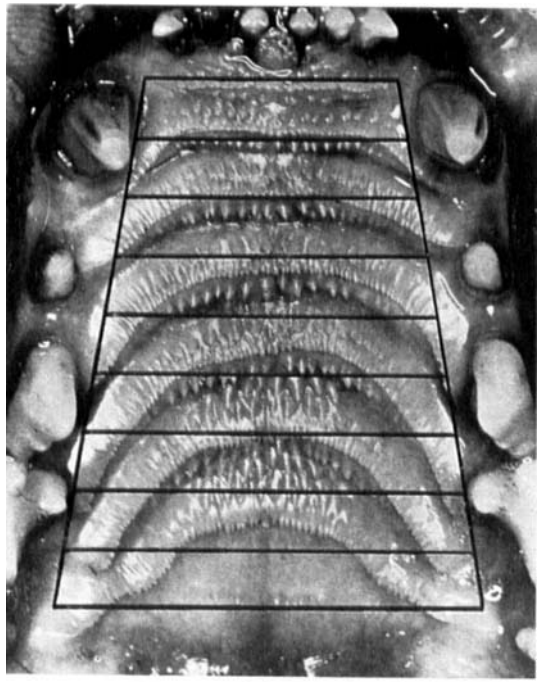

Fig. 1. Palatal mucosa with 9 indicated sections. The area between the soft and hard palate which is rich in glandular material was not used (section 9).

A search of the literature failed to reveal any study of ultrastructural changes in adult oral mucosa maintained as organ culture in chemically defined media. It was therefore considered of interest to compare the light microscopic and the electron microscopic findings in oral mucosa maintained in two different chemically defined media, *viz* medium 199 (*Morgan, Morton & Parker,* 1950) and Eagle's MEM medium (*Eagle,* 1959).

MATERIAL AND METHODS

Culture technique

The culture technique used has been described in detail by *Bergenholtz* (1969). The procedure is briefly as follows.

Palatal mucosa was excised from two apparently healthy, anaesthetized, female cats, age 1 1/2 year. The animals were then killed by incision of the heart. Circumorally the cats were washed with glycerol-ethanol (20:80v/v), after which the palatal mucosa was carefully rinsed in Tyrode's salt solution.

With two parallel scalpel-blades mounted 3 mm apart incisions perpendicular to the sagital plane were made from just behind the incisal papilla. 8 such incisions were made in the hard palate (Fig. 1). After one incision had been made along the dental arch about 1 mm from the teeth, the palatal mucosa was dissected free from the underlying bone. The strips of mucosa were placed in chilled transport medium (Hanks' balanced salt solution NSBL[1]); see *Hanks*, 1946) supplemented with 1000 U bensylpenicillin (AB Kabi, Sweden), 50 μg streptomycin sulphate (Glaxo Laboratories Ltd, England) and 50 U Mycostatin (E. R. Squibb & Sons Inc., USA) per ml solution. The same solution was used for cleaning the excised strips. With the aid of a stainless-steel punch with parallel razor blades 1 mm apart the excised mucosa strips were divided into standardized rectangular pieces (1×3 mm epithelium surface), which were kept in chilled transport medium for about half an hour, during which the explantation was performed.

Before explantation the culture chambers were prepared. These were made of Plexiglas with one inlet and one outlet for gas exchange (cf. *Trowell*, 1954). A sterile plastic petri dish (A/S Nunc, Denmark), 50 mm in diameter, was placed in each chamber, which contained a platform (25×25 mm) supported by legs 3 mm long. The platform was made of titanium expanded metal. The chambers and the lids were greased with lanoline, 4 ml of culture medium was then added to the petri dish. A piece (27×27 mm) of lens paper (Green's 105 B, J. Barcham Green Ltd, England) was placed on the top of the platform. After the chambers had been sealed by the lids they were gassed for 15 minutes with a mixture consisting of 50 % O_2, 5 % CO_2, 45 % N_2, at a flow rate of 100 ml/ minute. The chambers were then placed in an incubator at 37°C for about two hours in order to ensure gas equilibration of the culture medium. Pieces of palatal mucosa were then explanted to the moist lens paper while the chamber was still being gassed to prevent loss of CO_2 and a subsequent increase of pH. The culture chambers were regassed once a day for 15 minutes with the above mentioned gas mixture and flow rate.

The investigations comprised two experiments — one cat was used in each.

Experiment 1. One randomly chosen piece of palatal mucosa was maintained in each petri dish containing medium 199 /NSBL/(*Morgan, Morton & Parker*, 1950) containing 1600 μg $NaHCO_3$, 100 U bensylpenicillin, 50 μg streptomycin sulphate, 50 U Mycostatin and 250 μg L-glutamin per ml medium. All together 36 cultures and chambers were prepared.

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[1]) NSBL = The National Swedish Bacteriological Laboratory

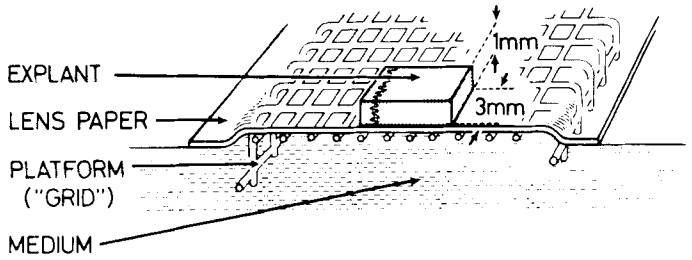


Fig. 2. The platform technique showing the position of the explant with the epithelial surface perpendicular to the grid surface.

Experiment 2. Three specimens of palatal mucosa were cultured on each platform in Eagle's MEM medium /NSBL/ (Eagle, 1959) containing 1600 μg $NaHCO_3$, antibiotics, fungostatic agent and L-glutamin, as in the first experiment. All together 30 culture chambers containing 90 explants were prepared.

Each explant was placed with the epithelium perpendicular to the surface of the lens paper (Fig. 2). Before and after culture the explants were examined ($\times$ 25—40) under a stereomicroscope (Zeiss Stereo III) in transmitted and reflected light to check that the explants were in proper position and to detect any changes in the explants after culture.

Specimens were taken at the time of the excision (control specimens), at the time of the explantation and after one, three and six days' culture.

Fixation and embedding technique

For light microscopy all explants were fixed in Bouin's solution (*Romeis*, 1948) for 3 hours and then kept in 70 % ethanol which was changed once a day for one week. After this the cultures were gently detached from the lens paper. The specimens were dehydrated in graded concentrations of ethanol and cleared in xylene and methyl-benzoate before being embedded in paraffin.

Two segments — one at the end and one in the middle — of each explant were serially sectioned. The sections were cut transversely-perpendicular to the epithelium with the microtome set at 5 μ. Conventional staining methods were used such as Mayer's hemalum-eosin and van Gieson-elastin stain. To facilitate orientation of the explants in each culture the top of the explant was marked with indian ink before fixation. This ink mark was visible in the final section.

Specimens for electron microscopy were divided in the middle perpendicular to the epithelium with a razor blade and immediately fixed by 2—3 hours' immersion in ice-cold 1 % osmium tetroxide buffered at pH 7.6

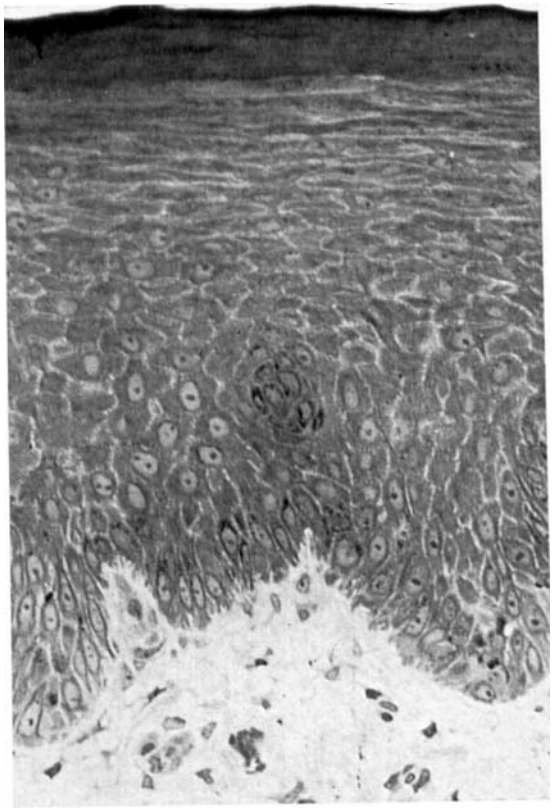


Fig. 3. Photomicrograph of uncultured palatal mucosa. Staining: Toluidine blue. × 330.

with phosphate buffer (*Millonig*, 1961) and then cut into smaller pieces. The specimens were then dehydrated in graded concentrations of ethanol and finally embedded in epon. For orientation purposes thick sections (1—2 μ) were cut from the epon blocks in an ultramicrotome. These sections were placed on ordinary glass slides and stained with toluidine blue. The specimens were then trimmed and sectioned in an LKB ultratome with glass knives. The thin sections were stained with uranyl acetate or double stained with uranyl acetate and lead citrate. The sections were examined in a Philips electron microscope EM 300*).

*) The electron microscope was kindly placed at the authors' disposal by G. Bloom, M.D., the Department of Histology, University of Umeå.

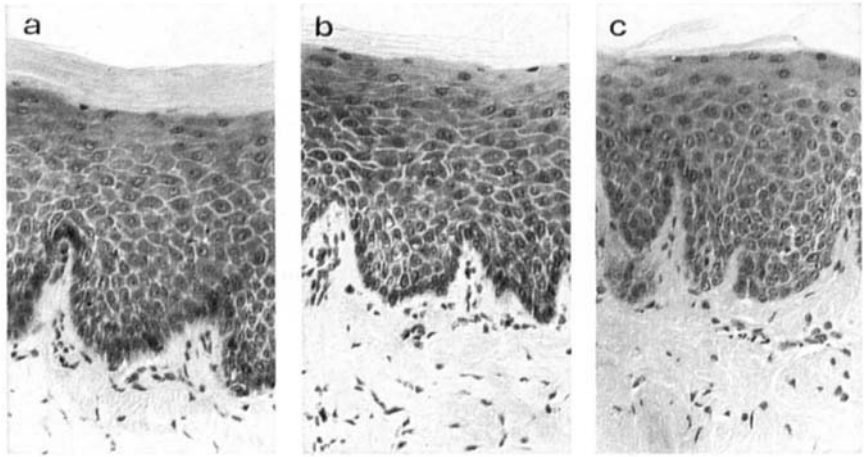


Fig. 4. Palatal mucosa cultured in MEM for a) 1, b) 3 and c) 6 days. No detectable histological alterations. Staining: Htx-eosin. × 310.

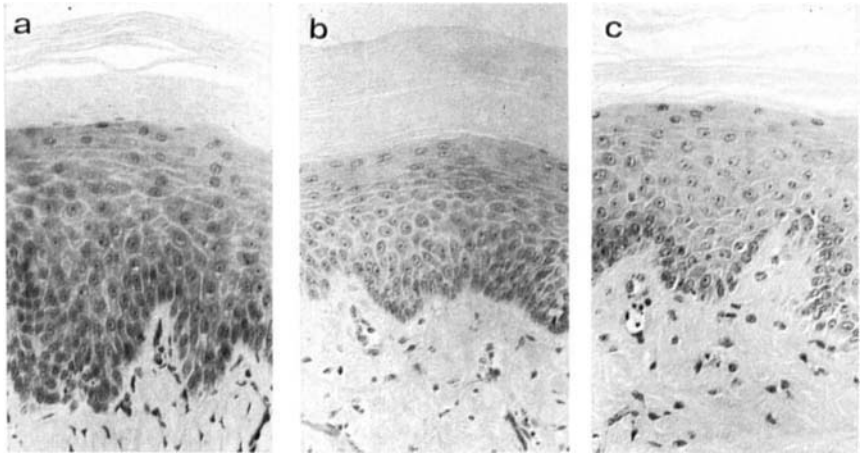


Fig. 5. Palatal mucosa cultured in medium 199 for a) 1, b) 3 and c) 6 days. No detectable histological alterations. Staining: Htx-eosin. × 310.

RESULTS

Light microscopy

In the control specimens (Fig. 3) the lamina propria was rich in collagen bundles and elastin fibers. The tissue was very vascular. The distal part of the palatal mucosa contained several glands.

The epithelium was of a stratified squamous keratinizing variety. The

basal layer consisted of a single row of columnar cells with spherical or ovoid nuclei. The spinous cell layer was 4—5 cells deep and contained polyhedral cells with a round nucleus. The cells became flatter as they approached the border to the stratum granulosum. Intercellular bridges between the cells in the basal and spinous layers were readily recognized. In the granular layer the cells were flat and were still flatter near the surface. The intercellular spaces were less distinct, which gave this layer a more homogenous appearance than the underlying ones. In more superficial parts, the cytoplasm contained keratohyaline granules. The border to the corneal layer was distinct. The stratum corneum consisted of crowded, flattened keratinized cells. This layer, which consisted of about 10 rows of cells, had a typical lamellar appearance. No difference in structure was observed when specimens taken at the time of excision and those taken at the time of explantation were compared.

After one day in culture (Figs 4a and 5a) the cut surface showed a thickening of the corneal layer and incipient proliferation of epithelial cells. This proliferation was seen on the top of the explants as well as in regions in contact with the medium. No difference could be observed between explants from the two media.

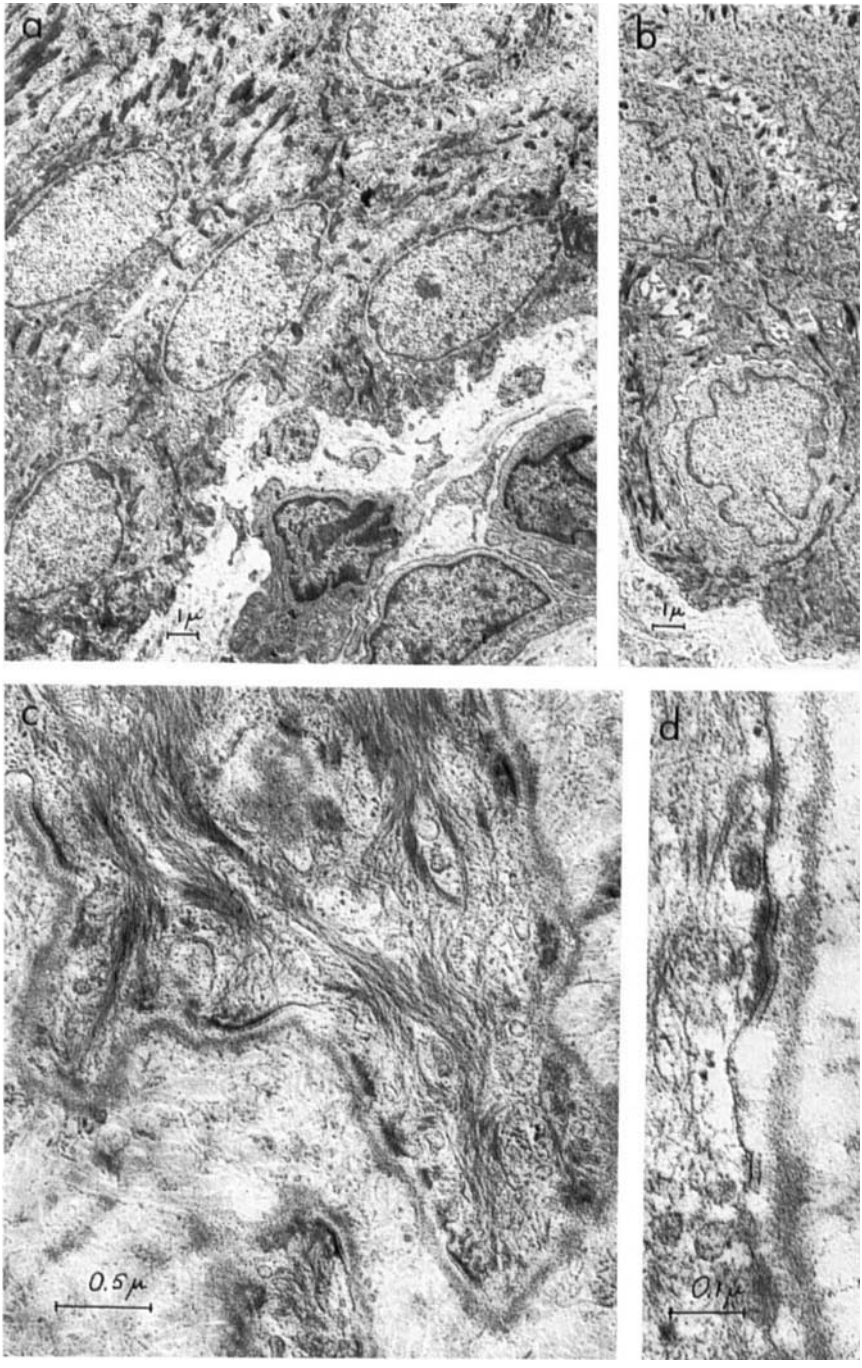
After three days' culture (Figs 4b and 5b) the stratum corneum was still thicker and had assumed a lamellar appearance. The proliferation of the cells had also increased, but in some specimens the cells showed pyknotic nuclei in that region which was in contact with the medium. There was still no significant difference between explants cultured in medium 199 and those maintained in Eagle's MEM medium.

When the specimens had been maintained for six days in Eagle's MEM medium the stratum corneum was very thick and lamellar (Fig 4c). Compared with those in the control specimens, the other layers were very well preserved. Explants maintained in medium 199 (Fig. 5c) often exhibited central necrosis of the epithelium characterized by pyknosis and acidophilic zones. Only some basal cells seemed to be intact. In the basal cell layer mitotic figures were more common in explants cultured in Eagle's MEM medium than in those maintained in medium 199. Epiboly was not observed in any explant.

Electron microscopy

Control specimens

Since this is apparently the first report on the ultrastructure of the palatal mucosa in the cat, it may be appropriate to give a detailed description of


a
1μ
b
1μ
c
0.5μ
d
0.1μ

the fine structure of the specimens immediately after they had been excised, *i.e.* a description of the appearance of the normal palatal mucosa in the cat.

Border zone epithelium and connective tissue (Fig. 6) was irregular and undulate. The two tissues were separated by a 400—700 Å thick osmiophilic layer, the lamina densa, which had a finely granular or sometimes fibrillar appearance. Between the lamina densa and the epithelial cells was a less electron dense zone, the lamina lucida. This rather clear homogenous zone was about 400 Å in width. The hemidesmosomes were numerous and were situated at irregular intervals. They had a typical lamellar appearance. Filamentous structures were often seen in the hemidesmosome area between the peripheral density and the lamina densa. Similar structures also extended from the connective tissue side of the lamina densa.

In the underlying connective tissue was a zone adjacent to the lamina densa in which the fibrils were rather sparse and not arranged in any particular fashion. Below this zone the fibrils were thicker and arranged in bundles (fibers). Blood vessels were numerous.

Stratum basale. The basal cells were columnar in type with a round nucleus. The nucleus often showed invaginations, which sometimes were fairly deep. In the cytoplasm the tonofilaments were rather sparse and did not mask the various organelles as they do in higher strata. The mitochondria were numerous but small and were irregularly distributed in the cytoplasm, with a slight tendency towards accumulation in the basal parts of the cells. Free ribosomes were numerous and diffusely distributed throughout the cytoplasm.

The tonofilaments were, as mentioned above, rather sparse and crossed the cytoplasm in bundles (tonofibrils) running in various directions. Some of these were connected with the attachment plaques of the desmosomes and the hemidesmosomes (Fig. 6c).

Intercellular spaces were rather wide and were therefore clearly seen even in views of the entire section. The periphery of the basal cell was very uneven and frequently interdigitated with cytoplasmic processes of different size and shape. The attachments between the epithelial cells consisted of

Fig. 6. Control specimen. From the basal layer.

a. *General view of the basal layer.* Capillaries run close to the lamina densa. × 5,700.
b. *From the basal layer.* A melanocyte between the epithelial cells. × 5,700.
c. *Border zone epithelium-connective tissue.* Hemidesmosomes and lamina densa are seen. × 37,200.
d. *Border zone epithelium-connective tissue in higher magnification.* Filamentous structures extend from the cell membrane and the peripheral density of the hemidesmosomes to the lamina densa. Small vesicles are present in the cytoplasm near and at the cell membrane. × 140,000.

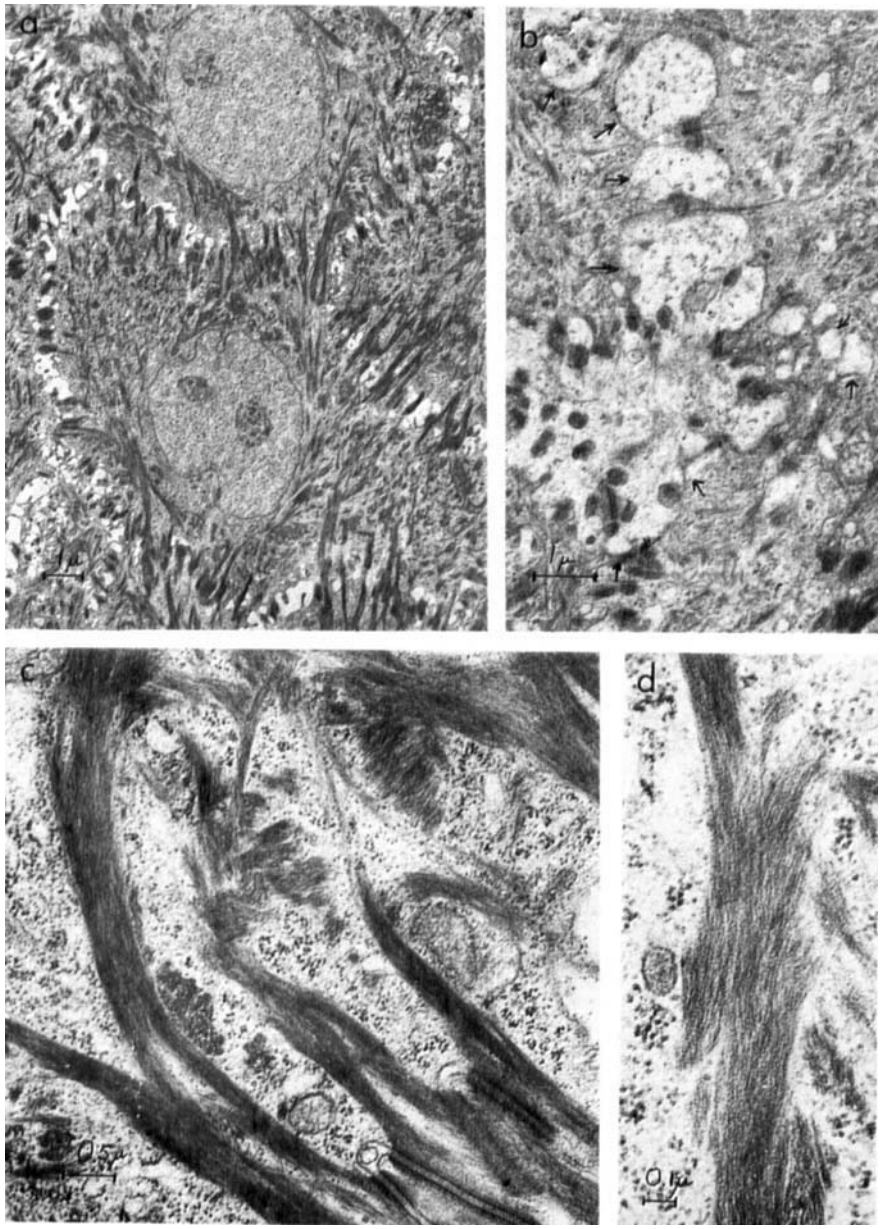


Fig. 7. Control specimen. From the spinous layer.

a. *General view.* The cells are relatively spherical. The nuclei have deeper invaginations than in the basal layer. × 8,000.

b. Cytoplasmic projections from clear cells in the basal layer are often seen between the spinous cells (arrows). × 13,500.

c. The tonofilaments are arranged in thick bundles and cross the cytoplasm in various directions. × 21,300.

d. In higher magnification the tonofilaments show a striated appearance. × 68,000.

desmosomes, tight junctions (zonula occludens) and intermediate junctions. The structure of these attachments is well known and will therefore not be described in detail here. The desmosomes were numerous and situated at different intervals. They showed a typical lamellar structure. Both tight junctions and intermediate junctions were common. There was very often an intermediate junction where the intercellular space ended in the lamina lucida.

Small invaginations partly filled with a rather osmiophilic substance were often seen in the cell membrane opposite the lamina densa. The cytoplasm near this part of the cell membrane often contained small vesicles with a similar content (Fig. 6d).

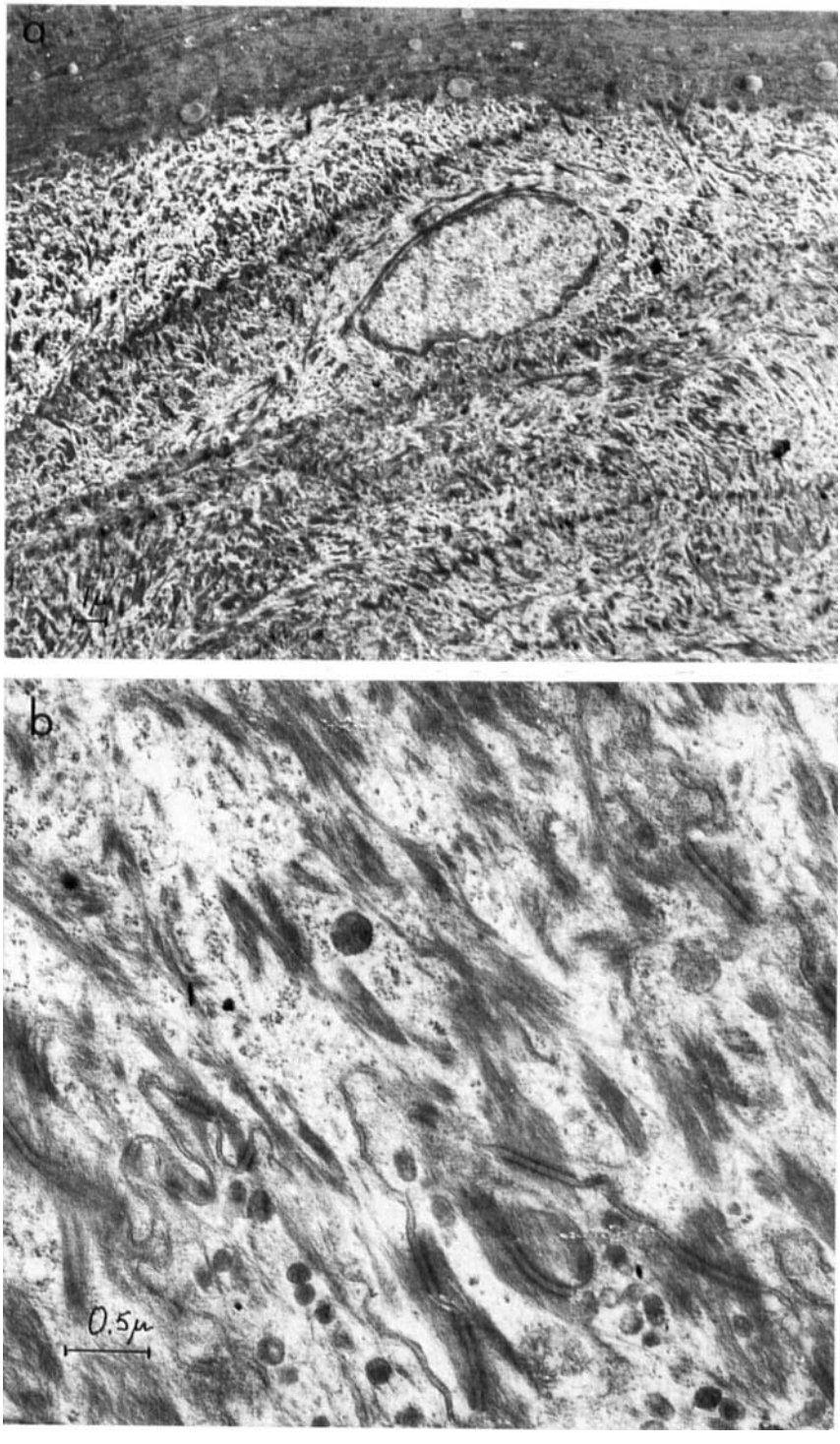
Melanocytes were interspersed between the epithelial cells (Fig. 6b). They lacked tonofilaments in the cytoplasm and the chromatin-rich nucleus showed deep invaginations. In most cases mature melanin granules were seen as osmiophilic spots. Besides these granules the cytoplasm contained numerous vesicles and granules of varying electron density, the latter constituting premelanosomes and melanosomes. Cytoplasmic projections from these cells were often found in the stratum spinosum. The intercellular space between these cells and adjacent epithelial cells was rather narrow and the cell membrane was more even than in the epithelial cell. The attachments consisted of intermediate junctions, no desmosomes or tight junctions were observed. The intercellular substance was homogenous and amorphous and of rather low electron density.

Stratum spinosum. The spinosum cells appeared rounded or stellate except in the layers adjacent to the stratum granulosum, where they were flattened with the longest diameter parallel to the tissue surface. The tonofilaments were abundant and arranged in thick bundles (Figs 7c and d). The usual organelles were often masked by this abundance of filaments. The nucleus was round with deep invaginations (Fig. 7a).

The intercellular space was of about the same width as that in the stratum basale. The cell membranes were highly convoluted and cytoplasmic projections interdigitated freely between cells. The desmosomes were numerous, they seemed to be more numerous here than in the stratum basale. Tight junctions and intermediate junctions were common.

Clear cells as well as cytoplasmic projections from melanocytes in the basal layer were interspersed among the epithelial cells (Fig. 7b). The clear cells of this layer showed the same characteristics as the above mentioned melanocytes except that they contained no demonstrable mature melanin granules.

Stratum granulosum. The cells were flattened and the higher they were


a
b
0.5μ

situated in this layer the flatter they appeared. The nucleus and the cytoplasmic organelles were intact in the lower part of the layer but higher up they began to disintegrate, and adjacent to the stratum corneum they were usually no longer demonstrable.

Keratohyaline granules, electron-dense and irregular in shape and form were abundant. They often seemed to be associated with the tonofilaments (Fig. 8b). Occasionally small osmiophilic granules were observed in the upper part of the stratum.

Intercellular spaces were narrower in this layer than in the lower strata. The desmosomes were numerous. At the border to fully keratinized cells the structure of the desmosomes changed and showed what has been called a composite structure. The inner layer of the cell membrane of the stratum corneum cell showed a continuous thickening which merged with its attachment plaque. On the stratum granulosum side there was no thickened inner layer outside the desmosome. Tight junctions and intermediate junctions were very common. Except for focally expanded parts, the intercellular spaces seemed to consist mainly of these three types of attachments.

Stratum corneum. The border between the stratum corneum and stratum granulosum was usually distinct (Fig. 8a). The cells were long, flat and arranged in even layers parallel to the tissue surface (Fig. 9a). The cytoplasm was characterized by crowded filaments in an osmiophilic amorphous ground substance (Fig. 9b). Sometimes nuclear remnants were observed in the lower layers but hardly ever in the most superficial layers. Oval, empty vacuoles were common in the cells. The cell membrane changed in appearance; in these layers it consisted of a single, thick, rather electron-dense band.

The intercellular spaces were very narrow and only focally widened. They ran a straighter course than deeper in the epithelium. The desmosomes showed a modified structure. They were very common and in the desmosome area the cell membranes of opposite cells ran parallel. As mentioned above, the cell membranes were thickened and no special attachment plaques could be distinguished. Between the thickened cell membranes there was a three-layered structure, a central, thick, weakly osmiophilic band bordered by two narrow, less electron-dense lines. Sometimes a denser intermediate

Fig. 8. Control specimen. From the granular and corneal layers.
a. The border between the stratum granulosum and the stratum corneum is rather distinct. × 5,700.
b. From the granular layer. Tonofibrils and keratohyaline granules are seen. The intercellular spaces are narrow. × 27,500.

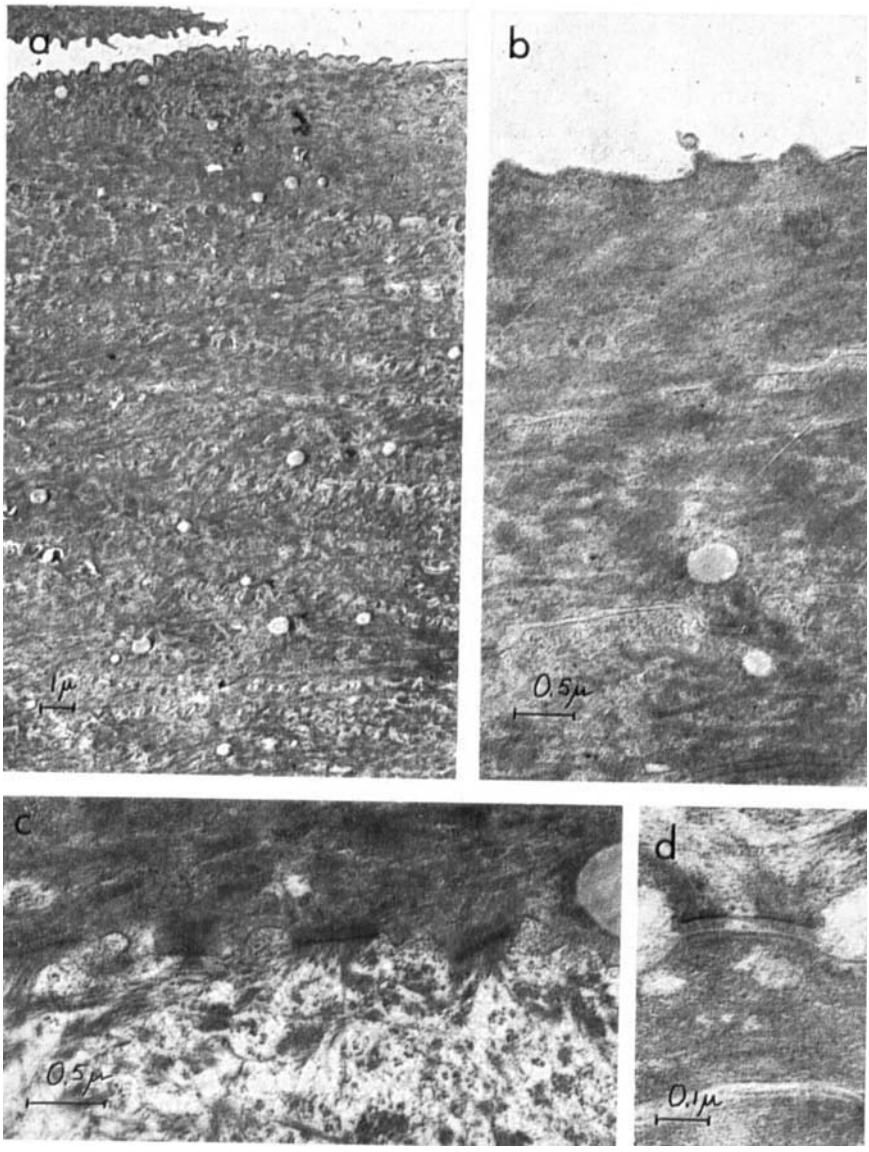


Fig. 9. Control specimen.

a. General view of the stratum corneum. The cells are long, flat and orientated parallel to the surface. × 5,700.

b. Stratum corneum in higher magnification. The cells consist of densely packed filaments in an osmiophilic amorphous substance. The intercellular spaces are narrow and rather straight. × 21,300.

c. From the border zone stratum granulosum-stratum corneum. At the border to fully keratinized cells the desmosomes show a composite structure. × 27,500.

d. A composite desmosome in higher magnification. For explanation see text. × 100,000.

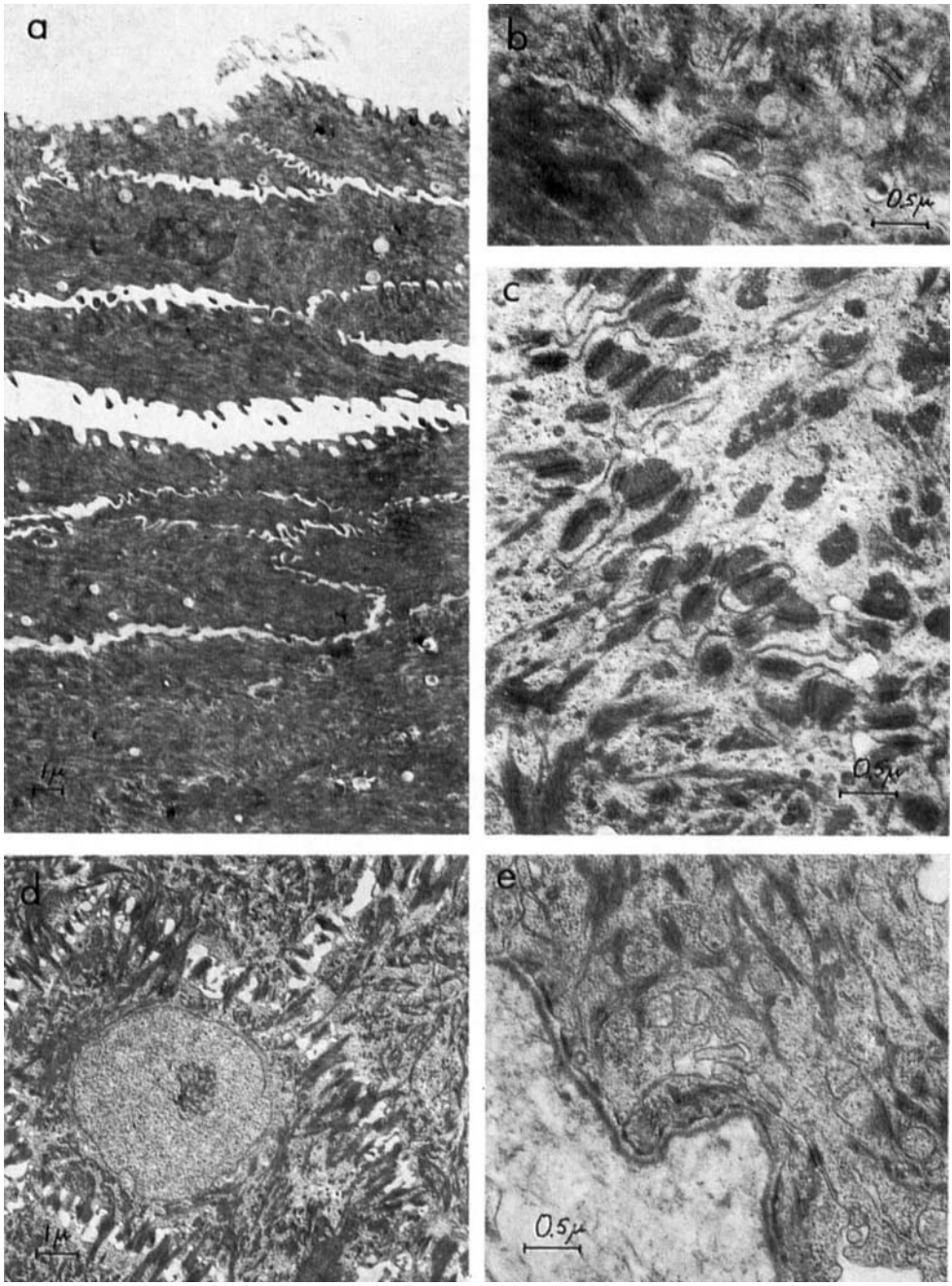


Fig. 10. One day culture. Medium 199.

a. General view of the upper part of the stratum corneum. There is an increase in thickness of this stratum, and the surface layers consist mostly of partly detached cells. × 5,700.

b, c, d, e. Except for this increase in thickness, no other structural changes could be observed: b. from the corneal layer (× 21,300), c. from the granular layer (× 21,300), d. from the spinous layer (× 8,000), and e. from the basal layer (× 21,300).

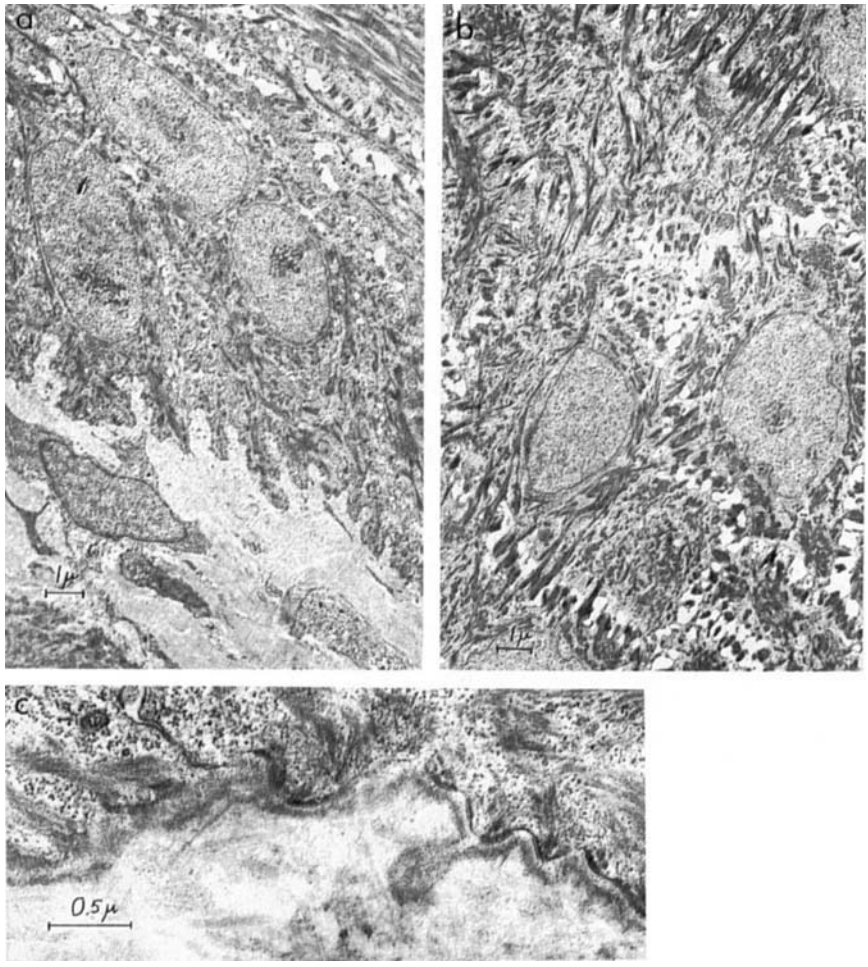


Fig. 11. One day culture. MEM.
Compared with the control specimens no structural changes could be observed in the basal layer (a, × 8,000 and c, × 37,200) or in the spinous layer (b, × 8,000).

line was seen in the central faintly osmiophilic band. Modified tight junctions were also common. At the orifices of the intercellular spaces at the tissue surface, modified desmosomes or tight junctions (Figs. 9c and d) were observed.

At time of explantation

No differences could be discerned between the specimens taken at time of explantation and the control specimens. The keeping of the pieces of mucosa

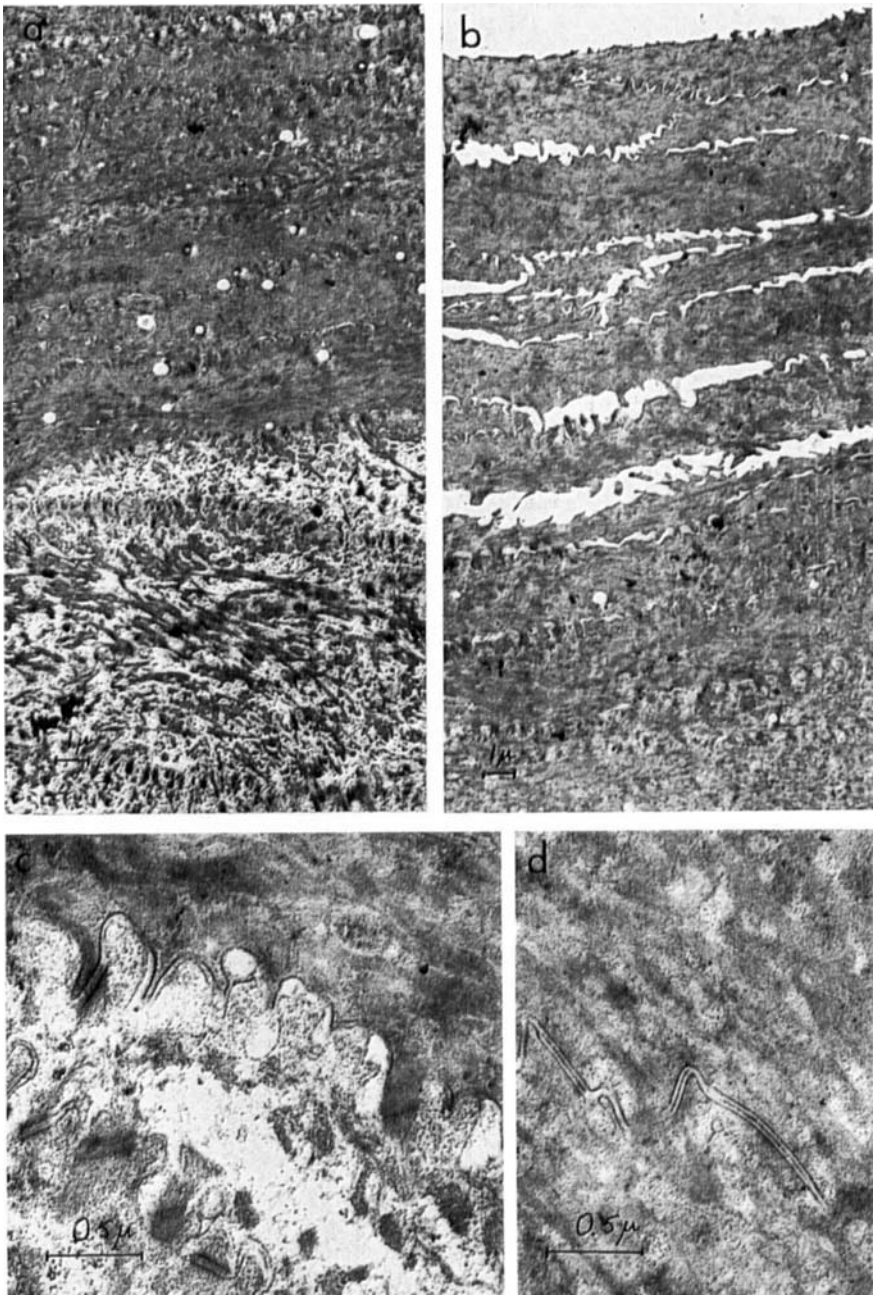


Fig. 12. One day culture. MEM.
The stratum corneum has increased in thickness (a, × 5,700 and b, × 5,700) and the cells of the outer layers are partly detached from each other. No other structural changes were observed in the corneal layer or the granular layer (c, × 37,200 and d, × 37,200).

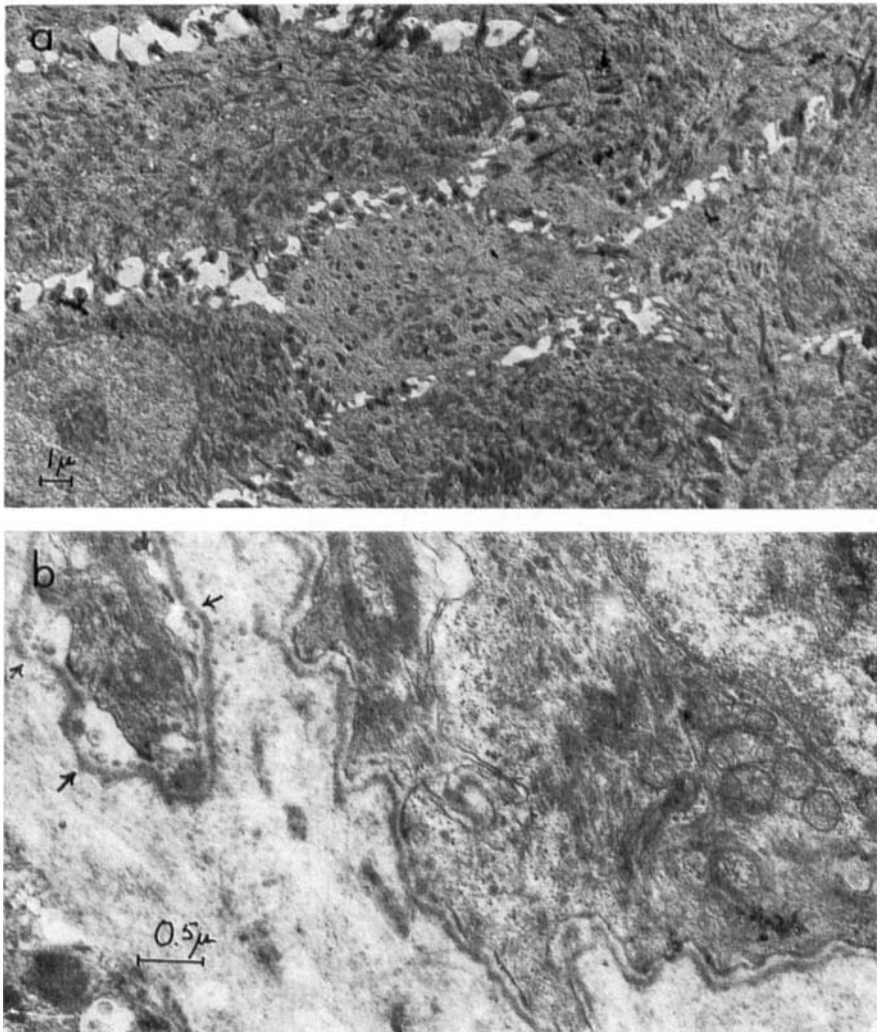
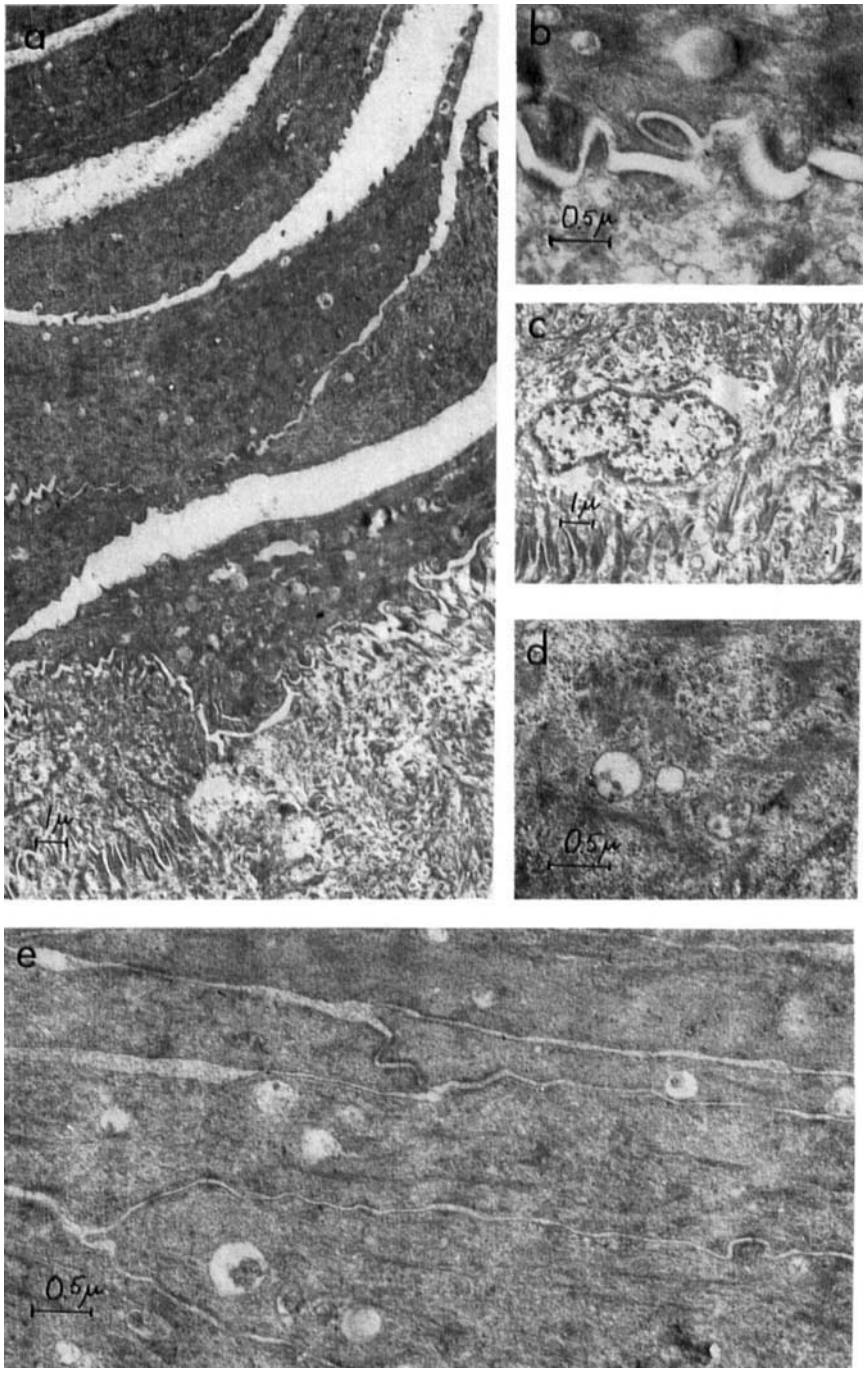


Fig. 13. Three day culture. Medium 199.

a. From the border stratum basale — stratum spinosum. The intercellular spaces are generally widened. × 5,700.
b. From the border zone epithelium — connective tissue. In the connective tissue there are local areas of oedema-like alterations. The clear zone between the cell membrane of the basal cells and the lamina densa is focally expanded (arrow). × 21,300.

Fig. 14. Three day culture. Medium 199.

a. Fissure-like formations in the stratum corneum down to the stratum granulosum. × 5,700.
b. Compared with the control specimens the cells of the stratum corneum are often more crowded. × 21,300.
c. From the stratum granulosum partial haloformation round the nucleus. × 5,700.
d. From the stratum basale. In some cells the number of mitochondrial cristae is reduced. × 21,300.
e. From the stratum corneum. In some areas like that illustrated in the figure, there are no changes in structure compared with that of the control specimens. × 21,300.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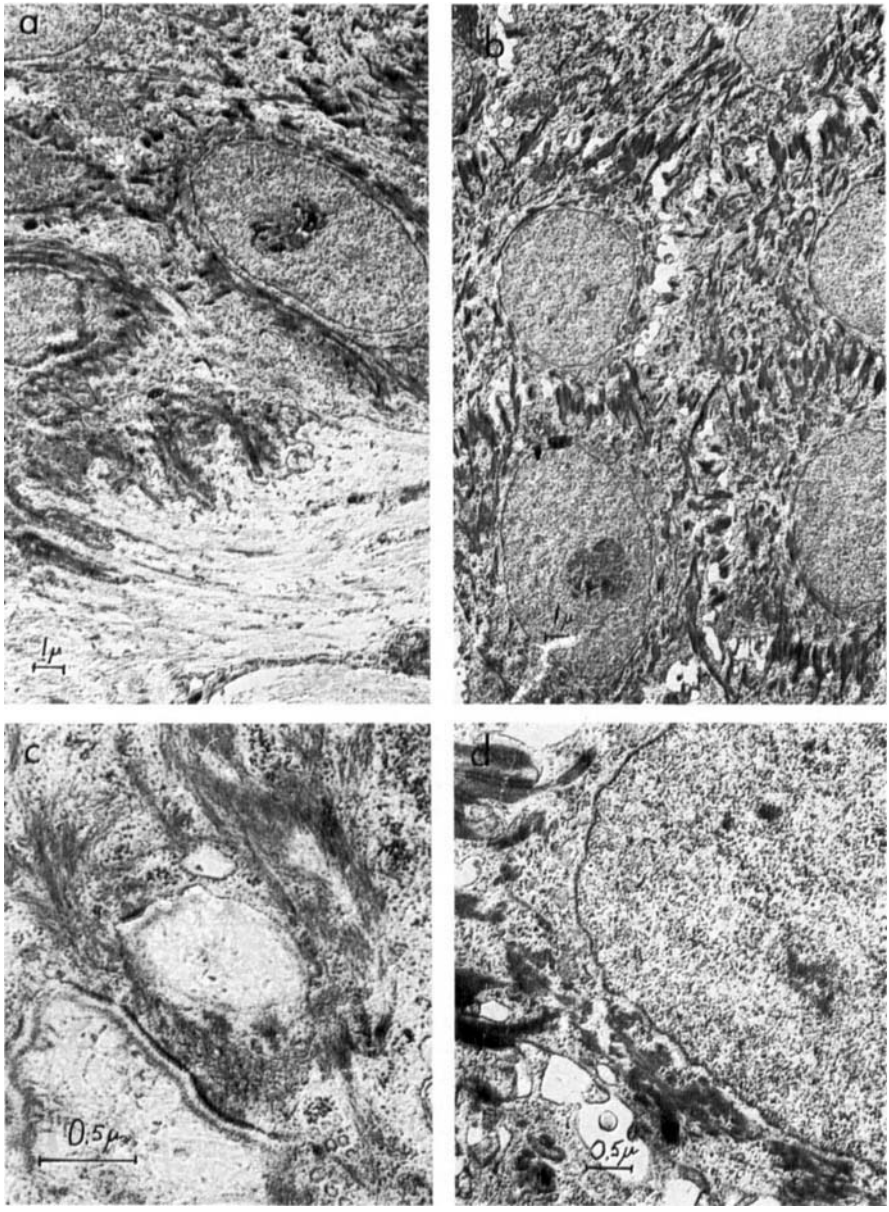


Fig. 15. Three day culture. MEM.
Compared with the control specimens no structural changes could be observed in the basal or the spinous layer.
a (× 5,700) and c (× 37,200) from the basal layer.
b (× 5,700) and d (× 17,000) from the spinous layer.

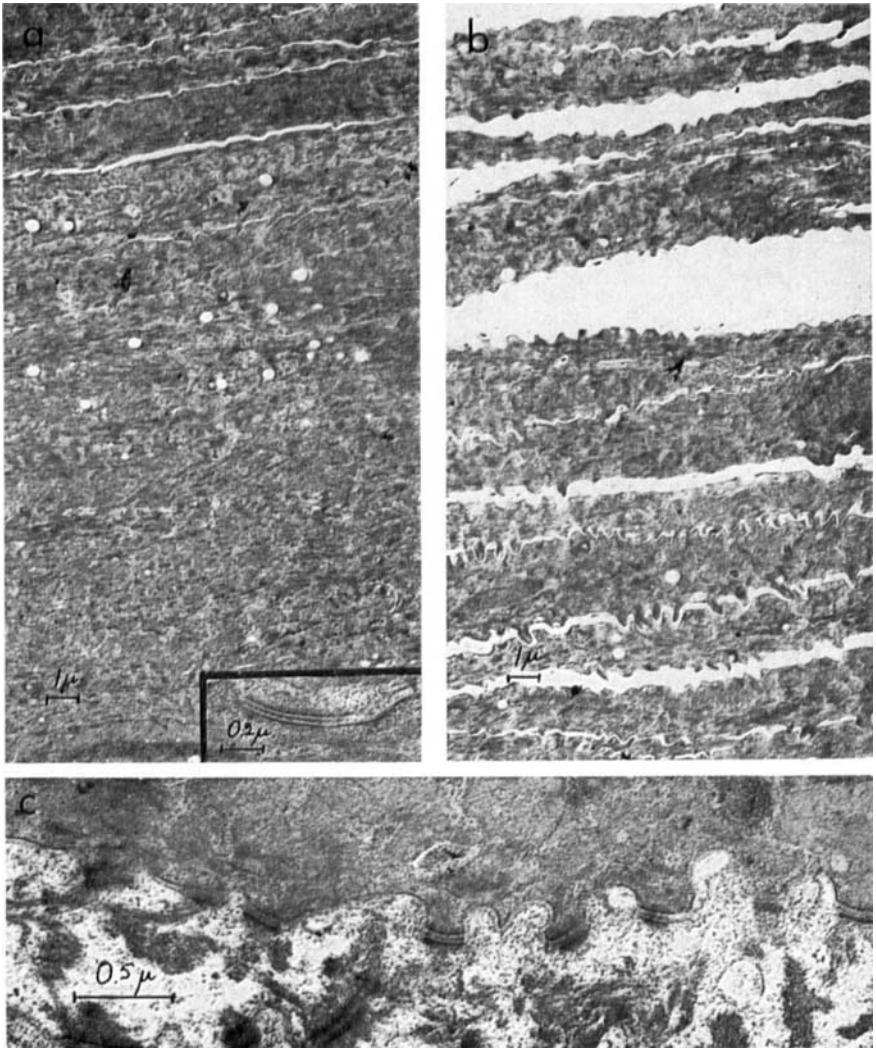


Fig. 16. Three day culture. MEM.
The corneal layer is thick with partly detached cells on the surface layers (a, b). The desmosome structure is quite normal (as inset).
c. The border zone stratum corneum — stratum granulosum is of quite normal appearance.
Magnification: a, × 5,7000; a, inset × 37,200,
b, × 5,700; c, × 37,200.

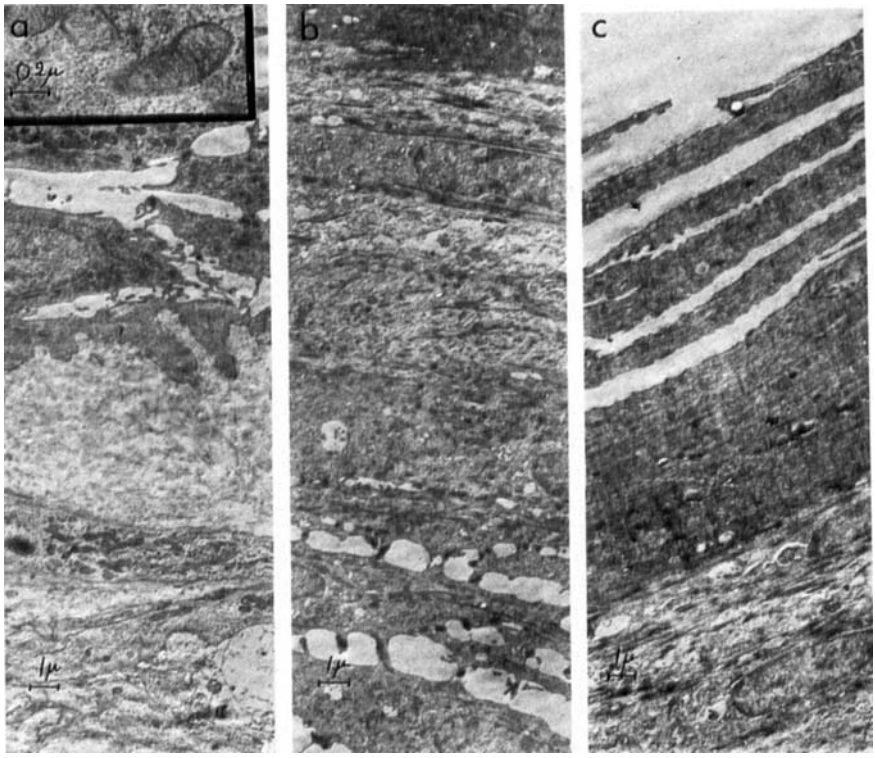


Fig. 17. Six day culture. Medium 199.

a. From the basal layer. The cells are flattened, the intercellular spaces are widened. The structure of the cell organelles is well preserved as are the mitochondria (a, inset). × 5,700. Inset × 37,200.
b. From the spinous layer. In the interior parts the intercellular spaces are widened. × 5,700.
c. From the stratum granulosum and the stratum corneum. The cells are very flat and the intercellular spaces are narrow except in the surface layers of the stratum corneum, where the cells are partly detached. × 5,700.

in the chilled transport medium between the excision and the explantation thus seemed to have no effect on fine structure.

Organ culture specimens

One-day-cultures. The only difference between the specimens in the two media and the control specimens was an increase in thickness of the stratum corneum in the cultivated specimens. This stratum was of typical lamellar appearance and the cells of the outer layers were partly detached from each other (Figs. 10, 11 and 12).

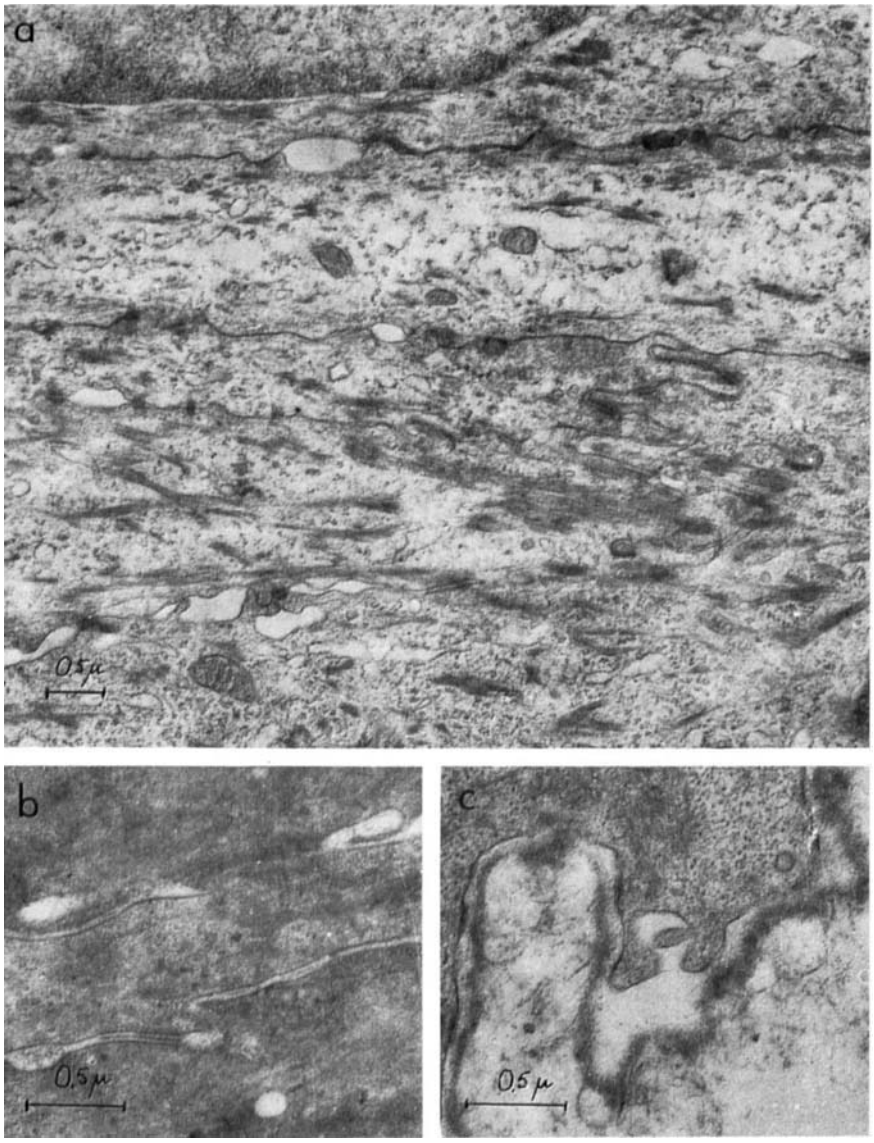


Fig. 18. Six day culture. Medium 199.

a. From the upper part of the stratum spinosum. The nuclei and the cell organelles are well preserved. The intercellular spaces are narrow. × 21,300.

b. Higher magnification of the lower part of the stratum corneum. Narrow intercellular spaces. × 37,200.

c. From the border zone stratum basale — connective tissue. The lamina densa is focally expanded from the basal layers. × 37,200.

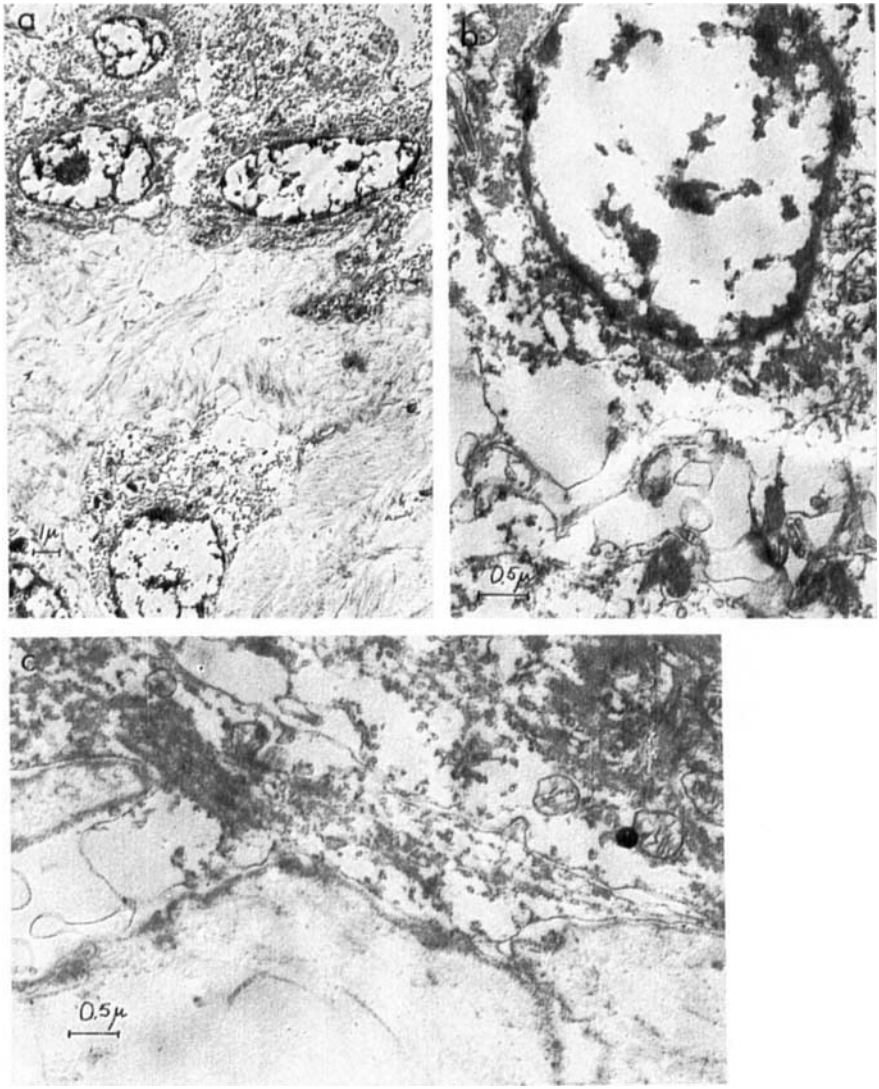


Fig. 19. Six day culture. Medium 199.
In some specimens the structure was severely changed, a. from the stratum basale (× 5,700), b. from the stratum spinosum (× 21,300) and c. from the border zone epithelium — connective tissue (× 21,300).

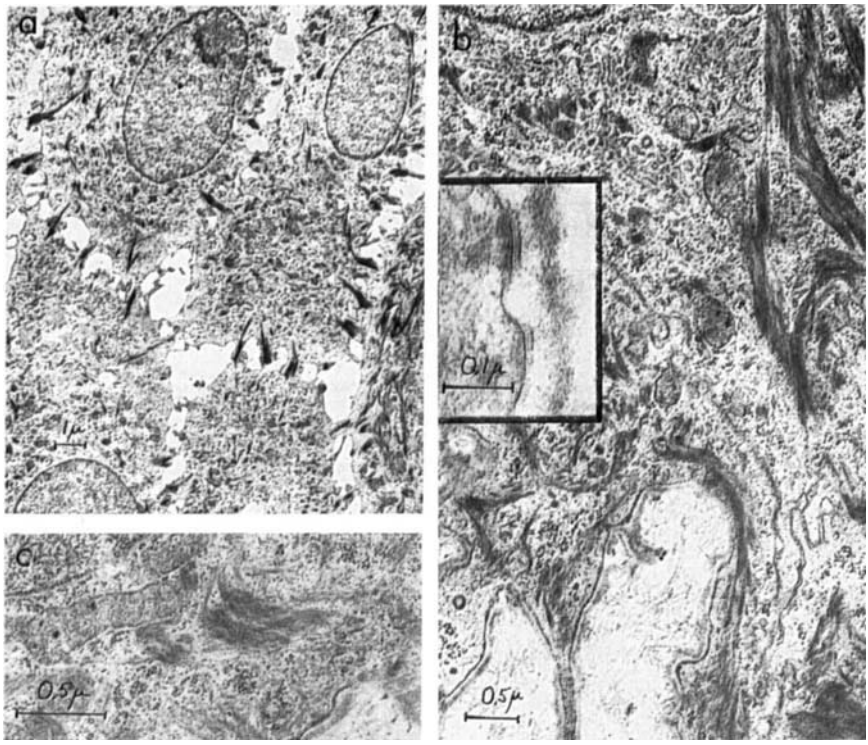


Fig. 20. Six day culture. MEM.
In stratum basale there are areas with widened intercellular spaces (a). In some areas the mitochondria are enlarged (c). Cells with apparently well preserved nuclei and cytoplasmic organelles are often seen adjacent to the stratum corneum, indicating delayed disintegration of these structures.
a. × 5,700.
b. In most areas at the stratum basale there were no changes in structure compared with the appearance of the control specimens. × 21,300. Inset × 140,000.
c. × 37.200.

Three-day-cultures. There were clear differences in structure between the specimens cultivated in the Eagle's MEM medium and those cultivated in the medium 199 (Figs. 13, 14, 15 and 16). The structural changes in the pieces cultivated in Eagle's medium were confined to the stratum corneum. Here the change was similar to that seen after one day's culture and consisted of a thickening of the corneal layer. Apart from the width, which can be characterized as the »normal» width of this layer *in vivo*, the cells were more or less detached from each other (Figs. 15 and 16).

The specimens taken from medium 199 showed structural changes in all layers. In the connective tissue beneath the epithelium there were areas of

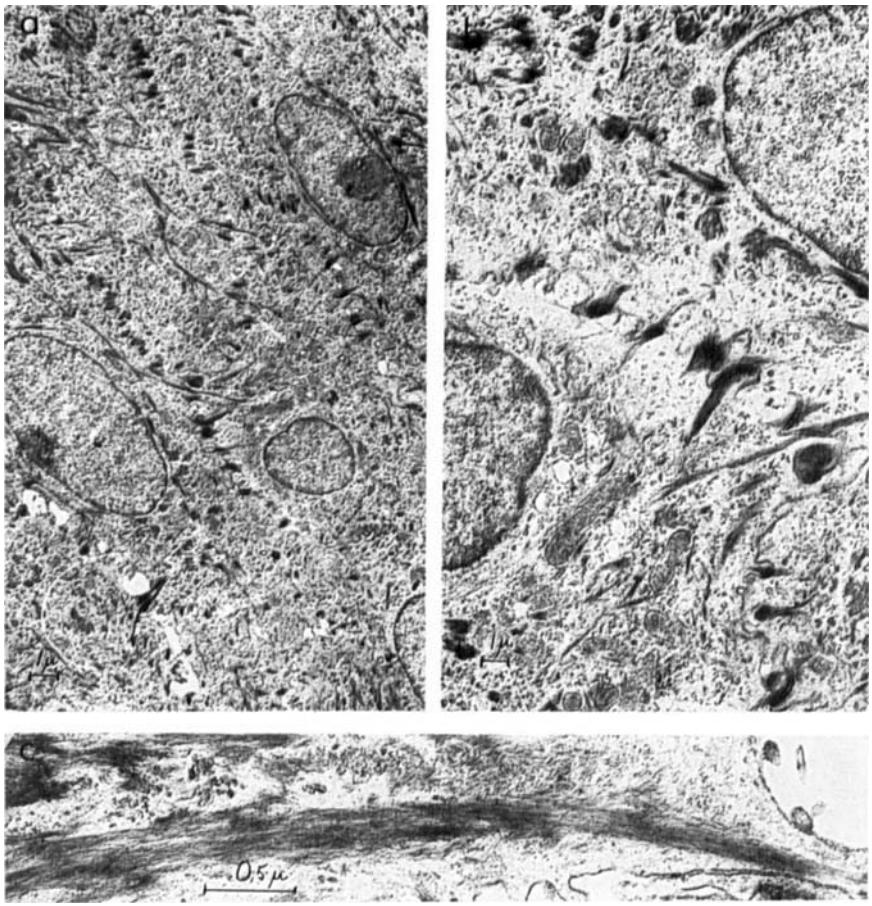


Fig. 21. Six day culture. MEM.
The cell structure are well preserved, a. from the basal layer (× 5,700), b. from the spinous layer (× 5,700), c. tonofibrils in the spinous layer (× 37,200).

oedema-like alterations (Fig. 13b). The clear zone (lamina lucida) between the cell membrane of the basal cells and the lamina densa was focally expanded and showed minute vesicular formations. The intercellular spaces were generally widened (Fig. 13a); in certain areas in the granular layer they became extremely wide. Where the widenings were rather limited the conventional set of attachments seemed to be intact, but in areas with very wide intercellular spaces in the stratum granulosum they were reduced in number. As in the one-day-specimen, the stratum corneum was thickened, but in some of the three-day-specimens the partial detachment of the cells produced

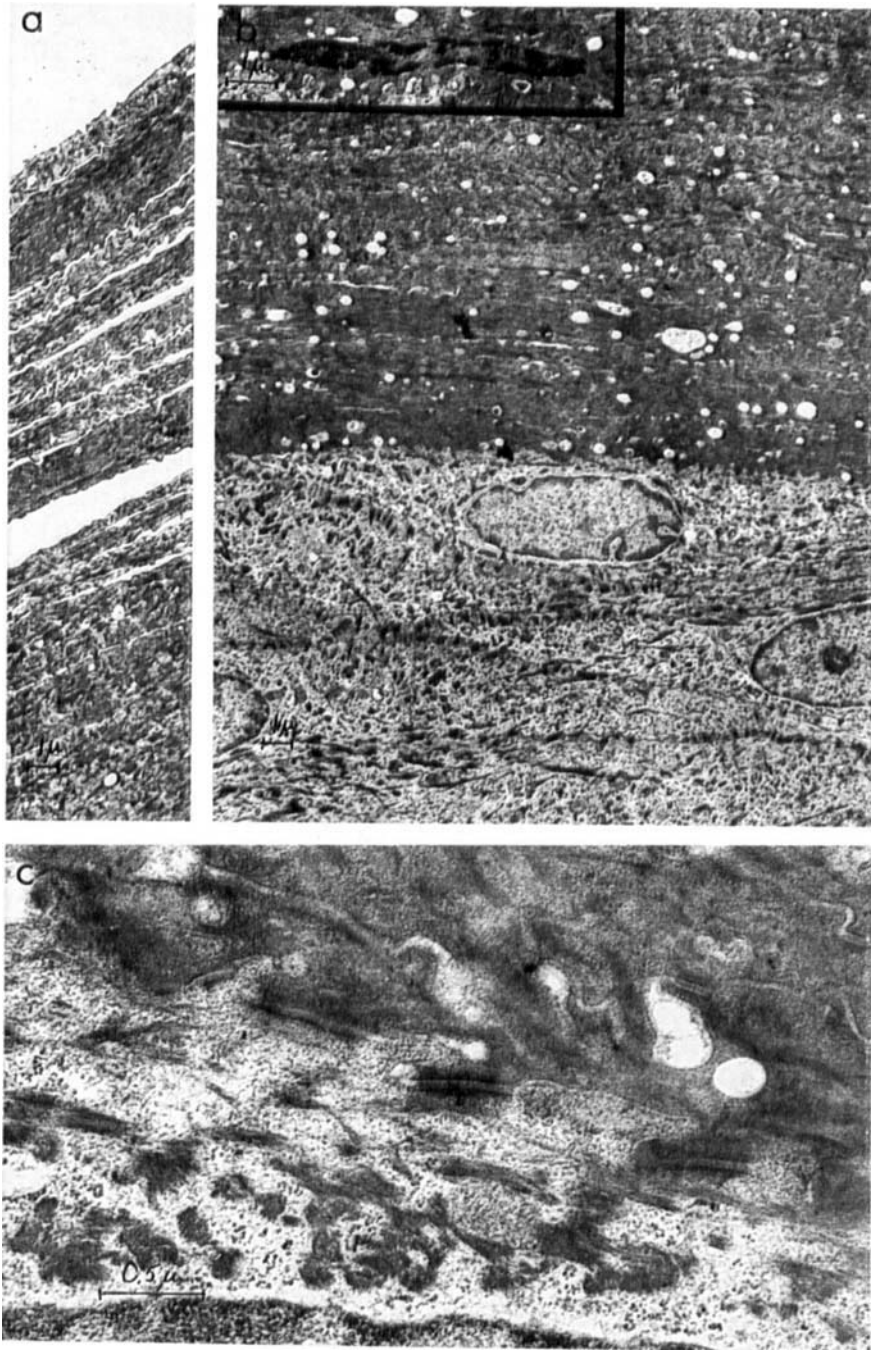
fissure-like formations down to the zone bording the stratum granulosum. In general, the cells of the stratum corneum were far less crowded than in the control specimens (Fig. 14b).

In these three-day-specimens from medium 199 there were also intracellular alterations. In some cells of the stratum basale and the stratum spinosum the endoplasmic reticulum was dilated and the number of mitochondrial cristae was reduced (Fig. 14d). In the stratum granulosum also other changes were occasionally seen in the form of partial haloformations (Fig. 14c) around the nuclei. These features were most often combined with changes in the nucleus. An incipient formation of vacuoles was occasionally seen in the cytoplasm of some cells of the basal and spinous layers. In the stratum granulosum these vacuoles were rather common, especially in the zone bordering on the stratum corneum. In this latter layer the usual oval or round vacuoles had increased in number, some of them seemed to be filled with a rather electron dense substance. The cells of this layer often contained remnants of the nuclei. There were, however, some areas in the specimens from medium 199 where the structure still resembled that of the control specimens (Fig. 14e).

Six-day-cultures. After six days' cultivation in Eagle's MEM medium rather small differences in structure could be discerned, compared with the picture of the control specimens (Figs. 20, 21 and 23). In the basal layer the intercellular spaces had increased in width in small areas (Fig. 20a). Here the conventional attachments had diminished in number. Some mitochondria seemed to have increased in size (Fig. 20c). These above-mentioned alterations could not be discerned in the spinous layer, where the structure in and between epithelial cells as well as clear cells seemed to be quite »normal» (Figs. 21b and c). The granular layer showed no distinct changes except a possibly delayed disintegration of nuclei and cytoplasmic organelles (Fig. 22c). In the stratum corneum the typical round or oval vacuoles had increased in number (Fig. 22b).

The appearance of the stratum corneum was largely the same as in the one-day and the three-day-specimens, there was a thickening of the layer and a partly detached, lamellar structure of the outer layers of cells (Fig. 22a).

The specimens which had been cultivated for six days in medium 199 showed changes of widely varying degree. The alterations varied from almost complete disintegration of the tissue to less pronounced or negligible changes. These areas with only negligible changes were mostly confined to the granular and corneal layers (Figs 17 and 18). The connective tissue part of the specimens was very sparse in fibrils and the cells were in a state of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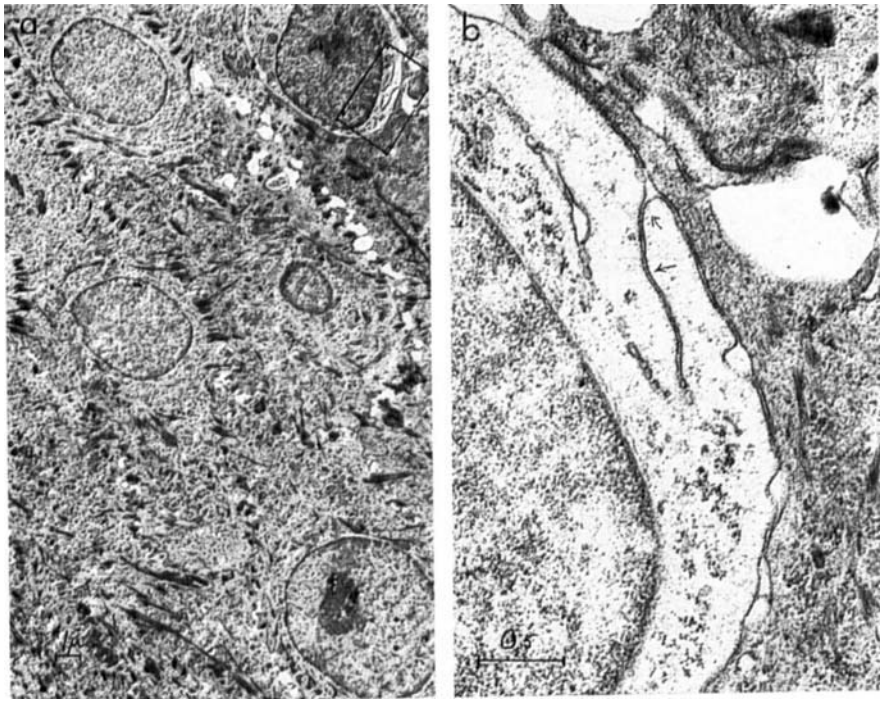

Fig. 23. Six day culture. MEM.

a. From the stratum spinosum. A clear cell is seen interspersed between the epithelial cells. × 5,700.
b. Higher magnification of the framed area in a. illustrating the relation between the endoplasmic reticulum and the cell membrane (arrows) × 37,200.

Fig. 22. Six day culture. MEM.

a. The stratum corneum is thickened with partly detached cells in the surface layers. × 5,700.
b. Cells with apparently well preserved nuclei and cytoplasmic organelles are often seen adjacent to the stratum corneum, indicating a delayed disintegration of these structures. The round and oval vacuoles have increased in number. Occasionally, nuclear remnants are observed in the stratum corneum (b. inset). × 5,700. Inset × 9,000.
c. From the border zone stratum corneum — stratum granulosum. The structure is very well preserved. × 37,200.

disintegration with a grossly changed nuclear and cytoplasmic structure. In some regions the lamina densa was largely intact, in others it had partly disappeared and focally, vacuoles of different size could be observed between the lamina densa and the cell membrane of the basal cells (Fig. 18c). In the epithelium the most marked changes were localized to the basal and the spinous layers (Fig. 19). In large areas the cells were more or less disintegrated with a severely vacuolized cytoplasm. The endoplasmic reticulum was, when it could still be discerned, increased in width, and most of the

mitochondrial cristae had disappeared. The nuclei contained only scattered osmiophilic remnants, mostly adjacent to the still visible nuclear membrane. The intercellular spaces had increased in width and of the conventional cell contact devices only desmosomes and scattered half-desmosomes could be observed. In other regions in the same or in other specimens the changes were not so obvious. Here the cell structure of the nuclei and cell organelles seemed to be rather well preserved. The cells were elongated and the cytoplasm poor in fibrils. The intercellular spaces were here markedly widened and the cell contacts consisted of a few desmosomes. In the stratum granulosum the cell structure was better preserved than in the underlying layers. The cells were flat, the intercellular spaces were narrow and only focally widened (Fig. 17c). They ran a rather straight course. The attachments were of ordinary types. The border to the stratum corneum was distinct. In a few areas the cells showed signs of incipient disintegration, as described above in the case of the underlying layers. The appearance of the stratum corneum was largely the same as that in the corresponding three-day-specimens. In general, the various layers consisted of fewer rows of cells compared with earlier specimens and the epithelial layer was therefore on the whole rather thin.

DISCUSSION

In previous investigations (*Bergenholtz*, 1969) the Trowell platform technique (*Trowell*, 1954) was found to be the method of choice when studying palatal mucosa in organ culture. Various technical factors found to improve the survival were also utilized in the present investigation. Thus, among tested chemically defined media medium 199 (NSBL) and Eagle's MEM medium (NSBL) were those which preserved the morphology and oxygen consumption best for up to 6 days in vitro. In the above mentioned investigation the results proved to depend on the oxygen concentration, and 50 % oxygen in the gas phase was found to be optimal. This concentration was accordingly used in the present investigation with medium 199 and Eagle's MEM medium.

In many respects the morphology (light as well as electron microscopy) of the palatal mucosa of the cat was found to closely resemble that of man (*Thilander*, 1968). *Gibbins* (1962) studied the ultrastructure of the palate of the albino rat and concluded »that this epithelium possesses many of the features of the stratified squamous epithelium found in other sites and in other animals», which is confirmed by *Stern* (1965), for example, as well as our findings.

Compared with the control specimens, the cultured palatal mucosa of the

cat showed an increase in the thickness of the stratum corneum with the duration of culture. This was observed in explants maintained in Eagle's MEM medium as well as in explants cultured in medium 199. *In vivo* there is normally a wear and replacement the rate of which is adapted to the prevailing physiological circumstances. Oral mucosa maintained *in vitro* does not show such mechanical wear and this probably explains the increased thickening and lamellation of the corneal layer. The thickening and lamellation should thus not be regarded as a sign of deterioration.

The increasing number of vacuoles in the stratum corneum observed after culture in each media may be difficult to explain. However, it is well known that endothelial tissue as well as epithelium close to vascular systems shows vacuolization. As the Trowell platform technique implies a close relationship between the tissue and the watery medium, the increased number of vacuoles may, perhaps, be due to an increased cellular uptake by pinocytosis. On their way to the epithelial surface it is plausible that small pinocytotic bubbles become greater by fusion and finally give rise to these vacuoles in the stratum corneum.

Judging from the light and electron microscopic findings, explants cultivated in Eagle's MEM medium seem to be intact up to at least six days after explantation. However, in some areas of the six day cultures, the mitochondria were enlarged, without any other alterations but of otherwise normal appearance. The enlargement may, perhaps, be the result of increased demands upon mitochondrial function. However, the mechanism leading to increased mitochondrial growth is not properly understood.

In earlier investigations explants cultivated in medium 199 have been shown to be almost as good as explants maintained in Eagle's MEM medium. The reproducibility of the results obtained with medium 199, however, seems to vary more from batch to batch of the medium and from one manufacturer to another, than that of the results obtainable with Eagle's MEM medium. In this investigation some of the specimens showed severe structural changes after six days cultivation in medium 199. This probably depends on the above mentioned factors. Judging from the present investigation, medium 199 does not seem to preserve palatal epithelium of the cat as well as Eagle's MEM medium. By comparing the results of light microscopy and those of electron microscopy no changes were observed in the light microscopy in explants cultured in three days in medium 199. However, changes in the ultrastructure were visible in the form of a focally expanded lamina densa forming minute vesicles. No such changes were observed in the specimens maintained in Eagle's MEM medium.

The present results show that it is possible to maintain palatal mucosa of

the cat in Eagle's MEM medium and a gas phase containing 50 % O_2 for at least 6 days without morphological deterioration. Good results were also obtained with medium 199. As better results were obtained with Eagle's MEM medium and as this medium is easier to prepare, it may be regarded as the medium of choice.

The results of this investigation and those of Bergenholtz (1969) show that oral mucosa can retain its physiological functions and structure *in vitro*. The differentiated cells in an organ culture represent the donor animal better than the dedifferentiated cells obtained by cell and tissue culture. By using the organ culture technique it may therefore be possible to study the direct effect of different factors or drugs on the differentiated target cell or target tissue.

SUMMARY

The ultrastructure of the palatal mucosa of the cat shows close similarities with that of human palatal mucosa.

Palatal mucosa of the cat was maintained in organ culture for a period of 6 days. Two different chemically defined media, Eagle's MEM medium and medium 199 were tested. In Eagle's MEM medium and 50 % oxygen in the gas-phase ultrastructural morphology is preserved for at least 6 days. Specimens cultured in medium 199 revealed alterations in the ultrastructure already after 3 days *in vitro*. After 6 days in medium 199 alterations were detectable also in the light microscope.

RÉSUMÉ

ÉTUDE AU MICROSCOPE OPTIQUE ET AU MICROSCOPE ÉLECTRONIQUE DE LA MUQUEUSE PALATINE DU CHAT ADULTE MAINTENUE EN CULTURE D'ORGANE DANS DES MILIEUX DE COMPOSITIONS CHIMIQUES DÉTERMINÉES

L'ultrastructure de la muqueuse palatine du chat présente une proche ressemblance avec celle de la muqueuse palatine humaine.

La muqueuse palatine du chat a été maintenue en culture d'organe pendant une période de 6 jours. Deux milieux différents de compositions chimiques déterminées ont été testés, le milieu MEM d'Eagle et le milieu 199. Dans le milieu MEM d'Eagle avec 50 % d'oxygène dans la phase gazeuse, la morphologie de l'ultrastructure était maintenue pendant au moins 6 jours. L'ultrastructure des fragments cultivés dans le milieu 199 présentait déjà des altérations au bout de 3 jours *in vitro*. Au bout de 6 jours dans le milieu 199, les altérations étaient aussi visibles au microscope optique.

ZUSAMMENFASSUNG

DIE ERHALTUNG DER SCHLEIMHAUT DER KATZE ALS ORGANKULTUR IN CHEMISCH DEFINIERTEN MEDIEN

EINE LICHT- UND ELEKTRONENMIKROSKOPISCHE UNTERSUCHUNG

Die Ultrastruktur der Schleimhaut der Katze zeigt grosse Ähnlichkeiten mit der der menschlichen Schleimhaut.

Die Schleimhaut der Katze wurde sechs Tage lang in einer Organkultur am Leben erhalten. Zwei verschiedene, chemisch definierte Medien, Medium Eagle MEM und Medium 199, wurden untersucht. In Medium Eagle MEM und 50 % Sauerstoff im Gaszustand wird die ultrastrukturelle Morphologie mindestens sechs Tage bewahrt. Schleimhautgewebe, die in Medium 199 gezuchtet wurden, zeigten schon nach drei Tagen *in vitro* Veränderungen der Ultrastruktur. Nach sechs Tagen in Medium 199 waren die Veränderungen auch im Lichtmikroskop erkennbar.

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