MORPHOLOGY OF CELL GROWTHS OF FETAL LENS ORGAN CULTURES

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When the integrity of the fetal lens in tissue culture is disturbed it undergoes dedifferentiation and incomplete differentiation of the nucleated cells of the lens. Cell growths which form under these circumstances resemble the cellular masses of the lens affected by cataracts [1-3]. The question arises: what is the role of integrity of the lenticular capsule and of the lens itself in the genesis of these abnormal cell growths. Existing information on organ cultures of the lens relates to short-term (1-3 days) cultures of adult animals, used for histophysiologic studies, and no morphological data are available [4]. There are likewise no data in the literature on more prolonged culture of mamalian fetal lenses. The investigation described below was carried out in order to fill this gap.

EXPERIMENTAL METHOD

Altogether 144 lenses from 72 pig fetuses 10-20 cm long were cultured in penicillin flasks closed with rubber stoppers. Eagle's or DMEM medium with the addition of 6% or 20% fetal calf serum was used. Penicillin and streptomycin were added to all media in a dose of 100 U/ml to prevent bacterial contamination. A lens was placed in each flask, covered with 3 ml of nutrient medium, and incubated at 37°C. The medium was renewed on the 2nd day of culture and thereafter every 2 or 3 days, with replacement of 3 and 4 ml throughout the period of culture (30-42 days). The lenses were studied daily with the naked eye to detect any opacity. Material for histological study was fixed on the 2nd-3rd and 6th-7th days, and thereafter at intervals of 1 week until the end of the experiment. Fixation was carried out in Carnoy's and Bouin's fluid or in a 10% solution of formalin. Sections 7-10 μ thick were stained with hematoxylin and eosin. Areas for electron microscopy were chosen under a binocular loupe, cut out, and treated by the usual method for electron microscopy. Very thin sections from the same block of epithelioid and fibroblast-like cells and from five blocks of vesicular cells were studied in the EVM 100BR microscope.

EXPERIMENTAL RESULTS

Original normal fetal lenses for culture were characterized by a simple cubical anterior epithelium, which change on the side of the equator into pseudostratified epithelium in the germinative zone. The cells were then arranged in meridional rows and later formed adult lens fibers (Fig. 1).

After culture for 24 h slight cortical opacity was observed in the zone of the anterior epithelium, and this partly disappeared by the 3rd day in culture. Throughout the period of culture speckled cortical opacities persisted, gradually widening and producing total opacity of the lens after 30-40 days of culture. Under the light microscope, opacities at the beginning of culture corresponded to cortical lacunae, containing fluid, between the cortical lens fibers. Later the opacities were due not only to lacunae, but also to cell growth starting to be formed at that time, in conjunction with lacunae. In Eagle's medium lacunae were found mainly in the equatorial part of the lens, whereas in DMEM medium they were most common in the posterior cortex.

Cells of the anterior epithelium of the lens in culture remained in a single layer, except in certain areas in which, starting with the 3rd day of culture, agglomerations of epithelioid and fibroblast-like cells appeared between the capsule and lens fibers, together with infiltrating cells migrating from the anterior epithelium and insinuating themselves

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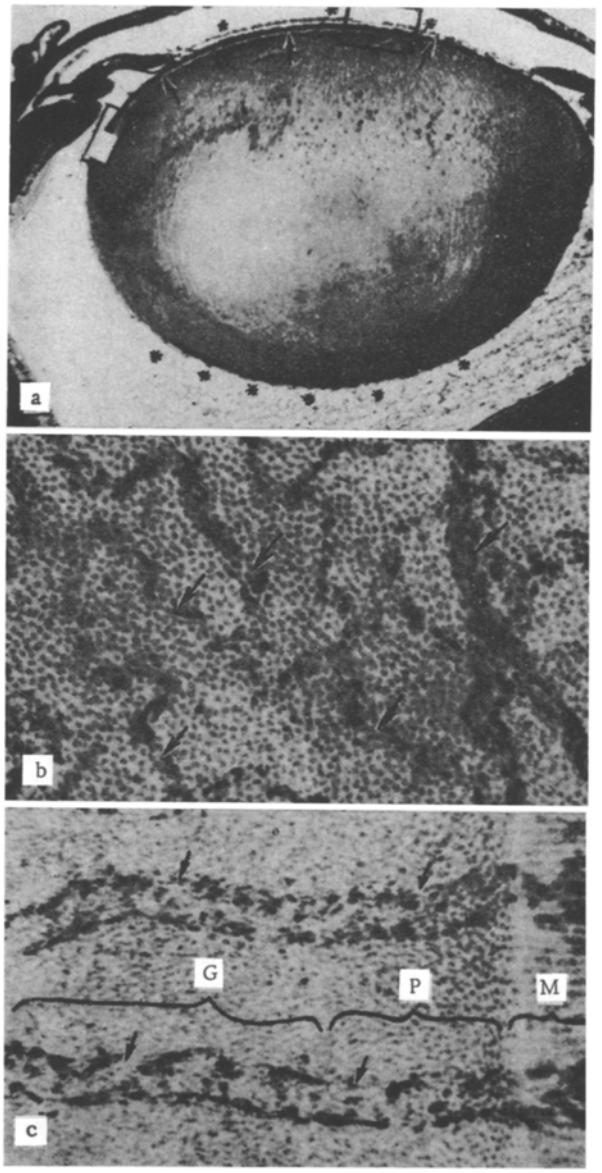

Fig. 1. Morphology of fetal lens. a) Slightly tangential section through fetal eye: simple anterior epithelium (arrows) and fragments of the vascular membrane around the lens (stars) can be distinguished. Carnoy's hematoxylin and eosin. Magnifying glass; b) film preparation of part of anterior epithelium; c) film preparation of part of equatorial region of pig fetal lens: dark cellular bands are blood vessels in vascular membrane of lens (arrows). G) Germinative zone; P) zone of postmitotic cells; M) zone of meridional rows. Hematoxylin and eosin. 200 ×.

between the cortical lens fibers. Some equitorial cells were converted into agglomerations of vesicular cells (Fig. 2). These cellular agglomerations appeared in nearly all the nutrient media used. The over-all prevalence of the different cell types was as follows: in 144 cultured lenses there were 11.1% with epithelioid and infiltrating cells, 20.8% of lenses with fibroblast-like, and 49.3% with vesicular cells. They could be found separately or in various combinations in one lens.

Agglomerations of epithelioid cells consisted of closely packed, haphazardly arranged cells (Fig. 2a), in whose cytoplasm a nucleus with dentate invaginations, a Golgi apparatus, a few mitochondria, cisterns of the smooth and rough endoplasmic reticulum, free ribosomes and polysomes, and microtubules were sometimes found; lysosomes, lipid drops, and microfilaments were often seen. By contrast with the other cells, epithelioid cells made contact with each other through interdigitations of cytoplasmic microvilli (Fig. 3).

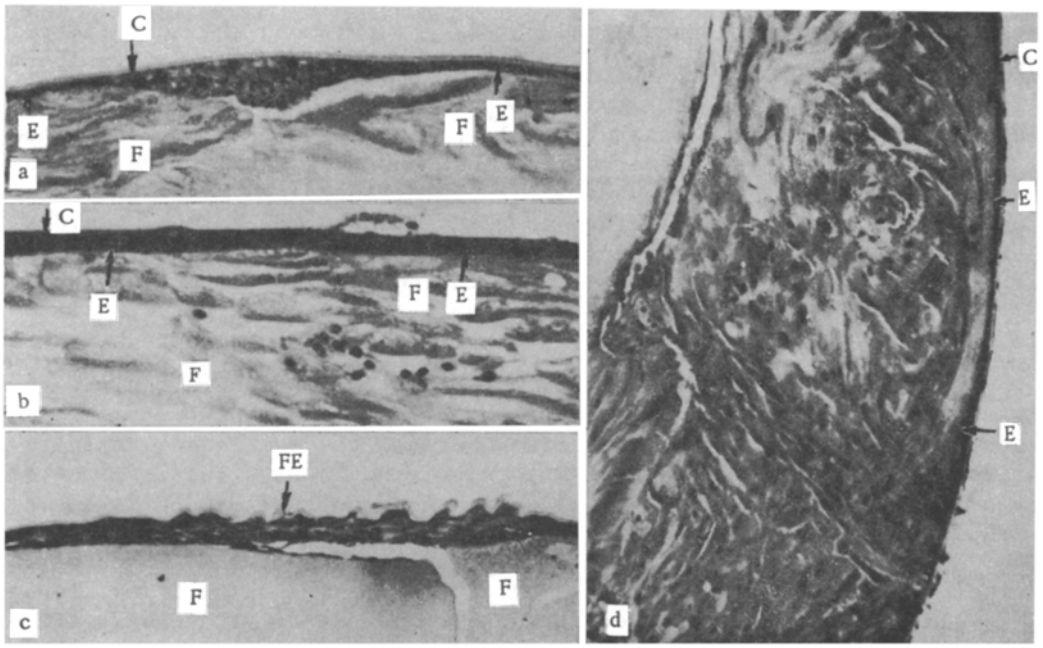

Fig. 2. Growths discovered in 21-day culture, formed of a) epithelioid, b) infiltrating, c) fibroblast-like, and d) vesicular cells. E) Epithelium, C) capsule, F) lens fibers. Hematoxylin and eosin. Magnification: a, c, d) 120, b) 200.

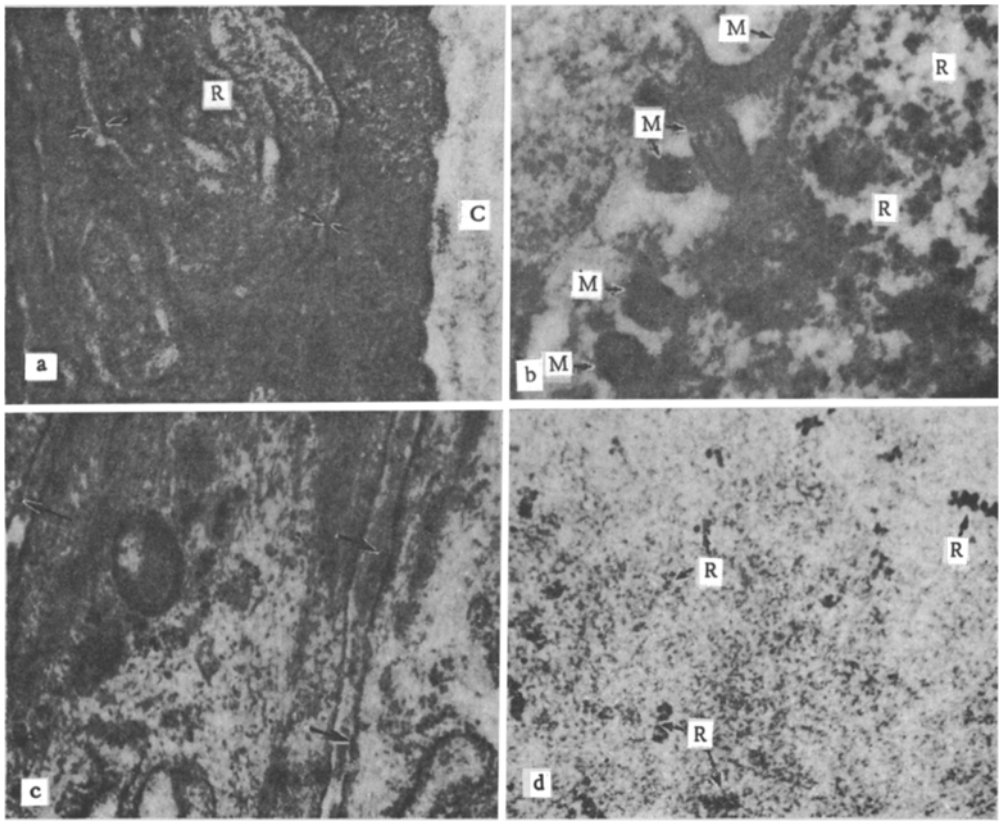

Fig. 3. Electron micrographs of regions of cytoplasm of different types of cells on 21st day of culture. a) Anterior epithelium: cells make contact through slit-like junctions (arrows). Ribosomes (R) can be seen in the electron-dense basic cytoplasm. 123,000 ×; b) Contact between epithelioid cells through interdigitation of microvilli (M). 113,500 ×. c) Ground substance between fibroblast-like cells (arrows). 58,600 ×; d) free and helicoid ribosomes (R) in pale basic cytoplasm of vesicular cell. 92,000 ×.

Infiltrating cells were arranged singly or in groups among the lens fibers, usually close to the anterior star of the lens. Infiltrating cells were round, fusiform, or stellate, with scanty cytoplasm (Fig. 2b).

Agglomeration of greatly flattened fibroblast-like cells (Fig. 2c) attained a diameter of 2-4 mm. Sometimes they were separated from the lens fibers by a layer of lenticular epithelium growing between them. The boundary between cells of the anterior epithelium and an agglomeration of fibroblast-like cells had no transitional cell forms. Besides ordinary intracellular organoids, the fibroblast-like cells also contained relatively many fat droplets and large bundles of microfilaments, which in the polarized light of the light microscope gave a picture of birefringence. The fibroblast-like cells differed from other abnormal cells in culture by the presence of a large quantity of ground substance between them.

Vesicular cells (Fig. 2d) differed from the other cell types in their very small number of the usual organoids (Fig. 3d). They made contact with one another through slit-like junctions, and occasionally 5-layered junctions between the membranes, characteristic of lens fibers, were found. Cell destruction in culture began with infiltrating cells in the 2nd-3rd week of culture. Later the fibroblast-like and epithelioid cells and cells of the anterior epithelium degenerated. The vesicular cells had the longest life of all in the culture. They were distinguishable even after total opacity of the lenses and degeneration of all the other cells. Comparison of the cell morphology of the original lenses and of the cell growth developing in culture showed that cell growths of epithelioid, fibroblast-like, and vesicular cells, characteristic of the pathological lens (affected by cataracts) [7], arise in organ cultures in which the integrity of the lens is preserved as well as in tissue cultures. Consequently, the decisive role in the appearance of abnormal cellular agglomeration in culture is played not by integrity of the lens, but by the medium surrounding the lens in culture.

Agglomerations of fibroblast-like cells appearing in culture are analogs, from the point of view of morphology and arrangement in the lens, of the cell growths of the anterior subcapsular cataract, whereas the vesicular agglomerations are analogs of vesicular or Wedl's cells [6] in the lens affected with cataract. Infiltrating cells found in culture are analogs of the similar cells in the lens of the Japanese quail when affected by cataracts [5]. Agglomerations of epithelioid cells constitute an independent cell type in culture, or some of these agglomerations give rise to fibroblast-like cells. In the late periods of culture both separate epithelioid and fibroblast-like cells and their agglomerations were found, so that both explanations may be true.

Long-term culture of fetal lenses may thus simulate, in a relatively short time, the process of cataract formation and the appearance of cataract-like growths similar to those in experimental animals and in man. The similarity between the cell growths of organ cultures and the lens affected by cataract is so close that long-term organ culture of the fetal lens is the most suitable model with which to study the histopathology of the lens, when compared with cell and tissue cultures. Data on the cell biology of these cataract-like growths, however, are still limited.

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