EFFECT OF HIGH OSMOTIC PRESSURE ON MORPHOLOGY AND CYTOSKELETON OF MOUSE FIBROBLASTS IN CULTURE

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KEY WORDS: osmotic pressure; cytoskeleton; lamella; cell-substrate contacts.

Fibroblasts cultured *in vitro*, on contact with adhesive substrates, spread out on them. The spreading of fibroblasts in culture has often been used as a convenient model with which to study the mechanisms of adhesion of cells to various surfaces. Spreading of cells in culture takes place through the formation of cell outgrowths (pseudopodia) and their attachment to the substrate. Lamellae, thin cytoplasmic plates located at the periphery of the cell, subsequently form from the attached pseudopodia. They have no large organelles but contain numerous bundles of actin microfilaments and microtubules. The lower surface of the lamella forms multiple contacts with the substrate of two main types: "focal" and "close." The dimensions and morphology of the lamella are important cell characteristics: In normal cells the lamella is usually well developed, whereas in transformed cells as a rule it is reduced.

An increased osmotic pressure in the culture medium is one factor which influences lamella formation; through its action the character of movement of receptors on the membrane is altered [1] and the throwing out of pseudopodia is inhibited [2, 3].

The object of this investigation was to study the effect of an increase in tonicity of the medium on the structure and functional characteristics of the lamella in mouse embryonic fibroblasts.

EXPERIMENTAL METHOD

Secondary cultures of embryonic fibroblast-like cells from C3HA mice were used. The cells were subcultured on basic Eagle's medium, diluted in the ratio 1:* with 0.5% lactalbumin hydrolysate, with the addition of 10% calf embryonic serum (from Gibco, USA). Before the experiment the cells were removed from the glass by consecutive treatment with 0.02% EDTA and 0.3% trypsin, suspended in growth medium, and seeded on coverslips measuring 12 × 12 mm^2, which were placed in glass flasks. Low-density cultures (seeded density 5×10^4-1×10^5 cells/cm^2, cultured for 24 h before the experiments began) were used. The tonicity of the solutions was varied by replacing the medium with fresh medium containing 0.25 or 0.5 M D-sorbitol (from Serva, West Germany). The sorbitol solutions were made up immediately before the experiment. The fixed cells were studied in an Opton light microscope fitted with a Nomarskii optical system for interference contrast. To discover the contact site between cell and substrate the method of interference reflection [4] was used. For immunofluorescence staining of the cytoskeletal structures, the cells were treated before fixation with the nonpolar detergent Triton X-100. This procedure and treatment with antibodies against actin and tubulin were carried out by the method described previously [5], using the same immune sera.

EXPERIMENTAL RESULTS

The fibroblasts in culture spread out widely and had large lamellae (Fig. 1a). If these cells were transferred for 1 h into medium containing 0.25 or 0.5 M sorbitol their morphology changed significantly: The area of the lamella was sharply reduced, and those parts of it which remained usually consisted of long outgrowths or had the appearance of a small fan at the edge of the cell. Sometimes perforations could be seen through the lamella of these cells, so that it resembled a wide-meshed net (Fig. 1b). A short stay in medium containing sorbitol caused several changes in the structure of the cytoskeleton. Such cells contained fewer microtubules, which lost their parallel orientation and became convoluted in form. Control cells were characterized by powerful bundles of microfilaments over 1 μ thick, running parallel to each other along the whole length of the cell (Fig. 2a). In cells treated with sorbitol the bundles of microfilaments were shorter, much shorter than the length of the cell, and their total number was reduced. Usually the thickness of these bundles did not exceed 0.2 μ (Fig. 2b).

Cells incubated for 1-2 h in medium containing 0.5 M sorbitol recovered their normal morphology when transferred back to the ordinary medium. Prolonged (24 h) exposure to 0.5 M sorbitol led to irreversible changes and death of the cells.

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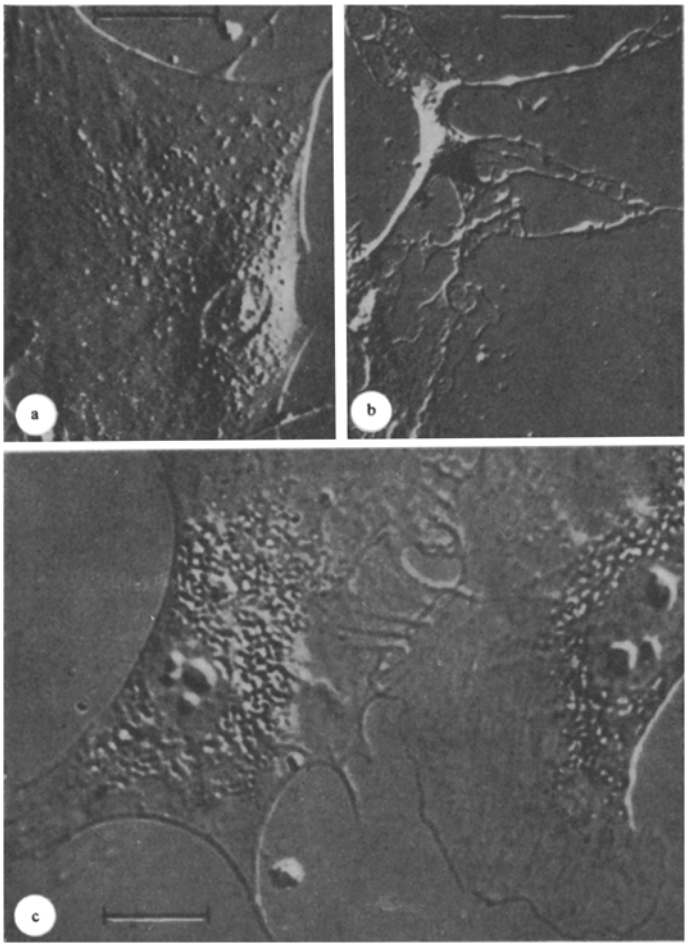

Fig. 1. Changes in morphology of mouse embryonic fibroblasts caused by incubation in medium containing sorbitol. a) Cells from control 24 h culture; b) cell from same culture after incubation for 1 h with 0.5 sorbitol; c) similar cells after incubation for 24 h with 0.25 M sorbitol. Nomarskii optical system. Scale 20 μ.

Conversely, if fibroblasts were incubated for 24 h with 0.25 M sorbitol the morphological changes observed after 1 h in this solution disappeared completely, so that in their shape and size the cell lamellae were indistinguishable from the control (Fig. 1c). However, compared with normal cells, these cells had acquired certain new properties. First, a change was observed in the structure of their actin skeleton. Powerful bundles of microfilaments, more than 1 μ thick and running parallel to each other along the whole length of the cell, disappeared. Instead short, thin bundles were found, just as during short exposure to sorbitol. Cells completely without any such bundles also were seen. Sometimes wide (up to 25 μ in diameter), more or less uniformly fluorescent actin-containing regions in which discrete thin bundles entered from both ends could be seen in the cytoplasm. A very characteristic feature of cells incubated for a long time in 0.25 M sorbitol was the presence of a semicircular band, about 10 μ wide, located at the edge of the cell and consisting of short, closely packed bundles. Second, a change was found in the character of the contacts with the substrate. Normal fibroblasts have both "focal" and "close" contacts, located chiefly in the lamella (Fig. 3a). After incubation of the cells for 24 h in medium with sorbitol the focal contacts changed their shape and became far fewer in number. The "close" contacts still persisted in these cells and the impression was obtained that they increased in area compared with normal (Fig. 3b). Because of the absence of focal contacts, cells exposed to the prolonged action of 0.25 M sorbitol were not less firmly attached to the substrate. On the contrary, to remove them from the substrate by means of standard solutions of EDTA and trypsin, a much longer time was needed than in the case of normal fibroblasts.

Microtubules which were partly destroyed in the cells as a result of short exposure to the action of sorbitol reacquired their original appearance after prolonged incubation in the same solution.

Cells with an altered structure of the lamella are adapted to increased osmotic pressure of the culture medium: They can exist for a long time under such conditions and can even multiply (although more slowly than normally) – during 5 days of culture in medium with 0.25 M sorbitol their number increased about fourfold, compared with about 16-fold in normal medium.

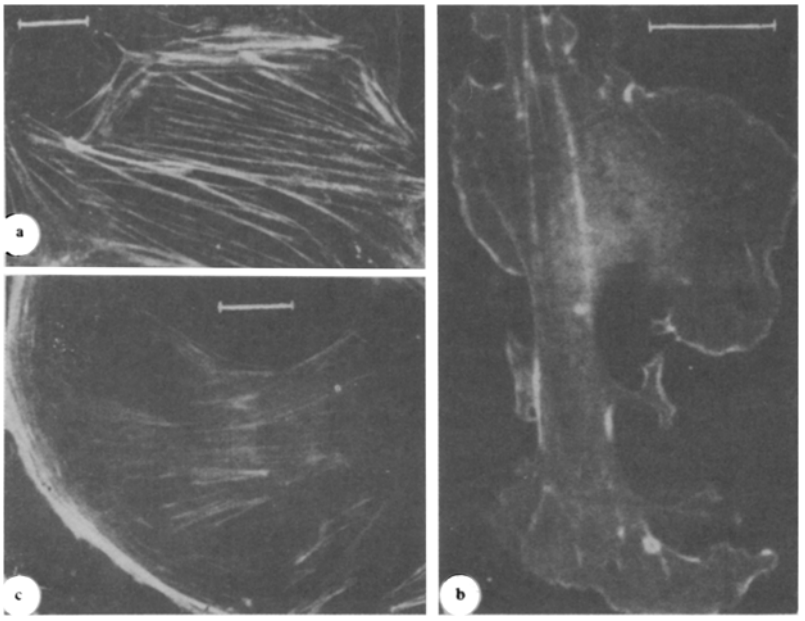

Fig. 2. Changes in structure of actin cytoskeleton following incubation in medium containing sorbitol. a) Cells from control 24-h culture; b) cells from same culture after incubation for 1 h with 0.5 M sorbitol; c) cell from similar culture after incubation for 24 h with 0.25 M sorbitol. Indirect immunofluorescence staining with antiactin. Scale: 20 μ.

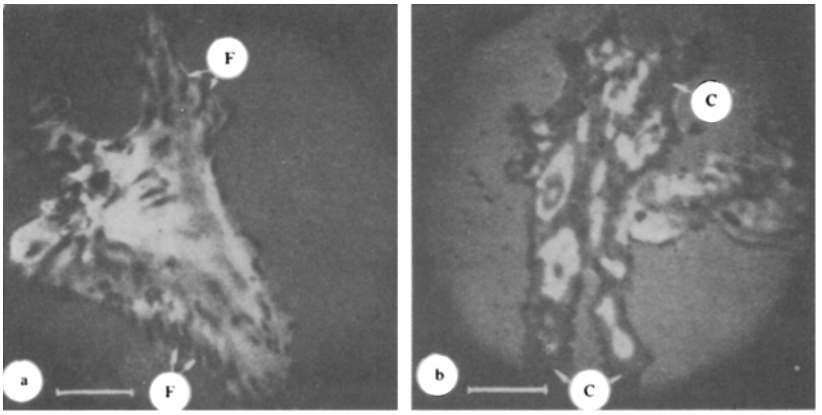

Fig. 3. Changes in character of cell-substrate contacts following incubation in medium with sorbitol. a) Cell from 24-h control culture; b) cell from similar culture after incubation for 24 h in medium with 0.25 M sorbitol. F) "Focal" contacts, C) regions of "close" contacts. Interference reflection method. Scale 20 μ.

It has hitherto been considered that the presence of long parallel bundles of microfilaments and of focal contacts is an essential feature of a well developed lamella [6-8]. The data described above show that cells cultured for long periods under conditions of an increased osmotic pressure form a lamella with a different structure, which contains neither actin bundles of the usual type nor focal contacts. Lamellae with this structure have not previously been described. The modified lamellae, like the ordinary type, evidently ensure firm attachment of the cells to the substrate and maintain the cells in the spread out state.

Further investigation of the properties of cells with lamellae of the same shape, but with differences in internal structure, may help toward the understanding of functions of bundles of microfilaments and cell-substrate contacts of different types.

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MORPHOLOGICAL AND FUNCTIONAL CHARACTERISTICS OF MOUSE LIVER MITOCHONDRIA (MORPHOLOGICAL AND BIOCHEMICAL STUDY)

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Mainly qualitative criteria of assessment are used in electron-microscopic investigations of cell organelles. However, some aspects of submicroscopic cytology can be investigated by quantitative methods of morphometry, which can be used to evaluate electron-micrographs [4, 5].

The object of this investigation was to study correlation between the dimensions of intact mitochondria and biochemical parameters of their activity.

EXPERIMENTAL METHOD

Hybrid male CBA × C57BL mice of the same age and weight (22 g) were used. Each point was represented by five mice. Mitochondria were isolated from the liver on two centrifuges (parallel for the control, normal state, and after γ-ray irradiation of the animals in a dose of 750 R at a dose rate of 641 R/min). Time in the experiment was counted from irradiation (5, 30, and 60 min after irradiation, thereafter every 60 min until 8 h). The experiment included two repetitions. Mitochondria were isolated by differential centrifugation in medium of the following composition: 0.3 M mannitol, 0.01 M Tris buffer, and 0.1 mM EDTA, pH 7.4. Respiration of the mitochondria was studied by a polarographic method, using the PO-4 polarograph (Denmark) with rotating platinum electrode in a 1-ml cell at 26°C. The composition of the incubation medium was: 0.2 M sucrose, 0.03 M Tris buffer, 0.01 M $MgCl_2$, 0.015 M KCl, 0.02 M KH_2PO_4, 0.2 mM EDTA, pH 7.4. The oxidation substrate was succinic and glutamic acids (5 mM). ADP (0.15 mM) was added. For counting the mitochondria a suspension of organelles was used after completion of their respiration cycle in a polarographic cell (i.e., in fact in stage 4 after Chance). Mitochondrial respiration showed the greatest fluctuations in Chance's state 3 (i.e., during phosphorylation of the added ADP), and for that reason data on the rate V3 of mitochondria isolated from the liver of irradiated mice are plotted on the graph in Fig. 1. The area of the mitochondria, calculated by the equation $S = \pi ab$, where S is the area of the mitochondria, their smallest radius, and b their greatest radius, was used as the morphometric test for assessing the state of the mitochondria. Altogether 1500 mitochondria were analyzed; a suspension of them was applied to a grid and negatively stained with 10% phosphotungstic acid solution.

The results of calculations for the test mitochondria were reduced to continuous variance series and broken down into classes by means of Stendzhes' equation $i = \frac{x_{max} - x_{min}}{1 + 3.32 \ln h}$, in which i is the class interval, x_{max} the maximal variant,

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