EFFECT OF POLYETHYLENE GLYCOL ON ULTRASTRUCTURAL EXPRESSION OF CELL FUSION IN RAT NEURINOMA CULTURE

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KEY WORDS: membranes; polyethylene glycol; fusion.

Polyethylene glycol (PEG), a high-molecular-weight polymer, is widely used in experiments in cell fusion in order to obtain cell hybrids [4, 5]. Most attention has so far been concentrated on the development of the technique of fusion and the establishment of optimal conditions for obtaining fusion, and the morphological aspects of the action of PEG on the cell at the ultrastructural level have been inadequately studied [7]. The object of this investigation was to make a dynamic study of the morphology of the plasma membranes during the action of PEG by transmission electron microscopy.

EXPERIMENTAL METHOD

Experiments were carried out on a transplantable cell line obtained from a neurinoma of the rat gasserian ganglion. The cells were cultured in Eagle's medium with the addition of 15% bovine serum, 15% of calf serum, and 5% chick embryonic extract.

Cell cultures at the 2nd-3rd day of growth were used for fusion. The fusion procedure was based on Hales' method [6]. PEG (mol. wt. 1000) was applied twice with an interval of 2 min to a residue of cells obtained by centrifguation and washed with Hanks' solution to remove serum. The first time a 41.7% solution of PEG, made up in RPMI medium containing 15% dimethyl sulfoxide was used, the second time a 33% solution of PEG. The total time of treatment was 6 min. The cell suspension treated at room temperature was diluted with serum-free Eagle's medium and incubated for 4 min to obtain better agglutination of the cells. One portion of cells was then fixed immediately, the other, diluted beforehand to a concentration of 150,000 cells/ml, was seeded in culture flasks on coverslips and incubated at 37°C for 10, 20, and 30 min and 1 and 2 h. The nutrient medium was changed after 2 h and subsequent fixation carried out after 3, 6, and 10 h. The cell suspension for electron-microscopic study was centrifuged at 1500 rpm for 3 min and fixed with 1.5% glutaraldehyde in 0.1 M cacodylate buffer, dehydrated, stained with 0.5% uranyl acetate in 70° alcohol, and embedded in a mixture of Epon and Araldite. The cell monolayer on the coverslips was fixed by a similar method and embedded in flat molds. The material was separated from the glass by placing the blocks in liquid nitrogen [1]. Ultrathin sections were cut on the LKB-111 Ultrotome, stained with lead citrate by Reynolds' method, and studied in the IEM-100B electron microscope. The glycocalyx was demonstrated with ruthenium red by Luft's method [2].

EXPERIMENTAL RESULTS

The experiments showed that in the early stages of the action of PEG agglutination of the cells takes place. After 10-15 min, a local arrangement of granules of electron-dense material measuring 30-35 nm was observed at sites of contacts between membranes of neighboring cells (Fig. 1: a, b). After dilution of the cell suspension with medium and incubation at 37°C, starting from 10-20 min the plasma membranes acquired indistinct, blurred contours and took on the appearance of a homogeneous layer in regions where they were locally close together (Fig. 1c). Near the fused plasmalemmas bundles of microfilaments were sometimes present. After 20-30 min common regions of cytoplasm appeared between the neighboring cells (Fig. 1d).

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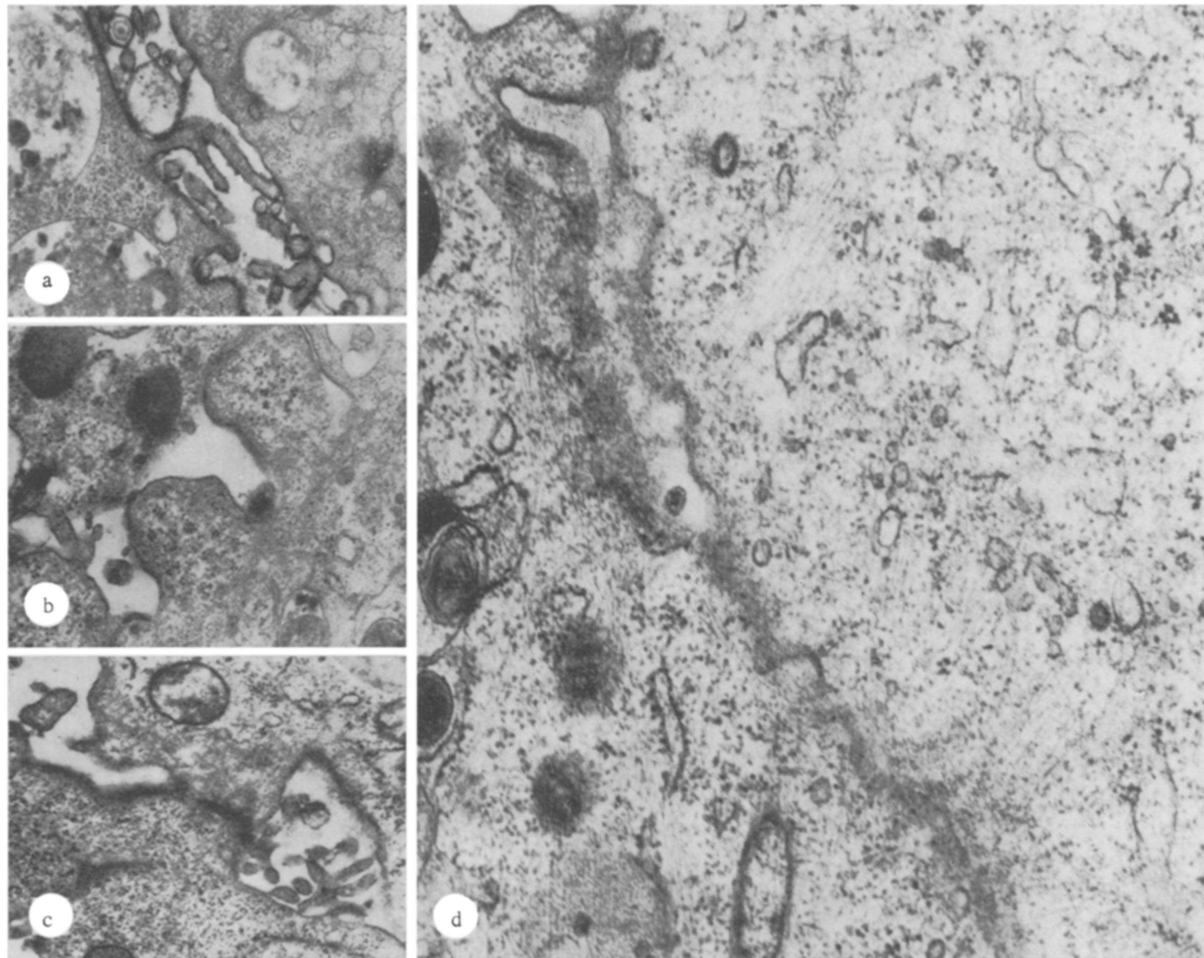
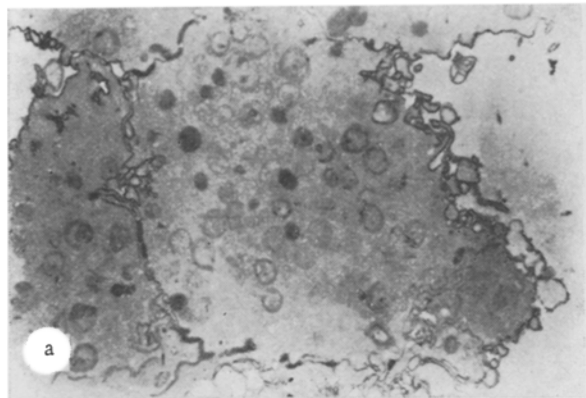


Fig. 1. Cells of rat neurinoma culture after treatment with PEG. a) 10 min after treatment; electron-dense material on surface on microvilli and on plasmalemma. 12,000 ×; b) electron-dense material in zone of contact of pseudopodia with smooth surface of neighboring cell. 20,000 ×; c) 10 min after incubation of cells at 37°C. Broad contours of plasmalemmas at sites of contact of pseudopodia of neighboring cells. 20,000 ×; d) 20 min after incubation at 37°C. Broad outlines of membranes; bundles of microfilaments near fused plasmalemmas. Common regions of cytoplasm between two cells. 45,000 ×.

The study of the state of the supramembranous surface layer of cells was very interesting. It has been suggested that this layer may prevent the process of fusion. The glycocalyx layer in a cell culture untreated with PEG appeared over the whole cell surface in the form of an electron-dense layer, and in isolated areas as a looser layer about 30 nm thick (Fig. 2a). After treatment of the cells with PEG the glycocalyx was no longer visible (Fig. 2b). Carbohydrates present on the cell surface evidently underwent certain changes or were eliminated, thus facilitating approximation of the subjacent membranous layers.

After 1 and 2 h numerous polykarya were observed in the culture (Fig. 3). In some cases the number of nuclei reached 100. This effect of PEG was possible only when dimethyl sulfoxide was added to the incubation medium; this substance increases the permeability of cell membranes [12].

PEG is known to cause agglutination of cells on account of adsorption of its molecules on the cell surface, through its hydrophilic properties and its ability to bind calcium ions [3]. The question of which structures are responsible for the initial stages of fusion has not yet been settled. Some workers consider that cell fusion is possible only by smooth surfaces free from microvilli [11], whereas others consider that the formation of structures resembling pseudopodia is essential [9]. Approximation of cells and the establishment of close contacts in local regions of plasmalemmas in the region of microvilli, pseudopodia, and smooth cell surfaces were observed.



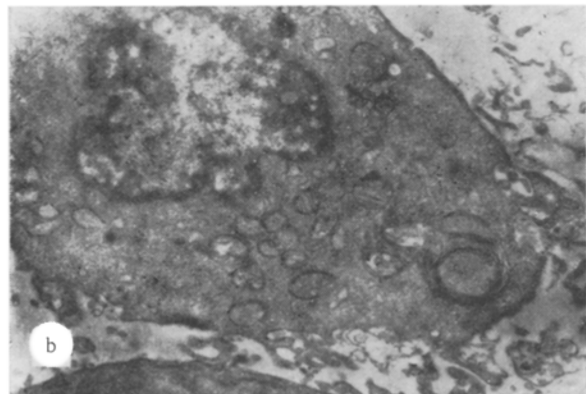


Fig. 2

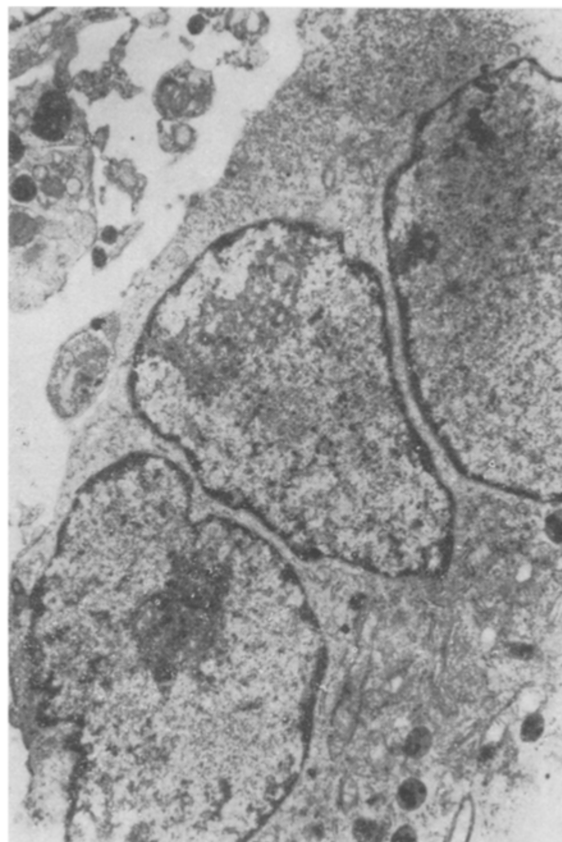


Fig. 3

Fig. 2. Cells of rat neurinoma culture. Stained with ruthenium red by Luft's method. a) Glycocalyx revealed on surface of cells not treated with PEG. 5000 ×; b) no reaction after treatment with PEG. 10,000 ×.

Fig. 3. Polykaryon. Trinuclear fragment. 10 h after treatment with PEG. 12,000 ×.

PEG molecules on the cell surface are considered to act as a barrier to their fusion, and the process itself takes place only after removal of this agent [10].

The electron-dense material found in the zone of serveral contacts in the early stages of the action of high concentrations of PEG before the beginning of incubation of the cells may perhaps be a complex of fusagen with the components of the cell after its removal, this material could not be detected.

It can accordingly be postulated that PEG molecules, after adsorption onto the cell surface, interact with the supramembranous surface layer and also, perhaps, with the intramembranous components of the cells. During washing with medium the PEG was evidently removed together with the components, exposing the subjacent hydrophobic layers, at which level, as many workers consider, fusion actually takes place [3, 8]. Morphologically this process consists in disorganization of the membranes at sites of contact and the formation of common regions of cytoplasm between neighboring cells.

The action of PEG on tumor cell membranes can thus be represented as several successive stages: adsorption of PEG on the cell surface, leading to agglutination of the cell; modification of the supermembraneous layer and its possible elimination in the course of washing off of the fusagen; disorganization of membranes at sites of local contact between neighboring cells and the formation of common regions of cytoplasm between them.

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QUANTITATIVE HISTOPHOTOMETRIC STUDY OF LIVER DEHYDROGENASE ACTIVITY DURING TEMPORARY LIMB ISCHEMIA

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One of the most serious complications of certain cardiovascular disease is arterial occlusion caused by thrombosis or embolism of the main arteries. The increase in the number of patients treated surgically for this condition has shown [6, 14, 17] that mortality after reconstructive vascular operations lies between 15 and 35% [10, 19]. Restoration of the circulation in ischemic limbs is known to lead to a systemic stress reaction, characterized by the development of a complex group of morphological and biochemical changes both in the affected tissue and in the body as a whole, and which in the recent literature has been called ischemic shock or the postischemic syndrome (PIS) [7]. The mortality from development of PIS is 25-40% [14, 17], and one cause of this is acute hepatorenal failure.

The liver occupies a central position in the regulation of metabolism, in the binding and neutralization of toxic substances of exogenous and endogenous origin. The morphological study of changes in hepatocyte metabolism in PIS is thus essential for the understanding of the key components of the pathogenesis of early postischemic disturbances. The important role of the liver in occlusion of the aorta and the main limb arteries and in states with similar pathogenesis has been stated repeatedly [1, 16, 18]. However, data in the literature on this problem are fragmentary [8] and the research has been done mainly at the biochemical level [11]. No reports of the quantitative enzyme histochemical study of changes in the liver in acute arterial occlusion of the limbs could be found in the accessible literature. Yet such an evaluation is necessary in order to elucidate the mechanisms of disturbance of hepatocyte activity under experimental and clinical conditions.

On the basis of previous experience of the use of a television image analyzer [4, 15] it was decided to undertake a quantitative study of the enzyme histochemical characteristics of changes in the liver during temporary limb ischemia followed by revascularization, and also during pharmacological correction in the ischemic and postischemic periods.

EXPERIMENTAL METHOD

Experiments were carried out on 31 mongrel dogs of both sexes (including five control dogs) weighing 13-18 kg. Occlusion of the trifurcation of the aorta was produced by the method of Zatevakhin et al. [5]. The duration of ischemia of the limbs was 3, 6, and 12 h, and the subsequent period of revascularization lasted 2 h (the surgical part of the experiment was performed by Candidate of Medical Sciences N. P. Istomin.). Correction was carried

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