of the thymus with respect to one of the determinant qualities of HSC proved to be unsuccessful. Removal of the thymus during its period of maximal activity does not affect an important parameter of HSC such as its proliferative potential. It is not clear, however, whether the thymus and its derivatives are in fact involved in the regulation of the property of HSC chosen for study, or whether a change in proliferative potential of HSC is not recorded under conditions of stable hematopoiesis.

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EFFECT OF HIGH ELECTROLYTE CONCENTRATIONS ON THE PLASMA MEMBRANE AND ITS GLYCOCALYX

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Among all the membranes that play an important role in the life of animal cells, several can be picked out and, in particular, the plasma membrane, which performs several functions including transport of nutrients and of inorganic ions into and out of the cell. An active role in these functions is played by the outer juxtamembranous layer, or glycocalyx, which has the function of a cation exchanger [1]. There are many different ways of isolating membranes in order to study their activity physicochemically. Most frequently these methods include destruction of the cell and isolation of fragments of the plasma membrane from homogenates in various ways, such as centrifugation in a density gradient [2]. In our investigations the observation and study of the behavior of these membranes were linked with investigation of the mechanism of halo formation around living cells. In our view, a halo is formed

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through conformational changes in the surface of central (halo-forming) cells, as a result of the reaction of living cells to the action of a high electrolyte concentration. We make allowance for the fact that under these conditions the electrostatic charge of the surface structure of the cell is in a dynamic state and is maintained by the metabolic activity of the cell [3]. Since in this particular case there is a shift (oscillation) of the electrically charged components of the plasma membrane and its outer juxtamembranous layer (the glycocalyx), we postulate that it is in these structures that the long-range electromagnetic forces involved in halo formation [4] are formed, as the theoretical and experimental studies of other workers have shown [5-8].

In the investigation described below an attempt was made to discover and study (or evaluate) the surface structures of the cell which may generate long-range electromagnetic oscillations in the process of formation of haloes with a radius of up to 100 μ.

EXPERIMENTAL METHOD

The investigation was conducted on a strain of human tumor cells (carcinoma of the ovary) and on blood leukocytes from 17 healthy donors.

Procedures carried out on the plasma membranes are based on the method of detection of halo-forming leukocytes, the essence of which is that to one drop of cell suspension (blood, cell culture) on a mixing slide is added 10 drops (volume 0.03 ml) of 15% NaCl solution with ink [9]. All are mixed, and one drop of the mixture (0.03 ml) was applied to a slide and covered with a coverslip, the specimen being isolated from the air with petrolatum. The specimens were examined in a Leitz light microscope under magnification of 100, 400, and 900 times.

EXPERIMENTAL RESULTS

Examination of the specimen after exposure of the cells to a high electrolyte concentration revealed: 1) the appearance of jets of liquid leaving the cell through the plasma membrane; 2) reversible shrinking of the plasma membrane and cell as a whole; 3) the formation of external vacuoles, i.e., the appearance of new plasma membranes near the surface of the cell in the form of hollow vesicles, filled with fluid; 4) the formation of a gel-glycocalyx (or swelling of the outer juxtamembranous layer) on the surface of the plasma membrane of the cell.

Analysis of the four different processes mentioned above shows that the jets of fluid arise immediately after contact between tumor cells and electrolyte (Fig. 1a). Against the dark background of particles of ink it could be seen that the height (length) of these jets varied from 0.8 to 1.8 μ. The bases of the jets were equidistant and 0.6 μ apart (estimated visually). We consider that the base of the jets consists of pores, arranged in a definite order in the plasma membrane, A process of destruction of the membrane in these cases was not observed.

The process of expulsion of the jets of fluid from the cell through the plasma membrane takes place as a result of compression of the cell by the high electrolyte concentration. Reversible compression of the cell takes place for a few seconds, and in this case it is natural to suggest that the sodium and other pumps in the plasma membrane do not function in a situation so stressful for the cell. Probably during the escape of fluid from the cell not only do the sodium pumps, dependent on the action of the enzyme ATPase, not function, but action potentials likewise are not observed.

They are perhaps completely blocked during the period of transfer of huge masses of water (up to 0.5 of the volume of the cell) outside the cytoplasm.

After 1-3 min the jets of fluid cannot be observed visually, i.e., in our view the jets of fluid dry up because of the establishment of osmotic equilibrium (according to the law of osmosis, part of the free water left the cell and part of the electrolyte NaCl entered the cell).

Next, vacuoles, i.e., large spherical "sacs," bounded by plasma membrane, which stains with methylene blue (Fig. 1b-d), begin to form on the surface of the plasma membrane of the tumor cells (or in the membrane itself or inside the cell).

The dynamics of the reaction of the (tumor) cells studied to the action of a high electrolyte concentration can be described as follows:

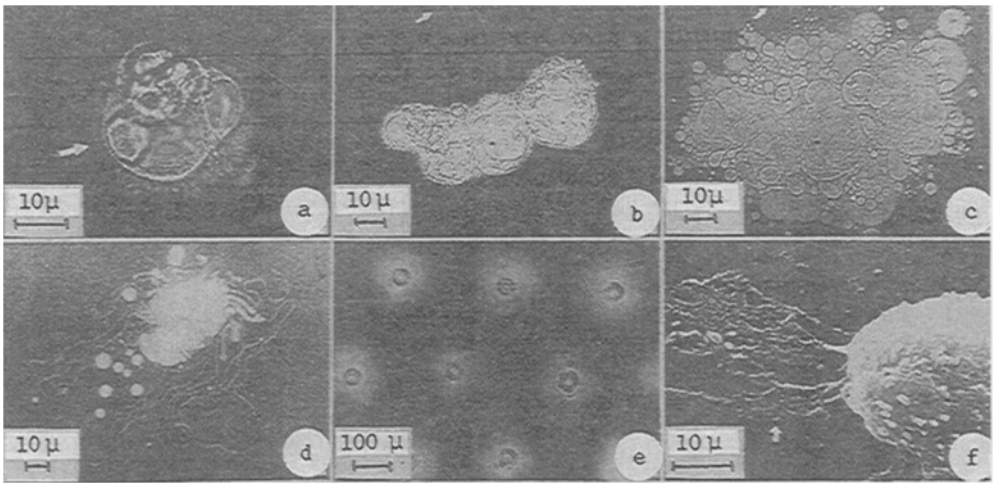

Fig. 1. Exposure of cell to high electrolyte concentration: a) jets of fluid above surface of cell (carcinoma of ovary); b, c) vacuole formation on cell surface; separation of vacuoles (coordinate indicated by arrow); d) destruction of vacuoles (formation of "ghosts"); e) formation of gel-glycocalyx near cell surface (leukemiccells); f) reticular structure of gel-glycocalyx near surface of donor's blood leukocyte (network indicated by arrow).

1 min – the fluid leaves the cell in jets, and vacuoles 1 μ in diameter appear;

3 min – the jets dry up and the vacuoles enlarge to 10-15 μ;

5 min – separate vacuoles, of varied diameter, are pinched off the surface of the plasma membrane as an independent unit with a diameter of between 1 and 10 μ;

40 min – the vacuoles increase in size to 10-20 μ and new ones appear;

20 h – the vacuoles enlarge to 20-80 μ;

136 h – the vacuoles enlarge to 100 μ in diameter, new vacuoles appear, some separate from the cell surface, which is followed by destruction of the vacuoles; their contents escape and "ghosts" of the plasma membranes remain,

Enlargement of old vacuoles and the appearance of new ones after exposure of the cell to high electrolyte concentration for 5 days indicates that the process of cell death (according to our observations, until the 10th day) is accompanied by enlargement of the vacuoles and the appearance of new ones.

This fact also confirms the view that the living cell resists the action of the electrolyte during all this time, and the mechanism of resistance is active synthesis of plasma membranes for the creation of vacuoles which contain water and the excess of NaCl for the cell.

Incidentally, besides the reaction of vacuole formation, cells, such as leukocytes (and leukemic cells), do not form vacuoles, but for up to 10 sec they form the gel-glycocalyx. This external juxtamembranous layer ("thick membranes") in isotonic medium measures 2-4 nm [10], but in hypertonic medium it enlarges to 20-30 μ in diameter, and forms a reticular structure (Fig. 1e, f).

Preliminary chemical analysis showed that the gel contains hyaluronic acid and proteins with varied molecular weight.

It can thus be concluded from the facts described above that the living cell, when placed in a high concentration of electrolyte, responds initially by a change in structure of the outer juxtamembranous layer (the formation of a gel-glycocalyx) and by active synthesis of plasma membranes. It can be tentatively suggested that the most correct role in the formation of long-range forces during halo formation is played by swelling of the glycocalyx and reduction in the size of the plasma membrane.

Swelling of the glycocalyx takes place for 4-12 sec as a result of electrostatic interaction of Na and Cl ions with the molecular components of the outer juxtamembranous layer (of hyaluronic acid type). The electrically neutral gel thus formed blocks the cell surface mechanically from the external electrolyte.

Reduction (compression) of the surface of the plasma membrane leads to approximation of the ionogenic groups and condensation of the electric charge. Displacement of electrically charged molecular components of the membrane surface induces the appearance of electromagnetic waves, emitted outside the cell and capable of forming haloes in the medium from erythrocytes for a period of 10-30 min, and with a radius of action of up to 100-150 μ.

Analysis and calculation of the physical parameters of the change in surface of the plasma membrane and its outer juxtamembraneous layer provides a practical approach to the substantiation of the hypothesis that electromagnetic forces are involved in halo formation.

To assess the specificity of the reactions described above, their mechanism, and their possible practical use, more intensive and extensive investigations are needed.

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