REDISTRIBUTION OF CONCANAVALIN A RECEPTORS ON THE SURFACE OF ENUCLEATED CELL FRAGMENTS

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Interaction between cell surface receptors and multivalent ligands leads to the combining of receptors into groups or patches, and later to migration of the patches into a definite region of the surface ("capping" or cleaning of the receptors). Later these capped receptors may undergo endocytosis [8]. The ability of the cell to redistribute its receptors is evidently decisively important in many biological processes: cell reactions to hormones and growth factors, adhesion of cells to other surfaces, and so on. Until recently capping has been observed only in experiments on nucleated cells (lymphocytes, fibroblasts, epithelial cells) [2, 6, 8]. However, the role of the nucleus and the various components of the cytoplasm in this process still remained unexplained: Is integrity of the cell structure essential for its implementation?

The aim of the present investigation was to study the ability of enucleated cells, preserving the principal cytoplasmic structures (cytoplasts), and of small fragments of cytoplasm to redistribute receptors of the plant lectin concanavalin A (Con A).

EXPERIMENTAL METHOD

A secondary culture of fibroblasts obtained by trypsinization of mouse embryos was used. The cells were spread out on glass or plastic disks with a density of 34×10^3 cells/cm^2 and cultured 24 h at 37°C in Eagle's medium with 45% lactalbumin hydrolysate and 10% bovine serum.

A modified method of Prescott et al. [7] was used for enucleation. The disks were placed with the cells underneath in a centrifuge tube with medium containing 10 μg/ml of cytochalasin B (from Feinbiochemica) and centrifuged for 20 min at 13×10^3 rpm and 37°C in a T24 centrifuge (from Nanetzki). After enucleation the disks with cells were transferred to medium without cytochalasin B.

Preparations for scanning electron microscopy were fixed in 2.5% glutaraldehyde, dehydrated in a series of ethyl alcohols, dried by the critical point crossing method in a Balzers Union apparatus, and sprayed with gold [3]. The technique of indirect immunofluorescence microscopy of the microtubules and microfilaments, which was described in detail previously [1], was as follows. To begin with membranes and the soluble part of the cytoplasm were extracted with 1% Triton in M buffer and 4% polyethylene-glycol (mol. wt. 40×1 10^3) and fixed in 4% formaldehyde, and in some cases additional extraction of the membranes was carried out with acetone. The preparations were then stained with antibodies against tubulin or actin.

Immunofluorescence detection of Con A receptors comprised successive incubation of cytoplasts and fragments with Con A (from Serva) for 10 min and with rabbit antibodies against Con A (from Calbiochem) for 10 min. The disks were then transferred for 40 min into medium, after which the cytoplasts and fragments were fixed with 4% formaldehyde. The fixed

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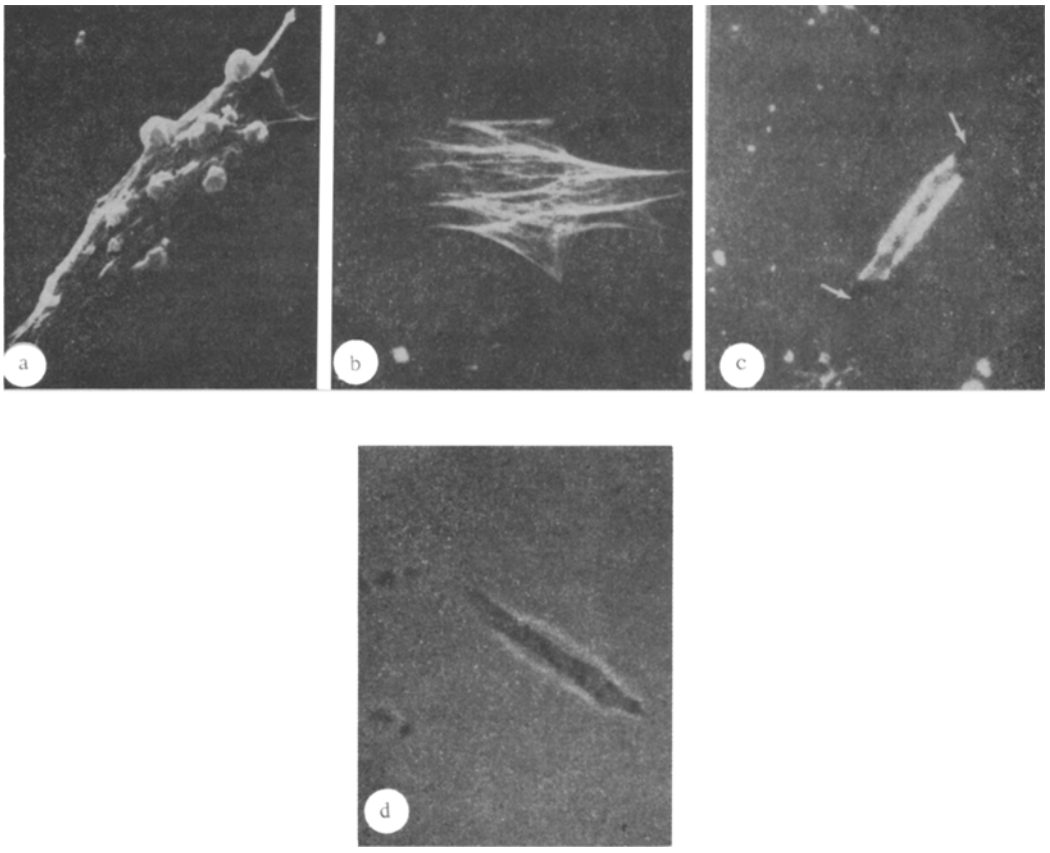

Fig. 1. Cell fragments: a) scanning electron microscopy, 4400 ×; b) microfilaments. Immunofluorescence microscopy, 3000 ×; c) capping of Con A receptors on the surface. Black areas on the ends of the fragment free from receptors can be seen (arrows). Immunofluorescence microscopy, 4000 ×; d) the same field of vision in the phase-contrast microscope, 4000 ×.

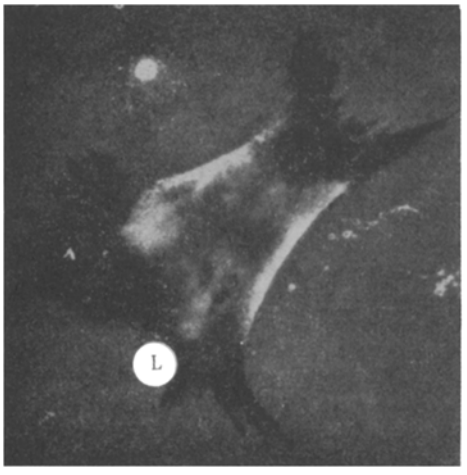

Fig. 2

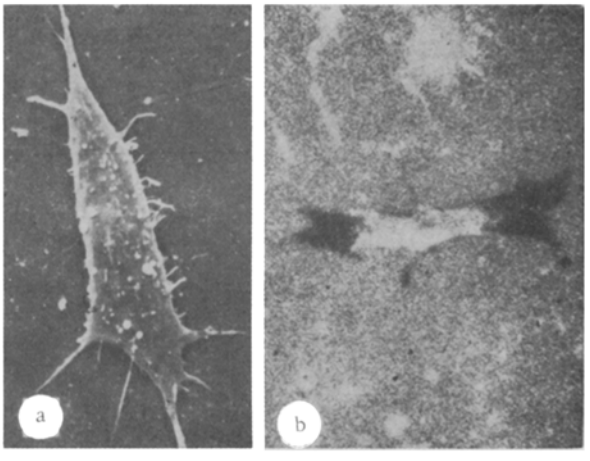

Fig. 3

Fig. 2. Capping of Con A receptors on surface of cytoplasts. L) Cleared lamellar. Immunofluorescence microscopy, 2500 ×.

Fig. 3. Cell fragments in colcemid (3 μg/ml, 6 h). a) Scanning electron microscopy, 900 ×; b) capping of Con A receptors on surface of fragment. Immunofluorescence microscopy, 400 ×.

preparations were stained with porcine antirabbit antibodies (from Miles), associated with fluorescein isothiocyanate (10 min). Preparations stained by the indirect immunofluorescence method were examined under the luminescence microscope (from Opton) in UV light, and also by phase-contrast microscopy. In the experiments with colcemid, cytoplasts and fragments were incubated for 6 h in medium containing 3 μg/ml of colcemid.

EXPERIMENTAL RESULTS

After enucleation, the enucleated cells (cytoplasts), resembling nucleated fibroblasts in shape and size, remained on the disks. Bundles of microfilaments and microtubules were found in the cytoplasm of the enucleated cells. The microtubules of the cytoplasts were more twisted than those of the control cells.

Besides cytoplasts, small cell fragments (from 10 to 50 μ) also were found on the disks (Fig. 1). Usually they were long, with small lamellar areas and with filopodia at their ends; spheres, microvilli, and folds could be seen on their dorsal surface. Bundles of microfilaments, arranged lengthwise, could be seen in the cytoplasm of the fragments. Immunofluorescence staining of tubulin showed single microvilli in large fragments, but none whatever in small fragments. The presence of bundles of microfilaments in fragments of cytoplasm of the fibroblast, preserving pseudopodial activity, also was observed by Albrecht-Buehler [4].

The original distribution of Con A receptors in the cytoplasmic membrane was diffuse in character: Receptors were uniformly distributed throughout the membrane. Incubation of the cytoplasts and fragments with Con A led to removal of receptors from the peripheral zones into the central region. During the immunomorphological study only the central region gave specific fluorescence, whereas dark areas free from receptors appeared at the periphery. Parallel phase-contrast microscopy of the same cytoplasts and fragments showed that the dark regions correspond to lamellar areas (Fig. 2).

Colcemid did not change the shape of the cytoplasmic fragments, which remained long, and pseudopodia and a lamella formed chiefly in the two polar regions (Fig. 3). The bundles of microfilaments and the direction of capping of the Con A receptors on the surface continued to be oriented lengthwise. Thus not only fibroblasts containing a nucleus, but also enucleated cells (cytoplasts) and even small cell fragments are capable of active redistribution of membrane receptors, bound together by a corresponding ligand (capping). This redistribution of receptors in the membrane is evidently a reaction that does not require the nucleus and also many cytoplasmic structures. In particular, the system of microtubules plays no part in the shift of receptors because capping takes place in cell fragments deprived of microtubules; colcemid, which destroys microtubules, moreover, does not inhibit movement of the receptors. Meanwhile all fragments taking part in capping contain bundles of microfilaments arranged lengthwise in the fragment. Groups of Con A receptors shift in the same direction during capping.

The results are in good agreement with views regarding the role of actin microfilaments in the movement of receptors over the surface. There is evidence to suggest that actin microfilaments attach themselves from inside to groups of receptors bound with a ligand, and later these microfilaments take part in the rearrangement of these groups [5, 9]. Cytoplasmic fragments may prove to be a convenient experimental system with which to study the mechanisms of this process.

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ANNUAL CYCLE OF CHANGES IN MITOTIC ACTIVITY OF THE GASTRIC MUCOSAL EPITHELIUM IN HIBERNATING RODENTS

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The digestive system undergoes marked seasonal changes in hibernating animals. However, the mitotic activity of the epithelium of the gastrointestinal tract of these animals has not been adequately studied. There are reports [3, 7, 8, 10, 12, 14, 15] of absence of mitoses during hibernation or a considerable reduction in their number. Under these circumstances it is suggested that the cell cycle is blocked in the G_0-, G_1-, and G_2-periods or in the course of mitoses.

The object of this investigation was to study mitotic activity and DNA syntheses in the mucosa of different parts of the stomach of the red-cheeked suslik *Citellus erythrogenus* Brandt in different seasons of the year.

EXPERIMENTAL METHOD

Active animals (May-June), animals in a state of deep hibernation (December-January), before the spring awakening (March-April), and at different times after awakening (1-2, 3-4, 5-7, and 14 days), and also animals kept during winter in a warm room (active in winter) were investigated. Each group contained 5-6 susliks. All the active animals were deprived of food for 24 h before sacrifice, which was always carried out in the morning. Material for investigation was taken from the fundus, body, and pylorus of the stomach. Paraffin sections were stained by the PAS reaction and counterstained with hematoxylin. Mitoses were counted at the level of the terminal portions (in the fundal glands in 3000 epithelial cells, in the pyloric glands in 6000 cells) and at the level of the pits and isthmus (in 3000 epithelial cells). A third level of counting also was used for glands of the body of the stomach, namely the lower portions of the neck of the glands (in 2000 epithelial cells). Diurnal fluctuations of mitotic activity were studied in summer in susliks with free access to food. The animals were killed in groups of six every 3 h. Mitoses were counted in the gastric glands at three levels: pits and isthmus, upper portions of the neck, and terminal portions (in 3000 epithelial cells in each case). Mitoses were always counted by one investigator in numbered preparations with a magnification of 1500 ×. The results were analyzed on the BÉSM-6 computer. To investigate DNA synthesis, autoradiography with [^{3}H]thymidine was used (injected intraperitoneally in a dose of 1.0 μCi/g, in 1, 2, 4, or even 16 injections). Pieces of mucosa also were incubated in Eagle's medium with 100 μCi/ml of [^{3}H]thymidine (30 min at 37°C, and in the case of animals killed during hibernation, at their body temperature). Paraffin sections were stained by the PAS reaction and coated with type M or type PR-2* emulsion. The sections were counterstained 4 weeks later with 1% toluidine blue in borate buffer, pH 9.2.

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