presynaptic dense projections. When aldehyde-fixed, non-osmicated brain tissue is block-stained in an ethanolic solution of phosphotungstic acid (E-PTA), according to the method of AGHAJANIAN and BLOOM[6], electron density of the paramembranous and intermembranous material at the synaptic junction is produced, leaving unstained the unit membranes of the cell and its organelles. The stain is thought to reveal basic proteins[7]. Thus, the E-PTA method makes feasible the quantification of numerical density of synaptic junctions and, further, facilitates description of configurational changes which develop with time in the maturing synapse[8,9] or as a consequence of altered environmental influences[10].

The purpose of this study was to qualitatively compare synaptic profiles, as revealed by the E-PTA method, of perinatally malnourished rats with those of well-nourished age mates. Male SPF albino outbred rats (Stock: ROROf) were used in all experiments. Malnutrition was induced according to the method of SHOEMAKER and WURTMAN[11]. Time-gestation rats were given free access to isocaloric diets consisting of 8.5% protein (malnourished animals) or 24% protein (control animals). The vitamin and mineral contents of the 2 diets were identical. The low protein regime was initiated on the 12th day post-conception and continued until the 35th day after delivery. Each litter was reduced to 8 pups on the day of birth. Offspring were left with dams until the 35th day after birth, at which time the ultrastructural studies were made. Only 51% of the malnourished rats survived; mean body and whole brain weights of this group were 20 and 80% respectively of controls.

Fixation for electron microscopy was carried out by aortic perfusion, under ether anaesthesia, of 5% glutaraldehyde, phosphate-buffered at pH 7.4, at 20 °C for 15 min. Area 10 of the frontal cortex[12] was isolated, cut into small cubes and, after immediate dehydration in ascending grades of ethanols, was block-stained for 1 h in 1% PTA in absolute ethanol containing 20 drops of 95% ethanol per 100 ml of staining solution. Tissues were then rinsed briefly in cold propylene oxide and, finally, the tissues were embedded in Epon.

Ultrathin sections were made perpendicular to the pial surface. Electron micrographs were taken in the plexiform layer of the frontal cortex, as indicated in Figure 1.

Repeated use of the E-PTA technique yielded consistent results in which synaptic junctions could be identified by the presence of presynaptic dense projections, intracleft material, and a postsynaptic band as illustrated in Figure 2. High magnification electron micrographs, Figures 3 and 4, contrast synaptic profiles from control and malnourished animals respectively. No obvious differences in the structural components of the synaptic profiles from malnourished and control animals at 35 days after birth were revealed.

Whether ultramorphological differences are more readily detectable at a later stage in development is not known. The mechanism of synaptic adhesion in the development of the synapse has not yet been clarified although the participation of proteinaceous material within the cleft in the establishment and maintenance of synaptic connectivity has recently been discussed[13].

*Summary.* The effect of a protein-deficient diet on E-PTA stained synapses in rat cerebral cortex was studied by electron microscopy. No significant difference was observed in synaptic morphology between control and malnourished animals at 35 days postnatal.

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[15] Acknowledgment. E.M.B. wishes to express appreciation for the kind hospitality found in the electron microscope laboratory of the Medical Research Department at Hoffmann-La Roche during the course of this study.

## Heparin Effects on Cultured Mammalian Cells[1]

Heparin, widely used as an anticoagulant[2], influences living cells in different ways. In high concentrations, it can be inhibitory to growth of tissue cells and might produce morphological abnormalities, but if the concentration is kept at a level that just inhibits coagulation (0.2 mg/100 ml), it seems to be relatively harmless[3]. In order to inhibit ribonuclease activity[4], heparin was included in the buffer used for isolation of polysomes from plasmacytoma cells grown in suspension culture[5]. When heparin was incidentally omitted from the buffer, higher concentrations of the non-ionic detergent Kyro EOB were necessary to obtain complete release of free polysomes. This prompted experiments on the influence of heparin alone on cells. The present communication reports on morphological alterations induced by heparin on cells in culture, synchronized as well as non-synchronized. Heparin, added to cultures of mouse plasmacytoma cells or mouse fibroblasts, was found to elicit an activity of intermittent bleb formation in these cells. This blebbing motion of the cell surface (called zeiosis, from the Greek word zeis meaning 'boil over') involves expansion of the

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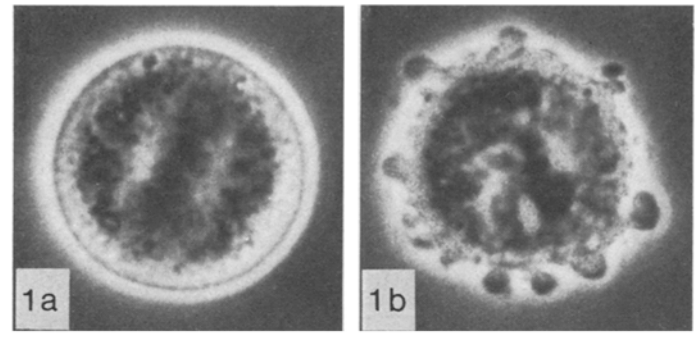


Fig. 1. Plasmacytoma cells from a non-synchronous culture exposed to heparin. ×1300. (a) A cell in mitosis displaying a smooth surface. (b) An interphase cell showing zeiotic blebs.

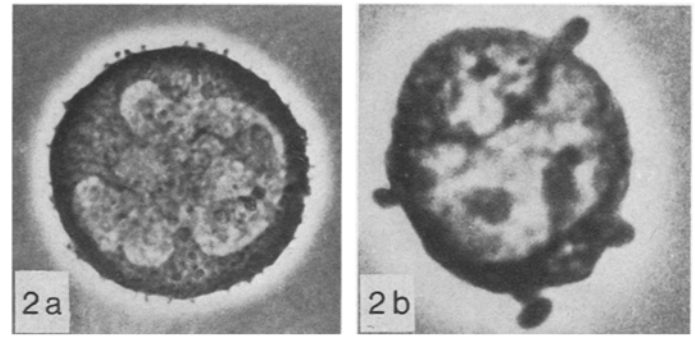


Fig. 2. Plasmacytoma cells from a synchronized culture: $G_1$ phase. ×1300. (a) From a control sample: The cell surface has tiny spines. (b) From a sample with heparin: The cell shows zeiotic blebs.

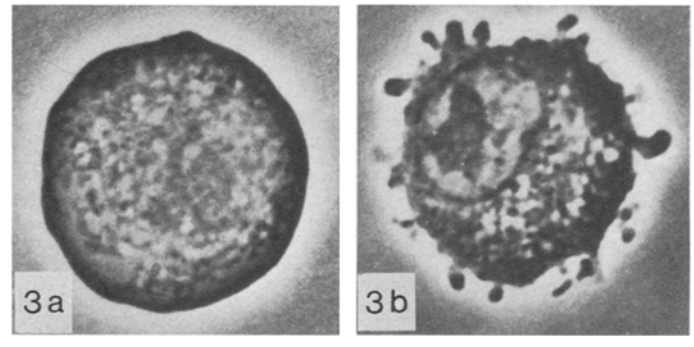


Fig. 3. Plasmacytoma cells in a synchronized culture: S phase. ×1300. (a) From a control sample: The cell surface is smooth and even. (b) From a sample with heparin: The cell shows zeiotic blebs.

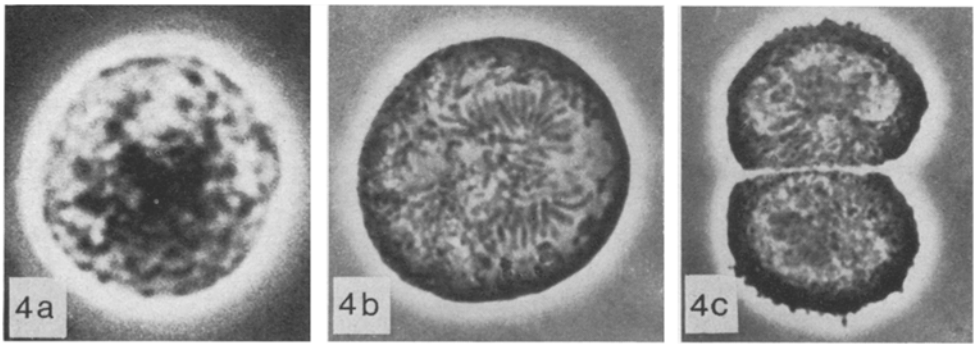


Fig. 4. Plasmacytoma cells from synchronized cultures at mitosis exposed to heparin. ×1300. (a) A cell in late $G_2$ phase without zeiotic blebs. (b) A cell in mitosis showing a smooth surface. (c) After completed cell division: The cell surface has tiny spines.

plasma membrane and is presumed to be an expression of abnormal environment[6].

*Material and methods.* Mouse plasmacytoma cells (MPC-11) grown in suspension culture, as described elsewhere[7], and mouse fibroblasts (L 929) grown in Eagle's minimum essential medium[8] were used. Plasmacytoma cells were synchronized by growth in isoleucine deficient medium. Early $G_1$ phase and S phase of the synchronized cultures were established, as previously reported[7]. Fibroblasts in monolayers were cultured in polystyrene flasks, either adhered to the bottom or on coverslips. Cells adhering to the culture flasks were exposed to trypsin for 2 min at 37 °C. After removal of trypsin, fresh medium was added to the flasks, which were gently shaken.

Heparin (147 USP units/mg) was purchased from Calbiochem, Inc. Trypsin, cell culture media, and serum were purchased from Bio-Cult Laboratories, Glasgow, Scotland.

Samples of the cell suspension were transferred from culture bottles to test tubes, and heparin (100 µg/ml)

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was added to every other tube. For phase contrast examination at room temperature, a drop of cell suspension was placed on a clean coverslip, which was subsequently inverted over the cavity of a depression slide. Also coverslips with adhering fibroblasts were exposed to heparin. These coverslips were carefully cleaned on the top before mounting on depression slides.

*Results and discussion.* The visible influence of heparin on cells seemed to be 'all-or-none'. The blebbing motion appeared when the heparin concentration had reached a certain level (100 µg/ml); a further increase did not alter the behaviour of the cells.

Most plasmacytoma cells in interphase, taken from non-synchronous cultures, revealed prominent zeiotic activity when treated with heparin (Figure 1b). In contrast, cells in various mitotic phases had a smooth and rounded surface (Figure 1a). These observations were confirmed when synchronous cultures were tested: Heparin induced zeiotic activity in cells in the $G_1$ phase (Figure 2b), S phase (Figure 3b), and early $G_2$ phase. No blebs were seen on cells in the late $G_2$ phase (Figure 4a) or during mitosis (Figures 4b and c).

Zeiotic blebs appeared to be punched out from the cell by the cytoplasm which filled and dilated them; through cytoplasmic return, the blebs were emptied and withdrawn. No rupture of the plasma membrane or detachment of blebs from the cells were observed under these conditions. The zeiotic effect produced by heparin might be amplified by the temperature drop during microscopic examination. The blebbing motion declined gradually and finally ceased after 10–15 min, presumably because the cells adapted to the new environment.

The changes in heparin sensitivity with cell cycle progression obviously reflect modifications in the character of the cell surface[9]. Fluctuations of the components

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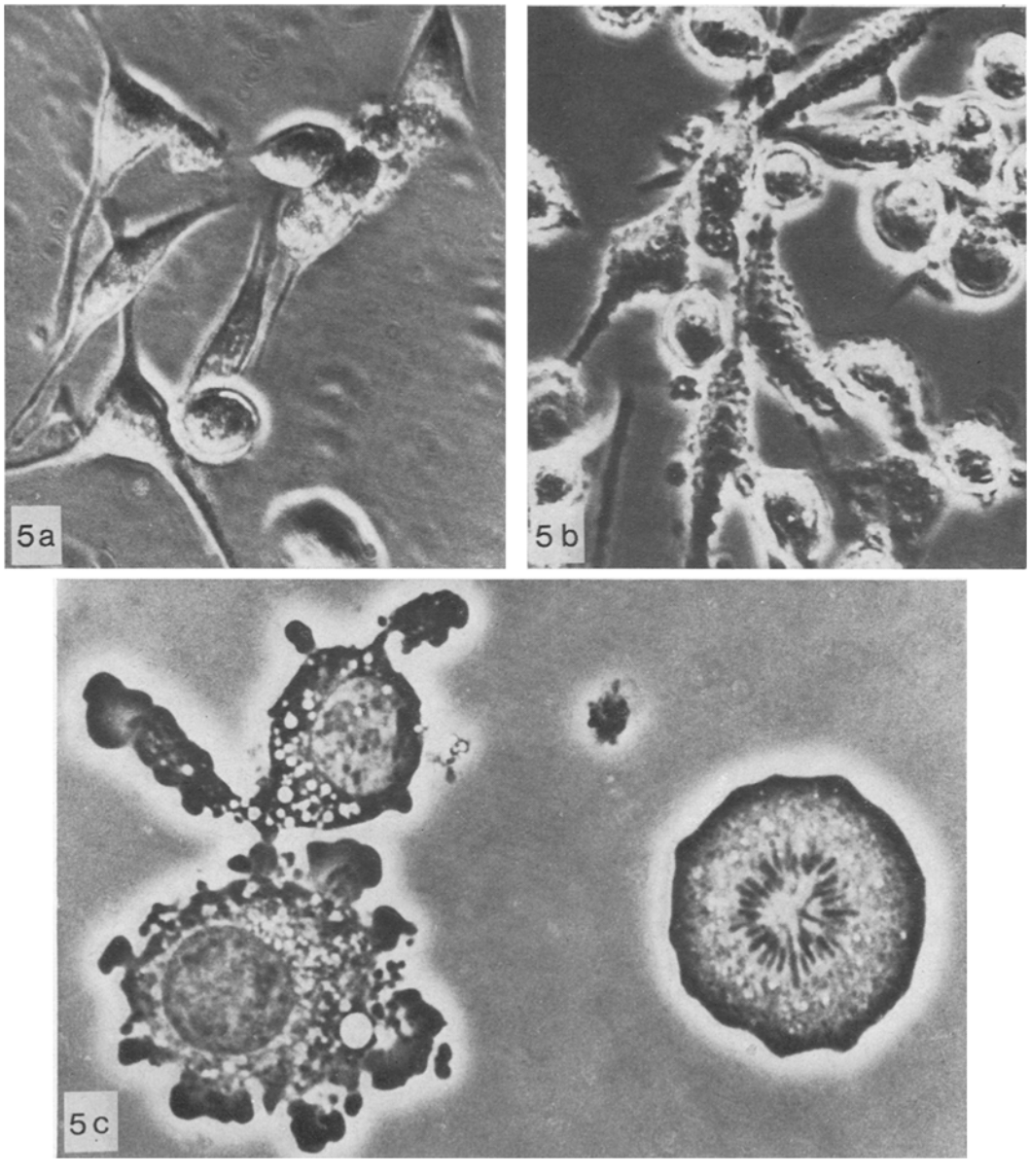


Fig. 5. (a) Fibroblasts, control. ×400. (b) Fibroblasts exposed to heparin: Cells show a blebbing surface. ×400. (c) Fibroblasts trypsinized before exposure to heparin: Extensive bleb formation of interphase cells (left); cell in mitosis with smooth surface (right). ×1300.

of the cell surface have been reported[10]. Previous ultrastructural studies on synchronized plasmacytoma cells demonstrated that cells in the $G_1$ phase have on their surface tiny slender cytoplasmic projections (seen also in the phase contrast microscope, Figures 2a and 4c); they disappear in the S phase[7]. This might be due to reduced stability of surface structures during $G_1$ phase. But it is as yet uncertain whether this phenomenon is related to the heparin sensitivity.

Zeiosis was also observed when monolayer cultures of fibroblasts were exposed to heparin (Figure 5a and b). After trypsinization, similar concentrations of heparin produced large non-reversible blebs in all cells except those in mitosis (Figure 5c). Fibroblasts tend to produce small reversible blebs after trypsinization; however, heparin seemed to accentuate the effect considerably. Preceding trypsinization made the cells fragile, and blebs detached easily.

Although heparin is known to produce morphological changes in cells[3], the mechanism behind such manifestations has not been explained. Fibroblasts and ascites cells incubated with heparin have been shown to adsorb this polyanion to the cell surface both reversibly and irreversibly[11]. The present observations suggest that the primary target for heparin is to be sought among components of the cellular periphery. The plasma membrane and its adjoining glycocalyx or 'cell coat', rich in heterosaccharide materials, are included within the concept of a larger functional complex[12]. As the necessary structural and functional information is lacking, the biology of the cell surface has been the subject for much speculation. The compounds of the cell surface are believed to play fundamental roles in cell-to-cell interactions in development and differentiation, cell transformation, and malignancy. It should be emphasized that heparin is structurally close to compounds of the cell surface. As trypsinized cells, presumed to have lost most of their glycocalyx[13] including their heparan sulphate[10], respond to heparin more vigorously than non-trypsinized cells, perhaps the target for heparin is to be found in the plasma membrane proper and not in the stabilizing glycocalyx.

*Summary.* Plasmacytoma cells exposed to heparin exhibited zeiotic blebs in the $G_1$ phase, S phase, and early $G_2$ phase. Zeiosis was not seen in mitotic cells. This heparin effect was reversible. Also fibroblasts were sensitive to heparin. After trypsinization of fibroblasts, heparin produced large non-reversible zeiotic blebs in the cells, except in those in mitosis. The primary target for heparin is apparently to be sought among components of the cellular periphery.

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## Histochemistry of the Lumenal Cell Surfaces of the Mucosa of the Oviducts and the Uterus of the Rat. Changes in Prepuberty, Estrous Cycle, Castration, Hormone Replacement and Pseudopregnancy

It was reported that the apical surface of the epithelial cells of the isthmus of the rat oviduct was more intensely PAS-positive than the ampulla and fimbriated end, but no changes were recorded during the estrous cycle[1]. Also, PAS-positive material was noted at the cell surfaces as well as in the apical cytoplasm of the epithelial cells of the guinea-pig uterus, varying during the cycle[2,3]. Similar observations were made on the mouse uterus[4], and on the prepuberal rat oviduct[5]. The purpose of the present study was to characterize further by histochemical techniques the surface coat of the rat oviducts and uterus at prepuberty, during the estrous cycle, after castration with and without hormone replacement and in pseudopregnancy.

*Materials and methods.* Albino female rats, kept with a 12 h schedule of light and darkness, were fed a balanced diet and water ad libitum.

1. *Prepuberal rats.* 16 normal rats were sacrified from the 10th up to the 30th day of age. Pseudopregnancy was induced in another group of 25-day-old rats. 26 rats were injected s.c. with 75 IU of PMSG (Eleagol, Elea) in 0.5 ml of 0.9% sodium chloride followed 60 h later with 25 IU of HCG (Endocorion, Elea) in 0.5 ml of 0.9% sodium chloride, s.c.[6]. 8 control rats received 0.5 ml of 0.9% sodium chloride. Animals were sacrificed between the 6th and the 21 th day after the last injection.

2. *Adult rats.* 21 rats, 2 to 4 months old, which showed a regular 4-day cycle controlled by exfoliative cytology, were sacrificed.

3. *Castrated rats.* 17 adult rats were bilaterally castrated. 15 rats were subjected to a sham operation. Groups of experimental and control animals were sacrificed 15 days after castration. 3 castrated rats received s.c. for 15 days 30 µg estradiol benzoate (Progynon B-Schering) daily. 3 castrated rats were injected daily with 2 mg progesterone (Prolution-Schering) for 15 days.

*Light microscopy.* Segments of the oviducts and uterus at the level of the uterine horns, were fixed in 10% neutral buffered formaldehyde pH 7.0 for 24 h at 4 °C. The following histochemical techniques for carbohydrates were applied to tissue sections: periodic acid-Schiff and diastase digestion[7]; colloidal iron[8]; alcian blue (pH 1.0 and 2.5)[8]; alcian blue (pH 1.0 and 2.5)-PAS sequence[8];

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