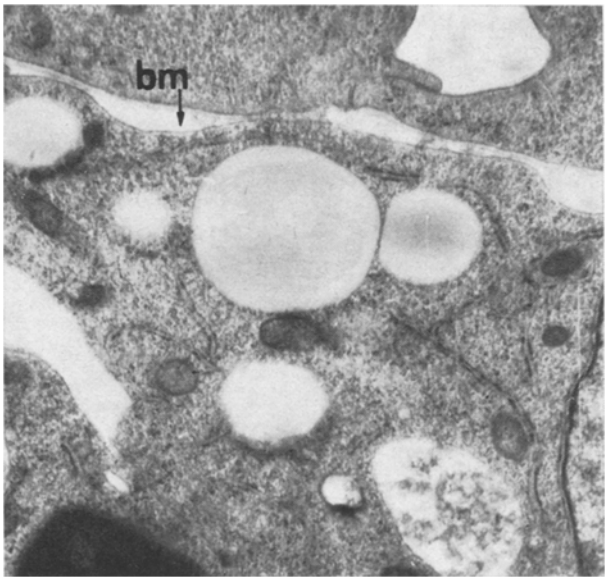

Fig. 5. A basement membrane (bm) is present lining the ventral ectoderm border. × 17,700.

Similar results are obtained by transmission electron microscopy (Figure 5). The ventral ectoderm border has raised cells with slight depressions where the cells meet. These cells are not joined by junctional contacts. The entire midline ectoderm ventral border anterior to Hensen's node is lined by a basement membrane except in the region of Hensen's node. Here the membrane is intermittent.

Discussion. In TEM studies the stage-5 mesoderm cells have usually appeared to have 3 or 4 filopodia which can only be traced a short distance in the micrograph[9]. The SEM photographs, however, allow the filopodia on an entire cell to be considered and their relationship to the ectoderm and endoderm cells to be considered. The mesoderm cells have filopodia on every part of their surface and one cell may have 6 or 7 cellular extensions.

Each mesoderm cell is also in contact with several other mesoderm and endoderm cells which may be important in allowing each cell to identify its position in the embryo as a whole[10]. Electrophysiological studies have also suggested these cells are in communication[11]. The importance of filopodia contact in primary neural induction is not clear. However, the numbers of filopodia present in contact with several different cells indicate a very close relationship between all the cell types present.

Although the SEM specimens allow an entire cell to be studied in relation to the embryo, the finer surface structures of the 3 cell layers have generally not been correlated with TEM studies. Several different junctional cell contacts are recognized by TEM[9] but at the present time most junctional cell contacts do not appear distinguishable by SEM[12].

The tufts of fibrous material present along the ventral ectoderm border correspond to the position of tufts of basement membrane as seen by transmission electron microscopy.

Summary. Normal primary neural induction has been studied by scanning electron microscopy and the results compared with those obtained by TEM. Mesoderm cells are usually in contact with several other cells, both mesodermal and endodermal in origin. By SEM the ectoderm layer has been shown to be in contact with the underlying mesoderm cells. Tufts of fibrous basement membrane are also present between the two cell types. TEM specimens also show an intermediate basement membrane.

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An Ultrastructural Investigation of the Effects of Perinatal Malnutrition on E-PTA-Stained Synaptic Junctions

The effects of malnutrition on the developing brain, in particular on synaptic ontogeny, have recently attracted much attention in scientific research[1, 2], in view of the key role of the synapse in the processing, storage and transfer of information within the nervous system. Studies of the effects of malnutrition[3, 4] have focused on quantitative aspects of synaptogenesis as revealed by standard preparative techniques for demonstration of fine structure. Results from these investigations are conflicting: while GAMBETTI et al.[4] reported a reduction in size and density of presynaptic endings in the cerebral cortex of malnourished rats compared to controls, earlier studies of CRAGG[3] revealed no significant change in these parameters but a marked reduction in the number of synaptic connections per neuron.

In order to investigate whether qualitative morphological alterations occur as a result of malnutrition, synaptic profiles were selectively stained with phosphotungstic acid, a technique first used by GRAY[5] to reveal

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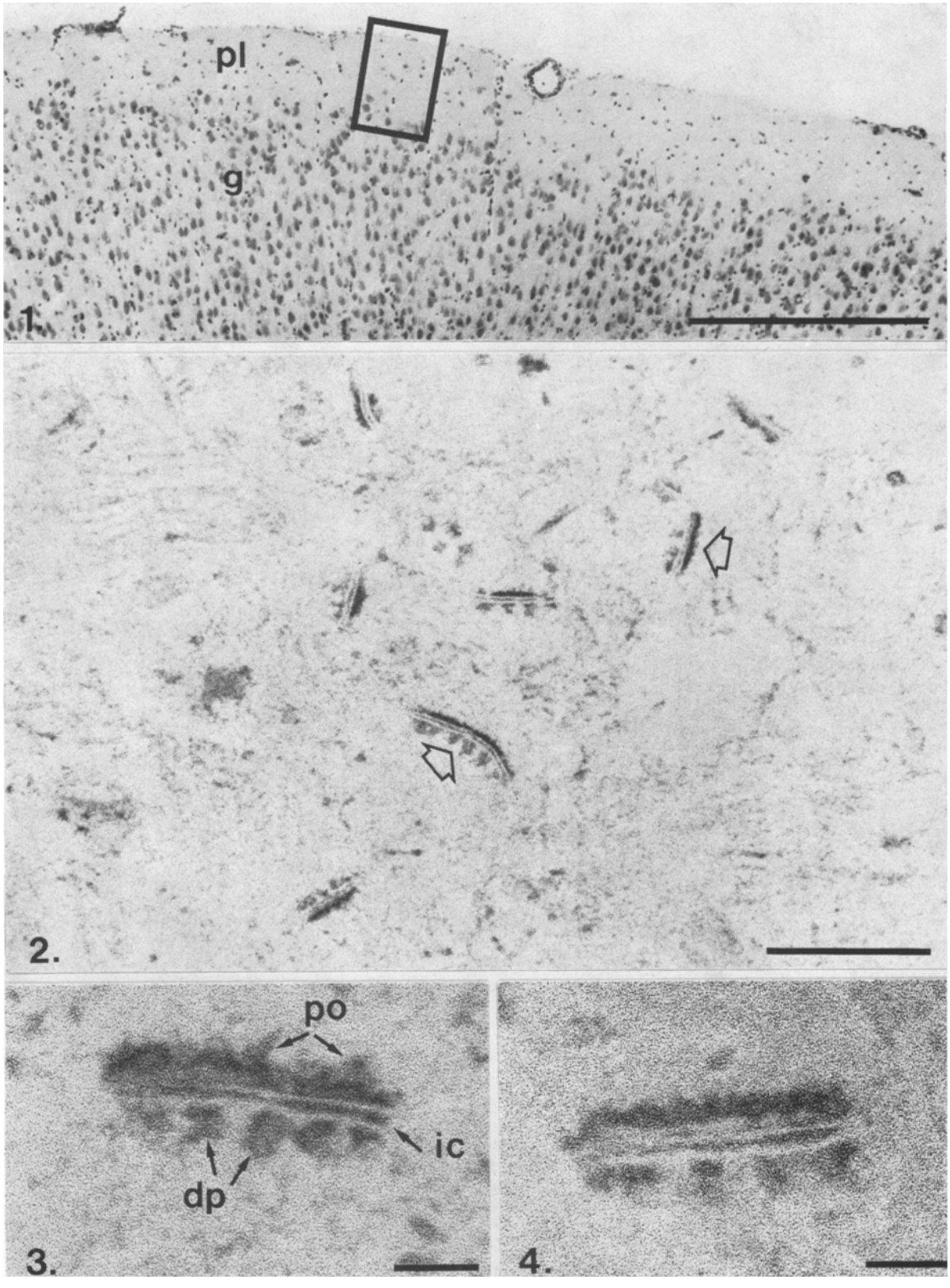

Fig. 1. Light micrograph of the rat cerebral cortex. Inset indicates the region examined by electron microscopy. g, outer granular layer; pl, plexiform layer. Scale: 0.5 mm. Cresyl violet-stained 16 μm-thick frozen section.

Figs. 2–4. Ultrastructural aspects of E-PTA-stained synaptic junctions in control (Figures 2 and 3) and malnourished (Figure 4) rats. The survey electron micrograph (Figure 2) illustrates the strikingly selective staining of synaptic junctions after E-PTA. Note the similarities between the structural components of the synaptic profiles in Figures 3 and 4. dp, presynaptic dense projections; ic, intracleft material; po, postsynaptic band. Scale: Fig. 2, 0.5 μm; Figs. 3 and 4, 100 nm.

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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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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