filamentous material, mitochondria and agranular endoplasmic reticulum (Figure 4). Lastly, other dense core vesicles are found within presynaptic axon terminals (Figure 5). When within these terminals, the dense core vesicles are frequently found in conjunction with translucent spheroidal vesicles. By the 17th and 22nd weeks of gestation, the pattern of dense core vesicle distribution remains unchanged. However, they are now more frequently seen, as by this period there has occurred a significant increase in synaptic development.

The results of the present study indicate that in the human cerebral cortex by the 15th week of gestation, dense core vesicles are found within axon cylinders, distal, dilated axon segments and within presynaptic axon terminals. Those vesicles within the axon cylinders are probably representative of synaptic vesicles in transit[2]. On the basis of morphological criteria established in other species[3-5], those dilated, distal axon segments containing dense core vesicles appear to be axon growth cones, and thus, the presence of dense core vesicles therein demonstrates that this vesicular component is present in the actively growing axon processes. Lastly, the identification of dense core vesicles within the presynaptic axon terminals seems of particular significance, for it suggests that these vesicles may be involved in the process of neural transmission. Dense core vesicles comparable to those described have been identified in the rodent cerebral cortex and such vesicles were identified as storage sites for the monoamine, norepinephrine[6,7]. Though it is attractive to speculate that the dense core vesicles of the present study are also linked with the storage of norepinephrine, it is impossible on the basis of purely morphological data to make such an assumption. Additionally, the fact that even cholinergic terminals contain occasional dense core vesicles[8], makes the correlation of these dense core vesicles with the monoamine, norepinephrine, all the more equivocal. Though the neurotransmitter linked with these dense core vesicles remains to be identified, the fact that these vesicles are present at all must be considered significant. The presence of dense core vesicles here at the 15th week of gestation indicates that in terms of the synaptic vesicle population, i.e., translucent and dense core vesicles, the typical synaptic complexes of the human cerebral cortex appear quite mature particularly in light of this very early stage of fetal development.

Summary. The present study clearly demonstrates that by the 15th week of gestation dense core vesicles appear within the human cerebral cortex. These vesicles can be identified within axon cylinders, axon growth cones, and axon synaptic terminals. The role of these vesicles is speculative, yet, their very presence at this early fetal stage seems to reflect an advanced state of synaptic vesicle development.

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Primary Neural Induction as Studied by Scanning Electron Microscopy

Primary neural induction has been studied extensively by light and transmission electron microscopy[1,2] and recent studies have demonstrated two morphological markers of primary neural induction in the early stage-5 embryo.

In the light microscope a band of nuclei can be seen immediately anterior to Hensen's node[3] and in the transmission electron microscope a ribosome free area has been observed[4].

The present study investigates the scanning electron microscopic (SEM) appearances during primary neural induction in the chick embryo, paying particular attention to the mesoderm cells and their relationship to the ectoderm layer.

Materials and methods. Stereoscanning electron microscopy (SEM). White leghorn chick embryos were incubated at 37.5 °C until early stage-5[5]. The eggs were opened and some of the albumen poured away. KARNOVSKY's fixative[6] was then gently injected below and above each embryo. The specimens were cut off the yolk and placed in KARNOVSKY's fixative for several h. They were then buffered in Cacodylate buffer[7] for several h, followed by 2% OsO_4 for 1 h and dehydrated in a graded series of acetone/water. In 70% acetone/water a narrow fracture was directed across the area pellucida through the region immediately anterior to Hensen's node. It was then possible to study mesoderm cell contacts in the area pellucida and anterior to the node. In some specimens, the endoderm was peeled off in the Cacodylate buffer to study the ventral ectoderm in this region.

The specimens were critical point dried by replacing the acetone with liquid CO_2 and mounted on Cambridge stubs with colloidal silver paint. They were then coated with gold and palladium approximately 40 nm thick and viewed on a Cambridge S4-10 scanning electron microscope.

Transmission electron microscopy (TEM). Specimens for study by TEM were fixed and buffered as described for SEM specimens. They were then dehydrated in a graded series of ethanol/water and embedded in Araldite[8]. Following ultrathin sectioning they were viewed on a Siemens Elmiskop 1B (80 KV) transmission electron microscope.

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Results. By scanning electron microscopy the midline ectoderm immediately anterior to Hensen's node is a compact layer several cells in thickness (Figure 1). The layer is a pseudostratified epithelium with the cells aligned dorso-ventrally. Along the ventral ectoderm border several mesoderm cells are present (Figure 2). Each mesoderm cell has several filopodia. They are of varying lengths and appear to contact other mesoderm (Figure 3) and endoderm cells. The filopodia similarly appear to contact the ectoderm layer. The mesoderm cell contacts in this region are similar to those in the adjacent area pellucida.

In the scanning electron microscope it was not possible to distinguish the various types of cell junctions as recognized in the transmission electron microscope. However, it is possible to see cells contacting one another. It is also possible to see tufts of fibrous material present in the region of the dorsal mesoderm and presumptive neural-ectoderm border.

The ventral ectoderm surface was examined on the SEM specimens in which the endoderm had been peeled away. The cells can be recognized as bumps with an encircling depression representing the cell perimeters (Figure 4). Basement membrane and cellular filopodia are present on this surface.

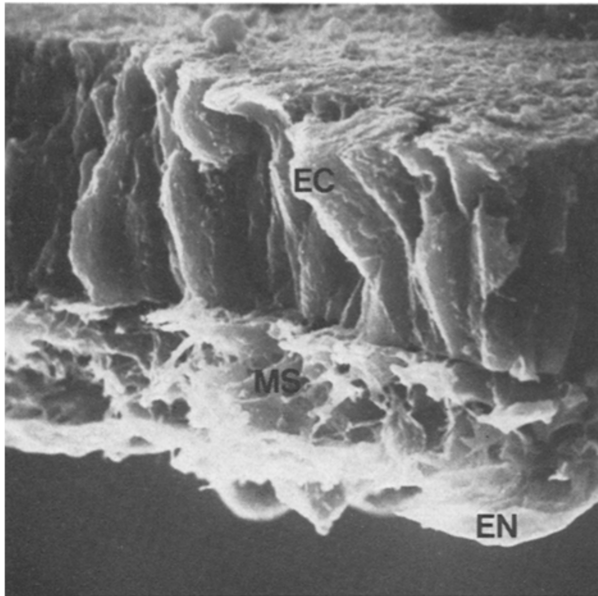

Fig. 1. Transverse section of a stage-5 chick embryo studied by SEM. The ectoderm cells (EC) are aligned dorso-ventrally. Mesoderm cells (MS) are present between the ectoderm and endoderm (EN) layers. ×1,520.

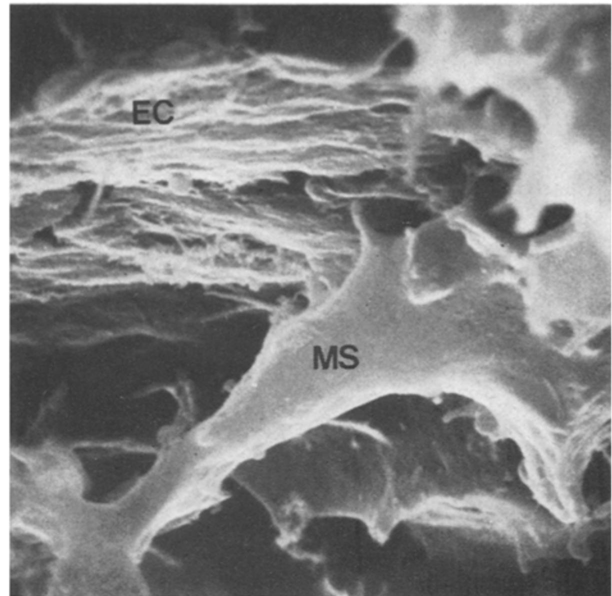

Fig. 3. A mesoderm cell (MS) ventral to the ectoderm border (EC) and in contact with another mesoderm cell. ×7,900.

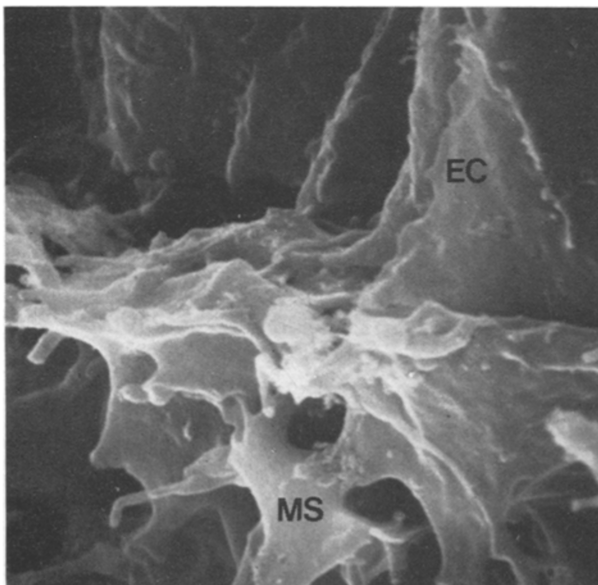

Fig. 2. Junction of the ectoderm (EC) and mesoderm (MS) cells in Figure 1. Several filopodia are present on the mesoderm cells. ×7,700.

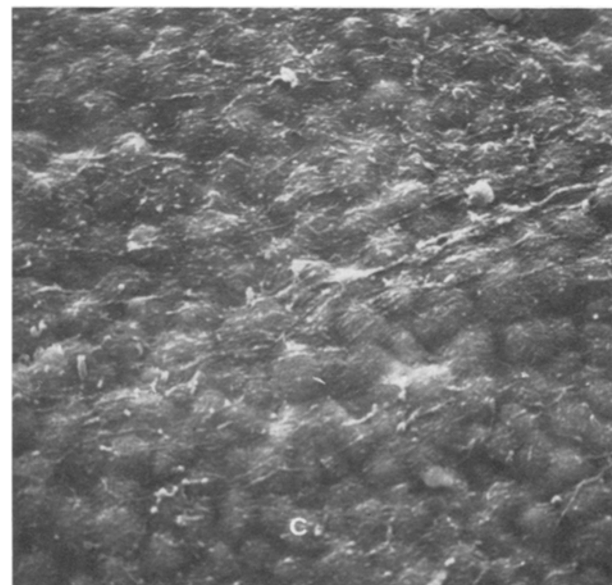

Fig. 4. The ventral ectoderm surface as studied by SEM. The cells (c) are present as swellings. Cell perimeters are seen as depressions. Several filopodia are on the surface probably remaining as the mesoderm cells were peeled away. ×1,100.

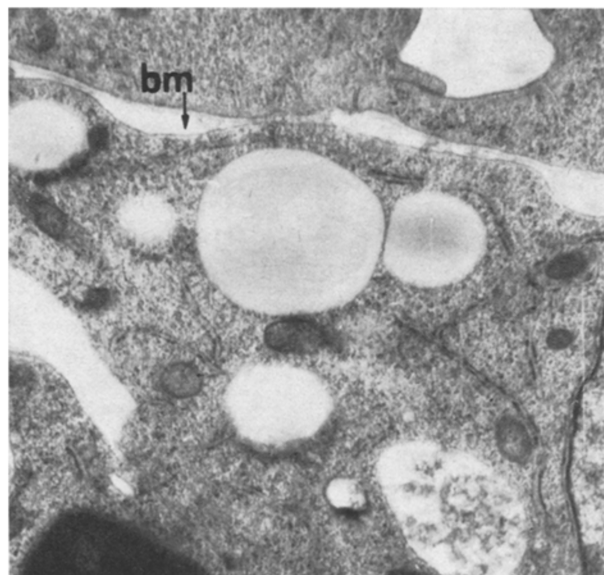

Fig. 5. A basement membrane (bm) is present lining the ventral ectoderm border. × 17,700.

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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