EDTA[5-7] and standardized bleedings[8], give about the same effect of raised mitose frequency of bone marrow and thymus. In the two last-mentioned experiments the hypercalcemia is secondary to an initial hypocalcemia. The Canadian scientists make it evident that the parathyroid glands are invested with the superior controlling function of the mitotic level of thymus and bone marrow. In our experiments, however, the bleeding caused by the bone marrow aspiration was insignificant.

The concept of chalones, on which a major literature exists[9], might also yield an explanation of the mitotic stimulation. Chalones are mitotic inhibitors normally fixed to the cell membranes, but which are thought to fall off, e.g. at traumatic incidents.

This report is merely preliminary, seeing that without further research it is impossible to say in which way the stimulation of mitoses is mediated.

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A Transmission and Scanning Electron Microscope Study of Primary Neural Induction

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Summary. Normal primary neural induction has been further studied by TEM and SEM. A single mesoderm cell is usually in contact with several ectoderm cells. The mesoderm cells are also contacting other mesoderm cells. It is suggested that ectoderm cells are induced in groups and that induction is synchronized by these contacts. At the points of contact between mesoderm and ectoderm cells cytoplasmic changes are present in the induced tissue.

Primary neural induction has been shown to occur in the very early stage-5 chick embryo[4,5]. At this point in development the ectoderm layer immediately anterior to Hensen's node has thickened. The presumptive notochord mesoderm cells are also present as a mass ventral to this area of thickened ectoderm and recent studies by scanning electron microscopy have demonstrated that the mesoderm and ectoderm cells are apposed at the time of induction[6].

The ectoderm and mesoderm cells have now been examined at the time of neural induction using both the SEM and TEM to obtain a more detailed assessment of the ultrastructural features. Particular attention has been paid to the cellular contacts and the changes in the cytoplasm at the junctional regions.

Materials and methods. White leghorn chick embryos were incubated at 37.5 °C until stage –5[7]. The eggs were then opened and the embryos cut off the yolk and mounted by New Culture[8]. The specimens were immediately placed in KARNOVSKY's[9] fixative for 12 h. They were then buffered in Cacodylate buffer[10] for 12 h.

SEM preparation. A narrow fracture was directed across the area pellucida immediately anterior to Hensen's node. This exposed the ectoderm, mesoderm, and endoderm in the region to be studied. In two specimens the mesoderm layer was sheared off slightly caudad to the ectoderm

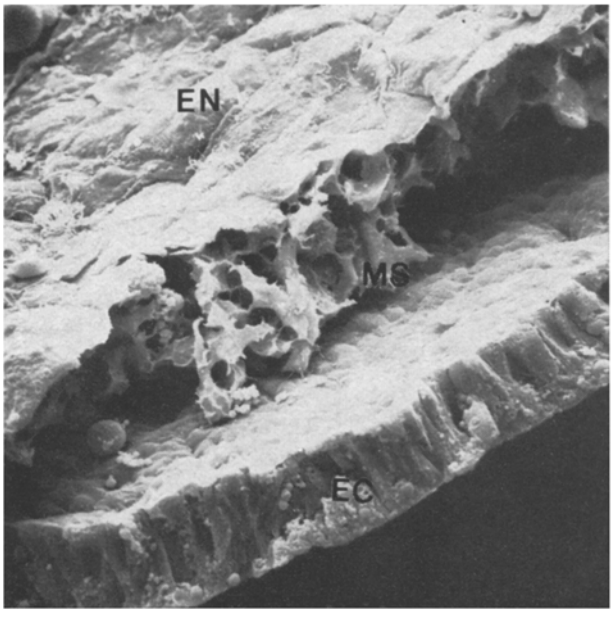

Fig. 1. The three embryonic layers anterior to Hensen's node. EC, ectoderm; MS, mesoderm; En, endoderm. ×219.

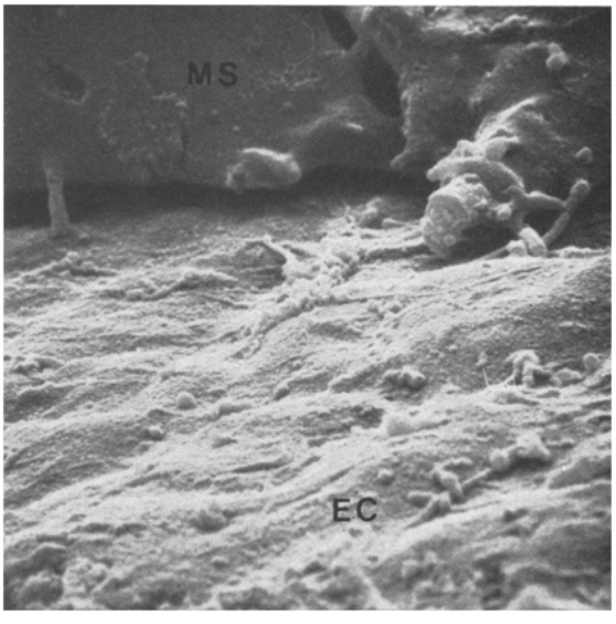

Fig. 2. The fibrils and filopodia on the ventral ectoderm layer. EC, ectoderm; MS, mesoderm. × 11,000.

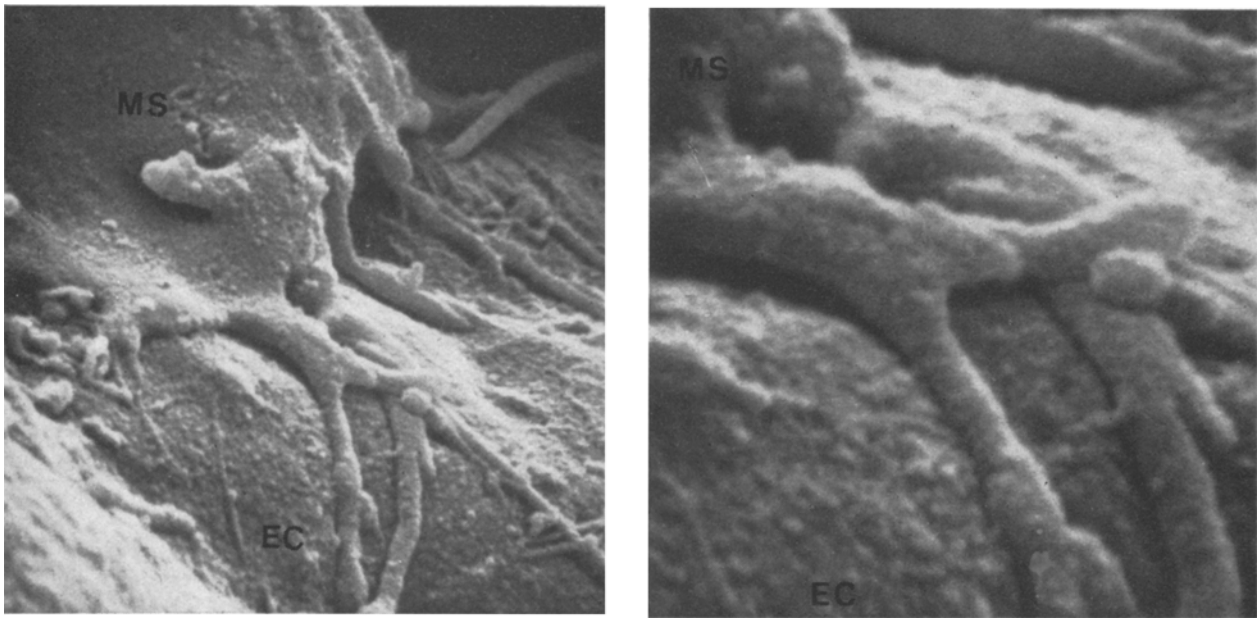

Fig. 3. A) and B). A mesoderm cell on the ventral ectoderm surface. The filopodia closely follow the contours of the ectoderm layer. EC ectoderm; MS, mesoderm. A) × 22,000; B) × 55,000.

layer. The resulting shelf of ectoderm allowed a useful study of the ventral ectoderm surface.

The specimens were critical-point dried by replacing acetone with liquid CO_2, mounted on Cambridge stubs with colloidal silver paint, coated with gold approximately 40 nm thick and viewed on a Cambridge S410 scanning electron microscope.

TEM preparation. TEM specimens were dehydrated in a graded series of ethanol/water and embedded in Araldite[11]. Ultrathin sections were prepared and viewed on a Siemen's Elmiskop IB (80 kV) transmission electron microscope.

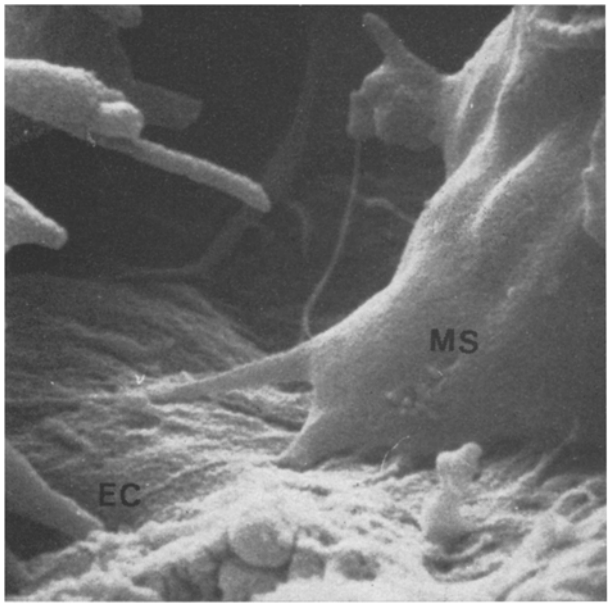

Fig. 4. A mesoderm cell adjacent to the ventral ectoderm layer. Note the numerous mesodermal filopodia fanning out on the ectoderm cells. EC, ectoderm; MS, mesoderm. × 22,000.

Results. SEM specimens. In transverse section, the ectoderm cells are aligned dorso-ventrally (Figure 1). Along the ventral border the cells appear as swellings and their perimeters as depressions. This border is covered with fine fibrils and several filopodia (Figure 2) which may have originated during specimen preparation. Some of the mesoderm cells are pulled away when the fracture is directed across the area pellucida though mesodermal filopodia remain on the ventral ectoderm surface. This surface is also covered with an incomplete layer of fine basement membrane material.

The mesoderm layer is composed of a meshwork of cells adjacent to the ectoderm layer. Some mesoderm cells immediately adjacent to the ectoderm are closely apposed to it (Figure 3). A single mesoderm cell may have several long filopodia which fan out on to the ectoderm layer so that most mesoderm cells contacted 4 or 5 ectoderm cells at the same time. However, a single mesoderm cell may be in contact with as many as ten ectoderm cells at the same time. Each mesoderm cell was also contacting four to 6 other mesoderm cells. Occasional mesoderm cells contacted ectoderm cells and mesoderm and endoderm cells. These mesoderm cells were present through the thickness of the embryonic layers from the ventral ectoderm to dorsal endoderm.

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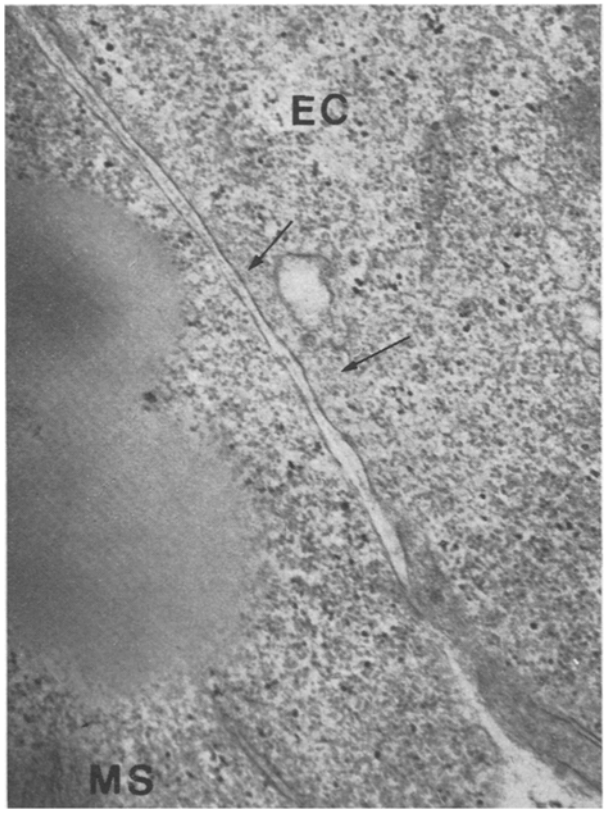

Fig. 5. A mesoderm cell adjacent to the ventral ectoderm during primary neural induction. Note the change in the cytoplasm (arrow). EC, ectoderm; MS, mesoderm. × 30,600.

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The mesodermal cell filopodia are closely apposed to the ventral ectoderm layer and follow its contours (Figure 4). The distal ends of the filopodia are usually narrower than their origins and a single filopodium may contact two or three cells by crossing the length of one surface to the next.

TEM specimens. In section the mesodermal filopodia can also be seen closely applied to the ectoderm border and there is a marked change in the cytoplasm of these cells when compared to adjacent ectoderm regions (Figure 5). The cytoplasm appears to be clear and the area is distinguished by its lack of ribosomes. In some regions the mesoderm cells are pressed against the ectoderm cells and if basement membrane material is present it is compressed against the ectoderm layer.

Discussion. The SEM studies described in the present paper show that mesoderm cells are in contact with several ectoderm cells at the same time. Electrophysiological studies also suggest that these cells are in communication with one another[12]. A single mesoderm cell contacting several ectoderm cells and several mesoderm cells suggests that primary neural induction may occur in groups of ectoderm cells. A single mesoderm cell contacting the ectoderm in this manner would possibly synchronize primary neural induction in a group of ectoderm cells. Induction of an area at approximately the same period would then be possible.

SEM studies of mesodermal filopodia show them to be contacting ectoderm cells. In the present TEM studies of filopodia in the same position, there is a distinct change in the cytoplasm. Ribosomes and cell organelles are not present in the region of the ventral ectoderm border. Previous TEM studies[13] have described a region anterior to Hensen's node in the early stage-5 embryo where primary neural induction is believed to occur. The cytoplasm in this region showed a marked change. Ribosomes and other cell organelles were not present in this region. The present investigation would suggest that primary neural induction is occurring in this region at this time.

Fine Structural Relation Between Pancreatic Excretory Ductules and Intercellular Spaces

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Summary. Horse-radish peroxidase injected into the femoral vein of intact rats, or infused at 30 cm H_2O pressure into the main pancreatic duct of intact dogs, entered easily the interstitial spaces surrounding acini and acinar cells. The latter are interconnected at their luminal segments by zonulae occludentes. These junctions form a barrier to tracer penetrating from the interstitium towards the lumen of terminal ductules. However, the intraductally infused peroxidase entered the interstitial spaces, probably through the pressure injured acinar cells, as did colloidal carbon particles when infused intraductally.

Pancreatic acinar cells are surrounded by interstitial spaces[1–4] easily permeated by horse-radish peroxidase (HRP), an ultrastructural protein marker[5] leaving the pancreatic endothelium rapidly[6]. At apical segments of these spaces, intercellular junctions (zonulae occludentes) are located and serve as local barriers to the passage of pancreatic enzymes from the lumen of terminal ductules towards the pancreatic interstitium[7,8]. The junctions between the ductular cells bordering the lumen fulfil the same function. Some of them are, however, permeated by HRP reaching the lumen from intercellular spaces[6].

The experiments reported here were designed to study pathways for HRP and colloidal carbon particles escaping the lumen of the pancreatic ductules and entering interstitial spaces.

Material and methods. Pancreatic tissue specimens from 10 pentobarbital anaesthetized, CFY intact male rats weighing 200 g, and 10 chloralose-anaesthetized intact male dogs weighing 15 kg on the average were used.

5 rats received 20 mg per 100 g b.w. of *HRP* (Sigma type II)[9], dissolved in 0.5 ml of saline, into the femoral vein. Specimens from pancreatic tissues were fixed