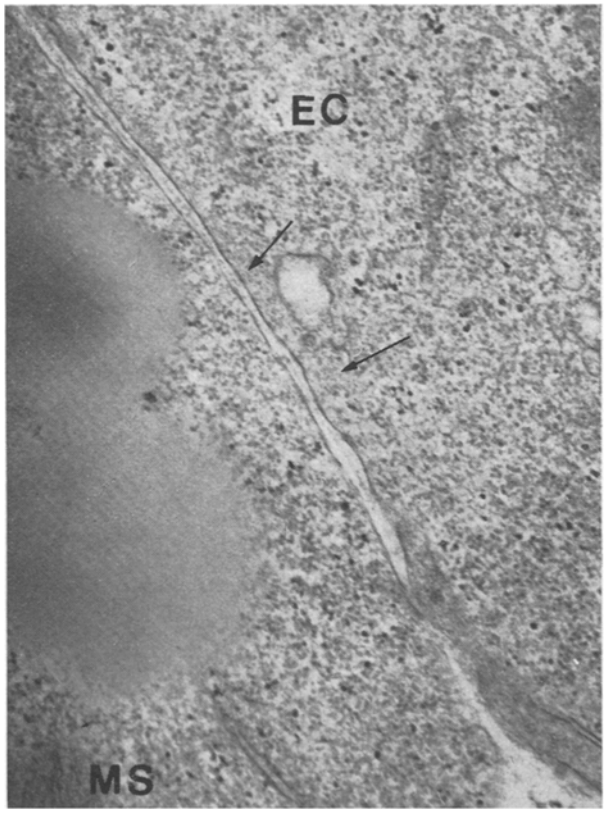

Fig. 5. A mesoderm cell adjacent to the ventral ectoderm during primary neural induction. Note the change in the cytoplasm (arrow). EC, ectoderm; MS, mesoderm. × 30,600.

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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

8–10 min after HRP injection. 5 rats not given HRP served as controls.

Polyethylene cannulas were introduced into the d. Santorini of 8 dogs fasted for 24 h; the free exit of their small duct into the duodenum was left intact. 5 mg of HRP per dog dissolved in 2 ml of saline was infused at a pressure not exceeding 30 cm H_2O into main pancreatic duct of 4 dogs during 30-minute periods. For 4 other dogs 2 ml of 1:1 saline diluted *colloidal carbon particles* (Pelikan) were infused as described before. The pancreatic

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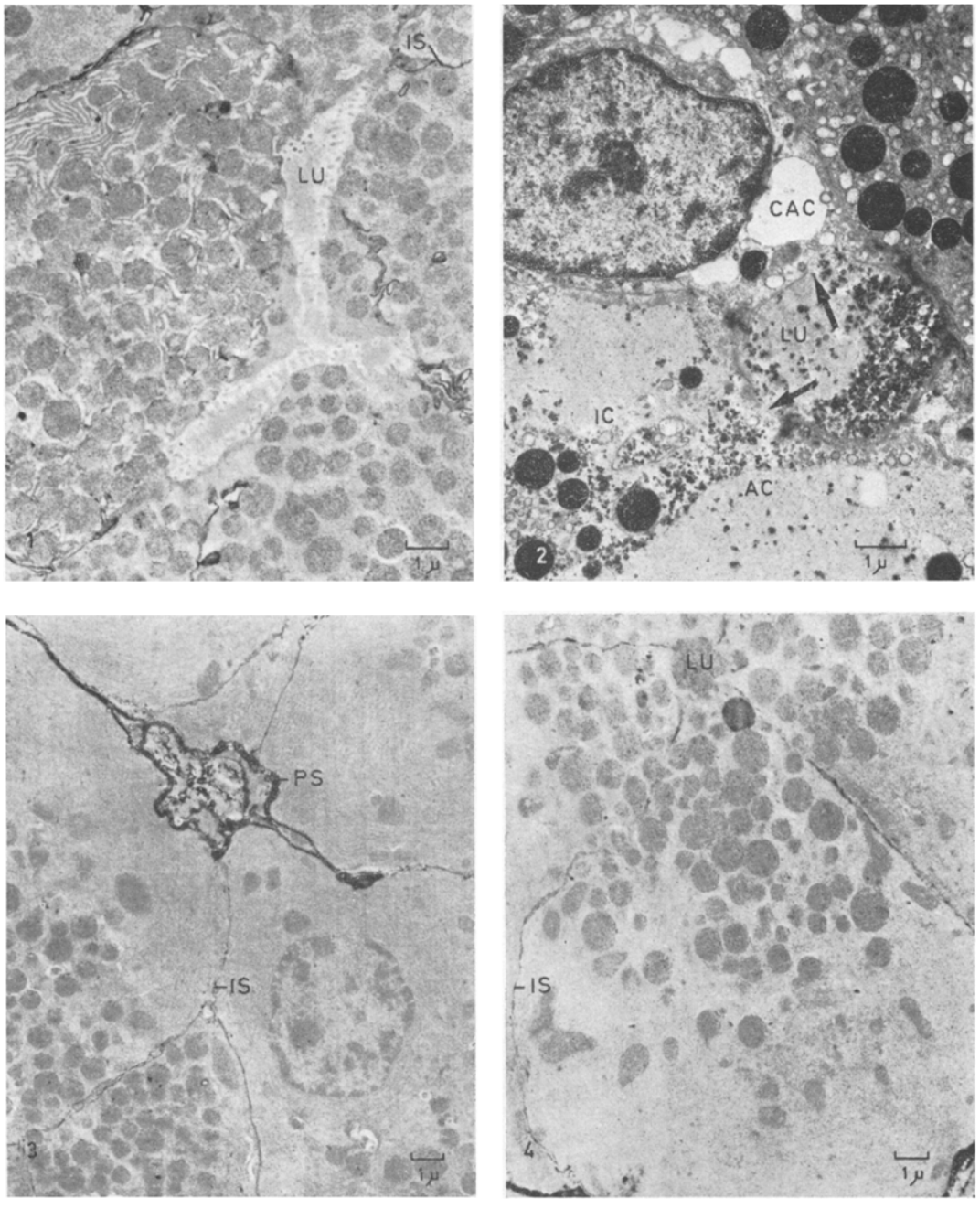

Fig. 1. Intact rat pancreas. HRP was injected intravenously. The electron micrograph shows intercellular spaces (IS) containing peroxidase reaction products which do not enter the excretory ductules lumen (LU).

Fig. 2. Intact canine pancreas. Colloidal carbon particles were infused by retrograde way. The electron micrograph shows the carbon particles containing central lumen (LU) and intercellular canaliculi (IC) of a pancreatic acini. The arrows indicate ruptures on the luminal surface membrane of a centroacinar (CAC) and acinar cell (AC).

Fig. 3 and 4. Intact canine pancreas, HRP was infused by retrograde way. Electron micrographs show peroxidase reaction products got into intercellular (IS) and periacinar spaces (PS).

secretion of 2 dogs in each of these groups was stimulated by 3 U/kg b.w. of secretin (Boots) injected into the femoral vein. HRP or carbon particles were infused intraductally when the effect of secretin had worn off. Two other dogs served as controls. Specimens from pancreatic tissues were fixed 30 min after intraductal infusions.

All the specimens from rat and dog pancreas were immersed into a +4 °C solution of 2% formaldehyde – 2.5% glutaraldehyde in 0.13 *M* sodium cacodylate-HCl buffer (pH 7.2). To demonstrate carbon particles, the fixed specimens were dehydrated and embedded in plastic (Durcupan ACM). Thin sections for electron microscopy were stained with uranylacetate and lead citrate. To demonstrate HRP, the fixed specimens were washed in 0.13 *M* cacodylate buffer, then cut into 50 µm slices and preincubated in the same buffer but containing 50 mg % 3, 3′-diaminobenzidine tetrahydrochloride (DAB) for 30 min. The slices were then incubated in the buffer containing 50 mg % DAB and 0.01% H_2O_2 for 30 min and fixed in 2% OsO_4 solution. All the specimens were dehydrated using ethanol and embedded in Durcupan ACM. Unstained ultrathin sections were examined.

Results. After the intravenous injection of HRP, peroxidase activity was occasionally seen in the pinocytotic vesicles of the acinar cells. A reaction product was, however, present in the interstitial space surrounding acini and acinar cells. The intercellular junctions of adjacent acinar cells obstructed the interstitial passage of HRP into the lumen (Figure 1). Carbon particles, however, infused by retrograde way entered secretin-stimulated acinar and centro-acinar cells. Despite the low infusion pressure, the luminal surface membranes were ruptured (Figure 2). By this way the intraductally infused HRP certainly entered the interstitial spaces around the acini and acinar cells (Figures 3 and 4).

Discussion. The crucial point in the pathogenesis of acute pancreatitis is: By which pathways can the digestive enzymes escape the excretory duct system and reach the interstitium in bulk? On the basis of histological observations it has been supposed that, due to the increased pressure, carbon particles or dyes injected intraductally may enter the interstitium through the acinar cells[10], intercellular gaps[10,11] or through intact cells bordering the isthmic segments of the excretory ductules[12]. The passage of different markers infused intraductally had been studied, and it was shown that carbon particles may enter seemingly intact acinar cells[13], thorotrast may reach the interstitium through spaces between acinar cells[14,15], without rupture of the excretory duct system, or intravenously given HRP can reach the lumen occasionally through some ductular cell junctions[6], because the interstitial spaces between acinar cells and most of spaces between centroacinar cells are occluded by zonulae occludentes[7,8].

The results presented here support the idea that enzymes may reach the gland interstitium even at low pressure through the ruptured acinar and centroacinar cells and possibly some interductular cell junctions[6]. However, another problem arises: Why is a rich ingested meal so rarely followed by pancreatic autodigestion though the pressure during digestion rises to the same or even to higher values[16] than we used in this experiment, resulting in the rupture of some acinar and centro-acinar cells and escape of ductal HRP or carbon particles into the interstitial spaces?

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Pyridine as an Unmasking Reagent for Lipoprotein Complexes in the Nervous System of Protein Deficient Squirrel Monkeys

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Summary. Acid hematin test with pyridine and Sudan black B controls was employed on selected areas of the brains of 115, 140 days fetuses, neonates and adult squirrel monkeys maintained on low and high protein diet. Our histochemical findings indicate that the reduction of phospholipids in the low protein fetuses and neonates is related to myelination, whereas in the adults, most of the lipids are bound to proteins and/or cerebrosides to form complexes, as revealed by the unmasking action of pyridine.

Preparations from a total of twenty-six animals were studied. A colony of 64 healthy female squirrel monkeys (*Saimiri sciureus*) were maintained in outdor cages for the purpose of breeding. Out of the 59 animals that became pregnant 35 were maintained on a diet low in protein content (4% and 8% protein calories) and 24 animals were fed a diet similar in composition, but high in protein content (25% protein calories) starting from 35 days after conception. Fetuses from animals maintained on low and high protein diets were removed at 115 days and 140 days after gestation. The neonates delivered at full term (175–178 days) and the young adults (3 years of age) were also used. The various histochemical tests employed for lipids are given in the table. The Baker's acid hematin test followed by its pyridine extraction control gave the most interesting results in the course of lipid formation during the period of brain development. In the 115 days and 140 days old fetuses (born to mothers given low and high protein diets), the acid hematin test gives moderate activity which persists more or less unchanged after pyridine extraction. At this stage some diffuse staining of the neuropil is also observed before pyridine extraction, which, as ALMEIDA and PEARSE[2] have stated, may indicate temporary storage of sphingolipid, to be used later in the process of myelination. By the time the animals are born at full term, the acid hematin staining with