

STRUCTURAL FEATURES OF THE EPITHELIO-MESENCHYMAL INTERFACE OF RAT DUODENAL MUCOSA DURING DEVELOPMENT

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ABSTRACT

In fetal rats 5-7 days before birth, the duodenal epithelium is separated from mesenchymal cells by a well-defined basal lamina. By 3-4 days before birth, when small rudimentary villi are first seen, direct contact between epithelial and mesenchymal cells occurs by means of epithelial cell cytoplasmic processes which project through gaps in the basal lamina into the lamina propria. At contact sites, the epithelial and mesenchymal cell plasma membranes were less than 100 Å apart but membrane fusion was not seen. In number and size these epithelial cell processes increase strikingly during the last 2 days of gestation, and they persist in large numbers until 7-10 days after birth. Thereafter, they decrease gradually in both number and size until 3-4 wk after birth, when the morphology of the epithelio-mesenchymal interface resembles that seen in adult rats, i.e., there are only rare epithelial cell processes which penetrate deeply into the lamina propria. The presence of a large number of epithelio-mesenchymal contact sites during the period of rapid growth and differentiation of duodenal mucosa may reflect epithelio-mesenchymal cell interactions which may facilitate the maturation of the duodenal mucosa.

INTRODUCTION

In their classic reports, Borghese (1) and Grobstein (2) documented that normal development and differentiation of salivary gland epithelium is dependent upon the interaction between epithelium and mesenchyme. Subsequently, it has been shown that epithelia from many organs and tissues, including the skin (3), pancreas (4), liver (5), thyroid (6), kidney (7), and the lens of the eye (8), require mesenchyme for normal cyto-differentiation.

The available data regarding this interaction between mesenchyme and epithelium during organogenesis have been the subject of several recent reviews (9, 10, 11), but it is clear that the exact nature of and the factors responsible for this interaction have not been fully defined.

Experiments in which developing epithelium and mesenchyme were cultured on opposite surfaces of Millipore filters suggest that high molecular weight materials produced by the mesenchymal cells may induce epithelial cell maturation. For example, Grobstein (12) has shown that the interaction of dorsal spinal cord and metanephrogenic mesenchyme decreases when the intervening membrane has a pore size of less than 0.5 μ or a width greater than 30 μ . It has been suggested that close contact between epithelial and mesenchymal cells in vivo may facilitate transfer of such inducers which stimulate growth and differentiation of the epithelium (13). Indeed, intimate contact between mesenchymal and epithelial cells has been demonstrated in several

developing and regenerating mammalian tissues including liver (14), tooth buds (15), and skin (16).

Virtually nothing is known about the interaction between mesenchyme and epithelium in developing small intestine. In a recent survey of the fine structure of the developing duodenum of the rat, we noted distinctive morphological features at the interface between epithelium and mesenchyme during the period of rapid growth and differentiation of the duodenal mucosa. These observations are detailed in this report.

MATERIALS AND METHODS

65 fetal and postnatal Sprague-Dawley rats ranging in age from 7 days before birth (15th day of gestation) to 32 days after birth and 4 adult rats were studied. Fetuses were obtained daily from the 15th day of gestation until the day of birth (22nd day of gestation) by laparotomy from timed-pregnant rats which had been anesthetized with ether. Fetuses and newborn animals up to 6 days of age were sacrificed by decapitation; older animals and adult rats were anesthetized with ether and killed after intestinal samples had been obtained.

Segments of mid-duodenum and distal ileum from fetuses and newborns up to age 6 days were removed and immersed immediately either in chilled 1% chrome-osmium fixative (17) for 1–2 hr or in chilled 2.5% glutaraldehyde in 0.1 M cacodylate buffer containing 0.9 mM calcium and 0.5 M sucrose for 3 hr. All fixatives and buffer solutions were adjusted to a pH of 7.4. In rats older than 6 days, the fixative solutions were instilled with minimal pressure into the intestinal lumen proximal to the site which was to be sampled. After 3–4 min of *in situ* fixation, segments of duodenum and ileum were removed, cut into 1 mm slices, and immersed in chilled fixative for 1–3 hr. Samples fixed in chrome-osmium were placed into a 10% neutral-buffered formalin for 30 min. Samples fixed in glutaraldehyde were rinsed in six changes of cacodylate buffer over a 1 hr period and then post-fixed in 1% osmium tetroxide in cacodylate buffer for 1 hr. Thereafter, all tissues were rapidly dehydrated in graded concentrations of ethanol and embedded in epoxy resin using Luft's (18) method. Embedded tissues were carefully oriented and mounted on aluminum rods for sectioning. Sections from the entire block were then cut 1 μ thick with a Sorvall MT-2 microtome, mounted on glass slides, and stained with toluidine blue (19). From these sections, areas were selected for electron microscopy and blocks were appropriately trimmed. Thin sections were cut on an LKB microtome with a diamond knife, mounted on uncoated copper mesh grids, and stained with uranyl acetate (20) and lead citrate (21).

Sections were examined with a Philips EM-300 electron microscope.

RESULTS

Growth and differentiation of the duodenum of the rat is rapid during the last week of gestation and the first 3–4 wk of extrauterine life. In fetuses 7 days before birth, the duodenum consists of a simple tube with a tiny lumen lined by stratified cells with no morphological evidence of differentiation. Over the next 7 days, the size of both the duodenum and its lumen enlarges rapidly. At birth, short but well-formed villi covered by a single layer of differentiated columnar absorptive cells have replaced the stratified epithelium, but crypts are still rudimentary and poorly developed. During the first 3–4 wk of extrauterine life, the villi increase in number and grow to the height found in adult rats. Concomitantly, crypt development proceeds and by 3–4 wk after birth the crypts are comparable in size to those of adult rats, and differentiated cells such as Paneth cells appear fully developed.

In the duodenum of fetal rats obtained 7–6 days before birth, the stratified epithelial cells were surrounded by a loose sheath of mesenchymal cells. Most mesenchymal cells were separated from each other by large intercellular spaces. A well-defined, apparently continuous basal lamina was interposed between epithelial and mesenchymal cellular elements and was closely applied to the basal plasma membrane of the deepest layer of epithelial cells (Fig. 1). In fetuses obtained 5 days before birth, aggregates of mesenchymal cells, closely applied to one another, were seen adjacent to the base of epithelial cells in some areas. However, a continuous basement membrane still separated the stratified epithelial cells from the mesenchymal elements.

Well-formed caveolae were present along the basal membrane and along the basal portion of the lateral plasma membrane of the epithelial cells adjacent to the mesenchyme (Figs. 1 and 2). In addition, a variable number of vesicles 100–250 m μ in diameter were seen in the cytoplasm adjacent to the basal plasma membrane in most epithelial cell sections (Fig. 2). Some of these vesicles were enclosed by a smooth membrane. The limiting membrane of others showed radially arranged, linear striations along the cytoplasmic or outer surface of the membrane (Fig. 2 B). Many of the vesicles had electron-lucent contents; others con-

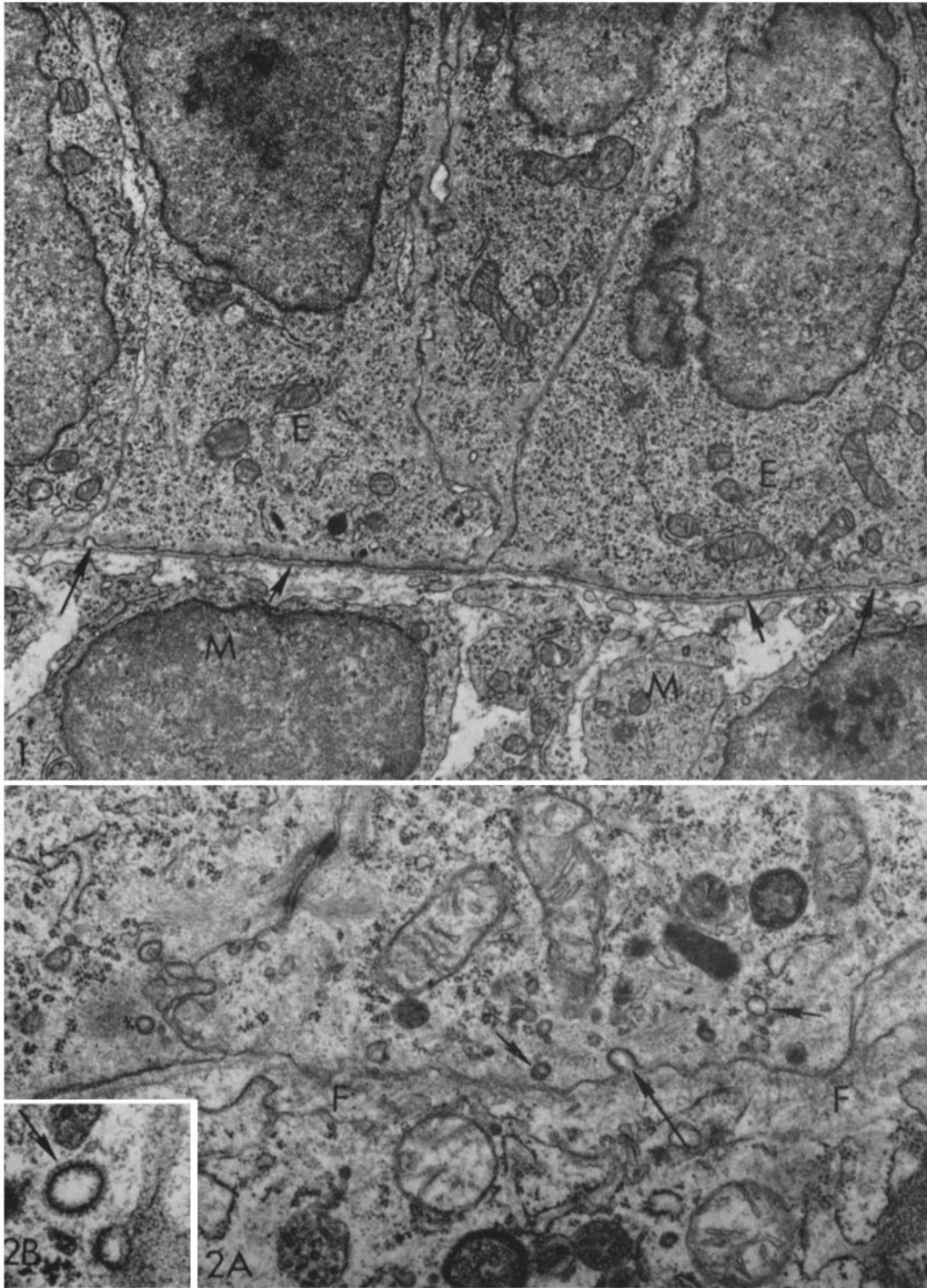


FIGURE 1 The epithelio-mesenchymal interface from the duodenal mucosa of a fetal rat 7 days before birth. A continuous basal lamina (short arrows) separates the base of epithelial cells (*E*) from the mesenchymal cells (*M*). The long arrows indicate caveolar indentations of the basal plasma membrane of the epithelial cells. $\times 8000$.

FIGURE 2 A Epithelio-mesenchymal interface from a fetal rat 5 days before birth. A caveolar indentation (long arrow) of the basal plasma membrane and some vesicles (short arrows) are seen at the base of the epithelial cell. Collections of microfibrils (*F*) are located at the interface between epithelium and mesenchyme. $\times 22,000$.

FIGURE 2 B A striated vesicle (arrow) is seen near the basal part of the lateral membrane. $\times 51,000$.

tained homogeneous material of moderate electron opacity.

Collections of fine fibrils 100–400 Å in width were interposed in many areas between the mesenchymal cells and the surface of the basal lamina facing the mesenchyme. These fibrils were more or less randomly arranged although some appeared to contact both the plasma membrane of mesenchymal cells and the basal lamina (Fig. 2). Occasionally, a periodicity of 640 Å was evident along the length of the wider fibrils.

In fetuses obtained 4 days before birth, when rudimentary villi first appear in the duodenum, morphological changes were evident at the epithelio-mesenchymal interface. The basal lamina, which was continuous in the duodenum of younger fetal rats, was discontinuous in many areas. In some of these areas, the basal plasma membrane of the epithelial cells and the plasma membrane of adjacent mesenchymal cells came into close contact (Fig. 3). In these areas the intercellular space between epithelium and mesenchyme was often less than 100 Å. Although fusion between epithelial and mesenchymal plasma membranes was not observed, condensations of electron-opaque material were seen occasionally along apposing surfaces of epithelial and mesenchymal cell plasma membranes (Fig. 3). These condensations resembled closely the membrane specializations interpreted by Overton as early desmosomes in developing chick blastoderm (22). In addition, small pseudopod-like cytoplasmic processes projected from the base of the epithelial cells through many of the gaps in the basal lamina into the lamina propria (Fig. 4). Many of these epithelial

cytoplasmic processes made direct contact with mesenchymal cells (Fig. 4). Those which did not, in a given section, may have been in contact with mesenchymal cells beyond the plane of section.

These cytoplasmic processes, first evident in fetuses 4 days before birth, became more abundant and increased in size during late fetal life. By the day of birth, numerous large pseudopods projected into the lamina propria from the base of many of the epithelial cells (Fig. 5). The pseudopod-like cytoplasmic projections were so prominent that they could be seen readily with a light microscope in toluidine blue-stained 1 μ sections. Fragments of basal lamina often were evident between adjacent projections (Figs. 5 and 7). The epithelial pseudopods contacted a variety of cell types in the lamina propria including fibroblasts, macrophages, eosinophiles, and, in older animals, even nerve elements. The processes were abundant along the length of the villi except at their extreme base, where they were seen only occasionally. Similarly, they were only occasionally found along the base of cells lining incompletely developed crypts. The few processes observed at the base of the villi and in the crypts were consistently smaller than those seen higher on the villi.

Although no basal lamina enveloped the plasma membrane lining the epithelial cell projections, a definite intercellular space, at times less than 100 Å wide, was always seen between epithelial and mesenchymal cell plasma membranes (Fig. 5). There was no fusion of epithelial and mesenchymal cell plasma membranes.

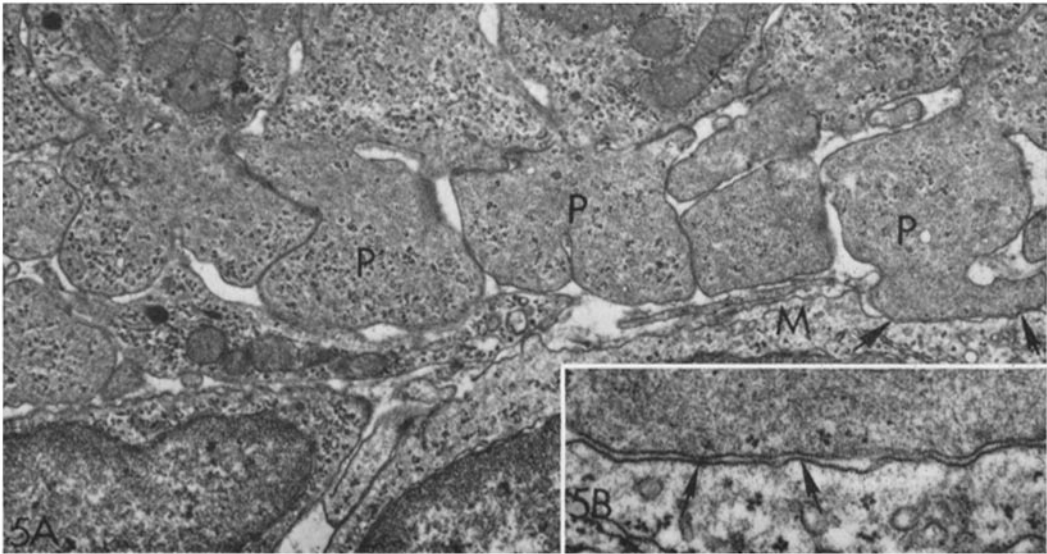
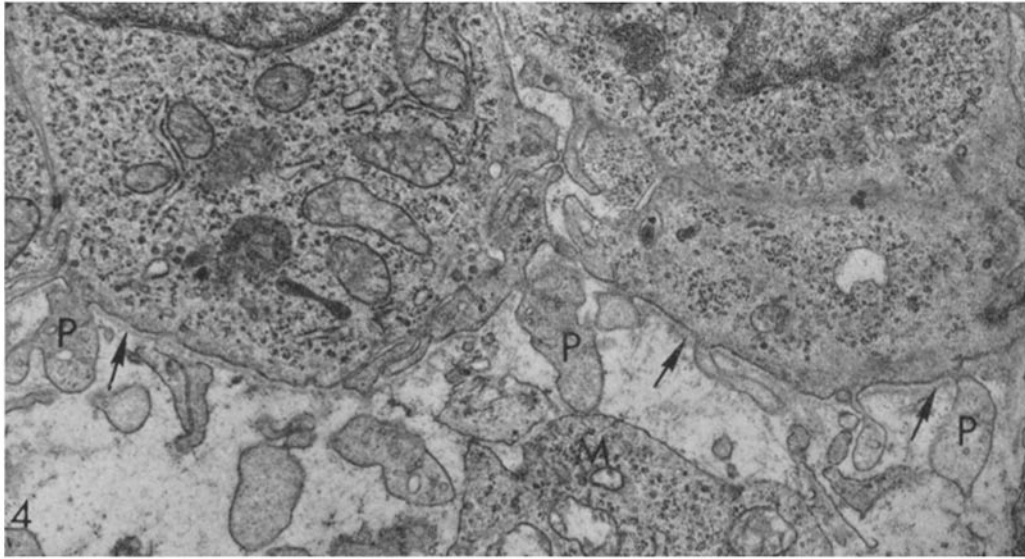
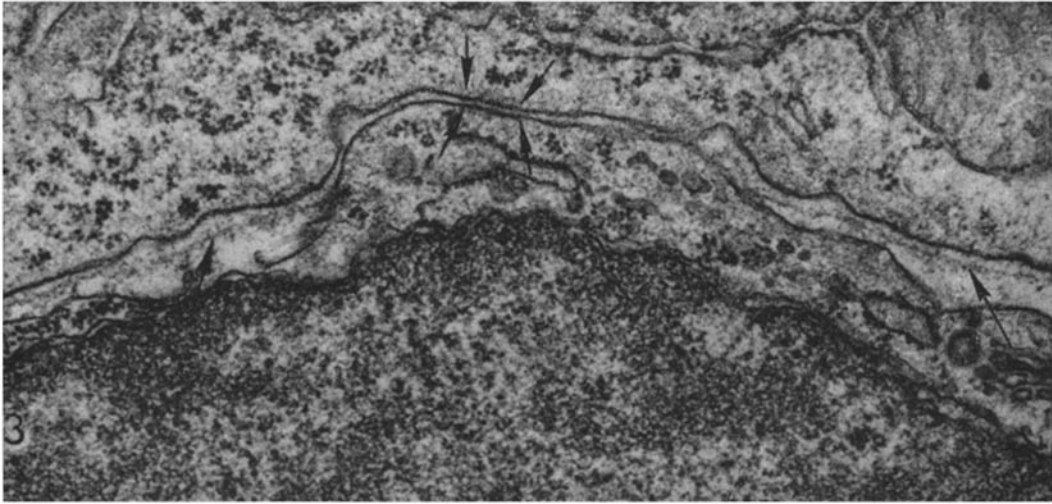
In older fetuses and in suckling animals, microfibrils were often seen in the extracellular space

FIGURE 3 Epithelio-mesenchymal interface of duodenal mucosa 3 days before birth. There is close apposition of the plasma membranes of an epithelial and a mesenchymal cell over a wide area where the basal lamina is deficient. The remaining basal lamina is indicated by long arrows. Note areas of increased electron opacity (short arrows) along apposed plasma membranes (? early desmosome). $\times 35,000$.

FIGURE 4 Epithelio-mesenchymal interface 4 days before birth. The basal lamina (arrows) between epithelial and mesenchymal cells is discontinuous. Small cytoplasmic processes (*P*) project from the base of the epithelial cells through these discontinuities and contact mesenchymal cells (*M*). $\times 13,000$.

FIGURE 5 A Epithelio-mesenchymal interface from a fetal rat killed just before birth. There are large cytoplasmic processes (*P*) with broad necks along the base of the epithelial cells. These project into the lamina propria and contact mesenchymal cells (*M*). $\times 10,000$.

FIGURE 5 B Higher magnification of the area of contact between the epithelial cell process and a mesenchymal cell indicated by arrows in Fig. 5 A. The plasma membranes are separated by a space of less than 100 Å in some areas (arrows). $\times 25,000$.



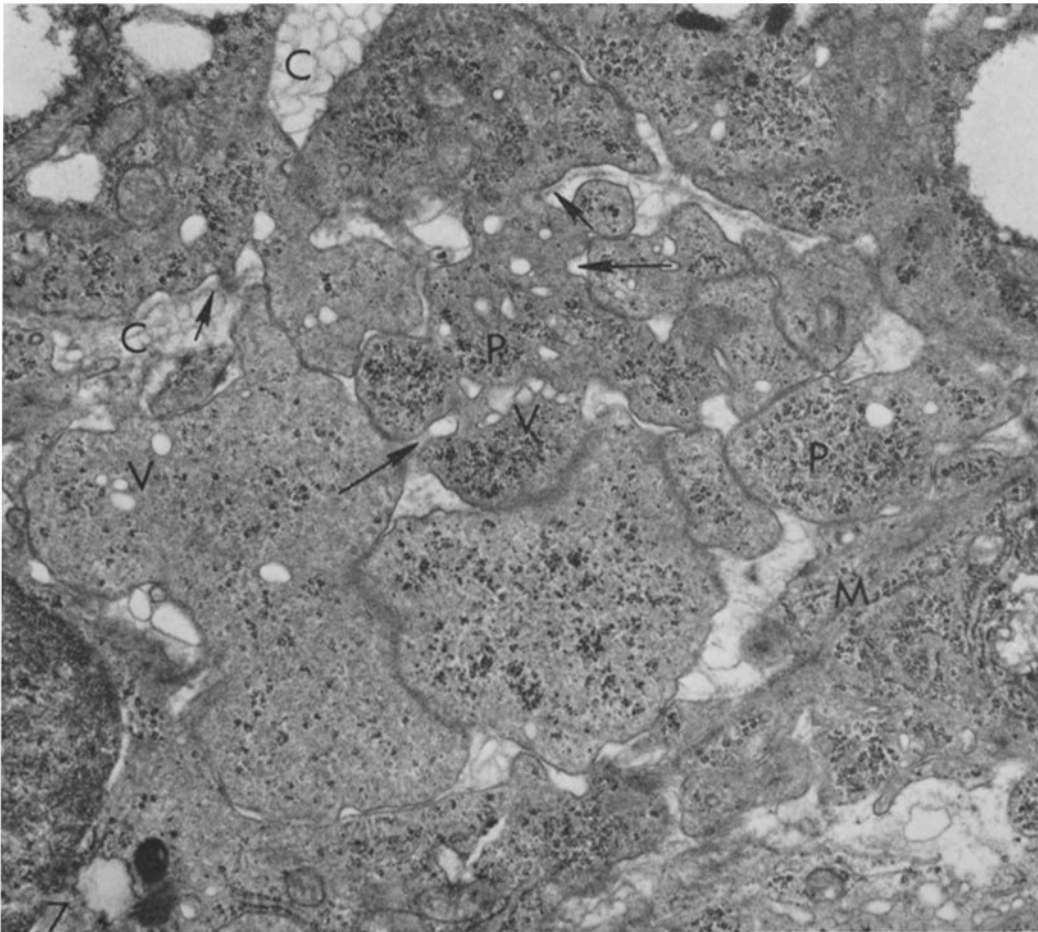
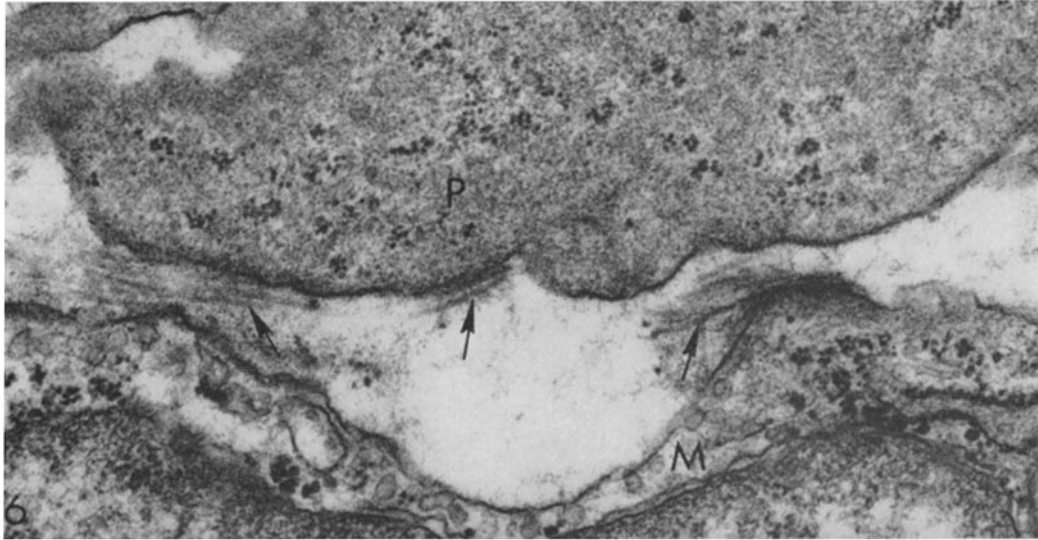


FIGURE 6 Basal aspect of an epithelial cell process (*P*) in lamina propria adjacent to a mesenchymal cell (*M*) from a fetal rat 1 day before birth. Tufts of microfibrils (arrows) which are more or less parallel to the surface of the process are located between it and the mesenchymal cell. $\times 36,000$.

FIGURE 7 Base of duodenal epithelium from a newborn rat killed a few hours after initiation of suckling. Several large cytoplasmic processes (*P*) project into the lamina propria through defects in the basement membrane (short arrows) and contact mesenchymal cells (*M*). Caveolae (long arrows) are seen along the plasma membrane of one of the processes. The processes contain unattached ribosomes and many vesicles (*V*), whose content is electron lucent. Chylomicrons (*C*) are seen in the intercellular spaces between epithelial cells and in the lamina propria. $\times 14,000$.

adjacent to the epithelial cell processes and the mesenchymal cells (Fig. 6). Most but not all of these fibrils were oriented parallel to the plasma membrane enclosing the epithelial cell processes, and some revealed a 640 Å periodicity along their length.

The cytoplasm of the epithelial cell processes contained abundant cytoplasmic ground substance, many unattached ribosomes, varying numbers of smooth-walled or striated vesicles, aggregates of glycogen, and, occasionally, lysosome-like dense bodies (Figs. 4, 5, 7, and 8). Caveolar indentations of the plasma membrane of the processes were seen occasionally (Fig. 7).

The epithelial cytoplasmic processes became most abundant a few hours after birth (Fig. 7) and persisted in large numbers until the animals were 7–10 days old. In animals killed during that time, many but not all epithelial cell sections showed this intimate morphological relationship between epithelial and mesenchymal cells. The processes were present along the base of goblet cells as well as absorptive cells (Fig. 8). They were not seen along the base of endocrine cells.

The number of cytoplasmic projections gradually decreased in animals older than 10 days. During the fourth week after birth, at which time crypt and villous morphology closely resembled that seen in adult rats, only rare absorptive cells possessed large processes which extended deeply into the lamina propria through gaps in the basal lamina. More frequently, much smaller epithelial cell processes extended into the lamina propria through gaps in the basal lamina at the lateral aspect of the cell base (Fig. 9). At such sites, cell processes of lymphocytes, macrophages, and eosinophiles from the lamina propria often projected into the intercellular space between adjacent epithelial cells through the gaps in the basal lamina (Fig. 9). Chylomicrons were often seen within the gaps in the basal lamina.

The epithelio-mesenchymal interface of the duodenum from adult rats was similar to that seen in suckling rats 3–4 wk of age. Small cytoplasmic processes at the lateral aspect of the cell base occasionally projected into the lamina propria, whereas the larger processes which extended several microns into the lamina propria (Fig. 10) were rare (approximately one to three such processes per longitudinal villus section).

In preliminary studies, we examined the epithelio-mesenchymal interface of the distal ileum

from fetuses obtained 4 days and 1 day before birth and from suckling rats killed 5, 10, and 15 days after birth. The large, basally located epithelial cell processes, which extended into the lamina propria in developing duodenum, were not seen in any of the samples of developing distal ileum.

DISCUSSION

Our findings, which are summarized in Fig. 11, indicate that distinctive morphological specializations appear at the interface between the epithelium and mesenchyme of the developing duodenal mucosa during its period of rapid growth and differentiation. 5–7 days before birth, the stratified duodenal epithelium of fetal rats appears completely separated from the underlying mesenchyme by a continuous basal lamina. When columnar epithelium and villi first become evident 4 days before birth, gaps appear in the previously continuous basal lamina. Large areas of epithelial and mesenchymal cell plasma membranes become apposed to one another at the level of the basal lamina where it is discontinuous. In addition, small pseudopod-like processes of cytoplasm project into the lamina propria through the gaps in the basal lamina and contact mesenchymal cells. In size and number these cell processes increase dramatically during the last 2 days of fetal life as villi grow in height and number, and they persist in large numbers until the suckling rat is 7–10 days old.

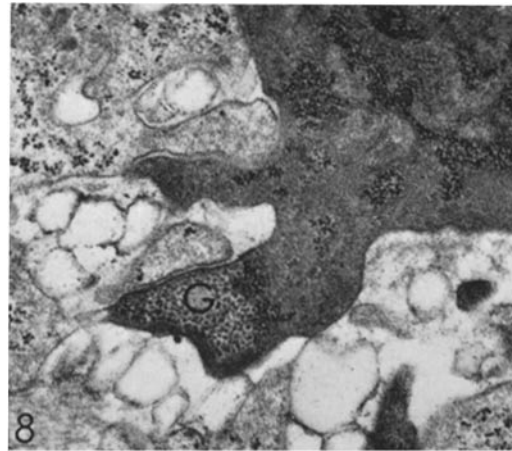


FIGURE 8 Base of a goblet cell from a newborn suckling rat, showing a cytoplasmic process projecting into the lamina propria. There are glycogen particles (G) in the process. $\times 22,000$.

Thereafter, they gradually decrease in both size and number until 3–4 wk after birth, at which time the duodenal mucosa appears fully developed, from a morphological point of view, with mature crypts and tall villi. At that time, the epithelio-mesenchymal interface resembles that found in adult rats, i.e., there are only rare gaps in the basal lamina through which epithelial cell processes project into the lamina propria as described previously in other species (23, 24).

The finding of an intimate morphological association between mesenchyme and epithelium in a rapidly growing, differentiating tissue is not without precedent. Similar contact between epithelial cell and mesenchymal cell plasma membranes through gaps in the basal lamina have been described in the developing tooth bud in cats (15) and in the developing liver of fetal mice (14). Contact between the epiblast and the mesoblast and between the hypoblast and the mesoblast has been noted in the young chick embryo (25). Direct contact between epithelium and mesenchyme has been described also in the regenerating skin of humans (16) and mice (26) during wound healing. An apparently identical morphological relationship, i.e. penetration of basal lamina by epithelial pseudopods and, in some instances, contact with mesenchymal cells, has also been observed in certain epithelial cell neoplasms including carcinoma of the colon (27, 28), carcinoma of the uterine cervix (29), and certain experimentally induced murine epithelial tumors (30, 31). In all these instances in which there is direct contact between epithelial and mesenchymal cells, there is rapid proliferation and growth of the epithelial cell populations.

The dependence of normal organogenesis and epithelial cell differentiation upon the interaction between mesenchymal and epithelial elements appears firmly established (2, 9). If the transfer of

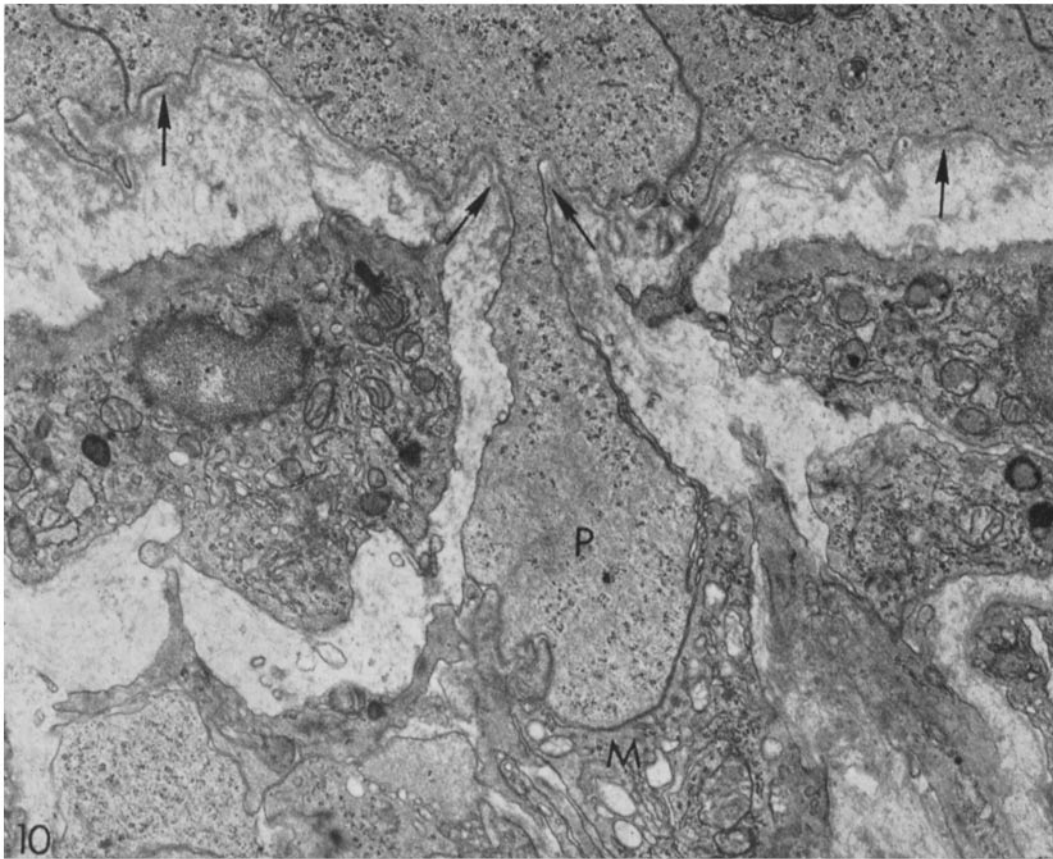
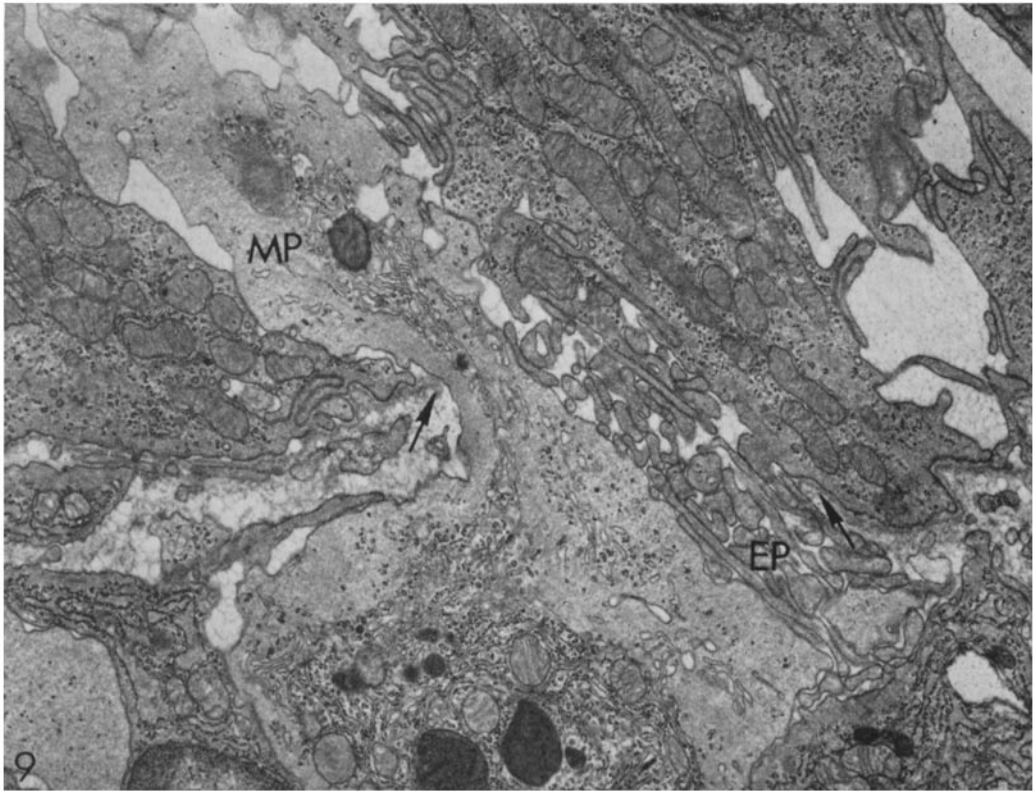
large molecules from mesenchyme to epithelium induces growth and differentiation as transfilter experiments suggest (12, 32), the morphological specializations observed by us at the epithelio-mesenchymal interface in developing rat duodenum may serve to facilitate such an interaction. The epithelial basal lamina, a potential barrier to the transfer of large molecules, is fragmented in the developing duodenum and permits extensive direct contact between epithelial and mesenchymal plasma membranes without intervening structures. Although the permeability characteristics of the basal lamina underlying the intestinal epithelium are not known, the basal lamina of other tissues such as the glomerulus (33) serves at least as a partial barrier to the transfer of large molecules such as ferritin.

The presence of caveolae along the apposed epithelial and mesenchymal plasma membranes and of vesicles in the cytoplasm near these membranes is consonant with the concept that transfer of materials may occur between these distinctive cell types. Caveolae and vesicles, especially striated vesicles, have long been implicated in the transport of large molecules (34).

Microfibrils, some of which have a longitudinal periodicity identical to that of collagen, were present at the epithelio-mesenchymal interface either adjacent to the epithelial cell basal lamina or closely applied to the surface of the plasma membrane of epithelial processes not covered by a basal lamina. Microfibrils and collagen, similarly disposed at the epithelio-mesenchymal interface, have been described during odontogenesis (35) and in transfilter cultures of salivary and pancreatic epithelium (36, 37). There is evidence that collagenous proteins are synthesized by the mesenchymal cells and polymerized at the epithelial surface (38, 39, 40). It has also been suggested that such collagenous proteins may be important

FIGURE 9 Epithelio-mesenchymal interface from a 22-day-old rat. A cell process (*MP*) from a mesenchymal cell, probably a macrophage, projects through a gap in the basal lamina (arrows) into the intercellular space between epithelial cells. In addition, many small cell processes (*EP*) project from the basolateral aspect of one of the epithelial cells into the lamina propria through the same gap in the basal lamina. $\times 12,000$.

FIGURE 10 Epithelio-mesenchymal interface from an adult rat. A large cytoplasmic process (*P*) projects from the base of an epithelial cell through a small gap in the basal lamina and is in close contact with a mesenchymal cell (*M*). Except for this single discontinuity, the basal lamina (arrows) is well-developed and separates epithelial and mesenchymal elements. $\times 12,000$.



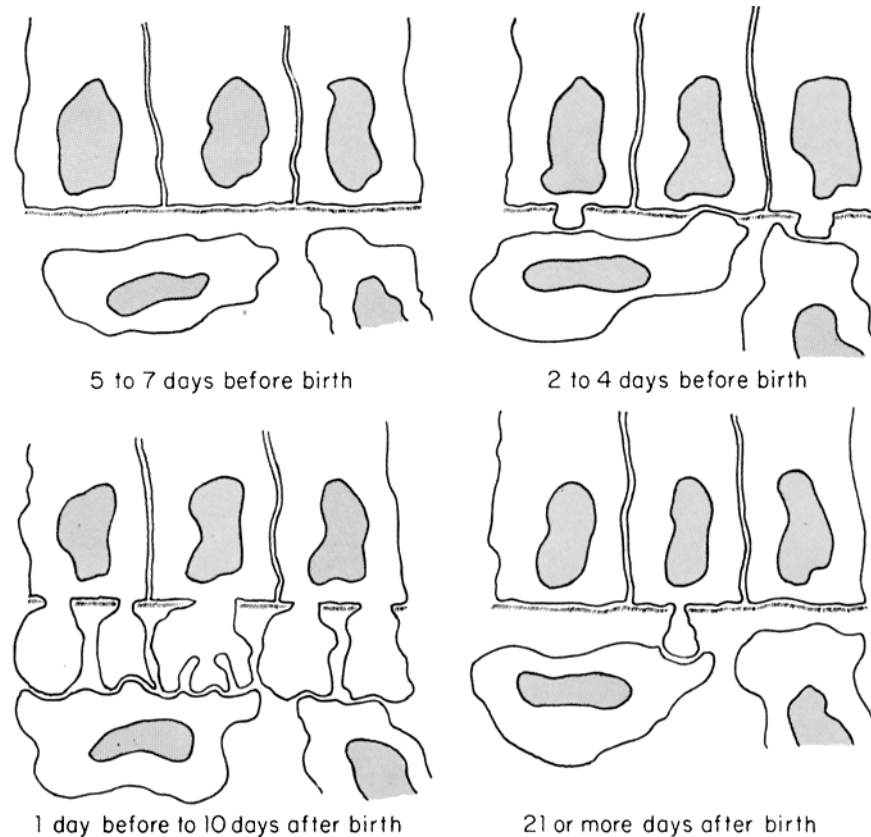


FIGURE 11 Schematic diagram in which the morphological features of the epithelio-mesenchymal interface during development of the duodenal mucosa are summarized.

in morphogenesis (38, 39) and may function as a mediator in epithelio-mesenchymal interactions (35, 37). However, recent studies indicate that transfilter passage of collagenous proteins from mesenchyme to epithelium is not dependent on epithelial morphogenesis (40).

It is puzzling that our preliminary studies of the epithelio-mesenchymal interface of the ileum do not show the morphological specializations evident in the duodenum during comparable stages of development. There is evidence that the function and morphology of the ileum may differ significantly from that of the duodenum in suckling rodents (41, 42). Clearly, further study of the morphological specializations at the epithelio-mesenchyme interface at various levels of the small intestine during development is needed.

Our morphological observations provide only indirect evidence that the epithelium and mesenchyme may interact in the duodenal mucosa during its period of rapid growth and differentiation.

To prove such an interaction, methods must be devised which will permit culture of isolated epithelial and mesenchymal elements of developing intestine so that the interrelationships between these tissues can be clarified. Thus, until additional information can be obtained, the possibility has not been excluded that the intimate morphologic relationship between epithelium and mesenchyme in developing rat duodenum may reflect some unknown function, quite apart from epithelio-mesenchymal interaction.

Dr. Mathan is the recipient of a Wellcome Trust Fellowship. Dr. Trier is the recipient of National Institutes of Health Career Development Award AM 47257.

This work was supported by National Institutes of Health Grants AM 14420 and AM 05005.

Received for publication 23 September 1971, and in revised form 1 November 1971.

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