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Letter to the Editor

GASTRIC EPITHELIUM UNDER ORGANOTYPIC PERFUSION CULTURE

Dear Editor:

A new tissue culture model for the investigation of gastric mucosa maturation was developed. The mature epithelium of the gastric mucosa is composed of different cell types with highly specialized functions. The embryonic stomach is covered by a single-layered, prismatic epithelium (5), whereas the mature gastric mucosa is characterized by numerous densely packed glands. The differentiation of the gastric glands is initiated with the invagination of the epithelium into the connective tissue beyond (4). It is further stimulated by a variety of soluble factors (9,15). At present little is known about the differentiation mechanisms which trigger the maturation of the individual glandular cell types as principal, oxcyntic and mucus-producing cells.

Cell and tissue culture models have been used to investigate the function of growth and differentiation factors during organ development (12,13). These culture models are widely used for toxicological and pathological testing (7,16). However, some of the gastric epithelial cells secrete highly aggressive substances which interfere with the preservation of the cultured tissue. Culture periods exceeding 24 h could be achieved only when serum was added to the culture medium or fetal tissue with less secretory activity was used (15). The serum supplement served as a buffer for secreted acid and proteases. On the other hand, serum includes a number of known as well as unknown growth and differentiation factors.

In search for a suitable culture model to investigate the influence of differentiation factors and nutrients on the maturation of the gastric mucosa, we established a new serum-free tissue culture model. We used tissue from newborn rabbits, taking advantage of the fact that the gastric mucosa of neonatal animals features an early developmental state. The organ-specific spatial organization and the organotypical extracellular matrix are essential for the coordinated development of the different tissue components (2,3). Consequently, pieces of the gastric wall were prepared by sectioning, and proteases were not applied. Thus, the cellular composition and the organ-specific extracellular matrix of the gastric wall remained intact. Tissue pieces were 1–2 mm thick and 13 mm in diameter.

To prevent curling up of the tissue samples, the pieces were mounted in tissue carriers. The holding ring of the carrier covered only a small rim of the tissue sheet; most of its surface remained accessible for the culture medium (Fig. 1 *a*). The use of tissue carriers is a prerequisite for optimal exchange of nutrients, secretory products and metabolites. Mounted tissue pieces were inserted in conventional culture dishes or in perfusion chambers (Fig. 1 *b*) and were cultured serum free over periods exceeding 5 d.

The comparison of stationary and perfusion-cultured gastric tissue revealed the following results: The gastric epithelium of the neonatal rabbit consists of a single cell layer which is folded and shows numerous shallow infoldings and dimples (Fig. 2). The cells are cuboidal to prismatic. At the time of birth only, some oxcyntic cells

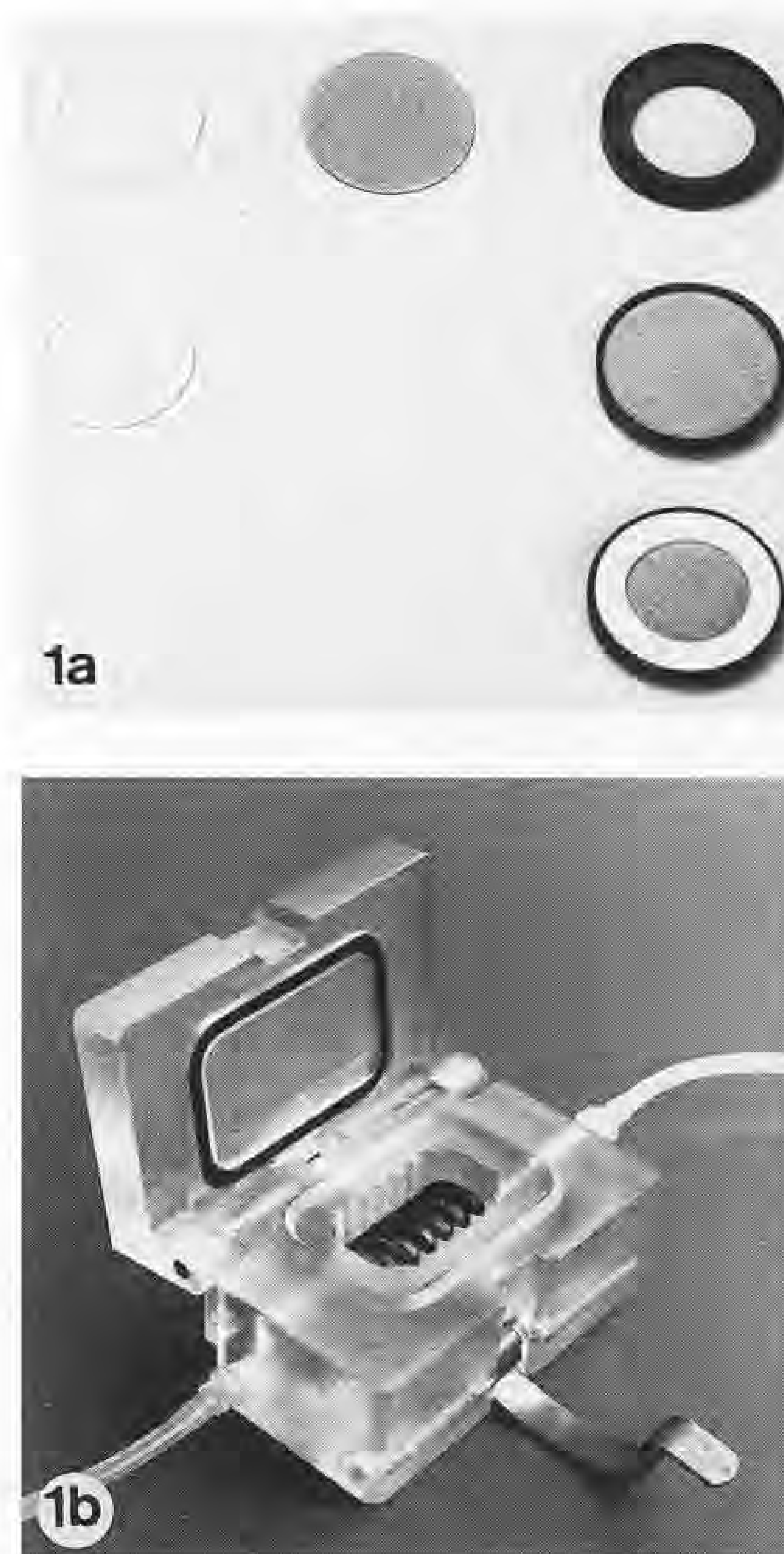

FIG. 1 *a*. Mounting of stomach tissue pieces in tissue carriers. The tissue was prepared from stomachs of 1- to 3-d-old rabbits. From the corpus region of the gastric wall pieces of stomach tissue 13 mm in diameter were cut. To prevent curling of the tissue pieces, the specimens were mounted in tissue carriers. The sample was placed in the black carrier and was fixed by insertion of the white ring. *b*, Perfusion culture. For culture under continuous medium flow, the mounted tissue pieces were inserted in a perfusion culture container (Minucells and Minutissue, Bad Abbach, Germany) and maintained at 37° C on a warming plate (Medax, Kiel, Germany) outside a CO_2 incubator. The specimens were kept under continuous medium flow (flow rate: 4 ml/h) for 5 d with serum-free medium (IMDM supplemented with an antimycotic mix composed of streptomycin, penicillin and amphotericin B; Gibco-BRL Life Technologies, Eggenstein, Germany). Magnification: ×0.8.

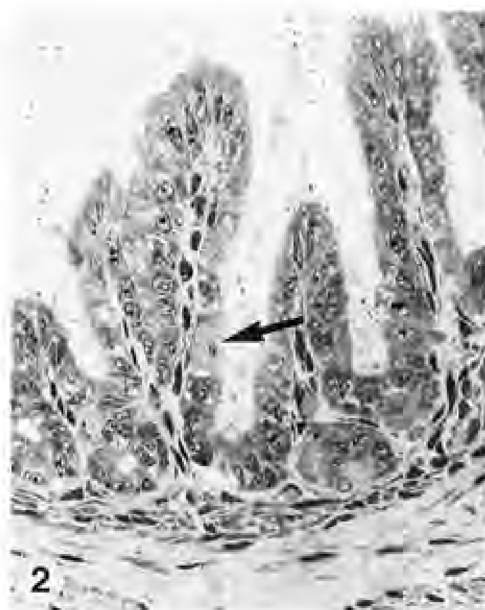

FIG. 2. Histology of the gastric epithelium of the neonatal rabbit. For morphological analysis, the tissue pieces were prepared for semithin sectioning. The samples were fixed, dehydrated and embedded in Epon. Semithin sections were stained with Richardson's solution (1% methylene blue in 1% Na-tetraborate; 1% Mallory's Azur II). The single-layered epithelium of the *tunica mucosa* was folded in shallow dimples. Typical gastric glands were not observed at this developmental stage. The epithelium consisted of prismatic cells. Few mucus granules were at the apical cell pole. Only few oxcyntic cells (*arrow*) were observed. At the base of the dimples, prismatic cells were detected which were darker than the mucus-secreting cells at the tips of the infoldings. Magnification: ×551.

interspersed between mucus-producing cells could be observed (Fig. 2). While the morphology of the oxcyntic cells resembled those found in the mature mucosa, most of the other epithelial cells appeared immature. They showed few dark-staining granules, the characteristic feature of mucus-producing cells. At the base of the infoldings some darker-stained cells were observed (Fig. 2), but fully matured principal cells could not be detected at this stage of development.

In a first set of experiments the mounted tissue pieces were placed in a conventional 24-well culture dish and cultured serum free with daily medium exchange. After 5 d the gastric epithelium had completely disappeared (Fig. 3 *a*). Fibrillar elements of the extracellular matrix and few spindle-shaped cells were the only remaining components of the gastric mucosa. Mucin production, which was detected by the viscous consistency of the culture medium, ceased after the first d of culture.

In a second set of experiments, the mounted tissue pieces were cultured under continuous medium exchange (Fig. 3 *b*). Again, the IMDM medium was not supplemented with serum. After 5 d of perfusion culture (flow rate: 4 ml/h), the surface of the specimen was covered by a continuous single-layered epithelium (Fig. 3 *b*). Numerous infoldings could be detected. The epithelial cells were of prismatic shape. Mucoid cells which included dark-staining granules beyond their apical cell membrane were frequently observed. Mucin secretion by these cells remained high during the whole culture period.

Organ development and the functional maturation of the parenchyme depend on a variety of external signals. Soluble growth and differentiation factors as well as components of the organ-specific extracellular matrix are involved in the complex developmental processes (3,8,9). The mature gastric mucosa consists of numerous densely packed glands and a covering epithelial layer which produces protective mucus. The requirements for gland formation are partial remodeling of the extracellular matrix, precursor cell proliferation, migration, and terminal differentiation. All of these steps have to be initiated, coordinated, and at least terminated.

Numerous soluble factors have been described which stimulate the proteolytic degradation of the extracellular matrix (1) or the syn-

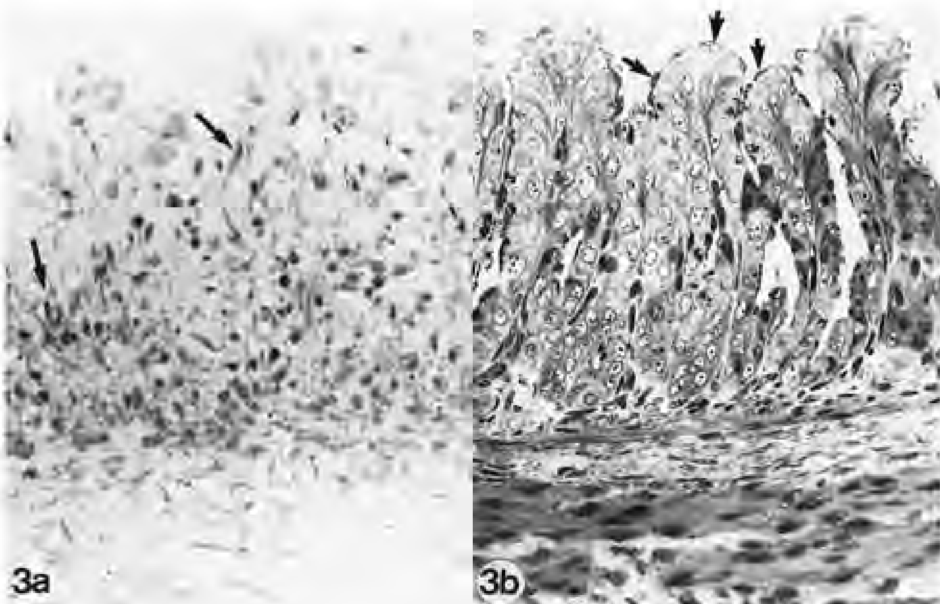

FIG. 3 *a*. The gastric epithelium after 5 d of stationary culture. Stomach explants mounted in tissue carriers were placed in 24-well culture dishes and kept in an incubator (Heraeus, Hanau, Germany; 5% CO_2, humid atmosphere, 37° C). The medium was changed daily. Specimens were cultured for 5 d in serum-free IMDM medium. Within the *tunica mucosa*, only some fibrillar tissue components and few spindle-shaped cells (*arrows*) remained. An epithelial cell layer was not detectable. Magnification: ×551. *b*, Histology of perfusion cultured gastric epithelium. The *tunica mucosa* was composed of prismatic epithelial cells. At the apical cell poles numerous dark-staining granules were found (*arrows*). The structure of the lamina propria remained unchanged. Magnification: ×551.

thesis of new matrix material (16). These factors may also induce cell proliferation and migration (12,15,16). Extracellular matrix components, proteases, and soluble paracrine factors are produced within the developing tissue. Thus, the cooperation of neighboring cells is an important factor for undisturbed organ development (3).

Bearing in mind the tremendous influence of intercellular communication (4,8,14) and the necessity of extracellular matrix compounds (6,13) for the development of typical cellular functions we sought a suitable tissue culture system for our investigations on the mechanisms of gastric mucosa development. As a first step, the application of proteases was avoided for the preparation of the tissue samples. The microsurgical preparation method retained the complex spatial tissue composition. Extracellular matrix and cell-to-cell contacts remained unchanged.

However, the production of highly aggressive, destructive molecules by some of the epithelial cells seriously interfered with the preservation of an intact tissue under stationary culture conditions. Rising concentrations of HCl and proteolytic enzymes in the culture medium caused severe damage even when the medium was changed daily (Fig. 3 *a*). This situation demanded a precise control of the culture environment. Therefore, a perfusion culture system was used which allowed for a continuous medium exchange over prolonged culture periods (11). The continuous elimination of harmful metabolites and secretory products resulted in unequaled tissue preservation. The tissue pieces were permanently supplied with fresh medium of defined composition of nutrients and oxygen. Using this system, the morphology of the cultured stomach pieces remained unchanged even after 5 d of culture.

A further important requirement for the investigation of the function of soluble growth and differentiation factors is a serum-free culture system. As shown previously, the perfusion culture system allows for the complete avoidance of serum supplementation of the medium (10). Soluble mitogens and morphogens now can be analyzed without interference caused by unknown serum components. Gastric tissue cultured without serum showed no necrotic lesions. Even the characteristic cellular function could be preserved when the tissue was supplied with basic medium alone. Perfusion-cultured epithelial cells secreted mucus over the whole culture period. At the morphological level, beginning maturation of the mucoid cells was indicated by the appearance of dark-staining granules.

The organotypic tissue culture model presented herein offers new opportunities for the investigation of the maturation of the gastric mucosa under defined and stable culture conditions. The influence of soluble differentiation factors and nutritional components on gastric maturation now can be investigated with this system. As in the developing organ, interactions between the different cellular tissue components and the supportive function of the organ-specific extracellular matrix can take place.

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