Effect of amniotic fluid and fetal bovine serum on the morphogenesis of mouse duodenal villi in organ culture[1]

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Summary. When duodenal explants of 15-day mouse foetuses are cultured in Trowell T8 medium during 72 h, villi do not develop. If mouse amniotic fluid (25%) is added to the same medium, short villi appear after 12 h of culture and medium size villi are seen at 48 h. Bovine amniotic fluid and fetal bovine serum promote cell growth but morphogenesis of intestinal villi is far less stimulated.

To what extent the amniotic fluid plays a role in the nutrition of embryos is still unknown. It has previously been shown in the rat that proteins[2] and horseradish peroxidase[3] injected into the amniotic cavity are absorbed by the foetuses. Moreover, a single dose of lactose into the amniotic fluid induces an increase in lactase activity in fetal rat jejunum[4,5]. Hence, the amniotic fluid may influence the differentiation of the intestinal mucosa in utero.

The aim of this work was to determine the effect of amniotic fluid and fetal bovine serum on the development of foetal mouse duodenum in organ culture. Special attention was given to the formation of duodenal villi and to the differentiation of absorptive cells.

Materials and methods. Swiss ICR mice embryos were removed under sterile conditions at 15 days of gestation. The culture method has been described elsewhere[6]. Baseline studies were done with the Trowell T8 medium (Grand Island Biological, Burlington, Canada). Fetal bovine serum (FBS) was added at a concentration of 10%; bovine amniotic fluid (BAF) and mouse amniotic fluid (MAF) were added at a concentration of 25%.

For morphological studies explants were examined after 1, 24, 48 and 72 h of culture. Tissues were fixed in 2.8% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2, rinsed and postfixed in osmium tetroxide. After dehydration, explants were oriented and embedded in Epon[7]. Thick sections were stained with toluidine blue and thin sections were contrasted with uranyl acetate and lead monoxide. The total number of epithelial cells per transverse section of properly oriented explants was counted in 5 explants at each time interval after the beginning of the culture.

Results and discussion. The number of epithelial cells per transverse section of explants was used to evaluate rapidly the behavior of the duodenal segments cultured with the different media. Figure 1 illustrates the percentage increase (or decrease) in the epithelial cell number per transverse section of 5 explants. Cultures supplemented with MAF were arrested at 48 h because of significant oedema in the explants.

With Trowell T8 medium, villi do not develop. The surface of the epithelium remains flat and the lateral edges of the explants are not covered with the epithelium even at 72 h of culture. When this same medium is supplemented with MAF, after 12 h small villi are seen over the entire free surface of the explants; after 24 h, medium size villi are observed (figure 2). In transverse sections, the serosal side of the explants is epithelialized; villi are seen on the entire free surface of the explants. After 48 h of culture with the same medium villi disappear on the lateral sides (figure 3) due to a severe oedema. When the explants are cultured with Trowell T8 medium supplemented with BAF, short

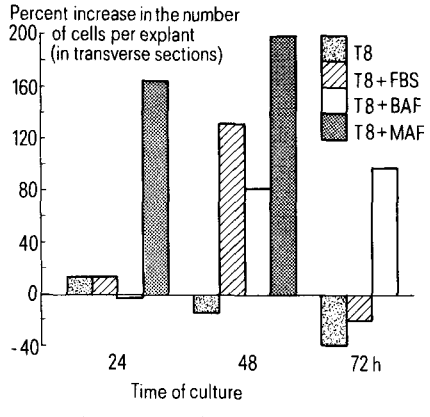

Fig. 1. Percentage increase (or decrease) in the number of epithelial cells per transverse section of 5 explants: T8 (Trowell T8 medium), FBS (Fetal bovine serum), BAF (Bovine amniotic fluid), MAF (Mouse amniotic fluid).

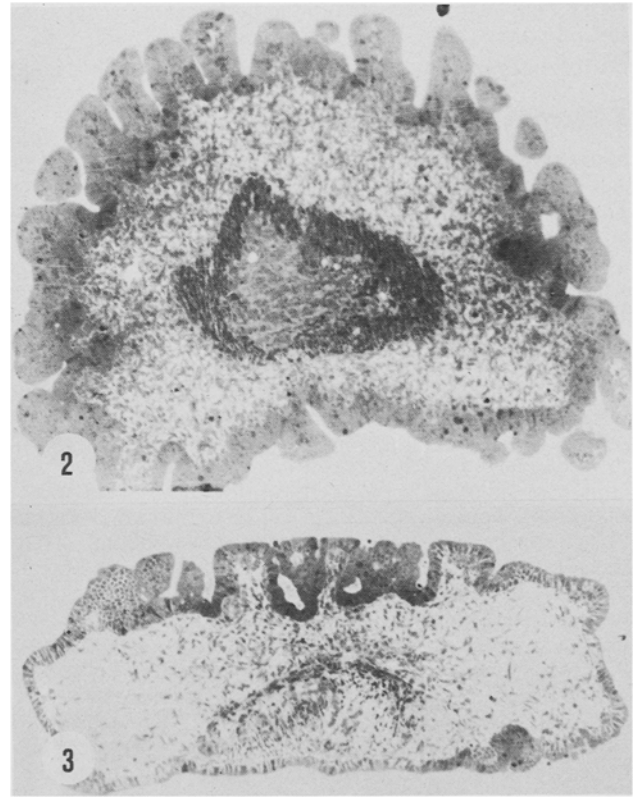

Fig. 2. Duodenal explant of a 15-day mouse fetus cultured during 24 h with Trowell T8 medium supplemented with mouse amniotic fluid. Medium size villi are present over the entire free surface of the explant. The serosal side seen in the lower part of the figure is epithelialized. × 180.

Fig. 3. After 48 h of culture, also with MAF, villi remain intact only in the uppermost area of the explant. On the lateral sides, villi have disappeared because of a severe oedema in the underlying tissues. × 180.

villi are found after 24 h of culture; here, the serosal side of the explants is not covered with the epithelium even after 72 h of culture, and oedema is absent. With FBS supplemented medium, short and irregular villi are formed only after 48 h of culture. In a few explants, the epithelium has reached the serosal side after 72 h.

At the fine structural level, endocrine and mucous cells seem to differentiate properly with all the media used. However, absorptive cells remain poorly differentiated.

Like serum, amniotic fluid is a very complex mixture. It is known to contain cortisol[9], somatomedin[10], dopamine[11], disaccharidases and lysosomal enzyme activities[12], glucose and potassium[13], fibronectin[14], oxytocin[15], diiodothyronines[16], epidermal growth factor[17], etc. For the moment it is not known if ingested amniotic fluid has any effect in vivo. However, in vitro, mouse amniotic fluid is able to promote cell growth in duodenal explants; furthermore, MAF also sustains the early stages in the formation of intestinal villi.

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Differences in phagocytic activity of methacrylate copolymer particles in normal and stimulated macrophages

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Summary. Methacrylate copolymer particles were found to be optimal for a study of phagocytosis in normal and stimulated macrophages, and, after their simple coating with antiserum, for a specific cell labelling with minimal spontaneous adhesion to the cell surface.

Phagocytosis of particles represents one of the several functional parameters used for characterization of macrophages. Besides bacteria and erythrocytes particles commonly classified as 'inert' have been employed. This group includes, e.g., colloid carbon, starch, silica, carbonyl iron or nickel, latex, etc. However, certain types of cells avidly form bonds with such particles exhibiting no specificity or ability for subsequent phagocytosis. It is beyond the possibilities of optical microscopy to differentiate between phagocytized particles, particles specifically bound to the surface of a phagocyting cell, and particles adhering spontaneously to cells of various types. A complete removal of the nonspecifically bound particles requires additional treatment (e.g., a trypsin-versene procedure in the case of the latex spheres[3]) which consequently limits the possibilities of a surface-marker study. These difficulties seem to be partly removed by the use of the methacrylate copolymer particles prepared in this laboratory. The nonspecific adherence of these particles to the cell surface is considerably reduced, while the binding of an antiserum or other ligands to these particles could be achieved using a simple procedure, thus opening up new possibilities for specific cell labelling.

Material and methods. Cells. Peritoneal exudate cells were obtained from peritonea of untreated, or thioglycollate-treated (TG), or proteose peptone-treated (PP) 3–4-month-old female mice of the A/J strain (breeding colony of the institute). The stimulation procedure consisted of an i.p. injection of 3 ml TG-medium (Institute of Microbiology, Prague) or 1.5 ml 10% PP (Difco) 3 days prior the cell harvest.

Particles. Methacrylate copolymer particles (mean particle diameter 0.5 µm) were prepared according to Rembaum and his coworkers[4,5] by gamma irradiation-activated (Co^{60}; 8 kGy) copolymerization of a nitrogen-bubbled 2% aqueous solution of a mixture consisting of 2-hydroxyethyl-

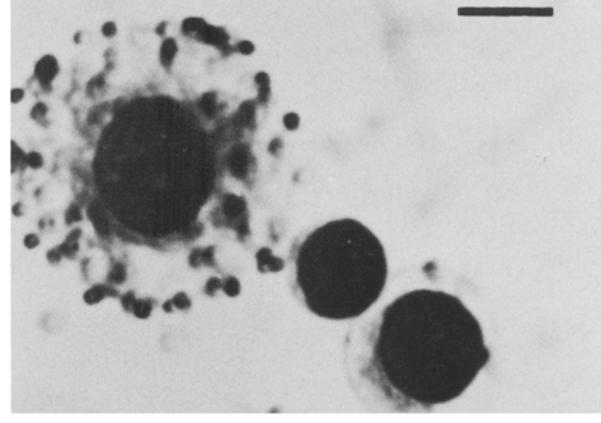

Fig. 1. Peritoneal exudate macrophage (stimulated with thioglycollate medium) with methacrylate copolymer particles as seen in the light microscope. 2 lymphocytes are negative (bar: 10 µm).