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Intestinal Explants of Fetal Mice: a Light and Electron Microscopic Study

Implantes Intestinales en Fetos de Ratones: Estudio al Microscopio de Luz y Electrónico

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Abstract: Intestinal explants of animal fetuses could be an useful instrument to study the development of the small intestine and gut infections. Various techniques have been described for the *in vitro* maintenance of intestinal tissue from adult, suckling or fetuses mice, but their use is limited by fast evolution to necrosis. In order to study in detail the effect of viruses infection on intestinal mucosa, we have developed an *in vitro* model using gut explants of mice fetuses. BALB/C mice fetuses where obtained by mid-line abdominal incision on days 18 to 20 of mice gestation. The intestines were collected and placed in cold complete medium (RIMI 1640, antibiotic and fetal bovine serum). They were incubated in petri dishes at 37°C in 5% CO_2. The results of light and electron microscopy observations showed that the explants may be cultured for up 5 days and that the enterocyte villi maintained the normal architecture similar to in uterus grown mice. Therefore, we conclude that this methodology could be used in the investigation of gut viral infections.

Key words: Intestinal explants, fetal mice, microscopy

Resumen: Los implantes intestinales de fetos animales podrían ser un instrumento útil para estudiar el desarrollo del intestino pequeño e infecciones intestinales. Se han descrito varias técnicas para el mantenimiento *in vitro* de tejido intestinal adulto, de lactantes o fetos de ratones, pero su uso está limitado por la evolución rápida a la necrosis. Para estudiar en detalle el efecto de infecciones virales sobre la mucosa intestinal, en este estudio se ha desarrollado un modelo *in vitro* usando implantes del intestino de fetos de ratón. Se obtuvieron fetos de ratones BALB/C mediante incisión abdominal los días 18 a 20 de gestación de los ratones. Los intestinos fueron colectados y colocados en medio completo frío (RIMI 1640, antibiótico y suero bovino fetal). Se incubaron en platos de petri a 37°C en CO2 al 5%. Los resultados al microscopio de luz y electrónico mostraron que los implantes pueden cultivarse hasta por 5 días y que las vellosidades de los enterocitos mantuvieron la arquitectura normal similar a los ratones crecidos en útero. Por consiguiente, se concluye que esta metodología puede emplearse en la investigación de infecciones virales intestinales.

Palabras clave: Implantes intestinales, ratones fetales, microscopía

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Introduction

The pattern of some *in vitro* experimental models to study morphological, physiological and immunologically the small intestine has been described by different groups (1-9, 11-15). So far none of these studies have shown an ideal model to study short term viral infections.

Previously we established an heterologous rotavirus infection in suckling mice *in vitro*, in order to get informations about SA-1 I rotavirus replication inside enterocytes and damages caused by this infection at an ultrastructural level of observation (10). In sequence we searched for a model that enables us to answer immunological as well as ultrastructural questions in intestinal viral replication. The utilization of small intestine explants may simulate the enterocyte-enteric pathogens interaction.

The study of gut *in vitro* culture systems has received much effort through the years with human gut (1, 3, 6, 8, 12, 15), pig gut (5), rat gut (13, 14), pony gut (2), but few workers have studied *in vitro* maintenance of mouse intestinal tissue (4, 7, 11). The culture of fetal mice small intestine have been done for up to 72 h by Calvert and Micheletti (4). A longer survival time of mice intestinal explants is necessary to study replication of viruses that multiply, besides 72 hours of culture.

The present paper describes the morphological appearance of explants of fetal mice intestinal mucosa up to five days in culture by laser scanning confocal and electron microscopy.

Materials and methods

Explant culture-BALB/C mice originally obtained from a colony maintained at the Instituto Nacional do Câncer (INCA), Rio de Janeiro, Brasil, were used. Eighteen to 20 days old mice fetuses were removed from the female by abdominal incision. After mid-line abdominal incision, the small intestines from fetuses were collected and placed into petri dishes containing RPMI 1640 medium with Hepes 2%, 10% fetal bovine serum, penicillin, streptomicyn and fungison, in ice bath. Intestines were transversally cut into 2 to 3 mm segments. The dishes were placed in incubators at 37° C in 95% O2 / 5% C02 gas mixture for 5 days.

Laser scanning confocal microscopy (LSCM)- For LSCM the fragments of small intestine were stained with Evans Blue dye in concave slides and observed in a Zeiss LSCM.

Electron microscopy (TEM). For TEM studies, samples of the intestine were fixed with 2% glutaraldehyde in 0.2 M cacodylate buffer pH 7.2. The segments were postfixed in 1% osmium tetroxide, dehydrated in graded acetone and embedded in Epon. Ultrathin sections were stained with urany acetate and lead citrate and examined with a Zeiss EM-900 electron microscope.

Scanning electron microscopy (SEM). For SEM samples were fixed with 2% glutaraldehyde in 0.2 M cacodylate buffer pH 7.2, postfixed in 1% osmium tetroxide and dehydrated in graded acetone as described above. Tire samples were dried in a critical point drying apparatus (Balzers) and coated with a 20 nm thick gold layer. Observations were made using a Zeiss DSM-940.

Results

Cultures of intestinal explants of fetal mice for up to 5 days were examined by SCLM, SEM and TEM.

At four days of culture, morphological observations by means of SCLM showed a well preserved intestinal explant architecture. The morphological aspect of the cultured intestinal explants was quite comparable to that of uncultured explants (Fig. 1). Enterocytes with well developed villi presented the nuclei frequently situated at the basal part of the cells (Fig. 2).

Uncultured intestinal villi observed by SEM showed well developed and regularly rounded tips (Fig. 3). Tire explants observed at three days in culture presented preservation of the cell structure similar to the uncultured intestine. Damaged cells were not detected at this time of culture (Fig. 4). At four days in culture, epithelial cells retained normal morphology, showing densely packed microvilli (Fig. 5). Gut explant cultures at five days showed also a good preservation of intestinal villi, nevertheless, microvilli of some absorptive cells started to be irregularly distributed (Fig. 6).

At TEM level the uncultured gut explants showed dense and uniformly stained cytoplasm, a layer of columnar epithelial cells with regularly placed microvilli, well preserved nuclei, mitochondria and endoplasmic reticulum (Fig. 7). Three explants at the fifth day in culture showed a preserved layer of enterocytes with regularly placed microvilli and mitochondria, similar to the observed in the uncultured explants. Nevertheless, the enterocytes contained some swollen organelles and reduced cytosol (Fig. 8).

Discussion

In order to get viral replication in mice enterocytes, the absorptive cells have to be differentiated, presenting well developed microvilli. Adult mice intestines were maintained in culture up to 48 hours as recommended by Ferland and Hugon (7), showing well preserved mucosa at light and TEM levels. In sequence, Malo et al. (11) used suckling mice small intestine cultures obtained well preserved ultrastructure of enterocytes for up lo 48 hours of culture. Calvert and Micheletti (4) analyzed fetal mice intestines using several culture media. The best result was obtained using RPMI medium, as used in our experiments. These authors observed fetal mice intestine ex-

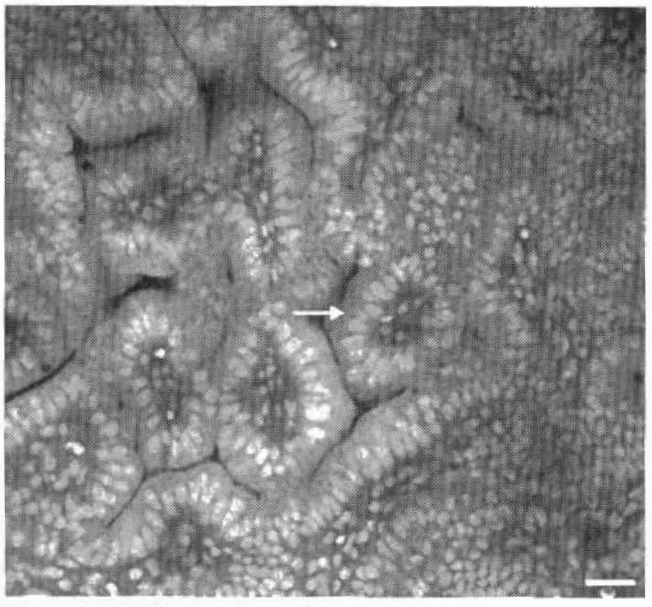


Figure 1. LSCM. Uncultured intestinal villi explant. The nuclei of the enterocytes are located at the basal part of these cells (arrow). Bar = 15,6 μm.

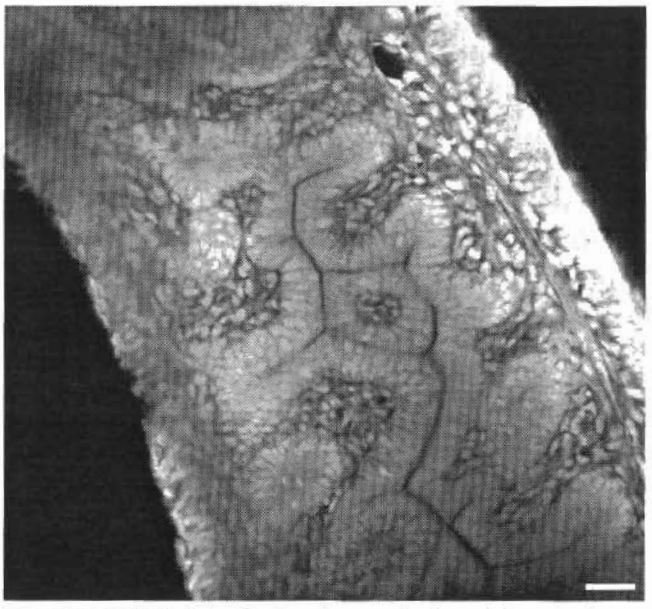
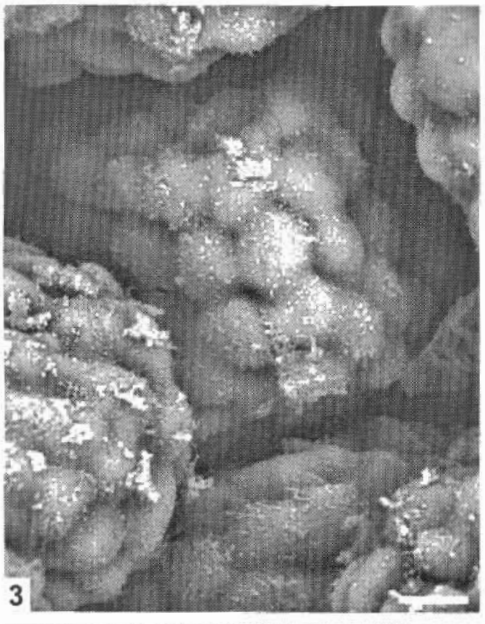
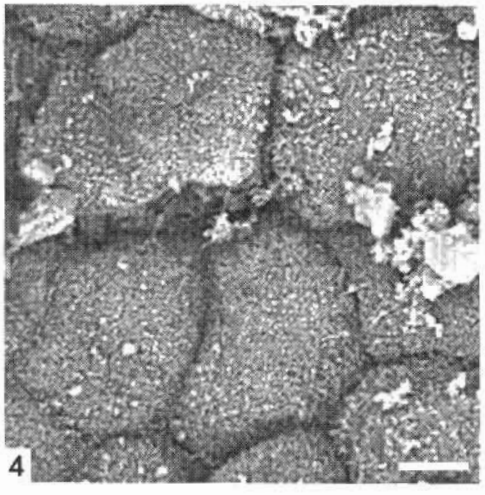


Figure 2. LSCM. Intestinal explant at the fourth day of culture. The nuclei are yet located at basal part of the enterocytes. Bar =31,2 μm.





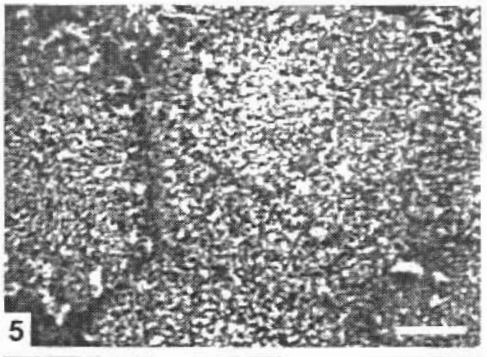


Figure 3. SEM. Uncultured intestine villi explant. The enterocyte surfaces present a regular pattern. Bar = 5,5 μm.

Figure 4. SEM. Intestinal explant at the third day of culture. Microvilli on enterocyte surfaces are well preserved (arrow). Bar = 1,8 μm.

Figure 5. SEM. Intestinal explant at the fourth day of culture. The microvilli are yet well preserved. Bar = 1,5 μm.

Figure 6. SEM. Intestinal explant at the fifth day of culture. The explant of intestine started to show irregularly distributed microvilli. Bar.= 1,6 μm.

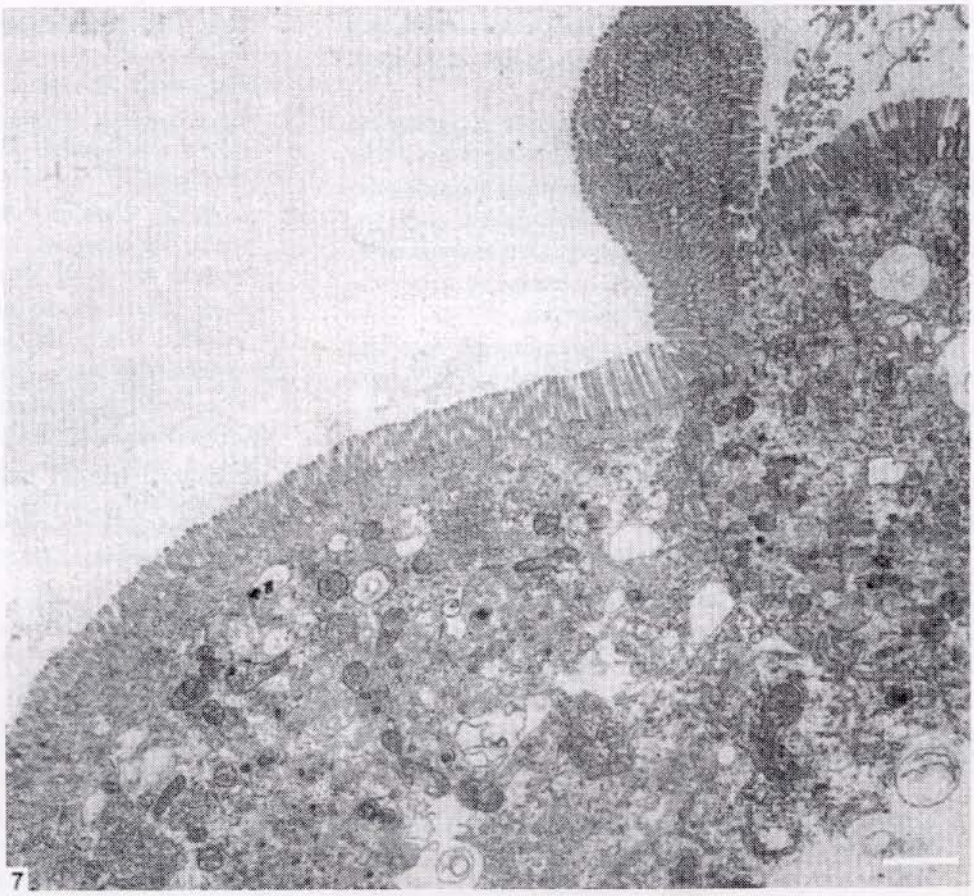


Figure 7. TEM. Intestinal explant at the fifth day of culture. Cell degeneration is starting by lack of cytosol. Bar.=1,6 μm.

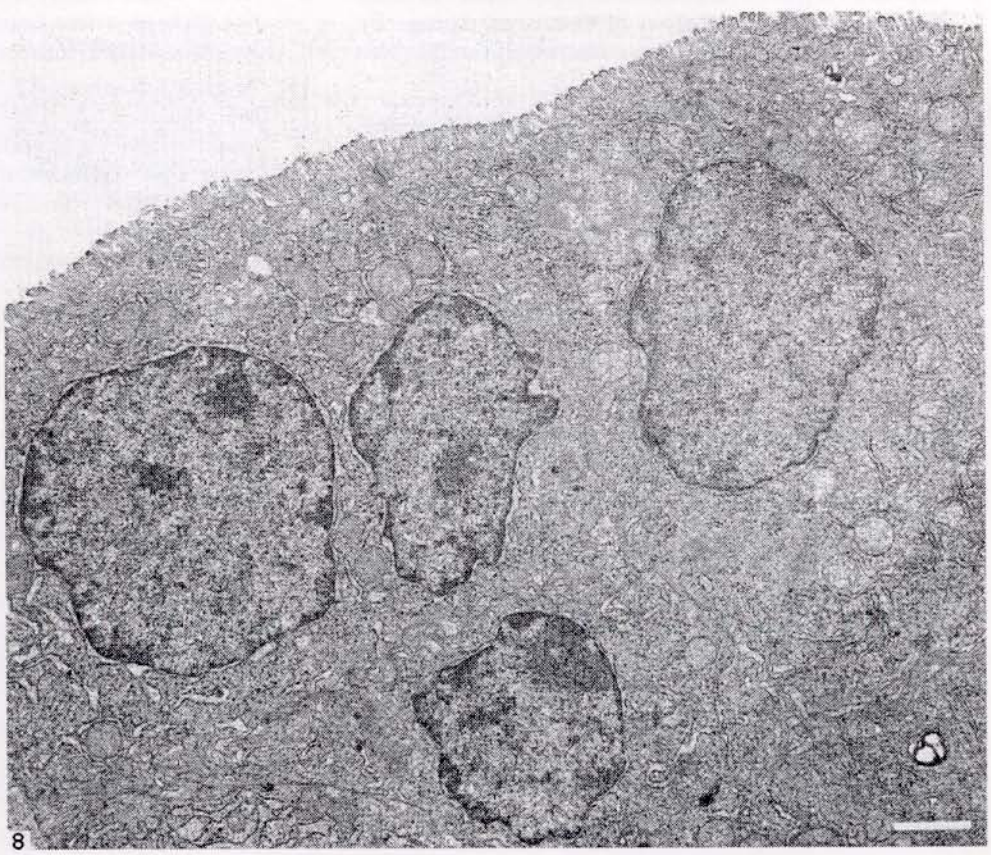


Figure 8. TEM. Uncultured explant intestinal enterocytes. The nuclei are located at the basal side of enterocytes. A well developed layer of structured and regularly distributed rnicrovilli is common of these cells. Bar = 1,6 μm.

plants of 15 days of gestation during 72 hours and found poor absorptive cell differentiation with very short microvilli.

In the present experiment, fetal mice intestine explants, at 18-20 days of gestation at 72 hours, showed that enterocytes are well differentiated showing normally developed microvilli. This aspect was maintained until the fifth day in culture. Although degeneration and autolysis of the cells became at fifth days in culture, the structural preservation of the villi was yet observed.

This fetal mouse model is contamination free and can enhance the understanding of human gastroenteric viruses and other microorganism infections, since the majority of them are short-term diseases.

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References

1. Batman PA, Fleming SC, Sedgwick PM, MacDonald TT, Griffin GE (1994). HIV infection of human fetal intestinal explant cultures induces epithelial cell proliferation. ***Aids*** 8: 161-167
2. Batt RM, Embaye H, van der Waal S, Burgess D, Edwards GB, Hart CA (1995). Application of organ culture of small intestine to the investigation of enterocyte damage by equine rotavirus. ***J Pediatric Gastroenterol Nutr*** 20: 326-332
3. Browning TH, Trier JS (1969). Organ culture of mucosal biopsies of human small intestine. ***J Clin Invest*** 48: 423-432
4. Calvert R, Micheletti PA (1981). Selection of a chemically defined medium for culturing fetal mouse small intestine. ***In Vitro*** 17: 331-344
5. Danielsen EM, Sjostrom H, Norén O, Bro B, Dabelsteen E (1982). Biosynthesis of intestinal microvillar proteins. ***Biochem J*** 202: 647-654
6. Dolin R, Blacklow NR, Malmgren RA, Chanock RM (1970). Establishment of human fetal intestinal organ cultures for growth of viruses. ***J Infect. Dis*** 122: 227-23 1
7. Ferland S, Hugon S (1979). Organ culture of adult mouse intestine. 1. Morphological results after 24 and 48 hours of culture. ***In Vitro*** 15: 278-287
8. Fleming SC, Kapembwa MS, MacDonald TT, Griffin GE (1992). Direct in vitro infection of human intestine with HIV-1. ***Aids*** 6: 1099-1104
9. Hirondel CI, Doe WF, Peters TJ (1976). Biochemical and morphological studies on human jejunal mucosa maintained in culture. ***Clin Sci Mol Med*** 50: 425-429
10. Majerowicz S, Kubelka CF, Stephens P, Barth OM (1994). Ultrastructural study on experimental infection of rotavirus in a murine heterologous model. ***Mem Inst Oswaldo Cruz*** 89: 395-402
11. Malo C, Arsenault P, Ménard D (1983). Organ culture of the small intestine of the suckling mouse in a serum free mediun. ***Cell Tissue Res*** 228: 75-84
12. Ménard D, Arsenault P (1985). Explant culture of human fetal small intestine. ***Gastroenterol*** 88: 691-700
13. Quaroni A (1985). Development of fetal rat intestine in organ and monolayer culture. ***J Cell Biol.*** 100 1611-1622
14. Simon PM, Kedinger M, Raul F, Grenier, JF, Haffen K (1982). Organ culture of suckling rat intestine: Comparative study of various hormones on brush border enzymes. ***In Vitro*** 18: 339-346
15. Wyatt RG, Kapikian AZ, Thornhill TS, Sereno MM, Kim HW, Chanock RM (1974). In vitro cultivation in human fetal intestinal organ culture of a reovirus-like agent associated with nonbacterial gastroenteritis in infants and children. ***J Infect Dis*** 130: 523-528