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Morphological Study of Chicken Cecal and Jejunal Mucosa During Epithelial Cell Isolation

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Epithelial cells from jejunum and proximal cecum of the chicken were isolated by using a sodium citrate containing medium. Cell dissociation during the isolation process was studied by scanning electron microscopy. Results after 5, 15 and 30 min incubation show a sequential cell detachment from the tip to the lower regions of the villi. Inspection of the cell suspension by scanning and transmission electron microscopy reveals that most cells are enterocytes that retain their characteristic cylindrical shape. The brush border of isolated cells maintains its architecture, while organelles remain intact.

Key words: Electron microscopy, Isolated enterocytes, Proximal cecum, Jejunum, Chicken.

Intestinal epithelial cell suspensions are being used with increasing frequency in studies on intestinal physiology. In a previous study (3), in search of a technique suitable for the isolation of viable epithelial cells from the chicken cecum and jejunum, three different isolation media containing either hyaluronidase, sodium citrate or EDTA were tested. Results showed that the best cell suspensions were obtained when using sodium citrate as isolating agent. In the present study, cell removal from the jejunal and cecal epithelium, by the citrate method, has been monitored throughout the isolating process by both scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The microscopic features of cell suspensions were also examined. Results show that the citrate-content medium yields cells with an excellent preservation of their structure.

Materials and Methods

Male white Leghorn chickens 5- to 7-wk old, obtained from «Cooperativa Comarcal de Avicultura de Reus (Tarragona), were used. Animals were fed a commercial diet (Gallina Blanca-Purina, Barcelona) and maintained in standard-

ized temperature and humidity conditions. Birds were killed by decapitation and a portion of the jejunum (yolk sac region) and of the proximal cecum (the cecal third nearest to the ileocecal junction) were removed, washed with ice-cold saline and opened lengthwise. Cell isolation was carried out as previously described (2, 3). Though the normal incubation time for cell isolation is 30 min, some intestinal segments were incubated for shorter periods (5 and 15 min) in order to monitor the process of cell removal from the mucosa by SEM. Cell suspensions obtained after 30 min incubation were washed twice with ice-cold medium of the same composition as the isolation medium except that sodium citrate was replaced by mannitol. Cells were then resuspended in a medium as previously described (3).

For either SEM or TEM observations, samples of both tissues and cell suspensions were fixed with 25 ml/l glutarade-

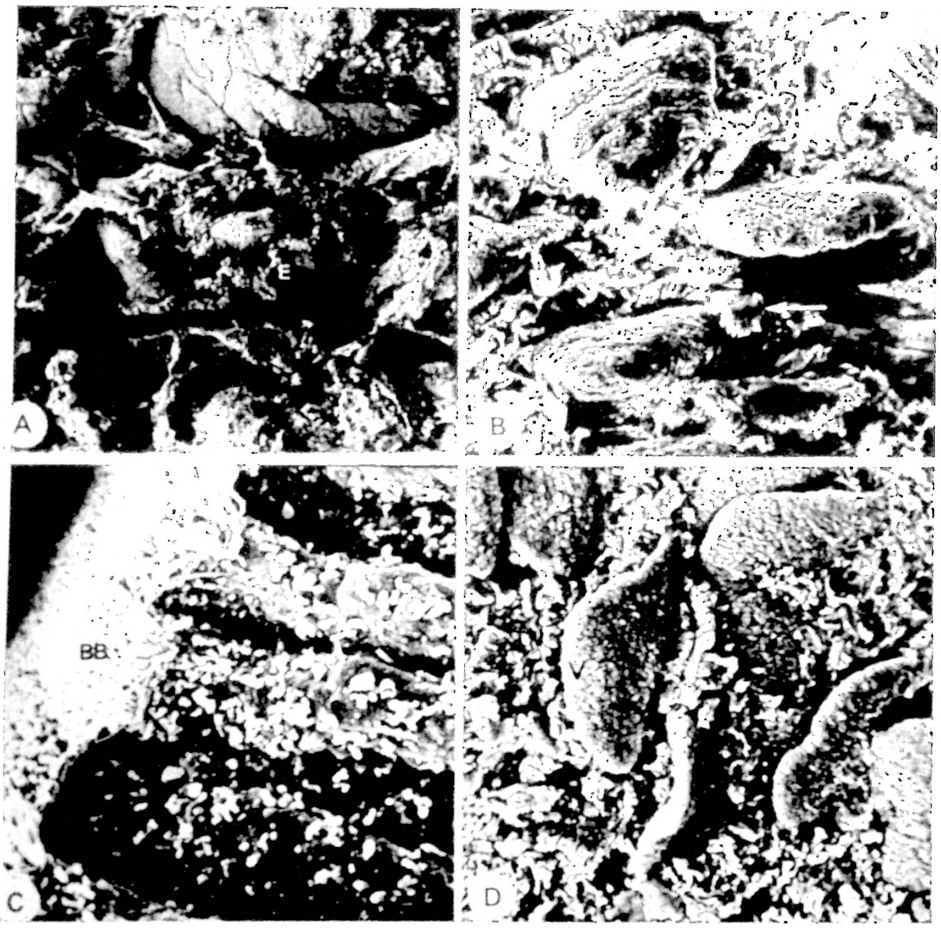

Fig. 1. *Scanning electron micrographs of the intestinal mucosa during the isolation process.* A: proximal cecum after 5 min incubation with the isolation medium; LP, lamina propria; E, epithelium (×110). B: proximal cecum after 15 min incubation (×102). C: detail of an epithelial sheet from a piece of jejunum incubated for 15 min; BB, brush border; e, enterocyte (×109). D: proximal cecum after 30 min incubation; V, villus (×2766).

hide in 1.0 mmol/l phosphate buffer (pH 7.4) and then washed in 0.2 mmol/l phosphate buffer. For TEM, specimens were postfixed in 0.2 mmol/l phosphate buffer containing 20 g/l OsO_4, dehydrated in acetone and further embedded in Araldite. Ultra-thin sections (60 nm) were stained with uranyl-acetate and lead citrate, according to REYNOLDS (10). Samples to be studied by SEM were dehydrated in ethanol, dried by the critical-point method, using CO_2 as transitional fluid, and subsequently coated with gold. Sample processing for microscopic observations was carried out at the «Servicio de Microscopía Electrónica of Universidad de Barcelona». Specimens were examined in Philips EM 200 (TEM) and Cambridge S.4 (SEM) electron microscopes, operating at 100 kV and 10-30 kV respectively.

Results

The morphological study of the intestinal mucosa by SEM during the isolation step shows that cell detachment from the villus is a progressive (sequential) process, with a pattern which is similar in the proximal cecum and in the jejunum. Figure 1A shows the cecal mucosa after 5 min incubation in the isolation medium. The epithelium of the tip-villus is detached and the lamina propria is exposed. After 15 min incubation (fig. 1B), the villi appear partially denuded, with mid- and lower-villus cells still attached to the lamina propria. The epithelium sloughs off from the mucosa in sheets (arrow). Figure 1C shows a high magnification of a sheet of epithelium from a sample of jejunum. This picture reveals a group of cylindrical cells, in a uniform parallel array, attached at the microvillus pole. Brush border of these cells appears undamaged. Figure 1D shows the appearance of cecal villi after 30 min incubation, denuded of epithelial cells.

SEM and TEM observation of cecal and jejunal cell suspensions collected after 30 min tissue incubation, reveals that the material removed from the intestine consists mostly of individual cells, small cell clumps, and sheets. Also, a few goblet cells and eritrocytes were observed but no other intestinal cell type or bacteria could be found.

Both cecal and jejunal enterocytes, observed by SEM, show the typical columnar shape though some spherical cells were occasionally found (figs. 2A, B). The brush-border architecture appears intact, with parallel-arranged microvilli. TEM observations of section of cecal isolated cells show that brush border is also intact and that the terminal web beneath the microvilli forms a constricting band around the luminal pole (fig. 2C). The figure also shows the nucleus localization, at the basis of the cell as in the intact epithelium (5), numerous and well-preserved mitochondria, and well developed Golgi membranes.

Discussion

The citrate-based isolation method enables sequential harvesting of epithelial cells in chicken jejunum and cecum. Similar time-dependent cell dissociation using citrate has been observed by LAWSON *et al.* (8) for the rat small intestine and by BROWN and SEPÚLVEDA (1) for rabbit jejunum. A premise of epithelial cell isolation methods, to be applied in transport studies, is that cell preparations are not contaminated by undifferentiated crypt cells. Contamination can be assessed both by hystological and functional studies. Inspection of figs. 1A, B and D indicate that there is a progressive disgregation of the epithelium but no indication of crypt damage is observed. In addition, isolated cells have well-developed microvilli, longer than those found in crypt enterocytes (4). Functional studies carried

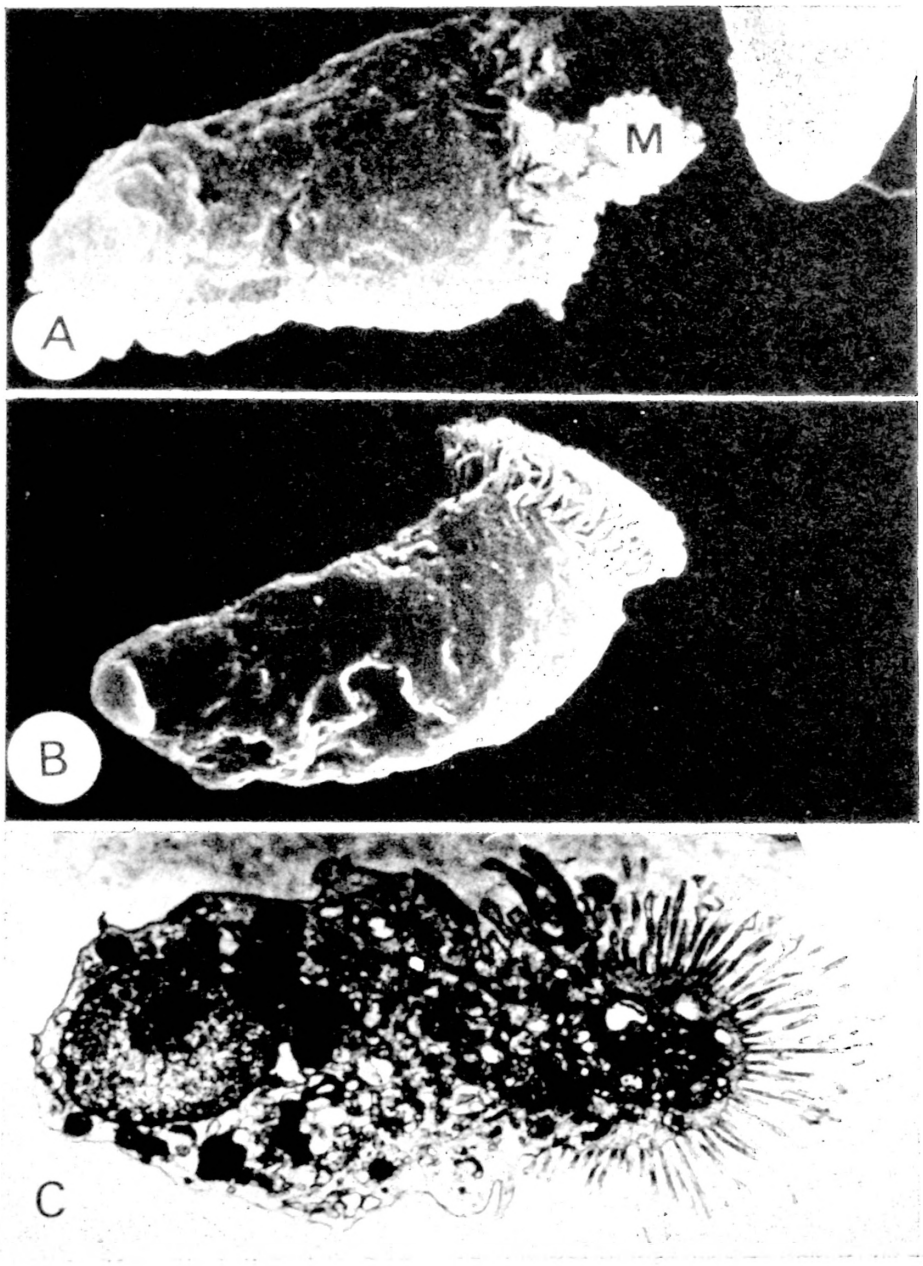

Fig. 2. *Photomicrographs of isolated enterocytes.*
A: enterocyte from the proximal cecum; M, mucus attached to the microvilli surface (SEM, ×5493). B: enterocyte from the jejunum (SEM, ×4395). C: enterocyte from the proximal cecum showing organelle distribution (TEM, ×8996).

out with cell suspensions from proximal cecum and jejunum (9) indicate that both are capable of supporting α-methyl-D-glucoside accumulation ratios higher tham those obtained by KIMMICH and RANDLES (7) who use a cell preparation originated from the upper villi of the small intestine (6). These results suggest that the contribution of non-transporting crypt cells to our cell population is small, if any. On the other hand, photomicrographs showing totally denuded villi (fig. 1D) indicate that the isolation procedure causes little damage to the supporting structures of the lamina propria, thereby avoiding connective tissue cell contamination of the suspension.

Microscopic observation of the cell suspensions indicates that cells exhibit a marked tendency to re-aggregate in sheets or clumps. However, they maintain their three-dimensional columnar shape and the ultrastructural features of the intact tissue. This observation, together with the optimal results obtained in viability studies (3), indicate that the citrate-based isolating method can be useful in *in vitro* physiological studies as well as starting material for subcellular fractioning.

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Resumen

Se estudian el proceso de aislamiento y las características de suspensiones celulares obtenidas de yeyuno y ciego proximal del pollo, mediante microscopía electrónica de barrido (MEB) y de transmisión (MET). El agente disgregante utilizado es el citrato sódico. Las fotografías obtenidas por MEB de la mucosa intestinal a los 5, 15 y 30 min de incubación demuestran que las células se desprenden secuencialmente, desde la punta a la base de la vellosidad. La observación de las suspensiones celulares por MEB y MET muestra que la mayoría de las células son enterocitos que mantienen su forma cilíndrica característica. Tanto las microvellosidades como los orgánulos celulares aparecen intactos.

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