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CULTURE OF HUMAN GASTROINTESTINAL EPITHELIAL CELLS

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Abstract. Human gastrointestinal (GI) epithelial cells and tissues have been propagated in vitro for several months. Viability of typical epithelial cells with minimal contamination by fibroblasts was best achieved by mechanical harvesting instead of using enzymes for dissociation or subculture. Individual cells generally adhered poorly to glass and plastic although they did attach to some coated substrates. Although standard formulations of culture base media were suitable, there was a continuous requirement for conditioned medium and cultures were readily propagated in suspension. Cultures had a propensity to grow as "islands" of epithelial cells consisting of central dividing regions and external "differentiated" regions. As yet undefined factors from yeast and brain (or pituitary) extracts were required for optimal growth.

Continuous culture of gastrointestinal (GI) epithelial cells has been largely unsuccessful, although viable cells can often be maintained for short terms in vitro, and some cell lines have been described (reviewed in 1-5). No typical human GI cells with the ability to grow and differentiate have ever been cultured, although such cultures would augment many aspects of gastrointestinal research. Extensive studies, detailed elsewhere (1) on culture of dog GI epithelium, and preliminary work with human GI tissue culture, were a prelude to this investigation. Studies described in this report were done to further optomize conditions for initiation and maintenance of human GI epithelial cells in vitro. The exciting and timely culmination of this work is the first description of "standard" procedures and culture conditions which have reproducibly permitted culture of typical GI epithelia in vitro for several months.

Materials and Methods. *Source of GI Tissue.* Thirteen sources of normal human gastrointestinal tissue were obtained. These included normal tissues from wide margins of resection of patients with cancer of the colon (n=5), small intestine (n=1), or stomach (n=1). Three colon segments were obtained from normal adults, and one from an infant, who required partial colon resection and repair. Fetal intestine was obtained from two (16 week) abortuses.

Tissue Harvesting, Rinsing, and Processing. Surgically resected sections of intestine or stomach were carefully cut and opened to expose the intraluminal mucosal surface. After several rinses with sterile saline, the mucosae was scraped away from the underlying submucosal layers with a sterile blunt-ended instrument such as a spatula or bone curette. The harvested tissues were immediately placed in sterile, autoclavable minimal essential medium (Autopow, Flow Labs) containing double concentrations of gentamycin (G; 50 μg/ml) and fungizone (Fz; 0.1 mg/ml). The volume of harvest medium used was generally 5 to 10 times the volume of tissue. Aseptic technique was employed throughout. Gentle mechanical dissociation of tissue was done using a stainless steel tissue homogenizer (EDCO, Inc.) followed by trituration with a pipette or syringe. The combined single cells and cell clusters were centrifuged and rinsed several times in harvesting medium. Approximately nine volumes of growth medium were added to the pellet which was resuspended and seeded into culture vessels. Trypan blue dye exclusion was used to test cell viability.

Culture Conditions and Characterization. Several base media and additives were analyzed in this study. Media

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were: Eagles' minimal essential medium with Earles' Salts (MEM), Dulbecco's modified MEM (D-MEM), L15, Ham's F-12, and low calcium MEM or D-MEM modified for suspension cultures. Media had pH values of 6.8 to 7.2. Additives tested were: 1-10% (v/v) fetal calf serum (FCS), G (25 μg/ml), and Fz (50 μg/ml). Culture media, sera and antibiotics were obtained from GIBCO or Microbiological Associates. Other supplements analyzed were ITS Premix [insulin (5 μg/ml); transferrin (5 μg/ml); selenium (5 ng/ml); Collaborative Research, Inc.], yeast extract, (YEx; 0.1 to 5%, v/v; Difco), L broth (NaCl, 5 g/l; Difco bactotryptone, 10 g/l; Difco yeast extract, 5 g/l); dexamethasone (0.04 μg/ml); newborn hamster brain extract (0.1 to 5% v/v), or bovine pituitary extract (PEx, Pel Freeze Inc.; 0.1 to 2% v/v). For some comparisons of growth, substrates were coated with fibronectin, type I collagen, yeast extract, brain extract, or GI "biomatrix" (i.e., dried lysates of freshly isolated GI epithelial mucosa rubbed over the surface of the culture substrate). Substrates were coated with minimal volumes then allowed to air dry for 2-6 hours. Several subculture and split ratios (1:1 to 1:10, v:v) were attempted. Cell suspensions were simply transferred in their conditioned medium to culture vessels containing various volumes of fresh medium. Alternatively, cells were dissociated with trypsin (0.25%), EDTA (ethylenediamine tetraacetic acid (0.02M); trypsin: EDTA (0.25%: 0.02M), or collagenase (50 μg/ml), rinsed, then resuspended in complete medium. Cells growing on substrates were removed by scraping with a rubber policemen, or by the various dissociating agents. Cultures were maintained in a 5% CO_2, 95% air atmosphere but those in L15 media could be grown without CO_2 present.

Cells were observed daily to assess morphology. Growth curves were done to determine population doubling times and ^{3}H-thymidine incorporation was used to monitor DNA synthesis. Standard methods for keratin and mucous stains and transmission electron microscopy to assess cell-cell associations were done, respectively, by the Histopathology Core Facility and D. Olmos, Surgery Dept. Immunofluorescence staining for fibronectin was done using monoclonal anti-fibronectin antibody kindly supplied by Dr. R.J. Klebe, Anatomy Dept., UTHSCSA.

Results. The basic parameters for culture of human GI epithelium were consistent for all the tissue sources. Fibroblasts were common and viability of epithelial cells was reduced to less than 10 or 20% in all primary and early cultures treated with the various dissociating agents. Thus, the most important requirement for maximizing viability of typical GI epithelial cells, while minimizing fibroblast growth, was the need to mechanically remove and gently dissociate the mucosal epithelium layer without the use of biochemicals.

After many permutations of the various culture media components, two culture media were (and continue to be) routinely employed: suspension culture MEM supplemented with 1% FCS, ITS, 2% L broth, 2% PEx; and L15 supplemented with 5% FCS, 5% L broth, 2% PEx. "Conditioned medium" comprising 25-50% of the culture medium, and yeast and brain extracts were necessary for growth in vitro. L broth, which contains yeast extract, and bovine pituitary extract (in lieu of brain extract) were excellent, readily available, sources of the needed growth factors.

The cultures generally consisted of various sized "islands" or clusters of cells which were often associated with mucous and grew well in suspension (Fig. 1). Although the cells adhered to GI biomatrix, YEx or PEx (but not collagen or fibronectin) coated substrates, no conditions that promoted formation of contiguous, complete monolayers were found. There was a similar appearance of fetal and adult intestinal cells in culture (Fig. 1). Morphologically, the various sized cell clusters were comprised of central regions of dividing, presumably "stem cells" with peripheral regions of a variety of differentiated cell types. Some small clusters had only dividing cells present, whereas others were comprised of only differentiated cells. Relative proportions of dividing and differentiated cell types varied in different cultures and upon subculture. As expected, cultures with more dividing cells generally had greater in vitro longevity. On uncoated substrates the more differentiated cell types adhered more often to the monolayers than did the dividing subpopulation. GI "biomatrix" provided a good substrate for attachment of dividing

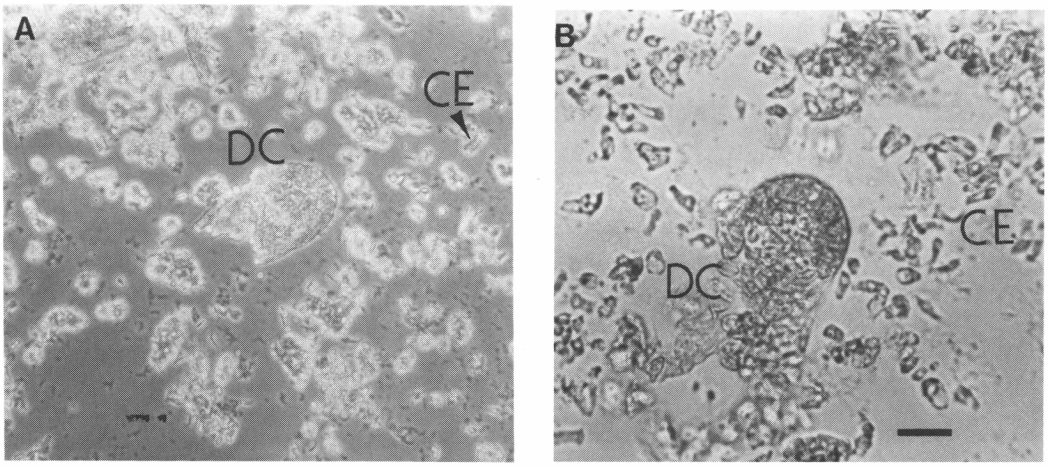

FIG. 1A. Suspension culture of fetal human intestinal epithelium. Cultures propagated for 8 weeks (2 subcultures) in L15 supplemented with 5% FCS, 5% L.B., 5% PEx and antibiotics. Note central regions of dividing cells (DC), and peripheral columnar epithelial (CE) cells. Bar is 100 μM.

FIG 1B. Culture of normal adult mucosal cells from descending colon (wide margins of resection for colon cancer) was maintained for 11 weeks as mixtures of cells in suspension or attached to pituitary extract-coated substrate. It was twice subcultured as described in the methods section. Note central colony of dividing cells (DC), and columnar epithelial (CE) cells present alone or as clusters. Bar is 100 μM.

and differentiated cells (Fig. 2).

Since the dissociating agents were cytotoxic, subcultures were done of cells in suspension or with rubber policemen. Split ratios greater than 1:4 were not successful. Population doubling times of the 13 cultures initiated ranged from 72 to 96 hr or more. The number of achievable subcultures and in vitro longevity also varied, from a minimum of 2 subcultures over 4 weeks to cultures which have been maintained more

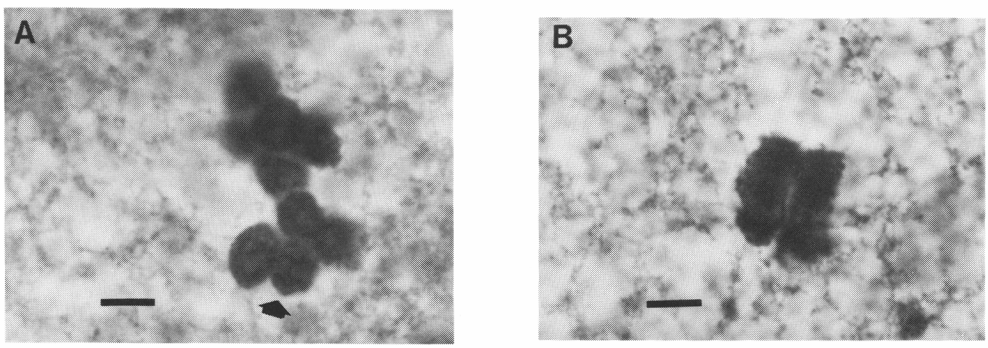

FIG. 2. Cells from adult human descending colon mucosa. (A) Colony of dividing cells; (B) Columnar epithelial cells. Cultures propagated for 6 weeks on "biomatrix" substrates of lysed cells. Cultures fixed with methanol and stained with Wrights stain. Arrow indicates an obviously mitotic cell. Bar represents 10 μM.

than 10 months and 20 subcultures.

Characterization studies (details to be published elsewhere) confirmed the epithelial origin of the cultured cells. Histological stains for mucous and keratin were positive, but the cells had little surface fibronectin. Ultrastructurally, the cells had epithelial plasma membrane junctional complexes.

Discussion. Successful cultivation of human GI epithelial tissue cultures required the use of novel harvesting procedures and growth factors. These alone might explain previous failures, but other reasons must be noted. The cytotoxicity of standard dissociating agents, the small size of the cells relative to cultured fibroblasts or cell lines, the propensity of the cells to grow in suspension, and the need for conditioned media are very relevant, since these cells would have been killed, overlooked, or discarded during attempts to culture these cells by "standard techniques". Although this paper describes the best present methods for culturing GI cells, the long population doubling times indicate that conditions are not optimal. More studies need to be done to develop defined media and to assess the functions of growth factors provided by the yeast and brain extracts. In addition, establishment of a permanent cell line has yet to be achieved. It must also be emphasized that these cells are more "delicate" than available cell lines. They require more time to adapt in vitro and careful observation is necessary for proper maintenance.

In summary, this new capability for long-term in vitro culture of GI epithelium should promote many exciting avenues of research on GI physiology, oncology, and differentiation.

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