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A Three-dimensional System for Long-term Culture of Human Colorectal Adenomas

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Studies of the adenoma–carcinoma sequence in the colon and rectum have been limited by the paucity of experimental models of adenoma growth and progression. Progress recently was reported in the development of monolayer culture systems. The principal objective of this study was to develop a primary culture system for colorectal adenomas that would simulate three-dimensional in vivo ***growth. We used a calcium alginate encapsulation technique that was previously described for established tumor cell lines. Briefly, fresh resected specimens were washed, minced into small multicellular particles called microadenomas, and encapsulated in 1% calcium alginate pellets. The pellets were maintained in minimum essential medium containing 10% fetal bovine serum at 37° C in humidified atmosphere of 95% air, 5% CO_2. Ten of eleven adenomas, including six tubular, three tubulovillous, and one villous have been successfully cultured for 34 to 162 days. Cell viability was confirmed histologically by light and electron microscopy. The cells were characterized as epithelial by morphologic features and ultrastructural studies, which showed a high degree of cellular differentiation, including villous brush borders and many desmosomes. Both tubular and villuslike structures have been observed*** in vitro, ***correlating in some cases with the histology of the parent adenoma. Measurements of proliferative activity by [^3H]thymidine autoradiography or immunohistochemical staining with the monoclonal antibody Ki-67 demonstrated growth fractions of 9% to 25%. A simple, highly efficient primary culture system was developed for the long-term maintenance of adenomas that promotes three-dimensional growth patterns and growth rates analogous to those seen*** in vivo. ***This model provides an opportunity to develop an experimental system for longitudinal studies of pathologic and molecular parameters in adenoma progression to carcinoma. (Am J Pathol 1990, 137:1539–1547)***

Colorectal adenoma is considered the precursor lesion of colon cancer.[1,2] Approximately 30% of the United States population older than age 45 years harbor one or several adenomas,[3] but only a fraction of these will undergo malignant transformation.[4] The factors governing this conversion are poorly understood. Adenomas, as potential cancer precursors, demand removal once identified. Therefore it is not possible to observe long-term adenoma growth, let alone experimentally manipulate them in humans. The experimental animal models of adenomas and colorectal carcinoma have severe limitations.[5,6] The dimethylhydrazine mouse model[5] shows perhaps the most convincing adenoma–carcinoma sequence but there are obvious limitations in extrapolating from this high-dose carcinogen-induced lesion to the sporadic human colorectal adenoma. The best solution to the study of the clinical, pathologic, and molecular parameters that affect the natural history of adenomas may be an *in vitro* model. Although *in vitro* techniques have proved valuable in the study of colorectal carcinomas,[7] there has been very limited success reported on the growth of colorectal adenomas in culture.[8–10] We report a new method of *in vitro* culture of adenomas that offers many advantages over previously described systems and simulates the three-dimensional growth and morphology of the parent adenoma *in vivo*.

Experimental Design and Methods

Primary Explant Cultures

Tissue was obtained by the pathologist from adenomas removed at colonoscopy or colectomy. Each tissue fragment was washed three times in 50 ml of phosphate-

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Table 1. *Parent Adenoma Characteristics and Duration of Culture*

Parent adenoma	Site	Size (cm)	Growth pattern	Dysplasia grade	Duration of culture (days)
1	Sigmoid	3.5	Tubulovillous	Low	162
2	Cecum	1.9	Tubulovillous	Low	94
3	Sigmoid	1	Tubular	Low	49
4	Sigmoid	1.5	Tubular	Low	52
5	Rectum	1	Tubular	High	52
6	Descending	1.2	Tubular	Low	68
7	Sigmoid	4	Villous	Low	3*
8	Sigmoid	1	Tubular	High	34
9	Ascending	4	Tubulovillous	High	63
10	Rectum	3.8	Villous	High	42
11	Cecum	4.8	Tubular	High	37

* The culture was discarded at this time due to contamination.

buffered saline (PBS), minced on 0.86-mm open wire mesh, pushed through the mesh with a sterile glass pestle, and the adenoma minceate resuspended in Dulbecco's Modified Eagle's Medium (DMEM). After centrifugation at 800 rpm for 6 minutes, the volume of the tissue pellet was estimated and the filtered minceate resuspended in 1% sterile (Cel-Prep™) sodium alginate (FMC Bioproducts, Rockland, ME) at a 25:1 (alginate:tissue) ratio. The alginate-adenoma suspension was added by drops to a sterile solution of 0.1 mol/l (molar) $CaCl_2$ at 4°C. The solid gel pellets then were washed and cultured in DMEM fortified with 10% fetal bovine serum (FBS), penicillin (100 μ/ml), streptomycin (100 mg/ml), 0.1 mol/l nonessential amino acids (Sigma Chemical Co., St. Louis, MO), and ampho-

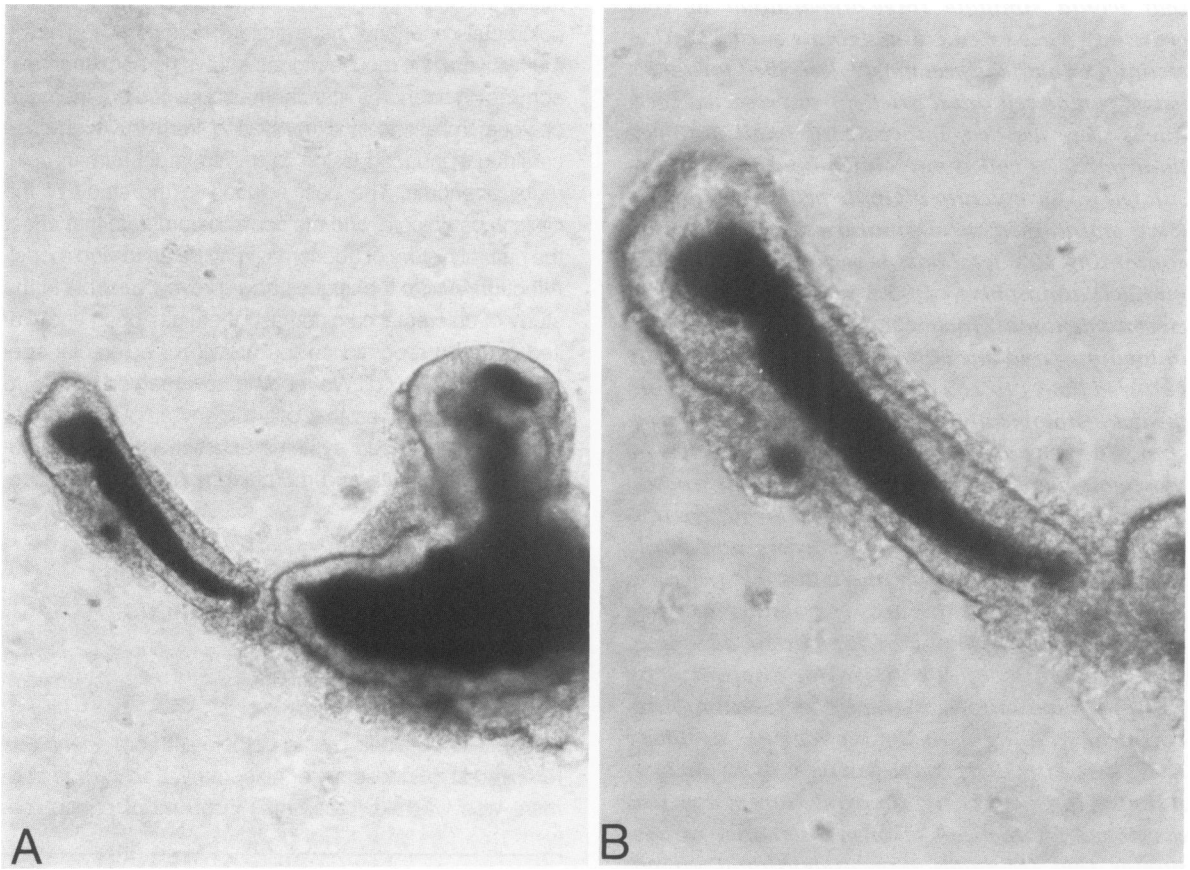


Figure 1. *Parent adenoma 2. Microadenoma in culture at 2 weeks after incubation.* **A:** *An ellipsoid structure with a broad based apical protrusion and a fingerlike extension at the opposite end can be identified (×25).* **B:** *A detail of the elongated extension with a lateral budlike outgrowth. This contour was retained up to 12 weeks after incubation (×100).*

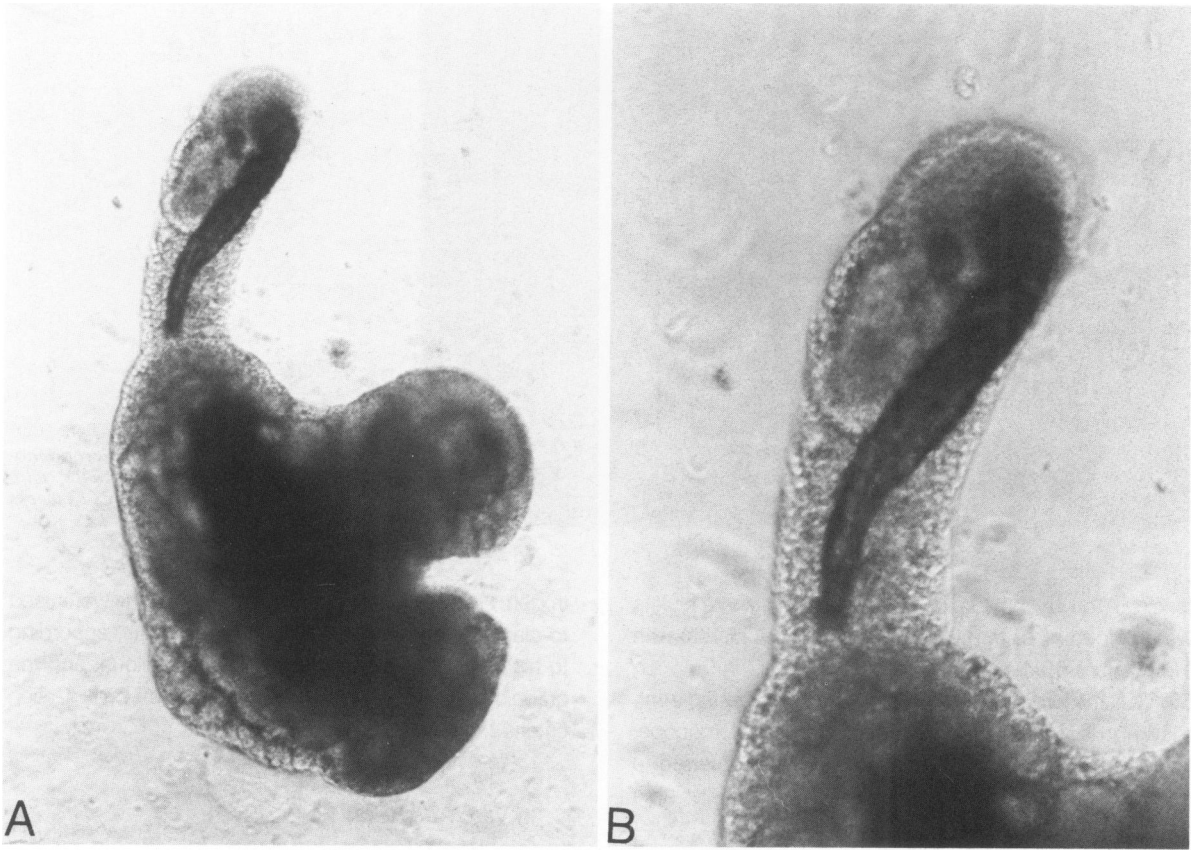
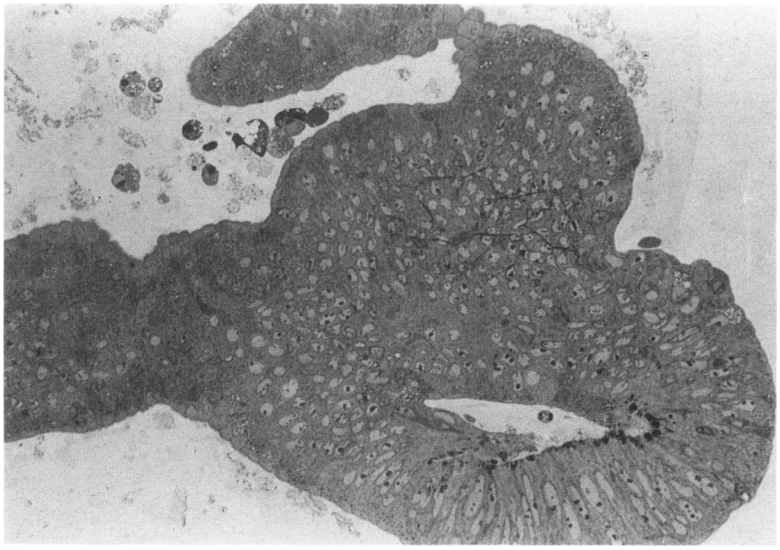


Figure 2. *Parent adenoma 2. Microadenoma in culture at 2 weeks after incubation.* **A:** *The same microadenoma has increased in size 10 weeks later but can (as can other microadenomas in separate pellets) be readily identified by its characteristic shape. Increased layered growth is apparent. The main change in the elongated structure is a symmetrical increase in bulk, with little overall increase in length (×25).* **B:** *The lateral budlike outgrowth identified 10 weeks earlier (Figure 1b) has become bulkier and more complex (×100).*

Figure 3. *Parent adenoma 2. Two-micron-thick section of a different microadenoma fixed 10 weeks after incubation and embedded in plastic. An eccentric tubular lumen with radial arrangement of epithelial cells can be identified with an elongated solid extension at one pole. The cells have uniform size and the nuclei are small, have evenly dispersed chromatin and small nucleoli (Richardson's blue stain; ×100).*



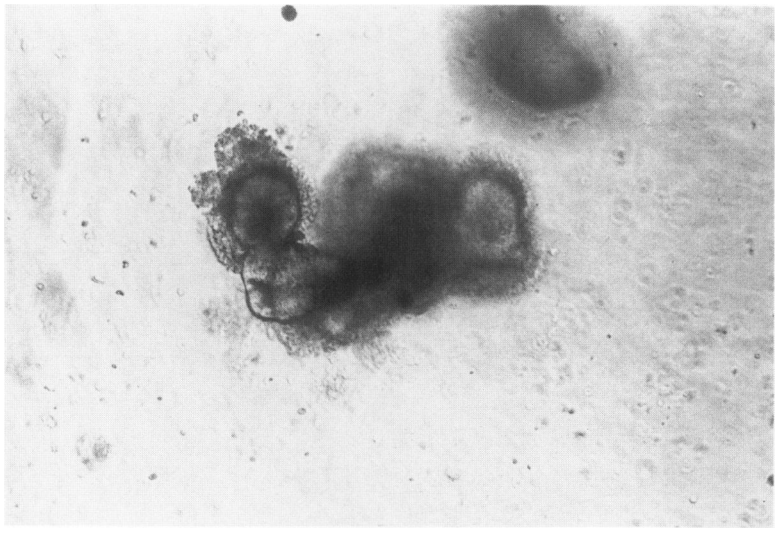


Figure 4. *Parent adenoma 1. Microadenoma with many budlike outgrowths, with lobulated grape-cluster contour (13 weeks after incubation, ×25).*

tericin B (2.5 μg/ml). One to three calcium alginate pellets were placed in 16-mm wells (Costar 24-well plates) with 1 ml culture medium and incubated at 37°C in 95% air, 5% CO_2 mixture. The 3- to 4-mm pellets are transparent, allowing daily monitoring using an inverted microscope. Fresh medium was added every 2 days. We referred to each tissue fragment within an individual gel pellet as a microadenoma.

Evaluation

Parent Adenomas

The parent adenomas were processed as routine surgical specimens in neutral buffered formalin after a small representative portion had been snap frozen in liquid nitrogen. Hematoxylin and eosin stained sections were used to classify the morphology of the adenomas, according to histologic type (tubular, tubulovillous, villous) and the grade of dysplasia, according to World Health Organization criteria.[11]

In Vitro *Adenomas*

Serial observations of individual microadenomas were made using inverted microscopy. Microadenoma volume and growth pattern, particularly size of buds or fingerlike projections from the main cell mass, were noted. Direct measurements were made using an ocular micrometer. Selected microadenoma images were stored in an ATT personal computer (ATT, Woburn, MA) using a digitized computer software program (Optel Telecommunications Telewriter III TGA, New York, NY) for future image analysis

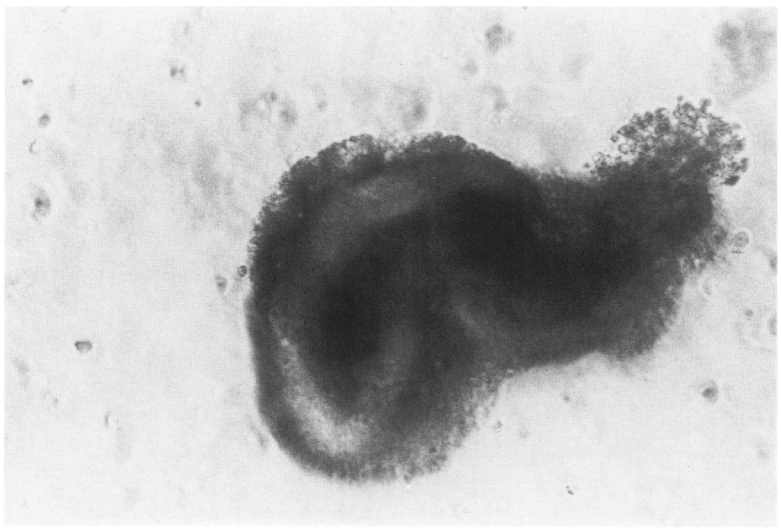


Figure 5. *Parent adenoma 1. This microadenoma has a coiled conch shell-like appearance, without prominent surface budding (13 weeks after incubation, ×40).*

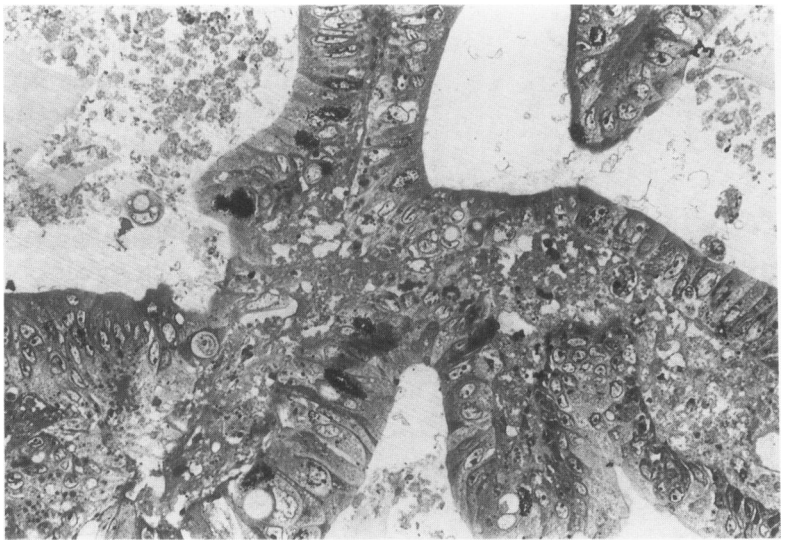


Figure 6. *Parent adenoma 10. Histoautoradiographs of microadenomas 2 weeks after incubation. The microadenomas were incubated with tritiated thymidine overnight before fixation and embedded in plastic. Sections were coated with emulsion and stored for 1 week, then developed and counterstained with Richardson's blue stain. Prominent fingerlike projections and no tubular growth is apparent. The proliferating nuclei are clearly identified by the many black grains over the nuclei. The proliferation index was 9%. Extensive debris is apparent in both upper left and upper right corners, with no apparent nuclei, round apoptotic-type bodies, and no thymidine labeling. These features are typical of the dead cell population of the microadenoma (Richardson's blue stain, ×100).*

studies. For microscopic examination, microadenoma pellets were embedded in plastic blocks. After initial fixation in four parts concentrated formaldehyde (40% solution) and one part 2.5% buffered glutaraldehyde for 4 to 6 hours, pellets were postfixed in 2% osmium tetroxide in cacodylate buffer (0.1 mol/l) for 1 hour, dehydrated with graded acetone, and embedded in Epon/Araldite. Sections (1 to 2 μ) were stained with Richardson's blue and serial sections were evaluated morphologically by light microscopy. For ultrastructural evaluation, ultrathin sections from plastic blocks were stained with uranyl acetate and lead citrate and examined and photographed with a Philips EM300 transmission electron microscope.

Viability and Cell Proliferation

The MTT (3-[4-5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide) assay was used to measure growth over time. MTT is a tetrazolium compound that is reduced by a mitochondrial reductase in viable cells to a blue formazan product that can be analyzed spectrophotometrically.[12] Microadenoma pellets[3] were placed in 16-mm wells with 1 ml of serum-free medium and 15 μg MTT and incubated at 37°C in 5% CO_2 for 4 hours. Dimethyl sulfoxide (200 μl) was added for 30 minutes to solubilize the formazan crystals and 100 μl of solution was transferred to microtiter wells. The optical density read at 570 nm was proportional to cell number.

Both tritiated thymidine uptake[13] and immunohistochemical staining with the Ki-67 antibody[14] were used to evaluate cell proliferation in separate pellets from the same parent adenomas incubated for similar periods of time. Pellets were incubated in 0.25 μC tritiated thymidine overnight, rinsed in PBS, incubated with cold thymidine for 2 hours, rinsed again, then fixed and processed as outlined above. Plastic sections (1 μ) were coated with emulsion (Kodak NTB2, Rochester, NY) and allowed to incubate at 4°C for 1 week. Slides were developed with Kodak D19 and stained with Richardson's blue. Proliferating nuclei had numerous (more than >50) overlying grains in contrast to background (less than 1 grain per nucleus). Labeling index was calculated as number of positive nuclei/total number of viable nuclei in a given section.

Sections (5 μ) of fresh microadenoma pellets were cut after snap freezing pellets in liquid nitrogen (−70°C). Cytospin preparations of other pellets were obtained by dissolving the alginate in 0.1 mol/l ethylene diamine tetraacetic acid at 37°C for 5 minutes, followed by deep dilution with PBS and centrifugation of the microadenomas at 800 rpm for 5 minutes. After resuspension in PBS, each specimen was centrifuged onto a polylysine-coated glass slide in a cytospin centrifuge (Shandon Cytospin 2, Pittsburgh, PA) for 5 minutes at 1000 rpm. The preparations were fixed in acetone/methanol (50:50) and stained with primary antibody (Ki-67, DAKO Corp, Carpinteria, CA) and an avidin-biotin modification of the immunoperoxidase technique with diaminobenzidine as chromogen. The Ki-67 growth fraction was calculated as the number of cells with any degree of positive nuclear staining as a percentage of the total number of viable cells.

Results

Eleven adenomas have been cultured for periods up to 5 months. Characteristics of parent adenomas and their duration of culture are presented in Table 1. The parent adenomas included 6 tubular, 3 tubulovillous, and two villous adenomas. Six had low-grade dysplasia and five high-grade dysplasia. The size ranged from 1 to 4.8 cm.

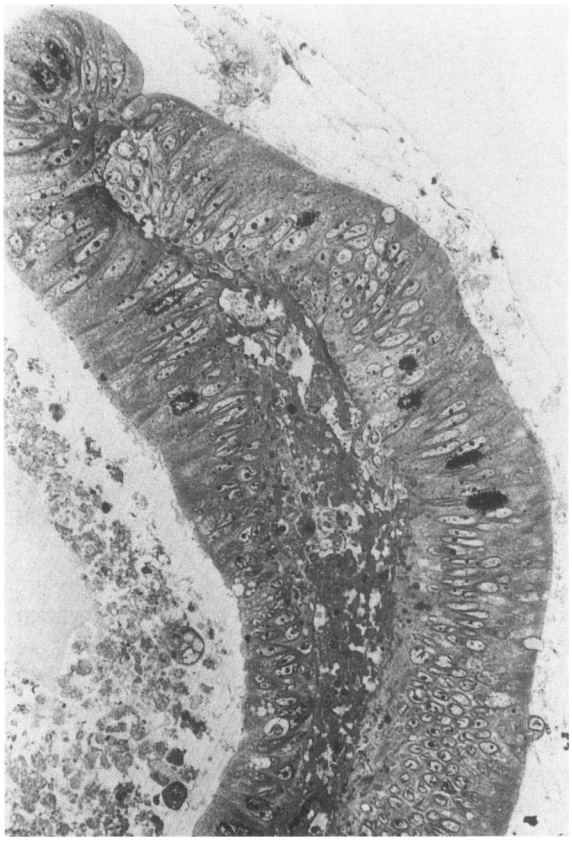


Figure 7. *Parent adenoma 10. Detail of microadenoma is shown in Figure 6. Villuslike projection. The epithelial cells are clearly viable and nuclear labeling is identifiable along the entire length of the tissue, including the tip. The N/C ratio is increased (compare to Figure 3) and the nuclei are more hyperchromatic, have denser, more irregular nuclear membranes, and more prominent nucleoli. There is still ordered growth, however, and some retention of polarity. The mesenchyme, in contrast to the epithelial cells, is completely degenerative. Mesenchymal obsolescence is a consistent feature in this culture system (Richardson's blue stain, ×250).*

One adenoma (#7) was discarded 3 days after incubation due to contamination. Each of the remaining 10 adenomas yielded at least 100 microadenoma pellets for primary culture. Adenomas were cultured for between 34 and 162 days. Criteria for viability included MTT assay and evidence of continued proliferation and viability, as determined by thymidine autoradiography and ultimately morphologic (light-microscopic studies) at the end of the culture period. All adenomas had well-preserved light-microscopic appearances with associated sloughed necrotic cells. Established viability at 30 days or longer was regarded as a successful culture. There was no apparent correlation between duration of culture and site, size, growth pattern, and grade of dysplasia in the parent adenoma. The microadenomas, at the outset, assumed a variety of shapes, frequently lobulated, with single or multiple projections. The major change with time was a symmetrical increase in bulk. Where projections were present soon after incubation, these tended also to increase in bulk rather than length with time. In some cases, shapes tended to reflect the growth pattern of the parent adenoma. Figures 1 and 2 show microadenomas from parent adenoma #2, with curved outgrowths and contours suggesting budding tubules. Light microscopy of another microadenoma from parent adenoma #2 revealed well-organized lumina with radially oriented cells and basal nuclei as well as fingerlike projections analagous to the tubulovillous growth pattern in the parent adenoma (Figure 3). Other growth patterns seen have included many budlike outgrowths from a solid center (Figure 4) or a coiled conchlike appearance, without prominent surface budding (Figure 5). Figures 4 and 5 are from parent adenoma #1 (tubulovillous). The pure villous adenoma (#10) showed a predominantly fingerlike growth pattern with no tubular growth apparent on light microscopy (Figures 6 and 7).

Morphologic correlation with the parent adenoma also appears to extend to the degree of dysplasia. Parent adenoma #2 showed low-grade dysplasia and adenoma #10 showed high-grade dysplasia. The microadenomas derived from the latter (Figures 6 and 7) had cells with higher nuclear:cytoplasmic ratios, and nuclei with more irregular chromatin, greater irregularity of nuclear membranes and larger, more prominent nucleoli, compared with the microadenoma showing low-grade dysplasia (Figure 3).

Ultrastructural studies performed on the microadenoma shown in Figure 3 revealed highly differentiated cells with features of both tubular and villous adenomas (Figures 8 and 9). Typical tubular adenoma cells showed short stubby microvilli, apical microvesicles, many desmosomes (more prominent apically), and irregular convolution of the lateral cell margin without distension of the intercellular spaces. These ultrastructural features were identified in cells from microadenomas forming tubular lumina (Figure 8). Elsewhere goblet cell differentiation was identified with more regularly arranged microvilli (Figure 9). Both light microscopy and ultrastructural studies showed epithelial cell proliferation only with obsolescence of the mesenchymal component and no fibroblast overgrowth (Figure 7).

The proliferation index of the microadenomas ranged from 7% to 10%. Thymidine uptake occurred throughout the microadenomas, with increased cell labeling in more solid areas. Ki-67 growth fractions ranged from 18% to 25%. Many dead cells and apoptotic bodies were identified around all the growing microadenomas. Serial measurements of growth over time were obtained for microadenomas from the same parent using MTT and thymidine labeling assays. Figure 10 shows change in MTT value over time (0 to 8 weeks). The initial value decreases to steady state of 30% of the original at 4 weeks, remains at a plateau until 6 weeks, and then increases to 90% original value at 8 weeks. The thymidine labeling indices

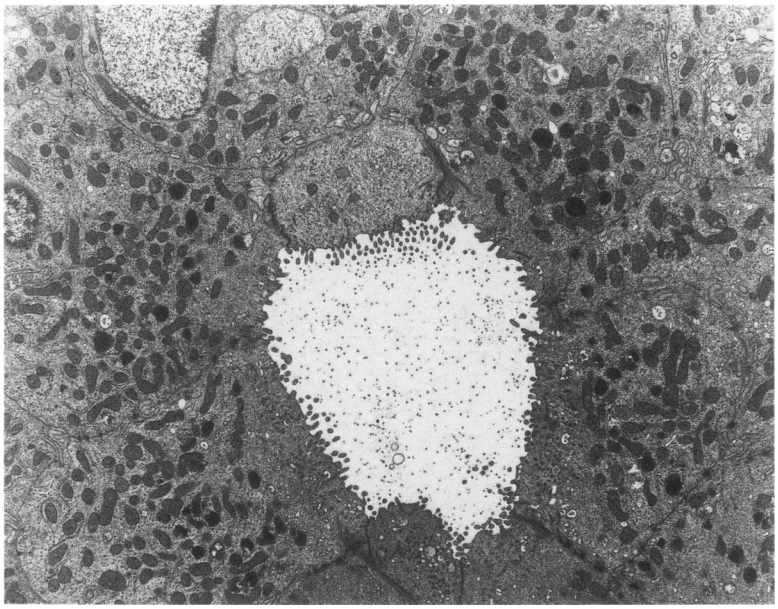


Figure 8. *Transmission electron microscopy of a cross-section of the elongated extension illustrated in Figure 3 shows a lumen surrounded by incompletely differentiated cells typical of tubular adenoma. The cells show short stubby microvilli, irregularly arranged, apical microvesicles, and many desmosomes. The nuclei are basally located and there are prominent mitochondria, some enlerged (×10,600).*

from corresponding microadenomas show similar results. At day 1 the labeling index was 14 ± 3% (mean ± SE). This decreased to 7 ± 3% at 1 week, 6 ± 2% at 3 weeks after incubation, and 4 ± 2% at 4 weeks incubation.

Discussion

Few reports of successful *in vitro* culture of colorectal adenomas have appeared in the literature. Friedman et al[8] described the primary culture of adenomas on a gelatin substrate and later a type 1 collagen-bovine serum albumen substrate. These short-term monolayer cultures were used to assess *in vitro* activity of tumor promoters on adenoma growth, cytoskeletal organization, and plasminogen activator secretion. Using methods similar to those described by Friedman et al,[8] both Wilson et al[9] and Paraskeva et al[10] derived cell lines from adenomas that retained typical epithelial morphology during *in vitro* passage. Further characterization of these cell lines has shown that, with rare exception, both tubular and villous adenoma cells are nonclonogenic, anchorage dependent, and nontumorigenic in athymic nude mice.[15]

The method described here for the *in vitro* culture of colorectal adenomas uses an alginate gel culture system previously used successfully for culture of carcinoma cell lines.[16] Cultured human pancreatic and colonic carcinoma cell lines have been maintained in the system for up to 49 days and have demonstrated organized growth and differentiation.[17] Other systems also have been claimed to allow three-dimensional growth of cells in culture. A number of these have relied on the spontaneous aggregation of cells in spheroid form[18] or the use of microcarrier Sephadex® beads.[19] The system most comparable to the alginate system is a collagen gel technique[20] in which epithelial cells, embedded in mouse tail collagen gel, grew to form three-dimensional structures resembling *in vivo* glands and ducts. In this system there was a significant problem of fibroblast overgrowth, which required the addition of cholera toxin for successful epithelial growth. In our system, epithelial growth is favored, with rapid obsolescence of mesenchymal elements. In no case to date has fibroblast overgrowth occurred in the alginate system.

The encapsulation of microexplants of adenomas promotes three-dimensional *in vitro* growth, which simulates *in vivo* growth. Three-dimensional growth is a particularly important attribute of an *in vitro* model of colorectal adenomas, in that it appears that the pattern of growth (ie, villous versus tubular) is an independent risk factor for malignant transformation in a given adenoma.[21] The biologic determinants of variation in growth patterns are not understood and this model may help to provide clues. Comparability of the model to the *in vivo* adenoma is also borne out by the preliminary electronmicroscopic studies, which showed ultrastructural features identical to those described in epithelial cells of tubular[22,23] and villous[24] adenomas, respectively.

Epithelial cell proliferation rates in the *in vitro* microadenomas studied also appeared comparable to those of *in vivo* adenomas[25] and, in addition, are similar to the values obtained by Friedman et al[26,27] in short-term monolayer culture. It is known that although cell proliferation rates of *in vivo* adenomas are high,[25] with growth fractions in the range of 25% to 60%, increase in size is slow. This has been attributed to differentiation and dehiscence of cells into the bowel lumen.[28,29] Ki-67 is a proliferation an-

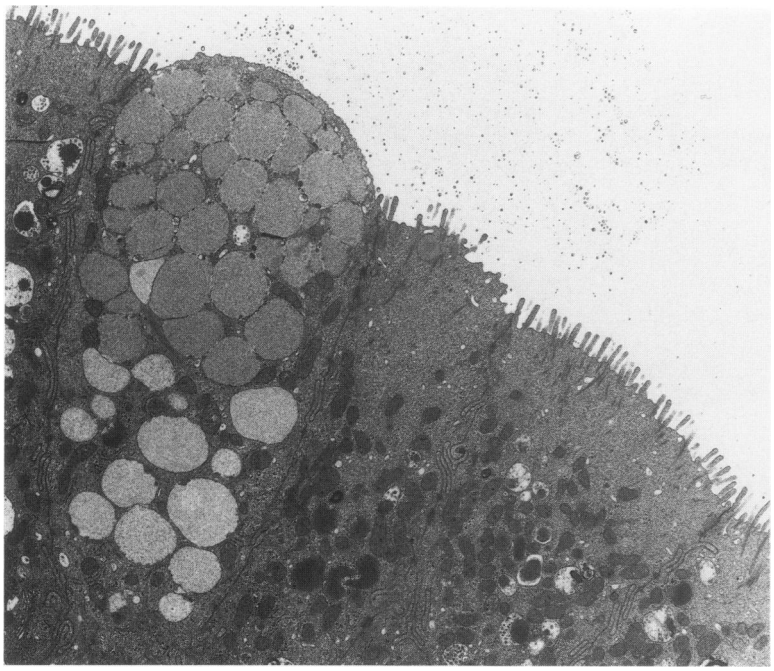


Figure 9. *Elsewhere the same adenoma shows focal goblet cell differentiation and cells with more regularly arranged microvilli. These features have been described in villous adenomas (×8800).*

tigen found in nuclei of cells in G_1G_2, S, and M phases of the cell cycle.[13] The ^{3}H-thymidine assay, on the other hand, identifies cells in S phase, thus accounting for the differences in their respective values as determined by the two methods. The alginate microadenomas had ^{3}H-thymidine–labeling indices of 7% to 10% and Ki-67 growth fractions of 18% to 25%, as determined by thymidine autoradiography and immunohistochemistry, respectively. The volume increase in the microadenomas also was less than would be expected from these growth determinations. A possible explanation is a high rate of cell loss reflecting a rapid progression to terminal differentiation, which is analagous to the *in vivo* phenomenon. We identified many dead cells and apoptotic bodies, both inside and outside the microadenomas, supporting this hypothesis and suggesting that the cell death is not a function of adverse culture conditions at the center of the tissue. Our current studies on the model focus on the systematic evaluation of microadenoma growth and proliferation under varied experimental conditions.

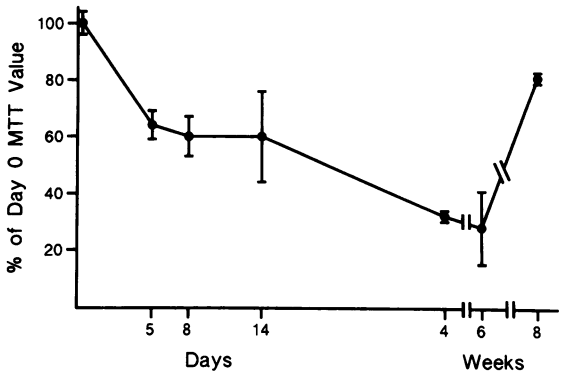


Figure 10. *Changes in growth rate of microadenomas over time assessed by MTT assay. Each value (± standard error) is the mean of three samples.*

We developed a primary culture system for the three-dimensional growth of colorectal adenomas in alginate capsules. Each parent adenoma provided at least 100 microadenomas for serial observation and assessment. Viability and continued proliferation *in vitro* has been maintained for up to 162 days. It has been possible to perform detailed morphologic studies on individual microadenomas and correlate gross microscopic immunohistologic and ultrastructural features. Previously we showed the capacity for diffusion of macromolecules from the capsule and their measurement in the culture medium.[16,17] Growth patterns analogous to tubular and villous histology have been observed and plastic sections have shown highly differentiated cells with well-formed lumina. Proliferation studies showed growth fractions comparable to those observed *in vivo*. Electron microscope studies have shown features typical of both tubular and villous adenomas. This model thus replicates the *in vivo* growth of colorectal adenomas and permits a wide range of morphometric, cell kinetic, and biochemical measurements to be performed serially and under varying experimental conditions.

References

1. Morson BC: The polyp-cancer sequence in the large bowel. Proc R Soc Med 1984, 67:451–457

2. O'Brien MJ: Cancer of the colon and rectum. Current concepts of etiology and pathogenesis. Ir J Med Sc 1988, 157(Suppl 7):5–15
3. Arminski TC, McLean DW: Incidence and distribution of adenomatous polyps of the colon and rectum based on 1000 autopsy examinations. Dis Colon Rectum 1964, 7:249–261
4. Eide TJ: Risk of Colorectal Cancer in adenoma-bearing individuals within a defined population. Int J Cancer 1986, 38: 173–176
5. Madara JL, Harte P, Deasy J, Ross D, Lahey S, Steele G: Evidence for an adenoma-carcinoma sequence in dimethylhydrazine-induced neoplasms of rat intestinal epithelium. Am J Pathol 1983, 110:230–235
6. Clapp NK, Lushbaugh CC, Humanson GL: Natural history and pathology of colon cancer in saguinus oedipus oedipus. Dig Dis Sci 1985, 30:107S–113S
7. Niles RM, Wilhelm SA, Steele GD Jr, Burke B, Christensen T, Daniel D, O'Brien MJ, Thomas SP, Zamcheck N: Isolation and characterization of an undifferentiated human colon carcinoma cell line (MIP-101). Cancer Invest 1987, 5(6):545–552
8. Friedman EA, Higgins PJ, Lipkin M. Tissue culture of human epithelial cells from benign colonic tumors. In Vitro 1981, 17: 632–644
9. Wilson JKV, Bittner G, Oberly T: Cell culture of human colon adenomas and carcinomas. Cancer Res 1987, 47:2704–2713
10. Paraskeva C, Buckle BG, Sheer D: The isolation and characterization of colorectal epithelial cell lines at different stages in malignant transformation from familial polyposis coli patients. Int J Cancer 1984, 34:49–56
11. Jass JR, Sobin LH: Histologic typing of intestinal tumors. World Health Organization International Classification of Tumors. Berlin, Springer Verlag, 1989, pp 29–31
12. Carmichael J, de Graaf WG, Gazdar AF, Minnr JD, Mitchell JB: Evaluation of a Tetrazolium-based semi-automated colometric assay: Assessment of chemosensitivity testing. Cancer Res 1987, 47:936–942
13. Simpson-Herren L: Autoradiography techniques for measurement of the labeling index. *In* Gray JW, Darzynkiewicz Z, eds. Techniques in Cell Cycle Analysis. Clifton, NJ, Human Press, 1987, pp 1–30
14. Gerdes J: Cell cycle analysis of cell proliferation associated with nuclear antigens defined by a monoclonal antibody Ki67. J Immunol 1984, 133:1710–1715
15. Wilson JKV, Bittner GN: In vitro models of human colonic adenomatous polyps. *In* Steele G Jr, Winawer SJ, Karr JP, eds. Basic and Clinical perspectives of Colorectal Polyps and Cancer. New York, Alan R. Liss, 1988; pp 346–356
16. Kupchik HZ, Langer RS, Haberern C, El Deriny S, O'Brien MJ. A new method for the three-dimensional in-vitro growth of human cancer cells. Exp Cell Res 1983, 147:454–460
17. El Deriny S, O'Brien MJ, Christensen TG, Kupchik HZ: Ultrastructural differentiation and CEA expression of butyrate treated human pancreatic carcinoma cells. Pancreas 1987, 2:25–33
18. Sutherland R, Carlson J, Durand R, Yuhas J: Spheroids in cancer research. Cancer Res 1981, 41:2980–2984
19. Hirtenstein M, Clark J, Lindgren G, Vretland P: Microcarriers for animal cell culture: A brief review of theory and practice. Devel Biol Stand 1980, 46:109
20. Yang J, Richards J, Bowman P, Guzman R, Enami J, McCormick K, Hamamobo S, Pitelka D, Nandi S: Sustained growth and three dimensional organization of primary mammary tumor epithelial cells embedded in collagen gels. Proc Natl Acad Sci 1979, 76:3401–3405
21. O'Brien MJ, Winawer SJ, Zauber AG, Gottlieb LS, Sternberg SS, Diaz B, Dickersin GR, Ewing S, Geller S, Kasimian D, Komorowski R, Szporn A, the National Polyp Study Workgroup: The National Polyp Study: Patient and polyp characteristics associated with high-grade dysplasia in colorectal adenomas. Gastroenterology 1990, 98:371–379
22. Kaye GI, Fenoglio CM, Pascal RR, Lane N: Comparative EM features of normal, hyperplastic and adenomatous human colonic epithelium. Gastroenterology 1973, 64:926–945
23. Fenoglio CM, Richart RM, Kaye GI: Comparative EM features of normal, hyperplastic and adenomatous human colonic epithelium. II: Variations in surface architecture found by scanning electron microscopy. Gastroenterology 1975, 69: 100–109
24. Imai H, Saito S, Stern AA: Ultrastructure of adenomatous polyps and villous adenomas of the large intestine. Gastroenterology 1965, 48:188–197
25. Johnson PJ, O'Brien MJ, Dervan PA, Carney DN: Immunohistochemical analysis of cell kinetic parameters in colonic adenocarcinomas adenomas and normal mucosa. Hum Pathol 1989, 20:696–700
26. Friedman EA, Steinberg M: Disrupted Communication between late-stage premalignant human colon epithelial cells by 12-0-tetradecanoylphorbol-13-acetate. Cancer Res 1982, 42:5096–5105
27. Friedman EA, Isaksson P, Rafter J, Marian B, Winawer S, Newmark H: Fecal diglycerides as selective endogenous mitogens for premalignant and malignant human colonic epithelial cells. Cancer Res 1989, 49:544–548
28. Deschner EE, Maskens AP: Significance of labelling index and labelling distribution as kinetic parameters in colorectal mucosa of cancer patients and DMH treated animals. Cancer 1982, 50:1136–1141
29. Camplejohn R, Bone G, Aherne W: Cell proliferation in rectal carcinoma and rectal mucosa. A stathmokinetic study. Eur J Cancer 1973, 9:577–581

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