GROWTH OF EXOCRINE ACINAR CELLS ON A RECONSTITUTED BASEMENT MEMBRANE GEL

CONSTANCE OLIVER[1], JUDITH F. WATERS, CAROLYN L. TOLBERT,
AND HYNDA K. KLEINMAN

Laboratory of Oral Biology and Physiology (C. O., J. F. W., C. L. T.) and Laboratory of Developmental Biology and Anomalies (H. K. K.), National Institute of Dental Research, National Institutes of Health
Bethesda, Maryland 20892

SUMMARY

Methods have been developed for culturing a dividing population of morphologically differentiated rat parotid, lacrimal, and pancreatic acinar cells in vitro. Isolated acinar cells were plated onto tissue culture dishes coated with a three-dimensional, reconstituted basement membrane gel. After attachment in Ham's nutrient mixture F12, the cells were cultured at 35° C in F12 supplemented with 10% heat inactivated rat serum, epidermal growth factor, dexamethasone, insulin, transferrin, selenium, putrescine, reduced glutathione, ascorbate, penicillin, streptomycin, and the appropriate secretagogue. Under these conditions, the cells attached rapidly and DNA synthesis was initiated within 2 to 3 d. Although the cells flattened on the substratum, they continued to maintain their differentiated morphology. The cells contained secretory granules, and the secretory enzymes peroxidase and amylase could be detected. The use of a reconstituted basement membrane gel proved critical for the attachment and growth of exocrine acinar cells.

Key words: pancreas; parotid gland; exorbital lacrimal gland; basement membrane.

INTRODUCTION

Exocrine acinar cells are highly differentiated epithelial cells specialized for secretion. In vivo, the apical portion of the adjacent cells are joined by junctional complexes which restrict the passage of material between the lumen and the lateral-basal intercellular space. Additionally, the cells are electrically coupled (44). These properties have made the acinar cells difficult to isolate and to maintain in a differentiated state in culture. Most of the methods used to isolate acinar cells (1,2,24,25,34) result in cultures that can be maintained only for relatively short periods and which do not undergo cell division. We have previously developed (39) an isolation method whereby differentiated, functional (7) acinar cells can be maintained in vitro for up to a month. However, these cells do not divide. Recently, Logsdon and Williams (32) reported that pancreatic acinar cells cultured on collagen gels in the presence of caerulein and other supplements will divide.

The present investigation was undertaken to define the conditions necessary to induce cell division and to maintain the morphologic differentiation of isolated exocrine acinar cells. Previous investigations on certain epithelial cells, such as mammary epithelium (12,13) and

sertoli cells (20), had suggested that attachment to a substratum of extracellular matrix might be required for maintenance of cell differentiation and for cell division. Therefore, several types of substrata in combination with variations in the media composition were examined for their ability to support cell division and differentiation. The morphologic appearance of the cells, with the retention of secretory granules was the primary criterion used in assessing differentiation in these cells.

MATERIALS AND METHODS

Male Sprague-Dawley rats (certified free of sialodacro adenitis virus and rat corona virus; Harlan-Sprague Dawley, Indianapolis, IN) weighing between 50 and 125 g were used. Exorbital lacrimal glands, parotid glands, and pancreas were removed, and acinar cells were isolated by a modified, previously published method (38). Briefly, glands were minced, placed into a spinner flask, rinsed twice in Ca^{++}, Mg^{++}-free Hank's balanced salt solution (HBSS), resuspended in 2 mM EDTA in Ca^{++}, Mg^{++}-free HBSS, and placed in a 35° C incubator with stirring for 15 min. The cells were then rinsed twice in Ham's nutrient mixture F12 (GIBCO, Grand Island, NY) supplemented with penicillin (100 U/ml; GIBCO)-streptomycin (100 μg/ml; GIBCO). The medium used for dissociation of the pancreas was also supplemented with soy bean trypsin inhibitor (0.1 mg/ml; Cooper Biomedical, Freehold, NJ).

After rinsing, the tissue was placed in a collagenase (1 mg/ml; type II; Cooper)-hyaluronidase (300 U/ml; chromatographically purified; Cooper) solution and returned to the incubator for 45 min. After rinsing in supplemented medium, the resulting cell suspension was passed through 500 and 100-μm Nitex filters (Tetko, Elmsford, NY). The cells were resuspended in F12 and layered over 15% heat inactivated bovine calf serum (GIBCO). After the cells were allowed to settle by gravity for 10 min, the majority of the supernatant fraction was removed, and the cells were pelleted, resuspended in F12, and layered over the serum again. After settling the second time, the cells were rinsed in F12 and exposed to 2 mM EDTA for 5 min before being plated into culture dishes. Approximately 1×10^5 cells were plated/35-mm dish. This procedure yielded primarily small (4 to 5 cell) aggregates and single cells. If larger aggregates were plated onto the gel, the cells in the center of the aggregates tended to become necrotic.

Culture conditions. Cells were plated onto either standard plastic tissue culture dishes or Primaria dishes (Falcon Labware, Becton Dickinson Labware, Oxnard, CA) either uncoated or coated with a reconstituted basement membrane gel matrix. The reconstituted basement membrane gel was prepared from an unfractionated extract of the EHS tumor (28). The extract polymerizes in 30 min at 37° C. For most experiments, 250 μl of the cold (4° C) extract, diluted 1:1 with Ham's nutrient mixture F12, was plated onto a 35-mm dish and allowed to polymerize. For some experiments cells were plated onto tissue culture or Primaria dishes coated with type I collagen (Vitrogen 100 collagen, Flow Laboratories Inc., McLean, VA) or with purified laminin (1 mg to 1 ng/ml). Growth medium consisted of Ham's nutrient mixture F12 supplemented with pencillin-streptomycin, dexamethasone (10 μg/ml; Collaborative Research, Lexington, MA), reduced glutathione (10 μg/ml; Sigma Chemical Co., St. Louis, MO) putresine (1 mM, Collaborative Research), L-ascorbic acid (50 μg/ml; GIBCO); 5 μg/ml insulin; 5 μg/ml transferrin; 5 mg/ml selenous acid (ITS); (Collaborative Research), epidermal growth factor (10 ng/ml; Irvine Scientific, Irvine, CA), 10% heat inactivated rat serum (GIBCO), and an appropriate
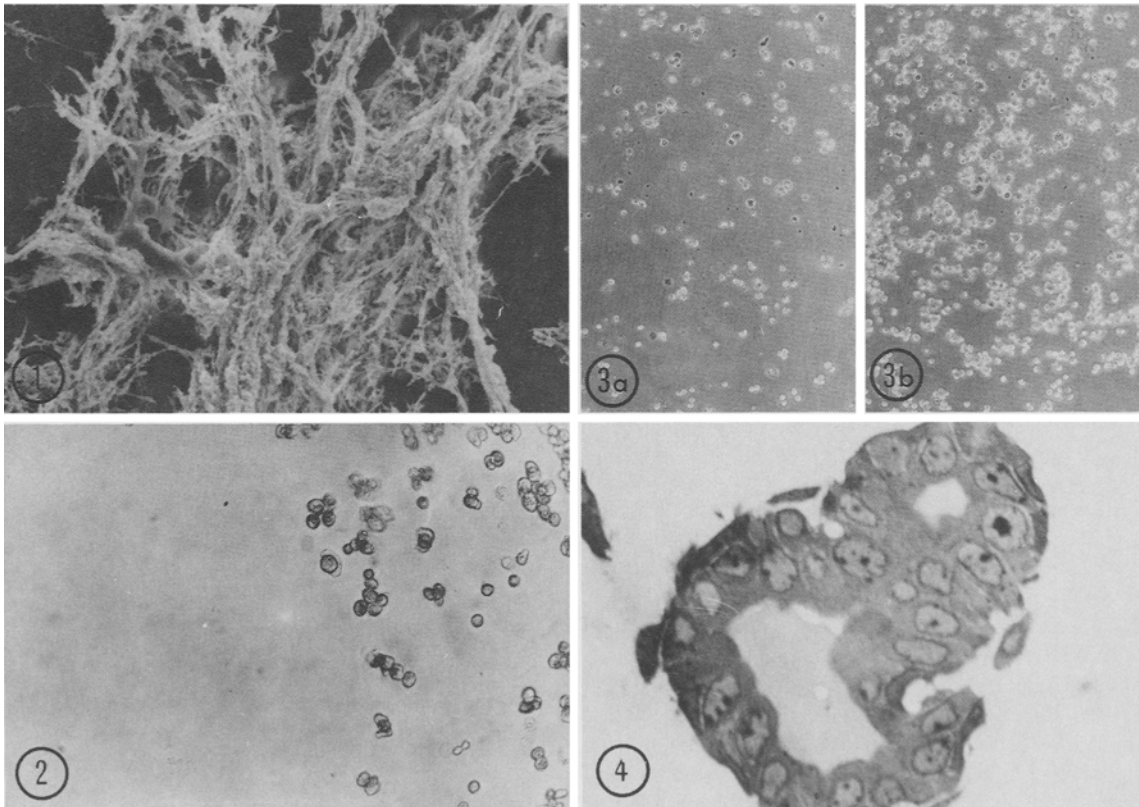

FIG. 1. By scanning electron microscopy, the fibrillar nature of the reconstituted basement membrane gel is apparent. ×783.

FIG. 2. Parotid acinar cells. Freshly isolated cells adhere only to the gel-coated side (*right*) of a tissue culture dish. ×150.

FIG. 3. Pancreatic acinar cells. Although some cells adhere to the surface of a Primaria dish (Fig. 3 *a*), many more cells attach when the surface is coated with the basement membrane gel (Fig. 3 *b*). ×75.

FIG. 4. Parotid gland, 7 d. When the cells are plated in the gel, ductlike tubules form. Here the cells were mixed with the basement membrane extract at 4° C before plating. The extract plus cells was pipetted onto a Primaria dish and allowed to polymerize for 30 min at 34° C. Medium was then added to the dish and the cells were cultured in the usual manner. ×300.

secretagogue (pancreas and exorbital lacrimal gland, 10^{-5} M carbamyl choline; Sigma and parotid gland 10^{-6} M isoproterenol; Sigma). Cells were cultured at 35° C in a humidified atmosphere of 5% CO_2 in air.

AR42J cells were grown on tissue culture dishes coated with the basement membrane matrix. They were cultured in Kaighn's nutrient mixture F12 supplemented with penicillin-streptomycin and 7.5% fetal bovine serum containing SerXtend (Hana Media, Inc., Berkeley, CA).

Microscopy

Light microscopy. Cultures were examined by either phase or bright field with a Leitz Diavert inverted microscope.

Transmission electron microscopy. For routine transmission electron microscopy cells were fixed either in situ or as suspensions after removal from the dish with a collagenase (1.25 mg/ml)-EDTA (2 mM) solution. The cells were fixed for 2 to 3 h in 2% glutaraldehyde (Ladd Research Industries, Burlington, VT)-2% formaldehyde (Ladd), rinsed in 0.1 M cacodylate buffer, pH 7.4, containing 7% sucrose (sucrose buffer), and stored overnight at 4° C in sucrose buffer. The cells were postfixed in 2% osmium tetroxide in sucrose buffer containing 1.5% potassium ferrocyanide (26), dehydrated through a graded series of ethanols, and embedded either in Spurr's resin (51) or in Epon 812. Thin sections were cut with a diamond knife, mounted on bare copper grids, stained with uranyl acetate and Reynold's lead citrate (47), and examined in a JEOL 100-CX electron microscope.

Scanning electron microscopy. Cells were grown either on glass cover slips or on Primaria dishes coated with the reconstituted basement membrane gel. Because the cells did not grow properly on the coated glass, the cover slips were only used to examine cells during their initial stages of attachment. Cells were fixed for 1 to 2 h in 2% glutaraldehyde (Ladd) in 0.1 M cacodylate buffer, rinsed in sucrose buffer, and dehydrated through a graded series of ethanols. The cells were then critically point dried with liquid CO_2. The samples were mounted on aluminum specimen mounts and coated with gold-palladium in a Polaron Sputter Coater. The specimens were then examined in a JEOL 35 CF scanning electron microscope.

Cytochemistry

For demonstration of peroxidase activity, the cells were fixed for 10 min in 0.5% glutaraldehyde (Ladd)-4% formaldehyde (Ladd), rinsed thoroughly in sucrose buffer and stored overnight at 4° C in sucrose buffer. Cells were incubated in diaminobenzidine-hydrogen peroxide containing medium (15) for 4 h at 37° C. After incubation, the cells were rinsed several times in sucrose buffer and either examined immediately by light microscopy or refixed in 2% glutaraldehyde-2% formaldehyde for electron microscopy. Those cells to be examined by transmission electron microscopy were then processed as described above.

Cell Growth

Cells were grown in 60-mm dishes coated with reconstituted basement membrane gel. Tritiated thymidine (1 μCi/ml; sp act: parotid 78.6 Ci/mmol; pancreas 20 Ci/mmol; NEN Research Products, Boston, MA.) was added 24 h before harvest. Cells were rinsed twice in Ca^{++}, Mg^{++}-free HBSS and exposed to a collagenase-EDTA solution for 10 min at 37° C. The cells were then removed from the dishes and rinsed twice in HBSS and once in phosphate buffered saline. All solutions used in harvesting the cells contained 100 mg/ml thymidine (Sigma). The cells were then disrupted by sonication in a 10% tetrachloroacetic acid (TCA) solution and total DNA/dish was determined (8). An aliquot of the hydrolyzed TCA precipitate was counted in a Beckman LS8100 liquid scintillation counter to determine thymidine incorporation.

Amylase determination. Amylase activity of cell homogenates was determined by the Sigma method (Sigma Diagnostics, St. Louis, Mo.) where one unit of α-amylase activity is defined as the amount of enzyme that results in the production of 1 μM of NADH/min in the coupled reaction system described by Pierre et al. (45).

RESULTS

Exocrine acinar cells from rat exorbital lacrimal gland, parotid gland and pancreas were successfully cultured on a reconstituted basement membrane gel which exhibited a fibrillar appearance by scanning electron microscopy (Fig. 1). Acinar cells from all of the tissues examined exhibited similar growth characteristics on the reconstituted basement membrane gel. However, the pancreas and parotid acinar cells adapted better to the culture conditions and grew more readily. Therefore, emphasis has been placed on these two cell types. Several different surfaces either uncoated or coated with the basement

TABLE 1

EFFECT OF SUBSTRATUM ON CELL GROWTH AND ATTACHMENT[a]

Surface	Attachment	Growth
Glass		
uncoated	−	−
gelcoated	+ +	+ +
Tissue culture dishes		
uncoated	−	−
gelcoated	+ +	+ +
laminin coated	−	−
Primaria dishes		
uncoated	+	+ +
gelcoated	+ +	+ + + +
laminin coated	−	−
Type 1 collagen gels	−	−

[a] Cells were grown in complete medium as described in Materials and Methods. Attachment: + + >75% of the acinar cells plated attach; + 50% of the acinar cells plated attach − 5% of the acinar cells plated attach. Growth: + + + + confluency in 6 d or less; + + + confluency in 10 d; + + significant growth, but cultures had not reached confluency in 10 d; − little or no growth.

membrane gel including glass, tissue culture dishes, and Primaria dishes were examined for their ability to promote cell attachment and cell growth (Table 1). Neither glass nor tissue culture dishes (Fig. 2) were suitable substrates for the cells because an even coating of the basement membrane gel could not be maintained on these surfaces. Gel-coated Primaria dishes proved to be the most suitable surface. Some cells adhered, however, to the uncoated Primaria dishes but a greater number of cells attached to the gel-coated surface (Fig. 3). With time in culture, the cells spread over the surface. Cultures were maintained on the gel-coated Primaria dishes for up to 21 d without significant detachment of the cells.

The attachment of acinar cells to type I collagen and laminin was examined. Cells that were plated on type I collagen gels failed to attach. Pretreating tissue culture dishes with laminin did not significantly increase cell attachment. However, pretreatment of Primaria dishes with laminin inhibited cell attachment.

Isolated acinar cells that were mixed with the gel instead of being plated on top of the gel showed rapid degeneration. Some cells resembling duct cells, however, proliferated and formed ductlike tubules within the gel (Fig. 4).

Morphology. As the acinar cells attached and adapted to culture, morphologic changes were observed. The cell aggregates initially attached to the gel at their exposed lateral-basal surface (Fig. 5). After 2 to 5 min, some cells assumed a tubular appearance as they attached to the gel surface (Fig 6). By 16 to 18 h after plating, individual cells, particularly at the edge of the clusters, began to
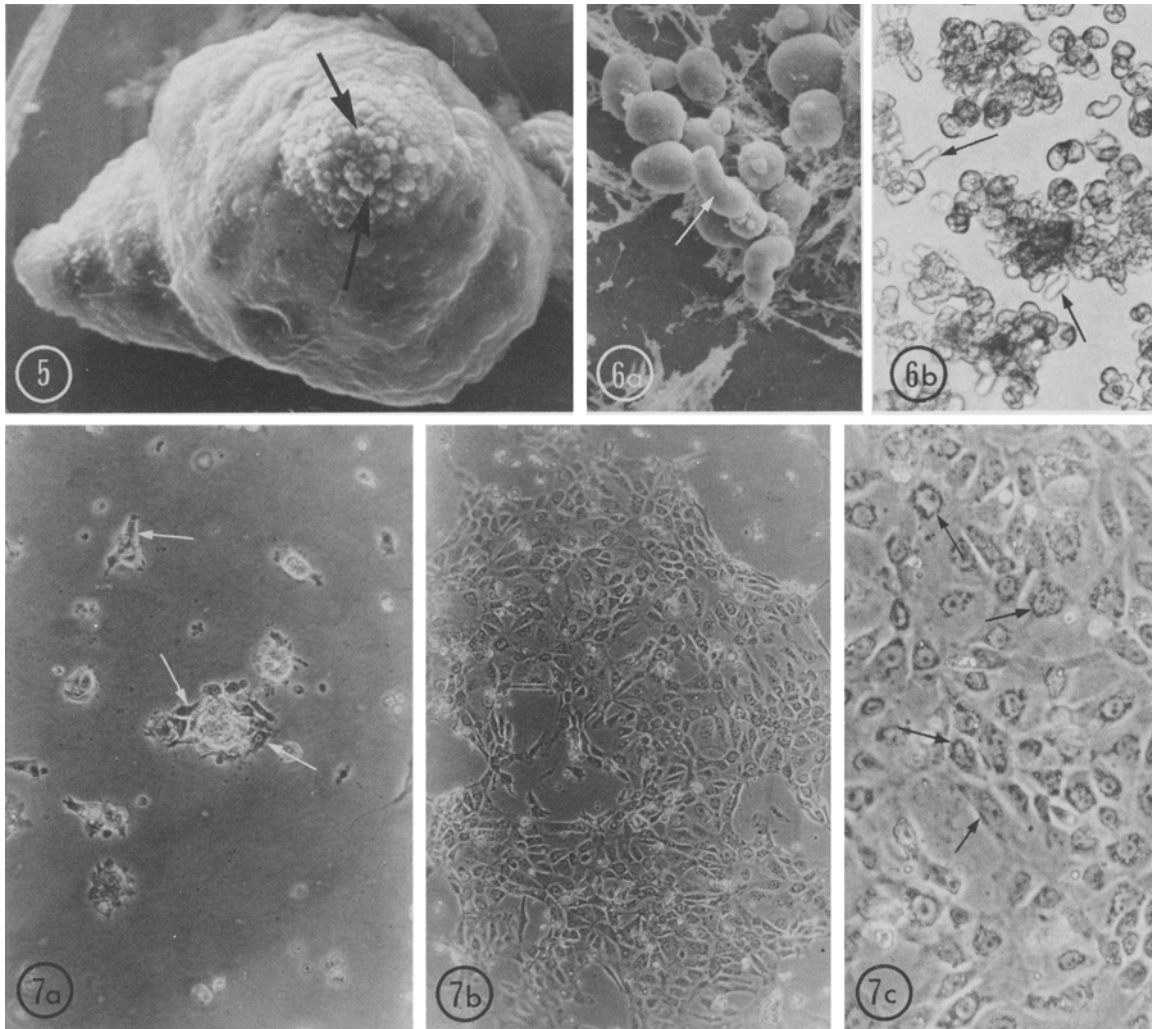

FIG. 5. Parotid acinar cells, 2.5 min. Acinar cells have attached to the gel at their basal surface. Note secretory granules (arrows) at the apical surface. X3182.

FIG. 6. Pancreatic acinar cells, 2.5 min. Immediately after plating onto the gel, many of the cells have a tubular profile (arrows). a, Scanning electron microscopy of the cells. X682. b, Light microscopy of the cells. X245.

FIG. 7. Parotid acinar cells. All micrographs are from the same culture. a, 18 h. Although most of the cells remain in clusters, some of the cells have flattened (arrows) and spread over the surface of the reconstituted basement membrane gel. X155. b, 3 d. By this time significant outgrowth of the cells has occurred. X155. c, 3 d. Numerous secretory granules (arrows) can be seen surrounding the nuclei. X275.

flatten and spread across the surface of the gel (Fig. 7 a). Significant outgrowth from clusters of acinar cells was apparent by Day 3 to 4 (Fig. 7 b), coinciding with initiation of DNA synthesis. Although these cells were flattened, they still possessed many of the morphologic features of differentiated acinar cells (Figs. 7 c and 8). They contained abundant secretory granules located in the perinuclear region and arrays of rough endoplasmic reticulum at the cell periphery (Fig. 8 a). Junctional complexes typical of epithelial cells were seen between the cytoplasmic processes of many adjacent cells (Fig. 8 c). During the first 3 to 4 d, the size of the secretory granules decreased. Although there was variation from cell to cell, the granules remained smaller in the cultured cells than in vitro. However, by enzyme cytochemistry, the secretory protein peroxidase could still be identified within the granules of parotid and lacrimal acinar cells (Figs. 8 a and 9). Biochemically, in the parotid and pancreatic acinar cells, amylase levels were initially high but decreased with time in culture (Fig 10). After 6 to 7 d in culture, cells with reduced numbers of secretory granules appeared. These cells tended to be more fibroblastic (Fig. 1 a) in appearance. When a well-characterized pancreatic acinar cell line, AR42J, was grown on the basement membrane gel, a similar morphology was observed, with less differentiated cells

radiating out from clusters of epithelioid cells (Fig. 11 b). To date, the cultures have been successfully maintained for up to 1 mo.

DNA synthesis. Significant DNA synthesis began on the 2nd to 3rd d in vitro (Fig. 12). After the 3rd d in culture, DNA synthesis was most pronounced on the day the cells were fed. When the cells were fed daily, DNA synthesis remained at a constant level until the cultures reached confluency, which was usually between 6 and 8 d.

Media composition. The medium employed was designed to promote cell growth while maintaining acinar cell differentiation. Cells were plated onto gel-coated Primaria dishes. The effect of a media component was assessed by the ability of the cells to form a confluent layer while maintaining a differentiated morphology. Differentiation was judged by the shape of the cells as well as their content of secretory granules. Various components were tested including dexamethasone, epidermal growth factor (EGF), putrescine, reduced glutathione, insulin, transferrin, selenium, ascorbate, and the secretagogues, isoproterenol and carbamyl choline. Only in the presence of fully supplemented medium, as described in Materials and Methods, were the cells able to grow and maintain their differentiation. The results with dexamethasone were somewhat equivocal. Although the
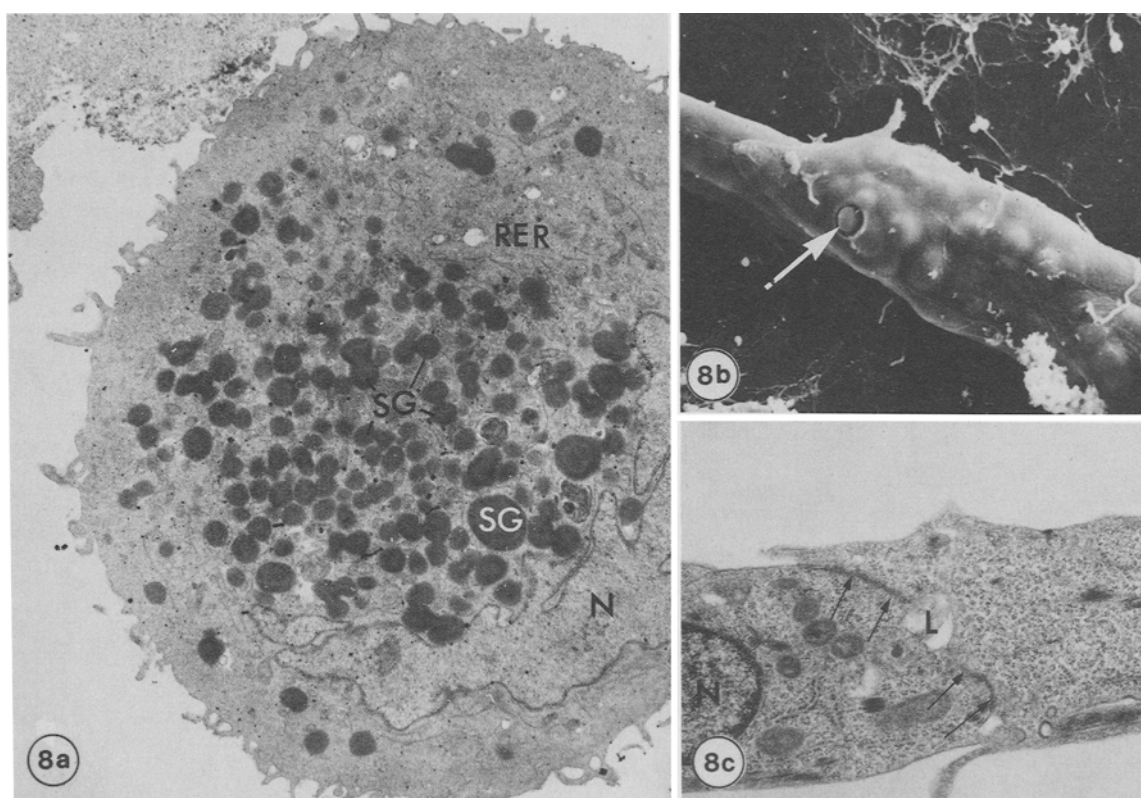

FIG. 8. Parotid acinar cells. a, 7 d. Peroxidase positive secretory granules (SG) lie adjacent to the nucleus (N). Segments of rough endoplasmic reticulum (RER) are present in the peripheral cytoplasm. ×3947. b, 3 d. The cells have flattened and elongated in culture. Secretory granules are present under the plasma membrane. One granule (arrow) is being released. ×3019. c, 3 d. A junctional complex (arrows) has formed between adjacent cells. (L, lumenlike space; N, nucleus) ×9434.
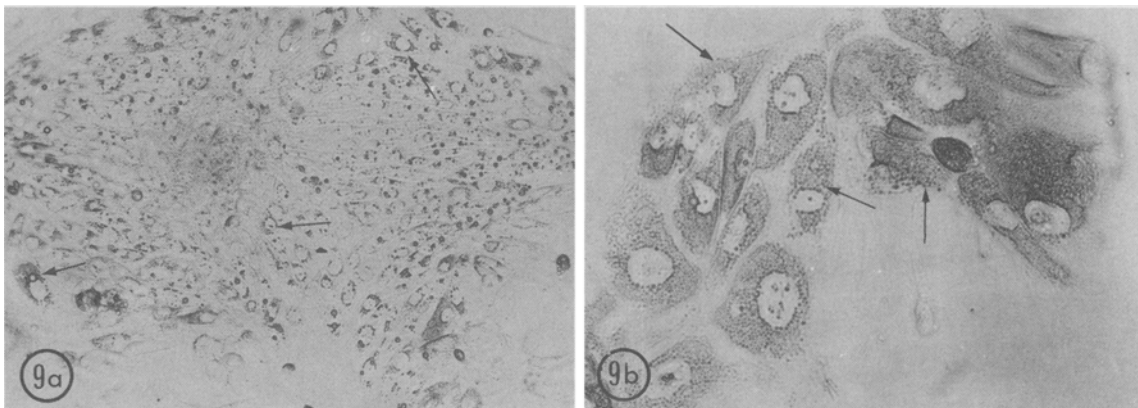
FIG. 9. Incubated for peroxidase activity. *a.* Parotid acinar cells. 10 d. Cells contain peroxidase positive secretory granules (*arrows*). ×138. *b.* Lacrimal acinar cells. 10 d. Peroxidase positive secretory granules (*arrows*) are clustered in the cell body surrounding the nucleus. ×603.

cells grew better in the presence of dexamethasone, there was a tendency for them to be less differentiated. Variations in serum concentrations were also tested (Table 2). Supplementing the medium with 10% rat serum resulted in optimum growth and differentiation of the acinar cells.

DISCUSSION

The use of a reconstituted basement membrane gel as a substratum for exocrine acinar cells has allowed their growth and retention of a differentiated morphology in vitro. Without attachment to a substratum, the cells could be maintained in culture, but they would not proliferate (39). In our experiments the basement membrane extract proved to be superior to any other substratum tested. In Logsdon and William's study (32), pancreatic acini attached to type I collagen gels would divide, but the lag time was longer (4 to 5 d) and the levels of digestive enzymes fell to about 4% of control values. In the present study, the pancreatic acinar cells began to divide at 2 to 3 d in culture and pancreatic amylase levels returned to 50% of the control levels by 6 d in culture. However, in the parotid acinar cells, amylase levels remained very low. Preliminary experiments in which ^{35}S-labeled secretory proteins were immunoprecipitated from cultures of both pancreatic and parotid acinar cells indicate that parotid amylase is the only secretory protein to be so affected. No differences between control and cultured cells could be seen for any other secretory proteins from either the pancreas or the parotid. Furthermore, in cells grown on the basement membrane extract, protein synthesis, as judged by ^{35}S incorporation, fell to 50 to 60% of control by Day 8, whereas [^{3}H]leucine incorporation fell to 16 to 25% of control when pancreatic acini were cultured on type I collagen gels (32). The ability of various substrata to promote differentiation and growth of epithelial cells in vitro is well documented (5,18,21,27). Since Ehrmann and Gey's (12) original studies on the effects of rat-tail collagen in enhancing the growth of cells in vitro, several types of substrata ranging from collagen extracts to extracellular matrix produced in vitro have been tested on a wide variety of cell types. In general, those substrata which most closely resemble the natural substratum of the cells
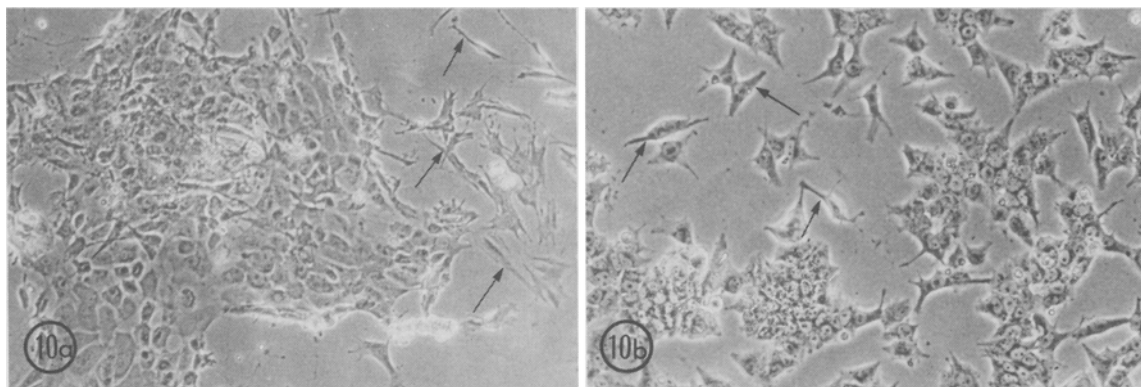

FIG. 10. *a.* Pancreatic acinar cells. 6 d. Cells with a more fibroblastlike appearance (*arrows*) are also present at this time. ×172. *b.* AR42J cells. Fibroblastlike cells (*arrows*) are present in the sparcer areas of the culture. ×207.
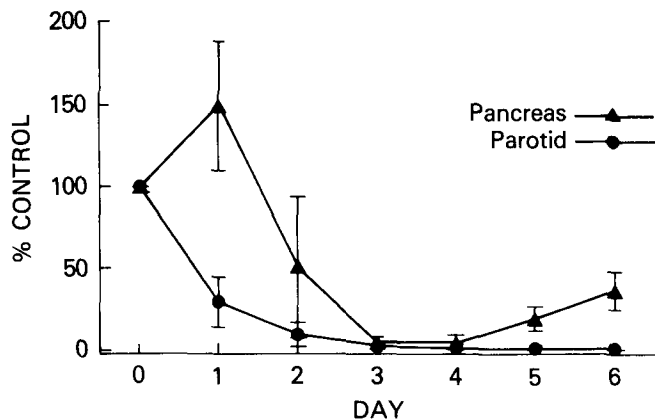
FIG. 11. Amylase activity is initially high but with time in culture drops to about 50% of control for the pancreas and to >1.0% for the parotid.

cells from streptozotocin-treated rats (29,30). However, the effects of any hormones or growth factors on cell division in exocrine acinar cells in vitro have not been previously investigated. The ability of both insulin (4,10,46,48,49) and EGF (4,9,10,35,46,49) to promote cell division in other cell types is well documented. Both will induce DNA synthesis alone and have a synergistic effect when used in combination. EGF may be stimulating DNA
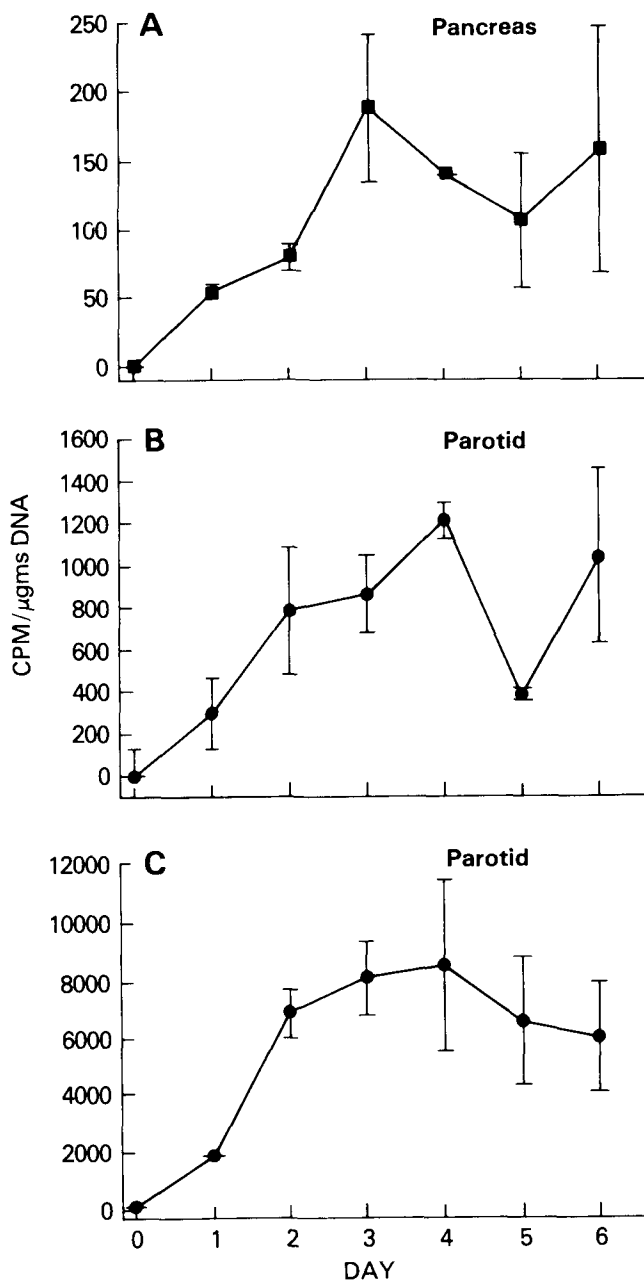
in vivo have been the most successful in inducing proliferation or maintaining differentiated functions in vitro or both. In the exocrine glands in vivo, the basal portion of the acinar cell is in contact with a basement membrane. Of the substrata examined in the present study, only the reconstituted basement membrane gel was able to support both attachment and proliferation. With all other substrata tested either the cells would not attach or only limited growth was observed.

The presence of an extracellular matrix has also been shown to lower cells' requirements for certain growth factors (18,19). In the present study, although it was necessary to supplement the medium to induce cell proliferation, there was a reduced requirement for serum. Under the culture conditions previously employed (39), it was necessary to add 20% serum to the medium for cell maintenance. When the cells were attached to the basement membrane gel, they could be maintained for limited periods (2 to 3 d) in serum-free medium and would proliferate in medium with 10% serum.

Mechanisms whereby the substrata exert their influence is unknown. The mechanical properties of the substratum seem to be important. Several studies have shown that mammary epithelia retain their characteristic morphology and function, i.e. secretion of milk proteins and production of basement lamina, only if cultured on floating collagen gels. Uncoated tissue culture dishes or attached gels would not support their differentiation (13,14,31,42). Likewise, floating gels which had been stabilized by glutaraldehyde fixation were unable to induce the cells to differentiate. Therefore, the flexibility of the underlying substratum seems to be an important factor.

The supplements to the medium used in the present study are all known to have an effect on cellular proliferation or differentiation in many cell types. In pancreatic acinar cells, both EGF and dexamethasone are required for cell maintenance in serum-free medium (7), whereas insulin has been shown to increase amylase activity in vitro, both in normal acinar cells (7) and in

FIG. 12. [³H]Thymidine incorporation into pancreatic and parotid acinar cells. When the cultures were fed every 48 h (Days 1, 3, 5), the peaks of DNA synthesis corresponded to the days on which the cultures were fed (A and B). If the cells were fed daily (C), once DNA synthesis was initiated the level remained constant until the cultures reached confluency (Day 6).

TABLE 2

EFFECT OF SERUM CONCENTRATION ON GROWTH AND DIFFERENTIATION OF EXOCRINE ACINAR CELLS

Serum Concentration[a]	Growth	Differentiation
20% Rat serum	+ + + +	+ + +
10% Rat serum	+ + + +	+ + +
5% Rat serum	+ + +	+ + +
0% Rat serum	+	+ + +
10% Calf serum	+ + +	+ + +

[a]Cells were grown in 24-well Primaria plates in complete medium as described in Materials and Methods. Growth: + + + + confluency in 6 d or less; + + + confluency in 10 d; + + significant growth, but cultures had not reached confluency in 10 d; + little or no growth. Differentiation (on Day 6): + + + >90% of cells are epithelioid and contain secretory granules; + + >50% of cells are epithelioid and contain secretory granules; + >25% of cells are epithelioid and contain secretory granules.

synthesis through its effect on polyamine biosynthesis (23,50). EGF has been shown to increase ornithine decarboxylase activity in other cultured cells (11,16,36,40,41). The exogenously added putrescine, which can be synthesized in vivo from ornithine by ornithine decarboxylase, may be acting directly on DNA synthesis or it may be acting to maintain the cytoskeletal framework of the cells (23,50,52). Transferrin also is essential for growth promotion in a wide variety of cell lines (6). Transferrin is effective only when bound to iron, and supplementation of iron-free medium with apotransferrin will not result in cell growth (43). Selenium is also essential for growth of many cell types (22). Selenium and reduced glutathione are both required for the action of glutathione peroxidase in protecting cells from damage by peroxides. Reduced glutathione serves as the hydrogen donor in the reaction catalyzed by glutathione peroxidase, a selenium-containing enzyme (37). Glucocorticoids, such as dexamethasone, have the ability to potentiate the effects of other medium components such as insulin and EGF. They not only affect DNA synthesis (17) but also the synthesis of proteins such as casein (38), collagen (54), and amylase (33,53). However, in the pancreas, only amylase and carboxypeptidase B production, but not that of other secretory proteins, was stimulated by dexamethasone. Finally, the requirement for ascorbic acid seen in this system was most likely related to the cells' need to synthesize collagen (27) for basement membrane formation. Mammary epithelial cells cultured on collagen gels in hormonally supplemented medium do synthesize their own substratum (54). It has not yet been determined if the exocrine acinar cells are synthesizing their own basement membrane in response to the culture conditions, but their requirement for ascorbate would indicate that they are.

The appearance of poorly differentiated cells at the periphery of the cultures seems to be a characteristic of exocrine cells cultured on the basement membrane gel, not the result of fibroblast outgrowth. AR42J cells, a well-characterized pancreatic acinar cell line, exhibited the same growth characteristics when cultured on the gel. Furthermore, components in the basement membrane gel are known to inhibit fibroblast growth (3). The fact that these poorly differentiated cells did not appear until 6 to 8 d in culture, and then only in regions that were contiguous with readily identifiable acinar cells and which were previously devoid of any cells, also makes it unlikely that these are fibroblasts.

The concentration of all of the factors added to the medium was determined by comparing the degree of differentiation with the growth rate of the acinar cells. What effect any of these constituents may have on specific proteins is not known. It is likely that a medium which is optimal for maintenance of differentiation may not promote cell growth. The ability to grow differentiated acinar cells in vitro should provide a useful system with which to study factors affecting the function of exocrine acinar cells.

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