Collagen Matrix Promotes Reorganization of Pancreatic Endocrine Cell Monolayers into Islet-like Organoids

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ABSTRACT To evaluate the capacity of pancreatic endocrine cells to reassociate in vitro according to the characteristic topographical pattern observed in the islets of Langerhans in situ, we cultured cells dissociated from neonatal rat pancreas within a three-dimensional collagen matrix. Cell monolayers grown on the surface of collagen gels were covered with a second layer of collagen. This induced the monolayers of endocrine cells to reorganize into smooth-contoured, three-dimensional aggregates, in which non-B cells (identified by electron microscopy and immunofluorescence) had a preferential distribution at the periphery, whereas B cells were concentrated in a central position. These results show that cultured pancreatic endocrine cells have the capacity to reassociate into islet-like organoids in vitro, and that collagen matrices may have a permissive effect on the expression of this potential.

The mammalian endocrine pancreas is a collection of microorgans, the islets of Langerhans, which are composed of four main endocrine cell types (for a review see reference 1). Within each pancreatic islet, the various endocrine cells are not randomly distributed, but have a characteristic topographical arrangement that is thought to play a crucial role in the coordination of the islet's secretory activity (2, 3).

The factors involved in the establishment and maintenance of the ordered distribution of islet cell types are not known; nor it is known whether dissociated islet cells retain the capacity to reassociate in vitro according to the specific pattern observed in situ. In monolayer cultures of the endocrine pancreas, a widely used in vitro system for the study of islet cell physiology (4–6), a characteristic arrangement of islet cells is not recognizable (cf. 7, 8), and the precise intercellular relationships present in situ are lost. To establish whether, under appropriate conditions, pancreatic endocrine cells can re-express the specific patterns of three-dimensional association found in vivo, we turned to the use of hydrated collagen gels (9) for the culture of these cells. Collagen gels provide a more physiological, isotropic environment which has been shown to promote the organization of different epithelial cell types into three-dimensional, tissue-like structures (10–15).

We show here that monolayers of pancreatic endocrine cells embedded in collagen gels reorganize into islet-like organoids, in which the specific, nonrandom distribution of the different endocrine cells found in vivo is re-established.

MATERIALS AND METHODS

Preparation of Collagen Gels: Type I collagen was solubilized by stirring adult rat tail tendons for 48 h at 4°C in a sterile 1:1,000 (vol/vol) acetic acid solution (300 ml for 1 g of collagen; 16). The resulting solution was filtered through a sterile triple gauze and centrifuged at 16,000 *g* for 1 h at 4°C. The supernatant was then extensively dialyzed against 1/10 Eagle's minimal essential medium (Gibco, Grand Island, NY) and stored at 4°C. Gels of reconstituted collagen fibers were prepared by simultaneously raising the pH and ionic strength of the collagen solution according to a modification (17) of the method originally described by Elsdale and Bard (9). This was achieved by quickly mixing 7 vol of cold collagen solution with 1 vol of 10 × Eagle's minimal essential medium and 2 vol of sodium bicarbonate (11.76 mg/ml) in a sterile flask kept on ice to prevent immediate gelation. The cold mixture was then dispensed into 35-mm plastic culture dishes (Falcon Plastics, Div. of Bioquest, Oxnard, CA) (~0.8 ml per dish) and allowed to gel for 10 min at 37°C.

Islet Cell Culture: Islet cells were isolated by enzymatic dissociation of neonatal rat pancreases according to procedures established in our laboratory (4, 5) and plated onto 100-mm plastic dishes in Eagle's minimal essential medium supplemented with 10% heat-inactivated fetal calf serum, 400 U/ml sodium penicillin, and 16.7 mM glucose. Since fibroblasts attach to the bottom of the dishes more rapidly than islet cells, after 16 h of incubation at 37°C the culture medium, which showed an increased ratio of islet cells to fibroblasts, was removed and replated in new dishes. Following a second "sedimentation" period of 6 h, the medium was transferred to 35-mm collagen-coated dishes, and the cells were allowed to attach and spread on the surface of the gels for 24 h at 37°C. After removing the culture medium and unattached cells, ~0.8 ml of the cold collagen mixture described above was poured on the top of the first gel and allowed to polymerize for 10 min at 37°C. Fresh medium was added after the collagen had gelled, and was renewed at 48-h intervals. The reorganization of the islet cell monolayers was monitored and photographed with a Zeiss inverted phase contrast photomicroscope. To release the reorganized clusters of endocrine cells from the surrounding collagen matrix, some cultures were incubated for 1 h at 37°C with 0.1% collagenase (CLS, Type I, Worthington Biochemical Corp., Freehold, NJ) in culture medium.

Processing for Light and Electron Microscopy: Following primary fixation in 2.5% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, the collagen gel cultures were either dehydrated in graded ethanols without postfixation in osmium tetroxide (for immunofluorescence) or processed as described (18) for electron microscopy. After EPON embedding, semithin and thin sections were cut either parallel or perpendicular to the culture plane. Thin sections were stained with uranyl acetate and lead citrate, and examined in a

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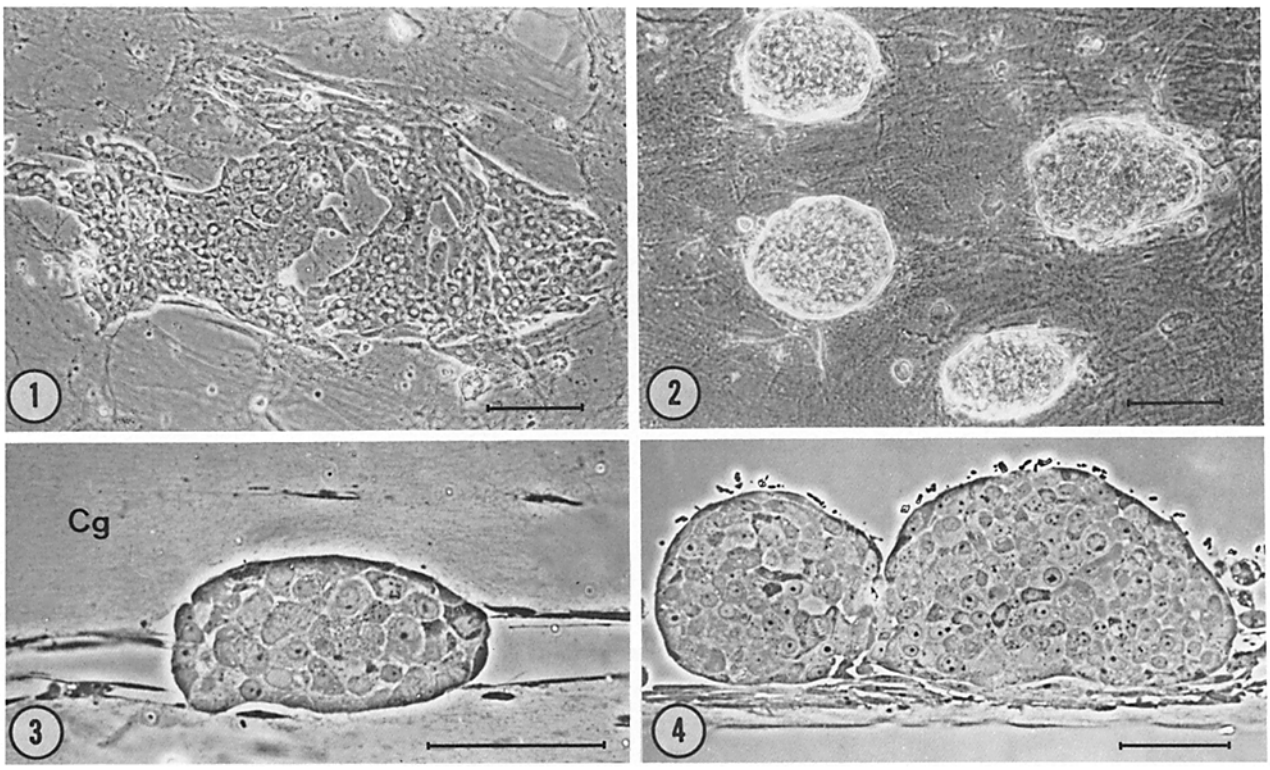
Philips EM 300 or a Zeiss EM 10 electron microscope. For immunofluorescence, serial semithin sections (1 μm) were treated to remove the embedding medium (19) and incubated for 2 h at room temperature in a moist chamber with one of the following antisera: (*a*) guinea pig anti-insulin antiserum (given by Dr. P. H. Wright, Indianapolis, IN), 1:200 dilution; (*b*) rabbit antiglucagon antiserum (given by Dr. A. Onheda, Sendai, Japan), 1:200 dilution; (*c*) rabbit antisomatostatin antiserum (given by Dr. M. P. Dubois, Nouzilly, France), 1:200 dilution; (*d*) rabbit antibovine pancreatic polypeptide antiserum (given by Dr. R. E. Chance, Indianapolis, IN), 1:1000 dilution. After washing in PBS, the sections were further incubated for 1 h with a 1:100 dilution of sheep anti-rabbit or anti-guinea pig IgG antisera labeled with fluorescein isothiocyanate (Pasteur Institut, Paris, France), washed again in PBS, and counterstained with 0.03% Evans blue. After washing in PBS, the sections were mounted in glycerol/PBS and photographed in a Zeiss photomicroscope equipped with epifluorescence optics.

RESULTS

Conventional monolayer cultures of pancreatic islet cells grown in plastic Petri dishes consisted of irregularly-shaped, flattened clusters of endocrine cells, as previously described (4, 5). Similarly, islet cells seeded on the surface of hydrated collagen gels formed flattened clusters (Fig. 1) but when overlaid with a second layer of gelling collagen solution they underwent a dramatic reorganization. The flattened clusters of endocrine cells sandwiched between the two collagen layers gradually changed (usually over a period of 3–8 d) into smooth-contoured, three-dimensional structures, 60 to 250 μm in diameter (Fig. 2). Examination of pictures of the same culture fields taken at daily intervals following collagen overlay showed that the reorganization process involved a progressive retraction and thickening of the endocrine cell clusters. As seen in semithin sections cut perpendicular to the culture plane, the reorganized clusters consisted of multilayered aggregates of cells (Fig. 3), in contrast to the typical monolayers formed either in plastic culture dishes or on the surface of collagen gels. After several days of culture, discontinuities sometimes appeared in the collagen gels, due to the strong tension (cf. 20–22) exerted on collagen fibrils by contaminating fibroblasts. This resulted in the denudation of some islet cell aggregates but, interestingly, these "denuded" aggregates still maintained their three-dimensional organization, even in the absence of a surrounding collagen matrix (Fig. 4).

In thin sections, the aggregates appeared as compact masses of well preserved and well granulated pancreatic endocrine cells (Fig. 5), even after 3 wk in culture. B-cells were usually concentrated in the central portion of the aggregates, while A and D cells were mostly located at their periphery (Fig. 5). This characteristic topographical distribution was confirmed by immunofluorescence studies showing that glucagon-, somatostatin-, and pancreatic polypeptide-containing cells were arranged at the periphery of a central mass of insulin-containing cells (distribution of insulin and glucagon shown in Fig. 6).

The islet cell aggregates could be easily released from the surrounding collagen matrix by incubating the culture with collagenase. The aggregates recovered in this way appeared to maintain their three-dimensional architecture (as seen in semithin and thin sections) as well as the specific pattern of distribution of endocrine cell types (Fig. 6).



FIGURES 1–4 Fig. 1: Pancreatic islet cells grown for 5 d on the surface of a collagen gel. The culture appears as a typical monolayer consisting of irregularly-shaped, flattened clusters of endocrine cells. The clusters are surrounded by fibroblasts. Fig. 2: Pancreatic islet cells embedded in a collagen gel. The cells were first allowed to attach and spread on top of a collagen gel for 24 h to form a monolayer (cf. Fig. 1), then overlaid with a second layer of collagen and grown for an additional 4 d. The reorganized clusters of endocrine cells appear as rounded cell aggregates. Fig. 3: Semithin section perpendicular to the culture plane. Pancreatic islet cells sandwiched between two collagen layers for 8 d have reorganized into a well delimited, multilayered cell aggregate. *Cg*, collagen gel. Fig. 4: Semithin section perpendicular to the culture plane showing two adjacent islet cell aggregates "denuded" of their surrounding collagen matrix (see text). Bars, 100 μm (Figs. 1 and 2); 50 μm (Figs. 3 and 4). × 120 (Figs. 1 and 2); × 460 (Fig. 3); × 280 (Fig. 4).

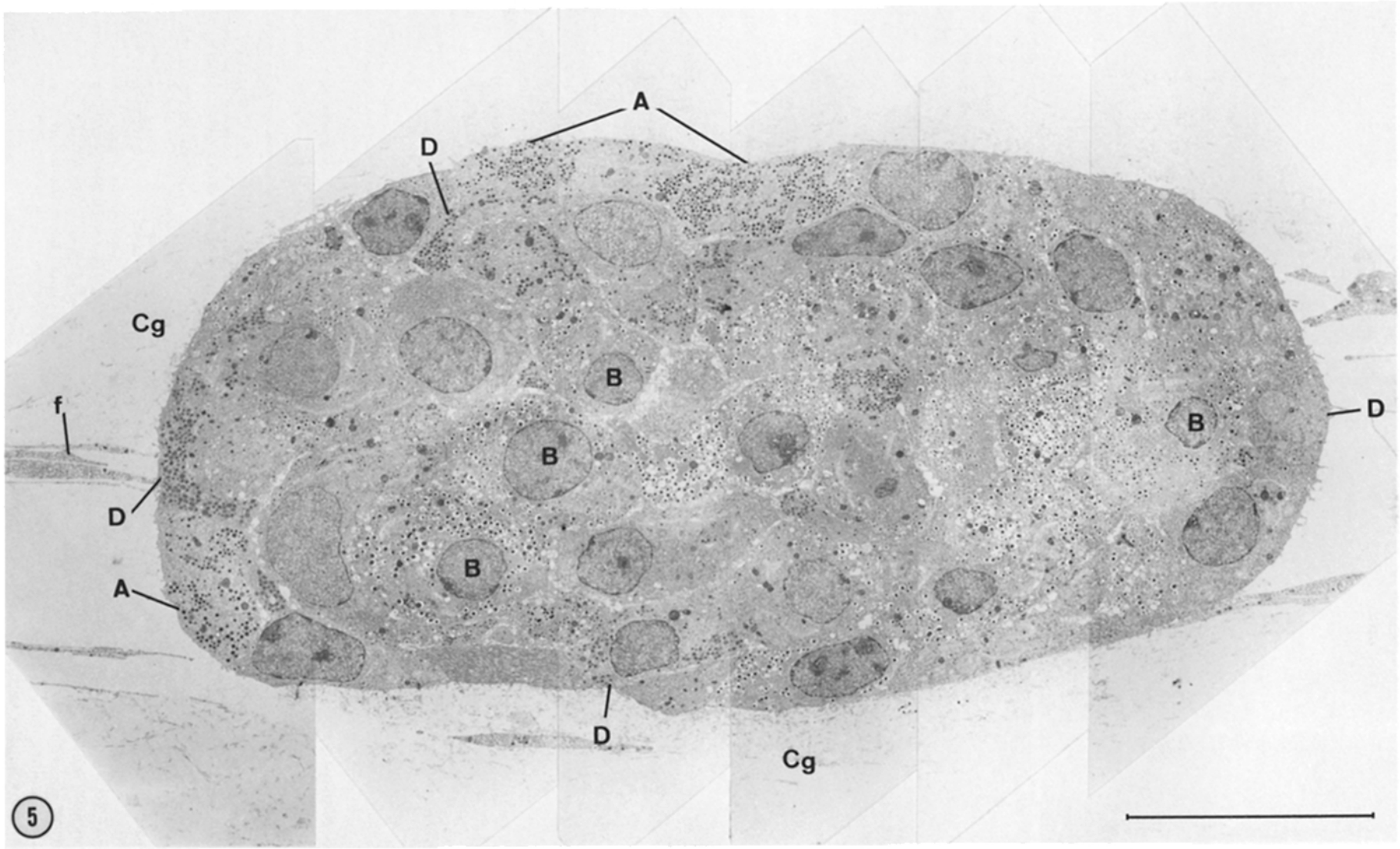


FIGURE 5 Thin section perpendicular to the culture plane showing a three-dimensional islet cell aggregate embedded in a collagen gel. B cells are concentrated in the central portion of the aggregate, whereas A and D cells are in a peripheral position. *B*, B cells; *A*, A cells; *D*, D cells; *Cg*, collagen gel; *f*, fibroblasts. Bar, 20 μm. × 2,100.

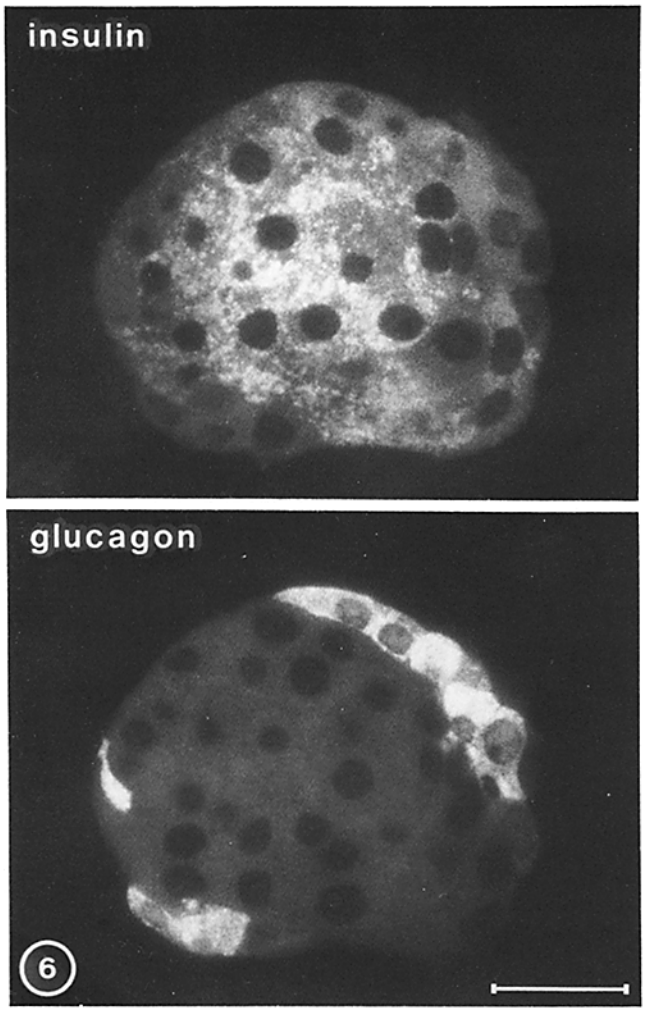


FIGURE 6 Consecutive semithin sections of an islet cell aggregate released from the surrounding collagen matrix by incubation with collagenase. The sections were stained by indirect immunofluorescence with anti-insulin and anti-glucagon antisera to visualize the topographical arrangement of the two endocrine cell types. Insulin-containing cells have a central location (upper panel), while glucagon-containing cells are distributed at the periphery of the aggregate (lower panel). Bar, 20 μm. × 860.

DISCUSSION

There is an increasing number of examples of the permissive effect of collagen matrices on the establishment of tissuelike structures by epithelial cells in culture (10–15). In all cases so far reported, cells have been isolated from cavitary organs and they give rise to cavitary structures when embedded in collagen gels. This is true for primary cultures of normal (11) and malignant (10) mammary gland cells, submandibular gland (14, 15), and thyroid cells (12), as well as established cell lines from dog kidney and mouse mammary gland (13).

Our present results, on the other hand, provide the first example of the in vitro reorganization of cells isolated from a solid, heterocellular tissue. Furthermore, in our system, the four endocrine cell types present in monolayer cultures reassociate to mimic their precise topographical distribution in rat islets of Langerhans in vivo, i.e., a central core of B-cells with the non-B cells in a peripheral position (1).

The factors controlling the ordered distribution of the various cell types is not known, but one possibility is that non-B cells have a greater affinity for interaction with the extracellular matrix compared with the B-cells. Nor is it known whether the formation of the islet-like organoids results solely from a redistribution of pre-existing cells, or whether mitotic activity is involved. In any case, it is likely that the interaction of the collagen gel with the "apical" surface of the monolayer creates new polarity requirements that result in an extensive re-modeling of intercellular relationships in the cell culture.

The existence of a precise distribution of endocrine cells within the islet of Langerhans (1) suggests that this is important for the integrated function of this microorgan (2, 3). It is becoming increasingly clear that the secretory response of B-cells to a glucose challenge requires their interaction with homologous and heterologous islet cell types (23, 24). The present observations could provide additional insight into this requirement, since the secretory behavior of the same endocrine cell population can now be examined both while the cells are in monolayer form and following their reorganization into islet-like organoids by collagen gel overlay. Apart from its use as a new in vitro model for the investigation of problems specific to the endocrine pancreas, this system provides a unique opportunity for the study of factors that contribute to the establishment and maintenance of three-dimensional cellular interrelationships in heterocellular tissues.

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