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IN VITRO CYTODIFFERENTIATION OF PERINATAL RAT ISLET CELLS WITHIN A TRIDIMENSIONAL MATRIX OF COLLAGEN

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SUMMARY

Cell suspensions prepared by collagenase digestion of pancreases obtained from rat fetuses (21.5 d old) and newborns (2.5 d old) were mixed with a collagen solution and inoculated on a collagen base layer. At the onset of the culture, most acinar cells became necrotic, whereas other epithelial cells proliferated. Most of the cell clusters arranged themselves into simple polarized structures composed of epithelial cells forming hollow spheres, and from these budded neoformed endocrine islets. Scarce fibroblasts were located close to these structures. Immunocytochemical localization of insulin and glucagon, as well as ultrastructural characteristics of the cell types revealed an intrainsular distribution similar to the in vivo localization. Tridimensional matrix of collagen offers, to perinatal pancreatic cells in culture, an environment close to the in vivo conditions: cells reorganize themselves in tissuelike structures and cell interactions concerned in the cytodifferentiation of pancreatic islets occur. This system allows for the study of undifferentiated epithelial cells—the presumed stem cells—differentiating and differentiated endocrine cells in the same preparation.

Key words: culture; collagen matrix; islet cells; differentiation.

INTRODUCTION

An intriguing question concerns the probable existence of a pool of precursor cells destined to differentiate into endocrine B-cells in the perinatal pancreas. It is now generally accepted that pancreatic endocrine cells develop from endodermic cells located in the primitive duct epithelium (1-3). The close relation between islet cells and exocrine ducts in the fetal rat pancreas (3-6) as well as in the regenerating adult rat pancreas (7, 8), or in the nesidioblastosis (9-11) corroborate this notion. Precursor cells should have a greater capacity for mitosis than differentiated endocrine cells and should constitute the main proliferative population during pancreatic ontogenesis. During the late fetal life of the rat, there exists a marked discrepancy between islet growth, representing a population doubling time of 48 h, and endocrine cell proliferation, as only 20% of the newly formed B-cells result from the divisions of preexisting B-cells (12). A similar observation was made concerning the regenerating endocrine pancreas of neonatal rat after partial destruction of the B-cells by streptozotocin (13, 14). These studies suggest a rapid formation of islet cells by multiplication and differentiation of committed cells which could be located in the acinar part and the ducts of the pancreas. Nevertheless, only indirect evidence has been offered as to the existence of this cellular pool. More extensive studies of the pool of precursor cells may provide information on the possible causes of B-cell inadequate formation later in life (15).

A limited number of in vitro experiments on fetal rat pancreas have produced additional data about the origin of islet cells. Based on morphologic criteria, some authors have observed the endocrine differentiation from pancreatic epithelial cells in culture (2, 16-19). However, no in vitro model, other than the complex organ culture originating from massive explants (20-22), can mimic the in vivo ontogenesis of islet cells.

In the present study, we describe the morphologic aspects related to the culture of rat pancreatic cell suspension composed of isolated cells and small aggregates within a tridimensional matrix of collagen. This culture provides an environment closer to the in vivo architecture, thereby allowing specific cell interactions to occur.

MATERIALS AND METHODS

Animals. The gestation period in our strain of Wistar rats lasted for 22 to 22.5 d. Fetuses 21.5 d old were collected from primiparous rats. Newborn rats were taken 2.5 d after delivery.

Tissue culture. The technique results from a combination of the culture method modified after Hellerström et al. (23) along with the preparation of collagen gel (24).

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The entire procedure, including the digestion of pancreatic tissue, was carried out in RPMI 1640 medium (Flow Laboratories, Irvine, Scotland) containing 10% heat-inactivated fetal bovine serum (GIBCO, Grand Island, NY) and antibiotics (penicillin: 200 U/ml, streptomycin: 0.2 mg/ml). The pancreases were removed aseptically, pooled by groups of 15, and placed in 2 ml of medium (4° C). They were then minced into small pieces and placed in 2 ml of medium containing 6 mg of collagenase (Boehringer, Mannheim, FRG) for fetal pancreases or 10 mg for newborn pancreases.

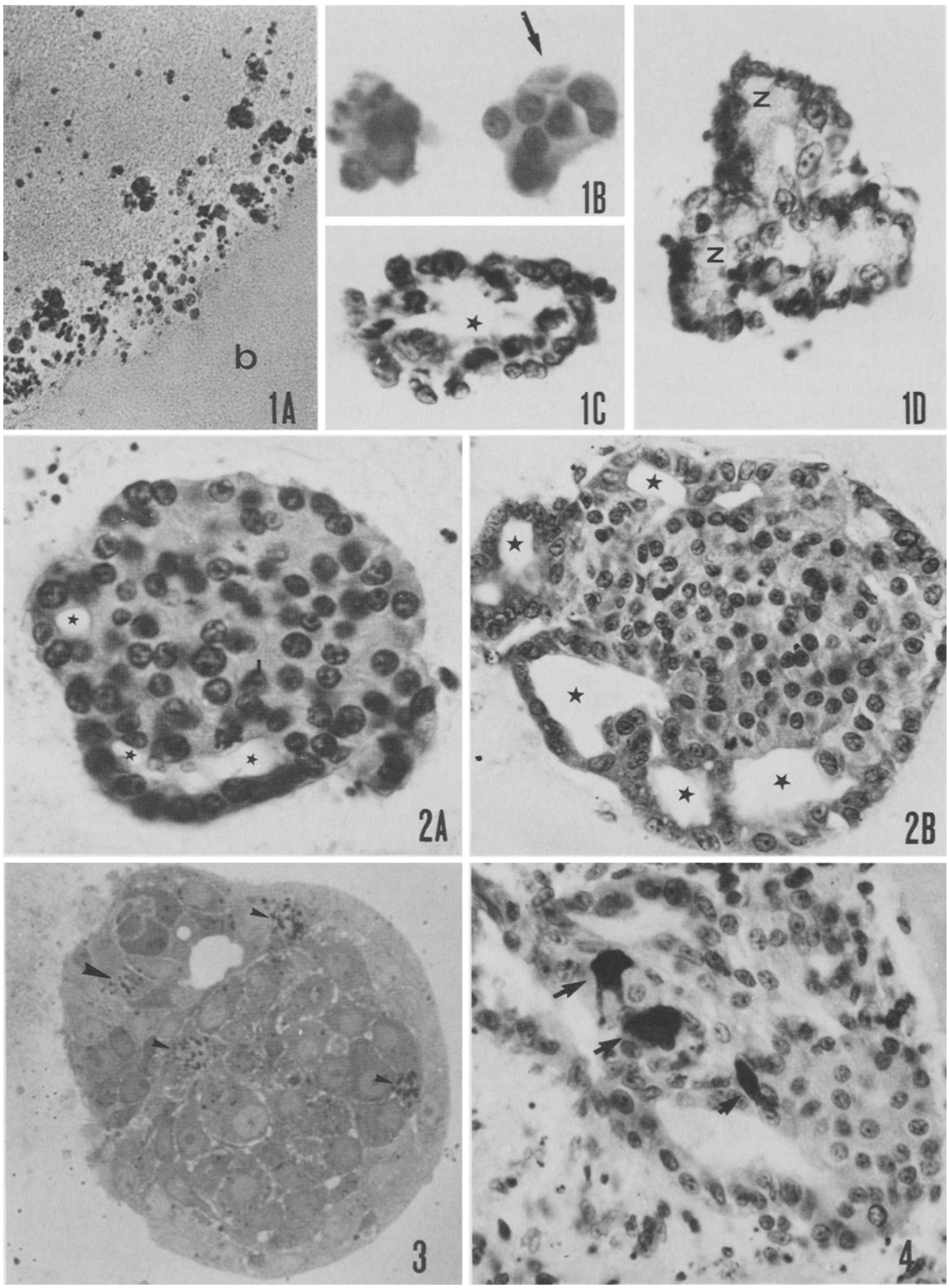

TABLE 1

Culture Period	1 h	1 d	4 d	7 d
Mean number of cells (± SEM)	5.5 ± 0.8	11.1 ± 1.3	28.8 ± 2.2	47.5 ± 5.1
Endocrine cells proportion (%)	15.2	28	41.8	41.6
n	60	60	100	64

Mean number of cells and proportion of the endocrine cells in sections of randomly selected clusters fixed at various times after the inoculation. Isolated cells were not taken into account in these computations.

The tubes were vigorously hand shaken at 37° C for 6 to 8 min, and the tissue was then washed three times with 5 ml of cold medium. The pellet was resuspended in 40 ml of medium, gently stirred for 60 min at room temperature to increase the cell dissociation, and then centrifuged.

The collagen gel was prepared by mixing two solutions. The A solution was composed of 1 vol of 10× concentrated RPMI, 1 vol of fetal bovine serum and antibiotics, and 1 vol of a mixture of NaOH (0.1 *M*) in $NaHCO_3$ buffer (0.25 *M*). The B solution was obtained by diluting 6 vol of purified collagen for tissue culture (Vitrogen 100, Flow Laboratories, Irvine, Scotland) with 1 vol of HCl 0.013 *M*. A base layer was poured into each 60-mm petri dish (Falcon 3006, Falcon Plastics, Los Angeles, CA) (1.5 ml/dish) and incubated for 10 to 15 min at 37° C until it gelled.

The cells were mixed with another aliquot of collagen gel solution (1 ml/fetal or 4 ml/newborn pancreas) and inoculated over the base layer (2.5 ml/dish). Gelation was achieved on warming at 37° C. Culture dishes were incubated in a humidified atmosphere (5% CO_2 in air) at 37° C. The growth medium was changed daily after the 2nd d up to 7 d.

Microscopy. Dishes were sampled and fixed in Bouin's solution at different times of culture. The collagen gel containing the tissue was embedded in paraffin, serially sectioned at a thickness of 7 μm, and stained according to Masson's trichrome method or with indirect peroxidase-antiperoxidase immunodetection (25) of insulin and glucagon.

Other dishes were fixed with 2.5% glutaraldehyde, postfixed in 1% O_sO_4 and processed for electron microscopy (26).

Autoradiography. On the 5th d of culture, 1 μCi/ml tritiated thymidine (deoxyribose-6[^{3}H]thymidine; specific activity: 7.3 to 7.6 Ci/mmol, Radiochemical Center Ltd, Amersham, UK) was added to the medium. After 24 h of incubation, the dishes were washed with Earle's solution containing 50 μg/ml cold thymidine, fixed in Bouin's solution and processed as above for light microscopy, or fixed in glutaraldehyde and Epon embedded. The paraffin or semithin sections were covered with a nuclear tract emulsion (Ilford K5). The slides were exposed for 1 wk, then developed and stained.

RESULTS

Cultures were fixed daily from the initiation of the experiment up to the 7th d and processed for light and electron microscopy. One hour after inoculation, isolated cells and small cell aggregates were dispersed within the collagen matrix. Five hundred of these structures were counted on randomly selected fields of light microscopy sections: 96.7% were composed of less than 6 cells, 3.1% contained 6 to 19 cells, and only 0.2% amounted to 20 cells or more. The cell aggregates consisted of incompletely dissociated islets, fragments of pancreatic ducts, clusters of acinar cells, and other heterogeneous structures (Fig. 1 *A-D*). During the first 2 d of culture, most acinar cells disintegrated after intensive vacuolization. Some may have lost their zymogen granules and taken on the appearance of ductlike cells. The remaining epithelial cells segregated from fibroblasts. More interestingly, numerous polarized structures appeared during the first 4 d of culture: epithelial (presumably duct) cells formed hollow spheroids from which budded endocrine clusters identified as neoformed islets (Fig. 2 *A*). The fibroblasts embedded within the collagen matrix remained few in number, most of them being preferentially located around epithelio-endocrine structures. On the 7th d of culture, the same cell arrangements were observed. However, the lumen of the epithelial spheres was enlarged, as were the endocrine islets (Fig. 2 *B*).

Cells were counted in randomly sampled epithelio-endocrine clusters (one section/cluster) after 1 h and 1, 4,

FIG. 1. *A-D*, pancreatic cells dispersed with collagenase were fixed 1 h after inoculation within collagen gel. Newborn rat, paraffin sections, Masson's trichrome. *A*, low power light micrograph of isolated cells and cell clusters in the collagen gel; B = base layer. ×190. *B*, incompletely dissociated islet (*arrow*). ×862. *C*, fragment of epithelial duct; * = lumen. ×862. *D*, clusters of acinar cells; *z* = zymogen granules in apical cytoplasm. ×733.

FIG. 2. *A*, *B*, epithelio-endocrine polarized structures within the collagen gel. Paraffin sections, Masson's trichrome. *A*, differentiated endocrine cells budded from epithelial layer (*). Fourth day of culture. Fetus. × 783. *B*, the lumina of ducts (*) were enlarged, presumably due to some secretion activity of the epithelial cells. Seventh day of culture. Newborn rat. ×450.

FIG. 3. Thymidine incorporation in S-phase nuclei of epithelio-endocrine structures during a 24-h period on the 5th d of culture revealed an autoradiographic labeling of epithelial (*large arrow*) and endocrine cells (*small arrows*), suggesting cell proliferation. Fetal rat, semithin section. ×817.

FIG. 4. Glucagon immunodetection on paraffin sections showed scattered glucagon cells (*arrows*) mainly located in the transitional zone between islet and epithelial drop. These cells frequently featured cell processes. Fourth day of culture. Newborn rat. ×450.

and 7 d of culture (Table 1). The results clearly demonstrated the growth of the clusters. The endocrine cells, identified on the sections by their rounded nucleus and granule content, markedly increased in proportion up to the 4th d.

Mitoses were identified in epithelial duct cells and to a lesser extent in budded islets. Thymidine incorporation during a 24-h period on the 5th d of culture revealed an autoradiographic labeling in both types of cells (Fig. 3). In the RPMI medium containing 200 m*M* glucose and 10% fetal bovine serum, the labeling index reached 10.4% (n = 1000 cells) for the endocrine cells. In other experimental series performed in a modified Eagle's medium containing 50 m*M* glucose and 1% fetal bovine serum, the labeling indexes for the endocrine and epithelial cells were 3.5% (n = 4500) and 6% (n = 4500), respectively.

Insulin and glucagon were detected by immunohistochemistry on the 4th and 7th d of culture. Scattered insulin and glucagon cells were inserted between epithelial ductlike cells. In budding endocrine islets, insulin cells comprised the bulk of the volume, whereas glucagon cells were mainly located in the transitional zone between the islet and epithelial sphere or in the periphery of islets (Fig. 4). Glucagon cells frequently featured cell processes.

By means of electron microscopy, the majority of cells surrounding the lumen of the epithelial sphere were observed as being undifferentiated. The light cytoplasm contained numerous ribosomes, scarce rough endoplasmic reticulum and Golgi, small mitochondria, lipid droplets, and prominent cytoskeleton. The plasma membrane facing the lumen bore short microvilli, and the lateral faces of neighboring cells were closed by apical junctional complexes (Fig. 5). In spite of the absence of cytologic evidence, the enlargement of the lumen with the duration of culture was probably due to some fluid secretion.

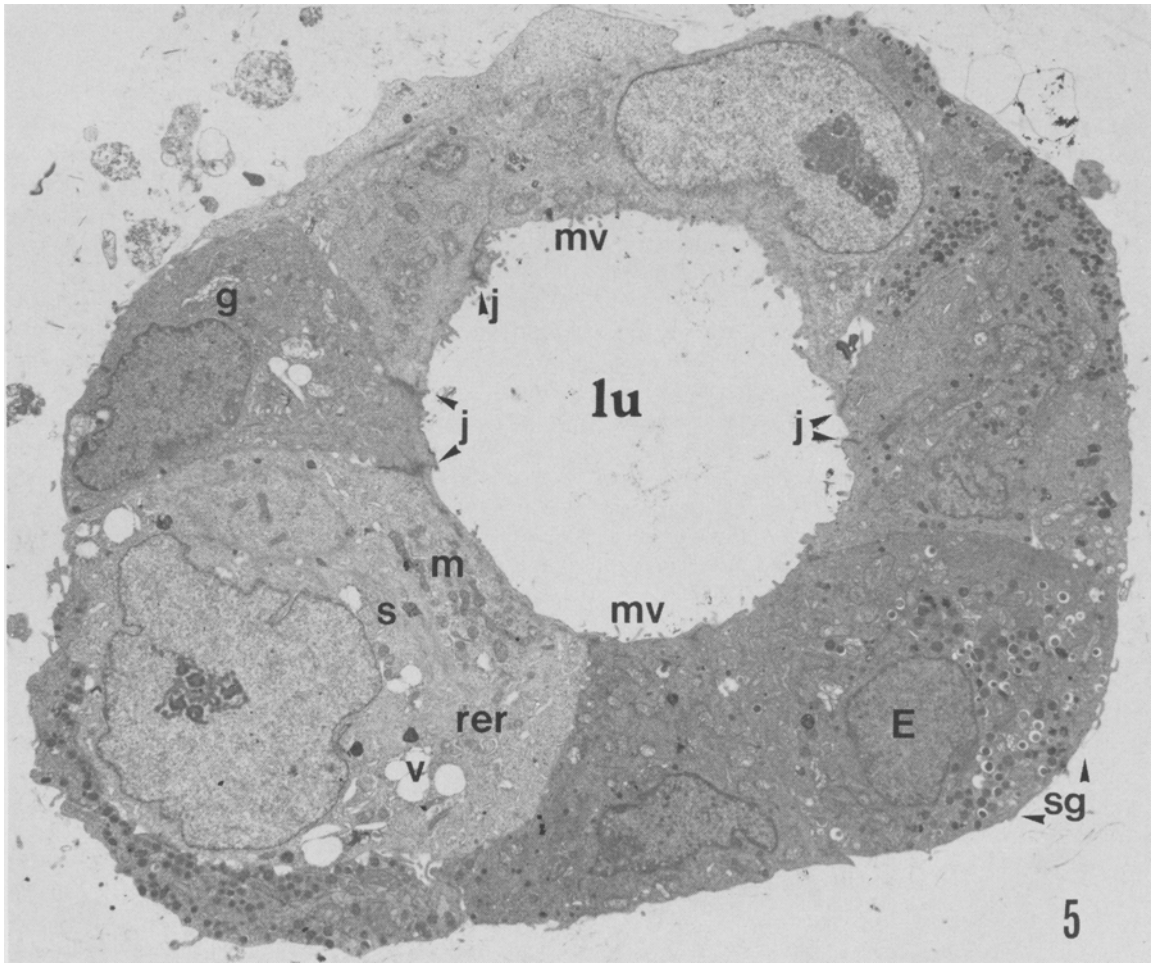

FIG. 5. Electron micrograph of an epithelio-endocrine structure on the 4th d of culture. Surrounding the lumen (*lu*) of the duct fragment, most epithelial cells did not feature differentiated characters; their light cytoplasm contained numerous ribosomes, scarce rough endoplasmic reticulum (*rer*) and Golgi (*g*), small mitochondria (*m*), vacuoles (*v*), and prominent cytoskeleton (*s*); *mv* = microvilli, *j* = junctional complexes. Some cells were engaged in endocrine cytodifferentiation (*E*) revealed by the presence of secretory granules (*sg*). Fetal rat. ×4172.

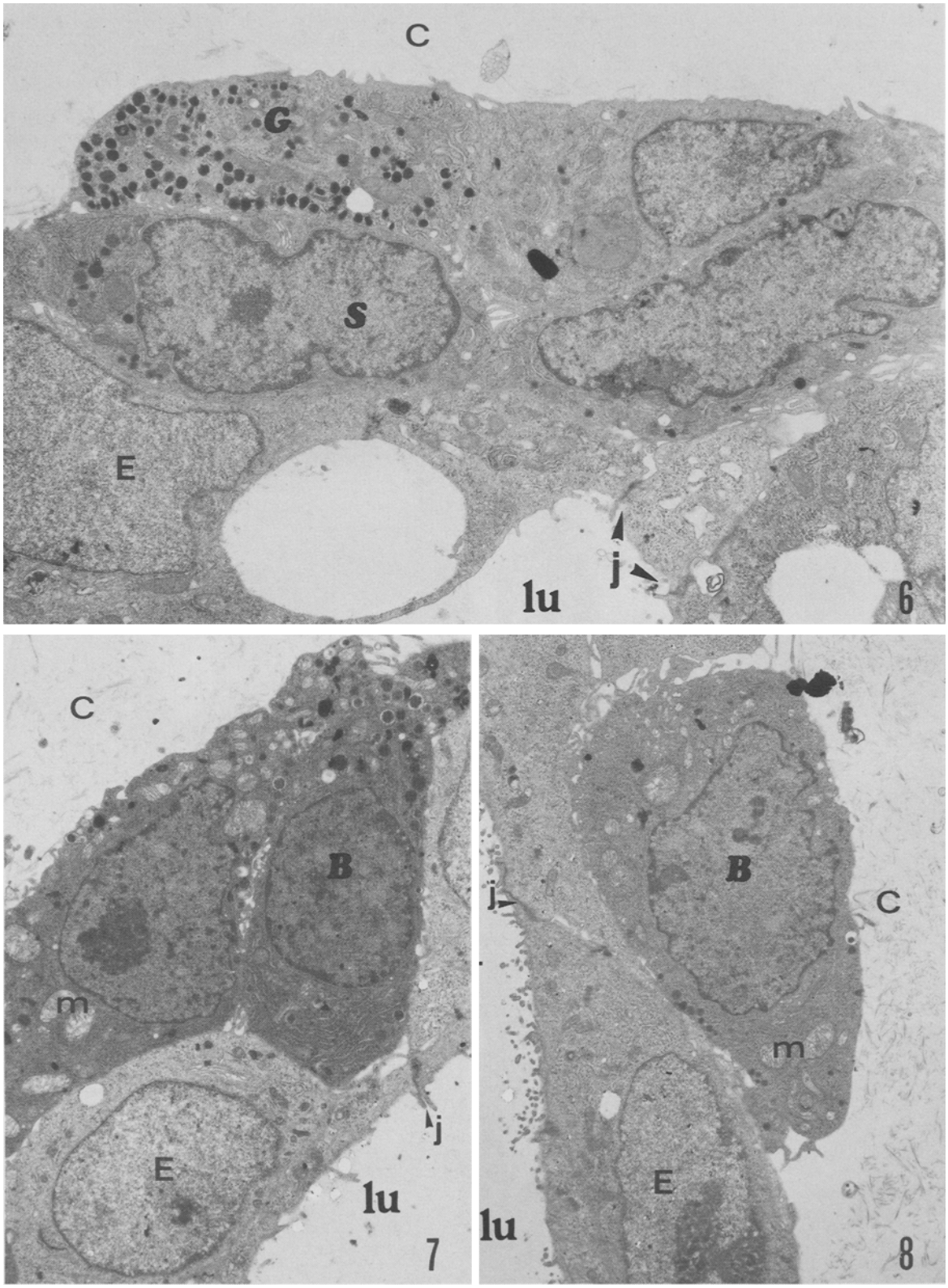

FIG. 6–8. Electron micrographs of endocrine cells close to the duct epithelium. FIG. 6. Presumable glucagon (*G*) and somatostatin (*S*) cells. Fetal rat, 4th d of culture. ×7699. FIG. 7, 8. Presumable B-cells (*B*), containing few secretory granules and unusual large mitochondria (*m*). Fetal rat, 6th d of culture. ×8850. Other legends: *lu* = lumen, *E* = epithelial cells; *j* = junctional complexes; *C* = collagen gel.

The endocrine cells inserted within the epithelial layer were generally separated from the lumen by cytoplasmic sheets of undifferentiated cells, but in some cases their apical plasma membrane was in contact with the lumen. The secretory granules of differentiating endocrine cells close to the ducts were of various known types: characteristic insulin, glucagon, somatostatin granules, and others remaining unidentifiable by the morphologic criteria (Fig. 6). Granules were often few in the latter cells (Fig. 7 and 8). Another feature was the presence of mitochondria of the exocrine type, i.e. large and showing numerous internal cristae (Fig. 7 and 8). Clusters of more differentiated cells containing an increased number of secretory granules and detached from the duct were observed (Fig. 9).

In the islet sprouting off the epithelial layers, electron microscopy revealed the absence of nonendocrine cells and confirmed the peripheral location of the non-B-cells. The penetration of cell processes originating from glucagon and somatostatin cells was also observed.

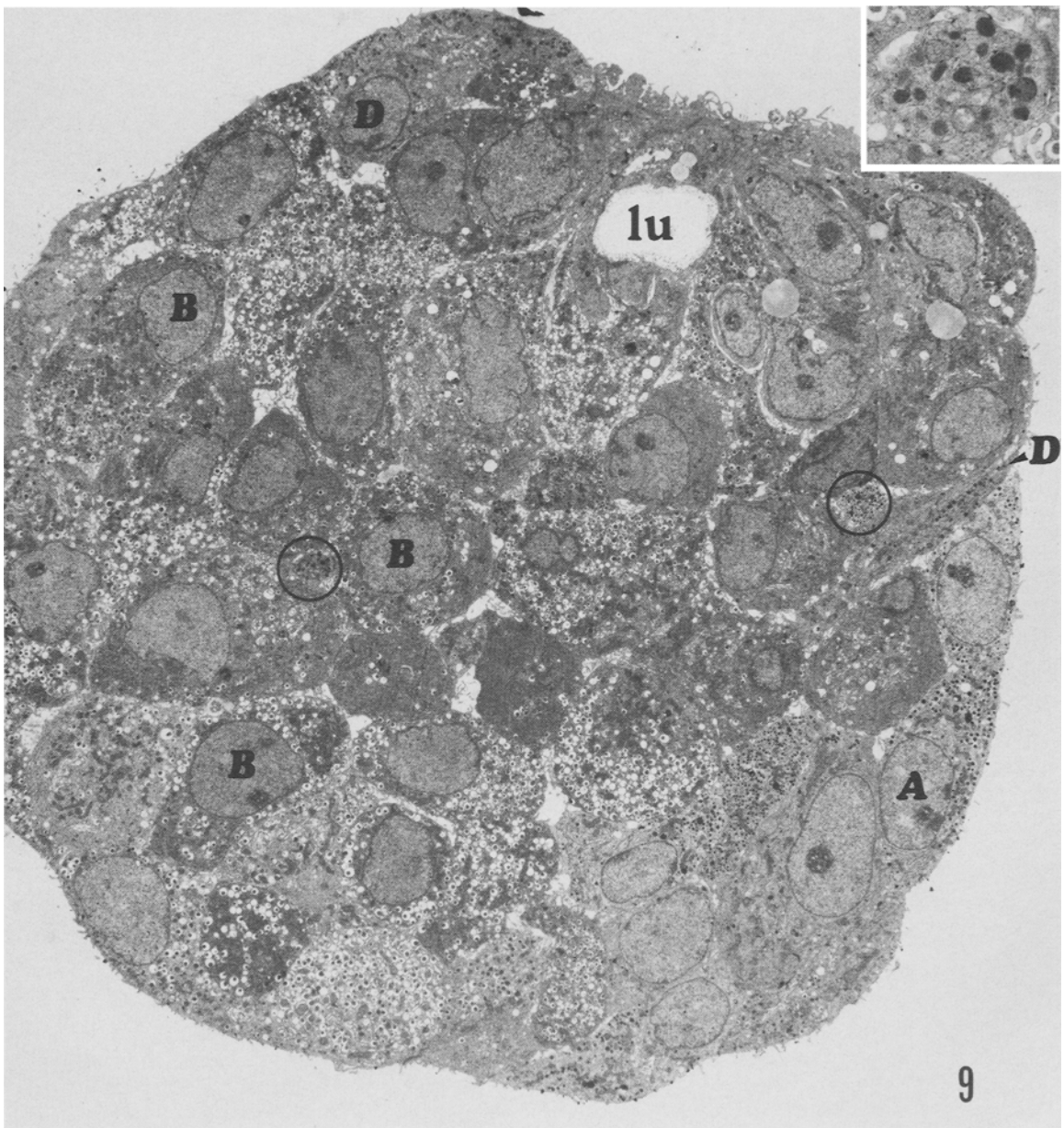

FIG. 9. Close to a large budding islet, the duct lumen (*lu*) is surrounded by cytoplasmic sheets of undifferentiated epithelial cells. Insulin (*B*) cells comprise the bulk of endocrine mass and the non-B-cells are distributed in the periphery. Some of their cell processes (*circles*) are directed toward the center. *A* = glucagon cells; *D* = somatostatin cells. 7th d of culture. Newborn rat. ×3103. *Inset:* non-B-cell process. ×13 793.

Insulin-secreting cells forming the major part of the islets were generally well granulated and displayed the cytologic characteristics of functioning B-cells (Fig. 9).

These descriptions apply to neonatal as well as fetal material.

Discussion

Perinatal pancreatic cell cultures on bidimensional support produce endocrine monolayers (19, 27, 28) or neoformed islets (16, 23, 29, 30) where observations of epithelio-endocrine affiliation have been only occasional. Dudek et al. (16) described the budding of islets from heterogeneous structures and noted a continuity between endocrine and epithelial duct cells. However, they did not provide ultrastructural description. Except for the complex organ culture originating from massive explants, no in vitro system exists that enables us to observe the differentiation of the pool of committed precursors into endocrine cells. As a consequence, few in vitro studies have dealt with the genesis of Langerhans islets.

From pancreatic cell suspensions prepared according to the method of Hellerström et al. (23) and cultured on bidimensional support, the endocrine cells first arrange themselves in monolayers, proliferate, and then reorganize into neoformed islets under the mechanical influence of fibroblasts, as was previously described (29, 30). The same preparation technique was used in the present study, except that the cells were inoculated in a collagen gel. The differentiated exocrine cells spontaneously disappeared by the 2nd to 3rd d after the inoculation. This was also the case on a bidimensional support (29, 30). By contrast, in the collagen environment, the behavior of other epithelial cells and fibroblasts was quite different from that observed in bidimensional cultures. The proliferation of fibroblasts was low, as was already observed with other tissues in tridimensional collagen matrix (24, 31). The endocrine and ductlike epithelial cells formed growing polarized structures recalling their in vivo ontogenic relationships, where the endocrine cells originate from duct cells and accumulate under the epithelium after rotation of their mitotic axis (1).

Contrasting to the present results, no endocrine islets budded off from adult pancreatic ducts cultured within an agarose matrix (32). It should be noted however that in collagen, as in agarose, the fragments of ducts sealed their cut ends and progressively enlarged due to fluid secretion into the lumen. The kind of secretion could not be identified, but was probably due to the specific secretory properties of the pancreatic duct cells. In another study (33), neonatal rat pancreatic cells overlaid with collagen also resulted in a rearrangement of epithelial cells into tridimensional organoid structures with typical lumen, but no appearance of endocrine cells was mentioned.

Preservation of or reorganization into tridimensional tissuelike architecture (a.o. by inoculation into a collagen gel) has been shown to promote or maintain differentiation in several cultured cell types, such as liver cells (34, 35), mammary epithelial cells (36–38), submandibular gland cells (39), thyroid cells (40,41), seminal vesicle cells (42), and corneal epithelium (43). Among the various factors that could favor the outgrowth of endocrine islets in the present culture system, the promotion of epithelial polarization and the plasticity of the collagen gel may be suggested. The contact of epithelium on collagen would maintain the cuboidal form of the cells and stabilize the basal pole, simultaneously destabilizing the apical pole (40,41,43) probably with movements of membrane receptors (35). On the other hand, the plasticity of collagen gel would permit the cells to change shape and to aggregate (37), thereby allowing endocrine islets to escape.

An extracellular matrix of collagen is known to increase the proliferation of epithelial cells (38,44–47). As far as islet cells are concerned, a bidimensional support of collagen favors the growth of the monolayers (28,29). However, when islets became spherical the presence of the collagen surrounding them did not seem to enhance their proliferative capacity, inasmuch as the labeling indexes of the endocrine cells after thymidine incorporation were roughly the same in the present study (10.4% in stimulating RPMI and 3.5% in nonstimulating MEM medium) and in islets neoformed on bidimensional support (12.4 and 3.5% in the two media, respectively) (48 and unpublished data).

Epithelio-mesenchymal interactions have been repeatedly evidenced during morphogenesis and cell differentiation (49–53). Insulin cells differentiate in organ culture from the pancreatic duct epithelium under the probable influence of mesenchymal factors (3). In the present system, fibroblasts surrounding the epithelial structures could meet the mesenchymal requirement, producing factors that could then reach the membrane of precursor epithelial cells and promote their differentiation.

In our cultures, differentiating insulin and glucagon cells were inserted between duct cells, and some of them were in contact with the lumen. Pictet and Rutter (1) also observed a few endocrine cells facing the lumen during rat pancreatic ontogenesis in vivo. This suggests that escape from the epithelial layer need not be a prerequisite for the cytodifferentiation of the endocrine cells. The secretory granule formation could occur simultaneously with and not exclusively subsequent to the liberation from junctional complexes. In collagen gel, the endocrine cells of the transitional zone between the duct and the islet usually displayed incomplete cytodifferentiation. Morever, they occasionally revealed the characteristic mitochondria of acinar cells. In the budding islets, non-B-cells tended to aggregate peripherally as in adult rat islets. The same topographic distribution was found in isletlike organoids obtained from monolayers grown on collagen and overlaid with a second layer of collagen (54), as was observed in islets neoformed after pancreatic cell cultures on bidimensional support (29,30). Cytoplasmic processes of non-B-cells were observed between the central B-cells in our cultures. Such processes were frequently exhibited by the somatostatin cells in adult human islets (55) and were related to their functional role as endocrine and paracrine unit.

In conclusion, the tridimensional matrix of collagen offers perinatal pancreatic cells in culture an environment conducive to cellular reorganization in tissuelike structures, which generally does not appear in monolayer culture. In this system, endocrine cytodifferentiation occurs in vitro, which allows for undifferentiated epithelial cells—the presumed stem cells—and differentiating and differentiated endocrine cells to be studied together.

References

1. Pictet, R.; Rutter, W. J. Development of the embryonic endocrine pancreas. In: Steiner, D. F.; Freinkel, N., eds. Handbook of physiology, sect. 7, vol. 1. Washington, DC:American Physiological Society; 1972:25-66.
2. Pictet, R. L.; Rall, L.; de Gasparo, M., et al. Regulation of differentiation of endocrine cells during pancreatic development in vitro. In: Camerini-Davalos, R. A.; Cole, H. S., eds. Early diabetes in early life. New York:Academic Press; 1975:25-39.
3. Rutter, W. J.; Pictet, R. L.; Harding, J. D., et al. An analysis of pancreatic development: role of mesenchymal factor and other extracellular factors. Symp. Soc. Dev. Biol. 35:205-227; 1978.
4. Fujii, S. Development of pancreatic endocrine cells in the rat fetus. Arch. Histol. Jpn. 42:467-479; 1979.
5. Sundler, F.; Alumets, J.; Hakansson, R. The endocrine pancreas. Morphology, ontogeny and hormone production. In: Oberg, K.; Boström, H., eds. Endocrine gastrointestinal tumours. Diagnosis and management. Upsala J. Med. Sci. Supplement 39; 1984:33-46.
6. Yoshinari, M.; Daikoku, S. Ontogenic appearence of immunoreactive endocrine cells in rat pancreatic islets. Anat. Embryol. 165:63-70; 1982.
7. Hultquist, G. T.; Karlsson, U.; Hallner, A. Ch. The regenerative capacity of the pancreas in duct-ligated rats. Exp. Pathol. 17:44-52; 1979.
8. Weaver, C. V.; Sorenson, R. L.; Kaung, H. C. Immunocytochemical localization of insulin-immunoreactive cells in the pancreatic ducts of rats treated with trypsin inhibitor. Diabetologia 28:781-785; 1985.
9. Barresi, G.; Tuccari, G. B-cell nesidioblastosis in the overweight newborn. Basic Appl. Histochem. 26:263-270; 1982.
10. Dutrillaux, M. C.; Hollande, E.; Rozé, C. Cytodifferentiation of exocrine cells in human nesidioblastosis. Biol. Cell. 38:65-70; 1980.
11. Pour, P. Experimental pancreatic ductal (ductular) tumors. In: Fitzgerald, P. J.; Morrison, A. B., eds. The pancreas. Baltimore, MD:Williams & Wilkins; 1980:111-139.
12. Swenne, I.; Eriksson, U. Diabetes in pregnancy: islet cell proliferation in the fetal rat pancreas. Diabetologia 23:525-528; 1982.
13. Bonner-Weir, S.; Trent, D. F.; Honey, R. N., et al. Response of neonatal rat islets to streptozotocin. Limited B-cell regeneration and hyperglycemia. Diabetes 30:64-69; 1981.
14. Dutrillaux, M. C.; Portha, B.; Rozé, C., et al. Ultrastructural study of pancreatic B cell regeneration in newborn rats after destruction by streptozotocin. Virchows Arch. B Cell. Pathol. 39:173-175; 1982.
15. Hellerström, C. The life story of the B cell. Diabetologia 26:393-400; 1984.
16. Dudek, R. W.; Freinkel, N.; Lewis, N. J., et al. Morphologic study of cultured pancreatic fetal islets during maturation of the insulin stimulus-secretion mechanism. Diabetes 29:15-21; 1980.
17. Hollande, E.; Giron, B.; Lehy, T., et al. In vitro secretion of gastrin, insulin and glucagon in tissue cultures of pancreas from a child with neonatal intractable hypoglycemia. Gastroenterology 71:255-262; 1976.
18. Popiela, H.; Moore, W. Islet neoformation in tissue culture. 68th annual meeting of endocrine society. Anaheim, CA:Endocrine Society; 1986:110.
19. Wallace, D. H.; Hegre, O. D. Development in vitro of epithelial cell monolayers derived from fetal rat pancreas. In Vitro 15:270-277; 1979.
20. Lazarow, A.; Wells, L. J.; Carpenter, A. M., et al. Islet differentiation, organ culture, and transplantation. Diabetes 22:877-912; 1973.
21. Schweisthal, M. R.; Céas, M. P.; Wells, L. J. Development of the pancreas of the rat embryo in vitro: islets and acini. Anat. Rec. 147:149-161; 1963.
22. Vecchio, D.; Gonet, A. E. Culture d'organe de pancreas foetal de rat: I. Effets du glucose, d'autres composants du milieu de culture, et d'un sulfamide hypoglycemiant. Helv. Physiol. Acta 25:103-122; 1967.
23. Hellerström, C.; Lewis, N. J.; Borg, H., et al. Method for large-scale isolation of pancreatic islets by tissue culture of fetal rat pancreas. Diabetes 28:769-776; 1979.
24. Elsdale, T.; Bard, J. Collagen substrata for studies on cell behavior. J. Cell. Biol. 54:626-637; 1972.
25. Sternberger, L. Immunocytochemistry. Englewood Cliffs, NJ:Prentice Hall; 1974.
26. Remacle, C.; De Clercq, L.; Delaère, P., et al. Organ culture of the islets of Langerhans from young and senescent rats. Cell Tissue Res. 207:429-448; 1980.
27. Orci, L.; Like A. A.; Amherdt, M., et al. Monolayer cell culture of neonatal rat pancreas: An ultrastructural and biochemical study of functioning endocrine cells. J. Ultrastruct. Res. 43:270-297; 1973.
28. Thivolet, C. H.; Chatelain, P.; Nicoloso, H., et al. Morphological and functional effects of extracellular matrix on pancreatic islet cell cultures. Exp. Cell Res. 159:313-322; 1985.
29. Masquelier, D.; Amory, B.; Mourmeaux, J. L., et al. Cell interactions during the in vitro neoformation of fetal rat pancreatic islets. Cell Differ 18:199-211; 1986.
30. Mourmeaux, J. L.; Remacle, C.; Henquin, J. C. Morphological and functional characteristics of islets neoformed during tissue culture of fetal rat pancreas. Mol. Cell. Endocr 39:237-246; 1985.
31. Sarber, R.; Hull, B.; Merril, C., et al. Regulation of proliferation of fibroblasts of low and high population doubling levels grown in collagen lattices. Mech. Ageing Dev. 17:107-117; 1981.
32. Githens, S., III; Holmquist, D. R. G.; Whelan, J. F., et al. Morphologic and biochemical characteristics of isolated and cultured pancreatic ducts. Cancer 47:1505-1512; 1981.
33. Chen, J.; Stuckey, E. C.; Berry, C. L. Three-dimensional culture of rat exocrine pancreatic cells using collagen gels. Br. J. Exp. Pathol. 66:551-559; 1985.
34. Guguen-Guillouzo, C.; Seignoux, D.; Courtois, Y., et al. Amplified phenotypic changes in adult rat and baboon hepatocytes cultured on a complex biomatrix. Biol. Cell. 46:11-20; 1982.
35. Rubin, K.; Höök, M.; Obrink, B., et al. Substrate adhesion of rat hepatocytes: mechanism of attachment to collagen substrates. Cell 24:463-470; 1981.
36. Foster, C. S.; Smith, C. A.; Dinsdale, E. A., et al. Human mammary gland morphogenesis in vitro: the growth and differentiation of normal breast epithelium in collagen gel cultures defined by electron microscopy. Monoclonal antibodies and autoradiography. Dev. Biol. 96:197-216; 1983.
37. Shannon, J. M.; Pitelka, D. R. Influence of cell shape on the induction of functional differentiation in mouse mammary cells in vitro. In Vitro 17:1016-1028; 1981.
38. Yank, J.; Larson, L.; Flynn, D., et al. Serum-free primary culture of human normal mammary epithelial cells in collagen gel matrix. Cell Biol. Int. Rep. 6:969-975; 1982.
39. Yang, J.; Flynn, D.; Larson, L., et al. Growth in primary culture of mouse submandibular epithelial cells embedded in collagen gels. In Vitro 18:435-442; 1982.
40. Chambard, M.; Gabrion, J.; Mauchamp, J. Réorganisation en culture des cellules folliculaires thyröidiennes. Influence du collagène sur l'orientation de la polarité cellulaire. C. R. Hebd. Acad. Sci. Ser. D 1:79-81; 1980.
41. Garbi, C.; Nitsch, L.; Wollman, S. H. Embedding in a collagen gel stabilizes the polarity of epithelial cells in thyroid follicles in suspension culture. Exp. Cell Res. 151:458-465; 1984.

42. Tomooka, Y.; Harris, S. E.; Mc Lachlan, L. A. Growth of seminal vesicle epithelial cells in serum-free collagen gel culture. In Vitro 21:237-244; 1985.
43. Hay, E. D. Interaction of embryonic cell surface and cytoskeleton with extracellular matrix. Am. J. Anat. 165:1-12; 1982.
44. Gospodarowicz, D.; Delgado, D.; Vlodavsky, I. Permissive effect of the extracellular matrix on cell proliferation in vitro. Proc. Natl. Acad. Sci. USA 77:4094-4098; 1980.
45. Richards, J.; Pasco, D.; Yang, J., et al. Comparison of the growth of normal and neoplastic mouse mammary cells on plastic, on collagen gels and in collagen gels. Exp. Cell Res. 146:1-14; 1983.
46. Wessels, N. K. Tissue interaction and development. Menlo Park, CA: Benjamin Cummings Publishing; 1977:212-229.
47. Wicha, M. S.; Lowrie, G.; Kohn, E., et al. Extracellular matrix promotes mammary epithelial growth and differentiation in vitro. Proc. Natl. Acad. Sci. USA 79:3213-3217; 1982.
48. Reusens-Billen, B.; Remacle, C.; Daniline, J., et al. Cell proliferation in pancreatic islets of rat fetuses and neonates from normal and diabetic mothers. An in vitro and in vivo study. Horm. Metab. Res. 16:565-571; 1984.
49. Cunha, G. R.; Bigsby. R. M.; Cooke, P. S., et al. Stromal-epithelial interactions in adult organs. Cell Diff. 17:137-148; 1985.
50. Haffen, K.; Kedinger, M.; Simon-Assman, P. M., et al. Mesenchyme-dependent differentiation of intestinal brush-border enzymes in the gizzard endoderm of the chick embryo. In: Bruger, M. M.; Weber, R., eds. Embryonic development part B: cellular aspects, vol. 85B. New York:Alan R. Liss; 1982:261-270.
51. Kusakabe, M.; Sakakura, T.; Sano, M., et al. Early development of mouse anterior pituitary: role of mesenchyme. Dev. Growth Differ. 26:263-271; 1984.
52. Schechter, J.; Gash, D.; Ahmad, N. Mesenchymal influences on the development of the adenohypophysis in the rat. Cell Tissue Res. 241:67-76; 1985.
53. Slavkin, H. C. Combinatorial process for extracellular matrix influences on gene expression: a hypothesis. J. Cranofacial Genet. Dev. Biol. 2:179-189; 1982.
54. Montesano, R.; Mouron, P.; Amherdt, M., et al. Collagen matrix promotes reorganization of pancreatic endocrine cell monolayers into islet-like organoids. J. Cell Biol. 97:935-939; 1983.
55. Grube, D.; Bohn, R. The microanatomy of human islets of Langerhans with special reference to somatostatin (D-) cells. Arch. Histol. Jpn. 46:327-353; 1983.

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