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Survival of Functional Pancreatic Acinar Tissue in Circumfusion Organ Culture Enhanced by Chemically Defined Medium with Hydrocortisone[1]

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Summary

Pancreatic explants from perinatal or 1-week-old rat circumfusion organ cultured with an insulin-free variant of Trowell's Medium T8 survive functionally, as judged from tissue amylase content, for about 3 days. When hydrocortisone 21-sodium succinate, 1.0 mg/liter, is added to the chemically defined medium, high levels of amylase persist for longer periods. Explants from 7-day-old animals, circumfusion cultured with hydrocortisone-supplemented medium, maintain tissue amylase levels equal to or greater than those of uncultured control pancreas for at least 5 days of culture, and over this period they release amylase into culture medium at a stable rate. Methods for maintaining functional pancreatic acinar tissue in culture provide a new biological model for *in vitro* analysis of the early effects of potential chemical carcinogens on this target organ.

Introduction

Experimental induction of pancreatic neoplasia has been demonstrated in guinea pigs (18), hamsters (17), and rats (10). Maintenance of functional pancreatic acinar tissue in organ culture could provide an experimental model through which at least the early changes induced by exposure of the organ directly to potential carcinogens might be explored. Such studies could contribute substantially to our now inadequate understanding of the etiology and development of pancreatic neoplasias.

Several investigators have successfully maintained embryonic or fetal pancreatic tissue in organ culture (3, 16, 19), although typically postnatal pancreas suffers almost total autolysis within the 1st 24 hr of culture (20). The normal pancreatic acinar cell produces trypsin, chymotrypsin, amylase, and other enzymes. In standard culture systems, the tissue literally digests itself. Circumfusion organ culture, by removing the destructive materials as they are released, prevents the development of toxic microenvironments and alleviates the early autolysis (14).

Long-term maintenance of functional, fully differentiated acinar pancreas requires careful attention to a host of factors most of which have yet to be explored. The analysis will be simplified greatly if a chemically defined culture medium, rather than one containing such undefined and highly variable (2, 5, 21) components as embryo extracts and serum, can be used. In this paper, we present data demonstrating improved functional survival of pancreatic acinar tissue in circumfusion organ culture with a simple chemically defined medium containing hydrocortisone.

Materials and Methods

That portion of the pancreas deep to the stomach and medial to the spleen (splenic pancreatic lobe) was aseptically removed from perinatal (birth ± 24 hr) or 7-day-postnatal random-bred Sprague-Dawley rats of either sex. Each splenic pancreatic lobe was placed in culture medium and, with minimum physical trauma, trimmed to a final size of 6 ± 1 (S.D.) x 5 ± 1 mm. Each lobe thus trimmed was used as a single explant with a thickness (governed by the size of the organ in rats of the ages used) of about 1 to 2 mm. In each culture vessel, explants from 6 or more rats were supported at the surface of 10 ± 1 ml medium by stainless steel mesh.

Details of our circumfusion organ culture apparatus and methods have been reported (14). In the present work, 6 ± 1 ml fresh culture medium were added sir ultaneously with the removal of an equal volume of "old" medium from each culture vessel at 3-hr intervals for the initial 24 hr of culture and at 24-hr intervals thereafter. Each vessel was supplied with continuously flowing 95 to 98% water vapor-saturated 95% O_2 and 5% CO_2; temperature was maintained at 36 ± 1°.

Our chemically defined culture Medium T8b is similar to Trowell's Medium T8 (20), except that (*a*) L-methionine (7.5 mg/liter), L-phenylalanine (16.5 mg/liter), and L-threonine (24 mg/liter) replace the DL forms specified for T8; (*b*) chloramphenicol and insulin are omitted; and (*c*) 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer is added. A variant, Medium T8c, is Medium T8b supplemented with hydrocortisone 21-sodium succinate, 1.0 mg/liter. All media were prepared in our laboratory with the purest available chemical components and deionized, glass double-distilled water and sterilized by pressure filtration through prewashed 0.22-μm pore size membrane filters. During culture, pH was maintained at 7.4 ± 0.1.

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Except as specified, preparation and handling of cultures followed standard methods (12, 13).

Pancreatic explants were homogenized mechanically in calcium- and magnesium-free phosphate-buffered saline (12). Amylase in homogenates was measured by a commercial version (Amylochrome; Hoffman-La Roche, Inc., Nutley, N.J.) of the method of Klein *et al.* (8). Calibration against recrystallized α-amylase from both *Bacillus subtilis* and hog pancreas demonstrated that 100 Amylochrome dye units correspond to 58 ± 1 standard amylase units as defined by Bernfeld (1). Protein in tissue homogenates was determined by a micromodification of the method of Lowry *et al.* (11), using crystalline bovine serum albumin standards.

Results

After 5 days of circumfusion organ culture of pancreatic explants obtained from perinatal or 7-day-postnatal rats with Medium T8b, no amylase can be detected in tissue homogenates. However, if the medium is supplemented with hydrocortisone, amylase can be demonstrated in explants cultured for this period (Chart 1). The amount of extractable amylase is dependent on the amount of hydrocortisone added and is similar for explants obtained from perinatal or 7-day-postnatal animals.

Histological observations (not reported here) supported cultured tissue amylase determinations and suggested that both morphological and functional integrity of cultured tissues were best preserved by the incorporation of 1.0 mg hydrocortisone per liter of medium. In another series of cultures, we determined the tissue amylase contents in circumfusion cultures with both Medium T8b and Medium T8c for up to 5 days (Chart 2). The inclusion of the glucocorticoid clearly results in better maintenance of tissue amylase levels throughout this period. With Medium T8b, the enzyme cannot be detected after 3 days of culture, whereas with Medium T8c high levels persist through at least 5 days. There are clear-cut differences in the responses of cultures prepared from perinatal and from 1-week-old animals. Cultures from older animals characteristically have tissue amylase levels at or above those of comparable

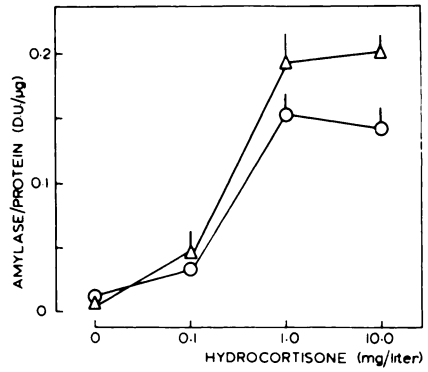

Chart 1. Effect of hydrocortisone supplementation of Medium T8b on tissue amylase:protein ratio after 5 days in circumfusion organ culture. Δ, pancreatic explants from 7-day-postnatal rats; O, explants from perinatal rats. *Points*, mean of 10 or more observations; S.E. indicated by symbol size or vertical line as appropriate. *D.U.*, Amylochrome dye units.

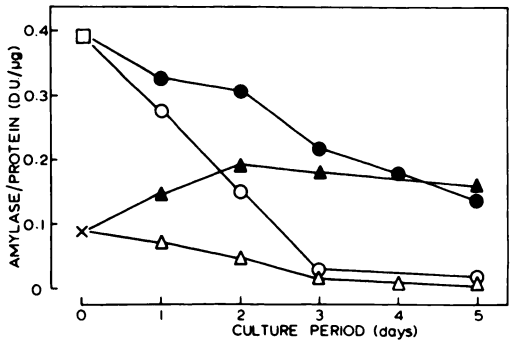

Chart 2. Tissue amylase:protein ratios over a 5-day culture period. □, uncultured perinatal rat pancreas; ×, uncultured 7-day-postnatal pancreas. ●, O, tissue cultured from perinatal pancreatic explants; ▲, Δ, from 7-day-postnatal explants. *Closed symbols*, Medium T8c; *open symbols*, Medium T8b. *Points*, mean of at least 5 observations. *D.U.*, Amylochrome dye units.

uncultured control tissues, while the enzyme level of perinatal tissues decreases throughout the culture period.

In vivo, amylase and other pancreatic acinar cell products are secreted into and released through the pancreatic duct system. In organ culture, the duct system is not physiologically intact, and the enzymes are released into the culture medium. Measurement of medium amylase levels provides an indication of functional survival beyond measurement of tissue amylase content, for it suggests preservation of cellular secretory mechanisms. Amylase can be detected in Medium T8c circumfused through cultures and collected at 24-hr intervals through 5 culture days. However, simultaneous addition and removal of medium at each circumfusion cycle necessarily results in intermixing of both new and old medium aliquots and precludes meaningful interpretation of amylase release rates.

Therefore, at the end of each 24-hr culture period, medium was removed from a series of cultures and replaced with fresh medium for a 30-min interval. Amylase contents of such 30-min samples were determined to estimate enzyme release rates during culture. Release patterns similar to those predictable from tissue amylase contents under a given set of culture conditions were observed. Explants from 7-day-postnatal animals cultured with Medium T8c released amylase at 11 to 14 Amylochrome dye units/explant/30 min; the rate was stable over 5 days of culture. Similar explants cultured with Medium T8b had amylase release rates that declined to undetectable levels after the 3rd culture day. These data suggest that basal enzyme release, presumably stimulated by component(s) of the culture medium, continues during culture.

Since explants from 7-day-postnatal animals contained higher amylase levels after 5 days of culture than were present in unclutured control pancreas and released the enzyme at appreciable levels for the same period, we conclude that net amylase synthesis continues during circumfusion organ culture.

Discussion

Glucocorticoids, or coculture with adrenal tissue, have been advocated for mouse pancreatic acinar organ culture

(4) and for fetal rat pancreatic islet organ culture (13). The media contained serum or embryo extracts, and therefore that work cannot be compared directly to the present experiments. More recently, mouse pancreatic islet cells have been maintained in cell culture on chemically defined media containing hydrocortisone, 0.09 mg/liter (9). Clearly, the optima must be defined for each system. That the glucocorticoids have demonstrated effects on preservation of insulin and amylase production in culture strongly suggests that the cultured pancreatic cells retain a functional cytoplasmic receptor protein specific for the glucocorticoid (6, 7).

Elsewhere, we report (14) pancreatic organ cultures using medium circumfusion at 3-hr intervals throughout the culture period. Tissue amylase levels similar to those reported here were obtained. Thus, the 1st 24 hr following explantation are critical for pancreatic acinar survival in culture. If medium is circumfused at 3-hr intervals for 24 hr, subsequent exchange rates may be lower and still permit functional survival. A few observations, chiefly histological, after 8 or more days of culture (14) suggest that pancreatic tissues remain viable for more than the 5-day interval studied in some detail. We have concentrated on the 5-day period in the belief that the basis for longer survival is established during that period.

Many variables in the circumfusion culture system remain to be explored. Our media have an osmolality of 300 ± 10 milliosmoles/kg, probably suboptimal for pancreas (15); the medium glucose level (400 mg/100 ml) is certainly far above the normal physiological range. No efforts have been made to define optimal levels of the majority of the medium components or to examine the utilization of those components during culture. Effects of known secretogogues such as pancreozymin are unknown in this system. The available data, although limited, suggest that acinar pancreatic tissue in organ culture may provide a biological model with which the early effects of chemical and other potential carcinogens on this target organ can be determined.

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