THE DEVELOPMENT OF THE

DORSAL AND VENTRAL MAMMALIAN

PANCREAS IN VIVO AND IN VITRO

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ABSTRACT

The origin, morphogenesis, and biochemical differentiation of the dorsal and ventral pancreas of the rat embryo have been investigated in order to ascertain the similarities and dissimilarities between the two lobes. We have utilized a culture system in which the primitive gut gives rise to a number of differentiated organs, including the dorsal and ventral pancreas. The two pancreases do not undergo fusion in these cultures, thus allowing independent analyses of the two lobes for comparison with in vivo results. The dorsal pancreas first appeared at the 23–25 somite stage while the ventral pancreas appeared approximately 12 hr later at the 29–30 somite stage. Guts from embryos as young as 12 somites were capable of developing both pancreases in vitro. In spite of the 12 hr difference between the times of their appearance, the dorsal and ventral pancreases exhibited identical patterns of morphological and biochemical differentiation. The two lobes contained the same exocrine enzymes and hormones, at similar levels, differing only in their glucagon content, the dorsal pancreas possessing a fivefold higher glucagon specific activity. The implications of these results are discussed.

INTRODUCTION

The embryonic pancreas has, during recent years, proven to be an excellent system for the study of development and differentiation. It is now known that an epithelial-mesenchymal interaction is necessary for pancreatic cytodifferentiation (Golosow and Grobstein, 1962) that this interaction can occur in the absence of cell contact (Grobstein, 1964), and that the accumulation of specific pancreatic proteins is biphasic; there is a rapid transition from low detectable levels to differentiated levels during a 3 day period of development (Rutter et al., 1967). The morphological (Kallman and Grobstein, 1964) and biochemical (Rutter et al., 1964; 1968) changes that occur during in vitro organ culture correlate precisely with the events occurring in vivo.

However, these experiments have been performed with the dorsal pancreas and provide no information on the development of the ventral pancreas. Although the ventral pancreas arises somewhat later than the dorsal diverticulum (see McEwen, 1949), and from the opposite side of the gut endoderm, it eventually forms a substantial portion of the adult organ.

In the present study, we have investigated the origin and morphogenesis of both the ventral and dorsal pancreases during their development in organ culture from isolated whole guts. A comparison of the changes in levels of several pancreatic proteins during development has been made between the dorsal and ventral pancreas, both in vivo and in vitro. Finally, the culture system has allowed an analysis of the levels of specific proteins in the dorsal and ventral lobes, in the absense of their fusion, at times beyond which fusion has begun in vivo.

MATERIALS AND METHODS

The tissues used in this investigation were dissected from Sprague-Dawley albino rat embryos. Virgin females were mated overnight with males, and the morning of discovery of the vaginal plug was designated as day 0. The number of days of gestation was used as the experimental embryonic age. All embryos younger than 12 days were further staged by somite number.

Whole-guts were dissected from 10- to 11-day rat embryos in a manner similar to that used by Wessells and Cohen (1967) on 8- to 9-day mouse embryos. The region taken was posterior to the position of the embryonic heart and anterior to the tail. The dorsal aorta, somites, and the other dorsal tissues, as well as the lateral body walls and any remaining cardiac tissue were removed with iridectomy knives. In addition, the primitive liver rudiments were dissected away, since liver development partially obscures ventral pancreatic morphogenesis. The remaining gut endoderm with its adherent mesoderm was designated as whole-gut. Gut endoderm was freed from its mesoderm by treatment with 3% trypsin-pancreatin (Wessells and Cohen, 1967). Whole-guts, endoderm, and endoderm-mesoderm recombinants were organ cultured on the upper surface of Millipore filter assemblies (Millipore Corporation, Bedford, Mass.), at the air-medium interface under an atmosphere of 5% CO_2 in air.

The media used for organ culture were Eagle's basal medium (BME), medium 199 (both obtained from Grand Island Biological Co., Grand Island, N. Y.) and modified Ham's F12 (Cahn et al., 1967). BME was supplemented with either embryo juice (EJ) or embryo extract (EE). EJ refers to a 3000 g supernatant of a homogenate of equal amounts (v/w) of Tyrode's and 9-day chick embryos, while EE refers to a 60,000 g supernatant of the same homogenate. Medium 199 was used unsupplemented, and modified F12 was used alone or supplemented with 10% fetal calf serum (Grand Island Biological Co.). All cultures were fed daily by a complete change of medium.

On day 13 in vivo, the ventral pancreas can be dissected out, as well as the dorsal pancreas. After dorsal-ventral fusion begins, on day 16, the two extremities were taken, and the transition region was discarded. Dissection of both lobes from whole-gut cultures was easily accomplished after 4–8 days in vitro. Dorsal and ventral pancreases of various ages were then frozen until assayed.

The tissues were disrupted by sonication, and protein determinations were made by Rutter's modification (1967) of the Lowry-Folin procedure. Standard curves of crystalline bovine serum albumin were linear from 0 to 10 μg. Amylase activity in tissue sonicates was detected by a modified Bernfield (1955) assay[1], measuring maltose equivalent release from starch in 0.05 M histidine Cl at pH 6.5. The proteolytic enzymes were assayed by micromodifications[1] of the spectrophotometric methods for chymotrypsin (Hummel, 1959), carboxypeptidase A (Folk and Schirmer, 1963), and carboxypeptidase B (Wolff et al., 1962). Prior to assays of proteolytic enzymes, the tissue sonicate was pretreated with crystallized trypsin (Worthington). In the case of chymotrypsin, 0.01 μg trypsin/μl sonicate was used for 2 hr at 0°C, while in the case of the carboxypeptidases 0.1 μg trypsin/μl sonicate was incubated at 0°C for 30 min before assays were done. Chymotrypsin activity on N-benzoyl-L-tyrosine ethyl ester was followed by an increase in absorbancy at 256 mμ. Carboxypeptidase A activity on hippuryl-dl-phenyllactic acid and carboxypeptidase B activity on hippuryl-L-arginine were both followed by an increase in absorbance at 254 mμ. Ribonuclease was measured by activity on ribonucleic acid and followed at 260 mμ by the procedure of Beard and Razzell (1964), as modified by Ball and Rutter[2]. Insulin levels were determined by a micromodification[3] of the double antibody procedure of Morgan and Lazarow (1963), and glucagon was measured by the double antibody procedure described by Hazzard et al. (1968).

Dorsal and ventral pancreases dissected from whole-gut cultures were processed for electron microscopy by conventional procedures. Following primary fixation in 2.5% glutaraldehyde with paraformaldehyde in 0.14 M phosphate buffer at pH 7.4 for 1 hr (modified after Karnovsky, 1965), the tissues were postfixed for 1 hr in 1% osmium tetroxide in 0.28 M Veronal buffer (pH 7.4) at 4°C. The tissues

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[1] T. G. Sanders and W. J. Rutter. 1970. The embryonic regulation of pancreatic amylolytic and proteolytic enzymes of the rat. Manuscript in preparation.

[2] W. D. Ball and W. J. Rutter. 1970. Manuscript in preparation.

[3] W. R. Clark and W. J. Rutter. 1970. Manuscript in preparation.

were dehydrated, embedded in Epon, polymerized, and thin-sectioned. Sections were stained with uranyl acetate and lead citrate (Venable and Coggeshall, 1965) and examined in a Hitachi HU-IIe-1 electron microscope.

RESULTS

Normal Development of Dorsal and Ventral Pancreas

Observations on isolated gut endoderm have allowed a determination of the time of appearance of the dorsal and ventral pancreatic diverticula in the rat embryo (Fig. 1). The dorsal pancreas first appears as a broad-based bulge at 23–25 somites (middle of day 11) in a position just posterior to the presumptive stomach. Although these events occur at *circa* 1.5 chronological days later in the rat, the time (in somite age) and position of appearance are identical with that observed in the mouse embryo (Wessells and Cohen, 1967). The ventral pancreas of the rat first appears at 29–30 somites, some 12 hr after the appearance of the dorsal pancreas. This ventral bulge of the gut endoderm occurs at the same anterior-posterior position as the dorsal diverticulum. The gross changes that occur with continued development are similar for both rudiments, the ventral pancreas remaining smaller over the next 3–4 days and undergoing such physical changes as lobulation 12–24 hr after the dorsal pancreas. However,
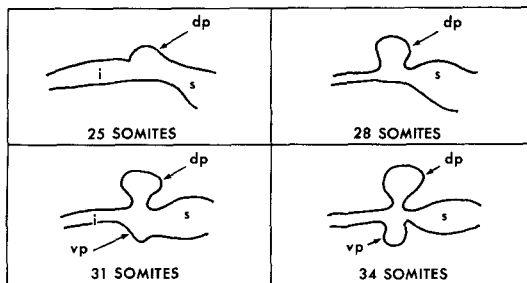

FIGURE 1 Schematic representation of the origin and early morphogenesis of the dorsal and ventral pancreatic diverticula. The developing liver anlage has been purposely left out of this scheme in order to focus on the ventral pancreas. The developing liver is normally in intimate association with and just anterior to the ventral pancreas. The observations for this scheme were made on trypsin-isolated gut endoderm from 11 to 12-day rat embryos ranging in stage from 20 to 35 somites. See text for description. *dp*, dorsal pancreas; *s*, stomach; *i*, intestine; *vp*, ventral pancreas.

there is visible opacity of acini, due to zymogen granule accumulation in both dorsal and ventral lobes by day 18 of gestation. Flexure and rotation of the stomach and intestine brings the two pancreases into juxtaposition by day 15 in vivo. Fusion between the lobes is first detected on day 16 and is fairly extensive on day 18.

In Vitro Development of Dorsal and Ventral Pancreas

The ability of gut endoderm and its adherent mesoderm (whole guts) to form dorsal pancreas in vitro (Wessells and Cohen, 1967) has been used for further analysis of the development of the dorsal and ventral pancreases.

Whole-guts isolated from 11-day rat embryos were found to give rise to a wide array of gut derivatives in organ culture. Since the primitive liver lobes were removed, liver tissue did not appear. However, differentiated intestine, stomach, esophagus, trachea, and lung did develop. Furthermore, the dorsal and ventral pancreases grew out from opposite sides of the gut. Pancreas morphogenesis in culture was identical with normal development: the dorsal pancreas appeared first, while the ventral diverticulum appeared 12–24 hr later. The continuity between the gut lumen and the lumen of the ventral pancreas was quite apparent in these cultures. With continued culture, the two diverticula formed large lobes which remained on opposite sides of the gut, eventually darkening in texture, presumably due to zymogen granule formation (Fig. 2). Careful dissection of 7-day cultures showed that the ventral pancreatic duct always entered the gut at the same level as or slightly anterior to the entrance of the dorsal pancreatic duct. Preliminary electron microscopic observations revealed the presence of typical exocrine acini in both lobes by day 6 of culture (Figs. 3 and 4). Some acinar cells already possess fully differentiated zymogen granules at this time. Furthermore, differentiated endocrine cells are present in both lobes and are recognizable by their characteristic secretory granules (Fig. 5). Measurements were also made to determine if this mophologically developed tissue had in fact undergone biochemical differentiation. After 7 days of culture, the pancreatic tissue was harvested and amylase determinations were made on pooled dorsal and ventral lobes. In other cases, glucagon determinations were made on the dorsal lobes.
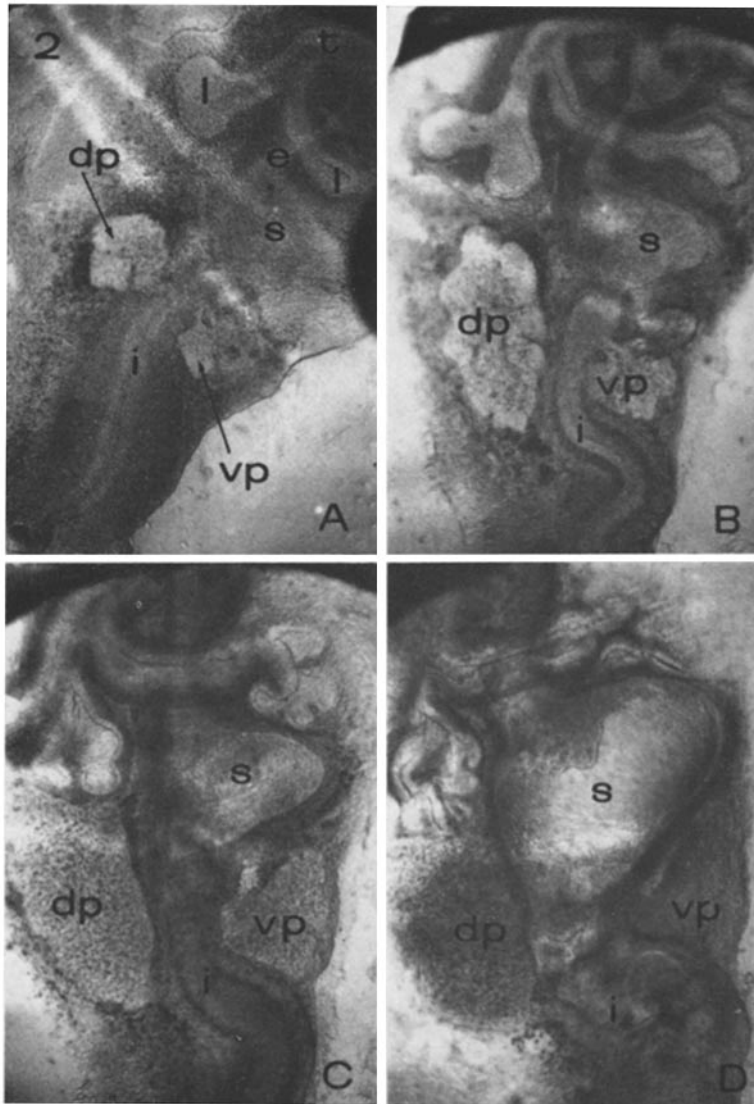
FIGURE 2 Dorsal and ventral pancreas development from an 11 day rat embryo whole-gut in vitro. (A) 2 days in culture. Both pancreas lobes have grown out from the gut. The stomach has begun to take shape, the lung buds have formed, and the trachea has separated from the esophagus. (B) 4 days in culture. The two pancreases have grown substantially and are both undergoing lobulation. The dorsal lobe still maintains a size advantage over the ventral lobe. The stomach and intestine are well formed and the lung buds are beginning to branch. (C) 6 days in culture. Further growth has occurred and both dorsal and ventral pancreas exhibit a distinct change in texture due to the presence of acini and the beginning of zymogen granule accumulation. Bronchial branching has continued. (D) 7.5 days in culture. The ventral pancreas is now only slightly smaller than the dorsal pancreas. Both lobes show intense optical opacity due to zymogen granule accumulation. The stomach appears to be filled with fluid. Smooth muscle has differentiated and contracts spontaneously throughout the gut and in the lungs. *dp*, dorsal pancreas; *vp*, ventral pancreas; *i*, intestine; *s*, stomach; *e*, esophagus; *l*, lung buds; *t*, trachea. Living culture, × 112.
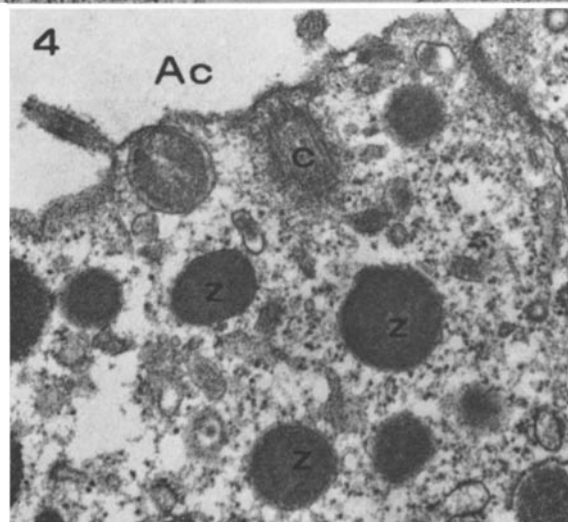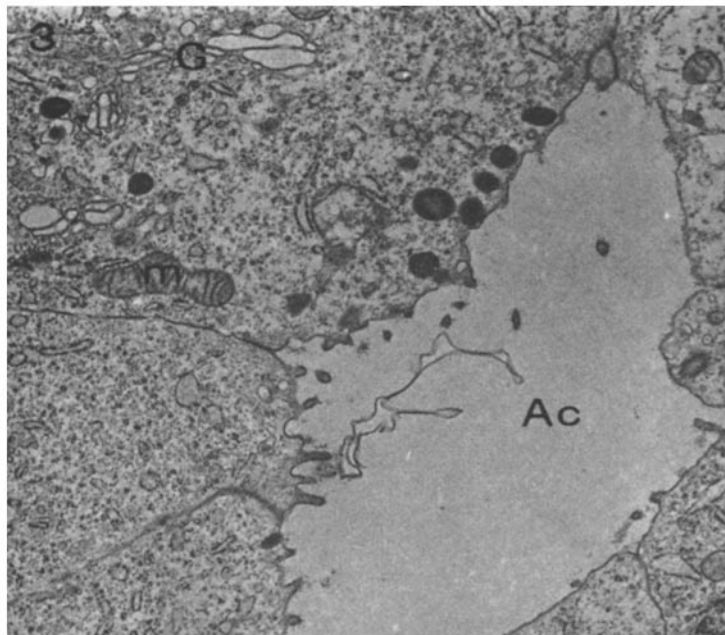
FIGURE 3 Electron micrograph showing a portion of an exocrine acinus in a ventral pancreas derived from an 11 day rat embryo whole-gut after 6 days of culture. The acinar cells have typical electron-opaque junctional complexes between them. Microvilli occur at the apical surface of the cells. The acinar cell at the upper left has a well developed Golgi complex and exhibits a number of zymogen granules in the apical cytoplasm. *Ac*, acinus; *G*, Golgi complex; *m*, mitochondrion. × 12,500.

FIGURE 4 Portion of an acinar cell in the dorsal pancreas of the same 6 day gut culture used in Fig. 3. Zymogen granules are present in the apical cytoplasm. The centriole present near the acinar lumen probably represents a basal body associated with a cilium. *z*, zymogen granule; *c*, centriole; *Ac*, acinus. × 32,300.
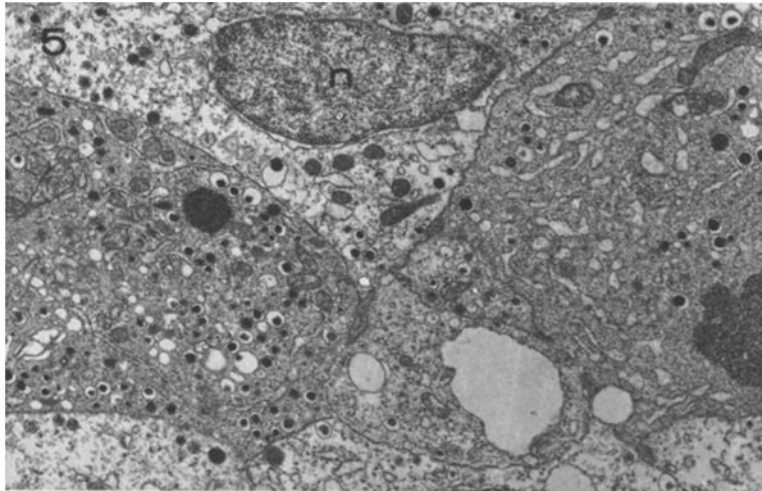
FIGURE 5   Electron micrograph of a section through several endocrine cells in the dorsal pancreas of a 6 day whole-gut culture. The cytoplasm of these endocrine cells contains numerous characteristic secretory granules. Identical cells are present in similar sections of the ventral pancreas. n, nucleus. X 7500.

TABLE I

*The Development of Exocrine and Endocrine Protein by Whole-Gut Cultures in the Presence of Various Media**

Medium	Supplement	Pancreatic protein	Specific activity	
			Amylase‡	Glucagon§
		µg/culture		
F12	None	5.1	3.3	—
F12	Fetal calf serum—10%	14	4.1	34
199	None	3.0	3.0	82
BME	Embryo juice—10%	3.3	3.8	56
BME	Embryo extract—10%	8.0	4.3	76
Specific activities at 18 days in vivo			5.0	26
Specific activities at 13 days in vivo			0.0053	15‖

* Cultures were terminated after 7 days in vitro.
‡ Amylase specific activity in pooled dorsal and ventral lobes (mg maltose released per mg protein/min).
§ Glucagon specific activity in dorsal lobes (µµg glucagon per µg protein).
‖ Glucagon value at 11 days in vivo.[4]

Amylase and glucagon were chosen as representative exocrine and endocrine proteins, and were found to be present at differentiated levels (Table I, line 4).

The possibility that this development was dependent on the presence of a particular complex culture medium was tested by culturing whole-guts in a variety of defined and complex media. Both dorsal and ventral pancreases developed in

[4] L. Rall and W. J. Rutter. Unpublished material.

all cases and possessed differentiated levels of amylase and glucagon (Table I). Thus, pancreatic morphogenesis and biochemical differentiation occur in gut cultures in the presence of either fully defined or complex media. Although there were no significant differences in amylase and glucagon specific activity, the total pancreatic protein was generally higher in complex media (e.g., approximately three times higher in F12 with fetal calf serum than in F12 alone), suggesting a general

growth effect. Modified F12 with 10% fetal calf serum has been previously used for clonal culture of differentiated thyroid cells (Spooner, 1970) and may be generally useful for culturing endodermally derived epithelial cells. It is interesting that rat pancreatic development was normal in the completely defined media, an observation not previously reported. The use of these media should allow an investigation of the effects of specific compounds, such as hormones, on pancreas development and secretory function.

In Vitro Gut Competency for Dorsal-Ventral Pancreas Development

The developmental time at which the gut becomes competent to form both dorsal and ventral pancreases has been explored by in vitro culture of guts of varying ages. By explanting guts from progressively younger embryos, one should be able to determine the age at which whole-guts have acquired the capacity for formation of the dorsal-ventral pancreatic set in the absence of the whole embryo. Such an analysis has, in fact, been made by Wessels and Cohen (1967) to determine the age of mouse gut competence for pancreas (presumably dorsal) formation.

Whole-guts were excised from 10- and 11-day embryos that had been staged by somite counts. The guts were cultured for 7–8 days and scored for the formation of dorsal-ventral pancreas sets. Dorsal and ventral pancreases were then dissected from the cultures, pooled, and assayed for amylase. The results are shown in Table II. Whole-guts from embryos as young as 12 somites were capable

of forming both dorsal and ventral pancreases in culture. The amylase specific activity reflected the differentiation that accompanied this gross morphogenesis (levels greater than 260 times the levels found in the dorsal pancreas at day 13 in vivo). Wessells and Cohen (1967) had previously shown that whole-guts from mouse embryos are capable of pancreas formation in vitro by 10 somites (circa 25–26 hr prior to the formation of the dorsal pancreatic diverticulum). The current results show that the rat gut is sufficiently stable to allow formation of both dorsal and ventral pancreases in vitro by at least 12 somites, an age circa 24 hr and 36 hr before formation in vivo of the dorsal and ventral diverticula, respectively. Although the results show that the capacity for dorsal and ventral pancreas formation is present by the 12 somite stage, we cannot experimentally determine the age at which these capabilities are first acquired. Prior to 12 somites, it was not possible to dissect out whole-guts without major damage, because the embryos had not yet turned to place the endoderm on the inner face of their "C"-shape and because of the general fragility of the tissues.

Wessells and Cohen have also demonstrated that gut endoderm from 15 to 20-somite mouse embryos can be isolated from its mesoderm with trypsin and will form pancreatic tissue if directly recombined with mesoderm. This observation has been extended to the rat embryo in the present study (Table II). However, we cannot distinguish dorsal or ventral pancreas in these recombinants. The endoderm forms simple gut vesicles with pancreas growing around or to the side of them.

TABLE II

*Ability of Whole-Guts or Endoderm to Undergo Pancreatic Morphogenesis and Cytodifferentiation in Vitro**

| Number of somites | Culture | Number of guts | Number of guts forming | | Amylase‡ specific activity | Amylase increase over day 13 dorsal level in vivo |
			Dorsal-ventral sets	Single lobes		
12	Whole-gut	20	17	3	1.4	264 times
15–20	Whole-gut	27	16	11	3.8	717 times
15–20	Recombination§	9	—	9	6.2	1170 times
21–25	Whole-gut	171	171	0	4.1	774 times
26–30	Whole-gut	175	175	0	3.0	556 times

* Cultures were terminated after 7 days in vitro.
‡ Mg maltose released per mg protein/min.
§ Endoderm was directly recombined with its own mesoderm.

Specific Protein Levels During Dorsal and Ventral Pancreatic Development

Rutter et al. (1968) have determined the changes in the levels of exocrine enzymes during the course of development of the dorsal pancreas in vivo and in vitro. Their results show distinct patterns of enzyme accumulation (developmental profiles) characterized by initial low levels of enzymes followed by a rapid transition to differentiated levels. The transition results in a 100–10,000-fold increase in enzyme levels. We have now determined the accumulation curves for several exocrine enzymes during normal development of the dorsal and ventral pancreas in vivo. In addition to the in vivo patterns for carboxy-

peptidase A, carboxypeptidase B, and amylase, we have studied the changes in amylase activity in both pancreas during in vitro morphogenesis from whole guts.

The in vivo accumualtion curves of carboxypeptidase A, carboxypeptidase B, and amylase were found to be virtuallv identical for the dorsal and ventral pancreases (Fig. 6). Thus, amylase and carboxypeptidase A entered the rapid transition period on the 14th and 15th days of development in both the dorsal and ventral pancreases, and carboxypeptidase B began its rapid rise in both lobes on the 16th day. The changes in enzyme levels did not vary between dorsal and ventral pancreas. During the rapid transition phase, the enzyme levels increase bv an order of magnitude
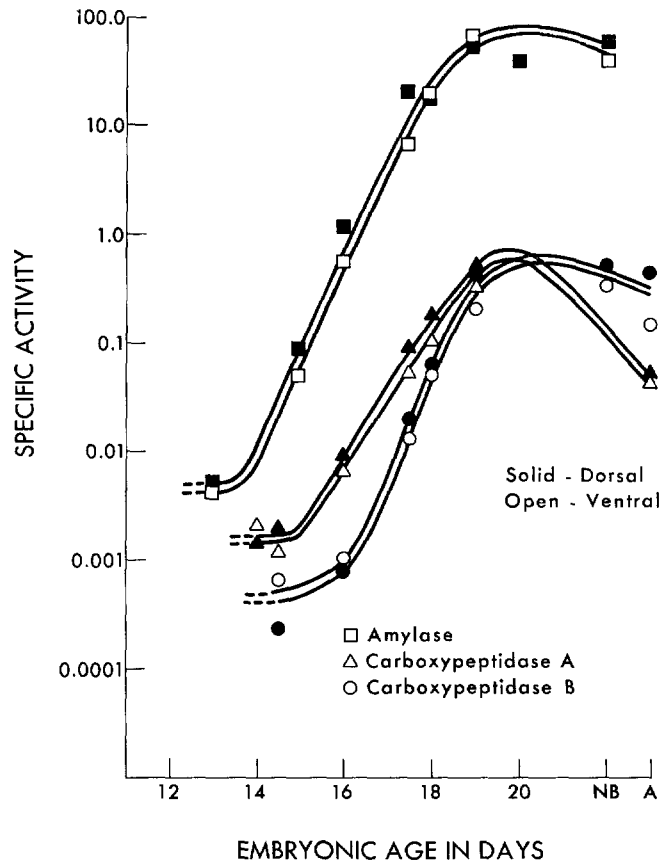

FIGURE 6 Accumulation of exocrine enzymes during development of the dorsal and ventral pancreas in vivo. See text for description. ■, amylase in dorsal pancreas; □, amylase in ventral pancreas; ▲, carboxypeptidase A in dorsal pancreas; △, carboxypeptidase A in ventral pancreas; ●, carboxypeptidase B in dorsal pancreas; ○, carboxypeptidase B in ventral pancreas. Specific activity: amylase, mg maltose released per mg protein per min; carboxypeptidase A, $\Delta A_{254\,m\mu}$ per min/mg protein; carboxypeptidase B, $\Delta A_{254\,m\mu}$ per min/mg protein.

TABLE III

Changes in Amylase Specific Activity with Increasing
Culture Age in Dorsal and Ventral Pancreas Derived
from Whole-Guts in Vitro

		Amylase specific activity*	
Age of explant	Days of culture	Dorsal pancreas	Ventral pancreas
13-day	None	0.0053	0.0043
11-day guts	4	0.070	0.17
11-day guts	6	1.1	1.1
11-day guts	7	5.6	4.3

* Mg maltose released per mg protein/min.

in each 24-hr period. If the 12 hr differential between the origin of the dorsal and that of the ventral pancreas was maintained during chemical differentiation, fivefold differences in enzyme levels would be expected during the rapid transection. The assays can easily discriminate such a difference, but one that large was never detected (see both Fig. 6 and Table III). These results demonstrate that the three enzymes are present in both lobes and that the pattern of exocrine cytodifferentiation is the same in the dorsal and ventral pancreases. Furthermore, the two lobes exhibited similar changes in amylase levels during culture (Table III). This critical in vitro confirmation of the in vivo result shows that fusion between the two lobes is not responsible for the correspondence in amylase levels, since fusion does not occur in these cultures.

Differentiated Levels of Specific Proteins in Dorsal and Ventral Pancreas

The correspondence between dorsal and ventral pancreas during morphogenesis and cytodifferentiation indicated that there were no differences in the two lobes. In order to clarify this possibility, we have compared specific protein levels in the dorsal and ventral pancreas at a time in development when these proteins should all be present at differentiated levels. The exocrine enzymes amylase, chymotrypsin, carboxypeptidase A, carboxypeptidase B, and ribonuclease have been compared, as well as the two endocrine hormones, insulin and glucagon. The age chosen was the 18th day of gestation in vivo, and the values were compared with those obtained from 7-day cultures of 11-day guts (chronologically 18 days). The results of this analysis are shown in Table

IV. There is good correspondence between the values obtained in vitro and those obtained in vivo, again demonstrating that the culture system is a true reflection of normal development. The exocrine proteins are present at similar levels in both the dorsal and ventral pancreases. The equivalence of the values is demonstrated by their ratios, in vivo and in vitro, which do not significantly deviate from unity. Insulin, like the exocrine proteins, is present in both dorsal and ventral pancreases at quantitatively similar levels. Furthermore, preliminary experiments indicate that both molecular species of insulin (Smith, 1964) are synthetized both in the dorsal and in the ventral pancreas. Insulin, ribonuclease, and chymotrypsin are also present at identical levels in both lobes on day 16 in vivo, suggesting identical patterns of development. Glucagon, on the other hand, is present in the two lobes in significantly different amounts. The dorsal pancreas has a glucagon-specific activity five- six-fold higher than that of the ventral pancreas in vivo and in vitro. This difference in dorsal-ventral glucagon specific activity has also been observed at 17 days in vivo and in 4-day, 6-day, and 8-day cultures (chronologically 15, 17, and 19 days of development). These results demonstrate a remarkable similarity, qualitatively and quantitatively, between the dorsal and ventral pancreas with respect to the exocrine enzymes and insulin. The two lobes were not identical, however, since they contain significantly different amounts of glucagon.

DISCUSSION

Observations on trypsin-isolated gut endoderm established that the ventral pancreatic diverticulum first appeared at 30 somites in the developing rat embryo, at the beginning of the 12th day of gestation, while the dorsal pancreas had appeared circa 12 hr earlier in 22–25-somite embryos, midway through day 11. The dorsal pancreas also first arises at 22–25 somites in developing mouse embryos (Wessells and Cohen, 1967), although the mouse is circa 1.5 days younger at this stage. Both pancreatic diverticula were found to originate from the endoderm just posterior to the presumptive stomach, one appearing on the dorsal surface and the other on the ventral surface. These observations were confirmed in culture with whole-guts taken from 11-day embryos. The early morphogenesis of both pancreases out of the gut wall could be clearly followed in such cultures.

TABLE IV

Levels of Specific Proteins in Differentiated Dorsal and Ventral Pancreas in Vitro and in Vivo

	AMY*	CHYMO‡	Cp-A§	Cp-B‖	RNase¶	Insulin**	Glu-cagon‡‡
Protodifferentiated§§	0.0053	0.0052‖‖	0.00031	0.00060	0.0004‖‖	0.14‖‖	15.0⁴
In vitro 11-day guts cultured 7 days							
Dorsal pancreas	5.6	3.2	0.035	0.016	3.2	7.0	76
Ventral pancreas	4.3	5.8	0.027	0.035	5.4	11	14
In vivo 18 days gestation							
Dorsal pancreas	5.1	3.8	0.09	0.020	4.7	5.0	26
Ventral pancreas	5.0	3.7	0.06	0.014	4.4	3.1	4.4
Dorsal pancreas/ventral pancreas in vitro	1.3	0.56	1.3	0.46	0.59	0.61	5.6
Dorsal pancreas/ventral pancreas in vivo	1.0	1.0	1.7	1.4	1.1	1.6	5.8

* Amylase: mg maltose released per mg protein/min.
‡ Chymotrypsin: ΔA 256 mμ per min/mg protein.
§ Carboxypeptidase A: ΔA 254 mμ per min/min protein.
‖ Carboxypeptidase B: ΔA 254 mμ per min/mg protein.
¶ Ribonuclease: ΔA 260 mμ per min/mg protein.
** Insulin: $\mu\mu g$ per μg protein.
‡‡ Glucagon: $\mu\mu g$ per μg protein.
§§ Dorsal pancreas values at day 13 in vivo for the exocrine enzymes, day 14 for insulin, and day 11 for glucagon.
‖‖ Recalculated from the data of Rutter et al., 1968.

With continued development, the ventral pancreas grows out from the gut, increases in size, forms acini, and accumulates zymogen granules in a manner identical to that of the dorsal pancreas. Initially, the ventral pancreas is smaller and begins lobulation 12–24 hr later than the dorsal pancreas, although they are similar in size and morphology by day 18 (day 7 in vitro). Fusion between the lobes began on day 16 in vivo and was fairly extensive by day 18. However, fusion did not occur in vitro and therefore could not account for the final similarity between lobes.

In vitro culture has been commonly used for studying morphogenesis of organ primordia. However, it has only rarely been used to study the initial formation of such primordia. The value of the technique has been demonstrated in the present study, and it should prove to be equally useful for analysis of the early morphogenesis of other organs derived from gut endoderm.

The time at which a population of cells becomes committed to a particular developmental fate is a difficult matter to experimentally ascertain. Some insight into this problem has been possible by use of the whole-gut culture technique. When whole-guts from embryos as young as 12 somites were grown in organ culture, both dorsal and ventral pancreases developed and eventually acquired levels of amylase corresponding to the differentiated state. The capacity to form both pancreases in vitro is, therefore, already present in whole-guts by 12 somites, 24 and 36 hr, respectively, before the initial appearance of the dorsal and ventral diverticula. Wessells and Cohen (1967) demonstrated by similar methods that the capacity to form pancreatic tissue (presumably dorsal) in the mouse is acquired between the 7- and 10-somite stages. It seems reasonable to speculate, therefore, that sufficient stability for in vitro development of the

dorsal-ventral pancreatic set is acquired in the gut between the 7- and 12-somite stages.

Attempts to assess the possibility of an inductive role for the gut mesoderm in the primary determination of the dorsal-ventral pancreatic set have not given definitive results. We have extended to the rat embryo the observation (Wessells and Cohen, 1967) that trypsin-isolated gut endoderm of 15–20-somite embryos will form pancreatic tissue when recombined with mesoderm. However, we do not know if the tissue is dorsal pancreas, ventral pancreas, or both. The difficulty is the complete lack of orientation in these recombinants, which are composed of simple gut vesicles and pancreatic tissue. There are no apparent markers in the system for identifying either dorsal or ventral gut wall.

In vivo analyses of the developmental profiles of carboxypeptidase A, carboxypeptidase B, and amylase revealed identical accumulation curves for the dorsal and ventral pancreases. In vitro amylase determinations showed the fusion between the two lobes was not responsible for their similar patterns. Rutter et al. (1967) have demonstrated that the dorsal pancreas goes through a "protodifferentiated" state, characterized by low levels of exocrine proteins, prior to a rapid transition to differentiated levels. Our finding that the dorsal and ventral pancreases have identical patterns of chemical differentiation imply that the "protodifferentiated" state is some 12 hr shorter in the ventral pancreas, since the ventral pancreas arises circa 12 hr later than the dorsal lobe. Furthermore, these data suggest the possibility of a common factor regulating cytodifferentiation in both tissues, thus resulting in the apparent simultaneity in development.

When measurements were made at a time when the tissue is differentiated, the dorsal and ventral lobes were found to contain quantitatively identical levels of amylase, chymotrypsin, carboxypeptidase A, carboxypeptidase B, ribonuclease, and insulin both in vivo and in vitro. Furthermore, both lobes contained detectable levels of glucagon. However, the lobes were not absolutely identical since they contained significantly different amounts of glucagon. The glucagon specific activity in the dorsal pancreas was five–sixfold higher than that in the ventral pancreas. This difference was found both in vivo and in vitro at several developmental ages. It is not yet known if this difference reflects the presence of different

numbers of glucagon-producing A-cells in the two lobes, or whether the hormone concentration per A-cell is higher in the dorsal pancreas.

The remarkable finding was that the dorsal and ventral pancreases are identical in their array of differentiated products, in spite of their originating from populations of cells on opposite sides of the primitive gut and undergoing their initial morphogenesis at different times. The development of the pancreases, then, could be accounted for by two identical primary inductive interactions occurring at different times, or by a single influence of relatively long duration with the responding endodermal cells achieving competence at different times. Alternatively, a single population of determined cells could, by migration and localization at the dorsal and ventral gut surfaces, account for the origin of both lobes.

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