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Reviews

Ontogenic development of peptide hormones in the mammalian fetal pancreas

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Introduction

The mammalian pancreas has been known for some time to synthesize and secrete insulin, glucagon, pancreatic polypeptide (PP) and somatostatin from distinct cell-types within the islets[28, 29]. More recently islet cells have been shown to store several structurally diverse peptides such as gastrin, peptide YY, thyrotropin-releasing factor (TRF), gastric inhibitory polypeptide and S-100 protein[6, 29, 42, 51, 55, 56, 97]. Various neuropeptides including vasoactive intestinal peptide (VIP), cholecystokinin (CCK), enkephalin and substance P are known to be present in nerve fibers innervating the pancreas[57]. In earlier studies, identification of islet cell-types was carried out by purely histochemical means and later by electron microscopy[7, 72, 77]. However, with the availability of specific antisera against insulin, glucagon, PP and somatostatin and with refinements in immunocytochemistry it is now possible to study the cellular and subcellular distribution of the four hormones with considerable reliability[9, 10, 58, 76]. Data from these studies have helped to modify the previous '1 cell - 1 hormone' hypothesis to the possibility of co-storage of more than one hormone per single cell-type[6, 44, 45, 49, 50, 105]. In the mammalian fetus, although several studies show that the fetal pancreas of advanced gestation contains insulin, glucagon, PP and somatostatin cells, information on the ontogenic development of the hormonal cells or their products is restricted to only a few species. Further, there is little data on the ontogeny of the additional peptides recently shown to exist in the adult pancreas. In this review, we summarize recent knowledge on the ontogenic development of insulin, glucagon, PP and somatostatin in the fetal pancreas of mammals.

Embryological development of the pancreas

The mammalian pancreas develops from two diverticula originating from the dorsal and ventral surface of the primitive gut which subsequently fuse to form the pancreas during fetal development[90]. The endocrine and exocrine cells are thought to be derived from a common pool of precursor cells. In the very early stages of gestation some of the ductule epithelial cells differentiate into isolated endocrine cells which gradually form clusters. During fetal development the four endocrine cell-types increase in number and undergo structural and functional maturation and attain a topography believed to be unique to each species[17, 31, 32, 43, 61, 80]. In the early stages the endocrine to exocrine ratio is higher by mass than in the more mature fetal tissue. However, mechanisms responsible for pancreatic organogenesis are far from clear.

Ontogeny of pancreatic hormones

Conventional histological techniques which are relatively insensitive were used in earlier studies to determine development of islet cell types[16, 24, 30, 61, 62, 71, 93]. Over recent years, immunocytochemical and radioimmunoassay procedures have been employed to study the ontogeny of insulin, glucagon, PP and somatostatin. The rat, human and pig are the only three mammalian species in which complete ontogenic examination of the four hormones has been reported[8, 22, 23, 40, 60, 98, 99, 101, 108]. These studies show that the fetal pancreas displays marked regional variation in the PP and glucagon content of islets, like in the adult tissue[64, 74, 75], which probably reflects the embryological origin of the dorsal and ventral lobes of the pancreas. Recent immunohistochemical and biochemical data have demonstrated that several pancreatic hormones are also stored in cells of the gastrointestinal tract[20, 21].

Rat

The chronological development of insulin, glucagon, PP and somatostatin in the rat has been studied immunohistochemically[40, 101, 108]. In their studies Yoshinari and Daikoku[108] reported that the first endocrine cells to be identified were those containing glucagon on day 11.5 followed by insulin on day 12.5 of gestation (gestational period in the rat: 21 days). On day 15.5, immunoreactive somatostatin cells were identified at the periphery of the primitive islets. These ontogenic data, however, are at some variance with those of Fujii[40] and Schweisthal et al.[93] who reported that insulin cells were present on day 14 and somatostatin on day 17. The detection of the two cell-types by these workers at a later stage of gestation may be a consequence of their use of hormonal antisera of different affinities with possible loss of immunocytochemical sensitivity. The ontogeny of PP in this species has been studied by Sundler et al.[101] and Fujii[40]. In the report by Sundler et al., PP cells were absent in the pancreas before birth, although single weakly staining cells were observed in the islet or exocrine cells just before birth. A significant increase in PP cells was not apparent until 5–7 days postpartum. On the other hand, Fujii[40] reported that PP cells were present on day 14. The use of the more sensitive immunoperoxidase procedure by Fujii[40] (compared with indirect immunofluorescence by Sundler et al.) may be a reason for the detection of PP cells at an earlier gestational stage by the former investigator. The ontogenic data of Yoshinari and Daikoku[108] appear to be in accord with previous radioimmunoassay analyses of hormone concentrations in the embryonic pancreas, which has been shown to contain low

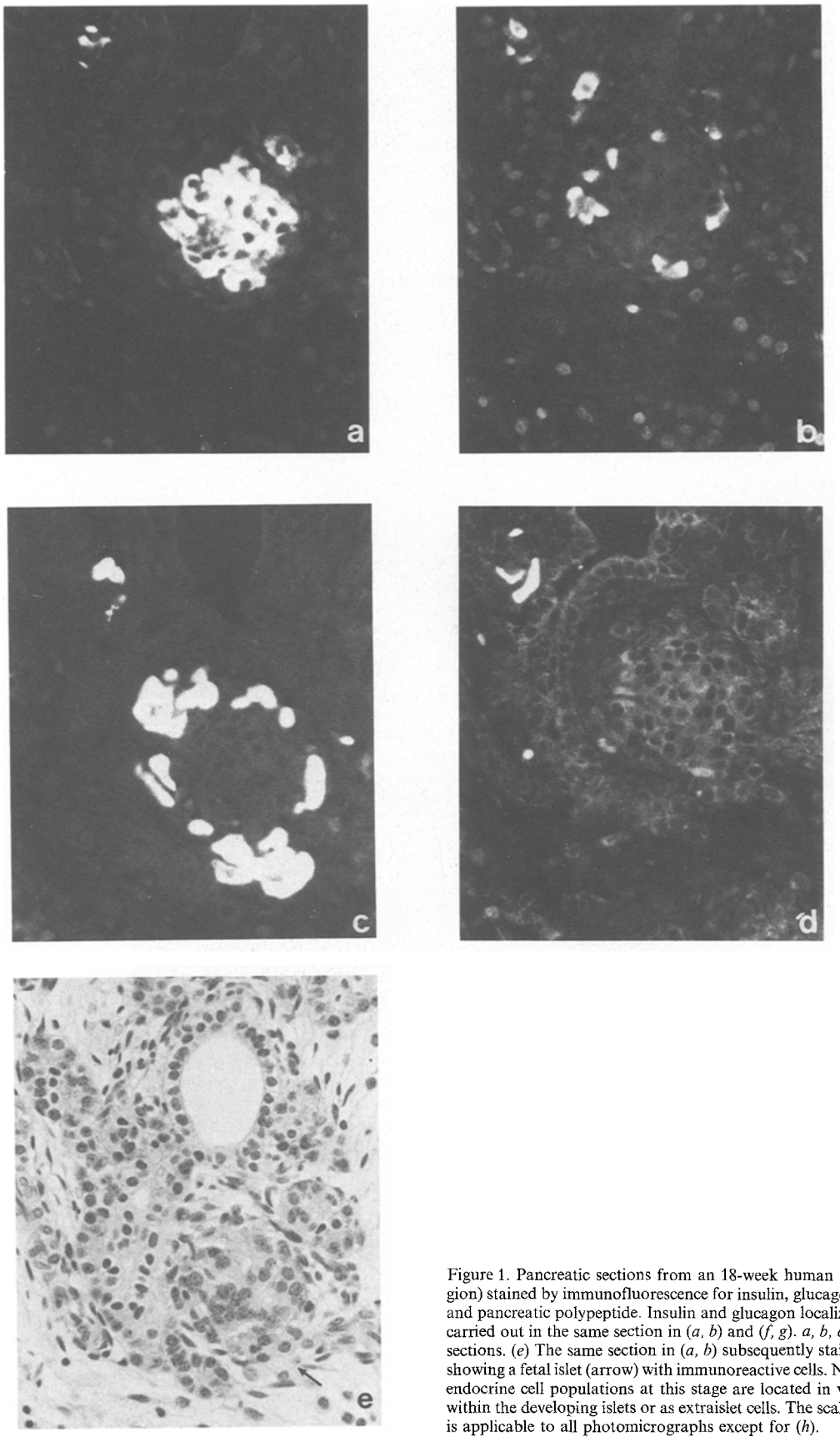
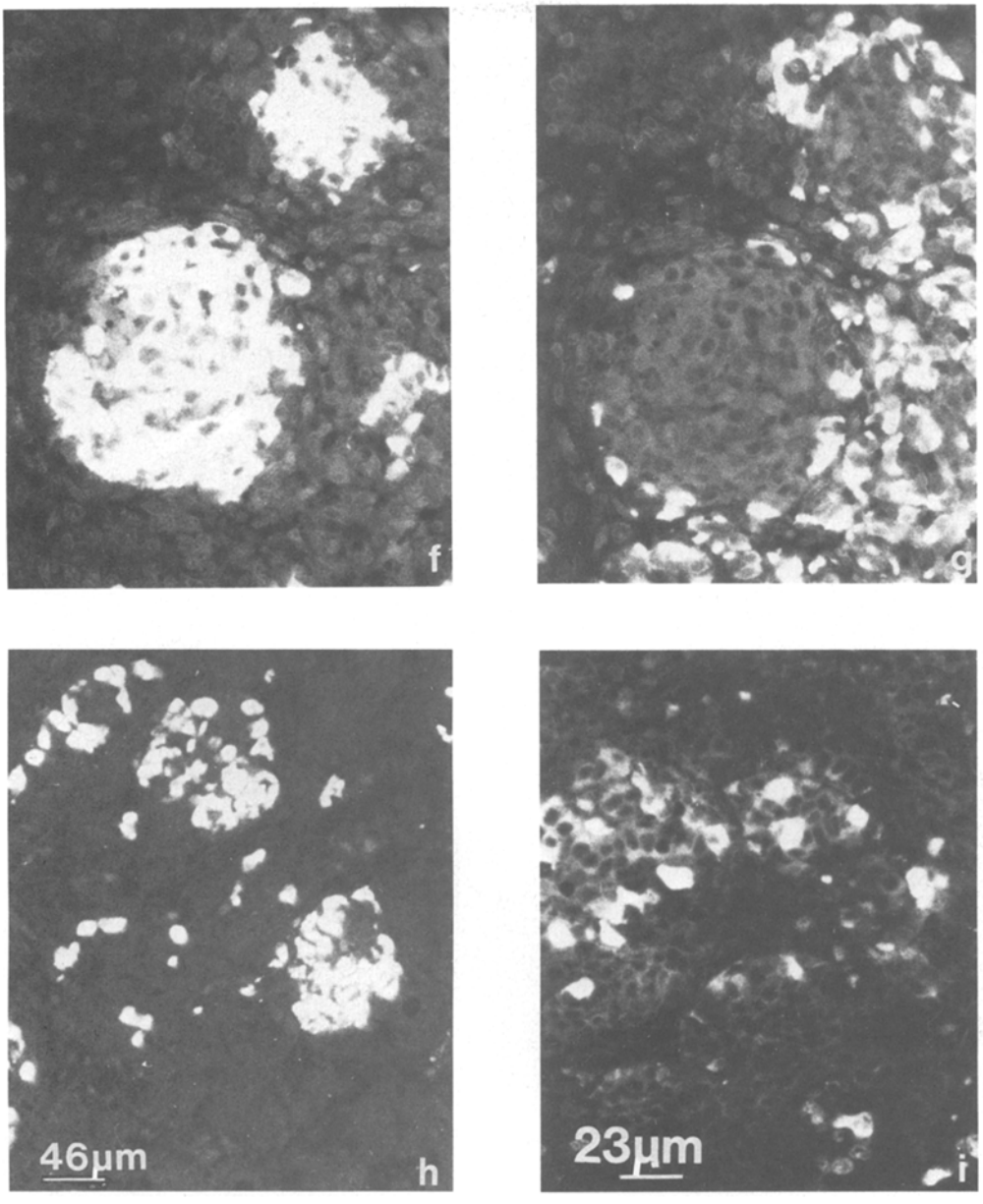


Figure 1. Pancreatic sections from an 18-week human fetus (splenic region) stained by immunofluorescence for insulin, glucagon, somatostatin and pancreatic polypeptide. Insulin and glucagon localization have been carried out in the same section in (*a, b*) and (*f, g*). *a, b, c* and *d* are serial sections. (*e*) The same section in (*a, b*) subsequently stained by H and E showing a fetal islet (arrow) with immunoreactive cells. Note that the four endocrine cell populations at this stage are located in varying numbers within the developing islets or as extraislet cells. The scale indicated in (*i*) is applicable to all photomicrographs except for (*h*).



concentrations of glucagon and insulin on days 11 and 12 respectively [71] and somatostatin on day 16 [26, 82]. Pancreatic concentrations of the three hormones have been shown to increase dramatically in the perinatal period and is consistent with morphometric data [26, 66, 67, 81, 82].

Human

There are numerous reports on the qualitative development of the human endocrine pancreas and their hormonal products in the fetus using histochemical and immunohistochemical procedures and electron microscopy [9, 16, 24, 27, 30, 61, 62, 75, 89, 104, 107]. More recently, the ontogeny of the four pancreatic hormones has been studied by morphometry after immunohistochemical staining [22, 23, 60, 98, 99]. In the 8–10-week-old fetal pancreas studied by Stefan et al. [98] a few insulin and somatostatin immunofluorescent cells were identified while glucagon and PP cells were extremely rare. From the twelfth week onwards, the total volume of insulin, glucagon, somatostatin and PP-containing cells in the pancreas increased with gestational age. Somatostatin cells were more abundant than glucagon and PP cells after 17 weeks. A sizeable proportion of endocrine cells at the 8–10-week stage reacting exclusively with anti-glicentin serum was noted. This cell population decreased and disappeared in later stages and was replaced by the adult glucagon/glicentin immunoreactive cell. However, the scarcity of glucagon cells during very early gestation and the presence of distinct glucagon and glicentin cell-types have not been emphasized by others [23, 60]. One possible reason for this discrepancy may be the use of antisera of different immunocytochemical specificities. Stefan et al. [98] also reported that PP cells were more numerous in the ventral rather than the dorsal region.

The distribution of insulin, glucagon, PP and somatostatin is shown in figure 1 where serial sections obtained from an 18-week human fetal pancreas (splenic end) have been stained for the four hormones by immunofluorescence. At this stage of gestation the endocrine cells are distributed within the developing islets and in the exocrine parenchyma. Pancreatic content of insulin, glucagon, PP and somatostatin determined by radioimmunoassay has been reported

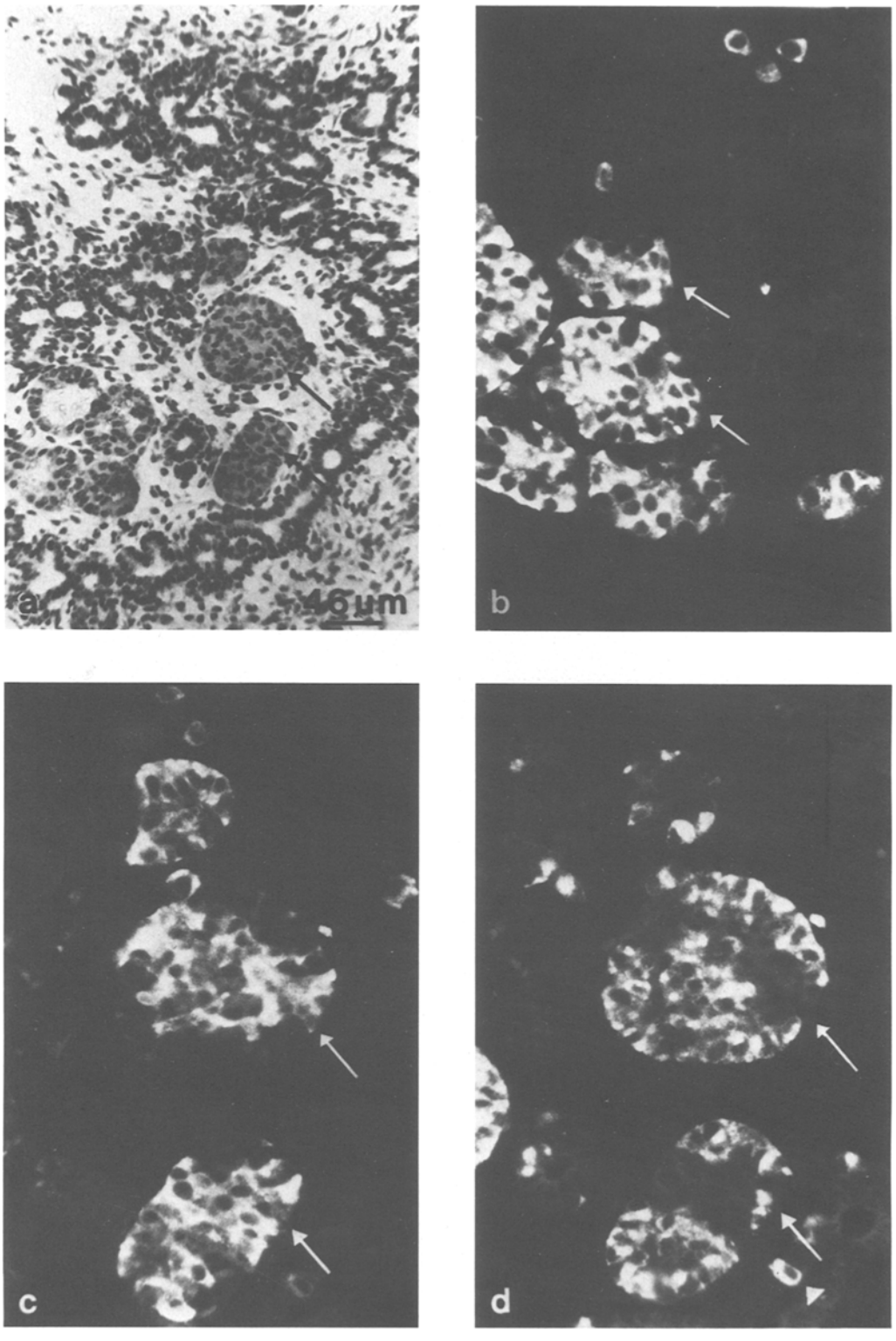
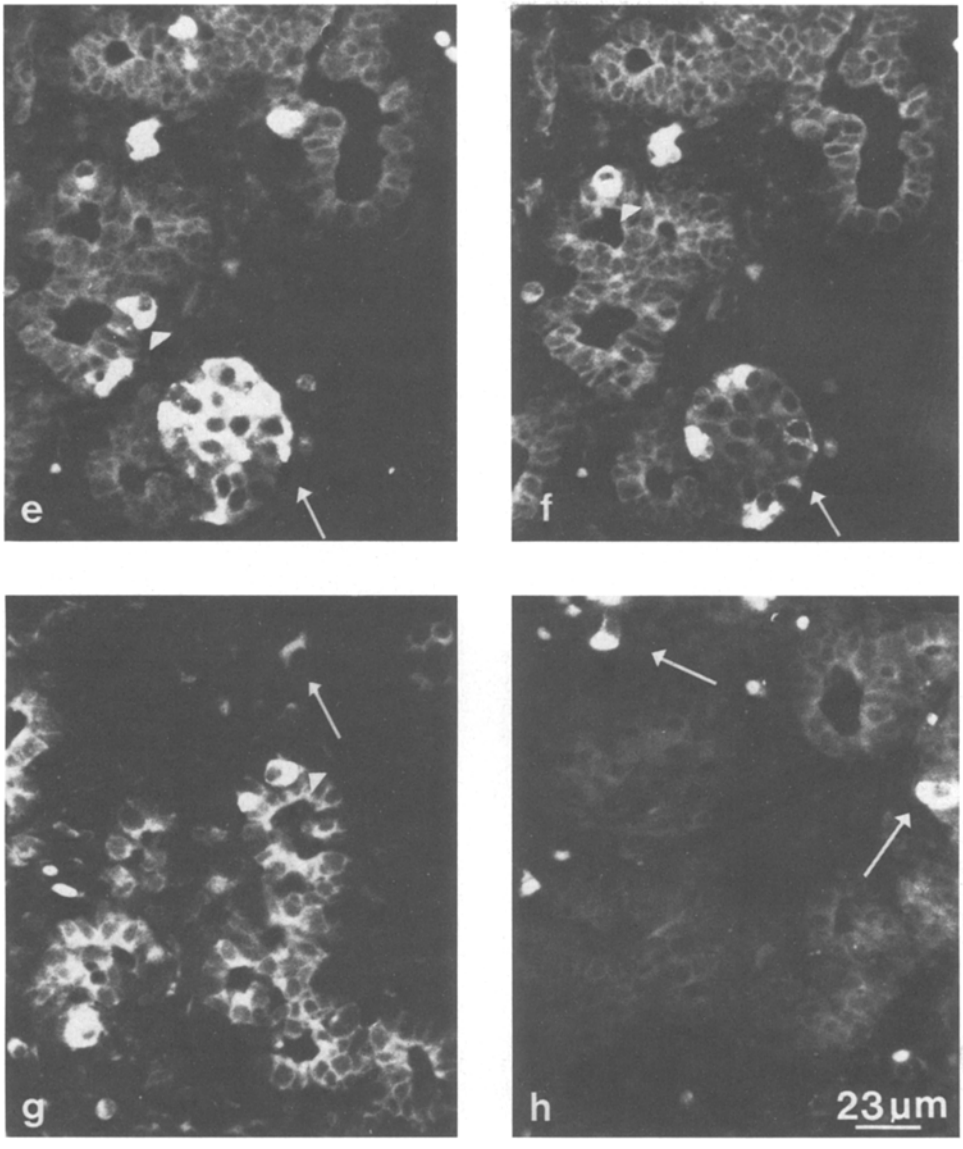


Figure 2. Pancreatic sections from a day 40–45 ovine fetus stained either by H and E (*a*) or by immunofluorescence for insulin (*b, c, e*), glucagon (*d, f*), pancreatic polypeptide (*g*) and somatostatin (*h*). All arrows point to fetal islets containing immunofluorescent cells. In (*c, d*) and (*e, f*) a single section has been stained simultaneously for insulin and glucagon cells. Islets indicated by arrows in (*a*) are identical to those in (*c*) and (*d*). Arrowhead point to immunofluorescent cells located in pancreatic ductules. The scale indicated in (*h*) is also applicable to (*b*)–(*g*). (Reproduced with permission from reference 84.)

previously[43, 63, 91, 107]. During the 12–19.5-week stage Goldman et al.[43] have demonstrated a progressive increase in the content of insulin, glucagon and somatostatin with advancing fetal age with an abrupt rise after 18 weeks. In contrast, no linear increase in tissue content of PP was demonstrated.

The ontogeny of gastric-inhibitory-peptide has been studied in the human fetal pancreas of the 5–24-week stage by im-



munocytochemistry and was first detectable at 14 weeks in the glucagon-containing cells[59]. Recently, the ontogeny of TRH in the pancreas of human fetus of 15–36 weeks of gestation[65] has been studied by radioimmunoassay. The hormone was first detected at 15 weeks and increased progressively until 34 weeks and declined subsequently.

Pig

The ontogenic development of endocrine cells in the porcine gut and pancreas has been recently reported by immunocytochemistry[8]. At four weeks of gestational age (gestational period in the pig: 120 days), insulin, glucagon and somatostatin cells were observed in the dorsal primordium but not PP cells. Somatostatin cells were less numerous than insulin and glucagon cells. At this fetal stage, PP cells were present in the ventral region which at 10 weeks also contained weakly staining insulin, glucagon and somatostatin cells. Pancreatic polypeptide cells increased in number in the ventral region with increasing fetal age but remained fewer than the glucagon cells.

Ruminants

In ruminants, ontogenic studies on the four major pancreatic hormones have been incomplete[25, 84–87]. By radioimmunoassay D'Agostino et al.[25] have recently reported that insulin content per wet weight of pancreatic tissue in the first trimester was similar to that in the adult animal and then increased progressively attaining a seven-fold increase in the third trimester compared with the adult tissue. In contrast, serum concentrations remained constant throughout gestation. Using immunofluorescence, we have recently studied the ontogeny of insulin, glucagon, PP and somatostatin in the bovine fetus from approximately day 100 of gestation to term[86] (bovine gestational period: 270 days). At day 100, the majority of endocrine cells stained for insulin and glucagon while somatostatin and PP cells were fewer in number. Somatostatin cells, however, became more numerous at later stages of gestation but PP cells remained low.

In the fetal sheep, low levels of serum PP are present in late gestation which rise rapidly to about 30% of adult value in the week before birth[95]. The localization of the four major

hormones during gestation has been studied in the fetal sheep pancreas recently in this laboratory[84, 85, 87]. At the earliest stage examined (day 40–45, gestation period in sheep: 150 days) insulin and glucagon immunoreactive cells were the predominant endocrine cell populations and were located either in the developing islets or as single scattered cells in the epithelium of the embryonic ductules (fig. 2)[84]. These cells became increasingly confined to the developing islets at later stages of gestation when PP and somatostatin cells also become more numerous. Earlier radioimmunoassay studies have demonstrated the presence of insulin and glucagon in plasma and pancreatic extracts of sheep fetuses throughout much of fetal life[2, 5, 12]. The hormone levels in fetal serum were found to be independent of maternal levels.

Other mammals

Previous studies by radioimmunoassay have indicated the presence of insulin and glucagon in the fetal pancreas and plasma of the rabbit of late gestation[14, 33, 68] (gestational period in the rabbit: 31 days). Pancreatic insulin was detected by day 20 with the tissue content being maintained until term[68]. During the same period plasma levels of fetal insulin remained essentially unchanged but glucagon levels increased significantly[14]. Plasma levels of both hormones were different from the mother suggesting that secretory activity of the fetal endocrine pancreas of the rabbit is functional during late gestation. In the fetal foal plasma insulin has been measured during the second half of gestation (gestational period in the horse: 335–350 days) and was found to be significantly less than the corresponding maternal value[36].

The cellular distribution of insulin, glucagon, somatostatin and PP has recently been examined immunohistochemically in the fetal pancreas of the guinea pig of late gestation[83] (gestational period in the guinea pig: 63 days). All four cell-types were present at the stage investigated. Insulin, glucagon and somatostatin immunoreactive cells were present not only in the fetal islets but also as scattered cells in the exocrine region. However, in the adult these cells were confined almost exclusively to the islets. The predominantly exocrine distribution of PP cells in the fetus was maintained in the adult. In the guinea pig as well as in several other mammalian species, complete ontogenic data, on the pancreatic hormones await further studies.

Biochemical studies of fetal pancreatic hormones

Studies on the control of biosynthesis of fetal pancreatic hormones, their translational processing and hormone structure are limited. There is, however, indirect evidence on the high degree of immunological and structural homology between maternal and fetal pancreatic hormones and also between mammalian species[100]. A significant degree of amino acid substitutions has been known for some time for insulin[96] and recently for glucagon[47] in the guinea pig. In the rat from about day 15 until birth there is a marked increase in the concentration of pancreatic insulin which is accompanied by a co-ordinate increment in pro-insulin mRNA[81]. During this stage glucose is believed to stimulate insulin biosynthesis at the translational level similar to the adult[19, 48]. Watts and Gain[106] have reported that while fetal pancreatic insulin in the rat is monomeric, it is complexed to a higher molecular weight form in serum with reduced biological activity. In other studies, fetal insulin in the rat has been shown to exist in two molecular forms as in the adult[41]. The biosynthesis of fetal glucagon has been examined in extracts of bovine pancreas where large glycoprotein-like precursor molecules have been demonstrated[103].

Physiological role of fetal pancreatic hormones

There is now some experimental support for the view that fetal pancreatic hormones, particularly insulin and glucagon may be physiologically important, at least in the later stages of gestation. Despite the experimental demonstration of the four major pancreatic hormones in the early fetus of the human[98], pig[8] and sheep[84], their role during this initial stage is unclear. The contribution by maternal insulin and glucagon to fetal development during early pregnancy may be indirect since these hormonal peptides have been shown not to cross the placenta[3, 4]. Insulin and glucagon secretion in the fetus in response to secretagogues such as glucose and amino acids have been examined in a number of species by in vivo and in vitro procedures[1, 11, 13, 15, 34, 35, 63, 69, 70]. Results from such studies have often been conflicting. In the fetal sheep before birth a range of amino acids has been shown to influence glucagon and insulin release[13]. In the chronically catheterized fetal sheep of advanced gestation arginine infusion elicited a rapid release of insulin whereas the insulin response to glucose was more variable and slower[35]. Studies carried out by others at later stages of gestation imply that pancreatic insulin release is attuned to circulating substrate concentrations in the fetus[78]. In the past, both in vivo and in vitro investigations have suggested that the fetal β-cell responds poorly to glucose[35, 46]. However, several other studies now suggest that fetal insulin release can be sensitive to glucose. In the rat the mechanisms of biphasic insulin release develop during the late fetal life[52–54]. In the chronically catheterized fetal foal[36] and fetal pig[38] β-cell response to exogenous glucose has been shown to increase with increasing fetal age during late gestation. In the human fetal pancreas of 14–20 week gestation, perifusion studies by Maitland, Parry and Turtle[64] suggest that the mechanisms involved in glucose-induced insulin release as well as synthesis are present although a number of previous studies did not support these findings[46, 73, 88]. The significant glucose-induced insulin secretion reported by Maitland et al.[63] is probably due to their use of the superior perifusion system to study insulin release with careful monitoring of the viability and secretory competence of the tissue. Despite the conflicting data concerning glucose-induced insulin release in the fetus there is strong evidence to suggest that insulin may be a physiological regulator of glucose metabolism and fetal growth. These postulated functions for insulin in the fetus are supported by studies on experimental fetal hypo- and hyperinsulinemia[18, 37, 39, 79, 102]. For example, surgical pancreatectomy in the fetal sheep causes severe growth retardation[37] and clinical studies show that diabetic infants or infants with pancreatic agenesis are small at birth[92, 94]. Conversely, experimental hyperinsulinemia in rat[79] and monkey fetuses[102] results in fetal hypertrophy.

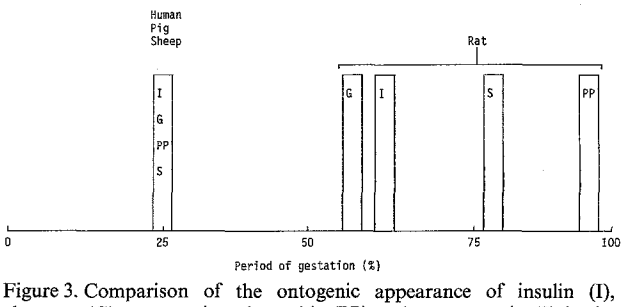


Figure 3. Comparison of the ontogenic appearance of insulin (I), glucagon (G), pancreatic polypeptide (PP) and somatostatin (S) in the human, pig, sheep and rat when the periods of gestation are converted to a uniform scale.

Summary

The ontogeny of insulin, glucagon, PP and somatostatin in the mammalian fetal pancreas has been examined in recent years largely by immunocytochemistry and in some instances by radioimmunoassay. Complete ontogenic data are available only for the rat, human, pig and sheep. Figure 3 compares the time of appearance of the endocrine cell-types within the fetal pancreas when the periods of gestation of the four species are converted to a uniform scale. The striking ontogenic difference in the rat probably reflects the immaturity of the rodent fetus at birth compared with the human, pig and sheep. In the fetal pancreas, differences in cell number of glucagon and PP cells in the dorsal and ventral lobes become apparent from an early gestational period.
Factors responsible for the functional and structural maturation of the fetal pancreatic endocrine cells and the processes involved in pancreatic organogenesis are poorly understood. Studies in these areas would have clinical implications since it may be possible in the future to employ agents for selective replication of fetal β-cells for transplantation in patients with Type I diabetes, bearing in mind that such cells must have the capacity to respond to normal stimuli and repressors when transplanted. The presence of the other islet cell-types may be obligatory for these appropriate responses. This would require a more complete knowledge of those factors which produce the normal selectivity of the four hormonal cell-types.

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An alternative regulatory pathway of the acute phase response: the role of fibroblast-derived interferon-β_2

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Bacterial infection and tissue injury both lead to a series of adaptive homeostatic reactions known collectively as the acute phase response. This includes fever, leucocytosis, increased secretion of glucocorticoids, transfer of amino acids from muscles to the liver, and drastic changes in synthesis of certain plasma proteins (acute phase reactants) such as fibrinogen (FBG), haptoglobin (HPT) and α_1-acid glycoprotein (AGP)[1]. Most of the phenomena of the acute phase response are attributable to the action of inflammatory cytokines, a group of low molecular weight proteins produced by monocytes and named originally leucocytic endogenous mediator or endogenous pyrogen, but more recently shown to be mixtures containing interleukin-1 (IL-1), tumor necrosis factor (TNF) and some other hormone-like factors[2, 3]. Because of the complexity of the intact organism, experiments in vivo cannot be expected to indicate unequivocally which of the cytokines are responsible for induced synthesis of acute phase proteins. However, recent work with primary cultures of rat and mouse hepatocytes or established lines of human hepatomas have shown that liver cells are stimulated to some extent by purified or recombinant IL-1 and TNF[4, 5]. Although active, these cytokines do not elicit synthesis, in the same system, of the whole range of acute phase proteins, as occurs when conditioned media from endotoxin-stimulated monocytes-macrophages are used[6, 7]. These observations strongly supported the existence of a separate hepatocyte stimulating factor (HSF) (or factors), as postulated earlier by several authors[8–12]. It has become clear that HSFs are distinct from IL-1 and TNF, and are produced not only by monocytes-macrophages but also by some leukemic cell lines[13], by keratinocytes and by human squamous carcinoma cells[10, 11]. The precise nature of any type of HSF remained unknown until, recently, Gauldie and co-workers[13] discovered that interferon-β_2 (IFN-β_2) produced by human fibroblasts elicits a strong acute phase response in cultured liver cells, thus qualifying as yet another HSF, or the main hepatocyte stimulating factor. The properties of IFN-β_2 appear to be very similar to monocyte-derived HSF. Despite its name IFN-β_2 shows rather low antiviral activity. However, because it enhances growth of B-lymphocytes and murine B-cell hybridoma and plasmocytoma it has also been named BSF-2[15–17].

In the experiment described by Gauldie et al.[14] human hepatoma cells Hep G 2, or rat hepatocytes as a monolayer, were cultured for 2 days with the addition of conditioned media from human peripheral blood mononuclear (PBM) cells, from human lung fibroblasts, or with preparations of human recombinant IL-1 β and human recombinant IFN-β_2. The typical acute phase proteins secreted by the hepatocytes or the hepatoma cells were determined by electroimmunoassay as described previously[9].

As shown in the table, conditioned media from mononuclear cells and fibroblasts, as well as Hr IFN-β_2, stimulated production of all those acute phase proteins tested, including