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Hepatocyte-like cells in the pancreatic islets: study of the human foetal pancreas and experimental models

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Summary. *Objective:* To determine the existence of hepatocyte-like cells in the human foetal pancreas. *Study design:* Foetal pancreas was examined in parallel with two experimental models involving pancreatic tissue regeneration. Foetal pancreases (n=20; 10 to 18 weeks) were obtained from spontaneous abortions and were histologically examined using haematoxylin-eosin and PAS staining. Tissues from food-deprived and copper-deficient female Wistar rats were studied following D-penicillamine administration, and tissue from female hamsters was evaluated following administration of a pancreatic carcinogen. Histological examination in animal studies included haematoxylin-eosin staining, and diaminobenzidine histochemistry. *Results:* The presence of a characteristic cell-type whose morphology was distinct from islets cells and exocrine pancreas cells was observed in human foetal pancreatic islets. These cells were morphologically similar to hepatocyte-like cells and were compatible to those observed in the experimental models. Topographical relationships suggest that these originate from stem cells which are related to the pancreas duct cells. *Conclusion:* We conclude that hepatocyte-like cells or precursors exist in the human foetal pancreas.

Key words: Foetal pancreas, Hepatocyte-like cells, Wistar rat

Introduction

Owing to the relatively few studies on the foetal pancreas to date, a number of questions still exist concerning the origin of endocrine islet cells, the time of their appearance during embryogenesis and their topographical organization. Several reports indicating the presence of hepatocyte-like cells in the pancreas of animal models following certain experimental or pathological conditions, such as metabolic disorders, carcinogenesis, as well as during normal embryogenesis have triggered a series of investigations on the histological structure of the foetal pancreas, and on the range of potential pancreatic cell differentiation (Like and Orci, 1972; Scarpelli and Rao, 1981; Reddy et al., 1984).

Nonetheless, several questions still remain regarding the histological structure of the foetal endocrine pancreas, including: 1) the nature of the stem cells of the endocrine pancreas; 2) whether the endocrine islet cells are derived from the small ductal cells of the exocrine pancreas; 3) whether there are cell types which express the structural and functional directions of the foetal endocrine pancreas, which may be either overshadowed or lost in the adult human pancreas; and 4) the physiological and pathological roles of these «stem» cells.

During embryological development, the mammalian liver and a substantial part of the pancreas are formed from the endoderm of a common embryonic structure derived from the wall of the ventral embryonic intestine (Dudek and Lawrence, 1988). The factors which direct the differentiation of cells, including the islet cells, remain to a great extent unknown. It is of interest than in some studies the appearance of hepatocyte-like cells in the pancreas of experimental animals has been observed under various experimental conditions, as well as during normal embryogenesis (Hoover, 1986). From both the published histological photographs and from morphological descriptions, it appears that these hepatocyte-like cells are directly associated with the islets of the endocrine pancreas. Furthermore, the presence of pancreas neoplasms with histological characteristics of liver neoplasms has also been reported (Hruban et al., 1987). The fact that these pancreatic hepatocyte-like cells always develop in a close relationship to the islets of the pancreas, suggest that these hepatocyte-like cells may be derived from cells initially found in the islets (Reedy et al., 1984).

The existence of hepatocytes or even hepatocyte-like cellular forms in the islets of the human pancreas has not been confirmed to date. This may be related to the fact that in normal physiological states intense regeneration

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of cell activity is absent, unlike certain experimental conditions where tissue restoration is active, such as during the period of rejuvenation following the application of carcinogens or food deprivation (Rao et al., 1983, 1986). However, conditions of intense regeneration and in general, of cellular activity exist in embryonic tissues. Hence, it is likely that the foetal pancreatic tissue may reserve greater multipotential capabilities when compared to the adult tissues. Since cells with hepatocyte-like characters or even other precursor or relative forms have not been described in the islets of the human foetal pancreas to date it was examined in order to isolate precursor or relative cellular forms, as well as completely differentiated pancreatic hepatocyte-like cells. To confirm the technical and morphological validity of the human foetal observation, these histological examinations are carried out in conjunction with experimental animal models known to demonstrate hepatocyte-like cells in the pancreas.

Materials and methods

Morphological study of human foetal pancreas

The pancreas of 20 human foetuses were examined at the Department of Anatomy, Histology and Embryology at the University of Ioannina, Medical School. Foetal tissue was obtained from spontaneous abortions from women who were hospitalized in the Clinic of Obstetrics and Gynecology at the University Hospital of Ioannina. The women did not suffer from diabetes or any other metabolic disease. Foetal weight and dimensions were used to calculate foetal age according to the method of Moore (Moore, 1974). The foetuses ranged from 10 to 16 weeks of age (10 weeks (n=4); 12 weeks (n=6); 16 weeks (n=5); and 18 weeks (n=5).

Foetuses were subjected to macroscopic and microscopic examination, and were then preserved in a solution of 7% formalin. The removal of the foetal pancreatic tissue was done following a vertical incision above and subumbilically. After removal, the pancreatic tissue was placed in a solution of 7% formalin. The tissue was then dehydrated and embedded in paraffin. Sections, 5-8 μm thick, were stained with haematoxylin-eosin. In addition, a diastase test for glycogen was performed by staining with PAS.

Experimental animal models

Pancreatic regeneration in experimental animal models appears to offer a greater possibility for examining cell differentiation of pancreatic hepatocyte-like cells. The histological and histochemical experiments were performed in the Department of Anatomy, Histology and Embryology according to the Guidelines established by the University of Ioannina Animal Welfare Committe for the care and use of non-human animals. The regeneration of the pancreas occurred after food deprivation, dosage and toxic and carcinogenic substances or deprivation of copper in rats, followed by a period of rejuvenation on a regular diet (Rao et al., 1983, 1986). In the present study, the following groups of experimental animals were used:

Group I: Penicillamine-Treated Rats

Female Wistar rats (220-270 g) were used to examine the effects of copper deficiency on pancreatic hepatocyte-like cell differentiation. Forty-five animals were fed *ad libitum* with rat chow, while forty rats were kept on half the normal diet for approximately 9 weeks. D-penicillamine (1250 mg) was administered every 48 hours by being dissolved in 200 ml of drinking water. At the end of 9 weeks, blood was taken from 20 rats and analyzed for copper content. After 9 weeks, the remaining 16 rats were returned to a regular diet and D-penicillamine administration was interrupted. These rats were studied after a rejuvenation period of three months. Five rats were used from the beginning of the experiment as controls. These animals were not subjected to any treatment and were sacrificed at the end of the experimental period.

Blood was taken from the left atrium of the heart in rats which had been anaesthetized in a chloroform chamber and placed in chilled, heparinized tubes. The determination of plasma copper levels was made in the Laboratory of Experimental Physiology of the University of Ioannina, Medical School. Plasma copper levels were determined by mixing plasma with an ionized solution of Na and K (25 mEQ/L and 50 mEq/L, respectively) and using atomic absorption spectrophotometry (Perkin-Elmer Model 560), as previously described.

Following blood collection, all rats were sacrificed and the pancreas was carefully removed and placed in a solution of formalin for histological examination. After processing and preparation, tissue sections were stained with haematoxylin-eosin, followed by diaminobenzidine (DAB) for the demonstration of peroxisomes, and finally stained with toluidine blue.

Group II: Hamsters

Female hamsters (25 g) were used to examine the effects of the pancreatic carcinogen on cell differentiation. Food (pellets, ELVIZ, Greece) was available *ad libitum* for control animals for the entire experimental period, while 17 experimental animals were deprived of food for six days. Water was available ad libitum for all animals. Experimental animals were injected hypodermically with DL-ethionine (500 mg/kg BW) daily. On day 7, experimental animals received an injection of L-methionine (800 mg/Kg BW, ip) and the animals were returned to a regular diet. Sixty hours after the methionine injection, the pancreatic carcinogen N-nitrosodiethylamine (N-NDEA) was administered hypodermically (45 mg/Kg BW). After 6 months, all animals were sacrificed and the pancreas was removed

and placed in a solution of formalin (7%). Sections were prepared from the tissues, stained with haematoxylin-eosin, followed by DAB for peroxisome detection, and finally stained with toluidine blue.

DAB Histochemistry for peroxisome detection

DAB histochemistry was performed as previously described (Novikoff and Goldfisher, 1969) with the following modifications developed by the Department of Anatomy, Histology and Embryology. The tissue was fixed for four hours in a cold fixing agent consisting of a glutaraldehyde solution (2.5%) in 0.1M sodium cacodylate buffer. After fixation, the tissue was washed (3 whases in 1 hour) and was kept until the following day in a buffer solution (0.1M sodium cacodylate and 0.02M sucrose). Following immersion in a DAB solution (50 mg DAB; 25 ml 2-amino-2-methyl-1,3 propranodiol buffer solution; and 0.5 ml of 1% H_2O_2; pH 9.0) at 37 °C for 1 hour, the tissue was then placed in a solution of 2% osmium tetroxide (OsO_4) in 0.1M S-collodine buffer, pH 7.4, for 1 hour. Finally, the tissue was embedded in EPON resin, and sections, 0.5-1 μm thick, were prepared and stained with toluidine blue. A Leitz Wetzlar Ortholux II microscope was used for microscopic examination.

Results

Human foetal pancreatic tissue

From the foetal age of 10 weeks up to the age of 18 weeks, when stained with haematoxylin-eosin, the pancreas showed small ducts with a wall consisting of a range of epithelial cells which had large, pale nuclei and little cytoplasm. These ducts were found within a connective tissue substrate. At the age of 10 weeks, this connective tissue was abundant. Later, probably due to the numerous developing acinar cells of the exocrine pancreas and to the islets of the endocrine pancreas, the connective tissue was gradually confined. Along the length of each duct, from various points on its wall, new, smaller ducts emerged at the edge of which, new acinar cells of the exocrine pancreas developed. These cells had a densely coloured nucleus and an intensely basophilic cytoplasm (Fig. 1A,B). Colonies of cells around the ducts eventually composed the islets of the endocrine pancreas.

The islets were usually paler in colour than the exocrine pancreas. These cells were rather small with a densely-coloured nucleus and a small amount of amphophilic cytoplasm. Connective tissue was found to surround the ducts, as well as the newly forming islets (Figs. 2, 3). The appearance of a distinct characteristic cell type in the islets which differed clearly from the other islet cells of the exocrine pancreas, as well as from the islet cells of the adult human pancreas, was confirmed. These cells had a large, round and diffusely-coloured nucleus with abundant cytoplasm and were clearly found in a greater number than any other islet cell type. The cytoplasm of these cells upon staining with haematoxylin-eosin became eosinophilic, whereas with PAS staining, they became diffusely red (positive reaction for glycogen) (Figs. 4A,B).

Staining for iron was negative in these cells. According to the previously described morphological elements, these cells appeared to be hepatocytes or hepatocyte-like cells (Hoover, 1986).

It is important to note that some of the cells which stained positively with PAS, lacked characteristics such as abundant eosinophilic cytoplasm and a large round, pale nucleus. Such cells may possibly represent stem cells which have not yet begun to differentiate. Particularly, at the age of 10 weeks, both the newly-forming ducts, as well as the islets were scarce in an abundant connective tissue substrate.

The hepatocyte-like cells were easily located and were stained diffusely with PAS. At the age of 12 weeks the islets were better formed and more numerous. The hepatocyte-like cells were few and their cytoplasm appeared either diffusely coloured with the PAS stain or at various locations within the cell.

At the age of 18 weeks the exocrine pancreas was well-developed and there were plenty of islets. Acinar cells and islets seemed to grow further from the duct. The hepatocytes were now exceptionally scarce and their cytoplasm was only locally and diffusely stained with the PAS dye. This suggests that the glycogen of the cytoplasm was reduced. At intermediage ages, the findings appeared to be transitional.

Wistar rat paradigm

Penicillamine administration in Wistar rats produced a significant reduction in plasma copper levels when compared to controls (mean±SEM 48.0±5.2 (n=20) vs 117.5±6.0 (n=5) μg/dl, respectively; $p< 0.05$).

Histological examination of pancreatic islets from rats which had received penicillamine treatment using haematoxylin-eosin staining, demonstrated morphological characteristics similar to those also observed in the hamsters. In some islets, usually around the periphery, small groups of cells with a diffusely-coloured nucleus were observed. In many cases, these cells demonstrated an abundant diffusely eosinophilic cytoplasm (Fig. 5). Following DAB histochemistry of the rat pancreas, the islets demonstrated a typical pale appearance in contrast to the exocrine ducts, which stained with an intense blue. The cells of the islets had diffusely-coloured nuclei with a moderate quantity of cytoplasm. Animals given penicillamine and sacrificed after three months showed pancreatic islets with features of hepatocyte-like cells. Under these experimental conditions, these cells were usually single or in small groups. They showed a large, round, diffusely-coloured nucleus which was larger than any other cell of the pancreas which had a distinct nuclear membrane and, in most cases, a distinct nucleolus.

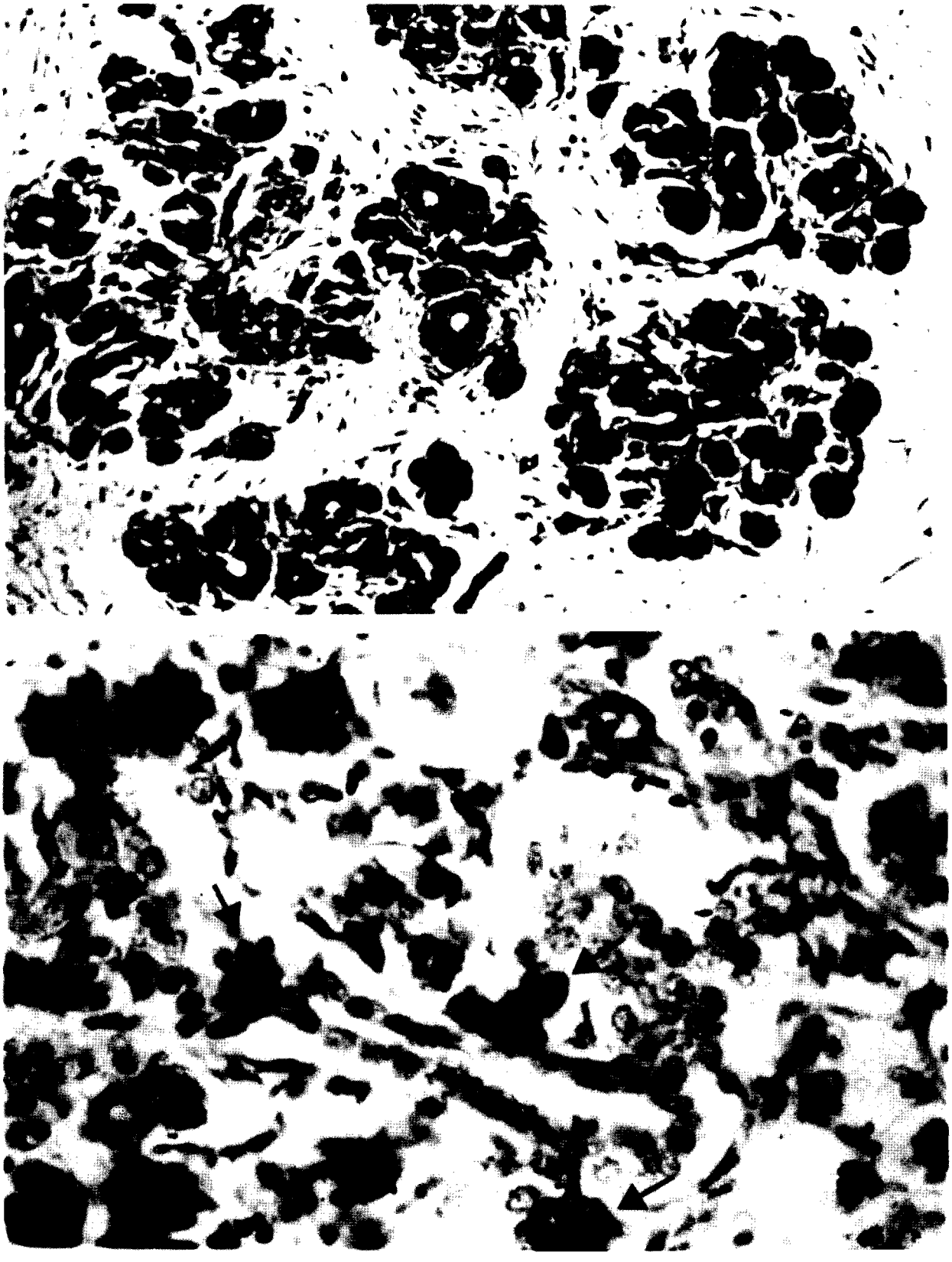

Fig. 1. A. Pancreas from a 10-week-old human embryo viewed with PAS staining. Ducts and adenocells are surrounded by abundant connective tissue (light area). x 16. **B.** Pancreas from 10-week-old human embryo viewed with haematoxylin-eosin staining. Dark-coloured cells representing adenocells (arrow) can be seen at the edge of a small duct in the lower middle portion of the photograph. x 400

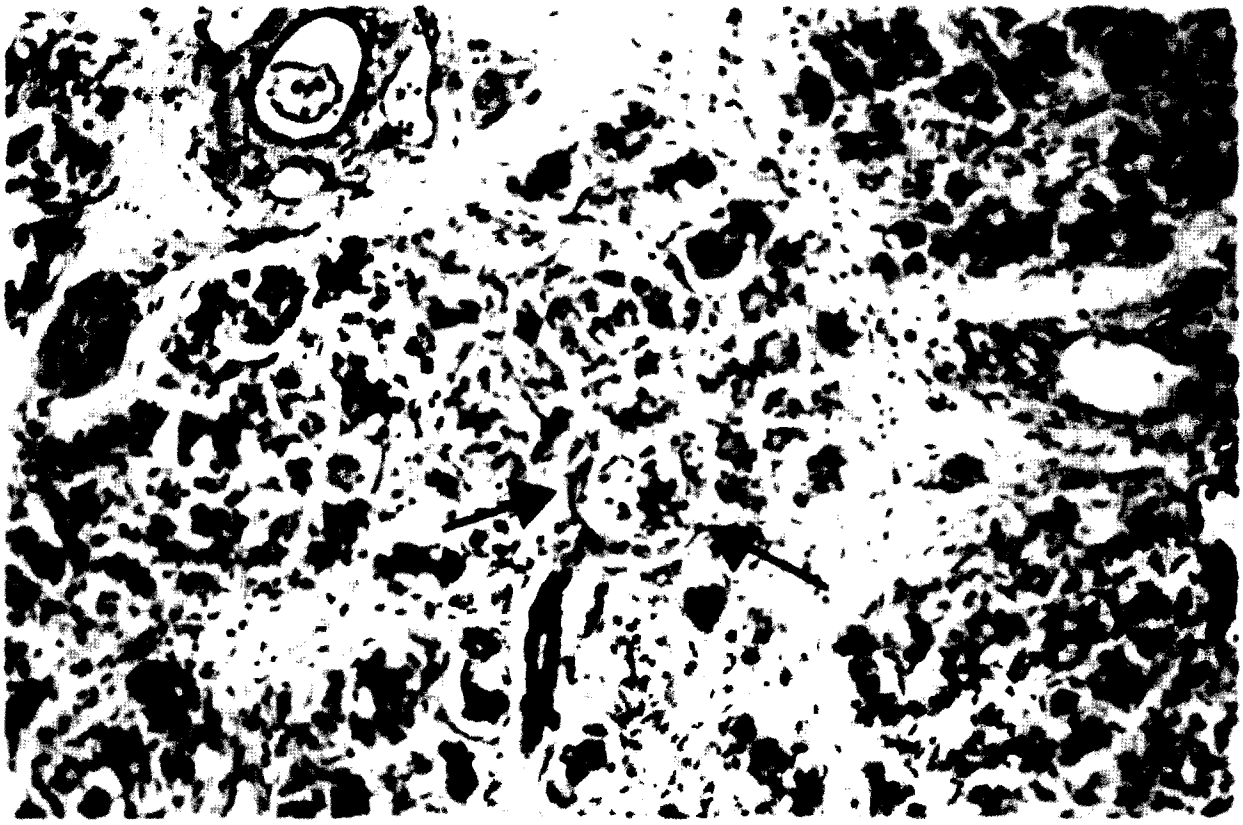

Fig. 2. Pancreas from a 12-week-old human embryo viewed with haematoxylin-eosin staining. Pancreatic islet emerging from a duct (middle and lower portion of the photograph) can be seen. Arrows indicate the periphery of the islet. x 160

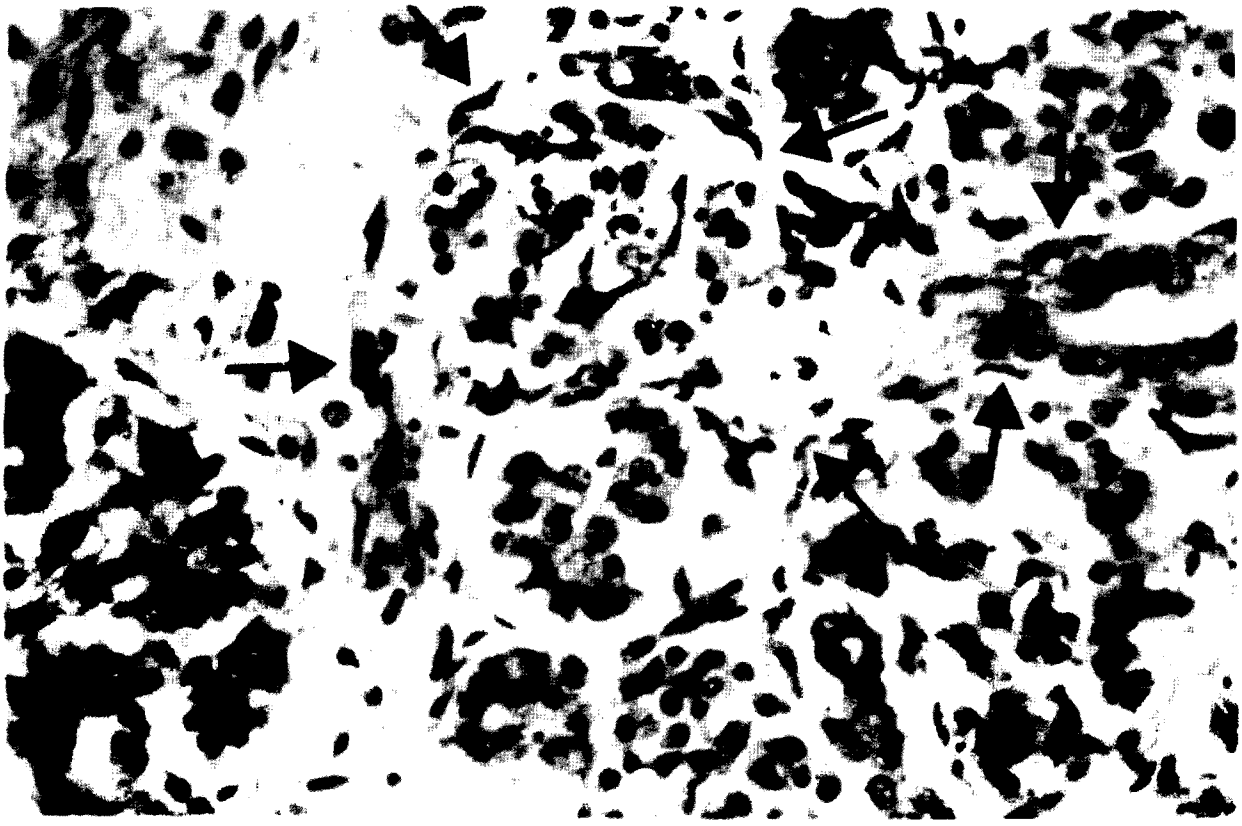

Fig. 3. Pancreas from a 12-week-old human embryo viewed with haematoxylin-eosin staining. A round pancreatic islet can be seen emerging from a duct in the right field. The connective tissue which surrounds the duct, also surrounds the newly-formed islet. Arrows indicate the fibroblasts of the connective tissue which surrounds the islet. x 400

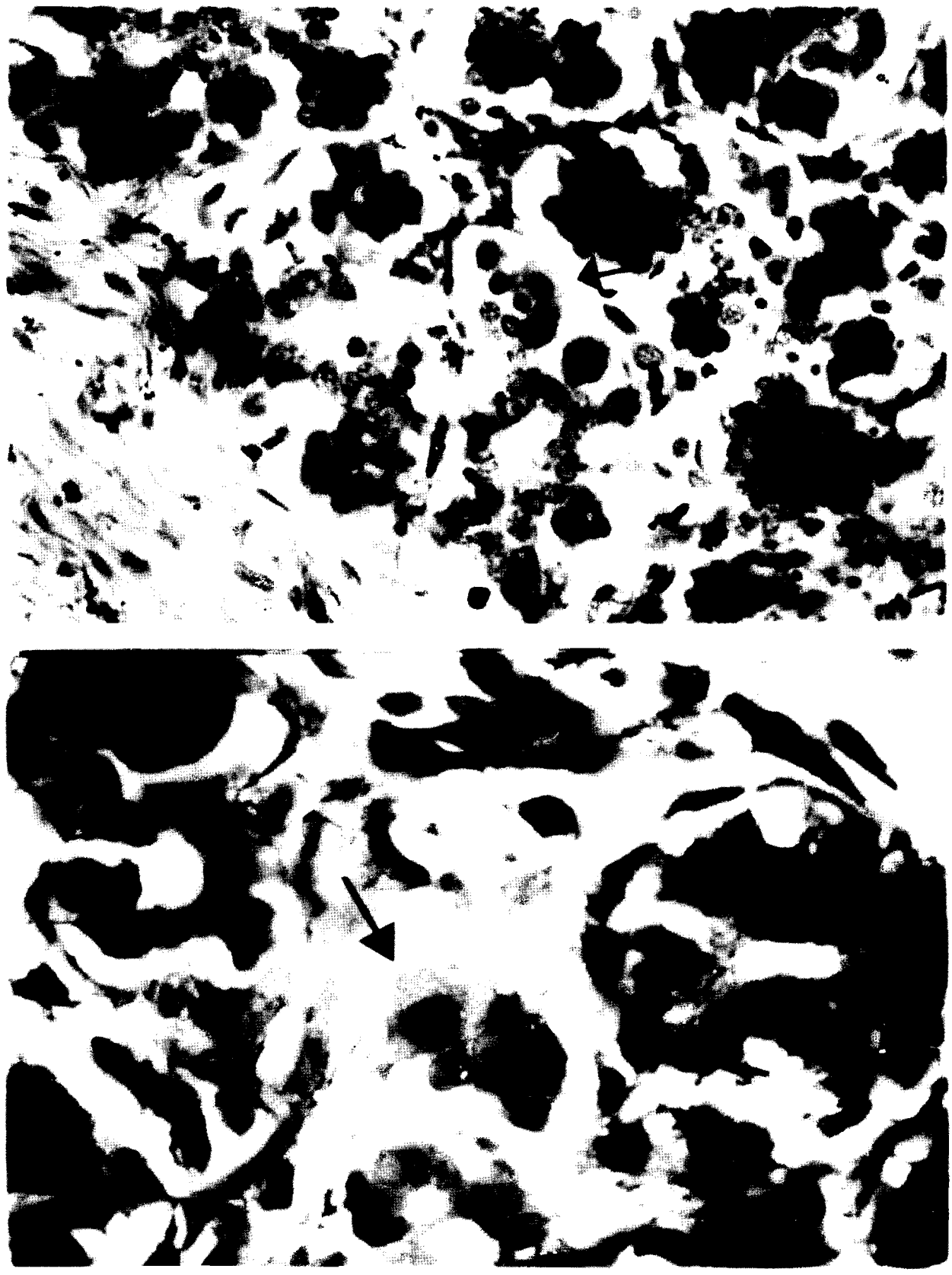

Fig. 4. A. Pancreas from a 16-week-old human embryo viewed with haematoxylin-eosin staining. A small islet can be seen in the centre of the photograph, representing a round grouping of pale cells, distinct from the dark-staining adenocells of the exocrine pancreas in the top right field. A hepatocyte-like cell (arrow), paler than most cells and with a large nucleus and abundant cytoplasm, is observed in the islet. x 400. **B.** Pancreas from a 10-week-old human embryo viewed with PAS staining. Groups of islet cells, which by definition are small and not densely stained, are seen in the centre. A hepatocyte-like cell (arrow) with a round nucleus and abundant cytoplasm which gave a positive PAS reaction, is seen among the islet cells. x 1,000

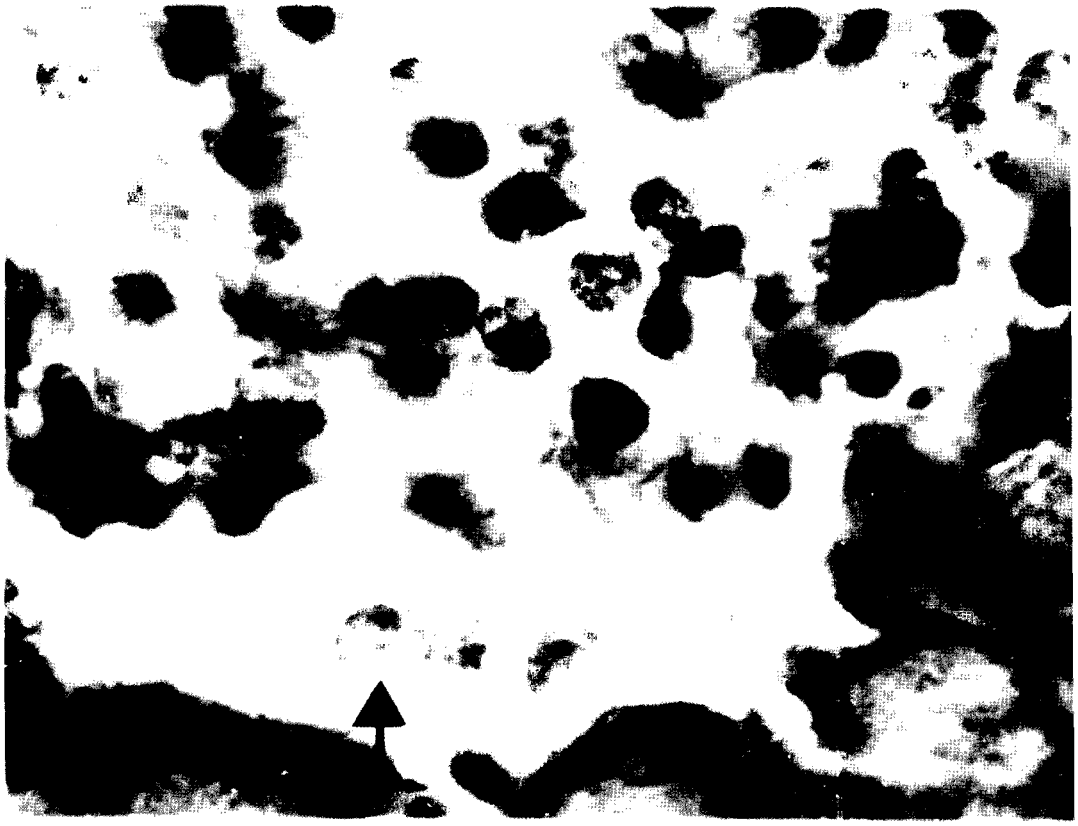

Fig. 5. Rat pancreas with haematoxylin-eosin staining. Portion of an islet (lower, light field) and portion of the exocrine pancreas (upper, dark field) are seen following D-penicillamine administration. Three cells with hepatocyte characteristics, including diffusely-staining nucleus, and distinct nucleolus are seen along the periphery of the islet (arrow). x 1,000

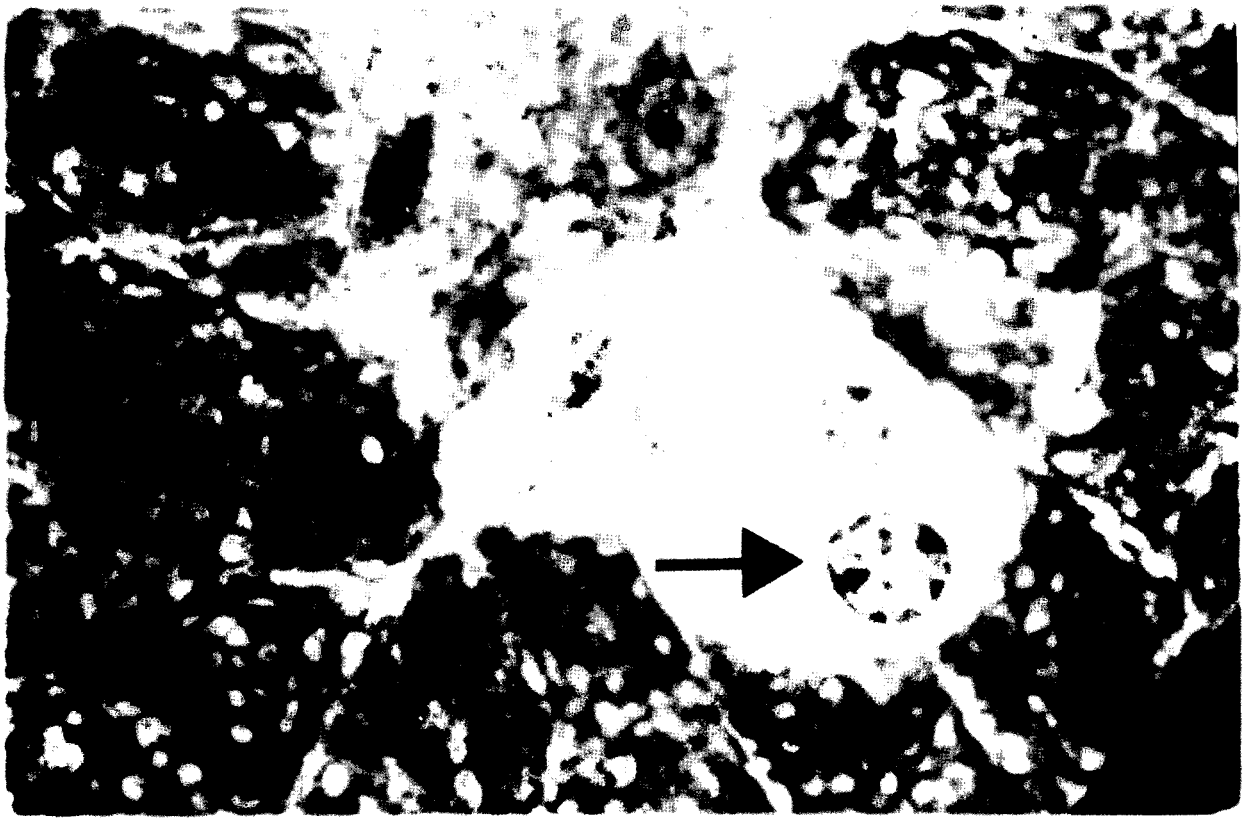

Fig. 6. Rat pancreas examined with DAB histochemistry and toluidine blue staining. Islet (light field) surrounded by exocrine pancreas is seen following D-penicillamine administration. An islet cell demonstrates hepatocyte characteristics, including large nucleus, distinct nucleolus and pronounced nuclear membrane. x 100

The cells also had an abundant, pale cytoplasm which in some cases demonstrated small black granules indicating the presence of peroxisomes. In addition, the cytoplasm in a number of cells showed large vacuoles (Figs. 6, 7). No significant differences were observed between rats in which penicillamine administration was interrupted and controls.

Hamster paradigm

Pancreatic islets from hamsters which were given nitrosodiethylamine, were examined after DAB histochemistry and toluidine blue staining. Cells with cytoplasmic granules with a positive reaction (dark staining) for peroxisomes were observed in some islets (Fig. 8). This was not observed in the section obtained from control animals.

Discussion

The presence of a characteristic cell-type with a morphology clearly distinct from islet cells and from cells of the exocrine pancreas was confirmed for the first time in the islets of the human foetal pancreas. According to their morphological features, these cells are classified as hepatocyte-like cells, although they may be a precursor/transitional form. This cell morphology is compatible with that observed in the experimental animal models described in the present study, as well as those previously reported (Scarpelli and Rao, 1987). Our observations suggest that the hepatocyte-like cells in the foetal endocrine pancreas may originate from stem cells. This would explain their topographical relationship with the pancreas islets (Dante et al., 1981; Rao et al., 1986). Our observations also indicate that pancreas islets may originate from pancreas ducts. With regard to this, it appears that the stem cells are most likely to be related to the duct cells (Rao et al., 1983).

Although a few authors have suggested that the original islet cells are visible at the end of the second month, most support the idea that the islet cells do not appear until the beginning of the third month (Like and Orci, 1972; Reiher et al., 1983; Grasso et al., 1986). According to Lui and Potler (1962), the initial islets are located in the intermediate connective tissue, while latter, they are located around the acinar cells. In the present study, the islets were always located very close to or even in contact with the ducts. In many cases, the connective tissue surrounded both the duct and the islet. In the early stages, the islet maintained its contact with the lower end of the duct.

The present study, as well as other studies, suggests

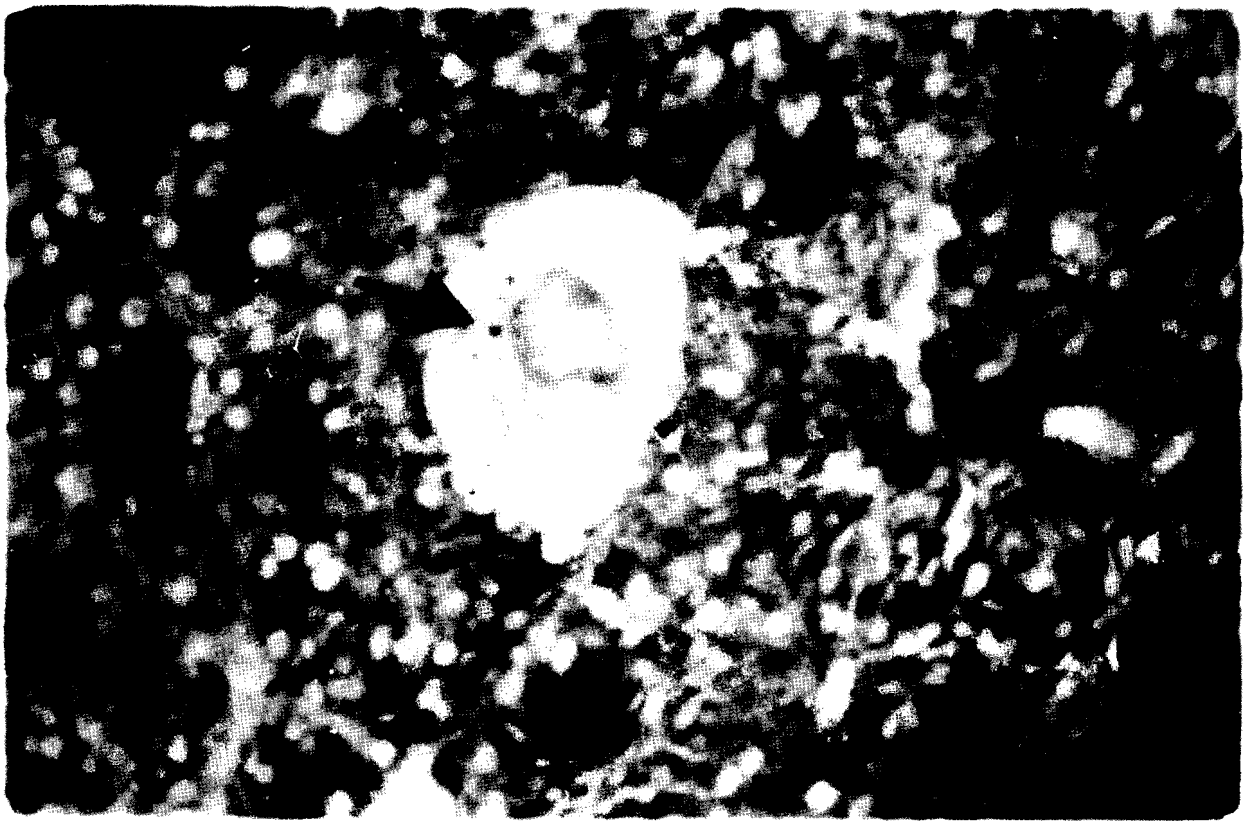

Fig. 7. Hepatocyte-like cell from the pancreas of a rat examined with DAB histochemistry and toluidine blue staining. Diffusely-staining nucleus, nucleolus, and abundant diffusely eosinophilic cytoplasm. Large granules which represent peroxisomes are seen in the cytoplasm (arrow). x 1,000

that non-differentiated stem cells may exist in the islets of the foetal pancreas. In this respect, cells whose cytoplasm gave a positive PAS test for glycogen were found in the forming islets in the 10th week. These may have been stem cells, since according to Von Dorsche et al. (1989), a characteristic feature of stem cells in the foetal pancreas is that they contain glycogen in their cytoplasm. In addition, some of these cells did not appear to have any of the morphological characteristics which would classify them as mature hepatocytes. These cells most likely originate from the neighbouring epithelial cells of ducts, and in this respect, non-differentiated epithelial cells may represent multipotent stem cells. Such stem cells have been identified in a small number in various tissues, including the foetal pancreas of the rat (Parsa et al., 1969).

Our observations indicate that, in general, the pancreatic islets of the human embryo at 10 to 18 weeks of age did not have many cells. However, there were some cells which gave a distinct positive PAS response for glycogen. In contrast to the rest of the islet cells which had small irregularly-shaped nuclei with little cytoplasm, these PAS-positive cells had a large round nucleus with ample cytoplasm, and demonstrated the morphological characteristics of hepatocyte-like cells. In order to confirm the identity and existence of these hepatocyte-like cells in the human foetal pancreas, experimental animal models known to produce this cell type were necessary for comparison.

Hepatocyte-like cells have only been confirmed so far in the pancreas of adult animal models under various experimental conditions, including metabolic disorders and generated cellular activity. These cells are localized primarily around the circumference of the islets and in contact with or close to the islets (Rao et al., 1986). Although the appearance of the hepatocyte in the pancreas of the adult experimental animal suggests the preservation of foetal stem cells, as well as the existence of hepatocytes in foetal pancreas, the existence of hepatocytes or hepatocyte-like cells in the human foetal endocrine pancreas has not been reported to date as far as we know.

The role of carcinogens-induced pancreatic hepatocytes is not well-understood. However, it has been known that carcinogens, such as nitrosamines, can induce the steady appearance of hepatocytes in the pancreas (Gaddock, 1973; Scarpelli and Rao, 1981). In the present study, cellular changes were not observed in the animals subjected to carcinogen or ethionine. However, when these animals were subjected to ethionine-induced submethylation of DNA, followed by carcinogen administration, some forms of hepatocytes were observed. Thus, it appears that a synergistic action of both may be necessary to provoke stem cell differentiation to hepatocytes. It is not clear why hepatocytes appear following a reduction of copper levels in this organism. One hypothesis is that a specific gene may be activated which transforms the pancreatic cell phenotype to that of hepatocyte-like cells (Lalwani et al., 1981; Di Bernardino et al., 1984). It is also

Fig. 8. Hamster pancreas examined with DAB histochemistry and toluidine blue staining. Portion of a pancreatic islet (right, light field) and portion of the exocine pancreas (left, dark field) are seen following nitrosodiethylamine administration. Some of the islet cells demonstrate hepatocyte characteristics, including large granules which represent peroxisomes (arrow). x 1,000.

possible that there is a reduction in the number of pancreatic cells, which consequently induces activation and multiplication of stem cells which under certain conditions favours the appearance of hepatocytes (Rao et al., 1986).

In the present study, we observed round groups of cells comprising islet formations which arose from the ducts. The fact that hepatocyte-like cells are usually found around the periphery of islets, as observed in this study, is probably related to the greater cell activity and multiplication in that particular area (Tsubouchi et al., 1987).

Others have proposed that pancreatic hepatocytes originate from an intermediate between the interphase of endocrine and exocrine pancreas (Melmed, 1979). These cells have mixed features of exocrine or endocrine cells including characteristics of both, such as the presence of both endocrine and exocrine granules. Sometimes their organelles also have features resembling hepatocytes, such as peroxisomes. In the present study, the morphological features of hepatocytes did not all coexist in the same cell in many cases. Thus, organelles such as peroxisomes were not observed in all cases. This phenomenon has also been reported by others who used several identification criteria for hepatocytes, including not only morphological characteristics, but also dye tests, histochemical reactions, accumulation of iron, hypertrophy of normal endoplasmic reticulum, etc (Rao et al., 1983).

It appears that cells which have been described elsewhere in the literature with other names, are most likely to be hepatocyte-like cells or related precursor/transitional forms which express a hepatocyte-like morphology (Scarpelli and Rao, 1981; Reddy et al., 1984). Their close association to islets in both the human foetal pancreas and in experimental models suggests that these pancreatic hepatocytes may emerge from the pancreatic islets or from the tissue which surrounds the islets. It also appears that these cells develop from stem cells during differentiation, which were observed more frequently in the 10th week and were scarce during the 18th week. Finally, the presence of such hepatocyte-like cells in the human pancreas may explain the appearance of neoplasms with a mixed pancreatic and hepatic character in the human pancreas.

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