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CONFERENCE REPORT

Pancreatic Hepatocytes

An *In Vivo* Model for Cell Lineage in Pancreas of Adult Rat

JANARDAN K. REDDY, MD, M. SAMBASIVA RAO, MD, ANJANA V. YELDANDI, MD, XIAODI TAN, MD, and RAMA S. DWIVEDI, PhD

Multiple foci of hepatocytes differentiate in the pancreas of adult rats subjected to a copper depletion–repletion regimen. Copper deficiency for seven to nine weeks causes an irreversible depletion of over 80% of the acinar cells in the pancreas. When transferred to a normal diet, these rats exhibit only a minimal and spotty acinar cell recovery. This disruption of tissue organization appears to trigger a profound change in cellular commitment, which leads to hepatocyte differentiation in the "oval cells" in the periductal interstitium and the epithelial cells lining the small pancreatic ductules. Pancreatic hepatocytes express several liver-specific genes including albumin, a_{2u}-globulin, carbamoylphosphate synthetase-I, and urate oxidase. Both carbamoylphosphate synthetase-I and glutamine synthetase, the ammonia-metabolizing enzymes, are expressed by all pancreatic hepatocytes; in liver, these are expressed by different populations of hepatocytes. The magnitude of hepatocyte differentiation in this model should facilitate studies on the molecular events regulating changes in cell lineage or differentiation commitment within the pancreas.

KEY WORDS: pancreatic hepatocytes; copper deficiency; pancreatic atrophy; stem cells; progenitor cells; cell lineage.

The differentiation of hepatocyte-like cells in the adult hamster and rat pancreas was first noted nearly 10 years ago (1, 2). In the hamster pancreas, hepatocyte-like cells appeared after the administration of a pancreatic carcinogen, *N*-nitroso-bis(2-oxopropyl)amine (BOP) during peak pancreatic acinar cell regeneration (1). In the rat pancreas, an occasional cluster of hepatocyte-like cells has been observed in a few animals that were fed for several months a diet containing Wy-14,643, a peroxisome proliferator (2). The hepatocyte-like cells in hamster and rat pancreas are morphologically indistinguishable from hepatic parenchymal cells, ie, hepatocytes in liver (1–4). They expressed albumin and responded to the peroxisome-proliferating effects of xenobiotics in a fashion analogous to that of hepatocytes in liver (3, 4). These extrahepatic liver cells are designated as pancreatic hepatocytes (4). Following early descriptions and characterization of these cells in hamster and rat pancreas (1–4), several reports describing the appearance of a few foci of pancreatic hepatocytes in an occasional aged rat and in a small percentage of rats exposed to certain xenobiotics in chronic carcinogenesis bioassays have appeared (5–7). Pancreatic hepatocytes have been observed in animals fed a methionine-deficient, ethionine-supplemented diet (8). In most

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From the Department of Pathology, Northwestern University Medical School, Chicago, Illinois 60611.

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Address for reprint requests: Dr. Janardan K. Reddy, Department of Pathology, Northwestern University, Medical School, 303 East Chicago Ave., Chicago, Illinois 60611.

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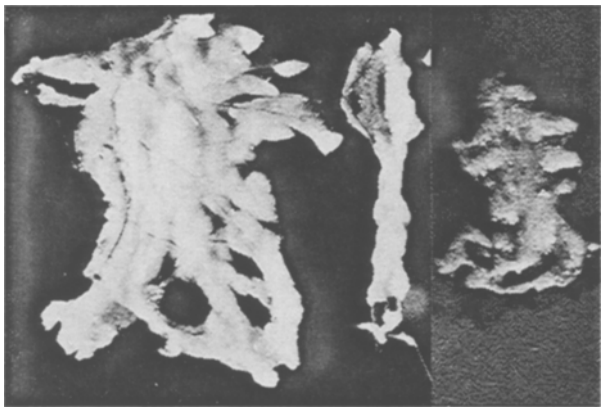


Fig 1. Gross appearance of pancreas: (A) normal rat; (B) pancreas of a rat fed copper-deficient diet containing trien for eight weeks; and (C) pancreas of a rat eight weeks after transfer to normal chow following copper deficiency-induced atrophy.

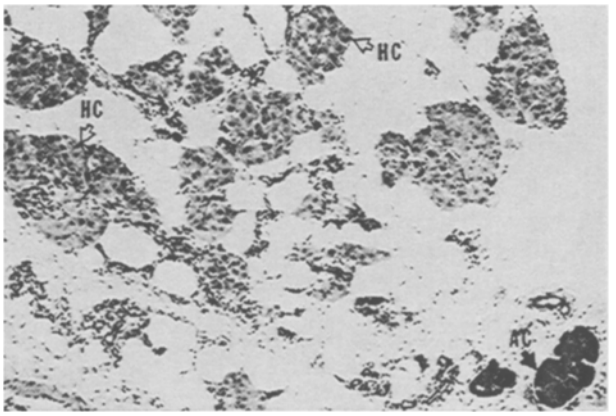


Fig 2. Pancreas of a male rat killed five weeks after transferring to normal chow following an eight-week feeding of a copper deficient diet containing 0.65% trien. Note the presence of numerous clusters of hepatocytes (HC), some surrounding the islets of Langerhans. The small ductules and oval-type periductal cells are present.

of these studies, the development of hepatocytes was unpredictable, as only a limited number of rats exhibit such hepatocytes. Furthermore, the number of pancreatic hepatocytes in the animals was confined to a few isolated clusters, in apparently otherwise normal pancreas with its abundant acinar tissue. Recently, a model system that yields large numbers of hepatocytes in rat pancreas severely depleted of its acinar cell population has been developed (9–11). This copper depletion–repletion model of pancreatic hepatocyte differentiation in the rat is described here. This model presents a unique opportunity to analyze the cellular and molecular aspects of cell lineage in an adult organ.

RAT MODEL OF PANCREATIC HEPATOCYTES

Rats maintained on a copper-deficient diet containing a copper chelating agent, trien, for seven to nine weeks develop marked pancreatic atrophy (Figure 1) with loss of pancreatic acinar cells but without apparent loss of their ductal/ductular cells or the islets of Langerhans (9–14). Following copper depletion and subsequent transfer to a normal diet, these animals display a potential for transdifferentiation leading to the appearance of numerous islands and sheets of hepatocytes within the pancreas (Figure 2). This nutritional model of conversion of pancreatic cells into hepatocytes in the adult rat pancreas offers several advantages and is superior to other such models. This model yields multiple foci of hepatocytes in a pancreas nearly devoid of acinar cells (Figure 2). This enrichment of hepatocytes in the pancreas enables one to study the liver-specific mRNAs in these cells (11). It also should facilitate a study of the events leading to the activation of dormant liver-specific genes in pancreatic tissue. The high incidence of animals showing this differentiation makes this an attractive system for a variety of experimental manipulations. Finally, this model should permit studies on the elucidation of the molecular controls that lead to a change in cell lineage, since the change in commitment from a pancreatic cell lineage to a hepatocyte lineage apparently occurs in the adult pancreas during the late stages of pancreatic acinar cell depletion (ie, between six and eight weeks on copper-deficient regimen) and persists for several weeks after transfer of these animals to a normal diet. This is a relatively long window of transition, compared to the situation in the embryonic development where the commitment of foregut diverticulum cells to differentiate into either hepatocytes or pancreatic cells occurs rapidly (15). This model should enable the isolation of cDNA clone(s) that control hepatocyte phenotypic differentiation.

EXPRESSION OF CERTAIN LIVER-SPECIFIC GENES IN PANCREATIC HEPATOCYTES

The light and electron microscopic features of pancreatic hepatocytes are well documented (1, 3, 4, 11). These hepatocytes are not arranged as one-cell-thick plates separated by sinusoids and do not present the classical architecture of a "liver acinus" (Figure 2). Typically several pancreatic hepa-

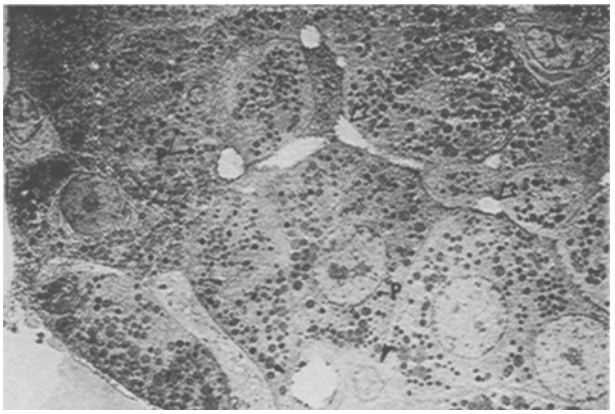


Fig 3. Electron micrograph of pancreatic hepatocytes. The tissue was processed for the cytochemical localization of peroxisomal catalase using the diaminobenzidine method prior to embedding in Epon. Morphological features of pancreatic hepatocytes are similar to those of liver parenchymal cells. Several bile canaliculi (arrows) are present. An occasional undifferentiated cell (arrow head) with ovoid nucleus and undeveloped cytoplasmic structures is present in between hepatocytes. Peroxisomes (P).

tocyte clusters are located close to the islets of Langerhans, often forming a multicell layer collar around the islets (Figure 2). The ultrastructural features of pancreatic hepatocytes are essentially similar to those of liver parenchymal cells. However, the clear-cut plasmalemmal domains are sometimes difficult to appreciate in pancreatic hepatocytes because of the disorganized pattern of the cells (Figure 3). Bile canaliculi are found frequently between sheets of adjacent pancreatic hepatocytes (Figure 3). Immunofluorescence studies with domain-specific antibodies clearly demonstrated the presence of sinusoidal, apical, and lateral plasmalemmal domains in pancreatic hepatocytes.

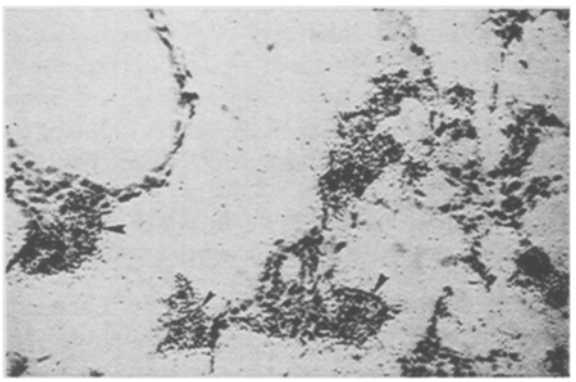


Fig 4. *In situ* hybridization of rat pancreas with hepatocytes for the localization of albumin mRNA. ^{35}S-labeled RNA (antisense) probe was used. The silver grains, representing albumin mRNA molecules, are found over the hepatocyte clusters (arrows).

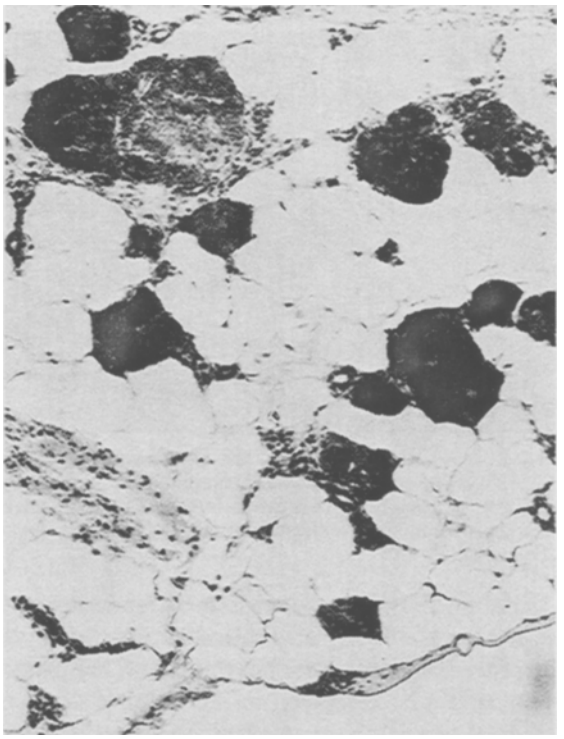


Fig 5. Immunoperoxidase localization of α_{2u}-globulin protein in pancreatic hepatocytes of a male rat. Note the intense reaction product in all hepatocytes.

Several liver-specific proteins and their mRNAs have been demonstrated in rat pancreatic hepatocytes (11). Albumin was found in these cells by immunohistochemical methods, and albumin mRNA has been demonstrated by *in situ* hybridization (Figure 4) and blot analysis of total RNA extracted from pancreas containing hepatocytes (11, 16). Recently, the presence of an α_{2u}-globulin protein by immunoperoxidase method in pancreatic hepatocytes (Figure 5) and α_{2u}-globulin mRNA transcript has been demonstrated in the pancreas of male rats containing hepatocytes (17). Moreover α_{2u}-globulin synthesis in pancreatic hepatocytes is under androgen regulation (17), similar to that seen in normal liver (18, 19).

In pancreatic hepatocytes, the ammonia-metabolizing enzymes carbamoylphosphate synthetase-I and glutamine synthetase can be demonstrated (16). In the mammalian liver, these two genes are expressed in two distinct populations of hepatocytes that are zonally demarcated in the normal liver acinus (20–23). In the liver, carbamoylphosphate synthetase-I is homogeneously distributed in all liver cells except for a single layer of hepatocytes surrounding the central veins, whereas the gluta-

mine synthetase is localized exclusively to the pericentral hepatocytes (Figure 6) that do not express the enzyme carbamoylphosphate synthetase-I (22, 23). Unlike normal liver, all pancreatic hepatocytes express both genes (Figures 7–9). Whether this is due to the absence of a portal blood supply to the pancreas or to other local environmental factors remains to be determined. The expression of both of these ammonia metabolizing enzymes in the same pancreatic hepatocytes makes them an interesting system for the study of factors that influence reciprocal regulation of these two genes.

HEPATOCYTE LINEAGE IN PANCREAS

Using *in situ* hybridization, albumin mRNA has been found in interstitial (periductal) cells and in epithelial cells lining small ductules in the pancreas of rats at the end of seven weeks of a copper-deficient diet (11). At this stage, the pancreas contains very few acinar cells. The presence of albumin mRNA in periductal and ductular cells during copper depletion implies that a change in commitment has occurred and that these cells are destined to differentiate into hepatocytes. Whether all or only a fraction of the cells containing albumin mRNA

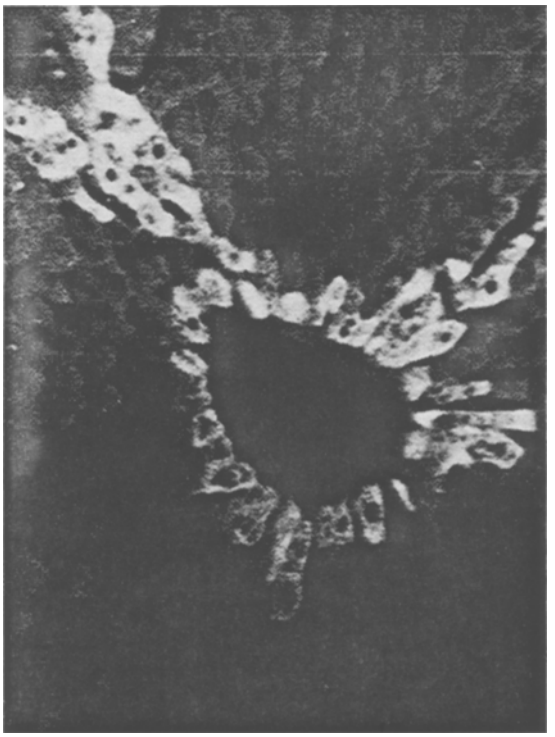
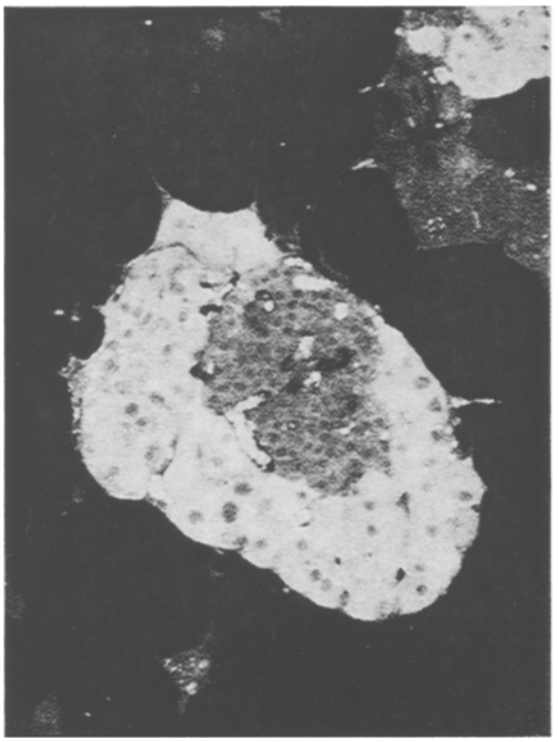
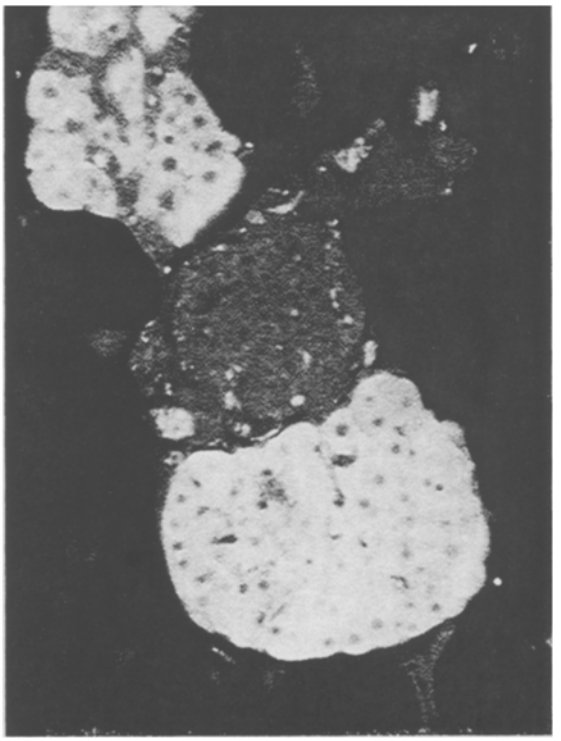


Fig 6. Immunofluorescence localization of glutamine synthetase in a male rat liver. Only a single layer of hepatocytes surrounding the central vein express this protein in the liver.





Figs 7 and 8. Immunofluorescence localization of glutamine synthetase in the hepatocytes that differentiated in the adult rat pancreas. All pancreatic hepatocytes express this gene.

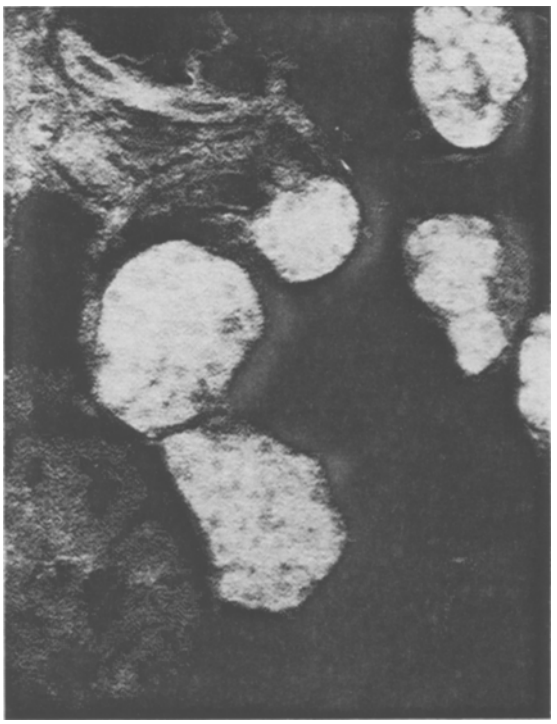
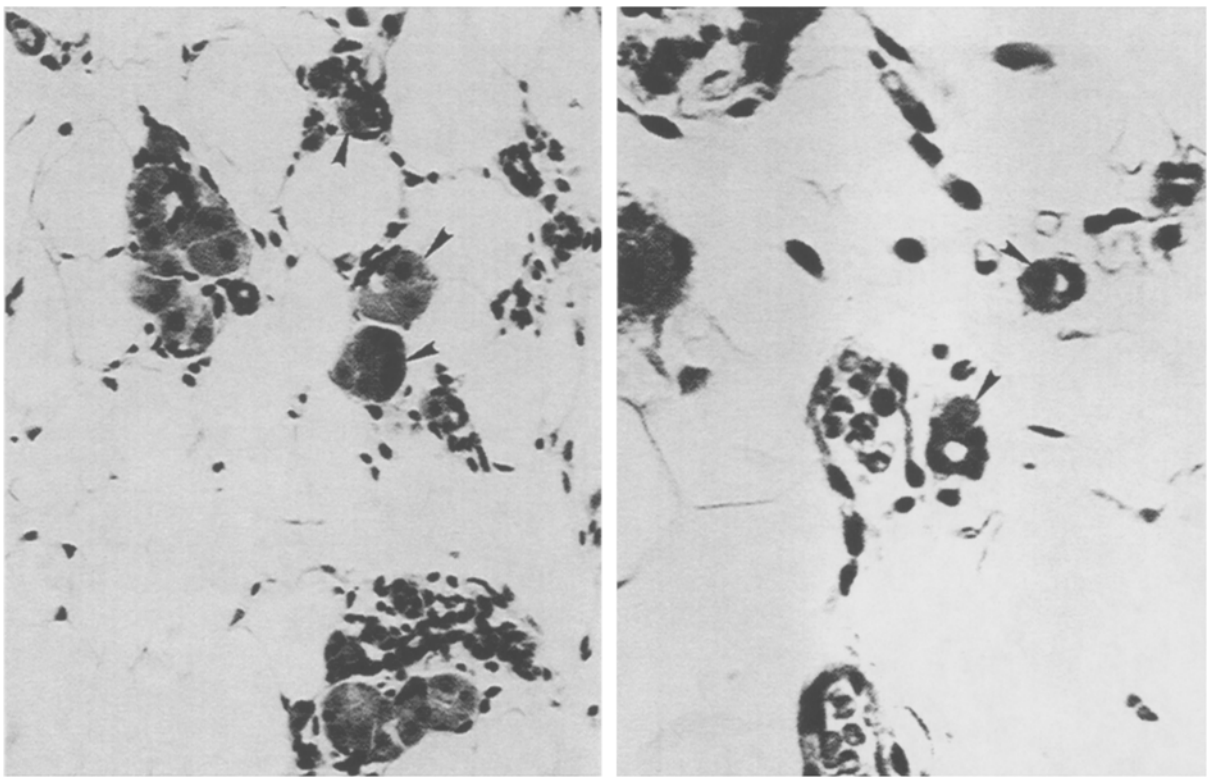


Fig 9. Immunofluorescence localization of carbamoylphosphate synthetase-I in pancreatic hepatocytes. Note that all pancreatic hepatocytes also express this protein.

differentiate into hepatocytes remains to be established. On Northern blot analysis of RNA, transforming growth factor-α, and the transforming growth factor-β_1 mRNAs can be found in the pancreas after eight weeks of copper deficiency (J.K. Reddy, unpublished). *In situ* hybridization studies are necessary to determine which cells in the degenerating pancreas actually express these genes in view of the recent evidence implicating a regulatory role for these growth factors in controlling liver cell regeneration (24, 25).

When the animals are transferred to a normal rat chow after eight or nine weeks on the copper-deficient diet, rapid phenotypic alterations occur in several cells within the interstitial and periductal areas and in some epithelial cells lining small ductules (Figures 10 and 11). These cells clearly differentiate into hepatocytes. Once the conversion occurs, the differentiated hepatocytes appear to expand clonally.

The periductal and epithelial cells lining the small ductules in the pancreas become very prominent and proliferate as a result of copper deficiency (Figures 12 and 13). During embryonic development, the presumptive hepatic and pancreatic en-



Figs 10 and 11. Several transitional forms (arrows) leading to the mature hepatocyte phenotype are discerned in the pancreas of rats during recovery from copper deficiency. These cells are present in the small ductules as well as in the interstitium.

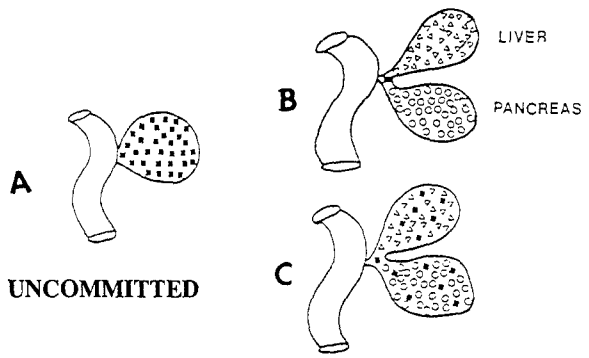


Fig 12. Uncommitted cells (■) in endodermal evaginations of the floor of the foregut (A) commit their descendants to differentiate into liver (△) or pancreatic (○) cells during embryogenesis (B). It is generally held that the adult organs do not contain uncommitted bipotential cells. We postulate the existence of uncommitted bipotential cells (■) capable of commitment to liver or pancreatic cell lineage in both adult liver and pancreas (C). These cells undergo proliferation and exhibit their commitment potential to differentiate when there is severe loss of cells and disruption of tissue organization.

doderms are derived from a common endodermal evagination of the floor of the foregut (Figure 12). At this stage, the cells of these evaginations are considered bipotential stem (uncommitted) cells. Very rapidly during fetal development, these cells become committed to give rise either to a hepatic

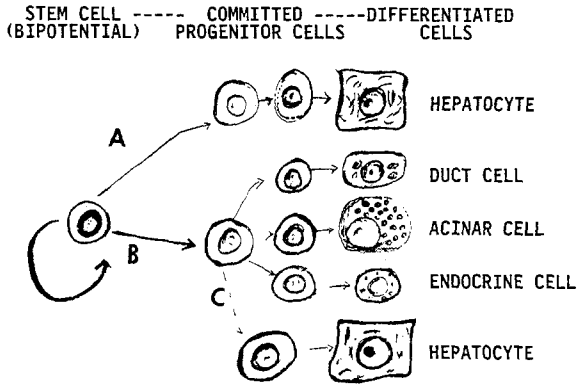


Fig 13. A model of pancreatic hepatocyte lineage in rats on copper depletion–repletion regimen. The marked reduction in acinar cell number and the disruption of normal architecture and cellular relationships resulting from copper deficiency causes proliferation of hitherto dormant stem (uncommitted) cells that are bipotential, ie, they are capable of generating progenitor cells that can be committed to differentiate into hepatocytes (A) or pancreatic cells (B). These bipotential stem cells presumably are located within the small ductules and in the periductal region. Alternatively, the epithelial cells lining the small ductules may have the progenitor potential to transdifferentiate into hepatocytes (C), when they are stimulated to proliferate when global loss of pancreatic acinar cells occurs. Why these progenitor duct cells do not follow the embryonic developmental program of differentiating into acinar, duct, and endocrine cells in the adult pancreas after copper deficiency is intriguing.

or pancreatic cell lineage. According to accepted developmental schemes, the cells in the pancreatic rudiment do not differentiate into hepatocytes, and the cells in the hepatic rudiment do not differentiate into pancreocytes (15). Thus in the adult pancreas and liver, uncommitted bipotential stem cells are not known to exist. Because of the recent examples of hepatocyte differentiation in the adult rat and hamster pancreas (1, 11), and differentiation of pancreatic acinar cells in rat liver (26, 27), we would propose that uncommitted bipotential endodermal cells do persist in the adult liver and pancreas (Figure 12C). During normal embryologic development and in the adult organ, these uncommitted cells remain dormant. However, they retain the capability to differentiate into either pancreatic or hepatic cells under conditions of experimentally induced stress. Thus the stability of these dormant stem cells depends on the organized state of the tissue and the local environment (28–31). A massive loss of acinar cells probably leads to the removal of growth-suppressive factors, thus leading to the activation of these dormant cells. The pressure to repopulate the atrophic pancreas leads to perturbations in cell lineage. However, in transgenic mice expressing diphtheria toxin in a cell-specific manner in developing pancreas, ablation of pancreatic acinar cells results in a rudimentary organ containing an underrepresentation of islet and ductlike cells (32). These results suggest that proliferation of the duct and islet cells may depend upon differentiation of acinar cells (32). In the copper-deficient adult pancreas, however, the irreversible loss of acinar cells appears to provide a stimulus for cell division by small ductular and periductal cells, suggesting that these cells may be the primitive stem cells (33–35).

PERSPECTIVE AND IMPLICATIONS

This model of pancreatic hepatocytes should enable investigators to study the role of copper deficiency in the ablation of pancreatic acinar cells in the adult pancreas, the molecular mechanisms involved in the alteration of cell lineage in an adult organ, and in evaluating the functional aspects of pancreatic hepatocytes. It is important to determine whether these hepatocytes can maintain essential hepatic functions in conditions where the homotopic liver is damaged. Whether copper depletion–repletion regimens or other conditions can induce

hepatocyte differentiation in man and nonhuman primates remains to be ascertained.

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REFERENCES

1. Scarpelli DG, Rao MS: Differentiation of regenerating pancreatic cells into hepatocyte-like cells. Proc Natl Acad Sci USA 78:2577–2581, 1981
2. Lalwani ND, Reddy MK, Qureshi SA, Reddy JK: Development of hepatocellular carcinomas and increased peroxisomal fatty acid β-oxidation in rats fed [4-chloro-6-(2,3-xylidino)-2-pyrimidinylthio]acetic acid (Wy-14, 643) in the semipurified diet. Carcinogenesis 2:645–650, 1981
3. Rao MS, Reddy MK, Reddy JK, Scarpelli DG: Response of chemically induced hepatocyte-like cells in hamster pancreas to methyl clofenapate, a peroxisome proliferator. J Cell Biol 95:50–56, 1982
4. Reddy JK, Rao MS, Qureshi SA, Reddy MK, Scarpelli DG, Lalwani ND: Induction and origin of hepatocytes in rat pancreas. J Cell Biol 98:2082–2090, 1984
5. US Department of Health and Human Services: Carcinogenesis bioassay of 2,6-dichloro-*p*-phenylenediamine in F344 rats and B6C3F1 mice (feed study). Public Health Service, National Institutes of Health, National Toxicology Program, NTP TR219, NIH Publication No. 82-1775, 1982
6. Chiu T: Focal eosinophilic hypertrophic cells of the rat pancreas. Toxicol Pathol 15:1–6, 1987
7. McDonald MM, Boorman GA: Pancreatic hepatocytes associated with chronic 2,6-dichlor-*p*-phenylenediamine administration in Fischer 344 rats. Toxicol Pathol 17:1–6, 1989
8. Hoover KL, Poirier LA: Hepatocyte-like cells within the pancreas of rats fed methyl-deficient diets. J Nutr 116:1569–1575, 1986
9. Rao MS, Subbarao V, Reddy JK: Induction of hepatocytes in the pancreas of copper-depleted rats following copper repletion. Cell Differ 18:109–117, 1986
10. Rao MS, Dwivedi RS, Subbarao V, Usman MI, Scarpelli DG, Nemali MR, Yeldandi A, Thangada S, Kumar S, Reddy JK: Almost total conversion of pancreas to liver in the adult rat: A reliable model to study transdifferentiation. Biochem Biophys Res Commun 156:131–136, 1988
11. Rao MS, Dwivedi RS, Yeldandi AV, Subbarao V, Tan X, Usman MI, Thangada S, Nemali MR, Kumar S, Scarpelli DG, Reddy JK: Role of periductal and ductular epithelial cells of the adult rat pancreas in pancreatic hepatocyte lineage. A change in the differentiation commitment. Am J Pathol 134:1069–1086, 1989
12. Muller HB: Der Einfluss Kupferarmer Kost auf das Pankreas. Virchows Arch (Pathol Anat) 350:353–367, 1970
13. Smith PA, Sunter JP, Case RM: Progressive atrophy of pancreatic acinar tissue in rats fed a copper-deficient diet supplemented with D-penicillamine or triethylene tetramine: Morphological and physiological studies. Digestion 23:16–30, 1982
14. Rao MS, Subbarao V, Yeldandi AV, Reddy JK: Pancreatic acinar cell regeneration following copper deficiency-induced pancreatic necrosis. Int J Pancreatol 2:71–85, 1987
15. Rutter WJ, Pictet RL, Morris PW: Toward molecular mechanisms of developmental processes. Annu Rev Biochem 42:601–646, 1973
16. Yeldandi AV, Tan X, Dwivedi RS, Subbarao V, Smith DD Jr, Scarpelli DG, Rao MS, Reddy JK: Coexpression of glutamine synthetase and carbamoylphosphate synthase-I in pancreatic hepatocytes of rat. Proc Natl Acad Sci USA 87:881–885, 1990
17. Dwivedi RS, Yeldandi AV, Subbarao V, Feigelson P, Roy AK, Reddy JK, Rao MS: Androgen regulated expression of the α_{2u}-globulin gene in pancreatic hepatocytes of rat. J Cell Biol 110:263–267, 1990
18. Kurtz DT, Sippel AE, Ansah-Yiadom R, Feigelson P: Effects of sex hormones on the level of the messenger RNA for the rat hepatic protein α_{2u}-globulin. J Biol Chem 251:3594–3598, 1976
19. Roy AK, Chatterjee B, Demyan WF, Milin BS, Motwani NM, Surendnath T, Schiop MJ: Hormone and age-dependent regulation of α_{2u}-globulin gene expression. Recent Prog Horm Res 39:425–461, 1983
20. Gebhart R, Mecke D: Heterogeneous distribution of glutamine synthetase among rat liver parenchymal cells *in situ* and in primary culture. EMBO J 2:567–570, 1983
21. Gaasbeek-Janzen JW, Lamers WH, Moorman AFM, deGraaf A, Los JA, Charles R: Immunocytochemical localization of carbamoyl-phosphate synthetase (ammonia) in adult rat liver. J Histochem Cytochem 32:557–564, 1984
22. Smith DD Jr, Campbell JW: Distribution of glutamine synthetase and carbamoyl-phosphate synthetase 1 in vertebrate liver. Proc Natl Acad Sci USA 85:160–164, 1988
23. Kuo CF, Paulson KE, Darnell JE, Jr: Positional and developmental regulation of glutamine synthetase expression in mouse liver. Mol Cell Biol 8:4966–4971, 1988
24. Mead JE, Fausto N: Transforming growth factor α may be a physiological regulator of liver regeneration by means of an autocrine mechanism. Proc Natl Acad Sci USA 86:1558–1562, 1989
25. Brenner D-A, Koch KS, Leffert HL: Transforming growth factor α stimulates proto-oncogene C-jun expression and a mitogenic program in primary cultures of adult rat hepatocytes. DNA 8:279–285, 1989
26. Kimbrough RD: Pancreatic type tissue in livers of rats fed polychlorinated biphenyls. J Natl Cancer Inst 51:679–682, 1973
27. Rao MS, Bendayan M, Kimbrough RD, Reddy JK: Characterization of pancreatic-type tissue in the liver of rat induced by polychlorinated biphenyls. J Histochem Cytochem 34:197–201, 1986
28. Rubin H: Molecular biology running into a cul-de-sac? Nature 335:121, 1988
29. Stent GS: Thinking in one dimension: The impact of molecular biology on development. Cell 40:1–2, 1985
30. DiBerardino MA, Hoffner NJ, Etkin LD: Activation of dormant genes in specialized cells. Science 224:946–952, 1984
31. Okada TS: Transdifferentiation in animal cells: Fact or artifact. Dev Growth Diff 28:213–221, 1986

32. Palmiter RD, Behringer RR, Quaife CJ, Maxwell F, Maxwell IH, Brinster RL. Cell lineage ablation in transgenic mice by cell-specific expression of a toxin gene. Cell 50:435–443, 1987
33. Rao MS, Yeldandi AV, Reddy JK: Differentiation and cell proliferation pattern in rat exocrine pancreas: Role of Type I and Type II injury. Pathobiology 58:37–43, 1990
34. Rao MS, Yeldandi AV, Reddy JK: Stem cell potential of ductular and periductular cells in the adult rat pancreas. Cell Dift Develop 29:155–163, 1990
35. Bartles JR, Rao MS, Zhang L, Fayos B, Nehme CL, Reddy JK: Expression and compartmentalization of integral plasma membrane proteins by hepatocytes and their progenitors in the rat pancreas. J Cell Sci (in press)