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Differentiation of regenerating pancreatic cells into hepatocyte-like cells

(regeneration/carcinogen)

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ABSTRACT **Differentiation is the process by which multicellular organisms achieve the specialized functions necessary for adaptation and survival. An *in vivo* model in the Syrian golden hamster is described in which regenerating pancreatic cells are converted into hepatocyte-like cells, as evidenced by the presence of albumin, peroxisomes, and a variety of morphological markers. These cells are stable after the conversion is triggered by a single dose of the carcinogen *N*-nitrosobis(2-oxopropyl)amine administered during the S phase in regenerating pancreatic cells. This suggests that, given the proper stimulus, regenerating cells in adult pancreas can be redirected into a totally different pathway of differentiation.**

Cell differentiation is the process by which progenitor cells having a common genotype give rise to the spectrum of cells having different phenotypes that characterizes the fully developed organism. Jacob and Monod (1) have defined differentiation in functional terms as ... present when cells with the same genome synthesize different proteins." Viewed from yet another perspective, differentiation may be considered as a progressive restriction of gene expression in cells whose nuclei contain all the genetic information necessary for the myriad functions characteristic of the whole organism (2). Despite the fact that differentiation has been the focus of much study, it is poorly understood. For example, questions regarding basic aspects of the process, such as the phenotypic stability of differentiation and the potential extent of its reversibility or redirection, remain largely unanswered (3–9). The present communication describes an animal model in which regenerating pancreatic cells can be induced to differentiate to cells resembling fully differentiated hepatocytes.

MATERIALS AND METHODS

Thirty-two male hamsters (Charles River Breeding Laboratories) (35–40 g each) were maintained on a full amino acid semisynthetic diet (10) for 3 weeks prior to induction of pancreatic injury by feeding a methionine-deficient diet and simultaneous daily injections of DL-ethionine (500 mg/kg of body weight) for 8 days. Regeneration was initiated on the ninth day by a single intraperitoneal injection of L-methionine (800 mg/kg of body weight) and returning the animals to the full amino acid diet. This leads to rapid restitution of pancreas accompanied by DNA synthesis that involves 22.4% of the acinar cells and a regain of 80% of control pancreas weight 8 days later (11). Sixty hours after initiation of regeneration, a single (30 mg/kg of body weight) subcutaneous injection of the pancreatic carcinogen *N*-nitrosobis(2-oxopropyl)amine (NBOP) (12) was administered. Twelve control animals were treated identically, except that carcinogen was omitted; after the dietary manipulations, all animals were maintained on the full amino acid diet until sacrifice. A second control group of 15 animals was maintained on a regular diet and pancreatic injury and regeneration were not induced; these were treated with a single dose (30 mg/kg of body weight) of NBOP.

Five experimental and three control hamsters were sacrificed at 2, 4, 8, and 10 months and full autopsies were performed. No gross or microscopic evidence of neoplastic disease was encountered in any of the animals. The pancreas was divided into three parts. One was fixed in buffered formalin, embedded, sectioned, and stained with hematoxylin and eosin and with periodic acid Schiff (PAS) stain with diastase-digested controls. The second was minced into 0.5-mm cubes and fixed in cold 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4), and an aliquot was fixed for 4 hr for cytochemical localization of peroxisomal catalase activity. The remainder was fixed overnight for routine electron microscopy. After fixation, tissue for cytochemistry was rinsed overnight in cacodylate buffer/0.2 M sucrose and then incubated at 37°C for 60 min in the 3,3′-diaminobenzidine oxidation medium of Novikoff and Goldfischer (13), using aminotriazole to inhibit catalase (14). Under the dissecting microscope at ×20 magnification, liver cell foci appeared brown and were easily distinguished from surrounding pancreatic tissue. Pancreatic lobules containing hepatocytes were isolated and postfixed for 1 hr in 2% OsO_4 in 0.1 M S-collidine buffer (pH 7.4) and processed for electron microscopy. This material was embedded in Epon and sectioned on an LKB ultratome; 1-μm-thick sections were examined in a Zeiss Ultraphot III microscope, and thin sections were examined in a Hitachi HU 12-B electron microscope. The remaining segment of pancreas was prepared for immunofluorescence according to the method of Sainte-Marie (15). The sections were incubated with rabbit antiserum (1:10 dilution) to the pancreatic enzymes α-amylase and carboxypeptidase A, whose specificity had been established by Ouchterlony double diffusion (16) and serum albumin (Cappel Laboratories, Cochranville, PA), for 1 hr at room temperature in a moist chamber. After three consecutive phosphate-buffered saline washes, slides were covered with goat anti-rabbit gamma globulin conjugated with fluorescein isothiocyanate (Behring, Somerville, NJ) and permitted to react for 30 min. The sections were examined in a Leitz fluorescence microscope equpped with a UV-Epiilluminator system (BP 340-380 exciting filter, LP 430 suppression filter, and 200-W mercury lamp). Specificity of immunofluorescence was ascertained by using nonimmunized rabbit serum and by omitting incubation with specific antibody.

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Abbreviations: NBOP, *N*-nitrosobis(2-oxopropyl)amine; PAS, periodic acid Schiff.

RESULTS

All animals that had received NBOP during pancreatic regeneration 2, 4, 8, and 10 months earlier showed moderate to severe pancreatic atrophy. Histologic study showed diminished acinar and islet tissue with replacement by adipose tissue. In addition to normal-appearing acini and islets, each pancreas contained numerous foci of eosinophilic cells. The foci consisted of polyhedral cells resembling hepatocytes with a centrally placed nucleus and finely vacuolated eosinophilic cytoplasm. Some foci contained entrapped pancreatic acini (Fig. 1 *Left*). Eosinophilic cells were intensely PAS-positive (Fig. 1 *Right*) and were negative after predigestion with diastase, confirming the presence of glycogen. These cells also showed strong cytoplasmic staining for catalase, which appeared to be localized to discrete organelles (Fig. 2) identified as peroxisomes by electron microscopy (Fig. 3). Immunohistofluorescence showed these cells to be stained intensely by antibody to hamster albumin; staining was localized to the cytoplasm (Fig. 4*A* and *B*). Adjacent acinar and islet cell tissues were negative. Staining with antibody to α-amylase (Fig. 4*C*) and carboxypeptidase A (Fig. 4*D*) showed localization limited to pancreatic acini. In every instance adjacent eosinophilic cells were negative. Observation of eosinophilic cells by electron microscopy showed morphologic characteristics identical with those of normal fully differentiated hepatocytes. These included patches of rough endoplasmic reticulum, rosette-like accumulations of glycogen, round to short rod-like mitochondria, peroxisomes, lysosomes, and bile canaliculi (Fig. 5). Some hepatocytes contained cytoplasmic foci of proliferated smooth endoplasmic reticulum (Fig. 6). In 10 control animals, pancreatic regeneration in the absence of NBOP resulted in an essentially normal-appearing pancreas in which no eosinophilic cells were present; in the remaining 2 animals, a few eosinophilic cell foci were observed. No such foci were present in the pancreas of the 15 animals comprising the group of controls treated with NBOP alone.

DISCUSSION

The present experiments, in which regenerating pancreatic cells are converted into stable hepatocyte-like cells, raise some interesting points. Although peroxisomes and albumin are not exclusive cell markers for hepatocytes (17–19), their presence in these cells, coupled with their characteristic morphology, is consonant with our interpretation that they bear a striking resemblance to hepatocytes.

Because the conversion occurred in regenerating pancreas of adult animals, a question arises concerning the identity of the precursor cells in which the change of gene expression was induced. Two views are currently held concerning cell renewal in organs. The first view involves the existence of stem cells in tissues that normally renew and maintain themselves by proliferating at a rate equal to that of cell loss (20, 21). This population responds to higher rates of cell loss and proliferates accordingly to replace the depleted tissues. Although the existence of stem cells in bone marrow, intestinal epithelium, and, perhaps, mammary gland of pregnant rats has been established, such cells have not been demonstrated in slow-growing tissues such as liver, kidney, and pancreas (22, 23). The second view holds that, under the proper stimulus such as normal or pathologic loss of cells, differentiated cells can undergo "retrodifferentiation," interrupt specialized functions, delete cytoplasmic structures, and undergo functional and morphologic simplification (24). Such cells acquire an increased capacity to divide and, by doing so, eventually replace the population deficit with cells identical to those that were lost. Although it could be argued that hepatocyte-like cells arise from undifferentiated

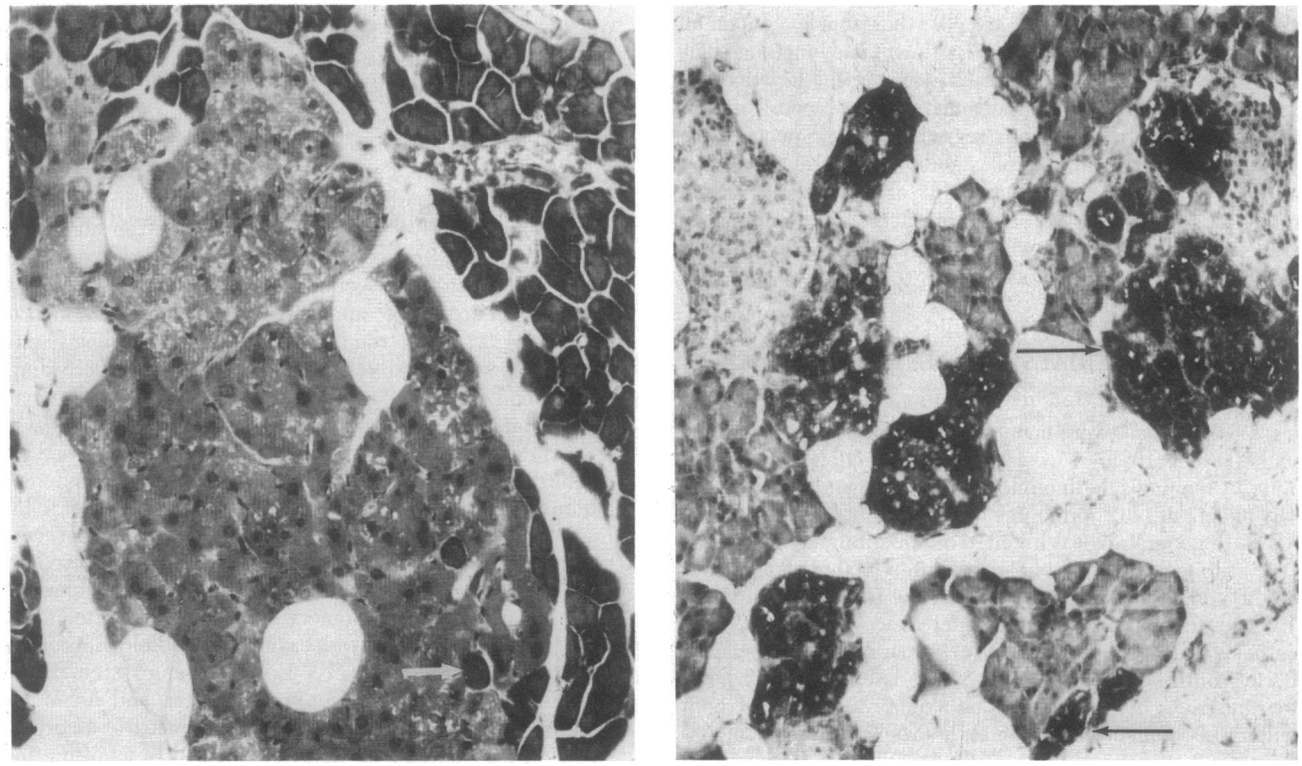

FIG. 1. Light micrographs of pancreas exposed to a single dose of NBOP during regeneration. (*Left*) Normal intensely basophilic acinar tissue is present at the upper right. Eosinophilic cells are present in a pancreatic lobule in the center. Entrapped pancreatic acini are at the lower right (arrow). (Hematoxylin and eosin; ×170.) (*Right*) Foci of cells intensely stained by the PAS reaction are evident, and some foci appear to follow the configuration of pancreatic lobules (arrows). (Hematoxylin and eosin–PAS stain; ×160.)

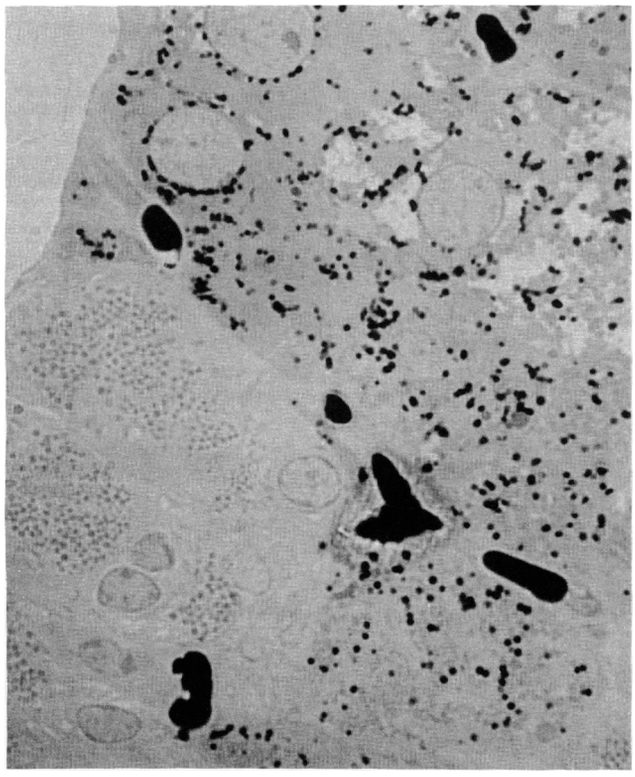

FIG. 2. Light micrograph of 0.5-μm-thick Epon section (not counterstained). Tissue was incubated in diaminobenzidine medium. Reaction product appears as black deposits in the cytoplasm of hepatocytes at the upper middle and right half of the figure. Compare the central large nuclei with those of adjacent acinar cells containing small basilar nuclei and apical zymogen granules. Blood vessels show an intense reaction due to hemoglobin in erythrocytes. (×1400.)

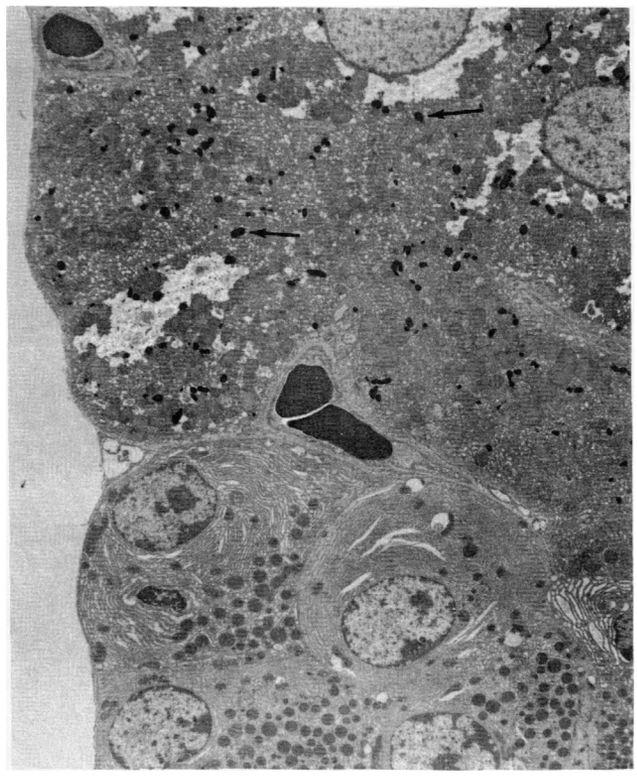

FIG. 3. Electron micrograph of section processed as in Fig. 2. Reaction product is localized to peroxisomes in the cytoplasm (arrows) of hepatocytes. These organelles are also shown at higher magnification (see Fig. 6). Electron-lucent areas are aggregates of unstained glycogen. Adjacent pancreatic acinar cells are evident at the lower left. (×2000.)

stem cells stimulated during regeneration, this is unlikely; in the regenerating hamster pancreas, the mitotic index of acinar cells reaches its peak (16.8 ± 5.0) 72 hr after regeneration, as compared with indices of only 1.8 ± 0.86 and 1.4 ± 0.6 for islet and duct cells, and similar differences are also observed with [^{3}H]dThd labeling. Moreover, no cells are identified during any period of regeneration that can be classified as undifferentiated (23). Although our findings differ somewhat from those of Fitzgerald in regenerating rat pancreas (22), he, too, could not iden-

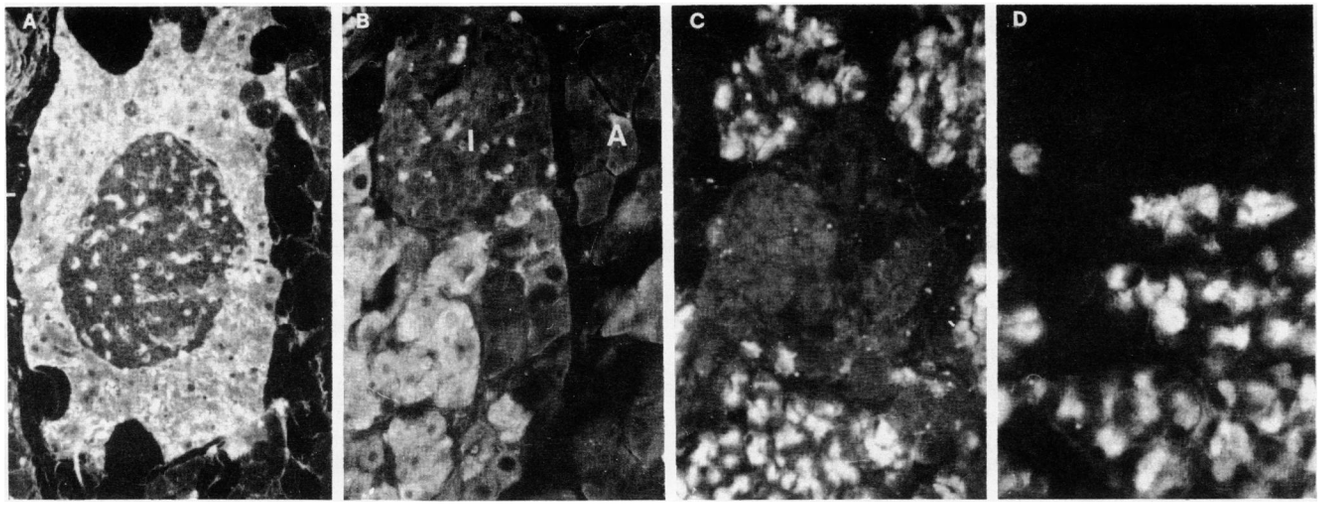

FIG. 4. Immunofluorescence microscopy of pancreas exposed to NBOP during regeneration. (*A*) Pancreas section that had been exposed to antibody against albumin showing islet in which cells are negative surrounded by a rim of hepatocytes that show an intense fluorescence. Staining in the islet is limited to capillaries containing albumin. (*B*) Pancreas section that had been exposed to antibody against albumin showing positive-staining hepatocytes in the lower half of the figure. A negative islet (I) and acini (A) are evident. (*C*) Pancreas that had been exposed to antibody against α-amylase. Staining is localized to pancreatic acini surrounding an island of hepatocytes that are negative. (*D*) Pancreas section that had been exposed to antibody against carboxypeptidase A. Staining of acini is present; adjacent liver cells in the upper half of the photograph are negative.

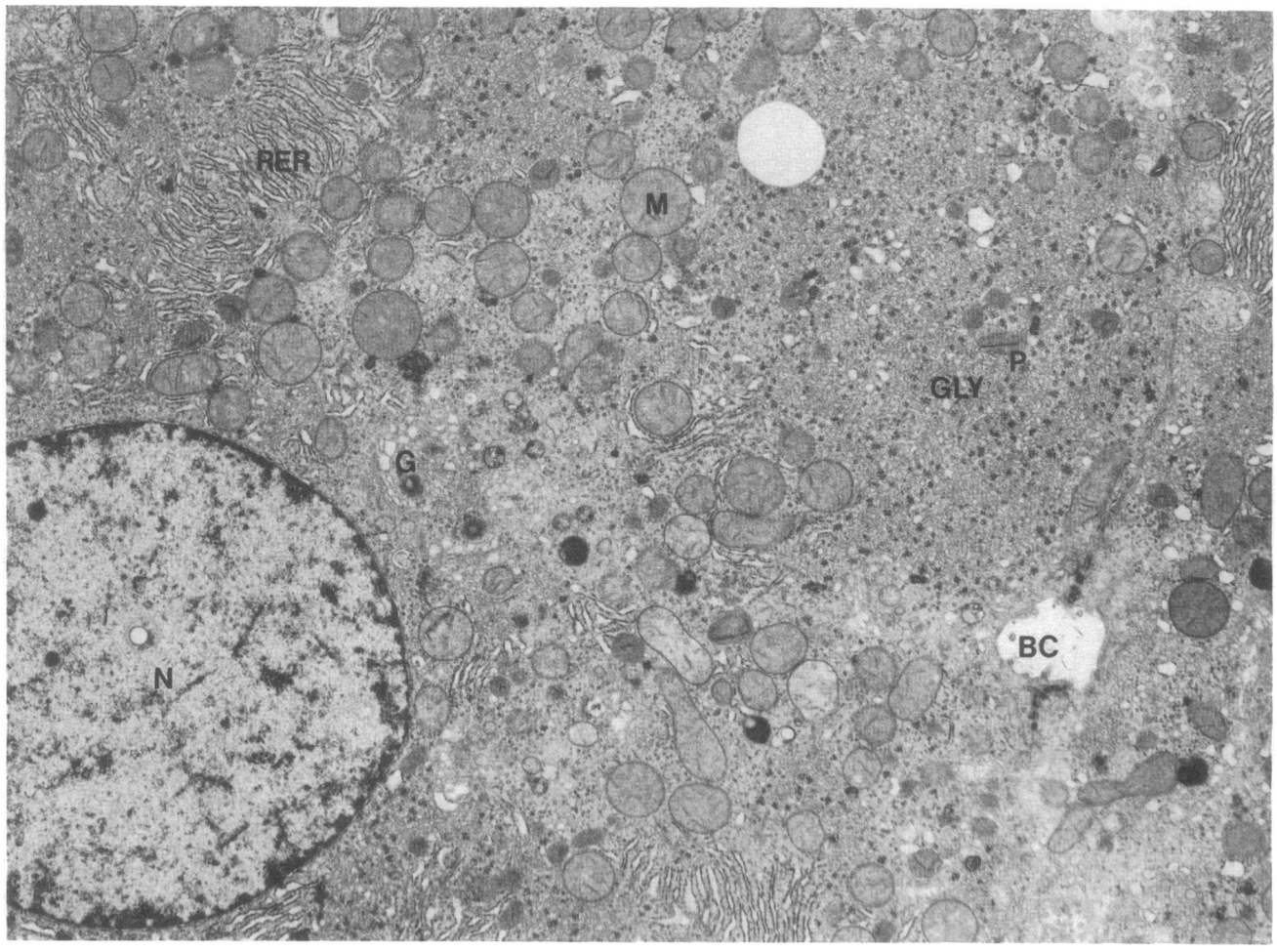

FIG. 5. Electron micrograph of an eosinophilic cuboidal cell with morphologic characteristics of a hepatocyte by light microscopy. A nucleus (N) is present at the left; in the cytoplasm, patches of rough endoplasmic reticulum (RER), mitochondria (M), peroxisomes (P), Golgi membranes (G), bile canaliculus (BC), and rosette-like aggregates of glycogen (GLY) are present. (×8100.)

tify an undifferentiated stem cell as the progenitor of acinar cells. He proposed that, during regeneration, acinar cells arise from preexisting acinar cells that survive ethionine toxicity or perhaps from ductular cells, which in the rat appear to divide significantly during regeneration. The foregoing is not in conflict with the view that pancreatic acinar cells may undergo retrodifferentiation during ethionine-induced injury and replicate to give rise to new acinar cells; accordingly, we have chosen this as a working hypothesis.

Eosinophilic cells have also been reported in pancreas of aged hamsters and were interpreted as a metaplastic alteration (25). The change occurs spontaneously, is uncommon, and varies considerably from one hamster colony to another. For example, in one study, only 4 (0.46%) of 877 animals (25) showed this change and, in a second, 26 (6%) of 426 animals did (26). As neither morphologic, cytochemical, nor immunochemical characterization of these cells are available, little can be said about their identity or relevance to the present findings. If these cells are identical to those we describe, their presence exclusively in aged animals and their infrequency suggest that they may represent a mutation-like change induced by an environmental mutagen.

It is significant that this model involves regenerating pancreas, because it supports the view of Holtzer *et al.* (27) that heritable changes in cell state occur only in association with mitosis. In this regard, the role of NBOP in the transformation of acinar cells to hepatocytes calls for further amplification. Although unexpected, the induction of such a change by exposure of regenerating pancreatic cells to NBOP during DNA synthesis is not surprising. The sensitivity of cells to the effects of a carcinogen is greatly enhanced when they are replicating (28–30). Previous studies have shown that a single dose of NBOP administered to regenerating hamster pancreas 60 hr after initiation of regeneration, when the maximum number of acinar cells are in the S phase, leads to a 20-fold increase in covalent binding of the carcinogen to DNA (11). The appearance of a few hepatocyte-like cell foci in animals treated with ethionine alone suggests that this carcinogen is also capable of altering gene expression. This is noteworthy in view of the limited extent (10^{-10} mol per nucleotide residue) to which ethionine binds to DNA (31, 32). If phenotypic alteration in the precursor cell is linked to interaction of carcinogen with DNA, then liver-specific gene activation may not require a high degree of carcinogen binding. This is further supported by the fact that, even though exposure of the precursor cell to ethionine occurred during toxic injury and necrosis of pancreas when DNA synthesis was minimal, there was sufficient binding of carcinogen to affect the conversion. The appearance of hepatocyte-like cells in pancreas is of interest because both organs share a common ancestry, both arising from gut entoderm. Repressed liver-specific genes are apparently capable of derepression under the proper stimulus. From a mechanistic point of view, interaction of carcin-

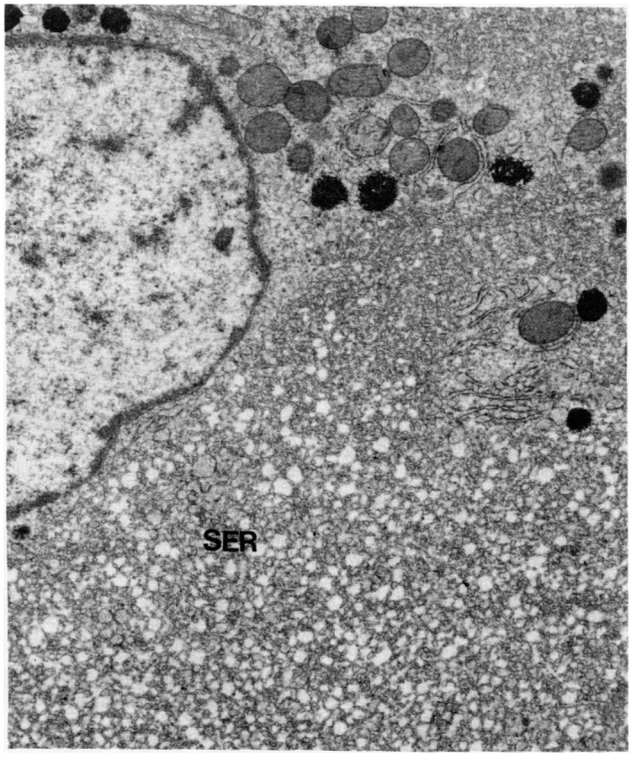

FIG. 6. Electron micrograph of an eosinophilic cell in pancreas processed as in Fig. 3. Reaction product is localized in peroxisomes (arrows). Abundant smooth endoplasmic reticulum (SER) is present in the lower half of the micrograph. (×6700.)

ogen with DNA activates a particular battery of structural genes coding for the synthesis of products and structural proteins that result in the morphological and chemical markers that identify the transformed cells as hepatocytes. The simultaneous appearance of morphologic and synthetic phenotype suggests that activation of the battery of liver-specific genes is probably closely coordinated by an integrator gene, as postulated by Britten and Davidson (33).

Transformation of one cell type to another is well documented and has been encountered in a wide variety of tissues. In the context of this communication, it should be noted that hepatocytes also possess a considerable capacity for inappropriate differentiation into other epithelial cell types, as evidenced by the development of intestinal epithelium after treatment with the hepatic carcinogen 3′-methyl-4-(dimethylamino)azobenzene (34, 35) and pancreatic and salivary gland tissue after long-term exposure to polychlorinated biphenyls (36).

The hamster pancreas model appears to be promising. The change occurs rapidly and reproducibly after a short exposure to carcinogen, and the suspected precursor cells and the transformed cells possess different and specific chemical and morphologic markers, which should facilitate study of many aspects of gene activation and repression.

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