

# Further Characterization of Carcinogen-Induced Hepatocytelike Cells in Hamster Pancreas

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Regenerating pancreatic cells of the Syrian hamster treated at the peak of S phase with the pancreatic carcinogen N-nitrosobis(2-oxopropyl)amine (BOP) are converted into stable cells with morphologic and functional characteristics that are strikingly similar to those of differentiated hepatocytes. In this article the authors further document their hepatocytelike nature. Seventy-two hours after subtotal hepatectomy, pancreatic hepatocytelike cells responded with an 8-fold increase in labeled nuclei ( $105.8 \pm 4.04/1000$  cells) which had incorporated  $^3\text{H}$ -thymidine and a 5-fold increased mitotic index ( $3.8 \pm 1.5$  mitoses/1000 cells), as compared with similar cells in the pancreas of control animals that had

undergone sham operations. Chronic administration of phenobarbital induced a 31-fold increase in the level of aryl hydrocarbon hydroxylase (AHH) in pancreas containing such cells, as compared with normal control pancreas, and caused marked proliferation of smooth endoplasmic reticulum (SER). These cells also showed an enhanced capacity for the accumulation of iron during acute iron excess, as compared with adjacent acinar cells. Collectively, these findings support the view that carcinogen-induced cells in pancreas bear a close functional resemblance to hepatocytes. (Am J Pathol 1983, 110:89-94)

ADMINISTRATION of the pancreatic carcinogen N-nitrosobis(2-oxopropyl)amine (BOP) to Syrian golden hamsters at the peak of acinar cell DNA synthesis in regenerating pancreas leads to the development of hepatocytelike cells in this organ. The foregoing, coupled with the fact that such cells are often located in the midst of pancreatic lobules surrounded by acinar cells, has led us to speculate that they may arise from preexisting acinar cells. These cells are morphologically indistinguishable from normal hepatocytes, contain albumin and glycogen,<sup>1,2</sup> respond to methyl clofenapate, a peroxisomal proliferation, in a fashion identical to liver cells,<sup>3,4</sup> and lack the acinar cell marker proteins  $\alpha$ -amylase and carboxypeptidase.<sup>1,2</sup>

We designed the present experiments to further characterize hepatocytelike cells by studying their response to partial hepatectomy, phenobarbital, and iron overload.

## Materials and Methods

### Induction of Hepatocytelike Cells

Hepatocytelike cells were induced in the pancreas as described previously.<sup>2</sup> Forty male hamsters (Charles

River Breeding Laboratories, Wilmington, Mass) weighing 70-90 g were maintained on a full amino acid semisynthetic diet<sup>5</sup> for 3 weeks prior to induction of pancreatic injury by the feeding of a methionine-deficient diet and simultaneous daily injections of DL-ethionine (500 mg/kg body weight) for 8 days. We initiated regeneration on the ninth day by a single intraperitoneal injection of L-methionine (800 mg/kg body weight) and returned the animals to the full amino acid diet. Sixty hours after initiation of regeneration, when DNA synthesis in acinar cells was at its peak,<sup>6</sup> a single (30 mg/kg body weight) subcutaneous injection of BOP was administered. Twenty-nine hamsters were available for the experiments that follow because of the death of some animals (27% mortality) during the induction of regeneration.

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### Partial Hepatectomy and Analysis of Liver Regeneration

Partial hepatectomy by removal of the median and left lobes were performed on 44 non-BOP-treated hamsters under light ether anesthesia and sterile conditions by the technique of Higgins and Anderson,<sup>7</sup> in which between 69% and 72% of the liver was removed. To avoid diurnal variations, partial hepatectomy was performed between 7 AM and 11 AM. Sham operations were performed on 4 control hamsters. These studies were necessary because there were no detailed reports documenting the kinetics of liver regeneration in the hamster.<sup>8</sup> The animals were sacrificed in groups of 4 at 6-hour intervals, beginning at 12 hours and extending to 48 hours after partial hepatectomy, and at 60, 72, 96, and 120 hours. Animals that underwent sham operations were sacrificed at 48 hours after laparotomy. All the animals were given a single intraperitoneal injection of tritium-labeled thymidine (<sup>3</sup>H-thymidine) (specific activity 2 Ci/mmol; Research Products International Corp., Elk Grove Village, Ill) at a dose of 1 Ci/g body weight 1 hour prior to sacrifice. Sections of liver were fixed in neutral buffered formalin and processed for light microscopy. Five-micron-thick paraffin sections were stained with hematoxylin and eosin (H&E). The extent of cell replication was assessed by autoradiography with the use of Kodak NTB<sub>2</sub> nuclear emulsion (Eastman Kodak Company, Rochester, NY).<sup>9</sup> Following 2 weeks of incubation at 4 C, slides were developed with Kodak D-19 developer for 4 minutes, rinsed, fixed, and stained with H&E, and we counted 1000 randomly selected cells to obtain the number of cells showing nuclear incorporation of <sup>3</sup>H-thymidine.

The effect of partial hepatectomy on hepatocyte-like cells in the pancreas was studied in 8 hamsters in which such cells had been previously induced as described above. Partial hepatectomy, subsequent sacrifice, autoradiography, and analysis were carried out as on both liver and pancreas as detailed above, except that beginning 42 hours after hepatectomy, all animals were given 3 intraperitoneal injections of <sup>3</sup>H-thymidine at a dose of 1 Ci/g body weight at 42, 60, and 70 hours and sacrificed 2 hours after the last injection. This schedule was based on the results obtained from the foregoing partial hepatectomy experiments.

### Effects of Phenobarbital on Pancreatic Hepatocytelike Cells and Hepatocytes

Eighteen hamsters in which hepatocyte-like cells had been induced were divided into 2 groups of 9 animals each. One group consisted of hamsters in which

0.1% phenobarbital in drinking water was administered *ad libitum* for 7 days; they were killed on the eighth day. The second group served as experimental controls. Eighteen normal hamsters were divided into 2 equal groups; one group received phenobarbital; the other served as normal controls. Aryl hydrocarbon hydroxylase (AHH) activity was measured as described previously<sup>10</sup> by the method of Nebert and Gelboin<sup>11</sup> in pancreatic tissue of all groups of animals and in the livers of phenobarbital-treated and untreated normal control hamsters. Portions of pancreas and liver from 2 animals in each group were fixed in 2.5% paraformaldehyde and glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) for 4 hours, postfixed in 1% OsO<sub>4</sub> for 1 hour, and processed for electron microscopy. Thin sections from selected blocks containing hepatocytelike cells were stained with uranyl acetate and lead citrate and examined with a Hitachi HU-12B electron microscope.

### Iron Overload Experiment

Three hamsters containing pancreatic hepatocyte-like cells were given iron-dextran complex (Imferon, Merrill International, Cincinnati, Ohio) subcutaneously (40 mg/100 g body weight) on alternate days for 8 days (4 doses) and were sacrificed 1 day after the last dose for a total dose of 160 mg/100 g body weight. Pancreas and portions of liver were fixed and processed for light microscopy; we stained 5- $\mu$ -thick paraffin sections with H&E and with the Prussian blue reaction to demonstrate intracellular iron.

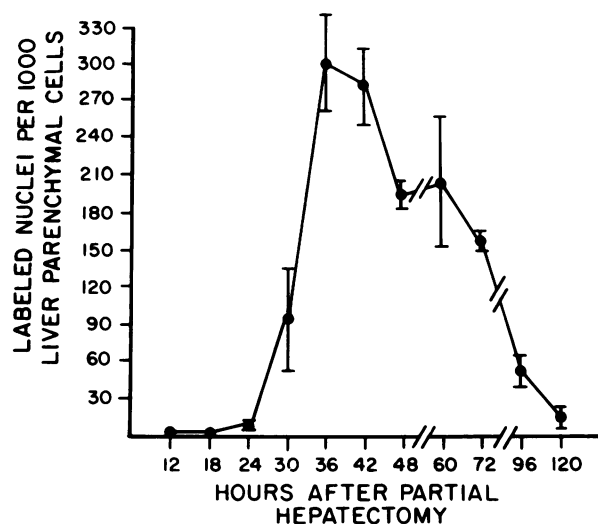


Figure 1—Kinetics of <sup>3</sup>H-thymidine labeling of hepatic parenchymal cell nuclei at different intervals following partial hepatectomy in the Syrian hamster.

## Results

### Hepatic Regeneration

Twelve and 18 hours after partial hepatectomy, the only changes encountered in the livers of normal non-BOP-treated hamsters were cytoplasmic fatty vacuolation and a slight diminution of nuclear labeling and mitosis. Increased nuclear incorporation of  $^3\text{H}$ -thymidine was first detectable 24 hours after hepatectomy and peaked at 36 hours, at which time about 30% of the liver cells were in the S phase of the cell cycle (Figure 1). Increased mitotic activity was first apparent 30 hours after partial hepatectomy and reached a maximum ( $37.3 \pm 10.1$  mitoses/1000 hepatocytes) at 42 hours and remained greater than that in control hamsters that underwent sham operations as long as 120 hours after surgery (Figure 2). Forty-two hours was the time point selected to begin labeling of hepatocytelike cells with  $^3\text{H}$ -thymidine during hepatic regeneration. After the initial peak of proliferation and beginning 48 hours after partial hepatectomy, there was a sustained level of DNA synthesis and cell replication, which began to diminish at 72 hours, as shown in Figures 1 and 2.

Hepatocytelike cells in the pancreas of hamsters that had undergone partial hepatectomy 72 hours previously responded, as shown in Table 1, with an 8-fold increase in labeled nuclei ( $105.8 \pm 4.04$  /1000 hepatocytelike cells) that had incorporated  $^3\text{H}$ -thymidine (Figure 3) and a 5-fold increased mitotic index ( $3.8 \pm 1.5$  mitoses/1000 hepatocytelike cells), as compared with hepatocytelike cells in the pancreas of control animals that underwent sham operations, where the indices were  $13.0 \pm 4.04$  labeled nuclei/1000 hepatocytelike cells and  $0.75 \pm 0.48$  mitoses/1000 hepatocytelike cells, respectively. Hepatocytes in the liver of hamsters bearing hepatocytelike cells in their pancreas responded to partial hepatectomy more vigorously than their counterparts in pancreas, with indices of  $351.7 \pm 73.4$  labeled nuclei/1000 hepatocytes and  $15.85 \pm 5.5$  mitoses/1000 hepatocytes a 63-fold and 19.8-fold increase, respectively, as summarized in Table 1.

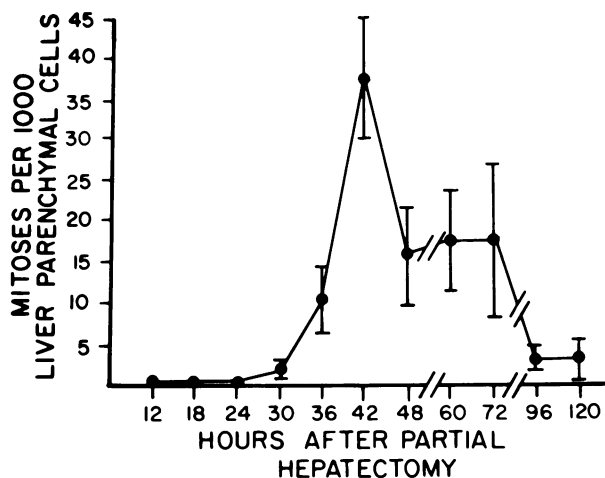


Figure 2—Kinetics of mitotic activity of hepatic parenchymal cells at different intervals following partial hepatectomy.

### Effect of Phenobarbital

Administration of 0.1% phenobarbital in the drinking water for 7 days increased AHH activity in hamster pancreas bearing hepatocytelike cells 31-fold over that present in normal control pancreas, 11.5-fold more than that in normal pancreas chronically exposed to phenobarbital, and 8.19-fold more than the level measured in pancreas containing hepatocytelike cells that had not been chronically exposed to phenobarbital (Table 2). As expected, the AHH activity of hepatocytes in the liver of phenobarbital-treated animals was increased 4-fold over that in normal control animals. Hepatocytelike cells (Figures 4 and 5) responded to chronic exposure to phenobarbital in a fashion identical to that of normal hepatocytes by proliferation of smooth endoplasmic reticulum (SER).

### Response to Iron Overload

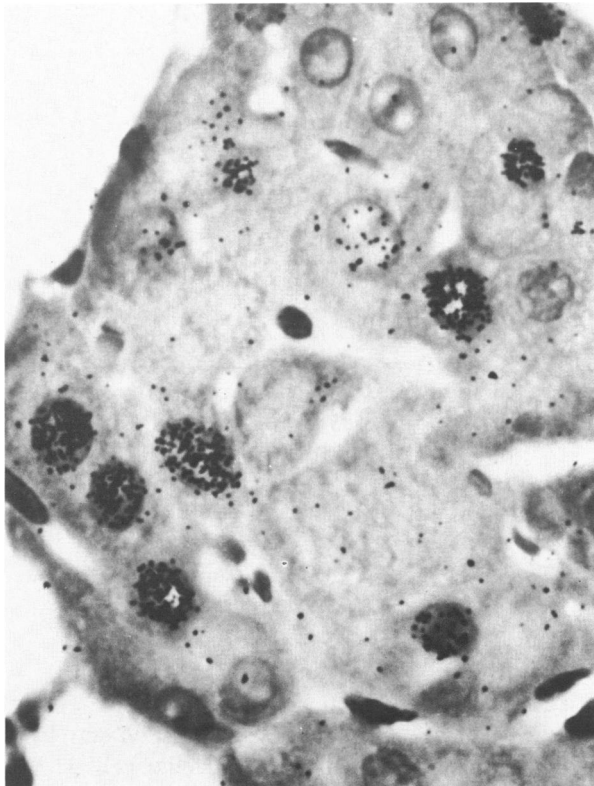
Iron-dextran complex administered over a period of 8 days led to the accumulation of iron in the cyto-

Table 1—Mitotic and Labeling Index of Hepatocytelike Cells in Pancreas\*

Group	Labeling index/1000 cells		Mitotic index/1000 cells	
	Liver parenchymal cells	Hepatocytelike cells	Liver parenchymal cells	Hepatocytelike cells
Control	$5.50 \pm 0.29$	$13 \pm 4.04$	$0.8 \pm 0.5$	$0.75 \pm 0.48^\dagger$
Hepatectomy	$351.7 \pm 73.4$	$105.8 \pm 20.9$	$15.85 \pm 5.5$	$3.8 \pm 1.5$

\* Hamsters containing hepatocyte-like cells were hepatectomized or sham operated and administered 3 doses of  $^3\text{H}$ -thymidine (1 Ci/g body weight) at 42, 60, and 70 hours after operation. All animals were killed 2 hours after the last dose.

† Mean  $\pm$  standard error (n = 4 hamsters).



**Figure 3**—Autoradiograph of pancreas from an animal injected with 3 doses of (1 mCi/g body weight) of <sup>3</sup>H-thymidine at 42, 60, and 70 hours following partial hepatectomy. Nuclear silver grains are evident in many of the hepatocytelike cells. (H&E, × 1000) (With a photographic reduction of 8%)

plasm of hepatocytelike cells and connective tissue cells in the interstitium. Acinar, islet, ductular, and duct cells were uniformly negative for stainable iron (Figure 6).

**Discussion**

This study further documents the equivalence of hepatocytelike cells induced in regenerating hamster

**Table 2**—AHH Activity in Hepatocytelike Cells in Pancreas

Group	Organ*	Specific activity† (pmol/mg protein/30 min)
Control	Liver	954 ± 48.00
	Pancreas	0.44 ± 0.08
Phenobarbital‡-treated	Liver	3929 ± 53.00
	Pancreas	1.19 ± 0.38
Regeneration + BOP	Pancreas	1.67 ± 0.81
Regeneration + BOP + phenobarbital	Pancreas	13.68 ± 2.24§

\* In all groups there were 9 animals; pancreases and slices of liver from 3 animals were pooled for preparation of postmitochondrial fraction for AHH estimation.

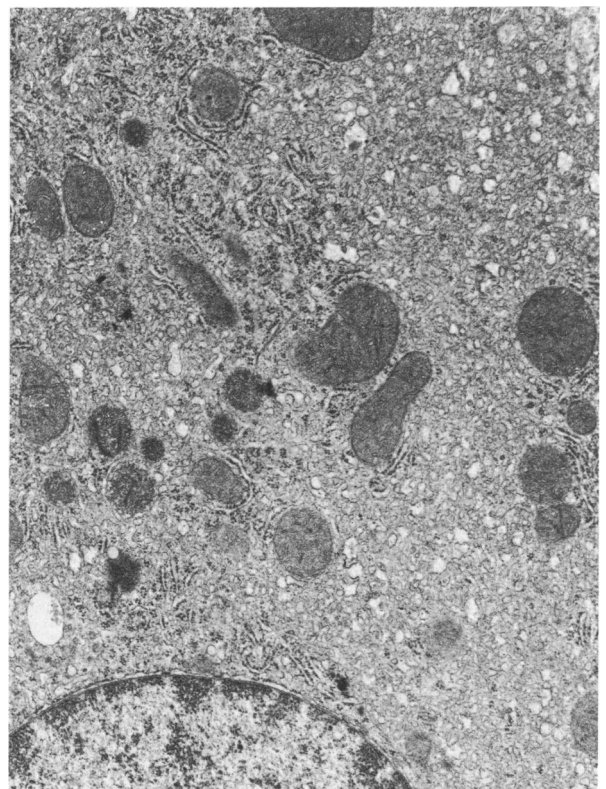
† Mean ± standard error.

‡ Phenobarbital was given in drinking water (0.1%) for 7 days, and the animals were killed on eighth day.

§ Significantly increased over the control group; P < 0.005.

pancreas by BOP with true hepatocytes by their proliferative response following partial hepatectomy, induction of AHH and proliferation of SER by phenobarbital, and their selective accumulation of iron during iron excess.

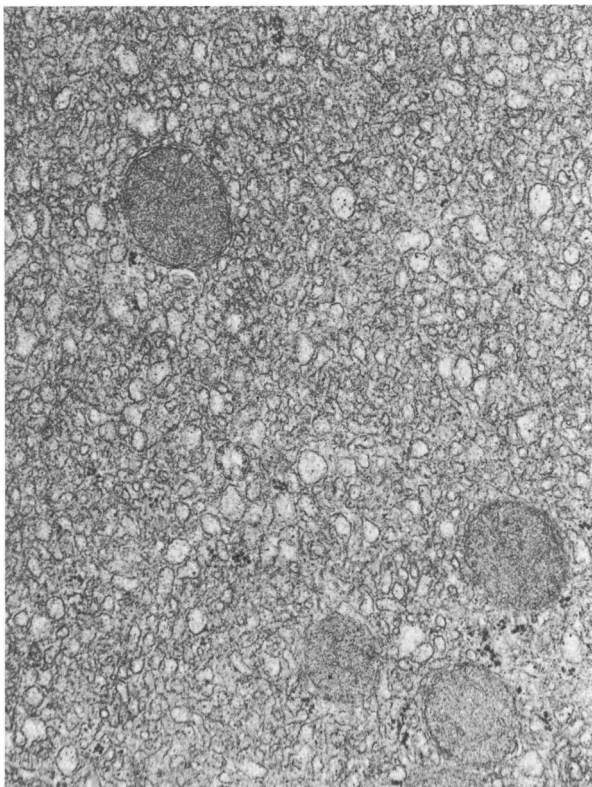
The response of hepatocytelike cells in the pancreas of hamsters subjected to partial hepatectomy was what would be expected if they indeed were hepatocytes. Although the responses were qualitatively similar, they were not similar in magnitude. Hepatocytelike cells incorporated 8-fold less <sup>3</sup>H-thymidine and exhibited 3-fold less mitotic activity, as compared with regenerating true hepatocytes in the same host animals. This is similar to the diminished regenerative response of rat liver cells that transplanted to extrahepatic sites, such as anterior abdominal wall<sup>12</sup> or interscapular fat pad.<sup>13</sup> Although the reason(s) for this differential response is not clear, Leong et al<sup>12</sup> suggested some years ago that the diminished mitotic activity of transplanted hepatocytes following subtotal hepatectomy might be due to dilution, by blood from other parts of the body, of a humoral liver-derived factor produced during regeneration, before it reached extrahepatic hepatocytes. This notion is given cre-



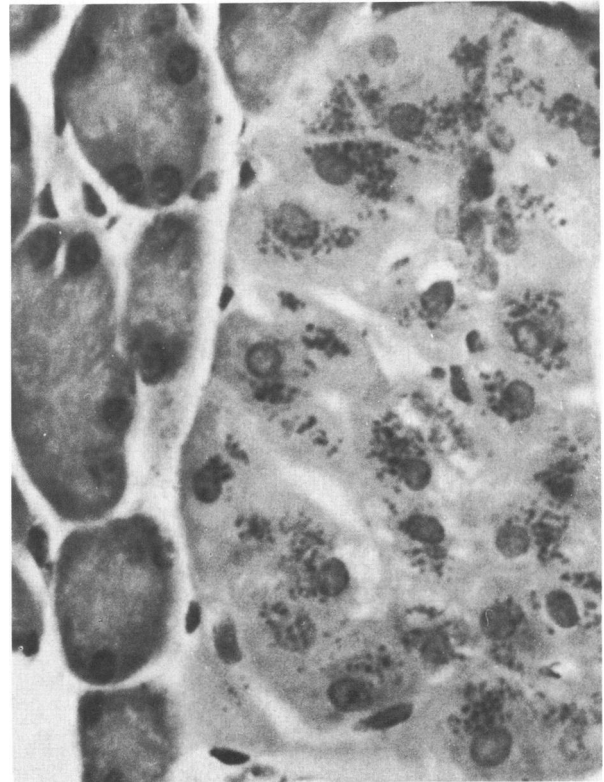
**Figure 4**—Electron micrograph of a hepatocytelike cell from an animal allowed free access to drinking water containing 0.1% phenobarbital for 1 week. Marked proliferation of SER is evident. (Uranyl acetate and lead citrate, × 12,500) (With a photographic reduction of 8%)

dence in view of the recent isolation of a 38,000-dalton glycoprotein from the plasma of partially hepatectomized rats that is capable of modulating liver cell proliferation.<sup>14</sup> Further, the present study demonstrates that the diminished response of pancreatic hepatocytelike cells despite the nearby presence of insulin and glucagon, hormones necessary for DNA synthesis by hepatocytes.<sup>15</sup> This finding is additional evidence in support of the view that these hormones are capable of modulating rather than initiating regenerative liver cell growth.

Metabolism of potentially toxic foreign compounds to innocuous metabolites is a protective adaptive reaction localized in organs that serve as portals of entry for the organism, such as the lung, the liver, and the gastrointestinal tract.<sup>16</sup> Among these, the reaction seems most highly developed in the liver. The synthesis of AHH, a cytochrome-P-450-linked enzyme in liver cells that is responsible for the oxidative metabolism of polycyclic hydrocarbons is readily induced by phenobarbital.<sup>17</sup> In the present experiments, as contrasted to liver, AHH in pancreatic cells was not appreciably induced by phenobarbital. Pancreas appears to be more responsive to the inductive effects



**Figure 5**—A higher magnification of the cytoplasm of a hepatocytelike cell following exposure to 0.1% phenobarbital for 1 week, showing numerous crowded vesicular and tubular profiles of SER. (Uranyl acetate and lead citrate,  $\times 26,500$ ) (With a photographic reduction of 8%)



**Figure 6**—Hepatocytelike cells from hamsters injected subcutaneously on alternate days with 4 doses of iron-dextran complex (40 mg/kg body weight). The cells contain cytoplasmic aggregates stained by the Prussian blue reaction for iron; adjacent acinar cells are negative. ( $\times 720$ ) (With a photographic reduction of 8%)

of 3-methylcholanthrene,  $\beta$ -naphthoflavone, and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.<sup>10</sup> It is of interest to note that the basal level of AHH activity in pancreas increased 3-fold following the appearance of hepatocytelike cells, indicating that the transformation of pancreatic cells to hepatocytelike cells is accompanied by augmented gene expression for the synthesis of AHH. Their similarity to hepatocytes is further supported by an 8-fold increase in AHH activity following phenobarbital treatment, as compared with the basal level in untreated hepatocytelike cells. The biochemical change induced by phenobarbital in these cells is further confirmed by morphologic evidence of proliferation of SER.

It has been amply documented that excessive intake of iron by either dietary or parenteral routes leads to cytoplasmic accumulation of ferritin and hemosiderin in parenchymal cells and reticuloendothelial cells of various organs.<sup>18</sup> To some extent, accumulation depends on the high iron requirements of normal parenchymal cells. Thus, liver and heart are major sites of pathologic iron deposition, although accumulation is first evident in liver. When the organism is subjected to higher levels of iron or when exposure is

prolonged, additional cells, especially acinar and islet cells of pancreas, tubular epithelium of kidney, and cells of the adrenal gland, accumulate iron. With the dose level and schedule of iron-dextran injection employed in these studies, iron deposition was encountered only in liver cells and hepatocytelike cells and connective tissue cells in the interstitium of the pancreas. Acinar and islet cells were totally devoid of stainable iron. It is hoped that the findings presented here will eventually allow us to refer to these cells as hepatocytes rather than hepatocytelike cells, because thus far, they appear to be strikingly equivalent to hepatocytes both morphologically and functionally.

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