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Liver Scarring Induced by Polychlorinated Biphenyl Administration to Mice Previously Treated with Diethylnitrosamine (42406)

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Abstract. Exposure of mice for 8 weeks to drinking water containing diethylnitrosamine (DEN) was accompanied by alterations in hepatocyte structure and varying degrees of liver nonparenchymal cell (NPC) proliferation. Eighteen and a half weeks after cessation of DEN exposure, there was a 47% incidence of hepatocellular nodules. Centrilobular hepatocyte hypertrophy occurred consistently in mice given intraperitoneal injections of the polychlorinated biphenyl (PCB) mixture Aroclor 1254. PCB administration to mice previously treated with DEN was not accompanied by increases in gross liver nodule incidence above that induced by DEN, but many more developing microscopic nodules within the liver were observed in DEN-treated mice given Aroclor 1254 than in mice treated only with DEN. Aroclor 1254 administration over a 16-week period to mice previously treated with DEN was accompanied by an 83% incidence of severe distortion of liver structure resulting from nodule formation, uneven patterns of hepatocyte growth, and extensive deposition of scar tissue containing proliferating bile ducts. Morphological evidence of intestinal metaplasia was observed in proliferating bile duct-like structures during an early stage of liver adenofibrosis.

The administration to laboratory animals of complex halogenated hydrocarbons, including polychlorinated biphenyls (PCBs), produces hypertrophy of centrilobular liver parenchymal cells, induces liver drug-metabolizing enzyme systems (LDMES), and promotes liver tumor formation in animals previously treated with an initiator of liver tumorigenesis (1–8). The administration to mice of large doses (50 mg/kg/day) of the PCB mixture, Aroclor 1254, over long time periods results in prominent intrahepatic bile duct proliferation in which the proliferating elements take on an adenomatous appearance (9). Considerable quantities of collagen are deposited in apposition to the proliferating bile ducts forming a lesion which has been termed adenofibrosis (9). Similar lesions have been observed following hepatic injury by a number of other chemicals including diethylnitrosamine (DEN) and 2-acetylaminofluorene (2-AAF), and have been called cholangiomatosis (10, 11) or cholangiofibrosis (12).

Proliferation of liver nonparenchymal cells (NPC's) in laboratory rodents occurs in response to the administration of a large group of chemicals including many carcinogens [extensively reviewed in (13)]. Continuous long-term administration to mice or to rats of carcinogenic dialkyl nitrosamines such as dimethylnitrosamine (DMN) and diethylnitrosamine is accompanied by moderate to extensive proliferation of "oval" cells, bile duct-like structures, and presumably fibroblasts, since collagen also is present in conjunction with the proliferating bile duct-like epithelium (14–17). We report here that the polychlorinated biphenyl mixture Aroclor 1254 when administered periodically to mice following a short period of DEN exposure results in liver scarring, which does not occur as the result of exposure to the nitrosamine or as the result of treatment with the PCB alone.

Methods. Male CD-1 mice weighing 25 g obtained from Charles River Canada, St. Constant, Quebec, Canada, were maintained in the central animal facilities in plastic cages containing Sani-Cel bedding and were given constant access to water and to food (Purina Laboratory Chow). Aroclor 1254 was obtained from Foxboro Laboratories, North Haven, Connecticut. Diethylnitrosamine was purchased from Eastman Kodak Corporation, Rochester, New York.

Aroclor 1254 was dissolved in tricaprylin and then in corn oil to provide a ratio of tricaprylin/corn oil of 1/4, v/v. DEN was dissolved in drinking water at a concentration of

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4 mg DEN/100 ml of solution, and fresh solution was prepared twice a week.

Mice were given the drinking water containing DEN for a period of 8 weeks providing a DEN dose of approximately 8 μg/g body weight/day. Control mice were maintained on drinking water containing no additions. DEN exposure was stopped after this 8-week period, and $2\frac{1}{2}$ weeks later, the administration of Aroclor 1254 was begun. One half of the control mice and one half of the DEN-treated mice were given the PCB mixture intraperitoneally in a dose of 100 μg/g body weight every other week for a total of eight doses over a period of 16 weeks. The remaining control and DEN-treated mice were treated similarly with the tricaprylin/corn oil vehicle.

Groups of mice were killed at the end of 8 weeks of Aroclor 1254 administration, after a total of four doses of the PCB mixture or of the vehicle, and at the end of the 16-week treatment period with Aroclor 1254, after a total of eight doses of the PCB mixture or of the vehicle. Mice were weighed at each of these intervals and each mouse was killed by decapitation, exsanguinated, and then autopsied. The liver was removed, weighed, and sections were placed into 10% neutral buffered formaldehyde solution (NBFS). Liver sections were prepared for examination by light microscopy and were stained with hematoxylin–eosin, with the Masson–Trichrome stain, with Alcian blue, or with Prussian blue.

The histological classification of mouse liver nodules was made with the Becker system for classifying mouse liver tunors (18). The term "nodule" as used in this report refers to tissue in the liver, clearly demarcated from liver tissue itself, usually beige in color, and which has the histiological features described by Becker (18).

Statistical analyses were the following: differences in body weight and liver weights among the treatment groups were compared using a computer-based nonparametric analysis of variance; the significance of the differences in the incidences of liver nodules among the treatment groups was determined with a 2×2 χ^2 analysis.

Results. *Body weight and liver weight changes.* There was a significant decrease in the body weights of mice initially given DEN and subsequently treated with the tricaprylin/corn oil vehicle, but no other significant changes in body weights were observed (Table 1). The administration of Aroclor 1254 over

TABLE I. BODY WEIGHTS AND LIVER WEIGHTS OF MICE TREATED WITH DEN FOLLOWED BY AROCLOR 1254

Treatment	*N*	Body wt (g)	Liver wt	
			g	As % body wt
(A) Mice treated with DEN followed by the vehicle or Aroclor 1254 (PCB) over an 8-week period				
Control + Vehicle	8	43.7 ± 1.5	2.4 ± 0.13	5.5 ± 0.09
DEN + Vehicle	8	39.1 ± 1.2[a]	2.3 ± 0.15	5.9 ± 0.11
Control + PCB	8	43.9 ± 1.1	2.9 ± 0.07[b]	6.6 ± 0.06[a]
DEN + PCB	8	41.6 ± 0.9	4.0 ± 0.25[c]	9.6 ± 0.14[d]
(B) Mice treated with DEN followed by the vehicle or Aroclor 1254 (PCB) over a 16-week period				
Control + Vehicle	18	42.9 ± 0.7	2.3 ± 0.09	5.2 ± 0.07
DEN + Vehicle	19	38.7 ± 0.7[a]	2.8 ± 0.17	6.9 ± 0.37
	(10)			(5.6 ± 0.28)
Control + PCB	18	41.4 ± 0.7	3.8 ± 0.11[c]	9.0 ± 0.27[c]
DEN + PCB	18	41.3 ± 0.7	5.7 ± 0.20	14.3 ± 0.49[d]
	(8)			(14.0 ± 0.72)

Note. Data are expressed as the means ± 1 standard error of the mean. *N* = number of mice. Numbers in parentheses are data from mice which did not have liver nodules (see Table 2).

[a] $P < 0.01$ compared to Control.

[b] $P < 0.015$ compared to Control and to DEN.

[c] $P < 0.001$ compared to Control, to DEN, and to DEN + PCB.

[d] $P < 0.001$ compared to Control, to DEN, or to Control + PCB.

an 8- or a 16-week period to control mice was accompanied by a significant increase in liver weight, whether expressed in grams or as percentage body weight (Table I). A further significant increment in liver weight accompanied the administration of Aroclor 1254 to mice which had been treated with DEN; liver nodules were not a factor in these added increases in liver weights (Table I).

Liver lesions. The incidence of liver nodules is summarized in Table II. The difference in grossly observed liver nodule incidence was not significant when mice given DEN and then the vehicle over 8 or 16 weeks were compared with mice given DEN and then Aroclor 1254 over 8 or 16 weeks.

The liver from one of the eight DEN-treated mice given the tricaprylin/corn oil vehicle over 8 weeks had a coarse surface; a similar change in the gross appearance of the liver was seen in 6 of 19 DEN-treated mice which were given the tricaprylin/corn oil vehicle over a 16-week period and in one of 18 control mice given Aroclor 1254 over the 16-week period. Much more striking was the severe structural distortion of the livers of 15 of the 18 mice which initially had been treated with DEN and subsequently with Aroclor 1254 (Table II, Fig. 1).

TABLE II. INCIDENCE OF GROSS LIVER CHANGES IN MICE TREATED WITH DEN FOLLOWED BY THE VEHICLE OR AROCLOR 1254 (PCB)

Treatment	*N*	Number of Mice with: Liver nodules	Number of Mice with: Scarred livers
(A) Mice treated with DEN followed by the vehicle or Aroclor 1254 (PCB) over an 8-week period			
Control + Vehicle	8	0	0
DEN + Vehicle	8	2	0
Control + PCB	8	0	0
DEN + PCB	8	2	0
(B) Mice treated with DEN followed by the vehicle or Aroclor 1254 (PCB) over a 16-week period			
Control + Vehicle	18	0	0
DEN + Vehicle	19	9[a,b]	1
Control + PCB	18	1	1
DEN + PCB	18	10	15

Note. *N* = number of mice in each group.
[a] $P < 0.001$ when compared with Control or with Control + PCB.
[b] $P < 0.7$ when compared with DEN + PCB.

Histological changes. Liver nodules were made up of Type I, Type II, or more commonly a mixture of Type I and Type II tissues. Liver sections from each of three DEN-treated mice given the vehicle over the 16-week period contained one well-demarcated area of Type I tissue suggesting early nodule formation.

The livers of DEN-treated mice given the tricaprylin/corn oil vehicle over 8 or 16 weeks contained variable numbers of oval cells, bile duct-like adenomatous proliferation, and cyst-like structures which represented the distinctive and most common histological change. Sections of livers from two of these DEN-treated mice given the vehicle over the 16-week period contained a modest degree of collagen deposition which contained proliferating bile ducts (adenofibrosis) which usually occurred on or near the surface of the liver and contributed to some of the surface indentations, giving the livers their rough surfaces.

Centrilobular hepatocyte hypertrophy was the consistent light microscopic change in all livers of mice treated with Aroclor 1254 over an 8- or 16-week period.

Adenofibrosis was present in all liver sections from DEN-treated mice given Aroclor 1254 for 8 weeks. The lesion occurred most commonly just below the surface of the liver with extensions toward lesions developing within the liver substance (Fig. 2). Among the proliferating bile ducts were glandular structures containing goblet-like cells (Fig. 3), thereby resembling intestinal metaplasia which has been described in sections of livers from rats treated sequentially with 2-AAF and partial hepatectomy (12).

Adenofibrosis was increased dramatically in the livers of 15 of the 18 mice treated with DEN and subsequently with Aroclor 1254 over 16 weeks (Figs. 4, 5). Differential staining indicated that the adenofibrotic tissue contained significant quantities of collagen and additionally some glycosaminoglycan (presumably as proteoglycan). No evidence of iron accumulation in liver cells was observed. Neither steatosis nor inflammation, two common features of progressive alcoholic liver disease (19), nor large areas of necrosis were observed in livers taken from mice at any intervals of this investigation.

Intrahepatic aggregates of Type I and of Type II tissue were seen in all but one of the

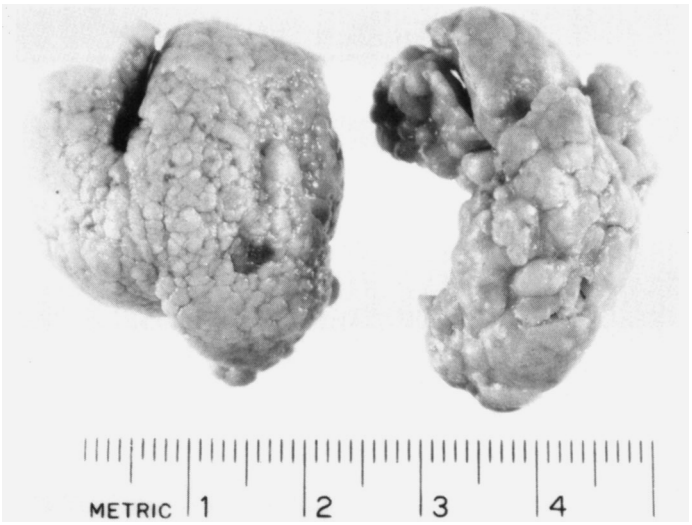

FIG. 1. Scarred livers from mice exposed to DEN for 8 weeks, given $2\frac{1}{2}$ weeks of no treatment, and then Aroclor 1254 over a subsequent 16-week period.

sections from mice treated with DEN and given Aroclor 1254 over the 16-week period. There were two or three of the Type I or Type II tissue aggregates in most of the sectons. The apparently uneven growth of the populations of liver cells, hypertrophied hepatocytes, re-

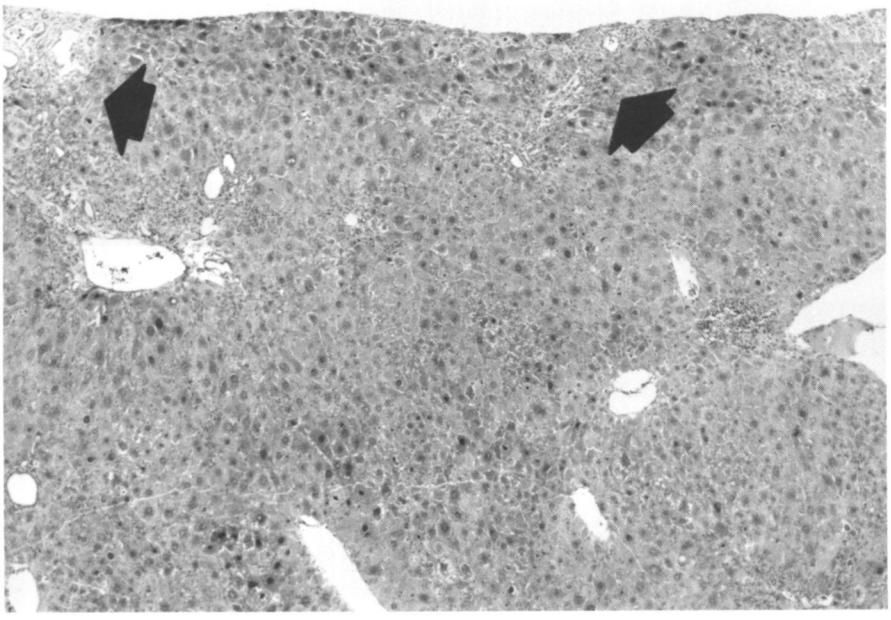

FIG. 2. Liver section from a DEN-treated mouse given Aroclor 1254 over an 8-week period. Areas of adenofibrosis, particularly at the surface of the liver (arrows), appear to be spreading into deeper zones. Large numbers of hypertrophied parenchymal cells occur throughout this section. Magnification 40×.

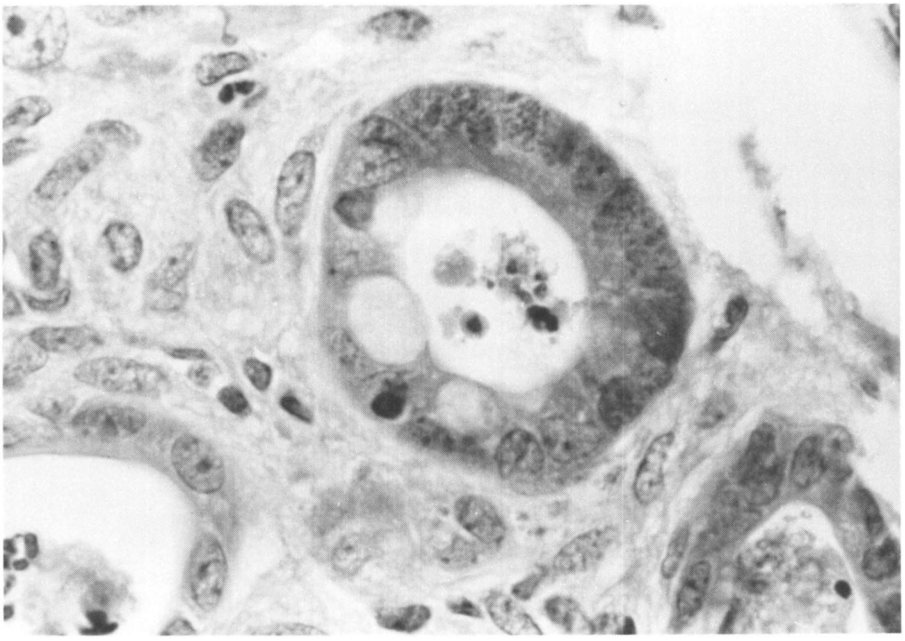

FIG. 3. High power magnification (800×) from an adenofibrotic lesion in the liver section from the mouse described in Fig. 2. A glandular structure contains goblet-like cells. Oval cells are present in the interstitial tissue.

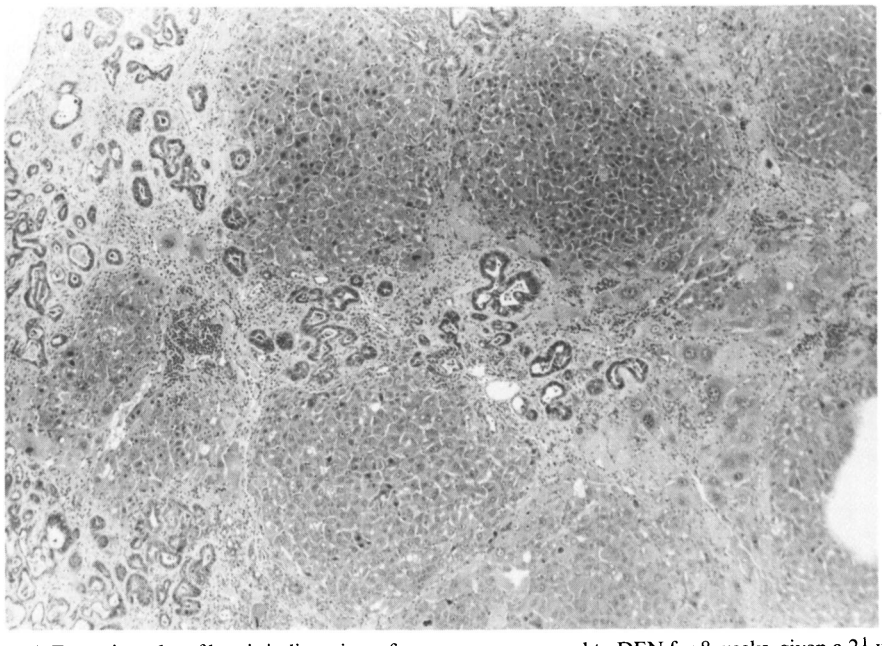

FIG. 4. Extensive adenofibrosis in liver tissue from a mouse exposed to DEN for 8 weeks, given a $2\frac{1}{2}$ week treatment-free interval and then treated with Aroclor 1254 over a 16-week period. Lobular aggregates of small hepatocytes are surrounded by adenofibrotic tissue. Magnification 40×.

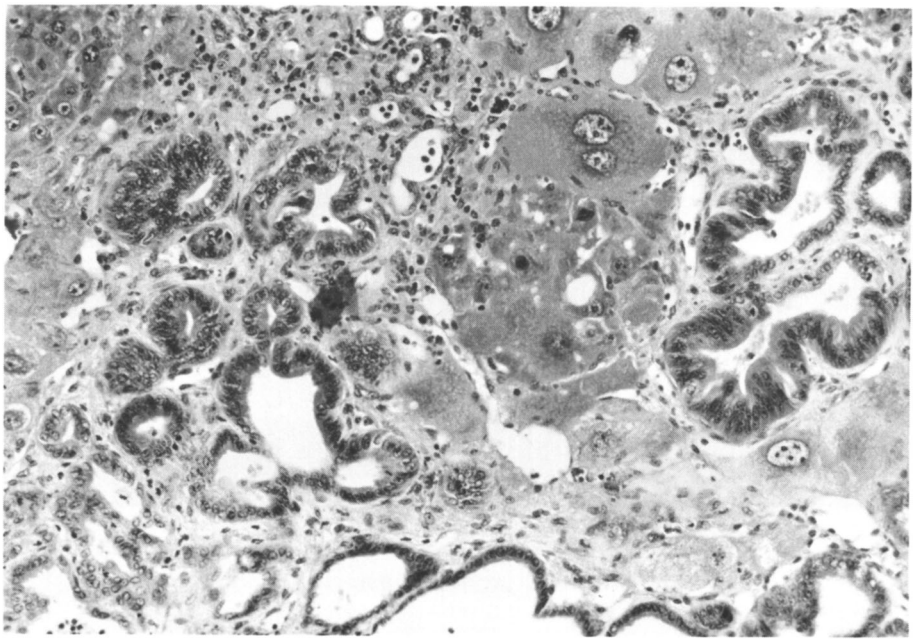

FIG. 5. Higher power magnification (400×) of a section of an adenofibrotic lesion. The proliferating bile ducts take on an adenomatous appearance. Collections of hypertrophied hepatocytes are present within the lesion.

generating liver cells, and nodular tissue formed irregular masses which protruded from the surface of the liver and contributed to the liver's distorted appearance.

Discussion. The extensive liver scarring described in this report has several features which differentiate it from human alcoholic or postnecrotic cirrhosis and from CCl_4-induced cirrhosis in rats. Most prominent is the widespread proliferation of adenomatous bile duct-like structures in the livers of DEN-treated mice subsequently given Aroclor 1254. This lesion has not been described in human alcoholic liver cirrhosis (19, 20) nor in liver cirrhosis of CCl_4-treated rats (21). Bile duct proliferation has been observed in the fibrous tissue of livers in human cirrhosis associated with idiopathic hemochromatosis (22) but iron accumulation did not occur in the scarred livers of mice.

Collagen synthesis in the pathophysiology of liver scarring may be induced by multiple stimuli and hepatocyte necrosis may not be an obligatory provoking factor (23). Tatematsu *et al.* have shown that the bile duct-like structures of livers of rats subjected to the AAF–hepatectomy regimen contained intestinal-like cells capable of secreting intestinal enzymes (12). The destructive effects of intestinal enzymes on connective tissue may be the adequate stimuli for collagen synthesis and deposition (12). This idea may explain the deposition of collagen in our example of chemically induced liver scarring in mice.

Body weight and liver weight changes similar to those reported in this study have been observed in mice given DEN and then fed a diet containing organochlorine insecticides (24) and in rats subjected to a DEN–PCB regimen (7). In both of these studies, feeding the chlorinated hydrocarbons was accompanied by significant increases in liver tumor incidence but no mention was made of liver scarring. The development of liver fibrosis and the lack of overt tumor promotion in DEN-treated mice given the PCB mixture may not have been the result of the intraperitoneal route of administration of Aroclor 1254. Most studies demonstrating the tumor promoting effects of PCBs used PCBs added to the diet of mice and rats (4, 5, 7), but Pereira *et al.* gave Aroclor 1254 by intraperitoneal injection to DEN-

treated mice and did not report either fibrosis or adenomatous bile duct proliferation (25).

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