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Proliferative Lesions of the Glandular Stomach and Liver in F344 Rats Fed Diets Containing Aroclor 1254

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Aroclor 1254 was fed to groups of 24 male and 24 female F344 rats, from 7 weeks of age, at dietary concentrations of 25, 50 and 100 ppm for up to 105 weeks. There was a dose-related depression of body weight gain for both sexes and decrease in survival for male rats. Histologically, an increased incidence of gastric intestinal metaplasia and adenocarcinoma was found in both sexes. Hepatocellular adenomas, carcinomas, and eosinophilic and vacuolated hepatocellular foci were usually found in dosed rats only. The number of these foci per unit area of liver was significantly increased in dosed rats, although eosinophilic foci were only found in rats exposed to Aroclor 1254. Basophilic hepatocellular foci were found in similar numbers per square centimeter of liver in controls and treated rats. This finding suggested that eosinophilic hepatocellular foci and tumors arose *de novo* rather than from the naturally occurring basophilic foci. The appearance of unique, potentially preneoplastic lesions and tumors in the liver and stomach in dosed rats which do not usually occur spontaneously in control rats would support the hypothesis that Aroclor 1254 induced or initiated these unique lesions *de novo* rather than promoted the growth of any naturally occurring lesions. Nonneoplastic hepatic lesions included degenerative hepatocellular changes and aggregates of macrophages with crystalline cytoplasmic structures and pigment granules.

Introduction

Polychlorinated biphenyls (PCBs) have been shown to induce liver tumors in rats (*1–5*) and mice (*6–8*) and to promote development of liver preneoplastic foci and tumors initiated by other carcinogens in liver (*9–19*). In the early 1970s, little was known of the carcinogenic potential of PCBs. The National Cancer Institute's former bioassay program selected Aroclor 1254 for testing for carcinogenicity in a large scale experiment to study the effects of combinations of chemicals. This large study of approximately 24,000 F344 rats was conducted at Stanford Research Institute under contract to the National Cancer Institute and was completed in 1974. This paper reviews the previous findings of the Aroclor 1254 bioassay (*4*) and re-evaluates hepatic and gastric lesions in light of recent cancer research finding.

Methods

Weanling male and female F344 rats were obtained from Simonsen Laboratory (Gilroy, CA) and from 7 weeks of age were fed a diet (Ralston Purina Co., St. Louis, MO) which contained 0, 25, 50 or 100 ppm of Aroclor 1254 (Monsanto Chemical Co., St. Louis, MO) for 105 weeks (Table 1) (*4*). The Aroclor 1254 was analyzed at SRI and found to be composed of 54.67% chlorine and to be a mixture of at least 18 isomers with from 4 to 7 chlorine atoms per molecule. The test chemical was dissolved in corn oil for addition to the diet.

A complete necropsy was performed on each rat in the experiment. Histopathologic slides were prepared

Table 1. Design of Aroclor 1254 chronic feeding studies in rats.

Sex and treatment group	Initial no. of animals[a]	Aroclor 1254 in diet, ppm[b]	Time on study, weeks	
			Treated	Untreated
Males				
Matched control	24	0		105
Low dose	24	25	105	
Intermediate dose	24	50	105	
High dose	24	100	105	
Females				
Matched control	24	0		105
Low dose	24	25	104–105	
Intermediate dose	24	50	104–105	
High dose	24	100	105	

[a]All animals were 53 ± 2 days of age when placed on study.
[b]All diets contained 3% corn oil.

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from all gross lesions, lungs, spleen, liver, testes, pituitary, kidney and brain. Generally, only one section of liver was prepared. In the original bioassay, few sections of the stomach were evaluated. In a subsequent review of the study, all stomachs were sectioned (*20*). Sections were stained with hematoxylin and eosin. Selected sections were stained by one of the following methods: periodic acid–Schiff method (PAS), Wilder's reticulin, Masson's trichrome, Alcian Blue–PAS. All liver sections from the study were reviewed by the author in August of 1983. Hepatic lesions were classified according to Ward (*21,22*).

For quantitation of focal proliferative liver lesions, the area of each liver section was determined using an automated image analyzer (Videoplan, Carl Zeiss, Inc., New York, NY). The number and type of foci and nodules were recorded and the number of foci per square centimeter was determined for each rat (*22*). The Mann-Whitney (Wilcoxon) test (*23*) was used to determine the significance of differences of the number of foci between groups. For analysis of liver tumor incidence, Fisher's exact test and the Cochran-Armitage test for trend were used (*24*).

Results

Clinical Signs, Body Weights, and Survival

There was a dose-related depression of body weight gain for both sexes, which persisted from week 10 until the end of the bioassay. Survival was dose-related for males but not for females: 92% of the control males, 83% of the low dose, 58% of the intermediate dose, and 46% of the high dose males survived to the end of the bioassay. The contributing causes of death for 13 male rats receiving the highest dose included leukemia (5), pneumonia (2), hepatic lesions (6), intestinal carcinoma (1), liver carcinoma (1) and brain abscess (1). For 10 males at the intermediate dose, the contributing causes of death included leukemia (4), pneumonia (6) and intestinal carcinoma (1). In several cases, more than one contributing cause of death was found. In some cases, the cause of early mortality of the males may have been Aroclor 1254 exposure (4) (hepatic lesions, intestinal carcinoma and liver carcinoma) while in other cases, natural causes were evident (leukemia and pneumonia). The Aroclor 1254 may have accentuated the natural deaths through immunosuppressive or other mechanisms.

Pathology

Many types of neoplastic and nonneoplastic lesions were found in rats in all groups. Except for those in the liver, stomach and intestine, no lesions were related to the administration of Aroclor 1254 (*4*).

Hepatic Lesions

The numbers and types of hepatic lesions were significantly related to the administration of Aroclor 1254 (Table 2). Focal hyperplasias (foci of cellular alteration) of the eosinophilic type were found only in rats given Aroclor 1254 (Table 2). Hepatocytes in these foci were larger than normal hepatocytes and had prominent pale eosinophilic cytoplasm (Figs. 1 and 2). They occurred randomly in the hepatic lobule and were multiple in many dosed rats. Some foci were 5 to 10 cells in diameter while others were larger than a lobule. When compression was found on two sides of the focus, the lesions were diagnosed as hepatocellular adenoma. Adenomas were usually large lesions, 1 to 5 mm in size. As in the eosinophilic foci, the hepatocytes in eosinophilic adenomas were larger than normal hepatocytes

Table 2. Hepatocellular foci and tumors in groups of 24 F344 rats fed diets containing Aroclor 1254.

Dose, ppm	Sex	Hepatocellular foci/cm^2 ± SD			Hepatocellular adenoma (number with lesion)				
		Eosinophilic	Basophilic (% with lesion)	Vacuolated	Eosinophilic	Basophilic	Vacuolated	Carcinoma	Any liver tumor
0	Male	0 (0)	1.48 ± 3.75 (29)	0 (0)	0	0	0	0	0
25	Male	0.93 ± 1.82* (29)†	0.27 ± 1.03 (8)	0.60 ± 1.80 (16)	1	0	0	0	1
50	Male	0.76 ± 1.29 (33)†	0.63 ± 1.70 (16)	0.58 ± 1.70 (16)	2	0	0	0	2
100	Male	1.24 ± 2.84* (43)†	1.58 ± 3.24 (37)	0.41 ± 0.66 (29)†	3	1	1	2	7*
0	Female	0 (0)	1.15 ± 3.30 (29)	0.09 ± 0.45 (4)	0	0	0	0	0
25	Female	2.74 ± 3.86* (54)†	0.44 ± 0.92 (20)	0.04 ± 0.21 (4)	0	0	0	0	0
50	Female	3.34 ± 3.49† (62)†	0.15 ± 0.73 (4)	0.10 ± 0.50 (4)	3	0	0	0	3
100	Female	1.27 ± 2.30* (41)†	0.87 ± 2.31 (29)	1.07 ± 1.62* (41)†	1	0	1	0	2

*<0.05 as compared with appropriate control.
†<0.01 as compared with appropriate control.

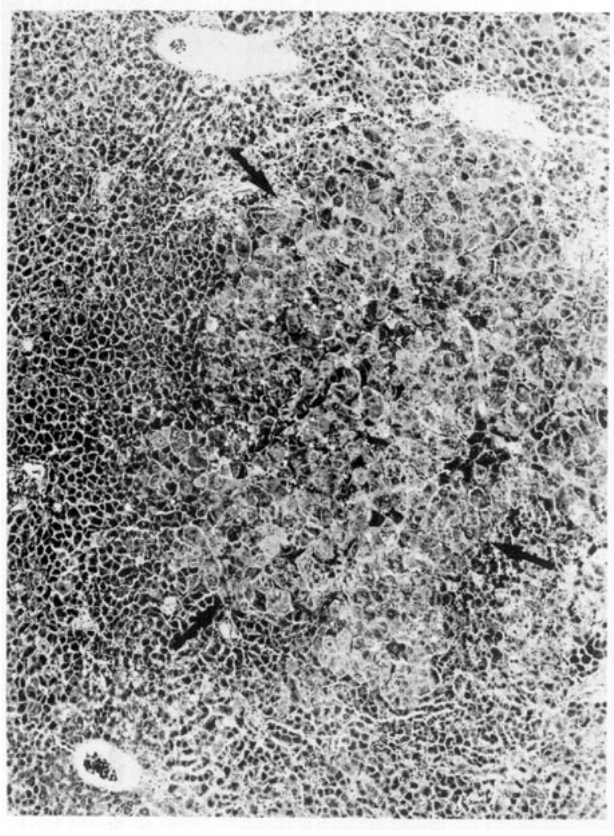

FIGURE 1. Eosinophilic focus (arrows) in liver of female F344 rat fed diet containing 100 ppm of Aroclor 1254. H & E; ×54.

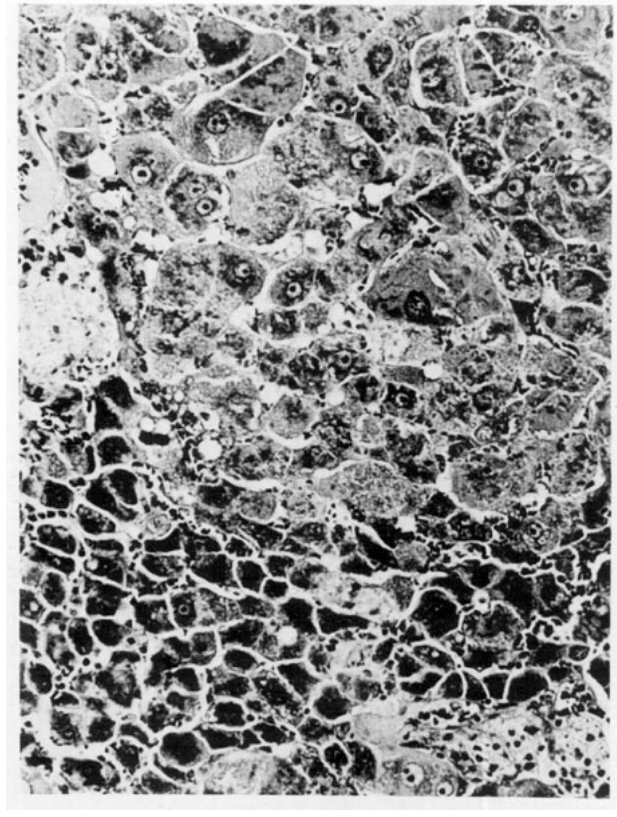

FIGURE 2. Higher magnification of Fig. 2, showing large hepatocytes in focus with prominent eosinophilic cytoplasm containing basophilic bodies. Note dark rounded hepatocytes in adjacent areas. H & E; ×220.

and had eosinophilic cytoplasm (Fig. 3). Seven adenomas were found in males and four in females. One male given the high dose and one female given the intermediate dose of Aroclor 1254 each had two eosinophilic adenomas. Hepatocellular carcinomas in two male rats receiving the highest dose of Aroclor 1254 were also composed predominantly of eosinophilic hepatocytes. Areas of prominent trabecular formations were seen in tumors of both rats and vascular invasion was seen in one tumor.

Basophilic foci were found both in control and in dosed rats (Fig. 4). They were not clearly dose-related. These foci were similar to those found in normal aging F344 rats. One basophilic adenoma was found in a high dose male rat. Focal areas of lipidosis (Fig. 5) were found primarily in dosed males and in dosed females. Two adenomas were composed of hepatocytes with prominent lipid droplets. When all types of liver adenomas and carcinomas were combined there was a dose-related trend in tumor incidence ($p < 0.01$) in male rats.

Other types of hepatic lesions were found (Table 3). Large granular lymphocyte (LGL) leukemia was dose related in males (control 3/24, low dose 2/24, intermediate dose 5/24, high dose 8/24) but not in females. Granulomas (composed of histiocytes and lymphocytes) in portal areas of the liver were sometimes associated with pigmented macrophages, but the former lesions were also seen in controls as well. Clusters of pigmented mac-

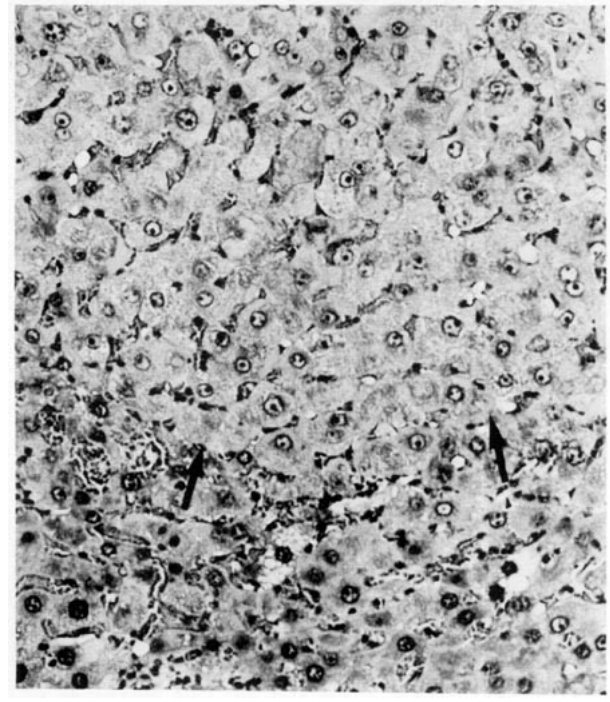

FIGURE 3. Portion of eosinophilic hepatocellular adenoma (arrows) in male rat receiving diet containing 100 ppm of Aroclor 1254. Note large size of cells as compared with normal hepatocytes. H & E; ×200.

rophages having crystalline structures within their cytoplasm were observed in dosed rats only (Fig. 5). More lesions with macrophages were found in females than in males.

Mild to moderate degenerative changes of hepatocytes occurred in a dose-related distribution. In high dose rats, hepatocytes were variable in size and individual cells were rounded. Foci of bile duct hyperplasia and oval cell (cholangiocellular) hyperplasia were seen in a few high dose rats. Oval cell hyperplasia was sometimes associated with eosinophilic foci.

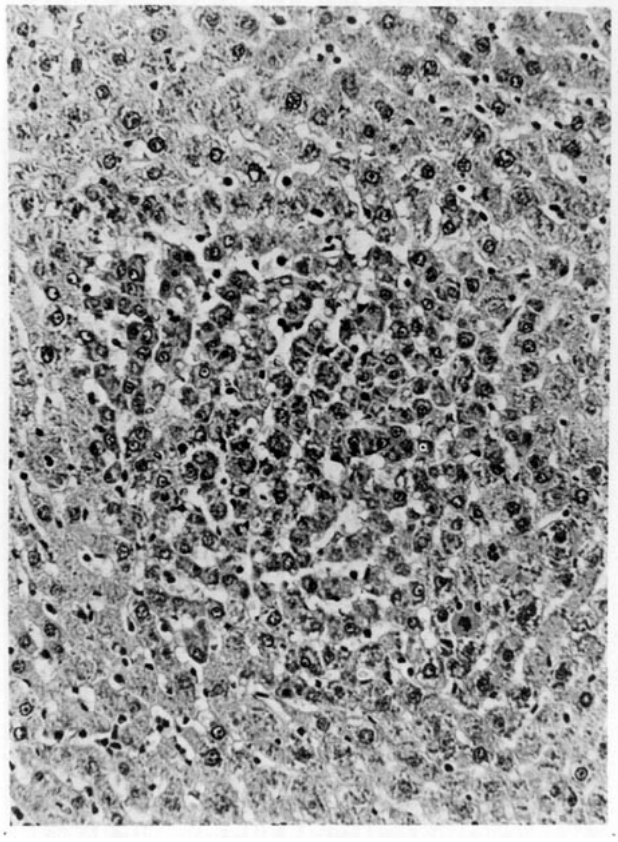

FIGURE 4. Focus of hepatocytes with basophilic cytoplasm (basophilic focus) in control F344 rat. H & E; ×220.

Gastric Lesions

Intestinal metaplasia and gastric adenocarcinoma were found in association with the feeding of Aroclor 1254 (Table 4). Because the necropsy protocol was not designed to detect gastric lesions, the actual incidence of these lesions may be higher. Three control rats (two males, one female) had small metaplastic lesions in association with lymphoid follicles in the pyloric region of the glandular stomach. The metaplastic lesions in dosed rats were different than those in the controls. Metaplasia was seen as the presence of many mucin-containing epithelial cells (Fig. 6). The lesions were usually not associated with lymphoid follicles and were

Table 3. Incidence of other hepatic lesions.

		No. of rats with lesion/no. of rats in group (%)		
Dose	Sex	LGL leukemia	Granulomas	Pigmented macrophages with crystalline structures
0	Male	3/24 (12.5)	4/24 (16.6)	0/24 (0)
25	Male	4/24 (16.6)	0/24 (0)	0/24 (0)
50	Male	6/24 (25.0)	4/24 (16.6)	4/24 (16.6)
100	Male	7/24 (29.1)	2/24 (8.3)	5/24 (20.8)*
0	Female	4/24 (16.6)	12/24 (50.0)	2/24 (8.3)
25	Female	7/24 (29.1)	6/24 (25.0)	7/24 (29.1)*
50	Female	7/24 (29.1)	8/24 (33.0)	16/24 (66.6)†
100	Female	4/24 (16.6)	11/24 (45.8)	23/24 (95.8)†

*$p < 0.05$.
†$p < 0.01$.

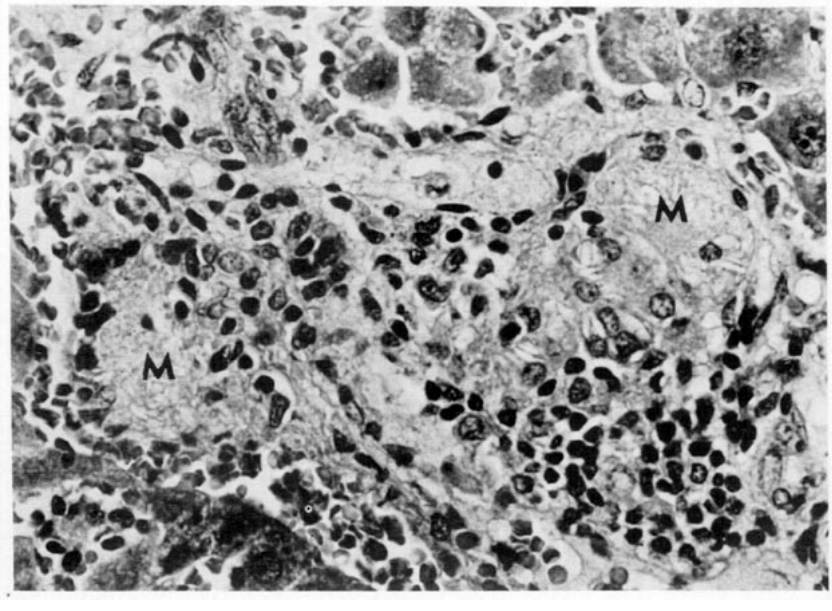

FIGURE 5. Two clusters of macrophages (m) with crystalline inclusions in liver of female rat fed Aroclor 1254. H & E; ×330.

Table 4. Intestinal metaplasia and gastric adenocarcinoma in male and female F344 rats fed Aroclor 1254.

Dietary concentration of Aroclor 1254, ppm	No. of rats with lesion/ no. of rats treated	
	Intestinal metaplasia	Adenocarcinoma
0	3/47	0/47
25	4/48	1/48
50	5/48	3/48
100	15/48*	2/48

*$p < 0.05$ as compared with control group.

FIGURE 6. Intestinal metaplasia in glandular stomach showing mucin in lesions (arrows) and none in adjacent normal epithelium. Alcian Blue-PAS; ×130.

frequently cystic (Fig. 7). The epithelium had areas containing mucus and was composed of single layers of basal, goblet or tall columnar cells. None of these lesions were apparent grossly at necropsy. Adenocarcinomas were usually seen at necropsy and invaded the stomach wall to varying degrees. Some tumors were cystic, and similar in morphology to the metaplastic lesions, except for piling up of multiple layers of epithelial cells (Fig. 8), increased anaplasia and invasion (Fig. 9).

Discussion

The bioassay of Aroclor 1254 in F344 rats provided evidence for the induction of proliferative lesions of the

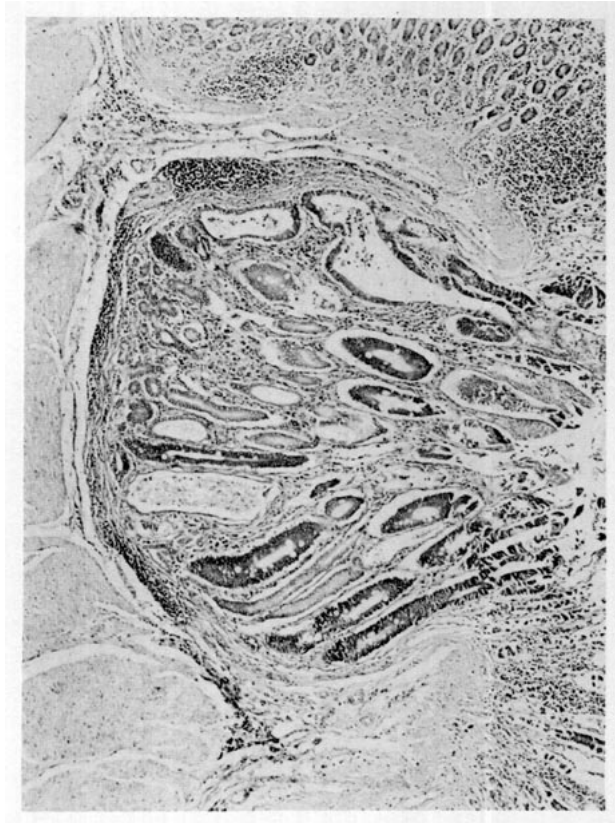

FIGURE 7. Intestinal metaplasia in rat showing focal cystic lesion with reactive inflammation. H & E; ×54.

FIGURE 8. Portion of invasive gastric adenocarcinoma with single layered and multiple layered epithelium and loss of epithelial cell polarity. H & E; ×330.

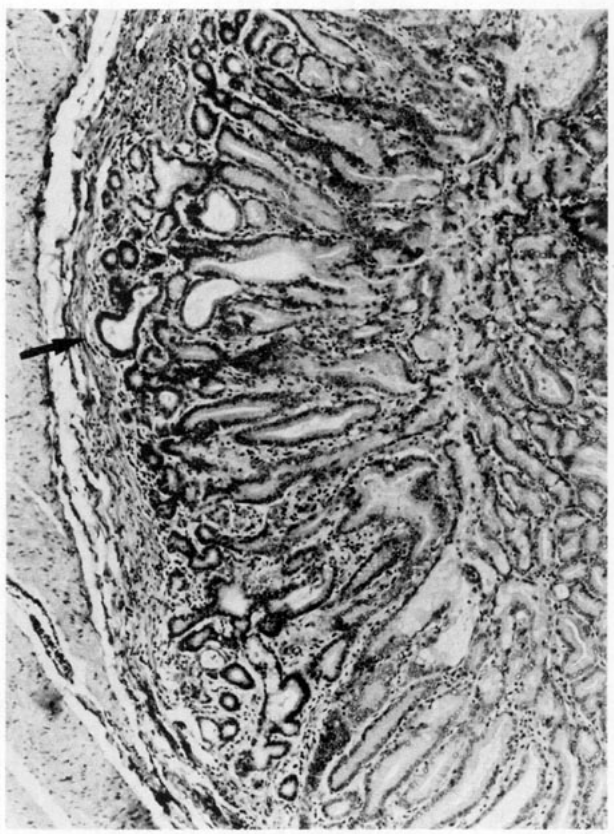

FIGURE 9. Portion of large gastric adenocarcinoma with early invasion into submucosa (arrow). H & E; ×80.

liver and stomach by the chemical. The evidence consists of unusual morphological lesions in rats exposed to Aroclor 1254. The low incidence of some of these lesions may depend, in part, on the dosages used in the study. Although body weight depression was adequate for a chronic bioassay, hepatic lesions were not as severe as one might expect from a liver toxin, even in other studies with PCBs (*24,25*). In another rat bioassay of Aroclor 1254 with doses up to 1000 ppm, no liver tumors were seen in the 8-month study (*26*). In mice given 300 ppm in the diet, liver tumors and adenofibrosis were seen (*7*). In studies of other polychlorinated biphenyls, liver tumors were found when dietary levels of from 100 to 1000 ppm were used (*1–5*).

Eosinophilic hepatocellular foci, like those found in this study, were also seen in another bioassay of Aroclor 1260 (*2*). The eosinophilic foci could be precursors of eosinophilic adenomas and carcinomas. Other chemicals have caused these unusual liver lesions. Eosinophilic foci and adenomas were seen in rats exposed to phenobarbital only (*22*) or after initiation by diethylnitrosamine and promotion by phenobarbital (J. Ward, unpublished observations). Since the foci in control F344 rats were basophilic (*21,22*) and foci in rats exposed to Aroclor 1254 were eosinophilic, the chemical may have induced these foci *de novo* or transformed the spontaneous basophilic foci to eosinophilic lesions. The fact that numbers of basophilic foci were similar in control and treated rats suggests that the eosinophilic foci were formed *de novo* or arose from other types of foci not readily visible or reported. Vacuolated or clear cell foci have been reported in aging F344 rats but are difficult to detect (*22*). Polychlorinated biphenyls were shown to be promoters of liver foci and tumors in short term *in vivo* assays (*9–19*). The findings in this study suggest that they may also be initiators. It has been suggested that nongenotoxic promoters would promote spontaneous foci rather than initiate new tumor populations (*27,28*). Aroclor 1254, like phenobarbital, induces the eosinophilic foci and tumors rather than promoting the spontaneous basophilic lesions. Both carcinogens may act directly on hepatocytes to cause proliferation of smooth endoplasmic reticulum, an effect which causes affected hepatocyte cytoplasm to appear eosinophilic (*26*).

Stomach lesions in F344 rats exposed to Aroclor 1254 were those of intestinal metaplasia and adenocarcinoma. We previously showed these lesions to contain alkaline phosphatase (*20*). The metaplastic lesions resembled those in monkeys exposed to PCB (*29,30*), but differed from them in some characteristics. Monkey lesions were diffuse while rat lesions were focal and usually singular. Stomach tumors were not found in monkeys. The finding of the unusual metaplastic lesions in rats suggests that Aroclor 1254 acted as an initiating agent rather than as a promoting agent, since the few metaplastic lesions in stomachs of control rats differed morphologically from those in dosed rats. The metaplastic lesions resembled precancerous lesions induced by gastric carcinogens (*31*), but the few invasive tumors seen suggest further study is needed of the effect of PCBs on the glandular stomach of rats.

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