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Enhanced Polychlorinated Biphenyl Lesions in Moloney Leukemia Virus-Infected Mice

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INTRODUCTION

Polychlorinated biphenyls (PCB) are potential environmental contaminants that are used in electrical capacitors and transformers, heat transfer systems, plasticizer applications, hydraulic lubricants, and other miscellaneous applications [1]. In 1968, the ingestion of K rice oil contaminated with PCB Kanechlor 400 (48% chlorine) produced the disease "Yusho" in Japan [2]. The disease was characterized by increased eye discharge, follicular accentuation, acne-form eruption, sweating palms, weakness, and pigmentation of the skin and nails. Polychlorinated biphenyls are hepatotoxic in mammals [3-5] but produce edema formation in birds [3].

Since PCBs are hepatotoxic and neoplasia produced by Moloney leukemia virus (MLV) may involve the liver, the present study was devised to determine if different formulations of PCB may affect induction of neoplasia by MLV or if MLV may affect the toxicity of PCB.

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MATERIALS AND METHODS

Balb/c male mice 28 days old were used in the experiment. The animals were divided into 10 groups. Nine groups of 100 mice each received PCB Aroclor 1221, 1242, or 1254 (Monsanto Co., St. Louis, Mo.) mixed in the feed (Ralston Purina Lab., St. Louis, Mo.) and 175 controls were given non-PCB-contaminated feed. The PCB formulations contained 21, 42, and 54% chlorine.

The PCB-exposed groups received either 375, 37.5, or 3.75 ppm of each Aroclor (1221, 1242, or 1254) (Table 1). Twenty-five mice in each of the nine PCB groups were inoculated intraperitoneally (IP) at 42 days of age with 0.3 ml of either 1×10^{0}, $1 \times 10^{-1.5}$, or 1×10^{-3} dilution of Moloney leukemia virus (MLV) (NCI, NIH by University Lab. Inc., Highland Park, N.J.), and the remaining 25 were inoculated IP with sterile physologic saline (Table 1). Fifty control mice (without PCB) were each inoculated IP at 42 days of age with 0.3 ml of 1×10^{0}, $1 \times 10^{-1.5}$, or 1×10^{-3} dilution of MLV, and 25 mice received neither PCB nor MLV.

The PCBs were fed for six months at which time one-half of the animals in each group were killed. Each mouse was weighed and necropsied. The liver, spleen, and kidneys were weighed and placed in buffered 10% formalin. Feeding of the PCB-contaminated food was discontinued at six months and mice that had been receiving 37.5 or 3.75 ppm Aroclor 1254, 375 ppm Aroclor 1242, and no PCB were fed another three months of PCB-free diet. These animals were then killed, weighed, and necropsied. The liver, spleen, and kidneys were weighed and placed in 10% formalin. The mice remaining in the other PCB groups were killed, examined for gross and microscopic lesions, and discarded after the six of months of feeding PCB since lesions had not occurred by that time.

Microscopically, hepatic lesions were graded from + to ++++. The most prominent lesions of each grade were as follows: + was represented by mild centrolubular granular degeneration and necrosis of hepatocytes and a slight variation in cell size; ++ was determined by moderate vacuolar degeneration and necrosis of the centrolobular hepatocytes (Fig. 1) and several hepatocytes were enlarged; +++ changes consisted of severe centrolobular cytoplasmic vacuolar degeneration and necrosis of hepatocytes that often involved the entire lobule, marked variation in cell size, and nucleoli that were often prominent; ++++ lesions were characterized by extensive bile duct proliferation and periportal fibrosis, marked variation in cell size but most all were enlarged, necorsis of hepatocytes, and the nucleoli were distinct with margination of the chromatin (Fig. 2). Fat droplets occurred in some hepatocytes at all grades of alteration, but there was never an abundant accumulation of fat.

The spleen was considered to be altered by Moloney leukemia virus

TABLE 1. Mean Liver Weights (gm) of Mice Given Three Different Aroclors of Polychlorinated Biphenyls for Six Months and Inoculated with Three Dilutions of Moloney Leukemia Virus[a]

PCB Aroclor	ppm	Dilution MLV: No virus		1×10^{0}		$1 \times 10^{-1.5}$		1×10^{-3}	
		6 mo.	9 mo.	6 mo.	9 mo.	6 mo.	9 mo.	6 mo.	9 mo.
1254	375	10.0		9.46		8.82		9.10	
	375[b]	5.48		6.01		5.79		6.05	
	37.5	2.57	2.01	2.47	1.96	2.60	1.86	2.55	1.91
	3.75	2.11	1.87	2.07	1.57	2.08	1.84	1.96	1.79
1242	375	3.11	1.98	2.85	2.43	2.79	2.56	3.27	2.41
	37.5	1.97		2.06		1.98		2.01	
	3.75	1.98		2.05		1.93		1.74	
1221	375	1.82		1.75		1.83		1.99	
	37.5	2.13		1.96		1.91		1.84	
	3.75	1.71		1.92		1.88		1.70	
Control	None	1.74	1.63	1.79	1.76	1.73	1.80	1.66	1.82

[a]Mean liver weights are included for three exposures of PCB in which PCBs had been removed for three months after the six-month exposure (9 mo.). Analysis of variance revealed the increase in mean liver weights were highly significant ($p < 0.01$) in each of the Aroclor 1254 groups and in the 375 ppm Aroclor 1242 group at six months. There was a highly significant ($p < 0.01$) decrease in mean liver weights when the PCB was removed from the diet for three months in the 37.5 ppm Aroclor 1254 groups and in the 375 Aroclor 1242 group.

[b]Died before six months.

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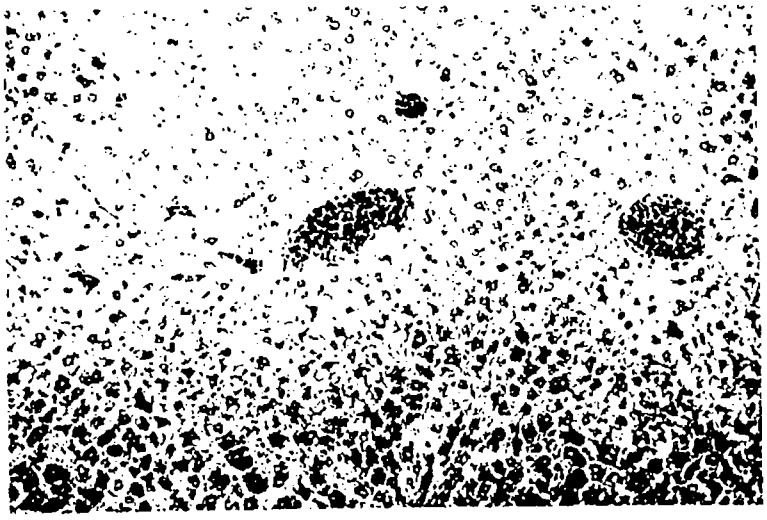

FIG. 1. Centrolobular necrosis of the liver that was considered as a ++ PCB lesion. Notice the vacuolated hepatocytes.

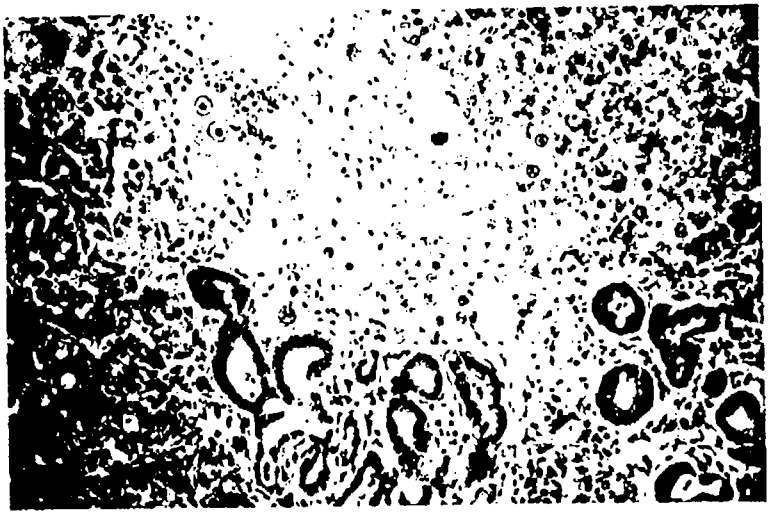

FIG. 2. Bile duct proliferation and early fibrosis that was typical of a ++++ PCB lesion. Notice the variation in cell size and the prominent nucleoli in some of the enlarged nuclei.

(neoplastic) when there was excessive hematopoesis, enlarged lymphoid nodules, or neoplastic lymphocytes in the red or white pulp. The spleen weights also increased when these alterations were evident. Since the spleen was considered to be the organ of origination of the neoplastic cells (lymphocytes), accumulations of neoplastic lymphocytes in the liver were considered as metastasis.

RESULTS

Many of the mice that received 375 ppm Aroclor 1254 died during the fifth and sixth month due to PCB toxicity. This dose of PCB was the only one that was toxic during the six-month feeding period. Those that died and were not inoculated with MLV had severe centrolobular to diffuse cytoplasmic vacuolar degeneration and necrosis of the hepatocytes. Many hepatic cells were enlarged but there was a marked variation of size throughout. The nucleoli were often prominent with margination of chromatin in cells that had enlarged nuclei. These histopathologic changes were considered to be +++.

Those mice that received 375 ppm Aroclor 1254 and 1×10^{0} or $1 \times 10^{-1.5}$ MLV that had spleens affected by the virus had increased liver damage from +++ to ++++ (Table 2). Histologically, there was usually extensive bile duct proliferation and periportal fibrosis of the liver. The hepatocytes varied considerably in size but most were enlarged. Necrosis of the cells was prominent and several contained hyalinized bodies. The nucleoli were distinct in the enlarged nuclei and the chromatin was marginated. These lesions were characteristic of a ++++ liver response to PCB (Fig. 2). Those livers from mice that did not exhibit splenic change were similar to the nonvirus group and had +++ liver change.

Only eight mice survived for six months on the 375 ppm Aroclor 1254 diet. These mice had ++++ liver lesions and seven had splenic involvement.

Mice given 37.5 ppm Aroclor 1254 without virus had + liver lesions characterized by a mild centrolobular granular degeneration and necrosis of hepatocytes and a slight variation in cell size. Those that were inoculated with MLV and had splenic involvement had ++ liver lesions (Table 2) represented by a moderate vacuolar degeneration and necrosis of the centrolobular hepatocytes (Fig. 1). Several hepatocytes were enlarged and variation in size was apparent. Liver lesions did not develop in the 3.75 ppm Aroclor 1254 and nonvirus group, but ++ liver lesions occurred when mice were inoculated with 1×10^{0} virus and exhibited splenic alterations.

Mice that received 375 ppm Aroclor 1242 without MLV had ++ liver lesions while those that were inoculated with 1×10^{0} virus and had splenic involvement had +++ liver lesions (Table 2). A similar effect

TABLE 2. Severity of Hepatic Lesions in Mice Exposed to Three Different Aroclors of Polychlorinated Biphenyls and Inoculated with Three Dosages of Moloney Leukemia Virus[a]

PCB Aroclor	ppm	Dilution of MLV: No virus 6 mo.	No virus 9 mo.	1×10^{0} 6 mo.	1×10^{0} 9 mo.	$1 \times 10^{-1.5}$ 6 mo.	$1 \times 10^{-1.5}$ 9 mo.	$1 \times 10^{-3.0}$ 6 mo.	$1 \times 10^{-3.0}$ 9 mo.
1254	375.0	++++		++++		++++		++++	
	375.0[b]	+++		++++[c]		++++		+++	
	37.5	+	+	++	+	+	+	+	+
	3.75	—	—	++	+	—	—	—	—
1242	375.0	++	—	+++	+ to +++	++	+ to +++	++	—
	37.5	—		++		—		—	
	3.75	—		—		—		—	
1221	375.0	—		—		—		—	
	37.5	—		—		—		—	
	3.75	—		—		—		—	
Control	none	—	—	—	—	—	—	—	—

[a] —, No hepatic lesions. +, mild centrolobular degeneration and necrosis hepatocytes, slight variation in size hepatocytes. ++, moderate centrolobular degeneration and necrosis hepatocytes, several hepatocytes enlarged. +++, severe centrolobular degeneration and necrosis hepatocytes, marked variation in cell size, nucleoli prominent. ++++, bile duct proliferation, periportal fibrosis, marked variation in cell size, necrosis of hepatocytes, distinct nucleoli with margination of chromatin.

[b] Died before six months.

[c] Increase in lesion severity for each virus-inoculated group occurred only in those mice that developed splenic lesions due to MLV.

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occurred at 37.5 ppm Aroclor 1242. Liver lesions did not occur unless splenic lesions were evident in the MLV-inoculated mice and then liver lesions developed.

At six months, regardless of the virus dose, whenever there was evidence of neoplasia in the spleen by histopathology and increased spleen weight, the PCB liver lesions were increased in severity by one +.

At nine months (three months after discontinuing PCB feed), mice in the 375 ppm Aroclor 1242 group without virus did not manifest liver lesions, but those inoculated with 1×10^{0} MLV had liver changes ranging from + to +++ (Table 2). Seventy-three percent had lesions; the majority (36%) had advanced lesions (+++). However in the $1 \times 10^{-1.5}$ MLV group, 71% had liver lesions ranging from + to +++ but the majority (43%) had only mild lesions (+). Microscopically, alterations did not occur in the spleens nor were their weights increased in the virus-inoculated animals as was observed in the six-month animals. Apparently, the virus enhanced PCB liver change, but did not produce neoplasia in the spleen as it had in other animals.

The livers from mice given 37.5 and 3.75 ppm Aroclor 1254 for six months and then removed from it for three months had lesions similar to those at six months, (Table 2), indicating that liver damage from PCB was not reversible in the three-month period. However, livers from mice given 375 ppm Aroclor 1242 had ++ PCB lesions at six months, but lesions were not present at nine months, indicating that the liver had regenerated.

The mice that lived for six months in the 375 ppm Aroclor 1254 group had the largest livers (Table 1). Those that died early also had livers that were markedly enlarged and that were three times larger than control livers. The liver weights in 37.5 and 3.75 groups decreased in size accordingly but remained larger than the controls. The livers were smaller in the 37.5 and 3.75 ppm groups at nine months than at six months, suggesting that the lesions had regressed.

The PCBs did not produce hepatic neoplasia in the six-month feeding period nor did MLV produce liver pathology other than infrequent metastasis of neoplastic cells to the liver from the spleen.

DISCUSSION

Hepatic toxicity produced by PCBs was enhanced by MLV. Hepatic lesions in the mice exposed to PCB for six months were increased in severity by MLV only when the virus produced evidence of neoplasia in the spleen. Regardless of the virus dose, whenever there was an increase in weight and histologic alteration of the spleen, the hepatic lesions were more pronounced. When MLV did not affect the spleen, the PCB liver lesions were similar to those of non-virus-inoculated

mice. Three months after removal from the PCB diet, hepatic lesions were again advanced by MLV. However, the spleens were usually free of histologic evidence of neoplasia and their weight was comparable with spleens from non-virus-inoculated control mice.

Liver weights corresponded with the dose of PCB given. The highest dose of Aroclor 1254 produced the largest livers, and the livers decreased in size as the dose diminished (Table 1). Only the highest dose of Aroclor 1242 (375 ppm) produced enlarged livers, while the other doses of 1242 and 1221 had no effect on liver weight. Histopathology coincided with liver weights since hepatic lesions were apparent only at those doses that resulted in increased liver weights. Liver weights decreased and returned near the non-PCB-exposed liver weights in those mice that were removed from the PCB feed at six months and were then fed a PCB-free diet for an additional three months. Histologically, the livers had recovered from the toxic hepatitis in the 375 ppm Aroclor 1242 group (Table 2), but hepatic lesions remained apparent in the 37.5 ppm Aroclor 1254 mice. Perhaps the residues of Aroclor 1254 are detoxified more slowly by the liver than those of Aroclors 1242 and 1221. However, since PCBs are stored in fat [6] and Aroclor 1254 appears to be more toxic than Aroclor 1221 and 1242 [5], small quantities of residues may be released continuously from fat depots, prolonging complete detoxification by the liver.

PCBs have promoted the induction of chemically induced neoplasms by benzene hexachloride in mice [7]. In the current study, PCBs did not promote the induction of neoplasia by MLV. Many environmental contaminants have been reported to be synergistic to infectious agents [8-13], but only two have been incriminated to activate an oncogenic virus [14]. Several of those contaminants [15-17], including PCBs [18, 19]; are apparently immunosuppressive. Since oncogenic viruses are often activated in immunosuppressed hosts [20, 21], and PCB increased the pathogenecity of a nononcogenic virus [8], it was anticipated that PCB would activate MLV. This did not occur in our experiment. Perhaps the immunosuppression, if it did occur, was not sufficient to activate the virus. These results conform with a recent study [13] in which methylmercury chloride increased the susceptibility of mice to a nononcogenic (encephalomyocarditis) virus, but not to an oncogenic (RLV) virus.

The PCBs, 500 ppm Kanechlor 500 and 300 ppm Aroclor 1254, produced neoplastic changes in livers of mice when fed for eight and eleven months, respectively [7, 22]. In the present study, neoplasia did not occur in mice fed 375 ppm PCB Aroclors 1254, 1242, and 1221 for six months.

The results of this experiment demonstrated that an oncogenic virus (MLV) enhanced PCB-induced hepatic toxicity but conversely, PCBs did not appear to activate MLV. It was also demonstrated that PCB-produced hepatic lesions (noncirrhotic) do regenerate after removal

of PCB from the diet. To my knowledge, this is the first report to indicate that an oncogenic virus enhances toxicity of an environmental contaminant.

SUMMARY

The hepatic toxicity produced by polychlorinated biphenyls (PCB) was enhanced in mice that were inoculated with an oncogenic virus, Moloney leukemia virus (MLV). Whenever there was neoplastic involvement of the spleen by MLV, the hepatic lesions produced by PCB were more pronounced than in those of non-MLV inoculated mice.

Mice were exposed to PCB Aroclors, 1254, 1242, and 1221 for six months. Aroclors 1254 and 1242 were hepatotoxic with Aroclor 1254 causing death. Aroclor 1221 did not affect the mice.

Liver weights in mice that were fed PCBs for six months and then maintained on a PCB-free diet for an additional three months were comparable with those of non-PCB exposed mice. These results suggest that the PCB-produced hepatic lesions (noncirrhotic) regenerate after removal of PCB from the diet.

Polychlorinated biphenyls did not affect (promote or induce) the oncogenesis of MLV in this study.

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