

# Lipopolysaccharide-induced Hepatic Injury Is Enhanced by Polychlorinated Biphenyls

Alan P. Brown,<sup>1</sup> A. Eric Schultze,<sup>2</sup> Wendy L. Holdan,<sup>1</sup> John P. Buchweitz,<sup>1</sup> Robert A. Roth,<sup>1</sup> and Patricia E. Ganey<sup>1,3</sup>

<sup>1</sup>Department of Pharmacology/Toxicology, Institute for Environmental Toxicology, Michigan State University, East Lansing, MI 48824 USA; <sup>2</sup>Department of Pathology, College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37901-1071 USA; <sup>3</sup>Department of Medicine, Institute for Environmental Toxicology, Michigan State University, East Lansing, MI 48824 USA

After intravenous administration of bacterial lipopolysaccharide (LPS) to rats, polymorphonuclear neutrophils (PMNs) rapidly accumulate in the liver, and midzonal hepatic necrosis is prominent by 6 hr. PMNs are required for the development of hepatic injury in rats. Certain polychlorinated biphenyls (PCBs) can activate PMNs, resulting in production of superoxide anion ( $O_2^-$ ) and release of cytolytic factors from granules. This raises the possibility that PCB exposure might enhance PMN-mediated tissue injury, such as LPS-induced hepatotoxicity. We treated female Sprague-Dawley rats with a minimally toxic dose of LPS in saline (2 mg/kg, intravenous) and 90 min later exposed them to Aroclor 1248 (50 mg/kg, intraperitoneal), a mixture of PCBs. The animals were killed 6 hr after LPS administration, and hepatic injury was assessed. Neither LPS nor Aroclor 1248 alone produced liver injury. Co-treatment with LPS and Aroclor 1248 resulted in pronounced liver injury as demonstrated from increased activities of alanine aminotransferase and isocitrate dehydrogenase in plasma. Histological evaluation indicated increased severity of hepatic necrosis in rats receiving both LPS and Aroclor 1248. Hepatic accumulation of PMNs, normally observed after LPS, was not altered by co-exposure to PCBs. Aroclor 1248 stimulated rat PMNs *in vitro* to produce  $O_2^-$  and to degranulate. In addition, PMN-mediated cytotoxicity to isolated rat hepatocytes in culture was increased upon addition of Aroclor 1248. PCBs activate PMNs *in vitro* and increase PMN-dependent hepatocellular damage *in vitro* and after LPS treatment *in vivo*. PCBs may act *in vivo* as an additional inflammatory stimulus to activate PMNs to become cytotoxic, resulting in increased tissue injury. **Key words:** Aroclor 1248, hepatotoxicity, liver, neutrophils, polychlorinated biphenyls. *Environ Health Perspect* 104:634-640 (1996)

The sequelae of gram-negative bacterial sepsis are a major cause of morbidity and a leading cause of death in the United States (1). Gram-negative bacteria are a normal constituent of the gastrointestinal tract, and pathogenesis from these organisms can arise during systemic exposure to either whole bacteria or their constituents. Such exposure can occur through increased translocation of bacteria from the gut to the portal circulation during surgery and during a wide range of disease states, including urinary tract infections, inflammatory bowel disease, obstructive jaundice, liver disease, and cirrhosis (2-4).

Lipopolysaccharide (LPS) is a major component of the cell wall of gram-negative bacteria and is likely responsible for many of the events that occur during sepsis (5). Exposure to LPS can produce an array of pathophysiological changes similar to those seen during sepsis, including hypotension and multi-organ failure involving damage to the liver, lungs, kidneys, heart, and gastrointestinal tract (6). Neutrophils (PMNs) have been implicated as playing a critical role in LPS-induced tissue damage (7,8). After an intravenous administration of LPS to rats, PMNs accumulate in the liver sinusoids within 1 hr, and multifocal, midzonal hepatic necrosis is prominent by 6 hr (8,9).

Depletion of circulating PMNs before LPS exposure protects against hepatic injury, indicating a requirement for these cells (8,10). Activation of PMNs is likely a necessary step in producing PMN-mediated tissue injury, and studies *in vitro* have demonstrated that activated PMNs can kill both endothelial cells and hepatocytes (11-13). Although the exact mechanisms of PMN-mediated tissue injury are unknown, they probably involve production of reactive oxygen species such as the superoxide anion ( $O_2^-$ ) and/or release of cytotoxic enzymes contained within granules (14).

Polychlorinated biphenyls (PCBs) are industrial chemicals that have been released into the environment. In experimental animals, these compounds produce an array of toxic responses including hepatotoxicity and alterations in the immune system (15,16). Humans accidentally exposed to high concentrations of PCBs expressed changes in immune system parameters such as alterations in lymphocyte subpopulations and suppression of cellular immunity (17-19). In addition, monocytes and PMNs obtained from individuals exposed to PCBs had lower percentages of cells bearing immunoglobulin and complement receptors (20). Primates exposed to PCBs have exhibited a wide range of

adverse responses including inflammatory lesions in the liver (21,22), and abnormal liver function tests have been reported in exposed human populations (19).

Although immunotoxicity due to PCB exposure has been described in some detail, less is known about the interactions of these compounds with PMNs or inflammatory responses. PCBs rapidly activate PMNs *in vitro* to produce  $O_2^-$  and to degranulate (23). PMN activation by PCBs occurs through signal transduction pathways involving phosphoinositide hydrolysis, inositol triphosphate production, phospholipase  $A_2$  activation, and  $Ca^{2+}$  mobilization (24-26). Activation of PMNs results in production and release of cytotoxic mediators into the extracellular environment. Moreover, exposure to PCBs *in vitro* alters PMN responses to subsequent stimuli (23). Enhanced PMN activation or responsiveness by PCBs could lead to increased tissue injury where PMN involvement occurs, such as during exposure to LPS resulting from bacterial infections or increased translocation of bacteria from the gut. Accordingly, since LPS-induced hepatotoxicity is PMN dependent, we hypothesized that co-exposure to PCBs would enhance LPS-induced liver injury. This hypothesis was tested by exposing rats to a minimally toxic dose of LPS followed by the PCB mixture Aroclor 1248 and assessing hepatic injury. Aroclor 1248 was chosen because this mixture of PCBs is composed predominantly of tetrachlorinated biphenyls and *ortho*-substituted congeners (27), which activate PMNs *in vitro* (26). Furthermore, *ortho*-substituted PCB congeners have been identified in human biological samples, and their potential health effects have yet to be determined (19).

Address correspondence to P.E. Ganey, Department of Pharmacology/Toxicology, Life Sciences Building, Michigan State University, East Lansing, MI 48824 USA.

This research was supported by NIH grant ES04911. A.P.B. was supported in part by a National Research Service Award from the National Institute of Environmental Health Sciences, NIH grant ES05684. R.A.R. was supported in part by a Burroughs Wellcome Toxicology Scholar Award. W.L.H. was supported by an American Society for Pharmacology and Experimental Therapeutics Undergraduate Student Fellowship.

Received 14 September 1995; accepted 29 February 1996.

## Materials and Methods

**Animals.** We used female Sprague-Dawley rats [CrI:CD BR(SD) VAF/plus, 175–220 g] for studies of LPS toxicity. Male Sprague-Dawley retired breeder rats were used as donors of isolated PMNs. Hepatocytes were isolated from male Sprague-Dawley rats (175–300 g). All animals were obtained from Charles River Laboratories (Portage, Michigan). Animals were maintained on a 12-hr light/dark cycle for at least 1 week before use. Food (rat chow, Teklad, Madison, Wisconsin) and water were provided *ad libitum*.

**Chemicals.** Aroclor 1248 was purchased from ChemService (West Chester, Pennsylvania). Lipopolysaccharide (*E. coli* 0128:B12 serotype), glycogen (type II from oyster), phorbol myristate acetate (PMA), superoxide dismutase (SOD), ferricytochrome C, cytochalasin B, guaiacol, dimethylformamide (DMF), *N*-formyl-methionyl-leucyl-phenylalanine (fMLP), and Triton X-100 were purchased from Sigma Chemical Co. (St. Louis, Missouri). Histochoice fixative was purchased from Amresco (Solon, Ohio). Collagenase type A was purchased from Boehringer-Mannheim Biochemicals (Indianapolis, Indiana). Williams' medium E and gentamicin were purchased from Gibco (Grand Island, New York). Fetal calf serum was purchased from Intergen (Purchase, New York).

**Treatment protocol.** Female Sprague-Dawley rats received either LPS (2 mg/kg) or sterile saline vehicle (2 ml/kg) via a tail vein. The animals then received either Aroclor 1248 (50 mg/kg) or corn oil vehicle (2 ml/kg) 90 min later by intraperitoneal injection. We used this dosing regimen to minimize potential effects on hepatocytes by the PCBs alone and to maximize exposure of both circulating and tissue-adherent (in the liver) PMNs to PCBs. Rats were killed 6 or 9 hr after LPS/saline exposure. The animals were anesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal), the abdominal cavity was exposed, and blood was drawn from the inferior vena cava into a syringe containing heparin. Livers were removed, and sections were prepared and immediately placed in fixative. We used an aliquot of plasma on the same day to determine enzyme activities.

**Assessment of hepatic injury.** We assessed hepatic injury by measuring activities of alanine aminotransferase (ALT) and isocitrate dehydrogenase (ICDH) in plasma by kinetic determination using a Gilford Spectrophotometer (Sigma kits no. 59-UV and 153-UV, respectively) (28). Liver sections were fixed, embedded in paraffin, cut at 6  $\mu$ m, stained with hematoxylin and

eosin, and coded and evaluated without bias by a veterinary pathologist. The severity of hepatic injury was graded on a scale from 0 (no significant lesions) to 5 (severe, coalescing necrosis).

**Quantification of hepatic PMN accumulation.** PMNs in liver sections were visualized using an immunohistochemical technique (9). Liver sections were fixed, embedded in paraffin, and sectioned at 6  $\mu$ m. Paraffin was removed from the tissue sections with xylene before staining. PMNs within the liver tissue were stained using a rabbit anti-rat PMN immunoglobulin. This anti-PMN immunoglobulin was isolated from serum of rabbits immunized with rat PMNs as described previously (8). After incubation with the primary antibody, the tissue sections were incubated with biotinylated goat anti-rabbit IgG, avidin-conjugated alkaline phosphatase, and Vector Red substrate to stain the PMNs within the tissue. We assessed hepatic PMN accumulation by averaging the number of PMNs counted in 20 mid-zonal, 400 $\times$  fields throughout 3 separate lobes using light microscopy. PMNs were identified by positive staining and morphology.

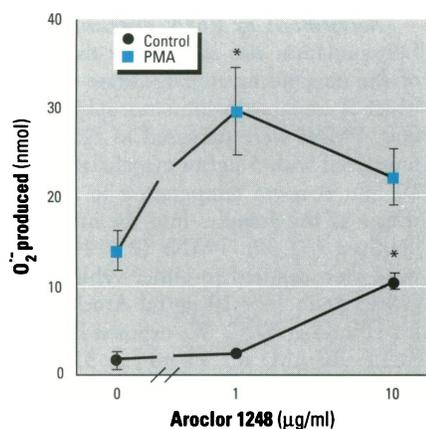
**Isolation of PMNs.** PMNs were isolated from the peritoneum of male Sprague-Dawley retired breeder rats by glycogen elicitation as described previously (29). Isolated PMNs were resuspended in Hanks' balanced salt solution (HBSS), pH 7.4. The percentage of PMNs in the cell preparations was routinely >95%, and the viability was  $\geq$ 95% as determined by the ability to exclude trypan blue. The PMNs were suspended in borosilicate glass test tubes, 12  $\times$  75 mm (VWR, Chicago, Illinois), at a final concentration of  $2 \times 10^6$  cells/ml. The isolation procedure was performed at room temperature.

**Detection of superoxide anion production.** PMNs ( $2 \times 10^6$  cells) were prepared in HBSS in a final volume of 1 ml and exposed to either vehicle (1  $\mu$ l DMF) or to 1 or 10  $\mu$ g/ml Aroclor 1248 for 30 min at 37°C. These concentrations of PCBs were chosen for their ability to affect PMN function in the absence of cytotoxicity (23,26). PMA (0 or 20 ng/ml) was then added for an additional 10 min at 37°C. We measured  $O_2^-$  generated during this 40-min incubation period by the SOD-sensitive reduction of ferricytochrome C (30). In a separate series of experiments, PMNs ( $2 \times 10^6$  cells) were incubated with LPS (10 or 100  $\mu$ g/ml) or vehicle (HBSS) for 10 min, followed by exposure to Aroclor 1248 (1 or 10  $\mu$ g/ml) or vehicle (DMF) for an additional 30 min. We detected  $O_2^-$  produced during this 40-min period as described above.

**Assessment of PMN degranulation.** Degranulation was assessed by the release of the enzyme myeloperoxidase (MPO), which is contained within azurophilic granules. PMNs were prepared in HBSS and pretreated with 5  $\mu$ g/ml cytochalasin B for 10 min at room temperature to facilitate release of the granules into the incubation medium (31,32). PMNs ( $2 \times 10^6$  cells) were then exposed to either vehicle (1  $\mu$ l DMF) or to 1 or 10  $\mu$ g/ml Aroclor 1248 for 15 min at 37°C. We exposed PMNs to fMLP (10 nM) for 15 min at 37°C as a positive control. fMLP is a chemotactic peptide derived from gram-negative bacteria that binds to a G-protein-coupled receptor, leading to PMN activation (33,34). The incubation was terminated by placing the cells in an ice-water bath followed by centrifugation at 4°C. The cell-free supernatant was collected, and MPO activity was measured by the  $H_2O_2$ -dependent oxidation of guaiacol (35,36). We measured the change in absorbance at 470 nm over 2 min at 25°C in a spectrophotometer and calculated MPO activity (U/l) using a molar extinction coefficient of 26,600 (36). Lactate dehydrogenase activity (LDH) present in the cell-free supernatant was measured according to the method of Bergmeyer and Bernt (37) as a marker of cytotoxicity.

**Hepatocyte-neutrophil co-cultures.** Hepatocytes were isolated according to the method of Seglen (38) as modified by Klaunig et al. (39). Briefly, rats were anesthetized with sodium pentobarbital (50 mg/kg), and the portal vein was cannulated. The liver was perfused with approximately 150 ml of  $Mg^{2+}$ -free,  $Ca^{2+}$ -free HBSS followed by perfusion with 250 ml collagenase type A (0.5 mg/ml). The resulting liver digest was filtered through gauze and spun in a centrifuge at 50g for 2 min. The hepatocytes were resuspended in Williams' medium E containing 10% fetal calf serum and 0.1% gentamicin. Using this isolation procedure, 98% of the cells in the final preparation were hepatic parenchymal cells with viability routinely >90% (13).

The hepatocytes were plated in six-well plates at a density of  $5 \times 10^5$  hepatocytes/well. After an initial 3-hr attachment period, the medium and unattached cells were removed, and either Williams' medium E containing 0.1% gentamicin or medium containing isolated PMNs was added. PMNs were isolated as previously described and plated at a density of  $5 \times 10^6$  PMNs/well, resulting in a ratio of 10 PMNs/hepatocyte. PMNs were allowed to attach for 30 min, then vehicle (2  $\mu$ l DMF) or Aroclor 1248 (1 or 10  $\mu$ g/ml)



**Figure 1.** Superoxide anion ( $O_2^-$ ) production by neutrophils during exposure to Aroclor 1248 and phorbol myristate acetate (PMA). Rat PMNs were exposed to either 0, 1, or 10  $\mu\text{g/ml}$  Aroclor 1248 for 30 min at 37°C. The PMNs were then exposed to either 0 or 20 ng/ml PMA for an additional 10 min at 37°C.  $O_2^-$  produced during the incubation period was determined as described in Materials and Methods;  $N = 3-4$ . \*Significantly different from respective value in the absence of Aroclor 1248, one-way ANOVA ( $p < 0.05$ ).

**Table 1.** Neutrophil degranulation in the presence of Aroclor 1248<sup>a</sup>

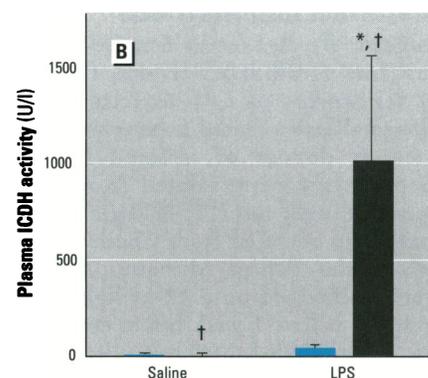
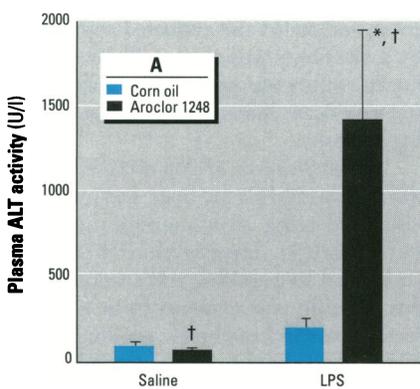
| Treatment                           | MPO activity (U/l) |
|-------------------------------------|--------------------|
| Vehicle control                     | 5.6 ± 3.2          |
| Aroclor 1248 (1 $\mu\text{g/ml}$ )  | 29.2 ± 19.1        |
| Aroclor 1248 (10 $\mu\text{g/ml}$ ) | 87.6 ± 42.9*       |
| fMLP (10 nM)                        | 291.8 ± 84.6*      |

Abbreviations: MPO, myeloperoxidase; fMLP, formyl-methionyl-leucyl-phenylalanine.

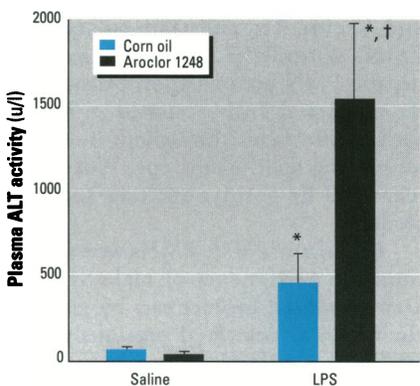
<sup>a</sup>Rat neutrophils were pretreated with 5  $\mu\text{g/ml}$  cytochalasin B for 10 min at room temperature. Neutrophils were then exposed to the various stimuli for 15 min at 37°C. Degranulation was determined by measuring the activity of MPO in the medium as described in Materials and Methods;  $N = 4$ .

\*Significantly different from vehicle control, one-way ANOVA ( $p < 0.05$ ).

was added to wells containing either hepatocytes alone or hepatocytes plus PMNs. After a 16-hr incubation at 37°C in 92.5%  $O_2/7.5\%$   $CO_2$ , the medium was collected. Previous studies have demonstrated that PMN-mediated hepatocellular toxicity in co-culture occurs by 16 hr after stimulation of PMNs (13). The cells remaining on the plate were lysed with 1% Triton X-100, followed by sonication. Both the medium and the cell lysates were spun in a centrifuge at 600g for 10 min. The activity of ALT in the cell-free supernatant fluids was determined (using Sigma kit no. 59-UV), and the activity in the medium was expressed as a percentage of total activity (activity in the medium plus activity in the cell lysates). ALT activity in PMNs is relatively small and is not changed upon exposure to Aroclor 1248 (data not shown).



**Figure 2.** Aroclor 1248 enhances hepatic injury 6 hr after lipopolysaccharide (LPS) exposure. Animals received either LPS (2 mg/kg, intraperitoneal) or saline followed 90 min later by treatment with either Aroclor 1248 (50 mg/kg, intraperitoneal) or corn oil vehicle. They were killed 6 hr after LPS or saline exposure, and plasma activities of (A) alanine aminotransferase (ALT) and (B) isocitrate dehydrogenase (ICDH) were determined;  $N = 5-11$ . \*Significantly different from respective value in the absence of LPS ( $p < 0.05$ ); †significantly different from respective value in the absence of Aroclor 1248 ( $p < 0.05$ ).



**Figure 3.** Aroclor 1248 enhances hepatic injury 9 hr after lipopolysaccharide (LPS) exposure. Animals received either LPS (2 mg/kg, intravenous) or saline followed 90 min later by treatment with either Aroclor 1248 (50 mg/kg, intraperitoneal) or corn oil vehicle. They were killed 9 hr after LPS or saline exposure, and plasma alanine aminotransferase (ALT) activity was determined;  $N = 4-11$ . \*Significantly different from respective value in the absence of LPS ( $p < 0.05$ ); †significantly different from respective value in the absence of Aroclor 1248 ( $p < 0.05$ ).

ALT is a sensitive and specific indicator of hepatocellular damage in the rat, and in the co-culture system release of ALT from hepatocytes correlates with cell death measured by uptake of trypan blue and electron microscopy (13). Therefore, we used ALT activity in the medium as an index of injury to the hepatocytes. To examine the mechanism by which Aroclor 1248 interacts with PMNs and kills hepatocytes, hepatocytes were cultured with cell-free conditioned medium from PMNs exposed to Aroclor 1248. PMNs in suspension ( $2.5 \times 10^6$  cells/ml Williams' medium E containing 0.1% gentamicin) were pretreated with cytochalasin B (5  $\mu\text{g/ml}$ ) for 10 min at room temperature and then exposed to

either vehicle (1  $\mu\text{l}$  DMF/ml cells) or Aroclor 1248 (10  $\mu\text{g/ml}$ ) for 30 min at 37°C. The cells were removed by centrifugation, the cell-free supernatant was collected and added to the hepatocytes (2 ml/well), and release of ALT was determined 16 hr later as described above (13).

**Statistical analysis.** All results are presented as means  $\pm$  standard error of the mean (SEM). For all results presented,  $N$  represents the number of individual animals and *in vitro* repetitions. Unless otherwise stated, data were analyzed by a two-way analysis of variance (ANOVA), and individual comparisons were performed using the least significant difference test. When variances were not homogenous, data were log-transformed before analysis. When appropriate, an outlier test (test for detection of extreme means) was applied (40). The criterion for statistical significance was  $p < 0.05$ .

## Results

Neither exposure of PMNs to vehicle nor to 1  $\mu\text{g/ml}$  Aroclor 1248 resulted in generation of  $O_2^-$  (Fig. 1). However, PMNs exposed to 10  $\mu\text{g/ml}$  Aroclor 1248 produced a significant amount of  $O_2^-$ . PMA alone caused an increase in  $O_2^-$  production. Exposure to 1  $\mu\text{g/ml}$  Aroclor 1248 before stimulation with PMA caused significant generation of  $O_2^-$ . The amount of  $O_2^-$  produced in the presence of 1  $\mu\text{g/ml}$  Aroclor 1248 and PMA was greater than the sum of  $O_2^-$  produced individually by these two agents. Pretreatment of PMNs with LPS did not alter  $O_2^-$  production in response to Aroclor 1248 (data not shown).

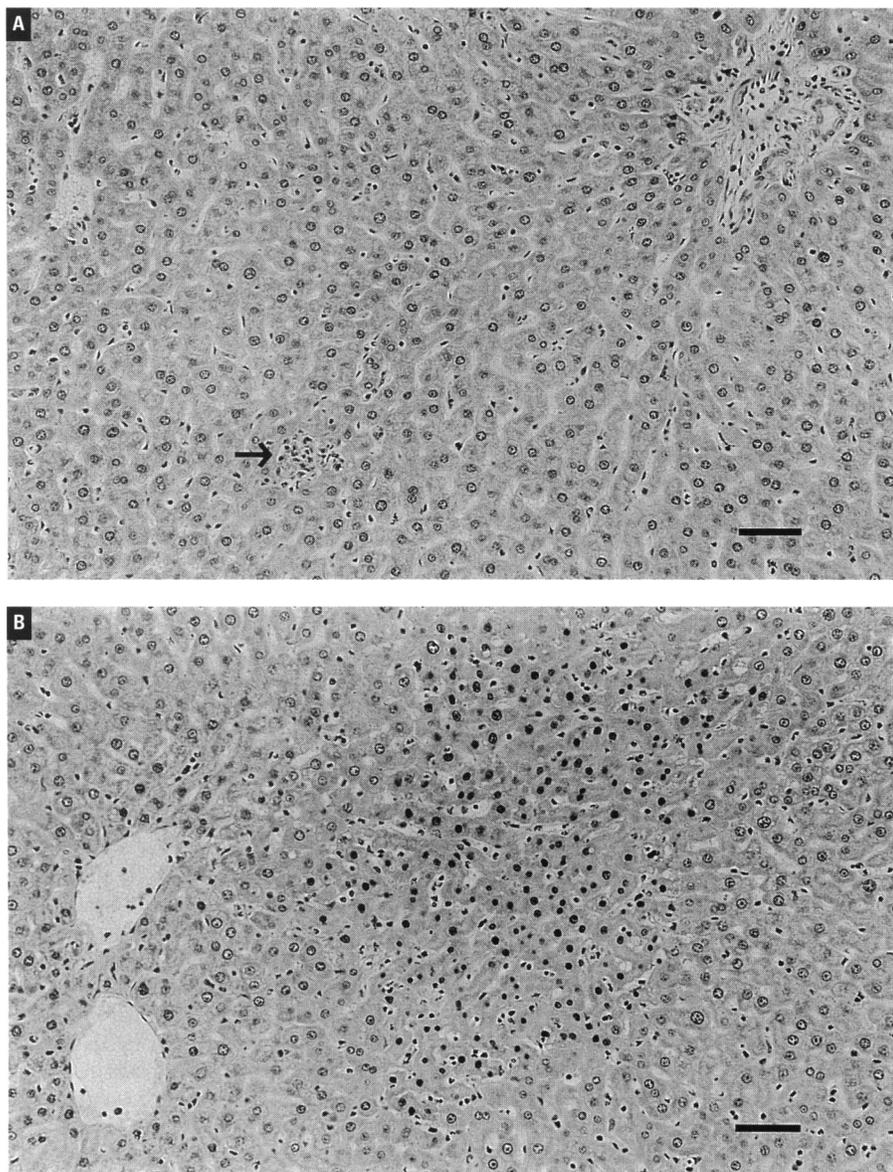
Exposure to 10  $\mu\text{g/ml}$ , but not 1  $\mu\text{g/ml}$ , Aroclor 1248 elicited a significant release of MPO from PMNs compared to vehicle control (Table 1). Release of MPO into the

**Table 2.** Aroclor 1248 potentiation of LPS-induced hepatotoxicity: histopathologic evaluation<sup>a</sup>

| Treatment       | N  | % Rats with histopathologic score |    |    |    |    |    |
|-----------------|----|-----------------------------------|----|----|----|----|----|
|                 |    | 0                                 | 1  | 2  | 3  | 4  | 5  |
| Saline/corn oil | 5  | 100                               | —  | —  | —  | —  | —  |
| Saline/Aroclor  | 5  | 100                               | —  | —  | —  | —  | —  |
| LPS/corn oil    | 11 | 27                                | 18 | 36 | 18 | —  | —  |
| LPS/Aroclor     | 9  | 11                                | —  | 11 | 33 | 22 | 22 |

LPS, lipopolysaccharide.

<sup>a</sup>Rats received either LPS (2 mg/kg, intravenous) or saline and 90 min later received either Aroclor 1248 (50 mg/kg, intraperitoneal) or corn oil vehicle. Animals were killed 6 hr after administration of LPS or saline, and liver sections were prepared from three different lobes. Severity of hepatic injury was graded on a scale of 0–5 reflecting the frequency and size of the hepatic lesions: 0 = no significant lesions; 1 = extremely mild necrosis; 2 = mild necrosis; 3 = moderate necrosis; 4 = marked necrosis; 5 = severe, coalescing necrosis.



**Figure 4.** Photomicrographs of liver sections taken 6 hr after lipopolysaccharide (LPS) administration. (A) Liver from corn oil-treated animal given LPS had sinusoidal neutrophilia and a small lesion (arrow). (B) Liver from animal treated with Aroclor 1248 and LPS had sinusoidal neutrophilia and marked midzonal necrosis. Bar = 50 µm.

medium occurred in the absence of cytotoxicity as determined by release of LDH (data not shown). PMN degranulation in response to fMLP was included as a positive control.

Six hours after exposure to LPS alone, activities of ALT and ICDH in plasma were not significantly elevated (Fig. 2). Administration of Aroclor 1248 (50 mg/kg) 90 min after LPS exposure resulted in significant hepatic injury, as demonstrated from increased activities of ALT and ICDH (Fig. 2). Exposure to Aroclor 1248 in the absence of LPS had no effect on ALT or ICDH activities in plasma. Nine hours after administration of LPS alone, plasma ALT activity was increased compared to controls (Fig. 3). Activity of ALT in plasma of rats treated with Aroclor following LPS was significantly greater than in rats receiving LPS alone. Co-administration of Aroclor 1248 with LPS resulted in lethality in 10% of the animals. Lethality was not seen in animals treated with LPS or Aroclor 1248 alone. In a separate study, administration of a smaller dose of Aroclor 1248 (10 mg/kg) did not affect LPS-induced hepatic injury as determined by activities of ALT and ICDH in plasma (data not shown). Exposure to a larger dose of Aroclor 1248 alone (100 mg/kg) did not produce hepatic injury, as evidenced from activities of ALT and ICDH in plasma ( $74 \pm 9$  and  $6 \pm 1$  U/l, respectively; not significantly different from vehicle controls) and histologic evaluation.

There were no significant lesions in the livers of vehicle-treated rats or rats exposed to Aroclor 1248 in the absence of LPS (Table 2). Six hours after LPS administration, livers from animals co-treated with either Aroclor 1248 or corn oil vehicle had lesions of varying degrees of severity (Fig. 4). The hepatic sinusoids of LPS-treated rats contained many PMNs, plump Kupffer cells, and small amounts of an eosinophilic, proteinaceous material. There were multifocal, irregularly shaped areas of midzonal hepatocellular necrosis. These lesions were characterized by hyper-eosinophilic parenchymal cells with small, pyknotic nuclei or by swollen, pale parenchymal cells with indistinct or absent nuclei and indistinct cytoplasmic borders. The necrotic foci contained degenerate PMNs. There were also foci of single-cell necrosis scattered throughout the tissue. Aroclor 1248 administration increased the severity of the hepatic lesions, but the nature of the lesions did not change.

PMNs were observed infrequently throughout the liver tissue from animals exposed to saline, regardless of co-treatment with Aroclor 1248 (Fig. 5). A marked

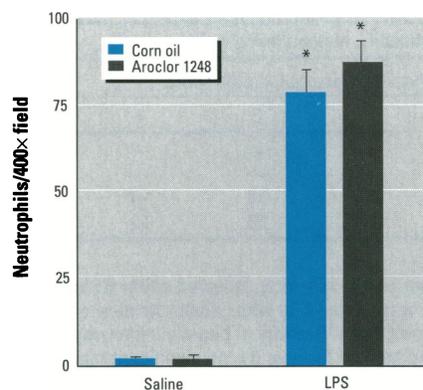
accumulation of PMNs was observed at 6 hr in livers from animals exposed to LPS, and this was not affected by co-administration of Aroclor 1248.

Isolated hepatocytes, either in the presence or absence of PMNs, were exposed to various concentrations of Aroclor 1248 for 16 hr (Fig. 6). Neither 1  $\mu\text{g}/\text{ml}$  nor 10  $\mu\text{g}/\text{ml}$  Aroclor 1248 was toxic to the hepatocytes. Addition of PMNs to the hepatocyte cultures did not produce cytotoxicity in the absence of Aroclor 1248. However, stimulation of the PMNs with either 1  $\mu\text{g}/\text{ml}$  or 10  $\mu\text{g}/\text{ml}$  Aroclor 1248 resulted in a significant release of ALT into the culture medium. In a similar experiment in which hepatocytes were incubated with cell-free conditioned medium from PMNs exposed to Aroclor 1248 or its vehicle, no significant differences were observed in these two groups (data not shown).

## Discussion

Under normal circumstances, the liver is exposed to small amounts of gut-derived LPS originating from gram-negative bacteria residing in the gastrointestinal tract. However, during sepsis or numerous disease states exposure may increase, and LPS may initiate a systemic inflammatory response leading to significant morbidity and mortality. PMNs have been demonstrated to play a key role in the development of organ injury during sepsis (6). In such situations, enhancement of PMN activation or responsiveness by xenobiotic agents could lead to increased tissue injury. The ability of PCBs to enhance organ injury during an inflammatory event was examined in a model of LPS-induced hepatic injury. In this model, PMNs rapidly accumulate in large numbers in the hepatic sinusoids after administration of LPS and are required for development of hepatic injury (8,10). At the dose used in this study, rats exposed to LPS alone and killed 6 or 9 hr later developed mild hepatic necrosis. Administration of Aroclor 1248 increased the severity of the hepatic injury as evidenced by elevated ALT and ICDH activities in plasma and increased severity of hepatic lesions determined histologically. The pathological changes observed are consistent with previous studies describing hepatic injury in rats treated with LPS (8,41). In addition, co-treatment with Aroclor 1248 produced lethality, whereas no mortality occurred in animals exposed to LPS alone. Exposure to Aroclor 1248 alone up to 100 mg/kg did not produce hepatotoxicity.

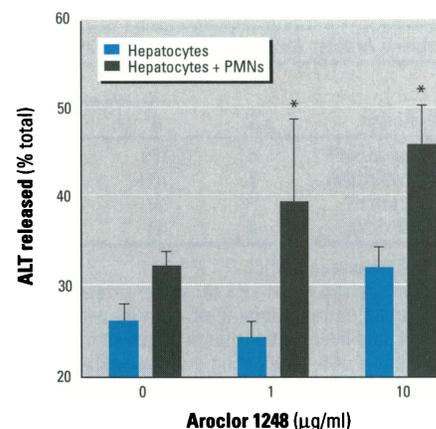
These results are consistent with previous studies describing sensitization to LPS-induced lethality by pretreatment with PCBs. Mortality after administration of



**Figure 5.** Hepatic neutrophil accumulation after lipopolysaccharide (LPS) exposure. Animals received either LPS (2 mg/kg, intravenous) or saline followed 90 min later by treatment with either Aroclor 1248 (50 mg/kg, intraperitoneal) or corn oil vehicle. They were killed 6 hr after LPS or saline exposure, the liver was removed, and sections prepared for immunohistochemistry as described in Materials and Methods. PMNs were counted in 20 400 $\times$  midzonal fields throughout 3 separate lobes and averaged for each individual animal;  $N = 5-11$ . \*Significantly different from respective value in the absence of LPS ( $p < 0.05$ ).

LPS was greater in mice fed Aroclor 1248 for 5 weeks compared to mice fed a control diet (42). Similarly, mice fed Aroclor 1242 for 6 weeks were more responsive to the lethal effects of LPS (43). Neither of these studies investigated damage to the liver or other organs. Other investigators have described a potentiation of LPS-mediated hepatotoxicity after exposure to compounds structurally related to some PCB congeners, i.e., 2,3,7,8-tetrachlorodibenzo-*p*-dioxin and polybrominated biphenyls (44-46). These studies involved treatment with halogenated biphenyls before administration of LPS so that effects on hepatic parenchymal cells, such as enzyme induction and alterations in gene expression, were likely to have occurred before LPS exposure and may have contributed to potentiation of toxicity. These effects may be related to Ah receptor-mediated events. In the present study, the direct effects of Aroclor 1248 on hepatic parenchymal cells cannot be ruled out; however, the treatment paradigm involved a short time (4.5 hr) between PCB exposure and evaluation of liver injury, so effects such as enzyme induction would be minimized.

PMNs begin accumulating in the hepatic sinusoids of rats within 30 min after LPS administration, and this event is a prerequisite for development of liver injury (9). Exposure to Aroclor 1248 did not affect hepatic PMN accumulation in LPS-treated rats; thus, potentiation of injury was not caused by increased numbers of PMNs in the liver. After absorption into the circula-



**Figure 6.** Aroclor 1248 stimulates PMN-mediated hepatocellular cytotoxicity in co-culture. Hepatocytes were cultured in the presence or absence of PMNs and exposed to either 0, 1, or 10  $\mu\text{g}/\text{ml}$  Aroclor 1248. The activity of alanine aminotransferase (ALT) was determined in the cell-free medium and the cell lysates 16 hr later, and the percentage of total ALT released was calculated as described in Materials and Methods;  $N = 5$ . \*Significantly different from respective value in the absence of PMNs ( $p < 0.05$ ).

tion, PCBs initially distribute in high concentration in the liver, which is a target organ for toxicity (15,16,47). Therefore, the presence of PCBs in the liver concurrent with PMN accumulation allows the possibility that PCBs affected the PMNs locally and that this contributed to increased hepatocellular damage. Indeed, isolated PMNs produced  $\text{O}_2^-$  and underwent degranulation upon exposure *in vitro* to Aroclor 1248, and Aroclor 1248 potentiated the production of  $\text{O}_2^-$  in response to PMA stimulation. PMA directly stimulates protein kinase C, leading to activation of the NADPH oxidase system and  $\text{O}_2^-$  production (48). These data with Aroclor 1248 are consistent with previous studies involving Aroclor 1242 and individual congeners of PCB (23,26).

PCBs initiate PMN responses within 15 min by activating the cellular signal transduction pathways responsible for these functions (24,26), thus providing a time-course for PMN activation that is consonant with the development of hepatic necrosis *in vivo*. This proposed mechanism for the enhancement of LPS-induced hepatic injury by Aroclor 1248 was supported by the results obtained using an *in vitro* co-culture system composed of isolated hepatocytes and PMNs. Aroclor 1248 was not toxic to the hepatocytes in the absence of PMNs; however, hepatocellular injury, as evidenced by increased release of ALT into the medium, occurred when hepatocytes were co-cultured with PMNs activated upon the addition of Aroclor 1248. The co-culture system is a

simplified model of events occurring *in vivo*, where PMN accumulation and adherence occurs before administration of Aroclor 1248. Our experiments involving cell-free conditioned medium from Aroclor 1248-treated PMNs suggested that either cell-cell contact between PMNs and hepatocytes is required for the mechanism by which Aroclor 1248 elicits PMN-mediated hepatocellular injury and/or the factor responsible for cytotoxicity is not stable enough to survive the time and process involved in preparing cell-free conditioned medium. This is not altogether surprising because Aroclor 1248 stimulates production of  $O_2^-$  by PMNs, which is highly reactive and short-lived. A combination of both reactive oxygen species and degranulation products may be involved in PMN-mediated killing of hepatocytes upon PCB exposure.

The mechanisms by which PMNs injure hepatic tissue are not fully understood. Recent studies suggest that proteases released from PMN granules can kill both endothelial cells and hepatocytes *in vitro* (11,13). Reactive oxygen species, such as  $O_2^-$ , may also play a contributing role in PMN-mediated tissue injury (14). Because Aroclor 1248 can elicit PMN  $O_2^-$  production and degranulation, both mechanisms may contribute to potentiating PMN-mediated tissue injury. It is also possible that PCBs may adversely affect hepatic parenchymal cells in a manner resulting in greater susceptibility to injury upon exposure to PMN-derived cytotoxic mediators.

In summary, Aroclor 1248 increased the hepatic injury that follows LPS administration. Because PMNs play a critical role in liver injury from LPS, these findings are consistent with previous studies demonstrating that PCBs can activate PMNs *in vitro* and sensitize animals to LPS-induced lethality. PCBs may act in a fashion analogous to inflammatory stimuli by activating PMNs to become cytotoxic, resulting in enhancement of PMN-mediated tissue injury during an inflammatory event. Studies with individual PCB congeners suggest that *ortho*-substituted PCBs can activate PMNs *in vitro*, whereas coplanar PCBs are inactive (23). Because Aroclor 1248 is a mixture of PCB congeners, the respective roles for both *ortho*-substituted and coplanar congeners in the mechanism for enhanced liver injury upon LPS exposure is uncertain at this time.

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