Pharmacologic Effects of 4-Chlorophenol in Rats: Comparison to Clofibrate (42899)

SRICHAN PHORNCHIRASILP,[1] SUMAN T. PATEL, JOHN M. HANSON, HOWARD A. I. NEWMAN, DONALD T. WITIAK, AND DENNIS R. FELLER[2]

Colleges of Medicine and Pharmacy, The Ohio State University, Columbus, Ohio 43210

Abstract. **4-Chlorophenol (4-CP) is an identified trace contaminant in commercial clofibrate preparations and the pharmacologic effects of 4-CP have not yet been widely established. We have examined the dose-dependent effects of oral 4-CP and clofibrate administration on selected hepatic parameters and on serum glucose, cholesterol, and triglyceride concentrations in male rats. 4-CP treatment (0.00125–0.08 mmol/kg, twice a day) of rats for 2 weeks increased hepatic microsomal protein (20–30%) and cytochrome P-450 (20–190%) contents without changing liver/body weight ratios. Both 4-CP (0.0025 mmol/kg body wt, twice a day) and CPIB (0.4 mmol/kg body wt, twice a day) treatment to rats for 2 weeks caused significant elevations in microsomal cytochrome P-450 content and in the maximal activities of ethylmorphine, aminopyrine, and benzphetamine *N*-demethylase, but not in the activity of zoxazolamine 6-hydroxylase. With the same dose of 4-CP, time-dependent increases in hepatic microsomal protein, cytochrome P-450, and the activity of benzphetamine *N*-demethylase were observed for a 4-week period, and the induction of hepatic microsomal benzphetamine *N*-demethylase activity by 4-CP was associated with an increased enzyme synthesis. 4-CP treatment produced a marked morphologic change in liver cell ultrastructure, including a proliferation of mitochondria and endoplasmic reticulum at lower 4-CP doses. A clustering of intracellular organelles (mitochondria and endoplasmic reticulum) and a foamy cytoplasm were seen at doses greater than 0.01 mmol/kg, twice a day for 2 weeks, and at 0.0025 mmol/kg, twice a day for >4 weeks. The effects of 4-CP and clofibrate on fasting blood glucose and fasting serum lipid levels were also monitored throughout an 8-week period. Both 4-CP (0.005 mmol/kg body wt, twice a day) and clofibrate (0.2 mmol/kg body wt, twice a day) produced significant elevations in fasting serum glucose levels, but this dosage of 4-CP did not alter serum lipid and lipoprotein parameters, whereas clofibrate significantly reduced serum total cholesterol and high density lipoprotein cholesterol levels. These results lead us to conclude that 4-CP does not contribute to the antilipidemic effects of clofibrate.** [P.S.E.B.M. 1989, Vol 191]

Coronary artery disease has been defined as one of the major causes of death among the human population in industrial nations (1), and elevated cholesterol and low density lipoprotein (LDL)-cholesterol concentrations are now accepted as significant risk factors for the development of this disease (2). Clofibrate [ethyl-2(4-chlorophenoxy)-2-propionic acid, *p*-chlorophenoxyisobutyric acid ethyl ester, CPIB] and newer structurally related analogs continued to be valuable adjunctive agents with diet for the treatment of hyperlipidemic patients (3, 4). Although the exact mechanism by which hyperlipidemia produces coronary artery disease is still not clearly known, drugs that lower plasma LDL and/or very low density lipoprotein (VLDL) concentration or increase serum high density lipoprotein (HDL) levels reduce risk for coronary artery disease. Clinically, CPIB is more effective at reducing serum triglycerides of VLDL and chylomicron remnants than lowering total cholesterol or LDL-cholesterol concentrations; concomittant with the VLDL lowering, it increases HDL (3, 4).

Clofibric acid was first synthesized in 1947 (5) and the plasma lipid-lowering ability of the ethyl ester, CPIB, was first discovered by Thorp and Waring in 1962 (6). Clofibrate therapy is generally well tolerated; however, adverse side effects include a higher incidence of gastrointestinal diseases, myositis, and metabolic

[1] Current address: Department of Pharmacology, Faculty of Pharmacy, Mahidol University, Sri-Ayudhya Road, Bangkok 10400, Thailand.

[2] To whom requests for reprints should be addressed at Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, OH 43210.

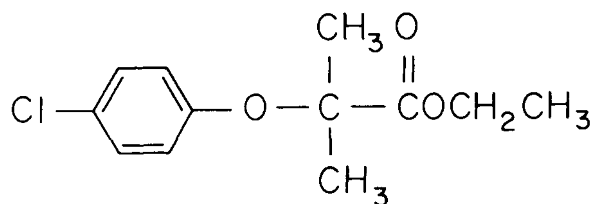
Received September 1, 1988. [P.S.E.B.M. 1989, Vol 191]
Accepted January 31, 1989.

0037-9727/89/1912-0139$2.00/0
Copyright © 1989 by the Society for Experimental Biology and Medicine

disturbances of the liver and pancreas (7, 8). In this regard, chronic administration of CPIB in experimental animals has produced hepatotoxicity associated with hepatocellular carcinomas, hepatomegaly, a proliferation of hepatic peroxisomes and endoplasmic reticulum, and an elevation in enzyme activities of these organelles (5, 8–10). Treatment of rodents with CPIB has been shown to increase hepatic microsomal protein, cytochrome P-450 content, and activities for the microsomal oxidation of selected Type I drug substrates (8, 11) and lauric acid (12, 13). Furthermore, studies in rodents, but not in humans, demonstrate that long-term CPIB treatment increased the incidence of malignant liver neoplasms (5, 7, 10). Subsequently, the Food and Drug Administration in the United States listed CPIB as a potential tumorigen (14).

Presently accumulated evidence in humans and laboratory animals indicates that CPIB is not converted to detectable amounts of 4-CP (4); however, closely related hypolipidemic analogs are reported to be metabolized to 4-CP *in vivo* (15, 16). These findings, coupled with the presence of trace quantities (0.003–0.4%) of 4-CP in commercial CPIB preparations (5, 17–20), suggest that long-term CPIB or CPIB analog treatment could result in an exposure of tissues to 4-CP (see Fig. 1).

4-CP is a locally acting antiseptic used in dental care (21); however, no *in vivo* studies on the pharmacologic activities of this compound have appeared. Thus, this study was undertaken to examine the comparative dose- and time-dependent effects of 4-CP and CPIB treatment in rats on liver cell ultrastructure and on selected hepatic (liver weight, microsomal protein, cytochrome P-450, and drug-metabolizing enzyme activities) and serum (glucose, cholesterol, triglyceride, and HDL-cholesterol concentrations) parameters.



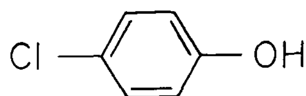


Figure 1. Chemical structures of CPIB and 4-CP.

Materials and Methods

Drugs and Chemicals. The compounds used in this study and their sources are 4-chlorophenol (Fisher Scientific Co., Fair Lawn, NJ); clofibrate (Ayerst Laboratories, NY, NY); glucose oxidase reagent (Beckman Instrument Inc., Fullerton, CA); A-Gent triglyceride reagent and A-Gent cholesterol reagent (Abbott Diagnostic Lab., Chicago, IL), benzphetamine hydrochloride (Upjohn Co., Kalamazoo, MI); and TRISMA, NADP, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase (Sigma Chemical Co., St. Louis, MO). 4-CP was purified by vacuum distillation, giving colorless crystals of a melting point = 43.2°C (literature = 43°C) (22).

Animals and Drug Treatment. Male Sprague-Dawley rats weighing 300–400 g were used in this study. Animals were purchased from Harlan Industries (Cumberland, IN) and were housed in a facility accredited by the American Association for the Advancement of Laboratory Animal Care. A 12-hr alternating light and dark cycle was maintained. Tap water and Purina rat chow were provided *ad libitum* except when overnight fasting of animals was required.

4-CP and CPIB were dissolved in corn oil (Mazola) and administered by oral intubation to rats at doses ranging from 0.00125 to 0.08 mmol/kg body wt, twice a day, and 0.2 or 0.4 mmol/kg body wt, twice a day respectively, for 1 to 8 weeks. Drugs were administered in a final volume of 1 ml/kg body wt. Control animals were fed with 1 ml/kg body wt corn oil.

Biochemical Assays of Hepatic Parameters. Control and drug-treated animals were killed by decapitation and the abdominal cavity was opened surgically. Livers were removed, blotted, and weighed, then placed in ice-cold 0.02 *M* Tris buffer containing 1.15% KCl. Livers were homogenized in 3 volumes of the 0.02 *M* Tris-KCl buffer, and liver microsomes were isolated by differential centrifugation as described previously (11). Microsomal protein and cytochrome P-450 were assayed according to the methods of Lowry *et al.* (23) and Omura and Sato (24).

Benzphetamine, ethylmorphine, and aminopyrine *N*-demethylase activities in liver microsomes were measured as described by Holtzman *et al.* (25). Reaction mixtures containing 5 mg of microsomal protein, drug substrate (10 μmol), and a NADPH-generating system (NADP, 1.6 mg; glucose-6-phosphate, 16 mg; glucose-6-phosphate dehydrogenase, 2 units; and $MgCl_2$, 25 μmol) were incubated with shaking at selected times (15, 20, or 30 min) at 37°C on a metabolic incubator. Liver microsomal zoxazolamine hydroxylation was measured as previously described (26) with 5 μmol of substrate. For kinetic studies, varying benzphetamine concentrations (50–1000 μM) were used, and the data were analyzed by the Cleland hyperbolic computerized program (27).

Preparation of Liver Samples for Electron Microscopy. Specimens from control and 4-CP animals were prepared by sectioning of liver into 0.5-mm^3 cubes and fixed in 0.02 *M* Tris-HCl buffer containing 4% glutaraldehyde for 1 hr. Tissues were washed with ice-cold buffer solution, and then stained with 1% osmium tetroxide for 1 hr and dehydrated with ethanol. The specimens were then placed into propylene oxide and embedded in Poly-Bed 812 using standard procedures. Silver ribbons were fished on copper grids, stained with uranyl acetate and lead citrate, and examined under an electron microscope (Zeiss model EM 9S).

Biochemical Assays of Serum Parameters. Rats were fasted overnight before each blood collection. Animals were anesthetized with ether and blood was drawn from orbital plexus through a heparinized capillary tube into a polyethylene tube. All blood samples were centrifuged at 2000*g* for 10 min to obtain a serum supernatant. Serum samples were assayed immediately or kept frozen at −20°C. Frozen serum samples were thawed at room temperature before analysis of serum glucose, triglyceride, cholesterol, or HDL-cholesterol concentrations.

Fasting serum glucose level was assayed enzymatically by glucose oxidase using the method of Kadish *et al.* (28). Serum samples were analyzed with the use of the Beckman Glucose Analyzer (Beckman Instrument Inc.). The sensor in this instrument measured the amount of glucose oxidase reagent in the presence of oxygen. The oxygen consumption by each serum sample was proportional to the glucose concentration. Known standards of glucose were used in the calibration of the instrument and the final glucose concentrations were expressed as mg/dl.

Fasting serum triglyceride concentrations were measured with A-Gent TG reagent on an ABA-100 using the method of Sampson *et al.* (29). Serum cholesterol was analyzed enzymatically by the method of Allain *et al.* (30) with A-Gent cholesterol reagent on an Abbott Biochromatic Analyzer (ABA-100) equipped with a 1:51 syringe plate. Serum HDL-cholesterol was determined by using a modification of the method described by Steele *et al.* (31). The assay of HDL-cholesterol was done as follows: a 100-μl serum sample was incubated with 10 μl of heparin-manganese chloride mixture (2:2.5 of 10,000 IU/ml heparin:1 *M* manganese chloride) in an ice bath for 30 min. Mixtures were then centrifuged at 4°C and at 5000*g* for 30 min to precipitate VLDL and LDL, leaving HDL in the supernatant. Aliquots of the recovered supernatant were analyzed for cholesterol as described above. Values obtained from each serum total lipid and lipoprotein determination were expressed as mg/dl.

Statistical Analyses. Data were analyzed by Student's *t* test with significance specified at $P < 0.05$.

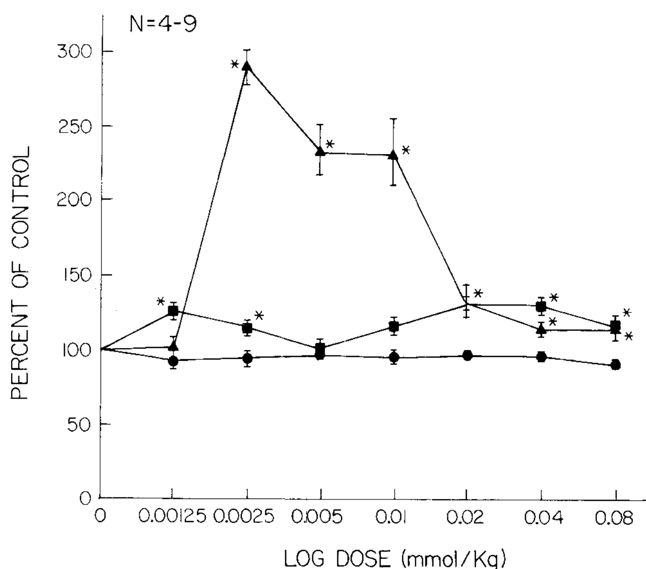


Figure 2. Dose-dependent effect of 4-CP on liver/body weight ratio (●), liver microsomal protein (■), and cytochrome P-450 content (▲) in male rats. 4-CP was dissolved in corn oil and administered at varying doses (0.00125–0.08 mmol/kg, twice a day), by oral intubation for 2 weeks. Each point represents the mean percentage of control ± SE from groups of four to nine rats. Control values for the measured parameters (mean ± SE) were liver/body weight ratio (%) = 5.05 ± 0.11, microsomal protein (mg protein/g liver) = 18.6 ± 0.9, and cytochrome P-450 (nmol P-450/mg protein) = 0.66 ± 0.02. Asterisks indicate that the treatment values are significantly different from the corresponding control values.

Results

Dose-and Time-Dependent Effects of 4-Chlorophenol on Hepatic Parameters. Pretreatment of rats for 2 weeks with 0.00125–0.08 mmol/kg, twice a day, significantly increased microsomal protein and cytochrome P-450 content throughout most of the dosage range (Fig. 2). A maximal elevation of microsomal cytochrome P-450 (290% of control) was obtained when rats were administered 0.0025 mmol/kg, twice a day. Although a progressively smaller elevation of cytochrome P-450 was found as the 4-CP dose was increased, at the highest dose, the content was still significantly higher than in the control. In contrast, liver/body weight ratios were unchanged by 4-CP treatment in this dose range. CPIB, at a dose of 0.4 mmol/kg, twice a day, produced significant ($P < 0.05$) elevations in liver/body weight ratio (23%), microsomal protein (28%), and cytochrome P-450 (45%) after a 2-week treatment period (data not presented).

A comparison of the effect of pretreatment of rats with CPIB or 4-CP on liver microsomal cytochrome P-450 and drug-metabolizing enzymes is given in Figure 3. Similar to CPIB, 4-CP elevated liver microsomal cytochrome P-450 to the same extent as the maximal rates of aminopyrine, ethylmorphine, or benzphetamine *N*-demethylation. For example, cytochrome P-450 and benzphetamine *N*-demethylation were increased by 150% and 147% of the corresponding con-

trol value by 4-CP treatment (Fig. 3, Experiment 3). At the doses used, neither CPIB nor 4-CP pretreatment modified microsomal zoxazolamine 6-hydroxylase activity.

The time course effects of 4-CP treatment (0.0025 mmol/kg, twice a day) in rats on various hepatic parameters were measured (Fig. 4). Oral administration of this 4-CP dose for 8 weeks showed no significant effect on liver/body weight; however, microsomal protein was increased after 2 weeks (117% of control) and remained elevated throughout the remainder of the treatment period. Microsomal cytochrome P-450 content was increased throughout 4 weeks of 4-CP treatment and diminished to below control levels after 6 and 8 weeks of drug administration. The observed 4-CP-induced increases in microsomal benzphetamine *N*-demethylase activity followed the same time course pattern as the changes in cytochrome P-450.

Kinetic studies of benzphetamine *N*-demethylation in microsomes were undertaken to further evaluate the nature of the induction by 4-CP in male rats. Pretreatment of rats for 2 weeks with 0.005 and 0.02 mmol/kg, twice a day, 4-CP produced a dose-dependent increase in the maximum rate of liver microsomal *N*-demethylation (133% and 151% of the control) without a change in the apparent Michaelis-Menten constant (94% and 106% of the control), respectively (data not presented).

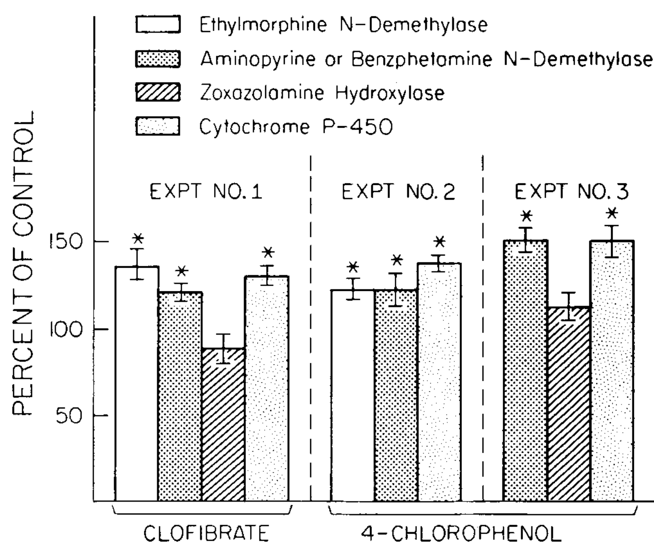


Figure 3. Comparative *in vivo* effects of clofibrate (CPIB) and 4-chlorophenol (4-CP) on rat liver microsomal cytochrome P-450 and selected microsomal drug-metabolizing enzymes. CPIB and 4-CP were given orally at doses of 0.4 mmol/kg, twice a day and 0.0025 mmol/kg, twice a day, respectively, for a 2-week period. Liver microsome isolation and measurement of enzyme parameters were done as described in Materials and Methods. Values represent the mean ± SE of n = 6–8 rats. Aminopyrine was used as a substrate in Experiment 1 and benzphetamine was used as a substrate in Experiments 2 and 3. Control values of measured parameters (mean ± SE) were ethylmorphine *N*-demethylase (nmol HCHO/mg/15 min) = 120.7 ± 4.8, aminopyrine *N*-demethylase (nmol HCHO/mg/20 min) = 33.5 ± 0.8, benzphetamine *N*-demethylase (nmol HCHO/mg/30 min) = 132.6 ± 9.4, zoxazolamine hydroxylase (nmol/mg/20 min) = 25.2 ± 2.7, and cytochrome P-450 (nmol P-450/mg) = 0.66 ± 0.01. Asterisks indicate that the treatment values are significantly different ($P < 0.05$) than the corresponding control values.

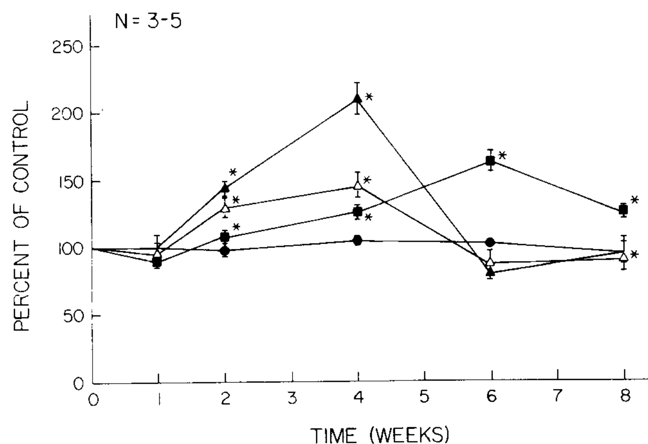


Figure 4. Time course effect of 4-CP (0.0025 mmol/kg, twice a day) on liver/body weight ratio (●), liver microsomal protein (■), cytochrome P-450 content (▲), and benzphetamine *N*-demethylase activity (△) in male rats. 4-CP was dissolved in corn oil and given by oral intubation twice daily for 8 weeks. Each point represents the mean percentage of control ± SE from groups of three to five rats. Control values for the measured parameters (mean ± SE of n = 3) at Week 1 were liver/body weight ratio (%) = 5.21 ± 0.08, cytochrome P-450 content (nmol P-450/mg protein = 0.61 ± 0.04, and benzphetamine *N*-demethylase (nmol HCHO/mg/min) = 3.1 ± 0.3. Asterisks indicate that the treatment values are significantly different from the corresponding control values ($P < 0.05$).

Effect of 4-CP Treatment on Liver Cell Ultrastructure. Liver specimens were prepared for electron microscopic examination from rats treated with 4-CP at various doses for 2 weeks or with 0.0025 mmol/kg, twice a day, throughout an 8-week period. As presented in Figure 5, a foamy cytoplasm and clustering of intracellular organelles (mitochondria and endoplasmic reticulum) were seen in 4-CP-treated cells, whereas these organelles were more randomly and uniformly distributed in the nondrug-treated (control) cells. Increased numbers of mitochondria and endoplasmic reticulum were seen at lower doses of 4-CP treatment (Fig. 5B). The 4-CP-induced foamy cytoplasm and clustering of intracellular organelles were seen in liver cells after longer term 4-CP treatment (>4 weeks with 0.0025 mmol/kg, twice a day; data not presented) or with higher doses of 4-CP (>0.01 mmol/kg, twice a day) over a 2-week period. The alteration of endoplasmic reticulum seen at low 4-CP doses appears to be correlated to the biochemical changes in drug metabolism and cytochrome P-450 levels.

Measurement of Fasting Serum Glucose Levels after 4-Chlorophenol and Clofibrate Treatment. Previous experiments demonstrated that substantial hepatic effects were produced by 4-CP at 0.005 mmol/kg, twice a day, and this dose was used in the subsequent experiments on the measurement of serum parameters. Rats were treated for 8 weeks with 0.005 mmol/kg body wt 4-CP or 0.2 mmol/kg body wt CPIB, twice a day,

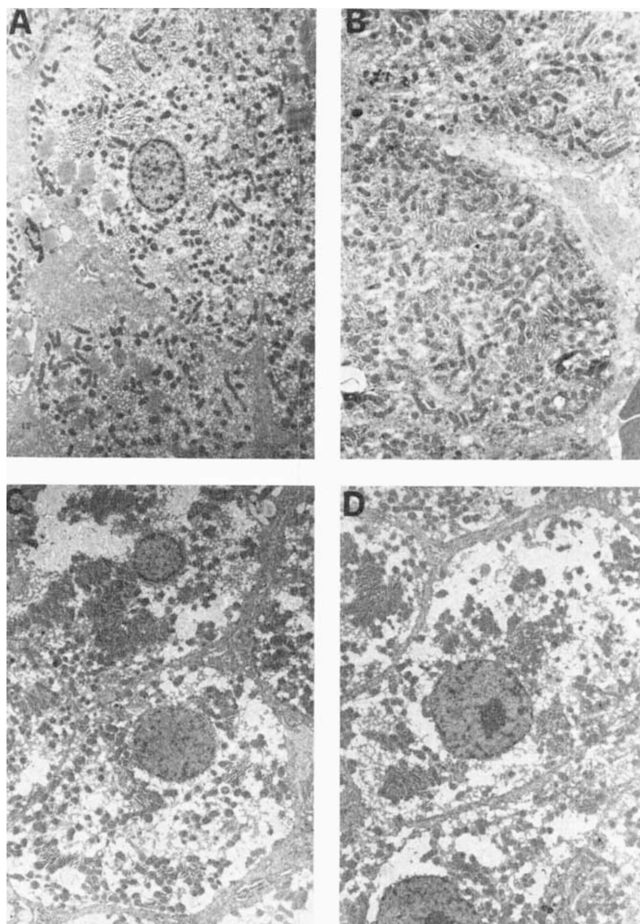


Figure 5. Electron micrographs (original magnification ×7000) of liver cells form control (A) and 4-CP treated (B–D) rats. (A) Control specimen. (B) 4-CP specimen (0.0025 mmol/kg, twice a day, 2 weeks). (C) 4-CP specimen (0.04 mmol/kg, twice a day, 2 weeks). (D) 4-CP specimen (0.08 mmol/kg, twice a day, 2 weeks).

and blood was sampled at various times. The effects of drug treatment on serum glucose concentrations are shown in Figure 6A. Chronic administration of CPIB and 4-CP elevated fasting serum glucose levels by 108–129%. A significant increase in serum glucose level was observed after 1 week of drug administration and continued treatment of the animals with both drugs maintained the elevated fasting serum glucose at levels which were significantly higher than the control values after 2, 3, 4, 7, and 8 weeks of treatment.

Measurement of Serum Lipid and Lipid-Lipoprotein Concentrations after 4-Chlorophenol and Clofibrate Treatment. Both 4-CP and CPIB were administered to rats and blood samples collected from overnight fasting animals were analyzed for triglyceride, cholesterol, and HDL-cholesterol levels. Daily administration of 0.4 mmol/kg body wt CPIB or 0.01 mmol/kg body wt. 4-CP did not alter fasting serum triglyceride concentrations throughout 8 weeks of treatment (Fig. 6B). In contrast to the effects on serum triglyceride levels, CPIB reduced total serum cholesterol concentrations to 68% of control level after 1 week of drug treatment (Fig. 6C). This cholesterol-lowering effect of CPIB remained significant for only 4 weeks. No significant reduction in serum cholesterol levels were observed with continued treatment of CPIB beyond 4 weeks.

The major cholesterol-bearing serum lipoprotein in rats is HDL and the observed decrease in serum cholesterol by CPIB may be directly related to a reduction in the cholesterol concentration of HDL (32, 33). Thus, the diminution of serum cholesterol found in CPIB-treated rats was further investigated and the results on HDL-cholesterol levels are presented in Figure 6D. Consistent with the serum cholesterol-lowering action of CPIB, serum HDL-cholesterol was also diminished by this drug. In contrast, 4-CP did not exhibit any hypocholesterolemic activity throughout the 8-week treatment period in rats.

Discussion

The overall objective of this study was to investigate whether 4-CP may mediate in part some of the adverse hepatic effects or desirable lipid-lowering activities of the parent drug, CPIB. Previous reports (8–11) have shown that CPIB administration causes hepatomegaly and increases liver/body weight ratio, liver microsomal protein, cytochrome P-450, and activities of microsomal drug-metabolizing systems. We have shown that pretreatment of rats with CPIB and 4-CP produced significant elevations in hepatic microsomal protein content; activities of ethylmorphine, benzphetamine, and aminopyrine *N*-demethylase; and cytochrome P-450 content. Treatment with both agents did not increase the microsomal activity of zoxazolamine 6-hydroxylase even though cytochrome P-450 content was significantly increased. The observed proliferation of endoplasmic reticulum in liver cells of treated rats also correlated with the dose-dependent effect of 4-CP on these hepatic microsomal parameters. More recently, other investigators have reported that chronic CPIB treatment selectively increases the activity of microsomal laurate 11- and 12-hydroxylase (10, 12, 13). The present experiments did not examine the effect of 4-CP treatment on the laurate hydroxylase enzyme activity. However, our studies have indicated that the 4-CP caused elevation of microsomal benzphetamine *N*-demethylase activity which were closely correlated to an increased cytochrome P-450 content and to a quantitative rather than a qualitative change in this *N*-demethylase enzyme system. Identical findings have been reported in rats administered CPIB at higher doses (11, 34). In contrast to CPIB, 4-CP did not produce an increase in liver/body weight ratios at the doses used in our studies.

A variety of halogenated hydrocarbons and insecticides are reported to induce the activity of the hepatic microsomal drug-metabolizing enzyme system (35–37).

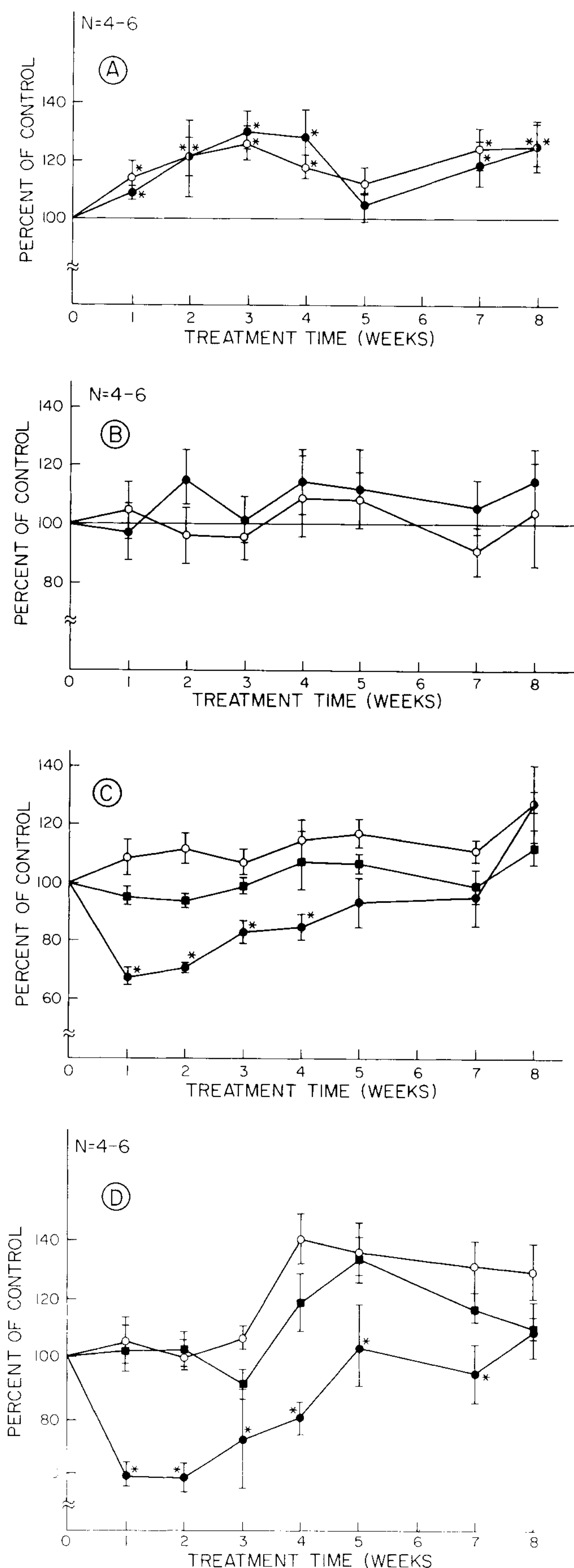


Figure 6. Effects of CPIB (0.2 mmol/kg body wt, twice a day) and 4-CP (0.005 mmol/kg body wt, twice a day) on fasting serum glucose (A), triglycerides (B), total cholesterol (C), and HDL-cholesterol (D) concentrations in rats. Control, ■; 4-CP, ○; and CPIB, ●. Data of fasting serum glucose and triglyceride were expressed relative to the corresponding control at each time of assay (A and B). Other data were expressed as a percentage of relative to Day −1 values. Drugs were given to animals by oral intubation and blood samples were obtained from overnight fasted rats. Serum parameters were measured as described in Materials and Methods. Each value represents the mean percentage of control ± SE from groups of four to six rats. Control values of measured parameters (mean ± SE of n = 5) at Weeks 1 and 8 were fasting serum glucose (mg/dl) = 90.8 ± 4.9 and 84.2 ± 2.5, triglycerides (mg/dl) = 100.6 ± 8.8 and 76.4 ± 12.0, total cholesterol (mg/dl) = 53.2 ± 7.0 and 61.8 ± 6.8, and HDL cholesterol (mg/dl) = 33.6 ± 4.6 and 35.6 ± 3.5, respectively. Asterisks indicate that the treatment values are significantly different from the corresponding control values ($P < 0.05$).

Notwithstanding the differential *in vivo* effects of 4-CP and CPIB on liver/body weight ratios, the changes in other measured parameters of the liver microsomal drug-metabolizing enzyme system were qualitatively similar. It is significant that the doses of 4-CP required to produce hepatic microsomal induction in the rats were from 20- to 167-fold lower than those of CPIB (see Figs. 2 and 3). Thus, our experiments suggest that 4-CP, if present as a minor contaminant of clofibrate (17–20) or as a metabolite of CPIB analogs (15, 16), may contribute to the *in vivo* hepatic effects seen during long-term administration. We do not propose, however, that 4-CP is an *in vivo* metabolite of CPIB. Many studies have been conducted with CPIB and neither 4-CP nor conjugates of 4-CP have been identified in tissue samples in humans or experimental animals to date (4).

Chronic treatment of rats with CPIB primarily produces a serum cholesterol lowering with little or no change in serum triglyceride concentrations (8, 33, 38). Previous work from our laboratory (33) indicates that CPIB lowers serum total cholesterol and HDL-cholesterol concentration in an identical dose-dependent manner in this species. Our present studies with CPIB treatment of rats confirm these earlier observations and further demonstrate that 4-CP, at the selected dosage (0.005 mmol/kg, twice a day), did not produce any changes in these serum lipid parameters (Fig. 6). At the selected doses, both 4-CP and CPIB produced significant elevations of fasting blood glucose concentrations. Based upon our preliminary studies, this 4-CP dose was chosen since marked changes were noted in liver cell ultrastructure (Fig. 5) and since higher doses (>0.01 mmol/kg, twice a day) of 4-CP produced undesirable effects on various hepatic parameters (Fig. 2) and reduced hepatic microsomal cytochrome P-450 and benzphetamine *N*-demethylase activities after 4 weeks of pretreatment (Fig. 4). In this regard, the 4-CP-induced morphologic changes in liver cell ultrastructure are analogous to those reported with CPIB (9) and the hepatocarcinogens, α-benzene hexachloride (39) and methapyrilene (40) in rodent species. We conclude that 4-CP is hepatotoxic at higher doses and that 4-CP does not contribute to the *in vivo* hypocholesterolemic activity of CPIB in rats. On the other hand, 4-CP appears to possess a greater *in vivo* potency than CPIB for elevating

fasting glucose levels. The mechanism for the blood glucose elevation by these agents is not clearly understood at this time. CPIB treatment is known to improve glucose tolerance in rats and in hyperliproproteinemic, hyperinsulinemic, or insulin-dependent patients (3, 4, 8) and is currently not contraindicated in diabetic patients (41).

Previous work (11, 33, 34) from our laboratories has shown that treatment of rats with CPIB (at doses >0.1 mmol/kg, twice a day) lowered serum HDL-cholesterol and cholesterol and induced various parameters of hepatic drug metabolism. In this regard, 4-CP exhibited qualitatively similar biochemical effects, and was considerably more potent than CPIB in inducing the hepatic microsomal drug-metabolizing system (0.6–5% of the CPIB dose) and in elevating blood glucose levels. The observed changes in the hepatic effects of 4-CP and CPIB appear to be due to the chlorophenyl group since nonchlorinated analogs of CPIB failed to exhibit such effects (42). From our studies, it is also clear that in rats, 4-CP does not lower serum total cholesterol or triglycerides at doses which produce significant alterations of other parameters (hepatic and blood glucose effects). Thus, it appears doubtful that 4-CP is a pharmacologically active metabolite or contaminant which contributes to the serum lipid- and lipoprotein-lowering effects of CPIB.

This work was supported by a grant from the Public Health Service (HL-12740).

1. National Institutes of Health Consensus Development Panel. Lowering blood cholesterol to prevent heart disease. J Am Med Assoc **253**:2080–2086, 1985.
2. Grundy, SM. Pathogenesis of hyperlipoproteinemia. J Lipid Res **25**:1611–1618, 1984.
3. Brown MS, Goldstein JL. Drugs used in the treatment of hyperlipoproteinemias. In: Gilman AG, Goodman LS, Rall TW, Murad F, Eds. The Pharmacological Basis of Therapeutics. 7th Ed. New York: MacMillan, pp 827–845, 1985.
4. Hagerman LM, Newman HAI, Feller DR, Witiak DT. Antilipidemic agents. In: Verderame M, Ed. CRC Handbook of Cardiovascular and Anti-inflammatory Agents. Boca Raton, FL: CRC Press, pp225–260, 1986.
5. WHO, International Agency for Research on Cancer (IARC). IARC Monographs on the evaluation of carcinogenic risk of chemicals to humans (some pharmaceutical drugs). Lyon, France: IARC, Vol **24**:pp39–58, 1980.
6. Thorp JM, Waring WS. Modification of metabolism and distribution of lipids by ethyl chlorophenoxyisobutyrate. Lancet 1:1323–1326, 1962.
7. Committee of Principal Investigators. WHO cooperative trial on primary prevention of ischaemic heart disease with clofibrate to lower serum cholesterol: Final mortality follow-up. Lancet **2**:600–604, 1984.
8. Witiak DT, Newman HIA, Feller DR. Clofibrate and related analogs: A comprehensive review. In: Grunewald G, Ed. Monographs in Medicinal Research. New York: Marcel Dekker, Inc., Vol 7:pp1–287, 1977.
9. Hess R, Staubli W, Riess W. Nature of the hepatomegalic effect produced by ethyl-chlorophenoxyisobutyrate in the rat. Nature **208**:856–858, 1965.
10. Reddy JK, Lalwani ND. Carcinogenesis by hepatic peroxisome proliferators: Evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans. CRC Crit Rev Toxicol **12**:1–58, 1983.
11. Lewis NJ, Witiak DT, Feller DR. Influence of clofibrate (ethyl-4-chlorophenoxyisobutyrate) on hepatic drug metabolism in male rats. Proc Soc Exp Biol Med **145**:281–285, 1974.
12. Gibson GG, Orton TC, Tamburini PP. Cytochrome P-450 induction by clofibrate: Purification and properties of a hepatic cytochrome P-450 relatively specific for the 12- and 11-hydroxylation of dodecanoic acid (lauric acid). Biochem J **203**:161–168, 1982.
13. Parker GL, Orton TC. Induction of oxyisobutyrates of hepatic and kidney microsomal cytochrome P-450 with specificity towards hydroxylation of fatty acids. In: Gustafsson J, Duke JC, Mode A, Rafter J, Eds. Biochemistry, Biophysics and Regulation of Cytochrome P-450. Amsterdam: Elsevier-North Holland, pp373–377, 1980.
14. US Food and Drug Administration, FDA Drug Bulletin. Rockville, MD, August, p14, 1979.
15. Loiseau GP, Millischer RJ, Lohier PY, Mardiguian JS, Gilede AMP, Ginocchio AV. On the metabolism of clofibride, a hypolipidaemic drug. J Pharm Pharmacol. **32**:483–491, 1980.
16. Almirante L, Bruseghini L, Fazi L. Dati sul metabolism in alcune specie animali di derivati dell acidico (4-clorofenossi)-isobutirrico. Boll Chim Farm **108**:292–298, 1969.
17. United States Pharmacopeal Convention, Inc. The US Pharmacopeia, 21st revision, Rockville, MD, p230, 1985.
18. Takubo E, Shigeoka S, Terajima K, Miyatake N. On the quality of medicinal medicines (1) clofibrate capsules. Ann Rep Tokyo Metrop Res Lab (Public Health) **24**:125–130, 1973.
19. Tanno K, Watanabe K, Morikawa E. The quantitative analysis of *p*-chlorophenol in clofibrate. J Hygienic Chem **16**:307–310, 1970.
20. Johansson E, Ryhage R. Gas chromatographic-mass spectrometric identification and determination of residual by-products in clofibrate preparations. J Pharm Pharmacol **28**:927–929, 1976.
21. Proudfoot SG, Davdani BH. Interaction of 4-chlorophenol and phenol with phosphatidylethanolamine monolayers in relation to antibacterial action. J Pharm Pharmacol 26(suppl):121, 1974.
22. The Merck Index. In: Windholz M, Ed. 9th edition. Rahway, NJ: Merck and Co., Inc., p274, 1976.
23. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with Folin phenol reagent. J Biol Chem **193**:265–275, 1951.
24. Omura T, Sato R. The carbon monoxide-binding pigment of liver microsomes. J Biol Chem **239**:2370–2378, 1964.
25. Holtzman JL, Gram TE, Gigon PL, Gillette JR. The distribution of the components of mixed function oxidase between the rough and smooth endoplasmic reticulum of liver cells. Biochem J **110**:407–412, 1968.
26. Trevor A. Correlation of drug disposition with pharmacologic actions. In: La Du BN, Mandel HG, Way EL, Eds. Fundamentals of Drug Metabolism and Drug Disposition. Chap 29. Baltimore: Williams & Wilkins, pp591–599, 1971.
27. Cleland WW. Statistical analysis of enzyme kinetic data. Methods Enzymol **63**:103–138, 1979.
28. Kadish AH, Little RL, Sternberg, JC. A new and rapid method for the determination of glucose by measurement of rate of oxygen consumption. Clin Chem **14**:116–131, 1968.
29. Sampson EJ, Demers LM, Krieg AF. Faster enzymatic procedure for serum triglycerides. Clin Chem **21**:1983–1985, 1975.
30. Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem **20**:470–475, 1974.
31. Steele BW, Koehler DF, Azar MM, Blaszkowski TP, Kuba K,

Dempsey ME. Enzymatic determinations of cholesterol in high density lipoprotein fractions prepared by a precipitation technique. Clin Chem **22**:98–101, 1976.
32. Alexander C, Day CE. Distribution of serum lipoproteins of selected vertebrates. Comp Biochem Physiol [B] **46**:295–312, 1973.
33. O'Brien M, Patel ST, Mukhopadhyay A, Newman HAI, Feller DR, Kokrady SS, Witiak DT, Lanese RR, Rice JC. Hypolipidemic effects of clofibrate and selected chroman analogs in fasted rats. Lipids **16**:903–911, 1981.
34. Mellon WS, Golderg AP, Witiak DT, Feller DR. Differential effect of clofibrate on hepatic drug oxidation and cholesterol 7α-hydroxylation. Biochem Pharmacol. **25**:2403–2406, 1976.
35. Triolo AJ, Coon JM. The protective effect of aldrin against the toxicity of organic anticholinesterases. J Pharmacol Exp Ther **154**:613–623, 1966.
36. Lacombe R, Brodeur J. The effect of pretreatment with dieldrin on certain in vivo parameters of enzyme induction in mice. Toxicol Appl Pharmacol **27**:70–85, 1974.
37. Oesch F, Friedberg T, Herbst M, Paul W, Wilhelm N, Bentley P. Effects of lindane treatment on drug metabolizing enzymes and liver weight of CF1 mice in which it evoked hepatomas in nonsusceptable species. Chem Biol Interact **40**:1–14, 1982.
38. Cayen MN, Dubuc J, Dvornik D. Effects of clofibrate on lipid metabolism in streptozotocin treated diabetic rats. Proc Soc Exp Biol Med **148**:752–757, 1975.
39. Tsukada H, Gotoh M, Mechizuki Y, Furukawa K. Changes in peroxisomes in preneoplastic liver and hepatoma of mice induced by α-benzene hexachloride. Can Res **39**:1628–1634, 1979.
40. Reznik-Schuller HM, Lijinsky W. Morphology of early changes in liver carcinogenesis induced by methapyrilene. Arch Toxicol **49**:79–83, 1981.
41. Physicians' Desk Reference, Product Information. 42nd ed. Oradell, NJ: Medical Economics Co., Inc., pp638–639, 1988.
42. Lewis NJ, Feller DR, Poochikian GK, Witiak DT. Differential effects of benzodioxane, chroman, and dihydrobenzofuran analogs of clofibrate on various parameters of hepatic drug metabolism. J Med Chem **17**:41–45, 1974.