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# PLASMA DISAPPEARANCE AND BILIARY EXCRETION OF SULFOBROMOPHTHALEIN AND PHENOL-3,6-DIBROMPHTHALEIN DISULFONATE AFTER MICROSOMAL ENZYME INDUCTION*

CURTIS D. KLAASSEN

Department of Pharmacology, Clinical Pharmacology and Toxicology Center, Kansas Medical Center, Kansas City, Kans. 66103, U.S.A.

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**Abstract**—The ability of various microsomal drug metabolizing enzyme inducers to enhance the plasma disappearance and biliary excretion of sulfobromophthalein (BSP) and phenol-3,6-dibromphthalein disulfonate (DBSP) was compared to the ability of these agents to increase biliary flow. Phenobarbital (PB) treatment produced the greatest increase in biliary flow and also had the greatest effect in enhancing the plasma disappearance and excretion of BSP and DBSP into the bile. With chlordane, phenylbutazone, nikethamide and chlorcyclizine treatment, no statistically significant changes in biliary flow were produced prior to dye administration. These agents produced a significant decrease in the plasma concentration of BSP or DBSP only at the 30-min sample and produced a slight increase in the excretion of these 2 dyes into the bile. With 3-methylcholanthrene and 3,4-benzpyrene, there was no apparent increase in biliary flow, plasma disappearance of the dyes, or excretion rate into the bile. Therefore, there appears to be no direct correlation between the ability of these agents to induce rates of drug metabolism and the ability to enhance the disappearance of these dyes. However, there generally appears to be a good correlation in the ability of these agents to increase biliary flow in rats and their ability to increase the plasma disappearance and biliary excretion of BSP and DBSP.

CANALICULAR bile flow appears to play an important role in the rate of secretion of organic anions into the bile. O'Maille *et al.*[1] were the first to demonstrate that an increase in biliary flow produced by taurocholate would increase the biliary excretion of sulfobromophthalein (BSP). Enhanced biliary flow produced by bile salt administration also increases the excretion of bilirubin.[2, 3]

Biliary flow is also increased after phenobarbital (PB) treatment. An increase in the plasma disappearance of bilirubin and biliary excretion has been reported in laboratory animals after PB treatment.[4, 5] PB also decreases the plasma bilirubin levels in patients with congenital unconjugated hyperbilirubinemia.[6–9] Bilirubin levels in newborn infants can be diminished by giving PB during pregnancy or to the infant.[10, 11] It is not clear if this decrease in plasma bilirubin levels after PB treatment is because of enhanced bilirubin conjugation or secretion, but most investigators favor the former hypothesis.

Enhanced plasma disappearance of BSP has also been demonstrated after PB treatment,[12] and increased biliary flow appears to play a major role in producing this

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effect.[13] Klaassen[14] has recently reported that PB was the only one of several known microsomal drug metabolizing enzyme inducers that significantly increased biliary flow. With chlordane, nikethamide, phenylbutazone and chlorcyclizine treatment, there was a trend towards an increase in biliary flow but the increases were not statistically significant. Biliary flow was not altered in rats pretreated with 3,4-benzpyrene and 3-methylcholanthrene (3-MC).

The purpose of the present investigation was to determine if a correlation exists between the ability of these inducing agents to increase biliary flow and their ability to enhance the plasma disappearance and biliary excretion of BSP. These parameters were also examined using an analog of BSP, phenol-3,6-dibromphthalein disulfonate (DBSP), which is not biotransformed before its excretion.[15, 16]

## METHODS

*Animals and treatments.* Simonsen Sprague–Dawley male rats (250–350 g) were used throughout. The following agents known to stimulate microsomal drug metabolizing enzymes[17, 18] were employed: phenobarbital sodium (75 mg/kg), gamma-chlordane (50 mg/kg), nikethamide (50 mg/kg), chlorcyclizine hydrochloride (25 mg/kg), sodium phenylbutazone (125 mg/kg), 3-methylcholanthrene (20 mg/kg), and 3,4-benzpyrene (20 mg/kg). The agents were given intraperitoneally once daily for 4 days in aqueous solution, except chlordane, 3-methylcholanthrene (3-MC) and 3,4-benzpyrene which were dissolved in corn oil. The aqueous solutions contained the proper dosage in a final volume of 0·01 ml/g and the corn oil solutions in a volume of 0·005 ml/g.

*Surgical procedure.* The rats were anesthetized with pentobarbital sodium (45 mg/kg, i.p.), supplemental doses being given as needed. The femoral vein and artery were cannulated with polyethylene tubing (PE-50) for administering the dyes and obtaining the blood samples respectively. The bile duct was surgically exposed by a midline incision and cannulated with PE-10 tubing. The rectal temperature of the anesthetized rat was maintained at 37° with a heat lamp in conjunction with a temperature regulator to prevent hypothermic alteration in biliary flow.[19]

*Sample collection.* Before the administration of the drugs, a 10-min bile sample was collected. BSP (120 mg/kg), or an equimolar dose of DBSP (96 mg/kg), was then given intravenously. Blood samples (0·3 ml) were obtained from the cannulated femoral artery 2, 10, 20, 25 and 30 min after administration of the dyes. Bile samples were collected at 10-min intervals for 40 min after administration of the dyes.

*Analytical methods.* The concentration of BSP and DBSP in the plasma was determined using a Spectronic 20 spectrophotometer at 580 mμ after an aliquot (100 μl) of the plasma was diluted with an appropriate volume of 0·1 N NaOH solution. The concentration of BSP and DBSP in the bile (50-μl aliquots) was determined similarly after the bile volume for each collection period was measured with a graduated pipet.

*Statistics.* The means of the various groups were compared by the Student *t*-test as described by Steel and Torrie.[20]

## RESULTS

*BSP plasma disappearance.* Figures 1 and 2 depict the plasma disappearance of BSP in control rats and rats treated with the various microsomal drug metabolism enzyme inducers for 4 days. No differences in the plasma BSP concentrations between

the control and treated groups were demonstrated for the 2-min collection periods, which indicates that the initial volume of distribution of BSP in the groups was similar. PB produced the greatest enhancement of plasma BSP disappearance. The plasma BSP levels were significantly lower in the PB-treated rats at the 10-, 20-, 25- and 30-min collection periods.

Chlordane, phenylbutazone and nikethamide treatment also significantly enhanced the plasma disappearance of BSP in rats, but not to the degree that PB did. The plasma BSP levels in the chlordane, phenylbutazone and nikethamide-treated rats were significantly lower than the control group only at the 30-min collection. With the

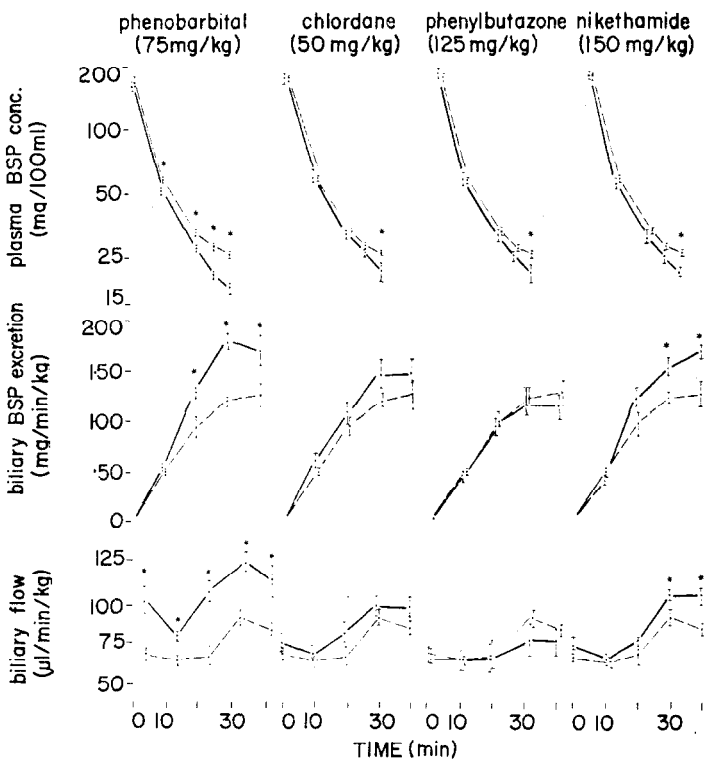


FIG. 1. Plasma disappearance of BSP, biliary excretion of BSP, and biliary flow of control rats and rats treated with various microsomal drug metabolizing enzyme inducers for 4 days. The slashed line represents the control group and the darker line the pretreated group. Each point represents the mean ± S.E. of six rats. The asterisk indicates the values are significantly different from the controls ($P < 0.05$).

chlorcyclizine, 3-MC, and 3,4-benzpyrene-treated rats no differences in plasma BSP levels between the control and treated groups were detected at any time interval examined.

*BSP biliary excretion.* The biliary excretion pattern of BSP in the rat after the intravenous administration of 120 mg/kg of BSP is seen in Figs. 1 and 2. PB significantly enhanced the biliary excretion of BSP at the 20-, 30- and 40-min collection period. The maximum rate of excretion of BSP was 1·25 mg/min/kg in the control

group and 1·80 mg/min/kg in the PB-treated group. Similar results have been reported by Klaassen and Plaa[13] for BSP Tm values in the 2 groups when BSP was continually infused at a rate greater than its excretion.

Nikethamide was the only other treatment that significantly enhanced the biliary excretion of BSP. However, its effect differed from that of PB. The increase was not significantly increased until the 30-min collection period, while in the PB group it was significantly increased at the 20-min period. At the 30-min collection period, the

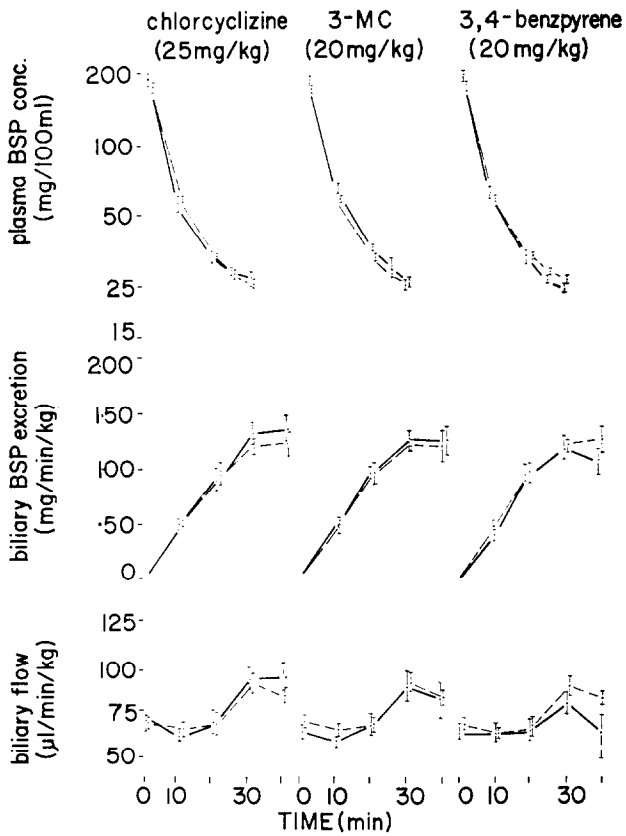


FIG. 2. Plasma disappearance of BSP, biliary excretion of BSP, and biliary flow of control rats and rats treated with various microsomal drug metabolizing enzyme inducers for 4 days. The slashed line represents the control group and the darker line the pretreated group. Each point represents the mean ± S.E. of six rats. The asterisk indicates the values are significantly different from the controls ($P < 0.05$).

biliary excretion of BSP was 1·52 mg/min/kg in the nikethamide group in comparison to the 1·80 observed in the PB group. In the nikethamide group the biliary excretion of BSP was still increasing during the last collection period while in the PB group it was beginning to decline. However, even during this last collection period, the rate of secretion was slightly greater in the PB group than in the nikethamide group. With the other agents there was no significant increase in the biliary excretion of BSP over the control group.

*Biliary flow.* Figures 1 and 2 also demonstrate the biliary flow before and after the administration of BSP in control rats and rats treated with the various microsomal enzyme inducers. The biliary flow in control rats was approximately 68 μl/min/kg and remained relatively constant for the first 20 min after the administration of the dye. At the 30-min collection, the choleretic effect of BSP was seen. In the PB-treated rats the biliary flow was 99 μl/min/kg before the administration of BSP; this rate was significantly greater than that of control rats. At all collection periods the biliary flow was greater in the PB-treated rats than in the controls.

Prior to administration of the BSP, no other treated group had biliary flow rates significantly different from controls. However, after the administration of BSP, a significant increase in biliary flow over the control group was seen in the nikethamide treated group. This increase was demonstrated only at the 30- and 40-min collection period.

*DBSP plasma disappearance.* Figures 3 and 4 demonstrate the plasma disappearance

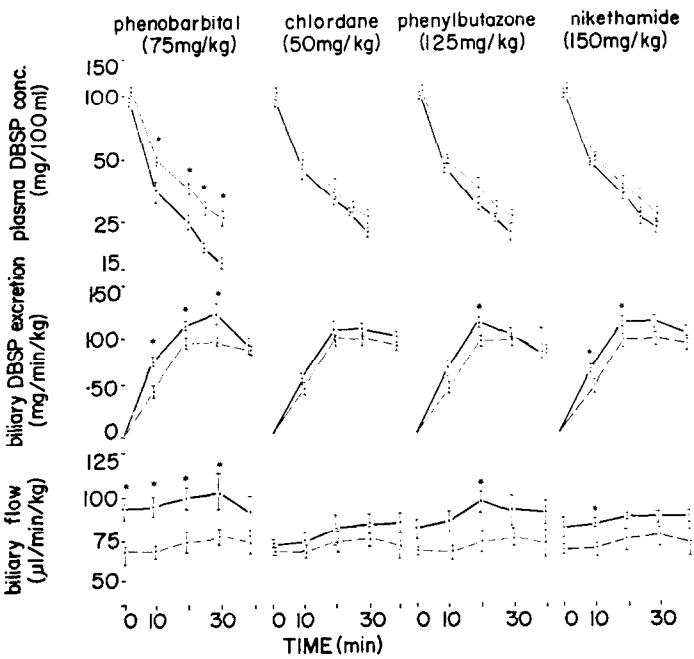


FIG. 3. Plasma disappearance of DBSP, biliary excretion of DBSP, and biliary flow of control rats and rats treated with various microsomal drug metabolizing enzyme inducers for 4 days. The slashed line represents the control group and the darker line the pretreated group. Each point represents the mean ± S.E. of six rats. The asterisk indicates the values are significantly different from the controls ($P < 0.05$).

of DBSP in control rats and rats treated with the various microsomal drug metabolism enzyme inducers. PB was the agent that produced the greatest enhancement of DBSP disappearance with significantly lower levels of DBSP at 10, 20, 25 and 30 min after the intravenous administration of the dye.

Chlorcyclizine was the only other agent studied that significantly enhanced the plasma disappearance of DBSP. This was significant only at the 30-min collection

period. With a number of the other agents there was a tendency towards enhanced plasma disappearance of DBSP but the values were not statistically different from controls. With 3-MC and 3,4-benzpyrene treatment the plasma disappearance of DBSP was equal to or slightly slower than that of control rats.

*DBSP biliary excretion.* The results of the excretion of DBSP into the bile after the intravenous administration of DBSP (96 mg/kg) are seen in Figs. 3 and 4. This dosage

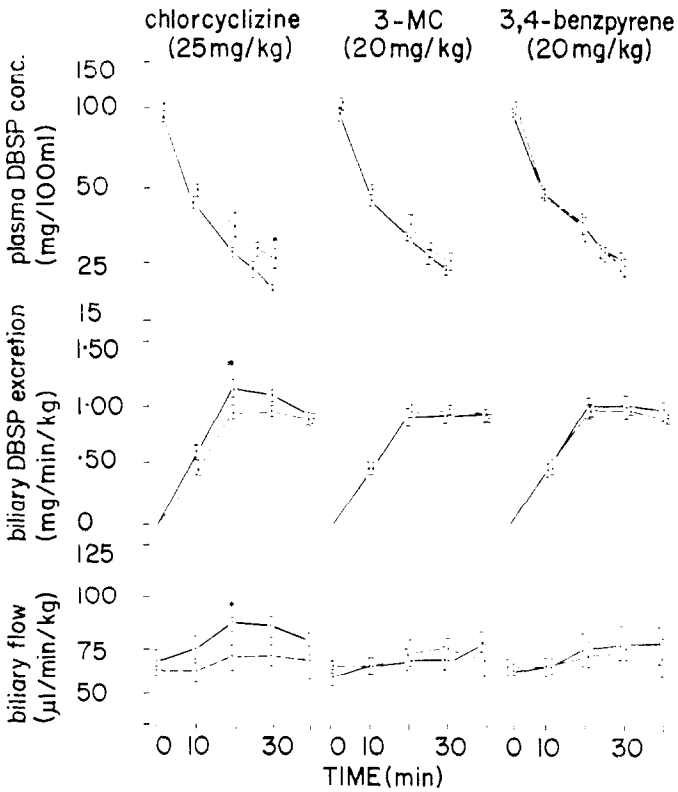


FIG. 4. Plasma disappearance of DBSP, biliary excretion of DBSP, and biliary flow of control rats and rats treated with various microsomal drug metabolizing enzyme inducers for 4 days. The slashed line represents the control group and the darker line the pretreated group. Each point represents the mean ± S.E. of six rats. The asterisk indicates the values are significantly different from the controls ($P < 0.05$).

of DBSP produces a rate of biliary secretion which is comparable to that obtained during infusions of DBSP.[16] It should be noted that maximal rates of biliary excretion of DBSP occur as early as 20 min after the intravenous administration of the dye. With BSP, maximal rates of excretion are not observed until 30 or 40 min after administration.

PB treatment significantly enhanced the excretion of DBSP into the bile at the 10-, 20- and 30-min intervals. Enhanced DBSP Tm values after PB treatment have also been reported during infusion of DBSP after PB.[13] Increased biliary secretion of

DBSP after PB treatment was increased as early as 10 min after the i.v. administration. The increase is not observed as rapidly with BSP.

Significant increases in the biliary excretion of DBSP were also demonstrated after phenylbutazone, nikethamide and chlorcyclizine treatment. However, in contrast to PB, the increases were not as large and were significant at only 1 or 2 collection periods. With chlordane, 3-MC, and 3,4-benzpyrene, no significant increases in the excretion of DBSP were demonstrated at any of the time intervals.

*Biliary flow.* Figures 3 and 4 also demonstrate the biliary flow in control and treated rats before and after the i.v. administration of DBSP. Only a slight choleretic effect was seen after the administration of DBSP in contrast to the marked choleretic effect seen after BSP. The PB-treated rats had a significantly higher biliary flow rate before the administration of the dye and remained significantly elevated for all collection periods after DBSP except for the last collection period.

With the phenylbutazone-, nikethamide- and chlorcyclizine-treated rats, biliary flow was significantly increased at 1 collection period. With the rats treated with 3-MC and 3,4-benzpyrene, there was no increase in biliary flow.

## DISCUSSION

The duration of action of most drugs in the body is usually determined by the speed at which they are metabolized and/or the rate at which they are excreted. In the last few years, considerable attention has been given to the importance of altered microsomal drug metabolizing activity and drug action.[17, 18] PB is an example of an agent which enhanced the microsomal enzyme activity and thus accelerates the rate of biotransformation of a large number of drugs and thereby reduces the biological half-life of many drugs. However, PB treatment also results in an enhanced biliary flow.[4] This increase in biliary flow after PB treatment appears to play an important role in the accelerated plasma disappearance and biliary excretion of BSP and possibly other drugs.

Klaassen[14] has recently reported that other known microsomal enzyme inducers do not appear to increase biliary flow to the extent that PB does. In the present study similar results were obtained. PB significantly enhanced biliary flow both before and after the dyes were given. With none of the other agents was there a significant increase in biliary flow before the dyes were given. After injection of the dyes there was a significant increase in biliary flow at two collection periods for nikethamide after injection of BSP, and a significant increase in biliary flow at one collection period for phenylbutazone, nikethamide and chlorcyclizine after DBSP administration. The influence of these microsomal enzyme inducers on biliary flow might be summarized as follows: PB treatment produces a significant increase in biliary flow of about 50 per cent; chlordane, phenylbutazone, nikethamide and chlorcyclizine treatments have a tendency to produce an increase in biliary flow, but this is usually not significantly different from controls; and 3-MC and 3,4-benzpyrene have no apparent effect on biliary flow.

The ability of these various agents to alter the plasma disappearance of BSP and DBSP generally corresponds to their effect on biliary flow. PB which produces the greatest increase in biliary flow also produced the greatest enhancement of the plasma disappearance of BSP and DBSP. With the agents that only had a tendency to increase biliary flow (chlordane, phenylbutazone, nikethamide and chlorcyclizine) there was

only a slight enhancement (significant only at 30-min collection period) or a tendency in the direction of an enhanced plasma disappearance of the two dyes. One definite exception to the correlation in biliary flow and plasma disappearance of the dyes was demonstrated with phenylbutazone after BSP injection; a slight enhancement in the plasma disappearance was demonstrated but no increase in biliary flow was apparent. No alteration in BSP and DBSP plasma disappearance was demonstrated for 3-MC and 3,4-benzpyrene. However, these last two agents produced significant increases in drug metabolizing activity, as demonstrated by a decrease in zoxazolamine paralysis time,[14] and yet produced no apparent enhancement in the disappearance of BSP and DBSP. Therefore, microsomal drug metabolizing induction itself does not result in the enhanced disappearance of these dyes. The reason why the other microsomal enzyme inducers enhanced the biliary excretory function, at least to a small degree, and 3-MC and 3,4-benzpyrene did not, is not known, but these latter two agents do not increase the amount of hepatic smooth endoplasmic reticulum and do not increase the metabolism of as many drugs as do the other agents.[17, 18]

The ability of the various agents to increase the rate of excretion of BSP and DBSP into the bile generally correlates with their ability to increase biliary flow and plasma disappearance of the 2 dyes. Enhanced biliary excretion was demonstrated only when there was an increase in biliary flow. PB produced the greatest increase in biliary excretion of the dyes into the bile. With the agents that only had a tendency to increase biliary flow (chlordane, phenylbutazone, nikethamide and chlorcyclizine) significant excretion of BSP into the bile was demonstrated only with nikethamide. With DBSP, significant excretion was exhibited only at one or two collection periods with phenylbutazone, nikethamide and chlorcyclizine. With 3-MC and 3,4-benzpyrene, which produced no apparent increase in biliary flow or plasma disappearance of BSP and DBSP, no increase in biliary excretion was demonstrated. Therefore, there generally appears to be a good correlation in the ability of these agents to increase biliary flow in rats and their ability to increase the plasma disappearance and excretion of BSP and DBSP.

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