*Biochemical Pharmacology*, Vol. 34, No. 12, pp. 2129–2135, 1985.
Printed in Great Britain.

0006-2952/85 $3.00 + 0.00


# SECRETION OF THE ORGANIC ANION HARMOL SULFATE FROM LIVER INTO BLOOD

## EVIDENCE FOR A CARRIER-MEDIATED MECHANISM

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(*Received* 7 *March* 1984; *accepted* 14 *January* 1985)

**Abstract**—In the liver, drugs with phenolic groups can be converted to sulfate or glucuronide conjugates and are then transported into bile or back into the bloodstream. The mechanism for transport of drugs and drug conjugates from the hepatocytes into the blood at the sinusoidal side of the cell are not well defined. In the case of carrier-mediated transport of these strongly polar conjugates, saturability of transport and mutual competition between structurally related compounds would be anticipated. This competitive aspect was investigated by using two organic anions, dibromosulfophthalein (DBSP) and harmol sulfate. The latter compound was generated by the hepatocytes from harmol, which was continuously infused into the rat *in vivo* and in isolated perfused rat livers. In addition we loaded the perfused rat livers with preformed harmol sulfate and studied its efflux rate to the perfusate as influenced by DBSP. In steady state, about 80% of harmol was sulfated and 20% was glucuronidated. Harmol sulfate was mainly excreted in the urine, the glucuronide was equally excreted in urine and bile. DBSP lowered the urinary excretion of harmol sulfate by about 30% which was due to a decrease in plasma concentration. However, renal clearance of harmol sulfate ($3.2 \pm 0.2$ ml/min) was unchanged. At the same time DBSP doubled the biliary clearance of harmol sulfate ($1.0 \pm 0.1$ and $2.2 \pm 0.2$ ml/min in controls and DBSP studies respectively). DBSP decreased glucuronide excretion both in urine and bile. The increase in biliary output and decrease in urinary excretion of harmol sulfate is explained by competitive interaction between the organic anion DBSP and harmol sulfate at the sinusoidal level. Efflux experiments in single pass perfused isolated livers showed a clear decrease of harmol sulfate transport from liver into plasma by DBSP and provided evidence for such an inhibitory phenomenon ($t_{\frac{1}{2}}$ of efflux was $3.58 \pm 0.21$ compared with $2.46 \pm 0.07$ min for controls). These results indicate that organic anion transport from the hepatocyte into the blood stream is very likely carrier-mediated. A decrease in renal output of drug conjugates therefore may not only be due to a decrease in the conjugation process but also to a lower liver to blood transport rate which at the same time may produce a higher biliary output.

Drugs with phenolic groups that are taken up in the hepatocyte are often converted to sulfate or glucuronide conjugates and subsequently transported into bile or back into the blood [1]. Much more is known about the mechanisms responsible for the excretion of compounds into bile [2] than about those responsible for transport from the liver cells back into the blood stream [3]. It is uncertain whether the latter process is carrier-mediated or occurs by simple diffusion. However, because of the polar character of such conjugates, carrier-mediated transport seems to be a probable mechanism. If transport across the sinusoidal membrane is carrier-mediated, one would expect substrate specificity, saturability of transport and mutual competition between structurally related compounds for excretion into the blood stream.

In this study we investigated this competitive phenomenon using two organic anions: harmol sulfate (HS) generated from harmol in the hepatocyte and dibromosulfophthalein (DBSP). DBSP is a frequently used model-compound which is not metabolized in the rat and can be easily detected spectrophotometrically [4–7]. The kinetics of formation and elimination of harmol sulfate have been extensively studied by Mulder and his co-workers [8–12] and this compound is quite useful because of its very sensitive fluorimetric determination. Harmol is conjugated in the rat by glucuronidation and sulfation only. Harmol glucuronide (HG) is excreted predominantly in bile, whereas harmol sulfate is equally well excreted in bile and urine in this species [8, 9]. We studied the influence of DBSP on the hepatic efflux of endogenously formed metabolites of harmol into bile and blood in the rat and studied the sinusoidal transport process more in detail in isolated perfused rat livers preloaded with harmol sulfate.

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## MATERIALS AND METHODS

Harmol hydrochloride was obtained from Sigma Chemical Co. (St. Louis, MO), dibromosulfophthalein (DBSP) from Société d'Etudes et de Recherches Biologiques (SERB, Paris, France) and albumin (demineralized bovine serum albumin) from Poviet (Oss, The Netherlands).

All other chemicals were obtained from Merck (Darmstadt, West Germany), Harmol sulfate was synthesized from harmol HCl [11].

All infusions were given by means of a Braun (Melsungen, West Germany) constant infusion pump.

In vivo *experiments*. Male Wister rats (200–300 g body wt), which had free access to food and water were used. All animals were anaesthetized prior to surgery by an intraperitoneal injection of sodium pentobarbital (60 mg/kg). During the experiments they were artificially respirated through a trachea canula. The animals were kept at 38° (±0.5°) by means of an electrically heated pad. The carotid artery, the jugular and femoral veins, the bile duct and urine bladder were cannulated. A solution of 100 mg/ml D-mannitol in saline was infused at a rate of 1.9 ml/hr to ascertain constant urine flow [13]. After a priming dose of 10 μmoles/kg of harmol, a solution of harmol in saline (25 μmoles/kg/hr) was infused through the jugular vein during 190 min. Ninety minutes after the start of the harmol infusion a priming dose of 60 μmoles/kg of DBSP was given followed by an infusion of DBSP (200 μmoles/kg/hr) through the femoral vein for 100 min. Bile and urine were collected in fractions of 10 or 15 min. Blood samples were taken at 85 min and 190 min after the start of the harmol infusion in heparinized plastic tubes. Plasma was obtained by centrifugation.

*The isolated perfused liver preparation*. Livers from male Wistar rats (290–310 g body wt) were isolated and perfused as described elsewhere by Meijer *et al.* [14]. The perfusion medium was a Krebs-bicarbonate buffer (pH 7.4) with 0.1% (harmol sulfate experiments) or 1.0% of albumin (harmol experiments). The flow through the liver was 35 ml/min. The medium was gassed with a mixture of 95% of $O_2$ and 5% of $CO_2$. Temperature was kept at 38°. The pH of the perfusion medium was maintained between 7.35 and 7.45 [14]. Liver weights at the end of the experiments ranged from 10.3 to 11.8 g.

*Perfusion experiments with harmol sulfate*. In order to load the liver with HS a dose of 12.4 μmoles of HS in 1 ml of 0.01 N NaOH solution was added to the perfusion reservoir, which contained 100 ml of medium. In the experiments with DBSP, 9 min after administration of HS a dose of 54 μmoles of DBSP was added. The liver was perfused during 35 min. Samples of the perfusion medium were taken and bile was collected at regular time intervals. After 35 min the liver was switched to a single pass perfusion with freshly oxygenated medium (pH 7.4) without HS or DBSP. The efflux of HS from the liver was measured by taking samples of the outflowing perfusate during 10 min. Bile was collected for 20 min in fractions of various time intervals.

*Perfusion experiments with harmol*. An infusion of harmol of 125 nmoles/min preceded by a priming dose of 4.5 μmoles was administered into the main reservoir which contained 100 ml of medium. Harmol was dissolved in saline. Eighty minutes after the start of the harmol infusion an infusion of DBSP (1000 nmoles/min) preceded by a priming dose of 30 μmoles was given into the main reservoir. Samples of the perfusion medium were taken and bile was collected at regular time intervals.

*Analytical methods*. The conjugates of harmol in bile and urine were quantified fluorimetrically after thin-layer chromatography by the method of Mulder and Hagedoorn [8]. Plasma of *in vivo* experiments and samples of perfusate obtained from experiments with harmol were mixed with an equal volume of methanol in order to precipitate proteins. After centrifugation 150 μl (for plasma) and 100 μl (for perfusate) of the supernatant was applied to silicagel TLC plates; after TLC, the spots were removed and eluted with 0.1 N HCl for one night. After centrifugation the fluorescence of the supernatant was read in a spectrofluorimeter [8]. DBSP was determined spectrophotometrically at 575 nm in 0.1 N NaOH [5]. The unbound concentration of harmol sulfate in Krebs-bicarbonate buffer with 0.1% albumin was determined in the absence and presence of 200 and 550 nmoles/ml of DBSP using equilibrium dialysis (24 hr, 37°) in Teflon cells.

*Pharmacokinetic analysis*. The renal ($Cl_{renal}$) and biliary ($Cl_{biliary}$) clearance were calculated by $(dA/dt)/C_p$, where $dA/dt$ = excretion rate (nmoles/min) in urine and bile respectively and $C_p$ = plasma concentration. The harmol sulfate plasma disappearance curves were analyzed according to a two compartment model. The clearance from plasma to liver ($Cl_{12}$) was calculated from $k_{12}$ (the rate constant for plasma to liver transport) × $V_1$ (the volume of the central compartment). The volume of the peripheral compartment was calculated by $k_{12}/k_{21} \times V_1$, where $k_{21}$ = rate constant for transport from liver to plasma. The hepatic distribution volume in steady state experiments was calculated by $A_H^{ss}/C_p$, where $A_H^{ss}$ is the amount in the liver in steady state and $C_p$ is the plasma concentration.

## RESULTS

### In vivo *experiments*

The biliary and urinary excretion rate of the harmol conjugates HG and HS during infusion of harmol are shown in Fig. 1. Within 90 min 96.4% of the amount infused was recovered as the conjugates in bile and urine (Table 1). The excretion patterns indicate that steady state conditions were obtained. When the DBSP was added the excretion rate of HS via bile increased considerably, while the urinary excretion rate clearly decreased. The total urinary HS excretion rate decreased, probably because the plasma concentration of HS was lowered following DBSP infusion (Table 1). Indeed, renal clearance of HS was unaffected by DBSP, whereas biliary clearance was elevated (Table 1). Both the biliary and urinary excretion rate of HG decreased during DBSP infusion.

Recovery of harmol as its conjugates was 96.4% before and 83.4% during DBSP infusion of the infusion rate of harmol. The biliary and urinary

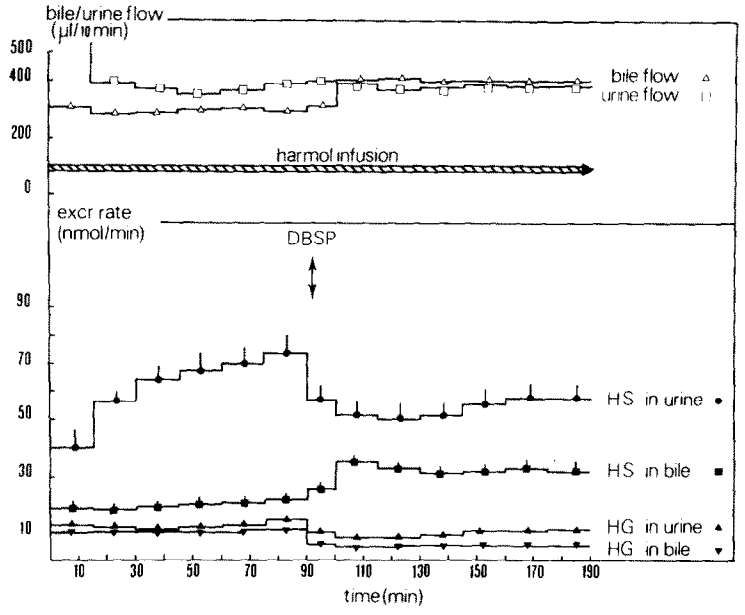


Fig. 1. Harmol conjugation *in vivo*. Biliary and urinary excretion rate of HG and HS (lower panel) and bile and urine flow (top panel) during infusion of 125 nmoles/min of harmol for 190 min, preceded by a priming dose of 4.5 μmoles. An infusion of 1000 nmoles/min of DBSP, preceded by a priming dose of 28 μmoles, was started 90 min after the start of the harmol infusion until the end of the experiment. The mean value ± S.E.M. are given (N = 6).

excretion rate of DBSP reached steady state within 20 min (not shown).

The increased biliary output of HS combined with the decreased urinary excretion of the organic anion can be explained either by a stimulatory effect of DBSP on biliary transport of HS, or alternatively, to an inhibition by DBSP of the liver to plasma transport of HS. In addition, a possible effect of DBSP on HS protein binding should be taken into account. Therefore we designed experiments to study the effect of DBSP on the hepatic efflux and the albumin binding of HS in more detail.

*Effect of DBSP on harmol sulfate kinetics in the perfused rat liver*

To exclude possible influences of DBSP on conjugation and transport processes outside the liver we repeated these experiments in the isolated perfused rat liver preparation. After 60 min of harmol infusion HS is excreted into the medium at a constant rate of 52 nmoles/min, as indicated by a linear increase in the harmol sulfate medium concentration in time (Fig. 2A). Biliary excretion rates of the harmol conjugates are fairly constant at that time (Fig. 2B). As was found in the *in vivo* studies, the excretion rate into bile of HS clearly increased during DBSP infusion. Initially the excretion rate of HG into bile was decreased, but after 40 min it tended to reach a value equal to the one before DBSP infusion. The excretion rate of HS into the medium was only slightly reduced by DBSP (50 nmoles/min).

*Pharmacokinetics of preformed harmol sulfate in the perfused liver*

To study the process of hepatic efflux of harmol sulfate in more detail we investigated the wash-out pattern after preloading isolated perfused livers with preformed HS. In Fig. 3A the medium concentration and the biliary excretion rate of HS in these loading experiments are shown. After a rapid uptake phase the medium concentration of HS reached a constant value of 40 nmoles/ml, which remained at that level for at least 35 min. After 35 min the total liver load, calculated from the amounts present in the medium and excreted into bile, was 8.4 μmoles of the 12.4 μmoles dose administered. The volume of the central compartment $V_1$ was calculated to be 97 ml, in agreement with the volume of perfusion medium (100 ml). We analyzed these curves according to a two compartment model. $Cl_{12}$ (the clearance for plasma to liver transport) was calculated to be about 35 ml/min, which is equal to the flow through the liver. The volume of the peripheral compartment $V_2$ was 204 ml; since liver weight is about 10 g this indicates considerable intracellular binding or alternatively concentrative uptake of HS into this organ.

After loading the isolated perfused livers with HS, the transport out of the liver into the perfusate was studied by switching to a single pass perfusion and measuring concentrations in the effluent medium (Fig. 4A). The concentration of HS in the outflowing perfusate is plotted on a semi-logarithmic scale in Fig. 4A for both the control and the DBSP experiments. In livers preloaded with DBSP the $t_{\frac{1}{2}}$ for efflux of HS was significantly longer, clearly demonstrating the inhibitory effect of DBSP on the efflux of HS. In experiments with different liver contents of HS, the $t_{\frac{1}{2}}$ of efflux appeared to be independent of the initial amount of substrate in the liver, indicating first-order kinetic conditions (Table 2). Fifty percent of the original load was recovered in the perfusate after 20 min perfusion both in absence and in pres-

Table 1. Pharmacokinetics of harmol sulfate and harmol glucuronide *in vivo*

| | Before DBSP infusion | | During DBSP infusion | |
|---|---|---|---|---|
| | HS | HG | HS | HG |
| $C^{ss}_{plasma}$ (nmoles/ml) | 22.2 ±0.9 | n.d. | 15.4* ±0.8 | n.d. |
| $Cl_{renal}$ (ml/min) | 3.2 ±0.2 | — | 3.2 ±0.1 | — |
| $Cl_{biliary}$ (ml/min) | 1.0 ±0.1 | — | 2.2* ±0.2 | — |
| Total excretion rate (nmoles/min) | 94.0 | 26.5 | 86.8 | 17.5 |
| Recovery (%) | 75.2 | 21.2 | 69.4 | 14.0 |

Plasma concentration ($C^{ss}_{plasma}$), renal ($Cl_{renal}$) and biliary clearance ($Cl_{biliary}$) and total excretion rate of HS and HG before and during DBSP infusion. (For further details see legend Fig. 1.)
Mean values ± S.E.M. (N = 5).
* Significantly different from control $P < 0.005$ (Wilcoxon's test).

ence of DBSP. The biliary excretion rate of HS during these wash-out studies is also plotted on a semi-logarithmic scale in Fig. 4B. In the presence of DBSP the $t_{\frac{1}{2}}$ of the excretion rate curve was significantly longer than in control experiments. The mean $t_{\frac{1}{2}}$ of efflux to plasma was about a factor of 2 smaller than that of the biliary excretion rate (see Figs. 4A and 4B), suggesting that transport into these directions may occur from different pools (intracellular or zonal).

*Effect of DBSP on the storage of HS in isolated perfused liver*

When we administered DBSP into the medium 9 min after the administration of HS we observed an

Table 2. Half life time for efflux of HS in the outflowing perfusate from livers preloaded with HS

| Liver load* (μmoles) | $t_{\frac{1}{2}}$ of efflux into the perfusate (min ± S.E.M.) | |
|---|---|---|
| 8.4 | 2.46 ± 0.07 | ($N = 5$) |
| 6.3 | 2.21 | ($N = 1$) |
| 4.2 | 2.58 | ($N = 1$) |
| 3.5 | 2.51 | ($N = 1$) |

* Liver load was calculated from the amounts added initially, present in plasma at the end of perfusion and excreted into bile.

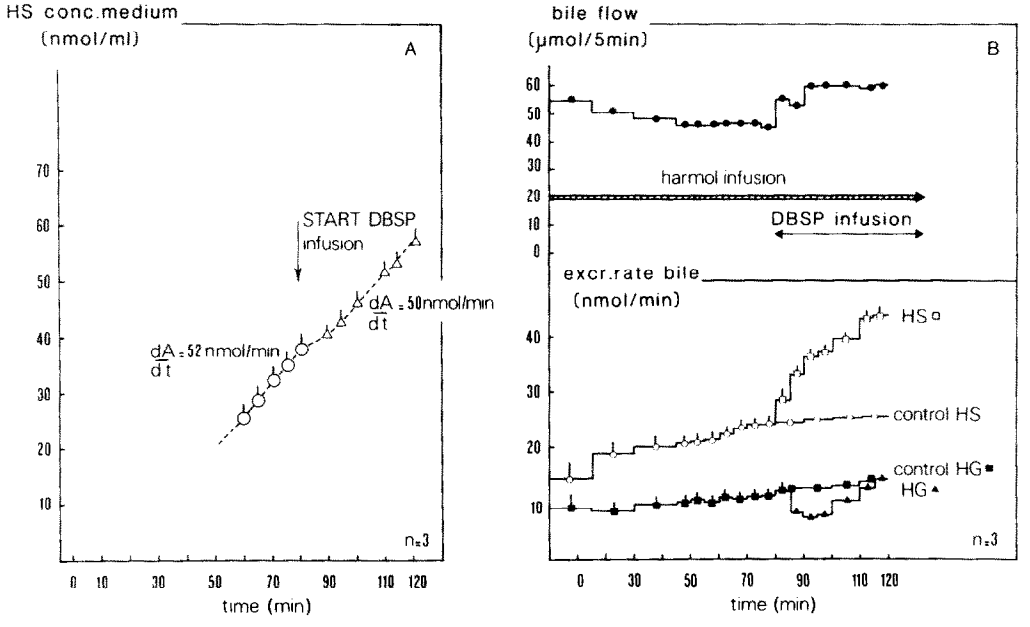


Fig. 2. Harmol conjugation in isolated perfused rat liver. (A) The concentration of harmol sulfate (HS) in the perfusion medium during infusion of harmol (125 nmoles/min), and (B) The biliary excretion rates of harmol glucuronide (HG) and harmol sulfate (HS). At $t = 80$ min an infusion of DBSP (1000 nmoles/min) preceded by a priming dose of 30 μmoles was started. The mean values ± S.E.M. are given (N = 3).

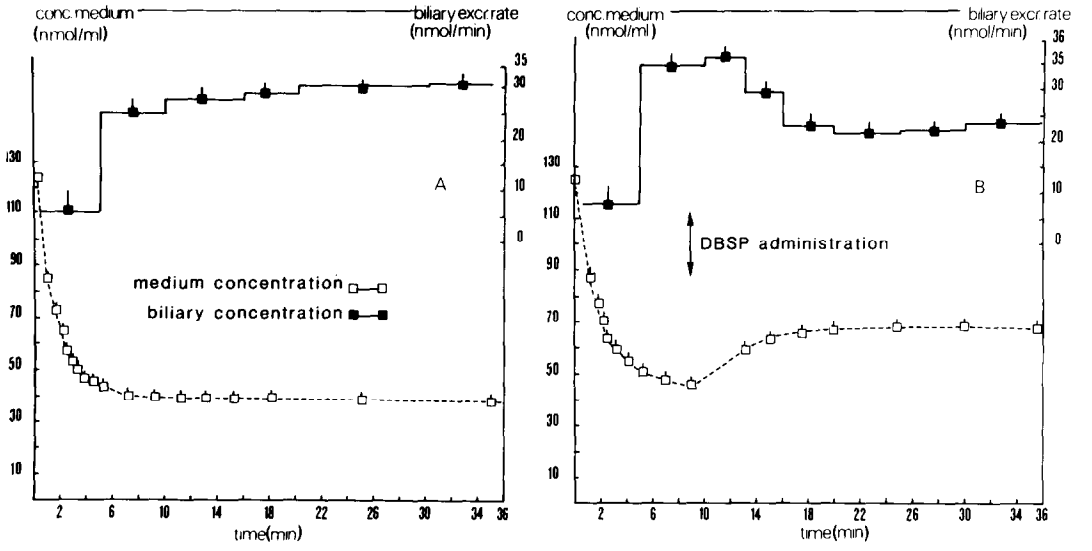


Fig. 3. (A) Disposition of harmol sulfate in the isolated perfused rat liver. Biliary excretion rate and concentration in the perfusion medium after the administration of 12.4 μmoles of HS in the isolated perfused liver. The mean values ± S.E.M. are given (N = 6). (B) Influence of DBSP on hepatic disposition of harmol sulfate during recirculating liver perfusions. Biliary excretion rate and medium concentration after the administration of 12.4 μmoles of HS in the isolated perfused liver. After 9 min a dose of 54 μmoles of DBSP was administered to the reservoir of the perfusion apparatus. The mean values ± S.E.M. are given (N = 6).

increase in the medium concentration (from 45 to 66 μM) (Figs. 3A and 3B respectively) and in the last 30 min of the experiments a decrease (from 37 to 22 nmoles/min) in the biliary excretion rate of HS (Figs. 3A and 3B respectively). Immediately after DBSP administration a short stimulating effect is seen on the biliary clearance of HS. The calculated HS liver content decreased: 10 min after DBSP administration the total liver load was calculated to be 5.5 μmoles as compared to 8.4 μmoles before DBSP administration. Steady state biliary clearance ($dA/dt$ over $C_p$) and hepatic distribution volume ($A_H^{ss}/C_p$) decreased 55 and 56% respectively.

*Effect of DBSP on the albumin binding of harmol sulfate*

Albumin binding of HS in Krebs-bicarbonate buffer with 0.1% albumin was measured in the concentration range 20–1000 nmoles/ml HS and the percentage unbound HS appeared to range from 80 to

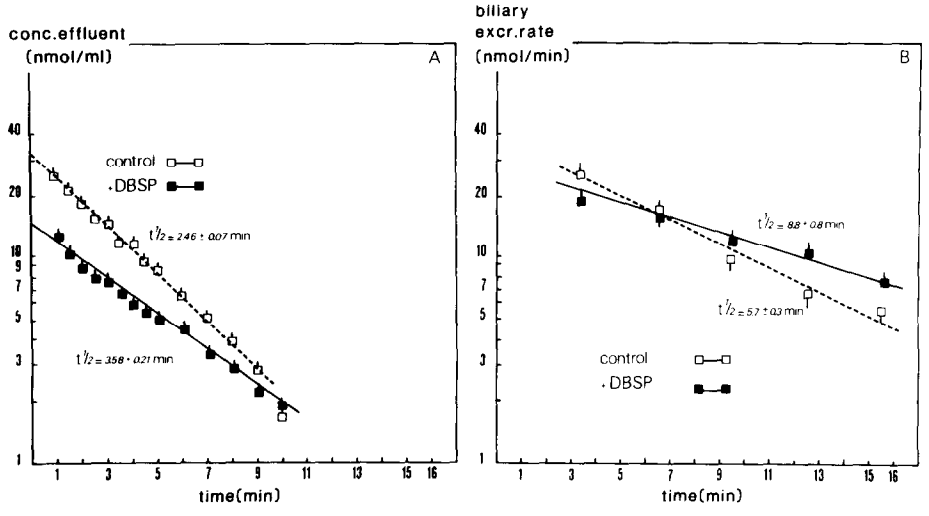


Fig. 4. (A) Wash-out studies with harmol sulfate in single pass liver perfusion. Concentration of HS in the outflowing perfusate of a liver preloaded with HS and of a liver preloaded with HS and DBSP. The mean values ± S.E.M are given (N = 6). (B) Biliary excretion rate of HS during a single pass perfusion of a liver preloaded with HS and of a liver preloaded with HS and DBSP. The mean values ± S.E.M. are given (N = 6).

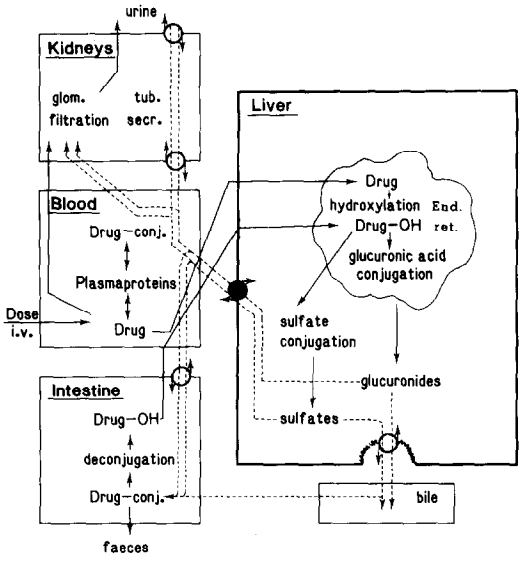


Fig. 5. Schematic representation of drug conjugation and transport of drugs (full lines) and drug conjugates (dashed lines) in the body. Liver, kidneys and intestine are pictured as single compartments. Exchange of drug conjugates between these organs and blood is supposed to be carrier-mediated as indicated by the circles. Biliary, intestinal and urinary secretion of glucuronides and sulfates are pictured to be mediated by single carrier-systems. Possible heterogeneity in these carrier processes or in zonal localization of the conjugation processes in the liver are not represented in the scheme.

95%. Upon addition of 200 and 550 nmoles/ml of DBSP the unbound percentage rose from 83 to 93% and from 85 to 91% respectively.

## DISCUSSION

The finding that administration of the organic anion DBSP during infusion of harmol *in vivo* increased the biliary excretion of another organic anion, harmol sulfate, and at the same time decreased its plasma concentration and urinary excretion rate was a puzzling observation and in fact the motive for the present study. In principle a number of interaction sites can be anticipated:

*A possible influence on the conjugation processes in the liver*

Inhibition by DBSP of one of the conjugation processes should be compensated by an increase of the other conjugation process [10] and an increased total excretion of the latter conjugate. The excretion rate of both harmol glucuronide and harmol sulfate is slightly reduced during DBSP infusion (Table 1) and although a slight inhibition by DBSP of the conjugation of harmol could not be excluded, this cannot explain the observed results.

*A possible influence of DBSP on the intracellular binding of the conjugate*

An influence on binding within the cells and thus the hepatic distribution volume would only result in temporary changes in HS excretion, since steady state clearance and excretion rate in steady state conditions are independent of the distribution volume. Also both transport into bile and into plasma should increase if only displacement from binding sites would occur.

*A possible influence of DBSP on protein binding of HS*

Although the plasma protein binding *in vivo* was not directly measured, an increase in the unbound fraction ($f_u$) of HS by DBSP should increase both the biliary and the renal clearance. In fact an unchanged renal clearance was found (Table 1). Its value strongly suggests the occurrence of tubular secretion for HS (glomerular filtration in the absence of protein binding in a rat of 300 g is about 1.0–2.0 ml/min [15]). In the single-pass perfusion experiments, where medium with 0.1% albumin was used, protein binding of HS was low (5–20%) and was only very slightly changed by the presence of DBSP. Thus competition of DBSP for protein binding of HS cannot explain the observed inhibition of HS efflux from the liver.

*Interference with membrane transport at the bile canaliculus*

Since biliary output of HS was clearly increased by DBSP, competitive inhibition in the case of the sulfate conjugate, at first sight, is not a likely mechanism. However such an effect cannot be fully excluded as discussed later on. Such an inhibitory action could play a role in the biliary excretion of HG (see Fig. 1), confirming the study of Uesugi and Ikeda [16], who showed a marked decrease in the biliary excretion rate of thiamphenicol glucuronide by bromosulfophthalein (BSP), a compound closely related to DBSP. Stimulatory influences of DBSP on HS excretion at the canalicular level could be due to the increased bile flow induced by DBSP [5]. However Jorritsma *et al.* [11] found that choleresis had no effect on the biliary excretion of HS in rats with intact kidneys.

*Interference with sinusoidal transport from liver into plasma*

Inhibition of transport into plasma of HS formed in the liver can explain a number of observations:

(a) The increased biliary output during DBSP infusion. Biliary excretion rate will be determined by the intracellular concentration of HS; a decrease in efflux into the plasma compartment will increase the intracellular concentration and thus the driving force for excretion into bile.

(b) The decreased plasma concentration of HS *in vivo* during DBSP infusion, which is also reflected in the lower urinary excretion rate (unchanged clearance).

(c) The inhibition of efflux of HS by DBSP from single pass perfused rat livers preloaded with HS (Fig. 4), which provided the definitive clue for our conclusion that the primary interaction between DBSP and HS lies in interference with the sinusoidal transport from liver into plasma.

The increase in the medium concentration of HS upon addition of DBSP in the recirculating perfused rat liver (Fig. 3B) can be explained as being the net

result of inhibition by DBSP of both HS uptake and efflux across the sinusoidal membrane. This might indicate that exchange between plasma and liver is a bidirectional and even a symmetrical carrier-mediated process. The influence of DBSP on the $t_{\frac{1}{2}}$ for excretion of HS into bile in the wash-out experiments (Fig. 4B) indicates that DBSP, apart from its influence on the HS transport across the sinusoidal membrane also can inhibit the biliary transport of HS. The outcome of the DBSP interaction may merely depend on its free concentration outside and inside the cell but also on the site of exposition of HS: formed in the hepatocyte, or administered exogenously. The influence of DBSP on the sinusoidal efflux of HS, leading to an increased intracellular concentration and consequently a higher excretion into bile, in fact could be partly masked by a simultaneous inhibitory influence at the canalicular transport. However, in the experiments where HS is formed from harmol within the cells the stimulatory effect clearly prevailed. If harmol sulfate itself was administered, the effect of DBSP on hepatic uptake was clearly expressed (Fig. 3B). The possible competition between HS and DBSP and between HG and DBSP for excretion into bile could be imagined if a common excretion pathway into bile for HG and HS would exist as Mulder and Pilon suggested [10]. On the other hand, Peterson and Fujimoto [17] and Smith and Peterson [18] assumed different biliary excretion pathways for the sulfate and glucuronide conjugates of morphine. Such a multiple excretory mechanism could explain the much greater influence of DBSP on HG excretion compared with HS excretion. At first sight the observations that during harmol infusion DBSP lowered the plasma concentration of HS *in vivo* (Table 1), but did not affect this parameter in the liver perfusion set-up where harmol was infused (Fig. 2), seem to be conflicting. However, it should be realized that HS *in vivo* is efficiently removed from the circulation by renal clearance whereas the compound will accumulate in the perfusion medium *in vitro*. Therefore re-uptake of HS *in vitro* is quantitatively more important. In view of the above mentioned interaction at the uptake level, inhibition of sinusoidal liver to plasma transport could well be masked by inhibition of the uptake of HS in the hepatocyte and therefore results in unchanged medium levels. Since albumin concentration *in vitro* is four times less than *in vivo*, unbound concentrations of DBSP outside the liver are much greater in the *in vitro* studies and this can be expected to strengthen the inhibitory influence on hepatic uptake of HS.

In conclusion, the present results demonstrate an interaction at the level of sinusoidal membrane transport from the hepatocyte into plasma for organic anions. Competition between DBSP and HS for carrier-mediated transport could play a role. Further studies among others with different doses of DBSP and HS to show mutual competition and saturation kinetics are in progress to substantiate this hypothesis. Also possible zonal differences in the hepatic efflux process are studied. Our results indicate that major changes in intracellular concentrations of drug conjugates may occur if their secretion from the cells into blood is affected. Such an interaction may also lead to marked changes in the excretion of drug conjugates in the urine, a body fluid which is often the object for bioanalysis of drug conjugates in man. A decrease in excretion of sulfate conjugates in the urine can, apart from inhibition of its formation, be due to a more efficient hepatobiliary clearance if a competing substrate would inhibit the excretion of drug conjugate from the liver into the blood stream (see Fig. 5).

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