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Phenol Sulphotransferase and Uridine Diphosphate Glucuronyltransferase from Rat Liver *in vivo* and *in vitro*

2,6-DICHLORO-4-NITROPHENOL AS SELECTIVE INHIBITOR OF SULPHATION

By GERARD J. MULDER and EGBERT SCHOLTENS
Department of Pharmacology, State University of Groningen, Groningen, The Netherlands

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Microsomal UDP-glucuronyltransferase and cytosolic sulphotransferase share many substrates, such as phenols and hydroxamic acids. In a search for a selective inhibitor of sulphation, several phenolic compounds were tested. 2,6-Dichloro-4-nitrophenol is introduced as a selective inhibitor of sulphation *in vivo*, having no effect on UDP-glucuronyltransferase activity. As substrate for both conjugating enzymes the phenolic drug harmol (7-hydroxy-1-methyl-9*H*-pyrido[3,4-*b*]indole) was used. In the rat *in vivo* 2,6-dichloro-4-nitrophenol caused almost complete inhibition of harmol sulphation after a single intraperitoneal injection (26 μmol/kg) for 48h; the percentage of harmol sulphated decreased from 75% in controls to 5% in the treated rats. The percentage of harmol glucuronidated increased from 25 to 95%. Pentachlorophenol was equally effective but also highly toxic. Salicylamide had only a very-short-lasting inhibitory effect on sulphation. *In vitro*, 2,6-dichloro-4-nitrophenol inhibited sulphation of harmol by a rat liver postmitochondrial supernatant completely at 1 μM, whereas even at 100 μM it had no effect on glucuronidation of harmol. It is concluded that 2,6-dichloro-4-nitrophenol is a selective inhibitor of sulphation and, further, that its long duration of action makes it suitable for studies on the regulatory role of sulphation in some biological processes.

Glucuronidation and sulphation are conjugation reactions by which a wide variety of substances are converted into glucuronic acid and sulphate conjugates, by UDP-glucuronyltransferase (UDP-glucuronosyltransferase, EC 2.4.1.17) and sulphotransferases (EC 2.8.2.) respectively. Since many drugs and toxic compounds contain acceptor groups for these conjugation reactions, and because usually the biological effects of the substrates disappear after the conjugation, they are considered to be 'detoxifying reactions'. However, it has become increasingly clear that in some cases the conjugation actually increases toxicity of the substrate, especially sulphation of hydroxamic acids such as *N*-hydroxy-2-acetylaminofluorene (DeBaun *et al.*, 1968, 1970) and *N*-hydroxyphenacetin (Mulder *et al.*, 1977). The former reaction has been implicated in the generation of the ultimate carcinogenic metabolite of 2-acetylaminofluorene, and the latter has been shown to lead to a reactive arylating species. In both cases the *N*-*O*-glucuronide conjugate is much less toxic than the *N*-*O*-sulphate conjugate, most likely because of a higher chemical stability, i.e. less reactivity. In these cases a glucuronide can be considered as a detoxification product, as compared with the sulphate conjugate of the same substrate.

The availability of a selective inhibitor of sulphation that has no effect on glucuronidation would be extremely helpful in clarifying the role of sulphation in the generation of highly reactive, eventually carcinogenic, sulphate conjugates *in vivo*. Further, it might be used to study the role of sulphation in the metabolism of steroid hormones and some neurotransmitters in brain.

We have tried to find a compound that inhibits selectively sulphation of a substrate and thereby increases the percentage of the dose that becomes glucuronidated after administration. Since sulphate conjugates of hydroxamic acids are extremely unstable and therefore cannot be measured, we have only used a phenolic substrate, harmol (7-hydroxy-1-methyl-9*H*-pyrido[3,4-*b*]indole), as the substrate of both phenol sulphotransferase (EC 2.8.2.1) and UDP-glucuronyltransferase, to characterize the effect of potential inhibitors of sulphation. Previously we have studied conjugation of harmol and excretion of its conjugates in bile and urine in the rat *in vivo* (Mulder & Hagedoorn, 1974; Mulder *et al.*, 1975; Mulder & Pilon, 1975). UDP-glucuronyltransferase and phenol sulphotransferase 'compete' for harmol, and inhibition of one conjugation will increase the amount conjugated by the other. Properties of these con-

jugating enzymes have also been studied *in vitro* (Mulder, 1975).

In the present study we show that 2,6-dichloro-4-nitrophenol is a very effective inhibitor of harmol sulphation that has no inhibitory effect on glucuronidation, and that is well tolerated by the rat *in vivo*.

Materials and Methods

Rats

Male Wistar rats (290–310g body wt.) were used in the studies *in vivo*. They had free access to food and water. In most experiments the protocol was as follows. The experiment was started with an intraperitoneal injection of the drug to be investigated as inhibitor of sulphation, dissolved in propane-1,2-diol ('propylene glycol'); the controls received the solvent only. Then 5min later pentobarbital sodium (60mg/kg) was given intraperitoneally for anaesthesia, and a further 15min later the operation was performed, consisting of ligation of the kidneys, application of artificial respiration through a trachea cannula, and bile-duct cannulation; the operation was completed within 10min. The temperature of the rats was kept between 37.5 and 38.5°C with a heating lamp. Finally, 45min after the injection of the drug to be tested as inhibitor, harmol was injected intravenously in the vena femoralis in a dose of 20μmol/kg body wt. In some experiments the sulphation inhibitor 2,6-dichloro-4-nitrophenol [dissolved in alkaline 0.9% (w/v) NaCl, pH8.0] was given intravenously; it was given in the vena femoralis such that harmol and the inhibitor were injected into opposite venae. Bile was collected for various periods as indicated in the Tables or Figures. The volume of bile was determined by weighing. In previous experiments we used a different strain of rats; the rats used in the present study had a somewhat lower rate of harmol sulphate excretion in bile, but the percentage of harmol becoming sulphated was the same in both strains of rats (i.e. about 75% of the dose).

Chemicals

Salicylamide was obtained from Interpharm, Den Bosch (The Netherlands); the other phenols tested as inhibitors of sulphation were obtained from Aldrich Chemical Co., Beerse (Belgium). Harmol and the other chemicals were from sources as reported before (Mulder, 1975; Mulder *et al.*, 1977).

Determination of harmol conjugates

A sample of bile (usually 10μl) was applied to a silica-gel t.l.c. plate and the conjugates were separated and determined as described previously (Mulder & Hagedoorn, 1974). None of the other compounds used in the experiments gave rise to fluorescent metabolites or interfered with the measurement. The conjugates were determined fluorimetrically.

Harmol conjugation in vitro

The assay of harmol conjugation *in vitro*, and the inhibition of glucuronidation and sulphation by the drugs tested, was as described previously, the postnuclear supernatant of rat liver being used as source of both phenol sulphotransferase and UDP-glucuronyltransferase (Mulder, 1975). UDP-glucuronyltransferase was activated by the addition of the detergent Triton X-100 to the postnuclear supernatant (Mulder, 1975). The inhibitory phenols were added in ethanol/water (1:1, v/v). Adenosine 3'-phosphate 5'-sulphatophosphate, the co-substrate of sulphation, was prepared in a preincubation from ATP and sulphate (Mulder, 1975); its concentration in the incubation was 75μM.

Sulphation assay with phenol and N-hydroxyphenacetin as substrate

This assay was performed by the method of Gregory & Lipmann (1957) as modified by Mulder *et al.* (1977). In this assay *p*-nitrophenyl sulphate is used as sulphate donor to the acceptor substrate; this transfer is mediated by adenosine 3',5'-bisphosphate, presumably by its conversion into adenosine 3'-phosphate 5'-sulphatophosphate by phenol sulphotransferase itself. The assay is performed at pH 8.0 and the increase in A_{400} (due to the release of free *p*-nitrophenol) is used as a measure of sulphation rate of the acceptor substrate, i.e. phenol or *N*-hydroxyphenacetin. As sulphotransferase preparation a partly purified rat liver phenol sulphotransferase was used, purified by the method of McEvoy & Carroll (1971) as far as the $(NH_4)_2SO_4$ precipitation and subsequent dialysis.

Results and Discussion

Harmol conjugation in vivo and the biliary excretion of its conjugates

After intravenous injection in the rat, harmol was completely recovered in the form of its two conjugates, harmol sulphate and harmol glucuronide, in bile in about 8h (Table 1). In the rat, therefore, sulphation and glucuronidation are the only biotransformation reactions for this phenolic compound. Both *in vivo* and in the perfused rat liver it is completely cleared from the blood or the perfusion medium within 5min (Mulder & Hagedoorn, 1974; Mulder *et al.*, 1975). The glucuronide conjugate is excreted mainly in bile; the sulphate conjugate appears in both urine

Table 1. *Biliary excretion of harmol as harmol sulphate and harmol glucuronide in the rat*

Harmol (20 μmol/kg) was injected intravenously in rats with ligated kidneys. Bile was collected and the conjugates were determined in bile. The percentages of the dose excreted in the form of the conjugates is given as the mean ± S.E.M. ($n = 5$). Bile was collected in two periods, from 0 to 2 h and from 3 to 8 h after injection of harmol.

Period (h)	Percentage of dose excreted as Harmol glucuronide	Harmol sulphate
0–2	18.0 ± 0.7	22.5 ± 1.6
3–8	5.1 ± 0.3	52.6 ± 2.1
0–8 (total recovery)	23.1 ± 1.1	75.1 ± 2.0

and bile. When the kidneys are ligated, as in the present study, harmol sulphate excretion in bile increases and ultimately all harmol sulphate normally cleared by the kidneys is then found in bile (Mulder *et al.*, 1975). In the present study we used rats with ligated kidneys because we wanted to measure excretion rates in some experiments; whereas bile can easily be sampled in fractions taken every minute, this is very difficult with urine in the rat.

In the experiments *in vivo* we wanted to use excretion rates of harmol sulphate and harmol glucuronide in bile as an indication of amounts of harmol that become glucuronidated or sulphated. As shown in Fig. 1, the time courses of the excretion of harmol glucuronide and harmol sulphate in bile were very different. Harmol glucuronide was excreted very rapidly in the first 10–15 min of the experiment. Harmol sulphate, however, was excreted for several hours at a rate that declined only very slowly; its concentration in bile was initially much lower than that of harmol glucuronide, but later it became much higher. Table 1 shows that after the initial 2 h almost exclusively harmol sulphate was excreted, to a total of 75% of the dose administered, whereas about 25% was excreted as harmol glucuronide. Therefore an increase in harmol glucuronide excreted in the initial phase of the experiment implies that total harmol sulphate excretion is decreased by the same amount.

As shown below, the biliary excretion of harmol sulphate in the rat is rate-limiting in the process of harmol elimination as its sulphate conjugate. Therefore the harmol sulphate excretion rate is not a good estimate of the amount sulphated; a decrease in sulphation need not necessarily be followed by a decrease in biliary excretion rate of the sulphate conjugate during, e.g., the first hour, because the excretory process of harmol sulphate transport in bile

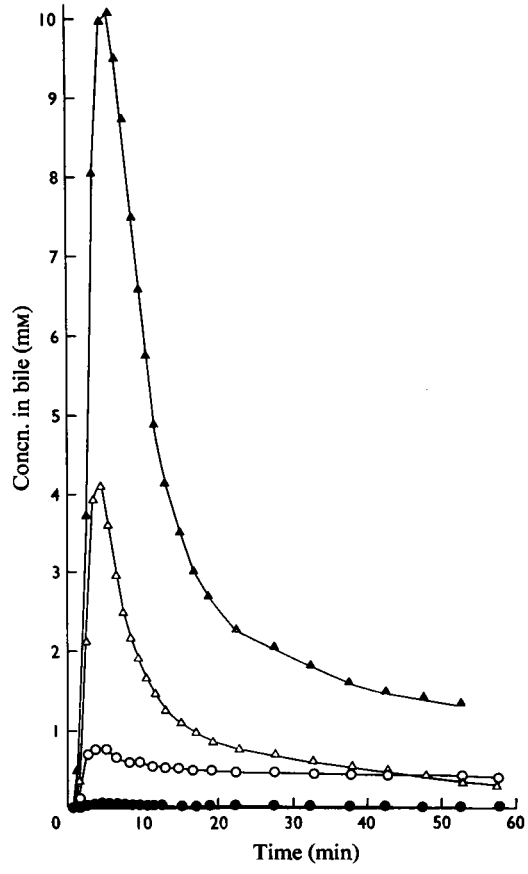

Fig. 1. *Time course of biliary excretion of harmol sulphate and harmol glucuronide in the rat with ligated kidneys; effect of 2,6-dichloro-4-nitrophenol*

Harmol (20 μmol/kg) was injected intravenously in a rat that had been pretreated with 2,6-dichloro-4-nitrophenol (●, ▲; 26 μmol/kg intraperitoneally, 45 min before the harmol injection) and in a control (○, △). The kidneys were ligated. Biliary excretion of harmol sulphate (○, ●) and harmol glucuronide (△, ▲) is shown as the concentration of the conjugates in bile. During the experiment bile production was constant.

may still be saturated, even at a lower supply of the conjugate. Harmol glucuronide, however, is excreted in bile as soon as it is synthesized. Therefore a relatively small decrease of sulphation may not give rise to a decreased excretion rate of harmol sulphate, but will be seen as an increase in biliary output (in the initial phase of the experiment) of harmol glucuronide; the latter is therefore a much more sensitive indicator of inhibition of sulphation of harmol. This will be further discussed below.

Table 2. *Effect of some phenols on glucuronidation and sulphation of harmol by the rat in vivo*

Harmol (20 μmol/kg) was administered intravenously. The rats were pretreated by an intraperitoneal injection of the compounds indicated, dissolved in propane-1,2-diol, 45 min before the harmol injection; salicylamide was also given only 5 min before harmol. The percentage of the dose excreted in bile in the form of the sulphate and glucuronide conjugates during 2 h after harmol injection is given. Means ± S.E.M. are given; *n* is the number of rats used. *, Significantly different from control [Wilcoxon's (1945) test] at $P<0.05$.

Pretreated with:	*n*	Dose (μmol/kg)	Percentage of dose excreted as: Harmol glucuronide	Harmol sulphate
—	8	—	17.1 ± 0.5	22.1 ± 1.8
Pentachlorophenol	5	8	29.9 ± 1.7*	17.8 ± 1.2*
Pentachlorophenol	5	39	52.8 ± 1.5*	5.3 ± 0.9*
2,6-Dimethyl-4-nitrophenol	4	26	20.4 ± 1.2*	22.2 ± 1.0
2,6-Dichloro-4-nitrophenol	4	26	61.9 ± 2.3*	2.2 ± 1.0*
3-Methyl-2-nitrophenol	2	26	16.8	20.0
Salicylamide	2	26	18.3	22.1
Salicylamide (5 min before harmol)	5	26	32.4 ± 4.3*	19.5 ± 1.0

Effect of some phenols on metabolism in vivo and biliary excretion of harmol

Kobayashi *et al.* (1976) have reported that pentachlorophenol is a potent inhibitor of sulphation of phenol in liver slices. We have tested the effect of this compound on harmol conjugation *in vivo*. Pentachlorophenol clearly decreased the biliary excretion of harmol sulphate (Table 2) and caused an increase in the excretion rate of harmol glucuronide. A time course of the pentachlorophenol effect on biliary excretion of the conjugates is presented in Fig. 2; pentachlorophenol was given intraperitoneally 45 min before the intravenous harmol injection. In controls, the brief, high excretion rate of harmol glucuronide reached a plateau in the second 30 min period; the excretion of harmol sulphate was rather constant during the whole 2 h period. After pentachlorophenol treatment, however, harmol sulphate had almost completely disappeared from bile and the amount of harmol glucuronide was greatly increased. The total recovery of harmol (as percentage of the dose recovered in the form of both harmol sulphate and harmol glucuronide) during the 2 h after its injection was increased significantly in the presence of pentachlorophenol, owing to an increased synthesis of the glucuronide conjugate of harmol, which is more rapidly excreted in bile than the sulphate conjugate. A lower dose of pentachlorophenol was much less effective (Table 2).

Although pentachlorophenol seemed an effective inhibitor of sulphation, it was not very satisfactory because rats given the higher dose of pentachlorophenol died after about 2 days, during which they were very immobile and dizzy. Therefore we looked for a related phenol that might be less toxic *in vivo*. 2,6-Dichloro-4-nitrophenol has a similar 'chemical environment' of its phenolic hydroxyl group as pentachlorophenol. This phenol was even more effective than pentachlorophenol in inhibition of sulphation, and much less toxic; even a dose of 100 μmol/kg intraperitoneally did not affect the behaviour of the rats and did not kill them. For our studies we have chosen a dose (26 μmol/kg) that was of the same order as that used for pentachlorophenol. Fig. 1 shows the short-term effects of 2,6-dichloro-4-nitrophenol on biliary excretion of harmol sulphate and harmol glucuronide. Harmol sulphate again nearly completely disappeared from bile, whereas harmol glucuronide increased dramatically. Again there was a great increase in total recovery of harmol during the 2 h of the experiment (Table 2), in parallel with the increase in glucuronidation; recovery increased from 39% in controls to 64% in the 2,6-dichloro-4-nitrophenol-treated animals. Fig. 2 shows that pentachlorophenol and 2,6-dichloro-4-nitrophenol had similar effects on harmol conjugation and biliary excretion. In both cases the pretreatment took place 45 min before the injection of harmol.

At present the metabolism of 2,6-dichloro-4-nitrophenol *in vivo* is not known. *In vitro*, it is a very poor substrate of UDP-glucuronyltransferase (Mulder & Van Doorn, 1975). 2,6-Dimethyl-4-nitrophenol, which is a relatively good substrate of UDP-glucuronyltransferase, had no effect on biliary excretion of harmol sulphate and caused only a small increase of harmol glucuronide excretion (Fig. 2). Similarly 3-methyl-2-nitrophenol, which is an excellent substrate of UDP-glucuronyltransferase (Mulder & Van Doorn, 1975), had no effect. Salicylamide has been claimed to be an inhibitor of glucuronidation (Levy, 1971), but it is also a good substrate of phenol sulphotransferase (Davis, 1975). When given 45 min before harmol it had no effect; when, however, it was

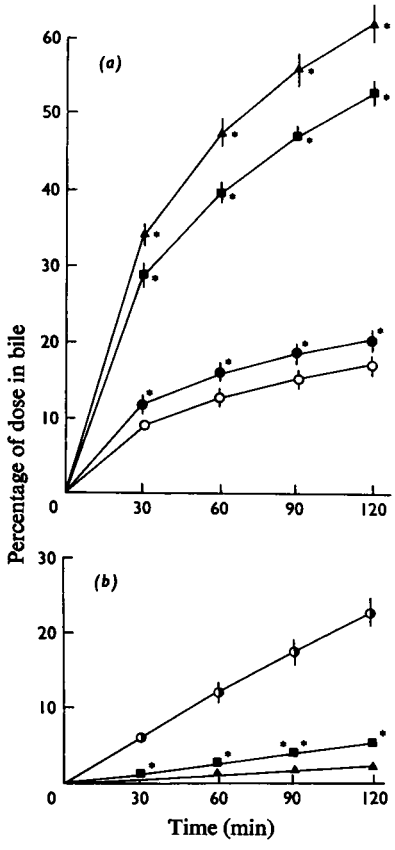

Fig. 2. *Time course of the effect of some phenols on biliary excretion of harmol conjugates in the rat with ligated kidneys*

The rats with ligated kidneys received harmol intravenously (20μmol/kg). The percentage of the dose excreted as either harmol glucuronide (*a*) or harmol sulphate (*b*) is shown cumulatively for the 2h of the experiment. 2,6-Dichloro-4-nitrophenol (▲; 26μmol/kg; $n=5$), 2,6-dimethyl-4-nitrophenol (●; 26μmol/kg; $n=4$) and pentachlorophenol (■; 39μmol/kg; $n=5$) were injected intraperitoneally 45min before the harmol administration; controls (○; $n=8$) received only the solvent. For the biliary excretion of harmol sulphate in the control and 2,6-dimethyl-4-nitrophenol-treated rats the same curve was found (◑). Results are means, the vertical bars indicating the S.E.M. *Statistically significant difference from control ($P<0.05$).

given only 5min before harmol, it clearly increased harmol glucuronide output, but had no effect on the excretion rate of harmol sulphate (Table 2). This short action of salicylamide may be due to its rapid conjugation and excretion. 2,6-Dichlorophenol and

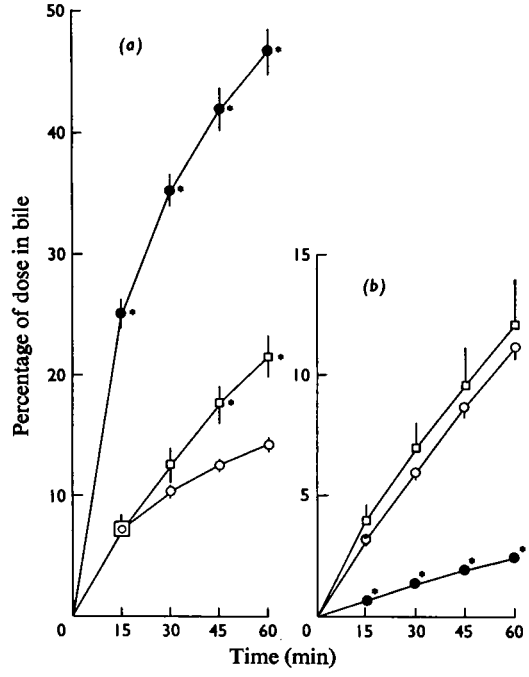

Fig. 3. *Effect of 2,6-dichloro-4-nitrophenol on harmol conjugation and biliary excretion when injected intravenously before or after the harmol injection*

2,6-Dichloro-4-nitrophenol was injected intravenously (5μmol/kg) either 2min before harmol (●) or 15min after harmol (□). The controls received alkaline 0.9% (w/v) NaCl, pH8.0 (○); 2,6-dichloro-4-nitrophenol was dissolved in alkaline 0.9% (w/v) NaCl, pH8.0. There were five animals in each group. (*a*) Harmol glucuronide; (*b*) harmol sulphate. For further details see the legend to Fig. 2.

4-nitrophenol similarly had no effect when given 45min before harmol (results not shown). Several reasons may explain the lack of effect of these compounds, one of which is illustrated by the example of salicylamide. Since we are interested in finding a long-acting inhibitor of sulphation we have further characterized the effect of only 2,6-dichloro-4-nitrophenol on harmol conjugation *in vivo* and *in vitro*.

Characteristics of the effect of 2,6-dichloro-4-nitrophenol in vivo

To determine the onset of action of 2,6-dichloro-4-nitrophenol we injected the inhibitor intravenously 2min before harmol. The result was a strong inhibition of excretion of harmol sulphate and a great increase in the excretion of harmol glucuronide (Fig. 3). Therefore 2,6-dichloro-4-nitrophenol seemed to work immediately. When it was injected intra-

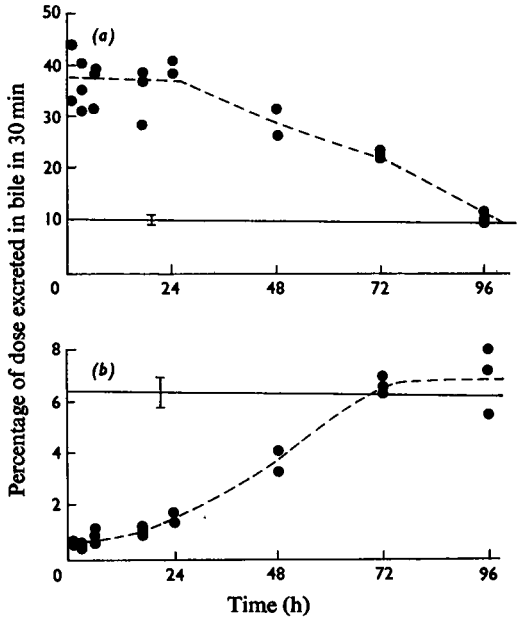

Fig. 4. *Duration of the effect of 2,6-dichloro-4-nitrophenol on conjugation of harmol by the rat in vivo*

2,6-Dichloro-4-nitrophenol was injected intraperitoneally in the rat (26 μmol/kg; dissolved in propane-1,2-diol). At different times after this pretreatment, harmol (20 μmol/kg; intravenously) was injected after the kidneys had been ligated and a cannula inserted in the bile duct by using the normal protocol (see the Materials and Methods section). The percentages of the dose excreted in bile during the first 30 min after the harmol injection have been plotted against the time elapsed between pretreatment and the harmol injection. The control values (±S.E.M.) are indicated in the Figure by the continuous line. Each point is the result for one rat. (*a*) Harmol glucuronide; (*b*) harmol sulphate.

venously 15 min after harmol, no subsequent decrease of the biliary excretion of harmol sulphate was seen during the next 45 min and only a small (but statistically significant) increase in the amount of harmol glucuronide in bile. This outcome proves first that 2,6-dichloro-4-nitrophenol does not affect biliary excretion of harmol sulphate. Secondly it shows that, in the 15 min after injection of harmol, already enough harmol sulphate had been synthesized to saturate the biliary excretion process for at least 1 h. It seems reasonable to assume that the extra amount of harmol excreted as harmol glucuronide in the 2,6-dichloro-4-nitrophenol-treated rats (2 min before harmol), that is about 18% of the dose during the first 15 min (Fig. 3), was converted into harmol sulphate in the absence of the inhibitor (i.e. in the controls) during these 15 min. Only 3% of this 18% was excreted in bile in the controls during these 15 min as harmol sulphate (Fig. 3), again indicating the rate-limiting character of the biliary excretion of the sulphate conjugate. Because harmol glucuronide is excreted rapidly in bile, an increase in glucuronidation results in a more rapid biliary recovery of harmol.

In some animals we have varied the period between administration of 2,6-dichloro-4-nitrophenol and harmol (Fig. 4). The inhibitor was given intraperitoneally in a single dose up until 4 days before the intravenous dose of harmol. After 24 h we still found the same effect of 2,6-dichloro-4-nitrophenol as after 45 min. Since the kidneys were ligated only 25 min before the harmol dose was given, there was ample opportunity for urinary excretion of the inhibitor during those 24 h. Since 2,6-dichloro-4-nitrophenol is highly lipid-soluble (Mulder & Van Doorn, 1975) its urinary excretion may be very slow, and the reason for this long-lasting effect may be a very slow elimination of the compound; even after 48 h it was almost completely inhibitory towards sulphation. Another possibility is that it inhibits phenol sulphotransferase irreversibly.

Effect of 2,6-dichloro-4-nitrophenol and pentachlorophenol on harmol conjugation in vitro

Sulphation and glucuronidation of harmol can be measured simultaneously with the postnuclear supernatant as enzyme preparation, containing both microsomal UDP-glucuronyltransferase and sulphotransferase from cytosol. This UDP-glucuronyltransferase preparation was activated with the detergent Triton X-100, which does not affect the sulphation rate of harmol (Mulder, 1975). Pentachlorophenol and 2,6-dichloro-4-nitrophenol inhibited sulphation of harmol at a much lower concentration than that for glucuronidation (Table 3). A concentration as low as 0.1 μM of the inhibitors was sufficient to inhibit harmol sulphation by about 50%, and 1 μM inhibited completely. On the other hand, even 100 μM-2,6-dichloro-4-nitrophenol caused almost no inhibition of glucuronidation; pentachlorophenol inhibited glucuronidation of harmol by 80% at that concentration.

We have also used phenol and *N*-hydroxyphenacetin as substrates for sulphotransferase. Unlike the experiments with harmol, we have used a partly purified sulphotransferase preparation for these assays. Moreover, there are several differences between incubation conditions of the two assay systems (see the Materials and Methods section). Pentachlorophenol had the same effect on sulphation of phenol and *N*-hydroxyphenacetin as on harmol

Table 3. *Effect of pentachlorophenol and 2,6-dichloro-4-nitrophenol on UDP-glucuronyltransferase and sulphotransferase activity*

Glucuronidation and sulphation of harmol were determined simultaneously with UDP-glucuronate and adenosine 3'-phosphate 5'-sulphatophosphate as co-substrates of glucuronidation and sulphation respectively, by using a postnuclear rat liver supernatant as enzyme preparation; the incubation was at pH7.3 and 37°C. Sulphation of phenol and *N*-hydroxyphenacetin was measured at pH8.0 and 30°C with *p*-nitrophenyl sulphate + adenosine 3',5'-bisphosphate as adenosine 3'-phosphate 5'-sulphatophosphate-generating system, by using a partly purified sulphotransferase preparation. Substrate concentrations were 150 μM for harmol, 1.0 mM for phenol and 0.5 mM for *N*-hydroxyphenacetin. The inhibitors were added in aqueous 50% (v/v) ethanol such that the final concentration of ethanol was 5% (v/v) in the incubation. Abbreviation: n.d., not determined.

		Percentage inhibition of			
		Harmol		Sulphation of	
Inhibitor	Concn. (μM)	Glucuronidation	Sulphation	Phenol	*N*-Hydroxyphenacetin
Pentachlorophenol	0.1	0	45	20	29
	1.0	0	95	73	80
	10	10	100	100	100
	100	80	100	n.d.	n.d.
2,6-Dichloro-4-nitrophenol	0.1	0	70	4	2
	1.0	0	100	20	9
	10	5	100	34	22
	100	10	100	n.d.	n.d.

sulphation (Table 3). However, 2,6-dichloro-4-nitrophenol was much less effective. So far we have no explanation for this lesser effect of 2,6-dichloro-4-nitrophenol.

Since some compounds activate UDP-glucuronyltransferase in native microsomal preparations (Dutton, 1975; Mulder, 1974) and since this might also occur *in vivo*, we had to exclude the possibility that our results could be explained by an activation of glucuronidation *in vivo* rather than an inhibition of sulphation. Therefore we have tested whether pentachlorophenol and 2,6-dichloro-4-nitrophenol could activate harmol glucuronidation in native microsomal preparations, by using a postnuclear supernatant that had not been treated with Triton X-100. From 0.1 to 100 μM neither compound had an activating effect on harmol glucuronidation. Inhibition of sulphation was the same in the absence of detergent as in its presence.

These results show that the effect of 2,6-dichloro-4-nitrophenol *in vivo* on harmol conjugation is most easily explained by an inhibition of sulphation, for which a very low concentration of the inhibitor is required. The long duration of action may be due to a very slow elimination rate for 2,6-dichloro-4-nitrophenol; however, an irreversible inhibition of sulphotransferase cannot be excluded. The specificity of the effect of 2,6-dichloro-4-nitrophenol towards the various sulphotransferases (Roy, 1971) needs to be further studied; disturbances of steroid metabolism may be expected if it also inhibits the steroid sulphation. In that case it can be used to study the role of sulphation in the regulation of steroid metabolism.

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