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SELECTIVE INHIBITION OF SULFATE CONJUGATION IN THE RAT

PHARMACOKINETICS AND CHARACTERIZATION OF THE INHIBITORY EFFECT OF 2,6-DICHLORO-4-NITROPHENOL

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Abstract—The pharmacokinetics of 2,6-dichloro-4-nitrophenol (DCNP) have been studied in the rat. Upon i.v. injection the plasma decay curve of DCNP showed a rapid distribution phase. After 30 min the plasma concentration reached a value that was constant for at least 90 min, indicating very slow elimination of DCNP. The volume of distribution was 88 ml/kg and a high degree of binding (over 99%) of DCNP *in vitro* to bovine serum albumin was found. The concentration of DCNP in the liver was between 30 and 50% of the plasma values. While *in vivo* the effect of DCNP persisted for a long time, its action was readily reversible in the single-pass perfused rat liver. *In vivo*, the effect of the dose of DCNP on the inhibition of sulfation of the phenolic compound harmol was investigated. Upon the i.v. injection of 26 μmole DCNP/kg an instantaneous and complete inhibition of sulfation of harmol was found. Using this property of DCNP, the rate of sulfation of harmol *in vivo* was evaluated in relation to the dose and the time after injection of the substrate. Saturation of sulfation apparently occurred because the consumption of inorganic sulfate was extremely small.

Sulfation and glucuronidation are the major pathways of metabolism for many phenolic compounds in vertebrates [1, 2]. These two conjugation reactions compete with each other for the same substrate, and a decrease in sulfation of a phenol is usually compensated by an increase of its glucuronidation. When the dose of a phenol in the rat is increased, usually sulfation reaches saturation first, which may result in a compensatory, more than proportional increase in glucuronidation as the dose increases further [2–4].

Sulfate and glucuronide conjugates of the same compound may have very different toxicological and physiological actions. For instance, sulfate conjugates of many hydroxamic acids are chemically very reactive and lead to covalent binding of the drug to nucleophilic groups in proteins and nucleic acids; the correspondant glucuronides, however, are more stable, and, therefore, much less toxic [5]. Sulfate conjugates of some steroids seem to be storage forms of these hormones while their glucuronide counterparts are usually excretion products [2]. Yet, the estrogen metabolite estradiol-17β-D-glucuronide [6] and the sulfate conjugate of glycolithocholic acid [7] have a toxic, namely cholestatic action.

The study of the role of the various conjugations *in vivo* will gain much from the availability of selective inhibitors of these conjugation processes. Recently, two very potent inhibitors of sulfation have been introduced: pentachlorophenol (PCP) and 2,6-dichloro-4-nitrophenol (DCNP). Both these compounds inhibit sulfation of various phenols and hydroxamic acids *in vitro* and *in vivo* [8, 9]. Their mechanism of action is competitive inhibition of phenolsulfotransferase [10, 11]. Their inhibitory potency depends on the substrate used: DCNP inhibited sulfation of harmol more strongly than that of phenol [8] or *N*-hydroxy-acetylaminofluorene [9], while PCP was more inhibitory towards the latter two substrates [8, 9]. DCNP seems to affect sulfate conjugation rather selectively, since other conjugation reactions and biliary excretion mechanisms were unaffected [12].

These inhibitors of sulfation are increasingly being used, e.g. in the study of the role of sulfation in isolated hepatocytes [13] to investigate the kinetic mechanism of sulfotransferase catalysis and specificity [13–15] and to study the role of sulfation in carcinogenesis of aromatic amines [9]. Also, it has been used in the study of the kinetics of sulfation and glucuronidation in the perfused rat liver [16]. Therefore, it was of interest to obtain more detailed information on the pharmacokinetics of DCNP. We have further characterized the inhibitory action of DCNP *in vivo* and in the perfused rat liver, and related the observed effects to the pharmacokinetic profile of DCNP. Finally, some applications of DCNP in the study of the rate of sulfation *in vivo* are indicated.

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MATERIALS AND METHODS

Materials. Harmol hydrochloride was obtained from Sigma Chemical Co. (St. Louis, MO). General tritium-labeled harmol was prepared by tritium gas-exposure labeling by New England Nuclear (Boston, MA) and was further purified as described below. 2,6-Dichloro-4-nitrophenol was obtained from Aldrich Europe (Beerse, Belgium). Dextran T-40 was from Pharmacia Fine Chemicals (Uppsala, Sweden). Demineralized bovine serum albumin (Code 452) was from Poviet (Boxtel, The Netherlands).

In vivo *experiments.* Male Wistar rats (290–310 g body wt), that had free access to food and water, were anesthesized with sodium pentobarbital (60 mg/kg i.p.). The trachea was catheterized for artificial respiration. The bile duct and urine bladder were catheterized for continuous collection of bile and urine. Through a catheter in the external jugular vein an infusion was given of D-mannitol (75 mg/ml in 0.9% w/v aqueous NaCl) to stimulate urine production; the rate of infusion was 1.9 ml/hr when 26 μmole harmol/kg was given and 9.5 ml/hr at higher doses of harmol to prevent precipitation of harmol sulfate in kidney and bladder [17]. It was found that the infusion rate of mannitol did not affect the conjugation pattern (unpublished observation). Harmol and DCNP were given as single doses in the femoral vein. Harmol–HCl was dissolved in 0.9% (w/v) aqueous NaCl. DCNP was dissolved in 0.9% (w/v) aqueous NaCl by adding 50 μl 4 N NaOH to 950 μl of the DCNP suspension in saline. The solution obtained was diluted once with 10% (w/v) albumin (in saline) to obtain the solution to be injected, which was 5% (w/v) in albumin; the presence of albumin was required to reduce the acute toxicity of DCNP. The volume injected was 3.3 ml/kg. Bile and urine were collected during 3 hr after harmol administration. In some experiments blood samples were taken from the carotid artery in heparinized blood collection tubes (Caraway capillary tubes), obtained from Sherwood Medical Industries (St. Louis, MO); in these rats, the D-mannitol infusion fluid was made 1% (w/v) in albumin to compensate for the loss of protein.

Liver perfusion. For the single-pass perfused rat liver, male Wistar rats of 290–310 g body wt, were used that had free access to food and water. Prior to surgery the rats were anesthesized with sodium pentobarbital (60 mg/kg i.p.). Liver weights at the end of the experiments were 8.9–10.8 g.

Surgery, perfusion method and perfusion equipment were the same as described by Pang *et al.* [18, 19]. The perfusion medium was a Krebs-bicarbonate buffer containing 1% (w/v) bovine serum albumin, 3% (w/v) Dextran T-40, 15% (v/v) washed sheep erythrocytes and 0.3% glucose [19].

Several conical flasks (1000 ml) with an outlet at the bottom served as reservoirs for the perfusion media. A constant concentration of [^{3}H]harmol (approximately 10^6 dpm/ml) was added to all reservoirs. DCNP was present in one reservoir at a concentration of 20 μM.

Purification of [^{3}H]*harmol.* This was accomplished by high performance liquid chromatography using a silicagel Lichrosorb Si 60 column (1 × 50 cm) (Merck, Darmstadt, West Germany) which was eluted with dichloromethane/methanol 80 : 20 (v/v) at a rate of 3 ml/min. Radiochemical purity was checked by thin layer chromatography using HPTLC-plates 60 F_{254} (Merck), which were developed in (a) dichloromethane/methanol 80 : 20 (v/v), (b) n-butanol/acetic acid/water 4 : 1 : 1 (v/v), and (c) chloroform/methanol/2-propanol/ammonia (25%) 90 : 10 : 95 : 5 (v/v). The radiochemical purity of [^{3}H]harmol obtained was more than 96%.

Drug and conjugate assays. Conjugates of [^{3}H]harmol were determined by radioactivity after thin layer chromatography to separate the glucuronide and sulfate conjugates [19, 20]. Unlabeled harmol conjugates were determined fluorometrically after TLC, according to the method of Mulder and Hagedoorn [20]. Unlabeled harmol was determined in blood by addition of 100 μl 0.2 M Na-phosphate pH 9.5 to 200 μl blood. This mixture was extracted with 3 ml ethyl acetate. The organic layer was extracted with 0.1 N HCl in which the fluorescence was measured [20]. The recovery was better than 95%.

DCNP in plasma was determined by adding 1 ml of 0.6 N HCl to 100 μl plasma. This mixture was extracted with 2.5 ml of ethylacetate (extraction was higher than 97%). The organic layer was used for high performance liquid chromatography. A μBondapack C_{18} column (0.39 × 30 cm) with a Corasil C_{18} precolumn (0.39 × 4 cm) (both from Waters Associates, Milford, MA) was eluted with methanol/0.02 M aqueous acetic acid 60 : 40 (v/v) at a flow rate of 1.5 ml/min. Detection was by absorbance at 280 nm.

In liver homogenates DCNP was determined by mixing 2 ml of a homogenate (20% in 0.154 M KCl) with 1 ml 2 N HCl. The mixture was extracted with 5 ml ethylacetate. DCNP was extracted into 1 N NaOH by extracting 3.5 ml ethylacetate layer with 2 ml 1 N NaOH. After removal of the ethylacetate and after clearing of the aqueous layer by filtration, the absorbance at 405 nm was measured.

Protein binding of DCNP. Protein binding of DCNP to bovine serum albumin was determined by equilibrium dialysis using Visking dialysis tubing. The dialysis lasted for 24 hr at 37°. DCNP was introduced on one side of the membrane and 1% albumin on the other (both in Krebs-bicarbonate buffer, pH 7.4). In addition, in duplicate incubations DCNP and protein were introduced at the same side of the membrane. After incubation these duplicates yielded the same results, indicating that equilibrium had been established. DCNP was determined by adding 200 μl methanol to 100 μl samples. After centrifugation (10 min, 1500 *g*) 900 μl of a 1 M glycine–NaOH buffer, pH 10, was added to 100 μl of the supernatant and the absorbance at 405 nm was measured.

RESULTS

Pharmacokinetics of DCNP in the rat in vivo. When a single injection of DCNP is administered intraperitoneally in the rat the inhibition of sulfation was previously shown to persist for at least 48 hr [8].

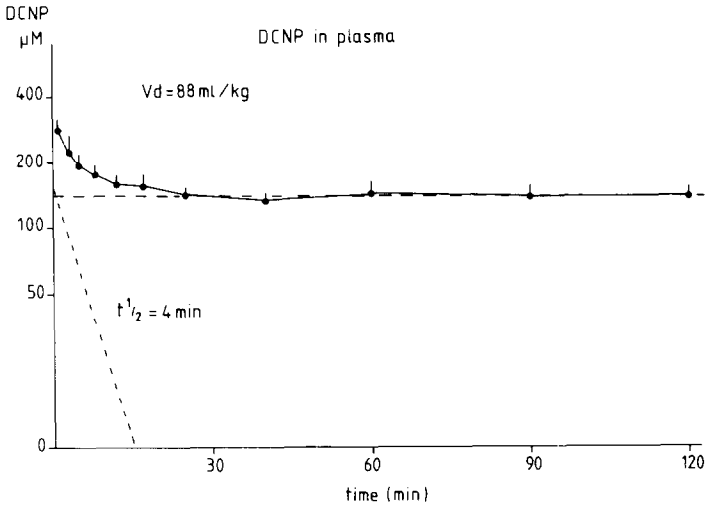

Fig. 1. Plasma concentration curve of 2,6-dichloro-4-nitrophenol (DCNP). Anesthesized rats received 26 μmole DCNP/kg i.v. while their bile duct and urine bladder were cannulated. Plasma samples were taken and analysed for DCNP. The date represent the results in four rats.

To elucidate whether this long duration of action was due to slow uptake from the peritoneal cavity or to slow elimination, DCNP (26 μmole/kg) was injected intravenously and the plasma concentration was recorded with time (Fig. 1). After an initial rapid distribution phase (t_i = 4 min) a value of about 140 μM was reached which remained constant for at least 90 min, indicating very slow elimination. The distribution volume was 88 ml/kg. *In vitro* experiments showed that erythrocytes did not take up any DCNP.

Concentrations in the liver were also determined. There was considerable scatter in the data, probably due to the fact that for each determination a different animal had to be used. The DCNP concentration in the liver (assuming homogeneous distribution over the total liver volume) was 30–50% of the plasma concentration (Fig. 2). Initially the concentration was about 80 nmole/g liver which decreased to about 35 nmole/g.

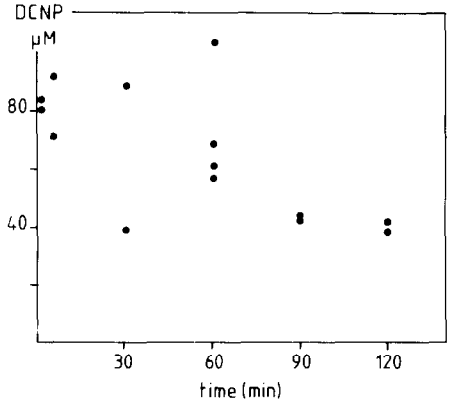

Fig. 2. Concentration of 2,6-dichloro-4-nitrophenol (DCNP) in the liver after i.v. administration of DCNP. Anesthesized rats received 26 μmole DCNP/kg; bile duct and urine bladder were catheterized. The livers were excised at the time indicated and analysed for DCNP.

Protein binding of DCNP. The small volume of distribution and the slow elimination could be due, among other factors, to a high protein binding of the compound in the plasma as has been found before for PCP [21]. Therefore, we determined the degree of protein binding to bovine serum albumin. In the concentration range between 50 and 400 μM DCNP, protein binding was more than 99%.

Rate of onset of inhibition of sulfation by DCNP. To investigate the rate of onset of inhibition by DCNP, DCNP (26 μmole/kg) was injected intravenously together with harmol (26 μmole/kg). Bile and urine were collected during 3 hr after the administration of harmol to collect the conjugates formed. Virtually no harmol sulfate was formed when DCNP and harmol were injected simultaneously, indicating that the inhibition by DCNP was instantaneous and complete. Therefore, apparently sulfation could be arrested completely at any time after harmol administration, thereby restricting sulfation to the time-interval between the administration of the substrate and the inhibitor. When this time-interval was varied (Fig. 3) the time-course of sulfation of harmol could be studied. An initial rapid phase was observed, after which the rate of sulfation decreased continuously, probably because of a decreased harmol supply. This is suggested by the blood decay curve of harmol (Fig. 4). Even at the high dose of 133 μmole/kg the initial rate of decrease of the blood concentration was extremely fast; after 5 min it was reduced to about 30% of its initial value.

Dose-dependence of inhibition of sulfation by DCNP. We studied the dose-dependence of the inhibitory effect of DCNP in order to find the lowest dose that gave satisfactory inhibition and a low toxicity. It was found that more than 90% inhibition was obtained with doses of DCNP higher than 13 μmole/kg (at a dose of harmol of 26 μmole/kg), (Table 1). In addition, a higher dose of harmol was tested (133 μmole/kg, i.v.), the amount of the sulfate conjugate produced was reduced from 62% of the dose in controls to 1% in the DCNP treated rats

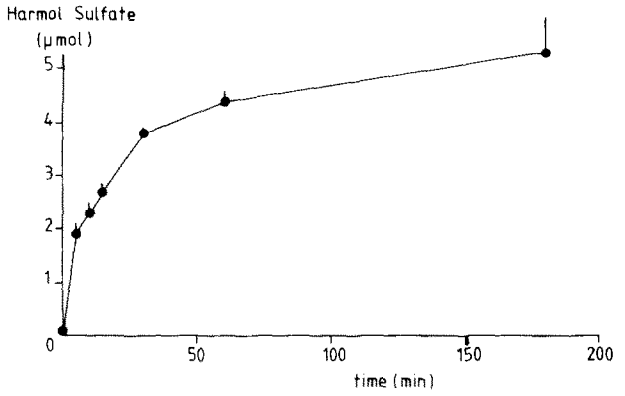

Fig. 3. Time-course of sulfation of harmol assessed by the use of DCNP. Harmol (26 μmole/kg) was injected i.v.; at various periods of time thereafter DCNP was injected (26 μmole/kg, i.v.). Each point represents four rats. Bile and urine were collected and analysed for harmol sulfate. The amount of harmol sulfate found in the period between injection of harmol and DCNP is plotted cumulatively.

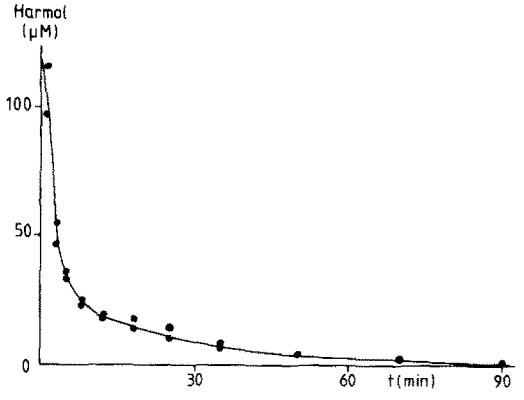

Fig. 4. Blood decay curve of harmol in the rat. Harmol (133 μmole/kg) was injected i.v. and blood samples, taken at the times indicated, were analysed for harmol. The data represent the results in two rats.

Table 1. Inhibition of sulfation of harmol by various doses of 2,6-dichloro-4-nitrophenol (DCNP)

DCNP dose (μmole/kg)	n	Harmol sulfate (μmole/rat)	Inhibition (%)
0	6	5.3 (0.7)	0
1.6	3	2.5 (0.2)	53
3.3	3	1.4 (0.3)	74
6.5	3	0.80 (0.06)	85
9	3	0.70 (0.07)	87
13	3	0.35 (0.09)	93
18	3	0.23 (0.03)	96
26	3	0.08 (0.02)	98

Anesthesized rats received 26 μmole/harmol/kg together with the indicated dose of DCNP (both intravenously). Bile and urine were collected during 3 hr thereafter and these were analysed for harmol sulfate. Values are given with their standard deviation between parenthesis; n is the number of rats used.

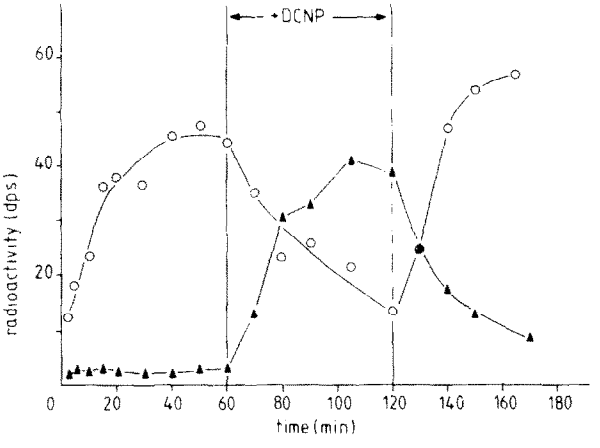

Fig. 5. Effect of 2,6-dichloro-4-nitrophenol (DCNP) on the sulfation of harmol in the single-pass perfused rat liver. A rat liver was perfused single-pass with a medium containing a tracer concentration of [^{3}H]harmol. Between t = 60 and 120 min, the medium contained also 20 μM DCNP. The effluent and bile were analysed for harmol conjugates. The data on the effluent are given in the figure (○ harmol sulfate; ▲, harmol glucuronide). Results of bile were as follows:

t = (min)	0–60	60–120	120–180
Harmol sulfate (% of dose)	19	14	15
Harmol glucuronide (% of dose)	1	19	11

when harmol was simultaneously injected with DCNP (26 μmole/kg, i.v.).

When DCNP was administered intravenously at a dose of 26 μmole/kg in saline, hematuria occurred. This could be prevented by injecting DCNP in 5% (w/v) bovine serum albumin in saline.

Reversibility of inhibition of sulfation by DCNP in the single-pass perfused rat liver. In a previous paper we have described the kinetics of sulfation and glucuronidation of harmol in the single-pass perfused rat liver [19]. We have used this system to study the reversibility of the inhibition of sulfation by DCNP. The liver was perfused with a tracer concentration of [^{3}H]harmol; within 60 min a steady-state of harmol conjugation was reached (Fig. 5). In the next perfusion period the liver was switched to a perfusion medium containing the same concentration of harmol and in addition 20 μM DCNP, while in the last period DCNP was omitted again from the medium. In the presence of DCNP the excretion of harmol sulfate in the effluent from the liver and in bile decreased considerably. The initially still high concentration of harmol sulfate in the effluent just after DCNP was added most likely reflects wash-out of preformed harmol sulfate. Steady-state was not yet reached during the DCNP-perfusion period, but the rate of sulfation in the presence of DCNP was below 30% of the control steady-state level. The sulfation rate increased immediately when DCNP was omitted from the medium in the third period. The concentration of harmol sulfate in the effluent reached a level above that in the first period. This was accompanied by a decrease of harmol sulfate excretion in bile as compared to the first period. These results show that the onset of inhibition of sulfation by DCNP is very rapid, and that this inhibition is readily reversible.

Use of DCNP to measure rates of sulfation at various doses of harmol. The rapid onset of inhibition of sulfation by DCNP was used to measure sulfation rates at various doses of harmol. So far the maximum capacity of sulfation *in vivo* could only be determined by the administration of very high doses of the substrates. These doses may consume a rather high part or even all of the amount of inorganic sulfate that is available for sulfation [22]; thus, it is often not clear whether the determined capacity was limited by the availability of inorganic sulfate or by the V_{max} of the sulfation process. However, when DCNP is used, sulfation can be limited to a short period after the administration of a single dose of harmol. The period in which the sulfation rate is maximal, was found to be the first 5 min period after harmol administration: DCNP administration 10 min after harmol (instead of 5 min) resulted in only slightly more harmol sulfate conjugate than when DCNP was administered 5 min after harmol (both at 26 and 133 μmole/harmol/kg). When the dose of harmol was varied between 17 and 133 μmole/kg and DCNP was administered i.v. 5 min after the i.v. injection of harmol, increasing amounts of harmol sulfate were excreted in bile and urine during 3 hr after harmol administration as the dose of harmol increased. This increase, however, was not linear with the administered dose of harmol, but a trend to saturation was observed (Fig. 6). The mean maximal rate of harmol sulfation, measured in this way was found to be 1.3 μmole/min/rat.

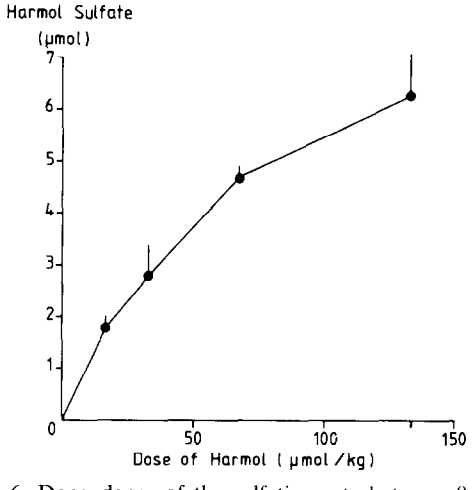

Fig. 6. Dependence of the sulfation rate between 0 and 5 min after injection on the dose of harmol. Harmol was injected i.v. in rats. After 5 min DCNP (26 μmole/kg) was administered i.v. Bile and urine were collected during 3 hr after harmol injection and were analysed for harmol sulfate. Each point represents the results of four rats.

DISCUSSION

The present results show that DCNP, when administered intravenously, inhibits the sulfation of harmol instantaneously and completely. This property of DCNP can be used to evaluate the sulfation rate of harmol *in vivo*. DCNP is rapidly distributed after i.v. injection but only very slowly eliminated, probably due to its high protein binding. No evidence of metabolism was found. In the single-pass perfused rat liver the inhibitory effect of DCNP on sulfation proved to be readily reversible. Binding to proteins in erythrocytes seems to be negligible because these cells did not take up measurable amounts of DCNP. Although DCNP seems to be readily soluble in biological membranes at physiological pH [23], protein binding apparantly prevents it from doing so to any major extent.

The over-all concentration of DCNP in the liver is of the same order of magnitude as that in plasma, especially when it is taken into consideration that the water content of the liver is only approximately 60% of the liver volume. The liver does not accumulate DCNP, which seems in agreement with the rapid reversibility of the inhibitory effect in the liver perfusion. Presumably protein binding of DCNP in the liver is about equally high as in the perfusion medium. The rapid wash-out of DCNP in the single-pass perfused rat liver indicates again that DCNP is able to pass biological membranes quickly and suggests that its distribution is mainly governed by protein binding.

The virtually complete inhibition of sulfation of harmol by DCNP when these compounds are administered simultaneously, indicates that DCNP reaches the sulfating enzymes as rapidly as harmol, or faster. However, when DCNP is given some time

after harmol, sulfation may continue during the time lapse required to build up an inhibitory concentration of DCNP at the site of the sulfotranferase; the above results indicate that this time lapse will be very short.

Using DCNP to arrest sulfation, we found that sulfation of harmol proceeds very rapidly during the first 5 min after harmol administration, while the rate decreases considerably thereafter. This was found both at a high and a low dose of harmol. Since the distribution phase of harmol, when it is injected intravenously, is also very rapid, these findings suggest that the sulfation rate may be determined by substrate supply of harmol: the concentration of the substrate at the sulfation site presumably decreases very rapidly by conjugation and further distribution. Sulfation during the first 5 min after harmol administration tended to become saturated at higher doses of harmol. Usually the maximal capacity of sulfation *in vivo* is measured by giving high doses or high infusion rates of the substrate and measuring the total sulfate conjugate production. This unavoidably implies that much inorganic sulfate required for sulfation is consumed, thus, the sulfate availability is strongly decreased during such an experiment. However, in our experiments at most 7 μmole of inorganic sulfate was consumed during the 5 min that sulfation occurred, while about 60 μmole inorganic sulfate was available [22]. Therefore, it is very probable that the trend to saturation that we observed was caused by saturation of the sulfotransferase with harmol; exhaustion of inorganic sulfate cannot have occurred. However, we cannot exclude that the intracellular availability of inorganic sulfate or the concentration of the cosubstrate in sulfation 3′-phosphoadenosine-5′-sulfatophosphate (PAPS) may decrease at higher doses of the substrate.

It is interesting that the highest sulfation rate we observed *in vivo* (6.7 μmole/5 min = 1.3 μmole/min) was of the same order of magnitude as that which was found in the single-pass perfused rat liver [19] where a maximum rate of 1 μmole/min was found. Moreover, when we plotted our data according to a Lineweaver–Burk plot (reciprocal of rate against reciprocal of dose) a straight line resulted (correlation = 0.997), and a V_{max} for sulfation of 2.1 μmole/min was found. Again this value is close to what has been found before (1.6 ± 0.5 μmole/min) in liver perfusion experiments.

In conclusion, DCNP proves to be a useful compound in the study of sulfation *in vivo* because of its immediate action upon intravenous injection. Also its very slow elimination makes one single injection effective for a long period of time, which is very convenient if the effect of long-term inhibition of sulfation has to be evaluated.

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REFERENCES

1. R. T. Williams, *Biochemistry of Phenolic Compounds* (Ed. J. B. Harborne), p. 205. Academic Press, New York (1964).
2. G. J. Mulder, in *Sulfation of Drugs and Related Compounds* (Ed. G. J. Mulder), Chapter 6. CRC Press, Boca Raton (1981).
3. R. T. Williams, *Detoxication Mechanisms*, Chapter 5. Chapman & Hall, London (1974).
4. J. G. Weitering, K. R. Krijgsheld and G. J. Mulder, *Biochem. Pharmac.* **28**, 757 (1979).
5. J. R. de Baun, E. C. Miller and J. A. Miller, *Cancer Res.* **30**, 577 (1970).
6. M. Meyers, W. Slikkers, G. Pascoe and M. Vore, *J. Pharmac. exp. Ther.* **214**, 87 (1980).
7. J. M. Yousef, B. Tuchweber, R. J. Vonk, D. Massé, M. Audet and C. C. Roy, *Gastroenterology* **80**, 233 (1981).
8. G. J. Mulder and E. Scholtens, *Biochem. J.* **165**, 553 (1977).
9. J. H. N. Meerman, A. B. D. van Doorn and G. J. Mulder, *Cancer Res.* **40**, 3772 (1980).
10. M. W. Duffel and W. B. Jakoby, *J. biol. Chem.* **256**, 11123 (1981).
11. A. Baranczyk-Kuzma, R. T. Borchardt, C. S. Schasteen and T. C. L. Pinnick, in *Phenolsulfotransferases in Mental Health Research* (Eds E. Usdin and M. Sandler), p. 55. Macmillan, London (1981).
12. H. Koster, E. Scholtens and G. J. Mulder, *Med. Biol.* **57**, 340 (1979).
13. M. Koike, K. Sugeno and M. Hirata, *J. Pharmaceut. Sci.* **70**, 308 (1981).
14. R. D. Sekura, M. W. Duffel and W. B. Jakoby, in *Methods in Enzymology* (Ed. W. B. Jakoby), Vol. 77, p. 197. Academic Press, New York (1981).
15. G. Rein, V. Glover and M. Sandler, in *Phenolsulfotransferases in Mental Health Research* (Eds. E. Usdin and M. Sandler), p. 98. Macmillan, London (1981).
16. H. Koster, I. Halsema, E. Scholtens and G. J. Mulder, manuscript in preparation.
17. H. Koster, I. Halsema, E. Scholtens, M. Knippers and G. J. Mulder, *Biochem. Pharmac.* **30**, 2569 (1981).
18. K. S. Pang and M. Rowland, *J. Pharmacokin. Biopharm.* **5**, 655 (1977).
19. K. S. Pang, H. Koster, I. C. M. Halsema, E. Scholtens and G. J. Mulder, *J. Pharmac. exp. Ther.* **219**, 134 (1981).
20. G. J. Mulder and A. H. Hagedoorn, *Biochem. Pharmac.* **23**, 2101 (1974).
21. U. G. Ahlborg and T. M. Thunberg, *C.R.C. Crit. Rev. Tox.* **7**, 1 (1981).
22. K. R. Krijgsheld, E. Scholtens and G. J. Mulder, *Comp. Biochem. Physiol.* **67A**, 683 (1980).
23. G. J. Mulder and A. B. D. van Doorn, *Biochem. J.* **151**, 131 (1975).