Biochemical Pharmacology, Vol. 31, No. 10, pp. 1941–1944, 1982.
Printed in Great Britain.

0006-2952/82/101941-04 $03.00/0

The effect of fasting on sulfation and glucuronidation in the rat *in vivo*

(*Received* 19 *October* 1981; *accepted* 21 *December* 1981)

Many interactions have been observed between nutritional factors and the biotransformation of drugs in animals and man, which may profoundly affect drug efficacy and toxicity [1, 2]. Much of this work has been about the effect of the diet on oxidative, Phase I metabolism [3–5], but much less is known about diet-related changes in conjugation, Phase II reactions. Such changes in conjugation *in vivo* may occur both at the level of the transferases involved, and the availability of co-factors for conjugation.

Fasting is the most drastic nutritional change, certainly when it lasts for several days. Body weight and liver weight decrease rapidly, while many physiological processes are affected; drug metabolism obviously will also adapt. In the literature various reports have touched briefly on the effect of fasting or a low-protein diet on conjugation reactions, especially sulfation and glucuronidation [6–9]. Protein-deficiency did not affect the *in vitro* determined activity of phenol sulfotransferase in rat liver, while UDP-glucuronosyltransferase activity was somewhat enhanced [10–12]. Sulfation requires a sufficient supply of inorganic sulfate to synthesize the co-substrate of sulfation, 3′-phosphoadenosine 5′-sulfatophosphate (PAPS), while glucuronidation uses UDP-glucuronate (UDPGA) as co-substrate. Decreases in sulfate or UDPGA availability have been shown to decrease the rates of sulfation and glucuronidation, respectively [13–15].

It has been suggested that glucuronidation is dependent on the carbohydrate reserves in the liver [6, 9]. Indeed, during fasting a decrease in the UDPGA concentration in the liver was observed [16, 17]. To increase the UDPGA availability glucose administration has been used. The results of these experiments are conflicting as far as the effect on glucuronidation is concerned: Reinke *et al.* [9] observed no increase in glucuronidation of *p*-nitrophenol in the isolated perfused rat liver, while Eacho *et al.* [18] found a pronounced increase in glucuronidation of the same substrate in isolated hepatocytes (both taken from fed rats). In isolated hepatocytes taken from fasted rats glucose increased glucuronidation [7, 9]. Of course, the conflicting findings may be due to the differences in preparations used and in the experimental protocols. On the other hand, UDPGA availability is not only determined by UDPGA concentration, but also by the rate at which it can be resynthesized; we recently demonstrated that during steady-state glucuronidation in the single pass perfused rat liver a glucuronidation rate of 150 nmol/min/g of liver could be reached and maintained for at least 30 min [19, 20], while only 300 nmol UDPGA is present per gram of liver [17]. Further, the experiments reported above used *in vitro* preparations with the inherent difficulty of translating the findings to the *in vivo* situation. Therefore, we have investigated sulfation and glucuronidation of harmol in rats *in vivo* that had been fasted for 72 hr. The results surprisingly showed no difference between control and fasted rats in the conjugation pattern of harmol, not even when a harmol infusion was administered for several hours.

Materials and methods

Animals. Rats were either fed a normal commercial rat chow, or fasted for 72 hr in the animal rooms. All rats had free access to water. The bodyweight of the rats was between 280 and 330 g; the control rats were matched with the bodyweight of the rats that had been fasted for 72 hr (see Results). For the experiments the rats were anesthesized with sodium pentobarbital (60 mg/kg i.p.), and catheters were inserted in the trachea (artificial respiration), bile duct (bile collection), bladder (urine collection) and the external jugular vein (mannitol or harmol infusion), as described elsewhere [21]. The mannitol infusion (75 mg mannitol/ml saline; infusion rate of 9.5 ml/hr) was administered to ensure sufficient, continuous urine flow. In some experiments an infusion of taurocholate was administered (53 μmol/hr; taurocholate was dissolved in the saline) in the external jugular vein. The body temperature of the rats was always held between 37.5 and 38.5°. Only male rats were used in this study.

Harmol determination. Harmol conjugates were determined as described before [22] after separation by thin layer chromatography, by their fluorescence.

Materials. Harmol HCl was obtained from Sigma Chemical Co., St Louis, MO (U.S.A.); harmol sulfate was synthesized essentially as described before [23]. It was completely pure judged by thin layer chromatography.

Results

Effect of a 72-hr fast on the condition of the rats. When the rats were fasted for 72 hr they lost about 14% of their body weight (from 330 g initially to approx. 285 g after 72 hr). In this study we have used as controls rats of 285 g that had not been fasted, and we have administered the same dose of harmol per unit body weight. During the fast the liver weight decreased also, from around 8.3 g in (285 g) controls to 6.8 g in the fasted animals of the same body weight (an approx. 18% loss). Concomitantly, the bile flow decreased considerably, from 0.95 ± 0.08 ml/hr (mean ± S.D.; $n = 6$) in rats with cannulated bile ducts, to 0.65 ± 0.08 ($n = 4$) in fasted rats (a 32% decrease). No differences in mannitol-stimulated urine flow were observed between the groups. The body temperature under pentobarbital anesthesia of the fasted rats tended to drop more rapidly than that of controls, as judged by the fact that the fasted rats required more heating than the controls to keep their body temperature at 37.5–38.5°.

An i.v. dose of 133 μmol of harmol per kilogram body weight, that was well tolerated by control rats, caused acute death in the fasted rats. This suggests that some aspect of the rat's ability to withstand the toxic effects of harmol had deteriorated during the fast, and, hence, we used lower doses of harmol for this study.

Effect of a 72-hr fast on the conjugation of a bolus injection of harmol and on excretion of the conjugates in bile and urine. When a bolus injection of harmol (52 μmol/kg) was administered i.v., we did not find any difference between the fasted and control rats in the ratio between the amounts of the sulfate and glucuronide conjugates synthesized and excreted during the 4 hr past injection (Table 1). The only effect was a shift of the excretion of harmol sulfate from bile to urine; no such shift was seen for harmol glucuronide. Similar experiments at a lower dose of harmol (26 μmol/kg) showed the same results. The time courses of the excretion of the conjugates in bile and urine were almost exactly the same in controls and fasted animals. For instance, the half-life of harmol sulfate excretion in bile and urine was approx. 40 min for both groups; a similar identity was observed for the excretion of harmol glucuronide.

Effect of a 72-hr fast on the conjugation of an infusion of harmol and on biliary and urinary excretion of the conjugates. Because we wanted to know whether still higher doses of harmol than could be administered as a bolus injection were conjugated alike in control and fasted rats, we gave an i.v. infusion of 1 μmol harmol/min to rats (approx. 3.5 μmol/min/kg) for 4 hr, and collected bile and urine during the infusion. When steady-state could be reached this would yield data on the activities of UDP-glucuronosyltransferase and phenol sulfotransferase. As

Table 1. Effect of a 72-hr fast on sulfation and glucuronidation of a bolus injection of harmol, and biliary and urinary excretion of the conjugates

Conjugate	Excretory pathway	Controls (% of dose)	72-hr fasted (% of dose)
Harmol sulfate	Bile	17 ± 2	5 ± 2*
	Urine	47 ± 6	60 ± 10*
Harmol glucuronide	Bile	22 ± 1	19 ± 3
	Urine	8 ± 2	9 ± 1
Total recovery	Bile + urine	93 ± 6	91 ± 14
(Ratio sulfate to glucuronide conjugate)		(68:32)	(70:30)

Harmol was administered i.v. as a bolus injection of 52 μmol/kg; the rats received an i.v. infusion of mannitol as detailed in Materials and Methods. Bile and urine were collected for 4 hr after the injection of harmol. Mean ± S.D. are given for $n = 4$ in each group.
* Significantly different from control ($P < 0.025$).

has been reported before [24, 25], such steady-state of conjugation is relatively short-lasting (as judged by the excretion rates of the conjugates in bile and urine), from 1 to 2½ hr after the start of the infusion. However, steady-state is not reached properly, because sulfate is depleted during the infusion due to sulfation of harmol [25], and therefore, sulfation starts to slowly decrease after 2½ hr [24, 25]; glucuronidation compensates for that by a slow increase. During this period the concentration of unconjugated harmol in plasma did not increase, indicating that somewhere in the body harmol was sequestered, since only 60–70% of the infused dose was recovered as conjugates. This was about the same in both groups of rats. We have taken the data of three consecutive ½-hr periods from 1 to 2½ hr after the start of the harmol infusion and calculated the 'steady-state' sulfation and glucuronidation rates. As can be seen from Table 2, again no change in the ratio between sulfation and glucuronidation of harmol is seen, although clearly the rate of conjugation is slower in the fasted rats than in the controls. Yet, this is not reflected in a change in the conjugation pattern. The second difference is again a somewhat lower excretion of harmol sulfate in bile in the fasted rats.

Effect of a 72-hr fast on the biliary excretion of i.v. injected harmol sulfate. Because the main effect observed was the decreased biliary excretion of harmol sulfate, we have studied the pharmacokinetic behaviour of chemically synthesized harmol sulfate in the rat *in vivo*. The results of Table 3 confirm that the biliary excretion of harmol sulfate is decreased by a 72 hr fast; however, almost 90% of the dose is excreted by the kidneys in the urine.

Since the effect of the fast on biliary excretion was only small, we have ligated the kidneys in a series of rats, to leave only the biliary excretion route for harmol sulfate intact. In this case the difference between the biliary excretion of harmol sulfate became very pronounced: from 72% of the dose in 4 hr in controls, to only 28% in the fasted rats (Table 3).

In these experiments the volume of bile excreted had also decreased considerably in the fasted rats (Table 3). Since we had shown previously [23] that bile flow may affect the biliary excretion of harmol sulfate, we have administered an i.v. infusion of taurocholate to fasted rats in order to stimulate bile flow to the same level as in controls. Yet, the biliary excretion of harmol sulfate was not increased by the taurocholate infusion (Table 3).

Discussion

In spite of the many changes resulting from a 72-hr fasting period, no change was observed in the conjugation pattern of harmol although in the infusion experiment the total rate of conjugation of harmol was somewhat decreased: the same ratio between sulfation and glucuronidation of harmol was found, even when a high infusion

Table 2. Effect of a 72-hr fast on steady-state conjugation of harmol during an i.v. infusion of harmol

Conjugate	Excretory pathway	Steady-state conjugation rate (μmol/min) (Control)	(72-hr fasted)
Harmol glucuronide	Bile	0.21 ± 0.03	0.18 ± 0.03
	Urine	0.07 ± 0.01	0.06 ± 0.01
		0.28 ± 0.03	0.23 ± 0.02
Harmol sulfate	Bile	0.09 ± 0.01	0.03 ± 0.00*
	Urine	0.35 ± 0.04	0.31 ± 0.04
		0.43 ± 0.03	0.33 ± 0.04*
(Ratio of sulfate to glucuronide)		61:39	60:40

An i.v. infusion of harmol (1 μmol/min/rat) was given for 4 hr, together with a mannitol infusion. The steady-state levels of sulfation and glucuronidation were calculated from the data on excretion of the conjugates in bile and urine in the period from 1 to 2½ hr after start of the infusion. Mean ± S.D. is given, for $n = 4$ in both groups.
* Significantly different from control at $P < 0.025$.

Table 3. Elimination of harmol sulfate after i.v. injection in bile and urine

	Control	72-hr fasted	72-hr fasted with taurocholate infusion
A. Rats with intact kidneys (32 μmol harmol sulfate/kg)			
Percentage of dose in bile	8 ± 3%	4 ± 2%*	–
Percentage of dose in urine	85 ± 15%	87 ± 5%	—
Volume of bile (4 hr)	3.9 ± 0.6 ml	3.1 ± 0.1 ml	
Volume of urine (4 hr)	36.1 ± 1.4 ml	30.9 ± 2.4 ml*	—
B. Rats with ligated kidneys (15 μmol harmol sulfate/kg)			
Percentage of dose in bile	72 ± 5%	28 ± 4%*	34 ± 3%*
Volume of bile (4 hr)	4.2 ± 0.3 ml	2.6 ± 0.2 ml*	4.2 ± 0.3 ml

In the rats with intact kidneys a mannitol infusion was given. In the rat with ligated kidneys no infusions (control and 72-hr fasted) or an infusion of taurocholate (the third group) were administered. The dose of harmol sulfate was 32 μmol/kg for the rat with intact kidneys, and 15 μmol/kg for the rat with the ligated kidneys. Bile and urine were collected for 4 hr after the harmol sulfate injection. Means ± S.D. are given.

* Significantly different from control ($P < 0.025$). $n = 4$ for all groups, except the taurocholate group, where $n = 3$.

rate of harmol was applied. This seems to indicate that the sulfation and glucuronidation processes are not easily disturbed; moreover, that the activities of both transferases *in situ in vivo* have not changed during the fasting for 72 hr (unless two opposing effects have cancelled each other). The literature data [7, 9, 17] suggested that UDP-glucuronosyltransferase activity might have increased, and phenol sulfotransferase activity decreased after fasting or a low-protein diet [10–12]; the data about the effect of fasting, however, are very incomplete and in part, conflicting. As yet there is no explanation for the (in part) apparently conflicting findings from the literature data and the present work; part of the problem may be the inherent difficulties of the (membrane-bound) UDP-glucuronosyltransferase assay (what reflects the *in situ* activity of the enzyme: activated or 'native'? See for review Dutton [17]), and the assay of phenol sulfotransferase in impure cytosolic preparations (see for review, Ref. [26]).

As far as the co-substrates are concerned, a decreased availability of inorganic sulfate was to be expected since little inorganic sulfate is excreted anymore in the urine [8]; the glucose effect in hepatocytes and the perfused rat liver on glucuronidation [7, 9, 18] had implied a decreased UDPGA availability. Yet, our *in vivo* data indicate that still enough PAPS and UDPGA can be generated to conjugate the harmol at the rates at which it has been supplied in this study.

The only consistent effect observed was the decreased biliary excretion of harmol sulfate in the fasted rat. This clearly is not due to a decreased bile flow, since a taurocholate infusion did not result in an improvement. It may be related to uptake of harmol sulfate from blood by the liver; if that were impaired, harmol sulfate excretion in bile certainly would decrease. The fact that normally harmol sulfate is mainly excreted in urine indicates that under normal conditions the major part of harmol sulfate synthesized in the cells (mainly the liver?) is eliminated via the blood.

These data demonstrate that the effect of fasting may be less severe than would have been anticipated from the *in vitro* data. It remains to be seen, however, if the same applies to man.

Department of Pharmacology
State University of Groningen
Groningen, The Netherlands

GERARD J. MULDER*
TOON J. M. TEMMINK
HENK J. KOSTER

REFERENCES

1. T. C. Campbell and J. R. Hayes, *Pharmac. Rev.* **26**, 171 (1974).
2. J. F. Mueller in *Human Nutrition, A Comprehensive Treatise* (Eds. R. B. Alfin-Slater and D. Kritchevsky), Vol. 3B, p. 351, Plenum Press, New York (1980).
3. A. E. M. McLean and P. A. Day, *Biochem. Pharmac.* **24**, 37 (1975).
4. A. Alvares, K. E. Anderson, A. H. Conney and A. Kappas, *Proc. natn. Acad. Sci. U.S.A.* **73**, 2501 (1976).
5. C. A. Shively, R. J. Simons, G. T. Passananti, B. H. Dvorchik, E. S. Vesell, *Clin. Pharmac. Ther.* **29**, 65 (1981).
6. R. G. Thurman, L. A. Reinke, S. A. Belinksy, R. K. Evans and F. C. Kauffman, *Archs Biochem. Biophys.* **209**, 137 (1981).
7. L. R. Schwarz, *Arch. Tox.* **44**, 137 (1980).
8. K. R. Krijgsheld, E. Scholtens and G. J. Mulder, *Biochem. Pharmac.* **30**, 1973 (1981).
9. L. A. Reinke, S. A. Belinsky, R. K. Evans, F. C. Kauffman and R. G. Thurman, *J. Pharmac. exp. Ther.* **217**, 863 (1981).
10. B. G. Woodcock and G. C. Wood, *Biochem. Pharmac.* **20**, 2703 (1971).
11. J. Magdalou, D. Steimetz, A. M. Batt, B. Poullain, G. Siest and G. Debry, *J. Nutr.* **109**, 864 (1979).
12. E. Hietanen, *Gen. Pharmac.* **11**, 443 (1980).
13. G. J. Mulder and K. Keulemans, *Biochem. J.* **176**, 959 (1978).
14. P. Moldéus, B. Andersson and A. Norling, *Biochem. Pharmac.* **27**, 2583 (1978).
15. P. Moldéus, B. Andersson and V. Gergely, *Drug Metab. Dispos.* **7**, 416 (1979).
16. B. F. Felsher, N. M. Carpio and K. VanCouvering, *J. Lab. clin. Med.* **93**, 414 (1979).
17. G. J. Dutton, *Glucuronidation of Drugs and Other Compounds*, CRC Press, Boca Raton, FL (1980).
18. P. I. Eacho, D. Sweeney and M. Weiner, *Biochem. Pharmac.* **30**, 2616 (1981).
19. K. S. Pang, H. Koster, I. C. M. Halsema, E. Scholtens and G. J. Mulder, *J. Pharmac. exp. Ther.* **219**, 134 (1981).

* Author to whom correspondence should be addressed.

20. K. W. Bock and I. N. H. White, *Eur. J. Biochem.* **46**, 451 (1974).
21. G. J. Mulder, E. Scholtens and D. K. F. Meijer, in *Methods in Enzymology*, (Ed. W. B. Jakoby), Vol. 77, p. 21, Academic Press, New York (1981).
22. G. J. Mulder and A. Hagedoorn, *Biochem. Pharmac.* **23**, 2101 (1974).
23. J. Jorritsma, J. H. N. Meerman, R. J. Vonk and G. J. Mulder, *Xenobiotica* **9**, 247 (1979).
24. H. Koster, I. Halsema, E. Scholtens, M. Knippers and G. J. Mulder, *Biochem. Pharmac.* **30**, 2569 (1981).
25. K. R. Krijgsheld, H. J. Koster, E. Scholtens and G. J. Mulder, submitted for publication.
26. G. J. Mulder, (Ed.) *Sulfation of Drugs and Related Compounds*. CRC Press, Boca Raton, FL (1981).

Biochemical Pharmacology, Vol. 31, No. 10, pp. 1944–1946, 1982.
Printed in Great Britain.

0006-2952/82/101944-03 $03.00/0

The effect of activators of glucuronyltransferase in the streptozotocin-induced diabetic rat

(*Received* 1 *October* 1981; *accepted* 11 *January* 1982)

The activity of UDP-glucuronyltransferase has been shown to be decreased in the streptozotocin-induced diabetic male rat, although no change was observed in female rats [1]. Using hepatic "native" microsomes from streptozotocin-induced diabetic male rats glucuronidation of *p*-nitrophenol proceeded at only half the rate observed in "native" microsomes from control rats. Furthermore, treatment with insulin abolished the effect of streptozotocin, but had no significant effect on hepatic glucuronyltransferase activity in control rats. This communication discusses the mechanisms by which these changes may occur, using the results from "native" and activated microsomal preparations.

Materials and methods

Male Sprague–Dawley rats (180–220 g) were injected i.v. with 60 mg/kg streptozotocin in acetate buffer, pH 4.5. The rats were used five days after the injection of streptozotocin (blood glucose 380 ± 26 mg/100 ml) or of acetate buffer (blood glucose 99 ± 3 mg/100 ml). Hepatic microsomes were prepared by a modification of the conventional ultracentrifugation method [2] involving the addition of 15% glycerol to the buffer in which the liver is homogenised [3]. Glucuronidation of *p*-nitrophenol was measured by the disappearance of substrate using the method of Chhabra and Fouts [4]. This was validated by direct measurement of *p*-nitrophenolglucuronide by high pressure liquid chromatography [5].

The microsomal incubations, which contained 500 nmole *p*-nitrophenol, 125 nmole saccharo-1,4-lactone, 5 μmole UDP-glucuronic acid and 0.2 ml microsomal suspension (equivalent to 3–5 mg protein), were made up to a final volume of 2.5 ml with 0.1 M Tris buffer, pH 7.4. For activation the microsomes were either preincubated with Triton X-100 for 10 min at room temperature or UDP *N*-acetylglucosamine was added directly to the enzyme incubation in the presence of 1 mM $MgCl_2$. The effect of 1 mM $MgCl_2$ alone on glucuronyltransferase activity was also measured. The concentrations of Triton X-100 and UDP *N*-acetylglucosamine used are as indicated in the figures. All reactions, which were found to be linear with respect to time up to 30 min, were carried out for 15 min at 37° unless otherwise stated. Michaelis–Menten constants for the enzyme kinetics were calculated by non-linear regression analysis using BMDP/PAR [6].

Results and discussion

Maximal activation of glucuronyltransferase by Triton X-100 occurred at a concentration of 0.25% (v/v) Triton X-100 in the incubation mixture whereas for UDP *N*-acetylglucosamine activation was maximal at a 4 mM concentration, [Figs. 1(a) and 1(b)]. The activity of "native"

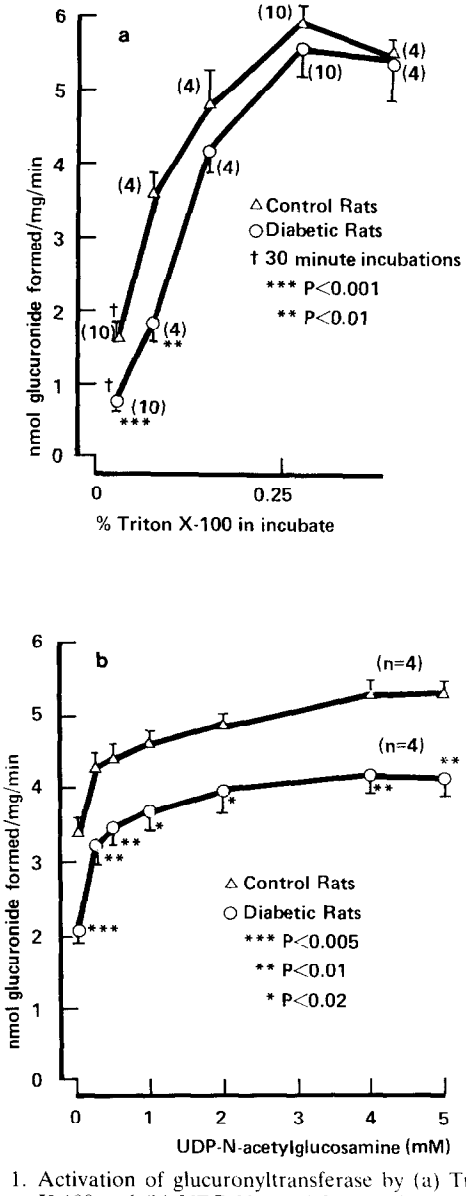

Fig. 1. Activation of glucuronyltransferase by (a) Triton X-100 and (b) UDP *N*-acetylglucosamine.