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THE INFLUENCE OF PHENOBARBITAL, 3-METHYLCHOLANTHRENE AND 2,3,7,8-TETRACHLORODIBENZO-*p*-DIOXIN ON GLUTATHIONE S-TRANSFERASE ACTIVITY OF RAT LIVER CYTOSOL

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Abstract—Specific activities and apparent Michaelis–Menten kinetic parameters were determined for glutathione (GSH) S-transferase activity (E.C. 2.5.1.18) in rat liver cytosol, towards styrene oxide (STOX), 1,2-butylene oxide (BOX) and 1-chloro-2,4-dinitrobenzene (CDNB) as electrophilic substrates, before and after pretreatment with the drug-metabolizing enzyme inducers phenobarbital (PB), 3-methylcholanthrene (MC) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). The measured GSH S-transferase activities appear to obey Michaelis–Menten kinetics. In non-induced animals the apparent K_m values of the transferase activities were equal for STOX vs GSH, but they differed by a factor of 2 for CDNB vs GSH and by a factor of 14 for BOX vs GSH. The apparent V_{max} values in each combination of GSH and electrophilic substrate were equal, but differed by one order of magnitude for the mutual substrate combinations. Pretreatment of the rats with MC resulted in enhancement of all measured activities expressed in terms of cytosol protein, while TCDD only enhanced the activities expressed as per gram body wt. PB enhanced both activities when STOX was employed as substrate, but when CDNB was used as the substrate, only the activity per gram body wt increased. All pretreatments increased the V_{max} values using CDNB as the substrate, while PB and MC had an enhancing effect using STOX; the V_{max} using BOX was enhanced after TCDD administration only. The K_m values using BOX as the substrate was lowered after MC pretreatment; TCDD pretreatment decreased the K_m using STOX, while it increased the K_m using CDNB. It is concluded that the GSH S-transferase system is inducible, but in contrast to the induction of the mixed function oxidase system, qualitative differences between the inducing effects of PB and MC were not observed. Use of TCDD as inducing agent, however, resulted in a different induction pattern, which may indicate that during induction with this agent different types of GSH S-transferases are involved.

GSH S-transferases (RX: glutathione S-transferase, EC 2.5.1.18) are enzymes catalyzing the conjugation of GSH with electrophilic and potential alkylating agents, which is the first step in mercapturic acid formation [1]. Hence these enzymes play an important role in the detoxication of xenobiotics and their reactive metabolites, which can be generated by metabolic activation under influence of e.g. the microsomal cytochrome P450 containing mixed function oxidase system [2]. The participation of GSH in the enzymic conjugation with xenobiotics is, however, only one aspect of its physiological function [3, 4].

The GSH S-transferases occur in the cytosol of cells from many tissues, generally predominantly in liver [5, 6], but some activity has also been reported to occur in other tissues such as kidney [7], lung [8], and small intestine [9]. Their presence is widespread in vertebrate species [10, 11] and insects [12].

The GSH S-transferases of human and rat liver comprise a group of at least five or six enzymes having different but overlapping second substrate specificities [6, 11, 13, 14]. Furthermore it was shown that the cytosolic, anion-binding hepatic protein ligandin (also called Y-protein) is identical with GSH S-transferase B in rat liver [15].

Previously it was thought that GSH S-transferases could not be induced [1, 16], but recent reports have demonstrated the induction by PB and polycyclic aromatic hydrocarbons of the enzymes from rodent liver [8, 17–19], mouse lung [8] and rat kidney and small intestine [9, 20].

The opinions concerning the steady state kinetics of GSH S-transferases are conflicting: Pabst *et al.* [21] and Mannervik and Askelöf [22] observed non-linear double reciprocal plots, although they differ in their conclusions, while in older [7] and recent investigations (cf. [17, 20 and 23]) linear kinetics are described.

The aim of the present investigation was to study the influence of some inducers of drug metabolism (PB, MC and TCDD) on GSH S-transferase activities in rat liver cytosol and to determine the effects on the apparent Michaelis–Menten kinetic parameters, using two epoxides (STOX and BOX) and

Abbreviations—BOX—1,2-Butylene oxide; CDNB—1-chloro-2,4-dinitrobenzene; DMSO—dimethylsulfoxide; DTNB—5,5′-dithiobis(2-nitrobenzoic acid); GSH—glutathione (reduced); MC—3-methylcholanthrene; PB—phenobarbital; STOX—styrene oxide; TCA—trichloroacetic acid; TCDD—2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

one aromatic chloro compound (CDNB) as the electrophilic second substrates.

MATERIALS AND METHODS

Chemicals. DTNB (Ellman's reagent) was purchased from Merck (Darmstadt, G.F.R) and BDH (Poole, U.K.); GSH (reduced) from Baker (Deventer, Netherlands) and Merck; MC was a purissimum grade reagent from Fluka (Buch., Switzerland), and TCDD was a generous gift from the Dow Chemical Co. (Midland, U.S.A.). All other reagents were of the best quality available.

Animals. Male rats of the laboratory-bred SPF–Wistar strain weighing 180–230 g were used. They were housed in groups of four animals on wood chips and fed a commercially available diet (Hope Farms, Woerden, Netherlands). Food and water were allowed *ad libitum*.

Group 1 received daily i.p. injections of 80 mg/kg of sodium phenobarbital for 14 days; group 2 was given MC dissolved in sesame oil via the same route of administration in daily doses of 20 mg/kg for 4 days. Group 3 received TCDD dissolved in 1,4-dioxane and diluted with sesame oil (1 part of dioxane to 40 parts of sesame oil) to a final concentration of 5 μg/ml: the rats were given an i.p. injection of 10 μg/kg on days 1 and 7. For each induced group a second group of four animals served as controls, receiving the dose vehicles only.

Enzyme source. Rats were starved overnight and killed by cervical dislocation between 8.30 and 10.30 a.m., 24 hr after the last injection of PB or MC. In the TCDD experiment this period was 6 days. The liver was perfused *in situ* with 0.25 M sucrose via the portal vein, removed, washed and homogenized in 5 vol. of 0.25 M sucrose with an all-glass Potter–Tenbroeck homogenizer. The homogenate was centrifuged for 10 min at 600 *g* to remove debris, followed by further centrifugation at 18,000 *g* for 15 min. Calcium chloride was added to the supernatant to obtain a concentration of 8 mM, and the supernatant was then centrifuged for 15 min at 27,000 *g* to separate the microsomes [24]. The particle-free supernatant was used as the enzyme source, and differences between this supernatant and a 100,000 *g* supernatant with respect to GSH S-transferase activities were not detectable. The whole procedure was performed at a temperature between 0–5°, and all livers were worked up and assayed separately.

Protein content was measured according to Lowry *et al.* [25], using bovine serum albumin as a standard.

Determination of enzymic conjugations. GSH S-transferase activity was assayed with three substrates: STOX, BOX and CDNB [13,26,27]. Routine incubations contained 0.5 mM of GSH and 0.5 mM of either substrate, but when BOX was employed, the GSH concentration was 2 mM and the BOX concentration was 40 mM. With the epoxides, the buffer was 0.13 M phosphate, pH 8.0, and the cytoplasmic protein content was 0.5 mg/ml. In the case of CDNB, 0.13 M phosphate buffer, pH 7.0, and 15 μg/ml of protein were used. Total incubation volumes were always 1.5 ml. Mixtures were preincubated for 2 min and the reaction was started by adding the substrate (epoxides in 50 μl of ethanol, CDNB in 50 μl of DMSO); incubation took place at 37° in a shaking water bath for 5 min. Under these conditions, product formation was linear with respect to time and protein concentration. The reaction was stopped by addition of 0.1 ml of a 33% aqueous TCA solution, followed by centrifugation at 600 *g* for 5 min. Appropriate controls in each experiment served as blanks and for determination of the spontaneous (non-enzymic) conjugation. All experiments were performed at least in duplicate.

Conjugations with STOX and BOX were calculated by measuring unreacted GSH with Ellman's reagent [28]: a sample (0.1 ml) of the deproteinized reaction mixture was added to 0.05 mM of DTNB in 0.1 M phosphate buffer (1.5 ml), pH 6.5, and thoroughly mixed. The absorbance at 412 nm was read after a fixed time interval [29]. When CDNB was the substrate, conjugate formation was directly determined by measuring the absorbance at 340 nm of the deproteinized mixture [13].

Enzyme kinetics. Assuming Michaelis–Menten kinetics, apparent K_m and V_{max} values of the GSH S-transferase activities were calculated using a computer program. In a V vs S plot this program fits a non-linear regression curve by using least squares for minimizing the deviations in V on the given S-values. For each set of observations, the program presents the values for the measured and calculated V (together with the difference in each of these pairs) and finally it provides the apparent K_m and V_{max} values as calculated from the fitted curve.

Lineweaver–Burk plots are depicted merely as illustration of the apparent kinetic behaviour.

Statistical analysis was performed with Student's 't' test; differences are assumed to be significant when $P < 0.05$.

RESULTS

Due to the wide variation in assaying GSH S-transferase activities as reported in the literature [5, 13, 18, 26, 27, 30–33], preliminary experiments served to establish optimal conditions for determining transferase activities in rat liver cytosol, resulting in the conditions outlined under Methods. Employing these conditions, the spontaneous as well as the enzymic conjugations were linear with respect to time. Using STOX as the second substrate the spontaneous conjugation was 9 per cent of the totally measured conjugation; using BOX or CDNB these figures were 42 and 5 per cent respectively.

The effect of substrate concentration on GSH S-transferase activity towards STOX, BOX and CDNB, each of them together with GSH, is illustrated using Lineweaver–Burk plots (Fig. 1). Within the limits of experimental error and concentration ranges studied, and in spite of the complex nature of this enzyme system, the Figure indicates that with these substrates the overall GSH S-transferase activity appears to follow Michaelis–Menten kinetics. Also it becomes clear that GSH S-transferase activity towards BOX is only measurable when concentrations of BOX as well as GSH are ap-

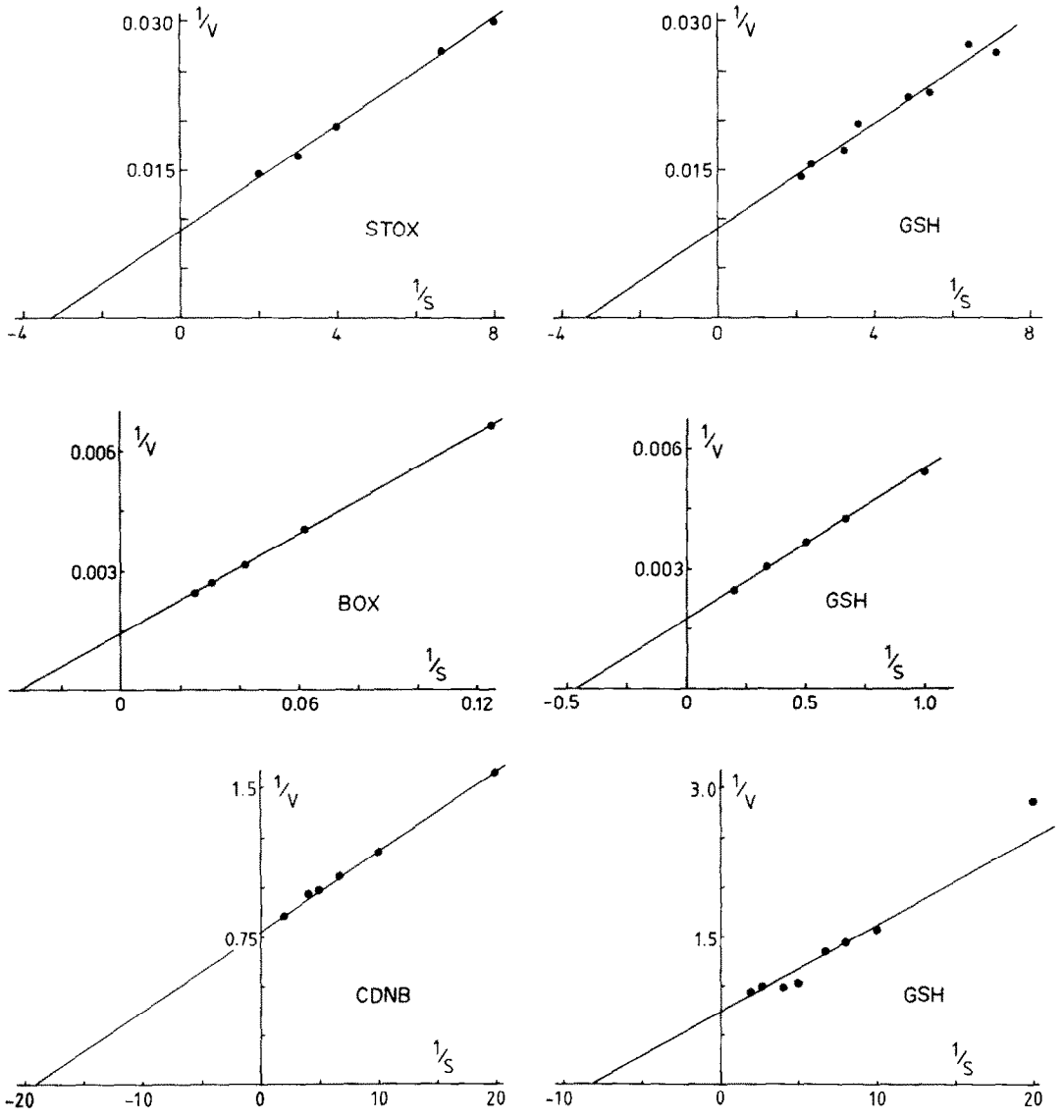

Fig. 1. Lineweaver–Burk plots showing the effect of substrate concentration on the reaction rate of rat liver GSH S-transferase activity.
Upper panel: styrene oxide (STOX) and GSH as substrate variables respectively (the second substrate concentrations were fixed at 0.5 mM).
Middle panel: 1,2-butylene oxide (BOX) and GSH as substrate variables respectively (for the left graph the GSH concentration was fixed at 2 mM, for the right one the BOX concentration was fixed at 40 mM).
Lower panel: 1-chloro-2,4-dinitrobenzene (CDNB) and GSH as substrate variables respectively (the second substrate concentrations were fixed at 0.5 mM).
Incubations were for 5-min at 37°, in 0.13 M phosphate buffer. Using STOX or BOX as substrate, the cytosolic protein content was 0.5 mg/ml, in medium buffered at pH 8.0; using CDNB as the substrate, the cytosolic protein content was 15 μg/ml, in medium buffered at pH 7.0.
1/S is expressed as mM^{-1}; 1/V is expressed as $nmoles^{-1}$ mg min for the substrates STOX and BOX, and as $\mu moles^{-1}$ mg min for the substrate CDNB.

preciably higher than when using the other two substrates.

Apparent Michaelis–Menten kinetic parameters were derived by computer calculation and are presented in Table 1 and indicate a more than 500-fold difference in enzyme affinity for the electrophilic substrate, and a 20-fold difference in affinity for GSH. The individual maximum initial rates in the three pairs of GSH and electrophilic substrate appears to be equal to each other; between the combinations the greatest difference is observed for STOX/GSH vs CDNB/GSH; however, the difference is not more than a factor of 12.

During the pretreatment of rats with enzyme inducing agents no signs of impairment of health became apparent. Body wt of control rats increased by 1.8 ± 1.0 per cent per day, for the groups treated with PB, MC or TCDD these figures were 3.1 ± 0.2,

Table 1

Apparent kinetic parameters of glutathione S-transferase activities towards styrene oxide, 1,2-butylene oxide, 1-chloro-2,4-dinitrobenzene and glutathione, in liver cytosol from untreated rats

	Substrates					
	Styrene oxide	Glutathione*	1,2-Butylene oxide	Glutathione†	1-Chloro-2,4-dinitrobenzene	Glutathione‡
K_m (mM)	0.30	0.30	28.9	2.1	0.052	0.120
V_{max} (nmoles/mg/min)	112	111	695	568	1280	1370

* 0.5 mM Styrene oxide, 0.5 mM glutathione.
† 40 mM Butylene oxide, 2 mM glutathione.
‡ 0.5 mM Chlorodinitrobenzene, 0.5 mM glutathione.

Table 2

Glutathione S-transferase activities of rat liver cytosol before and after treatment with enzyme inducing agents

		Substrates					
		Styrene oxide		1,2-Butylene oxide		1-Chloro-2,4-dinitrobenzene	
Pretreatments		Per mg of hepatic protein	Per gram of body wt	Per mg of hepatic protein	Per gram of body wt	Per mg of hepatic protein	Per gram of body wt
Phenobarbital	Control	72 ± 9	125 ± 14	456 ± 22	791 ± 77	1150 ± 200	1995 ± 251
	Induced	99 ± 4¶	181 ± 19‖	528 ± 81†	966 ± 128†	1650 ± 180§	3019 ± 368‖
Methylcholanthrene	Control	62 ± 4	106 ± 14	276 ± 48	472 ± 72	1390 ± 20	2378 ± 302
	Induced	127 ± 7†‡	148 ± 15§	410 ± 36‖	476 ± 52*	2110 ± 230¶	2451 ± 279*
Tetrachlorodibenzo-*p*-dioxin	Control	77 ± 6	145 ± 11	364 ± 19	685 ± 48	1210 ± 210	2279 ± 280
	Induced	85 ± 12*	222 ± 23**	400 ± 20‡	1044 ± 71**	1790 ± 240§	4670 ± 486**

Values are given as means of product formation in nmoles/min ± S.D.; for each pretreatment livers from four rats were individually assayed, at least in duplicate. Controls were treated with the vehicles only.
* $P > 0.5$, † $0.5 > P > 0.2$, ‡ $0.2 > P > 0.1$, § $0.1 > P > 0.05$ (not significant), ‖ $P < 0.05$, ¶ $P < 0.02$, ** $P < 0.01$, †† $P < 0.001$.

0.6 ± 0.4 and 2.3 ± 0.5 per cent per day respectively. Livers from PB treated rats were 33 ± 19 per cent heavier than livers from controls, for the MC and TCDD treated rats these values were 32 ± 20 and 39 ± 9 per cent respectively.

Pretreatment of rats with inducing agents has a marked influence on GSH S-transferases (Table 2). The results exhibit clear-cut statistical differences between the activities expressed as per mg of cytosolic liver protein and per gram of body wt, which is obvious for the MC and TCDD pretreated animals. These effects may be partly due to the decrease in cytosolic protein content per gram of wet liver wt following enzyme induction, which was observed for the groups pretreated with PB or MC only.

It becomes clear from Table 2 that MC pretreatment appears to have its greatest influence on the

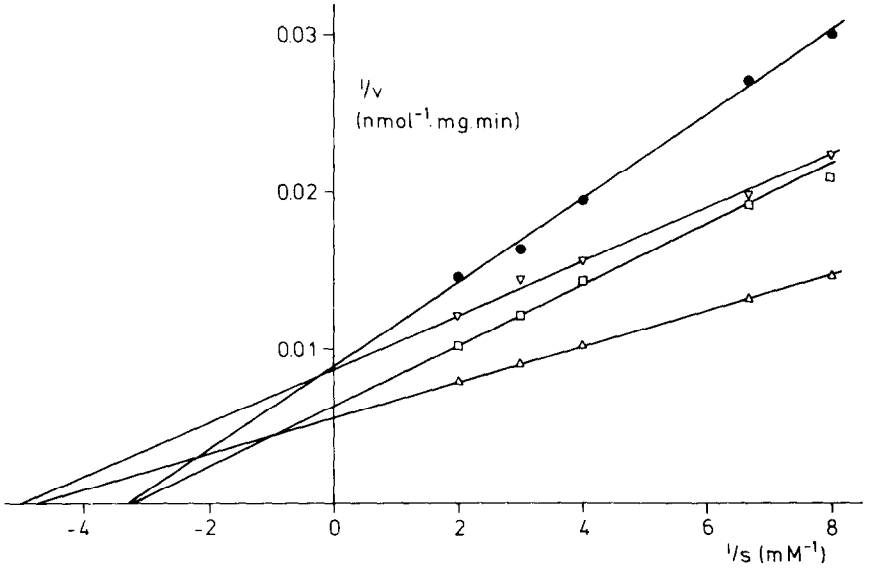

Fig. 2. Lineaver–Burk plots showing the effect of pretreatment with inducing agents on the rat liver GSH S-transferase activity using *styrene oxide* as the substrate variable. The induced activities are given together with the control activity.
●: Control; □: phenobarbital, △: methylcholanthrene, ▽: tetrachlorodibenzo-*p*-dioxin pretreated rats. Conditions were as in Fig. 1.

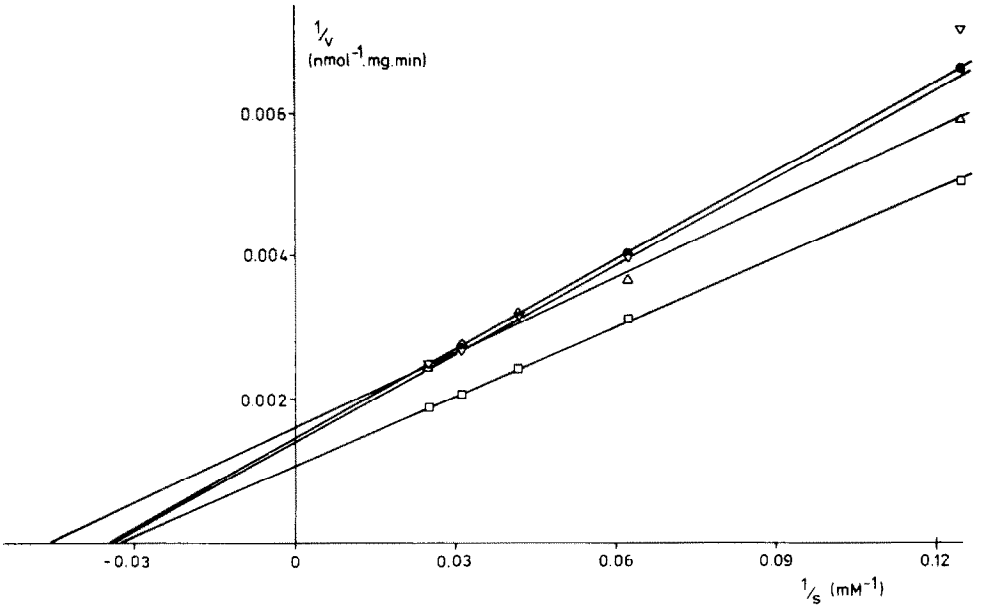

Fig. 3. Lineweaver–Burk plots showing the effect of pretreatment with inducing agents on the rat liver GSH S-transferase activity using *1,2-butylene oxide* as the substrate variable. The induced activities are given together with the control activity.
●: Control; □: phenobarbital, △: methylcholanthrene, ▽: tetrachlorodibenzo-*p*-dioxin pretreated rats. Conditions were as in Fig. 1.

sp. act. expressed as per mg of cytosolic liver protein, in contrast to the effect of induction with TCDD which exhibits its effect merely on the activities expressed as per gram body wt.

Not all observed differences are significant. It is, however, interesting to note that in all cases the various pretreatments resulted in increasing activities.

Figures 2–4 illustrate the apparent kinetic behaviour when STOX (Fig. 2), BOX (Fig. 3) and CDNB (Fig. 4) were used as the substrate variable in Lineweaver–Burk plots: in each of these Figs, GSH S-transferase activity in livers from untreated animals is depicted together with the activity in livers from animals treated with PB, MC or TCDD. Also after enzyme inducer pretreatment, the transferase activities appear to obey Michaelis–Menten kinetics.

The calculated apparent kinetic parameters before and after the pretreatments with inducing

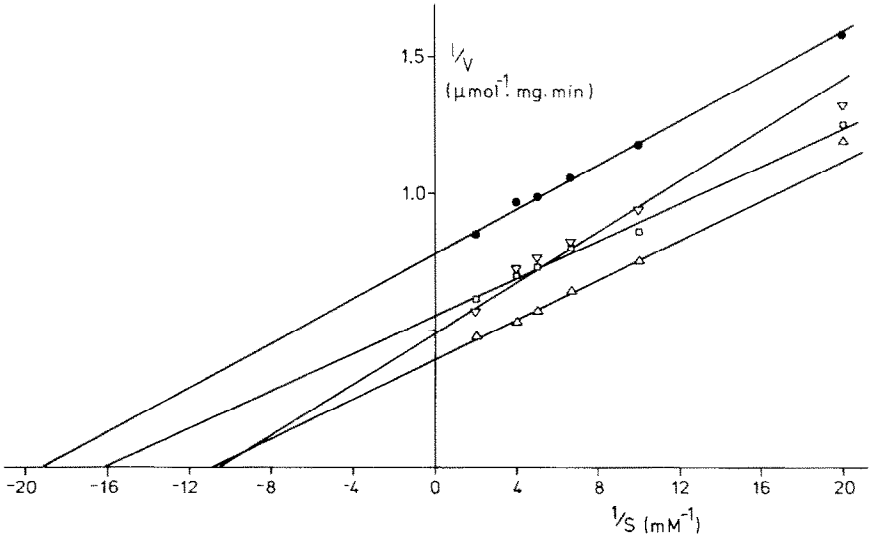

Fig. 4. Lineweaver–Burk plots showing the effect of pretreatment with inducing agents on the rat liver GSH S-transferase activity using *1-chloro-2,4-dinitrobenzene* as the substrate variable. The induced activities are given together with the control activity.
●: Control; □: phenobarbital, △: methylcholanthrene, ▽: tetrachlorodibenzo-*p*-dioxin pretreated rats. Conditions were as in Fig. 1.

Table 3.

Apparent kinetic parameters for glutathione S-transferase activities towards styrene oxide, 1,2-butylene oxide and 1-chloro-2,4-dinitrobenzene, in liver cytosol from rats pretreated with enzyme inducing agents

Substrate	Pretreatment		K_m (mM)	V_{max} (nmoles/mg/min)
Styrene oxide	Phenobarbital	Control	0.25 ± 0.18	109 ± 30
		Induced	0.31 ± 0.14*	159 ± 27‖
	Methylcholanthrene	Control	0.30 ± 0.10	93 ± 18
		Induced	0.21 ± 0.06†	180 ± 11††
	Tetrachlorodibenzo-*p*-dioxin	Control	0.35 ± 0.12	129 ± 23
		Induced	0.20 ± 0.05‖	116 ± 15*
1,2-Butylene oxide	Phenobarbital	Control	42.9 ± 21.1	939 ± 263
		Induced	29.7 ± 5.1†	924 ± 172*
	Methylcholanthrene	Control	39.6 ± 6.6	563 ± 142
		Induced	21.4 ± 5.0¶	616 ± 63*
	Tetrachlorodibenzo-*p*-dioxin	Control	24.4 ± 5.0	593 ± 69
		Induced	30.6 ± 3.0§	718 ± 35‖
1-Chloro-2,4-dinitrobenzene	Phenobarbital	Control	0.046 ± 0.008	1270 ± 240
		Induced	0.062 ± 0.015‡	1820 ± 230‖
	Methylcholanthrene	Control	0.079 ± 0.012	1540 ± 70
		Induced	0.092 ± 0.016†	2550 ± 280††
	Tetrachlorodibenzo-*p*-dioxin	Control	0.058 ± 0.016	1290 ± 240
		Induced	0.094 ± 0.022**	2040 ± 360††

Values are given as means ± S.D.; for each pretreatment livers from four rats were individually assayed, at least in duplicate. Control rats were treated with the vehicles only.

* $P > 0.5$, † $0.5 > P > 0.2$, ‡ $0.2 > P > 0.1$, § $0.1 > P > 0.05$ (not significant). ‖ $P < 0.02$, ¶ $P < 0.01$, ** $P < 0.005$, †† $P < 0.001$.

agents are given in Table 3. In contrast to Figs 2–4, this Table compares apparent kinetic parameters after enzyme inducer pretreatment to those of control rats, while in Figs 2–4, untreated (control) animals were compared with the induced ones.

The inducing agents influence the apparent Michaelis–Menten kinetic parameters towards the electrophilic substrates, as illustrated in Table 3. Using STOX as the substrate, a significant increase in the V_{max} values was found after induction with PB and MC respectively, whereas the K_m remained unchanged. TCDD led to a decrease of K_m, while V_{max} was unaffected. When BOX was employed as substrate, MC pretreatment resulted in a lower K_m and TCDD enhanced V_{max}, while PB produced no effects at all. Towards CDNB, an increase of the V_{max} values was observed following all pretreatments; only TCDD increased also the K_m value.

DISCUSSION

The detoxification of reactive intermediates in biologic systems is of utmost importance for the prevention of chemical lesions such as carcinogenesis, mutagenesis, tissue necrosis etc. Hence enzymatic mechanisms of detoxification pathways play an important role in the susceptibility of tissues or organisms to chemical lesions. The GSH S-transferase system (E.C. 2.1.5.18) is one of them; a second, especially acting on epoxides, is the epoxide hydratase (E.C. 4.2.1.63). The present study focused on the rat liver cytosol—the liver being the major metabolizing organ of mammalian organisms—in order to obtain information about the total GSH S-transferase capacity and the influence of inducing agents.

As appears from Table 2 values were found for the rat liver GSH S-transferase activity towards STOX of about 70 nmoles/mg protein/min. This resembles closely the activity reported by Mukhtar and Bresnick [18], but differs from the results of Marniemi and Parkki [32].

GSH S-transferase activity towards CDNB was found to be about 1100 nmoles/mg protein/min by Hales and Neims [19, 34] which is in good agreement with the values reported here.

Concerning BOX, to our knowledge reports dealing with this epoxide as a substrate for GSH S-transferase activity have not been published.

As to the Michaelis–Menten kinetic parameters, we found apparent K_m values for STOX as well as for GSH of 0.3 mM. Marniemi and Parkki [32] reported these values to be 0.38 and 0.84 mM respectively; more deviating values were found with enzyme preparations obtained from other species [30, 31].

Comparison of the GSH S-transferase activities towards the three substrates used in this study shows that there are considerable differences, in activities as well as in K_m and V_{max} values. Concerning the epoxides, obviously an important role is played by the physicochemical properties of the epoxide, especially the stability of the oxirane-ring is crucial. It is noteworthy, however, that the affinity for GSH also shows a clear difference, which could possibly be explained taking into account that here an overall activity is measured of a mixture of at least six iso-enzymes, while also the two-substrate enzymic mechanism can be of influence [21, 22, 35].

From the results compiled in Tables 1 and 2, it seems possible to conclude that an appreciable conjugation capacity is not primarily achieved by a relatively high affinity towards a certain substrate; the amount of the necessary set of iso-enzymes and the V_{max} appears to be of more importance.

The apparent kinetic parameters of the GSH S-epoxide transferase activity using STOX as the substrate indicate an increase of V_{max} values of 46 and 94 per cent after induction with PB and MC respectively, while no differences could be observed in the K_m values (Table 3). This agrees well with the findings of Kaplowitz *et al.* [17]. Marniemi and Parkki [32] observed an increase in the sp. act. after PB, but not after MC pretreatment. Interestingly the

inducing effects using the other epoxide substrate (BOX) are quite different: only MC appears to decrease the K_m value (Table 3).

For the transferase activity towards CDNB, the V_{max} after PB and MC pretreatment increased by 43 and 66 per cent respectively, in agreement with Kaplowitz *et al.* [17] and Hales and Neims [19, 36]. Here too there are no effects of these inducing agents on the K_m, but the K_m and V_{max} of the MC-pretreated control rats are increased in comparison to the analogous values of the other controls. This phenomenon was also observed by Hales and Neims for 1,2-dichloro-4-nitrobenzene [19], dissolved in corn oil. The most plausible explanation for these enhancements seems to be that they are caused by the oil injections.

In general it can be concluded that GSH S-transferases are inducible, but induction with PB or MC hardly affects the apparent K_m, whereas the apparent V_{max} is enhanced. These results point to an increase of the total amount of specific enzymes per mg of cytosolic protein following PB or MC pretreatment, while only the observations using BOX as the electrophilic substrate give slight indication of a possible change in enzyme characteristics. Hence a fundamental distinction between the effects of PB and MC in inducing the GSH S-transferase system cannot be made.

In contrast to the inductive effects of PB and MC are the present findings of the inductive capacity of TCDD. This highly toxic substance is reported to be a potent stimulator of microsomal mixed function oxidases [37] and UDP-glucuronyl transferase [38]. The only observation on effects of TCDD on GSH S-transferases was made by Kirsch *et al.* [39], who stated that it increased the total amount of rat hepatic and renal ligandin (GSH S-transferase B). Concerning mixed function oxidase, TCDD is thought to act especially on cytochrome P448 (P,450) and the related aryl hydrocarbon hydroxylase activity, in a manner comparable to, but 30,000 times more potent than MC [40, 41].

The results presented here indicate that after two doses of TCDD the apparent K_m of the GSH S-transferase activity towards epoxides seems to decrease, while the V_{max} is not changed. In contrast, the apparent K_m of GSH S-transferase activity towards CDNB is enhanced, accompanied by an increase of the V_{max}. These findings suggest that following TCDD treatment different forms of GSH S-transferases are induced, which fits well into such a postulate suggested by Poland *et al.* [41, 42]. The effect of TCDD in our experiments, however, is clearly distinct from that of MC, and in that respect not in agreement with the effects of MC and TCDD on aryl hydrocarbon hydroxylase induction [40–42]. On the basis of the present findings it cannot be excluded that more complex processes are involved.

In view of the role of GSH S-transferases in detoxifying processes, the study of the role of inductive effects on these transferases, especially by TCDD, seems to be a powerful tool for extending the knowledge of the underlying mechanisms.

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REFERENCES

1. L. F. Chasseaud, *Drug Metab. Rev.* **2,** 185 (1973).
2. E. Boyland and L. F. Chasseaud, *Adv. Enzymol.* **32,** 172 (1969).
3. I. M. Arias and W. B. Jakoby (Eds), in Glutathione–metabolism and function, Raven Press, New York (1976).
4. A. Meister and S. S. Tate, *Ann. Rev. Biochem.* **45,** 559 (1976).
5. J. Booth, E. Boyland and P. Sims, *Biochem. J.* **79,** 516 (1961).
6. M. J. Pabst, W. H. Habig and W. B. Jakoby, *Biochem. biophys. Res. Commun.* **52,** 1123 (1973).
7. M. K. Johnson, *Biochem. J.* **98,** 44 (1966).
8. H. Mukhtar and E. Bresnick, *Chem. Biol. Interact.* **15,** 59 (1976).
9. G. Clifton and N. Kaplowitz, *Cancer Res.* **37,** 788 (1977).
10. P. L. Grover and P. Sims, *Biochem. J.* **90,** 603 (1964).
11. K. Kamisaka, W. H. Habig, J. N. Ketley, I. M. Arias and W. B. Jakoby, *Eur. J. Biochem.* **60,** 153 (1975).
12. A. J. Cohen, J. N. Smith and H. Turbert, *Biochem. J.* **90,** 457 (1964).
13. W. H. Habig, M. J. Pabst and W. B. Jakoby, *J. biol. Chem.* **249,** 7130 (1974).
14. W. H. Habig, M. J. Pabst and W. B. Jakoby, *Archs Biochem. Biophys.* **175,** 710 (1976).
15. W. H. Habig, M. J. Pabst, G. Fleischer, Z. Gatmaitan, I. M. Arias and W. B. Jakoby, *Proc. Natl. Acad. Sci., U.S.A.* **71,** 3879 (1974).
16. F. Oesch, *Xenobiotica* **3,** 305 (1973).
17. N. Kaplowitz, J. Kuhlenkamp and G. Clifton, *Biochem. J.* **146,** 351 (1975).
18. H. Mukhtar and E. Bresnick, *Biochem. Pharmac.* **25,** 1081 (1976).
19. B. F. Hales, A. H. Neims, *Biochem. Pharmac.* **26,** 555 (1977).
20. G. Clifton, N. Kaplowitz, J. D. Wallin and J. Kuhlenkamp, *Biochem. J.* **150,** 259 (1975).
21. M. J. Pabst, W. H. Habig and W. B. Jakoby, *J. biol. Chem.* **249,** 7140 (1974).
22. B. Mannervik and P. Askelöf, *FEBS Lett.* **56,** 218 (1975).
23. N. Kaplowitz, G. Clifton, J. Kuhlenkamp and J. D. Wallin, *Biochem. J.* **158,** 243 (1976).
24. D. L. Cinti, P. Moldeus and J. B. Schenkman, *Biochem. Pharmac.* **21,** 3249 (1972).
25. O. H. Lowry, H. L. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193,** 265 (1951).
26. T. A. Fjellstedt, R. H. Allen, B. K. Duncan and W. B. Jakoby, *J. biol. Chem.* **248,** 3702 (1973).
27. T. Hayakawa, S. Udenfriend, H. Yagi and D. M. Jerina, *Archs Biochem. Biophys.* **170,** 438 (1975).
28. G. L. Ellman, *Archs Biochem. Biophys.* **82,** 70 (1959).
29. A. J. Baars, E. W. van Dongen and D. D. Breimer, *Pharm. Weekbl.* **112,** 1117 (1977).
30. T. Hayakawa, R. A. Lemahieu and S. Udenfriend, *Archs Biochem. Biophys.* **162,** 223 (1974).
31. M. O. James, J. R. Fouts and J. R. Bend, *Biochem. Pharmac.* **25,** 187 (1976).
32. J. Marniemi and M. G. Parkki, *Biochem. Pharmac.* **24,** 1569 (1975).
33. E. Boyland and K. Williams, *Biochem. J.* **94,** 190 (1965).
34. B. F. Hales and A. H. Neims, *Biochem. J.* **160,** 223 (1976).

35. J. Jakobson, P. Askelöf, M. Warholm and B. Mannervik, *Eur. J. Biochem.* **77,** 253 (1977).
36. B. F. Hales and A. H. Neims, *Biochem. J.* **160,** 231 (1976).
37. A. P. Poland and A. S. Kende, *Fedn Proc.* **35,** 2404 (1976).
38. G. W. Lucier, O. S. McDaniel and G. E. R. Hook, *Biochem. Pharmac.* **24,** 325 (1975).
39. R. Kirsch, G. Fleischer, K. Kamisaka and I. M. Arias, *J. clin. Invest.* **55,** 1009 (1975).
40. A. P. Poland and E. Glover, *Molec. Pharmac.* **10,** 349 (1974).
41. A. P. Poland, E. Glover, J. R. Robinson and D. W. Nebert, *J. biol. Chem.* **249,** 5599 (1974).
42. A. P. Poland, E. Glover and A. S. Kende, *J. biol. Chem.* **251,** 4936 (1976).