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# HUMAN PLACENTAL GLUTATHIONE *S*-TRANSFERASE-MEDIATED METABOLISM OF METHYL PARATHION

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**Abstract**—The ability of human placental glutathione *S*-transferase (GSHTr) to metabolize methyl parathion (MeP) was examined. MeP was found to be a substrate for both partially purified pre-term and highly purified term placental GSHTr. The characterization of the reaction by high performance liquid chromatography revealed the presence of desmethyl parathion (DesMeP) as the sole metabolite. Term placental GSHTr activity towards MeP ranged from 2.22 to 3.53 nmoles DesMeP formed $\cdot$ min$^{-1}$ $\cdot$ mg$^{-1}$ while an activity of 0.60 to 1.12 nmoles DesMeP formed $\cdot$ min$^{-1}$ $\cdot$ mg$^{-1}$ was observed with the pre-term placental enzyme. The absence of the O-dearylation reaction by pre-term and term placental GSHTr represents a major species- and/or tissue-specific difference.

In the past few years, OPs§ have replaced the persistent organochlorine pesticides. Residues of OPs are now found in the water [1] and the food we consume [2, 3]. Other sources of exposure include the work environment [4], transfer from contaminated laundry equipment [5], and drift from aerial application [6]. Reports have documented the exposure of a pregnant women [7] and transplacental passage [8] of OPs.

MeP was selected as the representative of OPs because: (1) it is used extensively (present in 609 EPA registered formulations); (2) transplacental passage of MeP has been shown in rats [9, 10]; (3) MeP is both embryotoxic [9] and teratogenic in laboratory animals [11, 12]; and (4) several investigators, using various *in vitro* test systems, have demonstrated that MeP alone or in combination with other OPs is mutagenic [13–16]. Chromosomal aberrations have also been reported in pesticide formulators and applicators exposed to MeP [17, 18]. These reports on reproductive and genetic toxicity raise serious questions regarding the safety of the human conceptus from maternal exposure to MeP.

It has been shown repeatedly that human placenta contains very little cytochrome P-450 and minimal cytochrome P-450-mediated monooxygenase activity toward specific xenobiotics [19]. Consequently, the activation of MeP to the corresponding oxon or the oxidative formation of *p*-nitrophenol is expected to be negligible. In contrast, a high titer of GSHTr is known to be present in human placenta [20, 21]. Hence, the predominant pathway of biotransformation of MeP is most likely to proceed via conjugation with GSH. GSHTr-mediated metabolism represents a major *in vivo* detoxification pathway in mammals and is the subject of several reviews [22, 23]. A vast amount of information has been amassed on GSHTr reactions with OPs; however, not a single study to date has addressed the *in vitro* GSHTr-dependent biotransformation of MeP by human tissues. Since previous studies on human placental GSHTr have been restricted to model compounds [20, 21], its potential for possible metabolism of pesticides has never been explored. Therefore, our investigation has focused on the human placental metabolism of MeP, in order to gain an understanding of the potential human fetotoxicity of OPs.

Hollingworth *et al.* [24] suggested the involvement of different isozymes of rat hepatic GSHTr in the biotransformation of OPs. Subsequent studies [25, 26] have demonstrated that MeP can undergo O-dealkylation and/or O-dearylation by different isozymes purified from rat liver. Therefore, it appears that the type of reaction observed is dependent upon the form of GSHTr. Although both these reactions are generally considered as important *in vivo* detoxification pathways [27, 28], formation of a metabolite containing a *p*-nitrophenolic moiety by dearylation may represent an activation reaction, since phenolic compounds have been reported to be teratogenic [29].

Therefore, the present study was undertaken to determine whether MeP is a substrate for human placental GSHTr and to examine the types of reaction MeP may undergo. Preparations of highly purified, homogeneous term placental GSHTr [30] and partially purified pre-term GSHTr were used in these studies.

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§ Abbreviations: OPs, organophosphorus insecticides; GSHTr, glutathione *S*-transferase (EC 2.5.1.18); MeP, methyl parathion; HPLC, high performance liquid chromatography; DesMeP, desmethyl parathion; GSH, reduced glutathione; CDNB, 1-chloro-2,4-dinitrobenzene; and MeOH, methanol (HPLC grade).

## MATERIALS AND METHODS

MeP (*O,O*-dimethyl *O-p*-nitrophenyl phosphorothioate) was provided gratis by the Monsanto Co. (St. Louis, MO). DesMeP (*O*-methyl *O-p*-nitrophenyl phosphorothioic acid) was synthesized according to the method of Whetstone *et al.* [31]. Para-phenylphenol was procured from Eastman Organic Chemicals (Rochester, NY). GSH was obtained from United States Biochemical (Cleveland, OH). Sodium acetate (HPLC grade) was purchased from Fisher Scientific (Livonia, MI). CDNB was procured from Aldrich (Milwaukee, WI). All other chemicals were obtained commercially.

*Enzyme isolation.* Pre-term placentas were obtained following early second trimester abortions. The procedure was performed surgically (dilatation and evacuation) under general and local anesthesia. Samples were placed immediately on ice and processed within 2 hr of pregnancy termination. Subsequent procedures were carried out at 4° unless specified otherwise. The tissues were weighed, minced, and washed several times with 50 mM Tris buffer, containing 0.25 M sucrose, pH 7.4 (Buffer A), to remove residual blood components. A 20% homogenate in Buffer A was centrifuged at 100,000 *g* for 60 min. The post-microsomal supernatant fraction containing GSHTr was further purified by affinity chromatography, using a Sepharose 4B-GSH affinity column (1.3 × 4.0 cm) as previously described [30]. Term placentas were obtained at the time of elective Cesarean deliveries. The purification of term placental GSHTr was performed as previously described [30]. The enzyme preparations used were found to be apparently homogeneous (Fig. 1) by polyacrylamide gel electrophoresis [30].

*Enzyme assay.* The ability of partially purified human pre-term and purified term placental GSHTr to metabolize MeP was examined. The incubation mixture (0.5 ml) consisted of 0.25 mM MeP, 4 mM GSH, and 150 μg GSHTr in 0.1 M potassium phosphate buffer, pH 7.4. The reaction was allowed to proceed at 37° for 30 min. The incubation vessel was placed on ice to terminate the reaction. Control assays were performed with boiled GSHTr. In the inhibition studies, prior to the addition of GSH and initiation of the reaction with MeP, GSHTr was preincubated with the specific inhibitor for 3 min at 37° in order to eliminate any possible interaction of GSH with the inhibitor. Protein content of placental cytosol was determined according to the method of Lowry *et al.* [32], whereas the dye binding assay of Bradford [33] was used to determine that of affinity and HPLC-purified GSHTr preparations. In the purification experiments, GSHTr activity towards CDNB was determined as previously described [30].

*HPLC analysis.* Once the reaction was terminated, 10 μl of internal standard, *p*-phenylphenol (0.5 mg/ml), was added to the incubation mixture and a 20-μl aliquot of the reaction mixture was analyzed by HPLC. An Altex model 420 microprocessor equipped with two Isco model 825 metering pumps, a Rheodyne model 7125 sample injector, and a SpectroMonitor III variable UV detector, set at 277 nm, was used. Separation was achieved with a Hamilton PRP-1 column (4.1 × 150 mm) maintained at 40° with a circulating bath. The mobile phase consisted of 50 mM acetate buffer, pH 4.0, and MeOH. MeP and DesMeP were resolved using a MeOH gradient (54–100%). The elution program employed was as follows: a flow rate of 1 ml/min and 54% MeOH at time 0; percent MeOH was increased to 100 in 2 min, beginning at time 8 min; flow was increased to 1.7 ml/min in 0.2 min at 9 min; and re-equilibration began at 18 min, at which time the percent MeOH was decreased to 54 over 2 min and the flow rate was further increased to 2 ml/min and maintained for 7.6 min. The amount of DesMeP produced was calculated from standard curves generated by plotting the ratios of peak height of DesMeP to *p*-phenylphenol versus the concentrations of DesMeP.

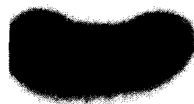

Fig. 1. Polyacrylamide gel electrophoresis of term placental glutathione *S*-transferase. A 100 μg protein sample of purified placental GSHTr was applied.

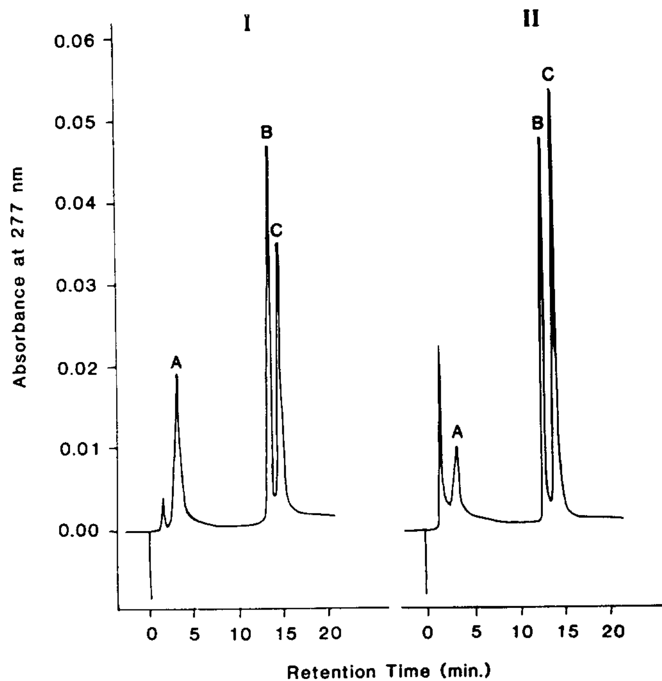


Fig. 2. High performance liquid chromatographic analysis of methyl parathion. (I) Elution profile of standards used: (A) DesMeP, 0.6 nmoles; (B) *p*-pheylphenol, 1.5 nmoles; and (C) MeP, 1.4 nmoles. (II) HPLC analysis of human placental glutathione *S*-transferase-catalyzed MeP metabolism. The reaction mixture consisted of 0.25 mM MeP, 4 mM GSH, and 150 $\mu$g GSHTr in 0.5 ml of 0.1 M potassium phosphate buffer, pH 7.4. The reaction was allowed to proceed at 37° for 30 min, at which time the reaction was terminated by placing the vessel on ice. After the addition of internal standard, *p*-phenylphenol, a 20-$\mu$l aliquot of the reaction was subjected to HPLC analysis.

## RESULTS

The chromatographic profiles of standard compounds and of the MeP utilized and DesMeP produced by placental GSHTr are presented in Fig. 2. The respective retention times (mean ± S.E.) for the standard compounds were 3.30 ± 0.04 for DesMeP (A), 13.40 ± 0.14 for *p*-phenylphenol (B), and 14.55 ± 0.13 min for MeP (C). The peaks observed in the HPLC profile of the incubation mixture containing GSHTr were 3.4, 13.2, and 14.6 min. HPLC analysis indicated the presence of a single metabolite. This metabolite was identified as DesMeP, based on similar retention time. The 1 : 1 ratio observed in the disappearance of MeP and formation of DesMeP confirms DesMeP as the sole metabolite of the term placental GSHTr-catalyzed reaction. No DesMeP production was observed when boiled GSHTr was used.

In the presence of 0.25 mM MeP and 4 mM GSH, the term placental GSHTr-mediated reaction was found to be linear up to 60 min (Fig. 3), with a 0.99 coefficient of determination. Since measurable amounts of DesMeP were detected, a 30-min incubation time was selected for subsequent experiments. Protein dependency of the reaction is shown in Fig. 4. Regression analysis of DesMeP production versus amount of GSHTr used exhibited a linear relationship (coefficient of determination = 0.99).

Term placental GSHTr activity toward MeP is presented in Table 1. The specific activity ranged from 2.22 to 3.53 nmoles DesMeP formed · $min^{-1}$ · $mg^{-1}$, suggesting relatively minor inter-individual differences in the GSHTr activity when purified preparations were used.

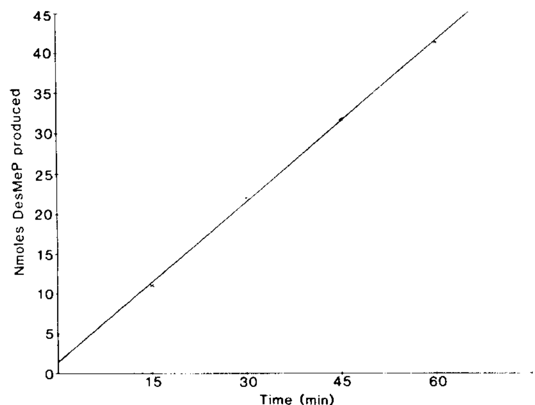


Fig. 3. Time dependency of human placental glutathione *S*-transferase-mediated O-dealkylation of methyl parathion. Assay conditions were 0.25 mM MeP, 4 mM GSH, 1.05 mg enzyme in 3.5 ml of 0.1 M potassium phosphate buffer, pH 7.4. The reaction mixture was incubated at 37°. A 500-$\mu$l aliquot was taken at the prescribed intervals and analyzed by HPLC.

Table 1. Activity of purified term placental glutathione *S*-transferase toward methyl parathion

| Sample No. | Specific activity (nmoles DesMeP formed · min$^{-1}$ · mg$^{-1}$) |
|---|---|
| 1 | 3.27 |
| 2 | 3.53 |
| 3 | 2.60 |
| 4 | 2.91 |
| 5 | 2.22 |
| 6 | 3.00 |
| 7 | 2.95 |
| 8 | 3.24 |
| Mean ± S.E. | 2.92 ± 0.14 |

Assays were performed as described in Materials and Methods.

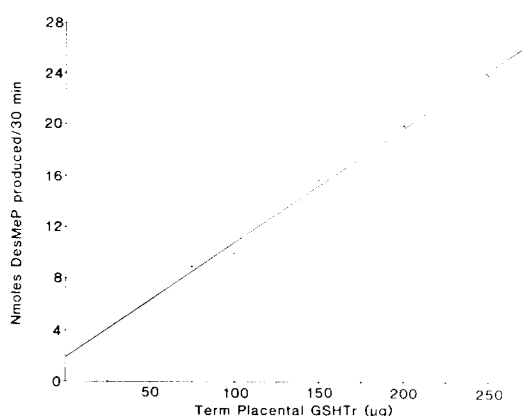


Fig. 4. Protein dependency of human placental glutathione *S*-transferase-mediated O-dealkylation of methyl parathion. MeP and GSH concentrations were maintained at 0.25 and 4 mM, respectively, while the amount of enzyme was varied as indicated. See text for further details.

Table 2 depicts the kinetic parameters of term placental GSHTr for MeP and GSH. Representative Lineweaver–Burk plots of the kinetic data (Sample 1, Table 2) are shown in Figs. 5 and 6. GSH concentration was kept constant at 4 mM for determination of $K_m$ and $V_{max}$ for MeP. At a MeP concentration of 0.25 mM, $K_m$ and $V_{max}$ values for GSH were assessed. The $K_m$ of MeP ranged from 0.20 to 0.51 mM, while the $K_m$ of GSH varied from 0.49 to 0.66 mM. The $V_{max}$ of MeP was more than twice that of GSH, suggesting that the concentration of MeP has a greater effect on the rate of reaction than GSH.

The *in vitro* inhibition of term placental GSHTr-mediated metabolism of MeP by inorganic heavy metals is given in Table 3. Based on preliminary experiments, inhibitor concentrations were chosen to produce about 50% inhibition. Cupric chloride appeared to be the most effective inhibitor, since a 4-fold lower concentration of cupric chloride resulted in about the same level of inhibition observed at 1.0 mM mercuric chloride or cadmium chloride. The observed inhibition caused by all three metals was statistically significant ($P \leq 0.05$) as compared to control.

The results of the purification of GSHTr from human pre-term placenta are given in Table 4. The number of samples examined thus far reflect the norm and variation in placental GSHTr activity at the corresponding gestational periods. An overall activity of 0.47 ± 0.06 μmoles · min$^{-1}$ · mg$^{-1}$ was observed towards CDNB with the crude fractions. The average specific activity of the 4B-GSH fractions was 91.68 ± 12.52 μmoles · min$^{-1}$ · mg$^{-1}$. A substantial, overall purification of 220-fold was achieved using affinity chromatography alone. Recovery of enzymatic activity averaged about 75%.

MeP metabolism studies were also conducted with pre-term placental GSHTr (Table 5). HPLC analysis showed the production of a single metabolite, DesMeP, indicating qualitative similarity between pre-term and term placental GSHTr in the metabolism of MeP. Data also suggest that pre-term placenta is capable of significant biotransformation of MeP. It must be noted that pre-term GSHTr preparations used were partially purified by affinity chromatography, due to lack of sufficient material for further purification by HPLC. In view of this, a direct comparison of term and pre-term activities toward MeP is precluded.

## DISCUSSION

Accurate assessment of MeP metabolism can only be accomplished with specific and sensitive assays. Most of the procedures previously used to quantitate

Table 2. Kinetic parameters of term placental glutathione *S*-transferase

| | Methyl parathion | | Glutathione | |
|---|---|---|---|---|
| Sample No. | $K_m$ (mM) | $V_{max}$ (nmoles DesMeP formed · min$^{-1}$ · mg$^{-1}$) | $K_m$ (mM) | $V_{max}$ (nmoles DesMeP formed · min$^{-1}$ · mg$^{-1}$) |
| 1 | 0.47 | 9.52 | 0.62 | 4.21 |
| 2 | 0.30 | 5.46 | 0.66 | 3.68 |
| 3 | 0.51 | 8.07 | 0.51 | 2.57 |
| 4 | 0.20 | 4.58 | 0.49 | 2.71 |
| Mean ± S.E. | 0.37 ± 0.07 | 6.91 ± 1.14 | 0.57 ± 0.04 | 3.29 ± 0.39 |

See text for further details.

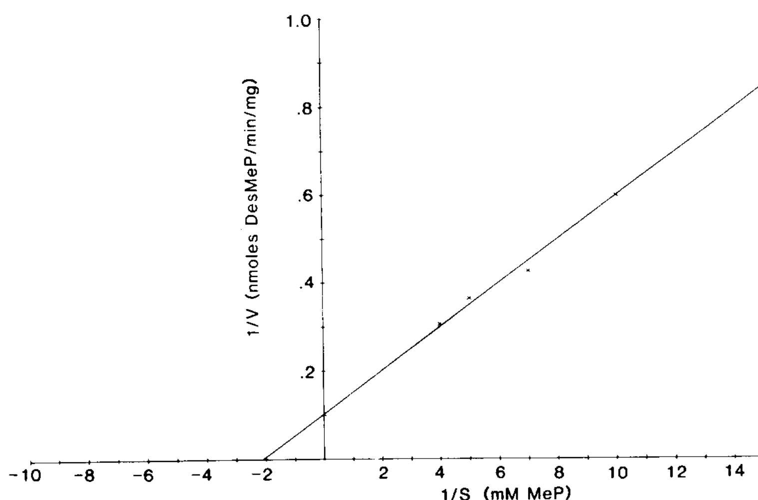


Fig. 5. Effects of methyl parathion concentration on the O-dealkylation activity of term placental glutathione *S*-transferase. Assays were performed in the presence of 4 mM GSH, 150 $\mu$g enzyme, and the indicated concentrations of MeP, as specified in Materials and Methods.

the biotransformation of MeP were based on either radiometry [34, 35] or gas–liquid chromatography [36, 37]. Besides being cumbersome and time consuming, these methods possess certain inherent problems, such as sample loss related to efficiency of extraction and tedious sample clean-up. A spectrophotometric assay for MeP has been reported [38], but it was not reproducible in our hands. Therefore, the HPLC method was devised, which allowed a direct measurement of both substrate depletion and product formation. Furthermore, the metabolite is amenable to further analysis. The HPLC method is extremely sensitive with lower limits of detection for DesMeP in the pmole range. Moreover, sample clean-up prior to analysis, the use of radiolabeled substrate, and laborious methodology are eliminated.

Recent studies [39, 40] have reported that placental GSHTr activity is not altered following exposure of pregnant women to the constituents of cigarette smoke. Animal studies have also indicated that placental GSHTr is refractory to the effects of such inducers as *trans*-stilbene oxide [41], phenobarbital, butylated hydroxyanisole, 2-acetylaminofluorene, and 3-methyl-4-dimethylaminoazobenzene [42]. These results suggest that the induction of human placental GSHTr upon exposure to xenobiotics is questionable. In addition, the reported presence of a single form of human placental GSHTr [20, 21, 30] would negate possible, selective induction of certain isozymes. Hence, exposure of pregnant women to xenobiotics is acknowledged, but is not of paramount concern in this study.

In contrast to the low cytochrome P-450-mediated

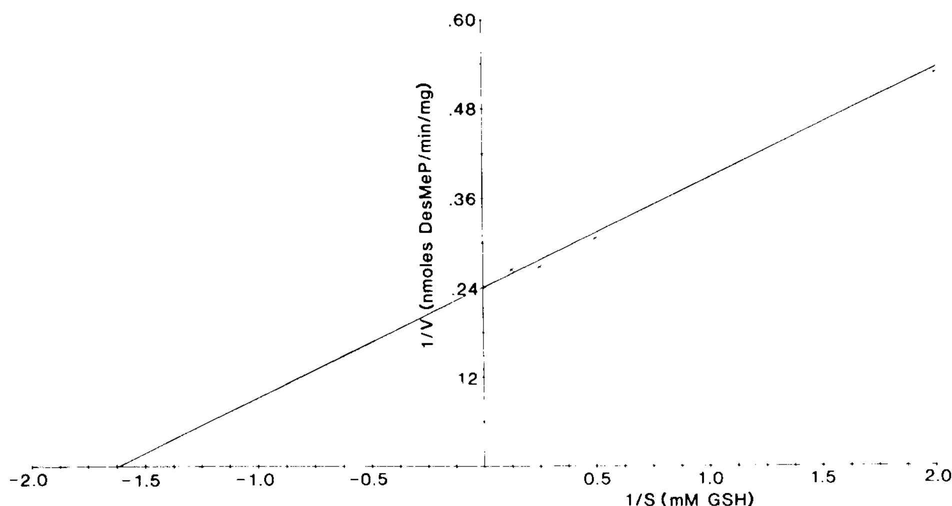


Fig. 6. Effects of glutathione concentration of the O-dealkylation activity of term placental glutathione *S*-transferase. Assays were performed in the presence of 0.25 mM MeP, 150 $\mu$g GSHTr, and the indicated concentrations of GSH. See text for further details.

Table 3. Inhibition of term placental glutathione *S*-transferase-mediated metabolism of methyl parathion by various heavy metals

| Sample | % Inhibition | | | |
|---|---|---|---|---|
| | Control | 1 mM $HgCl_2$ | 1 mM $CdCl_2$ | 0.25 mM $CuCl_2 \cdot 2H_2O$ |
| 1 | 0.0 (3.00) | 45.2 (1.65) | 49.0 (1.53) | 49.0 (1.53) |
| 2 | 0.0 (2.95) | 34.7 (1.93) | 45.6 (1.61) | 49.5 (1.49) |
| 3 | 0.0 (3.24) | 52.6 (1.53) | 50.4 (1.61) | 51.0 (1.59) |
| Mean ± S.E. | (3.06 ± 0.09) | 44.2 ± 5.2 (1.70 ± 0.12)* | 48.3 ± 1.4 (1.58 ± 0.03)† | 49.8 ± 0.6 (1.54 ± 0.03)† |

Specific activity values are expressed as nmoles DesMeP formed · $min^{-1} \cdot mg^{-1}$ and are given in parentheses. Experiments were performed as described in Materials and Methods.
* $P \leq 0.05$ as compared to control.
† $P \leq 0.005$ as compared to control.

Table 4. Purification of human pre-term placental glutathione *S*-transferase by affinity chromatography

| Gestation (weeks) | N | Crude spec. act.* | 4B-GSH spec. act.* | Purification† | % Recovery† |
|---|---|---|---|---|---|
| 13 | 1 | 0.44 | 65.18 | 149 | 93 |
| 14 | 1 | 0.33 | 36.97 | 112 | 149 |
| 15 | 4 | 0.49 ± 0.08 | 74.38 ± 26.43 | 153 | 68 |
| 16 | 6 | 0.62 ± 0.26 | 126.13 ± 43.61 | 246 | 61 |
| 17 | 1 | 0.50 | 135.94 | 271 | 110 |
| 18 | 2 | 0.32 | 70.03 | 236 | 76 |
| 19 | 2 | 0.27 | 104.15 | 405 | 65 |
| 20 | 1 | 0.26 | 35.00 | 135 | 45 |
| 21 | 1 | 0.67 | 65.80 | 98 | 91 |
| 23 | 1 | 0.37 | 92.13 | 252 | 65 |

See text for further details.
* Expressed in μmoles CDNB conjugate formed · $min^{-1} \cdot mg^{-1}$ (mean ± S.E., when applicable).
† Reported as an average whenever applicable.

Table 5. Pre-term placental glutathione *S*-transferase activity toward methyl parathion*

| Sample No. | Gestation (weeks) | Specific activity (nmoles DesMeP formed · $min^{-1} \cdot mg^{-1}$) |
|---|---|---|
| 1 | 15 | 0.85 |
| 2 | 16 | 0.64 |
| 3 | 16 | 0.72 |
| 4 | 17 | 1.12 |
| 5 | 18 | 0.99 |
| 6 | 20 | 0.60 |
| 7 | 23 | 0.82 |
| Mean ± S.E. | | 0.82 ± 0.07 |

* Transferase was purified solely by affinity chromatography.

monooxygenase activity [19], the placenta exhibits rather substantial GSHTr activity [20, 21]. The specific activity of the partially purified pre-term placental GSHTr towards CDNB (Table 4) does not differ statistically from that of term placenta [30], although the crude activity of pre-term placenta is approximately 2.5-fold greater than that of term [30].

Two independent groups [25, 26] have demonstrated that purified isozymes of rat liver GSHTr can catalyze O-dealkylation and/or O-dearylation of MeP, suggesting that the nature of the transferase may be responsible for differences in catalysis. The isozymes of rat liver GSHTrs obtained by further purification of fraction I [26], which did not bind the DEAE-cellulose and are recognized as basic isozymes [43, 44], were reported to catalyze ≥ 98% of the total metabolism of MeP via O-dealkylation.

In contrast, the fraction II isozymes [26], which did bind to DEAE-cellulose and are considered as acidic and/or neutral forms [45, 46], catalyzed both O-dealkylation and O-dearylation. The absence of the *O*-aryl conjugate suggests that placental GSHTr, an anionic protein, is catalytically different than the acidic and/or neutral forms of rat liver GSHTr.

The data presented in this paper constitute the first report of purified human GSHTr-mediated metabolism of a commonly used insecticide, MeP. The average specific activity of 2.92 nmoles $\cdot$ min$^{-1}$ $\cdot$ mg$^{-1}$ observed with term placental GSHTr toward MeP (Table 1) is comparable to the rates of 3.98 nmoles $\cdot$ min$^{-1}$ $\cdot$ mg$^{-1}$ reported for purified rat liver GSHTr [25] and 1.84 nmoles $\cdot$ min$^{-1}$ $\cdot$ mg$^{-1}$ for Sephadex G-50 gel supernatant fraction of rat liver [35]. However, the placental GSHTr activity is 3.7-fold less than that reported by Motoyama and Dauterman [26] for a presumably, basic isozyme of rat liver GSHTr. The observed activity of both term and pre-term placental (Table 5) GSHTr should be considered as significant, since extrahepatic metabolism of MeP in the rat is only about 2–10% of that observed in liver [35].

The average $K_m$ for MeP calculated with placental GSHTr (Table 2) is consistent with the values of 0.51 and 0.21 for mouse and sheep liver GSHTr [38] respectively. However, the mean $K_m$ value for GSH (Table 2) is approximately 2–3 times that reported for sheep and mouse liver GSHTr [38], suggesting major differences in the affinity of GSHTr from different sources for GSH. Comparison with values for human GSHTr is not possible due to lack of reported data.

Exposure to each of the metal salts examined resulted in a statistically significant inhibition of term placental GSHTr (Table 3). Since term placenta contains a single form of GSHTr, it is possible to compare inhibition data obtained for MeP with those reported for CDNB [21]. The necessity of such a comparison arises from the fact that studies on OP metabolism by human placental GSHTr have not been reported. The results for cadmium chloride are consistent with the reported value [21]. However, the percent inhibition observed with mercuric chloride is 50% less than that determined by Mannervik *et al.* [21]. Comparative data for cupric chloride were not available. Inhibition data support the contention that the cysteinyl residues of placental GSHTr are integral to catalytic function [20].

This is also the first paper that reports the purification of human pre-term placental GSHTr. A qualitative change in human placental GSHTr during gestation has not been documented. However, considering that one form exists in term placenta, the identical form is likely to be present in pre-term placenta. Besides the model compounds previously used [40, 47], the results of this study indicate that pre-term placental GSHTr can efficiently catalyze the metabolism of MeP.

In this study, we have shown that pre-term and term placental GSHTr catalyze the O-dealklyation of MeP. This ability of the placenta may protect the human fetus from the deleterious effects of maternal MeP exposure during gestation. Term placental GSHTr may have been at least partially responsible for the lack of fetotoxicity observed in a pregnant worker acutely exposed to a combination of mevinphos and phosphamidon [7]. These compounds should also undergo similar placental metabolism as MeP, since all are methyl ester OPs.

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