Biochemical Pharmacology, Vol. 30, No. 13, pp. 1859–1860, 1981.
Printed in Great Britain.

0006-2952/81/131859-02 $02.00/0

Effect of pregnancy on hepatic glutathione *S*-transferase activities in the rat

(*Received* 3 *November* 1980; *accepted* 8 *January* 1981)

The role of glutathione *S*-transferases (EC 2.5.1.18) in protecting against the toxic effects of highly reactive electrophiles has been largely recognized [1, 2]. These enzymes catalyse the conjugation of reduced glutathione (GSH) with many exogenous electrophilic compounds providing in this fashion a metabolic route of detoxication and subsequently excretion of a variety of alkylating agents [3, 4], even though some glutathione derivatives of dihaloalkanes have been found to be mutagenic [5, 6]. In rat liver there are at least six basic soluble GSH *S*-transferase forms with broad and overlapping substrate specificities [4].

Recently, we have identified and characterized the GSH *S*-transferase activity in human placenta cytosol [7, 8]. The enzyme, unlike liver transferases, is an anionic protein which appears to possess the same detoxifying properties assigned to the hepatic transferases.

Human placenta activity towards 1-chloro-2,4-dinitrobenzene (CDNB), the most common substrate of the transferases, is about 50 per cent of that found by Kamisaka *et al.* in human liver [7, 9]. Nevertheless, when we measured placental activity in the early stages of pregnancy (8–12 weeks) the value was twice that found at full-time term [7].

In this work an attempt was made in understanding whether pregnancy and the course of gestation have any effect on the maternal hepatic GSH *S*-transferase activities. The present communication reports that some GSH *S*-transferase activities contained in rat liver are significantly increased during gestation. We also give GSH *S*-transferase activities determined at mid- and late pregnancy in the placenta cytosol.

Female Morini origin rats (body weight 300–330 g), obtained from Istituto Zooprofilattico of Teramo (Italy), were maintained in laboratory cages and fed with synthetic diet and water *ad libitum*. Mating was carried out by placing one proven breeder into the cages containing four virgin females (all of the same age) for 24 hr. The removal of the male was considered to be the first day of gestation in those animals that were later found to be pregnant. Rats found not to be pregnant were employed as controls. After 10–11 and 19–20 days of gestation the animals were killed and 105,000 *g* supernatant fractions were prepared from liver and placenta. For the preparation of placenta cytosol 4–6 placentae from the same litter of 10–11 day and 19–20 day-pregnant rats were pooled. Samples were stored at −20° until assayed for enzyme activity. GSH *S*-transferase activities were monitored by using techniques previously described [7, 10] and cytosol as enzyme source. The following substrates were employed with GSH as co-substrate: 1-chloro-2,4-dinitrobenzene (CDNB), 1,2-dichloro-4-nitrobenzene (DCNB), *p*-nitrobenzylchloride (PNBC), and 1,2-epoxy-3-(*p*-nitrophenoxy)propane (EPNPP). Protein was determined by the method of Bradford [11]. Statistical evaluation of the results was made with the unpaired *t*-test.

The effects of pregnancy on GSH *S*-transferase activities in the liver are summarized in Table 1. A statistically significant increase (82%) in hepatic GSH *S*-transferase activity towards EPNPP was detected in pregnant rats, relative to non-pregnant controls, since mid-pregnancy. No comparable changes seem to occur in the same period for GSH *S*-aryl (CDNB and DCNB) and *S*-aralkyl (PNBC) transferase activities. When GSH *S*-transferase activities were measured in liver cytosol from 19–20 day pregnant rats a significant increase (57%) for CDNB and a decrease (22%) for DCNB were observed. Assessment of the ratio of transferase activities towards CDNB and DCNB is worthy of interest [12, 13]; it gives an indication of the fraction of conjugating activity in cytosol contributed by transferase B (ligandin), a protein which displays a multiple physiological function, i.e.: conjugation and binding properties [14, 15], 3-oxosteroid Δ^5-Δ^4-isomerase [16] and glutathione peroxidase activity [17] and prostaglandin endoperoxides conversion capacity [18]. Thus, a 101 per cent increase in activity ratio of CDNB/DCNB was noted at 19–20 days of gestation. GSH *S*-transferase activity towards EPNPP remained substantially unvaried, relative to mid-pregnancy, at full-term stage.

In accordance with the observations of Neale and Parke [19], liver weight in our experimental animals was augmented during mid- and late pregnancy by about 20 per cent and 35 per cent respectively.

Cytosol from rat placenta was tested with the four mentioned substrates, but an appreciable enzymic activity was recorded only with CDNB. The specific activity calculated

Table 1. Liver glutathione *S*-transferase activities at different stages of pregancy

Substrate	Control (n = 9)	Mid-pregnancy 10–11 days (n = 7)	Late pregnancy 19–20 days (n = 7)
1-Chloro-2,4-dinitrobenzene	26.3 ± 3.4	28.2 ± 3.6	41.5 ± 4.7*
1,2-Dichloro-4-nitrobenzene	0.42 ± 0.04	0.44 ± 0.06	0.33 ± 0.02†
p-Nitrobenzylchloride	0.70 ± 0.09	0.75 ± 0.10	0.63 ± 0.11
1,2-Epoxy-3-(*p*-nitrophenoxy)propane	0.17 ± 0.02	0.31 ± 0.03*	0.29 ± 0.03*
Activity ratio of 1-Chloro-2,4-dinitrobenzene/1,2-Dichloro-4-nitrobenzene	62.5	64	126

Activities are given in μmoles product formed/min/g tissue ± S.D.
Significant differences from controls are shown by * $P < 0.001$, † $P < 0.02$.

Table 2. Specific glutathione *S*-transferase activities in rat placenta at mid- and late-pregnancy

Substrate	Mid-pregnancy 10–11 days	Late pregnancy 19–20 days
1-Chloro-2,4-dinitrobenzene	0.090*	0.047
1,2-Dichloro-4-nitrobenzene	n.d.†	n.d.

Activity is expressed as μmoles/min/mg cytosol protein.
* Mean for group of three animals.
† Not detected under our experimental conditions.

for this arylchloride in the placenta during mid- and late-pregnancy was 4.1 and 7.9-fold lower than that measured in the liver at the corresponding gestational periods.

The results of our investigation indicate that rat liver GSH *S*-transferase activities increase during pregnancy in different fashion. Glutathione *S*-transferase activity towards epoxides, which may play an important action in inactivating epoxides formed by the metabolic activation of several classes of xenobiotics by microsomal oxygenases, was enhanced at mid-pregnancy. Significant increase in glutathione-transferase activity towards CDNB was observed only in the late stages of gestation. Conversely, data on the corresponding activity in full-term placenta indicate a decrease relative to mid-pregnancy. The decline in transferase activity for CDNB in the placenta with the ageing suggests a progressively decreasing importance of this enzymic system. On the other hand, it is noteworthy that the same activity in maternal liver, under the special physiological conditions as pregnancy, was significantly augmented in the late gestational stage. Tentatively, this occurrence could respond to a general function of supplying the foetus with a detoxicant mechanism efficacious during all phases of its development.

Summary. Rat liver GSH *S*-transferase activities have been studied during pregnancy. Glutathione *S*-transferase activity towards epoxides was significantly enhanced at mid-pregnancy and no further variation was recorded until delivery. A 100 per cent increase was observed for GSH *S*-transferase activity towards 1-chloro-2,4-dinitrobenzene only in the late stages of gestation. Placental GSH *S*-transferase activities determined at mid- and late-pregnancy are also reported.

Acknowledgements—This work was in part supported by a grant from Consiglio Nazionale delle Ricerche.

Institutes of Chemistry and Biological Chemistry
Faculty of Medicine
University of Chieti
66100 Chieti, Italy

GIOVANNI POLIDORO
CARMINE DI ILIO
ARDUINO ARDUINI
GIORGIO FEDERICI

REFERENCES

1. E. Boyland and L. F. Chasseaud, *Adv. Enzym.* **32**, 173 (1969).
2. G. J. Smith, U. S. OhL and G. Litwack, *Cancer Res.* **37**, 8 (1977).
3. L. F. Chasseaud, *Adv. Cancer Res.* **29**, 175 (1979).
4. W. B. Jakoby, *Adv. Enzym.* **48**, 383 (1978).
5. U. Rannug. A. Sundvall and C. Ramel, *Chem–Biol. Interact.* **20**, 1 (1978).
6. P. J. van Bladeren, D. D. Breimer, G. M. T. Rotteveel-Smijs and G. R. Mohn, *Mutation Res.* **74**, 341 (1980).
7. G. Polidoro, C. Di Ilio, G. Del Boccio, P. Zulli and G. Federici, *Biochem. Pharmac.* **29**, 1677 (1980).
8. G. Polidoro, C. Di Ilio, A. Arduini and G. Federici, *Biochem. Med.* in press.
9. K. Kamisaka, W. H. Habig, J. N. Ketley, I. M. Arias and W. B. Jakoby, *Eur. J. Biochem.* **60**, 153 (1975).
10. W. H. Habig, M. J. Pabst and W. B. Jakoby, *J. biol. Chem.* **249**, 7130 (1974).
11. M. M. Bradford, *Analyt. Biochem.* **72**, 248 (1976).
12. B. F. Hales and A. H. Neims, *Biochem J.* **160**, 223 (1976).
13. B. F. Hales and A. H. Neims, *Biochem. J.* **160**, 231 (1976).
14. W. B. Jakoby, W. H. Habig, J. H. Keen, J. N. Ketley and M. J. Pabst, in *Glutathione: Metabolism and Function* (Eds I. M. Arias and W. B. Jakoby), p. 189. Raven Press, New York (1976).
15. B. Ketterer, E. Tipping, D. Beale and J. A. T. P. Meuwissen, in *Glutathione: Metabolism and Function* (Eds I. M. Arias and W. B. Jakoby), p. 243. Raven Press, New York (1976).
16. A. M. Benson, P. Talalay, J. H. Keen and W. B. Jakoby, *Proc. natn. Acad. Sci. U.S.A.* **74**, 158 (1977).
17. J. R. Prohaska and H. E. Ganther, *Biochem. biophys. Res. Commun.* **76**, 437 (1977).
18. E. Christ-Hazelhof, D. H. Nugteren and D. A. Van Dorp, *Biochim. biophys. Acta* **450**, 450 (1976).
19. M. G. Neale and D. V. Parke, *Biochem. Pharmac.* **22**, 1451 (1973).