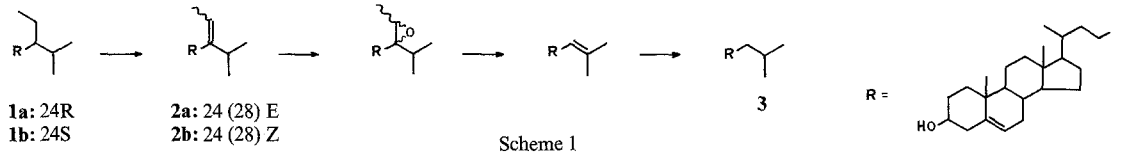


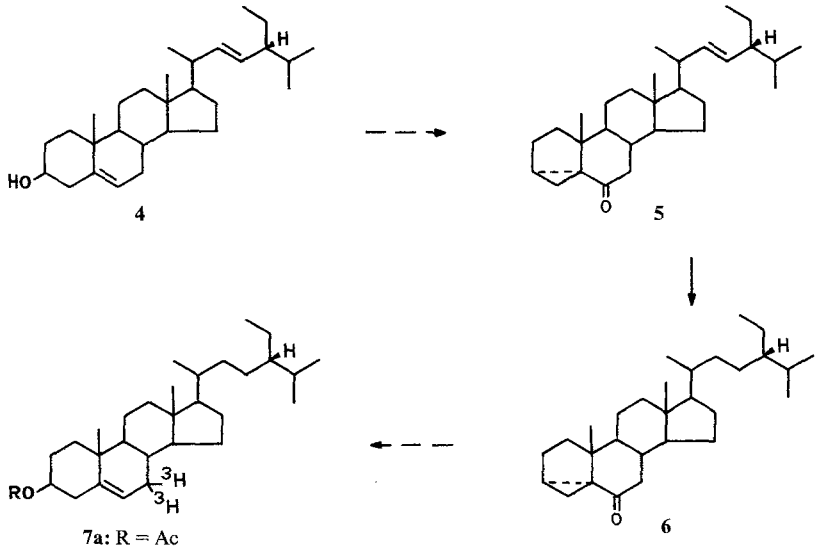
**1a:** 24R
**1b:** 24S

**2a:** 24 (28) E
**2b:** 24 (28) Z

**3**

R =

Scheme 1



**4** **5** **6**

**7a:** R = Ac
**7b:** R = H

Scheme 2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7 Compound (**5**): oily product, $\delta_H(CDCl_3)$ 0.2–0.6 (m, cyclopropyl protons), 0.74 (s, 18-Me), 0.83 (t, J 6 Hz, 29-Me), 0.85 (d, J 6 Hz, 26- and 27-Me), 1.01 (s, 19-Me), 1.04 (d, J 6 Hz, 21-Me), 5.01 (m, 22- and 23-CH). Compound (**6**): oily product, $\delta_H(CDCl_3)$ 0.2–0.6 (m, cyclopropyl protons), 0.68 (s, 18-Me), 0.83 (d, J 6 Hz, 26- and 27-Me), 0.85 (t, J 6 Hz, 29-Me), 0.94 (d, J 6 Hz, 21-Me), 1.02 (s, 19-Me).
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## Glutathione-related enzyme activities in pregnant rat liver

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*Summary.* The levels of GSH-related enzyme activities during pregnancy were determined. A significant increase in Selenium-dependent GSH peroxidase and GSH S-transferase E activity was observed. A concomitant increase in γ-glutamylcysteine synthetase was measured, which indicated an increased ability to synthesize the tripeptide.
*Key words.* Rat liver; liver, rat; pregnancy, rat; glutathione-related enzymes; enzymes, glutatione-related.

Glutathione (GSH) exerts a protective role in cells by forming conjugate derivatives and by acting as hydrogen donor for the reduction of peroxides. The conjugation process is catalyzed by a class of enzymes known as GSH S-transferases. These enzymes have been purified and characterized from several sources[1,2]. The removal of $H_2O_2$ and other peroxides is mediated by the action of GSH peroxidase[3]. The intracellular reduced GSH is formed from its precursor amino acids glutamate, cysteine and glycine. γ-Glutamylcysteine synthetase catalyses the dipeptide formation whilst GSH synthetase catalyses the tripeptide formation. The activity of γ-glutamylcysteine synthetase is rate-limiting, and the synthesis of GSH may be regulated by feedback inhibition of this enzyme[4]. Several investigations have indicated that the enzymes related to GSH metabolism gradually develop with age and can also be induced by numerous endogenous and exogenous substances[2,5–8]. In this context it was considered important to determine whether hepatic levels of GSH-related enzymes are in-

fluenced by the metabolic and hormonal changes taking place during pregnancy.

*Materials and methods.* Pregnant primipara Wistar rats on the 16th and 20th days of the gestation period and non pregnant females were obtained from Stefano Morini, Reggio Emilia, Italy. Animals were killed and the livers perfused with cold saline in situ. The perfused liver was then removed, minced with scissors and homogenized with 5 vol. of 0.15 M Tris-HCl buffer, pH 7.5 containing 1 mM EDTA and 2 mM $\beta$-mercaptoethanol in a Potter-type teflon-glass homogenizer. The homogenate was centrifuged for 1 h at 50,000 × g and the supernatant utilized for the determination of enzymatic activities. GSH peroxidase activity was measured according to Paglia and Valentine[9]. Selenium-dependent GSH peroxidase activity was determined using 0.25 mM of $H_2O_2$ as substrate whereas total GSH peroxidase activity was determined with 1.2 mM cumene hydroperoxide (CHP). Enzyme activity was expressed as µmoles of GSH oxidized/min/g of liver. GSH S-transferase activity was estimated according to Habig[10]. Enzyme activity was expressed as µmoles of GSH conjugate/min/g of liver. GSH reductase activity was determined as previously described[11]. Enzyme activity was expressed as µmoles of NADPH oxidized/min/g of liver. $\gamma$-Glutamylcysteine synthetase activity was measured as described by Sekura and Meister[12]. Enzyme activity was expressed as µmoles of Pi/min/g of liver. For the determination of GSH and free amino acids, perfused livers were homogenized with 5 vol. 0.15 m Tris-HCl buffer, pH 7.5, made 4% in sulfosalicylic acid and centrifuged for 1 h at 50,000 × g. GSH was measured by the method of Ellman[13]. Amino acids were determined with an LKB Alpha Amino Acid Analyzer. Protein concentration was determined by the method of Bradford[14].

*Results and discussion.* The enzyme activities obtained are reported in the table. A statistically significant increase (40%) in GSH peroxidase activity was observed on the 16th day of gestation, the value returning to non-pregnant control levels before parturition (20 days). It is well established that there are at least two enzymes which display GSH peroxidase activities in rat liver cytosol[15]. The selenium-dependent GSH peroxidase utilises $H_2O_2$ as well as organic hydroperoxides as substrates whereas the selenium independent form only reduces organic hydroperoxides. Since the percentage increase in GSH peroxidase activity towards either substrate was found to be the same, it appears that only the selenium-dependent activity was increased in maternal hepatic tissues.

The effect of pregnancy on the levels of GSH S-transferase activities was followed using three model substrates (table).

GSH and GSH-related enzyme activities in pregnant rat liver

| | Control (12) | 16th day preg (6) | 20th day preg (6) |
|---|---|---|---|
| GSH peroxidase | | | |
| H202 | 46.46 ± 4.6 | 66.6 ± 5.75* | 45.88 ± 8.3 |
| CHP | 52.3 ± 4.1 | 72.3 ± 6.7* | 57.6 ± 6.5 |
| GSH S-transferase | | | |
| EPNPP | 0.62 ± 0.19 | 4.33 ± 0.8* | 1.9 ± 0.66* |
| DCNB | 0.406 ± 0.035 | 0.29 ± 0.037* | 0.28 ± 0.046* |
| CDNB | 23.9 ± 2.7 | 29.5 ± 6.13 | 32.8 ± 2.7* |
| GSSG reductase | 1.98 ± 0.12 | 1.69 ± 0.19 | 1.73 ± 0.13 |
| $\gamma$-Glutamylcysteine Synthetase | 0.248 ± 0.02 | 0.434 ± 0.075 | 0.284 ± 0.074 |
| GSH** | 5.5 ± 0.4 | 5.41 ± 0.37 | 5.6 ± 0.28 |
| Glutamate** | 4.54 ± 0.47 | 4.02 ± 0.42 | 5.05 ± 0.9 |
| Glycine** | 1.81 ± 0.2 | 2.12 ± 0.38 | 1.73 ± 0.2 |
| Methionine** | 0.107 ± 0.008 | 0.09 ± 0.006 | 0.114 ± 0.009 |

Each value represents the mean ± SD of the numbers given in parenthesis. Activities are expressed as µmoles/min/g of liver. ** µmoles/g of liver; * $p < 0.001$.

The conjugating capacity towards 1,2-Epoxy-3-(p-nitrophenoxy)propane (EPNPP) was highly increased (up to 7-fold) on the 16th day of gestation and remained significantly enhanced (up to 3-fold) at the end of gestation. Conversely the activity towards 1,2-dichloro-4-nitrobenzene (DCNB) decreased to about 30% of non-pregnant controls. The activity towards 1-chloro-2,4-dinitrobenzene (CDNB) remained without appreciable change until almost the end of the gestational period when it was found to increase suddenly by 37%. GSH S-transferase in the rat liver occurs as several isozymes which are distinguished by their substrate specificities as well as differences in their isoelectric point. These forms have been isolated, characterized and named A, B, C, D, E, AA[1]. It is apparent from the data obtained that hepatic transferase activities towards the various substrates are differently regulated by pregnancy. Isozyme E activity, which is strongly enhanced in pregnant liver as indicated by the reactivity towards EPNPP, suggests that the maternal liver is equipped during the crucial phases of organogenesis with an extremely efficacious tool for intercepting highly reactive epoxides before they pass into the fetal circulation.

Although it would be expected that the enhancement of GSH peroxidase and GSH S-transferase activities would cause a net fall in the cellular level of GSH, no decrease was observed in the pregnant liver as compared to the non-pregnant controls. Also, no significant variation in the levels of the free amino acids utilized in the biosynthesis of GSH was noted. Similarly the activity of GSH reductase remained unaltered. However, the activity of $\gamma$-Glutamylcysteine synthetase was found to increase by 75% on the 16th day of pregnancy. It therefore appears that as a consequence of the increase in the activity of the enzymes that consume GSH an elevated adaptive capacity to synthesize the tripeptide occurs rather than a recovery through reductase. Similar observations made using the mammary gland of pregnant mice were in agreement with the results reported here. Lane and Medina[16] reported that the mammary glands of pregnant mice had a higher level of selenium-dependent GSH peroxide activity than did those of virgin mice, whereas Puente et al.[17] found an increase in $\gamma$-Gluthamylcysteine synthetase activity.


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