of SLI from spinal cord in acute multiple sclerosis. Increased lumbar SLI levels were reported in acute spinal lesions of other etiologies (Kohler et al., 1982). Our findings for SPlI suggest that SPLI is not closely related with multiple sclerosis or that the site of clinical damage is not at the site of release. These preliminary findings clearly suggest differential behaviour of CSF peptide gradients in neurological diseases which should be taken into account in interpretations of lumbar CSF studies.

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The purification of multiple forms of glutathione S-transferase from pig liver and their reaction with individual methyl linoleate hydroperoxides

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Lipid peroxidation is thought to be inhibited by certain glutathione-dependent enzymes, although the mechanism of inhibition and even the particular enzyme(s) responsible are still unclear (Bell et al., 1984; Tan et al., 1984). The three proteins implicated are glutathione S-transferase (EC 2.5.1.18) (Mannervik, 1985), selenium-dependent glutathione peroxidase (EC 1.11.1.9) (Christopherson, 1968) and a 'peroxidation-inhibiting protein' (Ursini et al., 1985). The transferase enzyme is able to conjugate 4-hydroxy-2-enals (one of a large number of secondary peroxidation products) with glutathione (Alin et al., 1985) and additionally, some transferase isoenzymes possess a glutathione-dependent activity for organic peroxide reduction (Prohaska & Ganther, 1977; Prohaska, 1980). This activity has been suggested to be of more importance than the selenium-dependent enzyme in controlling the build up of organic hydroperoxides in some tissues (McCay et al., 1981; Bell et al., 1984) and may be aided by the 'peroxidation-inhibiting protein' (Ursini et al., 1985). This protein protects phosphatidylcholine liposomes from peroxidative degradation under some conditions (Ursini et al., 1982). In this work, we purify five major and three minor forms of glutathione S-transferase from porcine liver and demonstrate that four of the five major forms are responsible for most of the organic peroxidase activity in this organ. Methyl linoleate hydroperoxides are used as a model system to assess the potential of glutathione S-transferase to reduce lipid hydroperoxides.

Soluble extracts from approx. 30 g of porcine liver contained 3820 units of transferase activity [with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate (Habig & Jakoby, 1981)] and 650 units of peroxidase activity [with cumene hydroperoxide as substrate (Habig & Jakoby, 1981)]. This was applied to a DEAE-Sephadex A50 column in 10 mm-potassium phosphate pH 6.5, which binds and removes the 'peroxidation-inhibiting protein' (Ursini et al., 1982). Most of the transferase activity (3480 units) and peroxidase activity (520 units) passed through this column and was applied to S-hexylglutathione-Sepharose (Mannervik & Guthenberg, 1981) elution with 5 mM-S-hexylglutathione/0.2 M-KCl.

Abbreviation used: CDNB. 1-chloro-2,4-dinitrobenzene.

yields almost pure glutathione S-transferase. The individual enzymic forms were resolved on a CM-Sepharose column in 10 mm-potassium phosphate pH 6.0, with a gradient of 0-0.2 M-KCI. The five major and three minor peaks of transferase activity (labelled I-VIII in order of elution from the column) appeared pure by SDS-gel electrophoresis (Table 1). The transferase/peroxidase ratios demonstrated that at least 75% of the activity for cumene hydroperoxide in the pig liver crude extract is associated with transferase forms IV-VII.

Methyl linoleate was autoxidized at 40 C for 5 days and the four isoforms (9-and 13-hydroperoxides, each with either the cis, trans or the trans, trans configuration of the conjugated diene) (Chan & Levett, 1977) were separated and purified by h.p.l.c. (Peers et al., 1981). The individual isoforms were tested as substrates for the five major transferase forms (I, IV, V, VI, VII) using an assay consisting of the following final concentrations in 0.5 ml at 25 C: 0.01 M-potassium phosphate pH 6.5, 0.04% Triton X-100, 10 mm-glutathione and 100 mm-lipid hydroperoxide. Enzyme (about 0.06 unit of transferase activity with CDNB) was added to start the reaction. After 2 min, the reaction was stopped by extraction of the lipid components into 1 ml of hexane/methyl tert-butyl ether (85:15). The amount and nature of the products were determined using h.p.l.c. The retention times were compared with those of the individual purified hydroperoxide substrates and to those of the corresponding hydroxides [produced by NaBH4 reduction of the hydroperoxides (Peers et al., 1981)]. By this method, an isomeric mixture of methyl linoleate hydroperoxides yielded only the corresponding hydroxides on enzymic reduction.

Table 1 summarizes the results when individual methyl linoleate hydroperoxide isoforms were incubated with each of the enzyme forms. The specific activities are compared with those with CDNB and cumene hydroperoxide as substrates. 'I showed no peroxidase activity with either lipid or aromatic hydroperoxides. In general, the cis, trans isoforms are better substrates than the trans, trans isoforms, as exemplified by the three-fold difference in rates of reduction of 9-cis, trans and 9-trans, trans by VI. In this context, it may be significant that most naturally occurring unsaturated fatty acids possess cis, trans double bonds.

Since the glutathione S-transferases exist in very high concentration (Habig et al., 1976), it is likely that these enzymes are effective scavengers of hydroperoxides. However, the relationship between the glutathione S-transferases,
Table 1. Properties of the major forms of glutathione S-transferase from pig liver

<table>
<thead>
<tr>
<th>Glutathione S-transferase</th>
<th>I</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
</tr>
</thead>
<tbody>
<tr>
<td>% of total transferase activity</td>
<td>6.1</td>
<td>12.1</td>
<td>5.9</td>
<td>38.6</td>
<td>31.6</td>
</tr>
<tr>
<td>Subunit Mr (SDS polyacrylamide-gel electrophoresis)</td>
<td>27 100</td>
<td>26 800</td>
<td>26 800</td>
<td>26 400</td>
<td>26 000</td>
</tr>
<tr>
<td>Specific activity (units/mg):</td>
<td>28 700</td>
<td>27 100</td>
<td>26 000</td>
<td>26 400</td>
<td>26 000</td>
</tr>
<tr>
<td>CDNB</td>
<td>24.4</td>
<td>7.90</td>
<td>14.8</td>
<td>45.1</td>
<td>47.1</td>
</tr>
<tr>
<td>Cumene hydroperoxide</td>
<td>0</td>
<td>3.16</td>
<td>4.77</td>
<td>5.71</td>
<td>11.8</td>
</tr>
<tr>
<td>H2O2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9-cis, trans</td>
<td>0</td>
<td>0.72</td>
<td>0.97</td>
<td>1.66</td>
<td>2.11</td>
</tr>
<tr>
<td>9-trans, trans</td>
<td>0</td>
<td>0.34</td>
<td>0.36</td>
<td>0.51</td>
<td>1.02</td>
</tr>
<tr>
<td>13-cis, trans</td>
<td>0</td>
<td>0.73</td>
<td>0.91</td>
<td>1.31</td>
<td>1.96</td>
</tr>
<tr>
<td>13-trans, trans</td>
<td>0</td>
<td>0.95</td>
<td>0.76</td>
<td>1.44</td>
<td>1.33</td>
</tr>
</tbody>
</table>

Selenium-dependent glutathione peroxidase and the 'peroxidation-inhibiting protein' in the inhibition of peroxidation in both simple lipids and in more complex lipids such as lecithins is unclear and needs further examination.

\begin{align*}
\end{align*}

\begin{align*}
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\begin{align*}
\text{Prohaska}, \text{J. R.} (1980) \text{Biochim. Biophys. Acta} \text{611}, 87 - 98
\end{align*}

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\begin{align*}
\text{Ursini}, \text{F.}, \text{Maiorino}, \text{M.} \& \text{Gregolin}, \text{C.} (1985) \text{Biochim. Biophys. Acta} \text{839}, 62-70
\end{align*}

\begin{align*}
\text{Ursini}, \text{F.}, \text{Maiorino}, \text{M.}, \text{Valente}, \text{M.}, \text{Ferri}, \text{L.} \& \text{Gregolin}, \text{C.} (1982) \text{Biochim. Biophys. Acta} \text{710}, 197-211
\end{align*}

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**Dye-affinity chromatography of CTP: cholinephosphate cytidylyltransferase**

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CTP: cholinephosphate cytidylyltransferase is believed to regulate the rate of phosphatidylcholine biosynthesis by the CDP: choline pathway in mammalian tissues (Peleck & Vance, 1984). Conventional purification techniques have produced a low recovery of cytidylyltransferase (Vance et al., 1981) making detailed study of the enzyme difficult. Alternative techniques such as dye-affinity chromatography have found application in the purification of a wide range of enzymes and other proteins (Lowe & Pearson, 1984) and consequently we have screened eight triazine-based dyes to evaluate their suitability as pseudo-affinity ligands for cytidylyltransferase.

Fetal human lung-derived fibroblasts were obtained from therapeutically aborted fetuses of 15-16 weeks gestation. Fibroblasts (100-200 mg wet weight) were disrupted by sonication for 3 x 10s at 14 microns in 5 ml of 0.145 M NaCl/50 mM-Tris/HCl (pH 7.4)/1 mM-EDTA/1 mM-dithiothreitol/1 mM-NaCN3/200 µM-phenylmethanesulphonyl fluoride. The microsomal fraction was recovered by centrifugation between 10000 g for 10 min and 100000 g for 60 min. The 100000 g for 60 min supernatants were incubated at 37°C for 2 h to allow cytoskeletal components to aggregate, which were then removed by a second 100000 g for 60 min centrifugation. Failure to remove these components resulted in interference with the dye–enzyme interactions. The supernatant from this second centrifugation contained the soluble enzyme used in subsequent experiments. Enzyme activity was measured by the incorporation of 9-3Hcholine phosphate into CDP: choline over 20 min at 37°C in the presence of 0.25 mM-phosphatidylglycerol (Stern et al., 1976). Selected triazine dyes were coupled to Sepharose CL-4B as described by Lowe & Pearson (1984). The dichlorotriazine dyes used were Procion dyes: Blue MX-R, Orange MX-G and Turquoise MX-G. The monoclorotriazine dyes were Procion dyes: Red H-7B, Blue H-B, Turquoise H-A, Green H-4G and the Levafix dye Brilliant Green E-5BNA. The ability of individual dyes to inhibit the enzyme was assessed after preincubation at 1 mol/l. Results suggest that enzyme inhibition by H-4G was not mediated by covalent modification at the active site of the enzyme.