CHAPTER III
5.1.0.0. INTRODUCTION

While lipid peroxidation is generally associated with widespread and irreversible damage to essential cell components, enzymatically controlled lipid peroxidation is involved in the biosynthesis of eicosanoids, hormone like substances required for maintaining tissue and cellular homeostasis. The immediate oxygenation products of arachidonic acid by the way of lipoxygenase and cyclooxygenase pathways include \( \text{PGG}_2 \) and HPETE respectively. Not only do these products serve as precursors for the biosynthesis of PGs and LTs, but they also have significant influence on the activities of various enzymes associated with arachidonic acid including cyclooxygenase and lipoxygenase. Thus, an enzyme system that can reduce these reactive hydroperoxides has the potential for modulating the arachidonic acid cascade.

Glutathione peroxidases, with the potential for reducing these hydroperoxides and endoperoxides, influence the biosynthesis of biologically active eicosanoids. Se-GSH Px catalyzes the reduction of both organic and inorganic hydroperoxides. In addition to these a group of Se-independent GSH Pxs (non-Se-GSH Px) associated with certain forms of GSTs are involved in the reduction of organic hydroperoxides (Lawrence and Burke, 1976). Both types of peroxidases are ubiquitously distributed in animals. The cellular Se-GSH Px is located primarily in the cytosolic and mitochondrial compartments, while the non-Se-GSH Px has been identified in both cytosolic and microsomal cell fractions (Reddy et al., 1981). The relative abundance of these two types of GSH Pxs within a given tissue is species specific. The relative contribution of these two enzymes in antioxidant defenses can best be studied by knocking out these enzymes one at a time. Induction of Se deficiency forms one such situation wherein Se-GSH Px activity is eliminated. As shown in the second chapter, Se deficiency and thus elimination of Se-GSH Px resulted in the induction of GSTs. The GSTs have been implicated in the synthesis of prostaglandins including \( \text{PGF}_{2\alpha} \). \( \text{PGF}_{2\alpha} \) is a biological mediator in a variety of physiological functions including contractions of
uterine smooth muscle and pulmonary arteries. Research from Reddy's group has provided evidence that the GSH Px activity of GSTs is associated with the reduction of PGH$_2$ to PGF$_{2\alpha}$, with the \textit{\textbf{\alpha-class}} or Ya-containing subunits being the most active (Burgess \textit{et al.}, 1987; Chang \textit{et al.}, 1987a; Hong \textit{et al.}, 1989). In addition to their role in PG biosynthesis, GSTs also play a role in lipoxygenase pathway at the level of reduction of hydroperoxides and in the formation of peptidoleukotrienes. GSTs, especially Yb containing subunits were shown to conjugate GSH to LTA$_4$ to produce LTC$_4$, the most potent vasoconstricting and bronchoconstricting compound (Chang \textit{et al.}, 1987b). Hence in the present study GSTs from the various dietary oxidant stress conditions were screened for their potential impact on the enzymatic lipid peroxidation pathways of the arachidonic acid.

5.2.0.0. RESULTS

5.2.1.0. CM-Cellulose column chromatography

The affinity purified GSTs from liver cytosol of +E+Se rats were further separated into different isozymes on CM-cellulose column chromatography. As shown in the typical chromatogram (Fig. 38) eight GST activity peaks, designated as peaks I to VIII according to the order of elution, were consistently resolved on the CM-cellulose column chromatography with two linear salt gradients used in succession, i.e., 0 to 75 mM KCl and 75 to 200 mM KCl, in 10 mM NaH$_2$PO$_4$, pH 6.0. Peak I isozyme, which did not bind to the CM-cellulose column is referred to as the anionic GST isozyme. The other isozyme peaks which were eluted by the salt gradient are referred to as the cationic GST isozymes.

SDS-PAGE analysis of each peak fraction demonstrated that all of them were electrophoretically pure (Fig. 39). Peak I, II, IV and VI were composed of Yb subunits only. Peak HI was a Ya homodimer. Peak VII and VIII were Yc homodimers. Peak V consisted of equal amounts of Ya and Yc subunits as previously reported for transferases (Bass \textit{et al.}, 1977; Tu and Reddy, 1985).
Fig. 38: Separation of affinity purified hepatic GST isozymes of +E-Se rats on CM-cellulose column chromatography.
Fig. 39: SDS-PAGE of liver GST isozymes +E+Se rats separated on CM-cellulose column chromatography

Lane: 1. Rat liver affinity purified GSTs
Peak fraction #
2. 21 3.58 4.85
5. 99 6. 107 7. 128
8. 143 9.227
10. Rat liver affinity purified GSTs
5.2.2.0. DE 52 cellulose column chromatography

The anionic form of GSTs obtained from the CM-cellulose column flow through fractions were subjected to anion-exchange chromatography on DE 52 cellulose column for further separation. No protein was observed in the effluent prior to the start of a linear gradient of 0-100 mM KCl in 10 mM Tris-HCl, pH 8.0. All the protein bound to the column was eluted at approximately 60 mM KCl as a single peak, coincident with the GST activity (Fig. 40).

5.2.3.0. GST catalyzed reduction of classical substrates

The substrate specificity studies have shown that, the isozymes containing Ya and/or Yc subunits can be clearly distinguished from the isozymes containing Yb subunits by their relatively low activities with DCNB and high non-Se-GSH Px activity (Table 15&16). These results indicate that all of the rat liver GST isozymes may be classified into two groups, Ya and Yc containing isozymes and Yb subunit containing isozymes according to their physical and chemical properties as well as biological activities. The YaYc heterodimeric protein is always eluted after the Ya homodimer and before Yc homodimer during CM-cellulose chromatography (Fig. 38). The activity levels with different substrates for peak V (YaYa) are approximately intermediate between those of peak III (YaYc) and peak VII (YcYc). Peak III (Ya homodimer) is characterized by having the highest activity with 1,2-dichloro-4-nitrobenzene; 1,2-epoxy-3-(p-nitrophenoxy)propane; Δ4-androstene-3,17-dione; bromosulphothalein, p-nitrophenyl acetate and 13-hydroperoxyoctadecadienoic acid (13-HPODE) as well as PGF2α formation (Table 15&16). Peak VII (Yc homodimer), on the other hand, showed the highest activity with ethacrynic acid as well as organic hydroperoxides (Table 15&16). In contrast to the Ya and/or Yc containing isozymes, peaks I, II, IV and VI all contained only Yb subunits. However, they eluted at different salt concentrations on CM-cellulose columns indicating their varied structural characteristics. For example, peak I was eluted in the CM flow through fractions, whereas peak II, IV & VI were eluted at different salt concentrations. In addition to their structural
Fig. 40: Anion exchange chromatography of +E+Se rat liver CM-cellulose column flow through fraction on DE 52 cellulose column chromatography
TABLE 15: SUBSTRATE SPECIFICITIES OF +E+Se RAT LIVER GST ISOZYMES SEPARATED ON CM-CELLULOSE COLUMN CHROMATOGRAPHY TO CLASSICAL SUBSTRATES

<table>
<thead>
<tr>
<th>Substrate</th>
<th>I  YbYb</th>
<th>II YbYb</th>
<th>III YaYa</th>
<th>IV YbYb</th>
<th>V  YaYc</th>
<th>VI YbYb</th>
<th>VII YcYc</th>
<th>VIII YcYc</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,2-Dichloro-4-nitrobenzene</td>
<td>0.17</td>
<td>1.10</td>
<td>0.82</td>
<td>1.07</td>
<td>0.6</td>
<td>1.96</td>
<td>0.06</td>
<td>0.07</td>
</tr>
<tr>
<td>1-Chloro-2,4-dinitrobenzene</td>
<td>3.66</td>
<td>14.44</td>
<td>21.0</td>
<td>17.1</td>
<td>19.6</td>
<td>8.88</td>
<td>2.17</td>
<td></td>
</tr>
<tr>
<td>Δ^5-Androstene-3,17-dione</td>
<td>0.01</td>
<td>0.02</td>
<td>1.0</td>
<td>0.03</td>
<td>0.8</td>
<td>0.05</td>
<td>0.04</td>
<td>0.03</td>
</tr>
<tr>
<td>1,2-Epoxy-3-(p-nitrophenoxy)propane</td>
<td>0.30</td>
<td>ND</td>
<td>0.10</td>
<td>0.095</td>
<td>0.1</td>
<td>0.07</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4-Nitropyridine-N-oxide</td>
<td>0.015</td>
<td>0.12</td>
<td>0.03</td>
<td>0.12</td>
<td>0.1</td>
<td>0.14</td>
<td>0.14</td>
<td>0.02</td>
</tr>
<tr>
<td>p-Nitrobenzyl chloride</td>
<td>0.78</td>
<td>3.27</td>
<td>1.86</td>
<td>3.84</td>
<td>1.3</td>
<td>0.83</td>
<td>1.20</td>
<td>1.01</td>
</tr>
<tr>
<td>Ethacrynic acid</td>
<td>0.15</td>
<td>0.36</td>
<td>0.70</td>
<td>0.44</td>
<td>0.9</td>
<td>0.13</td>
<td>1.25</td>
<td>0.9</td>
</tr>
<tr>
<td>p-Nitrophenyl acetate</td>
<td>0.20</td>
<td>0.2</td>
<td>0.38</td>
<td>0.42</td>
<td>0.3</td>
<td>0.57</td>
<td>0.20</td>
<td>0.17</td>
</tr>
<tr>
<td>Bromosulfophthalein</td>
<td>0.01</td>
<td>0.02</td>
<td>0.06</td>
<td>0.02</td>
<td>0.03</td>
<td>ND</td>
<td>ND</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Values expressed as μmoles/min/mg protein.
<table>
<thead>
<tr>
<th>Substrate</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>YeYe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumene hydroperoxide</td>
<td>0.56</td>
<td>0.040</td>
<td>5.12</td>
<td>1.05</td>
<td>5.43</td>
<td>1.42</td>
<td>8.0</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>t-Butyl hydroperoxide</td>
<td>0.05</td>
<td>0.21</td>
<td>1.32</td>
<td>0.41</td>
<td>1.4</td>
<td>0.71</td>
<td>2.25</td>
<td>1.90</td>
<td></td>
</tr>
<tr>
<td>13-HPODE</td>
<td>0.04</td>
<td>0.03</td>
<td>2.1</td>
<td>0.11</td>
<td>1.30</td>
<td>0.18</td>
<td>0.61</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>*PGH2 to PGF2α</td>
<td>15</td>
<td>125</td>
<td>1520</td>
<td>250</td>
<td>552</td>
<td>120</td>
<td>328</td>
<td>287</td>
<td></td>
</tr>
<tr>
<td>*LTA4, Me</td>
<td>123</td>
<td>7.9</td>
<td>6.2</td>
<td>69.7</td>
<td>1.6</td>
<td>ND</td>
<td>ND</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values expressed as μmoles/min/mg protein.

* Values expressed as nmoles/min/mg protein.
differences, they also showed varied functional characteristics. For example, peak I showed highest activity towards 1,2-epoxy-3-(p-nitrophenoxy)propane and LTA₄ Me; followed by peak IV with less activity and peak VI with no activity (Table 16). Similarly peak I, IV and VI showed relatively low, intermediate and very high activities respectively with DCNB, 4-nitropyridine n-oxide, Δ⁵-androstene-3,17-dione and p-nitrophenyl acetate (Table 15).

5.2.3.1. GST catalyzed reduction of cumene hydroperoxide

Se-GSH Px acts both on H₂O₂ and organic hydroperoxides whereas α-class GSTs act only on organic hydroperoxides which was designated as non-Se-GSH Px. The non-Se-GSH Px activity when measured with cumene hydroperoxide in the crude cytosolic fractions showed very high activity in the liver tissues of -E+Se (103 %), +E-Se (236 %) and -E-Se (304 %) animals when compared to the +E+Se animals (Fig. 41 A). Similarly, in lung tissue non-Se-GSH Px activity was induced in -E+Se (121 %), +E-Se (267 %) and -E-Se (572 %) animals (Fig. 41B). The activity levels in -E-Se were much higher when compared to that of +E-Se animals.

In the affinity purified GSTs, a clear induction of peroxidase activity is observed in liver and lung tissues of vit.E and/or Se deficient animals (Fig. 42A&B), however, the induction being much higher in the cytosolic fractions.

5.2.3.2. GST catalyzed reduction of 13-hydroperoxyoctadecadienoic acid (13-HPODE)

The total GSH Px activity measured with 13-HPODE in the crude cytosolic fractions decreased in the liver tissues of +E-Se (-64 %) and -E-Se (-61 %) animals when compared to that of +E+Se animals (Fig. 43A). Similarly in lung tissue, significantly lower levels of peroxidase activity were observed in +E-Se (-51 %) and -E-Se (-30 %) groups compared to that of +E+Se animals (Fig. 43B).
Fig. 41: Activity levels of GSTs with CHP in cytosolic fractions of liver and lung tissues of rats fed on vit.E and/or Se supplemented and deficient diets

Each value is the mean $\pm$ SD of six individual observations. Data was analyzed with a one way ANOVA followed by SNK test. Bars labelled with same letter are not significantly different from one another ($p < 0.05$).
Fig. 42: Activity levels of non-Se-GSH Px with CHP in the affinity purified GSTs of liver and lung tissues of rats fed on vit.E and/or Se supplemented and deficient diets

Each value is the mean ± SD of six individual observations. Data was analyzed with a one way ANOVA followed by SNK test. Bars labelled with same letter are not significantly different from one another (p < 0.05).
Fig. 43: Activity levels of GSTs with **13-HPODE** in cytosolic fractions of liver and lung tissues of rats fed on vit.E and/or Se supplemented and deficient diets

Each value is the mean + SD of six individual observations. Data was analyzed with a one way ANOVA followed by SNK test. Bars labelled with same letter are not significantly different from one another (p < 0.05).
Fig. 44: Activity levels of GSH Px with 13-HPODE in the affinity purified GSTs of liver and lung tissues of rats fed on vit.E and/or Se supplemented and deficient diets

Each value is the mean + SD of six individual observations. Data was analyzed with a one way ANOVA followed by SNK test. Bars labelled with same letter are not significantly different from one another (p < 0.05).
In contrast, the affinity purified GSTs showed a clear induction of peroxidase activity in liver tissues of -E+Se (21 %), +E-Se (50 %) and -E-Se (54 %) and lung tissues of -E+Se (140 %), +E-Se (197 %) and -E-Se (340 %) when compared to the corresponding tissues from +E+Se animals (Fig. 44A&B).

5.2.3.3 GST catalyzed reduction of 15-hydroperoxyeicosatetraenoic acid (15-HPETE)

Total GSH Px activity levels in liver tissues were significantly lower in +E-Se (-61 %) and -E-SE (-56 %) animals when compared to that of +E+Se animals (Fig. 45A). Similarly, significantly lower levels of total GSH Px were observed in lung tissues of +E-Se (-56 %) and -E-Se (-27 %) animals when compared to that of +E+Se animals (Fig. 45B). GSH Px activity towards fatty acid hydroperoxides decreased significantly in liver and lung crude cytosols Se deficient animals, but not in those of vit.E deficient animals.

In contrast, the affinity purified GSTs showed significantly increased peroxidase activity in liver tissues of all vit.E and Se deficient [-E+Se (21 %), +E-Se (32 %) and -E-Se (34 %)] animals when compared to +E+Se animals (Fig. 46A). Similarly higher peroxidase activity levels were observed in lung tissues of -E+Se (91 %), +E-Se (136 %) and -E-Se (249 %) animals compared to that of +E+Se animals (Fig. 46B).

5.2.3.4. GST catalyzed prostaglandin formation

Equal amounts of affinity purified liver and lung GSTs from vit.E and/or Se supplemented and deficient animals were analyzed for GST catalyzed prostaglandin formation. The prostaglandins were identified by the Rf values corresponding to the prostaglandin standards separated on TLC (Fig. 47).

The GST catalyzed \( \text{PGF}_{2\alpha} \) forming activity in the affinity purified GSTs were significantly higher from liver tissues of -E+Se (32 %), +E-Se (51 %) and -E-Se (92 %) animals compared to that of +E+Se animals (Table 17). This correlates well with the observation that the Ya subunit, which exhibits maximum
Fig. 45: Activity levels of GSTs with 15-HPETE in cytosolic fractions of liver and lung tissues of rats fed on vit. E and/or Se supplemented and deficient diets

Each value is the mean ± SD of six individual observations. Data was analyzed with a one way ANOVA followed by SNK test. Bars labelled with same letter are not significantly different from one another (p < 0.05).
Fig. 46: Activity levels of GSH Px with 15-HPETE in the affinity purified GSTs of liver and lung tissues of rats fed on vit.E and/or Se supplemented and deficient diets

Each value is the mean + SD of six individual observations. Data was analyzed with a one way ANOVA followed by SNK test. Bars labelled with same letter are not significantly different from one another (p < 0.05).
Fig. 47: Separation of prostaglandin standards on TLC
Table 17: Synthesis of prostaglandins catalyzed by liver affinity purified GSTs of vit.E and/or Se supplemented and deficient rats

<table>
<thead>
<tr>
<th>PG</th>
<th>-E+Se</th>
<th>+E-Se</th>
<th>-E-Se</th>
<th>+E+Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGD2</td>
<td>34.7 ± 6.93*</td>
<td>32.06 ± 7.57*</td>
<td>33.12 ± 5.46*</td>
<td>39.48 ± 9.1*</td>
</tr>
<tr>
<td>PGE2</td>
<td>42.5 ± 6.43*</td>
<td>43.06 ± 6.39*</td>
<td>46.47 ± 6.61*</td>
<td>41.66 ± 7.2*</td>
</tr>
<tr>
<td>PGF2α</td>
<td>22.8 ± 2.72*</td>
<td>24.84 ± 4.14*</td>
<td>30.40 + 4.1b</td>
<td>15.80 ± 3.75c</td>
</tr>
</tbody>
</table>

Values represent mean ± SD of five individual experiments and are expressed in terms of percent ratios of total PGs formed. Data was analyzed with a one-way ANOVA followed by SNK test. Significance was set at *p<0.05*. The same alphabet denotes no significant difference between the two groups in each row.

Table 18: Synthesis of prostaglandins catalyzed by lung affinity purified GSTs of vit.E and/or Se supplemented and deficient rats

<table>
<thead>
<tr>
<th>PG</th>
<th>-E+Se</th>
<th>+E-Se</th>
<th>-E-Se</th>
<th>+E+Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGD2</td>
<td>88.62 ± 15.8*</td>
<td>88.11 ± 15.5*</td>
<td>89.24 ± 19.11*</td>
<td>88.74 ± 22.4*</td>
</tr>
<tr>
<td>PGE2</td>
<td>8.52 ± 1.60*</td>
<td>8.88 ± 1.75*</td>
<td>8.34 ± 2.4*</td>
<td>8.37 ± 0.72*</td>
</tr>
<tr>
<td>PGF2α</td>
<td>2.84 ± 0.69*</td>
<td>2.99 ± 0.75*</td>
<td>2.56 ± 0.23*</td>
<td>2.89 ± 0.24*</td>
</tr>
</tbody>
</table>

Values represent mean ± SD of three individual experiments and are expressed in terms of percent ratios of total PGs formed. Data was analyzed with a one-way ANOVA followed by SNK test. Significance was set at *p<0.05*. The same alphabet denotes no significant difference between the two groups in each row.
peroxidase activity, was also induced significantly in the livers from vit.E and Se deficient animals (II chapter).

PGF$_{2a}$ forming activity was also investigated with rat lung affinity purified GSTs from vit.E and Se deficient and supplemented animals. In contrast to the results observed in liver, PGF$_{2a}$ synthesis was barely detectable in the lung tissue (Table 18). No significant changes were observed in PGE2 or PGD2 formation in both lung and liver tissues in response to vit.E and/or Se deficiency (Table 17&18).

5.2.3.5. GST catalyzed leukotriene formation

Among various rat liver isozymes, the anionic isozyme (peak I), a homodimer of Yb subunit, showed the highest LTC4 Synthase activity, followed by peak IV GST isozymes (Table 16). When 5,6 LTA$_4$ methyl ester was incubated with equal amounts (100 $\mu$g) of liver affinity purified GSTs from vh.E and/or Se deficient animals for 10 min in the presence of GSH, most of the LTA4 methyl ester was converted into an acid-resistant compound, LTC$_4$ Me, which eluted around 5 min on RP-HPLC (Fig. 48). The UV-absorption spectrum of the compound showed a typical LTC4 spectrum with maximum absorption at 280 nm and shoulders at 270 and 292 nm. Basing on the UV/VIS spectrum and co.chromatography with authentic standard, the compound with RT 5 min was identified as LTC$_4$ Me. In contrast to liver, the lung GSTs showed significantly higher LTC$_4$ Synthase activity. However, there were no significant differences in LTC$_4$ Synthase activity of GSTs in vit.E and/or Se deficient and supplemented animal tissues (Table 19).

53.0.0. DISCUSSION

5.3.1.0. Functional characterization of liver GSTs

The non-Se-GSH Px activity was significantly increased in liver cytosol fractions and affinity purified GSTs of vit.E and Se deficient animals (Fig.
Fig. 48: RP-HPLC analysis of GSTs catalyzed LTC₄ formation

Inset: UV/VIS absorption spectrum of 5,6 LTC₄ peak in methanol

Column: ODS, 1 Bondapak (Waters)
Solvents: Methanol Water: Acetic acid (65:35:0.1) pH 5.7
Flow rate: 280 nm
Table 19: \textit{LTC}_4 Synthase activity of affinity purified liver and lung GSTs of vit. E and/or Se supplemented and deficient rats

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>-E+Se</th>
<th>+E-Se</th>
<th>-E-Se</th>
<th>+E+Se</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>4.40 ± 0.8</td>
<td>4.6 ± 1.9*</td>
<td>4.64 ± 1.54a</td>
<td>5.6 ± 1.6a</td>
</tr>
<tr>
<td>Lung</td>
<td>12.8 ± 0.4*</td>
<td>11.6 ± 3.4a</td>
<td>15.4 ± 3.6a</td>
<td>12.6 ± 3.6&quot;</td>
</tr>
</tbody>
</table>

Values are mean ± SD of five individual observations and are expressed as \textit{nmoles/min/mg protein}. Data was analyzed with a one-way ANOVA followed by SNK test. Significance was set at \( p < 0.05 \). The same alphabet denotes no significant difference between the two groups in each row.
This increased non-Se-GSH Px activity correlated well with increase in the Ya and Yc subunit containing GSTs (chapter II). Also Ya and Yc subunits exhibit maximum activity towards CHP as substrate (Table 16).

In addition to Se-GSH Px, non-Se-GSH Px activity associated with GSTs also reduce ferry acid hydroperoxides. Fatty acid hydroperoxidase activity measured in the crude cytosol of vit.E and/or Se deficient and supplemented animal tissues with 13-HPODE and 15-HPETE showed significantly reduced activity levels in Se deficient animals (+E+Se; -E-Se) compared to Se supplemented animals (-E+Se; +E+Se) (Fig. 43&45). The fatty acid hydroperoxidase activity measured in the crude cytosol of Se supplemented animals (-E+Se; +E+Se) is contributed by both Se-GSH Px as well as non-Se-GSH Px activity of GSTs. Thus a more accurate measure of GSH Px activity would be achieved by subtracting H$_2$O$_2$ (Se-GSH Px) activity as is traditionally done in estimating non-Se-GSH Px activity with CHP. Indeed, such a calculation would closely approximate the value measured when the fatty acid hydroperoxidase activities were measured in the affinity purified fraction. Fatty acid hydroperoxidase activity of GSTs was increased by 54% with 13-HPODE and 34% with 15-HPETE in vit.E and/or Se deficient animals compared to supplemented groups. This induction of fatty acid hydroperoxidase activity in vit.E and/or Se deficient animals must have been contributed by Ya and Yc subunits in liver tissue as these isozymes exhibit maximum peroxidase activity (Table 15) and these are the subunits induced in liver tissues of vit.E and/or Se deficient animals (chapter II). It is to be noted that all fatty acid hydroperoxides are good substrates for Se-GSH Px, and is 50 times more efficient than non-Se-GSH Px in catalyzing the reduction of fatty acid hydroperoxides.

In addition to the fatty acid hydroperoxidase activity, GSTs also were reported to be involved in the biosynthesis of prostaglandins and leukotrienes. Ya subunits of GSTs were shown to be involved in PGE$_2$ and PGF$_{2\alpha}$ synthase activity (Chang et al., 1987a). In the present study liver affinity purified GSTs of +E+Se
animals when incubated with PGH₂ resulted in the formation of PGE₂, PGD₂ and PGF₂α, the relative abundance being in the same order (Table 17). In the vit.E and/or Se deficient animals, there was significantly higher formation of PGF₂α compared to that of supplemented animals with no change in the formation of PGE₂ and PGD₂ (Table 17). Hope et al (1975) have reported increase in the formation of PGE₂ and PGF₂α in rat blood in response to vit.E deficiency. Cooper and Carpenter (1987) found no effect of diet on PGE₂ production by sertoli cells isolated from the testes of rats maintained on vit.E altered diets. Hepatic PGF₂α was significantly increased along with GST activities in phenobarbital treated rats which suggests that induction of Ya containing GSTs may play a physiologically important role in the synthesis of PGF₂α, which may account, at least in part, for the tumor-promoting effects of phenobarbital (Hendrich et al., 1991).

No significant changes were observed in the leukotriene synthesis of vit.E and/or Se deficient animals compared to that of +E+Se animals (Table 19). The formation of LTC₄ has been demonstrated in mouse myocytoma cells, rat basophilic leukemia cells, rat mononuclear cells and in human and guinea pig lungs (Piper, 1984; Dahinden et al., 1985). GST Yn₁-Yn₁ purified from rat brain has the highest LTC₄ synthase activity so far found in rat cytosolic GSTs and accounts for the majority of LTC₄ synthase activity in the brain (Tsuchida et al., 1987). In the present study peak I with Yb₁Yb₂ subunits (CM-cellulose fractions) showed maximum LTC₄ Synthase activity in the liver tissue (Table 15). As shown in the chapter II, liver contains very little or no Yn₁Yn₁ isozymes and Yb₁Yb₂ is not induced in vit.E and/or Se deficiency. Thus GSTs induced by antioxidant deficiency are unlikely to alter LT synthesis directly.

The accumulated peroxides in Se deficiency could activate the transcription of the specific GST genes which would result in non-Se-GSH Px activity. This further implies that environmental factors and pathological conditions which result in increased lipid peroxidation or otherwise increased peroxide levels might also induce changes in GST expression, especially those
involved in their reduction and detoxification. In the present study induction of Ya and Yc subunits in the liver tissue of vit.E and/or Se deficient animals appears to be aimed at the reduction of hydroperoxides as well as endoperoxides. GSTs with Ya and Yc, more so with Yc, are more involved in the reduction of hydroperoxides while GSTs with Ya subunits being more specific for reduction of endoperoxides.

### 5.3.2.0 Functional characterization of lung GSTs

The lung tissue of vit.E and/or Se-deficient animals is under increased oxidative stress as evidenced by increased GSSG levels and reduced Se-GSH Px activity levels (I chapter, Fig. 21&19). The increased non-Se-GSH Px activity of GSTs with CHP as the substrate in vit.E and/or Se deficient animals observed in the present study (Fig. 41B&42B) may be a compensatory mechanism to protect the lung tissue from the increased oxidative stress. The induction of GSTs with Yc subunits in lung tissue of vit.E and/or Se-deficient animals (II chapter) supports such a possibility as they exhibit very high peroxidase activity. Fatty acid hydroperoxidase activity was measured with affinity purified lung GSTs with 13-HPODE as well as 15-HPETE (Fig. 44&46). Fatty acid hydroperoxidase activity was much higher in Se-deficient animal tissue compared to Se-supplemented ones. This pattern is very much similar to that observed for liver tissue except that it correlated with elevated Yc rather than predominantly Ya GSTs.

GSTs can reduce electrophilic substrates like reactive alkenes; a,P unsaturated aldehydes, including highly toxic 4-hydroxyalkenals and epoxides formed during lipid peroxidation. Among all the GST isozymes, GST 8-8 (YkYk) showed maximum activity towards 4-hydroxynonenal, which is a product of peroxidative degradation of arachidonic acid (Stenberg et al., 1992). Rat lung which contains YkYk isozyme, is thus involved in the reduction of hydroxyalkenals produced in lipid peroxidation. Even though Yk was not elevated in vit.E and/or
Se deficiency, the increased activity during oxidative stress could partially account for elevated GSSG in lung (chapter I).

Earlier studies have shown that glutathione peroxidase activity of GSTs was restricted to ferry acid hydroperoxides, which suggests that membrane phospholipids must be first cleaved by phospholipase A$_2$ before they could be utilized as substrates by GSTs (Tan et al., 1984). However the later studies have shown that the α-class GSTs including those of human lung can effectively reduce the intact phospholipid hydroperoxides through GSH Px activity (Singhal et al., 1992). Hence it can be suggested that the α-class GST isozymes (Yc & Yk) play a predominant role in detoxification of lipid peroxides particularly in lung tissue. GST n which constitutes about 90% GST activity towards electrophilic substrates, on the other hand is known for the detoxification of xenobiotics (Batist et al, 1986; Tsuchida and Sato, 1992; Zhang, 1994).

In rat lung, however, dietary vit.E and/or Se status had no effect on the conversion of PGH$_2$ to PGF$_{2\alpha}$ or in the formation of other prostaglandins (Table 18). This result can be explained by the observation that rat lung lacks Ya subunit (Reddy et al., 1982), which is known for endoperoxidase activity. This association of prostaglandin endoperoxide reduction with Ya subunit is consistent with the fact that rat brain cytosolic GSTs which lack this subunit also showed very little PGF$_{2\alpha}$ forming activity (Thyagaraju et al, 1986b).

Compared to liver tissue, lung tissue of +E+Se animals showed 3-fold higher LTC$_4$ synthase activity (Table 19). This may be due to the presence of GSTs with more of Yb subunits than in the liver tissue, which exhibit very high LTC4 synthase activity. LTC$_\infty$ synthase activity in vit.E and/or Se deficient animals showed no significant differences when compared with +E+Se animals. This observation coincides well with the data on GST Yb subunits, where no significant changes were observed (chapter II).
From these studies it can be concluded that, induction of Ya2 and Yc subunits in liver and Yc subunits in lung tissue with increase in peroxidase activity in vit.E and/or Se deficient animals compared to vit.E and Se supplemented animals, is a compensatory mechanism towards impaired antioxidant defenses as a result of nutritional deficiency.