Protective antioxidant mechanisms in rat and guinea pig tissues challenged by acute exposure to cigarette smoke

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Summary

Cellular damage from reactive intermediates formed during xenobiotic biotransformation is prevented by the presence of adequate levels of antioxidant chemicals in the tissues. Equally important for cell protection is the rate at which these chemicals are replaced if tissue stores are depleted. The present experiments, using adult male Sprague-Dawley rats and Hartley guinea pigs, were conducted to ascertain what effects mainstream (MS) and sidestream (SS) tobacco smoke would have on the water-soluble, cytoplasmic antioxidants, ascorbic acid (AA) and reduced glutathione (GSH). The animals were exposed by nose-only inhalation to varying doses (40, 120, 240 puffs) of a 1:5 dilution of a 35-ml volume of freshly generated MS from cigarettes made from different types of tobacco and delivered by a B.A.T-Mason inhalation apparatus. The animals were euthanized either immediately following exposure or at 3 and 6 h. The blood, lungs, liver, kidneys, heart and bladder were removed for the quantitation of AA and GSH following homogenization and deproteinization. Immediately following exposure to MS, dose-dependent decreases in pulmonary and renal GSH were observed in rats whereas, in guinea pigs, reductions in pulmonary, hepatic and renal GSH were observed only at the highest level of exposure. No reductions in tissue AA were observed in either species at any exposure level. In both species, blood levels of GSH and AA remained unchanged following exposure. Mainstream smoke (240 puffs) from flue-cured or dark air-cured tobaccos elicited a significant, immediate reduction in pulmonary and renal GSH, but MS from low tar, filter cigarettes was without effect. Within 3 h of exposure, GSH in all tissues had returned to pre-exposure levels. Whole-body, chamber exposure to concentrated SS, generated from smouldering cigarettes, caused a dose-dependent reduction in rat pulmonary, hepatic, renal, cardiac and bladder muscle GSH but only affected pulmonary GSH in the guinea pig. Lesser effects were observed in tissues of rats exposed to diluted SS. In the rat, a comparison of the results of diethylmaleate- and smoke-induced depletion of tissue GSH suggested that, even at exceptionally high levels of exposure, there was a significant store of GSH in tissues that did not interact with tobacco smoke.

Key words: Tobacco smoke; Mainstream; Sidestream; Tissue; Glutathione; Ascorbic acid

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Introduction

Acute and subacute exposure to cigarette tobacco smoke results in an enhancement of pulmonary aryl hydrocarbon hydroxylase (AHH) activity, the enzyme complex responsible for the activation of polycyclic aromatic hydrocarbons (PAHs) to potentially mutagenic and carcinogenic intermediates [1–6]. The ultimate reactivity of these intermediates depends largely on their efficient detoxification by such water-soluble, cellular antioxidants as ascorbic acid (AA) and reduced glutathione (GSH) [7–9].

Glutathione, a nucleophilic agent, protects tissues from the toxicity elicited by reactive metabolites formed by microsomal monooxygenases from halogenated alkanes and alkenes, PAHs, drugs, pesticides, industrial chemicals, etc. [10–13]. The availability of GSH rather than its rate of transfer to electrophilic agents by S-alkyl and S-aryltransferases may be a major factor in cellular protection [14]. The availability of GSH depends upon a balance between the tissue stores and the rate of synthesis (replacement in tissues) and the rate of conjugation with reactive intermediates [15]. Earlier studies noted distinct species differences, GSH being replaced in the liver and kidneys of the rat within 24 h of depletion by diethyl maleate (DEM) whereas, in the guinea pig, GSH levels reached normal values only 48 h after depletion [16]. Presumably, with a faster rate of GSH replacement than utilization, protection from the deleterious effects of reactive intermediates would be afforded to the tissues.

Lower levels of AA have been reported in the blood plasma and leukocytes of cigarette smokers, suggesting that this cellular constituent may play an antioxidant, protective role [17–19]. However, these observations are complicated by a known reduced dietary intake of AA by smokers [20]. This entire subject has recently been reviewed [21]. In animal studies, the exposure of rats to cigarette smoke for 3–7 days resulted in significant alterations in plasma and lung AA levels [8]. However, experiments with guinea pigs revealed that long-term exposure to cigarette smoke elicited a decrease in AA only in the adrenal gland [22]. Other chronic experiments, exposing rats and guinea pigs to cigarette smoke, revealed no effect on tissue (lung, liver, kidney, plasma) levels of AA [23].

Pulmonary tissue contains levels of GSH of the order of 1.0–1.2 μmol/g and AA in the range of 0.6–0.7 μmol/g [5,24]. In the present study, the potential interactions of tissue GSH and AA with absorbed cigarette tobacco smoke were investigated, measuring tissue depletion and replacement of these antioxidants following acute exposure.

Methods

Animals

Adult, male, Sprague–Dawley (Crl: CD(SD)BR) rats of 300–400 g body weight were purchased from Charles River Canada, St. Constant, Quebec and were acclimatized for 1 week in the animal care facility under controlled temperature (21–23°C), humidity (50%) and a light-dark cycle of 12 h. The animals were housed in standard plastic cages in groups of not more than five animals per cage and receiv-
ed standard Purina rat chow and water ad libitum. Non-fasted animals were used in all experiments and, because of the known diurnal variability in tissue AA and GSH residues, experiments were always conducted between 08:00–10:00 h. except for those animals held for a longer duration to examine the recovery of tissue levels of AA and GSH following exposure.

Young adult, male, Hartley (Crl:SH)BR) guinea pigs of 300–400 g body weight were purchased from Charles River Canada. St. Constant, Quebec and were acclimatized in the animal care facility, as described above, for 1 week prior to use. The animals were housed in groups of 3 animals per stainless steel cage with no bedding and received standard guinea pig food and water ad libitum. As with the rats, non-fasted animals were used in all experiments. these experiments being conducted between 08:00–10:00 h. except for those animals being held to examine the post-exposure recovery of AA and GSH residues in tissues.

**Treatment**

The B.-A.T.-Mason inhalation system was employed in the study. Rats and guinea pigs, in groups of six, were restrained in individual, cylindrical, plethysmograph-type chambers attached to a central inhalation chamber for nose-only exposure to freshly generated tobacco smoke from standard, plain-end cigarettes made from a blend of Canadian flue-cured tobaccos delivering approximately 20 mg of total particulate matter (TPM, water and nicotine-free) per cigarette as described previously [3,4]. A 1:5 dilution of a 35-ml sample of mainstream smoke (MS) was employed in the study, this level resulting in a concentration of approximately 11.43 mg TPM/l of air in the inhalation chamber [3]. When the smoke concentration in the exposure chamber was monitored during a 200-s (40 puffs) exposure, approximately 40 mg TPM were collected on Cambridge filter pads [3]. Exposures to higher concentrations (120, 240 puffs) of tobacco smoke were carried out intermittently, with a 5-min exposure to uncontaminated air following each 40-puff cycle, since 240 puffs was considered to be an excessively high level of smoke components for a relatively small animal. Control animals were treated in the same manner in all respects, except that room air was substituted for tobacco smoke.

In other experiments involving rats, two other types of cigarettes were used. One type contained a dark, air-cured, fermented tobacco yielding approximately 20 mg tar/cigarette. The other type was a flue-cured tobacco, filtered cigarette releasing approximately 1.0 mg tar per cigarette. Rats were exposed to 240 puffs of a 1:5 dilution of a 35-ml sample of MS smoke as described above. In additional experiments, animals were exposed nose-only to the vapor phase of tobacco smoke obtained from the standard cigarette, the particulate matter being removed by means of a Cambridge filter, in place before the effluent was passed into the inhalation chamber.

To deplete tissue stores of GSH, adult male rats were treated with diethyl maleate (DEM, Sigma Chemical Co., St. Louis, MO), each animal receiving a volume of 1.0 ml/kg body weight (5.8 mmol/kg) by intraperitoneal (i.p.) injection [16].

Experiments were conducted, exposing rats to sidestream smoke (SS) generated from 24 smouldering cigarettes within a sealed 1.0 m³ Perspex chamber, the smoke being constantly mixed with the aid of a fan mounted in the chamber. Sidestream smoke samples were collected via the B.-A.T.-Mason instrument over a 200-s time
period, diluting the 35-ml samples 1:5 before transferring it to the nose-only chamber for inhalation by the rats (treatment = 6 × 40 puffs with 5 min recovery period between exposures). In a second experiment, rats were restrained in the nose-only inhalation cones and were placed on the floor of the 1.0-m³ chamber for 50 min during the time period of SS generation as described above, thereby facilitating a comparison of diluted and undiluted SS.

At the end of the exposure to tobacco smoke, the animals were either euthanized immediately by an overdose of sodium pentobarbital or were returned to their cages for a duration of 3 or 6 h post-treatment recovery. At euthanasia, samples of blood were obtained and the lungs, livers, kidneys, hearts and bladders were removed immediately and were either stored frozen at −70°C until analyzed or were weighed and 10% w/v homogenates were prepared in cold 5.0% trichloroacetic acid (TCA) containing 10⁻³ M disodium ethylenediaminetetraacetic acid (EDTA) for GSH analysis or in cold 5.0% TCA alone for ascorbic acid analysis [16,18]. Whole-blood or plasma was treated in the same manner for the respective assays. The homogenates were centrifuged at 9000 × g for 5 min; aliquots of the clear supernatant were retained for analysis. Suitable aliquots of the deproteinized supernatants were diluted appropriately with 0.1 M phosphate buffer (pH 8.0), the non-protein sulhydryl groups (principally GSH) being quantitated by the addition of 0.05 ml of 10⁻² M 5-dithiobis-(2-nitrobenzoic acid) (DTNB), the yellow color being measured spectrophotometrically at 412 nm [16]. The concentration of GSH was calculated by relating the measured absorbance to a standard curve prepared with known concentrations of GSH in the TCA-EDTA solution and assayed as described above.

For the AA analysis, aliquots of the TCA-deproteinized supernatants were added to 2.5 ml of the working color reagent (50 ml of 5.0 M acetate buffer (pH 5.9), 0.5 ml of 0.048 M 2,4,6-tripyrinal-s-triazine (TPTZ), 0.4 ml of 0.02 M ferric chloride) with thorough mixing and measuring the developed purple color (reduced ferrous ion reacting with TPTZ) spectrophotometrically at 595 nm [25]. As described in the original paper, interference from uric acid was minimized by deproteinization and the use of the high molarity acetate buffer while precipitation of the developed colored complex was prevented by appropriate adjustment of the TPTZ and ferric iron concentrations.

The Student’s t-test was used to determine the statistical significance of values for smoke-exposed animals from those of control animals exposed to room air under identical conditions. The limit of statistical significance was accepted at P < 0.05.

Results

The exposure of adult male rats to varying concentrations of freshly generated MS resulted in significant reductions in pulmonary GSH concentrations in a dose-dependent manner (Fig. 1). At the 40- and 120-puff exposure level, the GSH was reduced by approximately 18% and 20%, respectively, compared to levels in control animals. At the 240-puff exposure level, the decrease in pulmonary GSH was 34%. Mainstream smoke effects on hepatic GSH concentrations were observed only at the 240-puff exposure level, with only moderate reductions of the concentrations being observed, although the values were statistically significant (P < 0.05). Significant
Fig. 1. The effect of nose-only inhalation of different levels of cigarette mainstream smoke or the gas phase of mainstream smoke on the reduced glutathione (GSH) concentrations in the lungs, livers and kidneys of adult, male, Sprague–Dawley rats immediately following exposure. The open and closed bars represent mean tissue GSH levels for air- and smoke-exposed animals, respectively. The vertical lines represent the standard deviations of mean values from 12 individual animals in each treatment group. The animals were exposed to 40 puffs (experimental duration = 200 s) of a 1:5 dilution of a 35-ml volume sample of freshly generated mainstream smoke from a standard cigarette. At levels of 120 and 240 puffs, the exposure was broken up into 3 and 6 40-puff cycles with a 5-min exposure period to uncontaminated air between treatments. For the gas phase experiments, the particulate matter was trapped on a Cambridge filter pad (pore size = 0.1 mm) prior to entry of the sample into the inhalation chamber. The animals were euthanized immediately and homogenates were prepared for analysis. The asterisk indicates GSH levels that are statistically significant ($P < 0.05$) from control values.
reductions in renal GSH were measured at both the 120- and 240-puff exposures. There was no change in whole blood GSH (control = 0.73 ± 0.009, exposed = 0.73 ± 0.15 μmol/ml; n = 6) or in blood plasma ascorbic acid (control = 0.06 ± 0.02, exposed = 0.06 ± 0.03 μmol/ml; n = 6) of rats following exposure to the 240-puff level. Treatment of rats at the highest exposure level of the gas phase of mainstream smoke resulted in non-significant reductions in the tissue GSH concentrations with the exception of the kidney where a 17%, statistically significant, decrease was measured.

Exposure of guinea pigs to the highest concentration (240 puffs) caused a modest but statistically significant reduction in pulmonary, hepatic and renal GSH levels (Fig. 2). However, levels detected at 3 and 6 h after exposure were comparable to

![Graph showing GSH and ascorbate levels in different tissues]  

Fig. 2. The effect of nose-only inhalation of cigarette mainstream smoke (240 puffs) on the reduced glutathione (GSH, upper panel) and on ascorbic acid (AA, lower panel) concentrations in lungs, livers, kidneys and bladder muscles of adult male, Hartley guinea pigs immediately following exposure. See methods or Fig. 1 for techniques. The open and closed bars represent mean tissue GSH or AA levels for air- or smoke-exposed animals, respectively. The vertical lines represent the standard deviations of mean values from 6 individual animals in each treatment group. The asterisk indicates values that are statistically significant (P < 0.05) from control values.
those measured in controls. In contrast to GSH depletion, the results demonstrated that the highest concentration of MS had no effect on guinea pig tissue AA levels, other than a slight, non-significant reduction in the pulmonary concentration.

Since the amount of potentially toxic compounds (gases, volatiles) was considered to be exceptionally high at the 240-puff exposure level (6 × 40 puffs with a 5-min interval between exposures), two other types of cigarettes in addition to the standard flue-cured product were used. One product was manufactured from a dark, air-cured, fermented tobacco yielding approximately 20 mg tar/cigarette. The rats were exposed to 240 puffs as described and were euthanized immediately after exposure or at 3 or 6 h following the termination of treatment. The results, shown in Fig. 3, demonstrated that smoke from the cigarette made from dark, fermented tobacco caused modest though statistically significant reductions in pulmonary and renal GSH concentrations comparable to the effects obtained with the standard flue-cured cigarette. Only slight, non-significant reductions in hepatic GSH were observed. The smoke from the low tar filter cigarette elicited little change in tissue GSH concentrations. All three tobacco products caused a slight though statistically non-significant increase in bladder muscle GSH. Levels of tissue GSH measured at 3 and 6 h post-exposure were comparable to those of the paired controls.

The distinct differences in chemical composition of MS and SS prompted a study in which animals were exposed to SS either (i) nose-only via the B.-A.T.-Mason system to a 1:5 dilution (6 × 40 puffs with a 5-min interval between exposures) or (ii) whole-body exposure to undiluted SS, the animals being placed inside the chamber for 50 min during which the SS samples were being generated. Marked decreases in tissue GSH levels were observed with both exposure techniques (Fig. 4). However, differences in response were anticipated since, in the nose-only exposure, the SS was diluted prior to transfer to the inhalation chamber. In all tissues except the bladder muscle, a more marked decrease in GSH concentrations was observed following the exposure to the concentrated SS, the average reductions ranging from 29%, 14%, 21%, 22% and 40% for liver, kidney and heart, lung and bladder muscle, respectively.

![Graph showing GSH concentrations in various tissues](image_url)

**Fig 3.** The effects of inhaled mainstream smoke (240-puff level) freshly generated from cigarettes manufactured from different types of tobaccos on the reduced glutathione (GSH) concentrations in pulmonary, hepatic, renal and bladder muscle tissue immediately following treatment (See methods for details). The open and closed bars represent mean tissue GSH levels for air- and smoke-exposed adult male Sprague-Dawley rats, respectively. The vertical lines represent the S.D.s of the mean values from 12 animals in each treatment group. The asterisk indicates GSH levels that are statistically significant (P < 0.05) from control values.
Fig. 4. Adult, male Sprague–Dawley rats were exposed to: (i) uncontaminated air (control: open bars); or to sidestream smoke (SS) generated from smouldering, standard brand cigarettes in an enclosed chamber by (ii) machine-delivered, 1:5 dilutions of 35-ml volume samples of SS to a nose-only inhalation chamber for a 240-puff exposure level (solid bars) and (3) whole-body exposure by placing the animals in cages in the SS-generating chamber for a 50-min period (hatched bars). See text for details. The tissue levels of reduced glutathione (GSH) shown are mean values from 12 animals at each treatment while the vertical lines represent the S.D.s of the means. The asterisk indicates values that are statistically significant ($P < 0.05$) from control values.

While no changes were observed in blood plasma AA levels of rats exposed to the highest level in the earlier experiments, no measurement of tissue AA was carried out at that time. With the marked differences in tissue GSH observed following SS exposure, experiments were repeated, exposing the animals to MS (nose-only, 240-puff level) and to SS (whole-body, 50 min in chamber) (Table I). A comparison of the tissue AA levels of control, air-exposed animals with levels of MS- and SS-exposed animals revealed no significant changes in AA concentration.

The weak response of guinea pig tissue GSH and AA only to high concentrations of MS resulted in an experiment where the animals were exposed to undiluted SS. The animals were placed in the 1.0-m$^3$ chamber and were exposed (whole-body) for a period of 50 min to the SS generated from 24 smouldering cigarettes. The tissue GSH and AA concentrations were determined immediately following exposure and euthanasia, comparisons being made to levels measured in animals exposed to room air for the same duration. The results, presented in Table II, demonstrated that SS caused a significant reduction in GSH only in the lung and had no effect on tissue AA concentrations.

Treatment of rats with DEM, a chemical capable of depleting tissue stores of GSH, resulted in marked reductions in GSH content of the order of 45–77% depending upon the tissue examined (Fig. 5). While treatment of control animals at the
TABLE 1

ASCORBIC ACID LEVELS IN TISSUES OF SPRAGUE-DAWLEY RATS EXPOSED TO MAINSTREAM AND SIDESTREAM CIGARETTE SMOKE

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Ascorbic acid (μmol/g tissue)</th>
<th>Mainstreamb</th>
<th>Sidestreamc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.77 ± 0.13</td>
<td>0.75 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>Exposed</td>
<td>0.68 ± 0.10</td>
<td>0.73 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.21 ± 0.15</td>
<td>1.21 ± 0.13</td>
<td></td>
</tr>
<tr>
<td>Exposed</td>
<td>1.41 ± 0.18</td>
<td>1.16 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.94 ± 0.13</td>
<td>0.83 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>Exposed</td>
<td>0.78 ± 0.08</td>
<td>0.81 ± 0.12</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.23 ± 0.04</td>
<td>0.18 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Exposed</td>
<td>0.21 ± 0.05</td>
<td>0.18 ± 0.02</td>
<td></td>
</tr>
<tr>
<td>Bladder</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>0.16 ± 0.07</td>
<td>0.17 ± 0.09</td>
<td></td>
</tr>
<tr>
<td>Exposed</td>
<td>0.19 ± 0.08</td>
<td>0.15 ± 0.08</td>
<td></td>
</tr>
</tbody>
</table>

bAnimals were euthanized immediately after exposure (See methods).

Animals (n = 6) were exposed nose-only to 240 puffs (6 × 40 puffs with a 5-min recovery period between exposures) of a 1:5 dilution of a 35-ml volume sample of freshly generated mainstream smoke from a standard cigarette producing 20 mg tar/cigarette.

Animals were exposed whole-body to freshly generated sidestream smoke from 24 cigarettes smouldering in an enclosed 1.0-m³ chamber for a period of 50 min. See methods for details.

240-puff exposure level resulted in small but statistically significant reductions in pulmonary, hepatic and renal GSH, such treatment had little or no effect on further decreasing the tissue GSH levels in DEM-treated animals. Interestingly, in both the control and DEM-treated rats, an elevation of GSH levels in bladder muscle was observed, although it was markedly higher only in the control, smoke-exposed group. No smoke-related changes were observed in cardiac muscle.

Discussion

While the current theories implicate the production of highly reactive intermediates and their interaction with cellular membranes and nucleic acids in the initiation and promotion of carcinogenesis, there are mechanisms whereby such oxidants can be neutralized by normal body constituents including water-soluble AA and GSH [7,24,26]. However, if these antioxidants were depleted by high levels of the reactive intermediates, cellular toxicity should occur. The question is whether these antioxidant mechanisms function efficiently and whether or not the rate(s) of resynthesis or replacement is sufficiently rapid to prevent the deleterious effects of the reactive intermediates. Earlier research demonstrated that acute exposure to
TABLE II
GLUTATHIONE (GSH) AND ASCORBIC ACID LEVELS IN TISSUES OF GUINEA PIGS EXPOSED TO SIDESTREAM CIGARETTE SMOKE*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Glutathione (µmol/g)</th>
<th>Ascorbic acid (µmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.27 ± 0.19</td>
<td>1.32 ± 0.12</td>
</tr>
<tr>
<td>Exposed</td>
<td>2.62 ± 0.24*</td>
<td>1.26 ± 0.18</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.47 ± 0.24</td>
<td>1.70 ± 0.24</td>
</tr>
<tr>
<td>Exposed</td>
<td>7.47 ± 0.80</td>
<td>1.66 ± 0.16</td>
</tr>
<tr>
<td>Kidney</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>3.65 ± 0.25</td>
<td>1.03 ± 0.20</td>
</tr>
<tr>
<td>Exposed</td>
<td>3.87 ± 0.36</td>
<td>0.89 ± 0.13</td>
</tr>
<tr>
<td>Bladder</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.25 ± 0.39</td>
<td>1.18 ± 0.21</td>
</tr>
<tr>
<td>Exposed</td>
<td>1.46 ± 0.61</td>
<td>0.96 ± 0.07</td>
</tr>
</tbody>
</table>

*Animals (n = 6) were exposed whole-body to freshly generated sidestream smoke from 24 cigarettes smouldering in an enclosed 1.0-m³ chamber for a period of 50 min. The animals were euthanised immediately after exposure (See methods).

Values statistically different from those of controls at P < 0.05.

cigarette tobacco smoke resulted in marked increases in pulmonary AHH, the enzyme complex involved in the activation of PAHs into reactive intermediates [3]. Once formed, these intermediates should elicit a local action on the pulmonary tissue prior to any systemic action and, therefore, should exert some effect on tissue levels of endogenous antioxidant chemicals.

In the present studies, the inhalation exposure to levels of MS smoke high enough to elicit toxicity did not affect tissue stores of AA in either the rat or the guinea pig. While the rat may not be an appropriate model for such an antioxidant effect, the species not being dependent upon diet for AA, the guinea pig should have shown some response even in an AA-repleted state. The results suggest that either the tissue AA was replaced too quickly to be reflected in a transient decrease or that this endogenous chemical did not interact with reactive molecules in tobacco smoke. The present studies have confirmed the results of other investigators, exposure to cigarette smoke having little effect on tissue AA concentrations [22,23]. In both the human and the hamster, subchronic exposure to tobacco smoke resulted in a 2-fold greater accumulation of 14C-labeled AA in alveolar macrophages over concentrations measured in unexposed individuals [7]. Other studies have reported lowered AA levels in both blood plasma and leukocytes [21]. These results suggested that any protective antioxidant role in the lung might occur at the cellular (macrophage) level. Studies in humans have shown that AA levels in the plasma of smokers were significantly lower than those in non-smokers [19,27]. Partly, this was attributed to a reduced dietary intake of foods rich in AA by smokers but, even so, the inverse association between smoking habits and plasma AA appeared to be independent of dietary intake [19–21,28].
In contrast to AA, GSH was reduced in a dose-dependent manner in the rat following acute exposure to MS and SS tobacco smoke. At least one other study has reported this observation in vivo [29]. Pulmonary GSH was neither affected by excessively high exposure levels (240 puffs) of the gas phase of MS smoke nor the MS smoke from filtered cigarettes (Figs. 1,3), suggesting that it is the particulate fraction containing the electrophilic agents that is responsible for the observed response. However, as the present study has shown, the depletion of pulmonary stores of GSH and those of other tissues was transient, tissue concentrations returning to control levels within 3 h of terminating treatment. The dynamics of GSH are such that certain tissues, i.e. the liver, are synthesizers and exporters of GSH, secreting large amounts into the bloodstream, this extracellular GSH being taken up and used by other tissues, i.e. the kidney, the lung, that cannot synthesize it rapidly [30]. Agents eliciting a drastic depletion of hepatic GSH will also cause depletion in other tissues. In the present study, blood levels of GSH were not decreased and hepatic concentrations were only slightly and transiently depleted immediately following exposure to the highest level of cigarette smoke.

Treatment of the rat with DEM resulted in an immediate and much greater (66%) depletion of pulmonary GSH (Fig. 5). Given the fact that approximately 10% of
tissue GSH is reported to be associated with the mitochondria, this fraction would be less available for rapid interaction with reactive intermediates as would the cytoplasmic fraction [26]. Mitochondrial GSH has a tissue half-life of 30 h whereas the half-life of the cytoplasmic pool is 2 h [26,31]. Since the excessively high exposure resulted only in a maximum of 35% depletion of pulmonary GSH, there would appear to be a significant unreacted reserve of approximately 55% GSH in the pulmonary tissue. It should also be noted that the in-chamber, whole-body exposure to concentrated SS smoke resulted in only a 27% and 22% depletion in pulmonary GSH in the rat (Fig. 4) and the guinea pig (Table II), respectively. It would appear that the inhalation of high levels of MS and SS tobacco smoke did not 'stress' the antioxidant capabilities of pulmonary GSH, the amounts used being replaced within 3 h after treatment. Reports indicate that the subchronic exposure to cigarette smoke over a period of 35 days resulted in a stimulation of GSH synthesis, tissue levels being higher than in air-exposed control animals [5]. This rebound phenomenon has been reported following both single and repeated exposure to agents that are detoxified by the GSH conjugating mechanism [30,32].

Inhalation exposure to MS tobacco smoke results in the induction of AHH in other mammalian tissues, particularly in the kidney [3]. In view of this, it was not surprising to find a reduction in rat and guinea pig renal GSH following exposure to the MS tobacco smoke generated from plain-end cigarettes. Results in the rat liver were equivocal, significant reductions in hepatic GSH being observed only at the highest levels of exposure to MS and SS tobacco smoke (Figs. 1,4). However, as has been shown, there is a considerably larger amount of GSH in this tissue than in others, so that a reduced tissue response would be expected. Again, the 'lost' GSH was replaced within 3 h of terminating treatment. Cardiac muscle GSH appeared to be little affected by MS although modest, dose-dependent reductions were observed following exposure to the highly concentrated SS (Fig. 4). The interesting and consistent observations of increased bladder muscle GSH (Figs. 3,5) cannot be explained at present, although this was seen only with MS, the SS smoke-exposed animals showing a decrease in muscle GSH.

In summary, it would appear that AA plays no role in the deactivation of reactive intermediates generated during the combustion of tobacco products or formed in situ following the inhalation of the smoke by either the rat or the guinea pig. In contrast, the early post-exposure decrease of GSH in such immediate target organs as the lungs of both species suggests that this antioxidant chemical plays some protective role. Both MS and undiluted SS elicited a transient depletion of pulmonary GSH, although the rapid replacement of the tissue stores may be a major factor in the resistance of these species to target organ toxicity. However, long-term smokers with lung cancer did not show a significant depletion in pulmonary GSH compared to those levels measured in the pulmonary tissue of nonsmokers [33]. Despite the detection of high levels of pulmonary monooxygenases, such results suggest that tissue stores of GSH were replaced rapidly.

References


