Fetal Exposure to Low Protein Maternal Diet Alters the Susceptibility of Young Adult Rats to Sulfur Dioxide-Induced Lung Injury

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ABSTRACT The maternal diet is an important determinant of glutathione-related metabolism in rats. Glutathione (GSH) may play a major role in the detoxification of sulfur dioxide (SO₂) within the lungs. The effects of fetal exposure to a low protein maternal diet upon later susceptibility to pulmonary injury induced by chronic SO₂ exposure were evaluated in young adult rats. Pregnant rats were fed purified diets containing 180 g casein/kg (control diet) or 120, 90 or 60 g casein/kg (experimental diets). After parturition, all dams were fed a standard non-purified diet (189 g protein/kg diet). The pups thus differed only in terms of protein nutrition during gestation. At seven wk of age the male pups were housed in either room air or 286 μg SO₂/m³ for 5 h/d during a 28-d period. At the end of the final SO₂ treatment period, the rats exposed to 90 or 60 g casein/kg diets in utero exhibited significantly greater pulmonary injury, as assessed by bronchoalveolar lavage, than did those exposed to control diet in utero. Significant maternal diet–induced differences in activities of enzymes of the γ-glutamyl cycle were noted in the lungs and livers of rats which had not undergone SO₂ treatment. Furthermore, the response of these enzyme activities to SO₂ treatment was determined by prior exposure to the maternal diet. SO₂-treated rats exposed to control diet (180 g casein/kg) and low protein diet (60 g casein/kg), but not those exposed to 120 or 90 g casein/kg diets, tended to augment the activities, relative to rats not treated with SO₂, of enzymes which maintain tissue GSH status either through synthesis or recycling. Differences in susceptibility to SO₂-induced tissue injury may be related to programming of GSH metabolism by the maternal diet. Alternatively, impaired immune and acute phase responses to an inflammatory insult may account for a failure to resolve initial SO₂-induced injury in rats exposed to low protein maternal diets.

KEY WORDS: rats • maternal undernutrition • glutathione

Industrial emissions and other urban air pollutants pose a major threat to human health. Ozone, nitrogen dioxide, particulate matter and sulfur dioxide (SO₂) have all been implicated in causing damage to pulmonary tissues and in initiation of asthmatic symptoms (Gong 1992). SO₂ is a highly injurious agent which, in contact with moist alveolar membranes, will form sulfuric acid. Regulation of SO₂ production has reduced concentrations in urban air to approximately 1.4 μg/m³, but local variations and occupational exposures may result in asthmatics and other susceptible individuals suffering SO₂-related pulmonary injury (Gong 1992, Stjernberg et al. 1985, Tewari and Shukla 1991).

Within the lung SO₂ is detoxified through the sulfitolysis of oxidized glutathione (GSSG) (Kagedal et al. 1986, Mannervik et al. 1974). GSSG is generated from reduced glutathione (GSH) through the action of glutathione peroxidase (GPx) on free radical species (Lawrence and Burk 1976). Sulfitolysis of GSSG and the subsequent elimination of sulfitolysis products in the urine, prevents the regeneration of GSH through glutathione reductase (GRed) (Mannervik et al. 1974, Winell and Mannervik 1969) and results in the depletion of GSH from all tissues (Langley-Evans et al. 1996). This may leave individuals more susceptible to tissue injury caused by secondary agents, or to long term pulmonary disorders associated with chronic gas exposure. Chronic exposure to low SO₂ concentrations is implicated in the development of childhood asthma (Tseng and Li 1990).

Previous work with rat models in our laboratory has demonstrated that glutathione metabolism is determined in utero by aspects of the maternal diet (Langley et al. 1994, Langley-Evans et al. 1995). Activities of GPx and GRed are strongly associated with maternal protein intake in rats undergoing mild food restriction. In otherwise untreated weanling rats, maternal diet is a determinant of tissue GSH concentrations, pulmonary and hepatic GRed and γ-glutamylcysteine synthetase (GCS) activities (Langley-Evans et al 1995). Given the influence of maternal diet on GSH metabolism and the role of GSH in the detoxification of SO₂, we have proposed that maternal diet during pregnancy may determine the later susceptibility of the offspring to SO₂-induced tissue injury. In this...
article we report the effects of chronic SO₂ exposure on rats of
different gestational protein nutrition.

MATERIALS AND METHODS

Chemicals. All chemicals were obtained from Sigma (Poole, UK) and
were of reagent grade.

Animals. Experiments were performed on a total of 88 rats, in
accordance with the ethical requirements of the British Home Office
Animal Act (1986). Sixteen virgin female Wistar rats, bred in the
Southampton University Animal Unit were maintained in wire
mesh cages in a room with a 12-h light cycle at 22±2°C.

When body weight reached 200–225 g rats were fed purified diets
containing 180 g casein/kg diet (control diet) or 120, 90 or 60 g
casein/kg diet (experimental), as previously described (Langley et al.
1994). After habituation to the diets for 14 d, rats were mated and
maintained on the habituated level of dietary protein until parturi-
tion. Within 12 h of parturition all dams were transferred to standard
nonpurified diet (CRMX, Special Diet Services, UK: 189 g/kg protein,
23 g/kg corn oil), and the same food was used to wean the pups. These
rats differed, therefore, only in terms of prenatal protein nutrition.

Sulfur dioxide exposure. At seven wk of age, male rats were ran-
domly divided into groups of 4 to 16 and were exposed to room air
(atmospheric control, <1 μg SO₂/m³) or 286 μg SO₂/m³ within an
airtight perspex chamber, 0.5 m³ volume, with a wire mesh floor. Gases
were fed into the chamber at a rate of 7 L/min and vented through
an outlet tube. All gassing treatments were completed between
0900 and 1600 h each day for 28 d, and no food or water was
provided in the gas chamber. In preliminary experiments, the pres-
ence of such materials and sawdust within the chamber was found to
significantly modulate gas concentrations (data not shown).

The gas treatment protocol of 5 h/d for 28 d was designed to
resemble occupational exposures in humans. A concentration of 286
μg SO₂/m³ had been demonstrated in preliminary studies to induce
measurable lung injury. To achieve the specified SO₂ concentration,
gas delivered from cylinders containing 1144 μg SO₂/m³ (BOC,
Guilford, Surrey, UK) was mixed with compressed air (BOC) using
a clinical gas mixing unit. Concentration of SO₂ was monitored using
an in-line monitor (Neotox, Harlow, Essex, UK).

Bronchoalveolar lavage and tissue collection. At the end of
the final 5-h SO₂ treatment period, rats were killed with sodium pento-
barbital. Bronchoalveolar lavage was performed as previously de-
scribed (Kelly et al. 1991), using five 2-ml aliquots of sterile saline
(9 g NaCl/L). No determination of lung volume was performed, but in
all bronchoalveolar lavages, 80–85% of instilled fluid was
recovered. Blood (2–3 mL) was collected by heart puncture imme-
diately after lavage, and lungs and liver were carefully removed
and weighted. A 200-mg portion of each tissue was retained for
glutathione determination, and another 200-mg section of lung and
liver was taken for immediate preparation of cytosol for assay of
glutathione S-transferase activity. The remaining tissue was frozen
in liquid nitrogen and stored at −80°C for later enzyme analyses.

Glutathione determination. Glutathione concentrations were
determined in fresh tissue, blood and bronchoalveolar lavage fluid
(BALF), using the method of Tietze (1969) as modified by Langley
and Kelly (1992). Oxidized glutathione was determined by the
method of Griffith (1980), but because concentrations were typically
below the detection limit of the assay (0.1 μmol/g tissue), data are
not shown.

Enzyme assays. Liver and lung homogenates were prepared as
previously described (Langley and Kelly, 1992). The activities of γ-
glutamyl transpeptidase [EC 2.3.2.1] (GT), glutathione peroxidase
[EC 1.11.1.9] (GPx) and glutathione reductase [EC 1.6.4.2] (GRed)
were determined using the methods of Langley and Kelly (1992).
γ-Glutamyl transpeptidase (GT) activity was assayed using the
method of Langley et al. (1994). Activity of glutathione S-trans-
ferase [EC 2.5.1.18] (GST) was determined in 100 000 g centrifuga-
lion preparations of lung and liver, using 1-chloro-2,4-dinitrobenzene
(CDNB) as substrate (Jones et al. 1988). This substrate will react
with GST forms α, π and μ. All enzyme activities are expressed per
mg of tissue protein, determined by the method of Smith et al.
(1985).

Cell counting. Total leucocyte numbers in blood and BALF were
counted using a hemocytometer as reported previously (Kelly et al.
1991). Staining of blood smears and of lavage fluid prepared using a
cytospin, with May, Grumwalds and Giemsa stains, was used to quan-
tify specific leucocyte populations.

Statistical analyses. All data are presented as means ± SEM for
4 to 18 observations per group. Two way analysis of variance followed
by a Tukey test (Williams 1993) was used to determine statistically
significant differences among groups (P < 0.05).

RESULTS

Markers of tissue injury. The typical recovery of fluid in-
stilled into the lungs during the bronchoalveolar lavage proce-
nure was 70–95%. The total number of leucocytes recovered in
lavage fluid was related to diet experienced in utero (P <
0.0001). Markedly fewer cells were recovered from the lungs of
rats exposed to 90 or 60 g casein/kg maternal diets (Table 1). In
all of the maternal diet groups the majority of leucocytes
recovered were macrophages (88–97%); consequently, the
same pattern of maternal diet-related differences (P <
0.0001) in cell number was observed. The proportion of cells identified
as macrophages was less (P < 0.05) in the control diet (180 g
casein/kg) group than in the experimental diet groups because
significantly more (11- to 22-fold) neutrophils were present in
the lungs of control rats. SO₂ Treatment had no major effects
upon alveolar leucocyte populations recovered in lavage fluid, other-
than to reduce neutrophil numbers (P < 0.05) in the
control diet group (180 g casein/kg) to the equivalent of the
other dietary groups.

Consistent with maternal diet-related differences in leuco-
cyte populations in the lung, circulating cell populations also
varied among rats born of dams fed different amounts of protein
during pregnancy (Table 2). The interaction of maternal
diet with SO₂ treatment influenced total cell number (P <
0.01), and maternal diet, SO₂ and their interaction influenced
lymphocyte number (P < 0.05). Although rats exposed in utero to the 60 g casein/kg maternal diet had numbers of lymp-
ghocytes and neutrophils in circulation similar to controls,
rats exposed in utero to 120 g casein/kg maternal diet had
higher total leucocyte counts, and rats exposed in utero to 90
 g casein/kg maternal diet had significantly fewer lymphocytes.

Treatment with 286 μg SO₂/m³ reduced total cell numbers in
the 180 g casein/kg and 120 g casein/kg maternal diet groups,
but not in rats prenatally exposed to lower levels of maternal
dietary protein. This effect was attributable to large SO₂-indu-
duced declines in lymphocyte populations. No significant ef-
fects of SO₂ on circulating leucocytes were observed in rats
exposed to lower maternal dietary protein concentrations.

BALF protein concentration provides a measure of damage
to capillary epithelial cells in the lungs because such damage
allows leakage of vascular fluid into the alveoli. There was no
significant variation in baseline BALF protein concentrations
among the different maternal diet groups that were not treated
with SO₂ (Figure 1). SO₂ Treatment did, however, modulate
BALF protein concentrations (P < 0.005). Following SO₂
 treatment, significantly higher BALF protein concentration
was observed in rats exposed to 90 or 60 g casein/kg diets in
utero, relative to the air-treated controls of the same maternal
dietary groups. No significant differences were noted in the
120 g casein/kg or 180 g casein/kg maternal diet groups. In
the 60 g casein/kg maternal diet group the concentration of
protein in BALF following SO₂ treatment was significantly
higher than in SO₂-treated control diet (180 g casein/kg mat-
nunal diet) rats (P < 0.05). This was not true of SO₂-treated
rats of the 120 or 90 g casein/kg maternal diet groups. In the
GSH concentrations in the lungs and BALF were unaltered by either gestational protein nutrition or SO2 exposure (Table 3). In the liver however, maternal diet (P < 0.05) and SO2 (P < 0.01) influenced GSH concentrations. GSH concentration was significantly lower in livers of rats exposed to the 120 g casein/kg maternal diet than in 180 g casein/kg maternal diet control rats. Conversely, rats exposed to 60 g casein/kg maternal diet had higher hepatic GSH concentrations than did the 120 g casein/kg maternal diet group. Treatment with 286 μg SO2/m3 had no effect on hepatic GSH concentration in the 180 or 90 g casein/kg maternal diet groups. In the 60 g casein/kg maternal diet group, hepatic GSH concentration was significantly lowered by SO2 treatment. In contrast, rats exposed to 120 g casein/kg maternal diet had significantly greater hepatic GSH concentrations in response to SO2 treatment.

Blood GSH concentrations were significantly modulated by maternal diet (P < 0.02), SO2 treatment (P < 0.001) and their interaction (P < 0.03). In air-treated rats, blood GSH concentrations were similar among the 180, 120 and 90 g

<table>
<thead>
<tr>
<th>Maternal diet</th>
<th>Treatment</th>
<th>n</th>
<th>Total cells</th>
<th>Macrophages</th>
<th>Neutrophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>180 g casein/kg</td>
<td>Control 18</td>
<td>12.1 ± 1.2a</td>
<td>10.7 ± 1.2a</td>
<td>1.11 ± 0.36a</td>
<td></td>
</tr>
<tr>
<td>SO2 6</td>
<td>14.0 ± 2.2x</td>
<td>13.7 ± 2.2x</td>
<td>0.12 ± 0.03*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>120 g casein/kg</td>
<td>Control 4</td>
<td>11.8 ± 1.8a</td>
<td>11.5 ± 1.7a</td>
<td>0.10 ± 0.03b</td>
<td></td>
</tr>
<tr>
<td>SO2 6</td>
<td>9.5 ± 2.0xy</td>
<td>9.2 ± 2.0xy</td>
<td>0.06 ± 0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 g casein/kg</td>
<td>Control 18</td>
<td>5.8 ± 0.9b</td>
<td>5.6 ± 0.9b</td>
<td>0.05 ± 0.03b</td>
<td></td>
</tr>
<tr>
<td>SO2 6</td>
<td>7.0 ± 1.0y</td>
<td>6.9 ± 1.0y</td>
<td>0.07 ± 0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 g casein/kg</td>
<td>Control 4</td>
<td>7.5 ± 0.5b</td>
<td>7.3 ± 0.5b</td>
<td>0.10 ± 0.02b</td>
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</tr>
<tr>
<td>SO2 10</td>
<td>9.4 ± 0.8xy</td>
<td>9.1 ± 0.8xy</td>
<td>0.12 ± 0.04</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2-way ANOVA
Effect of diet | P < 0.0001 |
Effect of SO2 | P < 0.0001 |
Interaction of diet and SO2 | P < 0.05 |

1 All data are shown as mean ± SEM for n observations. Different superscripts a, b for values in columns indicate significant differences among air treated groups, prenatally exposed to different diets (P < 0.05). Superscripts x, y indicate significant differences among SO2 treated groups exposed prenatally to different diets (P < 0.05). *Indicates a significant difference between air and SO2 treated rats of the same dietary group (P < 0.05).

TABLE 2
Leukocyte populations in circulation of rats exposed in utero to maternal diets of different protein concentrations, in response to sulfur dioxide treatment

<table>
<thead>
<tr>
<th>Maternal diet</th>
<th>Treatment</th>
<th>n</th>
<th>Total cells</th>
<th>Lymphocytes</th>
<th>Neutrophils</th>
</tr>
</thead>
<tbody>
<tr>
<td>180 g casein/kg</td>
<td>Control 18</td>
<td>4.73 ± 0.29b</td>
<td>3.97 ± 0.27a</td>
<td>0.54 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>SO2 6</td>
<td>3.60 ± 0.37y*</td>
<td>2.76 ± 0.34y*</td>
<td>0.75 ± 0.02</td>
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<td></td>
</tr>
<tr>
<td>120 g casein/kg</td>
<td>Control 4</td>
<td>5.70 ± 0.17a</td>
<td>4.65 ± 0.18a</td>
<td>1.02 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>SO2 6</td>
<td>4.20 ± 0.21xy*</td>
<td>3.44 ± 0.19xy*</td>
<td>0.74 ± 0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90 g casein/kg</td>
<td>Control 18</td>
<td>3.59 ± 0.28b</td>
<td>2.89 ± 0.27b</td>
<td>0.53 ± 0.14</td>
<td></td>
</tr>
<tr>
<td>SO2 6</td>
<td>4.27 ± 0.74xy</td>
<td>2.85 ± 0.48y</td>
<td>1.24 ± 0.32</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 g casein/kg</td>
<td>Control 4</td>
<td>4.33 ± 0.27bc</td>
<td>3.50 ± 0.31ab</td>
<td>0.78 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>SO2 10</td>
<td>4.88 ± 0.30x</td>
<td>3.80 ± 0.24x</td>
<td>1.04 ± 0.18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2-way ANOVA
Effect of diet | NS |
Effect of SO2 | P < 0.01 |
Interaction of diet and SO2 | P < 0.05 |

1 All data are shown as mean ± SEM for n observations. Different superscripts a, b, c for values in columns indicate significant differences among air treated groups, prenatally exposed to different diets (P < 0.05). Superscripts x, y indicate significant differences among SO2 treated groups exposed prenatally to different diets (P < 0.05). *Indicates a significant difference between air and SO2 treated rats of the same dietary group (P < 0.05).
casein/kg maternal diet groups, but elevated in the 60 g casein/kg group relative to the 180 and 90 g casein/kg groups (Table 3). Following SO\textsubscript{2} treatment, rats exposed to 180 and 60 g casein/kg maternal diet had lower blood GSH concentrations than did untreated controls. No alterations due to SO\textsubscript{2} were noted in the 120 and 90 g casein/kg maternal diet groups.

Figure 2 shows pulmonary activities of GPx, GRed, GCS and GT. Maternal diet had no influence on GPx or GCS activities in this tissue. Gestational exposure to the 120 g casein/kg maternal diet however, led to a postnatal elevation of lung GRed and GT activities relative to control and 90 g casein/kg maternal diet groups. Treatment with SO\textsubscript{2} had a number of maternal diet-specific effects on pulmonary enzyme activities. Rats born of dams fed 120 g casein/kg diet had markedly lower lung GPx activity following SO\textsubscript{2} treatment. No other maternal diet groups responded in this manner. Lung GCS activity was elevated significantly by SO\textsubscript{2} exposure in 180 and 60 g casein/kg exposed rats ($P < 0.05$), but GRed and GT activities were unaltered. Lung GST (Fig. 3) activity was not responsive to maternal diet. Following exposure to SO\textsubscript{2}, GST activity was between 29 and 40% lower in all maternal diet groups, except the 60 g casein/kg group in which the effect was not statistically significant ($P = 0.18$).

In the liver (Fig. 4), maternal diet influenced activities of GCS and GRed. Activity of GCS was elevated in the 120 and 60 g casein/kg maternal diet groups relative to 180 g casein/kg and 90 g casein/kg maternal diet groups. Liver GRed activity was elevated in rats born of dams fed 60 g casein/kg diet relative to dietary controls. Treatment with 286 mg SO\textsubscript{2}/m\textsuperscript{3} had no effect on hepatic GST, but GCS activity was significantly greater due to SO\textsubscript{2} exposure in rats whose dams were fed 180 and 90 g casein/kg diet. Following SO\textsubscript{2} treatment liver GRed activity was higher in 120 and 90 g casein/kg maternal diet groups than in their corresponding air exposed (untreated) controls, and GPx activity was similarly higher in SO\textsubscript{2} treated rats in the 60 g casein/kg maternal diet group.

**DISCUSSION**

We have examined the possibility that gestational nutrition may be an important determinant of later susceptibility to

![Figure 1](https://academic.oup.com/jn/article-abstract/127/2/202/4728739)

**FIGURE 1** Protein concentrations in bronchoalveolar lavage fluid from rats exposed in utero to maternal diets of different protein concentration, following sulfur dioxide treatment. All data are mean ± SEM for $n$ indicated in the tables. Different letters indicate significant differences among groups ($P < 0.05$), as described in the tables.

**TABLE 3**

<table>
<thead>
<tr>
<th>Maternal diet g casein/kg</th>
<th>Treatment</th>
<th>$n$</th>
<th>Lung $\mu$mol/g</th>
<th>Liver $\mu$mol/g</th>
<th>BALF $\mu$mol/g</th>
<th>Blood $\mu$mol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>180</td>
<td>Control</td>
<td>18</td>
<td>1.08 ± 0.06</td>
<td>5.69 ± 0.32\textsuperscript{a}</td>
<td>1.11 ± 0.26</td>
<td>507 ± 26\textsuperscript{b}</td>
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<td></td>
<td>SO\textsubscript{2}</td>
<td>6</td>
<td>0.95 ± 0.06</td>
<td>4.89 ± 0.56\textsuperscript{y}</td>
<td>1.09 ± 0.48</td>
<td>328 ± 25\textsuperscript{x}</td>
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<tr>
<td>120</td>
<td>Control</td>
<td>4</td>
<td>0.97 ± 0.11</td>
<td>4.64 ± 0.11\textsuperscript{b}</td>
<td>1.02 ± 0.52</td>
<td>532 ± 54\textsuperscript{ab}</td>
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<tr>
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<td>SO\textsubscript{2}</td>
<td>6</td>
<td>0.97 ± 0.04</td>
<td>5.20 ± 0.9\textsuperscript{x}</td>
<td>1.20 ± 0.14</td>
<td>464 ± 32\textsuperscript{x}</td>
</tr>
<tr>
<td>90</td>
<td>Control</td>
<td>18</td>
<td>1.03 ± 0.05</td>
<td>4.88 ± 0.41\textsuperscript{ab}</td>
<td>1.54 ± 0.29</td>
<td>498 ± 22\textsuperscript{b}</td>
</tr>
<tr>
<td></td>
<td>SO\textsubscript{2}</td>
<td>6</td>
<td>1.07 ± 0.10</td>
<td>4.16 ± 0.37\textsuperscript{y}</td>
<td>1.43 ± 0.40</td>
<td>522 ± 17\textsuperscript{x}</td>
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<tr>
<td>60</td>
<td>Control</td>
<td>4</td>
<td>1.37 ± 0.14</td>
<td>6.00 ± 0.30\textsuperscript{a}</td>
<td>2.01 ± 0.78</td>
<td>599 ± 50\textsuperscript{a}</td>
</tr>
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<td></td>
<td>SO\textsubscript{2}</td>
<td>10</td>
<td>1.15 ± 0.07</td>
<td>4.40 ± 0.17\textsuperscript{xy}</td>
<td>0.84 ± 0.27</td>
<td>453 ± 46\textsuperscript{xy}</td>
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</table>

2-way ANOVA

<table>
<thead>
<tr>
<th>Effect of diet</th>
<th>NS</th>
<th>$P &lt; 0.05$</th>
<th>NS</th>
<th>$P &lt; 0.02$</th>
<th>NS</th>
<th>$P &lt; 0.001$</th>
<th>NS</th>
<th>$P &lt; 0.03$</th>
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</thead>
<tbody>
<tr>
<td>Effect of SO\textsubscript{2}</td>
<td>NS</td>
<td>$P &lt; 0.01$</td>
<td>NS</td>
<td>$P &lt; 0.001$</td>
<td>NS</td>
<td>$P &lt; 0.03$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Interaction of diet and SO\textsubscript{2}</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>$P &lt; 0.03$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^1$ All data are shown as mean ± SEM for $n$ observations. Different superscripts a, b, c for values in columns indicate significant differences among air treated groups, prenatally exposed to different diets ($P < 0.05$). Superscripts x, y indicate significant differences among SO\textsubscript{2} treated groups exposed prenatally to different diets ($P < 0.05$). *Indicates a significant difference between air and SO\textsubscript{2} treated rats of the same dietary group ($P < 0.05$).
smaller secondary influx of neutrophils and macrophages was observed. There was no apparent evidence of capillary epithelial damage, and GSH was depleted by 25–40% in all tissues.

In this study the pattern of injury just described was not observed, despite using the same SO2 treatment regimen. A preliminary histological examination of the lungs of two rats from each of the eight groups revealed no evidence of inflammatory cell infiltration of the airways, which parallels the lack of differences in cell numbers recovered by bronchoalveolar lavage. Tissue GSH concentrations were largely maintained in most SO2-treated rats, a phenomenon which appears to stem from SO2-induced changes in enzyme activities discussed below. In addition to differences in maternal diets, an important contrast between this study and our earlier work (Langley-Evans et al. 1996) is the timecourse over which the injury was studied. In this study, SO2 exposure was for 28 d, and by this time elements of the injury, for example the influx of inflammatory cells, may well have been resolved. A transient neutrophil influx into the lungs with later resolution was noted in guinea pig neonates exposed to a hyperoxic atmosphere over 28 d (Phillips 1995). Moreover, the variation in numbers of particular cell types recovered in BALF and in blood was high, possibly masking trends within the data. In BALF in particular, this variability has been previously noted (Kelly et

FIGURE 2  Enzyme activities in lung tissue of rats exposed in utero to maternal diets of different protein concentration, following sulfur dioxide treatment. Top right: γ-Glutamylcysteine synthetase (GCS); Top left: Glutathione peroxidase (GPx); Bottom right: γ-Glutamyl transpeptidase (GT) Bottom left: Glutathione reductase (GRed). All data are mean ± SEM for n indicated in the tables. Units of enzyme activity are nmol NADH or NADPH hydrolyzed per min. Different letters indicate significant differences among groups (P < 0.05), as described in the tables.

pulmonary injury caused by an air pollutant. Epidemiological studies have indicated that in humans, incidence of chronic pulmonary disease and impairment of lung function may be related to characteristics at birth (Barker et al. 1991). The primary finding of our more specific investigation in rats is that fetal exposure to a mild restriction of maternal protein intake during pregnancy may lead to more pronounced SO2-induced injury in later life.

The injury induced by SO2 is, however, somewhat difficult to define. The variability of the response is most likely attributable to the magnitude and duration of the exposure. Human studies indicate that acute exposures induce bronchoconstriction in the vulnerable population (Sandstrom et al. 1989a and 1989b, Stjernberg et al. 1985), while chronic exposure, such as the SO2 treatment employed in this study, may be responsible for the development of asthmatic symptoms (Tseng and Li 1990). In rats, very high (2288 μg SO2/m3) exposure also leads to bronchoconstriction and damage to the upper airways (Stratmann et al. 1991) characterized by mucous metaplasia and sloughing of tracheal epithelial cells (Basbaum et al. 1990). Previous studies that utilized the offspring of rats fed non-purified diets (Langley-Evans et al. 1996), indicated that over 7–14 d exposure to 14–286 μg SO2/m3 for 5 h/d, the lung was invaded by neutrophils. After resolution of this response a

FIGURE 3  Glutathione S-transferase activities in lung tissue of rats exposed in utero to maternal diets of different protein concentration, following sulfur dioxide treatment. All data are mean ± SEM for n indicated in the tables. Units of enzyme activity are μmol conjugate formed per min. Different letters indicate significant differences among groups (P < 0.05), as described in the tables.
and was subsequently repaired, or whether it is a late-stage phenomenon which occurs first in the more susceptible, low protein-exposed groups. The latter seems likely, because in rats exposed to SO$_2$ concentrations of between 14 and 286 $\mu$g SO$_2$/m$^3$, no evidence of increased capillary permeability was apparent after 7, 14, 21 or 28 d exposure (Langley-Evans et al. 1996). By comparison, guinea pigs chronically exposed to hyperoxia develop such an injury early in their exposure and subsequently repair the epithelial damage (Phillips 1995). Although statistically significant, the relationship between maternal protein intake and post-SO$_2$ BALF protein concentration was weak. Maternal protein intake accounted for only 14% of the variation in BALF protein concentration. This relationship, however, considered only BALF protein concentration following SO$_2$ exposure. The relationship between the difference in protein concentration between SO$_2$ exposed and untreated rats and maternal dietary protein was much stronger ($r = -0.967$, $P = 0.03$) and explains 93% of the variation in BALF protein concentration. The remaining variability may be explained by different rates of repair and different time-courses of tissue damage. As stated, this study considers only the late phase of the injury elicited by SO$_2$ and may well overlook a complex development of injury and repair processes over a 4 wk time period.

It is possible that differences in SO$_2$-induced injury observed between this and earlier work (Langley-Evans et al. 1996) relate to maternal dietary influences on GSH metabolism. Our pilot studies utilized the offspring of rats fed non-purified diet, and such animals have lower hepatic and pulmonary GCS activities than do the offspring of rats fed casein-based purified diets (Langley-Evans et al. 1995). Since the maternal diet may broadly alter overall GSH metabolism, the subsequent utilization of GSH for the detoxification of SO$_2$ may also be modified. In this study, there was little evidence of the potent GSH depletion previously observed in SO$_2$-treated rats (Langley-Evans et al. 1996). This difference almost certainly relates to different responses to SO$_2$ exposure of rats born to dams fed different levels of dietary protein during pregnancy. $\gamma$-Glutamyl cycle enzymes in tissues of rats born to dams fed non-purified diet show no adaptive response other than to decrease liver GRed activity, which is likely to exacerbate GSH depletion (Langley-Evans et al. 1996). Rats exposed to purified diets in $\textit{utero}$, in addition to differing in baseline hepatic GRed and GCS activities (Langley-Evans et al. 1996), appear to increase GSH conserving activities.

These data are, however, broadly consistent with previous reports of intrauterine programming of $\gamma$-glutamyl cycle enzyme activities in rats (Langley et al. 1994, Langley-Evans et al. 1995). Gluthathione reductase appears to be most sensitive to maternal protein intake, most notably in the liver where higher activity was noted in rats exposed to the low protein diet (60 g casein/kg) in $\textit{utero}$. Tissue GSH concentrations fluctuate less consistently, but heart and liver appear to be susceptible to maternal influences (Langley et al. 1994, Langley-Evans et al. 1995). Tissue GSH concentrations represent a balance of the rates of synthesis, transport and utilization. Differences in GSH concentrations among the groups with different maternal protein intake may be attributable to previously observed (Langley et al. 1994) programmed changes in these processes. Earlier work indicated that in rats prenatally exposed to a 120 or 60 g casein/kg maternal diet, capacity to transport GSH is impaired.

The rats exposed to low protein maternal diet (60 g casein/kg) exhibited greater SO$_2$-induced lung injury, as assessed by recovery of protein in bronchoalveolar lavage, than did 180 g
casein/kg maternal diet—exposed rats. One of two explanations for this intrauterine modulation of susceptibility relates to GSH metabolism. GSH undergoes sulfitolysis with SO\(_2\) following oxidation to glutathione disulphide (GSSG) by GPx (Kagedal et al. 1986, Mannervik et al. 1974, Winell and Mannervik, 1969). The product of sulfitolysis, S-sulphogluthathione (GSSO\(_3\)) cannot be recycled through the GRed reaction and is excreted as thiosulfate. GSSO\(_3\) inhibits glutathione S-transferases (Pool-Zobel et al. 1990) and accordingly, pulmonary GST activities were markedly lower in the SO\(_2\)-exposed rats. Clearly all rats exposed to the four purified diets in utero were able to mount an adaptive response of the \(\gamma\)-glutamyl system to SO\(_2\) exposure, a feature absent in rats prenatally exposed to non-purified diets (Langley-Evans et al. 1996). As a consequence of this response, all rats exposed to SO\(_2\) were able to maintain tissue GSH levels, with the exception of the 60 g casein/kg group in which liver GSH fell by 27%. The wide variety of changes in enzyme activity observed in SO\(_2\)-treated rats shifted metabolism towards GSH conservation, and this response was apparently modified by the maternal diet. Rats exposed to the control maternal diet had greater GCS activity in both lung and liver after treatment with SO\(_2\). Rats of the 120 g casein/kg maternal diet group did not demonstrate SO\(_2\)-induced changes in GCS activity, but had high activity in liver prior to the gas exposure. The adaptive response of this group appeared dependent upon an elevation in GRed activity in liver that recycled GSH, and a lower GPx activity in lung that diminished loss of GSH through sulfitolysis of GSSG. Rats in the SO\(_2\)-treated 90 g casein/kg group had greater hepatic GRed and GCS activities than untreated rats. Thus, three of the diet groups maintained liver GSH concentrations in response to SO\(_2\) through alterations in the pattern of \(\gamma\)-glutamyl enzyme activities. In the 60 g casein/kg maternal diet group in which hepatic GSH concentrations were lower following SO\(_2\) treatment, pulmonary GSH did not respond; in the lung, only GCS activity was greater than that of untreated rats. Importantly, activity of hepatic GPx was elevated, which may have contributed to the observed depletion of GSH from liver. The role of the GSH system in determining susceptibility to injury is again difficult to interpret in view of the likely complexity of the timecourse of injury. It does appear, however, that in the case of the 60 g casein/kg maternal diet group which demonstrated the greatest level of pulmonary injury, the ability to conserve GSH was less than in other groups.

The mechanism underlying differential susceptibility to SO\(_2\)-induced injury may alternatively relate to the acute phase response mounted following initial tissue damage. An inflammatory response is elicited by SO\(_2\), demonstrated by recruitment of neutrophils into the airways. This response is presumably cytokine mediated. Cytokine production (Tippa et al. 1994) and the acute phase response to an endotoxin injection (Langley et al. 1994) are related in a non-linear manner to maternal dietary protein intake. Specific elements of the response to endotoxin are enhanced or blunted by intrauterine exposure to low protein diets maternal (Langley et al. 1994). The cell counts reported in this work may also reflect impairment of immune functions in these animals. Rats exposed in utero to 90 g casein/kg maternal diet had fewer circulating lymphocytes than did control animals. Additionally, as a proportion of the total cell number, rats from all the low protein groups tended to have a greater number of circulating neutrophils (control: 11%, 120 g casein/kg: 18%, 90 g casein/kg: 15%, 6 g casein/kg: 18% neutrophils). Moreover, such rats have also been observed in our lab to have low spleen weights. Others have demonstrated that the immune system is extremely sensitive to manipulations of the maternal diet during fetal development (Beach et al. 1982 and 1983).

The susceptibility of the young adult rat to sulfur dioxide induced lung injury, as evidenced by BALF protein concentrations, is programmed by aspects of the maternal diet. Fetal exposure to a low protein maternal diet increased the magnitude of injury, concomitant with modulation of glutathione metabolizing enzyme activities. As we have reported previously, rats of different prenatatal nutrition respond differently to an inflammatory stimulus. This may reflect the programmed state of the immune system and aspects of antioxidant defenses.

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LITERATURE CITED


