6. Experiments Following Arsenic Exposure and Methionine Supplementation

In this set of experiments arsenic as sodium arsenite was injected i.p. at a dose of 5.55 mg/kg body weight (35% of LD$_{50}$) per day for a period of 21 days. For methionine supplementation, some of the arsenic-treated rats were fed on an 18% protein diet supplemented with 0.8% methionine for five days prior to sacrifice.

6.1. Results

The results of blood glucose level represented in Fig. 3 demonstrate that after arsenic exposure, the blood glucose level decreased significantly (p<0.001). The decrease was found to be 42.8% of the control value. Methionine supplementation restored the blood glucose level to about 84.3% of control value. The glycogen content of liver was also reduced to 23.4% of control value (p<0.001) following arsenic treatment, and methionine supplementation appeared to have no significant effect in the restoration of liver glycogen level (Fig.4).

**Fig. 3. Changes in blood glucose following arsenic treatment with or without methionine supplementation**

- $p^a$ compared with pair-fed control group.
- $p^b$ compared with arsenic-treated group.
- $p^c$ compared with pair-fed control group.
Fig. 4. Changes in liver glycogen content following arsenic treatment with or without methionine supplementation

\[ p^b \text{ compared with arsenic-treated group.} \\
\[ p^c \text{ compared with pair-fed control group.} \\

Changes in the pyruvic acid content following arsenic exposure are depicted in Fig. 5. Arsenic treatment also decreased significantly the liver pyruvic acid level to 53.3% of control value, which appeared to be completely restored by methionine supplementation.

Fig. 5. Changes in liver pyruvic acid content following arsenic treatment with or without methionine supplementation

\[ p^a \text{ compared with pair-fed control group.} \\
\[ p^b \text{ compared with arsenic-treated group.} \\
\[ p^c \text{ compared with pair-fed control group.}
Table 5 reveals that arsenic treatment increased the free amino acid nitrogen content of liver by 53.9% (p<0.001) and decreased that of kidney by 27.4% (p<0.001). Methionine supplementation appreciably reversed the changes in free amino acid nitrogen contents found in both liver and kidney tissues. The restoration by methionine was found to be 98.4% and 98.8% in liver and kidney tissues, respectively.

Table 5: Changes in free amino acid nitrogen contents, GOT and GPT activities in liver and kidney following arsenic exposure with or without methionine supplementation

<table>
<thead>
<tr>
<th>Groups of animals</th>
<th>Free amino acid nitrogen (µg/100 mg tissue)</th>
<th>GOT activity (µmole of pyruvate produced/min/100 mg tissue)</th>
<th>GPT activity (µmole of pyruvate produced/min/100 mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Kidney</td>
<td>Liver</td>
</tr>
<tr>
<td>Pair-fed control</td>
<td>6.3±0.26</td>
<td>16.4±0.48</td>
<td>18.2±5.7</td>
</tr>
<tr>
<td>(6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>As-treated</td>
<td>9.7±0.29</td>
<td>11.9±0.62</td>
<td>16.8±11.5</td>
</tr>
<tr>
<td>(6)</td>
<td>*p&lt;0.001</td>
<td>*p&lt;0.001</td>
<td>*p&lt;0.05</td>
</tr>
<tr>
<td>As-treated+</td>
<td>6.2±0.34</td>
<td>16.2±0.9</td>
<td>17.6±6.3</td>
</tr>
<tr>
<td>Methionine-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>supplemented</td>
<td>(6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>*p&lt;0.001</td>
<td>*p&lt;0.001</td>
<td>*p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>*p&gt;0.05</td>
<td>*p&gt;0.05</td>
<td>*p&gt;0.05</td>
</tr>
</tbody>
</table>

Values are Means±S.E.M.
Figures in the parentheses indicate the number of animals.
*p compared with pair-fed control group.
*p compared with arsenic-treated group.
*p compared with pair-fed control group.

The results presented in Table 5 reveal the changes in the transaminase activities following exposure to arsenic with or without methionine supplementation. Methionine supplementation had no effect on unaltered GOT activities in both liver and kidney tissues following arsenic treatment. On the other hand, the diminished GPT activity (p<0.001) in the kidney tissues of arsenic-treated rats was found to be reversed by methionine supplementation (Table 5). The restoration by methionine was found to be 104.7% of control value. On the other hand, arsenic treatment had no significant effect on the liver GPT activity with or without methionine supplementation.
6.2. Discussion

The decreased blood glucose level and diminished liver glycogen and pyruvic acid contents following arsenic treatment, as noted in the present study, confirm our observations presented in earlier section. Several reports (Reichl et al., 1990, 1991) suggested that carbohydrate depletion is an important factor in arsenic toxicity. This contention is also supported by the present findings. The present study further reveals that dietary methionine supplementation appreciably counteracts the reduction of blood glucose level following arsenic treatment. But the change in liver glycogen level appears to be independent of methionine supplementation (Fig. 4). On the other hand, the diminished liver pyruvic acid level due to arsenic treatment was fully restored by methionine supplementation (Fig. 5). Thus, it appears that carbohydrate-depleting effect of arsenic can be partially prevented by methionine supplementation.

Methionine plays an important role in regulating gene expression including the expression of those genes that are essential for maintenance of blood glucose level (Hara et al., 2000). Methyl deficiency was found to be associated with many diseases, suggesting that disturbed methylation may be a risk factor in the development of cellular toxicity. Studies of Majano et al (2001) demonstrated that S-adenosylmethionine (SAM) grants protection to liver in pathological conditions associated with inflammatory components. This study further revealed that SAM administration alleviates experimental liver injury and increases survival of cirrhotic patients. Mikol et al (1983) showed that dietary deficiency of amino acids such as methionine, choline etc. exhibited good liver tumor promoting activity. The long-term ingestion of diets deficient in the amino acids, methionine and choline led to global hypomethylation of hepatic DNA (Poirier, 1986) as well as hypomethylation of specific oncogenes (Bhave et al., 1988; Zapisek et al., 1992), leading to carcinogenesis. It was shown that administration of SAM, the precursor of GSH, accelerates the clearence of ethanol and acetaldehyde in humans after ethanol intake (Di Padova et al., 1984). It was also proposed by Paredes et al (1987) that the beneficial action of SAM in alcohol intoxication is dependent on its capacity of increasing the synthesis of GSH. The beneficial role of SAM in lead intoxication was also demonstrated in experimental animals (Paredes et al., 1985). Flora and Seth (1999) investigated the beneficial effects of SAM in preventing inhibition of blood 5-aminolevulinic acid dehydratase (ALAD), alterations in blood and hepatic
glutathione (GSH) content, hepatic and brain malonaldehyde (MDA) formation, and uptake of lead following acute lead plus ethanol co-exposure in mice. The results suggest that supplementation of SAM may have beneficial effects in preventing alterations in some biochemical variables in blood, liver and other tissues during acute lead plus ethanol exposure in animals. From these observations, it is thus suggested that methionine plays an important role in protection of hepatic injury associated with DNA hypomethylation.

Various chemical agents including arsenic are considered as classical carcinogens, which alter either SAM level or DNA methylation (Lapeyre and Becker, 1979; Zhao et al., 1997). A number of investigators have suggested a role of metabolism of arsenic in its carcinogenic effects. In mammalian tissues, arsenic is metabolized to mono and dimethylated species by the enzyme arsenic methyltransferase. Methylation is involved in biotransformation of inorganic arsenic by rat liver, leading to production of monomethylarsonic acid and dimethylarsinic acid derivatives (Buchet and Lawerys, 1985; Thompson, 1993). SAM acts as the methyl group donor for the reaction. Thus, methionine deficient diet has a lower capacity of methylating and thereby detoxifying inorganic arsenic (Vahter and Marafante, 1987). Buchet and Lawerys (1988) demonstrated that this methylation reaction is stimulated by reduced glutathione (GSH). The stimulation of the first methylation reaction by GSH can only be evidenced at high inorganic acid concentration, because under these conditions, the second methylating enzyme can be sufficiently inhibited by inorganic arsenic to allow some accumulation of MMA. But a large excess of thiol groups may also prevent the methylation reactions probably by reducing the amount of free trivalent arsenic (Buchet and Lawerys, 1988). Studies by Takahashi et al (1990) revealed that the administration of arsenic trioxide to hamsters pre-treated intraperitoneally with 2 mg per kg body weight of SAM significantly enhanced excretion of dimethylarsinic acid through urine. These findings further suggest that SAM may be a very potent methyl group donor to inorganic arsenic. Diminished methylation of arsenic may, therefore, result in decreased urinary excretion of dimethylarsinic acid, the main metabolite of inorganic arsenic, and increase in arsenic retention in the body potentiating its toxic effects. It is, therefore, possible that methionine supplementation eliminates hypoglycemic effects of arsenic by diminishing the arsenic retention in the tissues through detoxification process. Similar explanation
can be suggested for reversal of arsenic-induced diminished liver pyruvic acid level by methionine supplementation.

In the present investigation arsenic treatment decreased the free amino acid nitrogen concentration in kidney and increased that of liver tissues. This also confirms our previous study (earlier section). In the present study methionine supplementation eliminated the arsenic-induced increased and decreased free amino acid nitrogen concentrations in liver and kidney, respectively. This suggests that mobilization of free amino acids from kidney to liver to provide substrates for increased gluconeogenesis due to arsenic treatment is counteracted by methionine supplementation. Methionine, due to its detoxifying capacity of arsenic, may enhance arsenic excretion and thereby reducing its adverse effects. Changes in transaminase activities reveal that liver transaminase activities (both GOT and GPT) remained independent of arsenic treatment with or without methionine supplementation. However, the decreased glutamate-pyruvate transaminase activity in the kidney following arsenic treatment was restored by methionine supplementation. This may be ascribed to the restoration of free amino acid nitrogen concentration in the kidney of arsenic-treated animals by methionine supplementation.

From these observations, it may be suggested that dietary methionine supplementation can prevent arsenic-induced hypoglycemia with associated decreased liver glycolytic activities.

6.3. Summary

Short-term exposure of arsenic produces carbohydrate depletion and hypoglycemia. Dietary deficiency of methionine causes impaired biotransformation of arsenic, which has been attributed to the pathogenesis of different diseases induced by arsenic. Accordingly, the effects of methionine supplementation on altered glucose homeostasis induced by arsenic have been studied. Arsenic (as sodium arsenite) treatment (i.p.) of male Wistar rats (weighing 80-100 g) at a dose of 5.55 mg/kg body weight (equivalent to 35% LD₅₀) per day for a period of 21 days caused significant diminution in blood glucose level and fall in liver glycogen and pyruvic acid contents. Methionine supplementation reversed the above changes except decreased liver glycogen
due to arsenic treatment. It may be suggested that the carbohydrate depleting effects of arsenic are partially counteracted by methionine. The free amino acid nitrogen content of liver was elevated while that of kidney was decreased after arsenic treatment. This may be due to rapid mobilization of free amino acids from kidney to liver to provide more substrates for gluconeogenesis. Methionine supplementation appreciably counteracted the changes of free amino acid nitrogen in both liver and kidney tissues, suggesting that arsenic-induced rapid mobilization of free amino acids from kidney to liver was retarded by methionine. Transaminase activities in liver and kidney were not significantly altered except that of GPT activity of kidney that decreased significantly after arsenic treatment. Methionine also restored the decreased kidney GPT activity. This may be due to restoration of free amino acid nitrogen content in the kidney tissue after methionine supplementation.

These partial protective effects of methionine against arsenic-induced carbohydrate depletion may be due to enhanced detoxification of arsenic by methionine. Methionine, being a potent methyl group donor, may participate in methylation process of arsenic, thus facilitating arsenic excretion from the body. Thus, some of the adverse metabolic effects of arsenic may be eliminated by methionine supplementation. From the present study, it may be suggested that hypoglycemia with associated decreased glycolytic activity induced by arsenic treatment at the present dose and duration can be partially counteracted by dietary methionine supplementation.