Evidence for arsenic essentiality

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Abstract

Although numerous studies with rats, hamsters, minipigs, goats and chicks have indicated that arsenic is an essential nutrient, the physiological role of arsenic is open to conjecture. Recent studies have suggested that arsenic has a physiological role that affects the formation of various metabolites of methionine metabolism including taurine and the polyamines. The concentration of plasma taurine is decreased in arsenic-deprived rats and hamsters. The hepatic concentration of polyamines and the specific activity of an enzyme necessary for the synthesis of spermidine and spermine, S-adenosylmethionine decarboxylase, are also decreased in arsenic-deprived rats. Thus, evidence has been obtained which indicates that arsenic is of physiological importance, especially when methionine metabolism is stressed (e.g. pregnancy, lactation, methionine deficiency, vitamin B₆ deprivation). Any possible nutritional requirement by humans can be estimated only by using data from animal studies. The arsenic requirement for growing chicks and rats has been suggested to be near 25 ng g⁻¹ diet. Thus, a possible human requirement is 12 μg day⁻¹. The reported arsenic content of diets from various parts of the world indicates that the average intake of arsenic is in the range of 12–40 μg. Fish, grain and cereal products contribute most arsenic to the diet.

Arsenic Deprivation Studies

Early attempts to prove the essentiality of arsenic were unsuccessful or had confusing results because the arsenic content of the arsenic-deficient diets was unknown or too high, or because the arsenic content of the control diets was too high. Also, the results of several studies were confounded by the use of diets that were nutritionally incomplete and thus even the control animals grew suboptimally.

The first strong evidence that arsenic is essential came from studies by Nielsen et al. (1975) and Anke et al. (1976). In the studies by Nielsen et al. (1975), rats were fed a diet based on dried skim milk and acid-washed corn. The basal diet contained approximately 30 ng As g⁻¹; control diets were supplemented with 4.5 μg As g⁻¹. Dams were placed on the diet within two days of being bred. The offspring of the arsenic-deprived dams displayed a rougher coat and a slower growth rate than the offspring from the controls. Males were more affected than females by arsenic deprivation. At 12–15 weeks, compared with controls, the arsenic-deprived males exhibited enlarged blackened spleens containing 50% more iron on a per gram basis. The spleens of arsenic-deprived females were enlarged to a lesser extent. Both sexes exhibited an elevated erythrocyte osmotic fragility.

In 1976 Anke et al. (1976) presented evidence for the essentiality of arsenic for goats and minipigs. A semi-synthetic diet containing less than 50 ng As g⁻¹ was fed to growing, pregnant and lactating goats and minipigs, and to the first and second generation offspring. Controls received 350 ng As g⁻¹ diet. Only 58% of the arsenic-deprived goats and 62% of the arsenic-deprived minipigs produced offspring; in comparison, 92% and 100% of the respective controls produced offspring. The offspring of the arsenic-deprived animals exhibited depressed growth and an elevated mortality rate. The slower growth was most marked in the second generation deprived goats. Some lactating arsenic-deprived goats died, apparently from myocardial abnormalities. Ultra-structurally there was an increase in mitochondrial membrane contours, and a fine granular and electron dense material in the membrane could be demonstrated. At a more advanced stage there was a rupture of the mitochondrial membrane (Anke, 1986).

Nielsen and Shuler (1978) reported findings indicating an essential physiological role for arsenic in growing chicks. Day-old cockerel chicks were fed a diet based on dried skim milk, acid-washed corn and high-protein casein. The basal diet contained approximately 15–25 ng As g⁻¹. Controls received the basal diet supplemented with 1 μg As g⁻¹. Some of the arsenic-deprived and arsenic-supplemented chicks were supplemented with 20 g arginine kg⁻¹ diet. After four weeks the arginine-supplemented, arsenic-deprived chicks weighed significantly less than comparable controls. Other findings in the arginine-supplemented groups included an elevated liver weight/body weight ratio, an elevated concentration of zinc and depressed concentrations of arginine, iron and manganese in the livers of arsenic-deprived chicks.

In retrospect, the study by Nielsen and Shuler (1978) was the first study performed in which arsenic deprivation was studied in animals with a stressed methionine metabolism. Guanidoacetate is formed in the metabolism of arginine. Guanidoacetate is methylated by S-adenosylmethionine forming creatinine. Thus, feeding high amounts of arginine can result in an induced methyl/methionine deficiency because of the required methylation in the metabolism of guanidoacetate. Shortly after the study of Nielsen and Shuler (1978), studies led by Nielsen and
Uthus focused on ascertaining the possible physiological importance of arsenic in methionine metabolism. The remainder of this paper will deal with these studies.

The effects in chicks and rats of methyl depletion as the result of high dietary guanidinoacetate (GAA) on the need for arsenic were studied in a series of experiments by Uthus and Nielsen (1986, 1987). In the experiment involving chicks, day-old cockerels were assigned to groups of similar weight in a fully-crossed, three factor design. The dietary variables were arsenic, as arsenate, 0 or 2 µg g⁻¹; choline, 0.65 or 1.3 g kg⁻¹; and GAA, 0 or 5 g kg⁻¹. The basal diet contained about 15 ng As g⁻¹. After 28 days, an interaction between arsenic and GAA affected body weight, haemoglobin and plasma total creatine-creatinine (Table 1). Regardless of the dietary choline supplement, GAA markedly depressed growth and haemoglobin; arsenic deprivation exacerbated the depression. GAA supplementation elevated plasma total creatine-creatinine; arsenic deprivation enhanced this elevation.

In our experiments involving rats, GAA was supplemented at 5 g kg⁻¹ diet and choline, folic acid and vitamin B₁₂ were all excluded from the diet (Uthus and Nielsen, 1987). In three separate experiments, arsenic deprivation depressed growth. In experiments that used caserin-based diets containing 0.58% methionine and 0.17% cystine, plasma taurine was decreased by arsenic deprivation in rats (Uthus, unpublished observation) and hamsters (Uthus, 1990) (Table 2).

In a study by Comatzer et al. (1983), the effect of arsenic deprivation on phosphatidylcholine biosynthesis in liver microsomes of the rat was determined. Beginning two days after breeding, pregnant Sprague-Dawley rats were fed either low arsenic (<15 ng As g⁻¹) or arsenic-supplemented (2 µg As g⁻¹) diets throughout gestation, parturition and lactation. After weaning, pups also were fed these diets. Male rats from the F₁ (age 71 days) generation were used to ascertain the effect of arsenic deprivation on phosphatidylcholine biosynthesis. Arsenic deprivation depressed the specific activity of PEM, and total liver microsomal activities of phosphatidyl-ethanolamine (PEM) methyltransferase, phosphatidyl-dimethyl- ethanolamine methyltransferase (PEM) and choline phosphotransferase (CPT) (Table 3).

Studies with chicks, rats and hamsters described above have demonstrated that the nature and severity of the signs of arsenic deprivation are affected by dietary manipulations which impact methionine metabolism and that many signs of arsenic deprivation include apparent perturbations in methionine metabolism. Because S-adenosylmethionine is of dominant importance in the metabolism of methionine and because it is involved in polyamine biosynthesis, the effect of arsenic deprivation on the polyamine content in rat liver was determined (Uthus et al., 1989). Also, the specific activities of liver S-adenosylmethionine decarboxylase and ornithine decarboxylase were determined. Arsenic deprivation resulted in putrescine being decreased to 62%, spermidine to 89% and spermine to 90% of respective control values (on a per g basis). S-adenosylmethionine decarboxylase was decreased to 83% of the control value by arsenic deprivation; ornithine decarboxylase tended to be

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### Table 1: Effect of arsenic, guanidinoacetate (GAA), choline and their interaction on body weight, haemoglobin and plasma creatine-creatinine.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>As (µg g⁻¹)</th>
<th>Choline (µg g⁻¹)</th>
<th>Body weight (g)</th>
<th>Haemoglobin (g 100mL⁻¹)</th>
<th>Plasma creatine-creatinine (mg 100mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.65</td>
<td>1.007</td>
<td>9.11</td>
<td>0.83</td>
<td></td>
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<tr>
<td>2</td>
<td>0.65</td>
<td>962</td>
<td>9.23</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1.30</td>
<td>1.027</td>
<td>9.08</td>
<td>0.83</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.30</td>
<td>1.031</td>
<td>9.28</td>
<td>0.78</td>
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<tr>
<td>0</td>
<td>0.65</td>
<td>612</td>
<td>5.62</td>
<td>5.05</td>
<td></td>
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<tr>
<td>2</td>
<td>0.65</td>
<td>754</td>
<td>6.37</td>
<td>4.28</td>
<td></td>
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<tr>
<td>0</td>
<td>1.30</td>
<td>645</td>
<td>5.27</td>
<td>4.32</td>
<td></td>
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<tr>
<td>2</td>
<td>1.30</td>
<td>765</td>
<td>6.38</td>
<td>3.62</td>
<td></td>
</tr>
</tbody>
</table>

* Amounts of arsenic, guanidinoacetate and choline supplemented to the basal diet.

### Table 2: Effect of arsenic deprivation on hamster and rat plasma taurine.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hamster (nmol mL⁻¹)</th>
<th>Rat (nmol mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>152 ± 17 (6)¹</td>
<td>126 ± 15 (12)²</td>
</tr>
<tr>
<td>1</td>
<td>199 ± 38 (7)</td>
<td>177 ± 36 (11)</td>
</tr>
</tbody>
</table>

* Amount of arsenic supplemented to the basal diet. Each value represents the mean followed by the standard deviation. Number of animals is in parentheses.

### Table 3: Effect of dietary arsenic on the activity of phosphatidyl-ethanolamine methyltransferase (PEM), phosphatidyl-dimethyl ethanolamine methyltransferase (PDM) and choline phosphotransferase (CPT).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PEM</th>
<th>PDM</th>
<th>CPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.100 ± 0.003 (18.5 ± 2.2)¹</td>
<td>2.29 ± 0.15 (423 ± 85)²</td>
<td>21.3 ± 2.7 (3943 ± 970)²</td>
</tr>
<tr>
<td>2</td>
<td>0.120 ± 0.003 (35.7 ± 3.9)¹</td>
<td>2.57 ± 0.32 (764 ± 151)</td>
<td>26.4 ± 3.7 (7858 ± 1693)</td>
</tr>
</tbody>
</table>

* Amount of arsenic supplemented to the basal diet. Values are means followed by standard deviations (n = 3 for arsenic-deprived groups and n = 4 for arsenic-supplemented group). Specific activity: nmol phosphatidylcholine formed/min/mg protein. Values in parentheses are nmol phosphatidylcholine formed/min/tot liver microsomes.

¹ p < 0.001; ² p < 0.05.
Table 4 Effect in rats of arsenic, methionine and their interaction on body weight, haemoglobin and mean corpuscular haemoglobin.

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Methionine (g kg⁻¹)</th>
<th>Body weight (g)</th>
<th>Haemoglobin (g 100mL⁻¹)</th>
<th>Mean corpuscular haemoglobin (pg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic (µg g⁻¹)</td>
<td>1</td>
<td>104</td>
<td>14.8</td>
<td>19.0</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>142</td>
<td>15.3</td>
<td>20.1</td>
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<tr>
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<td>5</td>
<td>221</td>
<td>14.5</td>
<td>20.3</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>211</td>
<td>15.3</td>
<td>20.4</td>
</tr>
</tbody>
</table>

Analysis of variance – p values

As | NS | 0.02 | 0.005
Methionine | 0.0001 | NS | 0.0006
As × Methionine | 0.03 | NS | 0.035
Error mean square | 624 | 0.04 | 0.21

* Amount of arsenic and methionine supplemented to the basal diet. n = 6 rats per group.

volume and mean corpuscular haemoglobin, total blood haemoglobin, haematocrit and tissue iron (all of which can be affected by changes in vitamin B₉ metabolism).

Thus, a 2 × 2 × 2 experiment was designed to ascertain whether dietary pyridoxine affected the response to arsenic deprivation by rats (Utthus and Poelot, 1991). Dietary variables were arsenic, 0 or 1 µg g⁻¹, methionine, 0 or 3 g kg⁻¹, and pyridoxine, 0 or 10 mg kg⁻¹. The basal diet contained less than 15 ng As g⁻¹ and 0.24% methionine. After ten weeks, growth was reduced by arsenic, pyridoxine or methionine deprivation. Both endogenous (−PP) and pyrdoxyl 5'-phosphate–stimulated (+PP) erythrocyte aspartate aminotransferase were decreased by pyridoxine deficiency. The ratio of +PP/−PP, known as the activation coefficient (AC), was affected by an interaction between arsenic and pyridoxine. Pyridoxine deficiency resulted in a less marked increase in AC in the arsenic-deprived rats than in the arsenic-supplemented rats. The iron concentration in plasma was slightly decreased by pyridoxine deficiency in the arsenic-deprived rats but increased by pyridoxine deficiency in the arsenic-supplemented rats. Plasma threonine and serine concentrations were increased by arsenic supplementation in the pyridoxine-deficient rats but there was no effect of arsenic supplementation on these amino acids in the pyridoxine-supplemented rats. The concentration of alanine in plasma was decreased by arsenic or pyridoxine deprivation. In pyridoxine deficiency, arsenic deprivation had no effect on plasma glycine concentration in the methionine-deficient rats but decreased the concentration of glycine in plasma of methionine-supplemented rats. In the pyridoxine-supplemented rats, arsenic had no effect on plasma glycine concentration, regardless of dietary methionine.

This study shows that arsenic can modify the effect of dietary pyridoxine deprivation. It is not known if this effect can be attributed to a direct or indirect physiological action of arsenic on vitamin B₉ metabolism. The findings do indicate that arsenic and pyridoxine can interact to affect amino acid metabolism.

Speculations

The signs of arsenic deprivation are numerous and have been described for the goat, minipig, rat, hamster and chick. The responses to arsenic deprivation have varied in nature and severity with alteration in dietary concentrations of numerous substances including arginine, choline, guanidoacetate, pyridoxine and methionine. These substances are all related because they affect methionine metabolism. There are several areas in methionine metabolism in which arsenic could play a physiological role. The areas under most consideration involve the biosynthesis and metabolism of taurine and the polyamines and the metabolism of labile methyl groups from methionine.

Arsenic deprivation has been shown to depress the concentration of taurine in hamster and rat plasma. The myocardium has a very high taurine content (Zelikovic and Chesney, 1989). Cardiomyopathy associated with low plasma taurine has been reported in cats fed a

decreased by arsenic deprivation.

Previous studies attempting to examine the effects of an altered methionine metabolism on arsenic deprivation depending on dietary supplements, including guanidoacetate acid or arginine, to induce methionine deficiency. Therefore, a 2 × 2 factorially arranged experiment was designed utilising an amino acid-based diet to ascertain the effects of a simple dietary methionine deficiency on arsenic deprivation. Dietary variables were methionine, 1 or 5 g kg⁻¹ and arsenic, as As₂O₃, 0 or 1 µg g⁻¹. The basal diet contained 0.14% methionine and about 10 ng arsenic/g. Results of feeding the experimental diets for six weeks are shown in Table 4.

Arsenic deprivation decreased growth and mean corpuscular haemoglobin in rats fed 1 g methionine kg⁻¹, but had no effect on those variables in rats fed 5 g methionine kg⁻¹; this resulted in the significance shown for the interaction between arsenic and methionine. An interaction between arsenic and methionine also affected mean corpuscular volume, bone concentrations of copper, potassium, magnesium and zinc, and the heart concentration of calcium (data not shown). Also, bone, heart and plasma iron concentrations tended to be increased by arsenic deprivation in the rats fed 1 g methionine kg⁻¹ (data not shown). These results indicate that there is a relationship between arsenic and methionine.

In experiments investigating arsenic deprivation, many of the findings suggested that arsenic affected enzymes or metabolites which are influenced by vitamin B₉ or that signs of arsenic deprivation were similar to signs of vitamin B₉ deficiency (Rapaport, 1971). Some of the responses to arsenic deprivation that indicated a possible relationship between arsenic and vitamin B₉ included the following: a decrease in the specific activity of liver cystathionase (Utthus, unpublished observation) and a tendency for a decrease in the specific activity of liver ornithine decarboxylase (Utthus et al., 1989) (both enzymes utilise pyridoxal–phosphate as a cofactor); a decrease in rat (Utthus, unpublished observation) and hamster (Utthus, 1990) plasma taurine (vitamin B₉ is needed for the synthesis of taurine); and changes in mean corpuscular
turnine-deficient diet (Zelikovic and Chesney, 1989). Taurine also apparently plays a role in the stabilization of cell membranes (Zelikovic and Chesney, 1989). It is possible that the rupture of heart mitochondrial membranes and subsequent death of arsenic-deprived goats could have involved myocardial taurine. It also is possible that changes in phosphatidylcholine biosynthesis could have affected the cellular membranes. Phosphatidylcholine is the major phospholipid found in the plasma, nuclear, mitochondrial and endoplasmic reticulum membrane of the cell. Arsenic deprivation can affect several enzymes involved in the biosynthesis of phosphatidylcholine (Cornatzer et al., 1983). Arsenic deprivation has been shown to increase erythrocyte osmotic fragility (Nielsen et al., 1975) which may be indicative of an aberrant membrane.

Arsenic may be involved in regulation of polyamine synthesis. Polyamines have an integral role in controlling cell proliferation and growth (Jäne et al., 1983). Chronic high intakes of arsenic have been associated with hyperkeratosis, hyperpigmentation and skin cancers (Benko, 1987). In experimental animals, exposure to physical or chemical stimuli that results in hyperproliferation or transformation of epidermal cells is accompanied by an early stimulation of polyamine synthesis (Jäne et al., 1983).

Although arsenic might affect gene expression through the polyamines, it is also possible that arsenic could have a similar effect through histone methylation. It is believed that inorganic arsenic is methylated by S-adenosymethionine as a detoxification step (Vahier and Marafante, 1988). However, because nutrients that affect methyl group metabolism affect the response to arsenic deprivation, arsenic may have an essential role in labile methyl metabolism involving methionine. Further support for this suggestion can be gained from the finding that arsenic can induce heat shock proteins (Desrosiers and Tanguay, 1986; vanBergen et al., 1987). The control of production of these proteins in response to arsenite appears to be at the gene level. Some studies have related the control to changes in the methylation of core histones (Desrosiers and Tanguay, 1986). Also, arsenic induces small stress proteins which can be detected by methionine incorporation; these proteins are not induced by heat stress (vanBergen et al., 1987). Signs of arsenic deprivation have been shown to be affected by dietary treatments (e.g. methionine deficiency, guanidoacetate supplementation) affecting labile methyl availability; several of the signs of arsenic deprivation indicate an aberrant methyl metabolism (e.g. changes in creatinine–creatinine and in methylating enzymes involved in phosphatidylcholine biosynthesis).

Summary

Studies have indicated that arsenic has an important role in the conversion of methionine to its metabolites taurine, labile methyl groups and polyamines. Thus, most likely arsenic affects sulphur amino acid metabolism. Also, arsenic deprivation signs may be more pronounced in situations where methionine metabolism has been stressed (e.g. pregnancy, lactation, methionine deficiency, vitamin B₆ deprivation).

Any possible nutritional requirement by humans can only be estimated by using data from animal studies. The arsenic requirement for growing chicks and rats has been suggested to be near 25 μg kg⁻¹ diet (Nielsen, 1990). Thus, a possible human requirement is 12 μg day⁻¹. The reported arsenic content of diets from various parts of the world indicates that the average intake of arsenic is in the range of 12–40 μg (Nielsen, 1990). Fish, grain and cereal products contribute the most arsenic to the diet (Nielsen, 1988).

References


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