

ENVIRONMENTAL ARSENIC TOXICOLOGY

Dr. Bruce Fowler

National Institute of Environmental Health Sciences

A B S T R A C T

It is important in discussing the toxicity of environmental arsenic that we distinguish between the different chemical forms of arsenic; arsenite, As^{+3} ; arsenate, As^{+5} ; and arsine gas, AsH_3 ; since organo arsenicals vary in their degree of toxicity. Arsenite is considered more toxic than arsenate, although arsenate is the more common environmental form. Arsine gas is a potent hemolytic agent of occupational concern in some industries.

This presentation is largely confined to a discussion of the mechanisms of inorganic arsenic toxicity to cellular respiratory systems. The first form to consider is arsenate. The toxic effects of arsenate on cellular respiration have been extensively used to study the uncoupling of oxidative phosphorylation in isolated mitochondria, and the formation of the unstable arsenate esters which substitute for phosphate esters during formation of ATP.

In a recent study done in collaboration with Martha Brown, Dr. Bonnie Rhyne, and Dr. Robert Goyer, we have reported¹ the *in vivo* ultrastructural and biochemical effects of arsenate on the rat kidney proximal tubule. In this particular study, male rats which were allowed access to drinking water containing arsenate at concentrations of 45, 85 and 125 ppm for a period of 6 weeks were used. Ultrastructural changes in the kidneys of these animals were correlated with oxygen electrode studies of renal mitochondria from the same rats. An electron micrograph of a proximal tubule cell from one of the rats exposed to the 85 ppm dose level of arsenate is shown in Figure 1. Notice the swollen mitochondria (see arrows) and the electron dense membranous lysosomal bodies which were increased in number in the proximal tubule cells of animals in the 85 ppm and 125 ppm dose groups.



Figure 1 Electron micrograph of a proximal tubule cell from rat exposed to 85 ppm dose level of arsenate.

The oxygen electrode data from these same animals are presented in Table I. One main effect of arsenate was observed as on the State 3 respiration where there was a decrease. The studies in Table I show that control animals are matched with the treated animals. The top number is the mean for the control group, the next number is the mean for the treated group, and the values below are the mean differences. The other major effect was on the respiratory control ratios which are also decreased.

TABLE I
RESPIRATORY PARAMETERS* MEASURED ON SUSPENSION
OF MITOCHONDRIA FROM CONTROL AND ARSENIC FED RATS

Arsenic µg/ml H ₂ O	40		85		125	
n	10		5		8	
Control versus Expected	C	As	C	As	C	As
	(D+S.E.)		(D+S.E.)		(D+S.E.)	
State 4 µg O/g prot./min.	29.6	29.6 (0.05+ 1.34)	27.7	26.4 (1.30+ 1.25)	30.4	29.1 (1.31+ 1.50)
State 3 µg O/g prot./min.	80.4	75.4 (4.7 + 2.33)#	76.3	67.3 (9.0 + 3.98)#	81.5	72.5 (8.75+ 2.81)#
ADP: 0	2.48	2.42 (0.06+ 0.05)	2.59	2.48 (0.10+ 0.18)	2.58	2.33 (0.24+ 0.11)#
Respiratory Control Ratio	2.73	2.78 (0.04+ 0.14)	2.99	2.55 (0.44+ 0.16)#	3.00	2.63 (0.37+ 0.13)#

*Respiratory parameters from a mitochondrial preparation from the kidney of a control and arsenic fed rat were measured simultaneously. Upper numbers are means of actual measurements; lower numbers are mean and S.E.M of paired differences between control and arsenic fed rats.

#Differs from control, $p < 0.05$

Looking at the total arsenic levels in the kidney, Table II, and in the washed mitochondria from these animals, there is a fairly extensive accumulation of arsenic in both the kidneys and the mitochondria. The leveling here between 85 and 125 ppm dose group is probably due to decreased water intake in the 125 ppm group.

TABLE II
MEAN TOTAL ARSENIC LEVELS OF KIDNEYS
AND WASHED MITOCHONDRIA (Mw)*

Arsenic g/ml H ₂ O	0	40	85	125
n	18	9	4	9
Kidney ($\mu\text{g/g} \pm \text{S.E.M.}$)	7.4 (± 2.1)	39.8# (± 6.5)	84.4# (± 18.2)	84.8# (± 10.0)
Mw ($\mu\text{g/g prot.} \pm \text{S.E.M.}$)	14 (± 4.6)	52 # (± 16.1)	105 # (± 9.8)	64 # (± 13.9)

*Concentrations expressed as ppm of wet weight
#p < 0.

The next form of arsenic to be examined is arsenite (As^{+3}), which is considered generally more toxic to cellular respiration than arsenate, although its mechanism of action is thought to be somewhat different. Dr. Reeves alluded to this rather substantially. In a preliminary study, higher renal levels of total arsenic in animals given arsenite in the drinking water were observed, as opposed to arsenate.

The data in Table III were from animals exposed to either 3 or 30 ppm of arsenate or arsenite in their drinking water for 14 weeks. Both male and female rats were used. The total arsenic levels in the kidney were significantly higher in rats given arsenite than in the animals exposed to arsenate. Another aspect, concerning toxicologists, is that male rats seem to accumulate higher renal levels of arsenic than females.

TABLE III
ARSENIC IN KIDNEYS OF RATS GIVEN DRINKING WATER
CONTAINING ARSENATE (As^{+5}) or ARSENITE (As^{+3}) FOR 14 WEEKS

CONTROL	Arsenate**		Arsenite**	
	3.0	30.0	3.0	30.0
M 1.098 $\pm$.845	6.571 $\pm$ 2.36	14.065 $\pm$.825	11.319 $\pm$ 4.75	91.038 $\pm$ 32.40
F 2.865 $\pm$ 1.05	4.199 $\pm$ 1.95	5.744 $\pm$.725	9.015 $\pm$ 2.30	30.118 $\pm$ 8.60

** Administered Dose Levels, Mean $\pm$ S.E.M. Renal Levels in ppm of total

One of the proposed general reactions for the interaction of arsenite with sulphhydryl groups of proteins is shown in Figure 2. Any group can be double bond O and P, protein. Notice that the arsenite can form bonds with the sulphhydryl groups and thereby inactivate them. In the case of the pyruvate oxidase system, its mechanism of action is thought to be through interaction with one particular molecule, namely lipoidic acid, which has 2 sulphhydryl groups on one chain, rather than where we have 2 separate proteins, as shown in Figure 2,

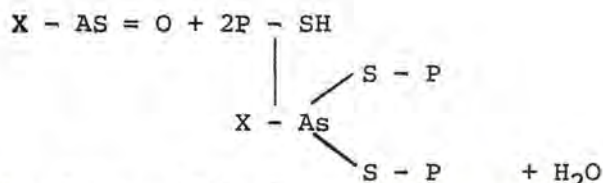


Figure 2 Proposed Reaction Between Trivalent Arsenicals and Proteins

A clear understanding of the mechanisms of arsenate or arsenite toxicity is complicated by their *in vitro* metabolism. Arsenate has been reported to be reduced to arsenite *in vivo* by renal proximal tubule cells of laboratory animals. Methylarsenic acid and dimethylarsine have also been reported in the urines of humans. It seems reasonable that a thorough understanding of arsenical toxicity is predicated on a complete understanding of their metabolism.

A third form of inorganic arsenic is arsine gas. Arsine gas is another form of potentially toxic arsenic, generated when arsenic is reduced by nascent hydrogen. This gas is a potent hemolytic agent which can cause massive hemolysis in ambient concentrations of about 5 ppm. The mechanism of hemolysis is unknown, but there is speculation that it is related to an oxidized metabolite such as arsenious acid. Hemolysis, without rigorous clinical intervention, is usually followed by death from renal failure. The renal toxicity of arsine has been attributed to anoxia or hemoglobin blockage of renal tubules. Studies by Hughes and Levy,² however, have also demonstrated arsine inhibition of respiration in both liver and kidney slices *in vivo*.

In conclusion, the toxicity of inorganic arsenic is highly dependent upon its chemical form and oxidation state. The inhibition of cellular respiratory systems is a common feature of all of these arsenicals. A precise understanding of the mechanisms by which these agents produce toxicity is, however, obfuscated in a chronic or subacute context by their metabolic "interconversions."

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A SYMPOSIUM

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Bertram W. Carnow, M.D.

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