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Interactions among Lead, Cadmium, and Arsenic in Relation to Porphyrin Excretion Patterns

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This paper reviews the effects of lead (Pb), cadmium (Cd), and arsenic (As) on the mitrochondrion with emphasis on alteration of mitochondrial heme biosynthetic pathway. The information was used to examine results of a Pb × Cd × As interaction study which employed urinary porphyrin excretion patterns as one assessment criterion. Data from the study showed that dietary Pb produced increased urinary excretion of aminolevulinic acid (ALA) and coproporphyrin. Dietary exposure to organic or inorganic As caused increased excretion of uroporphyrin and to a lesser extent coproporphyrin, while dietary Cd caused no significant changes in urinary levels of any of the porphyrins measured. The combination of Pb plus As produced an additive effect on coproporphyrin excretion but not that of either ALA or uroporphyrin. These data are discussed in relation to utilization of urinary porphyrins for assessing toxicity and elemental interactions.

Introduction

Man is exposed to a number of potentially toxic elements in his environment. Frequently the ability to discern effects of exposure to low levels of these metals is obscured by a lack of sensitive tests and by interactions between metals which alter measurable biological responses to exposure for a given element.

Arsenic, lead, and cadmium are common environmental pollutants, and many situations involve simultaneous exposure to more than one of these elements. In discussing elemental interactions, it is particularly important to specify the parameters or criteria by which any interaction is to be assessed since measurement of insensitive or nonspecific biological parameters will reduce the detectability of an interaction. Gross end points such as death or altered growth patterns, while important, are generally less sensitive than specific biochemical tests of target organ or organelle function.

Studies from our laboratories (*1–5*) have been concerned with the development of metal-specific biological response profiles based on a thorough understanding of the subcellular mechanisms of metal toxicity. This approach which involves correlations between ultrastructural morphometry and biochemistry permits delineation of effects on target organelles such as mitochondria in specific cell populations prior to the onset of overt toxicity. Measurement of circulating or excreted metabolites from these affected organelle systems may allow a rather specific estimation of the magnitude of the *in vivo* toxic effect.

To illustrate how this basic approach may aid in enhancing our understanding of metal interactions, the following discussion will briefly review some of the known effects of lead, cadmium, and arsenic on a highly sensitive cellular organelle system (mitochondria), and particularly the excretion patterns of metal-specific porphyrinurias. This information will hopefully provide a background for discussion of data from a recent lead, cadmium, arsenic interaction study which used measurement of porphyrin excretion patterns as one of the assessment criteria.

General Subcellular Effects of Lead, Cadmium, and Arsenic

It should be noted before focusing on the mitochondrial effects of lead, cadmium and arsenic that these elements are broad spectrum toxicants which

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are capable of altering many subcellular organelle systems when administered at sufficient dose levels. These other effects have been recently reviewed elsewhere (*6*) and hence will not be discussed here.

Effects of Metals on the Mitochondrion

Mitochondria (Fig. 1) are multifunctional organelles which have been found by many investigators to be markedly sensitive to metal toxicity. The specific individual biochemical effects of lead, cadmium, and arsenic on these organelles are summarized below on an elemental basis.

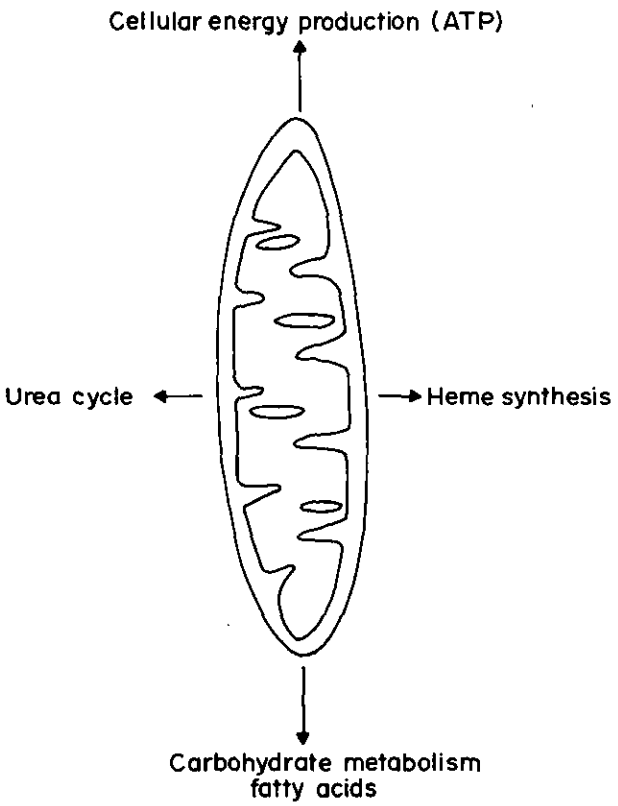

FIGURE 1. Schematic presentation of a mitochondrion showing general metabolic functions.

Lead

The effects of lead on mitochondria have been extensively studied. Accumulation of lead in the kidney mitochondria and the resultant swelling are early signs of nephrotoxicity from this element (*7*). Biochemical studies of lead poisoned mitochondria (*8*) have shown pyruvate/malate-mediated respiration to be more strongly inhibited than that supported by succinate. This effect is thought to occur by lead inhibition of the lipoic acid dehydrogenase complex. Lead has also been found to inhibit the mitochondrial heme biosynthetic enzymes δ-aminolevulinic acid (ALA) synthetase and ferrochelatase and the extramitochondrial enzyme δ-aminolevulinic acid dehydratase (*7*). This effect may in part account for the depression of mitochondrial cytochrome aa_3 content in lead-poisoned mitochondria (*9*). The important effects of lead on this organelle system are that it may cause cell death by impairment of energy production and increased urinary excretion of ALA and coproporphyrin due to inhibition of heme biosynthetic pathway enzymes (*8*).

Cadmium

In vitro studies with Cd^{2+} have shown that this ion has a high affinity for mitochondria (*10*) and is capable of inhibiting respiration and oxidative phosphorylation (*10, 11*). It is also known to interfere with the 1-hydroxylation of vitamin D by renal mitochondria (*12*). *In vivo* mitochondrial effects of cadmium are probably dependent upon the synthesis, availability and degradation of cadium metallothionein. The effects of cadium on mitochondrial porphyrin metabolism have not been studied, but this element has been reported to produce no alteration of ALA dehydratase in exposed subjects (*13*).

Arsenic

Arsenicals cause mitochondrial swelling and are known for their selective inhibition of pyruvate/malate linked mitochondrial respiration and for their uncoupling of oxidative phosphorylation (*2, 14–18*). This phenomenon, like that produced by lead, has been thought to result from arsenical inhibition of the lipoic acid activity needed as a cofactor for PDH activity, but recent biochemical studies (*19*) have suggested an alternative explanation based on altered regulation of PDH. Other effects of arsenicals on this organelle involve perturbation of mitochondrial marker enzyme systems, particularly those involved in heme biosynthesis (*2–4*) with a resulting dose-related uroporphyrinuria, a form of porphyrinuria which is distinct from that observed with methylmercury or lead (*5*).

On the basis of the above information, it should be clear that mitochondria are sensitive target organelles for the metals under discussion but that the mechanisms of mitochondrial toxicity with resultant metal-specific porphyrin excretion patterns vary between these elements.

Interactions Among Lead, Cadmium, and Arsenic with Respect to Porphyrin Excretion Patterns

In order to determine whether mitochondrial damage and specific porphyrin excretion patterns are altered by multi-element exposure, an interaction study employing lead, cadmium, and arsenic in either the organic (arsanilic acid) or inorganic (sodium arsenate) form was conducted. The diet and dose levels chosen were based on previous studies (*2, 20, 21*) to give regimens which produced no overt signs of toxicity. A total of 168 male Sprague-Dawley rats were fed a casein-based purified diet for 10 weeks. The animals were divided into groups by using a 2 × 2 × 3 factorial design. Lead as lead acetate was added to the diet for the lead-treatment groups at a concentration of 200 ppm. Cadmium as cadmium chloride was added at 50 ppm, while arsenic as either sodium arsenate (inorganic As) or arsanilic acid (organic As) was added to the diets of these treatment groups at concentrations of 50 ppm. A more detailed description of the study and specific ultrastructural and toxicological results have been presented elsewhere (*22*). Recently completed analyses of data on heme biosynthetic and porphyrin excretion patterns from this study have also been reported (*23*). A summary of the findings with respect to the impact of multi-element exposure on urinary excretion of ALA, uroporphyrin and coproporphyrin follows (Fig. 2).

The results of the study indicate that some lead–cadmium and lead–arsenic interactions do occur with respect to mitochondrial toxicity and to changes in porphyrin excretion patterns. Lead exposure produced significant increases in urinary ALA and coproporphyrin but not uroporphyrin. Concomitant administration of cadmium with lead caused a decrease in urinary ALA excretion but not that of coporphyrin. This effect may result from cadmium inhibition of formation of active metabolites of vitamin D (*12*) which appear to play a role in lead absorption (*22*). Cadmium by itself did not markedly alter urinary excretion of any of the measured porphyrins. Arsenic in either the organic or inorganic form produced no change in ALA excretion but caused marked increases in urinary uroporphyrin and to a lesser degree coproporphyrin, thus confirming earlier findings (*4*). The combination of lead plus arsenic produced an additive effect on coproporphyrin excretion but no alteration on the arsenic effect on uroporphyrin excretion indicating that the latter effect is rather specific. Other combinations of lead, cadmium and arsenic brought about porphyrin excretion patterns similar to those discussed above.

The conclusions to be drawn from this discussion are as follows:

(1) Mitochondria and their attendant biochemical systems are highly sensitive target organelles for the effects of lead, cadmium, and arsenic.

(2) Measurement of specific circulating or excreted metabolites (such as porphyrins) from damaged mitochondria in target organs may provide biological reflections of cellular dysfunction prior to the onset of overt toxicity.

(3) Some interactive effects between lead, cadmium, and arsenic with respect to those parameters

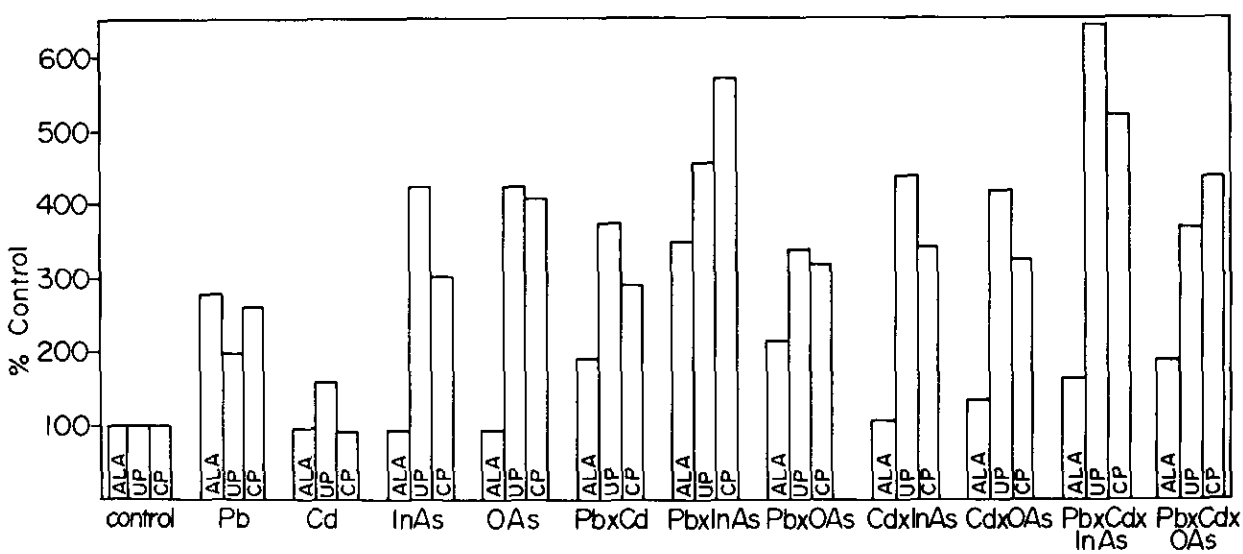

FIGURE 2. Schematic summary of porphyrin excretion profiles for rats exposed to combinations of lead (200 ppm), cadmium (50 ppm), and arsenic (50 ppm) for 10 weeks. Data are expressed as mean percent of control.

appear additive while others are apparently antagonistic. The basic specific porphyrin excretion patterns for some individual metals, however, remain discernible. Further basic research into the mechanisms of interaction among these elements in relation to toxicity is needed.

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