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INDUCTION OF MUCOSAL GLUTATHIONE SYNTHESIS BY ARSENIC

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Summary

In the rat, oral administration of 1 mg/kg arsenic (as As_2O_3) binds rapidly to mucosal glutathione such that effective glutathione concentration is reduced. In response to the binding of arsenic to glutathione, de novo synthesis of glutathione occurs in the mucosal cell, resulting in twice the normal concentrations of glutathione within 3 h. This finding may explain acquired tolerance to arsenic, as well as the protective effect of arsenic against selenium toxicity.

The acute toxicity of arsenic has been ascribed to the reaction of trivalent arsenic with sulfhydryl groups, and subsequent interference with cellular oxidation-reduction reactions [1]. Yet, tolerance to arsenic can be achieved if gradually increasing quantities are consumed, beginning with a very small dose. The famous 'arsenic-eaters' of Tyrol were able to tolerate easily doses of arsenic which would have been lethal to men not previously exposed [2]. The mechanism of acquired tolerance has never been explained.

Similarly, arsenic has long been known to exert a protective effect against selenium toxicity [3]. In appropriate amounts, arsenic is effective in counteracting or preventing the symptoms of experimentally-induced selenium poisoning [4]. The toxicity of selenium is due to the substitution of selenohydryl for sulfhydryl groups in several enzymatic processes [5]. Once again, the mechanism of the protective effect of arsenic has never been explained.

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We have investigated the effect of a small dose of orally-administered arsenic on glutathione synthesis in rat gastrointestinal mucosa. To our surprise, arsenic induced the synthesis of super-normal concentrations of glutathione. This effect may explain arsenic tolerance and the protective effect of arsenic against selenium toxicity.

Male albino Sprague-Dawley rats (250–400 g; Charles Rivers Lab., Wilmington, MA) were maintained on Purina Rat Chow and water ad libitum, but were fasted overnight prior to use and throughout the experiment. A solution of As_2O_3 (Matheson, Coleman and Bell, Los Angeles, CA) was prepared by dissolving 100 mg in 5 M NaOH. The volume was brought up to 50 ml, pH adjusted to 7.5 with conc. HCl, and the final solution brought up to a volume of 100 ml. Rats were administered 1 mg/kg As_2O_3 orally. At various time-points, groups of four rats were anesthetized with ether, the gut excised, and the mucosa of the anterior 8 cm of duodenum scraped onto a glass slide and rapidly homogenized in 5% trichloroacetic acid containing 0.01 M HCl and 2 mM EDTA. The next few cm were fixed in formalin and reviewed for histopathologic changes. Glutathione content was determined by reaction with 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB)]6] and protein determined by the Lowry method [7].

The administration of As_2O_3 resulted in an initial decrease in mucosal glutathione concentration, with subsequent rebound to twice the control value by 3 h; no obvious histopathology of the mucosa was observed. The glutathione concentration then gradually decreased, reaching control values by 18 h. (Fig. 1).

Since the DTNB method [6] of glutathione analysis could potentially have been detecting an elevation of some sulfhydryl compound other than glutathione, a second confirmatory method of duodenal glutathione analysis was performed. Additional rats were administered arsenic or the vehicle, kil-

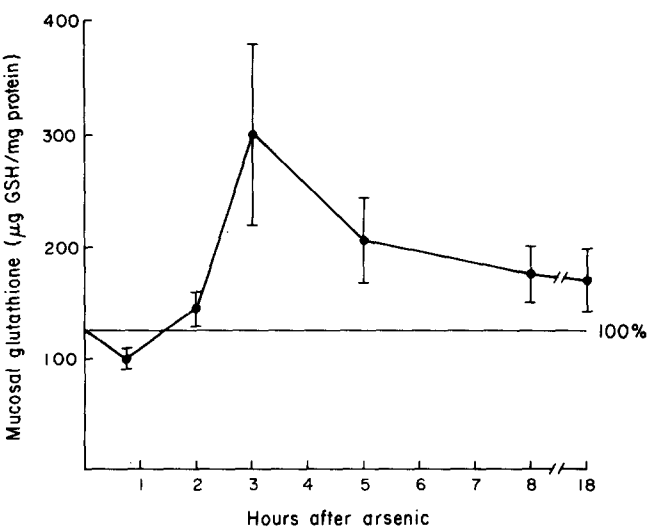

Fig. 1. Mucosal glutathione concentration. Effect of oral administration of arsenic (1 mg/kg as As_2O_3) on mucosal glutathione concentration, at various time intervals after arsenic administration. Groups of four rats were used at each time-point.

led after 3 h, and the duodenal mucosa removed by scraping, as above. The tissue was homogenized in 4 vols. of 10% sulfosalicylic acid, centrifuged, and the supernatant fractions analyzed for glutathione on a Durrum amino acid analyzer using the physiological fluid program [8]. Glutathione was determined as the 2-vinylpyridine derivative [9], which elutes on the analyzer in 98 min. Using this method, rats which received arsenic showed a 2-to-3-fold increase in glutathione concentration in comparison to the controls, thus confirming the data obtained by the DTNB method.

These experiments indicate that the oral administration of trivalent arsenic resulted in a decrease in assayable glutathione in the mucosa within 1 h. Subsequently, during the next 2 h, neosynthesis of glutathione to twice the initial concentration occurred, despite the fact that these animals still had not received food since the previous night. The induction of glutathione synthesis by arsenic could explain both arsenic tolerance and arsenic protection against selenium toxicity. Elevated intracellular concentrations of glutathione could act as a 'trap' for potentially toxic arsenic or selenium.

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