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Absence of Effect of Tocopherol on Acute Oral Toxicity of Sodium Selenite in the Rat. (31769)

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Selenium is now recognized to play two different roles in nutrition. At low levels it is an essential element whose function is closely related to that of vitamin E, although the exact mechanism is not thoroughly understood. At higher levels it is toxic. Evidence is accumulating that selenium and tocopherol administered together may be more effective in treating such disturbances as "white muscle disease" or "nutritional muscular dystrophy" than is either alone (1,2). The statement has been made that tocopherol also reduces the toxicity of selenium(3). The following experiments were undertaken to determine whether the administration of tocopherol with selenium did, in fact, alter the toxicity of selenium when it was provided to rats in a single dose orally as sodium selenite. The results indicated that it did not. Previous studies on the toxicity of selenium have been reviewed by Smith *et al*(4), Painter(5), Moxon and Rhian(6), and Rosenfeld and Beath (7), but the acute oral toxicity of sodium selenite in the rat seems not to have been investigated.

Experimental. Sodium selenite was dissolved in physiological saline to give a solution of final concentration 1.0 mg selenium per ml (2.19 mg sodium selenite per ml). The selenite-tocopherol combination was supplied by H. C. Burns Co.*

Male and female Long-Evans rats were maintained on a diet of laboratory chow (Purina Labena) and water, *ad libitum*. They were starved for 24 hours before administration of the selenite solutions by stomach tube. Dose was based on body weight of the animals (70 to 380 g range). Food was then made available and the rats were inspected frequently to note symptoms, weight changes, and time of death; autopsies were performed immediately thereafter. Control animals received physiological saline equal in volume to the selenite solutions. None of these died.

Results and discussion. Within 15 minutes after administration of selenite, with or without tocopherol, most of the rats became subdued, and developed a "garlicky odor." This odor is often used to identify selenium poisoning and is presumed to be due to di-

* Seletoc®, H. C. Burns Co., Oakland, Calif., reported to contain per ml: selenium (as sodium selenite), 1 mg; vit. E (as d-α-tocopheryl acetate), 50 mg; polyoxysorbitan mono-oleate, 10 mg; propyl p-hydroxybenzoate, 1 mg.

TABLE I. Acute Oral Toxicity to Rats of Selenium Administered as Sodium Selenite or Sodium Selenite Plus Tocopherol.

Dose (mg Se/kg)	Lethality*			
	Selenite		Selenite-tocopherol†	
	♀	♂	♀	♂
10.0	2/5	3/5	0/5	3/5
12.5	11/19	11/19	5/12	6/12
15.0	9/15	8/15	5/9	6/9

* No. of deaths within 48 hr to total number treated.

† Ratio of Se as selenite to d-α-tocopheryl acetate, 1/50 by weight, Seletoc (see footnote).

methyl selenide(8). Ganther(9) has recently demonstrated enzymatic synthesis of dimethyl selenide from sodium selenite in mouse liver extracts.

All animals continued to lose weight for at least 24 hours after selenium administration. Diarrhea was noted in a large percentage of the rats. Other symptoms included bleeding from the nose, yellow staining around the anal region suggesting internal bleeding, watering of the eyes, and extreme lethargy. On autopsy, some of the rats showed liver damage, hemorrhagic intestines and spleen, and accumulation of large amounts of fluid in the stomach. These symptoms are typical of those described by others(7) for acute selenium poisoning. Rats receiving selenite and those receiving selenite-tocopherol reacted similarly.

Surviving rats began to recover rapidly with 72 hours; after several days no ill effects of the selenium were seen. One rat developed a massive ascites tumor 3 weeks after dosage. This animal was extremely anemic; autopsy revealed a lumpy and atrophied liver and grayish-colored kidneys.

The selenite solutions were administered to approximately 100 rats. Preliminary observations indicated that the LD_{50} level was within the range of 10 to 20 mg selenite-selenium per kg. The data shown in Table I suggest that the true LD_{50} is approximately 12.5 mg per kg and that the presence of tocopherol did not appear to influence it. Schwarz (quoted by Burns(3)) found that the oral LD_{50} for selenite-tocopherol in rats was 12.5 mg per kg selenium. This was compared to an LD_{50} of 2.5 to 3.0 mg per kg for selenite-selenium alone. However, we could find no data in the literature for orally administered selenite in rats. The latter level does, however, correspond to the intravenous acute toxicity for rats reported by Smith *et al*(4), who also made the statement that ". . . 1.5 to 3.0 mgm per kilogram may be considered as the minimum lethal dose of selenium as sodium selenite or selenate in rats, rabbits, and cats, and the route of administration does not greatly affect its toxicity," without substantiating data concerning oral administration to rats. It also corresponds to the intraperitoneal acute toxicity found by Franke *et al*(10). Experiments not shown indicated that, when administered intraperitonally, the LD_{50} of selenite was similar to that of selenite-tocopherol, *i.e.*, about 3.0 mg selenium per kg.

These combined observations suggest that whatever may be the interrelationships between selenite and vit. E at physiological levels, the presence of tocopherol administered simultaneously does not modify the acute oral toxicity of selenite for the rat. No suggestion was obtained as to the cause of toxicity, which is usually ascribed to interference with normal sulfur metabolic compounds. Tocopherol apparently does not modify this mechanism. Jensen *et al*(11) recently showed that added tocopherol does not influence the distribution of selenium in the chick.

Summary. The acute oral toxicity of selenite-selenium in the rat is approximately LD_{50}, 12.5 mg/kg; this level is not changed by simultaneous administration of tocopherol as d-α-tocopherol acetate at 725 mg/kg.

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In vitro Production and Inhibition of Aortic Vasoconstriction by Mercuric, Cadmium, and Other Metal Ions.* (31770)

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Vasoactivity of the subgroup II elements, cadmium and mercury, has been demonstrated in three ways: First, intra-arterial injections of less than 0.05 mg of either element into anesthetized rats have produced acute, but transient increases in diastolic pressure(1). Although the pressor effects of the two ions were superficially similar, the mercuric ion did not alter cardiac output, whereas the cadmium ion increased it proportionally to the increase in pressure. Second, rats have exhibited prolonged hypertension after drinking water containing 5 parts per million of cadmium for several months(2) or following several weekly intraperitoneal injections of 0.2 mg of cadmium(3). Finally micromolar concentrations of mercuric ion have induced single slow contractions in spirally-cut strips of rabbit aorta, as described here. While cadmium ion produced no similar effect, it inhibited the effect of mercuric ion. It also inhibited epinephrine-induced, but not angiotensin-induced, contractions of aortic strips.

Methods. Aortic strips were set up according to the technique described by Furchgott and Bhadrakom(4) and modified in our laboratory(5): A large normal unanesthetized female New Zealand white rabbit was decapitated, its descending thoracic aorta quickly removed, trimmed of excess fat and connective tissue, and cut into four spiral strips approximately 2 mm wide and 25 mm long, with circumferential muscle fibers making an angle of about 15 degrees with the long axis of the strip. Each strip was mounted in an individual chamber where it was covered completely by 20 ml of Krebs Ringer bicarbonate solution† to each 100 ml of which 200 mg of glucose had been added. The temperature of the solution was maintained at 37 ± 0.1°C, and 100 ml per min of 95% O_2 and 5% CO_2 was continually bubbled through it. The bottom of a strip was fixed and the top attached to the lever of an ink-writing, gravity-type pen which amplified contractions 4-fold. The lever was counterweighted to exert constant tension of 1.7 g on a strip which was mounted so that, after 2 hours when it had completely relaxed, about 20 mm separated the top and bottom points of attachment. The responsiveness of each strip was tested by exposing it to 10^{-7} molar l-epinephrine and recording the maximum contraction. The solution containing epinephrine was then removed and replaced with fresh Krebs Ringer bicarbonate solution; this washing was repeated at 5-minute intervals until, after 15 to 30 minutes, the strip had completely relaxed and regained its previous responsiveness. Except for standardization with epinephrine, no strip was exposed to more than one metal or other test agent unless specifically indicated. All cited molarities apply to concentrations in the solution surrounding the strip.

Results. Approximately 20% shortening of the aortic strip was produced by 10^{-7} molar

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† The Krebs Ringer bicarbonate solution was composed of 100 parts of 0.90% (by weight) NaCl, 4 parts of 1.15% KCl, 3 parts of 1.22% $CaCl_2$, 1 part of 2.11% KH_2PO_4, 1 part of 3.82% $MgSO_4 \cdot 7H_2O$, and 21 parts of 1.30% $NaHCO_3$, all dissolved in deionized distilled water.