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Prolongation by selenium of pentobarbital hypnosis in the male rat

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Summary. Following treatment with sodium selenite, pentobarbital hypnosis was prolonged in male rats. The maximal effect occurred at 3–4 days following selenium treatment and the threshold dose for the effect was found to be 2.4 mg Se/kg (i.p.).

Selenium is now recognized as an essential trace element constituting an integral portion of the enzyme, glutathione peroxidase[2,3]. Until approximately 25 years ago, selenium was of concern primarily because of its toxicity. The toxicity induced by selenium exhibits many manifestations and depends upon many factors such as age, dietary constituents, species, and sex[4–6]. Toxicity of selenium can be induced acutely in laboratory animals or can occur following chronic exposure as is generally seen in farm animals eating a sufficient quantity of highly seleniferous plants[6].

The administration of many other metals, such as lead[7], cadmium[8], and manganese[9] produce hepatic effects such as inhibition of drug biotransformation which can lead to an altered responsiveness to other drugs including the barbiturates. This study was undertaken to examine the effect of acute selenium administration on drug response in the male rat.

Methods. Male, Sprague-Dawley rats weighing 160–240 g were obtained from Laboratory Supply Co. (Indianapolis, IN) and housed in community cages for at least 1 week prior to use. Animals were maintained in environmentally controlled rooms at approximately 22 °C under a 12-h alternating light-dark cycle (L) (06.00–18.00 h) with free access to laboratory food (Wayne Lab. Blox, Allied Mills, Inc., Chicago, IL) and tap water.

Sodium selenite (Na_2SeO_3) and pentobarbital Na solutions for injections were prepared using distilled, deionized water such that each animal received 1 ml/kg b.w, i.p. The duration of hypnosis was defined as the time elapsing from loss to recovery of the righting reflex. Statistical analyses were performed using analysis of variance (ANOVA) followed by Dunnett's test where appropriate. The acceptable level of significance was established as $p < 0.05$.

Results. In the first experiment the duration of hypnosis following the administration of pentobarbital (40 mg/kg, i.p.) to male rats treated 72 h prior to a challenge with selenium in doses of 0.8, 1.6, or 2.4 mg/kg was determined. These doses were chosen since they had been used by

Table 1. Dose-response of selenium prolongation of pentobarbital hypnosis in male rats

Selenium dose (mg/kg)	Duration of hypnosis (min ± SEM)
0	95 ± 9
0.8	97 ± 9
1.6	99 ± 7
2.4	150 ± 24*

Rats (8 per group) received pentobarbital (40 mg/kg, i.p.) 72 h after treatment with selenium in the designated doses and duration of hypnosis was determined. * Significantly different from control values ($p < 0.05$).

Table 2. Time-course of selenium prolongation of pentobarbital hypnosis in male rats

Time after selenium (h)	Duration of hypnosis (min ± SEM)
Controls	76 ± 7
1	66 ± 13
6	96 ± 16
12	78 ± 5
24	84 ± 7
48	116 ± 28
72	132 ± 20*
96	138 ± 11*

Rats (5–7 per group) received Na selenite (2.4 mg Se/kg) and at the specified time intervals received pentobarbital (40 mg/kg). The duration of hypnosis was measured. Controls received saline (1 mg/kg) 4 days prior to pentobarbital. * Significantly different from control values ($p < 0.05$).

others[10,11] as an antidote for heavy metal toxicity. As shown in table 1, the minimal effective dose of selenium which significantly prolongs the duration of pentobarbital hypnosis was 2.4 mg/kg.

In the next experiment the time-course of selenium-induced prolongation of hypnosis was examined. The animals were treated with selenium (2.4 mg/kg) and challenged at various time periods thereafter. The results of this experiment are shown in table 2. Although the duration of hypnosis was increased at 48 h, a significant prolongation of pentobarbital hypnosis was first observed at 72 h.This prolongation of effect was also observed at 96 h, the last time period examined. Thus, the effect of selenium on drug response does not occur immediately, but requires approximately 48–72 h to develop.

Discussion. The results of the present study indicate that selenium in a threshold dose of 2.4 mg Se/kg can alter drug response in the male rat. Furthermore, following this dose, drug response is not altered until 3 days following such treatment. A number of previous studies have shown that several metal ions can alter drug response in the rat. Such metals include cadmium[12], lead[7], arsenic[13], manganese[9], and the methylmercuric ion[7]. Such metal induced alterations of drug response are most likely to result from an influence on hepatic drug metabolism. Several studies have shown that these ions can decrease the levels of cytochrome P-450[14,15]. Indeed, Maines and Kappas[16] have reported that selenium administered in a dose of 7.9 mg/kg to male rats could effectively reduce cytochrome P-450 levels by 18.5% at 16 h following administration of selenium, thus, explaining the ability of selenium to prolong pentobarbital hypnosis.

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1,3,7-Trimethylxanthine (caffeine); a new natural fish fungicide

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Summary. During antifungal screening of products from higher plants 1,3,7-trimethylxanthine (caffeine), isolated from the seeds of *Coffea arabica*, was found to exhibit strong fungitoxicity. The fungicidal ability of caffeine has been tested in vitro on 4 fish pathogenic species of Saprolegniaceae. The applicability of this chemical has also been tested by conducting tolerance tests, using *Colisa lalia* and *Puntius sophore* as test-fishes. The present studies have established that caffeine is a potential fish fungicide.

Many chemicals have been studied for the purpose of controlling mycoses in fishes and their eggs[2–7]. However, there are few reports in the literature of the use of alkaloids in general and 1,3,7-trimethylxanthine in particular against any fish pathogenic fungi. Recently we have isolated 1,3,7-trimethylxanthine (caffeine) from the seeds of *Coffea arabica* and studied its insect-sterilizing, fungicidal and weed-killing activities[8–10]. The present communication deals with the fungicidal activities of caffeine against 4 fish pathogenic strains of Saprolegniaceae.

Experimental procedures. An ethanol extract of the seeds of *Coffea arabica* was dried in vacuo and its water soluble resudue was fractionated in petroleum ether, carbon tetrachloride, chloroform and n-butanol. Of these, the residue from the chloroform fraction was subsequently used for isolation of the pure compound by running the sample on a silica gel column with chloroform-methanol (98:2 v/v) followed by a TLC on a silica-gel plate using chloroform-methanol (96,25:3.75 v/v). Its identity with 1,3,7-trimethylxanthine was confirmed by m.p. determination (m.p. 235–236 °C), mixed m.p. determination (no depression in m.p.) as well as UV-, IR- and NMR-spectra (UV: λ max Me OH 225, 275 nm; IR: KBr, 3500, 3050, 2835, 2985, 1650, 1690 cm^{-1}; NMR: T2,6.14,6.68,6.89). The active principle was tested in vitro for its potential fungicidal activity on *Achlya* sp., *Achlya orion, Saprolegnia ferax* and *Aphanomyces laevis* by the usual methods[5] (table 1). Commercial caffeine was also tested in parallel on the same fungi for its potential fungicidal ability (table 2). For the applicability of this chemical as a chemotherapeutant to be