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

Table 1. In vitro studies on the effects of purified caffeine on the growth of 4 fish pathogenic fungi

Test fungus	Concentrations (%)	Diameter of the colony** (mm) with SD after 24 h	48 h	72 h	96 h	% inhibition at 96 h of growth
Achlya sp.	1.0	0	0	0	0	100
	0.5	0	0	0	0	100
	0.2	0	0	0	0	100
	0.1	*	7.2±0.97	10.3±1.00	13.8±1.23	30.0
	Control	7.5±1.00	10.5±1.30	14.1±1.60	19.4±1.90	No inhibition
Achlya orion	1.0	0	0	0	0	100
	0.5	0	0	0	0	100
	0.2	0	0	0	0	100
	0.1	*	6.8±0.90	9.7±1.12	12.9±1.20	40.9
	Control	5.8±0.87	9.1±1.00	12.8±1.32	21.4±2.10	No inhibition
Saprolegnia ferax	1.0	0	0	0	0	100
	0.5	0	0	0	0	100
	0.2	0	0	0	0	100
	0.1	*	5.0±0.80	7.9±1.05	11.8±1.12	36.8
	Control	7.0±1.20	9.0±1.20	13.0±1.24	18.7±1.87	No inhibition
Aphanomyces laevis	1.0	0	0	0	0	100
	0.5	0	0	0	0	100
	0.2	0	0	0	0	100
	0.1	6.5±0.99	8.8±1.00	14.6±1.32	20.0±2.05	21.4
	Control	9.5±1.24	15.6±1.40	20.5±2.20	26.0±2.74	No inhibition

*Unmeasurable growth; **Average of 30 readings (10 readings in each experiment performed in triplicate). Growth on modified SPS-medium (Prabhuji[11]) at 25–28 °C, Readings taken at every 24 h interval.

Table 2. In vitro studies on the effects of commercial caffeine on the growth of 4 fish pathogenic fungi

Test fungus	Concentrations (%)	Diameter of the colony** (mm) with SD after 24 h	48 h	72 h	96 h	% inhibition at 96 h of growth
Achlya sp.	1.0	0	0	0	0	100
	0.5	0	0	0	0	100
	0.2	0	0	0	0	100
	0.1	*	6.8±0.92	9.2±1.20	11.8±1.25	40.0
	Control	7.5±1.00	10.5±1.30	14.1±1.60	19.4±1.90	No inhibition
Achlya orion	1.0	0	0	0	0	100
	0.5	0	0	0	0	100
	0.2	0	0	0	0	100
	0.1	0	5.0±0.88	8.2±1.10	10.9±1.20	50.0
	Control	5.8±0.87	9.1±1.00	12.8±1.32	21.4±2.10	No inhibition
Saprolegnia ferax	1.0	0	0	0	0	100
	0.5	0	0	0	0	100
	0.2	0	0	0	0	100
	0.1	*	5.4±0.92	8.3±1.22	12.5±1.50	35.0
	Control	7.0±1.20	9.0±1.20	13.0±1.24	18.7±1.87	No inhibition
Aphanomyces laevis	1.0	0	0	0	0	100
	0.5	0	0	0	0	100
	0.2	0	0	0	0	100
	0.1	*	6.2±1.00	9.7±1.45	13.2±2.00	46.4
	Control	9.5±1.24	15.6±1.40	20.5±2.20	26.0±2.74	No inhibition

*Unmeasurable growth; **Average of 30 readings (10 readings in each experiment performed in triplicate). Growth on modified SPS-medium (Prabhuji[11]) at 25–28 °C. Readings taken at every 24 h intervals.

used in fishes, tolerance tests were also conducted using *Colisa lalia* Ham. and *Puntius sophore* Ham. as test fishes (table 3). All the experiments were performed at 25–28 °C.

Results and discussion. In vitro studies have shown (tables 1 and 2) that out of 6 concentrations tested, 0.2% caffeine was found to be the lowest effective concentration (LEC) exhibiting 100% inhibition. The difference in percentage inhibition by purified caffeine and commmercial caffeine (tables 1 and 2) is probably due to some fungicidal impurities in the purified caffeine. Further, it was found that both the test fishes could survive for more than an hour (table 3) in the LEC of the chemical. Caffeine is, therefore, suitable for dip treatments for short durations only and for topical application. In many earlier experiments[2–4,6] the identity of the fish pathogen or of the host was not always properly given; this information is essential for accurate assessment. However, a few communications[5,7] have given adequate details of experiments. The present investigations have established that caffeine is a potent fish fungicide. It was also revealed by performing tolerance tests that the toxicity of the chemical depends on the concentration of the chemical, length of exposure time and general vigour of the test fishes.

Table 3. Toxicity effects of 1,3,7-trimethylxanthine on test fishes

Concentrations (%)	Time of survival (min) *Puntius sophore*	*Colisa lalia*
1.0	7	6
0.5	30	27
0.2	100	92
0.1	150	120
Control	nontoxic	nontoxic

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Lithium and rubidium: Effects on locomotion of planaria *(Dendrocoelum lacteum)*

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Summary. The gliding locomotion of planaria *(Dendrocoeleum lacteum)* was suppressed similarly by LiCl and RbCl.

The use of lithium and rubidium as drugs to treat mental disorders has stimulated interest in their effects on behavioral processes in laboratory animals[3,4]. Invertebrates have been used occasionally to determine whether monovalent cations influence basic behavioral processes[4,5]. Previously, we studied effects of lithium and rubidium on rhythmic contractile movement of jellyfish[6], which have as simple a nervous system as can be found in the animal kingdom[7]. Now, we report on the influence of lithium and rubidium on locomotion in planaria, an animal with a very primitive CNS[5,8].

Materials and methods. We carried out experiments during August–December. Planaria of the species *Dendrocoelum lacteum* (Turbellaria, Tricladida, Pladudicola) were collected from lakes and streams in Aarhus, Denmark. They were 0.8–1.4 cm long and weighed 20–30 mg. They were housed together with free access to food (boiled egg yolk) in a beaker (18 cm diameter) containing 3 l of water from their natural habitat at room temperature (20 °C) with air bubbled vigorously in the beaker at all times. Fluorescent lights provided about 100 footcandles illumination in the vicinity of the planaria.

For tests, the planaria were placed individually in a Petri dish (10 cm diameter) containing 10 ml of water from the natural habitat (medium). A piece of white graph paper divided into squares (1 mm × 1 mm) by light blue lines was located under each Petri dish. Planaria that crossed 25–60 lines during 1 min after 15 min in the Petri dish (baseline conditions) were selected for experiments. 48 of the about 80 planaria examined met this criterion. After baseline recordings, the planaria were assigned at random to 1 of 4 equal groups. Each planaria was used only once. 3 groups had a solution (195 mmoles) of either NaCl, LiCl or RbCl added automatically to the medium in the center of the Petri dish by an infusion pump at a rate of 6 ml/h, while the 4th group received no treatment and served as a control. The media were not stirred because preliminary observations indicated that mechanical disturbances impaired activity of planaria. The number of lines crossed by each planaria was measured every 5 min for 1 min during 30 min. Analysis of variance was used to determine the statistical significance of the results. After test, 7 planaria in each group were selected at random, rinsed in saline, weighed and frozen. In addition, samples of media were taken at the center and along the edge of Petri dishes to which LiCl had been added. The concentration of sodium and potassium was determined by flame photometry and that of lithium and rubidium was measured by spectrophotometry.

Results. The figure presents the effects of lithium, rubidium and sodium on the rate of locomotion of planaria. Inspection of the data shows that lithium and rubidium reduced the rate of locomotion of planaria. Statistical analysis indicated significant differences for the overall effects of treatments (main treatment effect $p < 0.001$) as well as for pairwise comparisons between lithium and control, lithium and sodium, rubidium and control and rubidium and sodium (p's < 0.001), but not between lithium and rubidium or sodium and control. It is evident that locomotion tended to decrease as the concentration of lithium or rubidium increased, and statistical analysis demonstrated significant differences for the overall effect of the amount of salt added (main concentration effect $p < 0.001$) as well as for the amount of lithium or rubidium added (p's < 0.001). There was also a significant tendency for locomotion to decrease during the experiment in the control group ($p < 0.01$), but the decrease in locomotion in planaria treated with either lithium or rubidium was significantly greater than that observed in the controls (p's < 0.001).

Concentration of sodium, potassium, lithium and rubidium in planaria *(Dendrocoeleum lacteum)* treated with 195 mmoles solutions of NaCl, LiCl or RbCl added to 10 ml medium for 30 min at a rate of 6 ml per h. Values are means ± SEM for 7 planaria per group

	Cation concentration (mmole/kg)[a]			
	Sodium	Potassium	Lithium	Rubidium
Untreated	16.1 ± 2.5	30.7 ± 2.7	0	0
NaCl-treated	39.9 ± 8.8	36.5 ± 8.6	0	0
LiCl-treated	18.7 ± 1.9	36.2 ± 2.6	11.4 ± 2.0	0
RbCl-treated	16.5 ± 1.2	38.2 ± 1.5	0	10.5 ± 0.8

[a]The initial concentrations of sodium, potassium, lithium and rubidium in the medium were 1.5, 0.2, 0 and 0 mmoles, respectively.