series. The crystal type found in this series is a collection of fine crystals of less than 5 μm, and these fine crystals are sometimes accompanied with square or rectangular solitary crystals. This group includes all Japanese conifers, i.e., Taxaceae, Podocarpaceae, Cephalotaxaceae, Taxodiaceae, Cupresaceae, and Pinaceae.

Japanese gymnosperms, therefore, are divided into 2 large systems from their crystal form. Considering the embryological evidence on the crystal pattern, the pattern of the evolution advances in the direction of a fine crystal type → a solitary crystal type. Therefore, Pinaceae plants which contain a large quantity of solitary crystals seem to be a more advanced group of the conifers. The crystal patterns of *Abies* and *Tsuga*, which contain a large number of fine crystals but only a few solitary crystals, are transitional between the crystal patterns of *Cedrus* and *Pinus*, which contain a lesser number of fine crystals and a considerable quantity of solitary crystals, and the crystal patterns of Taxodiaceae and families other than Pinaceae in the conifers. This evidence suggests that evolution advances in the direction of conifers (excluding Pinaceae) → *Abies* and *Tsuga* → *Cedrus* and *Pinus*. However, these points require further examination, with reference to other characteristics, before a definite conclusion can be drawn.

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Metabolic studies of Hg-203 on *Chlamydomonas reinhardi*[1]

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Summary. Sterile cultures of *Chlamydomonas reinhardi*, WT^+, were treated with Hg-203 at 25 °C to identify probably formed volatile mercury compounds. Experiments were performed with living and dead cells under aerobic or anaerobic conditions, respectively, and the mercury concentration was measured in the system algae/nutrient medium. We found a timerelated decrease of mercury concentration in the cell suspension and the cell-free nutrient medium due to a reduction of Hg^{++} to Hg^0, probably caused by extracellular enzymes; monomethyl or dimethyl mercury could not be detected.

The increasing application of toxic elements in industry and agriculture causes environmental pollution and human diseases. Especially the highly toxic metal mercury attracts considerable attention on account of the accident in Minamata, Japan[3,4] and the effect of rather careless use of methyl mercury-containing seed dressings in Sweden[5–8]. Therefore extensive research has been performed on the fate of mercury in food chains; it could be shown that microorganisms are able to metabolize mercury compounds[9–15]. This reaction, regarded as a detoxification mechanism[16], can yield a product that is either more or less toxic to higher organisms. Ben-Bassat et al.[17] exposed *Chlamydomonas reinhardi* to mercury (II)-chloride and found a decrease of mercury concentration in the cell suspension; they supposed metabolization to volatile compounds, e.g. methyl mercury. Our work focused on the identification of probably formed volatile mercury compounds.

Materials and methods. Algae: The unicellular algae *Chlamydomonas reinhardi* was the organism employed in this work. Slant-agar stocks of WT^+ (wild type) were kindly given by R. Davies (John-Innes-Institute, Norwich, England). Pure cultures of the cells were grown asynchronously, i.e. by means of continuous illumination, at 25 °C and air bubbling through (1.5 l/h). Cultivating was done in 500 ml Erlenmeyer flasks containing 200 ml YAP[18] under sterile conditions; the final cell concentration was 5.10^6/ml (determinded by means of Thoma counting chambre).

Mercury: Mercury-203 was used as nitrate. This isotope was obtained by neutron activation (flux = 4.10^{13} n cm^{-2} sec^{-1}) of metallic mercury (analytical grade from Merck, Darmstadt, Federal Republic of Germany) in the ASTRA-reactor of the Research Centre Seibersdorf, Austria. After treating with concentration HNO_3 aqueous $^{203}Hg(NO_3)_2$ standard solutions were prepared (specific activity 1.9 μ Ci/ml). The initial concentration used in experiments was $8.2 \cdot 10^{-7}$ moles/1.

Measurement of mercury concentration in the system algae/nutrient medium. All experiments were performed at 25 °C under sterile conditions; the medium to be tested (cell suspension or YAP, respectively) was vigorously stirred and a stream of gas (air or nitrogen, respectively) was blown through it at a rate of 1.5 l/h. At certain time intervals 2 ml-samples were withdrawn, transferred into special counting tubes and measured on a single channel analyzer. In each case newly prepared sterilized YAP treated in the same way was used as control.

Cell suspension: The time-related decrease of mercury concentration in the cell suspension was studied on living and dead cells under aerobic (light/air (LA)) and anaerobic (dark/nitrogen (DN)) conditions as well. For DN-experiments using living cells 'preconditioning' (i.e. keeping under conditions of the following experiment) of cell suspension was done for 1 h before adding the heavy metal ion standard. In doing so respiration and photosynthesis were interrupted. Pre-conditioning was not necessary for LA-experiments. Experiments using dead algae required pre-conditioning for DN- and LA-experiments. Dead cells were obtained either by γ-irradiation (0.6 mrad/h, 5 h, ^{60}Co, Institute of Biology, Research Centre Seibersdorf, Austria) or steam sterilization (120 °C, 20 min).

Used YAP: The time-related decrease of mercury concentration was studied on the cell-free nutrient medium already used for cells grown until the end of their log-phase. 2 different methods were applied: 1. After centrifuging the living cells, both heat sterilized YAP (a) and non-sterilized

YAP (b) were tested. 2. The cell suspension was sterilized and the dead cells were centrifuged. In each case mercury was added after 1 h pre-conditioning (LA, DN).

Used YAP + trichloroacetic acid: We also investigated the influence of trichloroacetic acid ($8.2 \cdot 10^{-7}$ moles/l) on the time-related decrease of the mercury concentration in YAP already used for cell growing. Method (lb) was applied.

Studies of volatile mercury compounds. The stream of gas was tested as to the content of volatile mercury compounds it contained after having bubbled through the cell suspension. Experiments on mono- and dimethyl mercury were done on living cells in LA and DN. Experiments on Hg^0 were done on living and dead cells in LA.

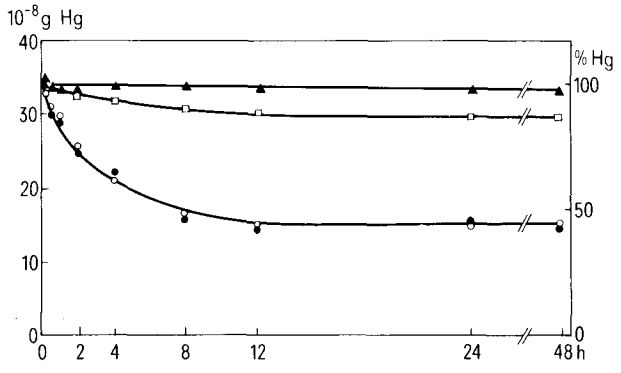
Monomethyl mercury: The stream of gas passed 3 wash bottles each containing 200 ml of a saturated toluene solution of dithizone. After 24 h these solutions were transferred into special flasks and after evaporating the toluene in vacuo to a final volume of 2 ml ^{203}Hg activity was measured in a single channel analyzer. Then the samples were tested by means of TLC. Therefore, the samples were collected in a separatory funnel containing 200 ml toluene and extracted 3 times with 75 ml sodium hydroxide (1% aqueous solution) to remove excess dithizone. The organic phase was washed twice with 50 ml aqua dest., dried over sodium sulphate, transferred into a special flask, concentrated to 1 ml and used for TLC (DC-Fertigplatte, Kieselgel 60 F_{254}, 0.25 mm, Merck, Darmstadt, Federal Republic of Germany). A mixture of n-hexane/acetone (93:7)[19] was used as solvent. The colored spots were scraped off, transferred into counting tubes and their γ-activity was measured.

Dimethyl mercury: While monomethyl mercury reacts fast with dithizone, formation of dimethyl mercury is slow to form the same dithizonate[19,20]. Dimethyl mercury is transformed to monomethyl mercury by the following reaction: $CH_3HgCH_3 + HCl \rightarrow CH_3HgCl + CH_4$. Raising of temperature and addition of copper(II)-chloride increase the reaction rate[8,21-23]. The apparatus for isolating monomethyl mercury was modified; the stream of gas passed 3 wash bottles, each containing 200 ml 5 N HCl + 1% $CuCl_2$ at a temperature of 65 °C before passing through the dithizone solutions. After 24 h 2 ml-samples of the acid solutions were withdrawn and measured in the single channel analyzer. Further treatment of dithizone solutions were analogous to the method described before for monomethyl mercury.

Elemental mercury vapor: Hg^0 was detected by means of atomic absorption spectrophotometry (AAS)[24,25]. 2 methods were used: 1. The stream of air passed a cooling trap. We used carbon dioxide 'snow'/ethanol as cooling agent. After 24 h the trap was heated to 80 °C and connected with the AAS. 2. The Erlenmeyer flask containing the culture was directly connected with the AAS for 4 h.

Studies on methyl mercury in algae and nutrient medium. To determine possibly formed methyl mercury in algae as well as in the nutrient medium, the filtration method of Macka et al.[26] was applied. Algae and filtrate were separately tested for methyl mercury according to a modified method[27] of Zelenko and Kosta[23].

Results and discussion. While in experiments with living cells (LA, DN) the mercury concentration in the cell suspension decreased by about 55% during the first 12 h (figure, a) and remained constant till the end of the experiment (48 h), dead cells (LA, DN) only effected a 1% decrease - analogous to the control (figure, b). The high decrease is ascribed to the metabolization of inorganic mercury to volatile mercury compounds already supposed by Ben-Bassat et al.[17]. Therefore we investigated the stream of gas after having passed the cell suspension to identify the volatile mercury compounds. We emphasized investigations on mono- and dimethyl-mercury because methylation of mercury is known as a detoxification mechanism[16] often to be found in microorganisms[10-12]. We neither found monomethyl nor dimethyl mercury as dithizonates but a dithizonate of inorganic mercury identified by TLC (corresponding to the 1% decrease). By means of AAS we could show that mercury ions were reduced to Hg^0 which does not react with dithizone. In the course of this reduction, no methyl mercury was formed as intermediate compound ($Hg^{++} \rightarrow CH_3Hg^+ \rightarrow Hg^0$): according to the method of Zelenko and Kosta[23] methyl mercury up to 10^{-11} M could neither be detected in the algae nor in the nutrient medium. By centrifugation experiments we found that the reduction takes place extracellularly (figure, a) probably caused by extracellular enzymes: 1. There was no decrease of mercury concentration after sterilization of 'used' YAP (methods 1a and 2) (figure, b). 2. Addition of trichloroacetic acid inhibited the reduction; there was only a 10% decrease of mercury concentration in YAP (figure, c). The corresponding control is equal to figure, b. A similar enzymatic reduction of inorganic mercury ions to Hg^0 was already found for *Escherichia coli*[28]. Reduction of Hg^{++} to Hg^0 by bacteria under these experimental conditions is to be excluded.



Time dependent decrease of mercury concentration. a) ●——● Cell suspension using living cells in aerobic (light/air) or anaerobic (dark/nitrogen) conditions. ○——○ Cell-free nutrient medium already used for cells grown until the end of their log-phase. b) ▲——▲ Control (newly prepared nutrient medium) under conditions of concerning experiment or cell suspension using dead cells in aerobic (light/air) and anaerobic (dark/nitrogen) conditions. Or cell-free nutrient medium already used for cells grown until the end of their log-phase, after sterilization. c) □——□ Cell-free nutrient medium already used for cells grown until the end of their log-phase, after addition of trichloroacetic acid (final concentration $8.2 \cdot 10^{-7}$ M).

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Behaviour of *Drosophila melanogaster* is affected by drugs

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Summary. Reserpine, colchicine and Rb^+ ions exerted specific effects on sexual behaviour and locomotor activity of the fruit fly. Neuroleptics and antidepressants were inactive.

Studies on the action of drugs on *Drosophila* have so far been limited to examination of their toxic and mutagenic properties. The single exception appears to be a recent study on toxicities of neuroleptics to *Drosophila,* in which the effect of the drugs on phototactic movement was also followed[2]. In this communication we present results of an introductory study on the effects of drugs specifically on behaviour of the fruit fly.

Materials and methods. A wild-type strain of *D. melanogaster* N 72 was grown on standard *Drosophila* medium seeded with live yeast at 25 °C in the dark. All experiments were also carried out at 25 °C. Freshly emerged virgin flies were sorted by sex and separately placed into test-tubes with a maintenance medium consisting of 5% glucose and 1% agar. After 3 days were transferred onto a fresh maintenance medium supplemented with the drug to be examined. The drugs were used at concentrations which proved non-toxic or only slightly toxic in preliminary assays. The flies were kept on this drug-supplemented medium for 24 h and then scored for locomotor and sexual activities. The tests were made in a box equipped with artificial dim light from 10.00 to 14.00 h when diurnal fluctuations in behaviour were found to be minimal.

Results and discussion. As shown in the figure, 3 unrelated substances affected sexual behaviour of the flies. The flies fed for 24 h on reserpine exhibited lower mating speed. The decrease in mating speed was essentially due to females: At a low concentration of reserpine, mating speed was almost normal if only males and not females were treated with the drug, and this tendency was apparent at a high concentration of reserpine as well. A reversed situation was observed in the case of flies fed on colchicine or rubidium chloride: Mating speed was almost as high as in the control if only females were treated with the drugs. If the males were treated and brought to copulate with either treated or control females, the mating speed was exceedingly low. Other alcali metal ions, Li^+ (1 mg/ml), Na^+ (up to 100 mg/ml), K^+ (up to 100 mg/ml), Cs^+ (1 mg/ml) did not affect the sexual behaviour of the flies.

An interdependence between mating speed and locomotor activity in *Drosophila* was repeatedly reported[3-5]. In the case studied here, the specific effects of reserpine and

Effect of drugs on locomotor activity of *D. melanogaster*

Drug	Concentration	Sex	Spontaneous activity (% of control)	Jumps (% of control before stimulation) Before stimulation	After stimulation
None (control)		♂	100	100	1857 ± 408
		♀	100	100	1800 ± 810
Reserpine	0.03 mg/ml	♂	149.3± 3.0	285.7± 94.1	928.6± 185.7
		♀	132.0± 13.2	245.5± 115.3	845.5± 397.4
	0.25 mg/ml	♂	168.0± 11.8	78.6± 70.7	880.6± 264.3
		♀	164.5± 3.3	129.1± 56.8	836.4± 368.0
Colchicine	5 µg/ml	♂	85.5± 9.3	380.9± 38.1	1619.1± 161.9
		♀	104.1± 2.1	27.2± 27.0	1009.1± 504.5
	10 µg/ml	♂	65.0± 6.5	0	714.3± 142.8
		♀	46.3± 5.0	0	0
RbCl	3 mg/ml	♂	123.0± 10.2	119.1± 48.2	10,380.9± 2415.2
		♀	20.8± 4.1	27.2± 4.9	1654.5± 185.2

Locomotor activity was assessed by Hay's time-sampling technique[7]. 5 individuals of the same sex were transferred into a Petri dish of 5 cm diameter, left quiet to accomodate for 30 min and then the number of momentarily moving flies were counted at 6-sec intervals for 60 sec. The measurements were repeated 10 times in succession and pooled. Care was observed to avoid the slightest stimulation of the flies either mechanically or optically. To score for stimulated activity, the dish was violently shaken in horizontal position for 6 sec and the number of jumps of all 5 flies within 6-sec intervals were counted for 60 sec. The figures are normalized with respect to controls and expressed with standard mean errors. The sex of males and females is denoted by ♂ and ♀ respectively.