action is monitored[1,16,17]. In conclusion, it may be stated that ouabain potentiates Ca release from SR by changing the ionic environment and that this phenomenon is responsible for the positive inotropic effect of ouabain.

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Purification and properties of a heat-resistant exotoxin produced by *Macrophomina phaseolina* (Tassi) Goid in culture

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Summary. A partially purified preparation of a water-soluble, heat-resistant, nonspecific exotoxin produced by a strain of *Macrophomina phaseolina,* isolated from *Phaseolus mungo* L. could reduce Cu^{++} ions and produced a red colour with 2,4-dinitrophenyl hydrazine reagent. It caused inhibition of seed germination, wilting of cut seedlings, stunted growth of young seedlings and loss of permeability of the cell membrane. Seedlings of *P. mungo,* grown in presence of the toxin showed a slight increase in the contents of protein and total RNA over control, but a significant increase in the specific activities of F-1, 6-BP aldolase and G-6-P isomerase.

An internally seed-borne fungus, found to cause serious damping-off of seedlings of *Phaseolus mungo,* was isolated and identified as *Macrophomina phaseolina* (Tassi) Goid. The fungus produced an exotoxin and was also strongly cellulolytic. Production of a nonspecific extracellular toxin by *M. phaseolina* and its role in pathogenesis and inhibition of seed germination have already been reported by Mathur[1] and Chan and Sackston[2]. The present communication deals with partial purification of the toxin, some of its physical and chemical properties, and some biochemical changes induced by it.

Material and methods. The fungus was isolated from surface sterilized (0.1% $HgCl_2$ solution) seeds of *P. mungo* L. (var. L22) and was brought into pure culture by dilution plating on Czapek's agar. It was identified as *M. phaseolina.* The toxin was produced in Czapek's liquid medium (pH 6.8) by the fungus in stationary culture. The culture filtrate was found to be also strongly cellulolytic. For isolating the toxin, the fungus was grown in 250-ml Erlenmeyer flasks containing 50 ml of the liquid medium at 30 °C for 15 days, and the mycelial mat was removed by filtration through Whatman No. 1 filter paper. The culture filtrate was treated with activated charcoal (Norit, E. Merck, 20 g/l) for 1 h at room temperature. The charcoal with the adsorbed toxin was collected by filtration and dried at 40 °C in an air-oven and then extracted with chloroform. The eluate was filtered and the filtrate was evaporated to dryness under reduced pressure at 30 °C. The residue was dissolved in a minimal quantity of ethanol and diluted with distilled water, filtered to remove insoluble matters and shaken with equal volume of diethylether in a separating funnel to eliminate fatty substances. The aqueous phase was collected and evaporated to dryness under reduced pressure at 80 °C to obtain a viscous liquid. The preparation so obtained was used for determining the properties of the toxin. It was free from cellulolytic activity.

The hypocotyl regions of treated and untreated seedlings were cut out and 1 g (fresh weight) of each was homogenized in a prechilled pestle and mortar with analytical sand. The homogenate was extracted with 5 ml of tris-HCl buffer (pH 7.4) and the extract after centrifugation at 12,000 × g for 10 min was dialyzed against the same buffer for 2 h at 4 °C. The dialyzed extract was used for enzyme assays and for protein determinations. F-1, 6-BP aldolase and G-6-P isomerase were assayed following the methods of Sibley and Lehninger[4] and Nosoh[5], respectively. Protein and total RNA were determined by Folin reagent[6] and the method of Markham[7], respectively. The isozyme patterns of the following enzymes were studied by polyacrylamide gel-electrophoresis, according to the methods of Arnisson and Boll[8]: peroxidase, polyphenol oxidase, catalase, glutamate dehydrogenase and malate dehydrogenase.

Results and discussion. The purified preparation of the toxin was found to be chromatographically homogenous, because it produced a single spot on thin-layer chromatograms using n-butanol:acetic acid:water (8:2:2), or ethanol:ammonia:water (80:5:15) as developing solvents and 5% chromic acid solution as spraying reagent. Paper chromatograms developed with the same solvent systems and sprayed with 2,4-dinitrophenyl hydrazine (DNPH) solution (0.1% in 2N HCl) followed by 10% NaOH solution also showed single spots, reddish in colour and having a R_f value of 0.85. That the spots were due to the toxin was confirmed by eluting parallel spots in distilled water and using the solution for seed germination test. The reaction with the DNPH solution indicated the presence of a carbonyl group in the toxin compound.

An aqueous solution of the toxin had a pH value of 4.8, and it did not lose its toxic property when autoclaved at 121 °C for 15 min. It was found to have reducing property, as indicated by its ability to reduce Cu^{++} to Cu^{+} ions using Somogyi's reagent[3].

The effect of the toxin on seed germination was tested on 4 cultivars of *P. mungo* (L22, H10, B76 and T9) by placing healthy surface-sterilized seeds on filter papers soaked in dilute solution of the toxin in sterile petri dishes. About 90% of the seeds so treated failed to sprout, whereas in the control (without toxin) germination percentage was 90 to 95%. The observation confirms that of Mathur[1].

The toxin caused wilting of cut seedlings of *P. mungo* and tomato. Seedlings (7–10-day-old) were cut under water and transferred immediately to dilute aqueous solution of the toxin in small tubes. Visible wilting started within 1–2 h and cuttings wilted completely within 4–8 h. On placing thin, thoroughly washed beet-root slices in similar toxin solution, the pigment leached out within 15 min indicating that the toxin caused loss of permeability of the cell membrane. On the other hand, intact seedlings did not show any wilting. When surface sterilized seeds of *P. mungo* were grown in dilute sterilized Knop's solution solidified with 1% agar and the toxin was added 3 days after germination, the seedlings showed stunting of growth in comparison to the control, but no wilting. The treated seedlings had also a significant swelling of the base of the hypocotyl.

The treated seedlings showed an increased protein and RNA contents. While the increase in protein content ranged from 5.5 to 10.0% in the different cultivars of *P. mungo*, the total RNA content increased by 9.7–12.2% over the untreated control. Increased protein and RNA contents are known to occur under pathogenic conditions[9,10]. It is of interest to note that similar changes also took place in toxin-treated seedlings, although the changes were of a smaller magnitude.

There were significant increases in the specific activities (enzyme activity/mg protein/min) of the aldolase and the isomerase in the treated seedlings over the control. The aldolase activity increased by 25–55% over control and the isomerase activity, 20–66% in the 4 cultivars studied. The results indicated that the presence of the toxin in the growth medium triggered a higher rate of glucose catabolism by the Embden-Meyerhof pathway. It has been postulated that during actual fungal infection, aldolase activity is stimulated to increase the synthetic rate of phenolic compounds[11]. There was no significant change in the patterns of the isozymes of any of the enzymes studied.

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Pimozide and p-chlorophenylalanine blockade in DL-amphetamine and pargyline-treated rats held at two environmental temperatures

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Summary. In rats, treated with DL-amphetamine + monoamine oxidase inhibitor, and held at an ambient temperature of 28.5 °C, hyperthermia was completely eliminated by treatment with pimozide + p-chlorophenyl-alanine. The same drugs markedly reduced the hypothermic effects in rats treated similarly at 4 °C. Results implied that serotoninergic and dopaminergic neurones were involved in the thermoregulatory effects of amphetamine.

Dexamphetamine, a sympathomimetic amine, has effects on body temperature that depend on ambient temperature[1,2]; hyperthermia develops at 20 and 37 °C and hypothermia at 4 °C. These effects are increased by a monoamine oxidase inhibitor. Changes in thermoregulation appear to be related to the release of dopamine in the central nervous system since they are reduced by pre-treatment with pimozide[1–4], a central dopaminergic receptor blocker[5]. However, other neurotransmitters such as 5-hydroxytryptamine (5HT, serotonin) may be involved in the effects of amphetamine[6–8].

The relationship of body temperature and amphetamine treatment in rats pre-medicated with various combinations of a monoamine oxidase inhibitor (pargyline), a depletor of brain 5HT, (DL-p-chlorophenylalanine, pCPA)[9] and pimozide are reported here.

Materials and methods. Male albino Wistar rats (n = 139) weighing 150–200 g were fed and watered ad libitum, and housed in a room at 21 °C. The room was brightly illuminated from 06.00–18.00 h, the rest of the period being dark. Rats of comparable body weights were grouped into 11 groups (n = 4–17) at each experimental temperature, 28.5 or 4 °C. Each experiment lasted 7.5 h and was done using groups of 13 rats comprising 3 controls and 2 treatment groups of 5 rats. Experiments were made on successive days. Pooled data have been used in the illustrations for control rats and in some other cases, e.g. pargyline + amphetamine treatment at 28.5 °C.

Pimozide (Ethnor Pty Ltd, Sydney, Australia) dissolved in 0.05 M tartaric acid in distilled water to a concentration of 1 mg/ml, was given i.p. at a rate of 10 mg/kg b.wt 3 h before amphetamine. DL-p-Chlorophenylalanine hydrochloride (pCPA, Sigma Chemical Co., St Louis, Mo., USA), suspended in 0.05% methyl cellulose solution, was given i.p. at a rate of 125 mg/kg b.wt each day for 3 days, the last dose being given 2 h before amphetamine. Pargyline hydrochloride (Abbott Lab., Sydney, Australia), dissolved in saline was given s.c. at the rate of 25 mg/kg b.wt 90 min before amphetamine. DL-Amphetamine sulphate (Smith, Kline & French, Sydney, Australia), with an optical rotation of zero, was dissolved in 0.09% saline and given i.p. at a rate of 15 mg/kg b.wt immediately after the rats were exposed to the experimental temperatures. Appropriate vehicles were injected into control rats in all experiments when the effects of the various chemicals were tested. Each animal was held in a restraining tube (23 cm long × 7 cm diameter) and rectal temperatures (Tre) were monitored with thermocouples.