HSDH) and glucose-6-phosphate dehydrogenase (G-6-PDH) as described earlier[12]. The spermatogenetic stages were identified as follows. Stage 0: primary spermatogonia; stage I: secondary spermatogonia, less than 10 cells in a cell nest; stage II: secondary spermatogonia, more than 10 cells in a cell nest; stage III: primary spermatocytes; stage IV: secondary spermatocytes; stage V: spermatids.

It is evident from table 1 that the average testis weight, testis diameter, tubule diameter and Leydig cell nuclear diameter were not affected by the short-term treatment with both 250 µg and 500 µg CPA. However, the quantitative analysis of the spermatogenetic stages (table 2) reveals that the number of cell nests of primary spermatogonia (stage 0) per seminiferous tubule cross section decreased significantly ($p < 0.001$) in the experimental groups. Thus, the development and/or proliferation of primary spermatogonia seems to be androgen dependent in *R. cyanophlyctis*. The other cell nests (stage I-V) were unaffected indicating, albeit indirectly, that androgens play a role in the early stages of spermatogenesis rather than the later stages in this species. In *Rana esculenta*[5,7] and *R. temporaria*[6] CPA blocked the formation of spermatids. However in the former species[5] spermatogonial multiplication was also partially affected. In the latter species[6], the author's conclusion that earlier stages of spermatogenesis are not affected is not supported by the quantitative and statistical analysis of the cell nest counts. To the best of our knowledge, the present work is the first report that shows the involvement of androgens at a very early stage of spermatogenesis in Amphibia.

In *R. temporaria* a regression of Leydig cells following CPA treatment has been reported[6]. However, in the present work on *R. cyanophlyctis* the Leydig cell nuclear diameter, 3β-HSDH and G-6-PDH enzyme activities remained relatively unaffected (table 1) possibly due to the short-term treatment with CPA. The androgen dependent thumb pads regressed significantly ($p < 0.001$, table 3) due to CPA treatment as reported in *R. esculenta*[2].

Table 3. Effect of cyproterone acetate on the thumb pad of *R. cyanophlyctis*

Group	Average height (µm ± SE) Epidermis	Glandular epithelium
Control	88 ± 3	23 ± 0.6
Treated with 250 µg CPA	67 ± 2 $p < 0.001$	15 ± 0.2 $p < 0.001$
Treated with 500 µg CPA	69 ± 2 $p < 0.001$	15 ± 0.7 $p < 0.001$

p-Values calculated by Student's t-test between control and experimental groups.

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Selective fungitoxicity of cyclohexylamine and related aromatic amines

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Summary. Cyclohexylamine, 10^{-1} mole/l, selectively inhibited the growth of various fungi in culture. *Pythium ultimum* was highly sensitive; *Fusarium oxysporum* was more tolerant. Phenylethylamine was toxic to these 2 fungi at lower concentrations than cyclohexylamine and hydroxiphenylethylamine.

The toxicity of cyclohexylamine (CHA) to mammals is well known, and the possible mutagenicity of neutralized CHA for human chromosomes has been widely discussed[2,3]. The phytotoxicity, and an apparently selective fungitoxicity, of volatile CHA have been demonstrated[4]. This study reports the effects of neutralized CHA and the closely related 2-phenylethylamine (PEA) and 2-(p-hydroxiphenyl)ethylamine [tyramine] (PEA-OH) on the mycelial growth of fungi in pure culture.

Material and methods. Single strains of thirteen species of fungi were selected from our collection. Solutions of CHA, PEA, or PEA-OH (analytical grade), neutralized or slightly acidified to pH 6.8 with 1 mole HCl/l, were sterilized by filtration and aseptically added to an autoclaved, concentrated culture medium. Final composition of this medium was: sucrose, 10 g; vitamin-free casamino acids (Difco), 2 g; yeast extract (Difco), 1 g; $MgSO_4 \cdot 7\ H_2O$, 0.5 g; $FeSO_4 \cdot 7\ H_2O$, 0.01 g; oligoelements[5], 1 ml; KH_2PO_4/K_2HPO_4 buffer (pH 6.8-7.0), 0.05 mole/l; adjusted to 1 l with distilled water. 50-ml portions of the final growth medium were distributed into 200-ml Erlenmeyer flasks. Inoculation was made with 0.1 ml of a suspension of conidia (10^5 spores/ml). For non-conidial species, inoculations were made with a disc (diameter 6 mm) obtained from a young culture on water agar (for *Pythium*) or water agar supplemented with 0.5% Difco malt extract. Cultures were kept at 25 °C in the dark and orbitally agitated (125 rpm). Dry weights of triplicate cultures were determined as

described previously[6]. Experiments were repeated 3 times, successively. The repetitions of a same expriment gave similar results. Therefore, the table and the figure present data from single, typical experiments.

Results. a) Effects of CHA on various fungi. Preliminary experiments showed that $2 \cdot 10^{-2}$ mole/l of CHA inhibited the growth of *Pythium ultimum, Phomopsis sclerotioides,* and *Rhizoctonia solani.* A concentration of 10^{-1} mole/l inhibited *Fusarium oxysporum* and *Gliocladium roseum* only slightly. Thus the selective fungitoxicity of CHA was studied with a concentration of 10^{-1} mole/l. As shown in the table, the most sensitive species were *Alternaria radicina, Cylindrocarpon destructans, G. virens, Paecilomyces farinosus, Ph. sclerotioides, Trichoderma viride, P. ultimum, Phytophthora citricola,* and *R. solani. Fusarium oxysporum, F. solani,* and *Aspergillus flavus* were the most tolerant, whereas *G. roseum* gave an intermediate response.

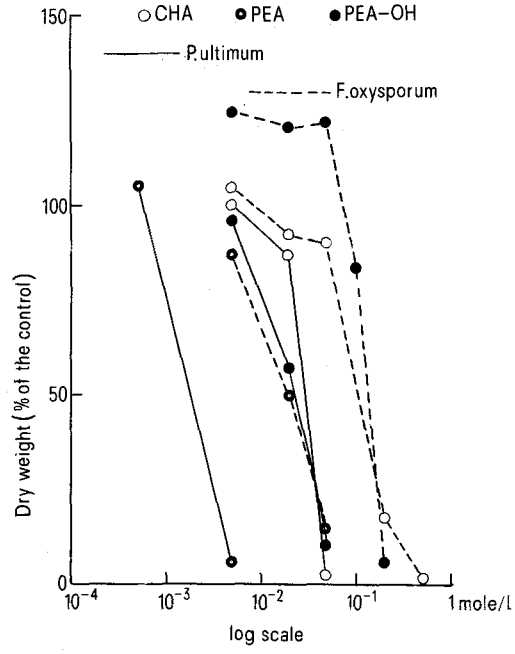
b) Effects of different concentrations of CHA, PEA and PEA-OH on one strain of Pythium ultimum and Fusarium oxysporum. Theses species were selected because of their great taxonomic differences, their importance as plant pathogens, and their opposite responses to CHA. The effects of concentrations from $5 \cdot 10^{-4}$ to $5 \cdot 10^{-1}$ mole/l of the 3 aromatic amines on the growth of both species are presented in the figure. A concentration of $5 \cdot 10^{-3}$ mole/l of PEA inhibited *P. ultimum.* The inhibitory concentration was $5 \cdot 10^{-2}$ mole/l for CHA and PEA-OH. *F. oxysporum* tolerated higher doses: inhibitory levels were $5 \cdot 10^{-2}$ mole/l for PEA, and $2 \cdot 10^{-1}$ mole/l for CHA and PEA-OH, CHA being the least toxic at this concentration. *F. oxysporum* was stimulated by concentrations of PEA-OH lower than 10^{-1} mole/l.

Discussion. In this study, neutralized CHA, PEA, and PEA-OH showed a selective fungitoxicity. These results complete previous data on the effects of volatile CHA[4]. Of the 3 compounds examined, PEA was the most toxic to *P. ultimum* and *F. oxysporum.*

The toxicity of the amines to living things is well known. The fungitoxicity of CHA has been evaluated for the control of electro-insulating materials[7]. Selective toxicity of anilines to blue-green algae has been reported[8]. However, the selective effects of the aromatic amines examined in this work were less well known. *Aspergillus niger* was inhibited by 0.1 g/l of an hypotensive compound considered by some authors as a polymer of CHA[9]. In this study, *P. ultimum,* one of the most sensitive fungi tested, was strongly inhibited by similar doses of PEA (0.06 [$= 5 \cdot 10^{-4}$ mole/l]–0.6 g/l [$= 5 \cdot 10^{-3}$ mole/l]).

Conjugation of a hydroxylated benzene ring with sugars represents an important pathway of detoxification of aromatic exogenous compounds by plants and animals[10,11]. It is interesting to note that PEA-OH was obviously less fungitoxic than PEA in this study (see fig.). It is known that aromatic amines such as PEA and PEA-OH may bind to single-stranded polyadenylic acid and decrease its circular dichroism amplitude[12]. The fungitoxicity of these aromatic amines may thus be partly related to their interference with protein synthesis.

An interesting use of these findings might be the improvement of selective media for isolating certain fungi. In



Effects of neutralized cyclohexylamine (CHA), phenylethylamine (PEA), and hydroxiphenylethylamine (PEA-OH) on the growth of *Pythium ultimum* and *Fusarium oxysporum* in liquid culture. Controls were grown in medium without amines.

Effect of 10^{-1} mole/l cyclohexylamine (CHA) on the growth of 13 species of fungi in liquid medium

Fungi	Dry weight[a] −CHA (control) (mg)	+CHA (mg)	Percent of control	Age of cultures (days)[b]
Alternaria radicina	68.2±8.9	1.5±1.5	2	6
Aspergillus flavus	206.0±2.7	179.9±9.2	87	6
Cylindrocarpon destructans	57.0±16.8	4.9±1.2	9	9
Fusarium oxysporum	179.9±7.7	178.1±27.2	99	4
Fusarium solani	63.0±18.3	47.0±1.5	75	5
Gliocladium roseum	152.2±14.2	65.2±13.8	43	9
Gliocladium virens	154.5±12.1	3.5±0.1	2	9
Paecilomyces farinosus	117.6±15.7	0.0±0.0	0	6
Phomopsis sclerotioides	210.0±42.4	0.0±0.0	0	10
Phytophthora citricola	79.2±6.8	7.5±0.5	9	9
Pythium ultimum	250.3±5.4	9.2±1.6	4	4
Rhizoctonia solani	211.3±16.2	14.6±10.9	7	10
Trichoderma viride	151.0±7.5	0.3±0.4	0	4

[a] Mean ± SE of 3 cultures in 50 ml medium. [b] Age determined as optimal (i.e. in the exponential phase of mycelial growth) for each taxon in separate experiments under the same environmental conditions.

preliminary dilution plate counts, high soil populations of *Chrysosporium* spp. were not recovered when nutritive agar contained 5 g/l of neutralized CHA ($\approx 5 \cdot 10^{-2}$ mole/l), whereas *Aspergillus, Fusarium, Penicillium* spp. as well as members of the Mucoraceae, and a grey sterile fungus, were isolated frequently when the CHA concentration was 20 g/l ($= 2 \cdot 10^{-1}$ mole/l). At intermediate concentrations, neutralized CHA eliminated species of *Acremonium, Gliocladium, Myrothecium, Paecilomyces,* and *Trichoderma.* It would also be interesting to know whether different strains of fungal species exhibit marked differences in their sensitivity to the aromatic amines examined in this study.

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Low temperature-induced contracture of depolarized smooth muscle and the effects of calcium and multivalent cations[1]

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Summary. Lowering of temperature caused tension development in the smooth muscle of the guinea-pig vas deferens, which was dependent on extracellular Ca. Mn^{2+} and La^{3+} reversed the effect and induced phasic contraction on rewarming.

It has been reported in skeletal and smooth muscle that rapid cooling caused contracture due to the release of intracellular Ca^{2}[2,3]. The contracture is also observed in depolarized preparations in which a change in membrane potential by cooling can be excluded. However, in the depolarized smooth muscle, the final effect of cooling varies with the type of smooth muscle. In the smooth muscle of *Taenia coli* and longitudinal muscle of the stomach of the guinea-pig, relaxation of depolarized preparations has been reported[4,5]. On the other hand, the smooth muscle of the urinary bladder and circular muscle of the stomach show a contractile response to lowering of temperature[5,6].

We have also observed that the smooth muscle of the guinea-pig vas deferens shows an increase in tension when cooling treatment is applied in the course of the tonic phase of contracture induced by high potassium concentration[1,7]. The present experiments were performed to investigate the effects of Ca and multivalent cations on the cooling-induced contracture of depolarized smooth muscle of the guinea-pig vas deferens.

Vasa deferentia were dissected from the abdomen and longitudinal preparations were made using the prostatic one-third of the vasa. The preparations were mounted in an organ bath filled with modified Tyrode solution of the following composition: NaCl, 137 mM; KCl, 2.7 mM; $CaCl_2$, 2.0 mM; $MgCl_2$, 1.0 mM, $NaHCO_3$, 11.9 mM; NaH_2PO_4; 0.4 mM; glucose, 5.6 mM; equilibrated with a gas mixture of 95% O_2 and 5% CO_2. The depolarizing solution (K-Tyrode solution) was made by replacing all NaCl in the solution with KCl. When Mn^{2+} and La^{3+} were used, Tris-buffer was used instead of bicarbonate buffer and the solution was gassed with 100% O_2. The change in pH due to the temperature alteration was not corrected, since a similar change in pH had no significant effect on the tonic contraction. In addition, no obvious difference in the tension development by cooling was observed between bicarbonate buffer and Tris-buffer, which is known to be more affected by the change in temperature than the former.

The developed tension was observed isometrically by a mechano-electronic transducer. The changes in membrane potential and membrane resistance were studied by the double sucrose-gap method[8]. The temperature was controlled by changing the temperature of the water surrounding the organ bath or the tube of test solution in the double sucrose-gap apparatus.

High-K induced contracture of the vas deferens was composed of a transient phasic contraction and a tonic contraction which was sustained for longer than 1 h without obvious change in tension. When the cooling treatment was applied during the course of the tonic phase, an increase in tension was observed; the membrane showed a slight further depolarization and the membrane resistance increased. By rewarming the preparation, the membrane potential and resistance returned to the initial level and the developed tension returned to the level of the tonic contraction. Although tension development by cooling was not observed in normal Tyrode solution (in contrast to other smooth muscles), this may be due to the difference in the speed and/or degree of cooling.

Since the highest tension development was obtained by cooling the preparations from 35 °C to 20 °C, most of the experiments were performed by lowering the temperature to 20 °C. At this temperature, the maximum tension was $179 \pm 10.6\%$ (mean $\pm$ SE, $n = 5$) of the control tonic contraction at 35 °C.

The tonic tension disappeared and no tension development